



The effect of transfusion of erythrocyte mass on clinical and haematological indicators of dogs with hemolytic anaemia caused by babesiosis

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Abstract. The relevance of the research is due to the spread of protozoal diseases of animals, which, in the absence of the necessary veterinary care, lead to death. In this regard, the aim of the study was to investigate changes in clinical and haematological parameters in dogs under complex treatment of babesiosis with simultaneous red blood cell transfusion. Transfusions were performed in five animals infected with *Babesia canis* by intravenous injection of red blood cells, the volume of which was calculated using the Sackmen formula. The diagnosis of babesiosis was made based on anamnesis, clinical symptoms (weakness, refusal to food, pallor of visible mucous membranes, fever up to 40°C), and confirmed by microscopic detection of babesiae in blood smears. Before transfusion, rapid tests were performed to exclude heartworm disease in donor dogs. The compatibility of the blood of the donor and recipient animal was determined using a large cross-test. The condition of the abdominal organs of the patients was assessed using ultrasound diagnostics. Clinical examination of the dogs' blood before treatment indicated the development of anaemia. The results of biochemical blood tests indicated an increase in the activity of alanine aminotransferase and gamma-glutamyl transpeptidase, which indicates a load on the hepatobiliary system. There were no contraindications to blood transfusion. After haemotransfusion, the clinical condition of the dogs was monitored based on the results of the examination of the animals, taking into account the anamnesis and haematological studies on the second, fifth, and tenth days of treatment. During the second and fifth days, a gradual increase in blood haemoglobin content and haematocrit value was noted. On the tenth day, there was a significant positive trend in the number of red blood cells, haemoglobin content and haematocrit to the reference values. It was established that the use of red blood cell transfusion in dogs with babesiosis as part of therapy contributed to the fastest recovery of the general condition of the animal. The material of the article is of practical value for the use of red blood cell transfusion in the treatment of dogs with babesiosis

Keywords: hemotransfusion; blood groups; blood components; hemoglobin; haematocrit; enzymes

Introduction

The practice of blood transfusion has a long history. Blood has been used for various diseases, blood loss, stimulation of various organs and systems (Zaremba *et al.*, 2022), detoxification, haemostasis, and parenteral nutrition (Muche *et al.*, 2023). Blood transfusion has been used in veterinary medicine since the 1950s and has become an important aspect of intensive and emergency therapy for dogs (Herter *et al.*, 2022). Scientific advances in recent years (Gul *et al.*, 2023) have contributed to the development and implementation of haemocomponent therapy.

Over the past decades, interest in transfusiology has been growing. Today, many dogs receive blood or blood components as part of their

treatment, if necessary. Plasma components, such as fresh frozen plasma, are used mainly for the treatment of coagulopathies. Plasma contains therapeutic levels of functional clotting factors, which are an important component of the treatment of many congenital and acquired coagulopathies. In allogeneic transplantation, healthy stem cells from bone marrow, peripheral blood or umbilical cord blood donors are used as a source to restore haematopoietic function in patients (Gul *et al.*, 2023). Haemotransfusion of red blood cells increases patients' haemoglobin levels and reduces hypoxia (Maliuk *et al.*, 2022). Transfusion of blood, plasma or moulded cells can cause potentially

life-threatening reactions in recipients (Herter *et al.*, 2022). Blood transfusion in animals is always associated with immunological risks. Factors affecting the effectiveness of transfusion include immune and non-immune factors (Malyuk *et al.*, 2023). It should be noted that transfusion-related complications occur despite the correct protocols for blood collection from donor animals, storage, and preparation for transfusion of blood and its components (in vitro immune reactions) to recipient animals (Maliuk *et al.*, 2022).

Blood transfusions are mainly performed on patients in intensive care. Therefore, additional complications arising from violations in the technology of donor blood storage should be avoided. Red blood cells undergo changes that are considered to be a series of interdependent processes, including metabolic disorders and oxidative stress. Haematological changes and oxidative stress are present as early as the first week of storage, leading to depletion of the antioxidant system and further accumulation of oxidation products, as well as erythrocyte haemolysis, which is most pronounced at the end of storage (Bujok *et al.*, 2022). Blood transfusion is increasingly used in veterinary medicine. Indications for blood transfusion are conditions associated with severe anaemia (Herter *et al.*, 2022). Anaemia is a condition of reduced oxygen carrying capacity of the blood, which can be caused by an absolute decrease in the number of red blood cells, haemoglobin content and haematocrit value. In recent years, there has been a spread of protozoal diseases in animals, which, in the absence of the necessary veterinary care, can lead to death. One of these diseases is babesiosis, which has been registered throughout Europe. This pathology causes changes in the circulatory system, which are manifested by direct damage to red blood cells (Penzhorn, 2020).

According to R. Zaremba *et al.* (2022), it was found that whole blood transfusion is a safe

and physiological way to eliminate acid-base and blood-gas disorders associated with severe babesiosis in dogs. In recent years, the issue of haemotransfusion in dogs has become more widely known and increasingly used in the practice of veterinarians. Taking into account all the above, the aim of the study was to investigate the effect of red blood cell transfusion on clinical and haematological parameters in dogs with haemolytic anaemia caused by babesiosis.

Literature Review

Babesiosis is an acute or chronic vector-borne disease that affects carnivores. The main symptoms inherent in this pathology are high body temperature, anaemia, jaundice of the mucous membranes, nervous system disorders and digestive system functions (Levytska *et al.*, 2020). Babesia is a protozoan that belongs to the Protozoa type and is the causative agent of Babesia canis. They can have different shapes (round, oval, pear-shaped, amoeboid) and are found in red blood cells. As a result of *B. canis* infection in dogs, the functions of all organs and systems are impaired, and morphological changes develop (Penzhorn, 2020). A. Almendros *et al.* (2023) indicate that *Babesia* spp. are intraerythrocytic, tick-borne haematozoan parasites that infect a wide range of mammals, including cattle, dogs, cats, and humans. Babesiosis is a complex of diseases caused by the infection of some hosts, in particular cattle and dogs, with certain Babesia species.

According to A. Ćoralić *et al.* (2018), canine babesiosis, caused by various Babesia species, is a protozoan tick-borne disease of worldwide distribution and global significance. Babesiosis infection is common among dogs not only in Europe (Penzhorn, 2020) but also on other continents (Yin *et al.*, 2022). According to D.H. Muguero *et al.* (2023), the prevalence in stray populations is up to 40%. Their recent study identified *B. gibsoni* as the most

common *Babesia spp.* infecting dogs in Hong Kong (>90%), followed by *B. vogeli*. The study by G. Baneth *et al.* (2019) showed that three completely different vector-specific parasite species existed in specific geographic regions, the response of animals to infection varied from subclinical to acute, and it was found that immunity to one parasite species did not necessarily protect against infection with other species. A.J. Birkenheuer *et al.* (2003) indicate that natural transmission in dogs occurs via Ixodes ticks, but horizontal transmission via fights, blood transfusions or transplacental transmission has been reported. Similar transmission routes are also found in cats. Although definitive evidence of regional tick species is lacking, D.H. Muguiro *et al.* (2023) suggest that the ticks *Rhipicephalus sanguineus sensu lato* and *Haemaphysalis longicornis* are likely to be vectors. A. Almendros & R. Burchell (2021) noted that infected dogs are often asymptomatic and the diagnosis of babesiosis infection is incidental.

A feature of the pathogenesis of the disease is the multiplication of the causative agent of babesiosis in the red blood cells of a susceptible animal. Most often, erythrocytes located in the vessels of the microcirculatory system are affected. The metabolic products of parasites released in this process often cause dysfunction of the hematopoietic organs, which leads to circulatory disorders. Research results show that the pathogenesis of this disease is dominated by the breakdown of red blood cells, which results in anaemia. As a result of the destruction of affected red blood cells in the liver, free haemoglobin appears in the blood, which is excreted in the urine in unchanged form or as urobilinogen (Schober *et al.*, 2018). R. Narurkar *et al.* (2017) note that one of the main causes of poisoning in babesiosis is the waste products of the pathogens of this disease. Against the background of rapid destruction of red blood cells, a hypoxic state develops in the body of animals. The activity of the

respiratory function of the blood decreases. The rate of aerobic biochemical processes and their total volume decrease. Energy expenditure is levelled out by the production of lactate (lactic acid) in the process of glucose breakdown. Lactic acid is produced in large quantities, which far exceeds the capacity of the buffer systems of cells. This causes the development of acidosis due to a shift in pH to the acidic side.

In allergic reactions, babesia, their waste products, and infected red blood cells (endoallergens) act as allergens. The cells of the immune system recognize them as foreign and destroy them. If the immune system also destroys non-invasive red blood cells, autoallergic processes develop, which lead to a sharp progression of anaemia. Y. Otsuka *et al.* (2002) and A.K. Ebel *et al.* (2020) reported that dogs can develop immune-mediated haemolytic anaemia (IMHA) and thrombocytopenia (IMTP), which is a secondary infection of Babesia. The results of J.P. Schoeman's (2009) research indicate that the main clinical manifestations in dogs with babesiosis are regenerative haemolytic anaemia and thrombocytopenia. S.Y. Wong *et al.* (2022) note that transfusion of blood and its components is effective in animals with anaemia of various genesis (post-haemorrhagic, haemolytic, aplastic). B. Davidow *et al.* (2013) point out that anaemia is quite common in animals. A state of decreased oxygen carrying capacity develops, which can be caused by an absolute decrease in the number of red blood cells, haemoglobin concentration or cell volume, which requires immediate intervention by veterinary specialists. E. Rozanski & A.M. de Laforcade (2004) stated in their work that indications for blood transfusion are conditions associated with severe anaemia – a decrease in the number of red blood cells, white blood cells, platelets, blood coagulation factors, as well as due to acute blood loss. Thus, the reaction of the immune system in recipient animals after transfusion is of concern.

The results of studies by I. Goralska & O. Pinsky (2016) indicate that blood is an excellent diagnostic test for diseases of various geneses. After all, haematopoietic organs are extremely sensitive, especially in the pathological state of the body, to the effects of various physiological factors. That is why the study of the morphological composition of the blood of dogs with babesiosis when using blood components during drug therapy of patients remains an urgent issue.

Materials and Methods

The studies were conducted at the Educational and Research Laboratory (ERL) "Animal Blood Bank" of the Department of Surgery and Pathophysiology named after Academician I.O. Povazhenko and at the Educational and Research Clinical Centre (ERC) "Vetmedservice" of the National University of Life and Environmental Sciences of Ukraine (NULES) during 2022-2023. The animal experiments were conducted in compliance with the requirements of the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (2006) and the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (1986). Permission to use animals in research according to the relevant scheme was obtained from the local bioethics' committee of the National University of Life and Environmental Sciences of Ukraine (dated 27.10.2020), protocol No. 31-1. The animals were kept, transfused, manipulated, and the studies were performed at the Animal Blood Bank of the Department of Surgery and Pathophysiology named after Academician I.O. Povazhenko of the National University of

Life Sciences of Ukraine and at the Vedmedservice Research Centre.

Sick dogs were clinically examined. The study was conducted according to generally accepted methods. First, anamnesis data was collected, and then the animals were thermometrically examined. The pulse rate and the number of respiratory movements per minute were counted, auscultation, percussion, palpation, etc. were performed. The animals were vaccinated and fed on premium commercial dry feed. To confirm the diagnosis of babesiosis, microscopic examination of thin smears from capillary blood stained with Leukodif 200 (LDF 200) stain (Erba Lachema, Czech Republic) was performed. The intensity of parasitemia in dogs was determined by the rate (%) of Babesia infection of erythrocytes. Haematological studies were performed using an automatic haematological analyser (MINDRAY BC-2300b, China). The morphological examination of dogs' blood included counting the number of red blood cells ($10^{12}/L$), leukocytes ($10^9/L$), platelets ($10^9/L$), and haematocrit. Biochemical analysis of blood plasma was performed on a semi-automatic analyser (StatFax 1904, Awarness Technology, USA).

To exclude structural changes in the abdominal cavity, all dogs underwent ultrasound examination (US). Visual methods of examination of the abdominal organs were performed using an Esaote Mylab 50 device (USA). For the study, a sample of 5 dogs of the same age group and with a similar clinical picture of babesiosis was made (Table 1). All animals underwent a general clinical examination, abdominal ultrasound, morphological and biochemical blood tests, and a native blood smear for babesia infection.

Table 1. Experimental dogs suffering from babesiosis (n=5)

The nickname of the dog	Age, years	Sex	Weight, kg	Body temperature, °C
Yuki	3.5	Female dog	28	39.7
Asya	4	Female dog	24	39.3

Table 1. Continued

The nickname of the dog	Age, years	Sex	Weight, kg	Body temperature, °C
Baghira	3	Female dog	29	40.0
Milady	3.5	Female dog	21	39.6
Moly	4.5	Female dog	27	39.8

Source: author's development

The blood of the donor dogs was examined for morphological and biochemical blood parameters (erythrocytes, leukocytes, haematocrit, and haemoglobin content), blood parasitic diseases (babesiosis and heartworm disease), and blood groups were determined. In preparation for haemotransfusion, the blood group of the recipient dog was determined, and *in vitro* compatibility tests were performed on the blood of the donor and recipient dogs. Thus, in order to avoid complications during the transfusion of blood components from donor

to recipient, group and individual compatibility was determined (large crossmatch).

The large crossover test was performed in a Micromed BV-4 water bath (Ukraine) at a temperature of 37°C. In this case, the serum of the recipient animal and the blood of the donor animal were mixed on a slide in a ratio of 1:5; 1:10. The reaction time was 5 min. After that, a microscopic examination was performed (Fig. 1). In the absence of agglutination, the biological sample was analysed for individual compatibility.

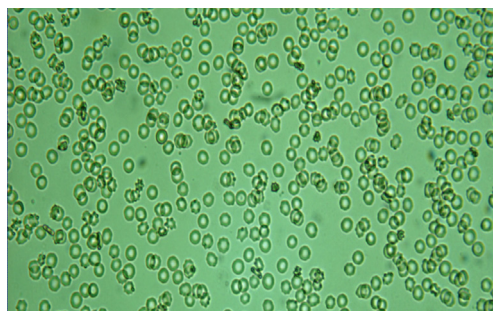


Figure 1. Large crossover sample. The agglutination reaction is negative (magnification $\times 40$)

Source: author's development

The blood group was determined using the RapidVet-H (Canine) test system (Italy). This assay is based on an agglutination reaction that occurs when the DEA 1.1 antigen, which is found on the surface of red blood cell membranes, interacts with a mouse monoclonal antibody that is lyophilized on a test plate. The monoclonal antibody was re-dissolved with a solvent to form an antiserum and thoroughly mixed with the patient's whole blood. All DEA 1.1-positive red blood cells react with the antiserum, causing agglutination. The antiserum

does not react at all with all DEA 1.1-negative erythrocytes. The results were determined visually. Dirofilariosis was tested using the Canine Heartworm Rapid Test (CHW Ag) by ZRBIO Ltd. Co. (China). The rapid test for the detection of specific antigens of mature females of heartworm (*Dirofilaria immitis*) is based on the immunochromatographic method (ICA), which includes the reaction between antigens (Ag) taken from a blood sample of a diseased animal and monoclonal antibodies (Ab) applied to a test strip in a cassette. A native smear test

was performed, the essence of which was that a drop of venous stabilized dog blood was examined under a microscope for the presence of live heartworm larvae of stage 1. Laboratory tests of blood were performed. The microscopic method

of diagnosing babesiosis was used. It is based on the direct detection of babesia in erythrocytes by light microscopy with oil immersion of thin and thick blood smears stained by the Romanowsky-Gimse method (Fig. 2).

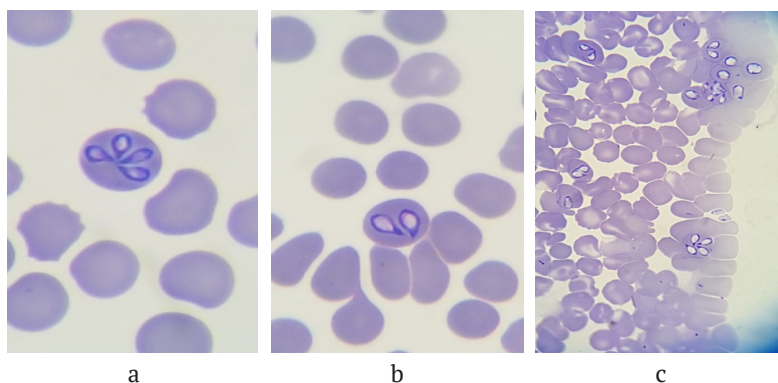


Figure 2. Lesion of erythrocytes *B. Canis* for microscopic examination of canine capillary blood smears

Notes: a, b – magnification $\times 1000$; c in – $\times 400$ magnification, manufacturing “Leukodif 200 (LDF 200)” (Erba Lachema, Czech Republic)

Source: author’s development

Treatment of sick animals was carried out according to the scheme: imkar (imidocarb dipropionate, 120 mg/mL) in a dosage of 0.5 mL per 10 kg of body weight subcutaneously, once; cyanocobalamin 0.02% in a dosage of 1 mL per animal, intramuscularly, once a day, for 5 days; prednisolone (30 mg/mL) in a dosage of 0.5 mg per 1 kg of body weight subcutaneously, once a day, for 3 days; tioprotectin 2.5%, 4 mL per animal, intramuscularly, once a day, for 5 days. The required blood volume (RBV) for hemotransfusion was calculated according to the formula given by Sackmen (1998).

$$RBV = \frac{K \times BW \times (Hbg - Hbr)}{Hbd},$$

where: K is a constant coefficient (K=85) for dogs, BW is the body weight of the recipient; *Hbg* – necessary haemoglobin; *Hbr* – recipient’s haemoglobin; *Hbd* – haemoglobin of the donor.

Allogeneic red blood cell transfusion was performed after administration of the antiparasitic drug. Red blood cells were administered to recipient animals once. The erythrocyte mass of donors was stored for 10 days in a pharmaceutical refrigerator “AEG-317” (Denmark) at $t 2-6^{\circ}\text{C}$ in the conditions of the educational and research laboratory “Animal Blood Bank”. Microscopic studies of cells and their photography were performed using a microscope – Sigeta Biogenic LED (China) with a built-in camera for a microscope, Sigeta MDC-560 CCD (China). The statistical processing of the obtained research results was performed using the computer program Microsoft Excel for Windows 2000 with the determination of the arithmetic mean (M), its error (m) and the level of probability (P) using the Student’s t-test table and the Tukey test with the Bonferroni correction.

Results and Discussion

According to the results of the clinical study of animals, it was found that the increase in body temperature of the studied dogs ranged from 39.3°C to 40.0°C. The results of the study coincide with the findings of I. Yeruham *et al.* (1998), who indicate that animals with babesiosis showed the appearance of fever, which can last for 8-10 days with a critical drop to normal or subnormal levels in the terminal stage of the disease. J.P. Schoeman (2009) explains this by the fact that the destruction of red blood cells releases a large amount of parasite waste products and heterogeneous proteins into the bloodstream. This is what leads to the development of general intoxication of the animal's body and the occurrence of a pyrogenic reaction.

The owners of the animals turned to the veterinary clinic with complaints of decreased appetite and apathy. There were no complaints of vomiting or digestive tract disorders. Superficial lymph nodes were not enlarged. Visible mucous membranes were pale or icteric. M.P. Prus *et al.* (2017) note that in their practice, doctors often encounter various symptoms of babesiosis. At the same time, jaundice and haemoglobinuria may often be mild or absent, whereas they were considered the main symptoms of the disease. Thus, clinically, babesiosis can manifest itself in different ways, its syndromes are unstable and in most cases insufficiently informative.

Auscultation of the chest revealed vesicular breathing without extraneous noise. The heart rate ranged from 85 to 105 beats per minute, the pulse was rhythmic, and respiration was 20-24 breaths per minute. A mild degree of dehydration was also observed in the experimental animals. The reaction to external stimuli was moderately reduced. No external visible injuries were found during clinical examination of the dogs. K.M. Young *et al.* (2019) note in their work that the clinical manifestation of babesiosis can be

observed in 3-5% of red blood cells. D.H. Muguiri *et al.* (2023) indicate that a prolonged immunosuppressive state (about 6 weeks) may occur. Without specific treatment, the mortality rate can be up to 80%. A long time after the disease, as found by Y. Otsuka *et al.* (2002) found that animals remain parasite carriers.

Ultrasound examination (UE) of animals with babesiosis revealed that the liver was not enlarged, with a smooth contour; medium-grained echostructure, homogeneous; echogenicity was typical; liver margin: sharp; hepatic veins: unchanged; no focal lesions were found. The gallbladder is of typical shape, the wall is not thickened, the cavity is visualized. No concretions with a complete distal acoustic shadow were detected, no parietal plaque was noted. There was no kink in the gallbladder floor. Internal and extrahepatic bile ducts were moderately dilated. The spleen was not enlarged, with a homogeneous echostructure and no neoplasms. The kidneys, both right and left, were not enlarged, with smooth, clear contours. They had a homogeneous, undeformed and non-compacted parenchyma. The pelvis was filled with fluid, with no signs of urinary outflow disorders. No concretions or cysts were found. The bladder was moderately filled, without calculi and neoplasms. The uterus was not visualized as the animals were sterilized. No free fluid or focal lesions were found in the abdominal cavity.

R. Narurkar *et al.* (2017) in their work expressed that in the cells of the body (mainly in the liver) in a state of energy starvation, the amount of neutralized toxic products decreases. Toxins begin to increase acidosis due to accumulation in tissues. In most internal organs, according to S.M. Radulescu *et al.* (2021), inflammatory and dystrophic processes develop against this background, leading to changes in their functional state. That is why the general

condition of animals deteriorates sharply in babesiosis with toxicosis. In this case, animals lose their appetite and the digestive process is disrupted. Often, the excretory capacity of the kidneys can also be sharply reduced, which sometimes leads to acute renal failure, which may be the result of clogging of the renal tubules by damaged red blood cells.

No structural changes and concomitant pathologies that could affect the statistical

reliability of the study results were recorded in the dogs selected for the study based on the results of ultrasound. The main contraindications to haemotransfusion for babesiosis, as noted by W. Zygnier *et al.* (2023) and R. Zaremba *et al.* (2019), are severe liver and kidney dysfunction, which were not diagnosed in the studied animals. Before starting the therapy, the animals underwent biochemical and clinical blood tests (Tables 2, 3).

Table 2. Biochemical analysis of blood plasma of dogs with hemolytic anaemia caused by babesiosis (n=5)

Indicator	Norm	Dogs, sick with babesiosis	The nickname of the dog				
			Yuki	Asya	Baghira	Milady	Moly
Total bilirubin, µmol/L	0.0-15.3	20.3±0.52*	18.9	20.2	22.0	21.4	19.0
ALT, U/L	9-42	47.8±0.91*	46.3	48.0	49.7	45.0	49.8
AST, U/L	5-40	34.4±1.48*	38.0	35.7	34.7	30.1	33.3
GGTP, U/L	1-6	6.9±0.09	6.9	6.7	6.5	7.0	7.4
Urea, mmol/L	2.0-8.3	7.5±0.36	7.7	8.0	6.4	8.2	7.0
Creatinine, µmol/L	26-120	97.2±3.72*	94.3	85.6	97.2	105.8	103.1
General protein, g/L	53-76	69.8±1.42	70.8	68.9	65.4	72.9	71.0
Glucose, mmol/L	3.5-7.3	5.7±0.30	5.4	6.3	4.8	6.1	5.9
Mg, mmol/L	0.72-1.12	0.8±0.02*	0.84	0.82	0.91	0.78	0.88
Ca, mmol/L	2.3-3.3	2.8±0.073	3.1	2.7	2.9	2.95	2.6
P, mmol/L	1.1-3.0	2.0±0.079	2.0	1.78	1.9	2.2	2.1

Notes: ALT – alanine aminotransferase; AST – aspartate aminotransferase; GGTP – gamma-glutamyltranspeptidase
* – $P < 0.05$ compared to pre-transfusion values using Tukey’s test with Bonferroni correction; SEM1 is the standard error of the mean (n=5)

Source: author’s development

The analysis of the results of biochemical studies made it possible to identify a number of quantitative changes in the activity of transaminases and the content of total bilirubin in the blood plasma during the acute course of babesiosis in dogs. For example, in sick animals with an acute course of this blood-borne disease, an average 30% increase in plasma total bilirubin levels relative to the norm was observed, which is associated with massive red blood cell breakdown (Pervushina *et al.*, 2018) and the release of a large amount of hemoglobin, which is broken down to bilirubin in the liver. An average 16%

increase in plasma gamma-glutamyl transpeptidase (GGTP) and alanine aminotransferase (ALT) – activity by an average 13% may indicate high hepatotoxicity of this disease and an additional burden on the hepatobiliary system. The plasma concentration of creatinine and urea in the group of diseased dogs corresponded to the limits of the reference values of the norm, which is a marker of the absence of impaired renal glomerular filtration capacity. Other parameters were within the reference values of the norm. Results of the general clinical blood test of dogs (Table 3).

Table 3. Indicators of the general clinical analysis of dogs for hemolytic anaemia caused by babesiosis (n=5)

Blood count	Norm (reference values)	Dogs, patients with babesiosis
Erythrocytes, $10^{12}/L$	5.50-8.50	1.74±0.42*
Hemoglobin, g/L	110-190	53.60±3.45*
Hematocrit, %	39.0-56.0	13.28±1.42*
Platelets, $10^9/L$	117-460	86.62±3.57*
Leukocytes, $10^9/L$	6.0-17.0	22.13±0.96*

Notes: * $P < 0.05$ compared with pre-transfusion values using Tukey's test with Bonferroni correction; SEM1 – standard error of the mean (n=5)

Source: author's development

As a result, a significant decrease in the number of red blood cells was found – by 3.2 times, haemoglobin content by 2 times, and haematocrit by 3 times. R. Narurkar *et al.* (2017) stated in their work that this is how the mechanical effect of the parasite on the body manifests itself. It is caused by the fact that when babesia gets into the bloodstream of animals, they enter red blood cells, which are destroyed and die as a result, which manifests itself in the form of progressive haemolytic anaemia.

I. Goralska & O. Pinsky (2016) noted that in the case of a decrease in the number of red blood cells, haemoglobin content and haematocrit, there is a violation of blood oxygenation in the body of the affected animal. General intoxication of the body develops due to the accumulation of underoxidised decomposition products, which leads to a deterioration in the clinical condition of the animal. A decrease in haematocrit, according to the results of studies by W. Zygnier *et al.* (2023), is a prognostic marker in dogs infected with *B. Canis*, and it is significantly lower in non-survivors compared to survivors. Anaemia in canine babesiosis is caused by intravascular and extravascular haemolysis. Extravascular haemolysis occurs in the spleen and in liver phagocytes, whereas intravascular haemolysis is the result of the parasite life cycle (merogony) and immune-mediated lysis of red blood cells. Allogeneic red blood cell

transfusion was performed in sick dogs, taking into account the results of clinical examination and changes in haematological parameters indicating the presence of anaemia (Fig. 3).



Figure 3. Transfusion of erythrocyte mass to a recipient dog with hemolytic anaemia caused by babesiosis

Source: author's development

After hemotransfusion, the clinical condition of the dogs was monitored, based on the clinical examination of the animals, a survey of the owners of the animals, and haematological studies on the second, fifth, and tenth days of treatment (Table 4).

Table 4. Hematological parameters of the blood of dogs suffering from babesiosis (n=5)

Indicator	Reference values of indicators	Day of treatment			
		the first before transfusion	the second	the fifth after transfusion	the tenth
Erythrocytes, 10 ¹² /L	5.50-8.50	1.74±0.42	2.43±0.58	4.13±0.75*	5.89±0.70*
Hemoglobin, g/L	110-190	53.6±7.73	58.2±8.52	96±3.31*	120.2±8.43*
Haematocrit, %	39.0-56.0	13.28±3.1	17.84±3.52	33.81±1.9*	41.66±2.7*
Platelets, 10 ⁹ /L	11.7-460	86.62±33.7	135±24.0	226.84±51.0*	303.2±38.7*
Leukocytes, 10 ⁹ /L	6.0-17.0	22.13±2.1	21.04±1.5	31.04±15.0	19.72±5.4

Notes: * – $P < 0.05$ compared with values before transfusion using Tukey's test with Bonferroni correction; SEM1 is the standard error of the mean (n=5)

Source: author's development

The general condition of the animals improved dramatically over the next 3-4 days. There was a decrease in clinical manifestations of anaemia. The appetite and motor activity of the animals returned to normal 5 days after component transfusion. Regarding changes in the parameters of the clinical blood test of dogs, it is possible to note a significant positive dynamics on the second and fifth days after red blood cell transfusion and the return of indicators to the normal reference values by the tenth day. One day after haemotransfusion, there was a 42% increase in the number of red blood cells and a 35% increase in the haematocrit. On the fifth day of treatment, a statistically significant increase in haematological parameters was observed compared to the values at the beginning of treatment, namely: the number of red blood cells – by 2.4 times, haematocrit – by 2.5 times, haemoglobin – by 80%. According to the results of the clinical blood test on the tenth day, a statistically significant positive dynamics and bringing the indicators to the reference values of the norm was noted, except for a slight increase in the number of leukocytes, which, compared to the second and fifth days of treatment, significantly decreased and approached the upper limit of the norm. This improvement in haematological parameters in dogs with

babesiosis on the fifth day of treatment with red blood cell transfusion is associated with the ability of red blood cells to bind oxygen to haemoglobin in the lungs, transfer and release it to tissues, and absorb carbon dioxide from tissues. It is the function of oxygen transport that is the main mechanism of action of transfused red blood cells.

M.A. Claus *et al.* (2022) note that red blood cells are involved in blood clotting and fibrinolysis due to an agent similar to factor PI in platelets, as well as erythrokinase, which activates and converts profibrinolysin to fibrinolysin. The large surface area of red blood cells (4000 m²), rapid turnover of non-deposited red blood cells (45 seconds), and their pronounced sorption capacity contribute to their detoxification function. Although it should not be forgotten that the main function of red blood cells is respiratory. According to O. Denysova *et al.* (2021), red blood cell transfusion is performed mainly for replacement purposes, in accordance with the modern concept of component haemotherapy. The advantages of transfusion of red blood cell components over transfusion of preserved donor blood, as shown by S. Gul *et al.* (2023), are as follows: increased oxygen transport activity in a small volume of the transfusion medium. J.L. Morris *et al.* (2021) note that the advantages

of using red blood cells include the absence or minimal content of sensitizing factors (plasma proteins, leukocytes, platelets). The results of studies by Y. Otsuka *et al.* (2002) showed that this ability is particularly pronounced in washed red blood cells. W. Zygnier *et al.* (2023) reported the absence or minimal content of decay products and vasoactive substances (potassium, sodium, nitrogen, serotonin, histamine) when using red blood cells. S.Y. Wong *et al.* (2022) found in their research that the use of red blood cells reduces the number of microaggregates, the absence of plasma coagulation factors and a significant number of platelets, and reduces the risk of contracting vector-borne infections.

Given the results of laboratory studies and clinical examination of animals with babesiosis, it can be assumed that red blood cell transfusion is a safe method of restoring the number of red blood cells in the body of recipient animals with babesiosis. It should be noted that the restoration of red blood cell count, haemoglobin content and haematocrit value leads to an improvement in tissue oxygen saturation in recipient animals, and as a result – to a decrease in general intoxication due to the normalization of redox processes.

Conclusions

During the clinical study of sick animals, it was found that the body temperature increased from 39.3°C to 40.0°C, appetite decreased, and apathy was observed. A mild degree of dehydration was observed. The reaction to external stimuli was moderately reduced. According to the results of ultrasound examination of the state of internal organs in the studied dogs, no abnormalities were found.

Under babesiosis in dogs, there was an increase in the number of leukocytes, indicating the course of an acute inflammatory process in the body of diseased animals. It was found that

in diseased dogs, there was a 13% increase in the plasma activity of alanine aminotransferase and a 16% increase in gamma-glutamyl transpeptidase. Such changes in enzyme activity indicate that sick animals are characterized by an additional burden on the hepatobiliary system. Allogeneic red blood cell transfusion in combination with specific and symptomatic treatment had a positive result in reducing the anaemic syndrome in dogs with babesiosis. Transfusion of red blood cell mass during the treatment of dogs with acute babesiosis contributed to the restoration of red blood cell count, haemoglobin content and haematocrit value, which had a positive effect on the elimination of anaemia, restoration of blood oxygenation and faster improvement of the clinical condition of dogs after the disease.

It was found that the use of red blood cell transfusion in sick dogs led to an increase in blood haematocrit and haemoglobin content, as well as the number of red blood cells in the first day. On the tenth day, these blood parameters were close to the reference values of the norm and almost twice as high as the number of red blood cells, haemoglobin content and haematocrit value in the peripheral cortex of the experimental animals before transfusion. The use of allogeneic red blood cell transfusion in dogs with babesiosis after antiparasitic treatment also led to a 2.5-fold increase in the number of red blood cells and haematocrit, and an 80% increase in haemoglobin. It was found that in dogs with babesiosis, the use of allogeneic red blood cells as part of transfusion therapy contributed to the rapid recovery of the general clinical condition of animals.

Given the positive result of the use of red blood cell mass in the treatment of dogs with acute babesiosis, the prospects for further research are to study the effectiveness of the use of blood components in recipient animals with anaemia of various geneses.

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Conflict of Interest

None.

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Вплив трансфузії еритроцитарної маси на клініко-гематологічні показники собак за гемолітичної анемії, викликаної бабезіозом

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Анотація. Актуальність наукового дослідження зумовлена поширенням протозойних захворювань тварин, які за відсутності необхідної ветеринарної допомоги призводять до загибелі. У зв'язку з цим, метою роботи було вивчити зміни клінічних та гематологічних показників у собак за комплексного лікування бабезіозу з одночасною трансфузією еритроцитарної маси. Переливання проводили п'ятьом тваринам, інфікованих *Babesia canis*, шляхом внутрішньовенного введення еритроцитів, обсяг яких для гемотрансфузії розраховували за формулою Sackmen. Діагноз на бабезіоз встановлювали з урахуванням даних анамнезу, клінічних симптомів (слабкість, відмова від корму, блідість видимих слизових оболонок, підвищення температури тіла до 40 °C) та підтверджували результатами мікроскопічного виявлення бабезій у мазках крові. Перед переливанням проводили експрес-тести на виключення дирофіляріозу у собак-донорів. Визначали сумісність крові донора і тварини-реципієнта за допомогою великої перехресної проби. Стан органів черевної порожнини хворих оцінювали з використанням ультразвукової діагностики.

Клінічне дослідження крові собак перед початком лікування свідчило про розвиток анемії. Результати біохімічного аналізу крові вказували на підвищення активності аланінамінотрансферази і гамма-глутамілтранспептидази, що констатує навантаження на гепато-біліарну систему. Протипоказань до переливання крові не виявили. Після гемотрансфузії проводили контроль клінічного стану собак за результатами огляду тварин з урахуванням даних анамнезу та гематологічних досліджень на другу, п'яту та десятю доби лікування. Впродовж другої та п'ятої діб відмічали поступове збільшення в крові вмісту гемоглобіну та величини гематокриту. На десятю добу відзначали достовірну позитивну динаміку щодо кількості еритроцитів, вмісту гемоглобіну та величини гематокриту до референтних значень. Встановлено, що використання трансфузії еритроцитарної маси собакам-реципієнтам за бабезіозу, як частини терапії, сприяло найшвидшому відновленню загального стану тварини. Матеріал статті становить практичну цінність для застосування переливання еритроцитарної маси при лікуванні собак за бабезіозу

Ключові слова: гемотрансфузія; групи крові; компоненти крові; гемоглобін; гематокрит; ферменти