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Differential leukocyte profile of *Rhinopoma microphyllum kinneari*

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Abstract. White blood corpuscle population in *Rhinopoma microphyllum kinneari* (both male and female) has been studied during October, November and December. The differential white blood corpuscles in male and female have been found to be significantly different. Fluctuations in the WBC population are evident during the three months. The white blood corpuscle populations have also been compared with other bat studies and are discussed in the paper.

White blood corpuscles, granulocytes, agranulocytes, *Rhinopoma microphyllum kinneari*

Introduction

Chiropterans (bats) are the only true flying mammals and have undergone various enigmatic modifications to meet the stress of flight adaptations. Though there are many myths regarding bats amongst common masses, they play important role in maintaining ecological balance. They are of economic or medical value to humans as well. They are of agricultural importance because of their diverse feeding habits. This is evident from the fact that fruit eating bats contribute to seed dispersal, bats feeding on nectar are responsible for fertilization/cross-fertilization of flowers, farmers use their guano as fertilizer, etc. Chiropterans may be harmful to humans if they are infected with rabies or any other virus or bacteria that may be transmitted by accidental cases of bat bites. Besides these contributions and diversities, bats have adapted themselves to different ecological niche as well (Wimsatt 1977, Meltzer& Rupprecht 1998, Winter 1999, Pape et al. 1999, Scott 2000, Ito et al. 2001). These adaptations suggest various physiological adjustments including blood composition and chemistry. From the perusal of literature it appears that the blood physiology of bats is as diverse as their diverse feeding habits e.g. insectivores, vampires, piscivores, frugivores etc. (Riedesel 1977).

Rhinopoma microphyllum kinneari Wroughton, 1912 is an insectivorous microchiropteran inhabiting diverse regions of the world. It is also a hardy microchiropteran and in addition to flight adaptations this bat faces the harsh conditions of the desert (Thar) state of Rajasthan (India). *Rhinopoma microphyllum kinneari* breed once in an annual cycle. Seasonal alterations in the histology and histochemistry of ovary and uterus of this bat have been studied. Active folliculogenesis and spermatogenesis is seen in ovaries and testes during October to January or February. A single Graafian follicle matures and ovulation occurs in March or early April. A single fetus

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develops from April to June. Parturition has been observed in June and July. Lactation period continues from this time onwards till August (Anand Kumar 1965, Lall 1986, Trivedi 1991).

Studies on effect of environmental stress on blood profile of bats have been carried out in various bat species (Riedesel 1977, Heard & Huft 1998). Perusal of literature indicates that no such study has been carried out in any Indian bat. This study was undertaken to study the differential leukocyte profile in male and female bats, as there is no preliminary account of WBC in any of the Indian bats.

Materials and Methods

Adult *Rhinopoma microphyllum kinneari* collected from fields surrounding Jodhpur (26° 19' N, 73° 8' E) in the months of October, November and December have been used for the study. Bats were kept in wire-net cages (approximately 12×16×12 inches) in the laboratory. Blood samples were collected from venipuncture (by sterile injection needle) of wings of five males and five females within 2–3 hr. of their collection. Blood smears stained by Giemsa stain have been used to carry out study of different white blood corpuscles. Differential count of five slides per bat has been done by two persons and reconfirmed by other two using binocular microscope (Olympus) and tabulated.

Note. References search on WBC profile of chiropterans was carried out on NCBI-PubMed search online till 11 th of November 2002. In addition to this literature search was also done at INSDOC and NIC at N. Delhi (India). All these searches gave only few references that were of any relevance and they have been included in this paper.

Results

The differential leukocyte counts of adult female and male have been computed separately and are given in the Tab. 1.

From the data it is evident that in female *Rhinopoma microphyllum kinneari*, the percentage of different white blood corpuscles varies during different months. Percentage of neutrophils is high in October, which decrease in the month of November and is extremely low in December. Eosinophils do not fluctuate in October and November but decrease in the month of December. Basophil counts are low in October, high in November and dip again in December. Fluctuations in the percentage of agranulocytes (small and large lymphocytes) are evident. Monocyte percentage is low in October but is slightly high in the months of November and December.

In males percentage of neutrophils does not vary during October and November but in December the percentage is very low. Eosinophil counts do not vary during the months of October and November but are higher in the month of December. Percentage of basophils, monocytes, small

Tab. 1. Differential WBC count (in %) in female and male *Rhinopoma microphyllum kinneari*
Tab. 1. Procentuální obsah krevních tělísek u samice a samce víkonosa asijského (*Rhinopoma microphyllum kinneari*)

	month	granulocytes			agranulocytes		
		eosinophils	neutrophils	basophils	monocytes	lymphocytes large	lymphocytes small
female	October	4.40 ± 1.19	60.20 ± 1.22	1.56 ± 1.29	2.40 ± 0.87	3.80 ± 0.71	28.40 ± 1.12
	November	4.64 ± 2.22	16.76 ± 5.76	3.88 ± 1.83	8.00 ± 3.81	10.80 ± 2.08	56.08 ± 11.43
	December	3.28 ± 2.13	2.48 ± 1.94	1.80 ± 3.07	8.64 ± 5.62	21.8 ± 9.27	64.36 ± 16.71
male	October	6.60 ± 0.82	67.32 ± 0.9	2.04 ± 0.68	3.20 ± 0.76	15.36 ± 1.68	4.92 ± 0.76
	November	6.84 ± 0.80	66.88 ± 1.05	1.96 ± 0.68	3.20 ± 0.82	16.16 ± 1.18	4.84 ± 0.62
	December	8.80 ± 6.46	3.16 ± 1.89	7.56 ± 10.55	14.80 ± 9.87	38.60 ± 16.54	28.16 ± 10.14

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and large lymphocytes during October and November do not vary but are elevated in the month of December.

On comparing the data of female and male, the values of eosinophils, neutrophils and large lymphocytes in males are more than those found in females during all the three months. Percentage differences of neutrophil in male and female are sharp only during November. Marked differences in percentages of basophils and monocytes in male and female are evident in the months of November and December. These differences are interesting because in November, females have higher values of basophils and monocytes whereas during December males have higher values. The values of small lymphocytes are high in females during all the three months.

From the present data it cannot be determined whether *R. m. kinneari* has predominance of lymphocytes or neutrophils. Total lymphocyte average (of three months) percentage indicates that in females it is higher than neutrophil percentage. However, in case of males, neutrophil average percentage is higher than the total average percentage of lymphocytes. The data on differential leukocyte counts in other bats is given in table – 2 for comparison with the present study.

Discussion

Chiropterans being the only true flying mammals are enigmatic animals as they have diverse adaptations. These adaptations may have effect on the blood composition and chemistry due to high oxygen demand; diverse eating habits and ecological niche that they occupy (Riedesel 1977).

The present study on insectivorous microchiropteran: *Rhinopoma microphyllum kinneari* reveals difference in the average white blood corpuscles count in both the sexes. The maximum difference amongst granulocytes is in the percentage of neutrophils where it is extremely high in males. Amongst the agranulocytes, there is a steep rise in the number of small lymphocytes in both the sexes but there seems to be an approximate difference of three times in the ratio of these cells of male and female. This difference is also evident in the percentage of large lymphocytes where there is approximately fifty-percent difference. Variations in the differential WBC counts are also evident during different months. The high standard deviations are probably due to higher replication error. The analysis of variance indicates F-ratio for gender and months to be highly significant (data not shown).

A comparison of this study with that of other bats reported by Riedesel (1977) reveals similarities of present data with some bats (Tab. 2). The average (of three months data) percentage of neutrophils (45.79) of male *Rhinopoma microphyllum kinneari* are comparable with *Phyllostomus discolor* (46.80), *Glossophaga soricina* (44.00), *Uroderma bilobatum* (42.30), *Arbeus lituratus* (45.90) and *Molossops temminckii* (47.00). The average percentage of basophil (3.85) and eosinophils (7.41) in male is not similar to any of the bats studied. Monocyte average percentage (8.00) of *Molossops temminckii* is comparable with that of *R. m. kinneari* (7.07).

In *R. m. kinneari* female, average percentage of neutrophil (26.48) is comparable with *Sturnira lilium* adult female (28.00) and *Uroderma bilobatum* subadult female (24.00). The percentage of basophils of female (2.41) are comparable with*Phyllostomus discolor* gravid and lactating females (mean=2.00) and *Artibeus lituratus* (mean=2.00). Average eosinophils (4.10) in *R. m. kinneari* female are similar to *Artibeus jamicensis* adult female (4.50). The average of monocytes in the present study (6.34) is similar to *Sturnira lilium* adult female (6.00).

The other data provided by Riedesel (1977) is not differentiated into data from male or female. This average data of neutrophil in *Myotis lucifugus* (42.00) and segment in *Nyctalus noctula*

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Tab. 2. Leukocyte counts (in %) in different species of bats (data taken from Riedesel 1977). Explanantion: M – male, F – female, ad. – adult, subad. – subadult, gr. – gravid, lact. – lactating, juv. – juvenil
Tab. 2. Procentuální objem bílých krvinek u rozných druhů netopýrů (údaje podle Riedesel 1977). Vysvětlivky: M – samec, F – samice, ad. – adultní, subad. – subadultní, gr. – gravidní, lact. – laktující, juv. – juvenilní

species	sex, age	eosinophils	neutrophils	basophils	monocytes	lymphocytes
Myotis myotis		0.75		0.46	2.30	37.00
Myotis nattereri		1.80		1.50	4.76	60.50
Nyctalus noctula		1.98		0.75	2.20	47.30
Plecotus auritus		0.83		0.63	3.00	21.20
Myotis lucifugus		0.40		0.60	2.80	54.20
Artibeus lituratus		0.30		0.40	1.50	38.50
Noctilio labialus		2.00			1.00	61.50
Phyllostomus hastatus		2.50			3.00	32.00
Molossus molossus		1.00			3.50	52.00
Phyllostomus discolor	M ad.	1.30	46.80		0.80	50.50
	F ad.	3.00	45.00	0.50	2.50	49.00
	F lact.	1.50	20.50	1.00	4.50	72.50
	F gr., lact.	5.00	13.00	2.00	1.00	79.00
Glossophaga soricina	M ad.		44.00		1.30	54.70
	F gr.		76.00			24.00
Lonchophylla robusta	F gr.	2.00	32.00		3.00	63.00
Carollia perspicillata	M ad.		61.30			38.70
	F gr.	2.00	34.00		2.00	63.00
	F lact.		40.00		1.00	59.00
Sturnira lilium	F ad.	1.00	28.00		6.00	65.00
Brachphylla cavernarum	M ad.	2.00	70.50	0.50	3.50	24.00
	F ad.	2.00	60.00	0.30	2.30	34.70
	F gr.	3.00	56.80		1.80	38.50
Uroderma bilobatum	M ad.	1.00	42.30		2.70	51.70
	F lact.	3.00	32.00			65.00
	F subad.	2.00	24.00		5.00	69.00
	F juv.	1.00	38.00		1.00	60.00
Artibeus jamaicensis	M ad.		53.30		1.00	45.70
(Colombia)	F ad.		34.00			56.00
	F gr.	0.20	49.20	0.40	0.80	49.40
	F lact.		60.00		4.00	36.00
Artibeus jamaicensis	M ad.	2.40	62.40	0.70	2.30	32.10
(St. Croix)	M subad.	4.00	70.00		4.00	22.00
	F ad.	4.50	54.50	1.00	3.00	37.00
	F gr.	5.00	60.00			35.00
Artibeus lituratus	M ad.	1.10	45.90	0.20	3.00	49.90
	M subad.	0.90	40.50	0.20	1.50	56.90
	F ad.	1.50	36.70	0.40	1.30	60.20
	F gr.	2.70	33.00	2.00	1.30	61.30
	F. juv.	1.00	65.00		5.00	29.00
Stenoderma rufum	F gr.	2.00	57.00	1.00	1.00	39.00
Erophylla bombiforns	F gr.	2.50	59.00	1.00	5.00	32.50
Myotis nigricans	M ad.		28.00		12.00	60.00
Eptesicus brasiliensis	F gr.	1.00	62.00		2.00	34.00
Molossops temminckii	M ad.	5.00	47.00		8.00	40.00
Molossus molossus	F ad.		62.00		1.00	37.00

(45.40) (data not shown); lymphocytes in *Myotis myotis* (37.00), *Phyllostomus hastatus* (32.00) and *Artibeus lituratus* (38.50) are comparable with *R. m. kinneari* male. Average percentage of neutrophils in *R. m. kinneari* female is comparable with *Myotis nattereri* (29.95).

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The available data in literature on the percentage of lymphocytes has not been separated into small and large lymphocytes. Hence, average of small and large lymphocytes of *R. m. kinneari* is taken to compare the data with other bats. Average of the total percentage (36.01) of small and large lymphocytes of *Rhinopoma microphyllum kinneari* males is comparable with *Carollia perspicillata* (38.70), *Artibeus jamaicensis* (32.10), *Molossops temminckii* (40.00) and *Molossus molossus* (37.00). In case of females the data on lymphocytes (61.74) matches with *Myotis nattereri* (60.50), gravid *Lonchophylla robusta* (63.00) and *Carollia perspicillata* (63.00), *Sturnira lilium* adult female (65.00), lactating *Uroderma bilobatum* (65.00) and *Artibeus lituratus* adult and gravid female (60.20 and 61.20 respectively).

In *Myotis velifer* (Caire et al. 1981), leukocyte parameters reveal that few eosinophils and basophils are found but basophil number is higher in male and only females have eosinophils. The highest percentages of leukocytes are the lymphocytes (30.00–57.00). Monocytes and stab cells are 2.00–10.00 and 0.00–6.00 respectively of the total leukocytes. Korine et al. (1999) have estimated biochemical alterations in blood parameters of *Rousettus aegyptiacus*.

Form these comparisons it appears that though some parameters do match with some bat species, yet at the same time they do not do so with others. Also in case of *Rhinopoma microphyllum kinneari* non-pregnant females, the data generally match with those of the gravid or lactating bats. Since breeding cycles of all bat species is highly diverse (Anand Kumar 1965, Lall 1986, Trivedi & Lall 1989, Trivedi 1991, Badwaik et al. 2000, Singh & Krishna 2000), it is possible that these parameters vary or seem similar with some reproductive stage in bats. December is the time of active folliculogenesis and spermatogenesis in *Rhinopoma microphyllum kinneari* (Anand Kumar 1965, Lall 1986) and possibly the gender differences observed in the present study could be due to the reproductive changes in the bats. If study of blood profile is carried out throughout the annual cycle in these bats only then such a comparison could be made. The higher percentage of lymphocytes, especially in case of the females could either be an indication of stress, infection or could be a normal phenomenon. This speculation draws support from studies in which correlation of stress or reproductive stage with leukocyte profile alteration has been observed (Pehlivanoglu et al. 2001, Silberman et al. 2002). However, to reach any comprehensive conclusion, there is need to investigate the white blood corpuscle profile in both the sexes during different season. This would enable to gauge whether there are seasonal variations in the profile investigated so far. This study also needs investigations with regards to reproductive stage specific changes especially in females.

However, these comparisons may not be highly significant according to Riedesel (1977); as it has been suggested that the blood collecting technique, time of capture and time of sacrifice may vary which may cause these differences or dissimilarities. Studies on *Pteropus hypomelanus*, indicate that physical restrain has greater influence on blood cell population as compared to use of anesthesia. Since the blood cell parameters change due to either kind of strain, blood samples should be drawn from the bats as soon as possible, preferably in the field itself (Heard & Huft 1998). Studies on other vertebrates also indicate that stress may influence blood profile (Maddock & Pariante 2001, Degabriele & Fell 2001, Mills et al. 2001, Pehlivanoglu et al. 2001, Silberman et al. 2002, Shive et al. 2002, Langston et al. 2002, Becker et al. 2002). Since the bats used in the present study were kept for some time in the laboratory in cages, it is possible that the data may be influenced by stress.

A comparison with human leukocyte values (neutrophils: 50.00–70.00; eosinophils: 1.00–4.00; basophils: 0.10; monocytes: 2.00–8.00 and lymphocytes: 20.00–40.00) (Guyton 1981, Vander et al. 1994) show very little similarity. Average values of only eosinophils and monocytes of female

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Rhinopoma microphyllum kinneari and monocytes and total lymphocytes of male *Rhinopoma microphyllum kinneari* are closer to human white blood corpuscles. Low number of neutrophils may be a normal phenomenon in female bats as compared to neutrophils found in males. However, a comparison of blood profile in other seasons would confirm this. If it were not a normal phenomenon, it would imply that either these bat colonies are affected in a manner where their neutrophil population is not sufficient to combat bacterial and or viral infections. High values of basophils in males and lower values in females may be in response to any allergy. However, this could be a normal phenomenon also because of active spermatogenesis and folliculogenesis after a lull of some months. Histamines and serotonin released from basophils (Guyton 1981) may be facilitating reproductive functions. But this theory needs confirmation.

Conclusion

From the study it is apparent that the white blood corpuscle population does undergo changes during the three months during which the study has been carried out. There are significant differences in the blood cell population verses the sex of the animal. Possibly these are either due to differences in the hormones of the sexes. Study of blood profile in different seasons may help in understanding whether the data observed in the present study are normal phenomenon.

In conclusion we stress that though there are many myths regarding bats amongst common mass and they may seem to be of no value to mankind, but it is far from truth. Comparison should be undertaken in order to understand the significance of alterations in blood profiles due to environmental changes, stress, infections or reproductive stage related effects. In this sense such monitoring may serve the purpose of bio-indicators of increase in environmental stress which may be due to either natural or man-made causes. Bats are important not only for agriculture but also for biological insect pest management, seed dispersal etc. Being mammals they are members of the same class to which *Homo sapiens* belong. Any harm to the survival of these organisms caused due to either natural or man-made reasons, may directly or indirectly affect mankind. Study and a careful watch of the blood physiology of these unique mammals may be of immense value not only to naturalists but to common mass as well. Little effort has been made to document even the basic blood profiles in these organisms especially in context of Indian species of bats. It is not easy to carry further work in this direction because of the stringent measures of the Indian Government imposed on research on animals especially wild life; using invasive methods. It is high time that such documentation is done before it is too late and we lose an important source of information that may help us solve some riddles of environmental stress. This study is only a small contribution in this direction and needs further work.

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Souhrn

Diferenciální profil bílých krvinek u víkonosa asijského (*Rhinopoma microphyllum kinneari*). V příspěvku je popsán profil populace bílých krvinek u víkonosa asijského (*Rhinopoma m. kinneari*), studovaných v průběhu října, listopadu a prosince, a to jak u samců tak i u samic. Diferenciální profil bílých krvinek samců se významně odlišoval od samičího. V průběhu uvedených tří měsíců je patrná fluktuace v populaci

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bílých krvinek. Zjištěné údaje o populaci bílých krvinek byly porovnány s údaji zjištěnými předcházejícími studiemi u dalších druhů netopýrů a v textu náležitě diskutovány.

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