

SECTION 1. ANIMAL FEEDING AND FEED TECHNOLOGY

1.1 Changes in hematological indicators of dogs in the acute stage of babesiosis invasion

Generalized lesion of inner organs and appropriate systems in the acute stage of babesiosis invasion in dogs substantially complicates its passing, that needs constant monitoring of diseased animal organism's functional condition. In this work there are generalized results of seven cases of Labrador dogs with babesiosis, aged 4 to 7 years. In the result of performed hematological examinations it is proved, that in dogs during the acute stage of babesiosis invasion the typical changes in morphological profile of blood are leukocytopenia with reducing the number of band neutrophils and simultaneous compensatory manifestation of basophilia and monocytosis, as well as erythrocytopenia, hypochromia, thrombocytopenia on the background of hematocrit and thrombocrit reducing. The characteristic changes of biochemical profile of diseased animals' blood plasma are the increasing of aminotransferase activity and hypochromia, hypoproteinemia, hypoalbuminemia, hyperazotemia, which indicates the aggravation of catabolic processes in cells of internal organs, primarily the liver. There are established patterns about qualitative and quantitative changes of hematological indicators at an early stage of babesiosis invasion, that are important for clarifying the pathogenesis of this pathology, defining the features of functional changes in the organism of diseased animals and developing of new approaches in their diagnostics, prevention of complications and treatment of animals.

Babesiosis is a transmissible, natural focal disease of dogs, cats, fur-bearing animals, and wild carnivores caused by protozoa of the genus *Babesia*, which are carried by ticks from the genus Ixodidae, and is common all over the world, including Ukraine [1]. The severity of the disease depends on lot's of factors, such as the type of involved *Babesia* forms, age and the immune status of the host [2, 3]. Due to the climate change, the active period of vectors of the disease increases, so babesiosis of dogs is registered more often in winter [4-6].

So, the Wildlife Conservation Society published the review «The Deadly Dozen: Wildlife Diseases in the Age of Climate Change», in which there are listed 12 diseases that are becoming more dangerous in the result of global warming process. Among the diseases, that can affect new territories, along with bird flu, Ebola fever, yellow fever, plague, cholera, tuberculosis and trypanosomiasis, babesiosis is also mentioned [3, 6].

People who are sick with babesiosis are also of concern. More than 400 human cases have already been described in the United States and 21 in Europe [7]. If we consider that after recovery, animals develop non-sterile immunity lasting 1–2 years, then the problem of further study of this invasion remains relevant [8, 9].

The problem of multiple organ failure in babesiosis invasion of dogs remains relevant at present, which prompts further study of the pathogenesis of the disease and the search for new informative methods for assessing the functional state of internal organs [9-12].

Now clinical laboratory diagnostics is distinguished by a significant arsenal of tests and methods for early and relatively accurate diagnosis of a significant part of the known forms of animal pathologies, including babesiosis, and also allows for tracking the course of the pathological process, the development of possible complications and the effectiveness of treatment [13-17]. In the case of babesiosis, changes in the biochemical parameters of biological material samples are not specific. However, comparing them with the norm indicators, it's possible to assess the degree and nature of intracellular metabolic disorders in the body of a sick animal, as well as the functional state of individual organs, their systems and the organism as a whole.

The purpose of the study was to determine the features of changes in hematological parameters of blood in dogs in the acute stage of babesiosis invasion.

Research methodology included a comprehensive analysis and comparison of the results of biochemical research of native blood/plasma of dogs in the acute stage of babesiosis invasion with clinically healthy animals. In experimental dogs, the diagnosis was confirmed microscopically by the presence of *B. canis* in erythrocytes of blood smears. To solve the tasks were formed two groups of Labrador dogs by the method of analogues, aged 4 to 7 years, with a body weight of 31.9-35.0 kg: control (clinically

healthy) and experimental (with an acute stage of babesiosis). Herewith, for hematological examinations the blood was taken from the anterior subcutaneous vein of the forearm. General and special methods of laboratory research were used in scientific work: microscopy, centrifugation, spectrophotometry and mathematical statistics, as well as modern laboratory equipment and devices, in particular: hematology analyzer «Dymind DF50» (China) and biochemical analyzers «StatFax 4500» (USA), «Mindray BS-230» (China) and «Rayto RT-9600» (China), reagents of the firm «Global Scientific» (USA).

General clinical analysis of dogs' blood in the acute stage of babesiosis invasion. During the acute stage of babesiosis development in Labrador dogs, a 38% decrease in the number of leukocytes in the blood (leukocytopenia) was noted, which was manifested against the background of a 1.9-fold decrease in the number of band neutrophils compared to the control. The established fact of leukopenia with a decrease in the relative number of young forms of neutrophils is an indicator of a decrease in the reactivity of the body in sick animals, which may be due to inhibition of the function of the organs of leukocytopoiesis (bone marrow, spleen, lymph nodes) in response to the infection of blood cells with babesia and the development of intoxication. At the same time, in animals of this group, compared to the control, there was a 1.8-fold increase in both the relative number of basophils (basophilia) and monocytes (monocytosis), which is a manifestation of the protective reaction of the dog's body and indicates the active development of phagocytosis of protozoa, products breakdown of erythrocytes and endotoxins (Table 1).

In addition, as a result of the morphological study of blood in sick dogs, anemia was diagnosed, which was confirmed for four out of six animals. At the same time, a decrease in the number of erythrocytes by 26% (erythrocytopenia) and hemoglobin content by 17% (hypochromia) was noted in the blood, while the hematocrit value decreased by 24% (Table 1).

Table 1. Morphological and biochemical parameters in the blood of dogs with the acute course of babesiosis invasion ($M \pm m$, $n = 7$)

Indicator	Reference limits	Control	Dogs with Babesiosis
Leukocytes, $10^9/L$	4.5–15.5	7.1 ± 0.9	$4.4 \pm 0.7^*$
Band neutrophils, %	0–3	1.7 ± 0.2	$0.9 \pm 0.1^*$
Segmented neutrophils, %	60–77	68.5 ± 2.7	70.2 ± 1.9
Eosinophils, %	2–10	2.4 ± 0.5	1.6 ± 0.4
Basophils, %	0–1	0.5 ± 0.1	$0.9 \pm 0.1^*$
Monocytes, %	0–10	7.3 ± 0.8	$13.0 \pm 1.5^*$
Lymphocytes, %	12–30	21.6 ± 2.8	15.4 ± 1.7
Erythrocytes, $10^{12}/L$	4.8–9.3	6.1 ± 0.8	$4.5 \pm 0.4^*$
Hemoglobin, g/L	120–180	127.0 ± 4.2	$105.1 \pm 2.1^*$
Hematocrit, %	36–55	43.5 ± 1.2	$33.0 \pm 1.7^*$
Platelets, $10^9/L$	170–500	339.3 ± 17.1	$51.3 \pm 2.2^*$
Thrombocrit, %	0.14–0.46	0.3 ± 0.1	$0.04 \pm 0.01^*$

Note: $*P < 0.05$, statistically significant differences compared to control.

Minor deviations from the reference boundaries of these indicators are explained by identifying the causative agent of babesiosis in dogs at an early stage of the development of the disease. At the same time, the most pronounced changes were established by the number of platelets in the blood of sick animals and the value of thrombocrit. So, in sick animals the number of platelets differed by values 7 times less from the control ones (thrombocytopenia), and the value of thrombocrit decreased by 8 times compared with clinically healthy animals. In particular, thrombocytopenia was detected in the blood smears of all studied dogs.

Therefore, in dogs with babesiosis, characteristic changes in the morphological profile of the blood are leukocytopenia (due to a decrease in the number of band neutrophils), basophilia, monocytosis, erythrocytopenia, hypochromia, thrombocytopenia, manifested against the background by a decrease in hematocrit and

thrombocrit. A significant decrease in the number of erythrocytes and hemoglobin content in sick animals as a result of intravascular hemolysis provokes the development of anemia and, consequently, circulatory and tissue hypoxia, which causes changes in homeostasis.

Biochemical parameters of dogs' blood plasma in the acute stage of babesiosis invasion. Due to the activity of *Babesia canis* in addition to pathological changes in the circulatory system of sick dogs, there is an increase in the activity of some enzymes in the blood plasma. Thus, in dogs with babesiosis during the acute stage of the invasion, hyperenzymemia of aspartate aminotransferase (AST) by 5 times, alanine aminotransferase (ALT) by 3 times, γ -glutamyl transpeptidase (γ -GTP) and alkaline phosphatase (ALP) by 2 times, respectively, were established compared with control (Table 2).

Accordingly, the most sensitive during the acute stage of babesiosis in dogs were enzymes AST and ALT. Since the studied enzymes belong to the nonspecific group, the established patterns indicate the generalized nature of structural and functional changes in the internal organs of sick dogs.

Moreover, they are relatively specific to organs such as the liver, heart, skeletal muscles, kidneys and brain, which indicates their involvement in the pathological process. Disorders of the functional state of these organs in this blood-parasitic disease are primarily associated with general intoxication of the body with waste products of babesia, damage to erythrocytes and their intravascular hemolysis, as well as severe hepatotoxicity of drugs.

Table 2. Blood plasma enzymes in dogs with an acute course of babesiosis invasion ($M \pm m$, $n = 7$)

Indicator	Reference limits	Control	Dogs with babesiosis
Aspartate aminotransferase, U/L	5–55	30.3 ± 0.8	$157.2 \pm 6.9^*$
Alanine aminotransferase, U/L	5–107	56.1 ± 1.9	$160.3 \pm 8.5^*$
γ -glutamyl transpeptidase, U/L	0–14	6.7 ± 0.6	$14.1 \pm 0.8^*$
Alkaline phosphatase, U/L	10–150	80.5 ± 2.6	$128.7 \pm 5.7^*$

Note: $*P < 0.05$, statistically significant differences compared to control.

As a result of native blood study, anemia was diagnosed in sick dogs, which was confirmed for four of the six animals. At the same time, a decrease in the content of hemoglobin by 17% (hypochromia) was noted in the blood (Table 3).

Along with this, among the biochemical parameters of blood plasma, the content of total protein undergoes probable changes against the background of hypoalbuminemia, which decreases by 15%, according to the control, which leads to a natural increase in urea concentration by 28% and is a reflection of the increased course of catabolic processes, primarily in hepatocytes (Table 3).

Due to the intensive development of tissue hypoxia, the main source of energy for parasites is anaerobic glycolysis, which intensive course leads to excessive lactate formation. Increased concentration of the latter in the blood causes the development of lactic acidosis. The occurrence of the latter also enhances the increase in vascular permeability, vasodilation, hypotension and blood stasis, which are typical for the pathogenesis of this blood-parasitic disease.

The shift of the active reaction of blood and intracellular environment to the acidic side also provokes a decrease in sensitivity to circulating catecholamines, an increase in lipid peroxidation of cell membranes, a violation of oxidative phosphorylation processes, a sharp decrease in adenosine triphosphate production, and, in general, affects the enzyme activity. As a result, there are changes in the direction and intensity of metabolic processes.

Further progression of this condition, as is known [4, 6, 12] leads to irreversible violations of the structural organization of specialized cells and the functional state of a number of internal organs (in particular, liver, heart, kidneys, brain). However, at this stage of the disease in dogs, the level of total bilirubin and its conjugated fraction remained unchanged (Table 2).

Table 3. Biochemical parameters of native blood/plasma in dogs with an acute course of babesiosis ($M \pm m$, $n = 7$)

Indicator	Reference limits	Control	Dogs with babesiosis
Hemoglobin, g/L	120–180	127.0 ± 4.2	$105.1 \pm 2.1^*$
Total protein, g/L	51–78	64.5 ± 1.4	$55.0 \pm 1.1^*$
Albumin, g/L	24–44	32.1 ± 1.1	$27.3 \pm 0.9^*$
Creatinine, $\mu\text{mol/L}$	44–138	79.2 ± 3.1	80.9 ± 2.2
Urea, mmol/L	2.5–9.6	6.1 ± 0.2	$7.8 \pm 0.5^*$
Cholesterol, mmol/L	2.4–8.4	4.8 ± 0.3	4.2 ± 0.2
Glucose, mmol/L	3.9–8.3	6.1 ± 0.3	5.5 ± 0.2
Total bilirubin, $\mu\text{mol/L}$	0–5	2.5 ± 0.3	2.9 ± 0.1
Direct bilirubin, $\mu\text{mol/L}$	0–3	1.5 ± 0.1	1.4 ± 0.2
Potassium, mmol/L	3.6–5.5	4.6 ± 0.2	4.4 ± 0.3
Calcium, mmol/L	2.2–3.0	2.6 ± 0.1	2.3 ± 0.4
Phosphorus, mmol/L	0.68–2.00	1.34 ± 0.15	1.43 ± 0.19
Magnesium, mmol/L	0.7–1.1	0.9 ± 0.1	0.8 ± 0.1

Note: $*P < 0.05$, statistically significant differences compared to control.

Conclusions on the research topic. Therefore, the peculiarities of the hematological profile in dogs in the acute stage of babesiosis invasion are leukocytopenia with a decrease in the number of band neutrophils on the background of compensatory manifestations of basophilia and monocytosis, as well as erythrocytopenia, hypochromia, thrombocytopenia with a decrease in hematocrit and thrombocrit, which indicates a decrease in the reactivity of the organism due to suppression of the functional capacity of leukocytopoiesis, the development of anemia and coagulopathy.

The most pronounced changes among the biochemical parameters of blood plasma in dogs in the acute course of babesiosis invasion were: the activity of aminotransferases - aspartate and alanine aminotransferases, hemoglobin, the content

of total protein, albumin, urea, which confirms the development of anemia and the presence of structural and functional changes in hepatocytes in this pathology and may be due not only to the toxic effects of babesia on the liver parenchyma, but also as a result of the use of potent drugs.

Established patterns of features of qualitative and quantitative characteristics of biochemical blood and plasma parameters are important to clarify the pathogenesis of babesiosis in dogs, determining the characteristics of functional changes in the body of sick animals in the acute stage of its course, which in the long run will help to develop new approaches in the diagnosis, prevention of complications and treatment of animals.