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# Features of Hepatopathy and Hematological Complications in Acute Spontaneous Babesiosis of Dogs

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**Abstract**. Spontaneous babesiosis in dogs is one of the most common and dangerous diseases. Erythrocyte damage conditioned upon pathogens is a trigger for hematological complications and lesions of the microcirculatory tract. The development of thrombotic processes leads to irreversible changes and subsequent death of the organism. The liver is one of the first organs to suffer damage. The aim of the study was to identify the links between the pathogenesis of hepatopathy and hematological disorders in the course of acute spontaneous babesiosis in dogs. To achieve this, two groups of dogs of 20 individuals were formed: the experimental group – animals with babesiosis, the control group – clinically healthy. It is established that acute spontaneous babesiosis occurs in successive forms – anemic and jaundice. Normochromic anemia, leukocytosis, thrombocytopenia were detected. Hemorheological parameters indicate increased aggregation of erythrocytes and platelets, which led to thrombotic conditions. The criterion of shock is established – decrease in the volume of circulating blood; its deficiency was 24% for anemic and 34% for jaundice. Also identified markers of disseminated intravascular coagulation syndrome (DIC) – hypofibrinogenemia, increased levels of soluble fibrin-monomer complexes, fibrin degradation products, including D-dimer. Changes in indicators increased as the disease progressed from anemic to jaundiced form. In the anemic form, shock is defined as subcompensated in moderate severity, and DIC syndrome – in the stage of consumption coagulopathy. Complications characterise a transitional state that is in unstable equilibrium. In the icteric form, shock is defined as decompensated severe, and DIC syndrome – in the stage of hypocoagulation. This condition is characterised as critical, with a pronounced tendency to irreversibility. Changes in liver parameters indicate hyperactivity of all indicator enzymes, hyperbilirubinemia and hyperuria. With the development of the disease, the rates increase significantly. DIC syndrome, shock and hepatopathy enter the vicious circle, in which hematological complications cause hepatopathy, and it, in turn, exacerbates them. Hepatopathy for babesiosis in dogs is considered a serious condition that requires intensive care

**Keywords**: shock, liver, DIC syndrome, hemorheology, hemodynamics, liver profile



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## INTRODUCTION

Babesiosis of dogs is an urgent problem of veterinary medicine today. The disease is characterised by severe course and causes significant damage to the animal's body, even life-threatening. In the seasons of tick activity mortality can reach 50%. The causative agents of this disease are blood spores, representatives of the series *Piroplasmida* Wenyon 1936 (Bilić *et al.,* 2018; Vishwakarma & Nandini, 2019). Known large forms of pathogens – *Babesia canis* Piana et Galli-Valerio 1895; *B. vogeli* Reichenow 1977; *B. rossi* Nutall 1910. The first two species are cosmopolitan, and the latter is more typical of regions of Africa. These forms are characterised by a fairly large size of zoites at the stage of paired pear-shaped formations. The length of such zoites exceeds the radius of the erythrocyte, so the paired forms are placed at an acute angle to each other. In the form of odd generations, zoites are also quite large (Vishwakarma & Nandini, 2019; Dubova *et al.,* 2020).

Representatives of babesia of small forms are: *B. gibsoni* Patton 1910; *B. vulpes* Baneth, Florin-Christensen, Cardoso & Schnittger 2015; *B. conradae* sp. nov. – a recently discovered species of "small" babesia in the United States, *B. microti-like* sp. nov. (Bilic *et al.,* 2018; Azagi *et al.,* 2021; Hussain *et al.,* 2021). In small forms of babesia, paired pear-shaped zoites have a length shorter than the radius of the erythrocyte. In the form of odd formations, such zoites resemble small round inclusions, which are microscopically different from prokaryotes in the existing nucleus. Pathogen *B. equi* Laveran 1901 (syn. *Theileria equi, Nuttalia equi* ) is an obligate parasite of horses, but cases of lesions in dogs have been identified, as confirmed by genetic identification of the pathogen (Vishwakarma & Nandini, 2019).

Babesia species are confined to certain vectors. Thus, for *B. canis* the relevant vector is *Dermacentor reticulatus*, which is common in Europe, in the temperate-continental climate zone. For *B. vogeli*, a specific vector is the brown dog mite *Rhipicephalus sanguineus*. It is widespread in the Mediterranean, prefers temperate climates, and is found in Central Europe and the British Isles (Bilić *et al.,* 2018; Vishwakarma & Nandini, 2019).

Small forms of pathogens (*B. gibsoni, B. microti-like, B. vulpis* ) use mites of the genus *Ixodes* as a biological vector – *I. hexagonus* Leach 1815, *I. canisuga* Johnston 1849, sometimes *I. ricinus*. Small pathogens do not occur in Ukraine (Vishwakarma & Nandini, 2019; Dubova *et al.,* 2020).

Conditioned upon its landscape and climatic characteristics, the Polissya Lowland Zone of the Western part of the Eastern European Plain is the optimal territory for the formation of a stable epizootic center of dog babesiosis. The structure of the soils of this area, climatic conditions, the presence of appropriate biocenoses are ideal for creating an area of Ixodes mites – vectors of babesiosis. In the region, specific vectors of large forms of *Babesia spp*. *Dermacentor reticulatus* Fabricius 1794 (main vector) and *Ixodes ricinus* Linnaeus 1759 (Dubova *et al.,* 2020) are the causative agents of canine babesiosis. Different types of pathogens have different virulence. There is information that among the largest forms of the most virulent species of *B. vogeli*. The virulence of small forms is still being studied (Bilić *et al.,* 2018; Vishwakarma & Nandini, 2019).

In the dog's body, the blood parasite begins its pathogenic effect after inoculation with the saliva of the tick. First of all, the pathogen destroys red blood cells. This leads to the development of hemolytic anemia, which results in most of the clinical symptoms of babesiosis (Azagi *et al.,* 2021; Ortiz *et al.,* 2020). Location *Babesia spp.* inside erythrocytes leads to the transformation of their discoid form, there are various pathological formations – spherocytes, planicites, fragments, etc. Such cells are actively destroyed in the liver and initially stimulate its hyperfunction, and then cause inflammatory and dystrophic processes (Bilić et al., 2018; Wesley *et al.,* 2021). At the same time, the activated blood coagulation cascade causes thrombosis in the microcirculatory tract of the liver, which is accompanied by the appearance of ischemic areas. Necrobiotic and subsequent necrotic changes in hepatocytes exacerbate liver failure, increase hypoxic, acidotic and intoxication processes, both in the liver and in the body as a whole (Gil, 2019; Liu *et al.,* 2019).

Finding out the pathogenetic mechanisms and processes of hepatopathy in babesiosis, the sequence of their manifestation and consequences for the body is an urgent problem of veterinary therapy. In the absence of pathogenetic treatment aimed at stopping the development of complications and eliminating their consequences, the course of acute spontaneous babesiosis in terms of prognosis has a clear tendency to an unfavorable end.

Thus, given the special danger of the acute form of spontaneous babesiosis of dogs and the severity of concomitant complications, there is a need for the formation of scientifically sound protocols of pathogenetic therapy. This will allow to determine the priorities and sequence of treatment of drugs against the development of severe complications, such as shock and disseminated intravascular coagulation syndrome (DIC).

*The purpose of the work* is to establish the pathogenetic links between hepatopathy and disseminated intravascular coagulation syndrome in acute spontaneous babesiosis of dogs.

## THEORETICAL REVIEW

The leading pathogenic factor of babesiosis is hypoxia due to erythrocyte damage. In addition, *Babesia spp.* have pyrogenic properties, have a toxic-allergic effect on the body and stimulate the emergence of immune complexes (Bilic *et al.,* 2018; Hussain *et al.,* 2021). The content of destroyed erythrocytes, and the damaged cells themselves are biologically active substances (Vishwakarma & Nandini, 2019). They react to the activation of immune processes: in particular, reactive changes in the vascular

endothelium, aimed at preventing the loss of circulating blood volume (Oaks *et al.,* 2020). The system of mononuclear phagocytes with the main function of protecting the body from invading agents is also activated. Indeed, mononuclear cells attack and destroy erythrocytes affected by babesia (Ortiz *et al.,* 2020; Roopali *et al.,* 2018). However, in the case of an autoimmune reaction, phagocytes will destroy completely healthy erythrocytes, which will greatly complicate the pathogenesis of the disease and the condition of the animal. Thus, as a result of a complex set of reactions in response to the invasion, all organs and systems are involved in the pathogenic process.

Previous studies have shown that the toxic-allergic effects of *Babesia spp.* causes severe complications that primarily affect the microcirculatory tract of organs (Ortiz *et al.,* 2020; Hildebrandt *et al.,* 2021; Rodeghiero *et al.,* 2019). The organs in which gas exchange and excretion of end products of metabolism (lungs and kidneys), and the liver, as the leading metabolic organ (Dubova *et al.,* 2020; Levi, 2018), suffer the most. In addition, pathogens spend some time of their prepatent period in liver cells and destroy them (Bilić *et al.,* 2018; Vishwakarma & Nandini, 2019). Thus, the pathogenesis of babesiosis is characterized by the development of complex multifactorial hepatopathy, which is exacerbated by the wrong circle of the axis of complication of the disease – DIC blood (Dubova *et al.,* 2020).

The destruction of erythrocytes causes the release of free biologically active hemoglobin and other substances into the blood plasma, which stimulates the activity of vascular endothelium and platelets (Roopali *et al.,* 2018; Gupta *et al.,* 2020). Their adhesive and aggregation properties increase, the process of activation of vascular-platelet hemostasis is started – the first link in the coagulation cascade (Quach & Li, 2020; Rayes *et al.,* 2018). Because the pathogenic stimulus *Babesia spp.* continues, the coagulation process becomes avalanche-like, further enhancing the primary vascular-platelet link of hemostasis (Tyutyumova *et al.,* 2019). Thus, a kind of vicious circle of activation of extensive blood clotting is created. DIC syndrome occurs throughout the bloodstream, and thrombosis and insufficiency of tissue perfusion develop at the level of microcirculation (Dubova *et al.,* 2020; Saini & Dunn, 2019).

Inadequate tissue perfusion, the development of hypoxia and ischemia is reflected in the processes of cellular metabolism. Necrobiotic and necrotic changes occur in cells. This condition is a pathomorphological criterion of shock. The development of shock changes at the level of organs shifts the local metabolism in the direction of toxic and hypoxic processes, which increases thrombosis and blood clotting. Thus, the next generalised vicious circle of shock is created (Dubova *et al.,* 2020; Liu *et al.,* 2019).

Conditioned upon the abundant blood supply network of the liver, thrombosis and shock changes, which initially occur only in hepatocytes, later spread to the liver lobes and the whole body. This leads to functional liver failure. Thus, the liver becomes unable to detoxify the body and perform its synthetic functions (Thakur *et al.,* 2018).

Thus, the study of the pathogenesis of complications of acute spontaneous babesiosis and their relationship with the development of functional and organic changes in the liver is necessary to understand the nature of hepatopathy and develop scientifically sound schemes of pathogenetic therapy of sick dogs.

## MATERIALS AND METHODS

The research was conducted during 2020-2021 on the basis of the educational-research-production clinic of veterinary medicine of Polissya National University, Zhytomyr, Ukraine.

The research was conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the General Ethical Principles of Animal Experimentation (Kyiv, 2001) and the Law of Ukraine "On the Protection of Animals from Cruelty". The compliance of the conducted research with the principles of bioethics and protection of animals from cruel treatment during scientific work was confirmed by the commission on bioethical examination of Polissya National University (Minutes No. 1 of 01/10/2022).

40 dogs of different breeds, age 2-4 years, body weight 20-30 kg were studied. 2 groups were formed – control and experimental, 20 animals in each, on the principle of pairs of analogues. The experimental group – dogs with acute spontaneous babesiosis. Control group – clinically healthy animals (no clinical signs, no history of contact with ticks, hematological and biochemical laboratory parameters were within the established standards).

***Clinical and microscopic studies.*** Clinical studies of animals were performed by general methods. The diagnosis of babesiosis was established by light microscopy of thin fixed blood smears stained by the method of Pappenheim on the device V-Chromer® III (West Medica, Austria). Microscopy was performed using a KERN OBE digital microscope (KERN, Germany). Generic identification of the pathogen and group size determination was conducted. Species identification of the pathogen has not been established. The intensity of parasitemia was determined by the percentage of affected erythrocytes.

***Hematological, hemorheological, hemodynamic studies.*** Venous blood samples taken from the right *vena subcutanea antebrachii* were examined. For this group of studies used Vacumed tubes with anticoagulant EDTA-K3, the concentration of which is 1.8 mg/ml.

The hemoglobin content, the amount of formed blood cells, the erythrocyte sedimentation rate (ESR) and the hematocrit were determined using an automatic hematology analyzer RT-7600 (Rayto, China). Spontaneous aggregation capacity of erythrocytes (spEAC) and platelets (spPAC) was determined by shaking a blood sample in a laboratory shaker S-3 MICROmed (MICROmed®, Ukraine) according to N. Tarasova (Barkagan & Momot, 2008).

Circulating blood volume (CBV) was calculated by diluting T-1824 blue Evans. Deficiency of blood volume was calculated by hematocrit using *Moore*'s formula (1):

$$BLV = CBV_{norm} \times \frac{(Ht_{norm} - Ht_{research})}{Ht_{norm}} \quad (1)$$

where $BLV$ – blood loss volume, ml; $CBV$ – circulating blood volume normal, ml; $Ht_{norm}$ – hematocrit value normal, L/L; $Ht_{research}$ – hematocrit value of the animals' research group, L/L.

***Investigation of the coagulation link of hemostasis.*** This series of studies was performed using a two-channel coagulometer RP-2202C (Rayto, China). Coagulogram parameters were studied: time of spontaneous coagulation of Lee-White blood, silicone time of blood coagulation, prothrombin time (prothrombin time) (PT), activated partial thromboplastin time [activated partial thromboplastin time] (APTT), and also determined the content of fibrinogen (Rayes *et al.,* 2018; Saini & Dunn, 2019).

***Determination of markers of DIC syndrome.*** The concentration of soluble fibrin-monomer complexes (SFMC) was measured colorimetrically by a quantitative variant of the orthophenanthroline method.

Fibrinogen/fibrin degradation products (FDP) and D-dimer were determined by enzyme-linked immunosorbent assay (Slater *et al.,* 2019).

***Biochemical research.*** For this group of studies, whole blood samples were taken from EximLab vacuum tubes with coagulation activator and demarcation gel. Obtained serum, which served as material for the study.

The studies were performed using a semi-automatic biochemical analyzer Chem 7 (Erba Mannheim, India).

The concentration of total and conjugated bilirubin (Malloy-Edeline method), the activity of the enzymes alanine aminotransaminase (ALT) and aspartate aminotransferase (AST) (Reitman-Frenkel method), gamma-glutamyltranspeptidase (GHT) method (phosphate method) Bodansky), lactate dehydrogenase (LDH) (Bergmeier method). The concentration of urea was determined by the Rashkovan method.

***Statistical analysis.*** Statistical processing of the obtained data was carried out using the application package SPSS Statistics 23.0 (IBM Company). ANOVA analysis of variance was performed to compare the variance caused by group differences with the variance caused by intragroup variability. The reliability of the obtained data was evaluated by Fisher's F-test at a confidence level of $p<0.05$.

## RESULTS AND DISCUSSION

The following clinical signs were observed in dogs with acute spontaneous babesiosis: pyretic fever, mucosal anemia, loss of appetite, intoxication syndrome (nausea, vomiting, abdominal pain). The primary acute process of development of the pathogen in the body developed without jaundice. Hemoglobinuria was observed in the period from the 1st to the 3rd day of the patent period.

Later in 3 days and later jaundice of mucous membranes and skin developed. The general condition of the animals deteriorated, the phenomena of intoxication intensified. Vomiting in the early stages was characterized by mucous and foamy masses, then there were impurities of bile, which soon turned reddish-brown.

In the area of the right hypochondrium palpation of the liver increased by 1.5-2 times. Manipulations were accompanied by a significant pain response of the animal. Subsequently, the dog went into a stupor-soporous state and coma. In the icteric form of acute spontaneous babesiosis, according to clinical statistics, mortality was about 40%.

Blood tests of dogs with acute spontaneous babesiosis were performed at different stages of the disease, which were defined as anemic (early) and jaundiced (late) forms. It was found that the changes found in sick animals are dynamically deteriorating and in the jaundiced form is more severe than in the anemic. The intensity of parasitemia remained throughout the observation period at the level recorded in the initial examination (Table 1).

***Table 1***. *Hematological parameters in acute spontaneous babesiosis of dogs, M±m*

| Indicator | Experimental group (n=20) | | Control group |
|---|---|---|---|
| | Anemic form | Jaundiced form | (n=20) |
| Hemoglobin, g/l | 98.6±4.2*** | 80.2±5.2*** | 128.9±6.3 |
| Erythrocyte sedimentation rate, mm/h | 18.3±3.3*** | 26.7±4.6*** | 4.4±0.18 |
| Erythrocytes. T / l | 3.4±0.6*** | 3±0.6*** | 6.8±0.36 |
| Leukocytes, G/l | 19.2±3.1*** | 13.6±2.1 | 10.2±0.7 |
| Platelets, G/l | 201±12.2*** | 164.4±10.8*** | 312±26.3 |
| Intensity of parasitemia, % | 13.4±1.8 | 11.2±1.2 | – |

***Note***: *Difference between investigated and control groups is significant (p<0.001)*

Anemia and progressive erythrocytopenia were noted in both forms of the disease ($p<0.001$). Anemia is normochromic in nature, and therefore has a hemolytic origin. In the anemic form of babesiosis hyperleukocytosis

was observed (p<0.001). In the jaundiced form, a tendency to return the number of leukocytes towards the reference values was observed. The number of platelets decreased dynamically in the progression of the disease (p<0.001), which was synchronized with the increase in spontaneous aggregation ability of the formed elements (p<0.001).

ESR increased during the development of babesiosis (p<0.001), and the number of platelets progressively decreased. Against the background of spPAC growth (Table 2), this picture indicates the release of platelets into blood clots and the area of the microcirculatory bed – thrombocytopenia "consumption".

***Table 2***. *Hemodynamic and hemorheological parameters in acute spontaneous babesiosis of dogs, M±m*

| Indicator | Experimental group (n=20) | | Control group (n=20) |
|---|---|---|---|
| | Anemic form | Jaundiced form | |
| Hematocrit, l/l | 0.34±0.07 | 0.27±0.08* | 0.45±0.04 |
| CBV, ml | 3386±57.2*** | 2943±66.5*** | 4508±89.6 |
| Specific CBV, ml/kg | 81.6±4.8*** | 76.2±6.3*** | 122.5±7.1 |
| Deficiency of blood volume, ml<br>% | 1100.3±96.3***<br>24 | 1526±88.6***<br>34 | – |
| spEAC, % | 37.3±5.6*** | 44.8±9.4*** | 8.4±1.8 |
| spPAC, % | 43.8±5.5*** | 61.2±7.3*** | 15.5±2.4 |
| The index of wetting the vascular wall | 0.72±0.04*** | 0.91±0.04*** | 0.31±0.02 |

***Note***: *difference between investigated and control groups is significant: *p<0.05; ***p<0.001*

In terms of hemodynamic and hemorheological parameters, progressive changes were observed in all parameters. CBV, respectively, and the specific CBV in the anemic form is significantly reduced relative to the control group. In the jaundiced form of the disease, all indicators are significantly lower (p<0.001) and much lower than the parameters of the anemic form.

SpPAC and spEAC were significantly increased throughout the course of babesiosis (p<0.001). Thus, there was a dynamic increase in the intensity of the shock process. It was expressed in the blockade of microcirculation conditioned upon the high aggregation capacity of blood cells (in particular, platelets) (Tyutyumova *et al.,* 2019; Ho-Tin-Noé *et al*., 2018), including the loss of circulating blood conditioned upon the release of liquid component in interstitial edema explained by increased vascular wall permeability (Levi, 2018; Saini & Dunn, 2019). The calculated CBV deficiency (p<0.001) determined the development of shock in acute spontaneous babesiosis (Dubova *et al.,* 2020; Liu *et al.,* 2019). In the anemic form, the shock was characterised as subcompensated to moderate, and in the jaundiced form – severe with a persistent tendency to decompensation (Dubova *et al*., 2020).

Indicators of coagulation hemostasis, established in different forms of acute babesiosis in dogs, confirmed the presence of DIC (Levi, 2018). Significantly altered and dynamically progressive index of vascular wall wetting indirectly is an indicator of electrophysical properties of the vascular wall and determines the state of the endothelium, which may be a strong accumulation of platelets and erythrocytes (Saini & Dunn, 2019). This figure increases with the growth of spPAC, spEAC.

In the anemic form of babesiosis in coagulation tests, which characterize the activity of blood coagulation by external (PT) and internal (APTT) mechanism (Roshal & Gil, 2019), there were divergent changes: reducing the role of external mechanism and increasing the role of internal (Table 3). This determined the second, transitional phase of DIC syndrome – consumption coagulopathy (Levi, 2018; Saini & Dunn, 2019).

***Table 3***. *Indicators of the hemostasis system in acute spontaneous babesiosis of dogs, M±m*

| Indicator | Experimental group (n=20) | | Control group (n=20) |
|---|---|---|---|
| | Anemic form | Jaundiced form | |
| PT, s | 22.7±0.3 | 32.4±0.6*** | 20.8±0.6 |
| APTT, s | 38.3±2.4 | 52.2±7.2 | 44±2.9 |
| Fibrinogen, g/l | 1.34±0.3*** | 0.83±0.13*** | 2.45±0.27 |
| FDP, g/l | 0.28±0.022*** | 0.32±0.043*** | 0.075±0.002 |
| D-dimer, μg/l | 0.42±0.08*** | 0.66±0.07*** | 0.15±0.02 |
| SFMC, g/l | 0.25±0.017*** | 0.51±0.03*** | 0.03±0.005 |

***Note***: *difference between investigated and control groups is significant (p<0.001)*

In the jaundiced form of the disease in both indicators there was a tendency to hypocoagulation, which characterised the beginning of the third phase of the syndrome – hypocoagulation and fibrinolysis.

Criteria for the diagnosis of DIC are the appearance of the following markers: hypofibrinogenemia ($p<0.001$), increase in FDP ($p<0.001$), including D-dimer ($p<0.001$), and SFMC ($p<0.001$). Fibrinogen is blocked as a result of its "consumption" in blood clots. Conditioned upon fibrinogen deficiency and "consumption" of other coagulation factors, full-fledged fibrin cannot be formed. Improper fibrinogen fermentation leads to increased levels of FDP, in particular D-dimer, and SFMC. Such markers are pathognomonic for DIC (Levi, 2018; Saini & Dunn, 2019 ). In the anemic form, these markers were reliable; in the jaundiced form, their numerical expression increased to the maximum. Thus, the degree of power of the DIC syndrome increased, the process tended to be irreversible.

Functional changes that occurred in the liver of sick dogs were clearly characterised by specific biochemical parameters of the "liver profile" (Table 4).

***Table 4**. Biochemical parameters of the liver profile in acute spontaneous babesiosis of dogs, M±m*

| Indicator | Experimental group (n=20) | | Control group (n=20) |
|---|---|---|---|
| | Anemic form | Jaundiced form | |
| Total bilirubin, μmol/l | 10.2±1.2*** | 26.7±4.2*** | 2.96±0.7 |
| Conjugated bilirubin, μmol/l | 2.8±0.4*** | 15.2±3.7*** | 0.01±0.002 |
| AST, od / l | 102±6.4*** | 139.2±7.4*** | 20.6±1.8 |
| ALAT, od/l | 122.4±8.4*** | 156.7±9.2*** | 17.6±1.7 |
| GGT, od/l | 28.5±2.6*** | 74.8±6.1*** | 5.2±0.7 |
| LDH, od/l | 382.7±3.1*** | 500.6±40.6*** | 120.4±11.3 |
| LF, od/l | 170.4±13.8** | 208±11.2*** | 125±8.3 |
| Urea, mmol/l | 15.3±2.7*** | 26.3±4.1*** | 4.9±1.2 |

***Note**: difference between investigated and control groups is significant: **p<0.01; ***p<0.001*

In the anemic form of the disease there was a significant increase in the level of total and conjugated bilirubin ($p<0.001$), hyperfermentation of transferases ($p<0.001$), GGT, LDH ($p<0.001$), LF ($p<0.01$), uremia ($p<0.001$). Thus, during the anemic form, functional disorders of the liver are pronounced and caused by the processes of destruction of hepatocytes. However, clinical signs and laboratory parameters indicated the development of subacute hepatitis. In this form of babesiosis, the functional state of the liver was within the reversibility of processes. Hyperactivity of indicator enzymes indicated the onset of insufficiency of bile production and bile excretion, and the tendency to develop toxic hepatitis.

In the jaundiced form of babesiosis, all indicators of the functional state of the liver significantly increased even in comparison with the anemic form. Increased activity of transaminases (7 and 9 times), GGT (14 times), LDH (4 times), LF (1.7 times), and hyperbilirubinemia (9 times), including an increase in the concentration of conjugated bilirubin (1.5 thousand times) indicated the course of reactive hepatitis of toxic nature, cholestasis, massive destruction of hepatocytes (Thakur *et al.,* 2018). Oversaturation of blood with bile pigments exacerbated intoxication and further stimulated the power of pathogenic secondary complications, such as shock and DIC (Dubova *et al*., 2020; Liu *et al.,* 2019). Jaundice was hemolytic-parenchymal in nature (Levi, 2018).

The increase in urea levels determined the state of uremia, which aggravated the general intoxication syndrome. At this stage, the liver was no longer able to perform detoxification functions, in particular, to dispose of hazardous and toxic to the animal residual nitrogen products (Ortiz *et al.,* 2020; Thakur *et al.,* 2018).

Liver damage in dogs with acute spontaneous babesiosis is primarily due to exposure *Babesia spp*., which, according to the ideas accepted today in science, spends a certain period of its development in this body (Bilić *et al*., 2018; Vishwakarma & Nandini, 2019). In addition, conditioned upon its functions, the liver becomes the center of pathogenetic events during the development of the disease (Vishwakarma & Nandini, 2019).

Summarising the results of our own research, we can note that the course of the pathological process of babesiosis in sick dogs becomes more powerful and is characterised first by anemic and then jaundiced form. One of the leading roles in the emergence of these forms is played by impaired liver function conditioned upon the development of severe processes with a persistent tendency to decompensation and irreversibility (Liu *et al.,* 2019; Saini & Dunn, 2019). The initial link of involvement in the pathogenic process of the vital organs of the body, including the liver, is the activation of the vascular-platelet link of hemostasis by the products of *Babesia spp*., immune complexes and consequences of erythrocyte destruction (Dubova *et al.,* 2020; Ho-Tin-Noé *et al.,* 2018).

According to the results of the obtained hematological parameters, the pathogenetic mechanism of

changes can be expressed as follows. The systemic inflammatory response of the whole organism is reflected by leukocytosis as the patent period of babesiosis progresses (Ortiz *et al.,* 2020; Tyutyumova *et al.,* 2019). The increase in ESR also confirms the redistribution of plasma proteins in favor of the globulin fraction, which once again indicates the development of a systemic inflammatory process (Rayes *et al.,* 2018). Decreased white blood cell counts in the jaundice stage of the disease may indicate that the body develops cytopenia explained by hyperstimulation of the spleen and the occurrence of hypersplenism (Levi, 2018; Saini & Dunn, 2019).

Anemia with erythrocytopenia develops conditioned upon the activity of the pathogen and is the basis of the pathogenesis of the disease. Destruction of erythrocytes with the release of hemoglobin into the blood plasma causes insufficient oxygen supply to organs and tissues, causing metabolic disorders and the development of hypoxia (Wesley *et al.,* 2021; Rodeghiero *et al.,* 2019).

The main arena of pathological processes is the microcirculatory bed of organs, which first suffers from thrombosis conditioned upon increased adhesive-aggregation properties of platelets and then erythrocytes (Dubova *et al.,* 2020; Levi, 2018). Given the abundant network of hepatic capillaries, thrombosis in the microcirculatory tract becomes the morphological equivalent of shock at the organ level (Liu *et al.,* 2019). At this time, the process of blood coagulation, activated by external and internal mechanisms, is intensifying (Levi, 2018; Saini & Dunn, 2019). DIC syndrome occurs. According to the pathogenesis of severe complications, such as DIC and shock, these processes cause and reinforce each other, forming vicious circles at different levels of the body (Dubova *et al.,* 2020; Liu *et al.,* 2019).

The release of platelets into blood clots determines "consumption thrombocytopenia" – one of the leading factors in the development of DIC syndrome (Levi, 2018). Circulatory platelet deficiency damages the vascular endothelium explained by the lack of vascular wall nutrition factor (Gupta *et al.,* 2020; Ho-Tin-Noé *et al.,* 2018). Loss of vascular elasticity enhances the adhesion-aggregation capacity of platelets, causing the above-mentioned vicious circle of the process. The release of the liquid component of the blood into the surrounding interstitial space leads to a decrease in CBV, resulting in a lack of tissue perfusion, as well as efficient heart function. Hypoxia is exacerbated (Liu *et al.,* 2019).

Hepatocytes due to hypoxia lose their metabolic functions. Enzymes capable of transporting bilirubin and utilising endotoxins are gradually being lost. Intoxication of the body is increasing, which in turn increases the intensity of DIC and shock syndrome (Levi, 2018; Saini & Dunn, 2019).

Conditioned upon the sinusoidal structure of the liver lobes, the body is able to resist vasoconstrictor effects, the formation of sludge and pooling as the main morphological criteria for shock, and thrombotic manifestations in the development of DIC (Liu *et al.,* 2019). However, if the etiological factor does not stop its action (babesia continues to multiply in the body), there is a depletion of the enzymatic capacity of the liver. There are organic changes, which are manifested in the death of hepatocytes with the formation of scars. The organ becomes a "shock liver", developing acute liver failure, which often ends in death (Levi, 2018; Liu *et al.,* 2019).

Thus, hepatopathies in acute spontaneous babesiosis of dogs occur as a result of secondary complications – DIC and shock. Complications develop as a universal response to the activity of *Babesia spp.*, and their pathogenetic mechanisms cause hemodynamic and hemorheological disorders in the hepatic vascular bed. As a result of reduced tissue perfusion of the organ, reduction of CBV and the development of hypoxia, there is a violation of the functional activity of the liver, distortion of enzymatic processes, which increases the intensity of complications. This is how the vicious circle "shock-syndrome DIC-liver failure" is formed, in which there is a mutual strengthening and interdependence of these pathological processes. Hepatopathy in the anemic form of canine babesiosis manifests itself as subacute hepatitis and functional liver failure. In the jaundiced form – as acute reactive toxic hepatitis and severe acute liver failure, which is prognostically life-threatening for animals.

## CONCLUSIONS

In the process of acute spontaneous babesiosis of dogs, hepatopathy develops, which is registered in the anemic form of the disease, and then progresses, causing a jaundiced form of the disease. Complications such as DIC and shock occur during babesiosis. In the anemic form, the shock is subcompensated, has a moderate degree, and DIC syndrome is in the stage of consumption coagulopathy. In the jaundiced form of severe shock acquires a steady tendency to irreversibility. DIC syndrome enters the stage of hypocoagulation/fibrinolysis.

Hepatopathy in the anemic form of the disease is in the form of subacute hepatitis and functional liver failure. In the jaundiced form develops acute reactive hepatitis of toxic nature, cholestasis, acute liver failure.

DIC syndrome, shock, hepatopathy are closely related and are involved in the vicious circles of increasing intensity of each other. The factor of activation of false circles is the DIC syndrome, which develops in response to the pathogenic influence of the pathogen.

Thrombotic lesions of the microcirculatory bed of the liver are a trigger for the development of shock and hepatocyte damage. In turn, pathological processes in the liver exacerbate the state of shock and DIC syndrome, closing the vicious circle.

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## Особливості гепатопатії та гематологічних ускладнень за гострого спонтанного бабезіозу собак

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**Анотація**. Спонтанний бабезіоз собак належить до найбільш поширених і небезпечних захворювань. Ураження еритроцитів внаслідок впливу збудників є пусковим фактором гематологічних ускладнень і ураження мікроциркуляторного русла. Розвиток тромботичних процесів веде до незворотних змін та подальшої загибелі організму. Печінка є одним з перших органів, які потерпають від пошкоджень. Мета досліджень полягала у виявленні зв'язків патогенезу гепатопатії та гематологічних розладів за перебігу гострого спонтанного бабезіозу собак. Для її досягнення сформовано дві групи собак по 20 особин: дослідна група – тварини з бабезіозом, контрольна – клінічно здорові. Встановлено, що гострий спонтанний бабезіоз перебігає у послідовних формах – анемічній і жовтяничній. Виявлені анемія нормохромного типу, лейкоцитоз, тромбоцитопенія. Гемореологічні параметри вказують на посилену агрегацію еритроцитів і тромбоцитів, що зумовило тромботичний стан. Встановлено критерій шоку – зниження об'єму циркулюючої крові; його дефіцит склав 24 % за анемічної форми та 34 % за жовтяничної. Також визначені маркери синдрому дисемінованого внутрішньосудинного згортання (ДВЗ) крові – гіпофібриногенемія, збільшення рівня розчинних фібрин-мономерних комплексів, продуктів деградації фібрина, в тому числі Д-дімеру. Зміни показників наростали у міру переходу захворювання з анемічної у жовтяничну форму. За анемічної форми шок визначений як субкомпенсований середньої важкості, а ДВЗ-синдром – у стадії коагулопатії споживання. Ускладнення характеризують перехідний стан, який перебуває у нестійкій рівновазі. За жовтяничної форми шок визначений як декомпенсований важкий, а ДВЗ-синдром – у стадії гіпокоагуляції. Такий стан характеризується як критичний, з вираженою тенденцією до незворотності. Зміни показників печінки вказують на гіперактивність усіх індикаторних ферментів, гіпербілірубінемію та гіперуремію. З розвитком хвороби показники значно збільшуються. Синдром ДВЗ, шок і гепатопатія вступають у хибне коло, за якого гематологічні ускладнення викликають гепатопатію, а вона, зі свого боку, їх посилює. Гепатопатія за бабезіозу собак вважається важким станом, що вимагає проведення інтенсивної невідкладної терапії

**Ключові слова**: шок, печінка, синдром ДВЗ, гемореологія, гемодинаміка, печінковий профіль