International Journal of Zoological Research 1 (1): 11-14, 2005
ISSN 1811-9778

Hematological Values in Hermann's Tortoise (*Testudo hermanni*) and Spur-thigted Tortoise (*Testudo greaca*) from Thrace Region (Turkey)

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Abstract: Fifteen adult *Testudo* specimens (*T. hermanni* and *T. graeca*) of Thrace Region (Turkey) were examined for 10 hematological parameters; Red Blood Cell count (RBC), hematocrit value (PCV), Mean Corpuscular Volume (MCV), Hemoglobin Concentration (HbC), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), blood cell (erythrocyte-leukocyte-thrombocyte) measurements and leukocyte formules. Of the investigation species, the widest and largest sized erythrocytes belong to *T. hermanni,* while the smallest sized erythrocytes is found *T. graeca*. Erythrocytes count and hemoglobin of *T. graeca* are found in more than *T. hermanni*. These hematological values were compared to those obtained from other Chelonia species.

Key words: *T. hermanni, T. graeca*, chelonia, hematology

INTRODUCTION

Today, hematology no doubt plays a large part in the diagnosis of disease in amphibians and reptiles. It is clear that unless more basic work is done to establish normal hematological ranges, this future will always remain distant. While some workers have attempted to show changes in some parameters due to specific disease, others have concentrated more on establishing base lines for these values. The majority of the references on the hematology of different reptiles are on blood cell counts and on cell sizes[1-7]. There is little available literature about clinical hematology for reptiles[8-10]. In Turkey, hematological studies have generally been conducted on some amphibians and reptiles. There is only one hematological study of the tortoise living in Turkey. But this study blood cell morphology and sizes in some turtle and tortoise were only given[6].

T. graeca and *T. hermanni*, have long been known in Turkey. The most populous and widely distributed species of Turkish land tortoise is *T. graeca*, which occurs from west to east, throughout Turkey with the exception of the eastern Black Sea coast. *T. hermanni* is limited to the European part of Turkey, the Thrace region, where it occurs sympatrically with *T. graeca*[11].

In this study, we analyze the hematological parameters of two *Testudo* species (*T. graeca* and *T. hermanni*) native to Turkey in an attempt to establish their normal reference intervals.

MATERIALS AND METHODS

The 15 adult specimens (5♀♀; 10♂♂) collected were brought alive to the laboratory. Blood sample of the live specimens were obtained in the laboratory within three days of their capture. The necessary blood samples were taken from the cardiac puncture[12] via syringe.

Blood samples for morphological characterisations of blood cells were obtained without any anticoagulant. The Red Blood Cell counts (RBC) were done utilizing a Neubauer hemocytometer, as diluting solutions, for erythrocytes the standard Hayem's solution was used.

The blood from each tortoise was placed into a heparinised tube and used to determine the hematological parameters. Hematocrit value (PCV) was determined by the micro-hematokrit method[13]. The tubes were then spun in a micro-hematocrit centrifuge for 5 min at 5 000 rev/min and the packet cell volume calculated with a hematocrit reader. Hemoglobin Concentration (HbC) was measured by the Sahli method[13]. The Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated mathematically from the results.

Blood smears stained with Wright's stain were used in measurements of the blood cells (erythrocytes, leukocytes and thrombocytes). Blood smears were prepared immediately and air-dried. Each cell type was measured and characterised. Cell measurements were done under a microscope with a Olympus ocular

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micrometer. On each blood smear 40 erythrocytes were randomly chosen; cell lengths and widths, together with the lengths and widths of their nuclei were measured in micrometers, then cell and nuclei sizes were computed with the following the formulas: $EL \cdot EW \cdot \pi/4$ and $NL \cdot NW \cdot \pi/4$, respectively. Leukocytes and thrombocytes were also measured in micrometers. Cell photomicrographs were taken with a Soif photomicroscope.

Data analysis: The results were analyzed by parametric descriptive statistics analysis and expressed as mean±SD. Comparison between groups was performed by one-way ANOVA test.

RESULTS AND DISCUSSION

The Red Blood Cells (RBC) of *T. graeca* and *T. hermanni* are nucleated, oval cells and their nuclei are also oval and centrally located like those of the other reptile species (Fig. 1). Since no significant differences were seen in the counts and sizes of the blood cells from males and females, data from both sexes were pooled.

Hematological parameters of species *T. graeca* and *T. hermanni* are shown in Table 1 and 2.

No comparison was possible concerning the thrombocyte and leucocyte counts, since we could not obtain reliable counts due to some technical difficulties.

The main purpose of this study was to accumulate information for comparison with existing and future results. Reptiles are heterogenous group of vertebrates with regard to their blood cell morphology. Hematological and biochemical measurements may very depending on factors such as gender, age, pregnancy, physical exercise, weather, stress, altitude, captivity[2,8,10,15-17]. In reptiles, erythrocyte sizes and counts very greatly.

We have established that there are some discernible differences between the investigated Testudo species from Thrace Region from the viewpoint of their hematological values.

Of the investigation species, the widest and the largest sized erythrocytes belong to *T. hermanni*, while the smallest sized erythrocytes is found in *T. graeca*. Erythrocyte count and hemoglobin of *T. graeca* is found in more than *T. hermanni*.

Hematocrit, or Packed Corpuscule Value (PCV), is the volume proportion of erythrocytes in whole blood. Because it is both easily measured and potentially informative about the condition and physiological of an tortoise. Seasonal variation in hematocrit occurs in tortoise.

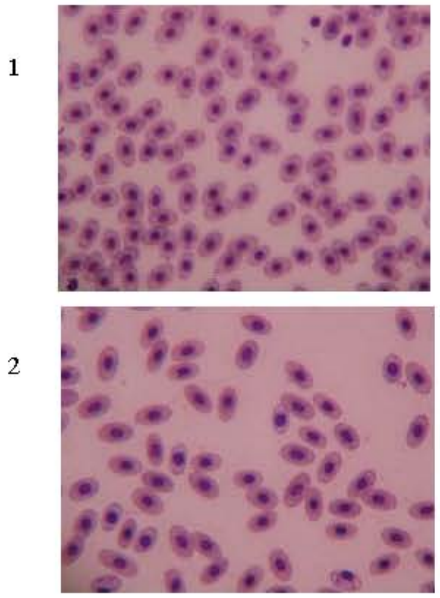

Fig. 1: The blood cells of *Testudo graeca* (1) and *Testudo hermanni* (2)

Table 1: Hematological data of the *T. graeca* and *T. hermanni*. N: Number of specimens, Ext: Extreme values, SD: Standard Deviations

	Testudo graeca (N: 5)			*Testudo hermanni* (N: 8)		
Hemogram determination	Ext.	Means	SD	Ext.	Mean	SD
RBC count (10^9 L^{-1})	536.0-946.0	758.40	147.00	493.0-750.0	649.10	89.50
Hemoglobin (g dL^{-1})	5.2-9.7	7.22	1.66	4.90-7.40	6.13	1.04
Hemotokrit (%)	24.0-36.0	28.80	4.43	21-34	27.87	5.08
MCV (gL)	360.0-447.7	383.80	36.59	328.57-607.14	435.22	95.02
MCH (pg)	80.0-100.0	94.05	7.99	71.42-125.00	95.40	16.70
MCHC (%)	21.66-27.14	24.61	2.58	18.84-29.04	22.21	3.01
Lymphocytes (%)	35.0-44.0	41.20	3.56	42-50	44.37	3.06
Monocytes (%)	20.0-28.0	23.60	3.50	20-28	23.87	2.23
Basophils (%)	5.0-20.0	11.20	5.44	8-15	11.12	2.29
Eosinophilis (%)	11.0-28.0	19.60	6.34	17.23	19.00	2.00

Table 2: The established measurements and sizes concerning the blood cells of *T. græca* and *T. hermanni* (μm). N: Number of specimens, n: Number of measurements/computings in each specimen, Ext: Extreme values, SD: Standard Deviations

	Testudo græca (N: 7)				*Testudo hermanni* (N: 8)		
Blood cells	n	Ext	Means	SD	Ext	Means	SD
Erythrocyte Length (EL)	40	16.26-19.31	17.93	0.96	17.43-19.06	18.11	0.61
Erythrocyte Width (EW)	40	9.25-10.62	9.86	0.42	10.06-10.84	10.46	0.26
Erythrocyte Size (ES)	40	118.19-161.53	139.38	13.48	139.2-159.4	149.06	6.98
Nucleus Length (NL)	40	5.39-6.00	5.67	0.25	5.28-7.12	5.72	0.59
Nucleus Width (NW)	40	2.92-3.87	3.44	0.34	3.53-4.87	3.88	0.41
Nucleus Size (NS)	40	12.53-18.32	15.54	2.02	14.89-27.47	17.73	4.07
EL/EW	40	1.77-1.87	1.81	3.237E	1.64-1.82	1.73	6.34
NL/NW	40	1.50-1.87	1.64	0.13	1.43-1.54	1.48	3.81
ES/NS	40	8.01-11.38	9.38	1.24	5.80-10.75	8.97	1.52
Lymphocyte (Large) diameter	20	10.50-12.87	11.28	0.85	10.37-12.62	11.06	0.68
Lymphocyte (Small) diameter	20	7.37-8.12	7.80	0.24	7.37-8.50	7.87	0.37
Monocyte diameter	20	11.56-15.00	13.37	1.17	12.37-13.75	12.95	0.55
Eosinophil diameter	20	10.62-13.56	12.17	0.96	11.75-14.37	12.69	0.99
Basophil diameter	10	8.25-10.00	9.10	0.63	8.12-11.50	8.84	1.10
Thrombocyte Length (TL)	20	7.37-9.87	8.92	0.79	7.62-10.56	9.65	0.89
Thrombocyte Width (TW)	20	4.00-5.25	4.81	0.51	4.65-5.81	5.25	0.39

Table 3: Erythrocyte counts, hemoglobin and hematocrit values of some *Testudo* species, according to several authors

Authors	Species	RBC (count)	Hemoglobin (g dL^{-1})	Hematocrit (%)
Alder and Huber[14]	*Emys orbicularis*	260.000-680.000	-	-
Hutchison and Szarski[15]	*Cleemys guttata*	475.000-750.000	-	-
Duguy[2]	*Testudo græca*	362.000-730.000	-	-
Wang[8]	*Chrysemys picta*	-	4.22	22.9
Dessauer[17]	*Emys orbicularis*	-	6.60	24.0
Dessauer[17]	*Emys orbicularis*	-	6.60	30.0

Hematological values of some *Testudo* species measured in this study (Table 3) which were similar (in both limits and central tendencies) to the published values for the species[8,15,18].

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