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Hematological profile of wild boar *Sus scrofa* (Linnaeus 1758) (*Suina, Suidae*) in the Kirov Region, Russia

Maria A. Perevozchikova*, Igor A. Domsky, Alexey A. Sergeev, Alexander V. Economov, Mikhail A. Dolgikh, Alexander V. Dolgikh

Professor Zhitkov Russian Research Institute of Game Management and Fur Farming, Kirov, Russia

* e-mail: mperevozchikova@mail.ru

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Abstract:
Physiological blood parameters help assess the health, feeding, immune, and reproductive status of wild animals. However, complicated sampling procedures make it difficult to establish the reference intervals for hematological parameters. Wild boar (*Sus scrofa*, Linnaeus 1758) is a popular game species. As a result, its population in Russia has been declining for the last decade. The wild boar is fertile and ecologically flexible; it responds well to biotechnical measures and have high population density. The research renders new data on the biology and physiology of wild boars.

The blood samples were obtained from 68 juvenile and adult wild boars in the Kirov Region in 2017–2023. The body weight varied from 30 to 211 kg. Blood from the jugular vein was collected into anticoagulant test tubes. The laboratory tests involved a veterinary version of a MicroCC-20 Plus automatic analyzer (High Technology, USA). The stained smears were examined using a MEIJI TECHNO light microscope (Japan) under an immersion system with a ×100 lens. The red blood cell parameters were measured using the Vision Bio software (Epi, Austria).

The research revealed the hematological profile of wild boars; the data were statistically processed, including, for the first time, the effect of sex and age on various hematological parameters. The significant differences ($p < 0.05$) between juvenile and adult females included the relative red cell distribution width by volume (standard deviation) and red blood cell thickness. The significant differences ($p < 0.05$) between juvenile and adult males were in hemoglobin, hematocrit, lymphocytes, and segmented neutrophils, as well as in such red blood cell parameters as total count, relative width by volume, area, perimeter, diameter, and sphericity index. The significant differences ($p < 0.05$) between juvenile females and males referred to hemoglobin and such parameters of red blood cells as total count, area, perimeter, and diameter. In adult males and females, it was the red blood cell thickness and platelet count. The research also yielded the lymphocytic profile of wild boar blood. The age affected such parameters as hematocrit ($p = 0.02$), segmented neutrophils ($p = 0.00$), and lymphocytes ($p = 0.00$). The body weight affected the hematocrit ($p = 0.02$) and mean red blood cell volume ($p = 0.04$).

The differences in *Sus scrofa* hematological profile depended on the physiological status, diet, minerals, age, sex, and stress. The reference intervals may help interpret the hematological profiles of other wild boar populations and optimize the game resource management.

Keywords: *Sus scrofa*, wild animals, hematology, blood cells, blood parameters, platelets, cell morphology

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INTRODUCTION

Hematological profile reveals the physiological status of mammals: its parameters characterize the exposure to infection, parasites, and ecotoxicants. Game managers and breeders use blood tests to determine the diet, immune, and reproductive status of the animals to take effective biotechnical, therapeutic, and preventive measures. Blood tests are especially important when other methods are unavailable [1–7]. Sampling becomes a very complex procedure in the wild, which makes it a serious limiting factor for ecological, veterinary, and biochemical studies [8–9]. Large, strong, dangerous, or nervous

animals are difficult to immobilize. In addition, drug administration and stress can affect the test results [10–12].

Establishing reference values for physiological parameters of wild animals are a difficult and expensive task. The strict standards for sampling make them time-consuming. Healthy animal physiology is affected by a number of factors, e.g., stress, food shortage, predators, injuries, diseases, anthropogenic triggers, migration, reproduction, etc. This list also includes procedures for capturing and handling [4]. Reference values for morphophysiological parameters have probably never been obtained for any biological species, including humans [13]. In this respect, the reference ranges we used in this research rely on the best approximation to the physiological norm and can be considered guidelines for interpretation.

The blood parameters for wild boars are rarely published in scientific journals [2, 4, 14–19]. As a rule, scientists focus on a limited sampling [14, 19], age group [2, 15], or parameter set. Since they usually pursue veterinary purposes [1–2], animals are anesthetized [4] and not classified by sex [1–2, 4, 10, 14–15]. The material obtained in this way has significant scientific value but cannot be considered representative.

Wild boar (*Sus scrofa*, Linnaeus 1758) is an important game species. In Russia, its population has halved since 2012 [20–22], mostly as a result of African swine fever [23].

The wild boar is a popular hunting object. Of all wild ungulates, wild boars are the most responsive to biotechnical measures. In addition, they are fertile, ecologically flexible, and have high population density. In semi-free conditions or an artificial habitat, veterinary care is necessary to prevent outbreaks that may be caused by the transfer of parasites or pathogens to native populations [2, 24–26]. On game farms, improper diet and poor reproduction management trigger metabolic changes that increase mortality as well as reduce fertility and life expectancy in the herd [27–28]. Unfortunately, metabolic changes are difficult to diagnose.

Hematological tests are the most informative ones in terms of physiological status and early diagnosis [29]. However, they are unpopular in nature management and conservation because the sampling complexity and wild habitat differences make their practical application particularly difficult.

Currently, the blood parameters of wild animals are taken mostly *postmortem* as venous blood from large vessels. This method provides high-quality samples in sufficient quantities. In wild boar studies, this method is simple, fast, reliable, clean, and human-safe.

Arenas-Montes *et al.* [3] offered an alternative sampling method. Based on dural venous system, it involved blood from intracavernous venipuncture from the cavernous sinus. This method is far from perfect because the delay between the death and the sampling may be several hours: blood tends to clot and becomes contaminated, which results in autolytic, cytotoxic, and/or hemolytic sera [30].

Blood parameter studies are important for wild boar breeding. This research provided an insight into the biology and physiology of wild boars, which made it possible to identify the reference values of hematological parameters in different age and sex groups in different environments.

STUDY OBJECTS AND METHODS

The study featured 68 wild boars shot in 2017–2023 by hunters issued with scientific collecting permits that authorized the lethal collection of wildlife. The animals lived on the scientific and experimental hunting grounds of the scientific and experimental hunting farm of the All-Russian Research Institute of Wildlife Management. The hunting grounds cover 66,250 ha in the Slobodskoy, Belaya Kholunitsa, and Zuevka districts of the Kirov Region, Russia (58.502270 N, 50.835894 E).

The animals were divided into those under 1 year of age (juveniles), which were even visually different from the adult animals (adults). The biomaterial was harvested during the hunting season, i.e., from October to December. The local climate is continental, with mild winters and warm summers. All wild boars moved freely within the hunting grounds. In addition to their natural diet, the boars received seasonal supplementary feeding, e.g., grain purchased from local farmers.

The blood obtained from these animals served as research material. The latest mandatory veterinary examination revealed no signs of disease, and all animals were considered clinically healthy.

The biomaterial was collected from the jugular vein (*v. jugularis*) into 4 mL UNIVAC vacuum tubes immediately after shooting. The tubes contained an anticoagulant, namely, dipotassium ethylenediaminetetraacetic acid (K2EDTA). The sampling, storage, and preparation followed Protocol no. 346н established by the Ministry of Health and Social Development, May 12, 2010 and adapted to wild animals [31]. Prior to laboratory tests, the tubes remained in a refrigerator at +4 °C.

The hematological studies were performed after 1–3 days using a veterinary version of a MicroCC-20 Plus automatic analyzer (High Technology, USA). The white blood cell count was obtained by the microscopy of blood smears stained with May-Grünwald's eosin methylene blue and Romanovsky's azure eosin (MiniMed-M-G, Russia).

The hematological profile consisted of the following parameters: total red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin content, mean corpuscular hemoglobin concentration, red cell distribution width, red blood cell distribution width (standard deviation), platelet volume, platelet distribution width, plateletcrit, platelet count, large platelet count, total white blood cell, proportion of lymphocytes, monocytes, band neutrophils, segmented neutrophils, granulocyte eosinophils, and basophils. The morphological parameters of erythrocytes included area, perimeter, diameter, thickness, and global sphericity index.

The red blood cell sphericity index was calculated by the standard formula:

$$IRS = D / T = D / (V/S)$$

where D is the mean diameter, μm; T is the mean thickness, μm; V is the mean volume, fl; S is the mean base area, μm.

The blood smears were air-dried and stained with May-Grünwald's eosin methylene blue and Romanovsky's azure eosin. Each stained smear was examined using a MEIJI TECHNO light microscope (Japan) under an immersion system with a ×100 objective. To determine the red blood cell parameters, we took 10–20 clear view fields with adequate morphology for each blood smear. The parameters were processed using the Vision Bio software (Epi, Austria). The total red blood cell pool more than 13,000.

The statistical analysis involved MS Excel (Office 2019) and Statgraphics (19-X64) with standard methods [32]. The hematological parameters were expressed as mean (M), error of the mean (m), standard deviation (SD), median (Me), 25 and 75% percentiles. Since the distribution of values in some cases differed from the standard, we used nonparametric Mann-Whitney U test and Kruskal-Wallis H tests to compare the parameters between groups.

The relationship between parameters was assessed by Spearman's correlation coefficient.

The one- and multifactorial analysis of variance (ANOWA-test) made it possible to assess the effect of age, sex, and weight on the hematological parameters. The effect was considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The body weight of wild boars varied from 30 to 211 kg. Young females tend to grow in weight faster than males, but eventually males become larger (Table 1).

Table 2 summarizes the red blood cell parameters in the wild boars under study.

We determined the area, perimeter, and diameter for 13,450 red blood cells. Table 3 shows their minimal and maximal values.

Table 4 shows the main parameters for 350 red blood cells, i.e., the mean values of area, perimeter, and diameter.

The red blood cell thickness and sphericity index were calculated for the blood samples that underwent a general blood test and had a smear taken (Table 5).

Table 6 describes the platelet indices, including the mean platelet volume, the relative platelet distribution width by volume, palatelcrit, platelet count, and large platelet content.

Table 7 gives the total count of white blood cells, as well as the share of lymphocytes, monocytes, band neutrophils, segmented neutrophils, eosinophils, granulocytes, and basophils.

The single- and multifactorial analysis of variance revealed the effect of such physiological factors as age, sex, and weight on the hematological profile. The age had a reliable effect on hematocrit ($p = 0.02$), segmental neutrophils ($p = 0.00$), and lymphocytes ($p = 0.00$). The weight affected such parameters as hematocrit ($p = 0.02$) and mean corpuscular volume ($p = 0.04$).

For hematological parameters, the reference intervals describe the dispersion of variables in animal blood samples and are true for 95% of all healthy individuals in the population [33]. However, healthy animals at different stages of ontogenesis may have variations in hematological parameter values even under stable conditions. This is true for most mammals, including humans. Accurate and adapted reference intervals provide the correct interpretation of blood tests from animals that lived in different parts of the habitat.

Reference intervals for hematological parameters in wild boars are difficult to determine because they depend on many factors associated with the high ecological plasticity of this species. These factors include age, season, habitat, diet, hunting, inheritance, pregnancy, lactation, etc. Available publications provide no stable reference values for hematological parameters in wild boars because they usually rely on small samplings and refer to different regions of the world.

To compensate for the lack of data, we compared our results with physiological values available both for wild boars and domestic pigs (Table 8).

Red blood cell parameters. Modern hematological analyzers show not only the red blood cell and platelet counts in peripheral blood, but also erythrocyte indices. Automatic calculation of red blood cell parameters provides more accurate information about hematological profile than manual counting.

We detected reliable differences ($p < 0.05$) between juvenile and adult animals for the following red blood cell parameters: cell count (males), hemoglobin (males), hematocrit (males), relative distribution width by volume (coefficient of variation, males), relative distribution width by volume (standard deviation, females), area (males), perimeter (males), diameter (males), thickness (females), and sphericity index (males) (Tables 2–6).

Both age groups demonstrated reliable sex differences: red blood cell count, hemoglobin, area, perimeter, and diameter in juveniles and thickness in adults.

Kukharenko *et al.* [14] studied the hematological profile of wild boars in the game reserves of the Amur Region, Russian Far East. The animals were classified by sex, not age. The blood tests revealed signs of anmia, i.e., low red blood cell count, hemoglobin, color index, and hematocrit. Moreover, they detected severe poikilocytosis, with a large proportion of spiky (23%) and indefinite shape (35.3%) red blood cells. As for the diameter, the red blood cells were represented by microcytes. Anemia, probably caused by bone marrow failure, reduces the population of Amur wild boars.

In this study, both adult and juvenile boars had higher values for red cell count: by 9.41 and 1.93%, respectively (Table 2). The concentration of hemoglobin was increased by 13.70% in the juvenile boars and by 28.78% in the adult boars. The hematocrit value was

Table 1 Body weight of wild boars from the Kirov Region, Russia

Parameter \ Group	Females		Males	
	Juveniles	Adults	Juveniles	Adults
n	13	9	21	25
M ± m	52.47 ± 5.54	105.00 ± 7.07	47.43 ± 11.35	127.50 ± 40.97
lim	42.50–60.00	100.00–110.00	30.00–64.50	80.00–211.00

Table 2 Erythrocyte parameters in juvenile and adult wild boars (n = 51)

Parameters \ Group	Females		Males	
	Juvenile (n = 8) M ± SD Me 25–75%	Adult (n = 9) M ± SD Me 25–75%	Juvenile (n = 14) M ± SD Me 25–75%	Adult (n = 20) M ± SD Me 25–75%
Red blood cells, 10^{12}/L	4.11–8.11 6.33 ± 1.31 [B] 6.13 5.77–7.12	4.44–7.16 5.61 ± 0.93 5.38 4.79–5.90	3.67–6.45 5.12 ± 0.86 [A, B] 5.30 4.37–5.59	3.67–9.40 6.18 ± 1.46 [A] 6.32 5.35–7.14
Hemoglobin, g/L	105.00–200.00 153.00 ± 30.18 151.50 [B] 137.75–162.75	109.00–169.00 133.33 ± 19.77 124.00 120.00–150.00	89.00–162.00 115.76 ± 22.28 115.00 [A, B] 96.00–132.00	89.00–210.00 147.10 ± 31.72 154.00 [A] 127.50–162.25
Hematocrit, %	26.70–44.40 37.02 ± 6.69 37.50 32.45–42.92	26.80–55.70 37.45 ± 9.52 39.80 28.50–42.60	20.00–46.30 33.00 ± 7.19 32.10 [A] 27.70–38.50	20.00–68.90 43.07 ± 12.01 42.95 [A] 34.95–52.10
Mean corpuscular volume, fl	48.20–74.30 60.36 ± 9.54 59.85 53.00–67.17	53.00–79.20 66.36 ± 9.35 67.80 58.30–72.60	49.70–73.60 64.25 ± 7.17 66.00 60.00–68.90	53.50–80.30 68.17 ± 7.12 70.35 63.27–73.42
Mean concentration hemoglobin, pg	22.60–25.50 24.07 ± 1.07 24.00 23.47–24.85	20.00–27.90 23.85 ± 2.89 23.60 22.20–26.10	16.60–31.00 22.78 ± 3.39 22.20 21.70–24.10	15.60–31.00 23.47 ± 3.74 24.15 21.70–25.27
Mean corpuscular hemoglobin concentration, g/L	341.00–471.00 406.00 ± 57.86 418.00 342.75–454.25	296.00–448.00 365.88 ± 54.52 352.00 342.00–382.00	241.00–495.00 359.23 ± 67.53 348.00 328.00–383.00	194.00–495.00 354.8 ± 74.93 368.00 320.50–387.75
Red blood cell distribution width (coefficient of variation), %	13.60–17.70 16.01 ± 1.59 16.25 15.12–17.30	12.40–17.80 15.12 ± 1.69 15.40 14.30–15.80	12.90–21.80 15.22 ± 2.24 14.50 [A] 14.00–15.60	12.90–20.50 16.22 ± 2.11 16.65 [A] 14.20–17.70
Red blood cell distribution width (standard deviation), fl	41.10–52.50 48.17 ± 3.59 47.80 [A] 47.30–50.40	39.20–50.60 44.41 ± 4.20 45.50 [A] 40.95–46.85	37.90–69.30 46.77 ± 9.00 45.50 41.65–47.22	38.70–54.70 45.43 ± 4.69 45.30 41.75–47.45

Reliable differences between juveniles and adults are marked with superscript A ($p < 0.05$); reliable differences between males and females are marked with superscript B ($p < 0.05$).

Table 3 Area, perimeter, and diameter of red blood cells in wild boars (n = 38): Minimal and maximal values

Group \ Parameters		min			max		
		Area, μm^2	Perimeter, μm	Diameter, μm	Area, μm^2	Perimeter, μm	Diameter, μm
Females	Juvenile	12.27–21.30	14.15–18.57	4.68–5.75	36.25–46.11	23.78–28.93	8.02–8.98
	Adult	17.38–21.65	16.29–18.56	5.27–5.95	42.19–45.55	26.47–28.72	8.43–9.45
Males	Juvenile	11.73–18.08	13.28–16.81	4.22–5.41	35.92–46.67	23.77–36.26	7.90–10.35
	Adult	16.97–22.34	16.15–19.21	5.08–5.89	37.20–46.64	26.14–28.98	8.44–9.51

Table 4 Area, perimeter, and diameter of red blood cells in wild boars (n = 38)

Parameters \ Group	Females		Males	
	Juvenile (n = 10) M ± SD Me 25–75%	Adult (n = 4) M ± SD Me 25–75%	Juvenile (n = 16) M ± SD Me 25–75%	Adult (n = 8) M ± SD Me 25–75%
Area, μm²	21.96–31.18 28.36 ± 2.76 29.13 [B] 27.36–30.29	26.95–33.11 29.67 ± 2.56 29.31 28.49–30.48	20.80–30.78 26.59 ± 2.37 26.42 [A, B] 25.77–27.32	27.80–32.63 29.19 ± 1.67 28.79 [A] 27.92–29.44
Perimeter, μm	18.51–22.92 21.41 ± 1.29 21.52 [B] 20.90–22.33	20.57–22.89 21.61 ± 0.95 21.50 21.26–21.85	18.21–22.12 20.56 ± 0.94 20.38 [A, B] 20.20–20.96	20.72–23.38 21.67 ± 0.89 21.46 [A] 20.98–22.08
Diameter, μm	6.03–7.13 6.77 ± 0.34 6.82 [B] 6.66–7.04	6.58–7.24 6.83 ± 0.28 6.76 6.71–6.88	5.77–6.95 6.51 ± 0.28 6.49 [A, B] 6.35–6.65	6.55–7.30 6.81 ± 0.24 6.80 [A] 6.62–6.87

Reliable differences between juveniles and adults are marked with superscript A ($p < 0.05$); reliable differences between males and females are marked with superscript B ($p < 0.05$).

Table 5 Thickness and sphericity index of red blood cells in wild boars (n = 21)

Parameters \ Group	Females		Males	
	Juvenile (n = 5) M ± SD Me 25–75%	Adult (n = 3) M ± SD Me 25–75%	Juvenile (n = 8) M ± SD Me 25–75%	Adult (n = 5) M ± SD Me 25–75%
Thickness, μm	1.74–3.02 2.33 ± 0.56 2.54 [A] 1.75–2.61	1.76–2.93 2.38 ± 0.59 2.45 [A, B] 2.10–2.69	2.16–2.86 2.56 ± 0.24 2.53 2.43–2.79	2.04–2.63 2.30 ± 0.23 2.25 [B] 2.15–2.42
Sphericity index	1.99–4.06 3.05 ± 0.94 2.61 2.54–4.05	2.23–4.11 3.03 ± 0.96 2.76 2.50–3.43	2.22–2.96 2.50 ± 0.25 2.45 [A] 2.32–2.60	2.48–3.41 3.02 ± 0.39 3.03 [A] 2.79–3.38

Reliable differences between juveniles and adults are marked with superscript A ($p < 0.05$); reliable differences between males and females are marked with superscript B ($p < 0.05$).

Table 6 Platelet parameters in juvenile and adult wild boars (n = 51)

Parameters \ Group	Females		Males	
	Juvenile females (n = 8) M ± SD Me 25%–75%	Adult females (n = 9) M ± SD Me 25%–75%	Juvenile males (n = 14) M ± SD Me 25%–75%	Adult males (n = 20) M ± SD Me 25%–75%
Platelets, 10^9/l	71.00–321.00 206.87 ± 97.95 227.50 124.00–291.00	37.00–174.00 116.55 ± 41.47 129.00 [B] 108.00–141.00	89.00–450.00 159.85 ± 93.21 138.50 103.25–174.50	65.00–436.00 198.55 ± 96.98 181.00 [B] 156.75–225.75
Plateletcrit, %	0.04–0.79 0.23 ± 0.24 0.15 0.08–0.26	0.02–0.17 0.10 ± 0.04 0.10 [B] 0.09–0.11	0.06–0.35 0.12 ± 0.07 0.10 0.07–0.12	0.05-0.46 0.18 ± 0.11 0.16 [B] 0.11–0.20
Mean platelet volume, fl	6.90–9.80 8.07 ± 1.03 8.05 7.15–8.70	3.40–10.60 7.91 ± 2.09 8.30 6.90–8.70	6.50–9.80 7.61 ± 0.91 7.50 7.02–8.02	5.90–72.10 10.89 ± 14.43 7.70 7.05–8.62
Platelet distribution width, %	3.20–22.80 13.00 ± 6.45 11.10 9.77–16.92	9.90–22.80 15.08 ± 4.74 13.90 11.00–18.30	3.40–23.20 13.11 ± 6.40 11.95 10.40–18.10	2.70–23.90 15.82 ± 5.51 16.80 14.00–18.82
Platelet large cell ratio, %	8.50–42.20 25.71 ± 13.20 23.4 16.10–36.90	13.30–55.70 35.50 ± 15.76 36.6 25.45–45.95	5.50–50.20 20.43 ± 14.59 14.3 11.20–28.80	10.20–44.30 21.97 ± 11.25 18.25 14.60–27.65

Reliable differences between males and females are marked with superscript B ($p < 0.05$).

Table 7 While blood cell parameters in juvenile and adult wild boars (n = 68)

Parameters \ Group	Females		Males	
	Juvenile (n = 13) M ± SD Me 25–75%	Adult (n = 9) M ± SD Me 25–75%	Juvenile (n = 21) M ± SD Me 25–75%	Adult (n = 25) M ± SD Me 25–75%
White blood cells, 10^9/L	4.00–7.40 5.51 ± 1.23 5.30 4.50–6.47	3.30–9.00 5.85 ± 2.24 5.50 4.20–8.10	1.20–15.30 6.98 ± 4.52 5.40 4.30–10.60	1.60–14.20 6.71 ± 3.30 5.60 4.42–9.10
Lymphocytes, %	28.00–93.00 75.54 ± 19.05 83.00 71.00–85.50	34.00–88.00 63.40 ± 26.36 71.00 37.00–87.00	62.00–95.00 82.93 ± 9.40 83.50 [A] 75.00–91.00	49.00–96.00 70.50 ± 10.96 68.50 [A] 63.75–76.00
Monocytes, %	0–4.00 1.54 ± 1.50 1.00 0–3.00	0–8.00 2.00 ± 3.39 1.00 0–1.00	0–3.00 0.81 ± 0.91 1.00 0–1.00	0–3.00 0.95 ± 1.04 1.00 0–2.00
Band neutrophils, %	0–2.00 0.72 ± 1.00 0 0–2.00	0–2.00 1.00 ± 1.00 1.00 0–2.00	0–4.00 1.31 ± 1.25 1.00 0–2.00	0–6.00 1.33 ± 1.71 1.00 0–2.00
Segmental neutrophils, %	2.00–66.00 17.27 ± 18.64 9.00 6.50–21.50	4.00–57.00 29.60 ± 24.96 22.00 10.00–55.00	2.00–28.00 10.75 ± 7.63 7.50 [A] 5.00–16.25	2.00–39.00 21.70 ± 9.42 23.00 [A] 15.50–28.25
Eosinophils, %	1.00–10.00 4.72 ± 2.57 4.00 3.50–5.50	0–8.00 3.60 ± 2.96 4.00 2.00–4.00	0–11.00 4.00 ± 2.68 4.00 2.00–5.00	0–16.00 5.37±4.16 5.00 2.75–6.25
Basophils, %	0–1.00 0.18 ± 0.40 0 0–0	0–1.00 0.40 ± 0.54 0 0–1.00	0–1.00 0.12 ± 0.34 0 0–0	0–2.00 0.20 ± 0.50 0 0–0

Reliable differences between juveniles and adults are marked with superscript A ($p < 0.05$)

Table 8 Hematological parameters in wild boars and domestic pigs across the world

M ± SD (min–max)		Species	Region	Reference
Juveniles	Adults			
Red blood cells, 10^{12}/L				
5.60 ± 0.00	5.30 ± 0.42	Wild boars	Amur Region, Russia	Kukharenko *et al*. [14]
6.40 ± 0.96	–	Wild boars	Iberian Peninsula, Spain, France	López-Olvera *et al.* [2]
–	8.00 ± 0.68 (6.87–9.03)	Wild boars	Croatia	Harapin *et al.* [15]
6.70 ± 1.38	6.02 ± 0.76	Feral pigs	Texas, USA	Shender *et al.* [1]
5.80 ± 0.35	5.60 ± 0.22	Domestic pigs	Amur Region, Russia	Kukharenko *et al.* [34]
♀ 5.70 ± 0.38 ♂ 7.90 ± 0.57	♀ 7.80 ± 0.99 ♂ 6.10 ± 1.20	Danish Landrace pigs	Amur Region, Russia	Semenova *et al.* [35]
5.71–8.47	♀ 5.46–7.20 ♂ 7.12–11.83	Large White pigs	European Russa	Gimadeeva *et al.* [36]
6.38 ± 0.93 (4.38–8.54)	–	Yorkshire pigs	Massachusetts, USA	Dimitrakakis *et al.* [37]
Hemoglobin, g/L				
115.00 ± 0.60	99.00 ± 5.00	Wild boars	Amur Region, Russia	Kukharenko *et al.* [14]
–	156.60 ± 17.32 (123.00–183.00)	Wild boars	Croatia	Harapin *et al.* [15]
126.87 ± 15.16 (101.00–172.00)	140.29 ± 13.24 (109.00–157.00)	Wild boars	Spain	Casas-Diaz *et al.* [4]
118.50 ± 21.6	120.30 ± 1.25	Feral pigs	Texas, USA	Shender *et al.* [1]

Continuation of Table 8

M ± SD (min–max)		Species	Region	Reference
Juveniles	Adults			
Hemoglobin, g/L				
92.00 ± 4.70	90.00 ± 5.40	Domestic pigs	Amur Region, Russia	Kukharenko *et al.* [34]
♀ 119.10 ± 9.77 ♂ 121.70 ± 3.00	♀ 122.80 ± 6.47 ♂ 118.30 ± 3.98	Danish Landrace pigs	Amur Region, Russia	Semenova *et al.* [36]
105.60–137.70	♀ 107.70–134.70 ♂ 106.40–162.30	Large White pigs	European Russia	Gimadeeva *et al.* [36]
	♀ 95.20–135.10	Domestic pigs	Chili	Baeza *et al*. [27]
99.90 ± 144.00 (80.00–140.00)	–	Yorkshire pigs	Massachusetts, USA	Dimitrakakis *et al*. [37]
Hematocrit, %				
36.40 ± 0.78	36.40 ± 1.72	Wild boars	Amur Region, Russia	Kukharenko *et al.* [14]
44.00 ± 6.00	–	Wild boars	Iberian Peninsula, Spain, France	López-Olvera *et al*. [2]
–	60.98 ± 4.20 (55.40–69.40)	Wild boars	Croatia	Harapin *et al*. [15]
36.00 ± 4.00 (30.00–51.00)	38.00 ± 3.00 (31.00–45.00)	Wild boars	Spain	Casas-Diaz *et al*. [4]
37.38 ± 6.85	37.86 ± 3.85	Feral pigs	Texas, USA	Shender *et al*. [1]
36.70 ± 1.74	36.90 ± 2.47	Domestic pigs	Amur Region, Russia	Kukharenko *et al.* [34]
42.67–57.00	♀ 41.55–49.94 ♂ 49.60–78.70	Large White pigs	European Russia	Gimadeeva *et al.* [36]
–	♀ 30.30–40.90	Domestic pigs	Chile	Baeza *et al*. [27]
34.47 ± 4.78 (25.25–47.00)	–	Yorkshire pigs	Massachusetts, USA	Dimitrakakis *et al*. [37]
Mean corpuscular volume, fl				
–	77.50 ± 5.13 (70.00–86.00)	Wild boars	Croatia	Harapin *et al.* [15]
Mean concentration hemoglobin, pg				
19.52 ± 0.58 (18.80–20.60)	21.13 ± 1.32 (18.10–22.90)	Wild boars	Spain	Casas-Diaz *et al.* [4]
13.64–18.62	♀ 17.91–20.68 ♂ 13.21–17.60	Large White pigs	European Russia	Gimadeeva *et al.* [36]
Mean corpuscular hemoglobin concentration, g/L				
347.10±11.80 (322.00–370.00)	355.00 ± 9.40 (339.00–376.00)	Wild boars	Spain	Casas-Diaz *et al.* [4]
Diameter, μm				
5.30 ± 0.60	7.20 ± 0.50	Wild boars	Amur Region, Russia	Kukharenko *et al.* [14]
Platelets, 10^9/L				
–	289.20–917.20	Domestic pigs	Chile	Baeza *et al.* [27]
359.00 ± 87.85 (180.00–559.00)	–	Yorkshire pigs	Massachusetts, USA	Dimitrakakis *et al.* [37]
White blood cells, 10^9/L				
21.50 ± 1.40	25.80 ± 1.90	Wild boars	Amur Region, Russia	Kukharenko *et al.* [14]
6.90 ± 2.94	–	Wild boars	Iberian Peninsula, Spain, France	López-Olvera *et al.* [2]
–	9.79 ± 4.27 (6.00–20.35)	Wild boars	Croatia	Harapin *et al.* [15]
22.10 ± 0.38	18.60 ± 0.54	Domestic pigs	Amur Region, Russia	Kukharenko *et al.* [34]
♀ 12.30 ± 2.25 ♂ 10.00 ± 0.57	♀ 10.90 ± 1.71 ♂ 7.90 ± 1.69	Danish Landrace pigs	Amur Region, Russia	Semenova *et al.* [35]
6.58–38.69	♀ 11.64–17.47 ♂ 5.46–24.78	Large White pigs	European Russia	Gimadeeva *et al.* [36]
–	♀ 11.06–19.64	Domestic pigs	Chile	Baeza *et al.* [27]
16.17 ± 3.33 (10.94–22.14)	–	Yorkshire pigs	Massachusetts, USA	Dimitrakakis *et al.* [37]
Band neutrophils, %				
8.50 ± 0.02	11.30 ± 1.60	Wild boars	Amur Region, Russia	Kukharenko *et al.* [14]
5.20 ± 0.02	4.00 ± 0.05	Domestic pigs	Amur Region, Russia	Kukharenko *et al.* [34]

End of Table 8

M ± SD (min–max)		Species	Region	Reference
Juveniles	Adults			
Segmental neutrophils, %				
29.00 ± 1.20	28.70 ± 3.80	Wild boars	Amur Region, Russia	Kukharenko *et al.* [14]
36.40 ± 1.21	33.20 ± 2.24	Domestic pigs	Amur Region, Russia	Kukharenko *et al.* [34]
–	43.50–76.20	Domestic pigs	Chile	Baeza *et al.* [27]
Monocytes, %				
6.50 ± 0.01	4.00 ± 0.60	Wild boars	Amur Region, Russia	Kukharenko *et al.* [14]
4.80 ± 0.23	4.20 ± 1.47	Domestic pigs	Amur Region, Russia	Kukharenko *et al.* [34]
–	1.40–7.60	Domestic pigs	Chile	Baeza *et al.* [27]
Lymphocytes, %				
52.00 ± 0.95	52.2 ± 4.60	Wild boars	Amur Region, Russia	Kukharenko *et al.* [14]
61.30 ± 1.56	55.90 ± 2.81	Domestic pigs	Amur Region, Russia	Kukharenko *et al.* [34]
	15.80–47.00	Domestic pigs	Chile	Baeza *et al*. [27]
Eosinophils, %				
4.00 ± 0.01	3.00 ± 0.10	Wild boars	Amur Region, Russia	Kukharenko *et al.* [14]
1.25 ± 0.04	1.40 ± 0.54	Domestic pigs	Amur Region, Russia	Kukharenko *et al.* [34]
	♀ 0–11.20	Domestic pigs	Chile	Baeza *et al*. [27]
Basophils, %				
0	0.70 ± 0.02	Wild boars	Amur Region, Russia	Kukharenko *et al.* [14]
0	0.50 ± 0.02	Domestic pigs	Amur Region, Russia	Kukharenko *et al.* [34]

lower by 4.51% in the juveniles and higher by 12.02% in the adults. The diameter of red cells was increased by 20.19% in the juveniles and reduced by 5.84% in the adults (Tables 3 and 4).

A comparative analysis of hematological parameters in the domestic pigs (*Sus scrofa domesticus*, Linnaeus, 1758) from the Amur Region [34] yielded similar results. However, the hemoglobin value in the Amur pigs (30.96%) was lower than in the wild boars from the Kirov Region (35.26%). The hematocrit in the juvenile Amur pigs was by 5.29% higher than in this study while in the adult pigs it was by 10.81% lower. The red cell diameter was by 20.19% smaller in the juveniles and by 5.84% larger in the adults.

The Iberian juvenile wild boars had higher red cell count (10.79%) and hematocrit content (21.00%) than in our research [2]. The Croatian adult boars demonstrated higher red cell count (26.88%), hemoglobin (11.24%), hematocrit (32.16%), and mean corpuscular volume (10.88%) [15].

The Texan feral pigs had higher red cell count: by 14.78% in the juveniles and 2.78% in the adults. The hemoglobin was by 11.07 and 13.46% lower in the juveniles than in the adults, respectively. The hematocrit was by 7.01% higher in the juveniles and by 8.49% lower in the adults [1].

The Spanish juvenile wild boars [2] demonstrated lower hemoglobin (by 4.70%) while the adults showed similar results to ours. The hematocrit was higher by 3.45% in the juveniles and by 8.15% lower in the adults. The mean content hemoglobin was lower by 12.94% in the juveniles and 10.70% in the adults. The hemoglobin concentration in red blood cells was also lower than in our studies: by 9.26% lower in the juveniles and 1.39% in the adults. The Spanish research did not classify the animals by sex. The boars were anesthetized with a combination of tiletamine-zolazepam (6 mg/kg) and xylazine (3 mg/kg).

The differences in hematological parameters could be associated with the habitat, e.g., the absolute geographic altitude (Fig. 1).

The Amur valley is about 250 m above sea level [14]. The Sant Llorenç del Munt i L'Obac Natural Park in the Northeast of Spain is 400–1,140 m above sea level. The Croatian hunting grounds of Sisak-Moslavina is over 500 m above sea level. The Texan feral pigs lived in the southeast of the state, 200 m above sea level. In our research, the wild boars lived at absolute altitudes of 160–200 m.

Adaptation to different altitudes, i.e. to the amount of oxygen available, causes anatomical, physiological, and biochemical changes [38]. High altitudes affect the oxygen transport system. At high altitudes, the partial pressure of oxygen in the air is insufficient to saturate the blood. As a result, the total capacity of the oxygen transporting system increases. Hemoglobin's lower affinity for oxygen requires an increase in the total amount of circulating hemoglobin. The regulatory effect of 2,3-diphosphoglycerate is enhanced to facilitate the release of oxygen by hemoglobin in tissues.

Behavioral and physiological adaptation pathways include the following mechanisms. Low muscle activity reduces the need for oxygen; increased heart rate and breathing raise the amount of oxygen delivered to tissues. However, these mechanisms are no longer necessary when adaptive shifts occur at the molecular level. The first change is an increase in the red blood cell count. It increases the ability of hemoglobin to carry oxygen to tissues as the concentration of 2,3-diphosphoglycerate in red blood cells grows [38].

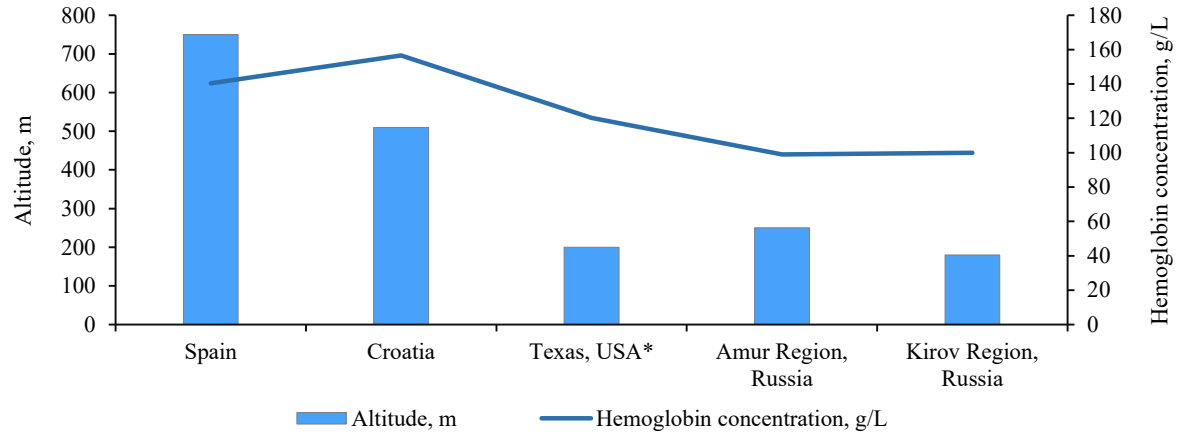

Figure 1 Mean hemoglobin concentration vs. habitat altitude based on our data and [1, 4, 14, 15]: Wild boars and Texan feral pigs*

A comprehensive comparative hematological analysis should take into account the difference in climatic conditions. The blood parameters in Table 8 were obtained from animals that inhabited different climatic zones, from the Mediterranean climate of the Iberian Peninsula, Spain, and Croatia to the oceanic climate of Massachusetts, USA, and the extremely continental climate of Russia's forest zone.

The hematology of domestic pigs depends on the low motor activity in farm stalls, dreary diet, population density stress, and regular veterinary and zootechnical measures. Yet, given the lack of information on the hematology of wild boars, domestic pigs remain a valuable source.

Juvenile domestic Landrace pigs from the Altai Region, Russia [35], demonstrated a higher red blood cell count in juvenile males (by 32.92%) and adult females (by 32.95%). Compared to the wild boars from the Kirov Region, Altai juvenile females and adult males had lower red blood cell counts (by 7.02% and 3.49%, respectively). As for hemoglobin, it was by 21.39, 6.36, and 23.19% lower in the juvenile females, adult females, and adult males, respectively. The data for young males, however, were similar to ours.

According to Semenova *et al.* [35], an age-related decrease in the hemoglobin level by 5.9–6.0% is a common feature shared by all pigs. At the age of 4–6 months, the hemoglobin level and red blood cell count in males are higher by 2.2–2.4 and 18.6–38.6%, respectively. At 8 months, the difference in red blood cell count between female wild boars and domestic pigs drops down to 1.6%. Pregnant sows have a slightly higher hemoglobin level (by 7.5%) because of increased metabolism.

The young Large White domestic pigs of both sexes and the adult females and males [35] from European Russia demonstrated much higher values of red blood cell count and hematocrit than those obtained in this study. On the other hand, the hemoglobin concentrations and mean hemoglobin content were lower.

Gimadeeva *et al.* [36] reported higher red blood cell counts, hemoglobin concentration, and hematocrit in adult males than in other age and sex groups. In the breeding boars, the red blood cell content was by 33.6, 21.4, and 20.9% higher in the barren sows, sows in recent pregnancy, and sows in late pregnancy, respectively. The pregnant sows had a 10.1–10.5% higher red blood cell content than the barren sows. The mean hemoglobin content per erythrocyte decreased by 6.8–7.6% while the hematocrit increased by 5.3–7.6%.

The lowest red blood cell count (3.43×10^{12}/L), hemoglobin (24.86 g/L), and hematocrit (8.71%) belonged to the suck-ling piglets. In terms of hemoglobin per erythrocyte, the suckling piglets exceeded other animals by 8.66–29.78%. The oxidation and reduction processes during active growth require a lot of red cells, white cells, hemoglobin, hematocrit, lymphocytes, monocytes, and granulocytes.

In the juvenile Yorkshire pigs from Massachusetts, USA [37], anesthetized with intramuscular injections of atropine (0.04 mg/kg), tiletamine-zolazepam (4.4 mg/kg), and xialzine (2.2 mg/kg), the red blood cell count was by 10.51% higher in our research while the hematocrit concentration was the same. The total hemoglobin concentration in the adult female domestic pigs from Chile [27] was lower than that in the wild boars from the Kirov Region whereas the hematocrit values were within similar ranges.

Baeza *et al.* [27] reported a decrease in hematocrit in older sows ($p = 0.00$). The underweight sows had lower hematocrit and hemoglobin levels ($p = 0.02$) than those with standard weight. The high hematocrit in the youngest sows could be caused by the increased bone marrow activity during growth [39]. Young animals might be more susceptible to stress, which triggers polycythemia [40, 41].

Other researchers [42, 43] see physiological anemia with a decrease in red blood cells, hemoglobin, hematocrit, and white blood cells as typical of young animals. The increased red blood cell count, erythrocyte mass, and hemoglobin in males compared to females are associated with erythropoietin and testosterone, especially during the mating season [44, 45]. The hematocrit fluctuations may depend on water consumption, intestinal disorders, etc. [44].

The adult wild boars from northeastern Spain demonstrated higher mean hemoglobin content, hema-

tocrit, and hemoglobin concentration in red blood cells than the juveniles [7], as was the case with the wild boars from the Kirov Region.

In Harapin *et al.* [15], the hematocrit and mean corpuscular volume in wild boars exceeded the upper limits for domestic pigs. Wild boars need more oxygen as they are more physically active [16]. When hunted, they experience tress and increased oxygen demand: the spleen contracts and raises the hematocrit level, as well as the total red cell count and hemoglobin. The same phenomenon is typical of canines [46].

For instance, the wild boars with helminthiasis demonstrated significant changes in blood parameters [47]. Poor diet also affects hematological parameters. The wild boars that lost a lot of weight had low hemoglobin and hematocrit concentration, especially during the late stages of starvation [48].

The fat content in bone marrow correlated with the concentrations of hemoglobin, hematocrit, albumin, total globulin, gamma globulin, alkaline phosphatase, and urea in the blood of 26 young boars [49]. This correlation was probably associated with the level of fat reserves and the efficiency of their use.

Platelet parameters. Hematological analyzers define platelet count in peripheral blood and such parameters as relative platelet distribution width by volume, mean platelet volume, and large platelet ratio vs. total platelets.

Automatic calculations provide reliable information on platelet count and their morphological properties [50–52]. Low mean platelet volumes and relative distribution width by volume indicate the predominance of microplatelets in the total platelet count, i.e., inhibited thrombopoiesis, while their growth indicates increased thrombopoiesis. The index of large platelet content indicates the rate of platelet formation in the bone marrow [53–58].

We found no publications on platelet count and their parameters in wild boars. We determined reliable sex differences ($p < 0.05$) in platelet count in adult wild boars (Table 6).

In the domestic pigs, the platelet count was 2–4 times as high as in the boars from the Kirov Region [27, 36]. The sows with insufficient body weight demonstrated below-standard platelet indices [27].

In humans, a drop in platelet count in the peripheral blood or changes in their morphology occur as a result of severe diseases, after surgery with complications, etc. [50].

Leukocyte parameters. We obtained new data for a comprehensive assessment of cellular immunity in wild boars (Table 7). Their lymphocytes were of average size, with a low share of small and large forms. In some juvenile females, the lymphocyte content exceeded 90%. Segmented neutrophils had polysegmented nuclei and poor granularity.

The males demonstrated reliable age differences ($p < 0.05$) in the percentage of lymphocytes and segmented neutrophils. Both juveniles and adults had fewer neutrophils than lymphocytes. The adults had more band and segmented neutrophils than the juveniles, which was consistent with other reports [10, 15].

Eosinophils contain a lot of small granules. Wild animals have more eosinophils than domestic ones [33] because eosinophils counteract parasitic infection. In this research, wild boars had ≥ 20% eosinophils.

The white blood cell count in the Kirov wild boars was at the bottom of the value ranges reported for boars from elsewhere [1–2, 10, 13, 15] and domestic pigs [27, 33, 35–36, 59]. In addition to environmental factors, the differences may be associated with the methods for determining the while blood cell count or the stress that the animals experienced during sampling.

The correlation between the white blood cell indicators, sex, and age was detected in domestic pigs [35, 41]. In the adult males, the leukocyte content in the peripheral blood was by 12–29% higher in females.

In the pre-weaning piglets, the white blood cell count was the lowest. Their body was protected mainly by the antibodies that they received with milk. As they grew and turned to adult diet, the count of blood cells that protected the body from pathogens increased. During this period, they had more leukocytes and lymphocytes than adults, but the count decreased with age [36].

Old females have a lot of lymphocytes, eosinophils, and monocytes [27]. During labor, sows develop neutrophilia and lymphopenia as a result of cortisol increase [59]. Lymphocytes and segmented neutrophils grow in number during early pregnancy, which is typical of other species [56–57, 60–61]. Probably, this phenomenon is connected with lower immune function of maternal cells, suppression of specific immune functions, and a compensatory increase in non-specific immunity [62–63]. Different ranges of eosinophils in farm pigs may be explained by different ventilation conditions because ammonia irritates the respiratory system [40].

Stress reactions. Physical restraint, transportation, chase, and trapping induce a biphasic pattern typical of stress reactions. The biochemistry of stress is described in [59]. Acute stress caused by physical struggle, restraint, or injury may cause significant changes in a number of hematological parameters [64]. Piglets demonstrate increased lymphocyte counts under stress [65]; stress affects the immune response in domestic pigs [1, 66]. Some publications report the effect of anesthetics during live capture on total blood counts in several wild animal species [4, 13, 67–70].

Baeza *et al.* [27] described young animals as more susceptible to stress: it makes the spleen contract, causing relative polycythemia [40]. This hypothesis is supported by the white blood cell count, which is higher in juveniles and may indicate a stress-induced change in the leukogram [39, 59].

Cortisol affects blood cells, reduces lymph flow, and increases segmented neutrophils and total while blood cell counts [71]. In this regard, leukogram stress is more neutrophilic than lymphophilic and is associated with increased hematocrit [59].

Stress during sampling is the largest source of hematological changes in pigs: it develops within minutes and affects the leukogram [4, 59]. In lactating animals, the

increase in segmented neutrophils may be explained by their role in eliminating pathogens in mammary glands by phagocytosis [72].

We obtained most blood samples from wild boars ambushed and killed at feeding sites, which eliminated the stress associated with fright and pursuit induced by other hunting methods.

Diseases and parasites. Parasites also affect hematological parameters; in case of wild boars, this issue remains understudied.

These studies [46, 73] indicated a possible connection between parasites and blood parameters in wild boars. In particular, *Metastrongylus* nematodes in the lungs cause metostrongillosis, which then affects hemoglobin concentration.

Statistically significant differences were established for white blood cells, triglycerides, lactic acid, creatinine, urea, aspartate aminotransferase, and lactate dehydrogenase in helminth-infected (*Ascaris* sp., *Physocephalus sexalatus*, *Ascarops strongylina*) and healthy boars [2]. Another study [1] revealed no changes in hematological parameters of feral pigs with helminths. However, the results may be caused by the differences in sampling methods. Some animals were shot while others were trapped and subjected to severe stress during capture and transportation.

The parasite-induced hematological and biochemical changes in blood serum are probably related to different nutrient absorption, physical activity, and immune response. Bacteremia may affect hematological parameters, in particular, *Borrelia* spirochetes in the blood. These spirochetes are quite common in wild boars and feral pigs, but this issue requires further study [74–77].

The hematology of wild boar depends on habitat, diet, age, and sex. Proteolytic enzymes of neutrophils are important for protein digestion while lipolytic enzymes of lymphocytes are important for fat digestion. In wild animals, the blood composition depends on the season [78]. In Spanish red deer (*Cervus elaphus*) [79], the seasonal hematological fluctuations in erythrocytes, white blood cells, and platelets could be connected with body weight, body growth, antler development, and reproductive cycle. A sudden weather change is stressful because it might complicate foraging process. As a result, lymphocytes enter peripheral blood. Red blood cell parameters increase in autumn rather than in spring as animals prepare for winter.

This paper describes the initial data on the hematology of wild boars from the Kirov Region, Russia. Comparative studies should take into account other limiting factors, e.g., different hematological analyzers, diagnostic methods, habitats, etc. [80]. Moreover, a gap of $\geq$ 72 h between sampling and analysis reduces the data for platelets, white blood cells, lymphocytes, monocytes, and basophils [81]. Their decrease explains the hematocrit growth.

Our results should be interpreted based on these factors. In our research, the morphological parameters depended on sex and age. The resulting clinical profile may help interpret other hematological profiles.

Our data demonstrate an urgent need for further comprehensive research of the hemogram parameters in wild boars.

CONCLUSION

Hemogram reference intervals expand our knowledge in the field of wildlife biology. In this study, we established the hematological profile for the wild boars (*Sus scrofa*, Linnaeus 1758) of different age and sex living in the Kirov Region, Russia. The research revealed reliable differences ($p < 0.05$) between the young and adult females in relative erythrocyte distribution width by volume (standard deviation) and red blood cell thickness. For the juvenile and adult boars, the reliable differences ($p < 0.05$) included the red blood count, hemoglobin, hematocrit, relative red cell distribution width by volume (variation coefficient), erythrocyte area, erythrocyte perimeter, erythrocyte diameter, erythrocyte sphericity index, lymphocyte, and segmented neutrophils. As for the juvenile females and males, the reliable differences ($p < 0.05$) included the red blood cell count and hemoglobin, as well as the area, perimeter, and diameter of red blood cells. In the adult males and females, the difference was in the thickness of red blood cells and platelet count. We also determined the lymphocytic profile.

The single- and multifactorial analysis of variance revealed the effect of age, sex, and weight on the hematological profile. The age affected hematocrit ($p = 0.02$), segmented neutrophils ($p = 0.00$), and lymphocytes ($p = 0.00$). The weight affected hematocrit ($p = 0.02$) and mean corpuscular volume ($p = 0.04$).

The comparative analysis with other domestic and foreign studies revealed certain features typical of wild boars from the Kirov Region, Russia. The variability of blood indicators depended on the living conditions, diet, age, and sex, as well as on the laboratory equipment.

The reference intervals identified in this research may help interpret hematological profiles of other wild boar populations to optimize the game resource management.

CONTRIBUTION

All the authors contributed equally to the study and bear equal responsibility for information published in this article. We also acknowledge the vital participation of A.V. Dolgikh in field research, collection and processing of scientific materials, and dedicate this article to his memory.

CONFLICT OF INTEREST

The authors declared no conflict of interest regarding the publication of this article.

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ORCID IDs
Maria A. Perevozchikova: https://orcid.org/0000-0003-3638-3712
Igor A. Domsky: https://orcid.org/0000-0003-1633-1341
Alexey A. Sergeev: https://orcid.org/0000-0002-9461-5131
Alexander V. Economov: https://orcid.org/0000-0002-0242-8954
Mikhail A. Dolgikh: https://orcid.org/0009-0004-2573-5721