UNIVERSITI PUTRA MALAYSIA

COMPARISON OF CLINICOPATHOLOGICAL FINDINGS OF LAR GIBBONS (*Hylobates lar*) IN DIFFERENT MANAGEMENT SYSTEMS, AGE AND SEX

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COMPARISON OF CLINICOPATHOLOGICAL FINDINGS OF LAR GIBBONS (*Hylobates lar*) IN DIFFERENT MANAGEMENT SYSTEMS, AGE AND SEX

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DEDICATION

This thesis is dedicated to

The memories of

Doggy

TJ

Roney

&

Chocky

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This project took a lot of time and energy to complete, and it would not have been possible without the generous help from many kind and humble people. I take this opportunity to thank the academicians who did not hold back any knowledge when it came to teaching. To my supervisor, Dr Azalea Hani Othman, working with you has made me feel the most confident and I am grateful for the trust you put in me to carry out this project. To Dr Azlan Che' Amat, I thank you for your kindness and patience with students and for your reassurance at times of uncertainty.

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ABBREVIATIONS

ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate aminotransferase
CK	Creatine kinase
GGT	Gamma-glutamyl transferase
GReP	Gibbon Rehabilitation Project
HCT	Hematocrit
LDH	Lactate dehydrogenase
MCV	Mean corpuscular volume
PCV	Packed-cell volume
RBC	Red Blood Cell
TP	Total Protein
WBC	White Blood Cell
CBC	Complete Blood Count
SBC	Serum Biochemistry
ISIS	International Species Information System

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar bagi memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Tahun Akhir.

PERBANDINGAN PARAMETER PATOLOGI KLINIKAL DALAM UNGKA TANGAN PUTIH *(Hylobates lar)* DARI SEGI SISTEM PENGURUSAN, UMUR DAN JANTINA YANG BERBEZA

oleh

Sathishwaran A/L Magis Paran

2023

Penyelia: Dr Azalea Hani Othman

Penyelia bersama: Dr Azlan Che' Amat

Walaupun terdapat beberapa kajian yang telah menggariskan kesan pengurungan terhadap tingkahlaku primat, perubahan hematologi dan biokimia serum sering kurang dikaji. Oleh itu, kajian ini bertujuan untuk membandingkan penemuan klinikopatologi bagi *lar gibbon* dalam sistem pengurusan yang berbeza, umur dan jantina dengan menganalisis parameter hematologi termasuk sel darah merah, hemoglobin, isi padu sel padat, min isi padu korpuskel, platelet dan kiraan perbezaan bilangan leukosit serta parameter biokimia serum iaitu natrium, kalium, klorida, kalsium, fosfat, jumlah protein (JP), albumin, globulin, urea, kreatinin, aspartat aminotransferase (AST), kreatina kinase (CK), alanina transaminase (ALT), fosfatase beralkali *(ALP), gamma-glutamyl transferase (GGT)*, amilase, glukosa, laktat dehidrogenase (LDH), bilirubin (terkonjugasi, tidak terkonjugasi, jumlah), kolesterol dan lipase. Sampel darah diperoleh melalui persampelan mudah daripada ungka

tangan putih yang sihat di tiga lokasi yang terdiri daripada Projek Rehabilitasi Gibbon Malaya (GReP) (n=11), Zoo Taiping (n=2) dan Zoo Melaka (n=3). Data retrospektif daripada ujian darah sebelumnya diperoleh daripada Zoo Negara (n=1). Maklumat tentang diet, spesifikasi kandang dan keadaan persekitaran telah diperolehi. Keputusan menunjukkan bahawa antara haiwan kurungan dan separa-kurungan, yang pertama menunjukkan aras monosit, natrium, JP, globulin, GGT dan kolesterol adalah lebih tinggi secara signifikan ($p<0.05$), manakala ungka separa-kurungan mempunyai kalium, klorida, albumin, urea, LDH, bilirubin (jumlah, tidak terkonjugasi), dan lipase yang lebih tinggi secara signifikan ($p<0.05$). Ungka dewasa mempunyai kiraan monosit yang lebih tinggi secara signifikan ($p<0.05$) berbanding dengan ungka adolesen, manakala nilai ALP yang lebih tinggi direkodkan daripada kumpulan juvenil. Dalam jantina betina merentasi jenis kurungan, ungka betina dalam kurungan mencatatkan JP, GGT dan kolesterol yang lebih tinggi secara signifikan ($p<0.05$) manakala ungka betina separa-kurungan mempunyai paras kalium dan klorida yang lebih tinggi secara signifikan ($p<0.05$). Kesimpulannya, dicadangkan iaitu sistem pengurusan yang berbeza barangkali menyumbang kepada kepelbagaian nilai biokimia serum dalam ungka, kemungkinan disebabkan oleh pemberian pemakanan yang berbeza. Maka, kajian ini boleh digunakan untuk mengubahsuai sistem pengurusan ungka tangan putih dari segi pemakanan dan habitatnya untuk meningkatkan usaha pemuliharaan dan pemulihan.

Kata kunci: *ungka tangan putih, hematologi, serum biokimia, pemakanan, habitat.*

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Final Year Project

COMPARISON OF CLINICOPATHOLOGICAL FINDINGS OF LAR GIBBONS (*Hylobates lar*) IN DIFFERENT MANAGEMENT SYSTEMS, AGE AND SEX

by

Sathishwaran A/L Magis Paran

2023

Supervisor: Dr Azalea Hani Othman

Co-supervisor: Dr Azlan Che' Amat

While few studies have outlined the effect of captivity on primate behaviour, haematological and biochemical changes are often understudied. Thus, this study aimed to compare clinicopathological findings of lar gibbons in different management systems, age and sex by examining haematological parameters including erythrocytes, haemoglobin, packed-cell volume (PCV), mean corpuscular volume (MCV), platelet and differential leucocyte count along with serum biochemical parameters namely sodium, potassium, chloride, calcium, phosphate, total protein (TP), albumin, globulin, urea, creatinine, aspartate aminotransferase (AST), creatine kinase (CK), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), amylase, glucose, lactate dehydrogenase (LDH), bilirubin (conjugated, unconjugated, total), cholesterol and lipase. Blood samples

were obtained via convenient sampling from clinically healthy lar gibbons in three locations which comprise of Malaya Gibbon Rehabilitation Project (GReP) (n=11), Zoo Taiping (n=2), and Zoo Melaka (n=3). Retrospective data from a previous blood test was obtained from Zoo Negara (n=1). Information on diet, enclosure specifications and environmental conditions were acquired. Results showed that between captive and semi-captive gibbons, the former displayed a significantly higher ($p<0.05$) monocyte, sodium, TP, globulin, GGT and cholesterol levels while semi-captive gibbons had significantly higher ($p<0.05$) potassium, chloride, albumin, urea, LDH, bilirubin (total, unconjugated), and lipase. Adults had a significantly higher ($p<0.05$) monocyte count in comparison to the adolescents, while higher values of ALP were recorded from the juvenile group. Within females across types of captivity, captive females recorded a significantly higher ($p<0.05$) TP, GGT and cholesterol while semi-captive females had significantly higher ($p<0.05$) levels of potassium and chloride. In conclusion, it is suggested that different management systems may have contributed to the variable values in overall serum biochemistry of lar gibbons, possibly due to different diet given. Hence, this study can be used to modify the captive lar gibbon's management system to improve conservation and rehabilitation efforts.

Keywords: *Lar gibbon, Haematology, Serum biochemistry, Diet, Habitat*

1.0 INTRODUCTION

1.1 Background of study

The lar gibbon *(Hylobates lar)* is an arboreal non-human primate that is characterised by the white-coloured hair that is characterised by the white-coloured hair on their hands and feet and pads on their rears called ischial callus (Beaman, 2014). In the wild, lar gibbons depend on the canopy layer in forests for their survival (Hankinson et. al., 2021) and they mainly feed on fruits, figs and leaves (Bartlett, 1999).

In a developing country like Malaysia, there is a huge threat on the survivability of the lar gibbons (Brockelman, 2020). Hence, this species are kept captive to protect from poachers and also as rehabilitation to be released back into the wild. There are two types of captivity management of gibbons in Malaysia: captive and semi-captive. Captive lar gibbons are housed in indoor-manmade enclosures with restricted space and are not restricted from exposure to humans. Semi-captive lar gibbons are housed in outdoor enclosures with restricted space and a higher resemblance to the wild habitat, on top of minimal human intervention (Modified from Hosey, 2005; Julien et. al., 2022).

Haematological studies are essential to comprehend how blood parameters correlate to the environment on an ecological and physiological level (Ovuru & Ekweozor, 2004). However, there has been limited to no research on how different types of captivities affect the lar gibbon's clinicopathological parameters. The lack of haematological data serves as a blockade to an in-depth comprehension of the effect of captivity and captive diet towards haematology and serum biochemistry of lar gibbons. Studies have reported significant differences in complete blood count and serum biochemistry parameters of animals in different habitat, age, and sex

(Trangerud, 2007; Shah et. al., 2022), but this is the first study of its kind in Southeast Asia on *Hylobates lar*.

1.2 Problem Statement

Lar gibbons are classified as “endangered” and its population is estimated to have declined by more than 50% throughout the last 45 years (Brockelman & Geissmann, 2020). These apes, native to Southeast Asia, including Peninsula Malaysia have been subjected to conservation and rehabilitation efforts from different organisations, locally and internationally. As a developing nation, Malaysia poses a huge threat to the survivability of the lar gibbon as more forests are cleared up for development and the remaining forests become fragmented, increasing likelihood of human-wildlife conflict at the same time constraining the gibbons in small, isolated areas. (Vellayan, 1981; Brockelman, 2020). To understand the lar gibbons’ physiology, haematology and serum biochemistry can be among the diagnostic tool to understand the effect of environment on this species on an ecological and physiological level (Ovuru & Ekweozor, 2004). However, there has been limited to no research on how different types of captivities affect the lar gibbon’s clinicopathological parameters.

1.3 Research Objectives

1. to examine the clinicopathological changes of lar gibbons in different management systems.
    a. to examine the clinicopathological changes of captive lar gibbons in comparison to semi-captive lar gibbons.
    b. to examine the clinicopathological changes of lar gibbons in different dietary management systems.
2. to examine the effect of age and sex on clinicopathological changes in lar gibbons

1.4 Research Questions

RQ1: Is there any significant difference in clinicopathological findings between lar gibbons in different management systems?

RQ2: Is there any significant difference in clinicopathological findings of lar gibbons of different sex and age?

1.5 Research Hypotheses

$H_0$1: There is no significant difference in clinicopathological findings between lar gibbons in different management systems.

$H_0$2: There is no significant difference in clinicopathological findings of lar gibbons of different sex and age.

1.6 Justification of the Study

This preliminary study is relevant to the conservation and rehabilitation efforts of lar gibbons as evidently there is a research gap in haematology of this species, particularly in Malaysia. This study also acts as an investigation onto the current reference values for lar gibbon haematology and serum biochemistry by the Species 360 database (Species360, 2023) while offering clinicopathological data to support findings from age, sex and dietary studies in this species. In addition, the comparison of clinicopathological findings done between different types of management systems may help determine the best management system for captive lar gibbons which may allow for improved conservation efforts for the currently endangered mammals. Improving the management system of lar gibbons will play a crucial role in saving this species from extinction.

Summary

This study aims to observe the effects of different management systems on the lar gibbon's physiology with a focal point on the haematology and serum biochemistry findings to allow early detection of abnormality in blood parameters, to assess the effect of human intervention on lar gibbons and to provide data to make necessary changes in management systems of lar gibbons.

2.0 LITERATURE REVIEW

2.1 Overview of Species

The lar gibbon (*Hylobates lar*) is an arboreal non-human primate and classified as lesser apes. The species belongs to the family of Hylobatidae (Bartlett, 1999). Lar gibbons, also known as white handed gibbons are characterised by the white-coloured hair on hands and feet as well as pads on its rears called ischial callus. Native to Southeast Asia, these mammals are currently endangered, and their population is estimated to have declined over 50% within the past 45 years (Brockelman, 2020). Locally, lar gibbons display a population density of 0.7-2.6 groups/km^2 in Kuala Lompat and Tanjong Triang (Ellefson 1968, 1974; MacKinnon 1977; MacKinnon and MacKinnon 1978; Bartlett 2007), while few smaller fragmented populations have been reported in north-western Malaysia (Brockelman, 2020). Their natural habitat lies in the canopy layer of forests (Hankinson et. al., 2021).

In the wild, lar gibbons spend their time feeding (32%), resting (27%), travelling (24%), performing social activity (11%), vocalizing (4%) and facing inter-group encounters (2%) as outlined in a study done in Khao Yai National Park, Thailand (Bartlett, 1999). Some anatomical specialisations of this species in contrast to other primates include a well-developed scapular spine, long forearms relative to both humerus and body size, and radii that are thicker sagitally than transversely, which are attributed to their arboreal lifestyle (Takahashi, 1990). The development of highways through protected areas, hunting of lar gibbons for the pet trade, exploitation of Aquilaria spp. trees, and commercial palm oil plantations are seemingly some of the major threats faced by the species. (Bartlett, 2007; Brockelman, 2020). The age groups for lar gibbons can be classified into five, as described by Brockelman et al (1998). The age classification is as described in Table 1.

Table 1. Age classification of lar gibbons.

Juvenile	*Infant:* 0-2 years. Carried by mother during travel.
	Juvenile: 2-5 years. Independent but small in size, tends to follow mother.
Adolescent	*Adolescent:* 5-8 years. Large juvenile but not quite fully grown
Adult	*Subadult:* 8 years – dispersal. Full grown but still within territory of parents or “step-parents”. Males sing solo near edges of territory.
	Adult: Mated with territory, sings duets.

2.2 Clinicopathological Findings of Wild Species

Clinical pathology encompasses microscopic examination of individual cells and tissues, laboratory testing of physiological fluids like blood, on top of recognising the abnormalities in those samples for disease diagnosis (Zynge, 2022). Studying the haematology and serum biochemistry parameters of a wild animal population could yield valuable information to assess health and nutritional status, as well as habitat quality (Hanks, 1981). Furthermore, the haematological findings can also be utilised to forecast survival of animals in reintroduction and translocation programmes (Mathews et al., 2006).

2.3 Diet of Lar Gibbons

An American survey found that in captivity, among the diet given to gibbons include carrots, legumes or beans, Mazuri primate biscuits, seeds or grains, sweet potato, oranges, and cucumber (Munir and Nealan, 2021). Figs and fruits like grapes, bananas, apples and pears ranked the highest in terms of food preference of captive lar gibbons (Jildmalm et. al., 2008).

In the wild, these frugivorous apes mainly feed on ripe, succulent small fruits (47%), young leaves (22%), figs (19%), insects (9%), vine (2%) and flowers (1%) with an average of 6.9 feeding visits per day (Bartlett, 1999; Vellayan, 1981),

2.4 Management System of Lar Gibbons

Gibbon management systems in Malaysia can be grouped into two, conservation and rehabilitation management systems. The former is defined as preservation of species and species diversity in the wild and in captivity while the latter brings a meaning of caring for sick, injured, or orphaned wild animals with the goal of releasing them back to the wild (Lu, 2009; Willette et. al., 2023). A conservation management system is often seen in zoos, while rehabilitation management systems are located at more remote areas with minimal human presence.

2.5 Clinicopathological Findings of Lar Gibbons

It is noted that haematological studies in lar gibbons are scarce, and near non-existent in Malaysia. A study of this kind in primates has been carried out in Indian rhesus macaques (*Macaca mulatta*) by Shah et. al., (2022) which reported significant differences in reference values and ranges of macaques from different source (wild-caught, inhouse), and different sex. Wild-born macaques displayed higher white blood cells (WBC), platelets, neutrophils, erythrocytes, hemoglobin, hematocrit (HCT), mean corpuscle volume (MCV), and total protein values in contrast to their captive counterparts, while sex-based differences were seen in levels of RBCs, hemoglobin, HCT, creatinine, calcium, phosphorus, albumin, and total protein (Shah et. al., 2022).

In 2002, the Interrnational Species Information System (ISIS, 2002) now known as Species 360, an international database for sharing of wildlife knowledge, including

blood reference ranges for wild species published a reference range on blood of lar gibbons. The Species 360 database later published an updated haematology and serum biochemistry reference range for Hylobates lar in 2023 (Species 360, 2023).

3.0 MATERIALS AND METHODS

3.1 Location of study and animals under study

This cross-sectional study was conducted in lar gibbons of two types of captivity, the captive lar gibbons, and semi-captive lar gibbons. Captive lar gibbons were housed in indoor manmade enclosures with restricted space, and were exposed to chronic presence of humans, throughout the year. Semi-captive lar gibbons were housed in outdoor enclosures with restricted space and a higher resemblance to the wild habitat with minimal human intervention (Modified from Hosey, 2005; Julien et. al., 2022). This study sampled a total of N=17 gibbons from four locations, namely the Malaya Gibbon Rehabilitation Project (Malaya GReP) (n=11), Zoo Taiping (n=2), Zoo Melaka (n=3), and Zoo Negara (n=1). Malaya GReP represented as a semi-captive management system while Zoo Taiping, Zoo Melaka, and Zoo Negara represented as captive management system.

From each location, signalment (age, sex), dietary information (type of food, frequency of feeding) and habitat specifications (enclosure size, presence of enrichment) were obtained via permitted zoo records and interviews with the keepers. Data on environmental conditions (temperature, humidity, rainfall) were obtained from Apple Weather. All gibbons sampled were fed twice a day. The categorisation of age groups of lar gibbons were modified from Brockelman et al (1998) due to the limited sample size of this study. The groups were merged to form only three groups, namely “juvenile”, “adolescent” and “adult” (Table 2). Other signalment data were categorised into sex (Table 3), description of the enclosures including the size and enrichments (Table 4), as well as environmental conditions (Table 5).

Table 2. Age of lar gibbons sampled from both types of management systems.

Location	Juvenile	Adolescent	Adult	Total
Semi-captive				11
Malaya GReP	4	5	2	
Captive				6
Zoo Taiping	-	1	1	
Zoo Melaka	2	-	1	
Zoo Negara	-	-	1	
Total	6	6	5	17

Table 3. Sex of lar gibbons sampled in different management systems.

Location	*Male*	*Female*	*Total*
Semi-captive			11
Malaya GReP	6	5	
Captive			6
Zoo Taiping	1	1	
Zoo Melaka	1	2	
Zoo Negara	-	1	
Total	8	9	17

Table 4. Description of lar gibbons' management systems.

Location	Sample size	Size of enclosure	Presence of enrichment
Semi-captive			
Malaya GReP	*11*	610cm x 610cm	Yes
Captive			
Zoo Taiping	2	520cm x 280cm x 238cm	Yes
Zoo Melaka	2	500cm x 250cm x 230cm	Yes
	1	378cm x 252cm x 268cm	Yes
Zoo Negara	1	1400cm x 748cm x 550cm	Yes

Table 5. The environmental conditions of the lar gibbons' captivity enclosure locations.

Location	*Temperature (°C)*	*Rainfall (mm)*	*Humidity (%)*
Semi-captive			
Malaya GReP	33	2	54
Captive			
Zoo Taiping	30	2	68
Zoo Melaka	30	1	74
Zoo Negara	31	1.6	70

This study has acquired a research permit from PERHILITAN and has obtained ethical clearance via Institutional Animal Care and Use Committee (UPM/IACUC/AUP-U035/2023).

3.2 Sample collection

A visual observation was done on the lar gibbons and clinically healthy animals were identified by the attending veterinarian. Only animals that did not display any clinical signs of disease were chosen for this study. Qualified lar gibbons were then sedated with Zoletil® (tiletamine and zolazepam) at a dose of 4-5mg/kg before handling. The cephalic vein or femoral vein was identified, and the site of venipuncture was prepared using an alcohol swab. Blood was collected into anticoagulant EDTA (whole blood) and plain tubes (serum) via venipuncture from the cephalic or femoral vein using a 23G needle. Whole blood samples were stored in ice box, while serum samples were allowed to clot in room temperature for 30 to 60 minutes before placing them in the ice box (Tuck, 2009). Thin blood smear was performed on glass slide and air dried immediately in the field for each sample.

3.2 Clinicopathological data interpretation

Thin blood smears, whole blood and serum samples were transported to the Veterinary Clinical Pathology Laboratory, Veterinary Laboratory Services Unit, Universiti Putra Malaysia where the serum samples were separated from the plain tubes and placed into Eppendorf centrifuge tubes. The serum samples were then centrifuged at 5,000 rpm for 5 minutes. A complete blood count and serum biochemistry analysis was done using Siemens Advia 2120i (Siemens Healthcare Diagnostics, Erlangen, Germany) and Biosystems BA400 (Biosystems, Spain) respectively. Thin blood smears were stained using Wright's stain for white blood cell differential count (Hoppe and Lassen, 1978).

A total number of 12 haematology parameters were analysed: RBC, haemoglobin, PCV, MCV, platelet, total leukocyte, band neutrophil, segmented neutrophil, lymphocyte, monocyte, eosinophil, and basophil. As for serum biochemistry parameters, a total of 23 parameters were measured, including sodium, potassium, chloride, calcium, phosphate, total protein, albumin, globulin, urea, creatinine, aspartate aminotransferase (AST), creatine kinase (CK), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), amylase, glucose, lactate dehydrogenase (LDH) bilirubin (conjugated, unconjugated, total), cholesterol, and lipase. The data obtained was tabulated and compared with a reference range provided by the Species 360 database (Species360, 2023).

3.3 Statistical analysis

The mean data for each parameter from each group was computed using Microsoft Excel. Mann-Whitney U and Kruskal-Wallis H tests were performed using SPSS version 26. Statistical significance is considered as $p<0.05$. The result interpretation was done by comparing means of clinicopathological findings to investigate for a statistically significant difference ($p<0.05$) between the groups compared. In addition, mean clinicopathological data obtained was also compared with a reference range from Species 360 to identify parameters that did not fall within the normal reference range. The parameters that showed a significant difference ($p<0.05$) between the groups, and abnormal finding of the reference range were regarded as ‘remarkable results’.

4.0 Results

4.1 Comparison across types of captivities

The comparison of lar gibbons' Statistical analysis of complete blood count (CBC) and serum biochemistry (SBC) data from lar gibbons of different types of captivity (Table 6) showed that between captive and semi-captive gibbons, the former displayed significantly higher ($p<0.05$) monocyte, sodium, total protein, globulin, GGT and cholesterol levels while semi-captive gibbons had significantly higher ($p<0.05$) potassium, chloride, albumin, urea, LDH, bilirubin (total, unconjugated), and lipase. Potassium and LDH levels were remarkably elevated beyond the reference range for semi captive lar gibbons in contrast to captive lar gibbons.

Table 6. Comparison of lar gibbons' blood parameters across type of captivities.

Parameters	Reference range	Captive (n=6)	Semi-captive (n=11)
RBC (x10^{12} cells/L)	5.11 - 8.34	7.37 ± 0.62	7.43 ± 0.73
Haemoglobin (g/L)	110 - 180	141.00 ± 21.18	147.27 ± 12.84
PCV (L/L)	0.35 - 0.56	0.48 ± 0.09	0.46 ± 0.04
MCV (fL)	54.4 - 75.7	64.19 ± 6.05	62.09 ± 3.88
Total leukocytes (x10^9 cells/L)	3.1 - 16.2	10.44 ± 2.48	7.44 ± 3.30
Band neutrophil (x10^9 cells/L)	0.00 - 0.91	0.00 ± 0.00	0.01 ± 0.02
Segmented neutrophil (x10^9 cells/L)	0.97 - 12.59	5.05 ± 1.65	4.50 ± 2.18
Lymphocyte (x10^9 cells/L)	0.33 - 5.33	3.83 ± 1.77	2.56 ± 1.34
Monocyte (x10^9 cells/L)	0.06 - 1.57	0.81 ± 0.48*	0.19 ± 0.12
Eosinophil (x10^9 cells/L)	0.00 - 0.39	0.07 ± 0.07	0.09 ± 0.09
Basophil (x10^9 cells/L)	0.00 - 0.37	0.14 ± 0.08	0.10 ± 0.12
Platelet (x10^9 cells/L)	47 - 413	214.17 ± 65.31	171.18 ± 57.51
Sodium (mmol/L)	134 - 151	143.67 ± 5.39*	136.55 ± 6.85
Potassium (mmol/L)	3.0 - 6.8	5.58 ± 0.92*	23.85 ± 6.13
Chloride (mmol/L)	98 - 116	102.83 ± 2.93*	113.09 ± 2.74
Calcium (mmol/L)	2.0 - 2.8	2.33 ± 0.17	2.14 ± 0.87
Phosphate (mmol/L)	0.29 - 2.23	1.58 ± 0.43	1.26 ± 0.38
Total protein (g/L)	51 - 77	69.23 ± 4.77*	62.31 ± 4.99
Albumin (g/L)	22 - 52	43.20 ± 5.78*	49.45 ± 4.55
Globulin (g/L)	9 - 41	26.03 ± 7.53*	12.86 ± 3.91
Urea (mmol/L)	1.8 - 13.6	2.94 ± 2.11*	4.78 ± 1.40
Creatinine (µmol/L)	44 - 141	89.87 ± 25.64	83.71 ± 12.96
AST (U/L)	9 - 69	32.88 ± 13.40	34.91 ± 8.49
CK (U/L)	49 - 1390	275.83 ± 13.54	237.23 ± 158.13
ALT (U/L)	12 - 82	35.43 ± 22.79	28.77 ± 8.82
ALP (U/L)	38 - 338	257.10 ± 203.42	312.56 ± 377.42
GGT (U/L)	3 - 37	20.04 ± 12.33*	7.94 ± 4.11
Amylase (U/L)	47 - 354	105.94 ± 29.48	134.65 ± 83.48
Glucose (mmol/L)	2.28 - 12.43	3.12 ± 2.29	1.60 ± 1.35
LDH (U/L)	53 - 666	280.44 ± 27.57*	726.73 ± 315.20
Conjugated bilirubin (µmol/L)	0.0-6.8	1.42 ± 0.29	2.54 ± 1.45
Unconjugated bilirubin (µmol/L)	0.0 - 8.6	1.67 ± 1.07*	3.88 ± 1.37
Total bilirubin (µmol/L)	1.7 - 12.0	2.85 ± 0.98*	6.42 ± 2.63
Cholesterol (mmol/L)	1.55 - 5.18	3.74 ± 0.20*	2.78 ± 0.71
Lipase (U/L)	12 - 168	47.09 ± 11.01*	92.54 ± 41.17

Data are presented as mean ± SD, n = number of samples, $p < 0.05$ is considered statistically significant.
* = indicates a statistically significant difference between captive and semi-captive animals.

4.2 Comparison across age groups

Comparison of lar gibbons' blood parameters between groups are as described in Table 7. Out of the three groups (juvenile, adolescent, adult), adults had a significantly higher ($p < 0.05$) monocyte count in comparison to the adolescents, while highest values of ALP were recorded from the juvenile group. A remarkable elevation was noted in the ALP levels of juveniles.

Table 7. Comparison of lar gibbons' haematology across age groups.

Parameters (units)	*Reference range*	*Juvenile (n=6)*	*Adolescent (n=6)*	*Adult (n=5)*
RBC (x10^12 cells/L)	5.11 - 8.34	6.93 ± 0.41	7.75 ± 0.70	7.58 ± 0.65
Haemoglobin (g/L)	110 - 180	135.67 ± 11.36	154.33 ± 11.47	145.20 ± 20.58
PCV (L/L)	0.35 - 0.56	0.44 ± 0.04	0.48 ± 0.06	0.48 ± .07
MCV (fL)	54.4 - 75.7	63.07 ± 3.25	62.31 ± 6.41	63.17 ± 4.76
Total leukocytes (x10^9 cells/L)	3.1 - 16.2	8.78 ± 4.41	7.21 ± 2.78	9.70 ± 2.32
Band neutrophil (x10^9 cells/L)	0.00 - 0.91	0.01 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
Segmented neutrophil (x10^9 cells/L)	0.97 - 12.59	4.53 ± 2.44	4.48 ± 2.01	5.15 ± 1.64
Lymphocyte (x10^9 cells/L)	0.33 - 5.33	3.56 ± 2.33	2.48 ± 1.28	2.98 ± 0.54
Monocyte (x10^9 cells/L)	0.06 - 1.57	0.53 ± 0.58	0.15 ± 0.09	0.56 ± 0.33*
Eosinophil (x10^9 cells/L)	0.00 - 0.39	0.08 ± 0.13	0.06 ± 0.05	0.11 ± 0.07
Basophil (x10^9 cells/L)	0.00 - 0.37	0.08 ± 0.07	0.16 ± 0.15	0.11 ± 0.08
Platelet (x10^9 cells/L)	47 - 413	204.50 ± 75.20	163.33 ± 52.44	192.20 ± 59.65

Data are presented as mean ± SD, n = number of samples, $p < 0.05$ is considered statistically significant.
* = indicates a statistically significant difference between age groups.

Table 8. Comparison of lar gibbons' serum biochemistry across age groups.

Parameters (units)	*Reference range*	*Juvenile (n=6)*	*Adolescent (n=6)*	*Adult (n=5)*
Sodium (mmol/L)	134 - 151	141.67 ± 6.47	135.00 ± 7.92	140.80 ± 5.76
Potassium (mmol/L)	3.0 - 6.8	13.85 ± 7.83	24.18 ± 10.12	13.54 ± 10.38
Chloride (mmol/L)	98 - 116	109.67 ± 6.80	111.50 ± 4.72	106.80 ± 5.54
Calcium (mmol/L)	2.0 - 2.8	2.45 ± 0.11	2.53 ± 0.14	2.01 ± 0.70
Phosphate (mmol/L)	0.29 - 2.23	1.64 ± 0.37	1.24 ± 0.32	1.23 ± 0.48
Total protein (g/L)	51 - 77	64.72 ± 8.97	64.87 ± 4.11	64.65 ± 3.94
Albumin (g/L)	22 - 52	46.85 ± 4.65	49.65 ± 6.07	44.82 ± 6.49
Globulin (g/L)	9 - 41	17.87 ± 11.33	15.22 ± 5.43	19.83 ± 8.08
Urea (mmol/L)	1.8 - 13.6	3.99 ± 2.53	4.60 ± 1.05	3.73 ± 1.94
Creatinine (µmol/L)	44 - 141	76.97 ± 19.01	85.20 ± 11.24	97.40 ± 19.57
AST (U/L)	9 - 69	34.75 ± 9.75	33.97 ± 10.32	33.80 ± 12.50
CK (U/L)	49 - 1390	305.44 ± 140.93	267.94 ± 137.09	185.67 ± 83.94
ALT (U/L)	12 - 82	26.32 ± 7.92	27.35 ± 11.01	41.42 ± 21.64
ALP (U/L)	38 - 338	554.33 ± 444.50*	171.80 ± 47.37	124.80 ± 34.65
GGT (U/L)	3 - 37	9.10 ± 3.10	9.02 ± 5.83	16.95 ± 15.14
Amylase (U/L)	47 - 354	116.98 ± 77.00	158.63 ± 80.03	89.28 ± 26.20
Glucose (mmol/L)	2.28 - 12.43	3.12 ± 1.55*	0.90 ± 0.54	2.26 ± 2.49
LDH (U/L)	53 - 666	642.34 ± 471.41	702.86 ± 197.28	488.98 ± 235.19
Conjugated bilirubin (µmol/L)	0.0-6.8	1.70 ± 0.46	2.63 ± 1.61	2.26 ± 1.73
Unconjugated bilirubin (µmol/L)	0.0 - 8.6	3.05 ± 1.47	3.71 ± 1.77	2.44 ± 1.77
Total bilirubin (µmol/L)	1.7 - 12.0	4.75 ± 1.57	6.34 ± 3.32	4.24 ± 3.25
Cholesterol (mmol/L)	1.55 - 5.18	3.19 ± 0.87	2.96 ± 0.43	2.89 ± 1.01
Lipase (U/L)	12 - 168	67.80 ± 43.15	78.34 ± 15.59	94.13 ± 64.13

Data are presented as mean ± SD, n = number of samples, $p < 0.05$ is considered statistically significant.
* = indicates a statistically significant difference between age groups.

4.3 Comparison across sex

Comparison of lar gibbons' blood parameters between sex is as described in Table 9 and Table 10. The glucose levels and leukocyte count of females were significantly higher ($p<0.05$) compared to males while total bilirubin was significantly higher ($p<0.05$) in males. Mean glucose from the male group was lower than the reference range.

Table 9. Comparison of lar gibbons' haematology across sex.

Parameters (unit)	*Reference range*	*Male (n=8)*	*Female (n=9)*
RBC (x10^12 cells/L)	5.11 - 8.34	7.62 ± 0.79	7.23 ± 0.53
Haemoglobin (g/L)	110 - 180	147.75 ± 17.62	142.67 ± 14.83
PCV (L/L)	0.35 - 0.56	0.48 ± 0.06	0.45 ± 0.06
MCV (fL)	54.4 - 75.7	62.83 ± 4.98	62.83 ± 4.71
Total leukocytes (x10^9 cells/L)	3.1 - 16.2	6.76 ± 2.21	10.04 ± 3.45*
Band neutrophil (x10^9 cells/L)	0.00 - 0.91	0.01 ± 0.02	0.00 ± 0.00
Segmented neutrophil (x10^9 cells/L)	0.97 - 12.59	3.87 ± 1.33	5.43 ± 2.23
Lymphocyte (x10^9 cells/L)	0.33 - 5.33	2.52 ± 1.32	3.44 ± 1.74
Monocyte (x10^9 cells/L)	0.06 - 1.57	0.25 ± 0.25	0.55 ± 0.49
Eosinophil (x10^9 cells/L)	0.00 - 0.39	0.06 ± 0.11	0.10 ± 0.06
Basophil (x10^9 cells/L)	0.00 - 0.37	0.15 ± 0.13	0.09 ± 0.07
Platelet (x10^9 cells/L)	47 - 413	196.88 ± 49.22	177.00 ± 73.27

Data are presented as mean±SD, n=number of samples, $p < 0.05$ is considered statistically significant.
*=indicates a statistically significant difference between male and female gibbons.

Table 10. Comparison of lar gibbons' serum biochemistry across sex.

Parameters (unit)	*Reference range*	*Male (n=8)*	*Female (n=9)*
Sodium (mmol/L)	134 - 151	137.88 ± 8.08	140.11 ± 6.45
Potassium (mmol/L)	3.0 - 6.8	19.84 ± 10.66	15.24 ± 9.95
Chloride (mmol/L)	98 - 116	110.13 ± 4.82	108.89 ± 6.68
Calcium (mmol/L)	2.0 - 2.8	2.48 ± 0.13	2.21 ± 0.60
Phosphate (mmol/L)	0.29 - 2.23	1.39 ± 0.37	1.37 ± 0.47
Total protein (g/L)	51 - 77	67.10 ± 5.26	62.67 ± 5.83
Albumin (g/L)	22 - 52	49.56 ± 5.34	45.18 ± 5.53
Globulin (g/L)	9 - 41	17.54 ± 9.20	17.49 ± 8.04
Urea (mmol/L)	1.8 - 13.6	4.38 ± 1.88	3.91 ± 1.91
Creatinine (µmol/L)	44 - 141	85.51 ± 17.96	86.21 ± 18.88
AST (U/L)	9 - 69	35.46 ± 6.96	33.07 ± 12.61
CK (U/L)	49 - 1390	261.90 ± 134.68	265.10 ± 131.85
ALT (U/L)	12 - 82	27.46 ± 9.66	34.38 ± 18.34
ALP (U/L)	38 - 338	411.36 ± 421.00	187.77 ± 154.46
GGT (U/L)	3 - 37	9.78 ± 4.32	12.38 ± 11.45
Amylase (U/L)	47 - 354	124.16 ± 66.32	127.19 ± 80.33
Glucose (mmol/L)	2.28 - 12.43	1.25 ± 1.12*	2.89 ± 1.98
LDH (U/L)	53 - 666	627.66 ± 222.97	634.53 ± 440.85
Conjugated bilirubin (µmol/L)	0.0-6.8	2.89 ± 1.54	1.49 ± 0.39
Unconjugated bilirubin (µmol/L)	0.0 - 8.6	3.91 ± 1.54	2.39 ± 1.46
Total bilirubin (µmol/L)	1.7 - 12.0	6.80 ± 2.98*	3.71 ± 1.61
Cholesterol (mmol/L)	1.55 - 5.18	3.06 ± 0.72	3.01 ± 0.82
Lipase (U/L)	12 - 168	82.14 ± 30.54	74.53 ± 50.38

Data are presented as mean±SD, n=number of samples, $p < 0.05$ is considered statistically significant.

*=indicates a statistically significant difference between male and female gibbons.

4.4 Other findings

Within females across types of captivity, captive females recorded a significantly higher ($p<0.05$) total protein, GGT and cholesterol while semi-captive females had significantly higher ($p<0.05$) levels of potassium and chloride. Comparison within age groups across different types of captivities and within males of different captivity computed no significant differences.

4.5 Summary

Overall, there were three key findings observed from this study. First, marked hyperkalaemia and elevated LDH levels were found in semi captive lar gibbons. Next, elevation in ALP was recorded in juveniles. Lastly, the males had lower glucose levels in comparison to the females.

5.0 DISCUSSION

5.1 Hyperkalaemia in semi-captive lar gibbons

Hyperkalaemia can be attributed to the high potassium diet fed to the semi-captive gibbons. This is in agreement with a study done by Dorsthorst et al. (2018), where healthy humans and renally impaired patients were both equally at risk of developing hyperkalaemia due to abnormally increased intake of high potassium foods. Some of the high potassium diet fed in the semi-captive facility includes red beans (1406mg/100g), dates (656mg/100g), guava (417mg/100g) and eggplant (229mg/100g). Due to scarce availability of dietary data in captive lar gibbons, a comparison is difficult to be done without further studies analysing the mineral contents of diet in captive lar gibbons.

5.2 Elevated LDH in semi-captive lar gibbons

Lactate dehydrogenase plays an essential role in carbohydrate metabolism by catalysing the reversible conversion of pyruvate to lactate with simultaneous oxidation of reduced nicotinamide adenine dinucleotide (NADH) to nicotinamide adenine dinucleotide (NAD) and vice versa. This process is the key in anaerobic respiration to ensure availability of NAD to maintain continuance of glycolysis in order to produce energy in the form of adenosine triphosphate (ATP) (Hicks et al., 2023; Farhana & Lappin, 2023). It has also been reported that a carbohydrate-rich diet significantly increases LDH-4 and LDH-5 (Yoshimura et al., 1986). LDH-4 and LDH-5 are isoenzymes of lactate dehydrogenase with the former primarily present in the kidneys with one heart and three muscle subunits (1H3M), while the latter largely present in liver and skeletal muscle with four muscle subunits (4M) (Farhana & Lappin, 2023). Considering the semi-captive gibbons were fed a high carbohydrate diet which comprised of market fruits like mangoes, oranges and pears, it is hypothesized that

a high carbohydrate intake by the semi-captive gibbons is the reason behind the elevation in their LDH levels. However, this hypothesis requires further investigation.

Amylase, an enzyme secreted in the salivary glands and pancreas plays a role in the digestion of carbohydrates by breaking down starch into smaller molecules to produce glucose molecules (Peyrot des Gachons & Breslin, 2016). While no significant findings were observed for the comparison of amylase levels, the reference ranges for serum amylase displayed an interesting finding. As shown in Table 11, the reference range from Species 360 (2023) used in this study reports a reference range of 47-354 U/L for serum amylase while a previous reference range done on lar gibbons in 2002 by International Species Information System (2002) published a range of 9-47 U/L. These reference ranges done on captive lar gibbons display a stark difference, where the normal serum amylase range has shown a marked increase over two decades. This observation suggests the captive gibbons may have acclimatized to the high carbohydrate diet fed which has subsequently increased their baseline serum amylase levels.

Table 11. Reference ranges for serum amylase of lar gibbons.

Year	*Serum amylase reference range*
ISIS (2002)	9 – 47 U/L
Species 360 (2023)	47 – 354 U/L

The elevation in the baseline amylase levels is not a species-specific phenomenon. Nakajima (2016) highlights the hypothesis that in humans, baseline amylase levels can potentially be a result of high carbohydrate diet consumed throughout multiple generations. In a study on haematology and serum biochemistry of amur leopards, a remarkable number of samples (14 out of 34) with abnormally elevated amylase levels was found (Bodgener & Lewis, 2017) when compared to the reference range

reported by Species 360 (ISIS, 2002). Bodgener & Lewis (2017) assert that the elevated amylase levels could be hereditary based on evidence from the studbook of the European Endangered Species Breeding Programme (EEP). The former authors also remarked that the consistent elevation in amylase levels on two separate sampling of one leopard which suggest the high amylase measurements could likely be a persistent elevation instead of a transient state for the animal.

5.3 Elevated ALP in juveniles

ALP is a membrane bound enzyme that facilitate the hydrolysis of organic phosphate esters in the extracellular space. This enzyme is found in various organs but over 80% of serum ALP traces back to the liver and bone.

With increasing age, a declining trend in ALP levels is seen which is in line with findings from a study on growth variables of dogs (Trangerud, 2007). An elevation in ALP is commonly seen in young domestic animals associated with normal physiology of growing animals. A similar phenomenon is observed in juveniles of orangutans, wild felids, marsupials, and carnivores (Foster and Cunningham 2009; Garcia et al., 2010; Latimer, 2011; Mendonça et al., 2016).

A physiologic elevation in ALP can be a consequence of greater osteoblastic activity exhibited in growing individuals. (Schmid and Forstner 1986; Turan et al., 2011) This is due to the fact that increased levels of ALP are frequently thought to be essential for the mineralization and synthesis of new bone. (Shu, 2022).

5.4 Decreased glucose levels in males

In this study, 11 out of 17 lar gibbons sampled in both male and female groups were hypoglycemic, and this could be attributed to fasting the day before blood sampling. Fasting was a necessary precaution to prevent any adverse effects like vomiting and

aspiration throughout sedation (Tardif et al., 2013). In humans, hypoglycemia can be seen as a result of fasting, but at a very low frequency (Tanaka, 2021). According to the Diabetes Control and Complications Trial Research Group (1993), human males are more at risk for severe hypoglycemia than females. This is in accordance with the findings of this study whereby the severity of hypoglycemia in males were marked ($\bar{x}$ = 1.25 mmol/L) while glucose levels of females ranged from low normal to slightly lower than the reference range ($\bar{x}$ = 2.89 mmol/L).

6.0 CONCLUSION

H_A1: There is a significant difference in clinicopathological findings between lar gibbons in different management systems.

Alternative hypothesis 1 is accepted. There is a significant difference in clinicopathological findings between lar gibbons in different management systems.

H_A2: There is a significant difference in clinicopathological findings of lar gibbons of different sex and age.

Alternative hypothesis 2 is accepted. There is a significant difference in clinicopathological findings of lar gibbons of different sex and age.

7.0 LIMITATIONS

Some of the major limitations of this study include limited time for research. This study also has a small sample size (N=17) which limits the strength of conclusions made in this study. The small sample size was due to a number of reasons, including the fact that lar gibbons are endangered globally. Obtaining permission from zoo and rehabilitation organisations to conduct sampling has to go through rigorous procedures as a study of this level of invasiveness may stress the gibbons, a phenomenon not in the caretaker's favour. In addition, sampling of blood in lar gibbons also require sedation which a number of risks like aspiration pneumonia (Tardif et al., 2013). This study was also limited by the type of enclosure the gibbons were housed in, where only animals in cages were accessible for sedation and blood sampling, while the semi-wild gibbons kept in higher trees within islands were not accessible due to the risk of sedating them at such heights, like inaccurate aiming and risk of gibbons falling to the ground.

Some blood smears were not of diagnostic quality which hindered the manual white blood cell differential count. Overlapping of cells, improper staining of slides and presence of debris on thin blood smears made on field were some of the major factors affected quality of thin blood smears. Some serum samples were lalso limited imited and were not adequate to measure all serum biochemistry parameters.

8.0 RECOMMENDATIONS

It is recommended that this study be repeated with a larger sample size, to obtain more robust statistical analysis and a stronger conclusion. A study incorporating fecal samples can allow a better understanding of the nutritional profile of lar gibbons. This can be done by examining the amount of nutrient within the feces to determine the apparent digestibility for each nutrient consumed (Coudrat & Cabana, 2019). Findings from this study suggest a need for reassessment of the dietary management system of lar gibbons kept in captivity, to maintain optimum levels of haematology and serum biochemistry parameters, as well as to maintain a good health status of these primates. It is also recommended that a reference range for local lar gibbons is established in order for more accurate assessments during health check ups and monitoring of gibbons.

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