Hematology Profile of Guinea Pigs [*Cavia porcellus* (Linnaeus, 1758)] Based on Sex and Age

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ABSTRACT
Guinea pigs or *Cavia porcellus* (Linnaeus, 1758) is small herbivorous rodent from South America. Guinea pigs (GP) are commonly used as experimental animals, pets, food, and animal-assisted therapy (AAT). Based on these benefits, the health of GP must be considered. Blood is essential biological sample as it describes the physiological condition and health status of the individual. Publication of the hematological profile of GP reared in foreign countries is numerous, otherwise Indonesia is very limited. This study aimed to provide data on complete blood counts (CBC) of GP based on sex and age. Sample animals were sourced from the local animal market in Yogyakarta. Sex and age were determined based on body weight and the development of the external genital organs. Blood sampling was collected from the orbital sinus after seven days of acclimatization in our animal facility. Assessment of CBC was performed using fully automated hematology analyzer Sysmex®XP-100. Results showed differences (some of which were significant) in the profile of erythrocytes, leukocytes, and thrombocytes between individuals of different sexes and ages. Our results are following the hematological profile of GP from references; however, we found some discrepancies and uniqueness in our work due to different environmental conditions, care management or husbandry as well as disease and nutrition.

Keywords: *Cavia porcellus, Complete blood count, Guinea pig, Health status, Hematology reference values.*

1. INTRODUCTION

Guinea pigs or *Cavia porcellus* (Linnaeus, 1758) are called "marmut" in Bahasa Indonesia. Based on morphological and phylogenetic studies, guinea pigs (GP) we know today are derived from the domestication of the wild type originated from Central Andean Mountains, South America, specifically the Peruvian highlands over 2500 years ago [1]. Local inhabitants use them as a daily food source, which the practice continues today [2]. Since then, GP has been distributed worldwide and become one of the popular exotic pets [3], and laboratory animals [4]. Recently, GP is involved in animal-assisted therapy (AAT) for physical and mental treatments as an alternative for patients who are arduous to treat with conventional therapeutic methods [5]. Regarding those uses of GP, appropriate care methods should be taken to keep their health. This animal lives in the wild as prey species based on their life history. Therefore, they do not exhibit early clinical signs until the illness becomes severe and too late to treat. Most health problems in domestic GP are related to maintenance factors in husbandry, especially nutrition and hygiene [6].

Hematology studies blood components, particularly blood cells and bone marrow, as the primary site of blood cells formation or hematopoiesis. One of the essential hematological examinations is routine complete blood count (CBC) that provides information about the number, composition, and structure of red blood cells (RBC or erythrocytes), white blood cells (WBC or leukocytes), and platelets (PLT or thrombocytes). Changes in their values describe certain physiological conditions [7]. Hematology profile can also be used in pathological diagnosis and screening diseases [8]. Hematological profile in small mammals

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like rats, mice, hamsters, gerbils, GP, chinchillas, rabbits, ferrets, and hedgehogs are influenced by many factors, such as strain, breed, sex, age, exercise, circadian cycle, nutrition, as well as pathologic factors [9].

Hematological profile of GP as pets has been published [10], consisting of adult males, adult females, and youngs in general without distinguishing between sex. Hematology profile of GP based on sex and age has been published [11]; however, samples were from Dunkin Hartley (albino). Hematological profile of non-albino or pigmented GP has been printed using specific strain 13/N breed [12] and Weiser-Maples [13], demonstrated that values are also associated with sex and age. However, those publications are all from foreign countries. There is only one publication of the hematological profile of GP in Indonesia [14]. Data are still presented in general, not categorized based on sex and age, and is old publication. Therefore, this study aimed to update and complete the hematological profile of GP based on sex and age. We hope that our work can be used as a baseline to establish the diagnosis of diseases and monitoring health conditions, as well as for reference or control in research.

2. MATERIALS AND METHODS

2.1 Ethical Clearance

The Research Ethics Committee of The Integrated Research and Testing Laboratory, Universitas Gadjah Mada (LPPT-UGM) approved all procedures related to the care and use of animals in this study, issuing Certificate of Ethical Clearance No. 00061/04/LPPT/VIII/2016.

2.2 Animals

Sample animals were sourced from the local animal market at Yogyakarta (PASTY). We chose GP of American/English type from available breeds for homogeneity. Sex and age were determined based on body weight and the development of external genital organs [15]. Young individuals are the ones with weight <350 grams, whereas adults have weight ≥ 350 grams [16]. We categorized them into four groups: young male (YM), adult male (AM), young female (YF), and adult female (AF). Each group consisted of sixteen individuals. Animal was acclimatized for a week in our animal facility (Animal House, Faculty of Biology UGM) to recover from transportation stress and habituation to the new environment [17]. We fed them with vegetables and fruits to match with the original conditions.

2.3 Blood samples

Blood sampling was performed after seven days (a week) of acclimatization. Animals are considered acclimatized when they are no longer stressed, as evidenced by their natural behavior [17]. Blood samples were withdrawn from the orbital sinus, collected in EDTA-coated microtubes. Animals were anesthetized using Ketamine 50 mg kg and Xylazine 5 mg/kg (IM) before bleeding [18]. Complete blood count (CBC) was performed automatically using hematology analyzer Sysmex®XP-100. Variables of hematology consisted of: **(1) Erythrocyte profile:** Erythrocyte count (RBC, x10^6/μL), Hematocrit (HCT, %), Hemoglobin (HGB, g/dL), Mean corpuscular volume (MCV, fL), Mean corpuscular hemoglobin (MCH, pg), Mean corpuscular hemoglobin concentration (MCHC, g/dL), erythrocyte distribution width (RDW-SD, fL); **(2) Leukocyte profile:** Total white blood cell count (WBC, x10^3/μL), Neutrophil count (NEU, x10^3/μL), Lymphocyte count (LYM, x10^3/μL), Monocyte-Eosinophil-Basophil count (MXD, x10^3/μL), Neutrophil percentage (NEU, %), Lymphocyte percentage (LYM, %), Monocyte-Eosinophil-Basophil percentage (MXD, %), ratio of neutrophil and lymphocyte (N/L); **(3) Thrombocyte profile:** Thrombocyte count (PLT, x10^3/μL), plateletcrit (PCT, %), mean platelet volume (MPV, fL), platelet distribution width (PDW, fL), platelet-large cell ratio (P-LCR, %).

2.4 Data analysis

Data were tabulated in Microsoft®Office®Excel®2019. Statistical analysis was performed based on one-way ANOVA followed by Tukey's HSD test (α=0.05) using IBM®SPSS®v.23. In addition, to determining the effect of sex and age on each variable of hematology profile, the analysis was undertaken to identify any significant interaction effect between variables [19]. Data were expressed as "mean±standard deviation." Reference intervals were constructed from the lowest and highest values for each variable as baseline [20].

3. RESULTS AND DISCUSSION

Figure 1. Colony of guinea pigs of American/English breed we used in this research

Based on length, texture, and direction of hair growth, GP can be classified into three main varieties as "types" or "breeds": (1) American/English, which have short, smooth, straight hair; (2) Abyssinian, also have short hair but coarser, and there are multiple hair growth centers (crown), so that hair appears to form rosettes; (3) Peruvian, which has long and silky hair [21]. When crossed with each other, these three breeds will produce offspring with various hair types, a mixture of their parents, or similar to the dominant parents.

In addition, to considering the homogeneity of samples, we chose American/English breed because it is the most frequently used in scientific activities. Abyssinian and Peruvian GP are mostly kept as pets due to their attractive appearance. The sample individuals we employed in this study are shown in Figure 1.

The actual age of GP used in this research cannot be known because they were not obtained through our breeding program but rather from the animal market. The term "age" in this study refers to the age of sexual maturity, which is divided into two categories: young (juvenile) and adult (mature). The size and position of testicles, the form of the scrotum, and the shape of the penis can all be used to determine male maturity in boars (male GP). Meanwhile, the vaginal opening, which occurs solely during the estrus phase, can be used to determine female maturity in sows (female GP) [15].

3.1 Erythrocyte profile

The shape of erythrocyte in GP is biconcave, identical to which in humans, size and volume are also relatively similar [22]. This species has the largest erythrocyte compared with other laboratory animal species [10]. Results as shown in Table 1, demonstrated that young GP has higher erythrocyte counts than adults. Young male GP also have higher values of hemoglobin and hematocrit than adults. However, the situation was in opposite in females. In rodents, males generally have a higher erythrocyte counts than females [23], similarly in GP [24]. However, in this study, results showed the contrary. In addition to sex and age, hematological values are influenced by physiological, psychological, and pathological status, environmental conditions, nutrition, care management, and husbandry [7].

Table 1. Comparison of body weight and hematology profile (complete blood count, CBC) in guinea pig [*Cavia porcellus* (Linnaeus, 1758)] from local animal market at Yogyakarta (PASTY).

VARIABLES	MALE		FEMALE	
	Young	Adult	Young	Adult
	n=16	n=16	n=16	n=16
BODY WEIGHT (g)	242.64±57.77 ^{a}	482.91±49.91 ^{b}	246.70±67.78 ^{a}	477.19±72.25 ^{b}
ERYTHROCYTE PROFILE				
RBC# (×10^{6}/µL)	4.82±0.16 ^{a}	4.76±0.22 ^{a}	5.02±0.48 ^{b}	4.97±0.43 ^{ab}
HGB (g/dL)	12.42±0.49 ^{a}	12.24±0.80 ^{a}	13.26±1.15^{ab}	14.19±0.91^{b}
HCT (%)	41.89±2.46 ^{b}	38.29±2.16 ^{a}	40.71±3.46 ^{ab}	41.54±2.49 ^{b}
MCV (fL)	79.06±2.40 ^{a}	80.38±1.71 ^{ab}	80.64±1.46 ^{ab}	83.65±0.57 ^{b}
MCH (pg)	25.79±0.46 ^{a}	25.63±0.74 ^{a}	26.21±0.68 ^{ab}	28.41±0.50 ^{b}
MCHC (g/dL)	30.12±0.79 ^{a}	31.88±0.65 ^{ab}	32.47±0.28 ^{ab}	33.98±0.65 ^{b}
RDW-SD (fL)	53.98±3.93 ^{b}	39.74±0.55 ^{a}	43.30±3.05 ^{ab}	44.76±0.87 ^{ab}
LEUKOCYTE PROFILE				
WBC# (×10^{3}/µL)	6.78±1.46 ^{b}	5.71±2.62 ^{a}	6.40±2.54 ^{b}	5.38±5.70 ^{a}
NEU# (×10^{3}/µL)	2.93±1.45 ^{a}	2.28±1.44 ^{a}	3.41±2.16 ^{ab}	4.38±4.71 ^{b}
LYM# (×10^{3}/µL)	4.68±1.58 ^{b}	3.09±1.78 ^{ab}	2.99±1.28 ^{b}	1.04±1.86 ^{a}
MXD# (×10^{3}/µL)	0.00±0.00 ^{a}	0.11±0.02 ^{ab}	0.00±0.00 ^{a}	0.32±0.04 ^{b}
NEU% (%)	44.21±5.31 ^{ab}	39.70±3.89 ^{a}	48.28±5.40 ^{b}	43.61±4.78 ^{ab}
LYM% (%)	55.75±5.32 ^{ab}	56.36±3.33 ^{b}	51.67±5.37 ^{a}	58.23±5.26 ^{b}
MXD% (%)	0.00±0.00 ^{a}	2.43±0.50 ^{ab}	0.00±0.00 ^{a}	0.42±0.05 ^{b}
N/L	0.74±0.41 ^{a}	0.79±0.49 ^{a}	1.08±0.24 ^{ab}	2.47±0.38 ^{b}
THROMBOCYTE PROFILE				
PLT# (×10^{3}/µL)	250.05±81.56 ^{a}	283.14±66.83 ^{ab}	280.88±100.17 ^{ab}	450.11±97.33 ^{b}

PCT (%)	0.21±0.05 [a]	0.20±0.04 [a]	0.18±0.04 [a]	0.34±0.02 [b]
MPV (fL)	8.03±0.25 [b]	7.22±0.41 [ab]	6.90±0.18 [a]	7.05±0.09 [a]
PDW (fL)	9.35±0.49 [b]	7.48±0.29 [a]	7.30±0.31 [a]	7.98±0.11 [a]
P-LCR (%)	13.53±0.52 [b]	8.76±2.33 [ab]	6.75±0.89 [a]	5.93±0.56 [a]

Note: Values are expressed as the mean±SD, ended with different superscript letters in a row for each variable indicate significant differences ($p<0.05$) from each other based on ANOVA test followed by Tukey's HSD test ($\alpha=0.05$).

Different from other rodents, GP has a unique erythrocyte profile. Even in normal or healthy individuals, their erythrocytes are unequal in size or anisocytosis [25]. This condition results in the erythrocyte indices (MCV, MCH, can MCHC) which also vary. Young male GP have a higher value of anisocytosis than the adults, indicated by a significant RDW-SD value. Meanwhile, the variation in erythrocyte size is not substantial in young and adult female GP.

3.2 Leukocyte profile

The types and functions of peripheral leukocytes in GP are similar to those in humans and other rodents, consisting of monocytes, lymphocytes, eosinophils, basophils, and neutrophils. Neutrophils in GP are referred to heterophils or pseudoeosinophils based on their characteristics as a response to Wright-Giemsa stain [10]. Our hematology analyzer detects monocytes, eosinophils, and basophils as a group or mixed cells (MXD). This is because their numbers are tiny compared to neutrophils and lymphocytes.

As shown in Table 1, young GP of both sexes have higher leukocyte count than adults, predominant by neutrophils. In adult GP, there is a shifting that lymphocyte becomes the main type, indicated by the increase of N/L value, similar to other rodents [9,25]. Adult GP of both sexes produces a significant number of different leukocyte types: monocytes, eosinophils, and basophils.

Sex and age affect immune responses and susceptibility to infections [26]. The older they get, the more exposed to various antigens. Therefore, their body will respond by producing several types of leukocytes to strengthen their immunity. Males are more susceptible to infection than females [26]. Data showed that males have a higher leukocyte count than females, which lymphocyte is significantly the highest percentage among all types of leukocytes. Therefore, they have a lower N/L value than females. To provide a better explanation, animals need to challenge with antigens to generate an immune response, which can then be compared between sexes.

3.3 Thrombocyte profile

Compared to the blood of humans and other rodents, GP blood coagulates faster with the result that the number of thrombocytes counted is relatively lower due to the clotting process [27]. The thrombocyte count in this sudy is following references [10-13]. Structural and physiological of thrombocyte in GP resembles that of humans [28]. Thrombocytes in GP show aggregation as in humans; this behavior is not found in rats and rabbits. Therefore, GP is suitable as a model in hemostasis research [27].

Results, as shown in Table 1, demonstrated that young male GP have a lower number of thrombocytes than adults, while other variable's values were higher. This result contrasts to that of female GP, where adults have a higher value than youngs. In humans, sex and age play a role in determining thrombocyte count, which females have higher counts than males. Thrombocyte count declined by age [29]. This also applies in GP, that both young and adult female GP have higher thrombocyte count than males. However, instead of declines, thrombocyte count in GP increased by age.

Values of thrombocyte profile are strongly influenced by the technique of phlebotomy, which commonly generates falsely low count. The number of thrombocytes in a blood sample may decrease when the blood collection process is not smooth, or bleeding occurs during sampling. Those activate thrombocytes to aggregate or partially clotted as a hemostatic mechanism [30].

In comparison to the references [10-14], the hematological profile of our GP exhibit relatively different values. One explanation is that the sample animals come in a variety of breeds. Although Zimmermann *et al.* [10] and Smith and Mangkoewidjojo [14] worked with similar GP breed type as we did (the American), however under different conditions and with additional care approaches. The guideline for the care or maintenance of GP and other laboratory animals are standardized [31]; however, the care methods, including housing, environmental factors, husbandry, nutrition, health monitoring, and disease management, are varied in each animal facility. Furthermore, evaluation methodologies and equipment have an impact on the outcomes. As a result, we propose that local reference values should be established based on where the animals are raised or reared.

The hematology profile of GP is associated with sex and age. Some of our findings are following the hematology profile of GP from references; however, we discovered some discrepancies and uniqueness in our work. This is because hematological values are influenced by many factors, including environmental conditions, care management or husbandry, as well as disease and nutrition. Because the number of publications on hematological profiles in GP based on sex and age is still limited, particularly in Indonesia, we hope this study will provide and complete the database.

AUTHORS' CONTRIBUTIONS

LF designed and directed the project; SL, ANI, NPW, and ASW carried out the experiment and data collection; LF supervised SL and ANI to perform data analysis. LF, NPW, and ASW contributed on additional references to support data provided by SL and ANI. LF wrote the paper with input from all authors.

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