

Investigation of blood cells of *Miniopterus schreibersii* (Mammalia: Chiroptera) in Turkey

Tuğba SARIÇAM^{1*}, İrfan ALBAYRAK²

¹Graduate School of Natural Applied Sciences, Kırıkkale University, Yahşihan, Kırıkkale, Turkey

²Department of Biology, Faculty of Science and Arts, Kırıkkale University, Yahşihan, Kırıkkale, Turkey

Received: 06.02.2019 • Accepted/Published Online: 27.08.2019 • Final Version: 01.11.2019

Abstract: This research was based on some characteristics of the erythrocyte and leukocyte cells of 9 *Miniopterus schreibersii* (♀♀) samples collected from Balıkesir Province in Turkey between July 2015 and September 2016. Bats were caught from an artificial cave using special nets. Blood was taken in heparinized hematocrit tubes from the forearm or tail membrane in the laboratory and the blood smears were prepared. The erythrocyte and leukocyte counts, hemoglobin concentration, and hematocrit values in the blood were determined. In *M. schreibersii*, the number of erythrocytes in 1 mm³ of blood was between 7.5 and 10 million, and the mean value was 8.8 million. The diameter values of the erythrocyte, monocyte, lymphocyte, eosinophil, neutrophil, and basophil cells were measured as 5.65, 9.66, 8.4, 8.66, 10, and 8.5 µm, respectively. Blood values of *M. schreibersii* were recorded for the first time in this study in Anatolia.

Key words: Hematology, blood values, *Miniopterus schreibersii*, Turkey

Worldwide, 22 species belonging to the genus *Miniopterus* have been recorded (Wilson and Reeder, 2005). Of these species, *Miniopterus schreibersii* is one of the 38 bat species living in Turkey (Albayrak, 2018). Many hematological studies have been made concerning vertebrate species but hematological studies related to bat species are limited (Jürgens et al., 1981; Başoğlu and Öktem, 1984; Bassett and Wiederhielm, 1984; Tanyer, 1985; Arevalo et al., 1987; Wolk and Bogdanowicz, 1987; Wolk and Ruprecht, 1988; Agar and Godwin, 1992; Ratnasooriya et al., 2005; Rodriguez et al., 2008; Schinnerl et al., 2011).

There are only 2 articles about bat hematology in Turkey. The first article was related to the white blood cells of *Rhinolophus ferrumequinum*, *R. hipposideros*, *R. mehelyi*, *R. euryale*, *M. capaccinii*, *Myotis myotis*, and *Miniopterus schreibersii* from Turkish Thrace (Paksuz et al., 2009). In the second study, some hematological values were recorded for sympatric species, such as *Myotis myotis* (greater mouse-eared bat) and *Myotis blythii* (lesser mouse-eared bat), belonging to the family Vespertilionidae in Turkey (Albayrak et al., 2016).

The purpose of this study was to determine the hematological values of *M. schreibersii* (long-winged bat), which is found commonly and has a large population in Turkey.

This research was based on the determination of erythrocyte and leukocyte characteristics of the blood cells of 9 *M. schreibersii* (♀♀) from Balıkesir Province during field studies conducted between July 2015 and September 2016. All of the specimens were from a young age group (Albayrak, 1985). In the laboratory, blood samples were taken from the veins of the upper arm or tail membrane of the bats. After blood collection, the animals were released back into their natural habitat within 2 days. The project was conducted with the approval of the Kırıkkale University Animal Ethics Committee (HADYEK), number 16/73.

The blood samples were obtained with heparinized hematocrit capillary tubes (Wimsatt et al., 2005). Hayem for the erythrocytes and Turkish solution for the leukocytes were used as the reconstitution solutions. The disseminated blood preparations to be used in the measurement and calculation of the blood cells (erythrocytes, leukocytes) were stained with Wright's dye (Başoğlu and Öktem, 1984). Counting of blood cells (erythrocytes and leukocytes) was performed with a Neubauer hemocytometer and measurement was done using Olympus 1–15× micrometric oxides.

The erythrocyte size and dimensions ($NU \times NG \times \pi/4$) were calculated according to the $EU \times EG \times \pi/4$ formula, by taking measurements of 40 randomly selected

* Correspondence: iralbayrak@yahoo.com

erythrocytes in each blood preparation (erythrocyte length - EU, erythrocyte width - EG, nucleus length - NU, and nucleus width - NG). The nucleus size was also calculated. Micrometric measurements were also made of the leukocytes. Blood cells were photographed with an Olympus CX31 microscope at 40× magnification. For the hematological analysis, samples were centrifuged at 6000 rpm for 4 min for hematocrit determination and the erythrocytes were precipitated with centrifugation of the hematocrit capillary blood. Values were read and recorded as percentages using millimetric paper. The hematocrit value was determined as the total volume fraction of the erythrocyte volume. The Sahli method was used for hemoglobin determination (Tanyer, 1985). Results of the mean cell volume (MCV), mean cell hemoglobin (MCH), and MCH concentration (MCHC) were calculated mathematically (Tanyer, 1985). The data of the specimens were compared with those in the literature and evaluated.

M. schreibersii is commonly distributed in Turkey (Schreiber's bat), in Havran artificial cave in Balıkesir, and *Myotis myotis* and *M. blythii* together constitute a summer colony of about 5000 individuals.

Mean values of blood cells of the *M. schreibersii* population were calculated and the erythrocyte cell diameter was 5.68 µm (5.45–593 µm). The diameters of the lymphocytes, monocytes, eosinophils, neutrophils, and basophils from the leukocyte cells were 8.95, 8.34, 8.78, 9.63, and 8.66 µm, respectively. The number of erythrocytes was between 7.5 and 10 million, with an average value of 8.8 million. The leukocyte count was in the range of 5000–9600 for all samples, with a mean value

of 7488. The hemoglobin value was between 11 and 15.60 g/dL for all samples, with an average value of 13.64 g/dL. The hematocrit value was between 41% and 58% for all of the samples, and the mean value was 47.75%. The MCV was between 43.88 and 64.44 fL for all of the samples, with an average value of 54.82 fL. The MCH was between 12.6 and 18.7 pg for all samples, with an average value of 15.56 pg. The MCHC was 24.83%–33.49% for all samples, with an average of 28.75% (Table 1).

In the specimens examined for *M. schreibersii*, the erythrocytes had an oval or round shape in the blood smear preparations stained with Wright's dye. The cytoplasm of the erythrocytes was observed as pink (Figure 1). The cytoplasm of the monocytes formed a large area. Some of the nuclei covered half of the cell in a structure that had collapsed inwards. The cytoplasm was observed as pale blue and the nucleus was observed as dark purple in the blood smear preparations dyed with Wright's dye (Figure 1).

There were very fine chromatin strands in the neutrophil cytoplasm. In the blood smear preparations stained with Wright's dye, the cytoplasm was observed as light blue and the nucleus was observed as dark blue. The nucleus had a lobed structure (Figure 2). The cytoplasm in the lymphocytes formed a narrow area. The nucleus was round and had a rather large structure, and it almost filled all of the cells. The cytoplasm of the lymphocytes was observed as light blue and the nucleus was observed as dark blue, due to the concentration of chromatin (Figure 2).

Table 1. Complementary statistics for the blood cell values and clinical hematology data of the *M. schreibersii* samples (N: number of specimens, min-max: minimum-maximum values, M: mean, SD: standard deviation).

Characters	Sex	N	Min-max	M	SD
Erythrocyte diameter (µm)	♀♀	9	5.45–5.93	5.68	0.15
Lymphocyte diameter (µm)	♀♀	9	8.25–10.00	8.95	0.60
Monocyte diameter (µm)	♀♀	9	5.80–10.00	8.34	1.36
Eosinophil diameter (µm)	♀♀	9	7.33–10.25	8.78	1.02
Neutrophil diameter (µm)	♀♀	9	9.33–10.00	9.63	0.24
Basophil diameter (µm)	♀♀	9	8.00–9.67	8.66	0.53
Number of erythrocytes	♀♀	9	7,500,000	8,805,555	-
Number of leukocytes	♀♀	9	5000–9600	7488.88	-
Hb (g/dL)	♀♀	9	11.00–15.60	13.64	1.44
HCT (%)	♀♀	8	41.00–58.00	47.75	5.20
OEH (fL)	♀♀	8	43.88–64.44	54.82	7.81
OEHb (pg)	♀♀	9	12.57–18.75	15.56	1.85
OEHbK (%)	♀♀	8	24.83–33.49	28.75	3.08

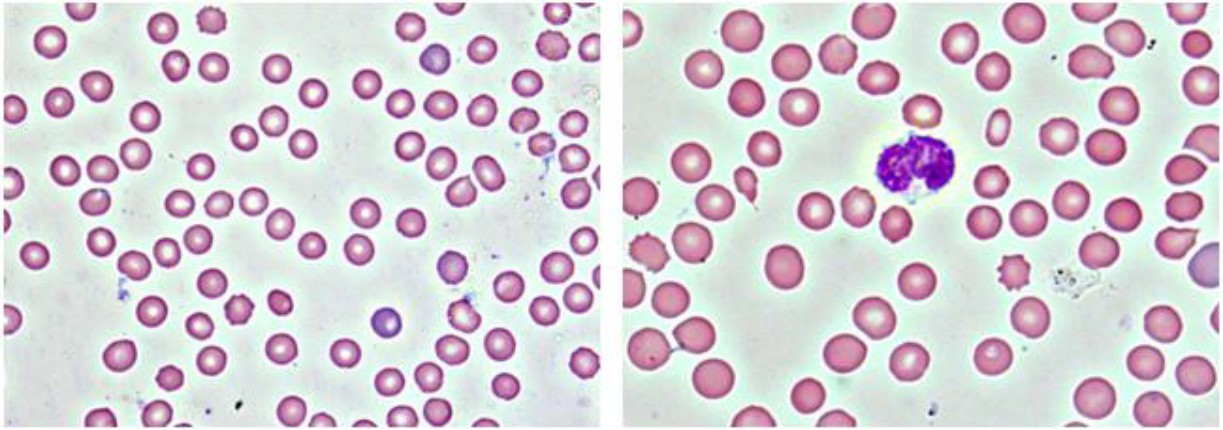


Figure 1. Erythrocyte (left) and monocyte (right) cells of *M. schreibersii*.

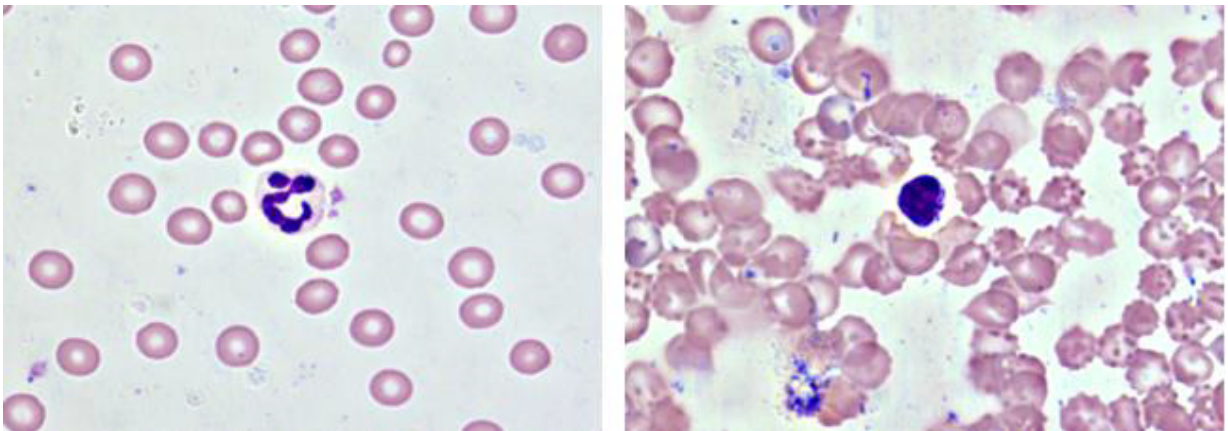


Figure 2. Neutrophil (left) and lymphocyte (right) cells of *M. schreibersii*.

The cytoplasm of the eosinophils was observed as light blue and the nucleus was observed as dark blue. The large, round, and red granules in the cytoplasm were a distinctive feature of these cells. The nucleus was observed as 2-lobed (Figure 3). Due to the dense granules in the cytoplasm of the basophils, the nucleus was not easily visible. The granules were observed as dark purple in the blood smear preparations (Figure 3).

In this study, the blood parameters of 9 (♀♀) individuals of *M. schreibersii* were evaluated. The findings obtained were compared with the blood cell morphology and clinically important hematological parameters of different bat species from previous studies (Table 2).

It can be seen from Table 2 that the number of erythrocytes of *M. schreibersii* differed distinctly from that of *Pipistrellus pipistrellus* in France (Jürgens et al., 1981) and was similar to that of *Myotis myotis* in Spain (Arveola, 1987). In terms of hemoglobin values, *M.*

schreibersii in Turkey was close to *Myotis myotis*, but distinct from *Pipistrellus pipistrellus* in France (Jürgens et al., 1981). Hematocrit values of the samples of this study were close to those of *Myotis daubentonii* in Poland (Wolk and Bogdanowicz, 1987) and *M. schreibersii* in Australia (Agar and Godwin, 1992), but distinct from those of *Myotis myotis* in Spain (Arevalo et al., 1987) and *Lasiurus borealis* (Rodriguez-Duran and Padilla-Rodriguez, 2008) in Antilles. In terms of the mean erythrocyte volume, it was close to *M. schreibersii* in Sri Lanka (Ratnasooriya et al., 2005), but distinct from *Eptesicus serotinus* in Poland (Wolk and Ruprecht, 1988). With regards to the mean erythrocyte hemoglobin, *M. schreibersii* in Turkey was close to *Myotis nattererii* in Spain (Arevalo et al., 1987), but distinct from *Pipistrellus pipistrellus* in France (Jürgens et al., 1981).

In terms of the number of erythrocytes, *M. schreibersii* in Turkey was close to that in Sri Lanka (Ratnasooriya et

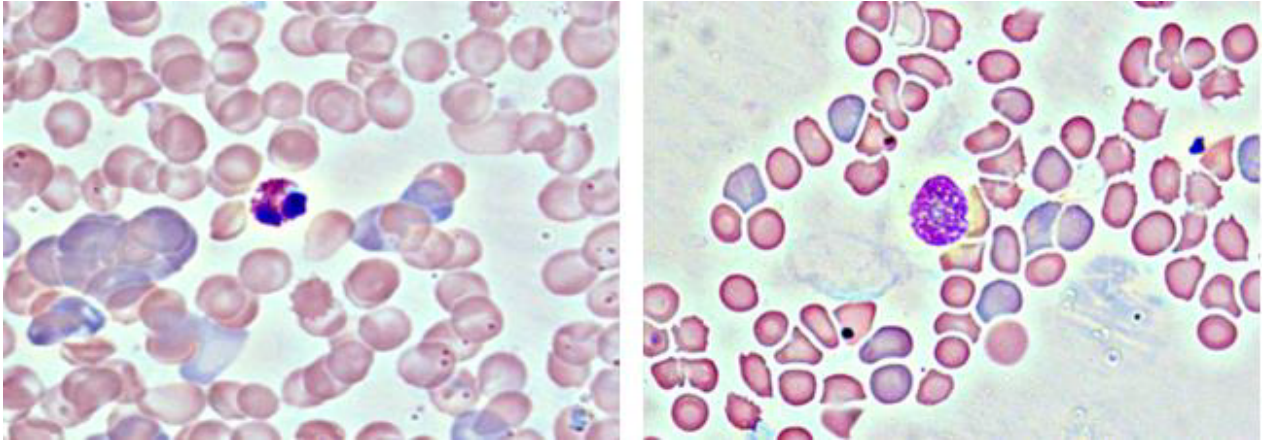


Figure 3. Cells of eosinophil (left) and basophil (right) cells of *M. schreibersii*.

Table 2. Hematological values of some bats belonging to the family Vespertilionidae according to various researchers (Hb: hemoglobin, Htc: hematocrit, MCV: mean cell volume, MCH: mean cell hemoglobin, MCHC: MCH concentration).

Source	Species	Number of erythrocytes ($\times 10^6/\text{mm}^3$)	Number of leukocytes (1 m^3)	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (%)
Jürgens et al. (1981), France	<i>Myotis myotis</i>	11.00	3300	18.40	51	46.40	16.70	36.00
	<i>Pipistrellus pipistrellus</i>	26.20	-	24.40	61.5	23.50	9.30	39.70
Basset and Wiederhielm (1984), USA	<i>Antrozous pallidus</i>	12.08	-	17.70	60.5	50.58	14.83	29.28
Wolk and Bogdanowicz (1987), Poland	<i>Myotis daubentonii</i>	10.55	5345	15.40	48.9	47.75	15.10	31.50
Arevalo et al. (1987), Spain	<i>Pipistrellus pipistrellus</i>	14.23	-	20.05	57.83	39.55	13.80	34.27
	<i>Miniopterus schreibersii</i>	12.93	-	16.44	55.06	42.86	13.04	30.70
	<i>Plecotus austriacus</i>	14.92	-	14.54	59.00	39.56	10.13	25.56
	<i>Myotis nattereri</i>	12.59	-	20.06	59.78	47.74	15.82	33.60
	<i>Myotis myotis</i>	9.31	-	15.80	43.30	46.79	17.08	36.73
Wolk and Ruprecht (1988), Poland	<i>Eptesicus serotinus</i>	14.90	-	20.10	56.20	38.00	13.60	35.70
Agar and Godwin (1992), Australia	<i>Miniopterus schreibersii</i>	10.90	-	19.40	48.40	44.40	17.80	40.60
Ratnasooriya et al. (2005), Sri Lanka	<i>Miniopterus schreibersii</i>	10.1	14346	17.60	53.00	54.60	-	33.20
Rodriguez-Duran and Padilla-Rodriguez (2008), Antilles	<i>Lasiurus borealis</i>	-	-	-	64.00	-	-	-
Schinnerl et al. (2011), Costa Rica	<i>Myotis elegans</i>	-	2045	-	56.50	-	-	-
	<i>Myotis nigricans</i>	-	5133	-	49.75	-	-	-
Albayrak et al. (2016), Turkey	<i>Myotis myotis</i>	11.35	4865	14.90	57.00	52.40	13.70	23.30
	<i>Myotis blythii</i>	14.52	4296	13.09	60.20	43.67	9.70	21.70
This study	<i>Miniopterus schreibersii</i>	8.80	7488	13.64	47.75	54.82	15.56	28.75

al., 2005), but distinct from that in Spain (Arevalo et al., 1987). In comparison to the number of leukocytes, the specimens here in Turkey had a lower value than that in the study carried out only in Sri Lanka (Ratnasooriya et al., 2005). In terms of hemoglobin values, *M. schreibersii* was close to that in Spain (Arevalo et al., 1987), but distinct from that in Australia (Agar and Godwin, 1992). With regards to the hematocrit values, the specimens here were close to those in Australia (Agar and Godwin, 1992), but distinct from those in Spain (Arevalo et al., 1987). With respect to the mean erythrocyte volume, *M. schreibersii* was close to that in Sri Lanka (Ratnasooriya et al., 2005), but distinct from that in Spain (Arevalo et al., 1987). When compared with the mean erythrocyte hemoglobin concentration, *M. schreibersii* was close to that in Spain (Arevalo et al., 1987), but distinct from *M. schreibersii* in Australia (Agar and Godwin, 1992). In terms of the mean erythrocyte hemoglobin concentration, *M. schreibersii* was close to that in Spain (Arevalo et al., 1987), but distinct from that in Australia (Agar and Godwin, 1992).

M. schreibersii in Turkey was close to *Antrozous pallidus* in North America (Bassett and Wiederhielm,

1984), but distinct from *M. schreibersii* in Australia (Agar and Godwin, 1992), with respect to the mean erythrocyte hemoglobin concentration. When our data were compared with the data of Albayrak et al. (2016), *M. schreibersii* was closer to *Myotis myotis* than to *Myotis blythii*.

As a result, hematologic values are likely to be influenced by the variations of the bats and the techniques of many different researchers. For this reason, the hematological values of *M. schreibersii* populations can vary due to some ecological parameters. The exact detection of hematological differences in populations of this species in different regions in Turkey will be possible with detailed research in the future. With these results, some measurements of the erythrocyte and leukocyte cells of young specimens of *M. schreibersii* were recorded for the first time.

Acknowledgments

We wish to thank Prof. Dr. M. Tosunoğlu for technical assistance during the laboratory studies. This study was a part of the MSc thesis of Tuğba Sariçam.

References

- Agar NS, Godwin IR (1992). Erythrocyte metabolism in two species of bats: Common bent-wing bat (*Miniopterus schreibersii*) and red fruit bat (*Pteropus scapulatus*). *Comparative Biochemistry and Physiology* 101B (1/2): 9-12.
- Albayrak İ (1985). Researches on bats of Ankara Province (Mammalia: Chiroptera). *Faculté des Sciences de L'Université d'Ankara, Série C: Biologie* 3: 1-20.
- Albayrak İ (2018). Some biological and acoustic features of *Pipistrellus pipistrellus*, pygmy bat (Mammalia: Chiroptera) in Turkey. In: *International Ecology 2018 Symposium Abstract Book*; Kastamonu, Turkey; 19–23 June 2018. p. 455.
- Albayrak İ, Özcan BH, Baydemir M (2016). Some hematological parameters in *Myotis myotis* and *Myotis blythii* (Mammalia: Chiroptera) in Turkey. *Turkish Journal of Zoology* 40: 388-391.
- Arevalo F, Perez-Suarez G, Lopez-Luna P (1987). Hematological data and hemoglobin component in bats (Vespertilionidae). *Comparative Biochemistry and Physiology* 88A (3): 447-450.
- Başoğlu M, Öktem N (1984). *Zoofizyoloji Praktikum*. İzmir, Turkey: Ege Üniversitesi Fen Fakültesi Kitaplar Serisi (in Turkish).
- Bassett JE, Wiederhielm CA (1984). Postnatal changes in haematology of the bat *Antrozous pallidus*. *Comparative Biochemistry and Physiology* 78A (4): 737-742.
- Jurgens KD, Bartels H, Bartels H (1981). Blood oxygen transport and organ weights of small bats and small non-flying mammals. *Respiration Physiology* 45: 243-260.
- Paksuz S, Paksuz EP, Özkan EP, (2009). Beyaz kan hücrelerinin (WBC) farklıyara (Chiroptera) türlerinin sayısı. *Trakya Üniversitesi Fen Bilimleri Dergisi*, 10: 55-59 (in Turkish).
- Ratnasooriya WD, Udagama-Randeniya PV, Yapa WB, Digana PMCB, Dharmasiri MG (2005). Hematological parameters of three species of wild caught microchiropteran bats, *Miniopterus schreibersii*, *Taphozous melanopogon* and *Hipposideros lanka diva* in Sri Lanka. *Journal of Science University of Kelaniya* 2: 27-40.
- Rodriguez-Duran A, Padilla-Rodriguez E (2008). Blood characteristics, heart mass and wing morphology of Antillean bats. *Caribbean Journal of Science* 44 (3): 375-379.
- Schinnerl M, Aydınonat D, Schwarzenberger F, Voigt CC (2011). Hematological survey of common neotropical bat species from Costa Rica. *Journal of Zoo and Wildlife Medicine* 42 (3): 382-391.
- Tanyer G (1985). *Hematoloji ve Laboratuvar*. Ankara, Turkey: Ayyıldız Matbaası A.Ş. (in Turkish).
- Wilson DE, Reeder DM (2005). *Mammal Species of the World. A Taxonomic and Geographic Reference*. 3rd ed. Baltimore, USA: Johns Hopkins University Press.
- Wolk E, Bogdanowicz W (1987). Haematology of the hibernating bat: *Myotis daubentonii*. *Comparative Biochemistry and Physiology* 88A (4): 637-639.
- Wolk E, Ruprecht AL (1988). Haematological values in the serotine bat, *Eptesicus serotinus* (Schreber, 1774). *Acta Theriologica* 33: 545-553.