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The functional state of service dogs under the influence of strong stress factors

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Abstract. Service dogs are more stress-resistant than other animals, but they are also susceptible to stress factors, which can lead to changes in their bodies and their ability to perform their tasks. In this regard, the aim of the study was to investigate the effect of excessive stress factors

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in the form of powerful ballistic shelling of the territory on the organism of service dogs at the physiological and behavioural levels and to identify markers of stress disorders in these animals. Visual examinations, morphological, biochemical, computational and statistical methods were used to determine the main criteria that help differentiate stress markers in service dogs. It was found that physiological criteria, such as heart rate and breathing rate, and behavioural reactions change even before the shelling due to the hypersensitive hearing and increased sensitivity to vibrations in service dogs, and quickly return to normal within an hour after the stressor has ceased. At the same time, the enhanced response to stressors does not affect the ability of service dogs to perform their work tasks. It was also found that service dogs aged 4-5 years are more resistant to stress, as confirmed by the absence of significant changes in serum cortisol levels during the study. Service dogs aged 1-1.5 years are more susceptible to stress factors, as evidenced by a 6.68-fold increase in cortisol levels ($P < 0.001$) during the first stress factor and a 1.69-fold increase ($P < 0.001$) after repeated exposure. Studying the condition of service dogs under stress factors will allow the development of effective methods for diagnosing and preventing stress in animals and maintaining their working capacity. The results of the study can be useful for veterinarians in developing diagnostic criteria and methods for monitoring and correcting changes in the body of animals caused by stress

Keywords: acute stress; chronic stress; diagnosis; behavioural changes; haematological parameters; cortisol

Introduction

Service dogs perform tasks to ensure human safety, are used in operational and investigative activities, to detect prohibited substances or explosives, explosive devices, small arms and ammunition, in areas affected by natural disasters, perform working (mine detection) tasks in combat zones, as well as security or transportation functions, act as guide dogs or companion dogs accompanying people with sensory or mental disabilities. The issue of maintaining the working capacity of service dogs is particularly acute, as animals, like humans, are also subject to stress factors. The level of excitability, adaptability to stressors and inherited behavioural characteristics of the animal play a key role in the working capacity of a service dog. Scientists, dog trainers, and canine therapy specialists have been studying the development of service dogs' resistance to stress factors for a long time. Many studies have been conducted on the level of stress experienced by working dogs while performing their tasks. However, most publications concern therapy dogs and companion dogs.

Thanks to their developed sense of smell, dogs recognise the odours of various substances, including the hormones cortisol, adrenaline and noradrenaline, whose levels are particularly elevated during a stress response. As pointed out by L. Kiiroja *et al*. (2024), dogs are able to distinguish volatile organic compounds (based on adrenaline and noradrenaline) that are released during stress in the exhaled air of humans with high accuracy. Thanks to this unique ability, service dogs can be used to search for missing people, people trapped under rubble or avalanches. In addition, there is a whole field of application for dogs in medicine. In particular, C. Wilson *et al*. (2022) pointed out that dogs are used to detect hypoglycaemia, seizures, dangerous bacteria, viruses (e.g. COVID-19) or parasites (e.g. malaria), epileptic seizures and even cancer in humans.

Service dogs play a special role in the rehabilitation of military personnel with post-traumatic stress disorder (PTSD). Military personnel who experience combat stress and prolonged traumatic effects require comprehensive treatment, including the use of therapy dogs for emotional support. As noted by S.C. Leighton *et al*. (2024), the use of therapy dogs helps to reduce the symptoms of PTSD in military personnel and improve their psychosocial functioning compared to conventional treatment practices. This is because assistance dogs have a unique mechanism for regulating stress indicators in veterans with PTSD by modulating the hypothalamic-pituitary-adrenal axis, which leads to the normalisation of cortisol (stress hormone) levels in the human body. In addition, as noted by L.O. Nieforth *et al*. (2024), positive interaction between dogs and humans leads to an increase in oxytocin (the happiness hormone) in both dogs and humans, which contributes to improved mood, reduced negative emotions, and a positive worldview. Studying the practice of using service dogs in the system of the State Emergency Service of Ukraine, H. Yatsenko (2022) noted that after 10-15 minutes of contact with a specially trained dog, a person experiences a reduction in the release of stress hormones at the neurovegetative level, normalisation of cardiac activity, decreased heart rate and pulmonary artery pressure, and an improvement in emotional state due to increased oxytocin levels, along with mobilisation of the body's internal resources.

At the same time, service dogs, like humans, are also exposed to stress factors, which may affect their performance of working tasks. Service dogs can also become fatigued, fall ill, experience low mood or sustain injuries. The cumulative triggering of stress factors may lead to refusal of the animal to perform its tasks or to atypical behavioural responses. It should be noted that dogs working in partnership with humans perceive the handler's emotional state and level of stress, which can also serve as an additional factor influencing the animal's performance. Stress resistance enables a service dog to respond appropriately to environmental conditions and stressors; however, even minor changes in the behaviour of a service dog must be detected in a timely manner and corrected.

The most common method used to assess stress in animals is the evaluation of behavioural responses and the analysis of cortisol samples in saliva, blood or hair. However, service dogs have specific characteristics compared with pet dogs, primarily due to their high stress resistance and adaptive capacity. E.A.E. van Houtert *et al*. (2023) studied dogs used for therapeutic purposes and found no significant differences in salivary cortisol levels, indicating the absence of an effect of work-related activity on physiological stress in these animals. At the same time, the number of publications assessing the physiological condition of search dogs or dogs working in combat zones remains limited. For example, in a study on the effects of sudden loud sounds on condition and behavioural responses, A.S. Mann *et al*. (2024) found that fireworks, thunder, gunshots and traffic noise are causative factors of increased stress in dogs. Fear of loud sounds of unknown origin, as demonstrated by S. Nichiporuk *et al*. (2023), is a primary aetiological factor in the development of PTSD in dogs during military operations. Stress experienced by service dogs can also have severe negative consequences, including loss of working capacity, which is often irreversible.

Given the lack of data in the literature on the impact of excessive stress factors in the form of intense and dangerous ballistic and cruise missile shelling on the nervous system and stress development in service dogs, it is necessary to conduct a detailed analysis of the degree of adaptability of service dogs to such stress triggers, to determine their functional capabilities and, where possible, to predict their responses during the performance of their service duties. Thus, the aim of this study was to analyse and compare the physiological condition of service search dogs at rest and under the influence of severe stress factors, in particular ballistic missile shelling and/or presence in combat zones, and to identify stress markers in these animals.

Literature Review

Stress is a state in which an animal's body responds to endogenous and exogenous threats that help it to cope with danger or adapt to new conditions. The causes of stress in animals may include various physical, chemical and biological environmental stressors. The mechanisms underlying the development of stress responses and the organism's reactions to them have been extensively studied and described in the literature. In particular, depending on the intensity and type of the stressor, the rhythm of metabolic processes in the animal's body is disrupted, adaptive forces are mobilised and, due to increased activity of the endocrine organs, immune system function is reduced and catabolic processes are activated, leading to a decrease in humoral defence factors. The adaptive nature of the stress response often itself becomes a damaging mechanism that negatively affects overall resistance and immunological reactivity of the animal's body.

The role of the endocrine system in stress modulation is particularly important. In restoring homeostasis in response to a stressor, as noted by S.D. Clark *et al*. (2019), two endocrine subsystems are involved: the sympatho-adrenal medullary (SAM) axis, which acts through the catecholamines adrenaline and noradrenaline, and the hypothalamic-pituitary-adrenal (HPA) axis, which acts through increased levels of the glucocorticoids cortisol and corticosterone. T. Kooriyama and N. Ogata (2021) reported that the SAM axis responds to a stressor almost instantaneously: within milliseconds after exposure, the sympathetic nervous system activates the adrenal medulla to release adrenaline and noradrenaline. This leads to a sudden increase in the animal's energy demand, i.e. an immediate "fight-or-flight" response. Oxygen consumption increases through an accelerated respiratory rate; heart rate and blood glucose levels rise; locomotor activity, alertness, sensory and learning functions, and memory are enhanced. In contrast, processes that are not immediately essential for survival (digestion, reproduction and growth) are suppressed, and pain perception is reduced.

S.D. Clark *et al*. (2019) also noted that during prolonged exposure to a stressor, another mechanism of the stress response is activated: the adrenal cortex, following stimulation by the pituitary gland via adrenocorticotropic hormone (ACTH), begins to release glucocorticoids within several minutes or hours after exposure, with cortisol concentrations in plasma or saliva reaching a peak 10-30 minutes after cessation of the stressor. The main metabolic effect of cortisol is energy mobilisation, which initially helps to restore homeostasis. However, prolonged elevation of cortisol levels contributes to maladaptive changes in the organism, eventually leading to immunosuppression and delayed growth and development of the animal. Sustained elevation of cortisol suppresses glucose utilisation by cells and impairs the function of macrophages, neutrophils, basophils and eosinophils, resulting in reduced immune system function. In particular, according to T. Kooriyama & N. Ogata (2021), prolonged stress reduces immune system effectiveness by 40-70%.

Exposure to high-intensity stressors (for example, explosions), or sequential stress situations that do not allow animals sufficient time to recover, generally leads to behavioural changes. At the same time, as noted by N.J. Rooney *et al*. (2016), fear responses develop in animals when negative events exceed their individual tolerance threshold. Sensitisation (a process in which an animal's response intensifies with repeated stimulation) occurs more frequently when the stressor is of high intensity or low predictability.

At the same time, animals may also develop habituation both to stressors and to the working environment. In particular, in a study of service dogs, S.D. Clark *et al*. (2019) noted that salivary cortisol levels were lower in dogs that participated in several therapeutic sessions per week compared with dogs that worked once a week or less frequently. In addition, older dogs also showed a lower cortisol response than younger ones. Taken together, these results indicate that dogs are capable of habituating to their working environment and, as a result, exhibit a lower cortisol response to work-related situations compared with less experienced dogs.

As reported by L. Townsend & N.R. Gee (2021), prolonged exposure to stressors in dogs may lead to trigger-based stress accumulation. Thus, it is necessary to pay attention even to subtle signs of stress in service dogs, which are manifested through body language, such as yawning, lip licking, tongue flicking, gaze avoidance, a lowered tail, and ears held back. If these behavioural indicators are not recognised in a timely manner, the stress effect in the animal will intensify (a cumulative effect), which may ultimately result in increased aggression or depression, body tremors, excessive coprophagia, increased vocalisation, inappropriate behaviour, and loss of working capacity of the service animal. Consequently, implementing measures to reduce stress levels in service dogs is essential, as they require a balance between work, play and rest.

B.M.G. Gormally & L.M. Romero (2020) indicated that the development of stress within the animal's body is directly reflected by changes in glucocorticoid levels and the excretion of their metabolites in saliva, faeces and urine; alterations in heart rate, heart rate variability and respiratory rate; metabolic disturbances (metabolic rate, changes in thermoregulation); cellular impairments; changes in the immune system; delayed development; and behavioural alterations (including stereotypic behaviours). Thus, the most commonly used biomarkers for interpreting stress states in animals are plasma cortisol and glucose concentrations, substances involved in adrenal cortex responses. Although measuring glucocorticoid levels is not equivalent to measuring stress itself, because these hormones mediate the physiological stress response, they serve as physiological markers of stress in animals. In addition to cortisol, other markers can be used to assess acute stress in dogs, including adrenaline, noradrenaline and chromogranin A (CgA). The latter is released together with catecholamines during acute stress but is more stable.

N.L.B. Corder-Ramos *et al*. (2019) reported that many researchers prefer non-invasive methods, including the assessment of cortisol concentrations in hair and claws, as well as cortisol, catestatin and vasostatin in saliva. R. Palme (2019) indicated that measuring cortisol/corticosterone metabolites in faeces can also be used as a non-invasive method for assessing glucocorticoid release and adrenal activity. E.H. Kang *et al*. (2022) demonstrated that the measurement of salivary alpha-amylase can likewise be considered an important non-invasive method for assessing pain-related stress in dogs. At the same time, as noted by L. Mesarcova *et al*. (2017), determination of cortisol concentration in urine cannot be considered a reliable indicator of stress in animals, since urinary cortisol reflects unbound, biologically active plasma cortisol, and measurement of free cortisol in urine has primary clinical significance in the diagnosis of Cushing's syndrome in dogs. Thus, the combination of behavioural changes and metabolic alterations in service dogs may indicate the development of cumulative stress, which in the future can lead to a decline in the working capacity of service dogs.

Materials and Methods

The study was conducted from October 2024 to June 2025 at the Department of Internal Diseases of the National University of Life and Environmental Sciences of Ukraine and at the Kyiv Region Canine Centre for service search dogs, within the framework of a research project carried out under contract No. BF/37-2021 dated 2 August 2021, "Scientific and practical foundations for ensuring animal health in Ukraine", in accordance with the task for 2025, "Effectiveness of natural-origin preparations in stress disorders caused by military actions in service dogs". Scientific research involving animals complied with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (1986) and the Law of Ukraine No. 3447-IV (2006). All necessary procedures involving animals were carried out in accordance with the ARRIVE recommendations (Kilkenny *et al*., 2010), without violating the guiding principles of Directive 2010/63/EU (2010) on the protection of animals used for scientific purposes.

The objects of the study were service search dogs from the canine centre. Fifteen clinically healthy dogs were examined and divided into three groups. At the first stage, under conditions of rest, a control group was examined consisting of five animals aged 4-5 years with a body weight of approximately 35.5 kg. At the second stage of the study, after exposure to excessive stress factors, five dogs from group 1 (German Shepherd Dogs and Belgian Shepherd Dogs, aged 4-5 years, with a body weight of approximately 35.5 kg) and five dogs from group 2 (German Shepherd crossbreeds aged 1-1.5 years, with a body weight of approximately 30 kg) were examined. The group of animals aged 1-1.5 years was added in order to differentiate whether age influences the manifestation of stress responses in service dogs.

The animals were housed at the service dog centre and were used for search work as intended. Dry feed Royal Canin Premium (France) was used for feeding. Observation of the animals was carried out periodically throughout the entire study period. Behavioural responses of the animals under resting conditions and under stress, as well as physiological indicators of functional status, were the subjects of observation. The animals were examined using generally accepted methods. The initial comprehensive examination included detailed history taking, assessment of general condition, visible mucous membranes, lymph nodes, the cardiovascular system, respiratory system, digestive system, urinary system and nervous system. Inspection, palpation, auscultation and laboratory blood tests were employed. To determine the functional state of the animals under the influence of excessive stress factors, a repeated examination was carried out. The stress factor consisted of intense, prolonged shelling of the territory of Ukraine with cruise and ballistic missiles by the aggressor. The repeated examination included assessment of body conformation, visible mucous membranes, lymph nodes, organs and systems, using inspection, palpation, auscultation and laboratory blood analysis.

Venous blood samples for analysis were collected from the superficial vein of the forearm in the morning before feeding. After shelling, blood sampling was performed within one hour after the end of the active phase of the attacks. For complete blood counts, 2 mL of blood was collected into Vacumed tubes containing the anticoagulant K_3EDTA for morphological studies. For biochemical analysis, 2 mL of blood was collected into Vacumed tubes with a clot activator, then centrifuged for 10 minutes at 3,000 rpm, after which the serum was transferred into clean Eppendorf-type tubes.

Complete blood count tests were performed using a Mindray BC-5000 automatic haematology analyser (China). The following parameters were determined: haemoglobin content – by photometric method; number of erythrocytes, thrombocytes, leukocytes, absolute number of granulocytes, monocytes and lymphocytes – by electrical impedance method; haematocrit, mean corpuscular volume, mean haemoglobin concentration in erythrocytes, and haemoglobin content in erythrocytes were determined using a calculation method. The qualitative composition of erythrocytes and the differential composition of leukocytes were studied in blood smears stained with "Leukodif-200" haematological dyes manufactured by "Erba lachema" (Czech Republic). The ratio of neutrophils to lymphocytes (N/L) was calculated by dividing the total number of neutrophils by the total number of lymphocytes in absolute values, and the ratio of platelets to lymphocytes (Tr/L) was calculated by dividing the total number of platelets by the total number of lymphocytes in absolute values.

Biochemical blood tests of dogs were performed on a semi-automatic biochemical analyser LabLine-010 (Austria) using Spine Lab reagents (Granum, Ukraine). The serum was tested for total protein, albumin, glucose, urea, creatinine, total bilirubin, calcium, inorganic phosphorus, alkaline phosphatase activity, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) were determined in the blood serum. During the biochemical study, standard methods were used: total protein content was studied by colorimetric method, based on the intensity of biuret complex formation, endpoint; albumin – by the bromocresol green reaction, end point; glucose – by the colorimetric method, enzymatic using oxidase, end point; ALT by a modified method of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), based on the rate of nicotinamide adenine dinucleotide (NADH) oxidation with measurement of absorbance decrease (kinetic); AST by a modified IFCC method based on the rate of NADH oxidation with measurement of absorbance decrease (kinetic); urea by the urease-glutamate dehydrogenase (GLDH) method (two-point); creatinine by a modified colourimetric Jaffé method (two-point); alkaline phosphatase by the p-NPP (p-nitrophenyl phosphate) kinetic method; calcium by the Arsenazo III method (end-point); and inorganic phosphorus by the phosphomolybdate reaction (end-point).

Serum samples (0.5 mL) were delivered within one hour to the veterinary laboratory "Bald" (Kyiv, Ukraine) for cortisol analysis. Serum cortisol concentration was determined using an ImmunoChem-2100 analyser (USA) by an enzyme-linked immunosorbent assay. The obtained results were processed statistically using Excel software, calculating the arithmetic mean, standard deviation and Student's t-test with significance levels of $P < 0.05$, $P < 0.01$ and $P < 0.001$, and comparing the probability of the animals' condition before and after shelling between groups of adult and young service dogs.

Results and Discussion

As a result of the examination of service search dogs at rest, it was established that at the beginning of the study, the physiological indicators corresponded to those of clinically healthy animals. Heart rate, respiration, and behavioural responses corresponded to the standards for service dogs (Levchenko *et al.*, 2008). There were no visible signs of depression in the animals. The dogs were active, non-aggressive, and easily performed training exercises. All service dogs, according to their purpose, underwent rigorous selection for breed characteristics and behavioural requirements, and possessed physical endurance, confidence, stress resistance, the ability to work in a team with humans, and a lack of fear of loud noises or aggression towards other animals and people. As a rule, dogs with average sensitivity were selected for work, as such dogs have balanced characteristics, are able to focus on the task and perform it. The morphological and biochemical blood parameters of the studied animals at rest also did not exceed the limit values, in accordance with the standards for healthy animals.

During the study, it was found that, thanks to their trained hearing, sense of smell and sense of vibration, service dogs began to get restless a few hours before the shelling, behaved excessively excitedly, ran around the enclosures, and exhibited increased vocalisation, which continued during the rocket fire. These symptoms were repeated during subsequent shelling. However, once the stressors had ceased, the service dogs quickly returned to their usual activities. After an hour, no signs of increased excitement or depression were observed in any of the animals; they were actively engaged in training exercises and ready to work. Evidently, engaging service dogs in constant physical activity and practising the necessary service skills allows service animals to reduce stress symptoms, as physical activity helps to release accumulated energy, reduce anxiety through the production of endorphins, and restore the animals' calm through interaction with the dog handler, which has a positive effect on the dog's well-being. The morphological indicators of service dogs' blood at rest and under stress also showed changes that characterise the animals' response to stress (Table 1).

Table 1. Morphological blood parameters of service dogs at rest and under the influence of stress factors (M ± m, n = 5)

Indicators	Control group	After severe stress factors, 24 days later		After repeated severe stress factors, 6 months later	
		Group 1	Group 2	Group 1	Group 2
Erythrocytes, 10^{12}/L	6.54 ± 0.93	8.06 ± 0.78	7.31 ± 0.48	7.59 ± 0.54	6.95 ± 0.50
Leukocytes, 10^9/L	7.92 ± 0.81	6.54 ± 0.85	9.24 ± 0.78•	8.45 ± 1.17	11.21 ± 1.35
Platelets, 10^9/L	105.33 ± 8.74	175.67 ± 27.18	108.05 ± 9.23•	212.25 ± 21.22*	179.0 ± 22.5**
Haemoglobin, g/L	154.75 ± 22.5	183.75 ± 18.21	162.35 ± 9.81	181.83 ± 11.89	163.67 ± 13.87
Haematocrit, %	52.15 ± 5.02	52.40 ± 8.0	48.10 ± 4.2	51.03 ± 3.2	47.32 ± 3.19
Neutrophils, 10^9/L	3.79 ± 0.42	4.92 ± 0.9	5.44 ± 0.36**	5.47 ± 0.55*	5.46 ± 0.04**
Lymphocytes, 10^9/L	2.76 ± 0.04	2.27 ± 0.61	1.99 ± 0.22	2.01 ± 0.16	3.17 ± 0.21
Monocytes, 10^9/L	0.58 ± 0.01	0.35 ± 0.03	0.84 ± 0.12*,••	0.38 ± 0.03***	0.57 ± 0.17
Eosinophils, 10^9/L	0.69 ± 0.01	0.56 ± 0.27	0.94 ± 0.32	0.54 ± 0.21	0.63 ± 0.26
Basophils, 10^9/L	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01*	0.01 ± 0.01	0.03 ± 0.01*

Note: ** – P < 0.05, ** – P < 0.01, *** – P < 0.001 compared with the control group; • – P < 0.05, •• – P < 0.01, ••• – P < 0.05 compared between groups 1 and 2*
Source: *developed by the authors*

As can be seen from Table 1, after stress exposure, the blood of animals in group 1 showed a 1.23-fold increase in the number of erythrocytes, a 1.67-fold increase in the number of thrombocytes, a 1.19-fold increase in haemoglobin content, and a 1.30-fold increase in neutrophils, while the number of leukocytes decreased by 1.21 times and monocytes by

1.66 times in absolute terms compared to the animals in the control group. However, no significant difference between the indicators was determined. In the blood of animals in group 2, there was a significant increase in the number of neutrophils by 1.44 times ($P < 0.01$), monocytes – by 1.45 times ($P < 0.05$), basophils – 3 times ($P < 0.05$), and a decrease in the number of lymphocytes by 1.39 times ($P < 0.01$) compared to the control group animals. In addition, there was a 1.41-fold increase in the number of leukocytes ($P < 0.05$), a 2.4-fold increase in the number of monocytes ($P < 0.01$), and a 3-fold increase in the number of basophils ($P < 0.05$) compared to group 1 animals. In a repeat study after exposure to a stress factor in service dogs, group 1 animals showed a 2.02-fold increase in the number of platelets ($P < 0.001$), a 1.44-fold increase in the number of neutrophils ($P < 0.05$), and decreased by 1.53 times ($P < 0.001$) compared to the control group. In the blood of animals in group 2, there was a 1.44-fold increase in the number of neutrophils ($P < 0.01$), a 3.0-fold increase in basophils ($P < 0.001$), and a 1.7-fold increase in platelets ($P < 0.001$) compared to the control group.

These data are consistent with N.L.B. Corder-Ramos *et al*. (2019), who indicated that during the development of acute stress in dogs, general leukocytosis developed, and a stress leukogram was recorded, characterised by neutrophilia, monocytosis, eosinopenia, and lymphocytopenia. The data obtained also consistent with the studies by E. Chmelíková *et al*. (2020), who noted that stress activates the hypothalamic-pituitary-adrenal axis, leading to the release of glucocorticoids and catecholamines, and then to an increase in the number of neutrophils and a decrease in the number of lymphocytes in the blood. In response to the action of glucocorticoids, circulating lymphocytes adhere to the endothelium of blood vessel walls and then migrate to other tissues, such as lymph nodes, spleen, bone marrow and skin, where they are sequestered. In addition, the release of glucocorticoids and catecholamines through cytokines promotes the production of acute phase proteins in hepatocytes, thereby increasing their levels in the blood serum.

The ratio of neutrophils to lymphocytes is also considered a marker of acute stress in animals. In service dogs, a change in the N/L ratio was observed after exposure to a stressor due to an increase in the number of neutrophils from 1.37 to 2.17 in dogs in group 1 and to 2.73 in dogs in group 2 during the first bombardment, and an increase in the N/L ratio to 2.72 in dogs in group 1 and to 1.72 in group 2 after repeated exposure to the stressor. These data are consistent with the results of a study by K. Radisavljević *et al*. (2015), who studied the effect of transport stress on dogs and found that the first physiological response is an increase in cortisol levels in blood plasma and saliva and an increase in the neutrophil/leukocyte ratio in the blood of animals. As noted by B.M.G. Gormally & L.M. Romero (2020), a change in the N/L ratio was recorded in the blood of animals 1-4 hours after exposure to the stress factor. In the bone marrow of dogs, the N/L ratio should be 1:1 or with a slight predominance of neutrophils, although variation is possible in adult animals. J.I. Cristóbal *et al*. (2022) established a range of reference values for the N/L ratio in healthy dogs, which was 0.74-5.62, and the platelet-to-lymphocyte ratio was 56.41-198.02.

The platelet-to-lymphocyte ratio (Tr/L) in dogs is an important indicator that reflects the presence of inflammation, infection, immune response and bleeding risk. An increased ratio may also be observed during the development of a stress response in the animal. In service dogs at rest, the Tr/L ratio was 38.2; under the influence of a severe stressor it was 77.4 in group 1 animals and 54.3 in group 2 animals. Following repeated exposure to the stressor, the ratio increased to 105.6 in dogs of group 1 and to 56.5 in animals of Group 2. However, despite the considerable differences in results, the values of these indices in animals of all groups remained within the established reference ranges for healthy animals.

Biochemical parameters of blood serum (Table 2) in dogs of group 1 were characterised by a significant twofold increase in glucose concentration ($P < 0.001$) compared with the control group. In the serum of dogs of group 2, glucose concentration increased by 1.78 times ($P < 0.001$), inorganic phosphorus by 1.63 times ($P < 0.005$), while creatinine concentration decreased by 1.34 times ($P < 0.01$) and bilirubin by 1.83 times ($P < 0.001$). Repeated serum analysis in dogs of group 1 was characterised by a 1.15-fold increase in bilirubin concentration ($P < 0.01$) compared with the control group, whereas in group 2 only an increase in inorganic phosphorus concentration was observed, by 1.5 times ($P < 0.01$) compared with the control group and by 1.62 times ($P < 0.01$) compared with group 1.

Table 2. Biochemical indicators of blood serum in service dogs at rest and under stress factors (M ± m, n = 5)

Indicators	Control group	After severe stress factors, 24 days later		After repeated severe stress factors, 6 months later	
		Group 1	Group 2	Group 1	Group 2
Glucose, mmol/L	2.18 ± 0.10	4.36 ± 0.27***	3.90 ± 0.35***	2.15 ± 0.07	2.33 ± 0.31
Total protein, g/L	61.68 ± 3.53	53.06 ± 5.05	55.8 ± 4.02	59.38 ± 6.19	52.88 ± 3.77
Albumin, g/L	33.15 ± 3.29	31.58 ± 5.53	32.5 ± 2.35	27.75 ± 2.53	30.5 ± 4.59
Total bilirubin, μmol/L	2.75 ± 0.07	1.32 ± 0.36	1.50 ± 0.26***	3.15 ± 0.11**	2.94 ± 0.18
Urea, mmol/L	4.95 ± 0.78	5.67 ± 1.1	4.26 ± 0.65	4.35 ± 0.30	4.02 ± 0.48
Creatinine, μmol/L	94.88 ± 4.31	89.04 ± 15.03	70.80 ± 5.63**	91.03 ± 8.86	101.67 ± 9.5
Calcium, mmol/L	2.40 ± 0.40	2.66 ± 0.35	2.3 ± 0.36	2.26 ± 0.33	2.43 ± 0.17
Inorganic phosphorus, mmol/L	1.21 ± 0.10	1.76 ± 0.21	1.97 ± 0.32*	1.12 ± 0.16	1.81 ± 0.12**,••

Note: ** – P < 0.05, ** – P < 0.01, *** – P < 0.001 compared with the control group; • – P < 0.05, •• – P < 0.01, ••• – P < 0.05 compared between group 1 and 2*
Source: *developed by the authors*

During the study of serum enzyme activity in dogs of group 1, a 2.49-fold decrease in AST activity ($P < 0.05$) was observed compared with the control group, while in the serum of dogs of group 2 a 2.11-fold decrease in AST activity ($P < 0.01$) and a 2.17-fold increase in alkaline phosphatase activity ($P < 0.001$) were recorded compared with the control group (Table 3). Interpretation of AST and ALT enzyme activity in serum is used to assess liver function in animals and hepatocyte damage, while increased alkaline phosphatase activity has high sensitivity (86%) but low specificity (49%) for canine liver disease. However, during the development of a stress response, the activity of these enzymes may also increase due to physiological reactions such as muscle tension and oxidative stress. Investigating hepatic enzyme activity in service dogs, N. Hadžimusić & D. Hadžijunuzović-Alagić (2024) found that the age of the animals does not significantly affect liver enzyme activity, except for alkaline phosphatase, which is elevated in young animals due to bone growth.

Table 3. Enzyme activity in the blood serum of service dogs at rest and under stress factors (M ± m, n = 5)

Indicators	Control group	After severe stress factors, 24 days later		After repeated severe stress factors, 6 months later	
		Group 1	Group 2	Group 1	Group 2
ALT, U/L	42.60 ± 3.04	20.6 ± 4.53	36.45 ± 6.25	32.02 ± 2.99*	29.47 ± 4.78*
AST, U/L	45.15 ± 5.42	18.1 ± 1.98*	21.36 ± 3.69**	27.1 ± 5.77*	23.98 ± 2.84**
Alkaline phosphatase, U/L	97.13 ± 6.72	166.7 ± 11.94	211.2 ± 14.59***	106.0 ± 12.16	189.4 ± 26.45**,••
GGT, U/L	10.52 ± 2.70	12.57 ± 2.53	8.5 ± 0.15	5.4 ± 0.56	6.1 ± 0.12

Note: ** – P < 0.05, ** – P < 0.01, *** – P < 0.001, compared with the control group; • – P < 0.05, •• – P < 0.01, ••• – P < 0.05 compared between groups 1 and 2*
Source: *developed by the authors*

A repeated examination after 6 months also revealed a decrease in ALT activity by 1.33 times (P < 0.05) in the blood serum of dogs in group 1 and by 1.45 times (P < 0.05) in dogs in group 2 compared with the control group. At the same time, AST activity in the blood serum of dogs in group 1 decreased by 1.67 times (P < 0.05), while in dogs in group 2 it decreased by 1.88 times (P < 0.01). It was noted that AST activity at the beginning of the study was slightly higher than the reference values for dogs (5-25 U/L); therefore, the reduction in its activity in the blood of dogs from both groups is a positive finding, indicating normalisation of liver function in the animals. In addition, an increase in ALP activity by 1.95 times (P < 0.01) was observed in the blood serum of animals in group 2 compared with the control group and by 1.79 times (P < 0.01) compared with group 1.

The intensive use of service dogs and significant muscular loads may also contribute to increased activity of liver enzymes, especially AST, since part of this enzyme is present in muscle tissue. Manifestations of chronic stress in animals may likewise affect the intensive functioning of the liver, leading to changes in hepatocyte membrane permeability and the release of these enzymes into the bloodstream. At the same time, T. Ochi *et al*. (2013) indicated that alkaline phosphatase activity may be a useful biochemical marker of transport-related stress in dogs, as changes in its activity have been demonstrated in animals after transportation. The increase in alkaline phosphatase activity during stress is associated with elevated cortisol levels, which stimulate the production of the C-ALP isoenzyme in dogs in response to increased endogenous or exogenous corticosteroids.

A key marker of stress in animals is the hormone cortisol, a glucocorticoid produced by the adrenal cortex under the influence of ACTH. It regulates the majority of physiological processes in the animal body and, in the short term, mobilises energy and various body systems to respond to an immediate threat. However, prolonged elevation of cortisol levels often leads to impairment of the immune system and behavioural changes. In addition, cortisol regulates overall metabolism, stimulates myocardial contractility, increases arterial blood pressure and blood glucose levels, and exerts anti-inflammatory and immunosuppressive effects, helping the body to cope with inflammation, but at excessive levels it may suppress immune function. Excess cortisol can lead to the development of Cushing's syndrome in animals, increase the risk of heart failure, anxiety, aggression or depression, reduced appetite, provoke vomiting or diarrhoea, and cause skin disorders such as eczema, ulcers and inflammation. Excessively elevated blood glucose levels may result in the development of diabetes.

Reduced cortisol levels, in turn, may lead to weakened immunity, increasing susceptibility to infections and slowing wound healing. M. Siniscalchi *et al*. (2013) reported that cortisol has a significant influence on mood, behaviour and overall well-being in animals by regulating their responses to the environment. Thus, the determination of cortisol is considered a reliable biomarker for assessing the overall level of stress and the temperament of a dog.

E.A.E. van Houtert *et al*. (2023) found that in many publications authors focus on non-invasive sampling methods for determining cortisol concentrations in saliva, hair, and even in claws and milk. Cortisol levels in hair vary depending on seasonality, coat colour, age of the animal, and reflect the state of chronic stress in animals. D. Oyama *et al*. (2014) noted that salivary cortisol reflects increases in plasma cortisol with a delay of 20-30 minutes, while during prolonged exposure to a stressor its concentration in saliva decreases. However, it remains unclear whether this reduction represents adaptive physiological changes or habituation to new environmental conditions. I. Schöberl *et al*. (2017) reported that salivary cortisol levels reflect the activity of the hypothalamic-pituitary- cortisol may serve as a useful indicator of stress-coping mechanisms in dogs, as higher variability is associated with better regulation of the HPA axis and more adaptive stress coping, whereas lower variability may reflect blunted cortisol responses, potentially indicating chronic stress in animals.

E. Chmelíková *et al*. (2020) demonstrated that the determination of cortisol in saliva also has limitations related to the timing, method of sample collection, and storage conditions. N.J. Russell *et al*. (2007) established that storage of blood samples in freezers at −20°C does not significantly affect cortisol levels, whereas storage at +4-5°C reduces cortisol concentrations by approximately 12.5%. Thus, the assessment of any stress markers should be complemented by observations of behavioural changes in animals. It should also be noted that stress responses in animals do not always positively correlate with cortisol production and depend on the baseline excitability of the nervous system.

In the present study, considering the selected stress stimulus, it was appropriate to determine cortisol concentrations in blood. At the beginning of the experiment, this parameter was relatively high, amounting to 15.25 nmol/L (4.39 ng/mL) (Table 4). This value is slightly higher than those reported in the literature for blood cortisol levels in dogs or companion dogs. However, it is consistent with the findings of J. Wojtaś *et al*. (2020), who investigated search-and-rescue dogs involved in disaster zones, where the mean salivary cortisol concentration ranged from 4.2 to 4.89 ng/mL.

Table 4. Cortisol hormone content in the blood serum of service dogs at rest and under stress factors (M ± m, n = 5)

Indicators	Control group	After severe stress factors, 24 days later		After repeated severe stress factors, 6 months later	
		Group 1	Group 2	Group 1	Group 2
Cortisol, nmol/L	15.25 ± 2.3	21.82 ± 0.69	101.88 ± 8.21***,●●●	16.13 ± 1.85	25.77 ± 0.39***,●●●

Note: ** – P < 0.05, ** – P < 0.01, *** – P < 0.001, compared with the control group; ● – P < 0.05, ●● – P < 0.01, ●●● – P < 0.05 compared between groups 1 and 2*
Source: *developed by the authors*

As shown in Table 4, the cortisol concentration in the blood serum of dogs in group 1 increased 1.43-fold after the first mass missile strike and showed almost no difference after the second, thus the results did not demonstrate a statistically significant difference. This may indicate that service dogs have a high level of stress resistance, which enables them to respond adequately to threats in dangerous situations. In contrast, in the blood serum of dogs from experimental group 2 a statistically significant increase in cortisol concentration was recorded, rising 6.68-fold ($P < 0.001$) after exposure to the first stress factor compared with the control group and 4.67-fold ($P < 0.001$) compared with dogs in group 1. This confirms the hypothesis that younger animals react more acutely to stressors and do not possess sufficiently developed stress resistance compared with adult service dogs. An increase in serum cortisol concentration in dogs of group 2 was also observed during the second mass missile strike. In particular, its level increased 1.69-fold ($P < 0.001$) compared with the control group and 1.6-fold ($P < 0.001$) compared with dogs of group 1 during this period of the study.

However, it is important to note that behavioural changes in dogs of group 2 did not reflect a stress response after the missile strikes. Despite the elevated serum cortisol concentration, the animals actively performed their tasks and interacted with their handlers. It is evident that the relationship with the human partner with whom the service dog works had an influence on the dog's physiological state. There is a hypothesis that reductions in cortisol levels in service dogs during stressful situations depend on dog-human interaction. T. Mitropoulos & A. Andrukonis (2025) pointed out that constant stress in the owner can lead to increased stress in service dogs. E. Chmelíková *et al*. (2020) indicated that the cortisol content in dogs' blood changes according to the circadian rhythm, with the highest peak observed in the morning after waking up and the lowest before bedtime.

A. Colussi *et al*. (2018) studied behavioral changes in dogs when they were played recordings of thunderstorms and thunder similar to the explosion of cruise missiles. Salivary cortisol concentrations in animals increased significantly 20 minutes after playback of the recording. The stress response to thunder sounds doubled salivary cortisol levels from a baseline of approximately 1.0 ng/mL to around 2.0 ng/mL. J. Wojtaś *et al.* (2020), studying service search dogs during tests simulating conditions after a natural disaster, found no increase in cortisol concentration in saliva. Elevated cortisol levels were not dependent on the dog's performance effectiveness or success during the examination. No stress-related behaviour was observed during therapeutic sessions either, indicating that the dogs did not experience excessive stress during the procedure. Thus, habituation through training is likely to help service dogs cope with excessive stress stimuli.

Investigation of changes in blood cortisol concentrations in service dogs can provide important insights into the animal's physiological health and behaviour, as well as improve understanding of how such dogs adapt to their environment and working tasks. Thus, monitoring clinical signs, as well as morphological and biochemical blood parameters in service dogs under conditions of increased stress load, is an essential component of the work of handlers and veterinary practitioners. The assessment of physiological and biochemical markers, such as cortisol levels and haematological and biochemical blood indices, enables effective evaluation of stress status in service dogs, contributes to understanding their adaptation to stressors and, when necessary, supports the maintenance of their functional capacity.

Conclusions

The present study described changes in the functional state of service dogs under the influence of excessive stress stimuli and identifies markers reflecting the manifestation of stress responses in these animals. It was established that exposure to excessive stimuli, in particular intense mass shelling with ballistic and cruise missiles, leads to increased anxiety and vocalisation in service dogs before and during shelling. Physical activity and training sessions with a handler minimise stress-induced behavioural changes, which helps to maintain the working capacity of service dogs.

Service dogs aged 4-5 years are more stress-resistant, which is reflected in both behavioural reactions and the results of morphological and biochemical blood tests. The manifestation of a stress response in these animals is indicated by a 2.0-fold increase in blood glucose levels ($P < 0.001$), a 1.44-fold increase in the number of neutrophils ($P < 0.05$), a change in the neutrophil/leukocyte ratio due to an increase in the number of neutrophils from 1.37 to 2.17 after the first bombardment and to 2.72 after the second. This is a manifestation of a stress leukogram in animals. At the same time, the absence of a reliable correlation between the cortisol content in the blood of these dogs and the optimal indicators of biochemical blood tests indicate a high level of adaptability of their organism, which was also observed after the second study.

Young service dogs aged 1-1.5 years are more prone to stress reactions. In particular, there was a 1.44-fold increase in the number of neutrophils ($P < 0.01$), a change in the neutrophil/leukocyte ratio due to an increase in the number of neutrophils from 1.37 to 2.73 after the first bombardment, and to 1.73 after the second; glucose content – 1.78 times ($P < 0.001$), alkaline phosphatase activity – 2.17 times ($P < 0.001$), cortisol level – 6.68 times ($P < 0.001$) under the action of the first stress factor. The same changes were observed in the repeated examination of animals in group 2. A sharp increase in serum cortisol levels in animals of experimental group 2, aged 1-1.5 years, compared to animals of experimental group 1 during the first and second bombardments, indicates a stronger stressful effect of excessive stimuli on animals of group 2 and, accordingly, a more powerful physiological response of the organism to stress factors.

Further research should focus on identifying behavioural changes in service dogs directly involved in combat zones, the mechanisms underlying the development of post-traumatic stress disorders in these animals, and the development of methods for their correction. In the context of intense shelling of Ukrainian territory, it is promising to study more profound changes in the immune system of service dogs under the influence of stress factors, correlations between hormones, cytokines, and other poorly studied indicators that would allow for the timely diagnosis of the development of stress conditions in service dogs, contributing to an understanding of their bodies' adaptation to stress factors. It will also be important to analyse and scientifically substantiate methods of reducing the impact of stress factors on service dogs under intense stress, which will ensure the preservation of the service dog's working capacity.

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Conflict of Interest

None.

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Функціональний стан організму службових собак за впливу сильних стресових чинників

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Анотація. Службові собаки є стресостійкішими, ніж інші тварини, однак вони також піддаються впливу стресових чинників, що може призвести до змін в їхньому організмі та здатності виконувати поставлені завдання. У зв'язку з цим, метою роботи було дослідити вплив надмірних стресових чинників у вигляді потужних балістичних обстрілів території на організм службових собак на фізіологічному та поведінковому рівнях і визначити маркери стресових розладів у цих тварин. Візуальними дослідженнями, морфологічними, біохімічними, розрахунковими та статистичними методами було визначено основні критерії, які допомагають диференціювати маркери стресу в службових собак. Встановлено, що фізіологічні критерії, такі як частота серцевих скорочень і дихання, поведінкові реакції зазнають змін ще до обстрілу завдяки надчутливому слуху та підвищеному відчуттю вібрацій у службових собак і швидко повертаються до норми впродовж години після завершення дії стресора. При цьому, посилена реакція на дію стресових чинників не впливає на здатність службових собак виконувати свої робочі завдання. Виявлено також, що службові собаки віком 4–5 років є стійкішими до стресу, що підтверджено відсутністю достовірних змін вмісту кортизолу в сироватці крові впродовж дослідження. Службові собаки віком 1–1,5 року сприйнятливіші до дії стресових чинників, що проявилося підвищеним вмістом кортизолу в 6,68 раза ($P < 0,001$) за дії першого стресового чинника та в 1,69 раза ($P < 0,001$) після повторної дії. Дослідження стану організму службових собак за дії стресових чинників дозволить розробити ефективні методи діагностики та профілактики стресових станів у тварин і збереження їх працездатності. Результати дослідження будуть корисними для лікарів ветеринарної медицини під час розробки діагностичних критеріїв та методів контролю і корекції змін в організмі тварин, викликаних стресовими явищами

Ключові слова: гострий стрес; хронічний стрес; діагностика; поведінкові зміни; гематологічні показники; кортизол