



Dogs' general response to babesiosis infection of various severities

Viktoriia Gryshchenko*

Doctor of Veterinary Sciences, Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
<https://orcid.org/0000-0001-6601-1392>

Olena Semenko

PhD in Veterinary Sciences, Associate Professor
Public University of Navarra
31006, "Arrosadia" Campus, Pamplona, Navarra, Spain
<https://orcid.org/0000-0002-6453-6192>

Diana Bilokur

Veterinarian
Veterinary clinic "Zoolux"
02000, 39 Dmytrivska Str., Kyiv, Ukraine
<https://orcid.org/0009-0007-3516-3204>

Abstract. Global climate change in recent decades has led to an increase in the activity and expansion of the range of many diseases. One of them is canine babesiosis. Therefore, it is becoming increasingly important to monitor the functional state of the organism in sick dogs and timely detection of kidney, liver, spleen, cardiovascular system, anaemia, and other disorders that complicate the course of babesiosis. The research aims to determine the peculiarities of metabolic and functional changes in dogs with different intensities of babesiosis infection. During the laboratory diagnostics, general and special research methods were used: light microscopy, centrifugation, spectrophotometry, and mathematical statistics. Based on the results of comprehensive microscopic, morphological, and biochemical studies of blood in dogs with different intensities of babesiosis infection, the most characteristic functional and metabolic changes in their body were identified. Thus, at different intensities of babesiosis infection in diseased animals, the features of the haematological profile are leukocytopenia against the background of eosinopenia (at a mild degree of infection) and

Suggested Citation:

Gryshchenko, V., Semenko, O., & Bilokur, D. (2023). Dogs' general response to babesiosis infection of various severities. *Ukrainian Journal of Veterinary Sciences*, 14(4), 59-73. doi: 10.31548/veterinary4.2023.59.

*Corresponding author



Copyright © The Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (<https://creativecommons.org/licenses/by/4.0/>)

lymphocytopenia with a simultaneous compensatory increase in the number of monocytes and neutrophils, as well as erythrocytopenia, hypochromemia, thrombocytopenia with a decrease in thrombocrit and haematocrit. In addition, these patients developed hyperfermentemia of aspartate and alanine aminotransferases, alkaline phosphatase, and γ -glutamyl transpeptidase, indicating structural and functional changes, primarily in the myocardium, liver, skeletal muscle, brain and kidneys due to their toxic damage by babesia waste products. At the same time, hypoproteinaemia, hypoalbuminemia and hyperazotemia were observed in the affected animals, indicating an increase in the intensity of catabolic processes in functional cells of organs and tissues, especially the liver. The most pronounced changes in the studied parameters were observed at high intensity of babesiosis infection. The established regularities allow the use of these haematological parameters as markers of functional and metabolic changes in the body of dogs at different intensities of babesiosis infection

Keywords: morphological blood parameters; haematocrit; thrombocrit; biochemical blood parameters; leukogram; enzymes

Introduction

Babesiosis is a blood-borne parasitic disease of domestic and wild carnivores and fur-bearing animals caused by protozoa of the genus *Babesia*, spread by ixodid ticks, and is recorded on all continents. In dogs, the disease is most often acute with a high mortality rate. The pathogenesis of severe babesiosis in dogs is primarily based on generalised, multiorgan changes, which require timely diagnosis and constant monitoring of the functional state of both individual organs and their systems, as well as the organism as a whole. Therefore, the research relevance lies in the need to determine marker indicators, in particular blood, for effective and timely laboratory diagnosis of functional disorders in dogs with babesiosis infection.

According to the researchers, due to significant climate change on the planet, there is an intensive increase in the period of activity of babesiosis vectors, and therefore this disease is increasingly recorded in dogs in winter. As a result, babesiosis, which used to be a localised disease, is now becoming widespread and should be subject to constant monitoring. V.A. Levytska *et al.* (2020) reported that the

territory of Ukraine is considered an epizootic focus of babesiosis in dogs. It should also be noted that infected animals develop non-sterile immunity for 1-2 years, and therefore J.A. Panti-May & R.I. Rodríguez-Vivas (2020) focus on the prospects for a comprehensive study of this infection in mammals. According to A. Hildebrandt *et al.* (2021), more than 400 such cases have already been recorded among the US population and 21 in Europe. According to V.A. Levytska *et al.* (2020), the most common causative agent of the disease in European countries is *B. canis*, but species such as *B. vogeli*, and *B. gibsoni* are also common. I.I. Torianyk (2021) also notes defects in the walls of blood vessels with dissection, haemorrhage, microvasculature and endothelial desquamation, the development of intravascular coagulation and thrombosis, microcirculatory disorders, and ischaemia. As noted by W. Zygnier *et al.* (2023), protozoa of the genus *Babesia* parasitise primarily erythrocytes, although they can be found in blood plasma and the cytosol of cells of the reticuloendothelial system. According to K. Rajamanickam & V. Leela (2023), *Babesia*

canis destroys red blood cells in diseased animals directly in the vascular bed, which is complicated by the development of hypotensive shock and generalised damage to functional cells of organs and tissues due to oxygen deficiency.

Among the most characteristic changes in red blood cells in the peripheral bloodstream, M. Liu *et al.* (2022) focus on the development of oligocythemia with anisocytosis and poikilocytosis, as well as the occurrence of oligochromemia, manifested by macrocytosis and a decrease in hematocrit. At the same time, significant changes in the morphological and functional state of erythrocytes are diagnosed in affected dogs. In particular, they affect the population composition of erythrocytes, namely the number of mature red blood cells that undergo rapid destruction predominates, and the number of young forms significantly decreases. D. Sojka *et al.* (2022) describe an increase in the content of free iron in the blood and a simultaneous decrease in the level of saturation of transferrin with this trace element. As a result of the described processes, haemoglobin synthesis at the level of red bone marrow is impaired, which leads to the development of anaemia, and subsequently jaundiced skin, visible mucous membranes, and haemoglobinuria. Among the established patterns, normochromic, normocytic, and non-regenerative anaemia is observed during the first day of clinical manifestation of the disease, and then, on the second or third day, hypochromic, macrocytic, with reticulocytosis.

During the active phase of the disease in dogs, according to V.A. Gryshchenko & D.S. Bilokur (2021), functional disorders of the cardiovascular, digestive, and urinary systems are most often detected, and in severe clinical forms, even cerebral pathology. Therefore, a general clinical blood test for babesiosis infection allows for determining the degree and extent of damage to internal organs in such

patients. In particular, J. Kuleš *et al.* (2023) note the need for an in-depth study of the features of the disease manifestation at the cellular level and the identification of new markers for assessing the functional state of both the whole organism and its internal organs. Modern laboratory diagnostics of animal diseases is based on the use of sensitive biochemical tests and methods for diagnosing most animal diseases, including babesiosis, which also allows for the control of the course of the pathological process, the occurrence of possible complications and determining treatment tactics.

Based on the aforementioned, the research aims to determine the general reaction of the Labrador dog to different intensities of babesiosis infection caused by *B. canis*. To achieve the aim of the study, the following tasks were set: to identify and differentiate the causative agent of canine babesiosis microscopically; to select animals with low and high intensity babesiosis infection; to study the effect of the blood parasite on the functional state of the organism and to determine marker changes in blood parameters in sick dogs with different intensities of babesiosis infection.

Literature Review

The clinical manifestation of Babesiosis infection in dogs has its characteristics depending on the type of *Babesia* species involved. As noted by V.A. Levytska *et al.* (2020), different strains of *Babesia canis* differ in pathogenicity. In some regions, the disease may be moderately expressed, while in others it is highly pathogenic. Dogs of all ages are susceptible, but puppies are more often affected. The incubation period for *B. canis* is 10-21 days, and in the case of *B. gibsoni*, it is 14-28 days. V. Djokic *et al.* (2021) described that the mortality rate from *Babesia spp.* reaches more than 12%, and in the case of *B. rossi*, up to 1%. There is no cross-immunity between *Babesia canis* and *Babesia gibsoni*.

According to B.K. Atkinson *et al.* (2022), in acute cases, the first clinical symptom of the disease is a significant increase in body temperature (up to 42°C). According to M. Torti *et al.* (2020), the most common symptom of babesiosis in dogs is the development of haemolytic anaemia caused, in particular, by the destruction of red blood cells due to increased osmotic fragility, extra- and intravascular haemolysis, erythrophagocytosis and immune-mediated destruction of red blood cells by parasitic antigens, parasite-induced cell membrane damage, and anaemia. Haemoglobin dysfunction, oxidative damage, contamination, and sequestration of red blood cells are also possible. O. Mazanny *et al.* (2020) noted an increase in haematocrit values as a result of relative haemoconcentration, which manifested itself simultaneously with increased haemolysis against the background of fluid movement from the intravascular to the extravascular component. Such animals were at risk due to the high threat of functional disorders of internal organs.

V. Djokic *et al.* (2021) note that the severity of the clinical course of babesiosis in dogs can vary significantly, from subclinical to acute or even chronic. Typical symptoms of this pathology include fever, depression, anorexia, anaemia of the mucous membranes and skin, splenomegaly, tachypnoea, and tachycardia. Clinical symptoms are caused by the development of anaemia and, as a result, tissue hypoxia in affected dogs, as well as the active release of cytokines, which provokes the manifestation of a systemic inflammatory response syndrome. Patients have no appetite, but thirst and general weakness are noted. Frequent manifestations include haemoglobinuria. M. Torti *et al.* (2020) point out that in chronic cases, body temperature is not too high and rarely lasts more than a few days. Anaemia and jaundice are less severe. In general, dogs become noticeably weaker and lose weight. Damage to the circulatory system is

manifested by vasodilation with a tendency to disseminate intravascular coagulopathy at the capillary level, increased permeability and may progress to hyperaemia and oedema. In case of central nervous system damage, movement disorders, paresis, epileptic seizures, etc. occur.

T. Rawangchue & S. Sungpradit (2020) indicate that *Babesia vogeli* in adult dogs causes an asymptomatic infection. Parasitaemia is extremely low, and babesiosis infection may not be diagnosed by blood smear examination. However, puppies show severe anaemia, and the subclinical course is mainly observed in adult dogs. B.L. Penzhorn (2020) reports the clinical manifestation of infection caused by *Babesia canis* shows greater variability in pathogenicity, intermediate between *B. vogeli* and *B. Rossi*, with most dogs showing anaemia. According to O. Teodorowski *et al.* (2022), *Babesia gibsoni* infection has a subacute, acute, or chronic course. As noted by the researchers, the most common is the acute course, which is manifested by the development of fever, haemolytic anaemia, severe weakness, splenomegaly, lymphadenopathy, and thrombocytopenia. The superacute state is rare. American Pit Bull and Staffordshire Bull Terrier disease is mainly transmitted through dog bites. PCR-positive subclinical infections with *B. gibsoni* have also been reported.

Materials and Methods

Haematological studies were conducted in 2022-2023 at the interdepartmental educational and scientific laboratory of veterinary diagnostic research at the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine and the laboratory of the Zoolux Veterinary Clinic (39 Dmytrivska Str., Kyiv).

The research methodology included a general clinical blood test and assessment of the biochemical profile of blood in dogs with acute babesiosis with different intensities of infection.

For scientific purposes, three groups of Labrador dogs aged 2 to 7 years and weighing 23.6-35.2 kg were formed. Experimental group 1 included dogs with a low-intensity babesiosis infection (4 animals with the names: Akita, Bruno, Jess, and Malysh), experimental group 2 included animals with a high-intensity infection (4 animals with the names: Danya, Bonia, Peta, and Charlie). The control group included 6 clinically healthy dogs (nicknames: Lisa, Fly, Boy, Day, Alma, Bars) aged 2 to 7 years, which underwent preventive examination at the Zoolux veterinary clinic (Kyiv). The results of a general clinical blood test in animals of this group corresponded to the reference range of physiological values typical for healthy dogs.

Blood samples for haematological studies were taken from the anterior saphenous vein of the forearm. The study design included the following steps:

- creation of smears of heparin-stabilised blood and determining the presence of babesia;
- identification of the pathogen and determination of the intensity of the babesiosis infection;
- blood centrifugation of stabilised ethylenediaminetetraacetic acid (EDTA) for biochemical tests at 3000-3500 rpm for 5 min;
- conducting a general clinical blood test using a Dymind DF51 haematological analyser (China) (examination of the number of formed elements, leukogram, haematocrit and thrombocrit, as well as haemoglobin content in whole blood);
- biochemical analysis of blood parameters on analysers “StatFax 4500” (USA), “Mindray BS-230” (China) (study of the activity of the following enzymes in blood plasma: alanine aminotransferase (ALT, ES 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1), alkaline phosphatase (ALP, EC 3.1.3.1), γ -glutamyl transpeptidase (γ -GTP, EC 2.3.2.2);
- a complex of biochemical parameters: total protein, albumin, creatinine, urea,

cholesterol, glucose, total bilirubin, direct bilirubin, potassium, calcium, inorganic phosphorus, and magnesium);

- research result summarisation and their analysis.

Laboratory diagnostic studies were performed using reagents from Global Scientific (USA). Microscopic assessment of the detection of intraerythrocytic parasites in stained blood smears was performed using the Romanowsky-Giemsa method. Light microscopy was used using a Micros microscope (Austria, 2012). Leukodif 200 stains (LDF 200, Czech Republic) were used to stain blood smears.

The intensity of erythrocyte damage by protozoa was determined per 100 cells and marked as “+”:

“+” – up to 1-2 erythrocytes affected by *Babesia* were assessed as low intensity of infection;

“++” – up to 3-4 erythrocytes affected by *Babesia* were assessed as medium intensity of infection;

“+++” – up to 1-4 erythrocytes affected by *Babesia* were assessed as severe infection.

At low intensity of infection, 1-2 *Babesia* were detected in one erythrocyte, and at high intensity – 4-6 or more protozoa in one cell. Experimental groups were formed from animals with only low and high-intensity of infection.

The results of the conducted research were processed using generally accepted methods of variation statistics with the involvement of computer programs. In this case, the values of arithmetic means (M) were calculated considering n – the size of a particular sample. The Student’s t-test was used to finally determine the existence of significant differences between the mean values for each indicator. Differences were considered significant at $P < 0.05$.

Experimental studies complied with the requirements of ISO/IEC 17025:2005 (2006). The animals were kept, and all manipulations were carried out following the provisions of the

Procedure for conducting experiments and experiments on animals by scientific institutions (Law of Ukraine No. 249, 2012), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European convention..., 1986).

Results

According to the results of clinical examination of dogs with babesiosis, the presence of hyperthermia (increase in body temperature to 40-41°C), anaemia of non-pigmented skin and visible mucous membranes was noted, which later in some animals with a high intensity of infection was replaced by icterus, loss of appetite, depression, exhaustion and the appearance of brown urine colour (in three animals named

Danya, Peta, Charlie). The latter may indicate the occurrence of a symptom characteristic of high-intensity infestation – haemoglobinuria. Generalised changes in most internal organs and their systems during this infection significantly affect the severity of its course, which requires regular monitoring of the functional state of the organism of sick dogs. Eight cases – 4 of low and 4 of high-intensity babesiosis infection in dogs caused by *Babesia canis* were described.

Microscopic picture of blood in dogs with different intensities of Babesiosis infection. Microscopic examination revealed *Babesia canis* in the blood smear. In four dogs – Akita, Bruno, Jesse, and Malysh – the infection was assessed as “+”, these dogs were included in the group with a low intensity of infection (Fig. 1).

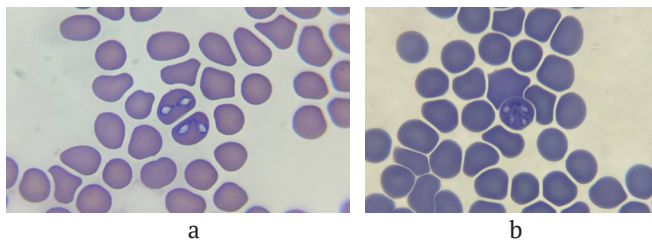


Figure 1. Blood sample analysis for babesiosis in dogs with low intensity of *B. canis*

Note: photos of blood samples: a and b, magnification ×400

Source: compiled by the authors

In four dogs - Danya, Bonya, Peta and Charlie – the infection corresponded to “+++”, which

was assessed as a high intensity of the lesion (Fig. 2).

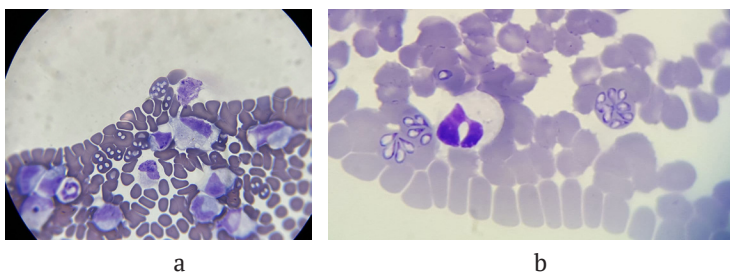


Figure 2. Blood sample analysis for babesiosis in dogs with high intensity of *B. canis*

Note: photos of blood sample preparations: a – ×200 magnification, b – ×360 magnification

Source: compiled by the authors

General clinical blood test in dogs with different intensities of babesiosis infection.

Morphological studies of the blood of Labrador dogs with babesiosis showed the presence of leukocytopenia, which was characterised by a decrease in the number of leukocytes by 31.0% at low and 60.6% at high intensity of infection. Lymphocytopenia was diagnosed in dogs of both experimental groups, in particular, a decrease in the relative number of lymphocytes by 2.2 times at low and 2.3 times at high intensity of babesiosis infection. The fact indicates a decrease in the resistant state of the organism in diseased animals, which may be a consequence of impaired functional capacity of leukocyte poiesis organs (bone marrow, lymph nodes, spleen) due to infection with the

pathogen of blood cells and progression of intoxication. At the same time, neutrophilia was observed in the blood of the diseased animals compared to the control, i.e., an increase in the relative number of segmented neutrophils by 1.1 times in both experimental groups and monocytosis, i.e., an increase in the relative number of monocytes both at low (2.0 times) and high (2.3 times) intensity of invasion, which reflects the body's defence response and indicates the phenomenon of active phagocytosis of protozoa, erythrocyte decay products, bacteria and endotoxins (Table 1). Eosinopenia was diagnosed only in animals with a low intensity of infection. In this case, the relative number of eosinophils was 3.0 times lower compared to the control.

Table 1. General clinical analysis of blood parameters in dogs with different intensities of babesiosis infection (M±m, n=4-6)

Indicator	Reference limits	Control	Dogs with low infection severity	Dogs with high infection severity
White blood cells, 10 ⁹ /L	4.5-15.5	7.1±0.5	4.9±0.4*	2.8±0.3*
Stick-nucleated neutrophils, %	0-3	1.7±0.2	0	0
Segmented neutrophils, %	60-77	67.5±2.4	75.6±2.1*	72.3±1.9
Eosinophils, %	2-10	2.4±0.3	0.8±0.2*	2.0±0.1
Basophils, %	0-1	0.5±0.1	0	0
Monocytes, %	0-10	7.3±0.8	14.3±1.0*	16.9±0.9*
Lymphocytes, %	12-30	20.6±1.8	9.3±0.8*	8.8±0.7*
Red blood cells, 10 ¹² /L	4.8-9.3	6.1±0.8	5.1±0.4	3.9±0.2*
Haemoglobin, g/L	120-180	127.0±4.2	113.2±3.3*	83.8±4.1*
Haematocrit, %	36-55	43.5±1.2	32.0±1.4*	23.0±1.2*
Platelets, 10 ⁹ /L	170-500	339.3±17.1	32.8±2.1*	25.8±2.4*
Thrombocrit, %	0.14-0.46	0.30±0.1	0.03±0.01*	0.02±0.01*

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

Source: compiled by the authors

The analysis of the results of morphological blood examination proved the presence of anaemia in diseased dogs. Thus, in the blood of animals with babesiosis at a high intensity of infection, a decrease in the number of red blood cells by 36.1% (erythrocytopenia) and haemoglobin content (hypochromemia) by

10.9% (at low intensity of infection) and 34.0% (at high intensity of infection) was observed with a simultaneous decrease in hematocrit value, respectively, by 1.4 and 1.9 times (Table 1). Significant changes were also noted in the number of platelets and thrombocrit in the blood of diseased animals. Thus, in

diseased animals, the number of platelets was reduced (thrombocytopenia) by 10.3 times (at low intensity of infection) and 13.2 times (at a high intensity of infection), and the value of thrombocrit decreased according to the intensity of infection by 10 and 15 times compared to the control.

Thus, in dogs with babesiosis, characteristic changes in the morphological profile of the blood are leukocytopenia against the background of eosinopenia (with a mild degree of infection) and lymphocytopenia, along with adaptive manifestations of monocytosis and neutrophilia. In addition, these animals were diagnosed with hypochromemia, erythrocytopenia (with high intensity of invasion), and thrombocytopenia with a decrease in thrombocrit and haematocrit. The intensive development of anaemia in sick dogs, especially pronounced at high intensity of infection, provokes the development of circulatory and tissue hypoxia, which causes shifts in the homeostasis

system and corresponding disorders of functional activity on the part of organs and their systems in the body, which significantly affect the biochemical profile of blood plasma.

Biochemical profile of blood plasma in dogs with different intensities of babesiosis infection. In dogs with babesiosis, in addition to changes in the morphological parameters of the blood, a significant increase in the activity of several enzymes was noted (Table 2). Thus, in dogs with babesiosis, hyperenzymemia was found: aspartate aminotransferase 1.7 times (at low intensity of infection) and 4.0 times (at high intensity of infection), alanine aminotransferase 1.1 times (at low intensity of infection) and 2.8 times (at high intensity of infestation); γ -glutamyl transpeptidase by 1.3 times (at mild intensity of infestation) and 1.5 times (at high intensity of infestation), alkaline phosphatase by 2.8 times (at mild intensity of infestation) and 4.6 times (at high intensity of infestation) compared to the control.

Table 2. Plasma enzyme activity in dogs with different intensities of babesiosis infection (M \pm m, n=4-6)

Indicator	Reference limits	Control	Dogs with low infection severity	Dogs with high infection severity
Aspartate aminotransferase, U/L	5-55	30.3 \pm 0.8	50.9 \pm 1.4*	120.3 \pm 3.7*
Alanine aminotransferase, U/L	5-107	56.1 \pm 1.9	62.6 \pm 1.3*	156.8 \pm 4.1*
γ -Glutamyl transpeptidase, U/L	0-14	6.7 \pm 0.6	9.0 \pm 1.1*	9.8 \pm 1.3*
Alkaline phosphatase, U/L	10-150	80.5 \pm 2.6	228.1 \pm 6.4*	366.1 \pm 7.1*

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

Source: compiled by the authors

In addition, in the context of the biochemical profile of blood plasma, a significant decrease in the content of total protein by 15% (at low intensity of invasion) and 29.9% (at high intensity of invasion) was noted compared to the control, against the background of hypoalbuminemia (according to the degree of invasion, a decrease in albumin level by 26.2 and 35.0%). This resulted in a significant increase in blood

plasma urea concentration by 70.5% (with severe infection), which reflected an increase in the intensity of catabolic processes, especially in liver cells (Table 3).

At the same time, the concentration of bile pigments – total and conjugated bilirubin, as well as other biochemical parameters – remained unchanged in the blood plasma of the affected dogs.

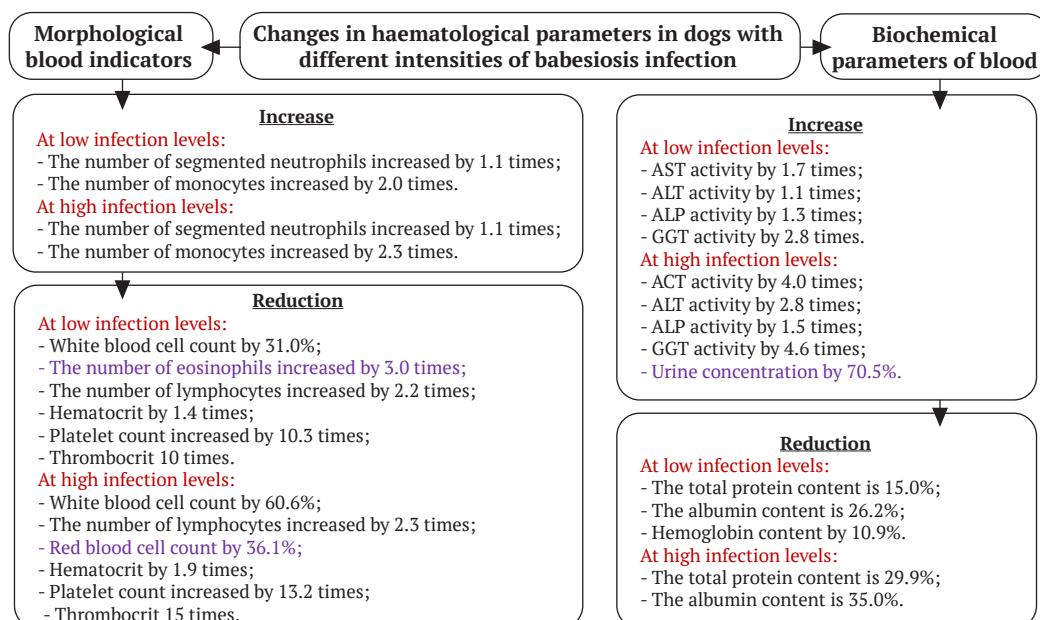
Table 3. Biochemical profile of dog blood plasma with different intensity of babesiosis infection (M±m, n=4-6)

Indicator	Reference limits	Control	Dogs with low infection severity	Dogs with high infection severity
Total protein, g/L	51-78	64.5±1.4	54.8±1.3*	45.2±1.1*
Albumin, g/L	24-44	32.1±1.1	23.7±0.8*	20.9±0.6*
Creatinine, µmol/L	44-138	79.2±3.1	74.6±2.3	73.1±3.4
Urea, mmol/L	2.5-9.6	6.1±0.2	4.9±0.6	10.4±0.9*
Cholesterol, mmol/L	2.4-8.4	4.8±0.3	5.2±0.3	4.3±0.8
Glucose, mmol/L	3.9-8.3	6.1±0.3	6.0±0.8	6.3±0.5
General bilirubin, µmol/L	0-5	2.5±0.4	1.8±0.3	2.2±0.4
Direct bilirubin, µmol/L	0-3	1.5±0.3	1.3±0.1	1.6±0.2
Potassium, mmol/L	3.6-5.5	4.6±0.2	4.0±0.3	4.3±0.4
Calcium, mmol/L	2.2-3.0	2.6±0.1	2.2±0.3	2.1±0.3
Inorganic phosphorus, mmol/L	0.68-2.00	1.3±0.2	1.0±0.2	1.5±0.4
Mangesium, mmol/L	0.7-1.1	0.9±0.1	0.7±0.2	0.9±0.2

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

Source: compiled by the authors

The results obtained are summarised and presented schematically in Figure 3, which shows the differences in the qualitative and quantitative characteristics of morphological and biochemical parameters of blood in dogs with different intensities of babesiosis infection.

**Figure 3.** Peculiarities of changes in morphological and biochemical parameters at different intensities of babesiosis infection in Labrador dogs

Note: Changes in indicators that are specific to a particular group of sick dogs are highlighted in purple

Source: compiled by the authors

As such, marker changes among the indicators of the biochemical profile of blood plasma in dogs with different intensities of babesiosis infection were observed: the activity of aminotransferases – aspartate and alanine aminotransferase, γ -glutamyl transpeptidase and alkaline phosphatase, the content of total protein, albumin, urea (at high intensity of infection), which confirms the development of destructive changes and functional disorders in the cells of the liver, kidneys and other internal organs. This may be not only the result of the toxic effects of the blood parasite's waste products on functional cells of internal organs but also the consequence of the use of potent medicinal products in sick dogs.

Discussion

The results of observation of dogs with babesiosis indicate the development of a symptom complex typical of this blood-borne disease. According to O. Mazanyy *et al.* (2020), almost 60-70% of affected animals showed the following clinical changes: characteristic of visible mucous membranes, haemoglobinuria, and hyperthermia, which confirms our study results. In most animals, the researchers also recorded other symptoms (weakness, loss of appetite, shortness of breath, thirst, etc.), the appearance of which depended on the intensity of the babesiosis infection and the severity of the disease. A similar clinical picture of the course of babesiosis in dogs is described by J.A. Panti-May & R.I. Rodríguez-Vivas (2020) and W. Zygnier *et al.* (2023), which may indicate the identity of the mechanisms of pathological effects of babesia on the body of dogs. It can be assumed that differences in the general manifestation of the disease in most cases correlate with the degree of pathogenicity of the pathogen, the intensity of its invasion and the individual characteristics of the resistant state of the dog's body. The results of comprehensive haematological studies in patients with babesiosis

infection of Labrador dogs indicate pathological changes in the structural and functional state of most internal organs, which generally proves the generalised form of the disease course at both low and high intensity of infection. This indicates the need for continuous monitoring of the functional state of the body, which is also mentioned by O. Dubova & A. Duboviy (2018), A. Henning *et al.* (2020), M. Karasová *et al.* (2022) and other researchers.

In particular, according to the results of haematological studies of blood smears from sick dogs with varying degrees of babesiosis infection, a decrease in the body's resistance state was noted, which may be due to a dysfunction of the functional capacity of leukocyte poiesis organs (bone marrow, lymph nodes, spleen) during infection with the pathogen of blood cells and progression of intoxication. At the same time, similarly to a study by F. Dantas-Torres *et al.* (2021), neutrophilia and monocytosis were observed in the blood of diseased animals compared to controls, the degree of which was determined by the intensity of babesiosis infection. The established patterns reflect the organism's defence reaction and are closely related to the phenomenon of active phagocytosis of protozoa, erythrocyte decay products, bacteria and endotoxins. According to the study, eosinopenia was diagnosed only in animals with a low intensity of infection.

Furthermore, during the course of babesiosis infection in dogs, all four enzymes studied were sensitive: aspartate and alanine aminotransferases, γ -glutamyl transpeptidase and alkaline phosphatase. This was particularly pronounced in animals with a high intensity of infection and confirms the results of similar haematological studies described by O. Shchebentovska *et al.* (2022). In particular, they noted the development of hyperfermentation of aspartate aminotransferase and aspartate aminotransferase, respectively, by 5 and

3 times compared to the control. O. Mazannyi *et al.* (2020) reported a more than 3-fold increase in aspartate aminotransferase activity in the blood plasma of sick dogs, with no abnormalities in alanine aminotransferase activity, creatinine, and urea concentrations. These facts indicate similar trends, and the differences relate only to numerical values, which are explained by different approaches to research. The study by V.A. Levytska *et al.* (2020) found that in the group of dogs with azotemia, a higher ratio of aspartate aminotransferase/alanine aminotransferase activity was recorded. This fact suggests that kidney damage causes an increase in the activity of aspartate aminotransferase in affected dogs.

The diagnosed changes in the activity of enzymes in the blood indicate a generalised nature of functional and structural disorders in the internal organs of sick dogs. In particular, V. Djokic *et al.* (2021) reported disorders of the structural organisation of specialised cells and the dependent functional state of several internal organs (heart, liver, brain, kidneys). These changes are also confirmed by A.S. Ubah *et al.* (2019), who added skeletal muscle and brain to the previous list. This suggests that the dysfunction of these organs is primarily associated with intravascular haemolysis of red blood cells affected by blood parasites, progressive hypoxia, and intoxication of the body with babesia waste products, as well as severe hepatotoxicity of potent therapeutic agents.

The development of circulatory and tissue hypoxia in dogs with babesiosis occurs against the background of the progression of haemolytic anaemia and pathological changes in the blood vessel wall. As noted by M. Torti *et al.* (2020), with babesiosis infection in dogs, due to a decrease in the partial pressure of oxygen in the tissues, anaerobic glycolysis, the main end product of which is lactate, becomes important in maintaining the energy balance in cells. An

increase in its concentration in the blood causes the development of lactate acidosis. According to these authors, acidification of the internal environment of the body in sick dogs provokes an increase in vascular wall porosity, vasodilation, blood stasis and a decrease in blood pressure, which are noted in the pathogenesis of this blood-parasitic disease. The development of lactate acidosis also causes a decrease in sensitivity to circulate catecholamines, an increase in the intensity of peroxidation of cell membrane lipids, disruption of oxidative phosphorylation, a sharp decrease in the production of adenosine triphosphoric acid, and, in general, affects enzymatic activity. As a result, according to T. Ullal *et al.* (2018), there are changes in the intensity of metabolic reactions and their direction, as well as disorganisation and disorders of the functional state of cell membranes of organs and tissues.

Thus, in the case of complex haematological studies in dogs with babesiosis with different intensities of *B. canis* infection, marker changes in the morphological and biochemical parameters of blood were determined. The obtained results are important for elucidating the pathogenesis of this disease in dogs both at the level of the whole organism and individual organs and their systems, as well as for determining the features of functional changes at low and high intensity of infection, which will contribute to the development of new approaches in their laboratory diagnosis, prevention of complications and increase the effectiveness of animal treatment.

Conclusions

Based on the results of comprehensive microscopic, morphological, and biochemical studies of blood samples taken from dogs with different intensities of babesiosis infection (mild and high) caused by *B. canis*, the most characteristic metabolic and functional changes in the

diseased organism were identified. Thus, depending on the level of babesiosis infection, the development of leukocytopenia was diagnosed in sick dogs, namely: a decrease in the absolute number of leukocytes in the blood by 31.0% at low and 60.6% at high intensity, which manifested itself against the background of eosinopenia (only at low intensity of infection) and lymphocytopenia with simultaneous monocytosis and neutrophilia and indicates suppression of leukocytopoiesis. In addition, these animals were characterised by erythrocytopenia, hypochromemia, and thrombocytopenia with a decrease in thrombocrit and hematocrit, indicating the development of anaemia and coagulopathy, which were more pronounced at a high intensity of infection.

The most sensitive biochemical parameters of blood plasma in sick dogs, especially at high intensity of babesiosis infection, were enzymes: aspartate and alanine aminotransferase, γ -glutamyl transpeptidase and alkaline phosphatase, indicating destructive changes in internal organs, in particular, liver, heart, skeletal muscle and kidneys. Sick dogs with varying intensity of infection, especially those with a high degree of its manifestation, are characterised by

hypoproteinemia, hypoalbuminemia and hyperazotemia, which proves the presence of homeostasis disorders and the prevalence of catabolic processes in the cells of internal organs, in particular hepatocytes. The observed patterns of changes in the morphological and biochemical parameters of blood in dogs with different intensities of babesiosis infection are important for clarifying the pathogenesis of this disease, determining the characteristic functional changes in the diseased organism, and developing effective methods of their laboratory diagnosis, effective prevention of complications and clarifying the strategy in the treatment of such patients.

A promising area for future research is to determine the peculiarities of the rehabilitation period for the restoration of functional activity of internal organs and metabolism in dogs suffering from various degrees of babesiosis infection caused by *B. canis*.

Acknowledgements

None.

Conflict of Interest

None.

References

- [1] Atkinson, B.K., Thompson, P., Van Zyl, E., Goddard, A., Rautenbach, Y., Schoeman, J.P., Mukorera, V., & Leisewitz, A. (2022). Kinetics of the inflammatory response during experimental *Babesia rossi* infection of beagle dogs. *Veterinary Parasitology*, 306, article number 109717. [doi: 10.1016/j.vetpar.2022.109717](https://doi.org/10.1016/j.vetpar.2022.109717).
- [2] Dantas-Torres, F., Alexandre, J., Miranda, D.E.O., Figueredo, L.A., da Silva Sales, K.G., de Sousa-Paula, L.C., da Silva, L.G., Valle, G.R., Ribeiro, V.M., Otranto, D., Deuster, K., Pollmeier, M., & Altreuther, G. (2021). Molecular epidemiology and prevalence of babesial infections in dogs in two hyperendemic foci in Brazil. *Parasitology Research*, 120, 2681-2687. [doi: 10.1007/s00436-021-07195-8](https://doi.org/10.1007/s00436-021-07195-8).
- [3] Djokic, V., Rocha, S.C., & Parveen, N. (2021). Lessons learned for pathogenesis, immunology, and disease of erythrocytic parasites: *Plasmodium* and *Babesia*. *Frontiers in Cellular and Infection Microbiology*, 11, article number 685239. [doi: 10.3389/fcimb.2021.685239](https://doi.org/10.3389/fcimb.2021.685239).
- [4] Dubova, O., & Duboviy, A. (2018). Hepathopathy and nephropathy in the dogs' babesiosis: pseudohepatorenal syndrome. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Veterinary Sciences*, 20(83), 102-107. [doi: 10.15421/nvlvet8320](https://doi.org/10.15421/nvlvet8320).

- [5] European convention for the protection of vertebrate animals used for experimental and other scientific purposes. (1986). Retrieved from <https://rm.coe.int/168007a67b>.
- [6] Gryshchenko, V.A., & Bilokur, D.S. (2021). The general reaction of dogs in the acute stage babesian invasion. *Ukrainian Journal of Ecology*, 11(5), 85-90. doi: 10.15421/2021_213.
- [7] Henning, A., Clift, S.J., & Leisewitz, A.L. (2020). The pathology of the spleen in lethal canine babesiosis caused by *Babesia rossi*. *Parasite Immunology*, 42(5), article number e12706. doi: 10.1111/pim.12706.
- [8] Hildebrandt, A., Zintl, A., Montero, E., Hunfeld, K.P., & Gray, J. (2021). Human babesiosis in Europe. *Pathogens*, 10(9), article number 1165. doi: 10.3390/pathogens10091165.
- [9] ISO/IEC 17025:2005. (2006). Retrieved from http://online.budstandart.com/ua/catalog/doc-page.html?id_doc=50873.
- [10] Karasová, M., Tóthová, C., Grelová, S., & Fialkovičová, M. (2022). The etiology, incidence, pathogenesis, diagnostics, and treatment of canine babesiosis caused by *Babesia gibsoni* infection. *Animals: An Open Access Journal From MDPI*, 12(6), article number 739. doi: 10.3390/ani12060739.
- [11] Kuleš, J., Rubić, I., Farkaš, V., Barić Rafaj, R., Gotić, J., Crnogaj, M., Burchmore, R., Eckersall, D., Mrljak, V., & Leisewitz, A.L. (2023). Serum proteome profiling of naturally acquired *Babesia rossi* infection in dogs. *Scientific Reports*, 13, article number 10249. doi: 10.1038/s41598-023-37312-9.
- [12] Law of Ukraine No. 249 “On the Procedure for Carrying out Experiments and Experiments on Animals by Scientific Institutions”. (2012, March). Retrieved from <https://zakon.rada.gov.ua/laws/show/z0416-12#Text>.
- [13] Levytska, V.A., Mushinsky, A.B., Zubrikova, D., Blanarova, L., Długosz, E., Vichova, B., Slivinska, K.A., Gajewski, Z., Gizinski, S., Liu, S., Zhou, L., & Rogovskyy, A.S. (2021). Detection of pathogens in ixodid ticks collected from animals and vegetation in five regions of Ukraine. *Ticks and Tick-Borne Diseases*, 12(1), article number 101586. doi: 10.1016/j.ttbdis.2020.101586.
- [14] Liu, M., Igarashi, I., & Xuan, X. (2022). *Babesia gibsoni*. *Trends in Parasitology*, 38(9), 815-816. doi: 10.1016/j.pt.2022.03.001.
- [15] Mazannyi, O., Prykhodko, Yu., Nikiforova, O., Fedorova, H., & Lyulin, P. (2020). Clinical and diagnostic studies babesiosis of dogs. *Bulletin “Veterinary Biotechnology”*, 37, 51-62. doi: 10.31073/vet_biotech37-06.
- [16] Panti-May, J.A., & Rodríguez-Vivas, R.I. (2020). Canine babesiosis: A literature review of prevalence, distribution, and diagnosis in Latin America and the Caribbean. *Veterinary Parasitology, Regional Studies and Reports*, 21, article number 100417. doi: 10.1016/j.vprsr.2020.100417.
- [17] Penzhorn, B.L. (2020). Don't let sleeping dogs lie: Unravelling the identity and taxonomy of *Babesia canis*, *Babesia rossi* and *Babesia vogeli*. *Parasites & Vectors*, 13, article number 184. doi: 10.1186/s13071-020-04062-w.
- [18] Rajamanickam, K., & Leela, V. (2023). [Low haemoglobin density as an anaemic indicator in canine babesiosis](#). *Indian Journal of Veterinary and Animal Sciences Research*, 49(1), 7-13.
- [19] Rawangchue, T., & Sungpradit, S. (2020). Clinicopathological and molecular profiles of *Babesia vogeli* infection and *Ehrlichia canis* coinfection. *Veterinary World*, 13(7), 1294-1302. doi: 10.14202/vetworld.2020.1294-1302.
- [20] Shchebentovska, O., Prus, B., & Danko, M. (2022). Pathomorphological changes in German boxer dog infected with *Babesia canis* parasites. Clinical case. *Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. Series: Veterinary Sciences*, 24(105), 10-17. doi: 10.32718/nvlvet10502.

- [21] Sojka, D., Jalovecká, M., & Perner, J. (2022). Babesia, theileria, plasmodium and hemoglobin. *Microorganisms*, 10(8), article number 1651. doi: [10.3390/microorganisms10081651](https://doi.org/10.3390/microorganisms10081651).
- [22] Teodorowski, O., Kalinowski, M., Winiarczyk, D., Dokuzeylül, B., Winiarczyk, S., & Adaszek, Ł. (2022). *Babesia gibsoni* infection in dogs – A european perspective. *Animals: An Open Access Journal From MDPI*, 12(6), article number 730. doi: [10.3390/ani12060730](https://doi.org/10.3390/ani12060730).
- [23] Toriyan, I.I. (2021). The role and significance of morphological changes in intraorgan vessels in animals during pathogenesis of babesiosis. *Actual problems of modern medicine: Bulletin of Ukrainian Medical Stomatological Academy*, 21(2), 160-164. doi: [10.31718/2077-1096.21.2.160](https://doi.org/10.31718/2077-1096.21.2.160).
- [24] Torti, M., Kules, J., Matijatko, V., Brkljacic, M., Kis, I., Gotic, J., Mrljak, V., & Iva, S. (2020). Acid-base status in canine babesiosis caused by *Babesia canis*. *Veterinary Archives*, 90(6), 603-610. doi: [10.24099/vet.arhiv.1230](https://doi.org/10.24099/vet.arhiv.1230).
- [25] Ubah, A.S., Abalaka, S.E., Idoko, I.S., Obeta, S.S., Ejiogor, C.E., Mshelbwala, P.P., Omeje, J.N., & Ajayi, I.E. (2019). Canine babesiosis in a male Boerboel: Hematobiochemical and anatomic pathological changes in the cardiorespiratory and reproductive organs. *Veterinary and Animal Science*, 7, article number 100049. doi: [10.1016/j.vas.2019.100049](https://doi.org/10.1016/j.vas.2019.100049).
- [26] Ullal, T., Birkenheuer, A., & Vaden, S. (2018). Azotemia and proteinuria in dogs infected with *Babesia gibsoni*. *Journal of the American Animal Hospital Association*, 54(3), 156-160. doi: [10.5326/JAANA-MS-6693](https://doi.org/10.5326/JAANA-MS-6693).
- [27] Zygnier, W., Gójska-Zygnier, O., & Norbury, L.J. (2023). Pathogenesis of anemia in canine Babesiosis: Possible contribution of pro-inflammatory cytokines and chemokines. *Pathogens*, 12(2), article number 166. doi: [10.3390/pathogens12020166](https://doi.org/10.3390/pathogens12020166).

Загальна реакція організму собак за різної інтенсивності бабезіозної інвазії

Вікторія Анатоліївна Грищенко

Доктор ветеринарних наук, професор

Національний університет біоресурсів і природокористування України

03041, вул. Героїв Оборони, 15, м. Київ, Україна

<https://orcid.org/0000-0001-6601-1392>

Олена Валентинівна Семенко

Кандидат ветеринарних наук, доцент

Державний університет Наварри

31006, кампус «Ароссада», Памплона, м. Наварра, Іспанія

<https://orcid.org/0000-0002-6453-6192>

Діана Сергіївна Білокур

Ветеринарний лікар

Ветеринарна клініка “Zoolux”

02000, вул. Дмитрівська, 39, м. Київ, Україна

<https://orcid.org/0009-0007-3516-3204>

Анотація. Глобальні зміни клімату, що відбуваються в останні десятиліття, призводять до збільшення активності та розширення ареалу розповсюдження багатьох хвороб. Однією з них

є бабезіоз собак. А тому, все актуальнішим стає здійснення моніторингу за функціональним станом організму в хворих собак та своєчасне виявлення уражень нирок, печінки, селезінки, серцево-судинної системи, розвиток анемії й інших порушень, що ускладнюють перебіг бабезіозу. Метою роботи було з'ясування особливостей метаболічних і функціональних змін в організмі собак за різної інтенсивності бабезіозної інвазії. Під час проведення лабораторної діагностики використовували загальні та спеціальні методи дослідження: світлову мікроскопію, центрифугування, спектрофотометрію та математичної статистики. За результатами комплексних мікроскопічних, морфологічних і біохімічних досліджень крові в собак за різної інтенсивності бабезіозної інвазії встановлено найхарактерніші функціональні та метаболічні зміни в їхньому організмі. Так, за різної інтенсивності бабезіозної інвазії в хворих тварин особливостями гематологічного профілю є лейкоцитопенія на тлі еозінопенії (за легкого ступеня інвазії) та лімфоцитопенії за одночасного компенсаторного збільшення кількості моноцитів і нейтрофілів, а також еритроцитопенія, гіпохромемія, тромбоцитопенія зі зменшенням величини тромбокриту і гематокриту. Крім того, у таких хворих фіксували розвиток гіперферментемії аспартат- і аланінамінотрансферази, лужної фосфатази, γ -глутамілтранспептидази, що вказує на структурно-функціональні зміни, насамперед, у міокарді, печінці, скелетних м'язах, головному мозку і нирках внаслідок їх токсичного ураження продуктами життєдіяльності бабезій. Водночас у хворих тварин відмічали гіпопротеїнемію, гіпоальбумінемію і гіперазотемію, що свідчить про зростання інтенсивності катаболічних процесів у функціональних клітинах органів і тканин, насамперед, печінки. Найвираженіші зміни досліджуваних показників встановлено за високої інтенсивності бабезіозної інвазії. Визначені закономірності дозволяють використовувати зазначені гематологічні показники в якості маркерів функціональних та метаболічних змін в організмі собак за різної інтенсивності бабезіозної інвазії

Ключові слова: морфологічні показники крові; гематокрит; тромбокрит; біохімічні показники крові; лейкограма; ензими