Hematologic markers of stress resistance of service Belgian Shepherd Dog (Malinois)

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Abstract. The morphological composition, the level of thyroxine and cortisol in the blood and the crystallogram of blood serum in 36 service Belgian shepherd dogs (malinois) of specialized military kennels of Russia were studied. The tendencies of changes in the blood formula depending on age, season, gender, category of use were identified. In a physiologically mature age, malinois has a higher platelet count, hemoglobin. In repair (training) animals older than two years, the leukogram and crystallogram are more stable, regardless of the season, while the correlation with the hormones thyroxine and cortisol is higher in younger animals. Belgian shepherd dogs in service categories have a tense adaptive status according to the Garkavi stress reactivity index. Significantly fewer monocytes and more lymphocytes in the raspberry mine search service, which corresponds to the reactivation reaction. According to the morphological picture of crystallograms in service malinois, a depressive and reactive state prevails. It is recommended to select malinois for official use more carefully.

1. Introduction
In the last decade, official dog training has sharply increased interest in using the Belgian Shepherd Dog (Malinois) as a special tool. Practitioners have found that dogs of this breed are not inferior, in a number of indicators in the service they even surpass German shepherds used in law enforcement agencies around the world [1]. First of all, these are good working qualities, quick response to stimuli, and high-quality training results [2]. At the same time, dog handlers note “emotionality”, “sensitivity”, “restlessness”, and the speed of phase transitions “excitation-inhibition” in dogs of this breed. According to foreign data, in the populations of European and North American military Malinois, a genetic pathology is detected associated with a disruption in the dopamine transporter gene, idiopathic epilepsy, the symptoms of which are revealed during the official use of the dog. Owners reported seizures and unpredictable behavioral changes: dog eye glazing, lack of response of dogs to environmental stimuli and loss of behavioral inhibition, including attacks directed at the owner and bites. Dogs with severe behavioral changes can be dangerous for humans and other dogs. Prevalence among dogs’ ranges from 0.5% to 5.7%. Behavioral symptoms, such as a blank look or lip smacking, may be the only manifestations of such non-generalized seizure activity. It was proved that in Malinois the occurrence of “pure” expression of this gene is significantly higher [3; four]. In behavioral observations, Malinois showed a high incidence of stress-related behaviors such as squats (low posture) and increased yawning. In this connection, it is not surprising that such evidence of stress-
expressed behavior in Malinois is also recorded in Russia. Along with this, service dogs in modern conditions are often used in harsh conditions, which is associated with their use in isolation from places of constant deployment, with transport transfers, climatic loads, changing feeds, and the use of special equipment. In addition, dogs of service breeds, traditionally cultivated in Russia, have accumulated such shortcomings as a decrease in general resistance to the action of foreign factors and stressors, a deterioration in the quality of preparation of animals in training courses, malfunctions, a decrease in physical strength and endurance, reproductive disorders [5; 6]. All this indicates a low stress resistance of service animals. In this regard, one of the paramount tasks of a dog handler, veterinarian, livestock specialist is the timely recognition of homeostasis disorders, presnosological diagnosis of possible deviations from the norm, and the assessment of adaptive reserves. This is of particular relevance with respect to the Belgian Shepherd Dogs (Malinois) for official use, since so far there are few scientific works on assessing the functional state of the Belgian Shepherd Dogs, and the popularity of the use of animals of this breed in the power structures of Russia is growing from year to year.

Adaptation, according to the ideas of G. Selye, is inherent in all known forms of life and is so comprehensive that it is often identified with life itself [7]. The transition from health to disease from the perspective of the adaptation direction is the process of reducing the degree of adaptation of the body to environmental conditions, the result of depletion and failure of adaptation mechanisms. At the same time, health from the point of view of adaptation theory can be characterized by the successful adaptation of the organism to difficult environmental conditions [8]. As it is shown by scientists, the study of the prospects for using the laws of adaptation is of direct practical importance [9; 10]. It became the main argument in choosing the direction of our research in relation to studying the functional state of service dogs, since the effectiveness of their practical application in military service depends on it. Based on the foregoing, the aim of the work was to study the stress reactivity of dogs of the Belgian Shepherd (Malinois) breed of specialized kennel of the National Guard of the Russian Federation with accessible objective physiological methods for assessing blood counts.

2. Object and methods of research
The object of the study was a domestic dog (Canis familiaris). We used dogs of the Belgian Shepherd (Malinois) breed from specialized kennels of the troops of the National Guard of the Russian Federation aged 1 year to 7 years in aviary. The first group consisted of 6 malinois of the military kennel of the city of Perm (Western slopes of the Middle Urals), the second - 30 malinois of the cynological center of the city of Moscow (the central zone of the East European Plain). The animals had a satisfactory history, underwent timely hatching, deworming, and vaccination. The conditions of keeping and feeding the studied dogs corresponded to regulatory requirements. The material for the study was whole blood and blood serum. We took into account the category of dogs, age, gender, season of the study. To assess stress reactivity, the following methods were used: a clinical blood test with a blood smear microscopy and a leukocyte formula; calculation of the lymphocytic index and assessment of the type of non-specific adaptive reactions according to L. Garkavi et al. [11]; chemiluminescent analysis method to assess the level of hormones cortisol and thyroxine; wedge-shaped dehydration of blood serum by the "open drop" method to establish the normative, reactive or depressive state [12-14]. The data obtained were subjected to statistical processing and correlation analysis using the criterion of reliability of student differences using Microsoft Excel and Stadia. The studies were carried out as part of the research work “Prospective directions for the activity of the cynological service of the national guard forces of the Russian Federation to study the functional state of service dogs in order to increase the efficiency of their use”.

3. Results and discussion
The most important physiological indicators of the dog’s body condition are evaluated by objective reliable methods of blood testing, as the most informative biological fluid, which is a “mirror of health” [8].
An important diagnostic value in medical and veterinary practice, as well as in determining adaptive capabilities, is a general blood test, and especially the leukocyte formula. It was found that the morphological composition of the blood of Belgian shepherds in some indicators has differences between the animals of the two studied samples, and in some cases, from generally accepted standards (table 1).

**Table 1. Hematological indicators of service malinois in Perm and Moscow.**

| Blood indicators | Perm | Moscow | Normal limits |
|------------------|------|--------|--------------|
| red blood cells, cells x 10^12 in 1 l | | | |
| hemoglobin, g/l | | | |
| white blood cells, cells x 10^9 in 1 l | | | |
| stab neutrophils | | | |
| segmented neutrophils | | | |
| eosinophils | | | |
| monocytes | | | |
| lymphocytes | | | |
| lymphocytic index (Garkavi) | | | |
| type of adaptation reaction | | | |
| platelets, number of cells x 10^7 in 1 l | | | |

Note: * - significant differences (p ≤ 0.05)

An analysis of the data on the blood composition of dogs in Perm suggests a trend in a number of characteristics depending on the season of the year, with reliability (p≤0.05) only by the lymphocytic index. By spring, the number of red blood cells and hemoglobin increased slightly (r = 0.9), and the number of platelets increased significantly. The erythrocytic characteristics in this case did not go beyond the reference intervals in both seasons. The concentration of hemoglobin in the blood was affected by climatogeographic conditions (season of the year, the Urals zone), age, and gender. In autumn and spring, the indicator is lower in females, which is explained by the effect on erythropoiesis, in addition to the main hematopoietins, male sex hormones [16]. A number of authors note that the content of red blood cells and hemoglobin decreases by the winter period and increases by the spring-summer period [17]. The objectification of our results according to these indicators can be argued by the fact that the initial studies were carried out at that time when autumn-winter conditions were already operating in the region (temperature conditions ranging from 0 °C to minus 7–10 °C, partial snow cover), therefore, the adaptive regime of animals works in tension. The platelet count was also recorded in autumn significantly lower than normal by 36%, a possible cause of such thrombopenia may be a latent infection in the transition period "autumn-winter", as it can contribute to the activation of latent infections and stimulation of certain hematological reactions. It is known that the blood system undergoes significant changes depending on the season of the year, climatogeographic and biogeochemical environmental conditions [18; 19]. Moreover, in the seasonal dynamics of peripheral blood parameters in humans, some researchers note its inconsistency and dependence on numerous factors acting on a person in his life [20]. We agree with the authors, since both human and dog make up two complex interacting systems with the environment.
With regard to leukocytes and their differential forms, the main criterion for exposure was probably the age-related development of malinois. With an equal average level of these cells, their total number in young animals varied ambiguously (in two of three dogs it increased, in one it fell sharply). Also, in two individuals of the older age group, on the contrary, its decrease was noted, in one male it was unchanged.

Evaluation of the leukocyte formula depending on the change of the season of the year, age or gender revealed some significant features. Both in autumn and spring, a high proportion of eosinophils was found (83% of dogs have higher normative limits) with a tendency to lower values in spring regardless of age and gender and normalization of the eosinophilic profile in 33% of dogs.

Since service dogs undergo timely examinations, deworming and hatching, the likely cause of an increased number of eosinophils may be an individual allergic reaction (possibly to the components of dry food, used as a treat for training). Seasonal dependence was found to increase the proportion of segmented neutrophils, and the number of younger forms, stab-neutrophils, positively correlated with the age-related development of dogs, having significantly increased by the age of 2 more than 2 times from 6% to 14.7%. A tendency towards a decrease in the number of lymphocytes in the leukogram by spring by 7% was noted. Taking into account the age criterion (figure 1), the drop in the level of data of agranular cells was more significant (p≤0.05).

![Figure 1. Age-related dynamics of the relative content of lymphocytes and stab neutrophils in the leukoformula of Belgian shepherd dogs in Perm.](image)

Studies of seasonal fluctuations in the leukoformula are described by different authors. So, M.M. Emkuzheva et al. [19] found similar fluctuations in rodents regardless of gender. Earlier, we found a similar picture of reliable seasonal fluctuations in the level of neutrophils in dogs of the German Shepherd breed [21]. The level of eosinophils, on average 35% higher than the normal limit (p ≤ 0.05), is especially significant in the group of investigated malinois PVI. Probably, such an “eosinophilic” status is associated with the originality of the breed as a whole and correlates with its archetypal status among other service breeds.

By the percentage of lymphocytes and the lymphocytic index, we estimated the seasonal fluctuations in the adaptive status of dogs. A signal indicator of adaptation reactions is the content of lymphocytes in the blood leukoformula, as well as a specific lymphocyte index [11], which reflects the ratio of neutrophils / lymphocytes. A correlation of the number of lymphocytes with the state of the immune and endocrine systems, biochemical parameters, and nonspecific resistance of the body has been established by many scientists. So, stress reactions are usually accompanied by lymphopenia and neutrophilia. On the contrary, the reactivation reaction is characterized by lymphocytosis and excessively high activity of the nervous and endocrine subsystems of the body with tight
synchronization, and then desynchronization of processes, with excessive hyperargicity, and is, like stress, a non-specific basis for many pathological processes [11; 22-24].

We have taken beyond the normal range an indicator of the level of lymphocytes of 14-25% [25-26]: adaptive reactions in dogs are differentiated by a stress response - less than 14% of lymphocytes; training reaction - 14–19%; reaction of calm activation - 19–21% and increased activation - 22–25%; reactivation reaction - above 25%. In the part of the sample, the adaptation reaction changed over the period from autumn to spring (table 2). In 83% of the sample in the spring, the type of reaction corresponds to the training reaction. However, in dogs of the older age group, in comparison with the autumn period, the reactivity did not change: in two - the reaction of training, and in one male, with relatively constant hematological parameters, “reactivation” was noted in autumn and spring. The achievement of physiological maturity by three animals contributed to the transition from increased activation and reactivation to the training reaction. According to the theory of non-specific adaptive reactions, the training reaction develops in response to a threshold, relatively small value of the acting factor. Consequently, climatogeographic conditions, as well as, probably, regular training loads by the end of May, when the study was conducted, affected this indicator and in young dogs the stress reactivity was switched so sharply.

The optimal type of adaptive state is the state of calm or increased activation, when all functional systems are in the operating mode of response to medium-sized stimuli, the level of nonspecific resistance is increased, which indicates a high activity of regulatory systems and their ability to respond flexibly and adequately to various environmental factors. With this type of reaction, the best performance is noted. Reactivation is characterized by high performance with an unexpected breakdown. For people, special methods of activation therapy have been developed [11], which cannot be said for service animals, since such a direction of research is new.

Table 2. Typology of adaptive reactions of the Perm’s Belgian Shepherd Dog in autumn and spring.

| Name and age of the dog | Autumn | Spring |
|------------------------|--------|--------|
| Up to 2 years old      |        |        |
| Zhanna                 | reactivation | training |
| Zhalo                  | increased activation | training |
| Zhak                   | increased activation | training |
| More than 2 years old  |        |        |
| Prime                  | training | training |
| Pallada                | training | training |
| Pride                  | reactivation | reactivation |

The reactivation reactions we discovered are a clear sign of tension - since the relative amounts of not only lymphocytes (more than 25%), but also stab neutrophils and eosinophils, go beyond the normative upper limits. The biological meaning of the training reaction identified in most of the sample is the preservation of homeostasis under the action of weak, insignificant stimuli. At the same time, anabolic processes predominate a little, relatively good working capacity is observed, but low work speed, decreased excitability, sleep and appetite are satisfactory, however, there are no significant suppressions or stimulation of the body’s protective subsystems.

When analyzing the ratio of "lymphocytes / neutrophils" in the leukoformula according to the age criterion, the mutual redistribution of these cells from autumn to spring is obvious, especially in dogs aged from one to two years (p ≤ 0.1). Such age dynamics of these hemogram parameters has been established for humans: the onset of age from puberty and older leads to a decrease in the proportion of lymphocytes and an increase in neutrophils [27]. Also, the proportion of these forms of white blood cells is described in specific indices. Simultaneously with the assessment of the adaptive non-specific reaction, the calculation of the corresponding lymphocytic index (the ratio of “lymphocytes / neutrophils”), often referred to as the stress reactivity index, showed that its values range from 0.25 units. up to 0.77 units in the fall and from 0.19 to 0.28 units. in 83% of dogs in the spring. Moreover,
the correlation of the index was logically negative for neutrophils, within the limits of average strength, and for lymphocytes there was a positive strong functional relationship ($r = 0.97$ and $r = 0.98$ in both periods of the study). This is a natural picture. As mentioned above, lymphocytes are the determining cells in the reactions of stress and nonspecific adaptability. Differentiating the influence of the age of dogs, an interesting fact was the decrease in the dependence of the index on the lymphoid population in young individuals by spring and the preservation of this relationship in adult animals at the level of $r = 0.98$. This, apparently, can be explained by the greater maturity, stability and stability of the most important functional system for maintaining homeostasis - the blood system in adult animals.

In addition, in the spring we also evaluated the level of the main stress hormone — cortisol and the “metabolic” hormone — thyroxine, since it is important to take into account the ratio of these hormones in the analysis of the typology of adaptation reactions. On average, the concentration of hormones in the blood was within the standard values, but thyroxine was closer to their lower border (table 1). The variability of the amount of cortisol was 42%, which indicates the likely normativity for the studied dogs of a state of increased reactivity, and they do not experience the stress of homeostasis support systems. Correlation was established between the lymphocytic index and hormone concentrations. The relationship is more significant depending on the age of the animals. If the correlation “thyroxine-cortisol” in two-year-old dogs was $r = -0.64$, then in older dogs $r = +0.82$, while the age of two years more directly affected the relationship between thyroxine and the number of leukocytes, neutrophils, to a lesser degree, back with a share of lymphocytes. For cortisol, the opposite picture is characteristic: to a greater extent, this hormone determines the level of lymphocytes than neutrophils and negatively correlates with leukocytes. As a result, in Malinois, two years old, the Garkavi lymphocytic index is interconnected with both hormones: $r = -0.91$ for thyroxine, $r = +0.90$ for cortisol. This is explained by the fact that cortisol is one of the leading regulators of stress: the higher the tendency for a reactivation reaction, the greater the concentration of this hormone. Concerning thyroxin, its role as a metabolic activator is obviously reflected: the lower its level, the less intense the metabolism, but the more chances the animal has to go into reactivation and break further into stress. In animals of the older prdgroup, the degree of correlation is much weaker, which is probably due to age-related stabilization of adaptation systems, despite the fact that lymphoid characteristics, as in young dogs, indicate a high reactivity.

The hematological indices of Malinois of the second group, Moscow, showed similar trends in the blood picture. The number of red blood cells did not differ from the first group, in the spring - platelets, hemoglobin levels are close (table 1). Slightly, 10% higher white blood cell count. Similarly to dogs of the first sample, eosinophilia was found: in 33% of dogs, eosinophils in the leukoformula ranged from 12 to 25%, significantly exceeding the norm.

Differentiation of data taking into account the Garkavi criterion showed that 13% of the second sample were in a state of stress, training and calm activation, 20% of the individuals were increased in activity, and the remaining 53% were in a state of reactivation at the time of the study, with 88% of malinois being reactivated - males. In addition, the hereditary dependence of such an adaptive state is monitored - many litter dogs had either a reaction of increased activation or reactivation. So, a significant number of animals are characterized by states of adaptation stress. Comparing the results with the literature, it was noted that A.V. Sevryukov [22], indicating the upper normative framework of lymphocytes in 30%, refers the 30–40% variant to reactions of increased activation, although the author of the theory of nonspecific adaptive reactions claimed that the excess of the upper limits of the level lymphocyte count is a reactivation. Consequently, so far there is no consensus regarding the study of dog blood by this criterion.

The lymphocytic index in dogs in Moscow averaged $0.41 \pm 0.033$ units, with limits of 0.13 units, up to 0.95 units and 45% variability. A similarly low number of lymphocytes in malinois compared with German shepherds, was noted in the work of O.S. Poptsova [1]. So, E.D. Sotnikova [24] in the results of work on modeling stress in dogs indicates that in 2-3-year-old dogs the index was 0.54 units before the experiment, and against the background of immobilization and pain stress fell sharply,
especially in the second case. Therefore, this proves that Belgian shepherd dogs are more vulnerable to stress, due to the low proportion of lymphocytes among all white blood cells.

To analyze the influence of the service type, the samples of Moscow and Perm dogs were combined into a single set with the criteria by type of service: patrol-search (PS), mine-search (MS), pedigree, repair (training) dogs. Statistical processing of the results showed that, in general, the sample is characterized by standard values of hemoglobin, the number of red blood cells, white blood cells, platelets, and lymphocyte index.

The least variability was observed in erythrocyte indices, especially in the group of breeding animals. There were few significant differences among the groups, but mine detection dogs were particularly prominent, with significantly higher numbers of leukocytes, red blood cells, and hemoglobin, and the lowest - platelets. This is probably due to the specifics of their application. Toxic explosive mimics or those that are used directly during mining when these dogs work, require hematopoiesis, activate leukemia and erythropoiesis, since the first group of uniform elements provides both nonspecific and specific protection against antigens and toxins, and erythrocyte activity the level of oxygenation for the implementation of detoxification reactions and, in addition, the latter is probably supported by the activity of the respiratory system in this type of use. This is also indicated by some thrombocytopenia. Significantly vary with respect to the average leukocyte indices, including the adaptation index. The highest values of the coefficient of variation are characteristic of eosinophils (especially in dogs of the MS group) and blood monocytes. As it can be seen from figure 2, the level of lymphocytes, as signal indicators of stress reactivity, is also higher in dogs of the MS group, which indicates reactivation. Similarly, to 50% of explosive search dogs, reactivation was noted for 60% of patrol detectives, 50% of repair and breeding animals. Mine search and repair malinois have a relatively low level of eosinophils (figure 2). The low number of monocytes in dogs for the search for explosives, combined with a high number of leukocytes, can prove the attraction of monocyte-macrophage cells to the mucous membranes to phagocytose adsorbed toxins, therefore, an outflow of monocytes from the blood is observed.

![Figure 2. The leukocyte formula of the Belgian shepherd depending on the type of use.](image)

So, the military malinois of Perm and Moscow are characterized on average by the following blood counts: red blood cells 6.4–6.8 x 1012 cells / l, hemoglobin 145–161 g / l, white blood cells 9 x 109 / l, platelets 160–185 x 1012 / l, leukocyte formula: neutrophils - 60–61%, eosinophils - 12.2%,
lymphocytes - 22.6%, monocytes - 4.59%. Age and seasonal differences in the studied blood parameters were found, dependence on the category of use was shown, correlative relationships with the lymphocytic index and the level of hormones cortisol and thyroxine were studied. The adaptive status of Belgian shepherds is in the range of reactions of increased activation and reactivation, in repair dogs in the spring - in the reaction of training.

The second block of the study was associated with the assessment of the crystallographic picture of dehydrated serum in 6 animals of the first and 22 of the second group. As described by scientists [11; 12; 14], they distinguish between types of systemic organization of blood serum facies, which make it possible to assess the intensity of adaptive reactions and the compensatory abilities of the body. In crystallograms of blood serum obtained synchronously with the assessment of the leukocyte formula, in 50% of individuals in the fall - “reactive”, 16.6% - “depressive” type of reaction, in two dogs under the age of 2 years - normative serum (figure 3). Moreover, one of the males who underwent research earlier, retained a depressive status, and in the second he became reactive. Consequently, in the Belgian Shepherd Dogs of the Perm Military Institute in autumn, only a third of the animals have normative physiological status, the remaining dogs have signs of pathological serum. The correlation between the lymphocyte index and the degree of normality of facies, as well as between the number of lymphocytes and the picture of facies, was of the opposite nature of average strength (r = - 0.6). By spring, the typology of facies also changed: in two repair dogs out of 6 dogs, serum testified to a normative state, in 50% - reactive, in one male - depressive. At the same time, the only male of the younger group, who reached the age of two by the spring, retained normativity.

Figure 3. Qualitative drawing of malinois serum facies: A - standard type, B - "reactive" type, C - "depressive" type (MBI-15 microscope, lens x 10).
The Moscow sample is characterized by 40% of dogs with a normative, 35% with a reactive, 24% with a depressed state. Depression occurs in dogs older than 2 years, and more so in males. This is probably due to heavy loads during the course of official activity and some vulnerability of males, their lower thresholds for irritants.

The interpretation of the effect on the adaptive typology of dried blood serum of the application category was performed in total in both samples. The differences in the occurrence of a depressive state in dogs of the MS group are obvious (figure 4), which clearly correlates with their lymphoid status described above. This convincingly suggests that it is these animals that can exhibit weak compensatory capabilities of the body, especially with regard to the specifics of their use. This most important discovery should be taken into account when using dogs of this category of this particular breed. It is very likely that liveliness of temperament, high natural reactivity and speed (obviously for repair dogs that are in the training course), attacking qualities that are intentionally restrained during preparation, and the requirement to strictly obey when explosives are found in turbulent regions disrupt adaptation support systems in these dogs.

Pedigree dogs stood out positively, which, taking into account the normality of the general blood test, indicates a physiological adaptive status. The group of shepherd dogs with patrol-specific specifics of application is also 50% represented by normative animals, 30% - reactive, 20% - depressed according to the typology of serum.

![Graph](image_url)

**Figure 4.** Occurrence of different types of serum facies among Belgian Shepherd Dogs (Malinois) of different application categories.

**4. Conclusion**

Thus, using objective physiological methods of blood testing, a picture of the adaptive state of dogs of the Belgian Shepherd (Malinois) breed of two specialized kennels of the national guard of the Russian Federation located in different climatic and geographical areas was obtained. In general, the stress reactivity of dogs of the Belgian Shepherd (Malinois) breed for official purposes can be estimated as quite intense, which was objectively confirmed by the leukocyte formula and crystallograms of blood serum. By the number of lymphocytes more than 50% of the malinois of the city of Moscow, a reactivation reaction is characteristic, which indicates the tension of the adaptation systems. In Perm, the reaction changed depending on the season of the year, in the spring, mainly, the reaction of
training. Expressed features were the leukogram and crystallogram of the Belgian shepherd mine search service. Cynologists need to be more attentive to the action of strong irritants in working with Malinois, especially carefully select Belgian shepherds for the mine search category. Our results should be taken into account in assessing the adaptive potential of Belgian shepherds and predicting the possibilities of their use in the service, avoiding exposure to extreme irritants, preventing stresses by using adaptogens, correct maintenance and care, and proper training.

Acknowledgements
The author is grateful to the staff of the training complex of the cynological faculty of the Perm Military Institute for their help in obtaining blood for service dogs and the preparation of equipment for blood testing.

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