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## Article

# Leukocytes of the blood of rodents from the “Kologrivsky Forest” Nature Reserve and Kostroma Forestry Site

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**Abstract.** The ecological, physiological and species-specific characteristics of the leukocyte profile of two species of cyclomorphic rodents, the bank vole *Myodes glareolus* (Schreber, 1780) and the pygmy wood mouse *Apodemus uralensis* (Pallas, 1811), were studied for the first time in the adjacent territories of the Kologriv and Kostroma districts of the Kostroma Oblast. It was established that the leukocyte profiles of the studied rodents are different in different species and vary significantly among individuals inhabiting the Kologrivsky Forest Reserve and the Kostroma Forestry. Multivariate analysis of variance showed that the white blood cell differential parameters largely correlate with the following factors: “location of capture,” “taxonomic species,” “gender,” “stage of the population cycle,” and “year of study.” The relatively high degree of deviation of the splenic index in the studied species indicates the heterogeneity of populations for this trait. At the same time, no reliable association was found between the risk of developing splenomegaly in mouse-like rodents, and the characteristics of their reproductive strategy and susceptibility to invasions.

**Keywords:** micromammals, bank vole, pygmy wood mouse, hematological parameters, variability, stress, spleen, splenomegaly, Kostroma Oblast

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*Научная статья*

## **Лейкоциты крови грызунов на территории заповедника «Кологривский лес» и Костромского лесничества**

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**Аннотация.** Впервые изучены эколого-физиологические и видовые особенности системы лейкоцитов крови двух видов цикломорфных грызунов – рыжей полевки *Myodes glareolus* Schreber, 1780 и малой лесной мыши *Apodemus uralensis* Pallas, 1811 на сопредельных территориях Кологривского и Костромского районов Костромской области. Установлено, что лейкоформулы исследуемых грызунов видоспецифичны и значительно отличаются у особей, обитающих на территории заповедника «Кологривский лес» и на территории Костромского лесничества. Многофакторный дисперсионный анализ выявил существенное влияние на лейкоформулу всех изученных факторов: «локация отлова», «видовая принадлежность», «пол», «стадия популяционного цикла» и «год исследования». Относительно высокая степень девиации индекса селезенки у исследуемых видов свидетельствует о неоднородности популяций по данному признаку. При этом достоверной сопряженности риска развития спленомегалии у мышевидных грызунов с особенностями их репродуктивной стратегии и восприимчивости к инвазиям не обнаружено.

**Ключевые слова:** микромаммалии, рыжая полевка, малая лесная мышь, гематологические показатели, изменчивость, стресс, селезенка, спленомегалия, Костромская область

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## Introduction

In recent years, blood parameters are increasingly used when studying the state of small mammal populations and assessing the influence of various factors (Emkuzheva et al., 2021; Moiseeva, 2016; Sabanova, 2008, 2010; Tarakhtii and Davydova, 2007; Tkachenko and Derkho, 2014). However, in contrast to laboratory studies, mammals in natural conditions are affected by many factors, which makes it difficult to interpret the results (Tarakhtii et al., 2007). There are attempts to characterize response in the blood systems to changes in the population cycle (Christian, Creel et al., 2012; 1961; Blondel et al., 2016; Boonstra and Boag, 1992). Hematological parameters to a certain extent reflect the physiological state of the body. They can be used to assess the degree of environmental impact and compile an ecological profile of both individuals and populations as a whole (Kozinets et al., 2007).

The immune system of the blood is an essential component of the complex mechanism of homeostasis and the most sensitive indicator of adverse effects (Moiseeva, 2000). Changes in the abiotic and biotic environment of organisms affect physiological processes, which changes characteristics of the circulating blood, enabling these indicators to be used to determine the functional state of the entire organism. In addition, the immune status of mouse-like rodents is an important environmental factor for parasitic organisms, in particular for helminths. Accelerated sexual maturation of the year's brood and the maximum involvement of females in reproduction leads to an increase in population size, while the mechanisms of induced immunity become suppressed, facilitating latent infections (Lazutkin, 2019; Lochmiller and Moshkin, 1999).

Thus, the result of assessing hematological parameters, can characterize the body's response to changing environments, and also provide information about these environments (Lokhmiller and Moshkin, 1999; Tarakhtii et al., 2007).

As leukocyte profile determines not only the immunological state, but also shows the effect of long-term stress (Davis et al., 2008), it can be used to monitor and predict the state of mammalian populations.

The method of morphophysiological indicators is used to assess the physiological state of small mammals (Ivanter et al., 1985; Schwartz et al., 1968). The spleen plays an important role in the regulation of the leukocyte profile being responsible for hematopoiesis, the formation of immunity, serving as a depot of hematopoietic elements and is involved in stress reactions (Salikhova, 2015). Pathological processes of this organ are usually accompanied by compensatory variability in the blood and immune systems (Bokov, 2015; Olenev et al., 2014).

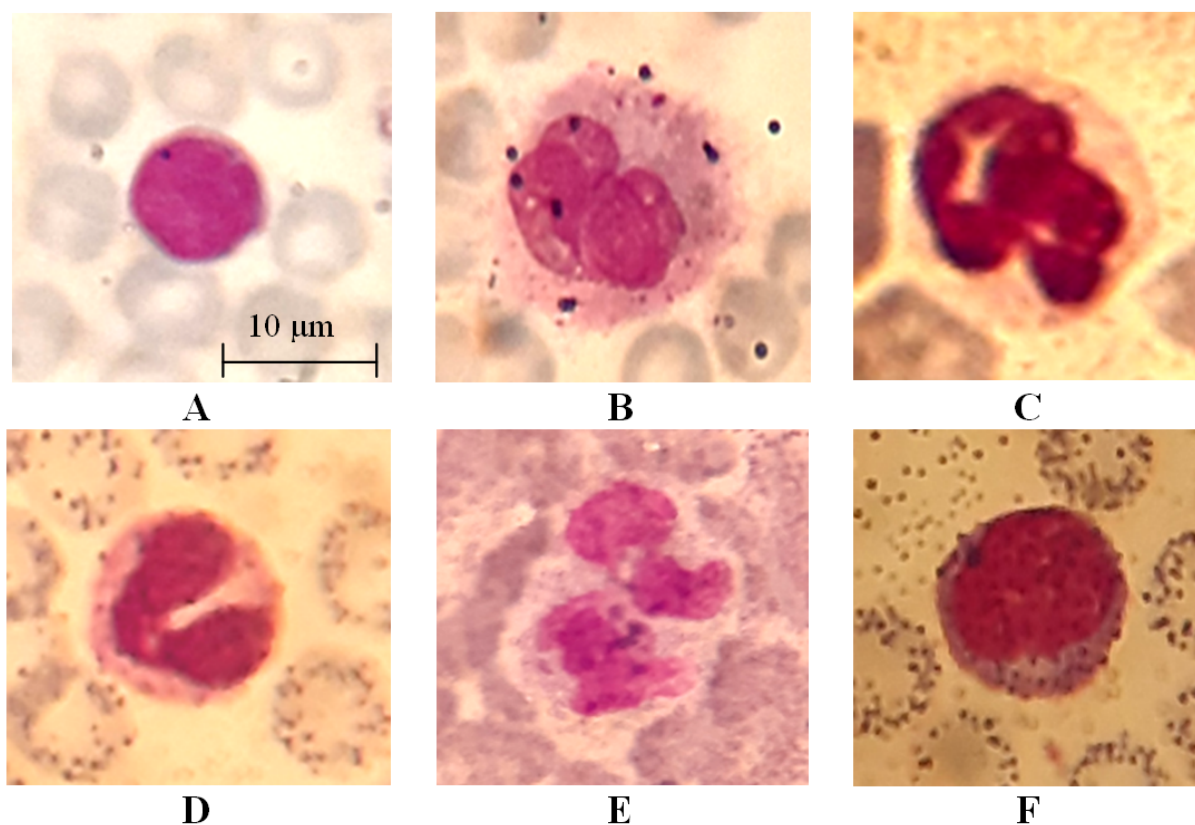
The populations of micromammals in the adjacent territories of the Kologriv and Kostroma districts of the Kostroma Oblast do not differ in dominance. The dominant species in both cases is the bank vole *Myodes glareolus* (Schreber, 1780) (79.41% of the population of mouse-like rodents living in the Kologrivsky Forest and 74.17% of the population of mouse-like rodents of the Kostroma Forestry in 2021–2023); the codominant is the pygmy wood mouse *Apodemus uralensis* (Pallas, 1811) (20.49% in the Kologrivsky Forest and 15% in the Kostroma Forestry in the same period).

The population dynamics of the bank vole living in the adjacent territories of the Kostroma Oblast are characterized by three-year cycles associated with endogenous regulatory factors. Changes in the abundance of the pygmy wood mouse over a long-term period are non-cyclical and primarily depend on environmental conditions. Moreover, the phases, periods and fluctuations of the cycles of the population dynamics of these rodents are separated in time (Klimova and Sirotnina, 2022). The purpose of this work is to use hematological indicators to assess the state of populations of the dominant species of mouse-like rodents living in protected and anthropogenically transformed areas of the Kostroma Oblast.

## Materials and methods

These studies are part of a comprehensive monitoring of the population organization of background species of cyclomorphic rodents on the territory of the Kologrivsky Forest Biosphere Reserve (Kostroma Oblast, Kologriv District), which is a southern taiga spruce forest not affected by economic activity, mainly of dark coniferous forest formations, and is characterized by the absence of anthropogenic pressure, as well as on the territory of the Kostroma Forestry of the Minskoe Farm (Kostroma Oblast, Kostroma District), which is a mixed forest adjacent to agricultural fields, household plots and the Kostroma-Krasnoe-on-Volga highway (Klimova and Sirotnina, 2022).

Hematological studies of 260 rodents were performed in the summer period 2021–2023. The micromammals were captured using live-traps, placed based on the trap-line method (Bobretsov, 2021; Sheftel, 2018; Tolkachev, 2019).



**Fig. 1.** Microphotographs of different types of leukocytes in the blood of a bank vole: **A** – lymphocytes; **B** – monocytes; **C** – segmented neutrophils; **D** – band neutrophils; **E** – eosinophils; **F** – basophils.

Blood was collected from research subjects by cardiac puncture after mild ether anesthesia (Amirov et al., 2020; Diehl et al., 2001; Sorokina et al., 2019). Vacuum tubes containing an anticoagulant (EDTA) were used to preserve blood samples for later analysis.

All manipulations with small mammals followed the International Recommendations (Code of Ethics) for Biomedical Research Using Animals (1985)<sup>1</sup>, as well as ethical standards approved by legal acts of the Russian Federation<sup>2, 3</sup>, and the international principles of the Basel Declaration “A call for more trust, transparency and communication on animal research” (2010)<sup>4</sup>.

The body weight of rodents (in grams) was determined by weighing them on a Scout SPU laboratory scale (Ohaus, Switzerland). To establish the specifics of the reproductive strategy and assign individuals to functional-physiological groups (FPGs), the age of rodents was determined by the degree of wear of the alveolar surface of the teeth and the tooth index and the type of ontogeny by the degree of sexual maturity of the individuals (Karaseva et al., 2008; Olenev, 2009). The mass of the internal organs of rodents was determined by weighing them on a SIERRA CX-298 electronic scale (measurement accuracy 0.01 g), after which the internal organ indices were calculated as the ratio of organ mass to body weight (Olenev and Grigorkina, 2019; Schwartz et al., 1968). Helminthological studies of the lungs, liver, stomach and intestines of rodents were performed in 2023 using the standard method of helminthological autopsy following Scriabin’s protocol. The identification of helminths was determined

<sup>1</sup> International Guiding Principles for Biomedical Research Involving Animals, 1985.

<sup>2</sup> Federal Law of December 27, 2018 No. 498-FZ “On the responsible treatment of animals and on amendments to certain legislative acts of the Russian Federation”.

<sup>3</sup> Recommendation of the EEC Board dated November 14, 2023 No. 33 “On the Guidelines for working with laboratory (experimental) animals when conducting preclinical (non-clinical) studies”.

<sup>4</sup> Basel Declaration. A call for more trust, transparency and communication on animal research, 2010.

by morphological characteristics using taxonomic keys (Anikanova et al., 2007; Ryzhikov et al., 1979). To characterize the invasion of cyclomorphic rodents, standard parasitological indicators were used: the extent of invasion, intensity of invasion and abundance index (Anikanova et al., 2007; Romashov et al., 2003).

Blood smears were prepared according to standard methods (Menshikov et al., 1987). For each individual, blood preparations were prepared in at least three replicates. To assess hematological parameters in rodents, the leukocytes were enumerated, leukocyte profile was determined, and leukocyte blood indices were calculated. Blood cells were identified using atlases of blood cells of farm and laboratory animals (Reagan et al., 2000; Simonyan and Khisamutdinov, 1995; Theml et al., 2004). Microphotographs of various types of leukocytes in the blood of bank voles are presented in Fig. 1. The leukocytes were counted in a Goryaev chamber, the leukocyte profile was determined from blood smears stained according to Pappenheim with May-Grunwald fixative dye and Romanovsky dye (MiniMed, Russia), using a Biomed-3 light microscope. To enumerate the leukocytes on blood smears, the types of leukocytes were visually determined by counting 200 cells. The obtained data were expressed as percentages and absolute values (Abrashova et al., 2013; Amirov et al., 2020; Kirillovsky and Tochilina, 2014; Poloziuk and Ushakova, 2019). The absolute number of different types of leukocytes was calculated based on their total number and percentage.

For statistical processing of the data, we used Microsoft Office Excel 2007 and Statistica 10 software package (Usmanov, 2020). Descriptive statistics included the arithmetic mean, error of the arithmetic mean, and coefficient of variation. The degree of reliability of intergroup differences was determined by Student's t-test; results were considered statistically significant at  $p < 0.05$  (Bavrina, 2021). For estimating the parameters, the Pearson parametric coefficient ( $r_p$ ) or the Spearman rank correlation ( $r_s$ ) were used. The method of multivariate analysis of variance (MANOVA) was used to determine the strength and significance of the influence of the location of the capture, the species, the gender of the rodent, and the stage of the population cycle on the blood parameters analyzed (Korosov and Gorbach, 2017).

## Results

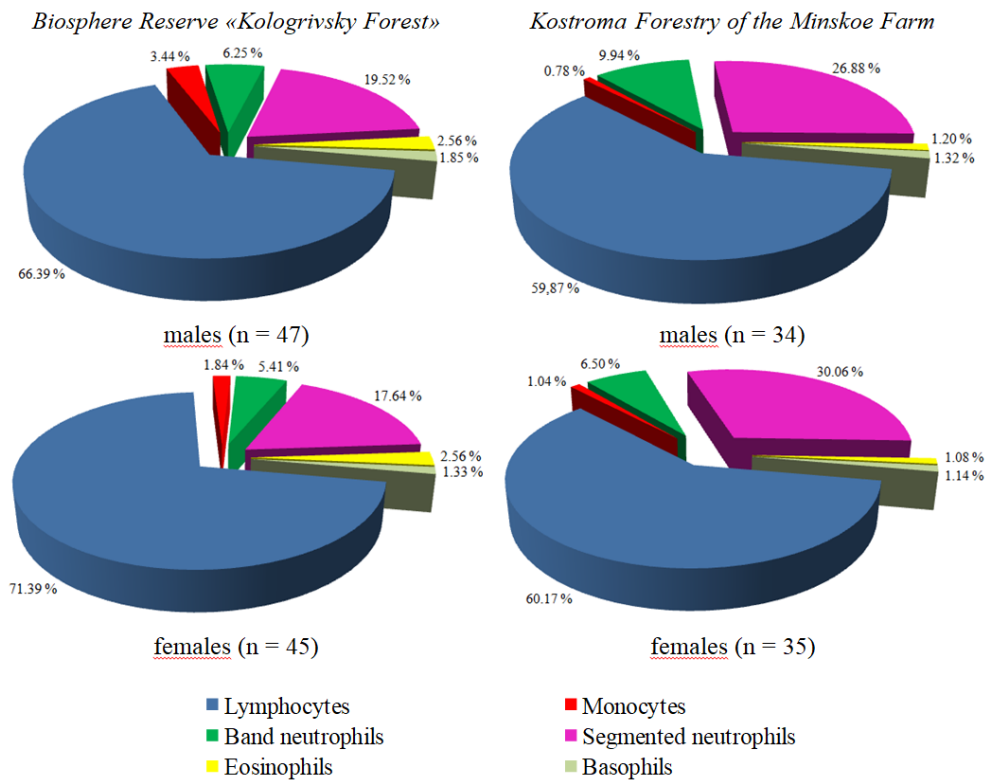
The comparative analysis of the absolute quantity of leukocytes in the blood of bank voles and pygmy wood mice in the examined territories did not show statistically significant differences (Table 1).

The leukocyte profiles of the blood of the rodents *Myodes glareolus* and *Apodemus uralensis*, presented in the form of leukograms on Figs. 2 and 3, give an idea of the relationship between the content of various forms of leukocytes in the blood and the special mechanisms of the immune protection of individuals living in the territories of the Kologriv and Kostroma districts of the Kostroma Oblast. Comparative analysis of leukograms showed that in rodents of both species living in the Kostroma Forest, the number of lymphocytes in the leukocyte profile is less than in rodents living in the biosphere reserve.

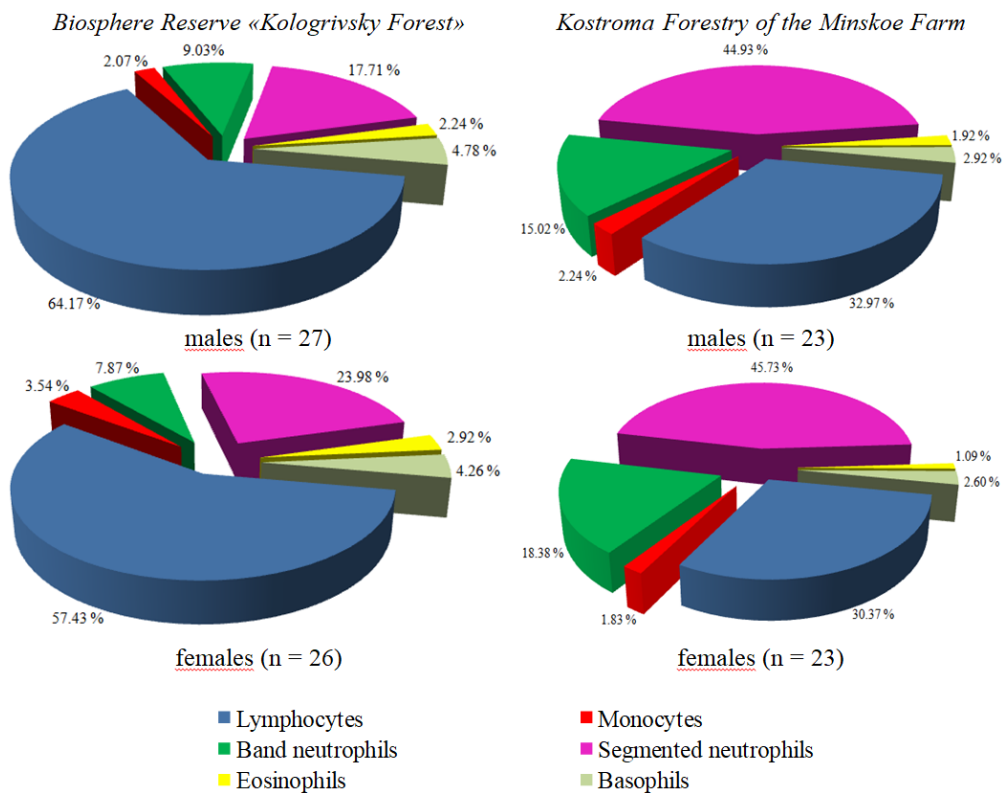
On the territory of the Minskoe Farm, the proportion of neutrophils in rodents has similar values compared to lymphocytes or even higher, while the severity of this phenomenon is different in males and females. According to Davis et al. (2008), an increase in the "neutrophil to lymphocyte ratio" in the blood indicates the body's response to various, usually long-term, stress. The content of neutrophils in the blood of bank voles and pygmy wood mice in the Kostroma forestry is 1.5 and 2.12 times ( $p < 0.01$ ) higher than the corresponding indicators for rodents living on the territory of the biosphere reserve.

**Table 1.** The content of leukocytes in the blood of mouse-like rodents in the Kostroma Oblast. X – arithmetic mean,  $S_x$  – error of the arithmetic mean, Cv – coefficient of variation; t – Student's t-test value.

Rodent species	Kologrivsky Forest Biosphere Reserve		Kostroma Forestry, Minskoe Farm		t
	X ± $S_x$ , thous. in mm <sup>3</sup>	Cv, %	X ± $S_x$ , thous. in mm <sup>3</sup>	Cv, %	
<i>Myodes glareolus</i> Schreber	17.50 ± 4.37	15.11	12.07 ± 1.64	11.22	1.16
<i>Apodemus uralensis</i> Pallas	14.14 ± 1.52	8.99	17.17 ± 2.62	22.09	1.00



**Fig. 2.** Leukogram of males and females of *M. glareolus* in the conditions of the Biosphere Reserve and Kostroma Forestry (2021–2023).



**Fig. 3.** Leukogram of males and females of *A. uralensis* in the conditions of the Biosphere Reserve and Kostroma Forestry (2021–2023).

In contrast, the content of monocytes, eosinophils and basophils in the blood of rodents is significantly higher in those living in the Kologriv Nature Reserve compared to similar indicators in the blood of individuals living in the territory of the Kostroma forestry: there are 2.9 times more monocytes in the blood of bank voles, eosinophils – 2.3 times, basophils – 1.3 times ( $p < 0.01$ ). In the blood of the pygmy wood mouse from the Kologrivsky Forest, the proportion of eosinophils is 1.71 times higher, basophils – 1.64 times, monocytes – 1.4 times higher than in the blood of individuals from the territory of the Kostroma Forestry ( $p < 0.01$ ).

It is noteworthy that myelocytes were not found in the blood of rodents of the studied populations, which is normal, since they are young cells of the granulocytic lineage and are located in the bone marrow.

Reliable correlations were established between the leukocyte profile in the blood of rodents and the location of capture, stage of the population cycle, gender, reproductive strategy, body weight, relative weight of the spleen and the presence of SM (Fig. 4). Due to species differences in changes in leukoformula, correlograms of the studied indicators in rodents were calculated separately for each species.

A number of correlations are quite expected and can be explained by the very specificity of the leukocyte profile, in which the majority of cells are represented by lymphocytes and neutrophils. These correlations are typical of both species studied. For example, a strong inverse correlation was found between the number of lymphocytes in the blood of rodents and band and segmented neutrophils (for the bank vole the correlation coefficient was  $-0.85$  and  $-0.83$ , respectively; for the pygmy wood mouse it was  $-0.81$  and  $-0.94$ ). In turn, the number of band and segmented neutrophils, as well as lymphocytes, strongly positively correlates with the indicator “ratio of segmented neutrophils to lymphocytes” (the correlation coefficient of lymphocytes is  $-0.83$  for the bank vole,  $-0.92$  for the pygmy wood mouse).

In addition, for the pygmy wood mouse population, an inverse correlation was established between the proportion of lymphocytes in the blood and the place of capture, as indicated earlier; their number is significantly higher on the territory of the biosphere reserve (the correlation coefficient is  $-0.84$ ). A direct correlation was recorded for the number of band and segmented neutrophils and the indicator “ratio of segmented neutrophils to lymphocytes” in relation to the place of capture (correlation coefficients are  $0.86$ ,  $0.87$  and  $0.87$ , respectively). At the same time, a weak correlation for these variables was established for the bank vole ( $r_s < 0.75$ ).

Although there is a positive correlation between the relative mass of the spleen and the splenomegaly index in both species, the correlation patterns with the participation of these indicators differ significantly between species. At the same time, no correlation between SM and leukocyte profile was found in the bank vole. In the pygmy wood mouse population, a weak positive correlation was established between the presence of SM and the number of lymphocytes in the blood of rodents (correlation coefficient equal to  $0.20$ ), and the opposite – with the number of band neutrophils ( $-0.22$ ). The observed trend suggests a possible increase in the number of lymphocytes and a decrease in the number of band neutrophils in the case of splenic hypertrophy in rodents. A significant positive correlation of splenomegaly in rodents was noted with the body weight of individuals (for the bank vole the correlation coefficient is  $0.59$ , for the pygmy wood mouse population it is  $0.60$ ).

The proportion of eosinophils in the leukocyte profile of both species on the territory of the reserve is significantly higher: 2.3 times in the bank vole ( $p < 0.01$ ) and 1.71 times in the pygmy wood mouse ( $p < 0.01$ ) compared with rodents living in the forest area. At the same time, a direct correlation between the spleen index and the level of eosinophils in the blood was established in the pygmy wood mouse (the correlation coefficient is  $0.22$ ), while in the bank vole it is inverse ( $-0.23$ ). A correlation between the presence of SM and the content of eosinophils in the leukocyte formula of rodents has not been established.

The mean values of the spleen index have a relatively high degree of deviation in rodents of the studied species suggesting heterogeneity of populations for this character (Table 2). It was noted that during the study period, the proportion of individuals with SM in populations of mouse-like rodents in total did not exceed 3.85% of the entire sample. In this case, the maximum of individuals with splenomegaly is observed mainly in breeding groups (3 FPG and 1 FPG) of populations of the species *M. glareolus*. In isolated cases, this phenomenon is recorded in the co-dominant species – *A. uralensis*. Correlation analysis showed the absence of a close connection between SM and the specific reproductive strategy of rodents



**Table 2.** Variability of the spleen index and the proportion of individuals with SM in populations of cyclomorphic rodents in the Kostroma Oblast in 2021–2023. Functional and physiological groups: 1 FPG – overwintered individuals, 2 FPG – non-breeding this year's brood, 3 FPG – breeding this year's brood.

Location	Species	n	Spleen index, ‰				Share of individuals with SM, %		
			min	max	$X \pm S_x$	Cv	I type of ontogeny		II type of ontogeny
							3 FPG	2 FPG	1 FPG
Kologrivsky Forest Biosphere Reserve	<i>M. glareolus</i>	92	0.01	13.00	1.81 ± 0.28	148.21	2.17	0.00	1.09
	<i>A. uralensis</i>	53	0.01	11.33	3.37 ± 0.41	88.84	5.66	0.00	0.00
Kostroma Forestry, Minskoe Farm	<i>M. glareolus</i>	69	0.01	41.18	3.54 ± 0.71	166.27	1.45	0.00	4.35
	<i>A. uralensis</i>	46	0.01	3.15	0.70 ± 0.11	102.47	0.00	0.00	0.00

**Table 3.** Indicators of infection of cyclomorphic rodents in the Kostroma Oblast (data for 2023). EI – extensiveness of invasion, II – intensity of invasion, AI – abundance index.

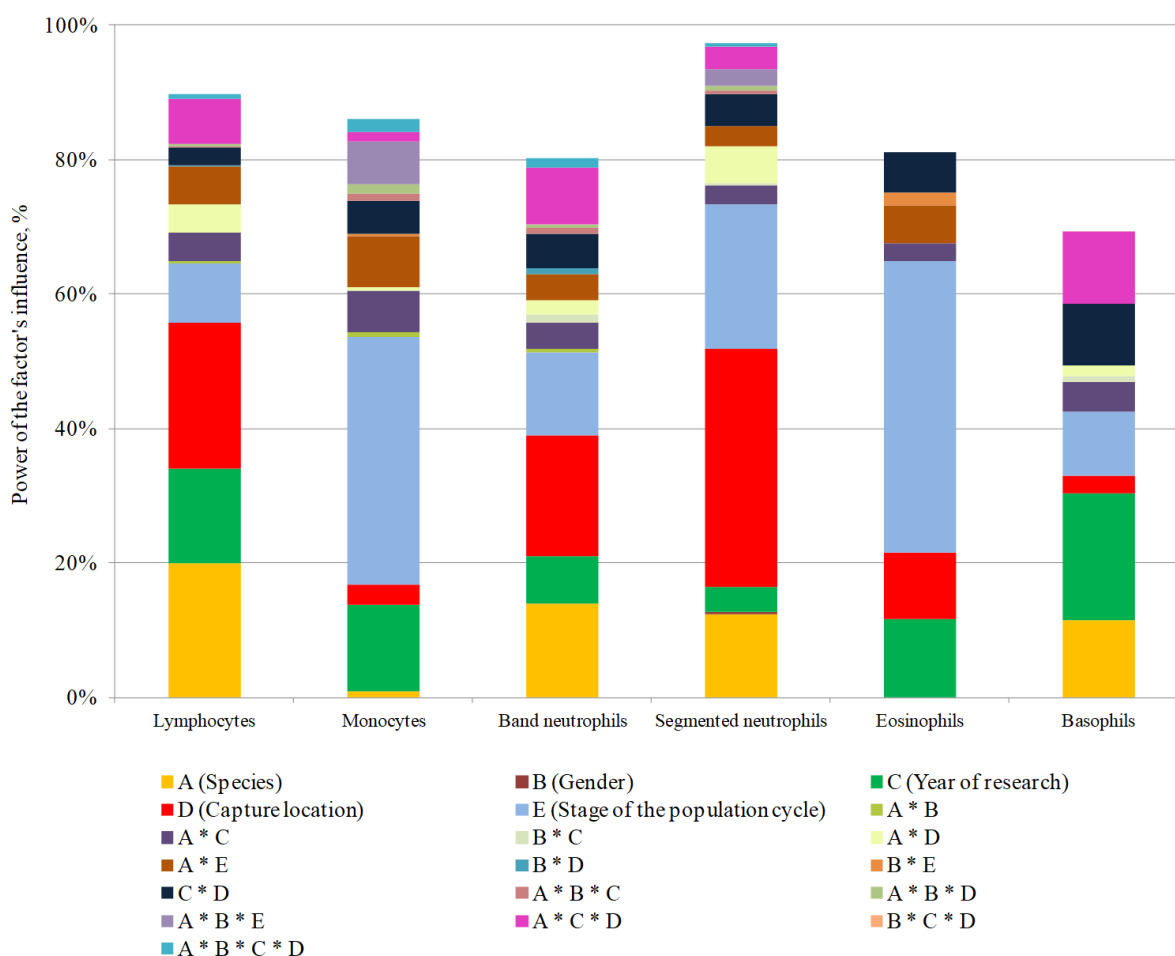
Index	Kologrivsky Forest Biosphere Reserve								Kostroma Forestry, Minskoe Farm	
	<i>M. glareolus</i> (n=33)				<i>A. uralensis</i> (n=16)				<i>M. glareolus</i> (n=29)	
	<i>Trichenella spiralis</i>	<i>Heligmosomum costellatum</i>	<i>Angiocaulus ryjkovi</i>	Cestoda (larval forms)	<i>Plagiorchis eutamiasis</i>	<i>Heligmosomum costellatum</i>	<i>Trichenella spiralis</i>	Cestoda (larval forms)	<i>Heligmosomum costellatum</i>	<i>Aspicularis tetraptera</i>
Number of infected rodents, specimens	2	20	1	7	1	1	1	3	2	2
EI, %	6.06	60.61	0.33	21.21	6.25	6.25	6.25	18.75	6.90	6.90
II, specimens	2.5	6.2	2	0.14	4	19	1	0.33	5.5	19.5
AI, specimens	0.15	3.76	0.06	0.21	0.25	1.19	0.06	0.06	0.37	1.34

## Discussion

One of the key issues when monitoring natural and anthropogenically transformed ecosystems is the choice of integral indicators of the state of bioindicator species, which can be used for comprehensive assessment of changes in ecosystems. The ecological and physiological features of the blood system and hematopoietic organs of the background species of mouse-like rodents reflect the natural processes of adaptation of the body to constantly changing environmental conditions. Thus, a shift in the profile of leukocytes in the blood of rodents can occur under the influence of various types of stressors: population processes, changes in biotic and abiotic environmental conditions, the presence of diseases and infections, etc. (Christian, 1950; Davis et al., 2008).

When animals respond to stress, the number of lymphocytes in the blood decreases, which can be accompanied by an increase in the number of neutrophils, which leads to an increase in the neutrophil to lymphocyte ratio of (Baker et al., 1998; Breuner et al., 2013; Davis, 2005; Hanssen et al., 2003; Lobato et al., 2005; Mueller et al., 2011). These processes are associated with changes in the rate of maturation of lymphoid cells, their release into the blood from hematopoietic organs, as well as cell migration into the lymphoid system (Brenner et al., 1998; Ottaway and Husband, 1994).

In the present study, rodents from the biosphere reserve showed a lymphocytic profile in their blood characteristic of most rodents, while in rodents from the Kostroma forestry, the proportion of lymphocytes decreases and is compensated by an increase in the level of segmented neutrophils, which indicates an adaptive response to stressors.



**Fig. 5.** The influence of various factors on the hematological parameters of cyclomorphmic rodents in the Kostroma Oblast (only reliable influences of factors according to analysis of variance data are presented).

Due to the high variability of the splenic index, in particular due to SM, it is not included among the classical morphophysiological indicators (Ivanter et al. 1985; Schwartz et al., 1968). However, some authors (Olenev and Grigorkina, 2019; Olenev et al., 2014, etc.) showed that the spleen can be considered an indicator of “ecological distress” and used as a marker of infection. In this study, the observed relatively high percentage of individuals with splenomegaly in populations of *M. glareolus*, mainly in breeding groups (3 FPG and 1 FPG) confirms the species-specific nature of the SM phenomenon and its association with the functional state of individuals, established previously (Olenev and Grigorkina, 2019; Salikhova, 2015; etc.). The frequency of occurrence of rodents with SM is higher on the territory of the Kologrivsky Forest Nature Reserve, which may be a consequence of a higher level of helminthiasis among rodents in this territory compared to the Kostroma Forestry. The absence of a reliable correlation between helminthiasis and the value of the splenic index in the present study can be explained by the capture of individuals in the initial period of invasion, when the animal's immune system has not yet reacted to infection, in particular, in the form of an increase in the splenic index to values above 10‰ (Olenev et al., 2014).

One of the factors that has a significant impact on the leukocyte profile in the Kostroma Oblast is the stage of the population cycle. During periods of peak population density of rodents, the proportion of segmented and band neutrophils in the blood of individuals increases, which characterizes the presence of chronic stress, which is caused by a possible increase in the frequency of individuals encountering each other, increased competition for shelter and food resources, and an increase in the proportion of helminthiasis and infectious diseases among rodents. This conclusion is confirmed by the results of other researchers who noted that with increasing animal population density, intensive production of stress hormones occurs, in particular glucocorticoids (Blondel et al., 2016; Boonstra and Boag, 1992; Christian, 1961; Creel et al., 2012), which lead to a decrease in the number of lymphocytes in the blood (Feldman et al., 2000; Stockham and Scott, 2002).

## Conclusions

It has been established that the variability of the leukocyte profile is species-specific and is associated with gender, reproductive activity of individuals, stage of the population cycle and location of capture.

Rodents from the Kostroma Forest biosphere reserve have a predominantly lymphocytic blood profile, which indicates the absence of tension in the immune system of the blood of individuals. At the same time, an increase in the ratio of neutrophils to lymphocytes was recorded in rodents from the Kostroma Forestry, which indicates the presence of an adaptive response to long-term stressors, ensuring the stable functioning of individuals. The relatively high degree of deviation of the splenic index values indicates the variability of this characteristic in the studied populations.

A direct correlation was established between the splenic index and the level of eosinophils in the blood in the pygmy wood mouse and an inverse correlation in the bank vole. A correlation between the presence of splenomegaly in rodents and the content of eosinophils in the leukocyte profile as an indicator of parasitic load has not been established. A significant correlation between the risk of developing SM in mouse-like rodents and the specifics of their reproductive strategy and susceptibility to invasions has not been confirmed.

Thus, enumeration of the blood cells of rodents can act as integral indicators of the state of populations, and their changes demonstrate the process of adaptation of rodents to constantly changing environmental conditions. Thus, the factor “species” has a significant impact on the proportion of leukocytes, neutrophils and basophils, the factor “location of capture” affects the same three indicators, as well as the level of eosinophils. The stage of the population cycle significantly affects all indicators of the leukocyte profile. The combined effect of these factors on the leukocyte profile in some cases can reach over 10%.

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