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Neutrophil to lymphocyte ratio is indicative of environmental heat stress in *Pteropus giganteus*

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Abstract : *Pteropus giganteus* is the giant Indian fruit bat that colonizes on tall canopies near water bodies exposed to environmental perturbations. Decline in the population of these bats as well as mortality during summer season has been reported by several authors but no reason has been suggested. Further, a potential marker for environmental heat stress (EHS) is still lacking. This work was therefore done for finding the association of EHS with neutrophil and lymphocyte ratio (N/L ratio). N/L ratio is used for studying heat stress in animals. Bats numbering 12 were captured and acclimatized for 15 days. The cage was placed under the colony and blood samples were collected from the bats. Blood smears were prepared and studied under the microscope. The result suggests N/L ratio shows an increasing trend beyond 30°C of ambient temperature and is also highly correlated to it (r = 0.67). Thus, it may be concluded that N/L ratio can be used for monitoring EHS. Future study may be done to evaluate the immunological changes for understanding the mechanism behind heterothermy in bats.

Keywords : ***Pteropus giganteus*, EHS,**

INTRODUCTION

Exposure to heat stress leads to physiological changes in organisms. Such alterations include elevation in circulating Neutrophils, lowering of circulating Lymphocytes, elevation in core body temperature, weight loss (due to water loss) increase in core body temperature and associated behavioral changes[1-2]. Changes in leucocyte profiles is an alternate method for measuring corticosterone as increase in glucocorticoid hormones cause changes in the leukocyte profile. Further, the leukocyte approach offers certain advantages over direct glucocorticoid measurement as it does not require prohibitively rapid sampling and is relatively inexpensive. The hematological response to stress is conserved across taxonomic groups, ensuring that this approach of measuring stress can be applied to most vertebrates. The results obtained from one taxonomic group should be useful for making predictions in others[3]. Leucocyte profiles have been used widely in biomedical studies as marker of infections, inflammation and stress. Corticosterone in plasma rises immediately under stress conditions, therefore its use in wild population becomes less appropriate[3-5]. N/L ratio has been used as a marker of corticosterone levels but the change in their ratio occurs gradually making it a more valuable tool in study of wild animals in their natural environment[3, 6-11]. N/L ratios have been used as a marker of heat stress in all taxa including humans with promising results. The effect of disease on leukocyte profiles is similar to that of stress as neutrophilia/heterophilia and lymphopenia are commonly observed in all taxa. Further, it has also been used as a marker of disease diagnosis and inflammation. Indeed, it is well-established that neutrophils/ heterophils, being phagocytic, proliferate in circulation to combat infections, and the increase in this cell type alone can cause elevated N/L ratios during infections. Therefore,

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interpreting changes in N/L ratio as an effect of heat stress only can be problematic when disease status is unknown. However, under disease influence monocytes proliferate. Elevation in monocyte numbers along with N/L ratio is indicative of stress due to infection[3]. Therefore along with N/L ratio number of monocytes must were considered in this study. An increase in the N/L ratio without any significant change in the number of monocytes reflects heat stress. Heat stress has been extensively studied on cattle from its causative factors to its effect on various aspects of animal health to the measures for coping with the adverse effects of heat stress. Heat stress affects the animal physiology and occurs in animals when there is an imbalance between heat production within the body and its dissipation. It is one of the wide varieties of factors which causes oxidative stress in-vivo and may cause alterations in metabolism, hormones, immunological, genetic and psychological as well. Lactation in cows has been shown to be hampered by heat stress and such effects can be expected in fruit bats also[6-11]. The effectiveness of N/L ratio in predicting stress caused by EHS is still unknown. This work was therefore done for finding the potential of N/L ratio in predicting the effect of EHS.

Pteropus giganteus is a fruit bat which uses tall open canopies to roost during day time and provides a wonderful opportunity to study the potential of N/L ratio in predicting the EHS effects on an organism[13-15].

MATERIALS & METHODS

Blood samples were collected on days with maximum temperature between 23- 28°C; 29-35 °C; 35-40°C; 40-45°C; >45°C and blood smears were prepared immediately. Slides were stained with leishman stain. Monocyte, neutrophil and lymphocyte percentages were ascertained and N/L ratio was calculated following standard protocol described elsewhere[17].

RESULTS & DISCUSSION

In the present study lymphocyte percentage of these bats ranged from 04.30 to 03.85, (Table 1, Figure 1). Whereas the neutrophil percentage ranged between 05.12 and 05.49°C, (Table 2, Figure1). The neutrophil to lymphocyte ratio varied from 02.30 to 05.99 (Table 3, Figure1). Monocyte percentage ranged from 03.67 to 03.50 (Tables 4; Figures 1). The fall in lymphocyte and increase in neutrophil as well as neutrophil/ lymphocyte ratio in response to heat stress has been well documented in many animals. Similar observations were found in this study as well. Lymphocyte percentage was found to be negatively influenced by Tamb (r= -0.47) while neutrophil (r=0.69) and N/L ratio (r=0.67) were positively influenced by Tamb. This may again denote the effect of EHS on the physiology of these bats. Most significant increase in lymphocytes was observed when the Tamb crossed 30°C. Tamb above 30°C is in the thermal discomfort zone of this species of bats. Most significant decrease in the number of Lymphocyte occurred when Tamb was above 45°C i.e., which is upper limit of thermal tolerance in these bats. The number of circulating neutrophils increases most significantly when the ambient temperature is beyond thermoneutral zone of these bats[18]. This may be caused by the sensitivity of neutrophils and lymphocytes to EHS. Thus it may be concluded that N/L ratio is indicative of EHS. This study thus gives some information for the cause of increase in mortality during summer season[14-16]. N/L ratio has also shown its relation with the thermal zones of comfort and discomfort in this species[18]. Further study is required to find the mechanism behind it.

Table 1- Showing lymphocyte percentage at various ambient temperature ranges and their correlation.

Sl. No.	Temperature ranges in °C	Mean	SD	SE	Correlation coefficient "r"
1.	23-30	04.30 a	0.24	0.15	
2.	30-35	04.13 ab	0.31	0.16	
3.	35-40	04.06 ab	0.34	0.17	
4.	40-45	03.97 b	0.37	0.18	
5.	>45	03.85 c	0.44	0.19	-0.47*

Means without a common alphabet are significantly different; * = significant to the level of p< 0.05

Table 2. Showing neutrophil percentage at various ambient temperature range and their correlation.

Sl. No.	Temperature ranges in °C	Mean	SD	SE	Correlation coefficient
1.	23-30	05.12 a	0.11	0.10	
2.	30-35	05.35 b	0.06	0.08	
3.	35-40	05.38 b	0.06	0.07	
4.	40-45	05.41 bc	0.06	0.07	
5.	>45	05.49 c	0.06	0.07	0.69*

Means without a common alphabet are significantly different;
* = significant to the level of p< 0.05.

Table 3. Showing N/L ratio at various ambient temperature range and thier correlation.

Sl. No.	Temperature ranges in °C	Mean	SD	SE	Correlation Coefficient "r"
1.	23-30	02.30 a	0.34	0.169	
2.	30-35	03.63 ab	01.31	0.34	
3.	35-40	04.07 abc	01.50	0.36	
4.	40-45	04.67 bc	01.94	0.41	
5.	>45	05.99 c	03.28	0.53	0.67*

Means without a common alphabet are significantly different;
* = significant to the level of <0.05

Table 4. Showing monocyte percentage at various ambient temperature range and their correlation.

Sl. No.	Temperature ranges in °C	Mean	SD	SE	Correlation Coefficient "r"
1.	23-30	03.67 a	02.39	0.45	
2.	30-35	03.47 a	02.07	0.42	
3.	35-40	03.59 a	02.07	0.42	
4.	40-45	03.84 a	02.04	0.42	
5.	>45	03.5 a	02.44	0.46	0.028*

Means without a common alphabet are significantly different;
* = significant to the level of p<0.05

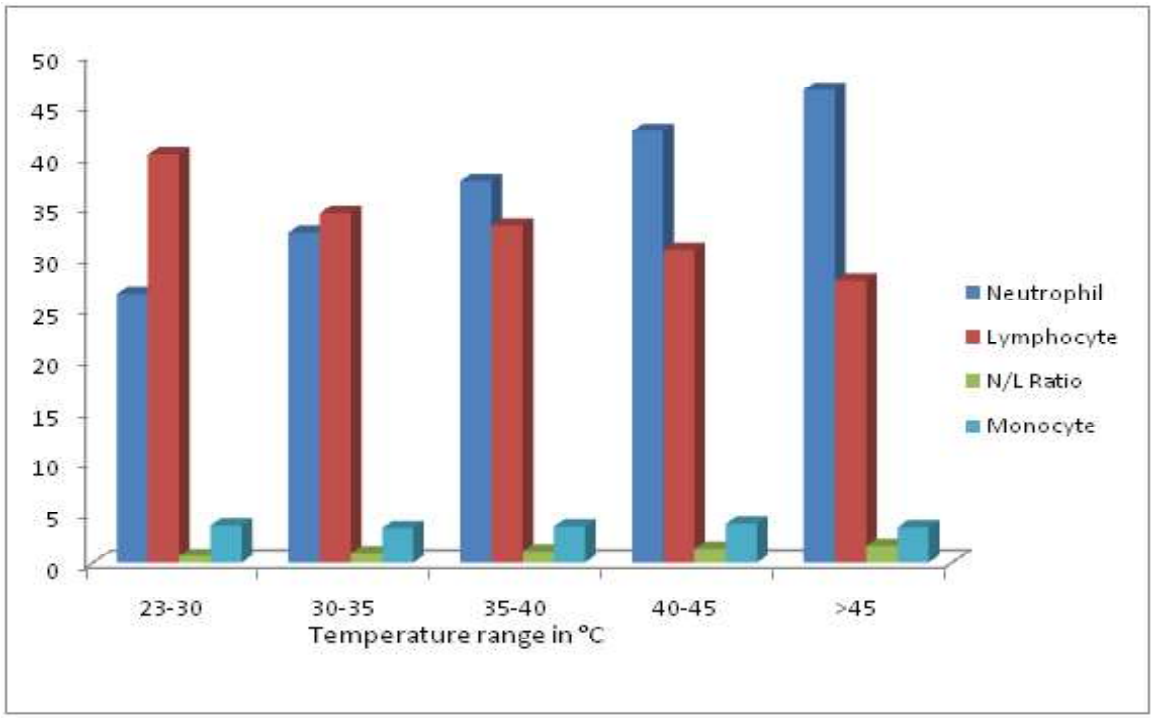

Fig.1- Showing neutrophil, lymphocyte, monocyte levels and N/L ratio in percent
N/L ratio = Neutrophil/Lymphocyte Ratio

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