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Morphological criteria for identifying coronavirus infection in companion animals

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Abstract. Due to the high level of urbanisation in Ukraine, during the years 2021-2023, there is a tendency for a rapid increase in the number of companion animals in large cities – dogs and cats. Humanity has not yet finally eliminated the SARS-CoV-2 coronavirus pandemic, as world scientists have established the involvement of these animals in its spread, which causes concern among specialists and requires a comprehensive investigation. The purpose of the scientific study was to determine marker changes in haematological parameters and haematopoiesis parameters under the influence of coronavirus infection in dogs and cats. For this purpose, spectrophotometric and refractometric methods of haematological studies were used: morphological and biochemical parameters of blood, the functional state of erythro- and leukocytopoiesis. Considering changes in blood morphological parameters, the main parameters of the identity of the pathogenic effect of coronavirus on the body of dogs and cats were established, namely: a decrease in the number of lymphocytes, glucose content, and protein coefficient. There was a significant increase in the activity of alkaline phosphatase in the blood and α-amylase, the content of total bilirubin, and the erythrocyte sedimentation rate. In particular, it has been experimentally established that the use of leukocyte indices increases the information content of the general blood test for coronavirus infection, and additional analysis of integral changes based on leukocyte blood parameters allows determining not only the state of reactivity of the body, but also the extent of endogenous intoxication. The results of changes in blood morphological parameters are somewhat comparable to those in humans, which indicates the need for an in-depth study of the genetic potential of coronavirus pathogens in companion animals and humans at the molecular and biological levels. Experimentally determined marker changes in haematological parameters for coronavirus infection in dogs and cats can be useful for its timely diagnosis and prediction of the severity of the disease

Keywords: coronavirus; erythrocytopoiesis; leukocytopoiesis; stabilised blood; blood serum; morphological and biochemical parameters

Introduction

Nowadays, in addition to solving problematic issues regarding the nature of the occurrence of SARS-CoV-2 and its pandemic properties and ways to overcome them, even more complex issues have arisen – its zoonotic nature and spread among pets (Kannekens-Jager *et al.,* 2022). Cases of natural coronavirus 2 infection with severe acute respiratory syndrome (SARS-CoV-2) in companion animals after contact with humans are rare. Infections in animals are usually subclinical or associated with transient respiratory or gastrointestinal diseases. At the same time, European researchers report that among companion animals, namely 2% of samples from dogs and 7.7% from cats are positive in the polymerase chain reaction (PCR) to SARS-CoV. Given the established transmission of the virus from humans to dogs and cats, the role of these animals in the spread of the virus is indisputable (Rotstein *et al.,* 2022).

Companion animals have the closest contact with humans, creating ample opportunities for common infectious diseases. According to the 2021-2022 national survey of pet owners, 70% of all U.S. households have a companion animal. Pets can have an impact on lowering blood pressure in humans, reducing the impact of stress factors, and reducing the rate of heart disease. Traditionally, the most common companion animals are dogs and cats

(Khan *et al.,* 2022). Recently, viral diseases have been increasingly diagnosed and one of the main pathogens is the coronavirus, which is registered quite often in companion animals (Felten *et al.,* 2022). Among mammals, pigs, cattle, dogs, cats and rodents are most often affected by the virus (Goralskii *et al.,* 2019).

Canine and feline coronaviruses are a group of acute infectious diseases caused by a virus of the family *Coronaviridae*, which is enveloped with a complex RNA genome and belongs to the subfamily *Orthocoronaviridae,* genus *Alphacoronavirus*. Coronaviruses are characterised by variable tropism to tissues and the ability to easily overcome interspecific barriers, causing diseases with noticeable differences (Felten & Hartmann, 2019; Sha *et al.,* 2022).

Coronavirus infections in dogs are clinically manifested in a respiratory form and sometimes with complications in the form of severe respiratory syndrome with an acute or asymptomatic course. In a third of sick animals, coronavirus causes damage to the gastrointestinal tract, which is manifested by the development of infectious gastroenteritis. The intestinal form has a latent course, but due to complications involving helminths or bacteria, the disease can be lethal (Vlasova *et al.,* 2022; Pandit *et al.,* 2023).

Coronavirus infections in cats have two different forms – feline intestinal coronavirus and infectious peritonitis. Feline infectious peritonitis (FIP) is extremely common. This is a relatively new disease, which is named due to a specific clinical symptom – peritonitis. One of the most sensitive to infectious coronavirus peritonitis is a domestic cat. Usually, cats suffer from infectious peritonitis quite seriously, possibly due to the initiation of multiple organ failure by the coronavirus, which usually leads to the death of the animal. Infectious peritonitis of coronavirus origin occurs in many members of the feline family (*Felidae*), even the wild fauna of Africa. This disease is one of the five most common infectious diseases of cats in many countries of the world (Guan *et al.,* 2020).

Coronaviruses exhibit unique molecular mechanisms of transcription and recombination. Due to the relatively high mutation rate, it was RNA-positive viruses that evolved. Due to biomolecular methods developed over the past two decades, new strains, serotypes, and subtypes of the virus have been identified. Given their virological characteristics, these viruses are capable of recombinant mutation and infection of several cell species and types (Krentz *et al.,* 2021).

According to the World Health Organisation, laboratory research methods provide more than 70% of diagnostic information about the patient's condition, and it is the blood test that is one of the fastest and most affordable diagnostic methods (Semotyuk & Kolesnyk, n.d.). Blood is a marker of the state of metabolism and the body as a whole, i.e., it reflects the functioning of all organs and systems. Accordingly, with any effect on the body, certain morphological changes occur in hemocytopoiesis. It is by quantitative and qualitative changes in haematological parameters that it is possible to assert the reactive nature provoked by an infectious agent, and to detect pathological processes at an early stage of the disease, even before the manifestation of clinical symptoms. A qualified interpretation of these indicators significantly increases the diagnostic value of a blood test (Jaroensong *et al.,* 2022).

At the initial stages of the pathological process, a complex of pathochemical shifts is formed, the generalisation of which occurs due to the intensive intake of toxic products from the primary lesions, which contributes to the synthesis of allergic antibodies, changes in immunological reactivity, and a decrease in non-specific resistance of the body. At the same time, leukogram indicators reflect the general nature and direction of haemostatic processes. That is why the calculation of leukocyte indices

based on leukogram indicators determines the effectiveness of the body's adaptive reactions and establishes the immunological reactivity of various parts of the immune system, making it possible to predict the course of the disease (Osadcha, 2021).

The purpose of the study is to establish identification parameters based on the determination of morphological parameters of blood, and indicators of erythrocytopoiesis and leukocytopoiesis in cats and dogs with coronavirus infection.

Materials and Methods

The research was conducted during 2020-2022 at the Faculty of Veterinary Medicine at the National University of Life and Environmental Sciences of Ukraine, and at the premises of veterinary clinics "Bahira" (Zhytomyr) and "Victoria" (Kyiv).

The study included pedigree and non-pedigree dogs and cats with coronavirus infection. During the research, the basic rules of GLP (Good laboratory practice) (Order No. 944..., 2009) and the provisions of the "General Ethical Principles of Animal Experiments", adopted by the National Congress on Bioethics (Kyiv, 2001) (On the Protection..., 2021) were observed. The entire experimental part of the research was carried out in accordance with the requirements of the international principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (1986), "Rules for conducting work using experimental animals", approved by Order of the Ministry of Health of Ukraine No. 281 (2000), in accordance with Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (2006). For the euthanasia of animals, medications were used to ensure a quick and painless death, in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (1986).

Diagnostic tests to confirm coronavirus were performed using VetExpert rapid test systems in dogs (CPV/CCV Ag, solid-phase immunochromatographic analysis for qualitative detection of the antigen Canine Parvovirus/Coronavirus) and in cats (FCoV Ag, solid-phase immunochromatographic analysis for qualitative antigen detection Feline Coronavirus) produced in Poland. Diagnostic studies for the presence of coronavirus antigen in dogs and cats were carried out on the premises of a private veterinary laboratory of the LLC "Bald" (Kyiv) using LLC "XEMA" (Ukraine) diagnostic preparations for detection of antibodies; Asan Korea, Megacor (Austria) and Agrolabo (Italy) – for detection of antigen along wigh enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) by Astrovir (Ukraine), Biocorp (Ukraine), DNA-Technology (Ukraine), Huarian Biologi (Taiwan).

For the diagnosis of coronavirus infection in veterinary clinics, diagnostic test systems based on solid-phase ELISA were used, which usually have sensitivity and specificity at the level of at least 95% and allow diagnostics without specialised skills and laboratory conditions in a fairly short period of time. In case of ambiguous results obtained during the immunochromatographic diagnostic test to clarify the diagnosis, biological material was sent to the laboratory for more specific studies using ELISA and PCR.

Morphological and biochemical parameters of blood were studied using a biochemical analyser "BioChem SA" (USA) using reagents of the company *High Tehnology, Inc*. (USA). To determine the deviation from the morphological parameters of the blood of clinically healthy animals, a control group of dogs and cats was established in the amount of 10 animals each. During the experimental study, 24 dogs and 27 cats were diagnosed with coronavirus infection. Biological material (stabilised blood and blood

serum) was collected from infected animals and subjected to haematological studies. Blood for the study was taken from the superficial forearm vein *v. Anterbrachium*, the medial saphenous vein or the subcutaneous vein of the calf *v.* Saphena.

General clinical blood test – the number of red blood cells and white blood cells was determined by the melange method in a chamber with a Goryaev grid; haemoglobin content in the blood – by the hemiglobincyanide method; haematocrit volume – by microcentrifugation according to Shklyar. Based on the results obtained, red blood indices were calculated – mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and mean cell volume (MCV).

Separate biochemical studies of blood serum were performed using the following methods: total protein was determined refractometrically, urea concentration – by colour reaction with diacetylmonooxime, aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) by the Reitman-Frenkel method, creatinine concentration – by the Jaffe reaction. The calculation of integral intoxication indices was carried out in accordance with the methodological recommendations "Integral haematological indices for assessing the degree of endogenous intoxication in dogs" (Goralskii *et al.*, 2019). Digital data were processed biometrically using generally accepted methods of variation statistics using STATISTICA 6.0 and Microsoft Excel 2007 software suites. The probability of differences between indicators was evaluated according to the Student's t-test. Three degrees of probability were calculated *P<0.05; **P<0.01; ***P<0.001.

Results and Discussion

Given that certain morphological parameters of blood can confirm the development of pathological processes provoked by viral agents, a study was conducted to investigate the degree of destabilisation of the body of dogs and cats under the influence of coronavirus. Morphological parameters of the blood of clinically healthy animals formed the basis for creating reference values. Considering the results of previous studies (Radzikhovskyi *et al.*, 2021) and foreign researchers (Decaro & Buonavoglia, 2008; Addie *et al.*, 2020), outbred or mixed-breed animals were most likely to contract coronavirus infection. This was taken into account when distributing animals to the control group. The results of the conducted studies are presented in Tables 1-3.

Table 1. Morphological parameters of blood in dogs and cats with coronavirus infection, M±m

Indicator		Control group, clinically healthy dogs (n=10)	Dogs infected with coronavirus (n=24)	Control group, clinically healthy cats (n=10)	Cats infected with coronavirus (n=27)
RBC, 10^6/μL		6.1±0.4	5.2±1.3	8.5±0.6	5.9±0.4**
HCT, %		45.4±2.4	35.2±3.8*	38.3±1.5	31.8±1.4**
PLT, 10^5/μL		252.3±19.7	343.6±34.1*	240.2±15.2	255.8±15.9
WBC, 10^3/μL		9.4±0.2	4.6±0.9***	8.4±1.2	16.6±1.8***
NEU, %	stab	3.1±0.2	1.6±0.4**	2.8±0.7	8.8±1.3***
	segmented	58.5±1.4	73.6±4.8**	46.2±3.2	61.2±4.1**
EO, %		1.8±0.3	3.1±0.8	1.9±0.5	1.7±0.4
MON, %		3.1±0.2	1.8±0.9	1.2±0.4	8.1±1.3***

Table 1. Continued

Indicator	Control group, clinically healthy dogs (n=10)	Dogs infected with coronavirus (n=24)	Control group, clinically healthy cats (n=10)	Cats infected with coronavirus (n=27)
LYM, %	35.1±1.3	18.4±3.2***	31.7±2.1	22.7±2.3*
ESR, mm/hr	4.4±0.5	7.2±1.1*	3.9±0.3	17.7±1.7***

Notes: **P<0.05; **P<0.01; ***P<0.001 compared to the control group of animals*
Source*: developed by the authors*

According to the results of hematologic examinations, erythropenia was diagnosed in the affected dogs and cats (Table 1). There was a decrease in the number of red blood cells in the blood of dogs by 15%, and in cats – by 31% (P<0.01) compared to the indicators of the corresponding control group. This fact indicates that the viral agent – coronavirus causes haemolysis of red blood cells, which was also established by F. Riemer *et al.* (2016), and leads to the development of anaemia. Along with this, it may indicate iron deficiency, which was confirmed by erythropenia and a decrease in haemoglobin concentration in animals of the study groups compared to the physiological limit (Table 1). In the blood of both dogs and cats, there was a significant decrease in the haematocrit volume, respectively, by 32.5% (P<0.05) and by 17% (P<0.01) compared to the control group, which also proves the presence of anaemia.

Coronavirus generally affects homeostasis in the body of sick animals and provokes the development of haemolytic anaemia. In addition, sick dogs showed a significant increase in the number of platelets in the blood by 36% (P<0.05) compared to the control group. In cats, the number of blood plates increased by 6.5% compared to the control group. Along with the development of thrombocytosis in these animals, the presence of inflammatory processes provoked by an infectious agent was confirmed.

T. Castro *et al.* (2013) reported leukopenia in puppies with coronavirus. Similar changes were found in the blood of adult dogs, namely, a likely decrease in the number of white blood cells by 2 times (P<0.001) compared to the corresponding control, which can occur against the background of immune suppression and the latent course of the disease. N.C. Pedersen (2014) and E. Gülersoy & M. Maden (2021) indicated that leukocytosis was observed in cats with coronavirus infection. As a result of the conducted studies, leukocytosis in cats (an increase in the number of white blood cells by 2 times) characterises a severe course of the disease with periodic waves of exacerbation. O.O. Melnyk (2020) noted that a similar trend in the development of leukocytosis in severe cases of coronavirus infection was recorded in humans. At the same time, leukocytosis was detected in 11.4% of patients with severe disease compared to 4.8% of patients with mild or moderate disease. Changes in the number of white blood cells were also noted on the quantitative indicators of stab neutrophils. Thus, in dogs, their number decreased by 48% (P<0.01), and in cats, it increased by 3 times (P<0.001) compared to the corresponding control. The development of segmented neutrophil leukocytosis with coronavirus infection was established. This was confirmed by a 26% increase in the number of segmented neutrophils in the blood of dogs (P<0.01) and a 32% increase in the number of segmented neutrophils in cats (P<0.01) compared to the control group. The consequence of this process is a shift in the leukocyte formula to the right. In dogs with coronavirus infection with simultaneous eosinophilia (an increase in the number of eosinophils by 72%), the presence of an infectious and toxic process was established, while in cats there was only a tendency

to slightly decrease the number of eosinophils compared to the corresponding control.

Important macrophages of the body – monocytes – respond to the presence of a viral antigen and monocytopenia has been observed in sick dogs. In the blood of such dogs, the number of monocytes decreased by 42%, and in cats, it increased by 6 times (P<0.001). S. Chen *et al.* (2019) and E. Gülersoy & M. Maden (2021) also characterised monocyte tropism for coronavirus in cats. The likely decrease in the number of lymphocytes in the blood of dogs by 48% (P<0.001), and in cats by 28% (P<0.05) compared to control animals, is probably caused by low molecular weight proteins – interferon, which are synthesised in response to viral infection. The rate of erythrocyte sedimentation in the blood of dogs increased by 1.6 times (P<0.05), and in cats – by 4.5 times (P<0.001) compared to the same indicator in healthy animals. This fact confirms the development of an inflammatory process in the body of sick animals.

According to biochemical parameters, it is possible to determine qualitative changes in the blood, get information about the state of health of the animal and the functioning of many organs, and identify pathological processes at an early stage. Therefore, at the next stage of the study, changes in blood biochemical parameters and indicators of the state of erythrocytopoiesis in animals with coronavirus infection were analysed (Table 2).

Table 2. Erythrocytopoiesis and biochemical parameters of blood serum in dogs and cats with coronavirus infection, M±m

Indicator	Control group, clinically healthy dogs (n=10)	Dogs infected with coronavirus (n=24)	Control group, clinically healthy cats (n=10)	Cats infected with coronavirus (n=27)
MCH, pg	23.6±1.6	25.3±1.8	15.6±1.3	14.6±1.4
MCHC, g/dL	33.9±1.4	38.5±1.7*	34.7±1.5	49.6±2.3***
MCV, fL	67.1±3.7	69.1±0.9	45.1±2.3	46.4±1.1
HGB, g/dL	145.0±12.5	131.1±27.2	132.3±8.3	105.5±4.3**
Total protein, g/L	71.1±1.8	39.6±6.8***	70.2±1.4	74.8±4.1
Albumins, g/L	36.5±0.5	11.3±0.1***	31.5±1.2	27.3±1.2*
Globulin, g/L	37.6±1.2	17.7±1.7***	36.8±1.4	40.7±1.9
Protein coefficient	0.97±0.04	0.63±0.10**	0.90±0.02	0.67±0.05***
Creatinine, μmol/L	97.1±3.9	48.8±14.2**	90.2±6.5	112.7±8.6*
Urea, mmol/L	5.3±0.2	4.2±0.4*	7.2±0.5	10.7±1.4*
Glucose, mmol/L	4.2±0.2	3.4±0.6	4.8±0.2	4.2±0.3
γ-GTP, U/L	4.1±0.2	4.9±0.4	3.1±0.7	5.2±0.7*
ALP, U/L	34.6±2.7	310.8±27.2***	27.2±1.7	68.8±10.1***
Total bilirubin, μmol/L	4.3±0.3	10.2±0.9***	4.4±0.3	14.1±2.2***
ALT, U/L	46.6±3.3	36.8±6.4	40.4±3.1	84.5±9.9***
AST, U/L	50.1±3.8	26.7±6.1**	21.6±2.5	57.8±8.9***
De-Ritis Ratio, ratio	1.2±0.2	0.7±0.02*	1.1±0.4	0.7±0.3
α-Amylase, U/L	1,635.2±76.7	1,918.6±96.7*	720.2±25.3	1,828.2±116.9***

Notes: **P<0.05; **P<0.01; ***P<0.001 compared to the control group of animals*

Source*: developed by the authors*

According to the data given in Table 2, an increase in the erythrocytopoiesis index was found, which characterises the intensity of maturation of red blood cells in the bone marrow and their saturation with haemoglobin. Thus, the mean concentration of haemoglobin in one red blood cell in dogs increased by 14% (P<0.05), and in cats – by 43% (P<0.001) compared to the corresponding control. Along with this, there was a decrease in the concentration of haemoglobin in the blood serum of dogs by 10%, and in cats – by 21% (P<0.01), which proves the development of anaemia in their body.

Albumin content (Table 2) in the blood serum of dogs decreased by 3 times (P<0.001), and in cats by 13% (P<0.05), in comparison with the indicators of the corresponding control groups. The content of total protein and globulins in the blood serum of dogs decreased, respectively, by 44% (P<0.001) and 53% (P<0.001), and in cats, a tendency to increase these indicators was observed. According to the above-mentioned changes in protein metabolism indicators, impaired liver function under the influence of a viral agent was established. S. Paltrinieri *et al.* (2002) and T. Castro *et al.* (2013) also indicate a decrease in serum albumin levels in dogs. N.C. Pedersen (2014) reported changes in protein metabolism indicators under the influence of coronavirus antigen on the liver of dogs. A significant decrease in the protein coefficient in the blood serum of dogs by 35% (P<0.01), and in cats by 26% (P<0.001) emphasises the infectious aetiology of liver damage.

To determine the effectiveness of renal excretory function under the influence of coronavirus infection, the final nitrogen-containing products of protein metabolism – creatinine and urea – were examined in the blood of companion animals. In particular, the concentration of creatinine in the blood reflects the filtration capacity of the kidneys. With coronavirus infection, the creatinine content in the blood serum of dogs decreased by 2 times (P<0.01), compared to healthy animals, which may indicate changes in muscle mass due to exhaustion of the body. At the same time, in sick cats, the creatinine content increased by 25% (P<0.05), which may indicate the development of renal failure.

The concentration of urea in the blood serum may indicate a violation of the hepatorenal system. This indicator is of key importance in the diagnosis of diseases of both the kidneys and liver. In dogs, the concentration of urea decreased by 21% (P<0.05), and in cats, on the contrary, it increased by 49% (P<0.05) compared to the control group. It is the concentration of urea that can be used to establish a balance between its synthesis in the liver and excretion by the kidneys. High indicators of urea concentration in the blood serum indicate pathology in the kidneys, which is associated with a violation of the outflow of urine from the organ, and low indicators suggest a violation of urea synthesis by the liver.

According to the results of the conducted studies, it was found that the concentration of glucose in the blood serum of dogs was marked by a tendency to decrease by 19%, and in cats – by 13% compared to healthy animals. The development of hypoglycaemia in sick animals may be a consequence of the occurrence of hepatorenal syndrome and damage to the gastrointestinal tract. When the level of glucose in the blood of animals is low, there is a compensatory increase in gluconeogenesis, which is primarily characteristic of the liver.

Alanine aminotransferase (ALT) is a biochemical marker of liver diseases. The activity of this enzyme decreased in the blood serum of dogs by 21%, which may indicate structural and functional changes in hepatocytes. Along with a simultaneous significant increase in the serum bilirubin level (2.4 times), this indicates

the development of complications from the hepatobiliary system. In contrast, in the blood serum of cats, ALT activity increased by 2 times (P<0.001) compared to the control, which indicates a pathological state of the liver and the development of cholestasis.

Aspartate aminotransferase (AST) – an enzyme, the largest amount of which is produced by heart and liver cells. Thus, in dogs, its activity decreased by 47% (P<0.05), compared with the control, which indicates damage to myocardial and liver cells. And in the blood serum of cats, an increase in the activity of this enzyme was noted by 2.7 times (P<0.001), which indicates structural and functional changes in myocardiocytes and hepatocytes.

Tendency to increase the activity of γ-glutamyltranspeptidase (γ-GTP) in the blood serum of dogs (by 20%), and in cats, a significant increase in its activity by 67% (P<0.05) compared to the corresponding control, against the background of a simultaneous increase in the content of total bilirubin in dogs by 2.4 times (P<0.001), and in cats – by 3.2 times (P<0.001), and a significant increase in the activity of alkaline phosphatase (ALP) in dogs – 9 times (P<0.001), and in cats – 2.5 times (P<0.001) indicates liver pathology, the development of obstruction of the bile ducts and impaired bile outflow.

The functional activity of the pancreas is characterised by α-amylase (Amyl, α-AM) (Sulehria *et al.,* 2020). This indicator, according to F. Riemer *et al.* (2016), is a marker of the acute phase of pancreatitis. In the blood serum of dogs with coronavirus, α-AM hyperemesis was observed – an increase in activity by 17% (P<0.05), and in cats – 2.5 times (P<0.001) compared to the corresponding control. This fact may also indicate the development of pancreatic complications in sick animals (Jaroensong *et al.,* 2022).

O.O. Melnyk (2020) and D.A. Lysenko *et al.* (2021) note that biochemical changes in animal blood serum, namely: increased α-AM activity, total bilirubin content, and alkaline phosphatase activity partially coincide with changes in the body in people with severe coronavirus infection.

Regarding the statistical diagnostic formula for the main morphological and biochemical changes in the blood of sick animals, the results obtained coincide with the findings of other researchers. In coronavirus infection in both dogs and cats, the most commonly observed changes are: an increase in the number of platelets and segmented neutrophils (Hazuchova *et al.*, 2017); an increase in the erythrocyte sedimentation rate and mean cell haemoglobin (Stranieri *et al.*, 2018); activity of γ-GTP, ALP, and α-AM; total bilirubin content and a decrease in the number of red blood cells and lymphocytes (Simsek *et al.*, 2022); haematocrit volume, haemoglobin concentration, albumin and glucose content, and protein coefficient value (Hazuchova *et al.*, 2017).

The main cells of the body's immune response to the presence of a foreign agent are white blood cells and their derivatives. As a result of the conducted studies, certain differences in the effect of coronavirus on the body of dogs and cats were established, namely, leukopenia was characteristic of dogs, and leukocytosis was characteristic of cats. A similar trend was observed in relation to stab lymphocytes and monocytes.

For a deeper understanding of the pathogenic effect of the virus on leukocytopoiesis, integral indicators of blood leukocytes were determined, which can be used to find not only the degree of reactivity of the body, but also to assess the level of endogenous intoxication, which is important for predicting the course of the disease. Ultimately, infectious agents in animal organisms cause intoxication and, as a result, are characterised by increased catabolism processes, and insufficient liver and kidney function (Table 3).

Table 3. Indicators of integral haematological indices in dogs and cats with coronavirus infection, M±m

Indicator	Control group, clinically healthy dogs (n=10)	Dogs infected with coronavirus (n=24)	Control group, clinically healthy cats (n=10)	Cats infected with coronavirus (n=27)
Lii	2.2±0.4	1.4±0.03	3.3±0.7*	1.6±0.04
Rrn	10.3±1.2***	2.1±0.1	2.2±0.5*	1.1±0.08
Blsi	2.3±0.3**	1.5±0.2	4.0±0.35***	1.9±0.04
Li	0.74±0.01**	0.96±0.07	0.20±0.02***	0.54±0.01
Irle	13.4±0.7**	16.7±0.8	6.0±0.2**	9.3±1.03
Ai	1.1±0.1***	2.4±0.1	0.7±0.01***	1.7±0.1
Ii	32.5±3.1	34.8±2.8	23.2±2.5**	39.8±4.2
Hi	0.40±0.01***	0.70±0.02	0.24±0.003***	0.60±0.01
Irlm to ESR	1.7±0.03***	8.4±0.9	2.9±0.1***	9.9±0.3

Notes: **P<0.05; **P<0.01; ***P<0.001 compared to the control group of animals*
Source*: developed by the authors*

Analysing the results of integral leukocyte indices for coronavirus infection in dogs and cats (Table 3), certain identities have been established, namely, an increase in such indicators in the blood as: leukocyte intoxication index (Lii) by 2 times (P<0.05) in dogs, which coincides with the study by L. Goralskii *et al.* (2019) and by 57% in cats (Radzikhovskyi *et al.*, 2021); reactive neutrophil response (Rrn) in dogs by 2 times (P<0.05), and in cats by 5 times (P<0.001), and the leukocyte shift index (Blsi) in dogs by 2 times (P<0.001), and in cats by 53% (P<0.01). T. Jaroensong *et al.* (2022) note that this indicates the development of endogenous intoxication against the background of immunosuppression and impaired immunoreactivity.

A significant addition to the state of immunoreactivity of the body shows a decrease in the blood value of such indicators as: leukocyte index (Li) in dogs by 63% (P<0.001), and in cats by 23% (P<0.01); lymphocyte-eosinophil ratio index (Irle) in dogs by 35% (P<0.01), and in cats by 20% (P<0.01); allergisation index (Ai) in dogs by 55% (P<0.001), and in cats by 59% (P<0.001); immunoreactivity index (II) in dogs by 42% (P<0.01), and in cats by 7%; the Harkavy's index (hi) in dogs by 60% (P<0.001), and in cats by 43% (P<0.001); and the ratio of lymphocytes and monocytes to the rate of erythrocyte sedimentation (irlm to ESR) in dogs by 70% (P<0.001), and in cats by 80% (P<0.001). A.R. Fehr & S. Perlman (2015) in their paper indicate that a decrease in these indicators indicates an inflammatory process due to the persistence of the virus. F. Riemer *et al.* (2016) noted that this trend indicates immunosuppression for lymphopenia, and L. Goralskii *et al.* (2019) indicated brain hypoxia for such indicators, because erythropenia and the dominance of delayed-type hypersensitivity processes were observed. L. Goralskii *et al.* (2019) noted the pathogenic effect of the virus on the indicators of "white blood" in viral diseases in dogs, and the results of studies by F. Riemer *et al.* (2016) point to similar changes in cats.

Summing up the results of this study, there is a need for a comprehensive laboratory diagnosis of coronavirus infection, since changes in the morphological parameters of blood indicate the complex pathogenesis and multiple organ

effects of the virus, which complicates treatment in the early stages of the disease.

Conclusions

Based on the results of comprehensive studies of blood parameters for coronavirus infection in companion animals, the following changes were established: an increase in the value of erythrocyte sedimentation rate from 1.5 to 4.5 times; the number of segmented neutrophils by 26-32%; mean cell haemoglobin concentration from 14 to 43%; total bilirubin content from 2.4 to 3.2 times; alpha-amylase activity from 17% to 2.5 times; γ-glutamyltranspeptidase from 20 to 67%; alkaline phosphatase from 2.5 to 9 times. It is necessary to consider a decrease in the value of such indicators as protein coefficient by 26-35%, glucose concentration by 13-20%; albumins by 14% and up to 3 times; haemoglobin content by 10-21%; haematocrit volume by 17-23%; the number of red blood cells by 15-30% and lymphocytes by 29-48%. Therefore, according to the conducted complex morphological and biochemical studies of the blood of companion animals, a certain identity of changes in coronavirus infection can be traced, which indicates a complex pathogenesis of the disease. Based on the parameters of statistical calculation of integral leukocyte indices, it was established that, being in the animal's body, the coronavirus contributes to the development of endogenous intoxication, depletion of the immune system, impaired immunoreactivity with the dominance of the cellular immune system and delayed-type hypersensitivity processes. In the blood of sick animals, the indicators of intoxication indices increased: Lii – from 57% to 2 times; Rrn – from 2 to 5 times; Blsi – from 53% to 2 times. As a result of the inflammatory process against the background of immune system suppression, there is a tendency to an incomplete immune response and a deficiency of cytokines of lymphocytic origin due to a decrease in non-specific reactivity indicators, such as: Li – from 23 to 63%; Irle – from 20 to 35%; Ii – from 7 to 42%; Hi – from 43 to 60%. By investigating the pathological effects of the virus on the body of dogs and cats, it is possible to improve the understanding of the pathobiology of coronavirus, which will allow establishing early intravital diagnostic markers. Thus, a certain set of changes in the morphological and biochemical parameters of blood can be considered a characteristic criterion in the differential diagnosis of coronavirus infection in companion animals. Further study is planned to investigate pathomorphological features of the impact of coronavirus on companion animals, which will expand diagnostic capabilities at different stages.

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Conflict of Interest

None.

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Анотація. У зв'язку з високим рівнем урбанізації в Україні, протягом 2021-2023 років відмічається тенденція до стрімкого збільшення у великих містах чисельності тварин-компаньйонів – собак і котів. Людство ще остаточно не ліквідувало пандемію коронавірусної

інфекції SARS-CoV-2, як світові науковці встановили участь цих тварин у її поширенні, що викликає занепокоєння у фахівців і потребує всебічного вивчення. Мета наукового дослідження полягала у визначенні маркерних змін гематологічних показників та параметрів гемопоезу за впливу на організм собак і котів коронавірусної інфекції. Для цього у роботі використовували спектрофотометричні та рефрактометричні методи гематологічних досліджень: морфологічних і біохімічних показників крові, функціонального стану еритро- і лейкоцитопоезу. Враховуючи зміни морфологічних показників крові, встановлено основні параметри ідентичності патогенного впливу коронавірусу на організм собак і котів, а саме: зменшення кількості лімфоцитів, вмісту глюкози та величини білкового коефіцієнта. Водночас відзначалось істотне підвищення в крові активності лужної фосфатази та α-амілази, вмісту загального білірубіну й величини швидкості осідання еритроцитів. Зокрема, експериментально встановлено, що використання лейкоцитарних індексів підвищує інформативність загального аналізу крові за коронавірусної інфекції, а додатковий аналіз інтегральних змін на основі лейкоцитарних показників крові дозволяє визначати не лише стан реактивності організму, але й величину ендогенної інтоксикації. Результати щодо змін морфологічних показників крові певною мірою є співставними з такими в людей, що свідчить про необхідність глибокого вивчення ще й генетичного потенціалу збудників коронавірусу в тварин-компаньйонів і людини на молекулярно-біологічному рівні. Експериментально визначені маркерні зміни гематологічних показників за коронавірусної інфекції у собак і котів можуть бути корисними для своєчасного її діагностування та прогнозування ступеня важкості перебігу захворювання

Ключові слова: коронавірус; еритроцитопоез; лейкоцитопоез; стабілізована кров; сироватка крові; морфологічні та біохімічні показники