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Enzyme-morphological criteria for diagnosing the stages of hepatorenal syndrome development in canine babesiosis

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The study presents the characterization of the stages of babesiosis manifestations depending on the sex of dogs, as well as enzymatic diagnostics of liver pathology and the determination of urea and creatinine concentrations in the blood serum of dogs with acute babesiosis. Additionally, we investigated the macro- and microscopic structure of the organs in dogs affected by acute babesiosis. Based on the observational data, specific features of the course of babesiosis in dogs were identified, indicating a stage-by-stage progression of the disease with a sequential transition from the incubation period to the period of clinical manifestation. According to the anamnesis data, the incubation period (from pathogen entry into the body to the appearance of the first clinical signs) lasted 5–8 days. This duration showed seasonal variation: 6–8 days in spring and 5–6 days in autumn. Clinical signs of acute babesiosis were identified in 45 spontaneously infected dogs of different breeds (German shepherd, Central Asian shepherd, and Rottweiler), aged 1.5–8.0 years, of both sexes, kept in individual households and some kennels in Zhytomyr. The intensity of *Babesia canis* invasion was reflected in the severity of clinical signs and depended on the duration of disease progression: The longer the pathological process persisted, the greater the intensity of invasion, which proportionally increased the severity of the disease. Based on the analysis of the obtained data, three stages of acute babesiosis were identified. Among the total group of spontaneously infected dogs (n = 45), 16 animals were assigned to stage I, 13 to stage II, and 16 to stage III. The degree of hepatic parenchymal damage depended on the nature of the injurious factor, duration of exposure, and individual susceptibility. Histological examination of the liver revealed disorganization of hepatic cords in certain lobular areas, focal hemorrhages, and congestion of capillaries. The interlobular connective tissue exhibited serous edema. Interlobular bile ducts were dilated and filled with bile. Most hepatocytes were in a state of granular (protein) dystrophy, with accumulation of brown pigment (hemofuscin) in the cytoplasm. These cells also showed signs of atrophy and severe destructive changes, leading to intrahepatic cholestasis. This was confirmed by a significant increase in the activity of the cholestatic enzyme gamma-glutamyl transpeptidase, which in the dogs at stage III of the disease exceeded normal values fourfold. Histological examination of the kidneys revealed granular dystrophy and necrosis of the epithelial cells of the convoluted renal tubules, as well as hemorrhages in both cortical and medullary regions. These changes led to impaired renal circulation and decreased filtration, excretory, and reabsorptive functions. The results of this study, along with the data from other researchers, allow us to identify the patterns in morphological changes in the organs and tissues during the acute course of the disease and contribute to the understanding of the functional significance of their structural components.

Keywords: pathomorphology; histological studies; liver; kidneys; pathogenesis and morphogenesis of disease.

Introduction

Among seasonal parasitic diseases of dogs, babesiosis (piroplasmosis) is particularly notable as a natural focal, vector-borne hemoparasitic disease caused by protozoan parasites of the genus *Babesia* (notably *Babesia* (*Piroplasma*) *canis*) which parasitize erythrocytes (Fasolya & Goralska, 2010; Kurman et al., 2011; Zygner et al., 2023). The causative agent of babesiosis is widely distributed across all continents of the world (Beugnet & Moreau, 2015). The disease is particularly common in southern countries such as Costa Rica, Brazil, and India. The African continent is characterized by endemic outbreaks of babesiosis (Bloch et al., 2018). In Nigeria, the disease is recorded throughout the year (El Haj et al., 2002; Matjila et al., 2004; Coralic et al., 2018). Animals of all species are susceptible to this infection.

Since the 1980s, endemic foci of babesiosis have been recorded in Ukraine, and the number of affected animals has been steadily increasing each year (Prus, 2001). According to statistical analyses, the incidence of canine babesiosis continues to rise across the entire territory of Ukraine (Semenko et al., 2017; Levytska et al., 2020). Zhytomyr Oblast is also considered an enzootic focus of this protozoan disease (Dubova & Soroka, 2005; Dubova, 2017).

At present, infection foci are found even in urban centers, including parks, garden squares, and dog-walking areas, where transmission to clinically healthy dogs occurs. The increase in the number of babesiosis cases in dogs is associated with the continuous and uncontrolled growth of both pedigree and stray dog populations, insufficient effectiveness of preventive measures, poor sanitary conditions in walking areas, and the cessation of acaricidal treatment of forested areas.

According to the results of various studies, dogs of different breeds are susceptible to babesiosis; however, males are affected 1.5–2.0 times more frequently than females. Animals aged 1–5 years are the most susceptible to the pathogen, whereas puppies are affected less frequently but tend to develop a more severe course of the disease (Prus et al., 2017).

The development of the pathological process in infected animals is determined not only by host susceptibility but also by the degree of pathogen virulence, which is associated with the toxic effects of *Babesia* on the central nervous system and the overall functional activity of the organism (Koster et al., 2015; Bartnicki et al., 2017). Researchers from various countries have also described transplacental transmission of the causative agent of babesiosis to puppies, indicating a vertical transmission mechanism (Karasová et al., 2024). In this case, transmission from the mother to the offspring may occur through direct contact, via the oocyte and its genetic apparatus, as well as through the placenta. As of now, based on the literature analysis and our own studies, the epidemiology of canine babesiosis in Ukraine

has been extensively investigated (Prus et al., 2000; Mazannyi et al., 2020). The pathogenic effects of the parasite and the characteristics of the host immune response to invasion have been studied and confirmed. Furthermore, treatment and preventive strategies have been developed and proposed (Goralska et al., 2017). Considerable attention has been paid to the study of the underlying mechanisms of the development and manifestation of immunity in canine babesiosis (Wężyk et al., 2023).

However, laboratory diagnostics of internal pathology in canine babesiosis still require improvement (Baneth et al., 2019). The limited availability of immunoassays and polymerase chain reaction (PCR) complicates accurate diagnosis of the disease. Particular attention should be paid to the diagnosis of complications during the acute course of babesiosis (Goralska & Pinsky, 2016). Clinical manifestations of liver and kidney pathology at early stages of the disease are often nonspecific, and laboratory analyses of blood and urine are not always accessible. In addition, the consequences of hemolytic anemia and complications developing during the acute course of babesiosis in dogs remain insufficiently studied (Hoenig & Ferguson, 2002).

The disease is characterized by disturbances in the cardiovascular and digestive systems, icterus of mucous membranes, and hemoglobinuria. Dogs experience the development of hemolytic anemia and tissue hypoxia, accompanied by damage to the kidneys and liver, which, in the absence of treatment, ultimately leads to their death (Winiarczyk et al., 2017). In the early stages of disease development, dogs exhibit lethargy and loss of appetite (Dubova & Duboviy, 2018). It should be noted that the prognosis for dogs affected by babesiosis depends on multiple factors, primarily including the state of the immune system, the age of the animals, and the type of pathogen. Early diagnosis and treatment play a crucial role and are decisive for successful recovery (Dubova et al., 2019).

The morphological forms of the parasite are diverse, including round, amoeboid, spindle-shaped, anaplasmoid, pear-shaped, and other. The diagnostic form of *B. canis* is considered to be a paired pear-shaped structure located in the center of the erythrocyte, with thin connected ends forming an acute angle and relatively larger dimensions. In small *Babesia* species, a predominantly round shape is observed (Zygner et al., 2023).

Data from morphological studies on internal organ pathology in animals affected by babesiosis remain inconsistent, and histological findings in dogs have been reported only in a limited number of sources. Morphological investigation of structural changes in the internal organs allows researchers to determine the pathogenesis and morphogenesis of the disease depending on functional disturbances, and also facilitates pathogenetic therapy and enables timely prevention of organ-specific pathology (Kondrakhin, 2006; Dunaievska et al., 2020).

The objective of this study was to investigate the structural organization of certain digestive organs (liver) and kidneys in dogs affected by babesiosis using a complex of anatomical, pathomorphological, morphometric, and statistical methods. Considering that babesiosis is associated with multiple-organ pathology, it is relevant to study the structural organization of parenchymal organs in both clinically healthy animals and those with an acute course of the disease.

Materials and methods

Experimental studies were conducted in accordance with the requirements of international principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the Rules for Conducting Work Using Experimental Animals in accordance with the Order of the Ministry of Health of Ukraine No. 281 as of November 1, 2000, "On Measures for Further Improvement of Organizational Forms of Work Involving Experimental Animals", and the Law of Ukraine "On Protection of Animals from Cruel Treatment" (No. 3447-IV, dated February 21, 2006, Kyiv) (Mishalov et al., 2007).

The objects of the study were dogs of the following breeds: Rottweiler, German shepherd, and Central Asian shepherd, aged from 18 months to 8 years. The animals were divided into two groups: clinically healthy dogs (n = 16) and spontaneously infected dogs at different stages of liver and kidney pathology associated with babesiosis (n = 45). The animals were kept in private households and several kennels in the city of Zhytomyr.

At the first stage of the study, the prevalence of canine babesiosis in Zhytomyr was analyzed based on veterinary clinic reports for the period 2018–2022. The timing (seasonality) of disease occurrence, the total number and percentage of affected animals admitted to stationary veterinary clinics, as well as the age, breed, and sex of the animals were examined.

The diagnosis of babesiosis in the dogs was confirmed by detection of the pathogen in blood smears stained according to Romanowsky–Giemsa. The functional state of hepatocytes was assessed by determining the activity of liver-specific enzymes in blood serum, namely gamma-glutamyl transpeptidase (GGT), using a kinetic reaction with α-γ-glutamyl-4-nitroanilide. Renal excretory function was evaluated based on serum urea levels (diacetyl monoxime reaction), while the glomerular filtration function was assessed by measuring creatinine levels using the Jaffé colorimetric reaction (Levchenko et al., 2010).

For histological and morphometric studies, tissue samples not exceeding 5 mm^3 were collected from the clinically healthy dogs and dogs that died due to acute babesiosis. The samples were fixed in 10% neutral buffered formalin and Carnoy's solution (Horalskyi et al., 2019). After fixation, the tissues were washed, dehydrated in graded alcohols, and embedded in paraffin. Histological sections (up to 10 μm thickness) were prepared using a sliding microtome (MS-2). The sections were deparaffinized and stained with Ehrlich's hematoxylin, Karazzi hematoxylin, and eosin, followed by mounting in balsam (Horalskyi et al., 2019).

Microphotographs of histological specimens were taken with a CAM V200 video camera (Inter Med, China, 2017) mounted on a Micros MC-50 microscope (Austria, 2012).

Statistical analysis of the data was performed using variation-statistical methods on a personal computer with the Statistica 6 software package. Results are presented as mean ± standard deviation ($x \pm SD$). Differences between control and experimental groups were assessed using Tukey's test and were considered statistically significant at $P < 0.05$.

Results

The course and development of babesiosis in the dogs were characterized by a pathogenesis and clinical signs typical of this disease. A specific feature of the disease progression was identified, indicating a stage-by-stage course with a sequential transition from the incubation period to the manifestation of clinical symptoms. According to the collected anamnesis data, the period from pathogen entry into the body to the appearance of the first clinical signs lasted 5–8 days. Its duration showed seasonal variability: 6–8 days in spring and 5–6 days in autumn. A clear age-related dependence was also observed: in young animals aged approximately 1.5 years, the incubation period was most often reduced to 5 days. The study of peripheral blood parameters in canine babesiosis represents a fundamental diagnostic approach, playing a decisive role in establishing the diagnosis and monitoring the course of the disease. Therefore, the diagnosis of babesiosis in the dogs was confirmed by detecting the pathogen in blood smears stained according to Romanowsky–Giemsa (Fig. 1).

Microscopic examination revealed the causative agent of canine babesiosis, *Babesia canis*, an intracellular protozoan organism exhibiting round, pear-shaped, amoeboid, and irregular forms, with the paired pear-shaped form being the most typical (Fig. 1).

Clinical signs of acute babesiosis were detected in 45 spontaneously infected dogs of different breeds (German shepherd, Central Asian shepherd, and Rottweiler), aged 1.5–8.0 years, of both sexes, kept in private households and several kennels in the city of Zhytomyr. The diseased animals were admitted for outpatient treatment throughout the entire disease season (spring and autumn). The control group consisted of 20 clinically healthy dogs selected according to the matching principle.

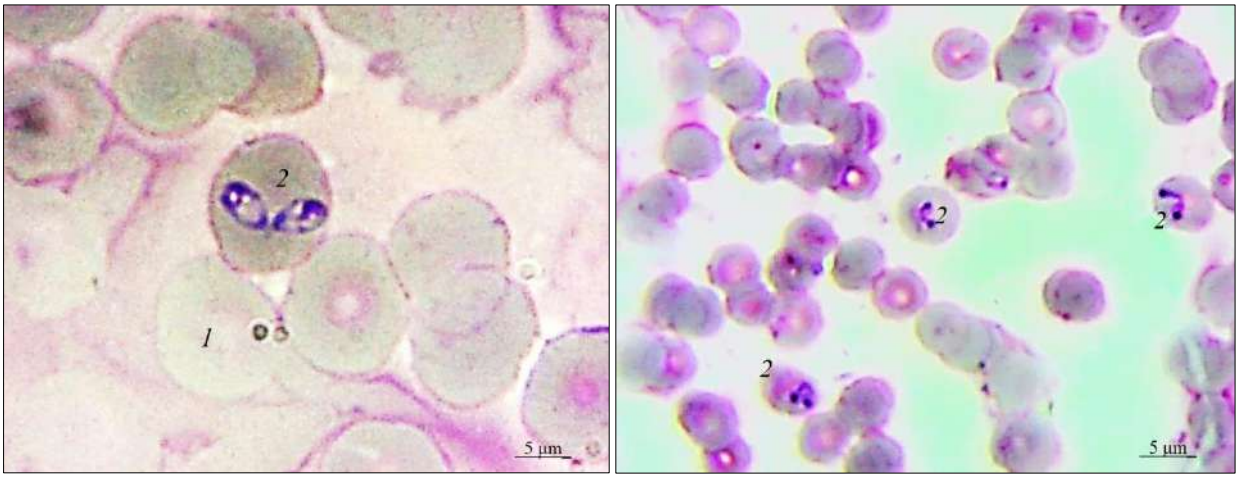

Fig. 1. Erythrocyte lesions in a dog caused by *Babesia canis*:
1 – erythrocytes; *2* – erythrocytes containing pear-shaped forms of *Babesia canis*; Romanowsky–Giemsa stain

The stages of acute babesiosis were determined as follows: the first stage – duration of the disease 1–2 days with an invasion intensity of 2–4%; the second stage – duration of 3–4 days with an invasion intensity of 5–8%; the third stage – duration of 5–6 days with an invasion intensity exceeding 9%. Among the total group of spontaneously infected animals (n = 45), 16 dogs were classified as stage I, 13 as stage II, and 16 as stage III (Table 1).

Table 1
Characteristics of babesiosis stages depending on the sex of dogs

Indicator	Total	%	Males (n)	%	Females (n)	%
Total number of diseased animals	45	100.0	27	60.0	18	40.0
Stage I	16	35.6	10	62.5	6	37.5
Stage II	13	28.8	8	61.5	5	38.5
Stage III	16	35.6	9	56.3	7	43.8

Gamma-glutamyl transpeptidase (GGT) activity in dogs is normally low (1–6 U/L). At the first stage of the disease, its activity did not exceed physiological limits in all the diseased animals. At the second stage, hyperenzymemia was significant (9.7 ± 0.7 U/L), being 2.6 times higher than in the clinically healthy animals ($P < 0.001$). The highest GGT activity in blood serum (14.7 ± 0.7 U/L) was observed at the third stage of babesiosis (Table 2).

Table 2
Enzymatic diagnosis of liver pathology
in acute canine babesiosis (x ± SD)

Group of animals		GGT, U/L
Clinically healthy, n =20		3.7 ± 0.3^a
Diseased animals by stages of the disease	Stage I (n = 16)	4.9 ± 0.2^b
	Stage II (n = 13)	9.7 ± 0.7^c
	Stage III (n = 16)	14.7 ± 0.7^d

Note: different superscript letters within the column indicate statistically significant differences ($P < 0.05$) according to Tukey's test.

Considering that an increase in gamma-glutamyl transpeptidase activity in blood serum results from impaired bile excretion or its release due to altered permeability of hepatocyte membranes, and given that the canine liver has a pronounced ability to enhance enzyme production in response to bile retention, the observed changes in enzyme activity can be regarded as indicative of cholestasis and significant damage to the hepatobiliary architecture caused by hyperbilirubinemia. Thus, hyperenzymemia reflects substantial alterations in the cellular and membrane structure of hepatocytes, which is further confirmed by pathomorphological findings.

Histological examination revealed disorganization of hepatic cords in certain areas of the liver lobules (Fig. 2). Also, focal hemorrhages and congestion of capillaries were observed. The interlobular connective tissue of the liver was in a state of serous edema. Interlobular bile ducts were dilated and filled with bile (Fig. 2).

In the interlobular connective tissue, particularly in the areas of the hepatic (portal) triads, mild lymphohistiocytic infiltration was observed (Fig. 2). The majority of hepatocytes were in a state of granular (protein) dystrophy with accumulation of brown pigment (hemofuscin) in the cytoplasm (Fig. 2). Among these cells, individual hepatocytes undergoing necrobiosis and necrosis were detected. At the periphery of the hepatic lobules, replacement of liver parenchyma by young connective tissue cells was noted. Hepatocytes in these areas were in a state of atrophy and underwent profound destructive changes (Fig. 2), which ultimately led to the development of intrahepatic cholestasis.

This is confirmed by the high activity of the cholestatic enzyme gamma-glutamyl transpeptidase (GGT), which in the dogs at the third stage of the disease exceeded normal values fourfold.

The main biochemical parametrs of blood serum reflecting renal functional impairment are urea and creatinine levels. Our studies demonstrated that in dogs at the first stage of babesiosis, the urea concentration increased to 7.5 ± 0.34 mmol/L ($P < 0.001$; Table 3). Subsequently, depending on the stage of the disease, the level of this parameter continued to rise. In particular, at the second stage of the pathological process, the average urea level reached 8.4 ± 0.31 mmol/L. Hyperazotemia was detected in 69.2% of the affected animals. However, the most pronounced hyperazotemia was observed in the dogs at the third stage of babesiosis – 24.1 ± 1.48 mmol/L, which was 4.2 times higher than in the clinically healthy dogs ($P < 0.001$; Table 3).

Table 3
Serum urea and creatinine concentrations
in dogs with acute babesiosis (x ± SD)

Groups of animals		Urea, mmol/L	Creatinine, µmol/L
Clinically healthy (n = 20)		5.8 ± 0.2^a	103.7 ± 4.0^a
Diseased animals at different stages	Stage I (n = 16)	7.5 ± 0.3^b	101.0 ± 3.1^a
	Stage II (n = 13)	8.4 ± 0.3^c	121.0 ± 2.9^b
	Stage III (n = 16)	24.1 ± 1.5^d	274.7 ± 12.1^c

Note: see Table 2.

The diagnostic indicator of the filtration capacity of renal glomeruli is the level of creatinine. In contrast to urea, this parameter of residual nitrogen increases mainly in severe destructive processes affecting the glomerular apparatus of the kidneys.

The serum creatinine level in the dogs at the first and second stages of babesiosis remained within normal limits (Table 3). At the same time, at the third stage of the pathological process, a pronounced hypercreatininemia was observed (274.7 ± 12.1 µmol/L), which was 2.6 times higher than in the clinically healthy animals (Table 3). Elevated creatinine levels were detected in 100% of the affected animals. Thus, a significant increase in residual nitrogen parameters in the blood serum of the diseased dogs indicates intoxication, the develop-

ment of renal failure, and decreased filtration capacity of the glomerular apparatus.

Pathoanatomical examination of the dogs at stage III of babesiosis revealed that the kidneys were enlarged in most cases and had a flaccid consistency. The surface of the kidneys appeared cyanotic. The cortical substance was hyperemic and had a reddish-brown coloration (Fig. 3), whereas the medullary substance exhibited a yellowish-clay color. Histological examination showed disruption of the structure of some convoluted renal tubules, with narrowing of their lumen. The epithelial cells of the renal tubules were in a state of granular dystrophy, and some of them exhibited necrosis. Hemorrhages were observed in both the cortical and medullary regions of the kidneys (Fig. 3). Small blood vessels were congested with blood. In certain areas, pronounced proliferation of lymphoid cells around renal glomeruli and blood vessels was observed. The connective tissue was in a state of edema. Morphological changes characteristic of nephrosis-nephritis were detected, including marked fatty degeneration of the epithelial cells of renal tubules (Fig. 3).

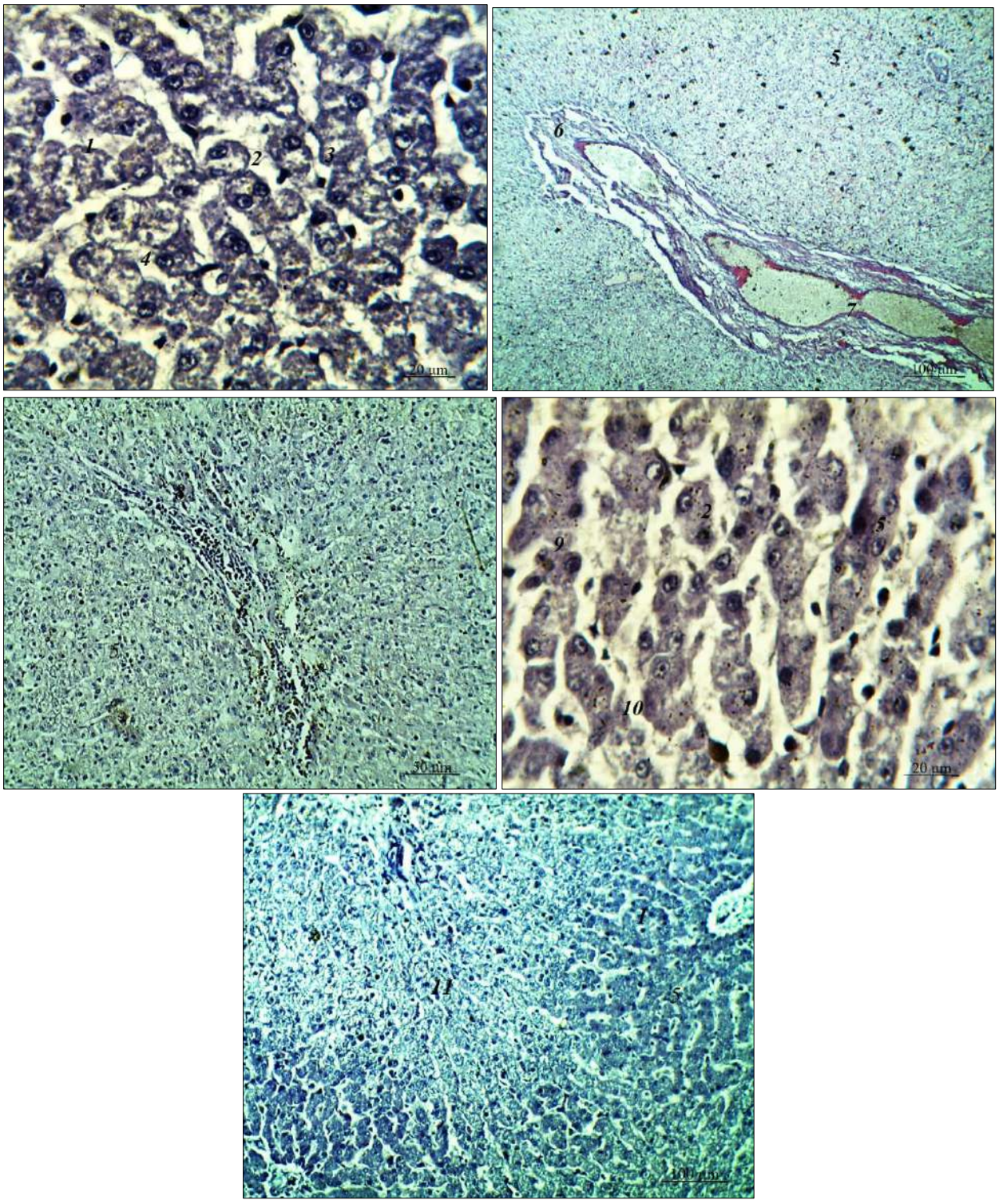

Fig. 2. Fragment of the microscopic structure of the liver of a dog with acute babesiosis: *1* – hepatocytes; *2* – hepatocyte nuclei; *3* – hepatic cords; *4* – disorganization of hepatic cords; *5* – hepatic lobule; *6* – interlobular connective tissue; *7* – dilation and bile accumulation in bile ducts; *8* – lymphohistiocytic infiltration in the area of portal triads; *9* – granular dystrophy of hepatocytes; *10* – pigment granules; *11* – atrophy of hepatocytes and their nuclei; hematoxylin and eosin staining

Discussion

In recent years, veterinary medicine has made significant progress in the development of diagnostic methods for diseases of small companion animals. The introduction of radiography, endoscopy, echography, computed tomography, and ultrasonography into clinical practice has substantially expanded clinicians' understanding of the disease development and enabled them to conduct detailed assessments of morphological changes in the organs.

Babesiosis develops following infection of dogs with the pathogen *B. canis*, transmitted through the bite of ixodid ticks, which serve as vectors of the disease. The danger of this condition lies in its high mortality rate and the development of complications that may persist even after treatment of the primary disease. Therefore, timely and accurate diagnosis of babesiosis remains a relevant issue for veterinary practitioners. The disease typically occurs in acute and subacute forms. The main clinical signs of canine babesiosis include fever, anemia, jaundice, hemoglobinuria, dyspnea, and rapid fatigue. However, in recent years, there has been an increasing tendency toward a higher number of chronic and atypical forms of the disease, as well as cases of coinfection involving atypical babesiosis and other infectious or invasive diseases, which often result in fatal outcomes (Karasová et al., 2022).

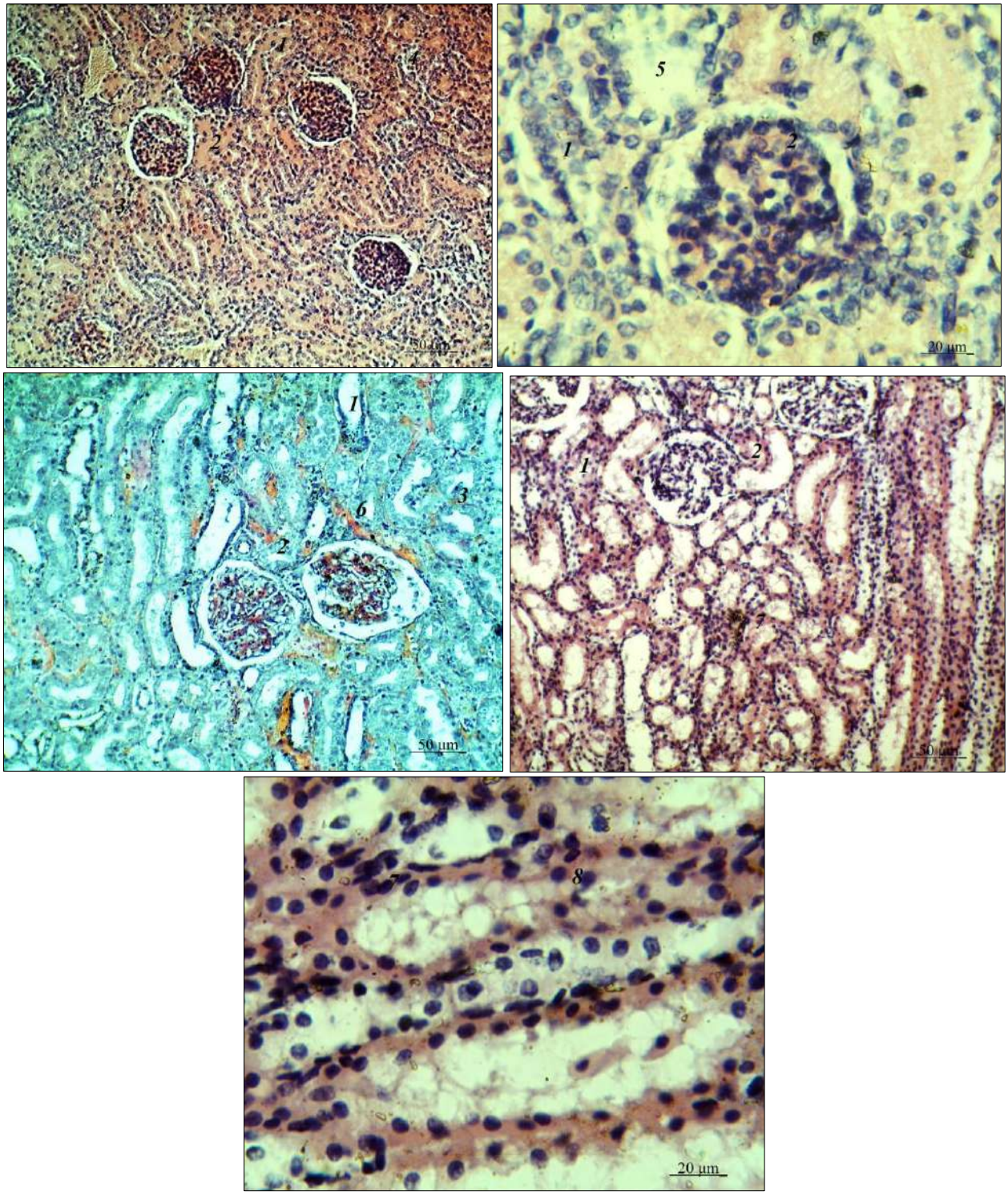

Fig. 3. Fragment of the microscopic structure of the kidney of a dog with acute babesiosis: *1* – cortical substance; *2* – renal corpuscles; *3* – convoluted renal tubules; *4* – hyperemia of the cortical substance; *5* – desquamation of epithelial cells of the convoluted renal tubules; *6* – hemorrhages; *7* – fatty degeneration of epithelial cells of renal tubules; *8* – medullary substance; hematoxylin and eosin staining

The challenge in controlling babesiosis is that the disease is accompanied by the development of hemolytic anemia (Goralska & Shpanarska, 2013), while disturbances in microcirculation in various organs lead to tissue hypoxia. Toxins formed as a result of hemolytic processes contribute to disease progression (Rajapakse & Bakirhan, 2021), and animals that have recovered from babesiosis often develop pathological changes in parenchymal organs (Kovalchuk, 2013; Kovalchuk, 2014). The goal of currently available diagnostic methods is the accurate and definitive identification of the causative agent and the assessment of the degree of organ involvement in affected animals. Equally important is the evaluation of the epizootic situation regarding this disease. In suspected cases of babesiosis, in addition to collecting anamnesis and analyzing clinical data, laboratory diagnostics are essential. These include microscopic examination of Romanowsky–Giemsa-stained blood smears, hematological analysis, and pathomorphological studies.

The latter allow for the determination of the pathogenesis and morphogenesis of the disease depending on functional disturbances, facilitates optimization of pathogenetic therapy, and enables timely implementation of preventive measures. Diagnosis of babesiosis is complex and requires consideration of multiple factors at early stages of disease development, including the epizootic situation, season, duration and severity of the disease course, clinical manifestations, pathoanatomical changes, and microscopic examination of peripheral blood.

In scientific works by both Ukrainian and international researchers, the complex of clinical symptoms and signs of babesiosis has been extensively described, along with detailed accounts of its epizootiology, and the development and implementation of new methods for treatment and prevention. However, there remain issues that require further in-depth investigation, particularly concerning the specific features of disease progression and the extent of damage to vital organs, especially the liver and kidneys.

The key to effective therapy and successful prevention of babesiosis is timely and accurate diagnosis (Beugnet et al., 2019). Diagnostic methods are classified into antemortem and postmortem approaches. The primary goal of all currently available diagnostic tools is to identify the causative agent and to determine the degree of damage it causes to the organs and systems of the animal organism (Radzikhovsky et al., 2020).

The degree of damage to the hepatic parenchyma depends on the nature of the injurious factor, the duration of its action, and individual susceptibility. One such factor is erythrocyte hemolysis, which develops during babesiosis in dogs. The metabolic products of *B. canis*, accumulating within erythrocytes, lead to dysfunction of the blood, nervous and cardiovascular systems, gastrointestinal tract, liver, kidneys, and other organs (Gryshchenko et al., 2023).

Despite the considerable compensatory capacity of the liver, the acute course of babesiosis in dogs is accompanied by damage to their parenchyma. Liver involvement is one of the most characteristic syndromes of this disease. A consequence of hemolytic anemia is jaundice, which indicates disturbances in the metabolism or excretion of bile pigments (Köster et al., 2015; Beri et al., 2021).

Moreover, despite the liver's significant compensatory potential, the acute course of babesiosis in dogs is already associated with structural damage to the hepatic tissue. Hepatic lesions represent one of the most prominent syndromes of the disease. The development of hemolytic anemia leads to jaundice, resulting in yellow discoloration of visible mucous membranes and skin, and reflects disturbances in bile pigment metabolism or excretion (Goralskyi et al., 2012).

Excessive destruction of erythrocytes caused by the penetration and multiplication of *Babesia canis* within them (Onishi et al., 1990; Zygner et al., 2023) is the primary cause of hemolytic anemia in dogs with babesiosis. The latter leads to complications such as accumulation (sequestration) of infected erythrocytes in the vessels of internal organs, including the kidneys, liver, intestines, brain, and other. Impaired microcirculation in the tissues and organs results in hypoxia and activation of anaerobic glycolysis with subsequent accumulation of lactic acid (Holovakha et al., 2018). Toxins formed as a result of renal failure, along with toxic products of protein degradation during hemolytic processes, represent key mechanisms in the development of pathology (Kovalchuk, 2014). During spontaneous diagnosis of the disease in 45 dogs (German shepherd, Central Asian shepherd, and Rottweiler) aged 18 months to 8 years, kept in private households and several kennels in Zhytomyr, the data on the acute course of the disease were collected depending on its duration, particularly during the first 6 days of clinical manifestation. In addition to anamnesis data indicating the removal of ixodid ticks from the skin and coat of animals, the most characteristic features were the rapid progression of the disease and the detection of *Babesia* in peripheral blood. The pathological process during the acute course of canine babesiosis was considered as a unified process, while the clinical development of the disease was differentiated according to its stages.

The duration of the disease was directly associated with the intensity of invasion. The longer the pathological process persisted, the greater the intensity of invasion, which contributed to the increasing severity of the disease course (Al Izzi et al., 2013).

Based on spontaneous detection of the disease (depending on its duration), the data on the acute course during the first 6 days of clinical manifestation were collected. Several authors distinguished multiple stages in the clinical progression of acute babesiosis (Baneth et al., 2019; Homer et al., 2000; Levytska et al., 2020). The intensity of *Babesia canis* invasion was reflected in the severity of clinical signs and depended on the duration of the disease (Dubova et al., 2019). The longer the pathological process lasted, the higher the intensity of invasion, which proportionally led to a more severe disease course.

Thus, based on the analysis of the obtained results of clinical, hematological, anatomical, histological studies, and morphometric parameters in dogs with acute babesiosis, it can be concluded that the pathogen *B. canis* exerts a detrimental effect on the organism of affected animals and leads to irreversible pathological changes in parenchymal organs.

The diversity of clinical manifestations of babesiosis in dogs, along with the polymorphism of disease severity, which often results in fatal outcomes in young animals, prompted us to conduct a more detailed and practically applicable clinical and laboratory investigation suitable for modern veterinary clinics and laboratories. Interpretation of clinical and laboratory parameters enables the assessment of the severity of the pathological process, prediction of disease outcomes, monitoring of disease progression, and evaluation of treatment efficacy. Considering that the organism functions as an integrated system, it is evident that pathology in any organ or system is accompanied by changes in other organs (Otranto et al., 2009). The generalization of such findings in babesiosis allowed us to identify liver and kidney pathology in the affected dogs.

One of the key approaches to evaluating the microscopic structure of the organs is the objective assessment of histo- and cytostructures through morphometric analysis (Dunaievska et al., 2024). This method has gained recognition as an objective and reliable tool for the analysis of morphological structures. Pathoanatomical examination revealed that the liver was enlarged, with a reddish-brown or yellowish-clay coloration, and a smooth surface. In some cases, petechial hemorrhages and anemic areas were observed on the liver surface. The gallbladder was often distended with thick yellow-brown bile. Hepatomegaly in dogs with babesiosis has also been reported by other researchers, who noted a smooth liver surface, occasionally with hemorrhages and anemic regions. On the section, the organ exhibited a flaccid consistency (Köster et al., 2015; Ubah et al., 2019). The microstructure of certain hepatic lobules was altered, with focal hemorrhages, as well as granular and fatty degeneration of hepatocytes and hemosiderosis. In summary, the results of this study are of both theoretical and practical importance. A deeper understanding of the mechanisms by which the causative agent of babesiosis affects the histoarchitecture of parenchymal organs and the pathogenesis of the disease will contribute to the development of more effective treatment strategies and preventive measures for canine babesiosis.

Conclusion

Based on the analysis of the obtained data, three stages of the acute course of babesiosis were identified. The first stage was charac-

terized by a disease duration of 1–2 days and an invasion intensity of 2–4%; the second stage by a duration of 3–4 days and an invasion intensity of 5–8%; and the third stage by a duration of 5–6 days and an invasion intensity exceeding 9%. Thus, among the total group of spontaneously infected dogs (n = 45), 16 animals were classified as stage I, 13 as stage II, and 16 as stage III. Hepatorenal syndrome was characterized by increased activity of gamma-glutamyl transpeptidase (GGT). Under normal conditions, GGT activity in dogs ranges from 1 to 6 U/L. At the first stage, its activity did not exceed physiological limits in all affected animals. At the second stage, hyperenzymemia was 2.6 times higher compared with the clinically healthy animals ($P < 0.001$), reaching 9.70 ± 0.66 U/L. The highest serum GGT activity was recorded at the third stage of babesiosis (14.70 ± 0.69 U/L). These findings indicate significant alterations in the cellular and membrane structure of hepatocytes, confirmed by pathomorphological studies.

Pathomorphological examination revealed that the liver was enlarged, with a smooth surface and a reddish-brown or yellowish-clay coloration. The microstructure of certain hepatic lobules was altered, with pronounced focal hemorrhages, as well as granular and fatty degeneration of hepatocytes and hemosiderosis. Interlobular bile ducts were dilated and filled with bile. The functional state of the kidneys was assessed based on residual nitrogen indicators (urea and creatinine). Their significant increase in the blood serum of affected dogs indicates intoxication, the development of renal failure, and a decrease in the filtration capacity of the glomerular apparatus. Thus, toxins produced as a result of the vital activity of *B. canis* accumulate in erythrocytes and lead to disturbances in the main functions of the blood, liver, and kidneys, as confirmed by our pathomorphological studies.

Prospects for further research are aimed at investigating hematopoiesis and the morphofunctional state of the liver in dogs with acute babesiosis, particularly in relation to protein, carbohydrate, pigment, and lipid metabolism, as well as the activity of indicator enzymes.

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