

# Evaluation of the Haematology Profile and Blood Chemistry of *Macaca nemestrina* (Linnaeus, 1766) at Primate Research Center, IPB University

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## Abstract

*Macaca nemestrina* (Linnaeus 1766) or pig-tailed macaque, is a primate successfully bred by Primate Research Centre (PRC) IPB University to conserve and use as a model animal in biomedical research. Pig-tailed macaque with ID. Tattoo 6180 is a male pig-tailed macaque  $\pm$  23 years old in a coral cage B which focuses on breeding purposes in captivity to produce offspring. Health evaluation of ID. Tattoo 6180 becomes one of the important things related to that goal. This study aimed to measure the haematological and blood chemistry profile of pig-tailed macaque with ID. Tattoo 6180. The method used in this research is descriptive observational to identify blood cell composition, leukocyte differentiation, and blood chemistry. Based on the hematologic profiles measurement results, it is known that the pig-tailed macaque can be diagnosed as having leukopenia (WCB value  $7.5 \times 10^3/\mu\text{l}$ ), hyperchromic macrocytic or macrocytic with stress (MCH and MCHC value 23.4 pg and 33.2 g/dL, respectively), thrombocytopenia ( $231 \times 10^3/\mu\text{l}$ ). The results of the differential leukocyte examination showed that the N/L value was 4.68, which indicated stress. In addition, blood chemistry measurements show that the pig-tailed macaque can be diagnosed with a muscle injury or heart inflammation (SGOT/AST value 119.1 U/L) and hypercholesterolemia (Cholesterol value 87 mg/dL). Results examination showed that the pig-tailed macaque with ID. Tattoo 6180 was diagnosed as macrocytic without anaemia, leukopenia, stress, thrombocytopenia, muscle injury/heart inflammation, and hypercholesterolemia. Further examination is needed to establish the pig-tailed macaque's health status.

Key words: blood parameters, hyperchromic macrocytic, pig-tailed macaque, stress, thrombocytopenia

## 1. Introduction

*Macaca nemestrina* (Linnaeus, 1766), or pig-tailed macaque, is a primate species spread in Indonesia, Malaysia, Brunei, Thailand, and Singapore (introduced). Based on the IUCN red list evaluation as of 2020, the conservation status of the pig-tailed macaque is categorised as vulnerable (IUCN, 2020). According to Anggraeni *et al.* (2009), the decline in the population of pig-tailed macaques is caused by habitat fragmentation, reaching 49% of the total habitat. Therefore, a conservation strategy for conducting captivity is very necessary. In addition, the high demand for Pig-tailed macaques as model animals in research provides an impetus to carry out captivity breeds.

Primate Research Center (PRC) is one of the research and community service institutions belonging to IPB, which was founded in 1990 based on the Decree of the Chancellor of IPB No.080/C/1990. One of the objectives of PRC IPB University is to provide advice in developing strategies, objectives, and policies in conservation areas and using primates as animal models in biological and biomedical research. PRC IPB University, accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), is one of the institutions that can meet these challenges. One of the primates successfully bred by PSSP IPB in captivity is *Macaca nemestrina* (Linnaeus 1766).

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PRC IPB University has conducted ex-situ captive breeding of Pig-tailed macaques to be bred as model animals. Pig-tailed macaque can be used as a model animal because it can provide an overview and conditions in humans or other species to understand and identify biological, physiological, and pathological phenomena (Sajuthi *et al.* 2016). It is widely used as a model animal in comparative neurobiology (Preuss *et al.* 1997), foetal development (Heath-Lange *et al.* 1999), and vaginal/rectal physiology, as well as in response to sexually transmitted disease transmission (Joag *et al.* 1997; Patton *et al.* 2004).

All procedures for captive breeding and use of animals carried out by PRC IPB University have obtained approval and are under the supervision of the Commission for the Welfare and Use of Research Animals (KPKPH) PRC IPB University with no. #08-B03-IR (Anggraeni *et al.* 2009). The pig-tailed macaques were housed in groups in a coral cage measuring 15×8×3 m with a male-to-female ratio of 1:10. Cage conditions are enriched in such a way as to provide opportunities for animals to behave naturally and reproduce.

Pig-tailed macaque with ID. Tattoo 6180 is a male (23 years) in a coral B cage. Coral B cage is a cage that focuses on breeding purposes in captivity to produce offspring. Health evaluation of ID. Tattoo 6180 becomes one of the important things related to that goal. Tattoo 6180 is the only male in the cage. In addition, based on internal PRC IPB University, ID. Tattoo 6180 has succeeded in breeding several times to produce offspring, even though he is already categorised as old age. Therefore, the health evaluation of ID. 6180 Tattoos become everyday essentials.

Evaluation of the physiological condition of ID. Tattoo 6180 can be used as a benchmark for developing breeding potential and evaluating current health conditions (Hasanah 2019). One of the basic data that can be used to determine and evaluate

the physiological condition of animals is blood parameters. Blood parameters can be used as a basic reference for further examination to support the diagnosis of a disease (Garcia-Feria *et al.* 2017).

Examining blood parameters includes evaluating the haematological and blood chemistry profiles against normal values (Canales-Espinosa *et al.* 2015). Evaluation of the haematological profile can be used as a reference in detecting haematological abnormalities seen from the number, composition, and function of blood cells (Hasanah 2019). In addition, blood chemistry evaluation can be used to detect and diagnose problems or abnormalities in organ function seen from the composition and concentration of products in plasma or serum (Tizard 2017). Balance disorders in blood parameters can indicate that the animal has certain health problems (Hasanah 2019).

Based on that background, this study aims to determine the health condition of the pig-tailed macaque with ID. Tattoo 6180 through evaluation of haematological and blood chemistry profiles. The evaluation was carried out to screen the current health condition of the animal as the only male in coral B cage. In addition, the evaluation results will be used as a reference in further examination and as a support for diagnosing a disease.

## 2. Materials And Methods

This research was carried out in two laboratory facilities of PRC IPB University, namely the Pathology Laboratory and Research Animal Facilities Lodaya (RAF-L). The research was carried out in January-March 2021. The method used in this research was descriptive. pig-tailed macaque with ID. Tattoo 6180 was purposively chosen as an object for taking blood samples.

The tools and materials used in this study include object glass, cover glass, test tube, micro

tube, micro tip, vortex, light microscope (Nikon YS100), micropipette (Fortuna), Class II B2 Biological Safety Cabinet, Centrifuge (Biobase), Haematology analyser MEK-6550 (Nihon Kohden), Photometer 5010 (Mezos), vacutainer tube with Ethylenediaminetetraacetic acid (EDTA), vacutainer tube without anti-coagulant (Plain), disposable syringe, blood sample with ID. Tattoo 6180, alcohol 70%, methanol, MDT staining reagent, blood chemistry reagent test (DiaSys), and other tools and materials support this research.

### 2.1. Blood Sampling

Blood sampling ID. Tattoo 6180 done by drh. Suryo Saputro, a veterinary attendant of RAF-L with procedures that have been known and approved by the supervision of the Commission for the Welfare and Use of Research Animals (KPKPH) PRC IPB University. Pig-tailed macaques were anaesthetised using 10% ketamine at 10-15 mg. Blood was then taken using a syringe intravenously in the femoral vein as much as 3 ml. The blood sample was then put into a vacutainer tube (EDTA) and a tube without anti-coagulant (plain). The blood sample is then stored in a cooler box to be transferred to the pathology laboratory for further procedures.

### 2.2. Haematology Analysis

Haematological analysis ID. The tattoo 6180 procedure refers to the standard protocol from manufacturing. The Haematology Analyzer is set up, and the sample ID is typed on the interface screen. Blood samples were quickly placed in the EDTA vacutainer tube and homogenised until homogeneous without clots. The tube is then placed in the aspirating section. Then the aspirating button is pressed, so the machine sucks in the blood. Haematology Analyzer will generate values for White Blood Cell (WBC), Red Blood Cell (RBC), haemoglobin (HGB), haematocrit

(HCT), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV) and platelets (Platelets; PLT).

### 2.3. Leukocyte Differential Examination

Leukocyte differentiation procedure based on Hasanah (2019) with modifications to the slide preparation. The blood in the EDTA vacutainer tube was taken as much as 10  $\mu$ l and then placed on a slide. The drops of blood are then carefully examined using another slide, and the slide is then dried. The dried slide was then fixed in methanol for 3 minutes and air-dried. The dried slide was then carried out with rapid staining using methylene blue for 1 minute and eosin for 1 minute. The dried slide was then observed under a microscope at a magnification of 400 $\times$ . The slide was calculated for the proportion of leukocytes per 100 cells observed.

### 2.4. Blood Chemistry Examination

The blood chemistry examination parameters refer to Hasanah (2019) with a modification of adding parameters, namely, albumin examination. The blood sample in the plain vacutainer tube was allowed to stand for 5 minutes while the EDTA vacutainer tube was centrifuged at a speed of 2500 rpm. for 5 minutes. The supernatant in the form of serum in a plain vacutainer and plasma in an EDTA vacutainer was then collected into different microtubes. After obtaining serum/plasma, blood examination procedures were carried out according to the manufacturer's protocol of the reagents (DiaSys) used as follows:

#### 2.4.1. SGOT/AST

As much as 50  $\mu$ l of blood plasma or serum was put into a test tube containing 500  $\mu$ l of SGOT reagent. The mixture was then homogenised using a vortex. The homogeneous solution was then examined using Photometer 5010 at an incubation temperature of 37°C for 5 minutes.

#### 2.4.2. SGPT/ALT

As much as 50 µl of blood plasma or serum was put into a test tube containing 500 µl of SGPT reagent. The mixture was then homogenised using a vortex. The homogeneous solution was then examined using Photometer 5010 at an incubation temperature of 37°C for 5 minutes.

#### 2.4.3. Total Protein

As much as 20 µl of blood plasma or serum was put into a test tube containing 1000 µl of total protein reagent. The mixture was then homogenised using a vortex and incubated for 10 minutes. The homogeneous solution was then examined using Photometer 5010 at 25°C for 2 minutes.

#### 2.4.4. Urea Examination (BUN)

As much as 10 µl of blood plasma or serum was put into a test tube containing 1000 µl of urea R1 reagent, then homogenised by vortex and incubated for 5 minutes at room temperature. After incubation, 1000 µl of urea R2 reagent was added to the solution, then homogenised and incubated for 5 minutes at room temperature. After incubation, the solution was examined using Photometer 5010 at 25°C for 2 minutes. After obtaining the results of the urea value, the BUN (Blood Urea Nitrogen) value can be calculated by the following formula Refers to DiaSys reagent protocol:

$$\text{BUN} = 0.466 \times \text{urea value}$$

#### 2.4.5. Calcium (Ca)

As much as 10 µl of blood plasma or serum was put into a test tube containing 1000 µl of calcium reagent. The mixture was then homogenised using a vortex. The homogeneous solution was then examined using Photometer 5010 at an incubation temperature of 25°C for 2 minutes.

#### 2.4.6. Phosphorus (P)

As much as 10 µl of blood plasma or serum was put into a test tube containing 1000 µl of phosphorus reagent. The mixture was then homogenised using a vortex and incubated for 5 minutes. After incubation, the solution was examined using Photometer 5010 at 25°C for 2 minutes.

#### 2.4.7. Cholesterol

As much as 10 µl of blood plasma or serum was put into a test tube containing 1000 µl of cholesterol reagent. The mixture was then homogenised using a vortex and incubated for 10 minutes. After incubation, the solution was examined using Photometer 5010 at 25°C for 2 minutes.

#### 2.4.8. Albumin

As much as 10 µl of blood plasma or serum was put into a test tube containing 1000 µl of albumin reagent. The mixture was then homogenised using a vortex and incubated for 5 minutes. After incubation, the solution was examined using Photometer 5010 at an incubation temperature of 25°C for 2 minutes. After obtaining the results of the albumin value, the globulin value can be calculated by the following formula referring to the DiaSys reagent protocol:

$$\text{Globulin} = \text{Total Protein} - \text{Albumin}$$

#### 2.4.9. Creatinine

As much as 50 µl of blood serum or serum was put into a test tube containing 500 µl of creatinine reagent. The mixture was then homogenised using a vortex. The homogeneous solution was then examined using Photometer 5010 at 25°C for 2 minutes.

#### 2.4.10. Glucose

As much as 10 µl of blood plasma or serum was put into a test tube containing 1000 µl of glucose reagent. The mixture was then homogenised using a vortex and incubated for 10 minutes. After incubation, the solution was examined using Photometer 5010 at 25°C for 2 minutes.

### 3. Results

#### 3.1. Haematology Profile

Based on the hematologic profiles measurement results, it is known that the pig-tailed macaque having some parameter categorised out of the normal range value specifically WCB ( $7.5 \times 10^3/\mu\text{l}$ ), MCH (23.4 pg), MCHC (33.2 g/dL), PLT ( $231 \times 10^3/\mu\text{l}$ ) that shown in Table 1. These results can be interpreted as several possible diagnoses experienced by pig-tailed macaque ID. Tatto 6180 namely leukopenia, hyperchromic macrocytic or macrocytic with stress, and thrombocytopenia when compared relative to the normal value of the hematological profile. This condition can be caused by several factors such as liver disorders or lack of folic acid and vitamin

B12 (Weiss and Wardrop, 2010), Stress conditions that will result in the secretion of catecholamines (Adinata *et al.* 2018), malaria and dengue fever (Livina *et al.* 2014; Asmara 2018), spinal disorders or abnormalities (Sianipar 2014), and also physiological and pathological factors (Tizard 2017).

#### 3. 2. Blood Chemistry

SGOT/AST is an enzyme found in organs, muscles, the heart, kidneys, and a little in the liver. Thus, the dynamics of SGOT/AST levels cannot be used as a specific indicator of liver damage (Adriani *et al.* 2014). Based on Blood chemistry measurements show that the pig-tailed macaque can be diagnosed with a muscle injury or heart inflammation (SGOT/AST value 119.1 U/L) that shown in Table 2.

Table 1. The results of the haematological examination with ID. Tattoo 6180.

| Parameters                                       | Unit               | ID.<br>Tattoo<br>6180 | Normal Value Reference  |                                           |                                           |                                        |                                         | nb     |
|--------------------------------------------------|--------------------|-----------------------|-------------------------|-------------------------------------------|-------------------------------------------|----------------------------------------|-----------------------------------------|--------|
|                                                  |                    |                       | PRC<br>Internal<br>Data | <i>Macaca<br/>nemestrina</i> <sup>1</sup> | <i>Macaca<br/>nemestrina</i> <sup>2</sup> | <i>Macaca<br/>leonina</i> <sup>3</sup> | <i>Macaca<br/>tonkeana</i> <sup>4</sup> |        |
| Leucocyte (WBC)                                  | $10^3/\mu\text{l}$ | <b>7,5</b>            | $9,6 \pm 3,4$           | $11,78 \pm 2,65$                          | -                                         | $14,54 \pm 3,82$                       | $10,6 \pm 5,0$                          | ▼      |
| Erythrocyte (RBC)                                | $10^6/\mu\text{l}$ | <b>5,35</b>           | $5,96 \pm 0,68$         | $5,95 \pm 0,57$                           | $5,7 \pm 0,1$                             | $6,55 \pm 0,55$                        | $6,32 \pm 0,53$                         | normal |
| Haemoglobin (Hb)                                 | g/dl               | <b>12,5</b>           | $11,6 \pm 1,1$          | $11,3 \pm 1,3$                            | $11,3 \pm 0,2$                            | $13,7 \pm 1,05$                        | $13,0 \pm 1,4$                          | normal |
| Haematokrit (HCT)                                | %                  | <b>37,7</b>           | $40,05 \pm 3,65$        | $41,8 \pm 4,82$                           | $36,7 \pm 0,65$                           | $45,45 \pm 3,36$                       | $44,3 \pm 4,5$                          | normal |
| Mean Corpuscular Volume (MCV)                    | fl                 | <b>70,5</b>           | $66 \pm 5$              | $70,4 \pm 6,2$                            | -                                         | $69,65 \pm 2,83$                       | $70,0 \pm 2,4$                          | normal |
| Mean Corpuscular Hemoglobin (MCH)                | pg                 | <b>23,4</b>           | $20 \pm 1,3$            | $19,0 \pm 1,7$                            | -                                         | $21,03 \pm 1,27$                       | $20,5 \pm 1,1$                          | ▲      |
| Mean Corpuscular Hemoglobin Concentration (MCHC) | g/dl               | <b>33,2</b>           | $29,1 \pm 1,6$          | $27,1 \pm 2,3$                            | -                                         | $30,25 \pm 1,03$                       | $29,3 \pm 1,1$                          | ▲      |

Source : <sup>1</sup>Rahlmann *et al.* (1967); <sup>2</sup>Rainwater (2006); <sup>3</sup>Pang *et al.* (2013); dan <sup>4</sup>Thierry *et al.* (2009).

Note : - : no data; ▲ : above normal value; ▼ : below normal value

Table 2. The results of blood chemistry measurements with ID. Tattoo 6180.

| Parameters         | Unit  | ID.<br>Tattoo<br>6180 | Normal Value Reference                |                                       |                                    |                                     | nb     |
|--------------------|-------|-----------------------|---------------------------------------|---------------------------------------|------------------------------------|-------------------------------------|--------|
|                    |       |                       | <i>Macaca nemestrina</i> <sup>1</sup> | <i>Macaca nemestrina</i> <sup>2</sup> | <i>Macaca leonina</i> <sup>3</sup> | <i>Macaca tonkeana</i> <sup>4</sup> |        |
| BUN                | mg/dl | <b>21,6</b>           | 19,6 ± 2,4                            | -                                     | -                                  | 75,31 ± 8,82                        | normal |
| Creatinine (Cr)    | mg/dl | <b>1,24</b>           | -                                     | 0,6 ± 0,2                             | 0,57 ± 0,12                        | 1,29 ± 0,26                         | normal |
| Total Protein (TP) | g/dl  | <b>6,7</b>            | 7,3 ± 0,5                             | 6,2 ± 0,2                             | 7,9 ± 0,41                         | 7,36 ± 0,4                          | normal |
| Albumin (A)        | g/dl  | <b>4,0</b>            | 3,7 ± 0,4                             | 3,8 ± 0,4                             | 4,5 ± 0,3                          | 3,9 ± 0,2                           | normal |
| Globulin (G)       | g/dl  | <b>2,7</b>            | 3,5 ± 0,5                             | 2,7 ± 0,5                             | -                                  | -                                   | normal |
| A/G Ratio          | -     | <b>1,5</b>            | 1,05 ± 0,22                           | 1,4 ± 0,45                            | -                                  | -                                   | normal |
| SGPT/ALT           | U/l   | <b>49,6</b>           | -                                     | 34,9 ± 20,9                           | 27,61 ± 15,88                      | 28,4 ± 17,0                         | normal |
| SGOT/AST           | U/l   | <b>119,1</b>          | -                                     | 40,2 ± 11,2                           | 36,83 ± 16,97                      | 51,6 ± 17,2                         | ▲      |
| Glucose            | mg/dl | <b>77</b>             | 73 ± 17                               | 78,7 ± 14,4                           | 96,39 ± 24,86                      | 65,4 ± 11,35                        | normal |
| Calcium (Ca)       | mg/dl | <b>7,0</b>            | -                                     | 9,5 ± 0,2                             | 38,91 ± 2,88                       | 41,80 ± 2,16                        | ▼      |
| Phosphorus (P)     | mg/dl | <b>37,9</b>           | -                                     | -                                     | -                                  | 21,08 ± 9,0                         | ▲      |
| Cholesterol        | mg/dl | <b>87</b>             | -                                     | -                                     | 54,05 ± 9,2                        | 55,49 ± 8,82                        | ▲      |

Source : <sup>1</sup>Rahlmann *et al.* (1967); <sup>2</sup>Rainwater (2006); <sup>3</sup>Pang *et al.* (2013); dan <sup>4</sup>Thierry *et al.* (2009).

Note : - : no data; ▲ : above normal value; ▼ : below normal value

Evaluation of other parameters such as plasma protein, BUN, and creatinine (Table 2), the pig-tailed macaques reveals did not experience kidney problems or damage. Therefore, the possible diagnosis is that the pig-tailed macaque has a muscle injury or heart inflammation.

The value of serum cholesterol pig-tailed macaque ID. Tattoo 6180 is 87 mg/dl (Table 2). Total cholesterol levels above the threshold are hypercholesterolemia (Wresdiyati *et al.* 2006). Low-Density Lipoprotein (LDL) is a type of cholesterol generally dangerous and can induce complications of diseases such as coronary heart disease, arteriosclerosis, atherosclerosis, and stroke. The Pig tailed macaque has been indicated to have hypercholesterolemia.

### 3.3. Differential Leukocytes

Leukocyte differentiation is a grouping of leukocyte cells based on the presence or absence of granules, the number of nuclear lobes, and the size

of cells observed in blood smear preparations which aims to determine the proportion of leukocyte cell types in the sample as diagnostic supporting data (Rosmanah 2015). The results of the differential leukocyte examination can be shown in Table 3. Pig-tailed macaque ID. Tattoo 6180 has neutrophils, lymphocytes, and monocytes respectively, 75%, 16%, and 9%. This value will be more meaningful in evaluating animal health if the diagnosis is made by analysing the value of the blood's neutrophil: leukocyte (N/L) ratio. The value of the N/L ratio can be used as a parameter to detect the severity of systemic inflammation, stress, bacteraemia, and oncological sepsis (Zahorec 2001). Pig-tailed macaque ID. Tattoo 6180 has an N/L ratio value of 4.68. According to Kannan *et al.* (2000), the value of the N/L ratio can indicate that the animal is experiencing stress if it has a ratio value of > 1.5. This condition indicates that the pig-tailed macaque is experiencing stress.

Table 3. The Results of differential leukocytes with ID. Tattoo 6180

| Parameter (%) | Pig-tailed macaque ID. Tattoo 6180 | Normal value* |
|---------------|------------------------------------|---------------|
| Lymphocyte    | 16                                 | 44,9 ± 3,9    |
| Monocyte      | 9                                  | 2,3 ± 1,7     |
| Neutrophils   | 75                                 | 50,1 ± 3,4    |
| Basophils     | -                                  | 0.7 ± 0,6     |
| Eosinophils   | -                                  | 2,0 ± 1,5     |

Source : \*Rahlmann *et al.* (1967)

Note : - : no data

#### 4. Discussion

According to Canales-Espinosa *et al.* (2015), examining blood parameters, which include the evaluation of haematological profiles and blood chemistry, can be used as supporting data in diagnosing infectious and non-infectious diseases. These data can be used to examine further the results of a temporary suspicion of disease (Garcia-Feria *et al.* 2017). In addition, veterinarians can also enforce animal health status based on blood parameter data obtained (Hasanah 2019).

The data in this study were compared with previously published clinical normal values of *Macaca nemestrina* (internal data of PRC IPB University; Rahlmann *et al.* 1967; Rainwater 2006), *Macaca leonina* (Pang *et al.* 2013), and *Macaca tonkeana* (Thierry *et al.* 2009). Selected clinical reference data based on species kinship relationships. According to Morales and Melnick (1998), *Macaca nemestrina*, *Macaca leonina*, and *Macaca tonkeana* have close relatives collected in a monophyletic clade based on mtDNA sequence analysis. Therefore, all clinical references are assumed to be used as a source of comparative information in this study. The results of the examination of the haematological profile using the Haematology Analyzer include White Blood Cells (WBC), Red Blood Cells (RBC), Erythrocyte Index, and platelets (PLT). The results of the examination of the haematological profile with ID. Tattoo 6180 is shown in Table 1.

RBC, Hb, HCT, and MCV pig-tailed macaque ID values. Tattoos 6180, respectively, are  $5.35 \times 10^6/\mu\text{l}$ ,

12.5 g/dl, 37.7%, and 70.5 fl. This value is still within the normal range compared to the normal clinical value, which can be seen in Table 1. Based on the RBC, Hb, HCT, and MCV values, pig-tailed macaque ID. Tattoo 6180 can be interpreted as not having anaemia. According to Jameel *et al.* (2012), animals or patients with anaemia will have HCT values below the normal range. Subsequently, the MCV value will be used to determine the type of anaemia based on the erythrocyte size if the patient/animal is diagnosed with anaemia (Hsieh *et al.* 2017).

Although pig-tailed macaque is not diagnosed with anaemia based on the values of RBC, Hb, HCT, and MCV, it is also necessary to observe the MCH and MCHC as a consideration in making the diagnosis. MCH is the proportion of haemoglobin weight in the average erythrocyte regardless of its size, while MCHC expresses the value of haemoglobin concentration per unit volume of erythrocytes (Adinata *et al.* 2018). The MCH and MCHC pig-tailed macaque ID values are based on the measurement results. Tattoos 6180, respectively, are 23.4 pg and 33.2 g/dl. These values are relatively high compared to normal clinical values, which can be seen in Table 1. Pig-tailed macaque with ID. Tattoo 6180 was not iron deficient due to its high MCH value. However, based on Laloan *et al.* (2018), MCH values that exceed the normal range may indicate that the animal has macrocytic.

Pig-tailed macaque with ID. Tattoo 6180 has a normal MCV value and a high MCH. Thus, it can be assumed that the pig-tailed macaque was macrocytic. Macrocytic is a condition where the animal has a high

MCH value (Fassah and Khotijah, 2011). It happens because the size of the erythrocytes is enlarged more than normal. This enlargement may or not affect the proportion of Hb in erythrocytes. In addition, the enlargement, in this case, did not significantly affect the value of Hb and MCV. According to Weiss and Wardrop (2010), macrocytic can occur due to liver disorders or lack of folic acid and vitamin B12.

The high MCHC value in the pig-tailed macaque is thought to be due to the haemolysis of blood cells. However, this did not happen to ID Tattoo 6180 because the Hb value was normal. The condition of the MCHC value above the normal range is referred to as hyperchromic (Bunga *et al.* 2019). The condition is caused by haemolysis. The Hb comes out into the blood plasma and is counted when measuring the concentration of Hb in the blood. Stress conditions of animals can also cause the high value of MCHC. Stress factors will result in the secretion of catecholamines (epinephrine/norepinephrine) into the blood, which causes Hb levels to increase (Adinata *et al.* 2018). Based on the evaluation of the values of RBC, Hb, HCT, MCV, MCH, and MCHC with ID. Tattoo 6180 may undergo hyperchromic macrocytic or macrocytic stress.

Value of leukocytes pig-tailed macaque ID. Tattoo 6180 was  $7.3 \times 10^3/\mu\text{l}$ . This value was categorised below the normal range value, as seen in Table 1. The physiological condition where the number of leukocytes has decreased is called leukopenia (Tilley and Smith 2011). According to Titisari *et al.* (2019), a decrease in the value of leukocytes can indicate that an animal is experiencing a stressed or stressed condition. Similar to Tizard's (2017) statement, a decrease in leukocytes can indicate an animal is experiencing stress or an attack by pathogens. However, other factors, such as physiological and pathological factors, can affect leukocyte levels. Physiological factors include animal

age, health status, and stress.

In comparison, pathological factors include infection or inflammation that occurs in animals (Guyton and Hall, 2014). In addition, an increase in total leukocyte levels is possible due to an increase in one or several types of leukocytes (Maheshwari *et al.* 2008). Thus, a differential leukocyte examination was performed to establish a diagnosis of the cause of leukopenia in this case.

The results of the differential leukocyte examination can be shown in Table 3. Pig-tailed macaque ID. Tattoo 6180 has neutrophils, lymphocytes, and monocytes, respectively, 75%, 16%, and 9%. All of these values are categorised as being above the normal clinical value. This value will be more meaningful in evaluating animal health if the diagnosis is made by analysing the value of the blood's neutrophil: leukocyte (N/L) ratio. The value of the N/L ratio can be used as a parameter to detect the severity of systemic inflammation, stress, bacteraemia, and oncological sepsis (Zahorec 2001). This is because the dynamic N/L ratio in the blood is one of the body's physiological efforts in maintaining homeostatic conditions (Binol *et al.* 2010). The N/L ratio of animals in several studies shows a slower change than detecting cortisol levels (Kannan *et al.* 2000; Khovifah, 2013; Titisari *et al.* 2019). According to Titisari *et al.* (2019), leukocyte levels change more slowly (in the range of 0.5-20 hours) than corticosterone levels in response to stress. Thus, compared to corticosterone levels, the N/L ratio can be used as a reliable parameter in indicating long-term stress.

Pig-tailed macaque ID. Tattoo 6180 has an N/L ratio value of 4.68. According to Kannan *et al.* (2000), the value of the N/L ratio can indicate that the animal is experiencing stress if it has a ratio value of  $> 1.5$ . The normal value of the N/L ratio for *Macaca nemestrina*, when viewed from the normal clinical



value of leukocyte differential. It is in the range of 1.09 to 1.1. This condition indicates that the pig-tailed macaque is experiencing stress. This stress condition can be triggered by cage transfer and lack of enrichment in new cages. Pig-tailed macaque ID. Tattoo 6180, which was previously in a group cage with an area that allows massive locomotion activity, is limited because it is in an individual cage. So, it is possible that this causes the pig-tailed macaque to have stress. It is because confinement simultaneously can increase the chances of the emergence of natural behaviour and reduce abnormal behaviour and self-harm activities compared to individual cages that can potentially cause abnormal behaviour (Sajuthi *et al.*, 2016). The same thing was reported by Titisari *et al.* (2019) in langurs, who stated that the increase in the N/L ratio was caused by moving cages due to discomfort. Another thing reported by Sajuthi *et al.* (2016) stated is that the size and shape of the cage could affect animal behaviour, leading to stress.

PLT value pig-tailed macaque ID. Tattoo 6180 was  $231 \times 10^3/\mu\text{l}$ . This value was categorised below the normal range value, as seen in Table 1. Rosmanah (2015) states that the physiological condition where PLT is below the clinically normal value is thrombocytopenia. The condition of thrombocytopenia in animals can be caused by certain disease factors such as malaria and dengue fever (Livina *et al.* 2014; Asmara 2018), decreased platelet production caused by spinal disorders or abnormalities, increased platelet damage in the blood caused by disease, and platelet sequestration (Sianipar 2014). Further examination of the cause of thrombocytopenia in pig-tailed macaque ID. Tattoo 6180 needs to be done in order to confirm the diagnosis of the disease and its treatment.

Pig-tailed macaque ID. Tattoo 6180 has an SGOT/AST value of 119.1 U/l. These values are categorised as high above the normal clinical values presented in Table 2. SGOT/AST is an enzyme found

in organs, muscles, the heart, kidneys, and a little in the liver. Thus, the dynamics of SGOT/AST levels cannot be used as a specific indicator of liver damage (Adriani *et al.* 2014). Hasanah (2019) stated that the enzyme could only show the occurrence of muscle injury, heart inflammation, and damaged tissue, not the liver. Based on the evaluation of other parameters such as plasma protein, BUN, and creatinine, the pig-tailed macaques did not experience kidney problems or damage. Therefore, the possible diagnosis is that the pig-tailed macaque has a muscle injury or heart inflammation. Enforcement of this possible provisional diagnosis requires further examination of creatinine phosphokinase (CPK) enzyme levels as a reference to determine whether the pig-tailed macaque has muscle injury or heart inflammation (Soeparno and Karangwangkal 2014).

The value of serum cholesterol pig-tailed macaque ID. Tattoo 6180 is 87 mg/dl. This value is above the clinically normal range, as seen in Table 2. Total cholesterol levels above the threshold are hypercholesterolemia (Wresdiyati *et al.* 2006). Low-Density Lipoprotein (LDL) is a type of cholesterol generally dangerous and can induce complications of diseases such as coronary heart disease, arteriosclerosis, atherosclerosis, and stroke. The pig-tailed macaque has been indicated to have hypercholesterolemia. There is a need for further examination to establish the diagnosis that occurs in order to carry out appropriate treatment to control serum cholesterol levels. Several things, such as a high-fat diet, genetics, liver damage, and lack of physical activity, can cause hypercholesterolemia. Setting a low-fat diet is one way that can be done to control cholesterol levels (Indriyani *et al.* 2018). Therefore, regulating animal feed composition is one of the keys to controlling animal homeostasis.

The examination results of blood parameters include haematological profiles and blood chemistry

measurements on a pig-tailed macaque with ID. Tattoo 6180 can be used as basic and supporting data to diagnose a certain disease. Based on the evaluation of blood parameters, pig-tailed macaque with ID. Tattoo 6180 was diagnosed as hyperchromic macrocytic without anaemia, stress, thrombocytopenia, muscle injury/inflammation of the heart, and hypercholesterolemia. However, further examination is needed to know the current state of animal health. Further examination will determine effective and efficient management for these animals. In addition, the data obtained in this study can be used as an evaluation for the PRC IPB University in managing cages, feed, and routine evaluations of animal health.

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