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HAEMATOLOGICAL PARAMETERS OF THREE SPECIES OF WILD CAUGHT MICROCHIROPTERAN BATS, *MINIOPTERUS SCHREIBERSII*, *TAPHOZOUS MELANOPOGON* AND *HIPPOSIDEROS LANKADIVA* IN SRI LANKA

W. D. RATNASOORIYA,* P. V. UDAGAMA-RANDENIYA, W. B. YAPA, P. M. C. B. DIGANA AND M. G. DHARMASIRI

Department of Zoology, University of Colombo, Colombo 3, Sri Lanka

* Corresponding author (E-mail: wdratna@zoology.cmb.ac.lk)

ABSTRACT

This study, for the first time in South Asia, examines haematological parameters of three species of wild caught Sri Lankan microchiropteran bats, belonging to three different families having different global zoogeographical distributions; *Miniopterus schreibersii* (Vespertilionidae; n = 12), *Taphozous melanopogon* (Emballonuridae; n = 19) and *Hipposideros lankadiva* (Hipposiderosidae; n = 15). Blood samples were obtained from the median vein and the total white blood cell (WBC) count, red blood cell (RBC) count, packed cell volume (PCV), haemoglobin content (Hb), mean corpuscular volume (MCV) and mean corpuscular haemoglobin content (MCHC) were determined using standard haematological techniques. Interspecies differences existed in some of the parameters monitored, *i.e.* PCV, MCV, WBC and RBC counts, % of neutrophils and lymphocytes. On the other hand, a gender discrepancy was recorded for *T. melanopogon* with respect to neutrophils and lymphocytes, and for *M. schreibersii* with respect to the total WBC count. The highest WBC count and the lowest PCV and MCV were found in *M. schreibersii*. *T. melanopogon* registered the highest percentage of lymphocytes and the lowest percentage of neutrophils, while *H. lankadiva* possessed the highest MCV. Haematological values were recorded also for a single lactating *T. melanopogon.*

Key words: haematology, Microchiroptera, *Miniopterus schreibersii*, *Taphozous melanopogon*, *Hipposideros lankadiva*, Sri Lanka.

INTRODUCTION

Baseline data on some aspects of haematological profiles are available for bats from nearctic and neotropical regions (Riedesel, 1977). Bats are unique in their energy requirements and exhibit high weight-specific basal metabolic rates. These animals overcome problems of flight and high weight-specific metabolism by presenting elevated blood oxygen transport properties, *i.e.*, high red blood cell counts, haematocrits and haemoglobin concentrations (Riedesel, 1977).

Blood oxygen transport properties of the African fruit eating megachiropteran, *Rousettus aegyptiacus* and microchiropterans such as the South American tropical *Phyllostomus discolor* and *Molossus ater*, and the European *Myotis myotis* and *Pipistrellus pipistrellus* were documented by Jurgens *et al.* (1981). The seasonal variation in the haematocrit and plasma protein content of the pallid bat, *Antrozous pallidus,* maintained in constant laboratory conditions in the USA had been reported by Bassett and Curt (1982). They also reported on the haematological changes occurring during postnatal growth and the haematological ontogeny of this bat (Bassett and Wiederhielm, 1984). The haematological values and haemoglobin electrophoretic results of bats belonging to the family Vespertilionidae from Spain were investigated by Arevalo *et al.* (1987). Essential haematological parameters having a decisive effect on capacity of the oxygen delivery system and leucocytic indices in hibernating *Myotis daubentoni* have been reported by Wolk and Bogdanowicz (1987). Standard haematological parameters and oxy-haemoglobin dissociation curves had been determined for the common bent-wing bat, *Miniopterus schreibersii* and the red fruit bat, *Pteropus scapulatus* in Australia (Agar and Godwin, 1992). Viljoen *et al.* (1997) had studied peripheral blood characteristics of gravid *Miniopterus schreibersii natalensis* from South Africa and concluded that haemoglobin concentration rather than erythrocyte counts should be taken as a reflection of oxygen carrying capacity as the high count of smaller erythrocytes represents diffusion and haemorrheological, rather than oxygen-carrying, adaptations.

The normal haematological values of bats from South Asia are scarce and blood profiles are non-existent for Sri Lankan bats. In an attempt to fill this lacunae, the present study was carried out to examine the haematology of three microchiropteran species of Sri Lanka belonging to three different families, namely *Miniopterus schreibersii* Hodgson, 1835 (Family: Vespertilionidae), *Taphozous melanopogon* Temminck, 1841 (Family: Emballonuridae) and *Hipposideros lankadiva* Kelaart, 1850 (Family: Hipposiderosidae). The basis for selection of these three species of bats was their variable global zoogeographical distribution: *H. lankadiva* is restricted to Sri Lanka and India; *T. melanopogon* is found only in the

oriental regions while *M. schreibersii* has a wide distribution encompassing all zoogeographical regions except the nearctic and neotropical regions (Bates and Harrison, 1997).

MATERIALS AND METHODS

Bats were caught from their day roosts at Wavulgalge, a natural cave in Wellawaya (6° 44'N; 81° 06'E) and Ponnampalamvanechar Hindu Kovil in Kotahena, Colombo (6° 57'N; 79° 51'E) using hand nets. *Miniopterus schreibersii* and *H. lankadiva* were captured at Wavulgalge in October, 1996 while *T. melanopogon* were caught from the Hindu Kovil in January, 1997. Each bat species was captured during a single sample collecting session to avoid multiple capture of the same animal. These two particular roosting sites were selected due to ease of multiple capture of bats that would minimize capture related stress. Bats were identified using Bates and Harrison (1997).

Adult bats were selected for this study based on the forearm length and body weight. Forearm lengths were 88.5-95.0 mm, 45.6-47.0 mm and 61.5-64.0 mm, and weights were 48-75 g, 11-12.5 g and 22-32 g in *H. lankadiva*, *M. schreibersii* and *T. melanopogon* respectively. The length of the forearm (radius & ulna) is often used to measure the postnatal growth of bats (Kunz 1987). The length of the right forearm was measured using a vernier calliper. Captured bats were weighed individually using a spring balance (range 1-100 g; Pesola, Switzerland). Pregnant females were detected by the presence of palpable fetuses in mid to late gestation. The lactating females were identified by the presence of pubic teats (McWilliam, 1987). Accordingly, haematological parameters of *M. schreibersii* (6 males, 6 females), *H. lankadiva* (6 males, 9 females) and *T. melanopogon* (9 males, 10 non pregnant females, 1 lactating female) were examined.

Within one hour of capture, blood samples (50 –100 µl) were obtained from the median vein of bats with heparinised micro-haematocrit tubes under manual restraint using aseptic precautions. Total white blood cell (WBC) and red blood cell (RBC) counts, packed cell volume (PCV) and haemoglobin content (Hb) were estimated, and mean corpuscular volume (MCV) and mean corpuscular haemoglobin content (MCHC) were computed using standard haematological procedures (Ghai, 1993). Thin blood smears were made, stained with Leishman's stain and differential counts (DC) of white blood cells were made for all three species by counting at least 100 leucocytes (Ghai, 1993). Following collection of blood, all bats were released.

Results are represented as mean ± SEM. Statistical comparison of parameters for sexes of the same species were made using Mann-Whitney U-test while Kruskal-

Wallis test followed by multiple comparisons (Siegel and Castellan, 1988) was used for comparisons between the three species of bats.

RESULTS

Overall values of haematological parameters calculated for the three species of bats are summarized in Tables 1 and 2. *M. schreibersii* had a significantly ($p<0.05$) higher WBC count than *T. melanopogon* (by 56%) and *H. lankadiva* (by 51%). No basophils and extremely few eosinophils were evident in the blood smears of all three bat species. On the other hand, a very low level (4%) of monocytes was recorded for *M. schreibersii* while no monocytes were detected in *T. melanopogon* and *H. lankadiva*. *T. melanopogon* had a significantly lower ($p<0.05$) neutrophil count and a higher ($p<0.01$) lymphocyte count than the other two species (Table 1). Significantly lower ($p<0.05$) values of PCV and MCV were observed in *M. schreibersii* compared to *T. melanopogon* (PCV by 12% and MCV by 9%) and *H. lankadiva* (PCV by 12% and MCV by 28%) (Table 2).

Table 1. Overall values of total and differential WBC counts (Mean ± SEM; ranges in parenthesis) of the three species of Microchiropterans studied

Species	Mass (g)	WBC (mm^{-3})	WBC Differential Counts				
			Basophils (%)	Eosinophils (%)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)
Miniopterus schreibersii ($n = 12$)	11.76 ± 0.16 (11.0 – 12.5)	14,346 ± 1212[a]* (8975 - 21,050)	0	0.6 ± 0.3 (0 – 2)	50.5 ± 6.0 (37 – 82)	44.8 ± 5.5 (16 –57)	4.1 ± 0.9 (2 – 7)
Taphozous melanopogon ($n = 18$)	26.44 ± 0.24 (22.0 – 32.0)	9214 ± 188 (7625 - 10,050)	0	0.1 ± 0.1 (0 – 1)	34.4 ± 3.1[b]* (18 – 53)	65.6 ± 3.1[b]** (47 – 82)	0
Hipposideros lankadiva ($n = 15$)	55.33 ± 0.26 (48.0– 75.0)	9500 ± 802 (5450 - 14,750)	0	0.3 ± 0.2 (0 - 2)	49.6 ± 1.3 (42 –56)	50.1 ± 1.4 (44 – 56)	0

*P< 0.05; **P<0.01

[a] Significantly different from *Taphozous melanopogon* and *Hipposideros lankadiva*

[b] Significantly different from *Miniopterus schreibersii* and *Hipposideros lankadiva*

Table 2. Overall values of erythrocyte related parameters (Mean±SEM; ranges in parenthesis) of the three species of microchiropterans

Species	RBC (10^6/mm^3)	PCV (%)	Hb (g/dl)	MCV (μm^3)	MCHC (g/dl)
Miniopterus schreibersii ($n = 12$)	10.1 ± 0.3 (8.4–13.6)	53.0±4.0[a]** (51.0-55.0)	17.6±0.2 (16.5-19.0)	54.6±2.3[a]** (42.3-67.5)	33.2 ± 0.5 (31.1-35.8)
Taphozous melanopogon ($n = 18$)	8.9 ± 0.6 (5.8–14.7)	59.2 ± 0.6 (53 – 63)	16.0 ± 0.3 (14.5-18.0)	59.33 ± 2.8 (39.0-77.0)	29.1 ± 0.7 (25.0-34.0)
Hipposideros lankadiva ($n = 15$)	8.9 ± 0.6 (6.6-14.8)	59.2 ± 0.7 (57 - 61)	NI	70.0 ± 4.0 (41.7-93.1)	NI

**P<0.01

[a] **Significantly different from *Taphozous melanopogon* and *Hipposideros lankadiva***

NI Not investigated.

Table 3. Values of total and differential WBC counts (Mean±SEM; ranges in parenthesis) of the different sexes of the three species of microchiropterans

Species	Sex (Mass [g])	WBC Count (mm^{-3})	WBC Differential Count				
			Basophils (%)	Eosinophils (%)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)
Miniopterus schreibersii	Male (11.58 ± 0.22) ($n = 6$)	11,567 ± 1373[d*] (9450-16900)	0	0.5 ± 0.3 (0 – 2)	58.1 ± 9.0 (42 – 82)	37.1 ± 7.5[b*] (16 – 51)	4.3 ± 1.5 (2 – 7)
	Female (11.93 ± 0.21) ($n = 6$)	17,125 ± 1223[b**,c**,d*] (13700-21050)	0	1.0 ± 1.0 (0-1.0)	40.3 ± 1.8[c**] (37 – 43)	55.0 ± 1.3[b**] (53 – 57)	3.7 ± 1.1 (3 – 6)
Taphozous melanopogon	Male (25.33 ± 1.18) ($n = 9$)	9,069 ± 288 (7625-9925)	0	0.1 ± 0.1 (0 –1 0)	47.9 ± 1.2[d**] (43 – 53)	52.0 ± 12[a*, d**] (47 – 56)	0
	Female (27.56 ± 1.2) ($n = 9$)	9358 ± 251[a**] (7675-10050)	0	0	22.2 ± 0.9[c**, d**] (18-27)	77.8 ± .9[a**,c**, d**] (73 – 82)	0
Hipposideros lankadiva	Male (52.0 ± 1.29) ($n = 6$)	9196 ± 1472 (5450-15975)	0	0.5 ± 0.34 (0 – 2)	51.3 ± 2.2 (44 – 56)	48.2 ± 2.4 (44 – 56)	0
	Female (57.55 ± 3.2) ($n = 9$)	9703 ± 981[a**] (6750-14750)	0	0.2 ± 0.2 (0 – 1)	48.5 ± 1.7[a**, b**] (42 – 56)	51.3 ± 1.6[b**] (44 – 58)	0

*P < 0.05; **P <0.01

a Significantly different from same sex of *Miniopterus schreibersii*

b Significantly different from same sex of *Taphozous melanopogon*

c Significantly different from same sex of *Hipposideros lankadiva*

d Significantly different between male and female of same bat species

Table 4. Values of some erythrocyte related parameters (Mean±SEM; ranges in parenthesis) of different sexes of the three species of microchiropterans

Species	RBC (10^6/mm^3)	PCV (%)	Hb (g/dl)	MCV (μm^3)	MCHC (g/dl)
Miniopterus schreibersii	Male 9.5 ± 0.3 (8.38-10.6)	52.8±0.6^{b*,c**} (51-54)	17.6±0.2^{b**} (17-18)	59.3±3.0 (53-67.5)	33.28±0.8^{b*} (31.5-35.3)
	Female 10.6±0.6^{b*,c*} (8.6-13.6)	53.2±0.5^{c**} (51-55)	17.6±0.3^{b*} (16.5-19)	51.5±2.8^{b*,c*} (42.3-58.9)	33.1±0.7
Taphozous melanopogon	Male 10.9±0.8^{d*} (7.95-14.68)	55.4±1.0^{a*,c*} (53-63)	16.1±0.3^{a**} (15-18)	53.3±3.5^{d*} (40-70)	29.0±0.8^{a*} (25-34)
	Female 8.6±0.6^{a*,d*} (5.9-12.9)	54.3±0.5^{c**} (53-56)	15.8±0.3^{a*} (14.5-23)	65.3±3.5^{a*,d*} (43-90)	29.2-±0.6 (27-42)
Hipposideros Lankadiva	Male 9.5±1.3 (6.6-14.8)	58.7±0.9^{a**,b*} (55-62)	NI	65.5±7.1 (41.7-87.6)	NI
	Female 8.5±0.6 (6.6-11.8)^{a*}	59.6±0.8^{a**,b**} (58-64)	NI	73.1±4.8^{a*} (50.22-93.1)	NI

*P < 0.05; **P < 0.01

a Significantly different from same sex of *Miniopterus schreibersii*

b. Significantly different from same sex of *Taphozous malanopogon*

c Significantly different from same sex of *Hipposideros lankadiva*

NI not investigated

Haematological values of the two sexes of each species are presented in Tables 3 and 4. The WBC counts of the females of all three species were generally higher than those of their male counterparts and this was conspicuous (by 48%) and significant ($p<0.05$) in the case of *M. schreibersii*.

Gender disparity of statistical significance ($p<0.05$) was detected in *T. melanopogon* for neutrophil and lymphocyte counts, RBC count and MCV. In this species the neutrophil count was significantly ($p<0.01$) higher in the males (116%) compared to females, while the lymphocyte count was significantly ($p<0.01$) lower (by 50%) (Table 3). A high RBC count was evident in male *T. melanopogon*, which was significantly ($p<0.01$) higher than in the females (by 27%). In addition, MCV of the female *T. melanopogon* was significantly ($p<0.05$) higher than in males (by 23%). *H. lankadiva* did not show a gender discrepancy in these blood parameters ($p>0.05$).

Interspecific differences in some blood parameters were apparent among females as well as males of the three bat species. The highest WBC count was detected in female *M. schreibersii* that was 83% higher ($p<0.05$) than that of *T. melanopogon* and 76% higher than *H. lankadiva* (Table 3). The neutrophil count of female *H. lankadiva* was significantly ($p<0.01$) higher than that of female *T. melanopogon* (by 118%) and *M. schreibersii* (by 20%). Female *T. melanopogon* had the highest lymphocyte count which was significantly ($p<0.01$) higher than that of female *M. schreibersii* (by 41%) and *H. lankadiva* (by 52 %). Among the males, a significant difference ($p<0.05$) existed in the lymphocyte count of *T. melanopogon* and *M. schreibersii,* the former having a 40% higher count than the latter (Table 3).

Further, the RBC count of female *M. schreibersii* was significantly ($p<0.05$) higher than that of female *T. melanopogon* (by 23%) and *H. lankadiva* (by 26%) (Table 4). The highest PCV was recorded in female *H. lankadiva*, which was significantly ($p<0.05$) higher than that of both males and females of the other two species: *T. melanopogon* (males by 8% and females by 10%) and *M. Schreibersii* (males by 13% and females by 12%). Both male and female *M. schreibersii* had significantly higher ($p<0.01$ and $p<0.05$, resepectively) Hb levels than that of the same sexes of *T. melanopogon*. The highest MCV was evident in female *H. lankadiva*, whilst the lowest was seen in the females of *M. schreibersii*. In addition, MCV of the female *T. melanopogon* was significantly ($p<0.05$) higher than both males (by 10%) and females (by 27%) of *M. schreibersii*. Male *M. schreibersii* had a significantly higher ($p<0.05$) MCHC than male *T. melanopogon* (Table 4).

The haematological values recorded for the single lactating *T. melanopogon* were as follows: WBC count – 7900 mm^{-3}, RBC count – 5.8x106 mm^{-3}, PCV – 54%, Hb 17.6 g/dl, MCV – 90 μm^3 and MCHC – 42 g/dl.

DISCUSSION

Haematological data of bats are valuable not only as a reference collection but will also be useful to understand the physiological adaptations of various species of bats in flight and in prognosis, diagnosis and treatment of captured bats maintained in zoological gardens (Conway, 1966; Viljoen *et al.*, 1997). Haematological parameters are very sensitive to environmental pollutants (Posin *et al.*, 1978; Fukumoto *et al.*, 1983; Dmowsky *et al.*, 1998). Therefore, blood parameters of bats from virtually unpolluted environments could be used as indices to evaluate the impact of pollution on bats.

Haematological values tend to vary with stress (Riedesel, 1977). In this study, stress was minimised by careful handling of captured bats. Furthermore, it is claimed that haematological assessments made for laboratory bred animals and captive bats do not provide realistic values for the species (Riedesel, 1977; Viljoen *et al.*, 1997). The results obtained from field caught healthy bats (in terms of external appearance and WBC counts) in this study, therefore, could be considered as realistic and reassuring. It is also important to have reference data on blood parameters of wild caught bats for maintaining normal healthy animals in zoological gardens and laboratory bred animals for experimental purposes. In Sri Lanka, numbers of several species of insectivorous bats, i.e., *H. lankadiva* and *Rhinolophus rouxi*, have declined significantly in the past fifteen years and some species may be extinct in the near future (Yapa *et al.*, 2001). Therefore, it is important to have such valuable physiological data on blood on record.

This is the first study carried out to investigate the haematology of Sri Lankan bats. Overall results show that species and gender differences exist among the blood parameters examined, which are in agreement with the results of other investigators (Riedesel, 1977 and references therein). However, one has to be cautious when interpreting such data based on a low sample size. Nevertheless, due to ethical reasons, the sample size had to be minimized. Interspecific differences were evident in overall WBC counts, percentages of neutrophils and lymphocytes, PCV and MCV (Tables 1 - 3). Further, significant disparities between the three bat species were apparent in RBC counts, Hb and MCHC when values were compared among the same gender of different species (Table 4). Gender differences were seen in *M. schreibersii* (WBC counts) and *T. melanopogon* (neutrophil and lymphocyte counts). Such differences are reported for other mammals too (Hawkey and Hart, 1983 ; Ratnasooriya *et al.*, 1990).

The highest WBC count was found in *M. schreibersii,* which is the smallest of the three species of bats examined. However, in fruit bats, WBC counts tend to

increase with increasing body weight (Riedesel, 1977). On the contrary, the results of the present study with insectivorous species do not support the above relationship, but seem to indicate a reverse association (Table 1). This species is a strict cave dweller, which usually roosts in large numbers (Brosset, 1962). The population of this species in the natural cave in Wavulgalge was infested with large numbers of ectoparasites (nycteribiid and streblid flies and ticks) as well as endoparasites (digenean flukes and nematodes) (Randeniya *et al.*, unpublished data). However, it seems unlikely that high WBC counts observed in *M. schreibersii* could have been a response to infection as there was no difference in the total WBC and differential counts between parasitized and parasite-free hosts of this species (data not shown). In the DC, there was no elevation of eosinophils arguing against a parasitic origin for high WBC counts. This may possibly be a consequence of parasite/host co-evolution that is widespread in ectoparasitic insects (Waage, 1979). High lymphocyte counts are usually indicative of viral infections (Roit *et al.*, 1998). However, infected bats generally show lethargic behaviour that was not evident in this colony. Moreover, Viljoen *et al.*, (1997) had observed that, in contrast to humans, lymphocytes represented the largest percentage of WBC in *M. schreibersii.* Lack of injuries and unimpaired health of captured *M. schreibersii* (as judged by their behaviour and high Hb and PCV values) further support this view. Also, the fact that the majority of haematological values recorded for gravid female *M. schreibersii natalensis* in Africa (Viljoen *et al.*, 1997) being similar to that of the present study, suggests that methodological errors are minimized.

In this study, low eosinophil and monocyte counts and moderately high numbers of lymphocytes and neutrophils were evident in the DC. These values are in agreement with the values recorded for other species of bats (Riedesel, 1977). This phenomeneon is also evident in birds, including greater and lesser flamingos (*Phoenicopterus roseus* and *P. minor*) (Hawkey *et al.*, 1985), and mammals e.g., axis deer (*Axis axis*), Pere David's deer (*Elaphurus davidianus*) (Hawkey and Hart, 1983) and Sri Lankan elephants (*Elephas maximus maximus*) (Ratnasooriya *et al.*, 1990). Further, in the DC, no basophils were detected in *H. lankadiva, T. melanopogon*, and *M. schreibersii*. This is in accordance with the basophil level recorded for neotropical fruit bats (Riedesel, 1977).

Moderately high RBC counts, PCV, Hb and MCHC were seen in females of *M. schreibersii* compared to the other two species of bats. High RBC counts and Hb content are generally considered to be an essential physiological adaptation for long distance and fast flight (Viljoen *et al.*, 1997). Females of *M. schreibersii* in the colony from Wavulgalge, fly 50 km to maternity colonies for parturition (Yapa *et al.*,

unpublished data). Paradoxically, the males of this species, which do not show this migratory behaviour, also had high RBC and Hb values. Currently, we have no explanation to offer for this observation. Furthermore, *M. schreibersii* in South Africa is known to fly long distances, as much as over 150 km, during the breeding period (Van der Merwe, 1975). This species was also observed to have low MCV values in concordance with other workers (Arvelo *et al.*, 1987; Agar and Godwin, 1992; Viljoen *et al.*, 1997), which may be due to the fact that smaller MCV favours the oxygen diffusion capacity into and out of the erythrocyte, as the smaller erythrocyte size is generally assumed to facilitate rapid diffusion. Values for erythrocyte related parameters for *M. schreibersii* from the current study were in par with published data for this species from Spain (Arvelo *et al.*, 1987) and Australia (Agar and Godwin, 1992).

Records of haematological values for lactating bats are virtually unavailable. Haemotological data obtained for a single lactating *T. melanopogon* captured under natural conditions are reported in the present study.

In conclusion, this study has provided some basic haematological data for wild caught adults of three insectivorous species of bats from Sri Lanka to supplement existing worldwide baseline data. This is the first record of blood related parameters of Sri Lankan bats.

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