ESTABLISHING HAEMATOLOGICAL PARAMETERS OF CAPTIVE ASIAN ELEPHANT (*ELEPHAS MAXIMUS*) IN MALAYSIA

BY

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A RESEARCH PAPER SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF VETERINARY MEDICINE

FACULTY OF VETERINARY MEDICINE

UNIVERSITY OF MALAYSIA KELANTAN

2024

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Establishing Haematological Parameters of Captive Asian Elephant (*Elephas maximus*) in Malaysia

ABSTRACT

In Malaysia, Asian elephants play a significant role in the history and culture of the nation. Interpreting haematology and blood chemistry data for Asian elephants (*Elephas maximus*) can be challenging due to the wide range of reference values available. This study examined the haematological and serum biochemical parameters of elephants across different age stages, including infants (0-5 years), juveniles (5-10 years), sub-adults (11-15 years), and adults (16 years and above), with a focus on sex differences. In the juvenile group, most haematological parameters showed no significant ($p > 0.05$) differences between males and females. There were no significant ($p > 0.05$) differences in most haematological parameters found in the adult group. For serum biochemical parameters, juvenile males had significantly ($p < 0.05$) higher total protein levels than females, but other parameters such as ALT, ALP, LDH, and cholesterol showed normal values in both sexes. Sub-adult females demonstrated consistent biochemical profiles, with no significant ($p > 0.05$) variation in parameters such as ALT, ALP, and cholesterol. In adults, most biochemical parameters did not differ significantly ($p > 0.05$) between sexes, but globulin levels were significantly ($p < 0.05$) higher in males. These results suggest that, while some sex-based variations exist, they do not consistently affect the haematological and biochemical profiles across different age groups in elephants.

Keywords: **haematology, serum biochemistry, elephants, sexes, age, infant, juvenile, sub-adult, adult**

Mewujudkan Parameter Hematologi Gajah Asia Tawanan (Elephas maximus) di Malaysia

ABSTRAK

Di Malaysia, gajah Asia memainkan peranan penting dalam sejarah dan budaya negara. Mentafsir data hematologi dan kimia darah untuk gajah Asia (*Elephas maximus*) boleh mencabar kerana julat luas nilai rujukan yang tersedia. Kajian ini mengkaji parameter biokimia hematologi dan serum gajah merentas peringkat umur yang berbeza, termasuk bayi (0-5 tahun), juvana (5-10 tahun), sub-dewasa (11-15 tahun), dan dewasa (16 tahun ke atas), dengan tumpuan kepada perbezaan jantina. Dalam kumpulan juvana, kebanyakan parameter hematologi tidak menunjukkan perbezaan yang signifikan ($p>0.05$) antara gajah jantan dan betina. Untuk parameter biokimia serum, gajah Jantan juvana mempunyai jumlah protein yang lebih tinggi secara signifikan ($p<0.05$) berbanding betina, tetapi parameter lain seperti ALT, ALP, LDH dan kolesterol menunjukkan nilai normal dalam kedua-dua jantina. Gajah betina sub-dewasa menunjukkan profil biokimia yang konsisten, tanpa perubahan ketara ($p>0.05$) dalam parameter seperti ALT, ALP dan kolesterol. Pada gajah dewasa, kebanyakan parameter biokimia tidak berbeza dengan ketara ($p>0.05$) antara jantina, tetapi paras globulin secara signifikan ($p<0.05$) lebih tinggi pada gajah jantan. Keputusan ini menunjukkan bahawa, walaupun beberapa variasi berasaskan jantina wujud, ia tidak secara konsisten mempengaruhi profil hematologi dan biokimia merentas kumpulan umur yang berbeza dalam gajah.

Kata kunci: hematologi, biokimia serum, gajah, jantina, umur, bayi, juvana, sub-dewasa, dewasa

CERTIFICATION

This is to certify that we have read this research paper entitled '**Establishing Haematological Parameter of Captive Asian Elephant (*Elephas maximus*) in Malaysia'** by Ahmad Naimie bin Mohammad Azlan and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirements for the course DVT 55204 – Research Project.

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ACKNOWLEDGEMENT

Special thanks to those who have given their support, guidance, advice and aid for the completion of this project paper:

Family

Department of Wildlife and National Park

Prof. Madya Dr. Mohd Farhan Hanif bin Reduan

Prof. Madya Dr. Choong Siew Shean

Dr. Mohammed Dauda Goni

Dr Ana Maria binti Abd Rahim

Dr Mohd Firdaus Ariff bin Abdul Razak

Pn. Rabi'atul Adawiyah binti Hashim

Pn. Nur Eizzati binti Badrul Hisham

Mysarah binti Ursilan Afandi

Ahmad Jafni bin Ahmad Hizad

Muhammad Amirul Shauqat bin Azaha

Irfan Fakhri bin Al-Itqam

DVM 5 class of 2020/2025

Thank You

DEDICATIONS

First and foremost, I would like to praise Alhamdulillah and be grateful for the health and strength granted throughout the research project, which allowed me to finish the research project successfully without any trouble.

I want to extend my deepest gratitude towards my loving parents, Mohammad Azlan Bin Ali Bashah and Ruzaina Binti Mohammed Zaki, who always gave me words of encouragement and utmost support throughout this process. My siblings, Ahmad Nafizi, Aimi Natasha, and Ahmad Nabil, have always been with me and provided any means necessary to help, leading me to where I am today.

Next, I would also like to dedicate this dissertation to my supervisor, Associate Prof. Dr. Mohd Farhan Hanif Bin Reduan, and my co-supervisor, Associate Prof. Dr. Choong Siew Shean, Dr. Mohammed Dauda Goni, and my field supervisor, Dr. Ana Maria Binti Abd Rahim and Dr. Firdaus Ariff Bin Abdul Razak, for their unconditional support and guidance. Special thanks also to Associate Prof. Dr. Mohd Farhan for allowing me to use his equipment and materials required for the research project and guiding me patiently during the process, which led to the completion of this thesis. I would also like to dedicate this work to the Clinical Pathology laboratory assistants of FPV UMK, Puan Nur Eizzati Binti Badrul Hisham, for her great help and support.

I would like to express my sincere gratitude to the Department of Wildlife and National Parks (PERHILITAN) for granting me permission to collect and analyze elephant blood samples in Peninsular Malaysia. This research would not have been possible without their support and trust. I am truly grateful for the opportunity to work in collaboration with such a dedicated team, and I hope this research will play a part in furthering the understanding and preservation of Malaysia's elephant populations.

Finally, I would also like to dedicate this spadework and give special thanks to my classmates, Mysarah Binti Ursilan Afandi, Ahmad Jafni Bin Ahmad Hizad, Muhammad Amirul Shauqat Bin Azaha, and Irfan Fakhri bin Al-Itqam, for always being there with me and supporting me endlessly throughout the research project.

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List of Abbreviations

NECC · National Elephant Conservation Centre

DWNP · Department of Wildlife and National Park

SDECC · Sungai Deka Elephant Conservation Centre

CBC · Complete Blood Count

WBC · White Blood Cell

PCV · Packed Cell Volume

RBC · Red Blood Cell

MCV · Mean Corpuscular Volume

MCH · Mean Corpuscular Haemoglobin

MCHC · Mean Corpuscular Haemoglobin Concentration

RDW · Red Cell Distribution Width

BUN · Blood Urea Nitrogen

AST · Aspartate Aminotransferase

ALP · Alkaline Phosphatase

ALT · Alanine Aminotransferase

CK · Creatinine Kinase

GGT · Gamma-Glutamyl Transferase

LDH · Lactate Dehydrogenase

EDTA · Ethylenediaminetetraacetic Acid

NAF · No Abnormal Findings

EEHV · Elephant Endotheliotropic Herpesvirus

List of Symbols

^{o}C · Degree celcius

% · Percentage

µ · Micro

± · Range of values

* · Indicates significance difference

CHAPTER 1

INTRODUCTION

1.0 RESEARCH BACKGROUND

Asian elephants (*Elephas maximus*) are members of the Proboscidea order and family Elephantidae. Asian elephants are thought to number between 48,323 and 51,680 in the wild and 15,000 in captivity worldwide (Menon and Tiwari, 2019). According to Menon and Tiwari (2019), they are dispersed throughout 13 Asian nations, including Bhutan, Bangladesh, Cambodia, China, India, Indonesia, Laos, Nepal, Sri Lanka, Myanmar, Malaysia, Thailand, and Vietnam. Asian elephants are known to exist in four subspecies: the Sumatran subspecies (*Elephas maximus sumatranus*), the Borneo subspecies (*Elephas maximus borneensis*), the Indian subspecies (*Elephas maximus indicus*), and the Sri Lankan subspecies (*Elephas maximus maximus*) (Fernando & Lande, 2000; Sukumar, 2003). The International Union for Conservation of Nature (IUCN) has listed the Asian elephant as endangered (red listed) (Williams *et al*., 2019).

In Peninsular Malaysia, Asian elephants play a significant role in both the history and culture of the nation. These days, they have become essential since most of the National Elephant Conservation Centre (NECC) captive elephants have been utilised for tourism and the relocation of wild elephants in the Malay Peninsular since May 11, 1974 (Ismail, 2023). According to Saaban *et al*. (2011), the elephant population in seven states Kedah, Perak, Pahang, Terengganu, Kelantan, Johor, and Negeri Sembilan, is estimated to be between 1,223 and 1,677. Less research has been done on establishing elephant blood haematology and biochemistry profiles. This is due to insufficient opportunities to collect adequate samples from animals kept in captivity (Ranjini *et al*., 2021).

The well-being of elephants kept in captivity is a serious concern. Under captivity, alterations in environmental, social, physical, and psychological factors can result in various health problems that may significantly increase the morbidity and mortality rate of elephants (Fowler & Mikota, 2006). Elephants often mask clinical symptoms, making it difficult to diagnose and treat diseases in them. For this reason, clinical pathological examinations are important diagnostic tools for early detection. The development and use of accurate reference values for blood parameters are more advantageous for the clinical management and healthcare planning of elephants (Fowler & Mikota, 2006). This study was carried out to ascertain the baseline haematological profile of the Asian elephants kept in captivity at the National Elephants Conservation Centre (NECC). Regionally specific data of this kind is beneficial for both treatment and health monitoring in the conservation of elephants in Malaysia.

1.1 RESEARCH PROBLEM STATEMENT

Interpreting haematology and blood chemistry data for Asian elephants (*Elephas maximus*) can be challenging due to the wide range of reference values available. Also, the values might not apply to the entire population due to various factors, including management, sex, geographic location, age and climate. Thus, the haematological and biochemical values of elephants may differ among them in different countries. Several countries, including Thailand (Janyamethakul *et al*., 2017), Myanmar (Santos *et al*., 2020), Sri Lanka (De Alwis *et al*., 2011, De Mel *et al*., 2014), and India (Gromadzka-Ostrowska *et al*., 1988), have provided data on Asian elephants. The blood parameters may be considered differently depending on location and nutritional sources.

1.2 RESEARCH QUESTIONS

I. What are the haematology parameters of captive Asian elephants (*Elephas maximus*) in Malaysia?

II. Are the haematology parameters of Asian elephants in Malaysia different from those of other countries?

III. Is there any difference between the haematology and serum biochemical parameters findings of Asian elephants of different sexes in Malaysia?

1.3 RESEARCH HYPOTHESIS

I. Haematology parameters of captive Asian elephants (*Elephas maximus*) in Malaysia are established.

II. The haematological parameters of Asian elephants in Malaysia differ from those of other countries.

III. There are differences in haematology and serum biochemical parameters in the findings of Asian elephants of different sexes in Malaysia.

1.4 RESEARCH OBJECTIVES

I. To determine the blood parameters reference values for Asian elephants (*Elephas maximus*) kept in captivity in Malaysia.

II. To compare the reference values of Asian elephants in Malaysia with those of other countries.

III. To differentiate haematological and serum biochemical parameters values between the sexes of Asian elephants.

CHAPTER 2

LITERATURE REVIEW

2.1 HAEMATOLOGICAL AND WHITE BLOOD CELLS FINDINGS IN ELEPHANTS

The complete blood count (CBC) is a fundamental component of the minimum diagnostic database for elephant management (Weisbrod *et al*., 2021). Regular haematological testing aids in the management of health. An animal's nutritional status, feed intake, and level of dehydration are aided by measuring its haemoglobin, packed cell volume, and red blood cell count (Franco Dos Santos *et al*., 2020). An accurate leukocyte differential count depends on correctly identifying white blood cell (WBC) types. Automated haematology analysers frequently carry out the complete blood count processing in domestic mammalian species, which can be more accurate and require less labour than manual techniques (Kjelgaard-Hansen and Jensen, 2006). Although there is currently no information regarding automated analyser accuracy, it has been hypothesised that the morphological similarities between elephant lymphocytes and bi-lobed monocytes may cause inaccurate differentiation by automated analysers (Stacy *et al*., 2017).

Elephants possess unique leukocyte morphology, challenging differentiation of cell types (Weisbrod *et al.*, 2021). Analysing white blood cells, particularly by looking at the leukocyte differential count, can help one understand an individual's immune response (Franco dos Santos *et al*., 2020). Elephants have heterophils instead of neutrophils because there are eosinophilic-staining granules in these cells,the cytochemical staining properties and functions of elephant heterophils are the same as neutrophils in other mammals (Fowler and Mikota, 2006). As phagocytic cells, neutrophils and monocytes participate in the innate response,

whereas lymphocytes are involved in adaptive immunity (Cheynal *et al*., 2017). Eosinophils promote immunity against internal parasites and contribute to the inflammatory response (Cheynal *et al*., 2017). As per Veeraselvam et al (2021) and Janyamethakul et al (2017), the differential count of white blood cells in the study indicated that heterophils were the most prevalent cell populations, followed by lymphocytes.

On the other hand, it contradicted the findings of Fowler and Mikota (2006), who stated that the predominant cell type most commonly encountered was the lymphocyte. According to Fowler and Mikota (2006), elephant monocytes come in two varieties. Unsegmented monocytes have the same appearance as any other mammalian species; the other is sometimes trilobed and bilobed. The typical monocyte type with a more rounded to reniform nucleus is commonly seen in blood films of domestic mammalian species, elephants also have a monocyte type with a bi-lobed nucleus that can be rarely tri-lobed (Allen *et al*., 1985; Harr *et al*., 2010). According to Harr et al. (2010), the elephant haematology literature has a history of misidentifying bi-lobed monocytes as lymphocytes due to their morphological similarity, resulting in an overestimation of lymphocyte proportions and an underestimation of monocyte proportions. The distinctive nuclear morphology of the uncommon tri-lobed monocytes makes it easy to distinguish them from lymphocytes. The haematological findings from different sources are shown in Table 1 below.

Table 1: Reference values of the haematological parameter from other literature

Haematology	Parameters	References		
		Fowler & Mikota (2006)	**T. Janyamethakul *et al.*, (2017)**	**Ranjini *et al.*, (2021)**
Red Blood Cell	**Packed Cell Volume (PCV**	30-40 %	27.8-43 %	29.76-31.88 %
	Haemoglobin	11-15 g/dL	9.8-15.6 g/dL	10.5-11.44 g/dL
	RBC count	2.5-5.0 x10^6/µL	1.9-3.2 x10^6/µL	2.29-2.7 x 10^{12}/L
	Mean Corpuscular Volume (MCV)	80-160 fL	104-127.2 fL	123.83-134.23 fL
	Mean Corpuscular Haemoglobin (MCH)	35-50 pg	N/A	40.09-48.42 pg
	Mean Corpuscular Haemoglobin Concentration (MCHC)	25-40 g/dL	29.9-38.9 g/dL	33.54-38.55 g/dL
	Red Cell Distribution Width (RDW)	28-32 %	N/A	14.79-15.43 %
White Blood Cell	**WBC count**	10-18 x 10^3/µL	7202.5-23220.5 cell/µL	16.93-17.82 x 10^9/L
	Band heterophil	N/A	N/A	0.039-0.083 x 10^9/L
	Segmented heterophil	2-4 x 10^3/µ	828.7-13514.3 cell/µL	4.84-5.63 x 10^9/L
	Lymphocytes	5-8 x 10^3/µL	1064.1-12032.8 cell/µL	4.65-5.42 x 10^9/L
	Monocytes	2-4 x 10^3/µL	0-1170 cell/µL	0.27-0.51 x 10^9/L
	Eosinophils	0.1-1 x 10^3/µL	0-1170 cell/µL	0.37-0.76 x 10^9/L
	Basophils	0.00-0.03 x 10^3/µL	0-142.6 cell/µL	0.025-0.071 10^9/L
Platelet	**Platelets count**	200-600 x 10^3/µL	101.6-598.7 x 10^3/µL	3.35-3.57 x 10^{11}/L

2.2 SERUM BIOCHEMISTRY FINDINGS

Serum chemistry analysis measured kidney function, including blood urea nitrogen (BUN) and creatinine, protein concentrations (total proteins including albumin and globulins), liver enzymes, including aspartate transferase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT) and gamma-glutamyl transferase (GGT), total bilirubin, glucose and amylase, lipid storage (triglycerides, cholesterol), muscle injury which is creatine kinase (CK), and total calcium levels. BUN is the end product of protein metabolism, and creatinine is the end product of creatine metabolism in the muscles (Franco dos Santos *et al*., 2020). The kidneys remove these complementary serum chemistry health parameters from the body (Fowler and Mikota, 2006). Electrolytes are in charge of normal cell function, electrochemical impulses, and acid-base equilibrium (Fowler and Mikota, 2006). In the study conducted by Ranjan *et al*. (2023), it was discovered that during the musth season of male Asian elephants, which is marked by a variety of biochemical alterations, there are various parameters linked to tests of liver and kidney function, including elevated levels of testosterone, alkaline phosphatase, and creatinine. The serum biochemistry findings between these literatures are shown in Table 2 below.

Table 2: Reference values of serum biochemical parameters from other literature

Haematology	Parameters	References		
		Fowler & Mikota (2006)	**T. Janyamethakul *et al*., (2017)**	**Ranjini *et al*., (2021)**
Blood Chemistry	Blood Urea Nitrogen (BUN)	5-20 mg/dL	3.1-27.2 mg/dL	15.38-15-95 mg/dL
	Creatinine	1.0-2.0 mg/dL	0.7-2.2 mg/dL	1.3-1.73 mg/dL
	Aspartate Transferase (AST)	15-35 IU/L	4.8-56.3 U/L	26.05-32.25 IU/L
	Alanine Transaminase (ALT)	1.5-3.0 IU/L	0-5.6 U/L	2.28-3.34 IU/L
	Alkaline Phosphatase (ALP)	60-450 IU/L	0-281.5 U/L	222.6-308.96 IU/L
	Gamma-glutamyl transferase (GGT)	4-35 U/L	N/A	N/A
	Total bilirubin	0.2-1.0 mg/dL	N/A	0.69-0.74
	Conjugated bilirubin	0.03-0.8 mg/dL	N/A	0.58-0.63 mg/dL
	Unconjugated bilirubin	0-0.6 mg/dL	N/A	0.088-0.118 mg/dL
	Glucose	60-116 mg/dL	N/A	79.05-80.7 mg/dL
	Amylase	N/A	N/A	N/A
	Creatinine Kinase (CK)	50-250 IU/L	N/A	N/A
	Lactate Dehydrogenase (LDH)	250-500 IU/L	N/A	N/A
	Total protein	6-12 g/dL	6.5-9.3 g/dL	6.94-7.21 g/dL
	Albumin	1.5-3.5 g/dL	N/A	2.41-2.61 g/dL
	Globulin	3.7-6.5 g/dL	N/A	4.43-4.62 g/dL
	Cholesterol	26-68 mg/dL	N/A	57.22-60.22 mg/dL
	Fibrinogen	100-400 mg/dL	N/A	N/A
Electrolytes	Sodium	120-140 mEq/L	N/A	N/A
	Potassium	3.0-6.0 mEq/L	N/A	N/A
	Chloride	100-115 mEq/L	N/A	N/A
	Calcium	9-12 mg/dL	N/A	N/A
	Phosphorus	4.0-6.0 mg/dL	N/A	N/A

2.3 DIFFERENCE IN VALUES OF BLOOD PARAMETERS BETWEEN SEXES

It has been documented that in many species, sex is one of the variables that can impact haematological values (Etim, 2014). Santos et al. (2020) noted there were notable sex differences in certain serum chemistry health parameters but no significant differences between males and females in haematological health parameters. According to research by Santos et al. (2020), females had lower creatinine, glucose, and alkaline phosphatase levels. All the analysed haematological parameters did not significantly differ between the male and female groups, except for alanine aminotransferase, band cells, and basophils (Ranjini *et al*., 2021).

CHAPTER 3

RESEARCH METHODOLOGY

3.0 MATERIALS AND METHODS

3.1 ETHICS CONSIDERATIONS AND PERMIT APPROVAL

Ethical approvals for using animals in the current study have been obtained from the Animal Ethics Committee, Faculty of Veterinary Medicine,University of Malaysia Kelantan (UMK/FPV/ACUE/FYP/016/2024). Permit approval was obtained from Department of Wildlife and National Park, Peninsular Malaysia (JPHLTN.600-6/1/4 JLD3 (26)).

3.2 SAMPLES COLLECTION AND PREPARATION

3.2.1 STUDY AREA

The study focused on all thirty elephants located at the National Elephant Conservation Centre (NECC), Kuala Gandah, Pahang, Malaysia and at the Sungai Deka Elephant Conservation Centre (SDECC), Hulu Terengganu, Terengganu, under the Department of Wildlife and National Parks (DWNP).

3.2.2 STUDY DESIGN

The study design was conducted using a cross-sectional study approach, with samples collected in the field within the specified period.

3.2.3 DATA COLLECTION OF MANAGEMENT

Management data from the conservation centre was collected, including identification (name), age, sex, feeding management, working hours, and physical appearance.

3.2.4 PHYSICAL EXAMINATION FINDINGS

The DWNP veterinarian performed a physical examination of the elephants to ensure they were healthy. This involved assessing the body condition score, and measuring the temperature by placing a thermometer in the centre of a fresh faecal ball. Additionally, the respiration rate was determined by feeling air currents at the end of the trunk, the mucous membrane (rosy pink) was examined, and the pulse rate was measured by placing the fingers on an auricular artery. The normal ranges of each parameter were as shown in Table 3 follows:

Table 3: Reference parameters of vital signs in elephant

Parameters	Range	References
Temperature	36–37 ºC	Fowler and Mikota (2006)
Respiration Rate	10–12 breaths per minute	
Pulse Rate	25–30 beats per minute (standing)	

3.2.5 BLOOD COLLECTION

The elephant was placed in a comfortable position for blood collection by its mahouts and a DWNP veterinarian, either standing (for a juvenile elephant) or in sternal or lateral recumbency. The DWNP veterinarian drew 9 ml of blood using a 21-gauge butterfly catheter and a 10 ml disposable syringe through the auricular vein. The drawn blood was transferred into one plain tube (6 ml) and one tube coated with EDTA (3 ml) for each elephant. To prevent clotting, the EDTA-coated tube was gently inverted. The plain tube was allowed to clot for 1-2 hours at room temperature before the serum was separated by centrifugation at 2500 rpm for 15 minutes. Additionally, blood smears were prepared and stained with Giemsa stain for differential counts, which were conducted under a microscope at the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, University of Malaysia Kelantan.

3.2.6 HAEMATOLOGICAL EVALUATION

The blood samples from the EDTA-coated tubes were analysed using the MYTHIC 18 haematology analyser to check parameters such as red blood cell count (RBC), haemoglobin, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, total leucocyte count, and the differential white blood cell count, including band heterophils, segmented heterophils, lymphocytes, monocytes, eosinophils, and basophils. The white blood cell differential count was also performed under a light microscope at the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, University of Malaysia Kelantan, Kelantan, Malaysia (Appendix B).

3.2.7 SERUM BIOCHEMISTRY FINDINGS

The plain tube was allowed to clot for 1-2 hours at room temperature before the serum was separated by centrifugation at 2500 rpm for 15 minutes. The serum was then transferred into a microtube for serum biochemical evaluation. The IDEXX Vet Test 8008 Veterinary Analyser® machine was used to analyse 1.5 microlitres of the serum samples, checking for parameters such as total protein, albumin, globulin, blood urea nitrogen (BUN), creatinine, aspartate transaminase (AST), creatinine kinase (CK), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), amylase, glucose, lactate dehydrogenase (LDH), conjugated bilirubin, unconjugated bilirubin, total bilirubin, cholesterol, sodium, potassium, calcium, and phosphorus.

3.2.8 DATA ANALYSIS

The mean and standard deviation (Mean ± SD) and minimum and maximum values were used to express the numerical variables. The quantitative variables within the male and female groups were compared using an independent sample t-test for normally distributed variables. The statistical analysis was conducted using SPSS version 29.0.

CHAPTER 4

RESULTS

4.1 PHYSICAL EXAMINATION FINDINGS

Thirty elephants were included in this study, consisting of 6 males and 24 females. The animal age ranges from 2 to 50 years. The physical examination was structured to cover several vital systems, ensuring a broad scope of health evaluation. The systems assessed include the lymphatic, mammary gland, urinary, reproductive, cardiovascular, respiratory, gastrointestinal, ears, eyes, musculoskeletal, nervous, integumentary (skin) and mucous membranes. Each of these systems plays a crucial role in the overall health of an individual, and their thorough evaluation provides insights into possible underlying conditions. This approach is particularly useful in identifying system-specific issues and understanding how they might impact the overall health and well-being of animals.

Throughout the data of physical examination (Appendix A), most system evaluations are labelled "NAF" (No Abnormal Findings), indicating that most individuals exhibit no significant health issues within each respective system. However, several specific observations are noted in cases where abnormalities were identified. These findings highlight a range of conditions and health concerns. For instance, some individuals are noted to have experienced constipation and clear ocular discharge. Other individuals show symptoms affecting the skin, such as pruritic (itching) and skin thickening, which could suggest dermatological conditions or allergic responses. These observations emphasise the importance of integumentary health in the overall well-being of individuals. Additionally, suspected pregnancy is noted in one of the individuals, which would be a key finding for reproductive health management. The identification of conditions like mucus discharge, which may imply respiratory concerns and

wounds, abscesses and fungal infections, further demonstrates the range of health issues assessed during these examinations.

The management of captive Asian elephants provided feeding four times a day: morning, afternoon, and evening. In the morning, the mahouts gave horse pellets mixed with water (50:2 ratio of pellets to water), with 8 kg for sub-adult and adult elephants and 4 kg for juveniles. In the afternoon, the mahouts provided two banana trunks, fruits (depending on the fruit season, such as pineapple, sugarcane, watermelon and papaya) twice and approximately 10 kg of Napier grass per elephant. Lastly, the mahouts gave 10kg of Napier grass twice for the evening meals, during dusk and midnight.

4.2 HAEMATOLOGY PARAMETERS

The parameters for haematology were divided into stages of age, which is infant (0-5 years old), juvenile (5-10 years old), sub- adult (11-15 years old) and adult (16 years old and above)

i) Infant stage

Based on the physical examination and established data record, no individual's elephants were below 5 years old.

ii) Juvenile stage

This category included 8 individuals, comprising 2 males and 6 females. The haematological parameters of juvenile male and female elephants were mostly similar, with a few minor differences (Table 4). Leukocyte counts were slightly higher in females (25.18 × 10^3/µl) compared to males (22.7 × 10^3/µl), though this difference was not statistically significant ($p>0.05$). Females also had a higher lymphocyte count (13.71 × 10^3/µl) compared to males (12.95 × 10^3/µl), but again, this was insignificant ($p>0.05$). The monocyte count was slightly higher in females, with males having a value of 1.39 × 10^3/µl and females 1.60 × 10^3/µl, but this difference was also not significant ($p>0.05$). Heterophil counts were higher in males (7.53 × 10^3/µl) compared to females (8.68 × 10^3/µl), though again, no significant ($p>0.05$) difference was found. Basophil counts were low in both sexes, with females having a slightly higher count, but this was insignificant ($p>0.05$). Eosinophils were more abundant in males (0.65 × 10^3/µl) than in females (0.33 × 10^3/µl), but the difference was not significant ($p>0.05$).

For red blood cell parameters, males also had slightly higher erythrocyte counts (3.15 × 10^6/µl) compared to females (3.23 × 10^6/µl), with no significant

difference (p>0.05). Haemoglobin levels were higher in females (11.38 g/dl) than males (10.55 g/dl), but this difference was not significant (p>0.05) either. Haematocrit values were similar between males (34.6%) and females (36.52%), with no significant difference (p>0.05). Males had slightly lowerr mean corpuscular volume (MCV) values (109.9 μm³) compared to females (113.27 μm³), but this difference was not significant (p>0.05). The mean corpuscular haemoglobin (MCH) was higher in males (33.5 pg) than in females (35.27 pg), but the difference was not significant (p>0.05). Similarly, the mean corpuscular haemoglobin concentration (MCHC) was slightly higher in females (31.18 g/dl) compared to males (30.50 g/dl), but there was no significant (p>0.05) difference. Red blood cell distribution width (RDW) values were nearly the same for both sexes, with males at 15.75% and females at 15.58%, and the difference was insignificant (p>0.05). The were no difference (p>0.05) in platelet counts, with females has higher mean platelet count (802.67 × 10^3/μl) compared to males (603.5 × 10^3/μl). Overall, the study suggests most haematological parameters were not statistically significant (p>0.05) between sexes in juvenile elephants.

Table 4: Results on the haematological parameters of juvenile stage in elephant

Parameters	Units	Male				Female				P-Value
		Minimum	Maximum	Mean	Standard Deviation	Minimum	Maximum	Mean	Standard Deviation	
Leukocytes	10^3/µl	22.5	22.9	22.7	0.28	18.1	33	25.18	5.61	0.086
Lymphocytes	10^3/µl	12.3	13.59	12.95	0.91	9.53	18.7	13.71	3.46	0.169
Monocytes	10^3/µl	0.38	2.4	1.39	1.43	0.54	3.63	1.60	1.11	0.699
Heterophils	10^3/µl	7.35	7.71	7.53	0.25	6.03	10.88	8.68	1.61	0.265
Basophils	10^3/µl	0.07	0.3	0.19	0.16	0.24	0.49	0.36	0.10	0.416
Eosinophils	10^3/µl	0.92	0.38	0.65	0.38	1.32	0.33	0.33	0.13	0.771
Erythrocytes	10^6/µl	2.95	3.34	3.15	0.28	2.78	3.70	3.23	0.36	0.412
Haemoglobin	g/dl	9.7	11.4	10.55	1.20	9.3	13.1	11.38	1.45	0.670
Haematocrit	%	31.9	37.3	34.6	3.82	29.6	41.9	36.52	4.66	0.705

Mean Corpuscular Volume (MCV)	μm^3	108.1	111.7	109.9	2.55	98.0	127.0	113.27	10.76	0.163
Mean Corpuscular Haemoglobin (MCH)	pg	32.9	34.1	33.50	0.85	30.8	38.8	35.27	2.77	0.279
Mean Corpuscular Haemoglobin Concentration (MCHC)	g/dl	30.4	30.6	30.50	0.14	30.3	32.3	31.18	0.71	0.166
Red Distribution Width (RDW)	%	14.9	16.6	15.75	1.20	14.3	17.4	15.58	1.19	0.825
Platelet	$10^3/\mu l$	380	827	603.50	316.08	573	1141	802.67	196.06	0.393

N.B: P value derived from independent t-test

iii) Sub-Adult stage

The table summarizes the descriptive statistics for various variables measured in a two female, including the minimum, maximum, mean, and standard deviation. The data reveals considerable variability across the variables, with means ranging from 0.09 to 120.8. For instance, the first variable shows a mean of 23.6 and a relatively high standard deviation of 9.62, indicating moderate variability within the group. In contrast, some variables, such as eosinophils with a mean of 0.48 and a standard deviation of 0.04, demonstrate low variability. The largest mean value is in platelet is 530.5, is associated with the widest spread, as evidenced by a standard deviation of 81.32. Notably, for all parameters the P-values are marked as "N/A," suggesting that no inferential statistical tests were performed and therefore no conclusions about significance can be drawn as no males group to be compared. Overall, the table provides useful descriptive insights but lacks information on statistical comparisons or significance.

Table 5: Results on the haematological parameters of the sub-adult stage in elephant

Parameters	Units	Male				Female				P-Value
		Minimum	Maximum	Mean	Standard Deviation	Minimum	Maximum	Mean	Standard Deviation	
Leukocytes	10^3/µl	N/A	N/A	N/A	N/A	16.8	30.4	23.6	9.62	N/A
Lymphocytes	10^3/µl	N/A	N/A	N/A	N/A	8.96	15.10	12.03	4.34	N/A
Monocytes	10^3/µl	N/A	N/A	N/A	N/A	0.50	1.92	1.21	1.0	N/A
Heterophils	10^3/µl	N/A	N/A	N/A	N/A	6.72	12.87	9.80	4.35	N/A
Basophils	10^3/µl	N/A	N/A	N/A	N/A	0.0	0.17	0.09	0.12	N/A
Eosinophils	10^3/µl	N/A	N/A	N/A	N/A	0.45	0.51	0.48	0.04	N/A
Erythrocytes	10^6/µl	N/A	N/A	N/A	N/A	2.78	3.0	2.89	0.16	N/A
Haemoglobin	g/dl	N/A	N/A	N/A	N/A	10.9	11.0	10.95	0.07	N/A
Haematocrit	%	N/A	N/A	N/A	N/A	33.5	36.1	34.8	1.84	N/A
Mean Corpuscular Volume (MCV)	μm^3	N/A	N/A	N/A	N/A	111.7	129.9	120.8	12.87	N/A

Mean Corpuscular Haemoglobin (MCH)	Pg	N/A	N/A	N/A	N/A	36.3	39.6	37.95	2.33	N/A
Mean Corpuscular Haemoglobin Concentration (MCHC)	g/dl	N/A	N/A	N/A	N/A	30.5	32.5	31.5	1.41	N/A
Red Distribution Width (RDW)	%	N/A	N/A	N/A	N/A	14.1	15.3	14.7	0.85	N/A
Platelet	$10^3/\mu l$	N/A	N/A	N/A	N/A	473	588	530.5	81.32	N/A

N.B: P value derived from independent t-test;

iv) Adult stage

The adult group comprises 20 elephants, including 4 males and 16 females. The haematological parameters of adult male and female elephants show some differences, but none are statistically significant (Table 6). For leukocyte counts, males had a mean of 22.53 × 10^3/µl, while females had a slightly lower mean of 22.0 × 10^3/µl, with a p-value of 0.30, indicating no significant difference between the sexes. Lymphocyte counts were also comparable, with males at 12.19 × 10^3/µl and females at 11.95 × 10^3/µl, showing no significant difference (p-value = 0.34). Monocyte counts were higher in males (1.75 × 10^3/µl) than in females (1.05 × 10^3/µl), but this difference was not significant (p-value = 0.78). For other parameters, heterophil counts were similar between males (7.87 × 10^3/µl) and females (8.12 × 10^3/µl), with no significant difference (p-value = 0.58). Basophil and eosinophil counts were also similar between males and females, with no significant differences found.

For red blood cell parameters, erythrocytes in males had a slightly higher mean value (3.21 × 10^6/µl) compared to females (3.22 × 10^6/µl), but again, no significant difference was found (p-value = 0.46). Haemoglobin levels were nearly identical, with males at 12.13 g/dl and females at 12.45 g/dl, with a p-value of 0.43. The mean corpuscular volume (MCV) was slightly higher in males (122.83 µm³) compared to females (119.85 µm³), but this difference was also not statistically significant (p-value = 0.16). The mean corpuscular haemoglobin (MCH) was similar between males (37.78 pg) and females (43.71 pg), with no significant difference (p-value = 0.36). The mean corpuscular haemoglobin concentration

(MCHC) was slightly higher in females (31.38 g/dl) compared to males (30.78 g/dl), but again, the difference was not significant (p-value = 0.47).

Red distribution width (RDW) values were quite similar between the sexes, with males at 14.6% and females at 15.98%, and no significant difference was found (p-value = 0.40). no difference ($p>0.05$) in platelet counts, with females has higher mean platelet count (783.50 × 10^3/µl) compared to males (606.75 × 10^3/µl). This indicates that, while most parameters showed no significant differences between adult males and females.

Table 6: Results on the haematological parameters of the adult stage in elephant

Parameters	Units	Male				Female				P-Value
		Minimum	Maximum	Mean	Standard Deviation	Minimum	Maximum	Mean	Standard Deviation	
Leukocytes	10^3/µl	19.0	25.3	22..53	2.63	12.1	30.1	22	4.63	0.30
Lymphocytes	10^3/µl	10.45	13.66	12.19	1.38	6.49	16.68	11.95	2.71	0.34
Monocytes	10^3/µl	0.93	2.78	1.75	0.87	0.25	2.12	1.05	0.52	0.78
Heterophils	10^3/µl	5.76	9.71	7.87	1.67	4.48	12.14	8.12	2.03	0.58
Basophils	10^3/µl	0.17	0.38	0.24	0.10	0.0	0.61	0.22	0.17	0.22
Eosinophils	10^3/µl	0.17	0.68	0.48	0.23	0.32	1.26	0.67	0.27	0.57
Erythrocytes	10^6/µl	3.03	3.43	3.21	0.18	2.43	3.73	3.22	0.35	0.46
Haemoglobin	g/dl	11.1	13.0	12.13	0.88	9.7	14.6	12.45	1.40	0.43
Haematocrit	%	36.3	42.7	39.43	3.06	32.7	47.1	40.06	4.18	0.48
Mean Corpuscular Volume (MCV)	µm^3	117.5	125.9	122.83	3.70	40.3	134.6	119.85	22.01	0.37

Mean Corpuscular Haemoglobin (MCH)	pg	35.9	38.7	37.78	1.30	35.5	116.1	43.71	19.40	0.36
Mean Corpuscular Haemoglobin Concentration (MCHC)	g/dl	30.4	31.3	30.78	0.39	29.7	35.7	31.38	1.29	0.47
Red Distribution Width (RDW)	%	13.6	16.1	14.6	1.06	13.6	30.8	15.98	4.15	0.40
Platelet	$10^3/\mu l$	540.0	722.0	606.75	82.30	523	1112.0	783.50	165.28	0.15

N.B: P value derived from independent t-test;

4.3 SERUM BIOCHEMICAL PARAMETERS

The mean, standard deviation, minimum and maximum values and P-value of the serum biochemistry parameters for each animal stage are presented in Tables 7, 8 and 9. The parameters consist of alanine Aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total bilirubin, globulin, albumin, total protein, cholesterol, creatinine kinase, glucose, urea, creatinine, ammonia, calcium and phosphorus were evaluated.

i) Infant stage

Based on the patient signalment during physical examination and data record, no individuals were below 5 years old.

ii) Juvenile stage

Table 7 compares serum biochemical parameters in juvenile which 2 males and 6 females. Most parameters show no significant ($p > 0.05$) differences between sexes. Alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), bilirubin, globulin, and albumin levels were similar between males and females, with p-values ranging from 0.14 to 0.95. Notably, total protein levels were significantly higher in males (mean: 7.5 g/dl) than in females (mean: 6.98 g/dl), with a p-value of less than 0.001. Cholesterol and creatinine kinase levels also exhibit no significant ($p > 0.05$) sex differences. For additional parameters such as glucose, urea, creatinine, ammonia, calcium, and phosphorus, both sexes show comparable mean values, with no significant differences, as indicated by p-values ranging from 0.22 to 0.73. Overall, the data suggest that except for total protein, the biochemical profiles of juvenile males and females are largely similar.

Table 7: Results on the serum biochemical parameters of the juvenile stage in elephant

Parameters	Units	Male				Female				P-Value
		Minimum	Maximum	Mean	Standard Deviation	Minimum	Maximum	Mean	Standard Deviation	
Alanine Aminotransferase (ALT)	u/l	8.5	12.2	10.35	2.62	7.9	12.1	9.47	1.65	0.41
Alkaline Phosphatase (ALP)	u/l	71.2	81.6	76.4	7.35	57	80.2	70.62	8.14	0.95
Lactate Dehydrogenase (LDH)	u/l	4.51	4.96	4.74	0.32	4.0	5.07	4.50	0.44	0.34
Total Bilirubin	mg/dl	0.1	0.1	0.1	0.0	0.1	0.3	0.18	0.08	0.14
Globulin	g/dl	4.6	4.6	4.6	0.0	3.2	4.8	4.02	0.53	0.19
Albumin	g/dl	2.6	3.2	2.9	0.42	2.1	3.8	2.97	0.57	0.73
Total Protein	g/dl	7.2	7.8	7.5	0.42	6.9	7.1	6.98	0.08	<0.001*
Cholesterol	mg/dl	26.0	34.0	30.0	5.66	14.7	34.0	24.78	7.68	0.26

Creatinine Kinase	u/l	181	190	185.5	6.36	163	200	180.33	13.34	0.31
Glucose	mg/dl	37.0	45.3	41.15	5.87	32.0	47.0	38.98	4.85	0.71
Urea	mg/dl	10	10	10.0	0.0	7	12	8.83	1.72	0.22
Creatinine	mg/dl	0.6	0.7	0.65	0.07	0.6	1.1	0.77	0.19	0.37
Ammonia	µmol/l	553	739	646.0	131.52	326	758	559.33	157.23	0.73
Calcium	mg/dl	9.1	12.5	10.8	2.40	8.2	12.9	9.92	1.74	0.62
Phosphorus	mg/dl	4.5	5.0	4.75	0.35	3.7	7.2	5.52	1.27	0.24

N.B: P value derived from independent t-test; * indicates significance difference; Total Protein

iii) Sub-Adult stage

Table 8 summarises the serum biochemical parameters of 2 sub-adult females, as data for males is marked as unavailable (N/A). The parameters include mean, standard deviation and statistical significance for various biochemical markers.

The table presents descriptive statistics for various variables measured in a female population, showing minimum, maximum, mean and standard deviation values. The results reveal a mix of low and moderate variability across the variables. For instance, alanine aminotransferase (ALT) has a mean of 9.1 with a standard deviation of 0.85, indicating relatively consistent measurements. Ammonia shows the highest variability, with a mean of 537 and a standard deviation of 4.24, reflecting a broader spread in the data. In contrast, total bilirubin and total protein display the least variability, with means of 0.15 and 7.15, respectively, and standard deviations of 0.07, indicating highly consistent results. Overall, the data highlights differences in variability and central tendencies across the variables, though the absence of P-values indicates that no inferential statistical analyses were conducted. Overall, the biochemical profile of sub-adult females shows consistent mean values across parameters, with no statistically significant differences observed. Data for males is unavailable for comparison.

Table 8: Results on the serum biochemical of sub-adult stage in elephant

Parameters	Units	Male				Female				P-Value
		Minimum	Maximum	Mean	Standard Deviation	Minimum	Maximum	Mean	Standard Deviation	
Alanine Aminotransferase (ALT)	u/l	N/A	N/A	N/A	N/A	8.5	9.7	9.1	0.85	N/A
Alkaline Phosphatase (ALP)	u/l	N/A	N/A	N/A	N/A	69.2	73.1	71.15	2.76	N/A
Lactate Dehydrogenase (LDH)	u/l	N/A	N/A	N/A	N/A	3.43	3.6	3.52	0.12	N/A
Total Bilirubin	mg/dl	N/A	N/A	N/A	N/A	0.1	0.2	0.15	0.07	N/A
Globulin	g/dl	N/A	N/A	N/A	N/A	2.7	3.8	3.25	0.78	N/A
Albumin	g/dl	N/A	N/A	N/A	N/A	3.4	4.4	3.9	0.71	N/A
Total Protein	g/dl	N/A	N/A	N/A	N/A	7.1	7.2	7.15	0.07	N/A
Cholesterol	mg/dl	N/A	N/A	N/A	N/A	17	20	18.5	2.12	N/A

Creatinine Kinase	u/l	N/A	N/A	N/A	N/A	175.2	180	177.6	3.39	N/A
Glucose	mg/dl	N/A	N/A	N/A	N/A	45.0	46.3	45.65	0.92	N/A
Urea	mg/dl	N/A	N/A	N/A	N/A	8	12	10.0	2.83	N/A
Creatinine	mg/dl	N/A	N/A	N/A	N/A	0.8	1.0	0.9	0.14	N/A
Ammonia	µmol/l	N/A	N/A	N/A	N/A	534	540	537.0	4.24	N/A
Calcium	mg/dl	N/A	N/A	N/A	N/A	9.0	9.3	9.15	0.21	N/A
Phosphorus	mg/dl	N/A	N/A	N/A	N/A	3.2	5.9	4.55	1.91	N/A

N.B: P value derived from independent t-test;

iv) Adult stage

Table 9 presents data on the serum biochemical parameters of 4 males and 16 females adults, showing mean, standard deviation, minimum, and maximum values, along with p-values to assess sex-based significance. Most parameters exhibit no significant differences between genders. For alanine aminotransferase (ALT), the mean values for males were 10.05 u/l and for females were 9.31 u/l, with a non-significant p-value of 0.32. Alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) showed mean values of 74.38 u/l and 4.07 u/l for males, and 70.67 u/l and 4.66 u/l for females, respectively, also without significant differences (p-values 0.40 and 0.32). Total bilirubin levels were slightly higher in females (0.25 mg/dl) compared to males (0.18 mg/dl), but this difference was not significant ($p = 0.56$).

Nevertheless, a significant ($p<0.05$) finding was observed in globulin levels, where males have a higher mean (4.38 g/dl) than females (4.13 g/dl), with a p-value of 0.02. In contrast, albumin and total protein levels did not show significant differences, with mean values being similar across sexes (p-values 0.24 and 0.37, respectively). Cholesterol levels were nearly the same in males (24.33 mg/dl) and females (23.94 mg/dl), with a p-value of 0.90. Creatinine kinase values were comparable, with no significant difference ($p = 0.06$). Glucose levels show mean values of 39.53 mg/dl for males and 39.79 mg/dl for females, with a non-significant p-value of 0.14.

Other parameters such as urea, creatinine, ammonia, calcium, and phosphorus also revealed similar mean values between sexes, with p-values ranging from 0.22 to

0.77, indicating no significant sexes-based differences. Overall, while most biochemical parameters show comparable results between adult males and females, globulin was the only parameter with a significant ($p>0.05$) higher mean value in males.

Table 9: Results on the serum biochemical parameters of adult stage in elephant

Parameters	Units	Male				Female				P-Value
		Minimum	Maximum	Mean	Standard Deviation	Minimum	Maximum	Mean	Standard Deviation	
Alanine Aminotransferase (ALT)	u/l	8.3	11.2	10.05	1.24	7.1	12.7	9.31	1.7	0.32
Alkaline Phosphatase (ALP)	u/l	71.8	81.2	74.38	4.56	61.5	90.0	70.67	7.97	0.40
Lactate Dehydrogenase (LDH)	u/l	3.29	4.79	4.07	0.62	3.18	5.74	4.66	0.79	0.32
Total Bilirubin	mg/dl	0.1	0.3	0.18	0.1	0.1	1.40	0.25	0.31	0.56
Globulin	g/dl	3.7	6.0	4.38	1.09	3.4	5.3	4.13	0.44	0.02
Albumin	g/dl	1.7	3.3	2.86	0.78	1.8	3.6	2.92	0.49	0.24
Total Protein	g/dl	7.0	7.7	7.25	0.33	6.5	7.4	7.06	0.23	0.37
Cholesterol	mg/dl	18.0	35.0	24.33	7.59	15.0	35.0	23.94	6.03	0.9

Creatinine Kinase	u/l	175.0	187.0	182.55	5.49	162	215	186.26	15.73	0.06
Glucose	mg/dl	37.9	40.6	39.53	1.17	35.0	42.0	39.79	2.22	0.14
Urea	mg/dl	9.0	11.2	10.55	1.04	6.0	12.0	9.23	1.77	0.22
Creatinine	mg/dl	0.7	1.4	1.03	0.33	0.7	1.6	0.9	0.25	0.30
Ammonia	µmol/l	315	587	474.0	118.10	370	685	522.44	81.80	0.41
Calcium	mg/dl	9.0	10.0	9.33	0.46	8.5	10.7	9.33	0.57	0.63
Phosphorus	mg/dl	3.9	5.1	4.73	0.56	3.8	7.0	4.98	0.74	0.77

N.B: P value derived from independent t-test;

CHAPTER 5

DISCUSSION

5.1 Haematology

There were no notable differences ($p>0.05$) in haematological values between males and females across any stages: juvenile, sub-adult, and adult elephants (Tables 1, 2, and 3). The RDW count observed in this study was below the reference range for European zoos (28–32%) as reported by Fowler and Mikota (2006) but aligned with the reference values documented in Sri Lanka (14.79–15.43%) by Ranjini *et al.* (2021). In this study, lymphocytes were the most predominant white blood cells, followed by heterophils, basophils, eosinophils, and monocytes. Similar findings have been reported in previous studies, where lymphocytes were consistently identified as the dominant white blood cell type (Nirmalin, 1967; Jainudeen, 1971; Allen, 1985; Silva, 1993; Janyamethakul *et al*., 2017).

The study revealed that, based on clinical-pathological observations, particularly thrombocytopenia, the mean platelet count during juvenile and sub-adult stages fell within the ranges reported by Fowler and Mikota (2006), Janyamethakul *et al.* (2017), and Ranjini *et al*. (2021). In contrast, the mean platelet counts in adults exceeded the values noted in the previous studies. Tatiana *et al.* (2021) identified elevated platelet levels (>1,000 K /ml) in elephants with inflammatory conditions. Regular monitoring of platelet concentrations is essential for tracking health trends and identifying cases of Elephant Endotheliotropic Herpesvirus (EEHV), as thrombocytopenia is a key indicator of this disease (Richman *et al.*, 2000). Further research on platelet estimates is necessary to establish reference ranges and better understand disease-related variations.

5.2 Liver enzymes

According to Fowler and Mikota (2006), ALT is an enzyme that facilitates the transamination of L-alanine and 2-oxoglutarate to produce pyruvate and glutamate. It is associated with muscle specificity in large animals, while in dogs, it is liver-specific. In this study, the mean serum ALT value was slightly higher than the levels reported by Fowler and Mikota (2006), Janyamethakul *et al*. (2017), and Ranjini *et al*. (2021) but fell within the range noted by Veeraselvam *et al*. (2023). Fowler and Mikota (2006) also suggested that serum ALT is generally not a useful diagnostic marker for elephants, as their serum ALT levels tend to be low. These findings align with observations by Kaneko (1989) and Sreekumar and Nirmalan (1992), who noted that the livers of mature horses, cattle, sheep, and goats contain minimal ALT, with only minor elevations observed due to hepatic changes in these species.

Alkaline phosphatase (ALKP) catalyses the hydrolysis of organic pyrophosphates and is most concentrated in the bone, liver, kidney, intestine, and placenta (Schoppet & Shanahan, 2008). In this study, the ALKP values were consistent with those reported in other studies, showing no significant ($p>0.05$) differences. Additionally, no significant variations ($p>0.05$) in ALKP levels were observed across different age groups. However, Fowler and Mikota (2006) noted that ALKP levels are generally higher in young, growing mammals, including elephants. The present study reflected this trend, where male juveniles showed the highest mean serum ALKP levels. Furthermore, the study found that males had slightly higher mean serum ALKP levels than females, which aligns with Niemuller's (1990) observation that musth bulls tend to have elevated levels of this enzyme compared to non-musth elephants.

5.3 Total bilirubin and cholesterol

Total bilirubin, a byproduct of haemoglobin breakdown, is commonly used to assess liver function across animal species. In this study, no significant ($p>0.05$) differences in total bilirubin levels were observed between males and females at any stage of life. Furthermore, the total bilirubin values were lower but within the ranges reported in other literature (Fowler and Mikota, 2006; Janyamethakul *et al.*, 2017; Ranjini *et al*., 2021). These lower values may be attributed to different geographical regions, as highlighted by Veeraselvam *et al*. (2023).

Cholesterol levels in this study were slightly lower than those mentioned by Fowler and Mikota (2006) and Ranjini *et al*. (2021). According to Veeraselvam *et al*. (2023), such variations in cholesterol levels could result from differences in feeding regimens and the stable availability of feed under captive conditions. In this study, the elephants were fed a diet comprising horse commercial pellets, Napier grass and seasonal fruits, which may have influenced these findings.

5.4 Serum total protein, albumin and globulin

The serum total protein values observed in this study were consistent with those reported by Fowler and Mikota (2006), Janyamethakul *et al*. (2017), and Ranjini *et al*. (2021). Despite, the total protein in juveniles exhibit lower p-values ($p<0.05$) because large effect sizes combined with high precision can lead to statistical significance even in studies with small sample sizes (Bangdiwala, 2016). Similarly, the albumin and globulin values fell within the ranges that Fowler and Mikota (2006) and Ranjini *et al*. (2021) reported. However, in this study, globulin levels were higher than albumin. Plasma proteins serve various homeostatic functions, including acting as carriers, clotting factors, complement components, acute-phase reactants, and immunoglobulins.

Fowler and Mikota (2006) also noted that elephants typically exhibit higher total protein and globulin levels and lower albumin levels than most other mammals. They attributed this to the high viscosity of elephant blood, nearly twice that of other domestic species, which could explain the elevated serum protein and globulin levels.

5.5 Muscle enzyme

Creatine kinase (CK), an enzyme indicative of muscle necrosis and damage, was also analysed in this study. CK is primarily found in skeletal muscle, cardiac muscle, and the brain. The CK values observed in this study were within the range reported by Fowler and Mikota (2006).

5.6 Serum glucose

The glucose levels in this study showed no significant ($p>0.05$) differences between sexes at any stage. However, the values were lower compared to those reported by Fowler and Mikota (2006) is 60-116 mg/dL, Ranjini *et al*. (2021) is 79.05-80.7 mg/dL, and Veeraselvam *et al*. (2023) is 89.05-99.13 mg/dL. Similarly, studies by Simon (1961) and Nirmalan *et al*. (1969) also reported lower glucose levels, which may be attributed to insufficient measures to prevent glycolysis after blood collection. Varley (1969) noted that glucose levels in whole blood can decrease rapidly if there is a delay between sample collection and analysis. Appropriate blood tubes containing sodium fluoride are highly recommended in measuring glucose blood level in animals, including elephants.

5.7 Urea, Creatinine and ammonia

In this study, the urea values fall within the range reported by Fowler and Mikota (2006) and Janyamethakul *et al*. (2017). However, the urea levels reported by Ranjini *et al*. (2021) and Veeraselvam *et al*. (2023) were slightly higher than those observed in this study. Their discussions suggest that the wide range of urea levels could be influenced by factors such as

dehydration, differences in protein intake, protein catabolism, and renal excretion. This study also found that younger elephants exhibited lower urea values compared to adults, which is believed to be associated with increased protein intake as animals age. Protein metabolism has been shown to be higher in adult animals than in younger ones (Rahman *et al*., 2018). Creatinine, a metabolite produced during muscle metabolism, is released into the blood and excreted by the kidneys. This study observed no significant ($p>0.05$) differences in creatinine levels between males and females at any stage of life. Additionally, the creatinine values fell within the range reported in studies by Fowler and Mikota (2006), Janyamethakul *et al*. (2017), Ranjini *et al*. (2021) and Veeraselvam *et al*. (2023).

5.8 Calcium and phosphorus

The calcium and phosphorus levels observed in this study were consistent with those that Fowler and Mikota (2006) and Veeraselvam *et al*. (2023) reported. According to Fowler (1986), serum calcium and phosphorus levels typically remain within normal ranges unless influenced by metabolic diseases or nutrient deficiencies. In this study, no significant ($p>0.05$) differences were found in calcium and phosphorus levels across different age groups, aligning with findings by Brown and White (1977), who also reported no variations based on age or sex in elephants. Veeraselvam *et al*. (2023) further suggested that diet intake and environmental factors significantly impact intestinal absorption and serum levels of these minerals.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

This study comprehensively analysed haematological and biochemical parameters in elephants at different life stages (juvenile, sub-adult and adult). The findings indicated no significant ($p>0.05$) differences in haematological and biochemical values between male and female elephants across all stages. The observed values for parameters such as RDW, platelet count, and various liver enzymes (ALT, ALKP) were generally consistent with previously reported reference values. However, some parameters, such as platelet count in adult elephants, exceeded previously reported levels, highlighting the importance of continuous monitoring, especially concerning diseases like Elephant Endotheliotropic Herpesvirus (EEHV). Biochemical markers such as total protein, albumin, and globulin were within expected ranges, with notable trends in protein composition suggesting higher globulin levels. Additionally, muscle enzyme levels, serum glucose, creatinine, calcium, and phosphorus concentrations aligned with established reference ranges, confirming the health and nutritional status of the elephants in the study.

It is recommended that regular monitoring of key health indicators, especially platelet counts, be carried out to ensure early detection of potential health issues like EEHV. Future research should aim to establish more specific reference ranges for platelet counts and coagulation parameters in elephants, as these values are vital for tracking the population's overall health status. Besides, serum acute phase protein analysis shall be studied to explore the use of these biomarkers in detecting early inflammation in elephants. The study also suggests that diet and environmental factors play a significant role in mineral and electrolyte concentrations, highlighting the importance of tailored nutritional management for captive elephants. Furthermore, continued collaboration between wildlife conservation centres and veterinary

institutions can enhance the accuracy of health assessments and provide valuable data for better conservation practices and elephant care.

REFERENCES

1. Allen, J. L., Jacobson, E. R., Harvey, J. W., & Boyce, W. (1985). Hematologic and serum chemical values for young African elephants (Loxodonta african) with variations for sex and age. *The Journal of Zoo Animal Medicine*, *16*(3), 98-101.
2. Bangdiwala, S. I. (2016). Understanding significance and p-values. *Nepal journal of epidemiology*, *6*(1), 522.
3. Cheynel, L., Lemaître, J. F., Gaillard, J. M., Rey, B., Bourgoin, G., Ferté, H., ... & Gilot-Fromont, E. (2017). Immunosenescence patterns differ between populations but not between sexes in a long-lived mammal. Scientific reports, 7(1), 1-11.
4. C. Salakij, J. Salakij, Narkkong, N. A., D. Tongthainun, K. Prihirunkit, & S. Itarat. (2007). Hematology, cytochemistry and ultrastructure of blood cells in common palm civet (Paradoxurus hermaphroditus). Witthayasan Kasetsat Witthayasat, 41(4), 705–716.
5. De Alwis, G. K. H., Wijesekera, R. D., Vithana, D., Neththasinghe, N., & Ratnasooriya, W. D. (2011). Serum levels of some biochemical constituents of captive Sri Lankan elephants (Elephas maximus maximus).
6. Dos Santos, D. J. F., Jackson, J., Phil, M., Aung, H. H., Nyein, U. K., Htut, W., & Lummaa, V. (2020). Sex differences in the reference intervals of health parameters in semi captive Asian elephants (Elephas maximus) from Myanmar. Journal of zoo and wildlife medicine, 51(1), 25-38.
7. Fowler, M. E. (1986). Elephants (Proboscidae). Zoo and Wild Animal Medicine, 884-923.
8. Fowler, M. E., & Mikota, S. K. (2006). Biology, Medicine, and Surgery of Elephants.
9. Fernando, P., & Lande, R. (2000). Molecular genetic and behavioral analysis of social organisation in the Asian elephant (Elephas maximus). Behavioral Ecology and Sociobiology, 48, 84-91.
10. Franco dos Santos, D. J., Berger, V., Cristofari, R., Htut, W., Nyein, U. K., Aung, H. H., ... & Lummaa, V. (2020). Seasonal variation of health in Asian elephants. Conservation Physiology, 8(1), coaa119.
11. Gromadzka-Ostrowska, J., Jakubów, K., Zalewska, B., & Krzywicki, Z. (1988). Haematological and blood biochemical studies in female domesticated Indian elephants (Elephas maximus L.). Comparative Biochemistry and Physiology. A, Comparative Physiology, 89(3), 313-315.
12. ISMAIL, A. I. (2023, November 4). Cabaran menjadi gembala gajah. Sinar Harian. https://www.sinarharian.com.my/article/632910/berita/semasa/cabaran menjadi-gembala-gajah
13. Jorge Ortega, Lori Eggert, Sukumar, R. 2003. The Living Elephants: Evolutionary Ecology, Behavior, and Conservation. Oxford University Press, New York, 478 pp. ISBN 0-19-510778-0, price (hardbound), $74.50, Journal of Mammalogy, Volume 85, Issue 3, June 2004, Pages 581–582, https://doi.org/10.1644/1383960
14. Jainudeen, M. R., McKay, G. M., & Eisenberg, J. F. (1972). Observations on musth in the domesticated Asiatic elephant (Elephas maximus).

15. Janyamethakul, T., Sripiboon, S., Somgird, C., Pongsopawijit, P., Panyapornwithaya, V., Klinhom, S., ... & Thitaram, C. (2017). Hematologic and biochemical reference intervals for captive Asian elephants (Elephas maximus) in Thailand. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 23(4).
16. Kaneko, J.J., 1989. Clinical biochemistry of domestic animals (4th Edn.). Academic Press, San Diego, 821–837.
17. Kjelgaard-Hansen, M., & Jensen, A. L. (2006). Is the inherent imprecision of manual leukocyte differential counts acceptable for quantitative purposes?. Veterinary Clinical Pathology, 35(3), 268-270.
18. Mel, R. K. D., Weerakoon, D. K., Ratnasooriya, W. D., & Dangolla, A. (2014). A comparative haematological analysis of Asian Elephants Elephas maximus Linnaeus, 1758 (Mammalia: Proboscidea: Elephantidae) managed under different captive conditions in Sri Lanka. Journal of Threatened Taxa, 6(8), 6148-6150.
19. Morfeld, K. A., Lehnhardt, J., Alligood, C., Bolling, J., & Brown, J. L. (2014). Development of a body condition scoring index for female African elephants validated by ultrasound measurements of subcutaneous fat. PloS one, 9(4), e93802.
20. Menon, V., & Tiwari, S. K. (2019). Population status of Asian elephants Elephas maximus and key threats. International Zoo Yearbook, 53(1), 17-30.
21. Nirmalan, G., & Nair, S. G. (1969). Biochemical studies on the blood of Indian elephants (Elephas maximus). *Research in Veterinary Science*, *10*(2), 176-180.
22. Ranjini, M., P. M. Deepa and Ashok, R. U. 2021. Clinico–Physiological, Haemato–Biochemical and Urinalysis of 20 Adult Captive Indian Elephants (Elephas maximus).Int.J.Curr.Microbiol.App.Sci. 10(2): 2091-2099.
23. Ranjan, A., Komal, Gattani, A., & Kumar, A. (2023). Blood biochemical profile of Asian elephant (Elephas maximus) in captive state.
24. Saaban, S., Othman, N. B., Yasak, M. N. B., Burhanuddin, M. N., Zafir, A., & Campos-Arceiz, A. (2011). Current status of Asian elephants in Peninsular Malaysia. Gajah, 35(1), 67-75.
25. Stacy, N. I., Isaza, R., & Wiedner, E. (2017). First report of changes in leukocyte morphology in response to inflammatory conditions in Asian and African elephants (Elephas maximus and Loxodonta africana). PLoS One, 12(9), e0185277.
26. Silva, I. D., & Kuruwita, V. Y. (1993). Hematology, plasma, and serum biochemistry values in free-ranging elephants (Elephas maximus ceylonicus) in Sri Lanka. *Journal of zoo and wildlife medicine*, 434-439.
27. Schoppet M, Shanahan CM. Role for alkaline phosphatase as an inducer of vascular calcification in renal failure? Kidney Int (2008) 73:989–91. doi: 10.1038/ki.2008.104
28. Veeraselvam, M., Selvaraj, P., Senthil Kumar, S., Senthilkumar, A., Senthilkumar, T. M. A., Sreekumar, C., & Jyothi Priya, R. (2021). Haematology profile of the captive Asian elephants (Elephas maximus) in the Tamil Nadu state of India. The Pharma Innovation Journal SP-10 (10), 355-358.
29. Veeraselvam, M., Selvaraj, P., Kumar, S. S., Kumar, A. S., Senthilkumar, T. M. A., Priya, R. J., ... & Yogeshpriya, S. (2023). Serum biochemical profile of captive asian elephants (Elephas maximus) in Tamil Nadu, India. *International Journal of Bio-resource and Stress Management*, *14*(5), 683-690.

30. Weiss, D. J., & Wardrop, K. J. (Eds.). (2011). Schalm's veterinary haematology. John Wiley & Sons.
31. Williams, C., Group), S. T. (IUCN S. A. E. S., Varun Goswami (Conservation Initiatives, I., Inc), S. de S. (Trunks & L., A Kumar (Nature Conservation Foundation, I., N Baskaran (A. V.C. College, T. N., Pdr), K. Y. (WWF L., & Vivek Menon (Wildlife Trust of India and Chair, I. S. A. E. S. G. (2019, September 18). IUCN Red List of Threatened Species: Elephas maximus. IUCN Red List of Threatened Species. https://www.iucnredlist.org/species/7140/45818198
32. Weisbrod, T. C., Isaza, R., Cray, C., Adler, L., & Stacy, N. I. (2021). The importance of manual white blood cell differential counts and platelet estimates in elephant haematology: blood film review is essential. Veterinary Quarterly, 41(1), 30-35.

APPENDIX A; Physical examination data

Physical Examination/ ID	SURIYA	KALA	MAS	KAK MAH	INDAH	AKIYA	MEK DA	WAWA	ELLY	RAMBAI	PYAN	MEK SOM	AIDIL	ANI	SIPUT
Age	24Y	49Y	18Y	17Y	27	3Y 8M	3Y	19Y	7Y	41Y	50Y	5Y	36Y	13Y	22Y
Sex	Female	Female	Female	Female	Female	Male	Female	Female	Female	Female	Female	Female	Male	Female	Female
Body Condition Score	4	4	4	3	4	3	3	4	4	4	5	4	4	4	4
Temperature	34.2	34.9	34.9	35.9	34.3	34.8	34.9	35.2	36	35.1	35.2	38	38.2	36.5	37.5
Respiration	12	16	12	8	12	12	12	8	12	8	8	12	12	12	8
System															
General appearance	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Mucous Membrane	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Integument	NAF	Pruritis/skin thick (mange?)	NAF	NAF	NAF	NAF	NAF	Right forelimb presence of wound	Right forelimb lost due to snare	NAF	NAF	NAF	Wound at the back	NAF	Right forelimb have laceration

								(with pus) due to snare [Non healing wound?]					due to sun		wound for one year. No pus was observed and the wound is dry
Nervous	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Musculoskeletal	NAF	NAF	Right Hind Limb	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Eyes	NAF	has clear discharge	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Ears	NAF	NAF	NAF	NAF	NAF	NAF	Right eyes hs discharge for 2 weeks (mucus discharge)	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Gastrointestinal	NAF	had constipation	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Respiratory	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Cardiovascular	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Reproductive	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF

Urinary	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Mammary Gland	NAF	NAF	Suspected pregnant	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Lymphatic	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF

Physical Examination/ ID	**LINTANG**	**KASTURI**	**ALAM**	**AYU**	**KELAT**	**GANDAH**	**MEKLA**	**SITI**	**LEPAR**	**ABOT**	**SHAH**	**LASAH 1**	**TIMUR**	**LANGSAT**	**INANG**
Age	5Y	49Y	26Y	43Y	18Y	6Y	2Y 6M	43Y	13Y	28Y	4Y	26Y	50Y	16Y	19Y
Sex	Female	Female	Male	Female	Male	Female	Female	Female	Female	Female	Male	Male	Female	Female	Female
Body Weight															
Body Condition Score	2	4	4	4	4	4	4	4	4	4	3	4	4	4	4
Temperature	35.4	35.9	35.8	36.2	35	36.2	36.2	36.7	36.5	36.8	36.5	36.8	35.4	36.6	35.9
Respiration	12	12	12	12	12	12	12	12	8	12	12	12	12	12	12
System															
General appearance	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Mucous Membrane	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF

Integument	NAF	NAF	NAF	Right hindlimb has abscess wound. Has wound since 2013	NAF	NAF	NAF	NAF	Presence of white spot at the back which probably due to fungal infection on the back.	NAF	NAF	NAF	Presence of white spot at the back which probably due to fungal infection	NAF	NAF
Nervous	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Musculoskeletal	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Eyes	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Ears	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Gastrointestinal	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Respiratory	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Cardiovascular	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Reproductive	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Urinary	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Mammary Gland	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF
Lymphatic	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF	NAF

APPENDIX B: Leukocyte morphology

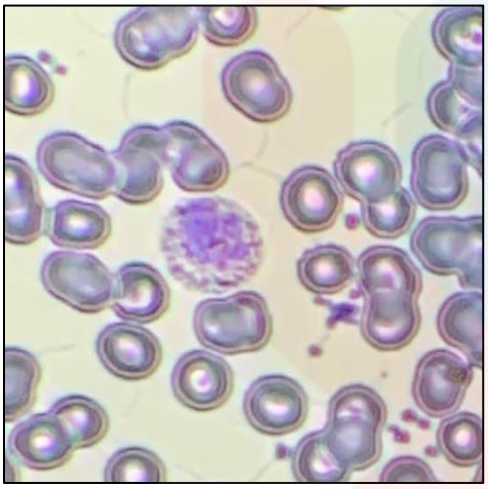

Figure 1: Presence of Basophil

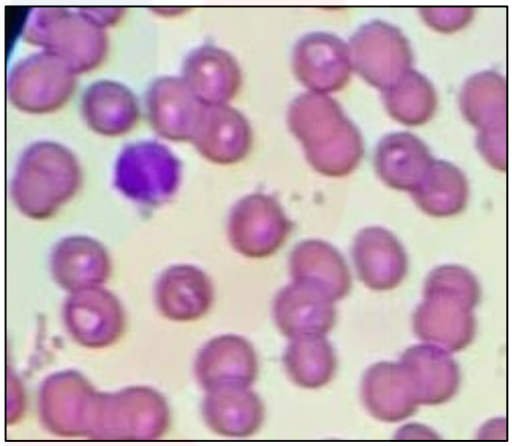

Figure 2: Presence of lymphocyte

Figure 3: Presence of heterophil

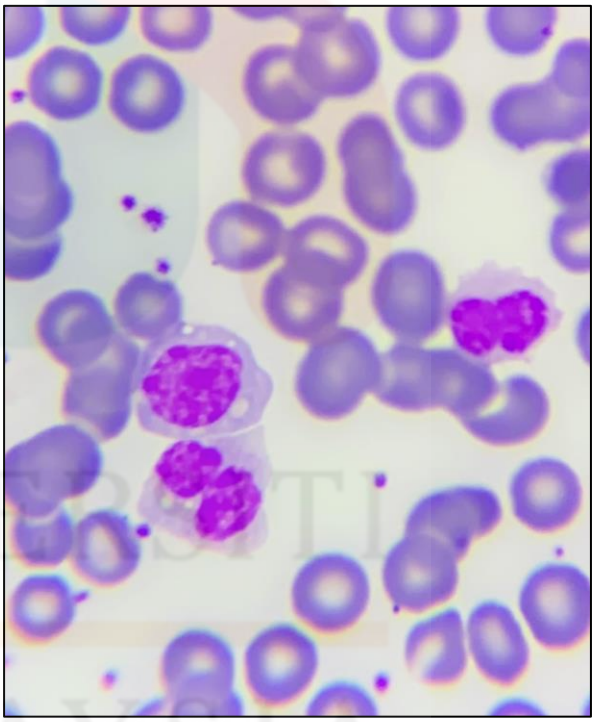

Figure 4: Presence of monocyte

APPENDIX C: Permit Approval Letter By The DWNP

IBU PEJABAT
JABATAN PERLINDUNGAN HIDUPAN LIAR DAN
TAMAN NEGARA (PERHILITAN) SEMENANJUNG MALAYSIA
HEADQUARTERS
DEPARTMENT OF WILDLIFE AND NATIONAL PARKS (DWNP)
PENINSULAR MALAYSIA
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Laman Web : www.wildlife.gov.my

Ruj. Kami: JPHLTN.600-6/1/4 JLD3 (26)
Tarikh : 15 Mei 2024

Ahmad Naimie Bin Mohammad Azlan
Universiti Malaysia Kelantan
1275 Jalan Padang Tembak
16100 Pengkalan Chepa
KELANTAN

Tuan,

KEPUTUSAN PERMOHONAN MENJALANKAN PENYELIDIKAN BERTAJUK *ESTABLISHING HAEMATOLOGICAL PARAMETERS OF ASIAN ELEPHANT (ELEPHAS MAXIMUS) IN MALAYSIA*

Dengan hormatnya saya diarah merujuk kepada perkara di atas dan keputusan Mesyuarat Jawatankuasa Penyelidikan Bil 05/24.

2. Sukacita dimaklumkan bahawa Jabatan **menyokong dan meluluskan** permohonan tuan untuk menjalankan penyelidikan seperti butiran di bawah:

Nama Pemohon	:	**Ahmad Naimie Bin Mohammad Azlan**
Institusi Pemohon	:	**Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan**
Tajuk	:	***Establishing Haematological Parameters Of Asian Elephant (Elephas Maximus) In Malaysia***
Tempoh	:	**Ogos 2024 – Disember 2024**
Lokasi	:	**a) Pusat Konservasi Gajah Kebangsaan (PKGK), Kuala Gandah, Pahang, Malaysia** **b) Pusat Konservasi Gajah Sungai Deka, Hulu Terengganu, Terengganu**

3. Sehubungan itu, tuan dipohon untuk melakukan beberapa perkara seperti berikut:

a) Menghubungi rakan saing dan *co-author* Jabatan yang dilantik iaitu Dr. Mohd Firdaus Ariff bin Abdul Razak, Pegawai Veterinar (Bahagian Konservasi Ex-Situ);

b) Berkongsi hasil penyelidikan laporan/penerbitan kertas saintifik/tesis dengan Jabatan melalui rakan saing;

c) Sampel tidak boleh dibawa ke luar negara;

d) Penyelidikan ini dapat memberi manfaat/ membantu kepada pengurusan analisa data hematologi Jabatan 2021 hingga 2024;

e) Menyumbangkan penulisan kepada Journal of Wildlife & Parks berkaitan perbandingan data hematologi Gajah Asia (*Elephas maxmius*) di dalam kurungan 2021 - 2024;

f) Perkembangan kajian perlu dimaklumkan kepada Jabatan pada setiap bulan Jun dan Disember daripada tarikh kajian bermula;

g) Mengemukakan satu laporan hasil penyelidikan yang lengkap kepada Jabatan dalam tempoh dua (2) bulan selepas tamat penyelidikan kepada Jabatan PERHILITAN;dan

h) Penyelidikan hendaklah diselesaikan dalam tempoh yang dinyatakan dalam kelulusan.

4. Sebarang pertanyaan mengenai perkara ini, tuan boleh berhubung dengan rakan saing yang telah dilantik atau Sekretariat Jawatankuasa Penyelidikan Jabatan PERHILITAN di talian 03-90866900 untuk maklumat lanjut.

5. Segala perhatian dan kerjasama tuan dalam perkara ini didahului dengan ucapan terima kasih.

Sekian.

"MALAYSIA MADANI"
"BERKHIDMAT UNTUK NEGARA"

Saya yang menjalankan amanah,

(DR. PAZIL BIN ABDUL PATAH)
Pengarah
Bahagian Konservasi Ex-Situ
b.p Ketua Pengarah
Jabatan Perlindungan Hidupan Liar dan
Taman Negara (PERHILITAN)

s.k.
Ketua Pengarah
Timbalan Ketua Pengarah (Konservasi)
Pengarah Bahagian Kawasan Perlindungan
Pengarah PERHILITAN Pahang
Pengarah PERHILITAN Terengganu

Ketua Pusat Konservasi Gajah Kebangsaan
Ketua Pusat Konservasi Gajah Sg. Deka

Dr. Mohd Firdaus Ariff bin Abdul Razak
Pegawai Veterinar
Bahagian Konservasi Ex-Situ
mfirdaus@wildlife.gov.my

BIL	MAKLUMAT UMUM	RINGKASAN PENYELIDIKAN
6.	**Tajuk Penyelidikan :** **P06/05/2024** Establishing Haematological Parameters Of Asian Elephant (Elephas Maximus) In Malaysia **Nama Pemohon :** Ahmad Naimie Bin Mohammad Azlan **Penyelia :** Dr Farhan Hanif Bin Reduan **Agensi :** Universiti Malaysia Kelantan **Jenis Penyelidikan :** Persampelan **Kategori Lokasi :** Luar kawasan perlindungan Dalam Kawasan Perlindungan **Subjek :** Hidupan Liar Jadual Kedua	**Objektif:** 1. Untuk menetukan nilai rujukan parameter darah bagi gajah Asia (*Elephas maximus*) yang dipelihara dalam kurungan di Malaysia. 2. Membandingkan nilai rujukan gajah Asia di Malaysia dengan negara lain. 3.Untuk membezakan parameter hematologi antara jantina gajah Asia di Malaysia **Lokasi:** 1. Pusat Konservasi Gajah Kebangsaan (PKGK), Kuala Gandah, Pahang, Malaysia 2. Pusat Konservasi Gajah Sungai Deka, Hulu Terengganu, Terengganu **Subjek:** Gajah Asia *(Elephas maximus)* **Sampel:** Darah **Metodologi:** 1. *Sample collection* The elephant will be placed in a comfortable position for blood collection by its mahouts and a DWNP veterinarian, either standing (for a juvenile elephant) or sternal or lateral recumbency. Next, the DWNP veterinarian will draw 9 ml of blood using a 21-gauge butterfly catheter and a 10 ml

	Tempoh & Tarikh Penyelidikan : Ogos 2024 – Disember 2024 **Punca Kuasa:** Akta 716 Seksyen 11 (d) & & Seksyen 10 (d) **Keperluan lesen/permit :** Permit Khas Penyelidikan	disposable syringe through the auricular vein. The drawn blood will be transferred into two tubes: one plain tube (6 ml) and one coated with EDTA (3 ml) for each elephant. To prevent the blood from clotting, the EDTA-coated tube will be gently turned upside down. The plain tube will be allowed to clot for 1-2 hours at room temperature before the serum is separated by centrifugation at 2500 rpm for 15 minutes. Additionally, blood smears will be prepared and stained with Giemsa stain for use in the differential counts carried out under a microscope at the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, University of Malaysia Kelantan. 8.3.4 Haematol 2. *Data analysis* The Mean and standard error (Mean ± SE) will be used to express the numerical variables. The quantitative variables within the groups will be compared using the Mann Whitney U test for non-parametric variables and the independent sample t-test for normally distributed variables. The SPSS version 29.0 will be used to conduct the statistical analysis. 3. *Stastical analysis*