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Seasonal Variation of Immunohematological Parameters of the Peripheral Blood in the Pond Bat *Myotis dasycneme* (Boie, 1825) of the Urals

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Abstract—Immunohematological parameters were for the first time studied in the pond bat *Myotis dasycneme* (Boie, 1825), the most common chiropteran species in the Urals, during seasonal periods of the annual life cycle. Multivariate nonparametric analysis of variance showed the absence of significant gender differences in red blood cell parameters ($p = 0.35$). Gender differences were observed in the counts of white blood cells, band neutrophils, segments neutrophils, and lymphocytes in the blood ($p < 0.05$). Males showed a higher development of innate immunity in summer compared with females ($p < 0.05$). A high lymphocyte level (50.6–53.5%) was observed in both males and females in the autumn–winter hibernation period, providing immune surveillance and specific reactive activation of the acquired adaptive immune response.

Keywords: bats, leukocytes, innate immunity, adaptive immunity

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INTRODUCTION

This work continues our cycle of studies on the integrating role that the bat blood system plays in the formation of the adaptive strategy that ensures functional stability of natural populations in the Urals [1–3]. Species specificity has been observed in the immune system and its protective and censoring functions in regulating the physiological processes in bats of the Urals [2]. The objective of this work was to comparatively study the seasonal variation in immunohematological parameters of the peripheral blood in the pond bat *Myotis dasycneme* (Boie, 1825).

MATERIALS AND METHODS

Myotis dasycneme (Boie, 1825) bats were captured in the Southern and Central Urals from 2013 to 2015. Collection sites included both sites where bats live in large numbers and reproduce in summer and sites where bat groups winter. Bats were captured with mist nets or collected manually from walls of their winter refugia in the region of the Maloe Miassovo Lake in Chelyabinsk Oblast (55°10′04″ N, 60°21′08″ E) and within and near the Smolinskaya Cove in Sverdlovsk Oblast (56°25′44″ N, 61°36′44″ E). Adult bats without signs of disease from natural populations were examined in the following seasonal periods of the life cycle: summer reproduction of the population (the second decade of July), autumn mass migration to wintering sites (the third decade of September), winter torpor (the third decade of February), and spring end of hypobiosis (the first decade of April). Daily average air temperatures in pond bat habitats ranged from 3 to 8°C in April, from 21 to 23°C in July, and from 5 to 7°C in autumn. In February, daily average air temperatures ranged from –16 to –20°C outside the cave and from 0 to 2°C within the cave with an extremely high humidity [4]. Bats ($n = 51$) were captured and kept in the laboratory in compliance with guidelines on the protection of animals used for experimental and scientific purposes [5]. To standardize the conditions, all bats were kept in a moderately cold container for one day; each bat was let to choose a roosting place, and no further locomotor activity was observed. BD Vacutainer sterile vacuum tubes with EDTA (United Kingdom) were used to collect the blood. Peripheral blood samples (400–800 μl) were tested using a BC–5800 hematology analyzer (Mindray, China) to mea-

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sure 17 parameters. The results were analyzed using the software package Statistica for Windows v. 10.0. Principal component analysis was carried out in the R statistical environment (R 3.1.2, the Ade4 package) [6].

RESULTS AND DISCUSSION

Red blood cell parameters of the peripheral blood showed no significant gender difference in bats by multidimensional nonparametric analysis of variance ($p = 0.35$), certainly reflecting that the adaptive response to maintaining homeostasis is universal in males and females. Substantial changes in functional activity of the blood system were observed in association with the seasonal periods of the life cycle in males and females ($p = 0.001$). All red blood cell parameters showed low variation in the periods of preparation to wintering and winter hibernation (autumn, winter, and spring) as compared with summer values. No significant difference between seasonal periods of the annual life cycle ($p > 0.05$) was observed for hemoglobin concentration (Hb, 167.9–187.2 g/L, $p = 0.08$) and related parameters: hematocrit (HCT, 47.2–51.5%, $p = 0.1$), mean corpuscular hemoglobin (MCH, 15.9–17.6 pg, $p = 0.31$), and mean corpuscular hemoglobin concentration (MCHC, 343.8–380.3 g/L, $p = 0.07$). High levels of hemoglobin, hematocrit, and red blood cells (RBC, 9.6–11.5 T/L, $p = 0.01$) were characteristic of the bat blood, pointing to a high intensity of the respiratory function in flying animals [7]. A summer decrease in mean corpuscular volume (MCV) increases the rate of oxygen absorption by hemoglobin and thus improves gas exchange in autumn (by 5% in comparison, $p = 0.05$), winter, and spring (by 9%, $p = 0.01$). In autumn, winter, and spring, the circulating platelet count (PLT = 256.2–271.3 G/L, $p = 0.001$) and plateletcrit (PCT = 0.16–0.17%, $p = 0.0002$) were twice as high as in active summer bats. Platelets are involved in immune and allergic responses and provide the first line of defense against seasonal pathogens at stably low daily average air temperatures in the region (from 3 to 8°C) and in the cave (from 0 to 2°C) [8, 9]. Certain fluctuations in particular seasons were observed for peripheral white blood cells (Table 1), which are responsible for both innate immunity and acquired adaptive immune responses [10].

Lymphocytes form a basis of humoral immunity, restrict the spreading of infections, perform the immune surveillance function, and are responsible for both nonspecific and specific immunity [11, 12]. The absolute lymphocyte count in females was significantly higher than in males in all seasons; the factors were 2.0 in summer, 2.3 in autumn, 1.7 in winter, and 1.8 in spring ($p < 0.05$) (Table 1). The count of monocytes, which produce proinflammatory cytokines, in females was significantly higher than in males by a factor of 4.3 in summer and a factor of 2.5 during deep winter hibernation ($p < 0.05$). The monocyte content increased during winter hibernation compared with summer in males by 166% and in females by 100% ($p < 0.05$), illustrating modulation of the immune system to maintaining the adaptive potential towards the pathogenic antigen spectrum characteristic of their low-temperature environment [13, 14].

Eosinophils showed a significant gender difference between males and females in spring (by a factor of 6, $p < 0.05$) and slight seasonal fluctuations during the annual life cycle ($p = 0.12$). Activation of eosinophilic granulopoiesis in the bat blood possibly indicates that antitoxin and antimicrobial reactions occur in the body as hypobiosis ends and bats leave their wintering caves. Basophilic granulocytes were not detected in the bat blood (Table 1). These cells are indicative of delayed-type inflammatory and allergic reactions. Granulocytic white blood cells (stab and segmented neutrophils) showed significant gender and seasonal differences during preparation to hypobiosis in autumn, winter torpor, and waking up from hibernation in spring ($p < 0.05$).

As for the lymphocyte–granulocyte composition of the blood, agranulocytes (54.1%) predominated in females and granulocytes (53.6%), in males in summer ($p < 0.05$). A higher content (50.6–53.6%) of agranulocytes, which ensure immune surveillance and specific reactivity (adaptive immunity), was observed in both males and females during hibernation in autumn and winter, providing a representative indicator of higher seasonal hypoxic pressure exerted by low positive and near-zero temperatures. No gender difference was observed in granulocyte content in the peripheral blood (47.1–49.4%, $p = 0.35$). Waking up from deep hypothermia in spring was accompanied by significant reactivity of the innate immunity system in both males and females (granulocytes, 53.2–54.2%, respectively; $p = 0.35$); this ensured nonspecific immediate defense, in particular, against virus invasion before specific defense mechanisms are developed by adaptive immunity [15].

Principal component analysis (PCA) was used to visualize the gender and seasonal specifics of the differential blood count in the pond bat; the results confirmed the above statistical data (Fig. 1). The first principal component accounted for 47.42% of the total seasonal variation in white blood cell parameters, and major contributions to the value were made by lymphocytes (28.71%), stabs (26.54%), and segmented neutrophils (24.91%). High coefficients of correlation with PC1 were observed for these parameters: 0.90, –0.87, and –0.84, respectively ($p < 0.001$). According to the parameter contributions, spatial differentiation of the bats into two seasonal groups, summer + winter and autumn + spring, was observed by PC1. Differentiation was especially distinct in females.

Seasonal bat groups were observed by PC2, which strongly correlated with juvenile neutrophils (0.80) and eosinophils (–0.60). PC2 accounted for 18.39% of the total variance in white blood cell parameters;

Table 1. White blood cell composition of the peripheral blood in the pond bat

Parameter	Gender	I. Summer n: ♂ (5)/♀ (5)	II. Autumn n: ♂ (7)/♀ (8)	III. Winter n: ♂ (9)/♀ (7)	IV. Spring n: ♂ (5)/♀ (5)	$\frac{F_{obs}}{p©}$
		$\bar{X}_{boot} \pm SE_{boot}$ [95% CI_{boot}]				
White blood cells, G/L	♂	1.64 ± 0.08 [1.49–1.81]	2.33 ± 0.26 [1.89–2.90]	1.20 ± 0.16▲ [0.88–1.51]	2.05 ± 0.27¶ [1.71–2.65]	$\frac{4.21}{0.01}$
	♀	2.84 ± 0.07@ [2.70–2.96]	4.69 ± 0.20*@ [4.34–5.11]	2.20 ± 0.14*▲@ [1.92–2.47]	3.69 ± 0.31*▲¶@ [3.11–4.29]	
Neutrophils, G/L	♂	0.87 ± 0.04 [0.79–0.96]	1.13 ± 0.14 [0.89–1.44]	0.56 ± 0.08▲ [0.40–0.71]	1.06 ± 0.10¶ [0.91–1.29]	$\frac{3.57}{0.02}$
	♀	1.34 ± 0.11@ [1.14–1.55]	2.23 ± 0.11*@ [2.02–2.45]	1.00 ± 0.08▲@ [0.84–1.14]	1.94 ± 0.19*¶@ [1.58–2.31]	
Juvenile, G/L	♂	0.14 ± 0.05 [0.05–0.24]	0.06 ± 0.02 [0.04–0.10]	0.02 ± 0.004* [0.01–0.03]	0.11 ± 0.01¶ [0.09–0.14]	$\frac{2.93}{0.04}$
	♀	0.07 ± 0.01 [0.05–0.10]	0.09 ± 0.01 [0.07–0.10]	0.08 ± 0.01@ [0.05–0.10]	0.14 ± 0.03 [0.09–0.20]	
Stab, G/L	♂	0.40 ± 0.05 [0.31–0.52]	0.46 ± 0.09 [0.32–0.65]	0.18 ± 0.03*▲ [0.12–0.24]	0.42 ± 0.04¶ [0.37–0.50]	$\frac{3.24}{0.03}$
	♀	0.93 ± 0.07@ [0.80–1.07]	0.92 ± 0.08@ [0.78–1.08]	0.32 ± 0.03*▲@ [0.27–0.37]	0.85 ± 0.10¶@ [0.66–1.05]	
Segmented, G/L	♂	0.32 ± 0.06 [0.19–0.43]	0.61 ± 0.08* [0.45–0.76]	0.36 ± 0.05▲ [0.26–0.45]	0.53 ± 0.06 [0.43–0.66]	$\frac{5.65}{0.004}$
	♀	0.34 ± 0.12 [0.11–0.59]	1.21 ± 0.03*@ [1.14–1.28]	0.60 ± 0.05▲@ [0.49–0.70]	0.95 ± 0.08*▲¶@ [0.81–1.11]	
Lymphocytes, G/L	♂	0.74 ± 0.06 [0.64–0.87]	1.07 ± 0.10 [0.88–1.29]	0.55 ± 0.07▲ [0.41–0.68]	0.79 ± 0.14 [0.60–1.10]	$\frac{8.60}{0.0003}$
	♀	1.40 ± 0.05@ [1.31–1.49]	2.43 ± 0.13*@ [2.21–2.71]	0.96 ± 0.08*▲@ [0.83–1.12]	1.39 ± 0.11▲¶@ [1.19–1.61]	
Monocytes, G/L	♂	0.03 ± 0.01 [0.002–0.06]	0.11 ± 0.03 [0.05–0.17]	0.08 ± 0.02 [0.05–0.11]	0.18 ± 0.04*¶ [0.12–0.26]	$\frac{3.31}{0.03}$
	♀	0.13 ± 0.02@ [0.08–0.17]	0.09 ± 0.02 [0.06–0.12]	0.20 ± 0.03▲@ [0.16–0.26]	0.29 ± 0.04*▲ [0.22–0.37]	
Eosinophils, G/L	♂	0.01 ± 0.005 [0.002–0.02]	0.02 ± 0.01 [0.01–0.04]	0.02 ± 0.01 [0.01–0.03]	0.01 ± 0.007 [0.00–0.03]	$\frac{2.08}{0.12}$
	♀	0.03 ± 0.01 [0.02–0.05]	0.04 ± 0.01 [0.02–0.05]	0.03 ± 0.01 [0.02–0.05]	0.06 ± 0.01@ [0.05–0.08]	

Seasonal differences between seasons (*) I and II, I and III, or I and IV; (▲) II and III or II and IV; and (¶) III and IV were significant at ($p < 0.05$); (@) gender differences were significant at $p < 0.05$. $\bar{X}_{boot} \pm SE_{boot}$, arithmetic mean and error of the mean of the bootstrap distribution; [95% CI_{boot}], confidence interval of the bootstrap distribution; © – $p = Pr(|Fran| \geq Fobs)$, two-factor analysis of variance with a permutation test (randomization).

significant contributions to PC2 were made by juvenile neutrophils (57.51%) and eosinophils (32.27%), which showed sufficiently high coefficients of correlation with PC2. The white blood cell parameters that correlated with PC1 accounted for more than 40% of the total variance, while those that correlated with PC2 accounted for approximately 20% of the total variance. The variation in blood parameters was

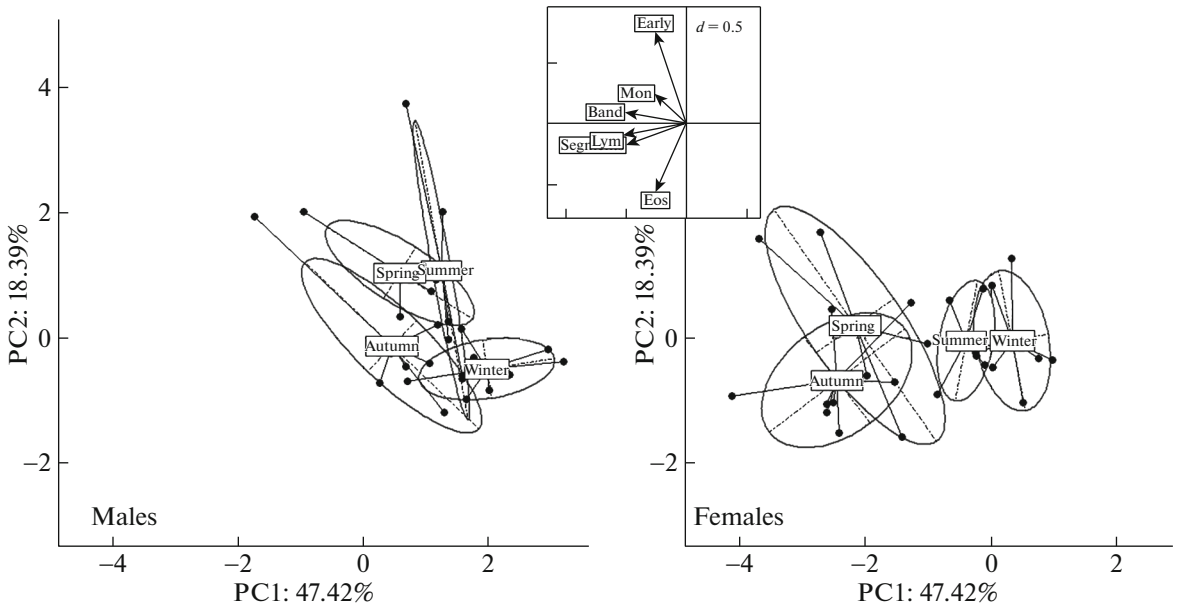

Fig. 1. Differential blood count parameters in *Myotis dasycneme* males and females in various seasons (summer, autumn, winter, and spring) in the space of the first two principal components. Designations: PC1 and PC2, axes of the principal components; %, portion of the variance that is explained by the principal component; arrows reflect the correlation of the principal components with initial variables (white blood cells); 95% confidence areas are shown with ellipses; early, band, segments, Lym—lymphocytes; Mon—monocytes; Eos—eosinophils.

caused by changes in the physiological state of bats and extreme conditions associated with their seasonal life cycle. Along with common regularities observed in homeostasis-maintaining mechanisms, certain specifics were detected in mobilization of mechanisms involved in the acute regulation of the lymphoid blood system in male and female bats.

To summarize, we for the first time report the seasonal variation in immunohematological parameters of the peripheral blood in the pond bat *M. dasycneme* (Boie, 1825) of the Urals. The reference immunohematological parameter values obtained in this work supplement and help to systematize the available data on the mechanisms that the bat blood system utilizes to adapt to seasonal environmental stress. Our findings may be used for long-term monitoring aimed to preserve the bat populations.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflicts of interest.

Statement on the welfare of animals. Bats were captured and kept in the laboratory in compliance with international guidelines of the Declaration of Helsinki on the protection of animals used for experimental and other scientific purposes [5].

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