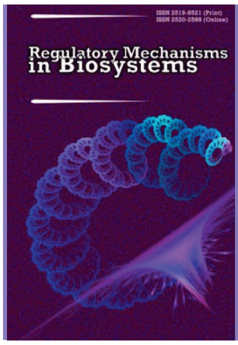

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Pathogenetic relationship between kidney pathologies and the microcirculatory capillary layer in dogs under the influence of *Babesia canis*

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With babesiosis in dogs, the capillary network is especially strongly affected due to the stimulation of the hemostasis system and the development of thrombus formation in the microvasculature. Acute renal failure and even death of animals are caused by shock phenomena and the syndrome of disseminated intravascular coagulation. Clinical, laboratory (hematological, biochemical, coagulometric, hemodynamic, enzyme immunoassay) methods of research were conducted. Dogs with babesiosis have fever, anemia, and hemoglobinuria, hematologically established anemia, systemic inflammation, thrombotic condition. Hemodynamic parameters determine the state of shock, which in dynamics becomes decompensated and irreversible. The appearance of poikilocytes in the blood of sick dogs indicates significant metabolic disorders in their body. The coagulogram reveals the syndrome of disseminated intravascular coagulation. Syndrome markers significantly changed with a trend to increase during nephropathy. In addition, uremia, hyperuricemia, hyperkalemia, hyponatremia, hyperphosphatemia, creatininemia were found in the sick dogs, which are reliable indicators and determine the development of an acute form of glomerulonephritis and progressive acute renal failure. According to the study of the general biochemical status in animals, hepatopathy in the form of acute hepatitis and pancreatitis, and general intoxication syndrome were established. In our findings: during acute spontaneous babesiosis, dogs developed nephropathy, which has two clinical stages: hemoglobinuria and oliguria/anuria. The pathogenetic basis for the development of glomerulonephritis and acute renal failure is the syndrome of disseminated intravascular coagulation in response to the influence of a pathogen, which causes shock, forming a vicious circle of shock. Under such conditions, renal failure increases and eventually leads to the death of the animal. The studied data will contribute to the expansion of the conceptual understanding of the pathogenesis of canine babesiosis and determine the leading role of complications in the development of kidney failure.

Keywords: glomerulonephritis; DIC syndrome; shock; uremia; hyperuricemia; hemoglobinuria; oliguria; anuria; renal failure.

Introduction

Ecological conditions in the zone of Ukrainian Polissia are optimal for the formation of biotopes of ixodid ticks. Representatives of the Ixodidae family, as temporary blood-sucking parasites, perform the functions of biological vectors of infections for many species of vertebrates (Estrada-Pēna et al., 2012; Mierzejewska et al., 2015; Blazhev et al., 2021). *Dermacentor reticulatus* Fabricius, 1794 and *Ixodes ricinus* Linnaeus, 1758 are adapted to parasitizing dogs. They are specific carriers of pathogens of the protozoan disease – babesiosis (Pawełczyk et al., 2022). *Babesia* spp. destroy erythrocytes, provoke the development of severe autoimmune lesions of various organs and systems of the animal body (Dubova et al., 2021; Bilić et al., 2023). Dogs of the Polissia region are parasitized by large babesia, the length of which is longer than the radius of erythrocytes. This is mainly *Babesia canis* Piana & Galli-Valerio, 1895, since the tick *D. reticulatus* is the relevant vector for them (Król et al., 2016a, 2016b; Daněk et al., 2022).

The destruction of erythrocytes is accompanied by the development of hypoxia in tissues. This condition leads to impaired functions and morphological changes in the organs of infected dogs (Konto et al., 2014; Akel & Mobarakai, 2017; Schetters, 2019). Shock states of various levels develop, as the microcirculatory field of vital organs suffers (Solano-Gallego & Baneth, 2011; Stowe et al., 2012; Bajer et al., 2022). Organs that directly interact with the external environment are especially sensitive to such violations. In particular, these are the kidneys, which, due to anatomical features, have fenestral capillaries (Defauw et al., 2012; Lobetti 2012; Kuleš et al., 2021).

Babesia spp. affect erythrocytes in such a way that they pathologically change their shape. In particular, erythrocytes lose their doubly concave discoid shape or cell fragments are formed. Distorted cells, when the body tries to dispose of them, stimulate hyperfunction of the liver, spleen and kidneys. Over time, degenerative processes develop in these organs (Konto et al., 2014; Akel & Mobarakai, 2017; Dubova et al., 2020).

The development of hepatopathy (Köster et al., 2015) extends the disease-causing process to functional disorders of the kidneys by the mechanism of pseudohepatorenal syndrome. Cases of acute renal failure can cause terminal conditions in dogs, often resulting in death (Defauw et al., 2012; Lobetti, 2012; Kuleš et al., 2021). The shock state and the closely related syndrome of disseminated intravascular coagulation (DIC) are universal secondary complications of babesiosis during severe pathologies. Pathogenetic mechanisms of these processes have fatal consequences for the functioning of vital organs. Lesions that develop in the pathogenesis of DIC blood syndrome as a result of microcirculation blockade can cause local ischemia, and subsequently ischemic necrosis, giving the organ a “shock” status (Goddar et al., 2013; Solano-Gallego et al., 2016; Mittal et al., 2019). Due to the peculiarities of the capillary’s system structure, it is the kidneys that are in the first place in the risk zone for the development of irreversible phenomena, moving into the category of “shock kidney”

(Brandt et al., 2014; Winiarczyk et al., 2019). Therefore, the study of these processes and connections involves the further inclusion in the official protocol of the babesiosis therapy in dogs, drugs aimed at eliminating the development of complications of the excretory system and carrying out anti-shock and stabilizing hemorheology measures.

The aim of work is to establish pathogenetic links between nephropathy and microcirculatory disorders, disseminated intravascular coagulation syndrome and shock in the course of acute spontaneous canine babesiosis.

Materials and methods

The research was conducted at the veterinary medicine clinic of the Polissia National University, Zhytomyr, Ukraine, during 2022. The research was coordinated within the norms of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), "General Ethical Principles of Experiments on Animals" (Kyiv, 2001) and the Law of Ukraine "On the Protection of Animals from ill-treatment" (2010). Compliance of the conducted research with the principles of bioethics and protection of animals from ill-treatment during scientific work was confirmed by the Commission for Bioethical Expertise of Polissia National University (Protocol No. 1 of 10.01.2022). Diagnostic procedures were carried out painlessly, and this didn't lead to a deterioration in the health of the dogs.

Dogs of various breeds, aged 1–5 years, in the amount of 30 animals, were selected as material for the study. Two groups were formed – the investigated and the control group, each with 15 animals. The investigated group included dogs suffering from acute spontaneous babesiosis with nephropathy symptom complex. Concomitant diseases in these animals have not been established. The control group was formed from clinically healthy dogs: those that did not have clinical signs of any diseases, and whose blood laboratory parameters were within reference limits.

Clinical studies of dogs were conducted using general methods. Venous blood samples were taken from the *Vena saphena medialis* of the right hind limb. F L Medical vacuum tubes were used for blood sampling:

1) with sodium citrate buffer solution 0.129 mol/L – for hematological and hemostasiological studies;

2) with gel and coagulation activator – for biochemical studies.

The basis for establishing the diagnosis was the indication and identification of pathogens using light microscopy of thin, fixed blood smears. Staining of smears was carried out by the method of rapid staining with Leukodif-200 using the V-Chromer®III device (West Medica, Austria, 2019). Light microscopy was performed on a Kern Obe digital microscope (Kern, Germany, 2021) (Satpathy et al., 2014). The intensity of parasitaemia was calculated by the percentage of affected erythrocytes in 10 fields of view. Using light microscopy, pathological forms of erythrocytes were also determined, which were identified by characteristic features (Dubova et al., 2020). Cytological studies of blood, hemoglobin content, determination of erythrocyte sedimentation rate and hematocrit value were carried out using an automatic hematology analyzer Abacus Vet5 (Diatron, Hungary, 2017).

Spontaneous aggregation ability of erythrocytes (spEAC) and platelets (spPAC) was determined by shaking blood samples using a laboratory shaker S-3 Micromed (Micromed®, Ukraine, 2020) according to N. I. Tarasova (Panzer & Jilma, 2011). Circulating blood volume (CBV) deficit was determined by Moore's formula (Pacagnella et al., 2013; Lopez-Picado et al., 2017). The series of coagulation hemostasis studies included the determination of Lee-White spontaneous clotting time, siliconized clotting time, prothrombin time (PT) and activated partial thromboplastin time (APTT), as well as fibrinogen content (Roshal, 2013a; Roshal, 2013b; Panteleev et al., 2015). We used a two-channel coagulometer RP-2202C (Rayto, China, 2019). The content of soluble fibrin-monomeric complexes (SFMC) was determined by the orthophenanthroline method using a spectrophotometer Granum 721 (Granum, Ukraine, 2017) (Rafaj et al., 2013; Panteleev et al., 2015). The level of D-dimer was determined using the method of solid-phase enzyme immunoassay using AccuBind IFA reagents (Monobind Inc.®, USA, 2021) (Rafaj et al., 2013; Gil, 2019).

Biochemical studies were performed using a semi-automatic biochemical analyzer Chem 7 (Erba Mannheim, India). The content of urea (Rashkovan method), uric acid (enzymatic uricase method), creatinine (Jaffe method), total protein (biuret method), albumins (reaction with bromocresol indicator), total and conjugated bilirubin (Van den Berg method), potassium, sodium and chlorine (indirect integrated multisystem technology method) were determined. Serum enzymes activity was also determined: aspartate aminotransferase (AsAT) and alanine aminotransaminase (AlAT) (Raitman and Frenkel method), gamma-glutamyltranspeptidase (GGT), lactact dehydrogenase (LDG), alkaline phosphatase (LF), alpha-amylase (kinetic and colorimetric method), pancreatic lipase (immunochromatographic method) using a rapid test cPL (IDEXX, USA, 2022) (De Loor et al., 2013; Kellum & Devarajan, 2014).

Statistical analysis was performed using the SPSS Statistics 23.0 software package (IBM Knowledge Center, USA, 2020), using the Fisher's variance analysis method. The reliability of the obtained data was assessed at the level of P < 0.05 according to Fisher's F-criterion.

Results

The manifest form of the babesiosis was pronounced by the following clinical signs: body temperature of 40–41 °C, loss of appetite, vomiting, apathy, dyscoordination of movements, pale mucous membranes, brown urine. During palpation of the kidneys in sick dogs, their enlargement was felt, and in the area of reflected pain sensations – clearly expressed. Subsequently, in some cases we could see yellowness of the sclera, visible mucous membranes and skin. Subsequently, the daily amount of urine decreased by 2–3 times, and in 6 animals (40.0%) urination stopped altogether. The animals fell into a state of stupor, ante-coma and coma. Blood tests of dogs with acute spontaneous babesiosis were performed at different stages of kidney damage – hemoglobinuria and oliguria/anuria (Table 1). Anemia and erythropenia were found at all stages of nephropathy. The nature of anemia was hyperchromic. Leukocytosis was detected, which decreased by 12.2% with the progression of nephropathy.

Table 1
Hematological indicators of dogs with the nephropathy as a result of acute babesiosis (x ± SE, n = 15)

Parameter	Investigated group		Control group
	hemoglobinuria	oliguria/anuria	
Hemoglobin, g/L	87.22 ± 4.46***	102.32 ± 7.63*	126.52 ± 7.06
Erythrocyte sedimentation rate, mm/h	24.63 ± 4.25***	28.35 ± 5.81***	5.13 ± 0.32
Erythrocyte, 10^{12}/L	3.04 ± 0.72***	4.34 ± 0.75*	7.26 ± 0.52
Leukocyte, 10^9/L	21.34 ± 2.11***	18.74 ± 1.82***	9.37 ± 0.82
Thrombocyte, 10^9/L	183.18 ± 13.63***	162.36 ± 11.64***	300.62 ± 18.26
Intensity of parasitemia, %	9.84 ± 1.74***	9.07 ± 0.62***	–

Note: * – P < 0.05; ** – P < 0.01, *** – P < 0.001 significant differences of the investigated indexes compared with those in control group.

Thrombocytopenia increased dynamically during the course of nephropathy. This indicator progressed synchronously with an increase in the spontaneous aggregation ability of platelets (spPAC) (Table 2). Dogs suffering from babesiosis with nephropathy symptoms had a reduced hematocrit (P < 0.05 at the stage of oliguria/anuria). The circulating blood volume deficit was 27.3% and 44.5%, respectively, spEAC and spPAC were significantly increased (P < 0.001), the wetting index of the vascular wall was increased by 2.4 and 3.0 times, respectively (P < 0.001).

Morphological assessment of blood cells revealed altered forms of red blood cells – poikilocytes, which are distorted cells that are not able to fully perform their function (Fig. 1). The main pathological forms were stomatocytes with one or several "mouths", which accounted for up to 75.5% of the altered forms. Echinocytes were also found – up to 17.3% of altered forms. The erythrocyte fragments are of particular importance – schizocytes (up to 10.2% of altered forms), which are pathognomonic forms of the blood DIC syndrome. In some cases, in dogs with signs of kidney failure, the presence of erythrocyte shadows was noted. These erythrocyte shadows are erythrocytes that were devoid of pigment and only their shells remained, as a result of hemolysis.

Table 2
Hemodynamic indicators and the state of vascular and platelet hemostasis in nephropathy of dogs with acute babesiosis (x ± SE, n = 15)

Parameter	Investigated group		Control group
	hemoglobinuria	oliguria/anuria	
Hematocrit, %	35.2 ± 0.63	26.4 ± 1.42*	47.4 ± 2.6
Specific circulating blood volume, mL / kg	90.5 ± 1.24***	67.9 ± 3.25***	122.8 ± 1.43
Blood loss volume, %	27.3***	44.5***	0
Spontaneous aggregation ability of erythrocytes, %	41.6 ± 2.36***	47.3 ± 5.24***	7.83 ± 0.75
Spontaneous aggregation ability of platelets, %	54.3 ± 3.84***	64.6 ± 6.28***	14.2 ± 2.72
The index of wetting the vascular wall	0.68 ± 0.03***	0.88 ± 0.08***	0.28 ± 0.03

Note: see Table 1.

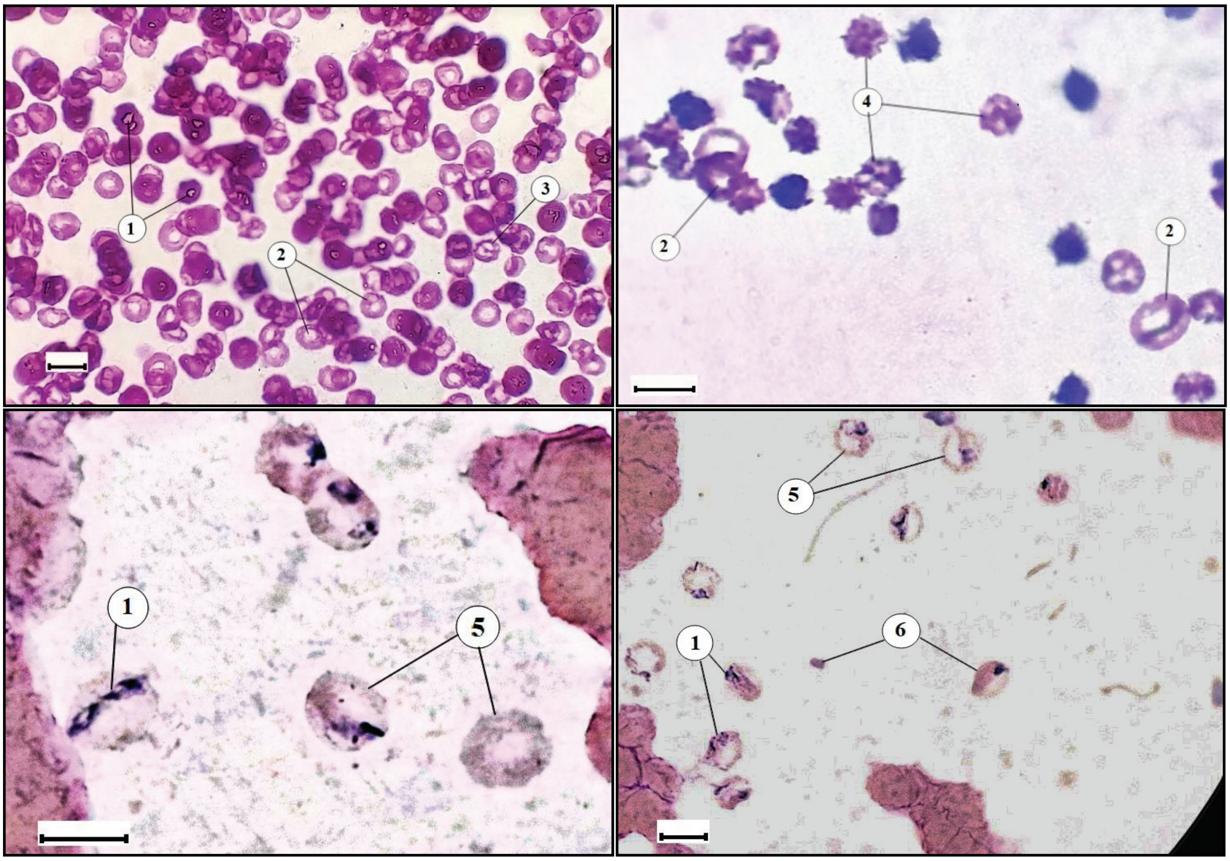

Fig. 1. Pathological forms of erythrocytes in a blood smear of a dog with acute spontaneous babesiosis: *1* – *Babesia* trophozoites; *2* – "one-mouthed" stomatocytes; *3* – "two-mouthed" stomatocyte; *4* – echinocytes; *5* – erythrocyte shadows; *6* – schizocytes; scale bars – 10 µm

Hypocoagulation in the PT test (P < 0.001) and hypercoagulation in the APTT test (P < 0.5) were found at the stage of hemoglobinuria, and hypocoagulation (P < 0.001) in the case of oliguria/anuria in both tests (Table 3). Hypofibrinogenemia, which progressively increases during the course of nephropathy (P < 0.001), a dynamic increase in the D-dimer level by 4 and 6 times, respectively (P < 0.001), and the SFMC level by 10 and 20 times, respectively (P < 0.001), were identified as markers of DIC syndrome.

Specific indicators of the renal profile expand the picture of functional changes in the kidneys of sick animals (Table 4). In sick dogs with a symptom complex of nephropathy, there were established indicators that progressed in their changes during the course of the disease: hyperuremia (P < 0.001), hyperuricemia (P < 0.001), hypercreatininemia (P < 0.001), hyperkalemia (P < 0.05 and P < 0.01), hyponatremia (P < 0.05), hyperphosphatemia (P < 0.001).

Assessment of the general condition of the animal's body can be carried out, based on general biochemical indicators that reflect the state of the main body systems (Table 5). For canine babesiosis with symptoms of nephropathy the following indicators were determined; progressive hypoalbuminemia (at the stage of oliguria/anuria P < 0.001), hypoalbuminemia (P < 0.05 and P < 0.001), hyperbilirubinemia due to conjugated bilirubin (P < 0.001), hyperfermentation of all significant enzymes (P < 0.001) – AsAT by 4.8 and 7.3 times, AlAT by 4.8 and 7.2 times, GGT by 3.8 and 7.2 times, LDG by 3.6 and 4.0 times, alkaline phosphatase by 2.0 and 3.4 times, alpha-amylase by 3.0 and 4.8 times, pancreatic lipase by 2.5 and 5.0 times.

Discussion

Parasitism of *Babesia* in the organism of dogs leads to destruction of the red blood cells, which is the leading trigger for the entire chain of pathological reactions. These reactions ultimately lead to functional disorders of organs and systems (Stowe et al., 2012; Solano-Gallego et al., 2016; Schetters, 2019).

In Ukraine on the territory of Polissia, outbreaks of acute babesiosis in dogs correspond to the periods of activity of ixodid ticks and occur in the spring (March–June) and autumn (end of August–November). In the summer, during the heat, and in the winter, during the frost, ticks are in a state of diapause of their biological cycle. On average, the prepatent period takes 2–3 days after infection. Established clinical signs characterize the intoxication syndrome. Febrile fever, vomiting, incoordination of movements, a state of apathy determines the general reaction of the body to the influence of the pathogen. In addition, these signs are caused by hypoxia due to a violation of hemoglobin transport. Anemia of the mucous membranes is one of the clinical criteria for the development of oxygen deficiency in the body (Solano-Gallego & Baneth, 2011; Konto et al., 2014;

Bajer et al., 2022). Hemoglobinuria is a characteristic symptom of babesiosis and confirms the release of hemoglobin outside the erythrocytes. It is also evidence of critical nephropathy with severe impaired glomerular filtration. As a result, the release of hemoglobin pigment into the urine becomes possible (De Loor et al., 2013; Kellum & Devarajan, 2014; Eichenberger et al., 2016). Acute glomerulonephritis develops, and the release of hemoglobin into the urine is one of the characteristic signs of this pathology.

Table 3
State of the hemostasis system in nephropathy in dogs with acute babesiosis (x ± SE, n = 15)

Parameter	Investigated group		Control group
	hemoglobinuria	oliguria/anuria	
Protrombin time, s	29.4 ± 0.48***	38.7 ± 0.53***	19.6 ± 0.14
Activated partial thromboplastin time, s	33.6 ± 4.23*	60.6 ± 3.28**	46.2 ± 3.25
Fibrinogen, g/L	1.63 ± 0.42*	0.71 ± 0.063***	2.82 ± 0.34
D-dimer, µg/L	0.54 ± 0.078***	0.76 ± 0.072***	0.13 ± 0.024
Soluble fibrin monomer complexes, g/L	0.30 ± 0.026***	0.60 ± 0.071***	0.028 ± 0.0024

Note: see Table 1.

Table 4
Indicators of renal blood serum profile in dogs with acute babesiosis (x ± SE, n = 15)

Parameter	Investigated group		Control group
	hemoglobinuria	oliguria/anuria	
Urea, mmol/L	14.7 ± 2.26***	23.6 ± 5.71***	5.12 ± 0.13
Uric acid, µmol/L	107.3 ± 6.91***	118.3 ± 8.36***	63.4 ± 2.76
Creatinine, µmol/L	356.7 ± 22.24***	570.2 ± 33.7***	97.2 ± 7.35
Potassium, mmol/L	8.2 ± 0.91*	11.2 ± 1.78**	5.6 ± 0.36
Sodium, mmol/L	132.4 ± 7.42	121.3 ± 6.84*	147.2 ± 7.26
Phosphorus, mmol/L	4.24 ± 0.31***	6.82 ± 0.81***	1.65 ± 0.083

Note: see Table 1.

Subsequently, oliguria develops, turning into anuria. These symptoms indicate acute renal failure and are life-threatening. At the same time, renal filtration does not occur, residual nitrogen products are retained in the body, and intoxication phenomena increase. Substances that are intended for removal have a toxic effect on the metabolism and cause functional disorders of various organs. As a result of these processes, the central nervous system is affected, severe depression of consciousness develops from a state of stupour to coma. In such conditions, the likelihood of death is extremely high. (Brandt et al., 2014; Winiarczyk et al., 2019).

Table 5
General biochemical parameters in dogs with acute babesiosis (x ± SE, n = 15)

Parameter	Investigated group		Control group
	hemoglobinuria	oliguria/anuria	
General protein, g/L	62.4 ± 3.4	48.2 ± 4.5***	72.3 ± 4.2
Albumin, g/L	21.6 ± 4.3*	16.2 ± 2.7***	35.9 ± 3.1
Total bilirubin, µmol/L	8.27 ± 1.77***	18.18 ± 2.37***	1.12 ± 0.09
Conjugated bilirubin, µmol/L	5.66 ± 0.16***	13.14 ± 3.36***	0.015 ± 0.003
Aspartate aminotransferase, IU/L	97.8 ± 9.2***	157.4 ± 15.5***	21.3 ± 0. 6
Alanine aminotransaminase, IU/L	86.3 ± 7.4***	129.6 ± 11.4***	18.0 ± 0.9
Gammaglutamyltranspeptidase, IU/L	18.2 ± 2.2***	34.8 ± 6.3***	4.8 ± 0.1
Lactate dehydrogenase, IU/L	453.2 ± 18.7***	513.1 ± 8.8***	126.3 ± 12.3
Alkaline phosphatase, IU/L	286.4 ± 14.2***	456.4 ± 23.3***	133.2 ± 7.1
α-Amylase, IU/L	3053.6 ± 28.4***	5016.7 ± 41.2***	1012.3 ± 15.8
Pancreatic lipase, IU/L	309.4 ± 14.4***	617.5 ± 23.2***	121.3 ± 8.2

Note: see Table 1.

Many studies (Vincent, 2013; Saini & Dunn, 2019; Goodman et al., 2021) have shown a close relationship between various terminal states and the development of complications – shock and DIC syndrome. Both processes are closely related, forming a "vicious circle". Our own laboratory tests were carried out in different stages of the course of nephropathy, which we tentatively defined as the stage of hemoglobinuria and the stage of oliguria/anuria. Hemoglobin content (Table 1) is significantly reduced simultaneously with erythropenia at the stage of hemoglobinuria, which is caused by the destruction of erythrocytes and the release of hemoglobin from them. At the stage of oliguria/anuria, the hemoglobin level increases. This can probably be caused by the exhaustion of the body's buffer systems, which lose the ability to adequately dispose of hemolyzed hemoglobin as the disease progresses. At the same time, the condition remains anemic. At the stage of hemoglobinuria, leukocytosis was noted, the intensity of which decreases as the nephropathy progresses. The rate of erythrocytes' sedimentation was stably increased by 5–6 times during the observations. The combination of these indicators indicates a systemic inflammatory response of the dog's organism to the destructive influence of *Babesia* spp. (Kirtz et al., 2012; Goddard et al., 2016; Rubić et al., 2022).

Thrombocytopenia, which progresses with the development of the disease synchronously with a reliable dynamic spPAC growth (Table 2), indicates a thrombotic state and determines a violation of the vascular-platelet hemostasis link. The interaction between the vascular endothelium and blood cells becomes inadequate, the nutrition of the vascular wall by blood plate factors does not occur, and the electrical relationship between the vessel and blood cells is changed. This often acts as a trigger for DIC syndrome. Thrombosis carries a special danger in the field of microcirculation of the organs, in particular, kidneys. A 3-fold increase in the wetting index of the vascular wall confirms the presence of a thrombotic state in the body (Barić Rafaj et al., 2013, Kuleš et al., 2017; Roopali et al., 2018).

The hematocrit value decreases significantly. At the stage of oliguria/anuria, it is almost two times lower than the control indicator. The estimated rate of circulating blood volume deficiency is 27.3% at the stage of hemoglobinuria, and 44.5% at the stage of oliguria/anuria. The hematocrit value decreases significantly at the stage of oliguria/anuria, it is almost two times less than the control indicator. The calculated indicator of BCC deficiency is 27.3% in the hemoglobinuria stage, and 44.5% in the oliguria/anuria stage. Taking into account the fact that there is no external bleeding, it is assumed that the liquid component of blood enters the tissues in the form of edema, and the formed blood elements are blocked in blood clots, mainly in the microcirculatory bed (Barić Rafaj et al., 2013, Kuleš et al., 2017; Roopali et al., 2018).

The stage of the shock state is determined by the deficit in the volume of circulating blood. Thus, the stage of hemoglobinuria includes subcompensated shock, while the stage of oliguria/anuria includes decompensated irreversible shock (Pacagnella et al., 2013; Lopez-Picado et al., 2017; Zygner et al., 2021). Such circumstances determine an extremely threatening prognosis for the animal's life.

At the first stages of conducting research, it is possible to establish qualitative changes in erythrocytes, which can serve as screening criteria for the condition of animals. Stomatocytes occupied up to 75.5% of the erythrocytes' changed forms (Fig. 1). These are erythrocytes of a changed shape, which are cup-like concave with the formation of a pelor that resembles a mouth. With babesiosis in dogs, stomatocytes with both one and two "mouths" are detected. They appear in the blood as a result of the accumulation of residual nitrogen products, which is caused by damage to the detoxification functions of the liver (Ford, 2013; Goddard et al., 2013; Dubova et al., 2020). After all, the mechanism of pseudohepatorenal syndrome consists in the secondary involvement of the kidneys in the pathological process and their overload during the filtration function (Mittai et al., 2019; Vishwakarma et al., 2019).

Up to 17.3% of the erythrocytes that changed forms were echinocytes – forms with an abnormal cell membrane containing a large number of small spiky protrusions (Fig. 1). The reason for the appearance of such cells is uremia and kidney disease. When changes in the membrane under the influence of residual nitrogen products become irreversible, the echinocyte turns into an acanthocyte (Ford, 2013; Akel & Mobarakai, 2017; Dubova et al., 2020). Fragments of erythrocytes – schizocytes – accounted for about 8–10% of the altered forms (Fig. 1). Such abnormalities in red blood cells occur in DIC syndrome, during which the generated fibrin threads in the bloodstream tear the cells, creating erythrocyte fragments (Kidd & Mackman, 2013; Akel & Mobarakai, 2017; Dubova et al., 2020). Erythrocyte shadows are abnormalities that occur during massive hemolysis and are membrane envelopes of cells. They also develop as a result of hemolysis caused by uremia (Ford, 2013). None of the changed forms of erythrocytes are able to fully perform their gas exchange functions. Thus, the formation of the altered forms found in erythrocytes is mainly caused by an increase in the concentration of residual nitrogen products in the blood, as well as the development of DIC syndrome.

In the coagulogram of sick dogs, multidirectional changes are presented (Table 3). Hypocoagulation was noted in the PT test at the stage of hemoglobinuria, while hypercoagulation was noted in the APTT test (Gil, 2019). This indicates the activation of blood coagulation by an internal mechanism, and the factors of such activation are both vascular changes and toxic substances in the blood circulation. Such multidirectional changes in the main coagulation tests indicate the consumption coagulopathy stage of DIC syndrome, which usually corresponds to the subcompensated shock we defined earlier (Roshal, 2013a, 2013b; Panteleev et al., 2015).

At the stage of oliguria/anuria, hypocoagulation was established in both coagulation tests, which indicates the stage of DIC syndrome's hypocoagulation and fibrinolysis. This syndrome's phase corresponds to decompensated irreversible shock established at the stage of oliguria/anuria.

The main markers of DIC syndrome (Table 3) increased during the progress of nephropathy: hypofibrinogenemia by almost 2 and 3 times, according to the stage; 4- and 6-fold increase in D-dimer; 10- and 20-fold increase in SFMC. Therefore, with the course of nephropathy, the DIC syndrome becomes more powerful, intensifying the vicious circle of shock (Kuleš et al., 2017; Gil, 2019; Goodman et al., 2021). Thus, the increase in the intensity of DIC syndrome is closely synchronized with the increase of shock and has a life-threatening prognosis for the animal.

The specific indicators used for functional assessment of the kidneys progressively changed during the development of nephropathy (Table 4). Uremia increased 2.5 times at the stage of hemoglobinuria and almost 4.5 times at the stage of oliguria/anuria. Together with progressive creatininemia (3.5 and 6.0 times, respectively), the indicator reflects the development of acute renal failure, which is life-threatening due to systemic disturbances in the water-electrolyte balance, acid-base balance, and intoxication (Lobetti, 2012; Kuleš et al., 2021).

Impairment of the kidneys' excretory function was also indicated by progressive uricemia (increase in the uric acid concentration by 1.5–2.0 times). It is a non-specific marker of the glomerulonephritis development (Yu et al., 2018). Hyperkalemia, hyponatremia, and hyperphosphatemia indicated impaired glomerular filtration against the background of hemorheological disorders of microcirculation, and the development of glomerulonephritis. In addition, hyponatremia is caused by fluid retention in the body, including the development of edema in the interstitial space during the development of shock and DIC syndrome (Palmer & Schnermann, 2015; Gumz et al., 2015; Suki & Moore, 2016). Decreased glomerular filtration rate and subsequent hyperphosphatemia is one of the functional criteria of acute renal failure. A 4-fold increase in the phosphorus concentration (Table 4) indicates the terminal stage of renal failure, in which the risk of animal's death due to acute heart failure increases (Prasad & Bhadauria, 2013; Suki & Moore, 2016).

For assessing the general biochemical condition of sick animals (Table 5) hypoproteinemia and hypoalbuminemia were established. A decrease in the protein content in the blood serum determines its loss due to the release of the liquid component of the blood into the tissues with the formation of edema. In addition, a significant decrease in serum albumins indicates an increase in the intensity of the systemic inflammatory process, which results in a multi-organ systemic inflammatory response (Akel & Mobarakai, 2017). Hyperbilirubinemia due to the conjugated fraction indicates acute hepatitis, and reliable hyperfermentation of indicator enzymes (AsAT, AlAT, LDG, alkaline phosphatase) proves the destructive processes occurring in cells. Consequently, along with nephropathy, dogs with babesiosis develop hepatopathy, creating pseudohepatorenal syndrome (Barić Rafaj et al., 2013; Akel & Mobarakai, 2017). The pancreas is also involved in the multiple organ systemic inflammatory response. Reliable hyperfermentation of indicator enzymes of organ functioning indicates the development of pancreatitis, which can be triggered by complications – DIC syndrome and shock (Barić Rafaj et al., 2013; Eichenberger et al., 2016). Consequently, complications of babesiosis in the form of disorders in the microcirculatory bed cause the development of DIC and shock syndrome, and these processes, in turn, involve various organs in the systemic inflammatory response, forming multiple organ failure. Despite the pronounced multiple organ pathology, the development of nephropathy is more severe prognostically, and functional changes in the kidneys characterize these organs as "shock" (Barić Rafaj et al., 2013; Eichenberger et al., 2016).

Thus, nephropathy in the course of acute spontaneous babesiosis can be characterized as progressive acute renal failure. It develops as a result of glomerulonephritis caused by disturbances in the field of the kidney glomeruli microcirculation. Such disorders are caused by the development of DIC syndrome and shock, and the triggering factor is the influence of *Babesia* spp. and its waste products on the vascular and platelet link of hemostasis. Acute renal failure is a life-threatening condition for an animal and is considered to require immediate emergency intensive care.

Conclusion

The development of acute spontaneous babesiosis in dogs is accompanied by nephropathy, which progresses and consists of two clinical stages – hemoglobinuria and oliguria/anuria. During the course of the disease, complications occur in the form of DIC syndrome and shock, which is confirmed by a decrease in the specific volume of blood circulation, a thrombotic state, as well as the presence of DIC syndrome markers – hypofibrinogenemia, an increase in the level of D-dimer and SFMC in the blood plasma. With hemoglobinuria, the shock is subcompensated, reversed (27.3% blood circulation volume deficit), and DIC syndrome is in the stage of consumption coagulopathy. With oliguria/anuria, the shock becomes decompensated, irreversible (BCV deficit 44.5%), DIC syndrome passes into the stage of hypocoagulation and fibrinolysis. Nephropathy due to acute babesiosis in dogs takes the form of glomerulonephritis with progression of acute renal failure. Shock, DIC syndrome, and acute renal failure are linked and create a vicious cycle. The vicious circle's starting factors are vascular-platelet link disorders of hemostasis and the development of DIC syndrome in response to the pathogenic influence of the causative agent. Shock and DIC syndrome, as the main complications, cause multiple organ failure, which, in addition to nephropathy, is also manifested by hepatopathy and pancreatitis.

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The authors declare that they have no conflict of interest.

References

Akel, T., & Mobarakai, N. (2017). Hematologic manifestations of babesiosis. Annals of Clinical Microbiology and Antimicrobials, 16, 6.

Bajer, A., Beck, A., Beck, R., Behnke, J. M., Dwużnik-Szarek, D., Eichenberger, R. M., Farkas, R., Fuehrer, H. P., Heddergott, M., Jokelainen, P., Leschnik, M., Oborina, V., Paulauskas, A., Radzijevskaja, J., Ranka, R., Schnyder, M., Springer, A., Strube, C., Tolkacz, K., & Walochnik, J. (2022). Babesiosis in Southeastern, Central and Northeastern Europe: An emerging and re-emerging tick-borne disease of humans and animals. Microorganisms, 10(5), 945.

Barić Rafaj, R., Kuleš, J., Selanec, J., Vrkić, N., Zovko, V., Zupančič, M., Trampuš Bakija, A., Matijatko, V., Crnogaj, M., & Mrljak, V. (2013). Markers of coagulation activation, endothelial stimulation, and inflammation in dogs with babesiosis. Journal of Veterinary Internal Medicine, 27(5), 1172–1178.

Bilić, P., Horvatić, A., Kuleš, J., Gelemanović, A., Ljubić, B. B., Mũnoz-Prieto, A., Gotić, J., Žubčić, D., Barić, R. R., & Mrljak, V. (2023). Serum and urine profiling by high-throughput TMT-based proteomics for the investigation of renal dysfunction in canine babesiosis, Journal of Proteomics, 270, 104735.

Blazhev, A., Atanasova, M., Kostov, K., Doychinova, T., Blazheva, S., & Karcheva, M. (2021). Estimation of *Ixodes ricinus* (Acari: Ixodidae) populations of Kaylaka Park in the Town of Pleven, Bulgaria. Insects, 12(9), 808.

Brandt, L. E., Ehrhart, E. J., Scherman, H., Olver, C. S., Bohn, A. A., & Prenni, J. E. (2014). Characterization of the canine urinary proteome. Veterinary Clinical Pathology, 43, 193–205.

Daněk, O., Hrazdilová, K., Kozderková, D., Jirků, D., & Modrý, D. (2022). The distribution of *Dermacentor reticulatus* in the Czech Republic re-assessed: Citizen science approach to understanding the current distribution of the *Babesia canis* vector. Parasites and Vectors, 15(1), 132.

De Loor, J., Daminet, S., Smets, P., Maddens, B., & Meyer, E. (2013). Urinary biomarkers for acute kidney injury in dogs. Journal of Veterinary Internal Medicine, 27, 998–1010.

Defauw, P., Schoeman, J. P., Smets, P., Goddard, A., Meyer, E., Liebenberg, C., & Daminet, S. (2012). Assessment of renal dysfunction using urinary markers in canine babesiosis caused by *Babesia rossi*. Veterinary Parasitology, 190(3–4), 326–332.

Dubova, O. A., Feshchenko, D. V., Bakhur, T. I., Dubovyi, A. A., Zghozinska, O. A., & Chala, I. V. (2021). Splenomegaly development and disseminated intravascular coagulation syndrome in acute canine babesiosis. Regulatory Mechanisms in Biosystems, 12(4), 670–675.

Dubova, O., Feshchenko, D., Zghozinska, O., Pinsky, O., Budnik, T., & Chala, I. (2020). Poikilocytosis under dogs' spontaneous babesiosis. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies, Series Veterinary Sciences, 22(97), 118–124.

Eichenberger, R. M., Riond, B., Willi, B., Hofmann-Lehmann, R., & Deplazes, P. (2016). Prognostic markers in acute *Babesia canis* infections. Journal of Veterinary Internal Medicine, 30(1), 174–182.

Estrada-Peña, A., Farkas, R., Jaenson, T. G., Koenen, F., Madder, M., Pascucci, I., Salman, M., Tarrés-Call, J., & Jongejan, F. (2013). Association of environmental traits with the geographic ranges of ticks (Acari: Ixodidae) of medical and veterinary importance in the Western Palearctic. A digital data set. Experimental and Applied Acarology, 59(3), 351–366.

Ford, J. (2013). Red blood cell morphology. International Journal of Laboratory Hematology, 35(3), 351–357.

Gil, M. R. (2019). Overview of the coagulation system. In: Shaz, B. H., Hillyer, C. D., & Gil, M. R. (Eds.). Transfusion medicine and hemostasis: Clinical and laboratory aspects. 3nd ed. Elsevier, Amsterdam. Pp. 559–564.

Goddard, A., Leisewitz, A. L., Kjelgaard-Hansen, M., Kristensen, A. T., & Schoeman, J. P. (2016). Excessive pro-inflammatory serum cytokine concentration in virulent canine babesiosis. PLoS One, 11(3), e0150113.

Goddard, A., Wiinberg, B., Schoeman, J. P., Kristensen, A. T., & Kjelgaard-Hansen, M. (2013). Mortality in virulent canine babesiosis is associated with a consumptive coagulopathy. The Veterinary Journal, 196(2), 213–217.

Goodman, A., Bilal, M., Amarnath, S., Gentile, T., & Shepherd, Z. (2021). The unusual case of babesiosis causing disseminated intravascular coagulation with hemophagocytic lymphohistiocytosis. Clinical Case Reports International, 9(9), e04744.

Gumz, M. L., Rabinowitz, L., & Wingo, C. S. (2015). An integrated view of potassium homeostasis. The New England Journal of Medicine, 373(1), 60–72.

Kellum, J. A., & Devarajan, P. (2014). What can we expect from biomarkers for acute kidney injury? Biomarkers in Medicine, 8, 1239–1245.

Kidd, L., & Mackman, N. (2013). Prothrombotic mechanisms and anticoagulant therapy in dogs with immune-mediated hemolytic anemia. Journal of Veterinary Emergency and Critical Care (San Antonio), 23, 3–13.

Kirtz, G., Leschnik, M., Hooijberg, E., Tichy, A., & Leidinger, E. (2012). In-clinic laboratory diagnosis of canine babesiosis (*Babesia canis canis*) for veterinary practitioners in Central Europe. Tierarztl Prax Ausgabe K: Kleintiere Heimtiere, 40(2), 87–94.

Konto, M., Biu, A. A., Ahmed, M. I., Mbaya, A. W., & Luka, J. (2014). Clinico-biochemical responses of dogs to experimental infection with *Babesia canis*. Veterinary World, 7, 113–118.

Köster, L. S., Lobetti, R. G., & Kelly, P. (2015). Canine babesiosis: A perspective on clinical complications, biomarkers, and treatment. Veterinary Medicine (Auckland, N.Z.), 6, 119–128.

Król, N., Kiewra, D., Lonc, E., Janaczyk, B., Chodorowska-Skubiszewska, A., Dzięcioł, M., Gola, M., Gruszka, R., Jackowska-Szlachcic, E., Jagiełło, M., Kawski, S., Łukaszewski, Z., Mizgalski, P., Narajowska, T., Niedzielska, J., Noczyński, M., Rudkowska, M., Rzepka, D., Samulska, K, Senze, M., Sieczko, P., Silny, A., Staniewska, A., Stańczyk, J., Stańczyk, W., Stasiak, M., Włodarczyk, M., & Zimniak, S. (2016a). *Dermacentor reticulatus* (Fabricius, 1794) and *Babesia canis* (Piana et Galli-Valerio, 1895) as the parasites of companion animals (dogs and cats) in the Wrocław area, South-Western Poland. The Annals of Parasitology, 62(2), 125–130.

Król, N., Obiegala, A., Pfeffer, M., Lonc, E., & Kiewra, D. (2016b). Detection of selected pathogens in ticks collected from cats and dogs in the Wrocław Agglomeration, South-West Poland. Parasites and Vectors, 9(1), 351.

Kuleš, J., Gotić, J., Mrljak, V., & Barić Rafaj, R. (2017) Blood markers of fibrinolysis and endothelial activation in canine babesiosis. BMC Veterinary Research, 13(1), 82.

Kuleš, J., Rubić, I., Beer Ljubić, B., Bilić, P., Barić Rafaj, R., Brkljačić, M., Burchmore, R., Eckersall, D., & Mrljak, V. (2021). Combined untargeted and targeted metabolomics approaches reveal urinary changes of amino acids and energy metabolism in canine babesiosis with different levels of kidney function. Frontiers in Microbiology, 12, 715701.

Lobetti, R. (2012). Changes in the serum urea: Creatinine ratio in dogs with babesiosis, haemolytic anaemia, and experimental haemoglobinaemia. Veterinary Journal, 191(2), 253–256.

Lopez-Picado, A., Albinarrate, A., & Barrachina, B. (2017). Determination of perioperative blood loss: Accuracy or approximation. Anesthesia and Analgesia, 125(1), 280–286.

Mierzejewska, E. J., Welc-Faleciak, R., Karbowiak, G., Kowalec, M., Behnke, J. M., Bajer, A. (2015). Dominance of *Dermacentor reticulatus* over *Ixodes ricinus* (Ixodidae) on livestock, companion animals and wild ruminants in Eastern and Central Poland. Experimental and Applied Acarology, 66(1), 83–101.

Mittal, M., Kundu, K., Chakravarti, S., Mohapatra, J. K., Singh, V. K., Raja Kumar, B., Thakur, V., Churamani, C. P., & Kumar, A. (2019). Canine babesiosis among working dogs of organised kennels in India: A comprehensive haematological, biochemical, clinicopathological and molecular epidemiological multiregional study. Preventive Veterinary Medicine, 169, 104696.

Pacagnella, R. C., Souza, J. P., Durocher, J., Perel, P., Blum, J., Winikoff, B., & Gülmezoglu, A. M. (2013). A systematic review of the relationship between blood loss and clinical signs. PloS One, 8(3), e57594.

Palmer, L. G., & Schnermann, J. (2015). Integrated control of Na transport along the nephron. Clinical Journal of the American Society of Nephrology, 10(4), 676–687.

Panteleev, M. A., Dashkevich, N. M., & Ataullakhanov, F. I. (2015). Hemostasis and thrombosis beyond biochemistry: Roles of geometry, flow and diffusion. Thrombosis Research, 136(4), 699–711.

Panzer, S., & Jilma, P. (2011). Methods for testing platelet function for transfusion medicine. Vox Sang, 101(1), 1–9.

Pawełczyk, O., Kotela, D., Asman, M., Witecka, J., Wilhelmsson, P., Bubel, P., & Solarz, K. (2022). The first records of canine babesiosis in dogs from *Dermacentor reticulatus* – free zone in Poland. Pathogens, 11(11), 1329.

Prasad, N., & Bhadauria, D. (2013). Renal phosphate handling: Physiology. Indian Journal of Endocrinology and Metabolism, 17(4), 620–627.

Rafaj, R. B., Kuleš, J., Selanec, J., Vrkić, N., Zupančič, M., Trampuš Bakija, A., Matijatko, V., Crnoqaj, M., & Mrljak, V. (2013). Markers of coagulation activation, endothelial stimulation, and inflammation in dogs with babesiosis. Journal of Veterinary Internal Medicine, 27(5), 1172–1178.

Roopali, B., Roy, M., & Roy, S. (2018). Clinico, haemato-biochemical changes and therapeutic management of canine babesiosis. Journal of Current Microbiology and Applied Sciences, 7(8), 1384–1388.

Roshal, M. (2013a). Activated partial thromboplastin time. In: Shaz, B. H., Hillyer, C. D., Roshal, M., & Abrams, C. S. (Eds.). Transfusion medicine and hemostasis: Clinical and laboratory aspects. 2nd ed. Elsevier, London. Pp. 805–807.

Roshal, M. (2013b). Prothrombin time. In: Shaz, B. H., Hillyer, C. D., Roshal, M., & Abrams, C. S. (Eds.). Transfusion medicine and hemostasis: Clinical and laboratory aspects. 2nd ed. Elsevier, London. Pp. 799–803.

Rubić, I., Burchmore, R., Weidt, S., Regnault, C., Kuleš, J., Barić Rafaj, R., Mašek, T., Horvatić, A., Crnogaj, M., Eckersall, P. D., Novak, P., & Mrljak, V. (2022). Multiplatforms strategies and metabolomics approaches for the investigation of comprehensive metabolite profile in dogs with *Babesia canis* infection. International Journal of Molecular Sciences, 23(3), 1575.

Saini, S., & Dunn, A. L. (2019). Disseminated intravascular coagulopathy. In: Shaz, B. H., Hillyer, C. D., & Gil, M. R. (Eds.). Transfusion medicine and hemostasis. 3rd ed. Elsevier, Amsterdam. Pp. 749–755.

Satpathy, S., Mohanty, A., Satpathi, P., Mishra, S. K., Behera, P. K., Patel, G., & Dondorp, A. M. (2014). Comparing Leishman and Giemsa staining for the assement of peripheral blood smear preparations in amalaria-endemic region in India. Malaria Journal, 13, 512.

Schetters, T. (2019). Mechanisms involved in the persistence of *Babesia canis* infection in dogs. Pathogens, 8(3), 94.

Solano-Gallego, L., & Baneth, G. (2011). Babesiosis in dogs and cats-expanding parasitological and clinical spectra. Veterinary Parasitology, 181(1), 48–60.

Solano-Gallego, L., Sainz, Á., Roura, X., Estrada-Peña, A., & Miro, G. (2016). A review of canine babesiosis: The European perspective. Parasites Vectors, 9, 336.
Stowe, D. A., Birkenheuer, A. J., & Grindem, C. B. (2012). Pathology in practice. Intraerythrocytic infection with organisms consistent with a large *Babesia* sp. Journal of the American Veterinary Medical Association, 241(8), 1029–1031.
Suki, W. N., & Moore, L. W. (2016). Phosphorus regulation in chronic kidney disease. Methodist DeBakey Cardiovascular Journal, 12(4), 6–9.
Vincent, J. L., & De Backer, D. (2013). Circulatory shock. New England Journal of Medicine, 369, 1726.
Vishwakarma, P., & Nandini, M. (2019). Overview of canine babesiosis. In: Bekoe, S. O., Saravanan, M., Adosraku, R. K., & Ramkumar, P. (Eds.). Veterinary medicine and pharmaceuticals. IntechOpen, London.
Winiarczyk, D., Michalak, K., Adaszek, L., Winiarczyk, M., & Winiarczyk, S. (2019). Urinary proteome of dogs with kidney injury during babesiosis. BMC Veterinary Research, 15, 439.
Yu, K. H., Chen, D. Y., Chen, J. H., Chen, S. Y., Chen, S. M., Cheng, T. T., Hsieh, S. C., Hsieh, T. Y., Hsu, P. F., Kuo, C. F., Kuo, M. C., Lam, H. C., Lee, I. T., Liang, T. H., Lin, H. Y., Lin, S. C., Tsai, W. P., Tsay, G. J., Wei, J. C., Yang, C. H., & Tsai, W. C. (2018). Management of gout and hyperuricemia: Multidisciplinary consensus in Taiwan. International Journal of Rheumatic Diseases, 21(4), 772–787.
Zygner, W., Rodo, A., Gójska-Zygner, O., Górski, P., Bartosik, J., & Kotomski, G. (2021). Disorders in blood circulation as a probable cause of death in dogs infected with *Babesia canis*. Journal of Veterinary Research, 65(3), 277–285.