HEMATOLOGICAL ANALYSIS AS A METHOD OF MONITORING PHYSIOLOGICAL STATUS OF MEDIUM CARNIVOROUS MAMMALS IN THE RUSSIAN FAR EAST

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Clinical blood analysis is considered not only a method of assessing the health status of individuals, but also their fitness and welfare, although the interpretation of the obtained data is complicated by the combined influence of various factors. For most wild carnivore species, there is insufficient information about the effect of environmental factors on hematological parameters. Mammals in the Far East are exposed to hard seasonal climate changes, and therefore have physiological adaptations that enable them to survive the cold season. The aim of this study was to estimate the hematological parameters of three widespread species of medium carnivorous mammals in this region, namely *Meles leucurus amurensis*, *Prionailurus bengalensis euptilura*, and *Nyctereutes procyonoides ussuriensis*, taking into account such factors as sex, age and season in the Ussuriisky State Nature Reserve (Russia), as well as to identify the most informative blood indicators of the animal physiological status. In spring and autumn of 2014–2023, blood samples were collected from 103 individuals of the studied carnivores. The animals were captured, immobilised, blood-sampled and released in the wild at the capture site. The number of leucocytes was counted in the field, and their ratio was detected on the blood smears in the lab. The absence of sex differences and the presence of age differences in the studied species were revealed, which corresponds to the already known data on the parameters of mammalian blood. In spring, *Nyctereutes procyonoides ussuriensis* had higher leucocyte count (14.77 ± 1.76 mln/ml and 12.33 ± 1.08 mln/ml (hereinafter, in spring vs. autumn, respectively)), the ratio of neutrophils to lymphocytes (4.87 ± 0.47 and 3.02 ± 0.28), and the percentage of segmented neutrophils (73.12 ± 1.77% and 62.95 ± 2.29%), and the lower percentage of lymphocytes (20.35 ± 1.45% and 25.64 ± 1.49%) compared to autumn. Similar blood patterns were observed in two other species. This may be the result of a decrease in body mass and poor condition of animals, i.e. physiological stress after a long winter period and a lack of food resources. In addition, *Nyctereutes procyonoides ussuriensis* had higher percentages of eosinophils, basophils, and band neutrophils (7.85 ± 1.61%, 3.69 ± 1.26%, 2.15 ± 0.34%, respectively) than *Meles leucurus amurensis* (2.55 ± 1.09%, 0.45 ± 0.17%, 0.55 ± 0.18%, respectively) and *Prionailurus bengalensis euptilura* (1.48 ± 0.59%, 0.30 ± 0.16%, 1.74 ± 0.47%, respectively). These differences are probably determined by the biological characteristics of *Nyctereutes procyonoides ussuriensis*, since this species is an ideal host and carrier of various diseases, and it also has specific morphological features of blood cells. Thus, our results demonstrate the importance of systematic blood tests and the necessity of considering both species-specific characteristics and environmental factors for assessing animal health.

Key words: Amur leopard cat, Asian badger, immune system, leucogram, raccoon dog, red blood cells, white blood cells

Introduction

The wildlife health monitoring has recently gained great importance due to the growing number of emerging problems that affect not only animals but also humans (Rahman et al., 2020; White & Razgour, 2020; Ellwanger & Chies, 2021). Almost all organisms are exposed to a wide range of potential pathogens in the environment, and therefore have developed complex physiological responses, including innate and adaptive immunity to counteract pathogens (Demas & Nelson, 2012). Variability in immune parameters can determine the population dynamics of diseases, which is becoming increasingly essential in the context of changes in environmental conditions, especially due to anthropogenic factors (Herrera & Nunn, 2019; Becker et al., 2020).

One of the priorities in ecological and zoological research is the assessment of the adaptive potential of individuals and populations, which has recently taken on increasing practical significance for the conservation of threatened animal species (Davis et al., 2008; Weiss & Wardrop, 2011; Davis & Maney, 2018). Traditionally, veterinarians are responsible for diagnosing animal health, but ecoimmunology suggests that conservation biology scientists can also raise wildlife health issues using some basic diagnostic procedures (Demas & Nelson, 2012; Maceda-Veiga et al., 2015). Particularly, clinical blood analysis is considered not only a method of assessing the health status of individuals, but also their fitness and welfare. Based on the quantitative and qualitative composition of blood cells, one can estimate the level of innate (neutrophils) and adaptive (lymphocytes) immunity (Weiss & Wardrop, 2011; van Lieshout et al., 2020), and the ratio of neutrophils to lymphocytes serves as one of the indicators of stress (Dhabhar et al., 1996; Davis et al., 2008; Davis & Maney, 2018; Pavlova et al., 2018). Additionally, examining blood smears can reveal the first signs of infection (e.g. an increase in the neutrophil count) or parasitosis (e.g. an increase in the eosinophil count) (Kirk et al., 2010b; Kido et al., 2011; Maceda-Veiga et al., 2015).

Nevertheless, the successful application of hematological analysis when working with wild animal species is challenging, especially in the wild, since the interpretation of the obtained data is complicated by the influence of many factors on individual blood parameters. Studies conducted on various systematic mammal groups have shown the relationship of blood cell composition with sex and age (Pastor et al., 2009; Manjerovic & Waterman, 2012; Stannard et al., 2016; van Lieshout et al., 2020; Soboleva et al., 2021), reproductive and social status (Macdonald et al., 1998; Kirk et al., 2010a), mating system (Nunn et al., 2000, 2003; Tian et al., 2015), presence of parasites, viral and bacterial infections (Kirk et al., 2010b; Pérez et al., 2015), method of collecting blood samples (Kusak et al., 2005; Davis et al., 2008; Pavlova et al., 2018), season (Gaspar-López et al., 2011; Pérez et al., 2015; Stannard et al., 2016; Xu & Hu, 2020), body size and mass (Downs et al., 2020; Naidenko & Alshinetskiy, 2020). However, for most wild species of carnivores, there is insufficient information about the effect of environmental factors on hematological parameters, making it difficult to objectively assess the fitness indicators of individuals and populations.

The Russian Far East, especially its southern part, including Primorsky Krai, is a region of unique biological diversity (Shvarts et al., 1995; Uphyrkina, 1996; Zhuravlev, 1997). Local animal species are exposed to hard seasonal climate changes that may lead to significant environmental consequences, in particular fires and floods (Korytny et al., 2007; Heim et al., 2019; Prikhodko et al., 2020). Changes in ecosystems result in habitat degradation and a sharp reduction in the number and distribution of forest-dependent species (Uphyrkina, 1996). Meanwhile, many animal species are endemic to this area and have physiological adaptations that enable them to survive the cold season (Zhuravlev, 1997).

Especially, members of three families are widespread among medium carnivorous mammals, namely *Meles leucurus amurensis* Schrenck, 1859 (hereinafter – Asian badger) (Mustelidae), *Prionailurus bengalensis euptilura* Elliot, 1871 (hereinafter – Amur leopard cat) (Felidae) and *Nyctereutes procyonoides ussuriensis* Matschie, 1907 (hereinafter – raccoon dog) (Canidae). These species also differ in their overwintering methods and reproductive strategies. Asian badgers hibernate in winter and use various breeding strategies depending on population density (Rozhnov et al., 2014; Zhou et al., 2017). Raccoon dogs are monogamous and hibernate in winter, but they often wake up and move, if necessary (Mulder, 2012; Mustonen & Nieminen, 2018). Amur leopard cats, like most Felidae species, demonstrate a promiscuous mating system and do not hibernate in winter, although their activity and movement patterns change (Erofeeva & Naidenko, 2020; Kovalzon et al., 2022; Erofeeva et al., 2023; Tkachenko, 2023). However, studies of the immune system for carnivores in this area are limited due to the presence and spread of various diseases (Goncharuk et al., 2012; Naidenko et al., 2018, 2019a,b; Gilbert et al., 2020; Seryodkin et al., 2020, 2023), which prevents a comprehensive understanding their health status.

Thus, the aim of this study was to estimate the hematological parameters of three species of carnivorous mammals in the Russian Far East, considering such factors as sex, age and season, as well as to identify the most informative blood indicators of the animal physiological status. We assumed that immune activity may be reduced in spring following a period of low activity during winter (the winter immunoenhancement hypothesis) (Martin et al., 2008; Xu & Hu, 2020), and immune activity in autumn may be higher due to frequent interactions with conspecifics and heterospecifics (Nunn, 2002; Nunn et al., 2003; Tian et al., 2015).

Material and Methods

The study was conducted in the Ussuriisky State Nature Reserve and the Land of the Leopard National Park (Primorsky Krai, Russia) (43.677778° N, 132.545555° E) in 2014–2023. The average altitude of this area is 300–400 m a.s.l. (rarely up to 600–700 m a.s.l.). The monsoon climate is characterised by low winter temperatures (with average January temperature of -17.9°C) and high summer temperatures (with average August temperature of +19.7°C). Annual precipitation varies from 500 mm to 1200 mm, averaging at 700–800 mm, with an average annual humidity ranging from 70% to 80%. The objects of this study were representatives of three families of carnivorous mammals: Asian badger (Mustelidae), Amur leopard cat (Felidae), and raccoon dog (Canidae). Animals were caught in spring (late March – early April) and autumn (late September – early October) using trapeze wooden or all-metal live traps with lures of fish or meat products. The summer seems to be an ineffective period for the capturing due to the high variety of prey for the target species and, consequently, low capture rate. Winter is unacceptable due to low air temperatures and, consequently, the risk of animal death, as well as low activity of target species (especially, Asian badger and raccoon dog). Traps were checked daily in the morning during the periods of animal capturing.

We chemically immobilised the captured individuals with a mixture of tiletamine hydrochloride and zolazepam hydrochloride (Zoletil, Virback, France, by estimated dose 10 mg/kg of body mass). Blood samples for hematologic analysis were collected from the femoral vein into a tube with K3 EDTA (MiniCollect, Greiner Bio-One, Austria) (0.25 ml). We weighted the animals on an electronic scale with 5-g precision and determined their sex and age. After all procedures were completed, the animals were administered atipamezole hydrochloride (Antisedan, Orion Pharma, Finland), ensuring a fast and safe recovery of the animals. The animal handling protocol was approved by the Regulatory Commission of Experimental Research (Bioethics Commission) of A.N. Severtsov Institute of Ecology and Evolution of the RAS, Russia (permission №67 of 16.03.2023). The study was conducted in accordance with the laws of the Russian Federation, the country where the research was performed. A total of 103 blood samples were collected, including 28 samples of Asian badgers (18 males, 10 females; 22 adults, 3 subadults), 26 samples of Amur leopard cats (18 males, 8 females; 16 adults, 10 subadults), and 49 samples of raccoon dogs (22 males, 26 females; 38 adults, 10 subadults). The sex and age of one raccoon dog, as well as the age of three Asian badgers, could not be determined. The animals were divided into subadults and adults according to the external condition of their teeth and the size of their canines (Mbizah et al., 2016; White & Belant, 2016).

We counted the total number of erythrocytes and leucocytes in a hemocytometer according to the standard microscopy method (Harvey, 2012) in the field to avoid time-dependent post-sampling changes. For the definition of leucocyte type counts, we also prepared blood smears, air-drying and fixing them with 100% methanol. The percentage ratio of various types of leucocytes was evaluated by identifying and classifying 100 leucocytes into specific morphologic types (lymphocytes, segmented and band neutrophils, monocytes, eosinophils, and basophils) after Romanovsky staining with a Leica CTR5000D microscope (Leica Microsystems, Germany/Switzerland) at a magnification of × 1000 (Harvey, 2012) at the Joint Usage Centre «Live collection of wild species of mammals» at the A.N. Severtsov Institute of Ecology and Evolution of the RAS (Russia). We determined leucocyte type counts as percentages to provide a differential profile for each individual and calculated absolute counts using the following formula: leucocyte number (counted by hemocytometer) × percentage of leucocyte types / 100. The differential profile reveals the relative abundance of each cell type, while the absolute number expresses how many cells of each type are present per volume (ml) of blood.

Statistical analysis was performed with STATISTICA ver. 10 (StatSoft, Tulsa, OK, USA). All means are given as a mean ± SE; significance level was set at $p < 0.05$. However, in some exceptional cases, p-values between 0.05 and 0.07 were also considered to be tendencies. Of the 15 hematological parameters, 11 ones were normally distributed based on Kolmogorov-Smirnov test ($p > 0.05$). As ANOVA is relatively robust to small departures from normality (Dillon & Goldstein, 1984), this was not an obstacle to applying parametrical tests. We used a factorial design of ANOVA with Tukey HSD post-hoc test (T) for each hematological parameter. Due to the heterogeneity of the available sample, the following methods were used for analysis of 84 values of the erythrocyte count (red blood cells, RBC), 89 values of the leucocyte count (white blood cells, WBC), 68 values of the absolute counts of various types of leucocytes, and 82 values of the percentages of various types of leucocytes and the ratio of neutrophils to lymphocytes (Neu:Lym). Preliminary analysis revealed no sex differences in hematological parameters (44 females, 58 males; ANOVA, not significantly), which allowed us to combine the samples for further analysis. We considered the effects of predictor factors such as age (subadults/adults), season (spring/autumn), and species (badger/cat/dog).

Results

The results showed that the total erythrocyte count did not differ depending on the species ($F_{(2,77)} = 1.58$, $p = 0.21$) (Table 1), age ($F_{(1, 77)} = 0.07$, $p = 0.79$) or season ($F_{(1, 77)} = 0.21$, $p = 0.65$). The total leucocyte count was significantly related to the species ($F_{(2, 82)} = 4.09$, $p = 0.020$), namely raccoon dogs had more cells than Asian badgers (T: $p = 0.037$), especially in spring (T: $p = 0.028$) (Fig. 1). Nevertheless, the effect of age was observed as a tendency ($F_{(1, 82)} = 3.40$, $p = 0.069$) for all the studied species, namely, the presence of 1.44 times on average more leucocytes in adults compared with subadults (T: $p = 0.047$).

Table 1. Hematological parameters in three species of medium carnivorous mammals captured in the Ussuriisky State Nature Reserve, Russia

Parameters	Asian badger			Amur leopard cat			Raccoon dog		
	N	M ± SE	Min–Max	N	M ± SE	Min–Max	N	M ± SE	Min–Max
Erythrocytes, mlrd/ml	24	11.32 ± 1.22	4.00–30.08	22	10.63 ± 1.04	3.26–26.88	38	9.05 ± 0.75	2.00–21.60
Leucocytes, mln/ml	24	9.78 ± 1.64	1.60–36.95	24	12.12 ± 1.61	3.75–34.65	41	14.69 ± 1.24	4.90–48.55
Lymphocytes, mln/ml	16	2.23 ± 0.43	0.46–5.70	21	3.29 ± 0.61	0.67–12.44	31	2.98 ± 0.29	0.69–7.62
Monocytes, mln/ml	16	0.06 ± 0.04	0.00–0.59	21	0.07 ± 0.02	0.00–0.35	31	0.07 ± 0.03	0.00–0.53
Eosinophils, mln/ml	16	0.48 ± 0.26	0.00–3.89	21	0.33 ± 0.21	0.00–4.49	31	1.12 ± 0.26	0.00–5.72
Basophils, mln/ml	16	0.11 ± 0.07	0.00–1.11	21	0.03 ± 0.02	0.00–0.26	31	0.39 ± 0.16	0.00–3.60
Segmented neutrophils, mln/ml	16	8.41 ± 1.67	2.21–28.08	21	8.57 ± 1.17	2.44–25.29	31	10.47 ± 1.36	2.48–42.24
Band neutrophils, mln/ml	16	0.07 ± 0.02	0.00–0.29	21	0.24 ± 0.11	0.00–2.42	31	0.27 ± 0.05	0.00–0.96
Lymphocytes, %	20	23.15 ± 2.05	7.00–48.00	23	25.70 ± 2.35	11.00–48.00	39	21.05 ± 1.41	7.00–42.00
Monocytes, %	20	0.50 ± 0.27	0.00–5.00	23	0.52 ± 0.16	0.00–3.00	39	0.46 ± 0.18	0.00–5.00
Eosinophils, %	20	2.55 ± 1.09	0.00–22.00	23	1.48 ± 0.59	0.00–13.00	39	7.85 ± 1.61	0.00–37.00
Basophils, %	20	0.45 ± 0.17	0.00–3.00	23	0.30 ± 0.16	0.00–3.00	39	3.69 ± 1.26	0.00–41.00
Segmented neutrophils, %	20	72.80 ± 2.22	51.00–91.00	23	70.26 ± 2.57	43.00–88.00	39	64.79 ± 2.51	36.00–92.00
Band neutrophils, %	20	0.55 ± 0.18	0.00–3.00	23	1.74 ± 0.47	0.00–9.00	39	2.15 ± 0.34	0.00–9.00
Neu:Lym	20	3.93 ± 0.57	1.06–13.14	23	3.60 ± 0.43	1.06–8.00	39	4.25 ± 0.50	0.86–13.14

Note: N – number of blood samples, M – mean value, SE – standard error of mean, Min – minimum value, Max – maximum value, Neu:Lym – ratio of neutrophils to lymphocytes.

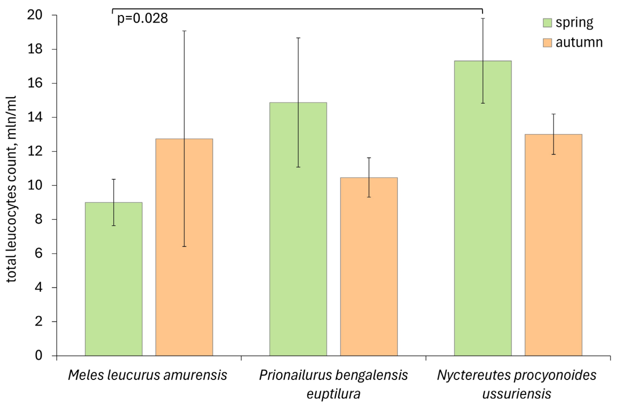

Fig. 1. The total leucocyte count in three species of medium carnivorous mammals captured in the Ussuriisky State Nature Reserve (Russia) in spring and autumn. Histogram bars represent mean values; vertical lines («whiskers») indicate standard errors of the means.

As a rule, the number of various types of leucocytes did not differ depending on the species, age and season. Nevertheless, there were some tendencies in the eosinophil count depending on the species and season ($F_{(2, 62)}$ = 2.90, p = 0.062), namely raccoon dogs had more cells than Amur leopard cats (T: p = 0.068), while the differences were significant in autumn (T: p = 0.015). In addition, the segmented neutrophil count tended to be more abundant in adults compared to subadults (T: p = 0.054).

However, some differences have been identified in percentages of various types of leucocytes. The percentage of lymphocytes varied depending on age ($F_{(1, 72)}$ = 8.38, p = 0.005), where it was 1.43 times on average higher in subadults than in adults (T: p = 0.0004). The percentage of lymphocytes was also associated with the season ($F_{(1.72)}$ = 4.09, p = 0.047), since it was 1.31 times on average lower in spring than in autumn (T: p = 0.003). At the same time, the percentage of monocytes (a second type of agranulocytes) did not depend on the species ($F_{(2, 72)}$ = 0.23, p = 0.79), age ($F_{(1, 72)}$ = 0.19, p = 0.66) or season ($F_{(1, 72)}$ = 2.27, p = 0.14).

The percentage of eosinophils was influenced by species ($F_{(2, 72)}$ = 6.35, p = 0.003) and season ($F_{(1, 72)}$ = 9.26, p = 0.003), as well as their combined effect ($F_{(2, 76)}$ = 5.00, p = 0.009) (Fig. 2). In raccoon dogs, the percentage of these granulocytes was higher in autumn than in spring (T: p = 0.0008) and in Asian badgers (T: p = 0.0005), and higher than in Amur leopard cats in both autumn (T: p = 0.0002) and spring (T: p = 0.018). The percentage of basophils did not depend on age and season, but differed as a tendency depending on the species ($F_{(2.76)}$ = 3.06, p = 0.053). The percentage of these granulocytes in raccoon dogs was slightly higher than in Amur leopard cats (T: p = 0.059) (Fig. 3).

The percentage of segmented neutrophils was influenced by the species as a tendency ($F_{(2, 72)}$ = 3.03, p = 0.055) and season ($F_{(1, 72)}$ = 10.80, p = 0.002), and also as a tendency by the combined effect of species and season ($F_{(2, 76)}$ = 2.92, p = 0.060). The percentage of these granulocytes in raccoon dogs in autumn was 1.31 times on average less than in spring (T: p = 0.0007). These differences were also significant for Amur leopard cats (T: p = 0.021) and Asian badgers (T: p = 0.001). In addition, the percentage of band neutrophils also differed depending on the species ($F_{(2, 72)}$ = 4.52, p = 0.014); e.g. raccoon dogs had a higher percentage than Asian badgers (T: p = 0.023).

The ratio of neutrophils to lymphocytes significantly differed between seasons ($F_{(1, 72)}$ = 9.24, p = 0.003) (Fig. 4). The values of this parameter were higher in spring compared with autumn (T: p = 0.001), especially in raccoon dogs (T: p = 0.008). In addition, although the ratio of neutrophils to lymphocytes did not depend on the age ($F_{(1, 72)}$ = 2.18, p = 0.14), adults had more than subadults (T: p = 0.022).

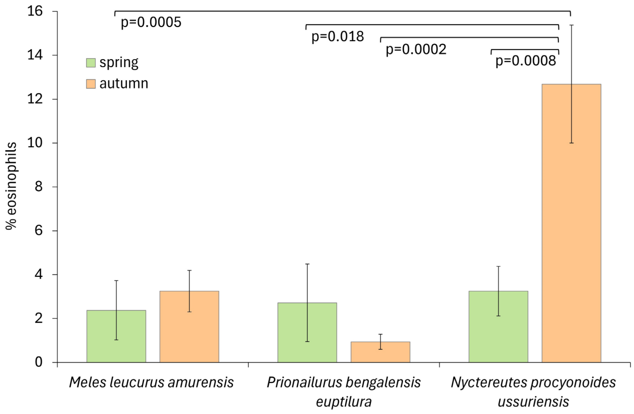

Fig. 2. Eosinophil percentage in three species of medium carnivorous mammals captured in the Ussuriisky State Nature Reserve (Russia) in spring and autumn. Histogram bars represent mean values, vertical lines («whiskers») indicate standard errors of the means.

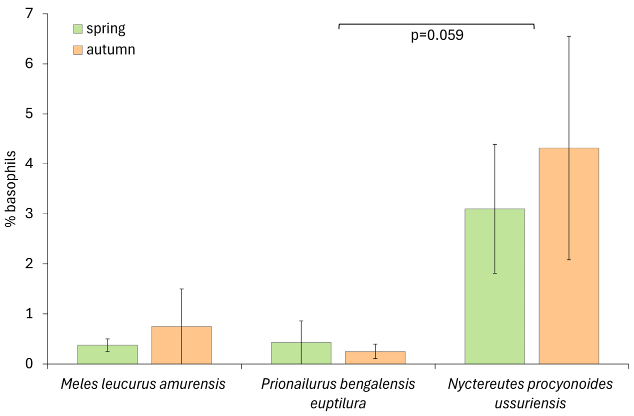

Fig. 3. Basophil percentage in three species of medium carnivorous mammals captured in the Ussuriisky State Nature Reserve (Russia) in spring and autumn. Histogram bars represent mean values. Vertical lines («whiskers») indicate standard errors of the means.

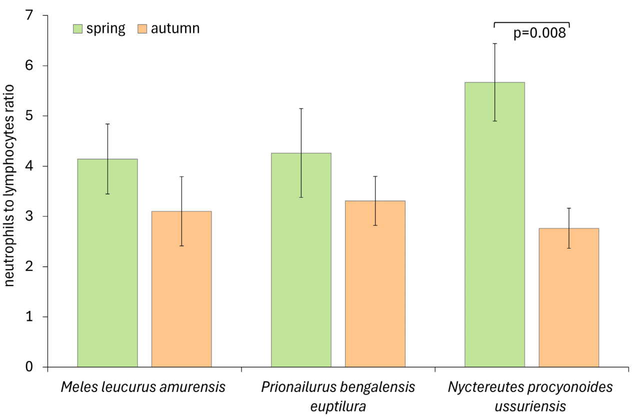

Fig. 4. Ratio of neutrophils to lymphocytes in three species of medium carnivorous mammals captured in the Ussuriisky State Nature Reserve (Russia) in spring and autumn. Histogram bars represent mean values; vertical lines («whiskers») indicate standard errors of the means.

Discussion

Even though various parameters of immunity are actively studied in carnivorous mammals, information on hematological parameters remains insufficient. Moreover, often only the values of blood parameters are described, without considering the influence of any factors, which may be especially important when assessing the physiological status of animals in wild populations.

Particularly, data on the hematology of the three species of medium carnivores studied by us are extremely fragmentary. There are no complete data for the Asian badger, but the blood parameters of *Meles meles* Linnaeus, 1758 in the wild (Winnacker et al., 2008) and in a wildlife rehabilitation facility (Gelli et al., 2024) were previously described. Compared with these data, the counts of erythrocytes, leucocytes, neutrophils, lymphocytes, and eosinophils in Asian badgers in our study were higher, while the monocyte count and the ratio of neutrophils to lymphocytes, on the contrary, were lower. Such significant differences both with individuals in the wild and in captivity suggest the presence of not only interspecific differences, but also immune reactions associated with the habitat conditions of the Asian badger (Becker et al., 2020; van Lieshout et al., 2020). For the Amur leopard cat, only the counts of erythrocytes and leucocytes, as well as the ratio of neutrophils to lymphocytes in captive individuals are known (Pavlova et al., 2018). In addition, since the Amur leopard cat is a subspecies of *Prionailurus bengalensis* Kerr, 1792 (hereinafter – Bengal cat), it is also possible to consider the hematological parameters described for this species in zoos (Salakij et al., 2010; Naidenko & Alshinetskiy, 2020). According to these studies, the erythrocyte count in Amur leopard cats in our study corresponds to the already available data (Salakij et al., 2010; Pavlova et al., 2018; Naidenko & Alshinetskiy, 2020). The leucocyte count and their various types, primarily neutrophils and lymphocytes, on the contrary, vary significantly. For example, the leucocyte count in the Amur leopard cat, both in captivity and in the wild, is higher than in the Bengal cat (Salakij et al., 2010; Naidenko & Alshinetskiy, 2020), which may be related to the body size (Downs et al., 2020; Naidenko & Alshinetskiy, 2020), since Amur leopard cats are larger than Bengal cats (5.0 kg (3.4–7.4 kg, our data) and 3.2 kg (3.0–3.5 kg, Salakij et al., 2010), respectively) or with the former adaptation to more severe living conditions (Becker et al., 2019). The ratio of neutrophils to lymphocytes in Amur leopard cats in the wild is 1.4 times higher than in individuals in captivity (Pavlova et al., 2018), and more than 2.5 times higher than in Bengal cats in captivity (Salakij et al., 2010; Naidenko & Alshinetskiy, 2020). This implies a significant shift towards neutrophils in individuals in the wild, which we consider below. As for raccoon dogs, hematological parameters have already been described in subadults on the farm and in adults in the zoo (Kido et al., 2011; Rui et al., 2011). However, in the individuals in our study captured in the wild, the counts of erythrocytes, leucocytes, neutrophils, eosinophils, and basophils, as well as the ratio of neutrophils to lymphocytes are significantly higher than in individuals in the zoo (Kido et al., 2011). An increased number of red blood cells may be associated either with exposure to frequent and prolonged stressors, or with the presence of blood parasites or ectoparasites (Johnstone et al., 2017). The possible causes of the increased leucocyte and granulocyte counts seem to reflect the living conditions of raccoon dogs in the wild and are listed below.

It should also be considered that the common procedure of collecting blood samples (handling time) in wild animals can be extremely stressful and involves immobilising animals with trapping and anesthesia for extended period, which can also affect blood parameters (Dhabhar et al., 1996; Kusak et al., 2005; Davis & Maney, 2018; Pavlova et al., 2018). If an increase in glucocorticoids level plays the role of a «messenger» for such stress reaction, then a change in the circulating leucocyte count and, accordingly, an increase in the ratio of neutrophils to lymphocytes is a «response» (or at least one of many physiological reactions) to this stress (Davis & Maney, 2018). This may explain the existing differences in hematological parameters in individuals in captivity and in the wild, however, using the same method of trapping animals and collecting blood samples in our study allows us to compare the obtained data considering sex, age, season and species.

Even though many studies describe differences in hematological parameters in males and females of various mammal species (Kusak et al., 2005; Pastor et al., 2009; Manjerovic & Waterman, 2012; Standard et al., 2016; van Lieshout et al., 2020), the effect of sex is not so clear. There are also many studies indicating the absence of sex differences (e.g. Chang et al., 2006; Barnes et al., 2008; Castellanos et al., 2010; Kirk et al., 2010a; Gelli et al., 2024), like in our case. The variability in the available data is likely related to the reproductive status of individuals. For example, Kirk et al. (2010a) have described differences between males and lactating females, but not non-lactating ones in *Ursus maritimus* Phipps, 1774. In addition, sex differences in blood parameters may be related to the biological characteristics of specific species (Hufschmid et al., 2014).

One of the indicators of animal health is the erythrocyte count and indices, in particular the concentration of hemoglobin and hematocrit (Johnstone et al., 2017). Erythrocytes can provide insight into aspects of physiology (e.g. aerobic function) that are quite difficult to investigate, especially in individuals in the wild. There is also some evidence that erythrocytes can perform direct functions in ensuring the immunocompetence of vertebrates, especially when they are formed throughout the life of an animal (Morera & MacKenzie, 2011). Particularly, some mammals, birds, reptiles and fish exhibit antimicrobial reactions based on erythrocytes, such as the formation of immune complexes and the production of reactive oxygen forms (Morera & MacKenzie, 2011). However, we found no differences in the number of red blood cells in carnivores depending on the species, age, or season in this study. Probably, this is likely due to the fact that mammals adapted or acclimatised to cold environments exhibit higher blood viscosity, defined as the ratio of blood plasma volume to blood cell volume, primarily erythrocytes, compared to species inhabiting a warmer climate (Deveci et al., 2001; Palenske & Saunders, 2002), and this viscosity is maintained at a relatively constant level. In this regard, the number of red blood cells does not change significantly during the year either, and in subadults it already seems to reach the level of adults. Definitely, this suggestion needs to be tested and the data on hematocrit values are obviously needed to confirm this assumption.

Nevertheless, the leucocyte count and the ratio of neutrophils to lymphocytes in subadults were lower than in adult carnivores. On the contrary, the percentage of lymphocytes was higher in subadult animals in our study. A similar blood pattern reflects the formation of the immune system in mammal ontogenesis, and it is typical not only for carnivorous mammals (Winnacker et al., 2008; Pastor et al., 2009; Kirk et al., 2010a; Græsli et al., 2014; Soboleva et al., 2021), but also for other species (Weiss & Wardrop, 2011; Harvey, 2012; Stannard et al., 2016; Xu & Hu, 2020). Accordingly, at the earlier stage of ontogeny, the percentage of lymphocytes is higher than in older animals and it affects the ratio of neutrophils to lymphocytes.

Animals in the temperate zone confront seasonal environmental fluctuations and experience changes in resource availability, climatic conditions, and the threat of infection throughout the year (Nelson, 2004; Martin et al., 2008). Immune function is usually enhanced to compensate for the immunosuppressive effects of stressors that occur in winter, such as low ambient temperature and limited feed availability (Demas, 2004; Martin et al., 2008). According to our assumption, the highest differences in the hematological parameters of carnivores in this study were revealed precisely in relation to the season and species. Particularly, individuals had a higher leucocyte count and the proportion of segmented neutrophils, and there was also a lower percentage of lymphocytes in spring compared to autumn. Accordingly, the ratio of neutrophils to lymphocytes was also higher in spring than in autumn. These patterns in seasonal changes in blood parameters relate primarily to raccoon dogs. In addition, this species had a higher percentage of eosinophils, basophils and band neutrophils compared to Asian badgers and Amur leopard cats. Moreover, the percentage of basophils in raccoon dogs was significantly higher than in the other two species in both seasons of the year, while the percentage of eosinophils was significantly higher in autumn than in spring. The raccoon dog is an omnivorous mammal which often scavenging large carnivores' prey. It may increase the contact of the animals with various pathogens (contained in meat) and, accordingly, increase the counts of neutrophils and eosinophils, which has been described in large cats (Naidenko & Alshinetskiy, 2020). This reasonable explanation, taking into account seasonal differences and an increase in the count of leucocytes (e.g. eosinophils) in autumn, confirms the assumption that the temperature in autumn was higher than in early spring that change the kill features faster.

It is believed that a similar blood pattern in some species may be associated with reproductive efforts during the breeding season (Fancourt & Nicol, 2019) or seasonal morphological changes (Gaspar-López et al., 2011). However, for animals in the temperate climate zone, the winter immunoenhancement hypothesis is more expected, suggesting that activation of immunity in the cold season helps animals to resist pathogens and, consequently, increases their survival (Martin et al., 2008; Xu & Hu, 2020). Nevertheless, the results of our study did not confirm this assumption and rather indicate the activation of the immune system to the weakened status of the animal organism after winter. A long period of forced rest (i.e. hibernation or a similar condition) and a shortage of food supply can lead to a decrease in body mass and poor condition of animals (Johnstone et al., 2012; Naidenko et al., 2014; Pérez et al., 2015; Xu & Hu, 2020). Thus, the presence of physiological stress and an increase in physical (muscular) activity in spring is characterised by an increase in leucocytes and neutrophils (Weiss & Wardrop, 2011), which were identified in our study, primarily in raccoon dogs. In addition, with the onset of warm weather, the number of contacts between conspecifics and heterospecifics increases, which implies a more active transmission of pathogens between individuals (Korenberg, 2000; Nunn et al., 2003; Lindenfors et al., 2007). The reproduction of pathogens may also increase under higher temperature and altogether it is probably the reason for the increase in the percentage of eosinophils and lymphocytes in autumn, since these cell types are responsible for fighting against parasitic and viral infections, respectively (Weiss & Wardrop, 2011; Harvey, 2012).

Special attention should be paid to the fact that most of the seasonal differences in hematology described by us primarily relate to raccoon dogs. Individuals of this species have a smaller percentage of segmented neutrophils and a larger percentage of eosinophils, basophils, and band neutrophils compared with Asian badgers and Amur leopard cats. It is considered that the biological characteristics of the raccoon dog make this carnivore an ideal host and carrier of various diseases (Sutor et al., 2014; Myśliwy et al., 2022), which probably affects the blood parameters of this species and may indicate inflammatory processes, as well as viral and parasitic infections. On the other hand, it was previously described that eosinophils in raccoon dogs have several morphological features compared to other mammal species (Kalinina et al., 2023). Probably, similar properties may also be characteristics of other types of leucocytes in this species, which causes the differences we found between the three species of carnivores and requires further study.

Conclusions

The use of clinical blood analysis to study wildlife is still not the most widespread method. However, it helps to expand the set of non-lethal procedures useful for monitoring the physiological status of wild animals. Systematic blood tests, particularly, can serve as early indicators of non-welfare of individuals in wild mammal populations due to stress or pathogens, significantly impacting reproduction and survival of animals. According to our study, the most informative hematological parameters are not only the leucocyte count, but also the percentage of lymphocytes, neutrophils, and eosinophils, and, consequently, the ratio of neutrophils to lymphocytes. Moreover, the most reliable results for individuals in the wild are provided precisely by the percentage of various types of leucocytes, which is estimated using blood smears. However, when analysing the data obtained, careful consideration of species, age, and seasonal differences in blood parameters is essential. Additionally, the role of diseases and infections in seasonal changes in hematological parameters requires further detailed study.

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ГЕМАТОЛОГИЧЕСКИЙ АНАЛИЗ КАК МЕТОД МОНИТОРИНГА ФИЗИОЛОГИЧЕСКОГО СОСТОЯНИЯ СРЕДНИХ ХИЩНЫХ МЛЕКОПИТАЮЩИХ ДАЛЬНЕГО ВОСТОКА РОССИИ

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Клинический анализ крови считается методом оценки не только состояния здоровья животных, но также их приспособленности и благополучия, хотя интерпретация полученных данных осложняется совместным влиянием разнообразных факторов. Для большинства диких видов хищных млекопитающих отсутствует информация о влиянии факторов окружающей среды на гематологические показатели. Млекопитающие Дальнего Востока подвержены резким сезонным изменениям климата, в связи с чем обладают физиологическими адаптациями, позволяющими им пережить холодное время года. Целью данного исследования было оценить гематологические показатели трех широко распространенных в этом регионе видов средних хищных млекопитающих, а именно *Meles leucurus amurensis*, *Prionailurus bengalensis euptilura* и *Nyctereutes procyonoides ussuriensis* с учетом таких факторов, как пол, возраст и сезон, в Уссурийском государственном природном заповеднике (Россия), а также выявить наиболее информативные параметры крови, отражающие физиологическое состояние животных. Весной и осенью в 2014–2023 гг. были собраны образцы крови у 103 особей исследуемых видов хищных. Животные были отловлены, обездвижены и после получения проб крови выпущены в дикую природу на месте отлова. Количество лейкоцитов подсчитывали в полевых условиях и определяли их соотношение на мазках крови в лабораторных условиях. Было выявлено отсутствие половых различий и наличие возрастных различий у всех трех видов, что соответствует уже известным данным о параметрах крови млекопитающих. Весной у *Nyctereutes procyonoides ussuriensis* было более высокое количество лейкоцитов (14.77 ± 1.76 млн/мл и 12.33 ± 1.08 млн/мл (здесь и далее – весной и осенью, соответственно)), соотношение нейтрофилов и лимфоцитов (4.87 ± 0.47 и 3.02 ± 0.28) и доля сегментоядерных нейтрофилов (73.12 ± 1.77% и 62.95 ± 2.29%), а также более низкая доля лимфоцитов (20.35 ± 1.45% и 25.64 ± 1.49%) по сравнению с осенью. Сходные изменения параметров крови наблюдались у двух других видов. Это может быть результатом снижения массы тела и худшего состояния животных, то есть физиологического стресса после длительного зимнего периода и нехватки пищевых ресурсов. Кроме того, у *Nyctereutes procyonoides ussuriensis* были более высокие доли эозинофилов, базофилов и палочкоядерных нейтрофилов (7.85 ± 1.61%, 3.69 ± 1.26%, 2.15 ± 0.34%, соответственно), чем у *Meles leucurus amurensis* (2.55 ± 1.09%, 0.45 ± 0.17%, 0.55 ± 0.18%, соответственно) и *Prionailurus bengalensis euptilura* (1.48 ± 0.59%, 0.30 ± 0.16%, 1.74 ± 0.47%, соответственно). Вероятно, данные различия определяются биологическими особенностями *Nyctereutes procyonoides ussuriensis*, поскольку этот вид является идеальным хозяином и переносчиком различных заболеваний, а также обладает специфическими морфологическими особенностями клеток крови. Таким образом, наши результаты демонстрируют важность систематических анализов крови и необходимость учитывать как видоспецифичные характеристики, так и факторы окружающей среды для оценки здоровья животных.

Ключевые слова: азиатский барсук, дальневосточный лесной кот, енотовидная собака, иммунная система, лейкограмма, лейкоциты, эритроциты