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Araştırma Makalesi / Research Article

WHITE BLOOD CELL (WBC) COUNT OF DIFFERENT BAT (CHIROPTERA) SPECIES

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Abstract: I*n this study, white blood cell counts and morphological characteristics of peripheral blood cells were determined. Bat specimens were collected from Koyunbaba Cave in Kırklareli Prov. in November 2007. The highest and lowest lymphocyte counts were determined in M. myotis (70.4±12.09) and R. ferrumequinum (53.3±14.83), respectively. When comparing the neutrophil counts of the species, it was seen that the highest value belongs to R. hipposideros (37.75±18.03) and the lowest one belongs to M. capaccinii (17.88±12.50). The highest percantage of monocytes was seen in R. ferrumequinum (12.55±5.77) where as R. hipposideros (7.75±3.89) has the lowest counts. The white blood corpuscle populations have also been compared with other bat studies.*
Key words: *bats, granulocytes, agranulocytes, white blood cell count*

Farklı Yarasa (Chiroptera) Türlerinin Beyaz Kan Hücresi Sayımı

Özet: *Bu çalışmada beyaz kan hücrelerinin sayısı ve periferik kan hücrelerinin morfolojik özellikleri saptanmıştır. Yarasa örnekleri, Koyunbaba Mağarası (Kırklareli)'ndan Kasım 2007'de toplanmıştır. En yüksek ve en düşük lenfosit sayıları sırasıyla M. myotis'te (70,4±12,09) ve R. ferrumequinum'da (53,3±14,83) belirlenmiştir. Türlerin nötrofil sayıları karşılaştırıldığında en yüksek değerin R. hipposideros'a (37,75±18,03), en düşük değerin ise M. capaccinii'ye (17,88±12,50) ait olduğu görülmüştür. En yüksek monosit oranı R. ferrumequinum'da (12,55±5,77) görülmüşken, R. hipposideros en düşük sayıya sahiptir (7,75±3,89). Beyaz kan hücresi sayıları ayrıca diğer yarasa çalışmaları ile karşılaştırıldı.*

Anahtar Kelimeler: *yarasalar, granülositler, agranülositer, beyaz kan hücresi sayımı*

Introduction

The biologists have been interested in the biology of bats for a long time. Bats are remarkable animals not only for their flight adaptations but also their position as a group in the class Mammalia whose feeding and reproductive strategies well known. The characteristic feature of bats is the presence of wings, making them the only mammals capable of powered flight. Because of their high metabolic needs and diverse diets, bats can impact the communities in which they live in a variety of important ways (Hill and Smith, 1984; Vaughan et al., 2000; Jones et al., 2003). 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. Bats have adapted themselves to different ecological niche. From the perusal of literature it appears that the blood physiology of bats is as diverse as their diverse feeding habits e.g. insectivores, frugivores etc. (Riedesel, 1977).

Haematological investigation is an important part of disease diagnosis especially when investigating individual animal disease. It may also be important when investigating diseases in groups of animals. Haematological investigation is essentially similar for all species. Examination of blood films is an important component of haematological investigation and provides useful information on erythroid, leukocytic and platelet/thrombocytic alterations. Interpretation of alterations is essentially similar for all species.

The white blood cell (WBC) differential is a percentage of different type of white blood cells based on a count of leucocytes on blood smears. Neutrophils, basophils, eosinophils, lymphocytes and monocytes are all included. The

WBC differential depends on concentration of different white blood cells in the blood. The WBC differential is a good, sensitive indicator of the animal's general health: a change can be noted when no other abnormalities are detected. It is the one of the most important tests to perform with smears of blood collected in the field. Changes in the WBC differential are often non-specific, however, reflect response of an organism to anyone stressful impact (various physiological and pathological factors). The blood profile is affected by various factors such as age, gender and reproductive state, by endogenic rhythms of various metabolites, as well as by external factors such as season, time of the day, food availability and quality (Westhuyzen, 1978; Hellgren et al., 1988; Wyk et al., 1993; Minematsu et al., 1995). Furthermore, the blood profile may also change due to stress, caused by capturing, handling and sampling the animal (Widmaier and Kunz, 1993; Kopman et al., 1995).

This study was undertaken to determine WBC count of different bat species belonging to Rhinolophidae, Vespertilionidae and Miniopteridae families. The white blood corpuscle populations have also been compared with other bat studies.

Materials and Methods

In this study, totally 6 (3♂♂, 3♀♀) individuals of *Rhinolophus euryale*, 9 (6♂♂, 3♀♀) of *R. mehelyi*, 10 (4♂♂, 6♀♀) of *R. ferrumequinum*, 2 (2♀♀) of *R. hipposideros* belong to the family Rhinolophidae and 13 (4♂♂, 9♀♀) of *Miniopterus schreibersii* belongs to the family Miniopteridae, 4 (2♂♂, 2♀♀) of *Myotis capaccinii*, 5 (2♂♂, 3♀♀) of *M. myotis* belong to the family Vespertilionidae were examined. The study was carried out on November 2007. Bats were collected by a hand net from Koyunbaba Cave in Turkey. Identification of the species was performed according to Dietz and von Helversen (2004).

Blood samples were collected from venipuncture (by sterile injection needle) of wings of males and females within 1-2 hour of their collection. Blood smears were prepared immediately and air-dried. Wright-stained blood smears were used in the counting and assessment of blood cells. Two blood smears were prepared per individual. 12 to 15 drops of Wright stain were dropped on the slides and allowed to remain on the slide for one and 30 sec before rinsing with a phosphate buffer (pH = 6.8). The slides were allowed to stand for ten minutes at room temperature and were then washed with distilled water and allowed to dry. Differential count (percentage of neutrophils, eosinophils, basophils, lymphocytes and monocytes) of two slides per bat were done using by binocular microscope (Olympus). To identify the numbers of different white cells, a blood film is made, and a large number of white cells (at least 100) are counted. This count gives the percentage of cells that are of each type. The blood cells were photographed by means of an Olympus microscope at a magnification 1000x.

Results

There are five types of WBC's: granulocytes - neutrophils, eosinophils, basophils and mononuclear cells - lymphocytes and monocytes (Fig. 1). The differential leukocyte counts of adult females and males were computed separately and were given in the Table 1. As seen from the table in all species, the percentage of lymphocytes is high while the percentage of basophils is low. The highest and the lowest lymphocyte counts are belong to *M. myotis* (70.4±12.09) and lowest in *R. ferrumequinum* (53.3±14.83), respectively. When we compare the neutrophils counts *R. hipposideros* (37.75±18.03) has the highest value. But *M. capaccinii* (17.88±12.50) has the lowest value. The highest percantage of monocytes was seen in *R. ferrumequinum* (12.55±5.77*)* where as *R. hipposideros* (7.75±3.89) has the lowest counts. The average percentage of eosinophils and basophils is too low in all species.

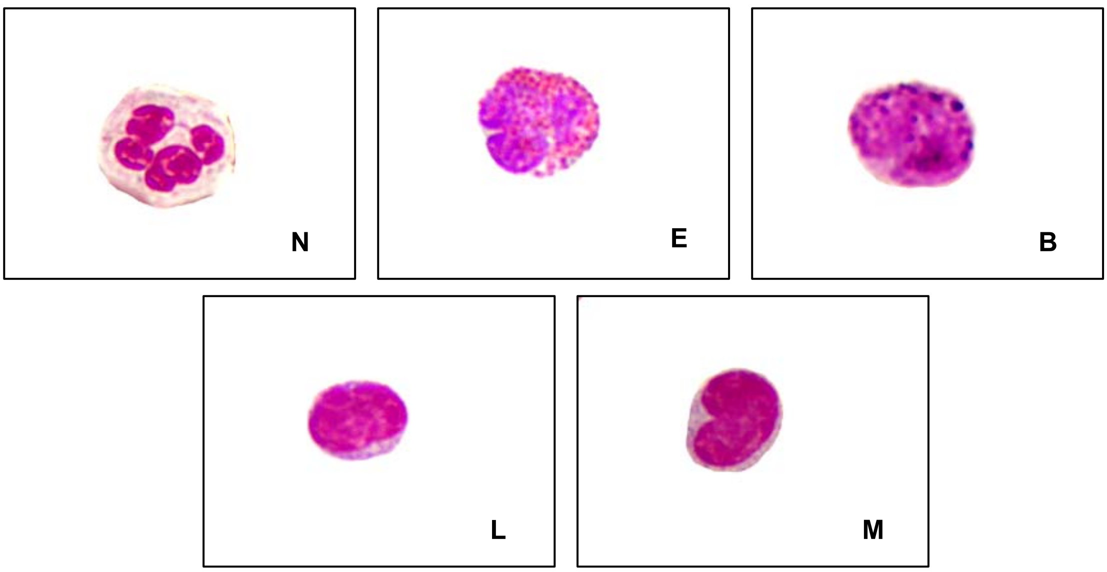

Figure 1. Blood cells of *Myotis myotis*: N, neutrophil; E, eosinophil; B, basophil; L, lymphocyte; M, monocyte; Wright, x1000.

Table 1. Differential white blood cell (WBC) count (%) in different species of bats.

		Granulocytes			Agranulocytes	
Rhinolophus euryale		Eosinophils	Neutrophils	Basophils	Monocytes	Lymphocytes
Female N=3	Maximum	3	32	4.5	16	68.5
	Minimum	1	8	1	8	56
	Mean	1.83±1.04	19.17±12.09	2.67±1.76	13.00±4.36	63.33±6.53
Male N=3	Maximum	1.5	23	1	16.5	77
	Minimum	0	15.5	0.5	6.5	65
	Mean	0.83±0.76	18.33±4.07	0.83±0.29	10.33±5.39	69.67±6.43
	Mean	1.33±0.98	18.75±8.08	1.75±1.51	11.67±4.62	66.5±6.75
Rhinolophus mehelyi						
Female N=3	Maximum	8.5	36.5	0.5	10.5	72
	Minimum	2	15.5	0	9	49
	Mean	5.17±3.25	27.17±10.69	0.17±0.29	10±0.87	57.5±12.62
Male N=6	Maximum	6.5	36.5	1.5	16.5	66
	Minimum	2.5	18	0.5	12	40
	Mean	3.92±1.83	24.75±6.52	1.25±0.42	13.5±1.97	56.58±8.73
	Mean	4.33±2.26	25.56±7.52	0.89±0.65	12.33±2.38	56.89±9.36
Rhinolophus ferrumequinum						
Female N=6	Maximum	1.5	46	1.5	23	72.5
	Minimum	0	13.5	0	7	33
	Mean	0.67±0.61	33.33±13.72	0.67±0.61	14.17±6.67	51.17±17.71
Male N=4	Maximum	4.5	43.5	1	14.5	69.5
	Minimum	0.5	18.5	0	7	47
	Mean	1.75±1.85	30.13±11.84	0.25±0.50	10.13±3.54	56.5±10.70
	Mean	1.1±1.29	32.05±12.42	0.5±0.58	12.55±5.77	53.3±14.83
Rhinolophus hipposideros						
Female N=2	Maximum	0.5	50.5	0.5	10.5	64
	Minimum	0	25	0	5	44
	Mean	0.25±0.35	37.75±18.03	0.25±0.35	7.75±3.89	54±14.14
Miniopterus schreibersii						
Female N=9	Maximum	12.5	50.5	2	12	71.5
	Minimum	1	14	0.5	3.5	37.5
	Mean	3.56±3.56	29±12.74	1.17±0.71	7.44±2.97	58.94±10.85
Male N=4	Maximum	12.5	59	3.5	10.5	66
	Minimum	3.5	16.5	0.5	7.5	25.5
	Mean	6.63±4.13	37±18.35	2.13±1.25	9.25±1.26	45±18.55
	Mean	4.5±3.86	31.46±14.39	1.46±0.97	8±2.65	54.65±14.47
Myotis capaccinii						
Female N=2	Maximum	2	18	0.5	18.5	71.5
	Minimum	1	8.5	0.5	9	70.5
	Mean	1.5±0.71	13.25±6.72	0.5±0.00	13.75±6.72	71±0.71
Male N=2	Maximum	3	35.5	1.5	14	78.5
	Minimum	2	9.5	1	8	47
	Mean	2.5±0.71	22.5±18.38	1.25±0.35	11±4.24	62.75±22.27
	Mean	2±0.82	17.88±12.50	0.88±0.48	12.38±4.85	66.88±13.72
Myotis myotis						
Female N=3	Maximum	5	43	0.5	6.5	82.5
	Minimum	0.5	13	0	1.5	50.5
	Mean	2.33±2.36	26±15.39	0.33±0.29	3.83±2.52	67.5±16.09
Male N=2	Maximum	0.5	12.5	1	17	76
	Minimum	0	8.5	0.5	10.5	73.5
	Mean	0.25±0.35	10.5±2.83	0.75±0.35	13.75±4.60	74.75±1.77
	Mean	1.5±2.03	19.8±13.88	0.5±0.35	7.8±6.16	70.4±12.09

Discussion

Chiropterans which are the only true flying mammals are enigmatic animals because of their diverse adaptations. These adaptations may have effects on the blood composition and its chemistry due to their high oxygen demand, diverse eating habits and ecological niches that they occupy (Riedesel, 1977).

In this study we studied insectivorous microchiropteran. The white blood cell counts in peripheral blood of adult *Rhinolophus euryale*, *R. mehelyi*, *R. ferrumequinum*, *R. hipposideros*, *Miniopterus schreibersii, Myotis capaccinii* and *M. myotis* were determined. The smears were analyzed for the WBC differential (percentage of neutrophils, eosinophils, basophils, lymphocytes and monocytes).

When we compare the results of this study with the results reported by Riedesel (1977), some similarities were found. The average percentage of neutrophils of *R. mehelyi* (27.17) and *M. schreibersii* (29.00) are comparable with *Sturnira lilium* adult female (28.00). The percentage of neutrophils of female *R. ferrumequinum* (33.33) is comparable with *Carollia perspicillata* (34.00), *Artibeus jameicensis* adult female (34.00) and *Artibeus lituratus* gravid female (33.00). In *R. hipposideros* female, average percentage of neutrophil (37.75) is comparable with *Uroderma bilobatum* juvenile female (38.00) and *Artibeus lituratus* adult female (36.70). Average neutrophils (13.25) in *M. capaccinii* female are similar to *Phyllostomus discolor* gravid and lactating females (13.00).

When we compare the data of *Rhinopoma microphyllum kinneari* with the data reported by Trivedi et al. (2003) the percentage of neutrophils of female *M. myotis* (26.00) is comparable with *R. m. kinneari* female (26.48). The average of eosinophils in *M. capaccinii* (1.50) is similar to *Phyllostomus discolor* lactating female (1.50) and *Artibeus lituratus* adult female (1.50). The percentage of basophil of female *M. capaccinii* (0.50) is comparable with *Phyllostomus discolor* adult female (0.50). For *R. euryale* females, the data on lympocytes (63.33) matches with *Lonchophylla robusta* gravid female (63.00). Average lymhocytes (58.94) in *M. schreibersii* female are similar to *Carollia perspicillata* lactating female (59.00). The average percentage of monocyte in female bats of this study is not similar to any of the bats studied. In males only average percentage of lymphocytes of *R. mehelyi* (56.58) and *R. ferrumequinum* (56.50) is comparable with *Artibeus lituratus* subadult male (56.90). Average neutrophils, eosinophils, basophils and monocytes of males in this study are not similar to any of the bats studied.

From these data it is evident that some parameters that belongs to the bat species in our study are similar to Riedesel (1977)'s results. Since breeding cycles of all bat species is highly diverse (Anand Kumar, 1965; Lall, 1986; Trivedi and Lall, 1989; Trivedi, 1991; Singh and Krishna, 2000; Badwaik and Rasweiler, 2001), it is possible that these parameters vary or seem similar with some reproductive stage in bats. Bats hibernate in deep torpor and give birth in summer when insect availability is highest (e.g. Racey and Swift, 1985; Rydell, 1992; Hickey and Fenton, 1996). In most temperate zones, vespertilionid bats mate in late summer and early autumn when sperm production reaches a peak and females are in oestrus (Racey and Tam, 1974; Encarnaçao et al., 2004).

Neutrophils and monocytes are part of the innate immune system, while lymphocytes are involved in adaptive immunity and recognition of parasites and pathogens. A high neutrophil count is seen in infections, some cancers, arthritis and sometimes during times when the body is under stress. A high eosinophil count often indicates allergies, skin diseases, or parasitic infections. Hence, variation in different WBC types may reflect different aspects of disease risk.

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. In the current study, most of the leukocytes were lymphocytes, while in human neutrophils are the predominant leukocyte. The higher percentage of lymphocytes could be result from stress and infection or this situation could be a normal phenomenon. Average values of eosinophils and basophils are closer to human white blood corpuscles. Low number of neutrophils may be a normal phenomenon in all bat species as compared to neutrophils found in human. However, a comparison of blood profile in other seasons would confirm this. If it were not a normal phenomenon, it would imply that there bat colonies are affected in a manner where their neutrophil population is not sufficient to combat bacterial and viral infections. The average percentage of monocytes is high in all species. A high monocyte count usually indicates an infection, but this may 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 (Pehlivanoğlu et al., 2001; Silberman et al., 2002). If study of blood profile is carried out throughout the annual cycle in these bats, the results will be more useful. Study of blood profile in different seasons may help in understanding whether the data observed in the present study are normal phenomenon. Comparison should be undertaken in order to understand the significance of alterations on 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 stres which may be due to either natura or man-made causes.

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