

Haematological Analysis of Leschenault's Leaf Toad Gecko, *Hemidactylus Leschenaultii* Dumeril and Bibron 1836

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Abstract

Haematology is useful in understanding the physiological features of lizards. The study was held to investigate the haemo profile of leschenault's leaf toad gecko in Odisha. The haematological parameters like Haemoglobin Concentration (HB), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Total Leucocyte Count (TLC), Total Platelet Count (TPC) and Differential Leucocyte Count (DLC) were calculated using standard procedures. Statistical analysis like correlation and t-test were done. The study revealed that the TEC and percentage of lymphocytes show significant difference ($p < 0.05$) between both sexes of lizard. The mean values of all other parameters are also showing difference between male and female of *Hemidactylus leschenaultii* Dumeril and Bibron 1836. The correlation coefficient varies in male and female with respect to the parameters analysed. Some parameters were positively correlated with each other and others were found to be negatively correlated. The data obtained could be a useful indicator for monitoring and managing the general health status of the species.

Keywords: Haematological Parameters; *Hemidactylus Leschenaultii*; Correlation; Significant Difference.

Introduction

The significant of haematological study for evaluating, analysing, examining and controlling the health status of animals is a prelude blueprint. The haematologic technique for assessment of body physiology has a great role being uncomplicated, serene, minimal invasive, productive first hand technique and economically sustainable.

Leschenault's leaf toad gecko is belonging to Class- Reptilia, Order- Squamata and Family Gekkonidae, found in warm climatic conditions throughout the world [1]. Haemocytological parameters are useful and widely used tools that assist in the diagnosis and monitoring of animal health [2]. The combinations of different haematological parameters detect the physiological conditions and clinical evaluation in reptiles [3,4]. The different external and internal factors also affect the haemoprofile of nonmammalian vertebrates [5,6]. Haematology of some saurians were studied [7,8,9]. The haemoglobin concentration, haematocrit value, mean cell volume, mean cell haemoglobin and mean

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Received on 29.01.2018, Accepted on 09.02.2018

cell haemoglobin concentration were studied in some reptiles [10,11,12]. Haematology in reptiles highly dependent on age as well as sex and it varies throughout life [13,14]. The blood cells were identified and different parameters were taken [15-20].

The data about haematology of reptiles is still challenging in comparison to other nonmammalian vertebrates. The literature available on haematology of this lizard specifically from the studied geographical region of Odisha is very less. The comparative scarcity of informations regarding the haematology of geckos provides a less evidences about

family Gekkonidae. The purpose of this study was to determine the reference intervals of haematology and morphometry of blood cells of normal and apparently healthy lizards. The findings will serve as base line data for future health assessment of lizards as well as for conservation and protection.

Materials and Methods

Animals

Fifteen lizards of each sex were collected from the coastal area of Rajnagar block of Kendrapara- 754 225, Odisha, located in 20° 20' N to 20° 37' N latitude and 86° 14' E to 87° 01' E longitude. They were caught at day time from the crevices of trees and sometimes at wall of boundaries and homes adjacent to the fields. Lizards were clinically healthy and in good condition transferred to animal house. The investigation of haematological profiles on *Hemidactylus leschenaultii* Dumeril and Bibron 1836 was carried out from 2014 to 2017.

Blood Collection

The venipuncture site was prepared aseptically prior to blood collection. Blood was collected from the ventral tail vein of lizards by inserting an insulin syringe (BD Ultra – Fine™ Needle 12.7 mm × 30G) at an angle of 45-60° between the scales on ventral midline [21,22]. Once blood appeared in the needle hub, held steady and a gentle negative pressure was applied to the syringe. The blood was kept in an EDTA vial and then transported in icebox to laboratory. The lizards were released to their natural habitat after collection of blood. Whole blood smear were obtained by push slide technique, air dried, fixed with methanol

and stained with Giemsa as protocol cited by Lillie [23].

Haematological Analysis

The blood parameters were studied using the procedure [24,25,26,27]. The concentration of haemoglobin was estimated as oxyhaemoglobin by Sahalis haemometer and expressed in g %, Packed cell volume (PCV) was determined by microhaematocrit method with a spun of microhaematocrit tube at 2500 rpm for 15 minutes. The quantification of RBCs and WBCs was performed by manual methods using haemocytometer, with Hayem's diluting fluid for RBCs and Turk's diluting fluid for WBCs. Erythrocyte indices like MCV, MCH and MCHC were calculated using standard formulae [28]. The percentage of different leucocytes as well as total platelet count determined [29,30].

Statistical Analysis

The data presented as Mean ± SE (Standard Error) for both sexes and MS office Excel 2007 was used for statistical analysis. The correlation analysis between the parameters and the significant difference (P<0.05) was taken using Student's t-test (assuming equal variances) with the help of Paleontological Statistics (PAST) version 2.17 (Natural History Museum, University of Oslo).

Results

Haematological parameters were analysed in case of adult lizard (Table1). Assuming a confidence level of 95%, significant differences were found in two

Table 1: Haematological parameters of *H. Leschenaultii* Dumeril and Bibron 1836

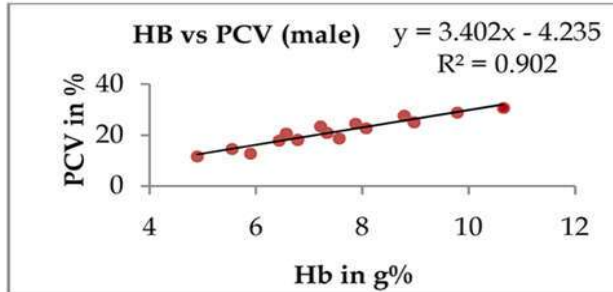
Sl. No.	Parameters	Unit	Range	Male Mean	SEM	Female Range	Female Mean	SEM	P value
1	Haemoglobin	g%	4.89 - 10.65	7.49	0.41	4.53 - 9.64	6.53	0.36	1.75
2	PCV	%	11.76 - 30.76	21.25	1.46	11.89 - 30.89	20.12	1.39	0.54
3	TEC	10 ⁶ mm ⁻³	0.87 - 2.34	1.43	0.14	0.72 - 1.86	1.14	0.12	2.27*
4	MCV	fl	91.51 - 267.48	161.41	13.55	105.87 - 304.61	185.33	14.14	1.26
5	MCH	pg	34.24 - 92.61	56.59	4.14	47.59 - 64.11	59.77	3.18	0.64
6	MCHC	%	24.36 - 45.76	35.17	1.32	23.36 - 47.1	34.16	1.72	0.43
7	TLC	10 ³ mm ⁻³	7600 - 14356	11362.13	493.92	8500 - 16540	12647.6	556.58	1.72
8	TPC	10 ³ mm ⁻³	15800 - 44356	30049.8	2182.46	16783 - 48900	34178.93	2525.89	1.31
9	Heterophils	%	50 - 79	65	2.03	50 - 78	60.8	2.41	1.24
10	Lymphocytes	%	17 - 38	26.73	1.81	21 - 42	33.8	1.99	2.73*
11	Eosinophils	%	1 - 15	6.2	0.92	2 - 7	4.33	0.45	1.59
12	Monocytes	%	2 - 4	2.13	0.32	0 - 4	1.73	0.24	0.89
13	Basophils	%	0	0	0	0	0	0	0

(Significant difference * p<0.05 for each haematological parameters)

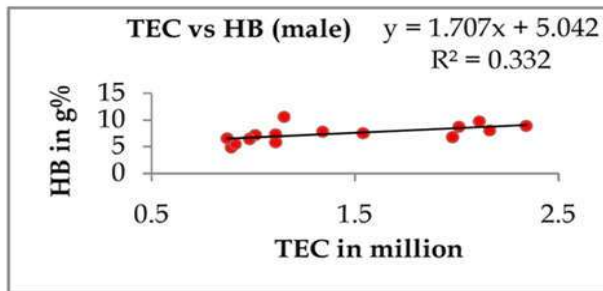
parameters throughout the investigation. The total erythrocyte count was found to be highest in male and lowest in female ($p < 0.05$). The percentage of lymphocytes is highest in female and lowest in male ($p < 0.05$). The highest mean value of haemoglobin and packed cell volume and mean corpuscular haemoglobin concentration was found in male and lowest in female while the mean values of other parameters were found to be highest in female. The

percentage of heterophils, eosinophils and monocytes was highest in male and lowest in female (Table 1). The correlation between haematological parameters of both male and female geckos was depicted (Figure 1). There is a positive correlation between Hb vs PCV (Fig.1a,b), Hb vs TEC (Fig.1c,d) and MCV vs MCH (Fig.1 k,l) in both male and females, but Hb vs MCV (Fig.1 e) is positively correlated in male and negatively correlated in female (Fig.1 f). A negative

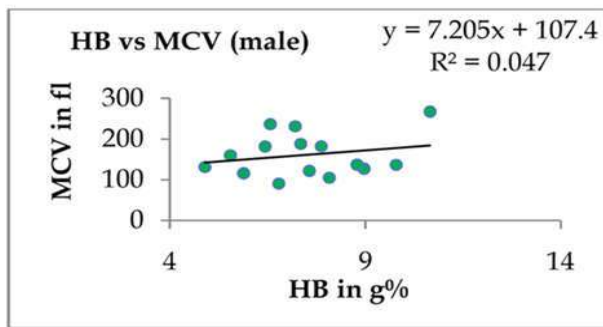
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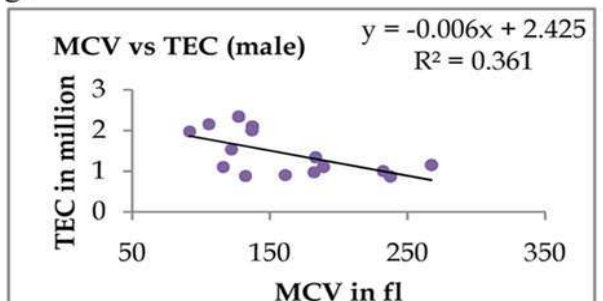
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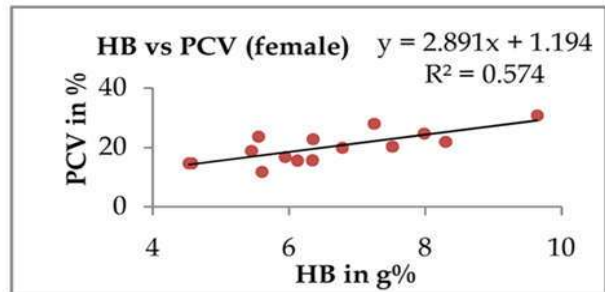
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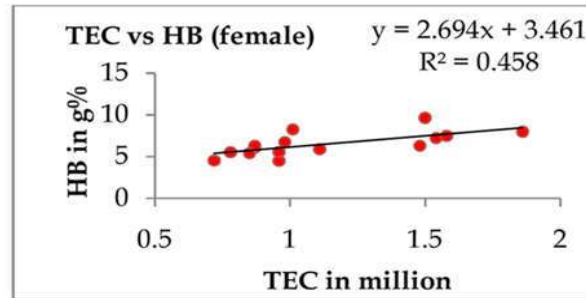
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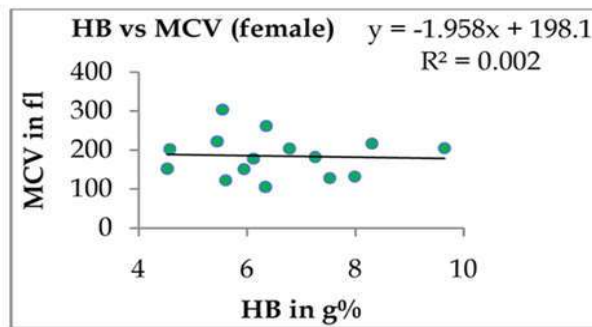
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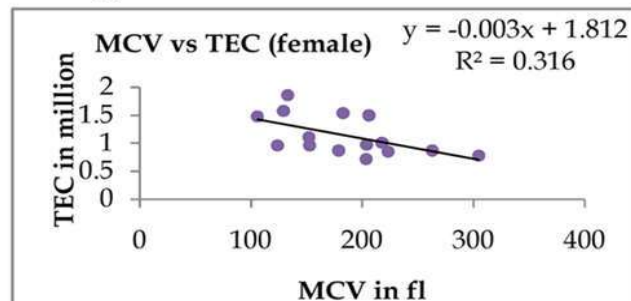
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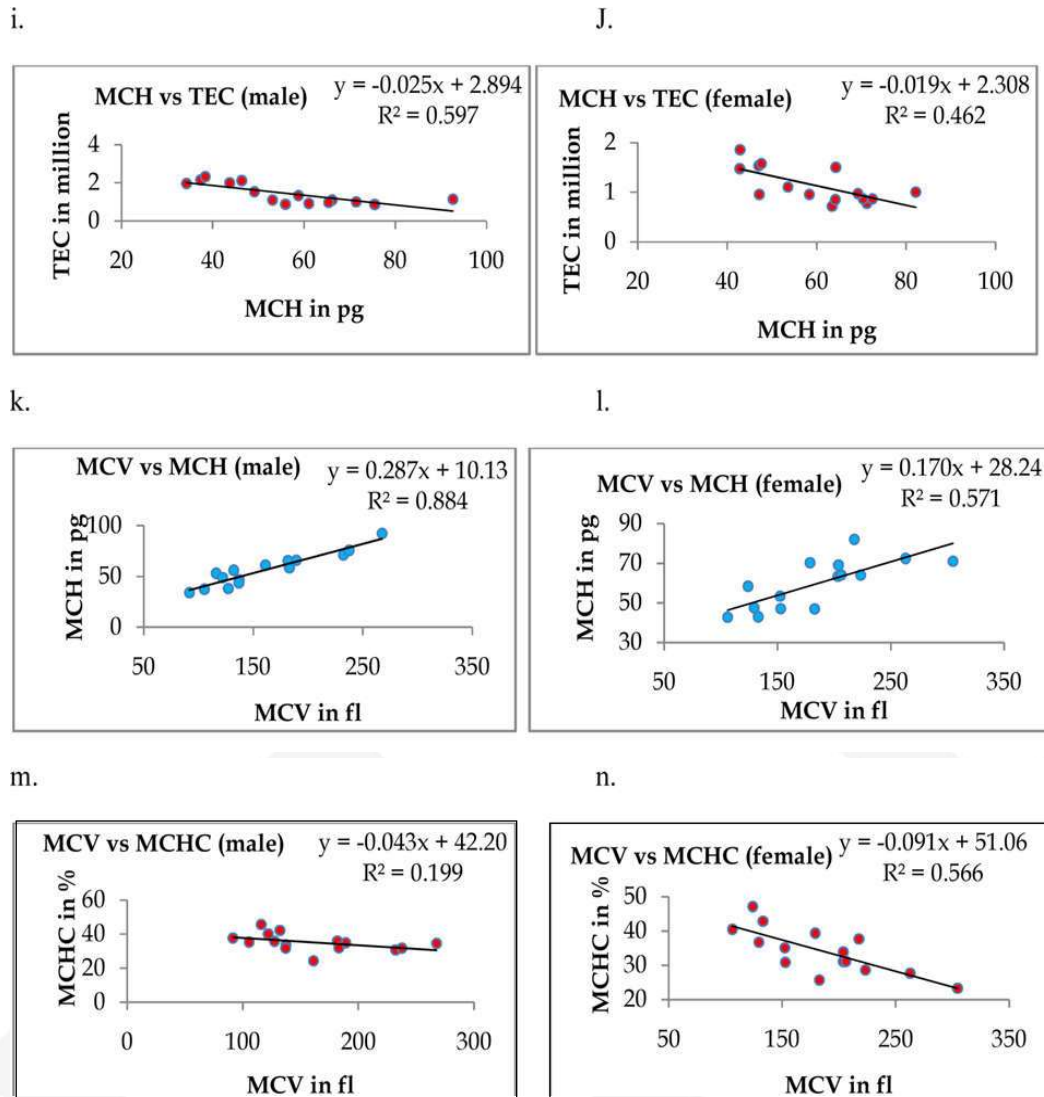


Fig. 1: Correlation between different haematological parameters (a-n) of *Hemidactylus leschenaultii* Dumeril and Bibron 1836

correlation was found in both sexes with respect to MCV vs TEC (Fig.1g,h), MCH vs TEC (Fig.1 i,j) and MCV vs MCHC (Fig.1 m,n).

Discussion

Haematological data are essential to correlate the health status of reptiles with their habitat. Haematology of reptiles provides a way to diagnose the animals [31]. There was no differences in total haemoglobin concentration in male and female lizards [32,33]. However the result of our investigation shows a difference in haemoglobin concentration. The difference in the value may be due to some seasonal factors in combination with age and sex of the individuals. According to [34] the PCV and TEC is

higher in male of Free living Mediterranean Pond Turtle and TEC in other reptiles [9,24]. Our data regarding PCV and TEC corroborate with this. The PCV and TEC found in this study is lower than the report by [35] for prehensile tailed skink. The TLC was higher in male and MCV was higher in female Mediterranean Pond Turtle [34]. But in case of Leschenault's leaf toad gecko TLC and MCV was found higher in female only. There was difference in mean values of haematological parameters between sexes of the studied gecko which is also seen in some agamidae [13], geckos, *Hemidactylus frenatus* [36]. Our findings of TEC and TLC show closeness with the finding of some agamidae lizards [37,38,39,40]. The result obtained in this study regarding the haematological parameters also fall within the range as studied in iguana by [41,42]. Haematological

parameters in reptiles vary with age sex and seasons [31,43]. The TEC, in both male and female is lower in comparison to *Psammophilus algirus* [44]. The MCV, MCH and MCHC in both male and female *Naja naja* were higher [45] in comparison to *Hemidactylus leschenaultii*.

The heterophil count, the lymphocyte count is higher in both male and female in this study in comparison to *Psammophilus blanfordians* and eosinophil and monocyte percentage falls within the range [13]. The monocyte, eosinophil and lymphocyte percentage is highest and heterophil percentage is lowest in *Trapelus lessonae* [46]. Generally the heterophils and lymphocytes are the highest occurred leucocytes followed by eosinophils, monocytes and basophils are rare occurrence [47,31,48].

In Leschenault's leaf toad gecko, the PCV shows a positive correlation with increase in haemoglobin concentration in both male and female, this indicates that TEC also directly proportional to concentration of haemoglobin. With increase in TEC, the PCV and concentration of haemoglobin, both increased in different sexes and are correlated positively. With increase in MCV, the MCH is increasing in male and female.

In case of male the MCV is increasing with increase in concentration of haemoglobin where as in female it is negatively correlated. This is an indication of poor health status like anaemia in case of female lizards. TEC shows a negative correlation with MCV and MCH in case of both male and female and the MCV is also negatively correlated with MCHC in both sexes. The relation between all the haematological parameters may be due to influences of different external like seasonal variations, diurnal variations, temperature and intrinsic factors including age, sex and body physiology.

Conclusion

Haematology is highly dependent on body physiology of lizards with respect to their surroundings. However, it provides the fact that the geckos showed different haemoprofile with respect to their sex. The most important thing is that, the less population and low body mass of geckos limit the data interpretation and further validation is necessarily required. The present study provides a base line reference value for the haematological parameters of *Hemidactylus leschenaultii*. This data may be useful for further study relating to impact of climate, environmental conditions, use of

microhabitat and seasonal fluctuations on haematology of lizards.

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