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Hemolytic anemia in dogs caused by the protozoan parasite *Babesia canis* and the impact of imidopyran and prednisolone

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Babesiosis is a tick-borne disease caused by apicomplexan hemoprotozoan parasites that infect red blood cells. The condition can develop from small species of *Babesia*, such as *B. gibsoni*, *B. conradae*, and *B. vulpes*, as well as larger species like *B. vogeli*, *B. canis*, and *B. rossi*. The number of *Babesia* infections has been increasing globally in the world, and that is a significant problem for wild and domestic animals and for humans. *Babesia* is found throughout the world. Climate change, including rising temperatures, altered precipitation patterns and prolonged warm spells, fosters tick reproduction and expands their habitats into new regions. This shift significantly increases the risk of babesiosis being transmitted to areas where it was previously uncommon. Additionally, climate change may affect the seasonal behavior of ticks, extending their active periods and thereby elevating the risk of infection for both humans and animals. The overall health of animals can also be compromised by climate change, making them more susceptible to infections, which further facilitates the spread of various diseases. Recent reports highlight that *Babesia canis* is the most prevalent species found in Ukraine. However, information regarding the hematological and biochemical changes in dogs naturally infected with *B. canis*, particularly in Dnipro city, remains scarce. This study focuses on a retrospective analysis of clinical cases of *Babesia* infection in dogs. For this research, a complete biochemical profile was obtained and analyzed for 25 dogs diagnosed with babesiosis. Treatment administered within the first 24 hours for dogs with *B. canis* infection demonstrated improvement in several biochemical parameters in the blood, including superoxide dismutase (SOD), inorganic phosphorus, lactate dehydrogenase (LDH), and various protein fractions. This treatment effectively reduced renal and oxidative stress, as well as improved electrolyte, protein, and lipid metabolism. While some indicators, such as urea, creatinine, potassium, albumin, cholesterol, calcium, and sodium, did not show significant changes, the overall trends suggest that the therapy was effective in stabilizing the dogs' conditions and alleviating symptoms of hemolytic anemia. Ongoing monitoring and possible additional therapeutic interventions may be required to achieve a complete normalization of biochemical parameters.

Keywords: hemolytic anemia; dogs; *Babesia canis*; babesiosis; tick-borne diseases; tick-pathogen; blood cells; oxidative stress; biochemical analysis; imidopyran; prednisolone.

Introduction

Vector-borne diseases (VBDs) are included in the list of emerging and re-emerging infectious diseases and pose a health problem for humans, livestock, wildlife, and companion animals (Kuleš, 2017). Several factors, such as global development, urbanization, climate change, increased international trade, animal movements, and translocation, influence the epidemiology and spread of tick-borne diseases (TBDs) (Baneth, 2012; Alvarez, 2017; Teodorowski, 2022). Ticks are known vectors of a wide range of microbial pathogens that are significant to public health and veterinary medicine (Drehmann, 2020; Efstratiou, 2021; Bajer, 2022). Babesiosis is a tick-borne disease that is distributed worldwide and is caused by hemoprotozoa belonging to the genus *Babesia*, which affect both farm and domestic animals (Boozer, 2003; Solano-Gallego, 2016). It was first recognized by Babes in 1888 as a cause of hemolytic anemia and mortality in cattle (Babes, 1888). There are many types of parasites affecting dogs, which can be morphologically classified into large and small forms. The large forms include *Babesia vogeli*, *B. canis*, and *B. rossi*, while the small forms include *B. gibsoni*, *B. conradae*, *B. vulpes*, and *B. annae* (Allison, 2011; Vannier, 2015; Antunes, 2017; Jalovecka, 2019). The moderately pathogenic *B. vogeli* is transmitted by *Rhipicephalus sanguineus* and has been found in Africa, Asia, North America, Brazil, and Australia.

The moderately pathogenic *B. canis* is transmitted by *Dermacentor variabilis*, which is widespread in Europe and Ukraine. The most pathogenic and fatal species is *B. rossi*, which is transmitted by *Hammondia ellipticus* and is prevalent in sub-Saharan Africa and South Africa (Schoeman, 2009).

Over the past 10 years, the number of studies on *B. canis* has increased significantly, highlighting the importance of this topic in modern medicine. For example, during the period from 2008 to 2016, 130 scientific articles related to *B. canis* were published, while from 2017 to 2024, this number increased to 242 articles. This indicates that the number of studies has increased by approximately 1.86 times (Fig. 1).

This increase in interest in the study of babesiosis is due to the expansion of knowledge about the prevalence of this disease, its impact on human and animal health, and the development of new diagnostic and treatment methods. *Babesia*, as intracellular parasites, have complex life cycles and a high capacity for adaptation, which makes them difficult to control. In addition, phylogenetic studies have recently attracted special attention, helping to better understand the evolutionary relationships between different species of *Babesia* and their hosts. For example, in a study in 2022 (Hirata, 2022), a phylogenetic analysis was conducted that shows the diversity and genetic heterogeneity of *Babesia* in different regions of the world.

Babesia canis is widespread throughout Europe and the Middle East, as evidenced by isolates from various regions, including Romania, other parts of Europe, and Israel. These isolates show a high level of genetic similarity, indicating that the *B. canis* genome is stable in these regions. This genetic stability may be the result of relatively recent evolutionary diversification of the species or limited variation in the genome due to specific environmental or geographic conditions. Understanding the phylogenetic structure of this group is critical for epidemiological studies, as it helps to identify the pathways of infection and possible sources of infection. In addition, such knowledge is necessary for the development of effective methods for the diagnosis and treatment of babesiosis in dogs, considering the regional characteristics of the pathogen.

On the territory of Ukraine, canine babesiosis caused by *B. canis* is constantly recorded. Previously, babesiosis in dogs was called a "forest disease" because the animal was attacked by infected ticks only during a walk in the countryside (Kjemtrup, 2009). In recent years, the situation has changed dramatically. Indeed, while in the 1960s and 1970s dogs were infected with babesiosis while hunting near summer cottages and in forests, in the late 1980s and early 1990s most cases of dog disease were recorded directly within the city (Djokic, 2021; Daněk, 2022). Most often, dogs get babesiosis after being attacked by ticks in city parks and squares, and even in yards. This was facilitated by the formation of biotopes of *Ixodes* ticks in urban areas and a sharp increase in their number in urban populations in the late 1980s (Djokic, 2021). In addition, the incidence of the disease in predominantly domesticated dog breeds has increased over the past few years, with outbreaks occurring twice as often (in spring and fall) and generally being sporadic. A significant number of cases have now been reported in dogs of different breeds and the disease is becoming increasingly widespread (Vannier, 2015).

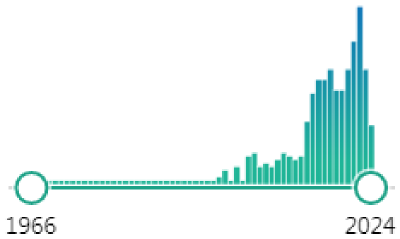

Fig. 1. The dynamics of the number of studies on *B. canis* for the period 1966–2024 (PubMed, 2024)

Babesia canis is mainly found in red blood cells and can be found in the plasma and cytoplasm of cells of the reticulo-endothelial system. Babesiosis is an obligately contagious disease. This is since the transmission of the pathogen occurs only through a specific vector – the *Ixodes* tick (Zahler, 2008). In addition to dogs, small ruminants and cattle, ungulates, and pigs are affected by babesiosis. Cases of babesiosis have also been reported in humans (Stegeman, 2003). Babesiosis in dogs has been known since the late 18th century. At the beginning of the 19th century, the disease was discovered in Ukraine (Aktas, 2015; Corduneanu, 2020). Today, this infection is very widespread among dogs on all continents of the world and in a wide variety of natural and climatic regions. This is largely due to the constant and uncontrolled increase in the number of dogs, especially stray dogs, the lack of effective preventive measures, and unsanitary conditions in walking areas (Adaszek, 2015; Panti-May, 2020; Kuo, 2020). After the cessation of pesticide treatment of forests, the reproduction of the *Ixodes* tick is practically not regulated, and its population continues to increase (Vannier, 2015). In addition, during babesiosis in dogs, pathological changes occur in red blood cells. This leads to the development of hemolytic anemia and deterioration of the general condition of the organism (Allison, 2011).

From a biochemical point of view, babesiosis has a significant impact on the metabolism and function of the dog's organs. The parasite *B. canis* destroys red blood cells, which leads to their degradation, which in turn indirectly increases bilirubin levels, as hemoglobin is divided into heme and globin (Schäfer, 2021). In addition, hypochromic microcytic anemia develops, in which the number of red blood cells decreases, hemoglobin levels and cell volume decrease. Changes in electrolyte balance occur during red blood cell destruction, the ratio of potassium-sodium balance changes (Eslahi, 2023). A decrease in albumin levels is commonly observed in babesiosis due to the loss of proteins from plasma or a decrease in their synthesis in the liver. There is an increase in globulin levels as a response to infection and inflammation. Disseminated intravascular coagulation (DIC) may also develop, causing microthrombosis and bleeding. There is a violation of kidney function. Elevated creatinine and urea levels indicate renal dysfunction, which may result from hemolysis and decreased renal perfusion. Thus, babesiosis has a serious impact on the dog's body, changing biochemical parameters and the functioning of many systems. Early diagnosis and treatment are important to reduce the severity of the disease and improve prognosis (O'Bryan, 2020; Penzhorn, 2020; Seleznova, 2020).

Therefore, the disease of dogs with babesiosis has not lost its practical importance for laboratory research. At this stage, it is important to investigate the effect of babesiosis in dogs on the functional state of organs and the development of hemolytic anemia, and how treatment affects the improvement of the general condition of the body. The aim of the study was to evaluate the effect of the *Babesia canis* parasite on biochemical profile of blood of the domestic dogs, as well as the effectiveness of treatment with imidopyran and prednisolone.

Materials and methods

The biochemical analysis of clinical cases of *Babesia* infection in dogs was performed. The study was based on well-documented laboratory records of the complete biochemical profile of 25 cases with positive disease caused by *Babesia canis* in blood smear tests. The dogs were examined at the time of the first manifestations of the disease. The study was conducted from February 2024 to April 2024 at the Veterinary Complex Peredovyi, Dnipro, Ukraine. Dogs with other diagnosed comorbidities or with poorly documented laboratory data were not included in the study. The animals were of different ages, breeds and sexes. Animal manipulation was carried out by the rules of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and "Regulations on the use of animals in biomedical research". All procedures were carried out following the ethical rules for manipulations with experimental animals and were allowed by the local Ethical Committee of Oles Honchar Dnipro National University (protocol No. 1, 2024).

Detection of *B. canis* parasites inside erythrocytes was performed using thin blood smears stained with LEUCODIF 200 fast dyes (Erba Lachema, Czech Republic) and examined under 100x magnification using a Leica DM4 optical microscope (Leica Microsystems, Germany) (Fig. 2).

Fig. 2. Blood smear of a dog with detected *Babesia canis* parasites

The main period of treatment of babesiosis in dogs was the first 24 hours with imidopyran/imidocarb dipropronate (Arterium, Ukraine, dose 7 mg/kg) and prednisolone (Darnitsa, Ukraine, 2.2 mg/kg) with simultaneous injection.

Blood for biochemical testing was drawn from the main vein / saphenous vein into a sterile tube, followed by centrifugation and serum separation. The obtained blood serum was used to determine the activity of superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), lactate dehydrogenase (LDH, EC 1.1.1.27), aspartate aminotransferase (AST, EC 2.6.1.1), alanine aminotransferase (ALT, EC 2.6.1.2), alkaline phosphatase (ALP, EC 3.1.3.1), different fractions of globulins, urea, creatinine, potassium, inorganic phosphorus, albumin, glucose, triglycerides, cholesterol, calcium, sodium, alkaline phosphatase, total bilirubin. The catalase activity was assessed based on the capacity of hydrogen peroxide to generate a stable-colored complex with molybdenum salts (Koroliuk, 1988).

The superoxide dismutase activity was determined by its capability to impede quercetin oxidation (Kostjuk, 1990). Determination of urea (Cat. No. HP018.02), potassium (Cat. No. HP024.01), calcium (Cat. No. HP013.05), sodium (Cat. No. HP029.03), protein fractions (Cat. No. HP006.01), LDH (Cat. No. HP015.01) was carried out using reagents from Felicit-Diagnostics (Felicit-Diagnostics, Ukraine) according to the standard assay protocols. The determination of creatinine (IN: 2-233), inorganic phosphorus (IN: 3-243), triglycerides (IN: 2-253), albumin (IN: 2-238), AST (IN: 7-314), ALT (IN: 1-216), ALP (IN: 7-312) was performed using reagents Cormay Diagnostics

(Cormay Diagnostics, Poland) following the manufacturer's instructions.

Glucose (HTI-G7521-125), cholesterol (HTI-C7510-125), total bilirubin (HTI-B7576-250) were determined using HTI reagents (USA) according to the standard study design. The study of biochemical parameters was performed using a semi-automatic analyzer BS-3000M (SINNOWA, China).

Descriptive statistics were used to determine the mean (x), standard deviation (SD), and variance of the pre- and post-treatment values. A two-way analysis of variance (ANOVA) test and Tukey post hoc test were applied to assess the differences between groups, considering P < 0.05 as statistically significant.

Results

It is widely acknowledged that the antioxidant system plays a pivotal role in preserving physiological functions and maintaining homeostasis. The antioxidant defense mechanism comprises a network of enzymes and molecules that counteract free radicals, thereby shielding cells from oxidative damage. Accordingly, in the initial phase of our research, we assessed the activity of the principal antioxidant enzymes under conditions of *Babesia* infection. Catalase activity (Fig. 3) in the blood serum of dogs of control group measured 10.00 ± 0.51 U/mg. In the first group, prior to treatment, catalase activity was markedly reduced to 6.28 ± 0.14 U/mg protein (P < 0.001; F < 0.001), indicating a significant decline in antioxidant capacity due to *Babesia* infection. Following 24 hours of therapeutic intervention (group 2), catalase activity rose slightly to 6.72 ± 0.10 U/mg protein, although it remained lower than in the control group. The observed difference between groups 1 and 2 was statistically significant (P < 0.05; F = 0.09), suggesting a partial restoration of enzymatic antioxidant activity post-treatment.

Superoxide dismutase (SOD) activity in the blood of the control cohort was 100.01 ± 4.18 U/mg protein. Infection with *Babesia* resulted in a substantial elevation of SOD activity in group 1, reaching 176.79 ± 26.22 U/mg protein (P < 0.01; F < 0.01). After 24 hours of therapy (group 2), SOD activity declined to 119.92 ± 15.33 U/mg protein, with a statistically significant difference compared to the untreated group (P < 0.05; F = 0.01). Despite this decrease, SOD level in the blood serum remained notably elevated compared to the control group, implying that oxidative stress persisted even after initial therapeutic intervention.

Lactate dehydrogenase (LDH) activity in the control group was 200.00 ± 9.94 U/L. Before treatment, activity declined to 177.33 ± 9.84 U/L, though this reduction was not statistically significant (P = 0.12; F = 0.96). Post-treatment, LDH activity significantly decreased to 109.37 ± 8.28 U/L (P < 0.005 vs. both control and pre-treatment; F = 0.38; F = 0.40), suggesting a marked decrease in cellular injury or metabolic changes following intervention.

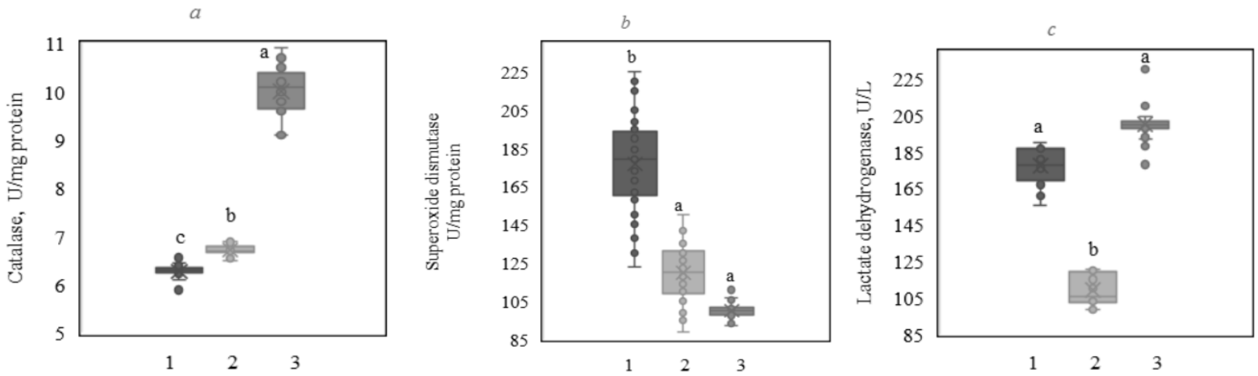

Fig. 3. Catalase (*a*), superoxide dismutase (*b*) and lactate dehydrogenase (*c*) activity in the blood serum of dogs under the influence of *Babesia canis* infection and treatment (x ± SD, n = 25): *1* – group of dogs infected with *Babesia*; *2* – group of infected dogs after the first 24 hours of treatment with imidopyran (7 mg/kg) and prednisolone (2.2 mg/kg); *3* – control group of dogs; [a, b, c] – mean values with unlike letters were significantly different between the groups (P < 0.05)

The next stage was to determine the key biochemical parameters that are most often studied in veterinary medicine.

Urea concentration (Fig. 4) in the blood serum of control dogs was 7.50 ± 0.35 mmol/L. Prior to treatment (group 1), urea level exhibited a slight increase to 7.82 ± 0.63 mmol/L, though the difference from the control group was not statistically significant (P = 0.72; F = 0.006). Post-treatment (group 2), the urea level marginally declined to 7.16 ± 0.52 mmol/L, yet again with no significant variation from the pre-treatment levels (P = 0.53; F = 0.34). These findings indicate that urea concentration remained relatively constant over the course of the study. In the control group of dogs, the creatinine level was 88.86 ± 3.69 μmol/L. Prior to treatment (group 1), the creatinine level rose to 103.96 ± 7.09 μmol/L (P = 0.09; F < 0.005), suggesting a potential impairment of renal function linked to *Babesia* infection. After 24 hours of therapy (group 2), creatinine levels decreased to 93.85 ± 5.85 μmol/L; however, this reduction was not statistically significant (P = 0.31; F = 0.35), indicating a modest response to treatment. The total bilirubin concentration in the control group was 4.40 ± 0.29 mmol/L (Fig. 8). In group 1, levels rose significantly to 11.00 ± 1.33 mmol/L (P < 0.001; F < 0.001), indicating pronounced liver dysfunction due to infection. After treatment, bilirubin levels declined to 7.52 ± 0.73 mmol/L, yet this reduction did not reach statistical significance (P = 0.06; F = 0.02), suggesting partial hepatic recovery.

Potassium concentration (Fig. 5) in the blood serum of control animals averaged 4.74 ± 0.06 mmol/L. In group 1, before treatment, the potassium level dropped significantly to 3.34 ± 0.14 mmol/L (P < 0.001; F < 0.001), suggesting a disturbance in electrolyte balance associated with the infection. Post-treatment (group 2), the potassium level improved to 3.48 ± 0.14 mmol/L, although the increase did not reach statistical significance (P = 0.41; F = 0.93), implying only partial recovery.

The inorganic phosphorus level in the control group was 1.35 ± 0.05 mmol/L. In group 1, prior to treatment, the level rose significantly to 1.86 ± 0.12 mmol/L (P < 0.001; F < 0.001). After 24 hours of treatment (group 2), phosphorus in the blood serum decreased to 1.69 ± 0.12 mmol/L, but the difference compared to group 2 was not statistically significant (P = 0.17; F = 0.75), indicating partial normalization.

The calcium level in the blood of control dogs measured 2.57 ± 0.07 mmol/L. In group 1, before treatment, the calcium level significantly dropped to 2.21 ± 0.20 mmol/L (P < 0.01; F < 0.001), reflecting a possible disruption in calcium metabolism. Following therapy, calcium level further declined to 2.09 ± 0.17 mmol/L, with a significant difference from control values (P < 0.001; F < 0.001), suggesting persistent hypocalcemia post-treatment. Sodium concentration in the control dogs was 145.20 ± 1.97 mmol/L. Prior to treatment, the sodium level fell dramatically to 115.55 ± 8.89 mmol/L (P < 0.001; F < 0.001), indicating severe electrolyte imbalance. After treatment, levels remained low at 114.80 ± 6.04 mmol/L, with no significant difference compared to pre-treatment values (P = 0.91; F = 0.06), signifying no early recovery of sodium homeostasis.

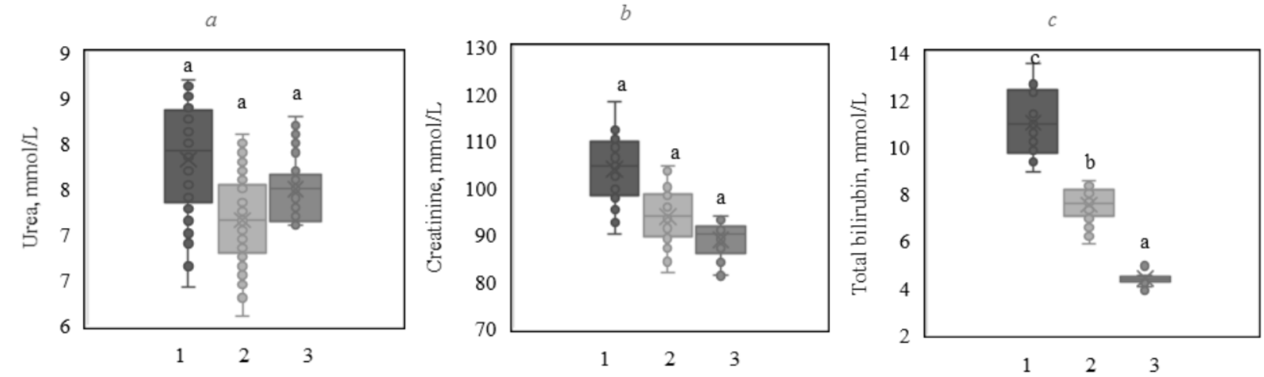

Fig. 4. The level of urea (*a*), creatinine (*b*) and the total bilirubin concentration (*c*) in the blood serum of dogs under the influence of *Babesia canis* infection and treatment (x ± SD, n = 25): see Fig. 3

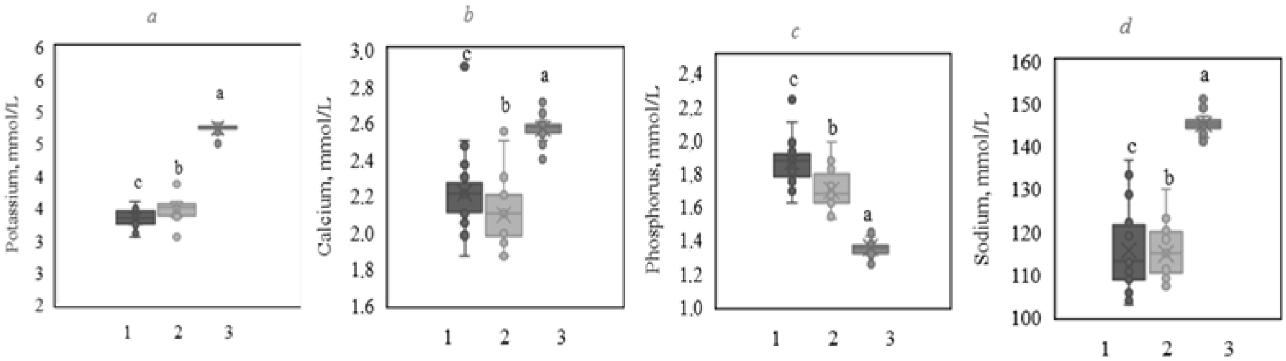

Fig. 5. Inorganic potassium, phosphorus, calcium and sodium levels in the blood serum of dogs under the influence of *Babesia canis* infection and treatment (x ± SD, n = 25): see Fig. 3

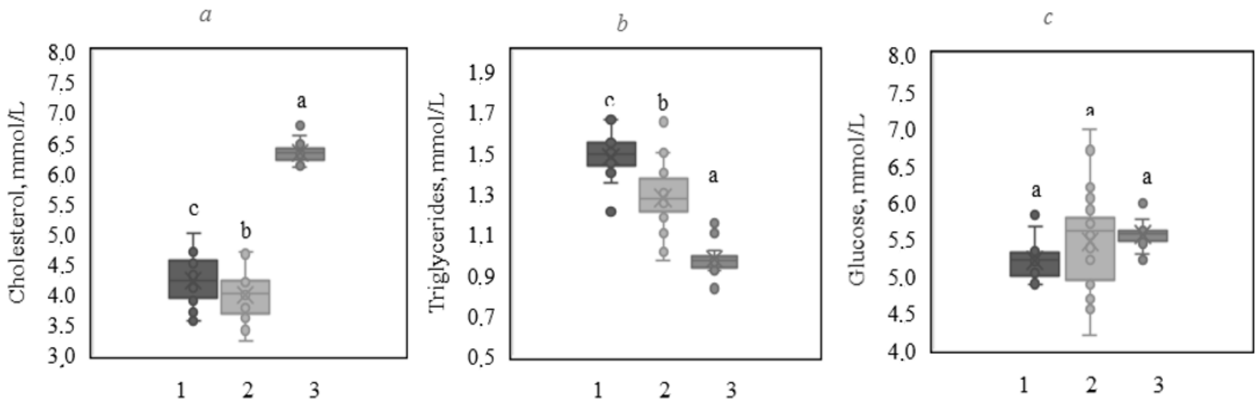

Fig. 6. Cholesterol, triglyceride and glucose levels in the blood serum of dogs under the influence of *Babesia canis* infection and treatment (x ± SD, n = 25): see Fig. 3

Cholesterol level (Fig. 6) in the blood serum of dogs of the control group was 6.32 ± 0.15 mmol/L. In group 1, prior to treatment, the cholesterol level dropped markedly to 4.21 ± 0.40 mmol/L ($P < 0.001$; $F < 0.001$). After therapy (group 2), the level further declined to 3.98 ± 0.41 mmol/L, remaining significantly lower than in controls ($P < 0.001$; $F = 0.92$), indicating that the treatment did not restore normal lipid profiles.

Triglyceride concentration in the control group was 0.98 ± 0.06 mmol/L. Before treatment, the level rose significantly to 1.48 ± 0.12 mmol/L ($P < 0.001$; $F < 0.01$), possibly due to infection-induced metabolic alterations. Post-treatment, the triglyceride level in the blood decreased to 1.28 ± 0.16 mmol/L, with a significant difference from pre-treatment ($P < 0.02$; $F < 0.01$), suggesting partial metabolic normalization.

Blood glucose in the control animals was 5.56 ± 0.15 mmol/L. Pre-treatment levels slightly declined to 5.21 ± 0.22 mmol/L, though the change was not statistically significant ($P = 0.15$; $F = 0.05$). After treatment, glucose level rose slightly to 5.46 ± 0.65 mmol/L, again without significant differences from either control ($P = 0.73$; $F < 0.001$) or pre-treatment values ($P = 0.22$; $F < 0.001$). These data indicate that glucose homeostasis remained relatively unaffected during the study period.

In the control dogs, serum albumin fraction accounted for 42.49 ± 0.84 % based on protein fraction analysis (Fig. 7). Before treatment, it slightly declined to 40.86 ± 3.34%, a change not considered significant ($P = 0.69$; $F < 0.001$). Post-treatment, the level rose to 42.25 ± 0.84%, indicating a trend towards recovery. However, this trend was less apparent in ELISA-based measurements.

The alpha-1 globulin fraction was 3.50 ± 0.11% in control animals. This increased significantly to 5.87 ± 0.53% before treatment ($P < 0.005$; $F < 0.005$), reflecting an inflammatory response to *Babesia* infection. Following treatment, the level decreased to 4.67 ± 0.66% ($P < 0.005$; $F < 0.005$), indicating a significant reduction in inflammation.

The alpha-2 globulin concentration in the control group was 9.50 ± 0.19%. Before treatment, the level remained nearly unchanged at 9.56 ± 1.83% ($P < 0.003$; $F < 0.001$), suggesting minimal impact from the infection. After treatment, it slightly decreased to 7.57 ± 0.89%, but the difference was not statistically significant ($P = 0.08$; $F < 0.001$).

In the control group, beta globulin made up 17.50 ± 0.59% of serum proteins. Prior to treatment, levels significantly rose to 23.00 ± 2.73% ($P < 0.005$; $F < 0.001$), indicating an immune activation. After therapy, the value slightly decreased to 22.21 ± 2.29%, remaining significantly elevated compared to controls ($P < 0.005$; $F < 0.001$).

The gamma globulin fraction in control dogs was 17.50 ± 0.65%. This rose modestly to 18.26 ± 1.99% before treatment ($P = 0.62$; $F < 0.001$). Following therapy, the level increased further to 21.37 ± 2.49%, with a significant difference compared to controls ($P < 0.001$; $F < 0.001$) and a near-significant difference compared to pre-treatment ($P = 0.06$; $F = 0.28$), suggesting an ongoing immune response.

Alanine aminotransferase (ALT) activity (Fig. 8) in the control group was 52.00 ± 3.64 U/L (Fig. 11). In dogs infected with *Babesia* (group 2), the activity dropped slightly to 40.76 ± 4.55 U/L ($P = 0.09$; $F = 0.28$). After 24 hours of treatment with imidocarb (7 mg/kg) and prednisolone (2.2 mg/kg), ALT levels further declined to 34.24 ± 3.25 U/L. The difference between groups 2 and 3 was significant ($P < 0.005$; $F = 0.58$), although there was no significant difference between groups 1 and 2 ($P = 0.31$; $F = 0.11$), indicating that ALT did not fully return to baseline levels.

In the control group, albumin concentration was 34.41 ± 1.01 g/L as determined by immunoenzyme assays. Prior to treatment, albumin levels in group 1 decreased to 30.45 ± 2.35 g/L ($P = 0.01$; $F < 0.001$). Following 24 hours of therapy (group 2), albumin levels showed a slight increase to 31.11 ± 2.51 g/L. However, the difference compared to pre-treatment values was not statistically significant ($P = 0.74$; $F = 0.75$), indicating only a modest recovery in protein concentration.

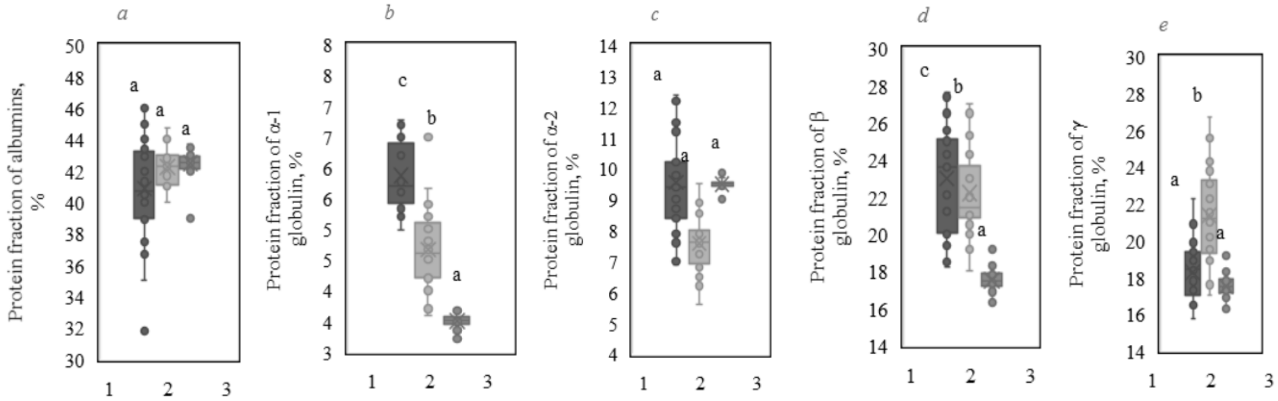

Fig. 7. The protein fractions: albumin, alpha-1 globulin, alpha-2 globulin, β-globulins and γ-globulins in the blood serum of dogs under the influence of *Babesia canis* infection and treatment (x ± SD, n = 25): see Fig. 3

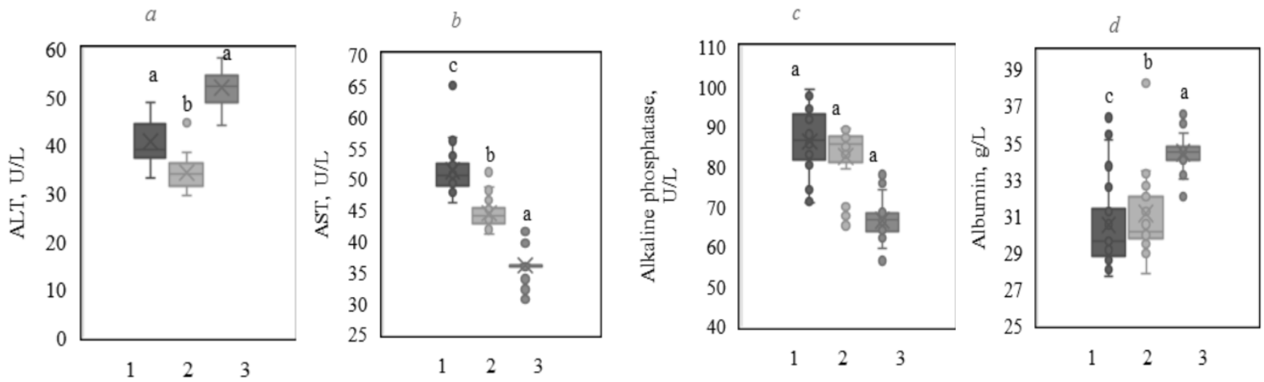

Fig. 8. Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase activity and albumin level in the blood serum of dogs under the effects of *Babesia canis* infection and treatment (x ± SD, n = 25): see Fig. 3

In the control group, Aspartate aminotransferase (AST) activity measured 35.98 ± 2.17 U/L (Fig. 9). Infected dogs (group 1) showed a significant elevation to 51.22 ± 3.79 U/L ($P < 0.001$; $F < 0.01$). Following treatment (group 2), AST dropped to 44.41 ± 2.29 U/L, though it remained above the control range.

Alkaline phosphatase (ALP) activity in the control group was 66.50 ± 5.32 U/L (Fig. 9). Before treatment, ALP levels increased to 86.19 ± 8.68 U/L ($P = 0.06$; $F = 0.02$). After treatment, the enzyme activity slightly decreased to 82.47 ± 7.58 U/L. No significant differences were observed across the groups, indicating that the ALP level remained largely stable during the study.

Discussion

Analysis and comparison of canine babesiosis data with existing literature. The analysis of data obtained in this study and its comparison with existing literature reveal significant similarities across multiple aspects. In Bosnia and Herzegovina, canine babesiosis is highly prevalent and is associated with severe anemia, oxidative stress, thrombocytopenia (89%), lethargy (100%), anorexia (95%), fever (66%), and elevated liver function markers (61%) (Coralic, 2018). In Serbia, the primary clinical signs of babesiosis in dogs include lethargy, anorexia, increased oxidative stress, fever, elevated liver enzyme levels, brown-red urine, pale mucous membranes, splenomegaly, and vomiting (Davitkov, 2015). Polish researchers evaluating babesiosis in dogs reported thrombocytopenia, low hematocrit (52%), and anemia (46%) in all affected animals. However, their findings suggest that while cardiac abnormalities are common in dogs with babesiosis, they are nonspecific and clinically insignificant (Bartnicki, 2017). Chinese studies identified the most frequent clinical signs of canine babesiosis as malaise (100%), anorexia (100%), pale mucous membranes or jaundice (80%), elevated liver enzyme levels, fever (70%), and brown urine (70%). Hematological abnormalities in infected dogs included anemia and thrombocytopenia (Yao, 2014). In France, the prevalence of canine babesiosis is also notably high. The primary clinical manifestations include weakness (98%), loss of appetite (98%), fever (>39 °C) (80%), pale mucous membranes (54%), and changes in urine color (45%) (Rene-Martellet, 2013). Ukrainian studies have indicated a twofold increase in α-amylase activity, a 3.7-fold increase in AST, and a 3.8-fold increase in ALT ($P < 0.001$) compared to control animals. Other parameters remained within normal ranges (Prus, 2017). Our findings also suggest increased AST activity and a general trend toward liver dysfunction.

The use of glucocorticoids in canine babesiosis treatment. The use of glucocorticoids, particularly prednisolone, in the treatment of canine babesiosis remains a subject of debate due to conflicting data on its efficacy and potential side effects. Babesiosis, a parasitic disease caused by protozoa of the *Babesia* genus, leads to red blood cell destruction, anemia, hemolysis, and immune-related complications (Jacobson & Clark, 1994). The key question is whether prednisolone can effectively control the inflammatory response driven by the immune component of the disease. Jacobson & Clark (1994) suggested that glucocorticoids can reduce inflammation caused by the immune response to *Babesia*-induced red blood cell destruction. However, prolonged prednisolone use suppresses the immune system, increasing the risk of secondary infections. Bilić et al. (2018) highlighted that prednisolone is beneficial for babesiosis cases complicated by severe immune-mediated conditions, such as thrombocytopenia and acute hemolytic anemia. Since these complications often arise from an excessive immune response against self-cells, prednisolone helps mitigate autoimmune aggression. However, its use should be strictly controlled to avoid adverse effects. Boozer & Macintire (2003) emphasized that while prednisone alleviates clinical symptoms, antiparasitic drugs such as imidocarb and atovaquone remain the cornerstone of babesiosis treatment. Prednisolone should only be used as an adjunct therapy in cases of severe systemic inflammation. Nevertheless, prolonged use poses immunosuppressive risks. Schoeman (2009) noted that prednisolone effectively reduces complications such as increased blood clotting and shock. By mitigating the cytokine response responsible for acute complications, prednisolone may improve clinical outcomes. However, accurate dosing is crucial, as excessive immunosuppression can exacerbate infections. A review by Solano-Gallego et al. (2016) concluded that prednisolone may be more justified in European regions where *Babesia canis* is highly prevalent. The authors recommend using it only in severe cases and under strict veterinary supervision to minimize risks. Overall, prednisolone is beneficial for managing severe inflammatory and immune complications of babesiosis, but its use should be limited and carefully monitorred (Jacobson & Clark, 1994; Loretti & Barros, 2005; Bilić et al., 2018). The primary treatment should rely on antiparasitic drugs, with prednisolone serving only as an adjunct in clinically indicated cases (Boozer & Macintire, 2005; Solano-Gallego et al., 2016).

The role of imidocarb dipropionate in babesiosis treatment. Imidocarb dipropionate, also known as imidopyran, is a primary drug for treating canine babesiosis. Numerous studies confirm its efficacy in eliminating *Babesia* spp. and preventing severe clinical manifestations. Brandão et al. (2003) demonstrated that imidocarb dipropionate effectively treats *B. canis* infections. It exerts antiparasitic effects, eliminating the pathogen and reducing the risk of relapses. However, side effects, including pain at the injection site and potential toxic reactions, have been reported. Jacobson (2006) corroborated imidocarb's efficacy in treating severe babesiosis cases, particularly those complicated by anemia and organ dysfunction. However, improper dosing may lead to relapses. Kjemtrup & Conrad (2006) emphasized the importance of accurate diagnosis when treating babesiosis with imidocarb, as different *Babesia* species may require alternative treatment approaches. Nonetheless, timely imidocarb administration significantly reduces mortality. Vial & Gorenflot (2006) conducted a comprehensive review of babesiosis chemotherapy, identifying imidocarb as one of the most effective therapeutic agents. It not only eradicates parasites but also provides long-term preventive effects, aiding disease control. However, hepatotoxicity has been observed in some cases, necessitating close patient monitoring. Brandão et al. (2003) confirmed imidocarb's high efficacy against *B. canis*, reporting rapid stabilization of severely affected dogs. However, overdose risks include decreased appetite, weakness, and liver toxicity, underscoring the importance of proper dosing.

In conclusion, imidocarb dipropionate remains one of the most effective treatments for canine babesiosis, directly targeting the parasite and helping control disease progression (Brandão et al., 2003; Jacobson, 2006; Kjemtrup & Conrad, 2006). Nevertheless, careful monitoring of potential side effects is essential, particularly with prolonged use or improper dosing (Vial & Gorenflot, 2006; Zygner et al., 2023).

The combination of imidopyran and prednisolone. The data published by scientists from various countries generally align with each other and with our results. Our findings support the effectiveness of treatment with imidopyran and prednisolone, demonstrating positive outcomes in reducing the impact of infection and improving several biochemical parameters. Notably, there was a significant decrease in lactate dehydrogenase (LDH) levels, suggesting reduced tissue damage and inflammation. Additionally, there was a partial improvement in antioxidant status, particularly in the activity of catalase and superoxide dismutase (SOD), indicating enhanced antioxidant defense mechanisms. The obtained results can be summarized in the following scheme (Fig. 9).

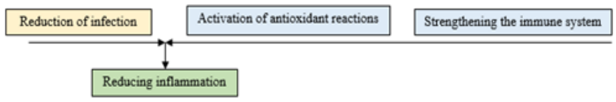

Fig. 9. Scheme of the mechanisms of imidopyran and prednisolone effects on the body of dogs infected with *Babesia*

Renal function remained stable throughout the treatment, as evidenced by unchanged levels of urea, creatinine, and potassium. Similarly, variations in inorganic phosphorus, albumin, and cholesterol levels were not statistically significant, suggesting that these parameters were not substantially affected.

Triglyceride levels continued to rise, while calcium and sodium levels declined after the first 24 hours of treatment. However, these changes were not statistically significant, indicating that a longer treatment duration may be necessary for full biochemical recovery. An increase in γ-globulin levels was observed after the first 24 hours of treatment, potentially reflecting an activated immune response. In contrast, β-globulin levels did not return to baseline values, and the changes in this parameter were not statistically significant. *Babesia canis* infection resulted in a decrease in alanine aminotransferase (ALT) activity and a significant increase in aspartate aminotransferase (AST) levels compared to the control group. This suggests potential liver and systemic organ damage related to enzyme metabolism. Moreover, our data indicate that treatment did not significantly reduce enzyme activity within the first 24 hours.

The combination of imidopyran and prednisolone demonstrated a beneficial impact on the body, as evidenced by a reduction in infectious burden, improvement in antioxidant status, and activation of the immune response. Imidopyran's antimicrobial properties contributed to a decrease in *B. canis* parasite load, while inflammation reduction facilitated faster recovery. The increased activity of antioxidant enzymes such as catalase and SOD helped mitigate oxidative stress, protecting cells from further damage. Prednisolone, as a glucocorticosteroid, not only reduced inflammation but also appeared to modulate immune function, particularly by increasing γ-globulin level. The observed decline in LDH and AST levels suggests decreased inflammation and improved tissue integrity.

Conclusions

Babesia canis poses a serious threat to the health of dogs in Ukraine and the world. The spread of ticks, in particular *D. reticulatus*, contributes to the expansion of the range of *B. canis*, which increases the risk of infection in new regions. *Babesia canis* can cause severe forms of the disease, including hemolytic anemia, fever, and even death without proper treatment. In many regions, including Ukraine, there is a lack of systematic research and diagnostic programs, which complicates the timely detection and treatment of the disease. The relevance of research in this area is due to the high prevalence of the infection, its clinical significance, and the need to develop effective prevention and treatment strategies. These findings underscore the potential of imidopyran and prednisolone as an effective therapeutic approach for canine babesiosis reducing the infectious load, improving antioxidant status, and activating the immune response, which leads to reduced inflammation and accelerated recovery. However, further research is needed to assess long-term biochemical changes and optimize treatment protocols for enhanced recovery outcomes.

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