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Characteristic Immunohematological Parameters of Migratory (*Vespertilio murinus* Linnaeus, 1758) and Resident (*Myotis dasycneme* Boie, 1825) Bat Species of the Ural Fauna

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Abstract—The results of the first comparative analysis of hematological parameters of bats from natural populations of the Ural region are presented: the pond bat (*Myotis dasycneme* Boie, 1825), which is a resident species, and the two-colored bat (*Vespertilio murinus* Linnaeus, 1758), which is a migratory species. Significant differences in the content of white blood cells ($p = 0.05$), red blood cells and platelets ($p = 0.001$) were shown. Interspecific differences were found in the leukograms of the studied bats both in the content of granulocytes ($p = 0.04$) and agranulocytes ($p = 0.05$). Migrating *V. murinus* are characterized by a significant contribution of the nonspecific protective system of the blood (54.9%) to the adaptive reactions of the body. In the pond bat, a pronounced lymphocytic profile (58.5%) indicates the activation of acquired adaptive immunity. Two-colored bats in comparison with pond bats are characterized by a higher level of innate immunity.

Keywords: bats, peripheral blood, granulocytes, lymphocytes

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The study of the adaptation mechanisms in bats is of particular interest due to their unique biological characteristics: adaptation to active flight, seasonal migrations, and hibernation, which are accompanied by physiological hypothermia and torpor state. The bat fauna in the Ural region has been studied quite fully [1]. However, despite significant factual material on their biology and ecology, the basic information on the functional capabilities of the blood system of insectivorous bats that provide the adaptive strategy of resident and migratory species is clearly limited [2–4].

This article deals with the problems of the formation of the adaptive strategy of bats in maintaining homeostasis of animals under the influence of permanently changing environmental factors. Comparative analysis of immunohematological parameters of the two species of ecologically contrasting insectivorous bats from natural populations of the Ural region (the pond bat and two-colored bat) is carried out for the first time.

Animals were caught by mist nets in the areas of mass habitation of summer colonies in the Chelyabinsk oblast (55°00′55″ N, 60°09′30″ E) in July 2014. The bat species that are numerous and widespread in the Ural region were taken as objects of research: the two-colored bat *Vespertilio murinus* Linnaeus, 1758 (mesophilic migratory species) was caught on the shores of Lake Bolshoi Kisegach, and the pond bat *Myotis dasycneme* Boie, 1825 (boreal resident and wintering species) was caught in the vicinity of Lake Maloe Miassovo. The pond bat mostly hibernates in caves from the second half of September to early May at temperatures ranging from 0 to + 2°C under extremely high humidity conditions. The two-colored bat arrives to the Southern Urals in May and migrates in the second half of August several thousand kilometers southwestward to the wintering grounds [1]. The animals without signs of disease were delivered to the laboratory in separate containers on the day of capture. Experimental groups ($n = 9$) are represented by underyearlings (*subadultus*). The bats were handled in accordance with the international principles of the Declaration of Helsinki [5].

Plasma was obtained by centrifugation of blood in a K-23D refrigerated ultracentrifuge (Germany) in Bekton Dickinson BP vacutainers (United Kingdom) with EDTA for 15 min at 3000 rpm. Peripheral blood parameters were determined using a BC-5800 hematology analyzer (Mindray, China). The differential leukocyte count was calculated in blood smears stained according to Romanovsky–Giemsa (for 100 leukocytes). Seventeen parameters of peripheral blood were studied in the experimental animals. The results were processed using the Statistica v. 10.0 software package. Principal component analysis (PCA) was imple-

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Table 1. Parameters of the peripheral blood in bats. Arithmetic mean ($X_{M\,boot}$), mean error (SE_{boot}) and confidence interval ([95% CI_{boot}]) of the bootstrap distribution

Indicators	Pond bat	Two-colored bat	*p*
Red blood cells (RBC), T/L	5.7 ± 0.4 [5.0–6.3]	9.2 ± 0.6* [8.1–10.6]	0.001
White blood cells (WBC), G/L	3.3 ± 0.5 [2.4–4.4]	5.9 ± 0.8* [4.1–7.6]	0.05
Hemoglobin (HGB), g/L	101.5 ± 5.6 [89.8–110.5]	127.1 ± 8.1 [111.8–142.8]	0.08
Hematocrit index (HCT), %	29.9 ± 1.9 [25.6–32.9]	33.3 ± 1.5 [30.6–36.5]	0.29
Mean erythrocyte volume (MCV), fl	52.3 ± 0.5 [51.6–53.4]	36.8 ± 1.1* [34.7–39.1]	0.001
Mean content of hemoglobin in erythrocyte (MCH), pg	17.8 ± 0.2 [17.4–18.2]	13.9 ± 0.4* [13.2–14.8]	0.001
Mean concentration of hemoglobin in erythrocyte (MCHC), g/L	340.9 ± 4.7 [333.0–350.3]	379.5 ± 7.3* [365.0–394.2]	0.001
Platelets (PLT), g/L	213.1 ± 28.0 [160.5–265.5]	476.3 ± 26.1* [421.2–522.0]	0.001
Mean platelet volume (MPV), fl	6.6 ± 0.1 [6.4–6.9]	5.2 ± 0.2* [4.8–5.5]	0.001
Thrombokrit (PCT), %	0.1 ± 0.0 [0.1–0.2]	0.3 ± 0.0* [0.2–0.3]	0.02

* Statistically significant differences between the groups, Permutation ANOVA, $p \leq 0.05$.

mented using the R statistical environment (R 3.1.2, the Vegan and Ade4 packages) [6].

The study of peripheral blood showed that the content of red blood cells in the two-colored bat was 1.6 times higher than in the pond bat (p = 0.001) (Table 1), which may have been related to the effective oxygen regime during prolonged flights at an increased mean concentration of hemoglobin in the erythrocyte. This is also favored by a decrease in the mean blood volume of red blood cells by a factor of 1.4 (p = 0.001) as well as a twofold increase in the number of platelets (p = 0.001) and thrombocrit (p = 0.02) in the peripheral blood of the migratory species in comparison with the blood parameters of the pond bat.

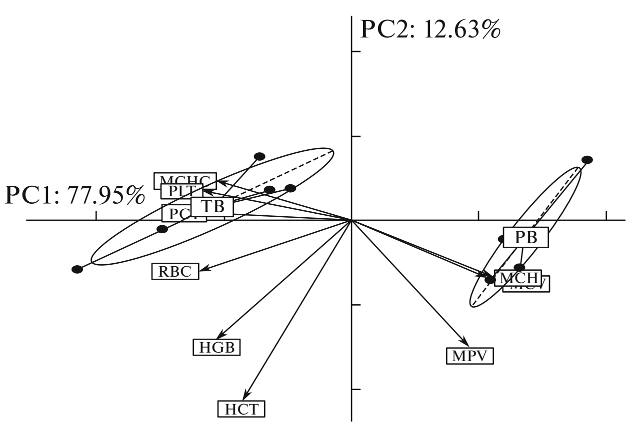

Fig. 1. Indicators of red blood of the two bat species in the space of the first two principal components. PC1, PC2 are principal component axes, % is the percentage of data variance accounted for by the principal component; arrows show the correlation of the principal components with the initial indicators; ellipses represent 95% confidence regions; PB is the pond bat, TB is the two-colored bat.

Researchers consider the activation of thrombocytopoiesis during physical exertion, exposure to low temperatures and hypoxia, exposure to anthropogenic, biotic stress factors as effectors of the immune system [7]. Participating in immune and allergic reactions along with macrophages, neutrophils, and eosinophils, platelets form the body's first line of defense against pathogens. The content of white blood cells in the two-colored bat is 1.8 times higher than in the pond bat (p = 0.05) (Table 1). White blood cells maintain homeostasis by forming blood and tissue barriers against microbial, viral and parasitic infections.

The principal component analysis (PCA) made it possible to visualize the species variability of the peripheral blood indices in the two-colored bat and pond bat (Fig. 1). The analysis showed that the first principal component (PC1) accounted for 77.95% of the total variance of red blood and platelet parameters and the second component (PC2) accounted for 12.63%. The greatest contribution to the interspecific variability of red blood indices for PC2 is made by red blood cells (13.49%), mean erythrocyte volume (13.01%), platelets (12.99%), thrombocrit (12.17%), mean hemoglobin content (11.88%), and their significant coefficients of correlation with PC1 are –0.97, –0.96, –0.95, –0.92, and 0.91, respectively (p = 0.001).

The first principal component for these variables clearly distinguishes resident (PB) and migratory (TB) species into independent groups (Fig. 1). The second principal component PC2 is strongly correlated (–0.70) with the hematocrit index (the contribution of 43.20%) and to a lesser extent with hemoglobin (the contribution of 18.86%), which also confirms the heterogeneity of individuals of the studied two ecologically contrasting species as regards these parameters.

The leukocyte composition of the bat blood is represented by granulocytes (neutrophils: immature,

Table 2. Leukocyte composition of the peripheral blood of bats. Arithmetic mean ($X_{M\,boot}$), mean error (SE_{boot}) and confidence interval ([95% CI_{boot}]) of the bootstrap distribution

Indicators	Pond bat	Two-colored bat	*p*
Neutrophils, %	39.8 ± 1.4 [37.0–42.5]	54.0 ± 3.3* [47.2–60.2]	0.01
• immature,%	2.5 ± 1.1 [0.5–4.8]	8.0 ± 1.3* [5.2–10.4]	0.05
• stab, %	26.0 ± 3.2 [18.8–30.0]	24.6 ± 3.0 [18.8–30.6]	0.77
• segmental, %	11.2 ± 2.9 [6.0–16.8]	21.4 ± 2.5* [17.6–27.0]	0.05
Lymphocytes, %	56.0 ± 1.8 [52.3–59.0]	43.4 ± 3.1* [37.6–49.2]	0.04
Monocytes, %	2.5 ± 0.8 [0.8–3.8]	1.6 ± 0.8 [0.4–3.4]	0.38
Eosinophils, %	1.3 ± 0.4 [0.5–2.0]	1.0 ± 0.3 [0.4–1.6]	0.39
Granulocytes, %	41.0 ± 1.8 [37.5–44.5]	54.9 ± 3.4* [48.0–61.4]	0.04
Agranulocytes, %	58.5 ± 2.1 [54.5–62.5]	44.9±3.5* [38.6–52.0]	0.05

* Statistically significant differences between the groups, Permutation ANOVA, $p \leq 0.05$.

stab, segmental neutrophils, and eosinophils) and agranulocytes (monocytes and lymphocytes) (Table 2).

Lymphocytes as the basis of humoral immunity limit the spread of infections by participating in an adequate immunological response of the body [8]. The resident species *M. dasycneme* is characterized by a high content of lymphocytes (56.0%), which provides a significant efficiency of cellular immunity (p = 0.04). Indicative is the active nonspecific defense of the body against toxic effects, viral and bacterial infections in the migratory species two-colored bat, which is provided by an increased level of neutrophils (p = 0.01) (Table 2).

No interspecific differences in the leukograms of the studied animals both in the content of monocytes (p = 0.38) and in the content of eosinophils (p = 0.39) were revealed, which indicates the identity of the adaptive mechanisms of the studied widespread bat species of the Ural fauna. It is known that monocytes, performing a phagocytic role, produce endogenous regulators of the immune response — proinflammatory cytokines [7, 9]. Eosinophils that actively circulate in the blood of the pond bat and two-colored bat provide the body's antihelminthic immune defense. It should be noted that peripheral blood leukocyte cells in bats, as in all vertebrates, are responsible for the manifestation of innate (natural) immunity and adaptive (acquired) immune responses [8, 9].

Significant interspecific differences in bats in the content of granulocytes and agranulocytes that reflect the interrelation of the effector mechanisms of the immune system of the animals were shown. The lymphocyte-granulocyte composition of the peripheral blood of the pond bat is dominated by agranulocytes (58.5%) that provide immune surveillance and specific reactivity of the body (adaptive immunity). In the migratory two-colored bat, granulocytes (54.9%) predominate, forming a nonspecific line of defense in case of activation of natural innate immunity. It is this innate immune defense system that responds faster and more efficiently to a wide range of biotic and abiotic environmental factors in comparison with adaptive acquired responses. Migratory two-colored bats are characterized by a higher level of innate immunity in comparison with the pond bat.

Thus, the results of our studies have allowed the first assessment of the hematological parameters of the two-colored bat and the pond bat, illustrating the specificity of the effector mechanisms of the immune system of the animals, which, in turn, are determined by the ecological and physiological characteristics of the resident and migratory bat species from natural populations of the Ural region.

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COMPLIANCE WITH ETHICAL STANDARDS

The capture and keeping of animals in the laboratory were carried out in accordance with the international principles of the Declaration of Helsinki.

CONFLICT OF INTEREST

The authors declare they have no conflicts of interest.

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