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Removal of heavy metals using sorbents and biochemical indexes in rats

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Abstract. A wide range of negative effects of heavy metals on the body of mammals predetermined the relevance of the search for ways to reduce the toxic effects of these chemical compounds. Solving this problem is possible by using sorbents. The research aims to determine the effect of sorbents on the content of heavy metals (cadmium, copper, lead, and zinc) in the rat tissues. Toxicological, physicochemical, and biochemical methods were used. Phillipsite sorbent reduced the content of copper and zinc in the blood of rats by 1.6 times, cadmium by 2 times and lead by 2.6 times. The content of copper and zinc in rat livers decreased by 1.4 times, and cadmium and lead – by 2 times. The content of the studied metals in kidney tissues decreased by half. A similar decrease in the level in the tissues was noted when using the chabazite sorbent for all heavy metals studied. Administration of the clinoptilolite sorbent into the body of rats contributed to a 2-fold decrease in the blood content of copper and zinc, cadmium, and lead by 2.6 and 3 times, respectively. A 1.6-fold decrease in copper and zinc levels and a 3-fold decrease in cadmium and lead levels were

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detected in the liver tissues. When mordenite sorbent was administered to animals, the content of copper and zinc in the blood decreased by 1.5 times, and cadmium and lead by 3 times compared to the control. The content of all studied metals in the liver of rats decreased by 2.4 times. The use of these sorbents in animal husbandry will contribute to the reduction of the content of heavy metals in animal tissues, which will ensure the production of high-quality and safe products, as well as contribute to the preservation of human health

Keywords: animal tissues; cadmium; copper; lead; zinc

Introduction

Man-made heavy metal environmental pollution requires studying the mechanisms of their action on humans and animals and developing new methods of protection against toxic effects. Sorbents are widely used to remove heavy metals and other foreign substances from the environment. The search for effective adsorbents that can reduce the concentration of heavy metals in human and animal organisms is currently relevant. Sorbents must be safe and effective so that they can be used regularly for preventive purposes (Ahmad *et al.*, 2020; Alengebawy *et al.*, 2021; Chen *et al.*, 2022). The increase of harmful pollutants in the environment has become a serious global problem. Zeolite materials have been widely used in the last decade to prevent heavy metal pollution due to their excellent properties of high ion exchange capacity and absorption capacity (Haemmerle *et al.*, 2021). Intensive pollution of the environment with heavy metals is quite significant and negatively affects the health of people and animals. The search for effective and safe means of detoxification of the human body from toxicants and the prevention of their adverse biological effects is one of the most urgent problems of modern biology, medicine, veterinary medicine, and toxicology (Kim *et al.*, 2019).

The unique physical and chemical properties of zeolite materials make them extremely useful for a wide range of applications, in agronomy, ecology, veterinary medicine, production and industrial processes. Clinoptilolite, natural zeolite, has been widely studied in veterinary and human medicine. The use of clinoptilolite products in vivo has increased dramatically due to their many health benefits, including detoxification. However, concerns about the safety of clinoptilolite for in vivo use have been raised. The conducted studies showed the high efficiency of clinoptilolite in various medical and veterinary applications *in vitro* and *in vivo*. Clinoptilolite from the group of zeolites has the best physical and chemical properties, thanks to which it is widely used in various fields of industry, medicine, and veterinary medicine. Different zeolite minerals can be found together in a particular rock. Zeolite mineralization is associated with several secondary and accessory minerals (feldspar, micaceous minerals, chlorite minerals, opal, cristobalite, zircon, tourmaline, iron, sulphur compounds, and clay minerals). Natural zeolites are subjected to appropriate activation during which zeolites can acquire better properties (Pabi´s-Mazgaj *et al.*, 2021).

Clinoptilolite is one of the most common and widely used zeolite minerals in the world. There are large deposits that are being developed and where zeolites are being mined, which have high selectivity and good temperature resistance. High ion exchange properties and molecular sieve properties make clinoptilolite

one of the inexpensive adsorbents for the reduction of heavy metals. At the same time, the low cost of natural zeolites makes it possible to use them in sorption processes that do not involve the regeneration of ion exchange resins (Burakov *et al*., 2018). Purified clinoptilolite tuff is used as a dietary supplement, which is made by applying a complex process to the substance to reduce the natural content of heavy metals and increase its effectiveness and ease of use. In addition, zeolites are natural ion exchangers and can combine the processes of adsorption and filtration (Tschegg *et al*., 2020). Chemical precipitation, membrane flotation, ion exchange, and solvent extraction are used to remove soluble heavy metals. These traditional methods either require heavy capital and operating costs or cannot reduce the content of heavy metals to the maximum permissible standards. This determines the current emphasis on the development and use of sorbents as alternative and inexpensive materials (Irannajad & Haghighi, 2021).

Natural zeolites are a promising type of sorbents, as there are currently more than 100 and about 40 synthetic ones. Having high adsorption, antitoxic, radioprotective, ion exchange, catalytic and immunomodulatory properties, they are increasingly used in various fields of science, industry, biology, medicine, veterinary medicine, agriculture, and ecology. These features of zeolites attracted the attention of scientists and were used to establish the versatile effectiveness of their use, but the issues of complex rehabilitation of mineral homeostasis and the influence of sorbents on metabolic processes remain unknown, which requires further research (Belviso, 2020; Morante-Carballo *et al*., 2021; Eberle *et al*., 2022).

The research aims to determine the effect of sorbents on the content of heavy metals (copper, zinc, cadmium, and lead) and biochemical indicators in animals.

Materials and Methods

The research was performed on nonlinear male rats of the same age, weighing 200-220 g, which were kept in separate cages under normal conditions of vivarium. A total of 40 animals were used, which were divided into five groups: I – control, II – animals with orally administered solution of copper sulfate at a dose of 3 mg/kg, which is 1/10 of LD_{50}, III – zinc sulfate at a dose 2 mg/kg, which is 1/20 of the LD_{50}, IV – cadmium sulfate at a dose of 1.7 mg/kg, which is 1/30 of the LD_{50}, V – lead nitrate at a dose of 2.0 mg/kg, which is 1/50 from LD_{50}. Intoxication was performed for 14 days, and then for 2 weeks zeolites (filipsit, chabazite, clinoptilolite and mordent) were administered at a rate of 0.25 g/kg of animal weight once a day. At the end of the experiment, rats were decapitated. Blood, liver and kidney tissues were taken for further studies. The experiments were performed following the Council of European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (European convention…, 1986).

The content of heavy metals in the blood, liver, and kidney samples was determined using a spectrochemical method using the absorption mode in an air-acetylene flame in atomic absorption spectrophotometer SpectrAA-55B ("VARIAN", USA). The standard samples of heavy metals solution served as control. They were produced at the Institute of Physical Chemistry, Academy of Sciences of Ukraine (Odesa). Biochemical parameters were determined in serum photometrically were performed using a semi-automatic biochemical analyzer Microlab 300 (Netherlands). Concentrations of urea, bilirubin (total and direct), creatinine, glucose, albumin, total protein, cholesterol, triglycerides, magnesium (Mg), inorganic phosphorus (Pi), calcium (Ca), chloride (Cl), Sodium (Na), Potassium (K) and the activity of alkaline phosphatase (ALP, EC 3.1.3.1), alanine aminotransferase (ALT, EC 2.6.1.2),

aspartate aminotransferase (AST, EC 2.6.1.1,), γ-glutamyltranspeptidase (γ-GTP, EC 2.3.2.2), lactate dehydrogenase (LDG, EC 1.1.1.27), cholinesterase (ChE, EC 3.1.1.8), α-amylase total (EC 3.2.1.1) were determined using Pliva Lachema test kits (Czech Republic).

Statistical analyses were carried out using "Microsoft Excel 2007". Data were shown as average ± standard deviation (SD). Comparisons between groups were performed with analysis of non-parametric tests. A value of $P<0.05$ was considered statistically significant (Kucherenko *et al.*, 2001).

Results and Discussion

In the liver, the concentration of copper and zinc decreased by a factor of 1.3, cadmium and lead by a factor of 2, and in the kidney tissues, the concentration of all of them at all studied decreased 2-fold. Similar changes were established when using chabazite for all the studied metals. When clinoptilolite was used, blood levels of copper and zinc decreased 2-fold, cadmium and lead 2.5 and 3-fold, respectively, while the liver showed a 1.5-fold decrease in copper and zinc and a 3-fold decrease in cadmium and lead. When using mordenite, the levels of copper and zinc in the blood decreased by 1.5 times, cadmium, and lead by 3 times, and in the tissues of the liver and kidneys, the content of all the metals studied decreased by 2.5 times (Figs. 1-3).

Figure 3 shows the results of the study. It shows the concentration of heavy metals in the tissues of the kidneys of rats.

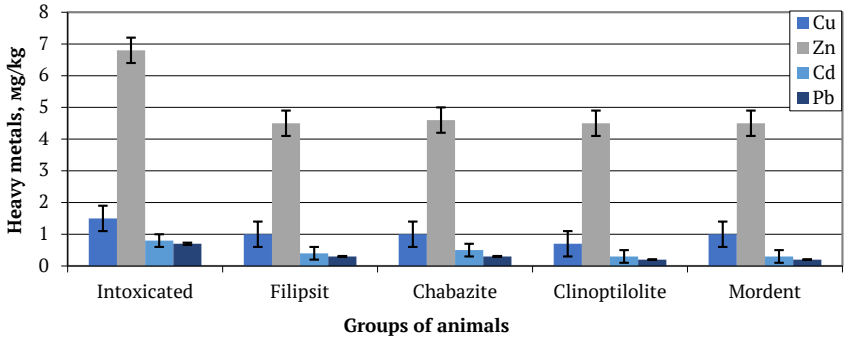

Figure 1. The concentration of heavy metals in the blood of rats with sorbents (M±m, n=8)
Source: *author's development*

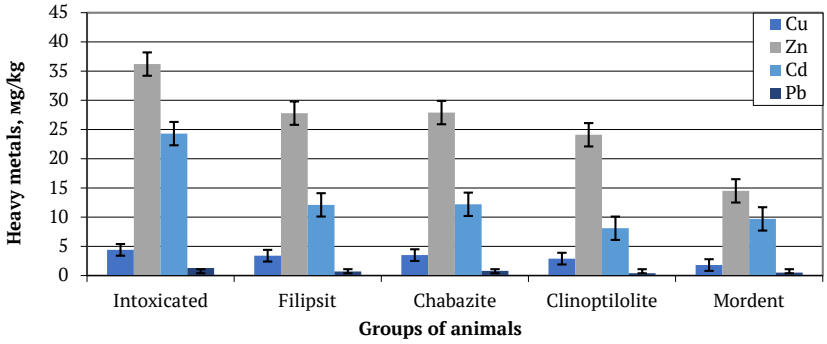

Figure 2. The concentration of heavy metals in the liver tissues of rats with sorbents (M±m, n=8)
Source: *author's development*

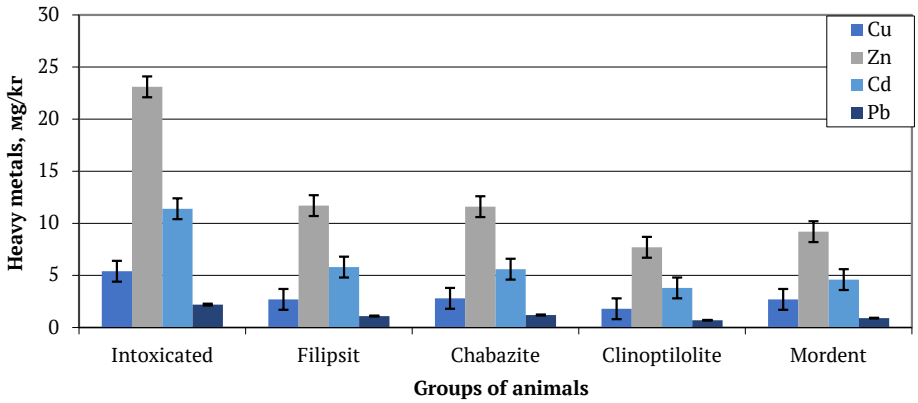

Figure 3. The concentration of heavy metals in the tissues of the kidneys of rats with sorbents (M±m, n=8)

Source: *author's development*

Biochemical indexes of blood indicate the condition of the liver in certain pathological processes, including heavy metal lesions (Tables 1-4). They can be used to determine deviations in the synthetic and detoxification functions of this organ. Functional disorders of hepatocytes are examined for the activity in the serum of indicator enzymes.

Table 1. Biochemical parameters of rat blood serum of copper-poisoned and with sorbents (M±m, n=8)

Indexes	Rats					
	Control	Copper-poisoned	Administered chemical			
			Filipsit	Chabazite	Clinoptilolite	Mordent
ALP, U/L	292.12±26.39	536.31±83.20*	436.12±45.22**	442.31±47.11**	402.62±42.42**	421.82±46.23**
ALT, U/L	78.31±6.33	132.72±10.72*	106.31±10.08**	108.13±10.03**	99.31±9.20**	102.21±10.01**
AST, U/L	163.22±15.85	254.51±20.31*	212.23±18.73	217.02±18.62	182.23±16.31**	196.32±18.26**
γ- GTP, U/L	25.63±1.95	40.13±2.90*	31.61±3.10	32.21±3.26	28.62±2.45**	30.13±2.42
LDG, U/L	324.41±31.11	652.21±53.73*	497.32±47.31**	496.73±48.28**	352.31±43.21**	482.52±45.20**
ChE, U/L	33.23±5.90	42.32±6.12	37.13±4.80	38.32±4.97	35.02±3.97	36.21±4.73
α-Amylase Total, U/L	518.71±89.83	747.21±115.11*	607.21±96.13**	609.51±97.31**	554.21±81.16**	597.12±94.27**
Bilirubin:						
Total, μmol/L Direct, μmol/L	4.11±0.20 0.82±0.02	4.72±0.19 1.31±0.03	4.72±0.18 0.83±0.01	3.63±0.20 0.91±0.03	3.62±0.18 0.91±0.02	3.63±0.21 1.02±0.02
Creatinine, μmol/L	69.21±6.11	102.32±8.92*	82.61±7.20**	83.32±7.67**	74.22±6.75**	81.31±7.42**
Urea, mmol/L	6.12±0.89	11.31±1.24*	9.02±1.08**	9.31±1.05**	7.31±0.97**	8.23±1.03**
Glucose, mmol/L	5.21±0.69	6.63±1.09*	5.61±0.71	5.73±0.80	5.03±0.61**	5.32±0.70
Albumin, g/L	42.53±3.30	33,71±2.51	40.12±2.84	39.62±2.70	41.21±3.93**	40.13±2.91
Total protein, g/L	74.62±3.71	61.23±2.42	71.14±3.18	69.71±2.63	72.61±3.41	71.21±3.20
Cholesterol, mmol/L	1.21±0.05	0.82±0.01*	1.02±0.02	0.82±0.02	1.03±0.03	1.12±0.01
Triglycerides, mmol/L	1.12±0.01	0.41±0.01*	0.51±0.01	0.53±0.02	0.82±0.03**	0.71±0.01
Chlorides, mmol/L	86.21±7.42	106.02±9.71	89.12±7.37	90.21±7.90	87.71±7.42	88.03±7.97

Table 1. Continued

Indexes	Rats					
	Control	Copper-poisoned	Administered chemical			
			Filipsit	Chabazite	Clinoptilolite	Mordent
Magnesium, mmol/L	1.61±0.10	2.23±0.20*	2.13±0.18	2.02±0.21	2.03±0.17**	2.12±0.16
Inorganic Phosphorus, mmol/L	2.42±0.21	1.31±0.09*	1.82±0.15**	1.73±0.13**	2.12±0.18**	2.01±0.17**
Calcium, mmol/L	1.83±0.12	2.81±0.19*	2.03±0.17**	2.01±0.21**	2.13±0.11**	2.13±0.16**
Sodium, mmol/L	144.11±12.32	127.21±11.80*	136.31±12.47	136.82±12.51	139.31±12.42	135.21±12.27
Potassium, mmol/L	5.23±0.29	7.32±0.59*	6.12±0.40	6.31±0.47	5.82±0.30**	6.12±0.38**

Notes: **P<0.05 compared with the intact rats; **P<0.05 compared with the intoxicated rats*

Source*: author's development*

Table 2. Biochemical parameters of rat blood serum of zinc-poisoned and with sorbents (M±m, n=8)

Indexes	Rats					
	Control	Zinc-poisoned	Administered chemical			
			Filipsit	Chabazite	Clinoptilolite	Mordent
ALP, U/L	291.12±27.39	537.21±86.10*	438.12±75.30**	440.21±76.25**	401.23±69.23**	422.31±71.28**
ALT, U/L	78.31±6.33	135.23±11.11*	105.51±9.17**	107.32±9.20**	98.51±8.50**	101.12±8.73
AST, U/L	162.22±14.85	254.82±22.40*	207.32±21.26**	210.23±19.33**	192.22±19.71**	198.63±20.13**
γ-GTP, U/L	24.63±2.20	40.41±4.18*	33.53±3.18**	34.02±3.41	29.61±3.20**	31.12±3.26**
LDG, U/L	323.41±32.11	653.32±55.22*	518.02±46.22**	522.31±47.17**	489.32±45.10**	501.53±46.17
ChE, U/L	34.23±4.90	40.03±5.02	37.13±4.13	37.82±4.75	35.13±4.30	36.02±4.96
α-Amylase Total, U/L	517.71±89.83	749.12±123.13*	617.31±112.21**	618.53±112.18**	604.02±117.23**	606.21±117.29
Bilirubin:						
Total, μmol/L Direct, μmol/L	4.11±0.20 0.82±0.02	4.81±0.22 1.12±0.01	4.12±0.19 1.03±0.02	3.72±0.20 1.03±0.01	3.51±0.18 1.02±0.02	3.62±0.20 1.03±0.03
Creatinine, μmol/L	69.21±6.11	104.21±9.21*	85.12±6.74**	87.21±6.68	78.32±6.22**	82.21±6.84**
Urea, mmol/L	6.12±0.89	11.23±1.14*	9.13±0.96**	9.32±0.98	7.73±0.70**	8.52±0.93**
Glucose, mmol/L	5.21±0.69	7.21±1.31*	6.21±0.91	6.23±0.95	5.32±0.51**	5.83±0.81
Albumin, g/L	42.53±3.30	34.82±2.72	37.12±2.40	37.81±2.71	41.13±3.02	39.71±2.70
Total protein, g/L	74.62±3.71	62.03±2.62	69.21±2.51	68.23±2.42	71.42±3.23	70.12±2.33
Cholesterol, mmol/L	1.31±0.05	0.82±0.02*	1.03±0.03	0.82±0.02	1.03±0.04	0.93±0.03
Triglycerides, mmol/L	1.12±0.01	0.41±0.02*	0.81±0.02**	0.81±0.01**	0.72±0.03**	0.71±0.02**
Chlorides, mmol/L	86.21±7.42	105.62±9.49	89.32±7.81	90.12±8.42	85.13±7.22**	86.62±7.30
Magnesium, mmol/L	1.61±0.10	2.31±0.23*	2.03±0.18	2.13±0.17	1.82±0.13**	1.83±0.15**
Inorganic Phosphorus, mmol/L	2.42±0.21	1.42±0.11*	1.72±0.16	1.72±0.15	2.01±0.21**	2.12±0.22**
Calcium, mmol/L	1.83±0.12	2.72±0.24*	2.51±0.21	2.61±0.24	2.12±0.17**	2.03±0.10**
Sodium, mmol/L	144.11±12.32	128.02±11.89*	136.23±11.81	135.12±11.73	139.21±12.41	137.31±11.97
Potassium, mmol/L	5.23±0.29	7.51±0.70*	6.72±0.65	6.83±0.62	6.13±0.50**	6.13±0.42**

Notes: **P<0.05 compared with the intact rats; **P<0.05 compared with the intoxicated rats*

Source*: author's development*

The liver ensures the neutralization of many endogenous and exogenous substances and is the central regulator of metabolic processes and regulation of homeostasis in the organism.

Table 3. Biochemical parameters of rat blood serum of cadmium-poisoned and with sorbents (M±m, n=8)

Indexes	Rats					
	Control	Cadmium-poisoned	Administered chemical			
			Filipsit	Chabazite	Clinoptilolite	Mordent
ALP, U/L	291.12±227.39	589.41±87.15*	462.02±72.22**	468.31±74.31**	434.62±69.72**	446.13±70.22**
ALT, U/L	78.31±6.33	176.12±12.30*	141.13±10.83**	148.42±11.20	135.21±10.23**	138.32±10.47**
AST, U/L	162.22±14.85	338.63±31.23*	273.3 ±27.52**	275.13±27.52**	252.23±27.08*	261.13±27.27**
γ-GTP, U/L	24.63±2.20	43.31±4.84*	36.23±3.71**	37.32±3.80	31.12±2.97**	34.21±3.41**
LDG, U/L	323.41±32.11	724.52±61.13*	602.41±57.02**	605.63±57.62**	561.31±54.10**	572.23±54.18**
ChE, U/L	34.23±4.90	43.13±5.93*	37.12±5.27	37.31±5.32	35.13±5.02	35.81±5.03
α-Amylase Total, U/L	517.71±89.83	809.21±141.24*	651.31±93.87**	653.21±94.11**	619.23±92.17**	632.62±93.06**
Bilirubin:						
Total, μmol/L Direct, μmol/L	4.11±0.20 0.82±0.02	4.71±0.25* 1.12±0.02*	4.42 ±0.18 1.13±0.02	4.51±0.21 1.02±0.03	4.12±0.11** 1.11±0.02	4.01±0.15** 0.92±0.03
Creatinine, μmol/L	69.21±6.11	118.43±11.53*	95.31±10.13**	94.73±10.17**	91.12±9.83**	92.31±9.85**
Urea, mmol/L	6.12±0.89	12.82±2.31*	10.63±2.08**	10.21±2.03**	8.31±1.61**	9.23±1.73**
Glucose, mmol/L	5.21±0.69	8.23±1.42*	7.12±1.23	7.32±1.27	6.42±1.22**	6.42±1.21**
Albumin, g/L	42.53±3.30	31.31±2.20*	37.13±2.42	36.71±2.81	39.31±2.87**	38.21±2.73**
Total protein, g/L	74.62±3.71	56.12±2.11*	65.31±2.18	64.12±2.23	68.32±2.71	67.23±2.68
Cholesterol, mmol/L	1.31±0.05	0.73±0.02*	0.82±0.03	0.83±0.02	1.13±0.07**	1.12±0.07**
Triglycerides, mmol/L	1.12±0.01	0.31±0.02*	0.43±0.02**	0.51±0.01**	0.62±0.02**	0.53±0.02**
Chlorides, mmol/L	86.21±7.42	112.23±10.91*	95.52±9.90	96.32±10.02	90.21±8.80**	92.21±9.11**
Magnesium, mmol/L	1.61±0.10	2.52±0.27*	2.41±0.20	2.43±0.21	2.12±0.16**	2.12±0.17
Inorganic Phosphorus, mmol/L	2.42±0.21	1.13±0.07*	1.21±0.08	1.32±0.12	1.53±0.13**	1.61±0.11**
Calcium, mmol/L	1.83±0.12	3.41±0.36*	2.72±0.25**	2.81±0.27**	2.41±0.17**	2.62±0.21**
Sodium, mmol/L	144.11±12.32	122.23±11.14*	134.41±11.02	132.23±10.63	130.12±10.43	131.31±10.22
Potassium, mmol/L	5.23±0.29	8.32±0.91*	7.93±0.65	7.62±0.72	7.23±0.53**	7.12±0.62**

Notes: **P<0.05, compared with the intact rats; **P<0.05, compared with the intoxicated rats*

Source*: author's development*

The liver ensures the neutralization of many endogenous and exogenous substances and is the central regulator of metabolic processes and regulation of homeostasis in the organism. The obtained data can be used as a marker of liver function issues in animals in case of heavy metal poisoning.

Table 4. Biochemical parameters of rat blood serum of lead-poisoned and with sorbents (M±m, n=8)

Indexes	Rats					
	Control	Lead-poisoned	Administered chemical			
			Filipsit	Chabazite	Clinoptilolite	Mordent
ALP,, U/L	291.12±27.39	554.61±86.93*	461.23±80.23**	464.12±80.27**	418.13±78.13**	422.21±79.01**
ALT, U/L	78.31±6.33	163.52±12.12*	134.21±11.83	158.63±12.07	117.21±10.24**	121.23±10.73
AST, U/L	162.22±14.85	285.31±23.11*	234.32±22.52	257.21±22.93	223.62±21.13**	227.32±21.41**

Table 4. Continued

Indexes	Rats					
	Control	Lead-poisoned	Administered chemical			
			Filipsit	Chabazite	Clinoptilolite	Mordent
γ-GTP, U/L	24.63±2.20	41.12±4.37*	33.63±4.08	37.43±4.26	29.31±3.22**	31.13±3.97**
LDG, U/L	323.41±32.11	692.31±55.72*	574.32±47.32**	618.72±54.21	524.03±45.32**	536.21±45.51**
ChE, U/L	34.23±4.90	42.23±5.17*	38.21±4.82	38.81±4.96	36.12±4.41	36.72±4.82
α-Amylase Total, U/L	518.71±89.83	792.31±134.22*	651.23±127.26**	708.42±132.17	605.31±126.17*	612.23±126.80**
Bilirubin:						
Total, μmol/L	4.11±0.20	4.62±0.27*	4.51±0.24	4.41±0.23	4.12±0.18	4.04±0.21
Direct, μmol/L	0.82±0.02	1.13±0.02*	1.02±0.07	1.03±0.06	1.12±0.03	1.13±0.03
Creatinine, μmol/L	69.21±6.11	112.51±10.14*	95.51±9.41	96.62±9.77	91.23±9.06	93.32±9.22
Urea, mmol/L	6.12±0.89	12.23±2.22*	10.23±1.73**	10.71±1.91**	9.71±1.61**	10.13±1.72**
Glucose, mmol/L	5.21±0.69	8.24±1.37*	6.82±0.95**	7.23±1.02	6.82±0.72**	6.81±0.81**
Albumin, g/L	42.53±3.30	32.02±2.31*	36.61±2.51**	34.12±2.40	36.43±2.33**	37.23±2.52**
Total protein, g/L	74.62±3.71	58.31±2.20*	65.23±2.83	60.71±2.39	67.21±3.20	66.12±2.63
Cholesterol, mmol/L	1.31±0.05	0.72±0.03*	0.72±0.02	0.73±0.03	0.82±0.03**	0.83±0.02
Triglycerides, mmol/L	1.22±0.01	0.31±0.01*	0.61±0.01**	0.42±0.02**	0.71±0.01**	0.72±0.02**
Chlorides, mmol/L	86.21±7.42	111.23±10.21*	95.23±9.40	96.31±9.31	90.13±9.80**	91.31±9.93**
Magnesium, mmol/L	1.61±0.10	2.62±0.24*	2.21±0.21	2.13±0.20	2.12±0.17**	2.23±0.21
Inorganic Phosphorus, mmol/L	2.42±0.21	1.23±0.11*	1.52±0.15	1.71±0.16	2.21±0.18**	2.12±0.22**
Calcium, mmol/L	1.83±0.12	3.21±0.43*	2.51±0.23	2.72±0.27	1.62±0.17**	1.81±0.17**
Sodium, mmol/L	144.11±12.32	123.12±11.74*	137.23±11.21	136.21±11.16	141.23±11.36	140.12±11.50
Potassium, mmol/L	5.23±0.29	8.03±0.83*	7.12±0.57	7.23±0.60	6.71±0.32**	7.03±0.64**

Notes: **P<0.05, compared with the intact rats; **P<0.05, compared with the intoxicated rats*
Source*: author's development*

The obtained biochemical characteristics of heavy metal poisoning will allow a better understanding of the molecular aspects of the mechanisms of their negative effects on the animal organism, which will in the future provide an opportunity to prevent negative effects on the human organism as well. The liver is actively involved in adaptive reactions during toxicological processes in the animal organism. ALT and AST are one of the most important indicators of the biochemical analysis of animal blood, directly indicating the state of internal organs. An excess of normal values indicates pathological processes occurring in such vital organs as the heart, liver, and kidneys. The transamination reaction is the transfer of an amino group from an amino acid to a fat metabolism product, a keto acid. As a result, a new amino acid is formed, synthesized directly in the animal organism, and α-keto acid. Transaminases are present in every cell of the animal organism. If the integrity of cellular structures is violated, these enzymes enter the bloodstream. Normally, aminotransferases are contained in the blood due to the presence of programmed cell death – apoptosis. This is the norm. However, with massive cell death and the release of a large number of enzymes, the indicators of biochemical research change, they can be exceeded tenfold, depending on the type of pathology and the size of the defect.

An increase in the activity of ALP and an increase in the concentration of bilirubin

indicates toxic damage to the liver with manifestations of cholestasis.

The main reasons for the rise in increased activity in α-amylase total, cholinesterase and creatinine blood levels are the development of pancreatitis, renal insufficiency, filtration pathology in renal glomeruli, development of nephritis as are the salt of development of toxic processes. Aminotransferase and LDG activity increased, cholesterol and triglycerides decreased, and glucose increased in the presence of γ-GTP, and ALP are the main reasons for the impaired excretory function of the liver with hepatocellular insufficiency.

The main reasons for the decrease in total protein and albumin in the blood serum in the heavy metal poisonings studied are the cellular damage to the liver and kidneys. Elevated serum urea levels are signs of increased protein catabolism and acute renal failure, indicating a change in the relationship between urea production and excretion in the urine. Urea is the largest reservoir of circulating of nitrogen and its production changes in parallel with the degradation of endogenous proteins. Urea transport is mediated by specific urea transport proteins, which play a central role in urine concentration (Wei *et al*., 2019; Saha & Paul, 2019; Treto-Suárez *et al*., 2020). Inorganic sodium and phosphorus levels decrease in all groups of poisoned animals. However, the concentration of chloride, magnesium, calcium, and potassium increased. All this points to nephritis and disorders of acid-base equilibrium disorder namely compensatory acidosis. The use of sorbents in intoxicated rats and comparison of the obtained results indicates their positive corrective effectiveness, especially when using clinoptilolite and mordent which is consistent with the data (Liu *et al*., 2018; Ma *et al*., 2018; Engwa *et al*., 2019). In the current study, normalization of almost all studied biochemical parameters of blood serum to such values as in intact animals was observed.

Agro-industrial wastes can be used as potential adsorbents of heavy metals. These residues were characterized to determine their structure and composition and to understand the adsorption mechanism. Adsorption is one of the most suitable technologies for removing heavy metals. The adsorbent efficiency and capacity were measured and compared. It is possible to affirm from the obtained results that all biomasses used are good alternatives to synthetic materials, with adsorption efficiencies greater than 50%. The heavy metals were immobilized, with efficiencies over 88.5% (Simon *et al.,* 2022). The results of the current research are consistent with the data presented in other studies in that intestinal adsorption is a very promising method for the removal of various toxins, both in emergencies and in planned detoxification of the organism. Intestinal adsorption is a simple and effective method of cleaning the organism utilizing adsorbent sand is used in the prevention and treatment of several diseases and poisonings, as well as in the amelioration of conditions related to endotoxemia and exotoxemia. Intestinal adsorbents are used to prevent toxic allergic reactions and reduce the metabolic load on excretory and detoxification functions. According to the World Health Organization's Anatomical Therapeutic Chemistry Classification System, enteric adsorbents belong to group A07B "Enteric Adsorbents". This section outlines recent scientific research on the clinical applications of enteric adsorbents, particularly for the removal of toxic metals from the human body. The latest classification of enteric adsorbents and their mechanisms of action are also presented (Fatullayeva *et al*., 2021).

The results are consistent with the data presented in studies on the efficacy of amphoteric cryogens as oral adsorbents (enerosorbents) for

the treatment of acute poisoning of small animals (rats) by heavy metals in vivo experiments. The morphological structure of the cryogels was examined by scanning electron microscopy/energy-dispersive X-ray analysis and confocal microscopy, and the use of cryogels to treat rats treated with aqueous solutions of LD50 amounts of $Cd(NO_3)_2$, $CsNO_3$, $Sr(NO_3)_2$, or $HgCl_2$ resulted in the rats treated with cryogel showed higher survival changes in comparison with the control group that did not receive such treatment. Histological and chemical analysis of the internal tissues of experimental animals and biochemical analysis of their blood showed that cryogel protects animals from the damaging effects of heavy metals on the organism, comparable to unithiol, a 2,3-dimercapto-1-propanesulfonate sodium salt-based chelating agent approved for the treatment of acute poisoning by several heavy metals the effectiveness of cryogel in protecting animals from the damaging effects of heavy metals on the organism was demonstrated (Baimenov *et al.*, 2021).

Several authors have established that the introduction of cadmium and lead salts into the organism of rats caused a decrease in organism weight gain compared to intact animals. Reduction of body weight gain in rats under heavy metal poisoning was accompanied by malnutrition and hypertrophy of organs. These changes are associated with the accumulation and sorption capacity of these metal ions, which contribute to the development of endogenous poisoning in the experimental group of rats. Chronic lead-cadmium toxicosis in rats was accompanied by erythrocytopenia and leukaemia with a simultaneous increase in erythrocyte volume and average haemoglobin content in erythrocytes. Which is also consistent with the results (Lopotych *et al.*, 2020).

Chronic effects of three groups of heavy metals on the body of rats were modelled, and biochemical parameters, as well as their changes, were measured and compared with the control group. The results are consistent with the results of this research. The study showed that when combined with chronic poisoning by heavy metal salts, biochemical indicators of the blood may be altered, and this is caused by functional violations of the liver, kidneys, and myocardium. On the background of toxic effects of heavy metals, "Ursodex" and "Schrot Rastoropshy" were used as biologically useful agents, which contributed to the reduction of metal toxicity. The level of adverse effects of these elements on biochemical indicators of blood in experimental animals was reduced (Tazitdinova *et al.*, 2018). Thus, the use of adsorbents showed good results regarding the elimination of the studied heavy metals and had a positive effect on biochemical indicators in rats.

Conclusions

In the case of intoxication of rats with heavy metals, the use of phillipsite, chabazite, clinoptilolite and mordenite was found to be effective for the removal of copper, zinc, cadmium, and lead from all studied tissues. Biochemical parameters of the rat blood indicate that the use of the studied sorbents had a corrective effect on the restoration of biochemical functions of liver cells. In this study, the adsorption behaviour of sorbents for various, health-related heavy metal cations was evaluated. The multifaceted properties of sorbents, especially high-silicon sorbents, indicate the possibility of their use as heavy metal detoxifiers. Conducting a comparative assessment of the results of research on the corrective effectiveness of the use of various sorbents indicates the possibility of their use as heavy metal detoxifiers. Zeolites are ecological absorbents of the active groups based on the way metal ions interact with the adsorbent, as well as their radius. Several factors control the adsorption process of heavy metals, including the type and distribution of active groups on

the adsorbent, the way the metal ions interact with the adsorbent, and the radius of the metal ion. The adsorbents proved to be perfectly effective with a high degree of removal from various animal tissues. The results show that the adsorbent can be used for the following purposes obtain ecologically clean products of animal husbandry, for animals located in regions polluted by heavy metals.

In further research, protein and lipid metabolism should be investigated more deeply. Further studies of metabolic indicators in animals can be continued with the aim of implementing them into practice for the study of pathological conditions that cause the toxic effects of heavy metals or other xenobiotics. Further continuation of research can be in animal husbandry, veterinary medicine, and toxicology.

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Conflict of Interest

None.

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Виведення важких металів за допомогою сорбентів і біохімічні показники у щурів

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Анотація. Широкий спектр негативного впливу важких металів на організм ссавців спрямовує дослідників на пошук способів зниження токсичної дії цих хімічних сполук. Вирішити цю проблему можливо завдяки використанню сорбентів. Мета наукового дослідження полягала у визначенні впливу сорбентів на вміст важких металів (кадмію, міді, свинцю та цинку) в тканинах щурів. Для цього використовували токсикологічні, фізико-хімічні та біохімічні методи. Встановлено, що застосування сорбенту філіпситу сприяло зниженню вмісту міді і цинку в крові щурів в 1,6 раза, кадмію в 2 рази та свинцю в 2,6 раза. У печінці щурів уміст міді і цинку зменшувався в 1,4 раза, а кадмію і свинцю – в 2 рази. В тканинах нирок уміст досліджуваних металів зменшувався вдвічі. Аналогічне зниження рівня в тканинах відзначалося і при використанні сорбенту шабазиту для всіх досліджуваних важких металів. Введення в організм щурів сорбенту клиноптилоліту сприяло зменшенню вмісту в крові міді і цинку в 2 рази, кадмію і свинцю в 2,6 і 3 рази відповідно. В тканинах печінки виявляли зниження рівня міді і цинку в 1,6 раза, кадмію і свинцю в 3 рази. За

введення тваринам сорбенту морденіту вміст міді і цинку в крові знижувався в 1,5 раза, а кадмію і свинцю в 3 рази порівняно з контролем. Уміст всіх досліджуваних металів у печінці щурів зменшувався в 2,4 раза. Застосування в тваринництві зазначених сорбентів сприятиме зменшенню вмісту важких металів у тканинах тварин, що забезпечить отримання якісної та безпечної продукції, а також сприятиме збереженню здоров'я людини

Ключові слова: тканини тварин; кадмій; мідь; свинець; цинк

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Morphological changes in the small intestine mesentery of cats with infectious peritonitis

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Abstract. The research relevance is determined by an insufficient study of morphological changes in the mesentery of cats with infectious peritonitis, even though their understanding is necessary to explain the mechanism of development of the main symptom of the disease – the effusion of fluid into the abdominal cavity. The research aims to establish gross and microscopic changes in the mesentery of the small intestine of cats with infectious peritonitis. The research employs gross and histological examination of the mesentery of the small intestine of cats at the infectious peritonitis.

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Slides of the mesentery were stained with haematoxylin and eosin. At dry and mixed forms of infectious peritonitis, gross and microscopic changes in the mesentery of the small intestine of cats are similar. In the mesentery grossly, small white spots were found, which protruded above the general surface and had a homogeneous appearance on the section. Microscopic changes in the mesentery of the small intestine of cats with dry and mixed forms of infectious peritonitis were also similar. When conducting histological studies, it was established that the mesothelium on the surface of the mesentery was necrotized or absent. The submesothelial layer of collagen fibres was necrotized or contained partially lysed and fragmented fibres. The loose fibrous connective tissue of the mesentery was swollen, necrotized in places, and infiltrated by lymphocytes, monocytes, and macrophages. Eosinophilic inclusion bodies were detected in the cytoplasm of some monocytes and macrophages. Foci of adipose tissue in the mesentery of the small intestine were infiltrated by lymphocytes and monocytes. Necrosis and destruction of their walls were found in blood vessels and destruction of endothelial cells in lymphatic vessels. Perivascular lymphoid nodules were markedly enlarged due to their swelling and an increase in the number of cells in them. In perivascular lymphoid nodules, expansion of lymphatic vessels and destruction of part of their endothelium cells were also established. Some of the lymphatic vessels of the mesentery were expanded and filled with lymph, which contained a significant number of lymphocytes, monocytes, and single neutrophils. The materials presented in the article are of practical value for anatomists, histologists and pathomorphologists, as well as for scientists who study the pathogenesis of infectious peritonitis in cats

Keywords: feline coronaviruses; perivascular lymphoid nodules; eosinophilic inclusion bodies; gross changes; microscopic changes

Introduction

Feline infectious peritonitis (FIP) is diagnosed worldwide. Some cat breeds (e.g., Bengals) and certain lines within breeds are more often affected. At the same time, infectious peritonitis is rarely reported in stray and feral cats (Thayer *et al*., 2022). The causative agent is a virus that belongs to the feline coronavirus group, which also includes feline enteric coronavirus (Haake *et al*., 2020). Feline enteric coronavirus is dominant in cat populations. It is low virulent. Infection of animals with this virus occurs through the faecal-oral route and the pathogen primarily enters the intestines. In this case, the disease does not occur at all or manifests itself only in the form of mild enteritis. However, in 4-5% of adult cats and 5-10% of kittens, due to mutations of the enteric coronavirus in the body of a particular infected animal, the pathogen of infectious peritonitis is formed, and this disease occurs. This usually occurs without horizontal transmission of the pathogen. In general, the incidence of infectious peritonitis in cats in their populations is low and rarely exceeds 5% of all cats infected with coronavirus. Kittens are protected from feline coronaviruses by maternal antibodies until 5-6 weeks of age, but after weaning, 90% of kittens exposed to coronaviruses become infected. Approximately 5-12% of these cats develop classic clinical symptoms of FIV (Khalaniia, 2020; Zappulli, 2020).

Feline infectious peritonitis is widespread among different cat populations worldwide. It's in vivo diagnosis is quite complex and is based on a combination of many clinical and laboratory

findings, including the detection of the FIV virus. At the same time, clinical symptoms (anorexia, lethargy, weight loss, pyrexia, ocular and neurological manifestations such as gait abnormalities, etc.) as well as laboratory test results are nonspecific (Felten & Hartmann, 2019). This disease is more commonly reported in animals under the age of one year. The postulated breed predisposition of cats to infectious peritonitis has been identified, as animals genetically related to certain family lines are more likely to be affected. However, in shelters, the disease also occurs in genetically unrelated cats. Factors in the cat's body that contribute to the occurrence of infectious peritonitis include the characteristics of the major histocompatibility complex, the quality of cytokine responses, and some others.

According to M.A. Kennedy (2020), histological examination and immunohistochemistry remain the best tests for the diagnosis of UC. However, intravital biopsy is difficult or impossible in some cases. Therefore, the conclusion about the death of cats from infectious peritonitis is made posthumously in some cases. When diagnosing IPC, it is necessary to keep in mind that tissues that do not replicate this virus contain only small amounts or no pathogen at all. Among the organs with the highest content of the causative agent of UTI are the omentum, mesenteric lymph nodes and spleen. Therefore, these tissues are the most useful for diagnosis. On the contrary, the kidneys, liver, lungs, myocardium, and popliteal lymph nodes contain little or no pathogen. Diagnostics based on the detection of antibodies also do not give a stable and reliable result. For example, the detection of antibodies to feline coronavirus in the CSF of animals with neurological infectious peritonitis was initially considered necessary for diagnosis. However, studies by S. Felten & K. Hartmann (2019) showed that the detection of antibodies in CSF, serum and effusion is not suitable for a definitive in vivo diagnosis. The sensitivity and specificity of antibody detection were compared with histological and immunohistochemical findings as a reference standard, and control groups included cats with only any type of neurological disease or both neurological and non-neurological disorders. A recent study included cats with neurological symptoms that were likely caused by infectious peritonitis, although the diagnosis was not confirmed by histopathological examination. In this study, cerebrospinal fluid from a large number of animals was analysed and the sensitivity of feline coronavirus antibodies for the diagnosis of infectious peritonitis was found to be low. However, it was hypothesised that the detection of feline coronavirus ribonucleic acid in the cerebrospinal fluid could confirm the diagnosis of infectious peritonitis, as it was found in all cats with a cerebrospinal fluid antibody titer of 1:640 or higher. Based on these results, it is concluded that a cerebrospinal fluid antibody titer of 1:640 or higher can be used to diagnose this infectious disease. However, these results are of limited value because control cats were not included in the study and the diagnosis of infectious peritonitis was not confirmed.

The international literature describes macroscopic and microscopic changes in spontaneous IPC and its experimental reproduction (Lisova & Kotliarov, 2022). All identified changes are summarised in several review studies (Thayer *et al.*, 2022). However, morphological changes in the intestinal mucosa of cats with infectious peritonitis have not been studied before. Currently, in human medicine, the mesentery is considered a separate organ that maintains the systemic continuity of all abdominal and pelvic organs. According to the currently accepted mesenteric model of abdominal anatomy, all abdominal and pelvic organs are organised into 2 domains: mesenteric and non-mesenteric. This model explains the

positional anatomy of all abdominal and pelvic organs and the associated vascular system. The microscopic structure of the mesentery is not fully understood. It has been established that this organ in humans and animals of some species is a mesothelium-covered lattice of loose fibrous connective tissue, with adipocyte populations in the cells. The loose fibrous connective tissue of the mesentery is made up of bundles of collagen elastic fibres and fibroblasts. The mesentery also contains blood vessels, lymphatic vessels, nerves, and perivascular lymph nodes (Coffey *et al.*, 2020). In modern human anatomy, the mesentery is divided into upper, middle, and lower sections (D'Souza *et al.*, 2020). Based on the abovementioned, the research aims to investigate morphological changes in the mesentery of cats that died from dry and mixed forms of infectious peritonitis.

Materials and Methods

The research was carried out at the Kasyanenko Department of Anatomy, Histology and Pathomorphology of Animals, Faculty of Veterinary Medicine, National University of Life and Environmental Sciences of Ukraine in 2019-2022. Histological examinations of the small intestine mesentery of 27 cats of different breeds and ages that died from the mixed form of the disease and 7 cats of different breeds and ages that died from the dry form of the disease were performed. During the studies, access to the abdominal organs was obtained by cutting the abdominal wall along the midline of the abdomen. The small intestine was removed along with the mesentery. The mesentery of the small intestine of each cat was examined and pieces of mesentery measuring 2 × 2 cm were cut out with scissors at an equal distance from the small intestine and mesenteric lymph nodes: in the duodenum – 1 piece (from the middle part of this intestine), in the jejunum – 3 pieces (from the middle part of the cranial, middle and caudal thirds), in the ileum – 1 piece (from the middle part of this intestine).

All selected pieces of the small intestinal mesentery were fixed in formalin for 5 days, then rinsed under running tap water for 24 hours, dehydrated in 60°, 70°, 80°, 96° and 100° ethanol, kept in each of them for 24 hours, and then embedded in paraffin. When embedded in paraffin, the pieces of small intestinal mesentery were first transferred from 100º ethanol to a mixture of 100º ethanol and chloroform 1:3 for 4 hours, then to a mixture of 100º ethanol and chloroform 1:1 for 4 hours, and then to chemically pure chloroform for 12 hours at room temperature. After that, the pieces of the mesentery of the small intestine were transferred to a mixture of chemically pure chloroform and paraffin 1:1 at 37°C for 12 hours in a thermostat TSO-80 (Ukraine), and later to molten paraffin at 56°C for 6 hours in a thermostat TSO-80 (Ukraine). After that, they were poured into a new portion of molten paraffin and cooled at +8°C until the paraffin completely solidified. Sections of the mesentery of the small intestine (9±2 µm thick) were obtained in cross-section on a sledge microtome MS-2 (Ukraine), after which they were stained with haematoxylin and eosin (Goralskij *et al.*, 2011). Histological sections were photographed under an MC 100 LED microscope (Austria) using a Canon EOS 550D camera (China). All experimental studies were conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European convention..., 1986).

Results and Discussion

In both dry and mixed forms of UC, small white spots were found in the thickness of the small intestinal mesentery, which protruded above the general surface of the mesentery, had a homogeneous appearance in the section and were located far from the mesenteric lymph nodes (Figs. 1, 2). Considering the presence of

these spots, a histological examination of the areas of the mesentery of the small intestine located far from the mesenteric lymph nodes was performed. According to the histological studies, it was found that the mesentery of cats is covered with a serous membrane represented by a layer of mesotheliocytes. Currently, as noted by J.C. Coffey & and D.P. O'Leary (2017), mesotheliocytes are an almost unexplored pool of stem cells.

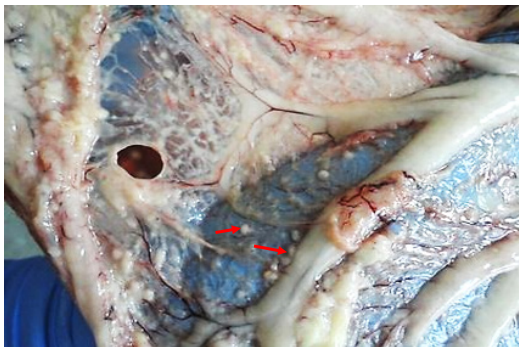

Figure 1. White spots in the mucosa of the cat's small intestine in the dry form of infectious peritonitis (arrows)

***Source**: developed by the author*

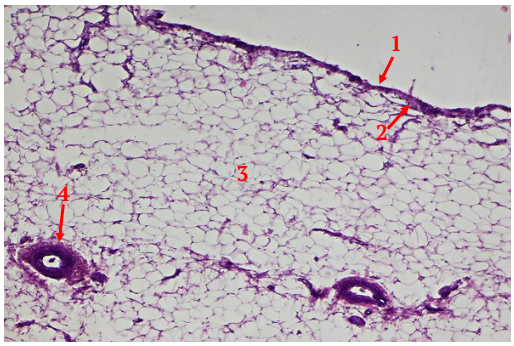

Figure 2. Mesentery of the small intestine of a control cat

***Note:** 1 – mesothelium; 2 – submesothelial layer of collagen fibre bundles; 3 – loose fibrous connective tissue; 4 – artery. Karatsi haematoxylin and eosin, ×100*

***Source**: developed by the author*

The study revealed that the mesothelium covering the mesentery of the small intestine, similar to that covering other internal organs of the abdominal and thoracic cavities, is represented by a single row of highly flattened, elongated cells with a rather large volume of cytoplasm, in the central, slightly thickened part of which there is a nucleus elongated along the long axis of the cell, containing distinctly basophilic condensed chromatin (heterochromatin) and 1-2 nucleoli. The long axis of mesothelial cells on the surface of the mesentery of the small intestine is oriented parallel to the outer surface of this mesentery. This orientation is also characteristic of mesothelial cells covering all abdominal organs (Fig. 3).

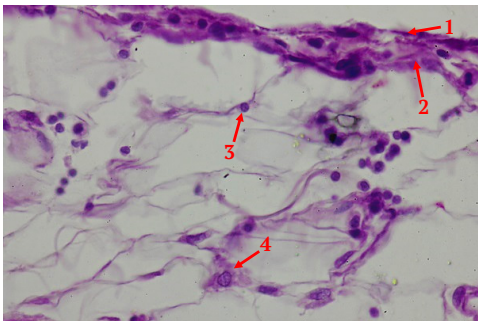

Figure 3. Loose fibrous connective tissue of the mesentery of the small intestine of a control cat
Note: *1 – mesothelium; 2 – submesothelial layer of collagen fibre bundles; 3 – fibrocyte with heterochromatin nucleus; 4 – fibrocyte with euchromatin nucleus. Karatsi haematoxylin and eosin, ×400*
Source*: developed by the author*

Beneath the mesothelium is a thin layer of rather thick and densely packed collagen fibre bundles (Figs. 2, 3). These bundles have a specific packaging. The vast majority of them are thick, densely packed bundles of fibres oriented parallel or almost parallel to the outer surface of the mesentery. These bundles are interconnected by a large number of slightly less densely packed and thinner collagen fibre bundles. It can be assumed that such a microscopic structure of the layer of collagen fibre bundles ensures the integrity and strength of the small intestine mesentery and enables this mesentery to maintain its integrity under various changes in position in the lumen of the abdominal cavity and under physical exertion due to peristaltic movements of the intestine.

Under the layer of collagen fibres, there is a loose fibrous connective tissue, which makes up the bulk of the mesentery tissue (Figs. 2, 3). This tissue is represented by a large number of fibrocytes located quite distantly from each other. These fibrocytes are connected by thin bundles of collagen fibres. Thus, fibrocytes together with collagen fibre bundles form a mesh-like structure with a fairly large mesh. Fibrocytes of the loose fibrous tissue of the mesentery of the small intestine have a typical microscopic structure for these cells. They are cells with a relatively large volume of oxyphilic cytoplasm and many processes of different lengths and thicknesses. In the central part of these cells are round or slightly oval nuclei, which contain mainly basophilic condensed chromatin (heterochromatin) and 1-2 nucleoli. Only in some fibrocytes do the nuclei contain uncondensed chromatin (euchromatin) (Figs. 3, 4).

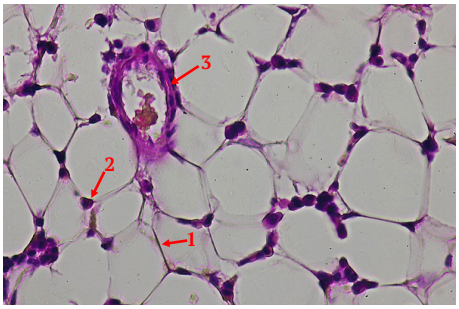

Figure 4. Adipose tissue of the mesentery of the small intestine of a control cat
Note: *1 – lipocyte cytoplasm; 2 – lipocyte nucleus with condensed chromatin; 3 – arterioles. Karatzi's haematoxylin and eosin, ×400*
Source*: developed by the author*

Focal accumulations of adipose tissue are scattered in the loose fibrous connective tissue of the mesentery of the small intestine without any noticeable pattern. This tissue is represented by densely packed fat cells (lipocytes) of rather large size (Fig. 4). They were characterised by a very narrow band of cytoplasm at the cell periphery. This band of cytoplasm was slightly widened in the area of nuclei. Lipocyte nuclei were small in size and, like the nuclei of mesotheliocytes and fibrocytes, contained distinctly basophilic, condensed chromatin (heterochromatin). The large size of mesenteric adipose tissue lipocytes was due to the presence

of a large fat droplet in each of them, which occupied almost the entire cell area in the plane of the histological section. Each accumulation of adipose tissue is separated from the surrounding loose fibrous connective tissue of the mesentery by a thin layer of collagen fibre bundles, among which there were few fibrocytes. These collagen fibre bundles are oriented parallel to the outer surface of each adipose tissue focus. The peculiarities of the microscopic structure of the mesentery of the small intestine of cats allow us to conclude that loose fibrous tissue together with foci of adipose tissue provides both the elasticity of the mesentery and the ability to easily stretch, and the ability to easily change its shape during the movements of the animal's body and the movements of its intestines and other abdominal organs. This microscopic structure of the mesentery of the small intestine of cats is assumed to provide the necessary mobility of the small intestinal loops of these animals. The microscopic structure of the mesentery of the small intestine of cats coincides with its structure described in the literature in other mammals, including humans (Sorenson & Brelje, 2014).

In addition, it was found that regardless of the form of infectious peritonitis, microscopic changes in the small intestinal mesentery of all cats studied were similar and concerned all types of tissues that make up this mesentery. These changes varied in different parts of the small intestinal mesentery, but there was no pattern or correlation between the nature and location of these changes. Mesothelium on the surface of the mesentery of the small intestine of cats killed by infectious peritonitis was necrotic, destroyed and underwent metaplasia in some areas, turning into cubic cells (Fig. 5). In other areas of the mesentery of this intestine, mesothelium was not detected at all (Fig. 6). This is likely caused by the fact that mesotheliocytes in these areas were destroyed in vivo before the cats died and their small intestinal mesentery was sampled for histological examination.

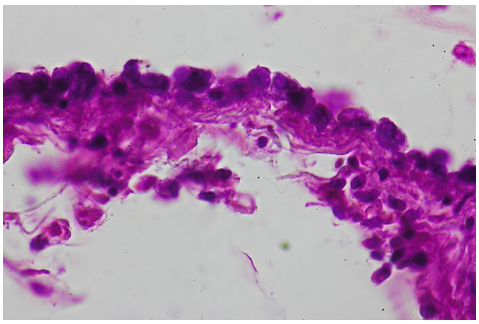

Figure 5. Cat's small intestine mesentery (a mixed form of infectious peritonitis)

Note: *1 – necrosis of mesothelium; 2 – destruction of mesothelium; 3 – cubic mesothelium; 4 – mesenteric edema Hematoxylin and eosin, ×400*

Source*: developed by the author*

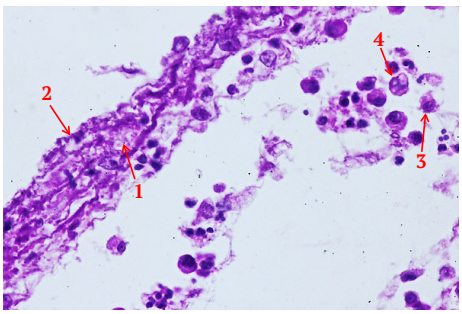

Figure 6. Cat's small intestine mesentery (dry form of infectious peritonitis)

Note: *1 – fragmentation and partial lysis of submesothelial collagen fibre bundles; 2 – absence of mesothelium on the outer surface of the mesentery; 3 – destruction of the monocyte; 4 – eosinophilic inclusion body in the vacuolated cytoplasm of the monocyte; Karatzi's haematoxylin and eosin, ×400*

Source*: developed by the author*

The layer of thick collagen fibre bundles located under the serosa was necrotic in some areas of the small intestinal mesentery, as well as the mesothelium, while in other areas these collagen fibre bundles were unevenly swollen,

partially lysed and fragmented (Fig. 6). The loose fibrous connective tissue underlying the layer of thick collagen fibre bundles was markedly swollen. Some of the cellular and fibrous components of this tissue were necrotic (Fig. 7). At the same time, unevenly granular oxyphilic substance or rather uniform oxyphilic substance with a small number of vacuoles in it was found in place of fibrocytes, and collagen fibre bundles turned into a structureless, heterogeneously stained mass. In many areas, the collagen fibre bundles were broken. The unevenly granular oxyphilic substance in the place of necrotic fibrocytes without any noticeable boundary turned into necrotic collagen fibre bundles, which made it impossible to differentiate the boundaries of the cellular and fibrous components of the loose fibrous tissue of the mesentery of the small intestine in most cases. Only in single necrotic fibrocytes the cell boundaries were visualised. In some places, foci of destruction of loose fibrous connective tissue were detected (Fig. 7). In such areas of the small intestinal mesentery, only small fragments of eosinophilic substance without any specific structure were detected, which were not structurally interconnected. This indicates that individual areas of loose fibrous connective tissue, which had previously undergone necrosis, were destroyed.

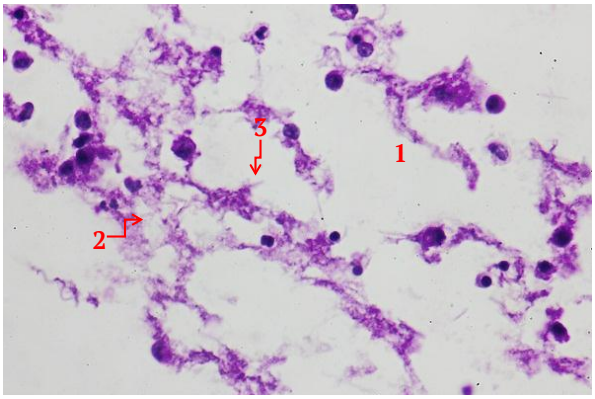

Figure 7. Cat's small intestine mesentery (dry form of infectious peritonitis)
Note: *1 – swelling of loose fibrous connective tissue; 2 – necrosis and destruction of collagen fibre bundles of loose fibrous connective tissue; 3 – necrosis of fibrocyte. Karatsi haematoxylin and eosin, ×400*

The loose fibrous connective tissue of the mesentery of the small intestine of cats that died as a result of infectious peritonitis was also unevenly infiltrated with lymphocytes and monocytes. These cells in the loose fibrous connective tissue were located both singly and in the form of clusters containing a different number of cells (Fig. 8). In this case, especially large clusters of these cells were located near the blood and lymphatic vessels of the mesentery. It should be emphasised that monocytes predominated in the cellular infiltrate in the loose fibrous tissue of the mesentery of the small intestine. Some monocytes were transformed into macrophages (Fig. 9). Since other researchers have found that such transformation occurs under the influence of various antigenic stimuli (Italiani & Boraschi, 2014; Yang *et al.*, 2014; Mysore *et al.*, 2022), the monocytes in the mesentery were in contact with the IPC virus or received appropriate signals from lymphocytes.

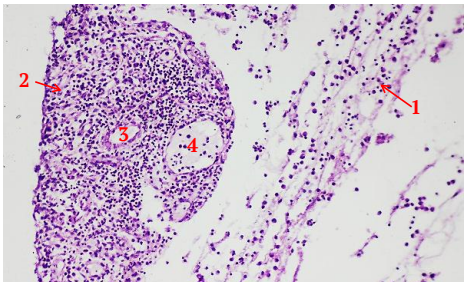

Figure 8. Cat's small intestine mesentery (dry form of infectious peritonitis)
***Notes**: 1 – infiltration of loose fibrous connective tissue with lymphocytes and monocytes; 2 – perivascular lymphoid nodule; 3 – venule; 4 – dilated lymphatic vessel. Karatsi haematoxylin and eosin, ×100*
***Source**: developed by the author*

A small number of small lymphocytes were detected between monocytes and macrophages infiltrating the loose fibrous connective tissue of the small intestinal mesentery (Fig. 9). At the same time, in addition to monocytes, macrophages and lymphocytes, neutrophils were also present in small numbers in the cellular infiltrate near the blood and lymphatic vessels of the small intestine mesentery. According to the results of microscopic studies, in the mesentery of the small intestine of cats killed by infectious peritonitis, vacuolation of the cytoplasm of some monocytes and macrophages infiltrating the loose fibrous connective tissue was also recorded (Fig. 9). According to S. Ohkuma & B. Poole (1981), such cytoplasmic vacuolation may indicate the activation of lysosomes of these cells as a result of their response to an antigenic stimulus or as noted by K. Miyata & K. Takaya (1983), about the accumulation of phagocytosed substances in the cytoplasm of monocytes and macrophages of the small intestine mesentery. The latter was primarily confirmed by the fact that according to the results of this work, phagocytosed basophilic material was found in the cytoplasm of some monocytes and macrophages (Fig. 10). The nature of this material could not be determined, as this requires electron microscopic examination. However, it can be assumed that these phagocytes phagocytosed fragments of destroyed cells and fibrous components (primarily collagen fibres) of the small intestinal mesentery tissues.

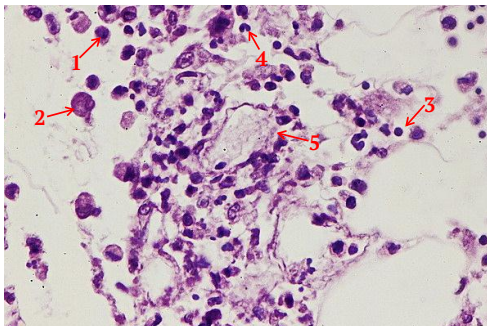

Figure 9. Accumulation of cells in the loose fibrous tissue of the mesentery of the cat's small intestine (a mixed form of infectious peritonitis)
***Note**: 1 – monocyte; 2 – macrophage; 3 – lymphocyte; 4 – segmented neutrophil; 5 – destruction of the lymphatic vessel wall. Karatsi haematoxylin and eosin, ×400*
***Source**: developed by the author*

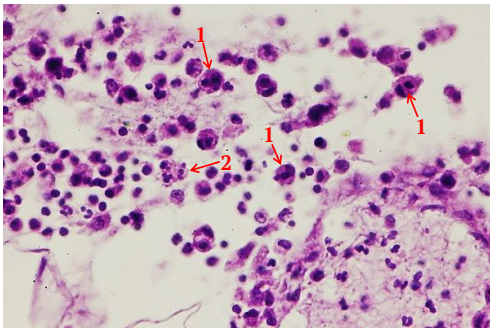

Figure 10. Accumulation of cells in the loose fibrous tissue of the mesentery of the cat's small intestine (dry form of infectious peritonitis)
***Note:** 1 – macrophages with phagocytosed basophilic material in the cytoplasm; 2 – apoptotic cells. Karatsi haematoxylin and eosin, ×400*
***Source**: developed by the author*

Some monocytes and macrophages were destroyed, resulting in the formation of apoptotic cells in their place (Fig. 10). The destruction of these cells is due to their direct damage by the infectious peritonitis virus. K. Shirato *et al*. (2018) found that this coronavirus is capable of infecting monocytes and macrophages, multiplying in these cells, and it is likely that the S gene mutations of the feline coronavirus occurred in these cells. Specific mutations of the S gene were identified during the full sequencing of the feline coronavirus genome. At the same time, other authors (Lewis *et al*., 2015) found that most feline coronaviruses of the pathotype that causes infectious peritonitis to have a mutation in a nucleotide position associated with a change in amino acids in the S protein putative fusion peptide and a substitution that leads to a change in the amino acid in the heptad repeat 1 (HR1) region. This can lead to a change in the fusogenic activity of protein S and, thus, to a change in cellular tropism.

According to the study, eosinophilic inclusion bodies were found in the cytoplasm of some monocytes and macrophages (Fig. 6). At present, domestic researchers consider such inclusion bodies to be microscopic changes specific to feline infectious peritonitis (Khalaniia, 2020), although it has not been confirmed that such inclusion bodies contain viral antigens or the feline infectious peritonitis virus itself. Other authors have found that viral inclusion bodies (also called viral non-proliferative complex or viral replication factories) are specialised structures that are built from viral and some cellular proteins and are a platform for viral replication (Zhang *et al*., 2017; Dolnik *et al*., 2021). Given these data, the presence of eosinophilic inclusion bodies in the cytoplasm of some monocytes and macrophages may indicate the replication of the causative agent of feline infectious peritonitis in these cells, although this is not direct evidence of replication of this particular virus, such as immunological methods of research.

At the same time, according to the available literature, eosinophilic inclusion bodies in the cytoplasm of cells in infectious peritonitis have not been studied. However, according to the world literature, in the case of the severe acute respiratory syndrome coronavirus (SARS-CoV), protein N was isolated from inclusion bodies (Li *et al*., 2021). As established by C. Chang *et al*. (2014), this protein is a nucleocapsid phosphoprotein that packages the viral genome into a helical ribonucleocapsid (RNP) and plays a fundamental role in virus self-assembly. The structures of this ribonucleoprotein (RNP) inside the virions of the causative agent of severe acute respiratory syndrome were also visualised.

The accumulation of adipose tissue in the mesentery of the small intestine in all cats that died as a result of infectious peritonitis was also unevenly infiltrated with lymphocytes and monocytes (Fig. 11). However, in contrast to the loose fibrous connective tissue of the mesentery, the transformation of monocytes into macrophages was not detected in this tissue. At the same time, the largest number of lymphocytes and monocytes in the foci of adipose tissue of the mesentery of the small intestine was localised in its peripheral parts, while the middle and peripheral areas of these foci were infiltrated with a significantly smaller number of lymphocytes and monocytes. From this, it can be concluded that lymphocytes and monocytes penetrated the foci of adipose tissue of the mesentery of the small intestine of patients with UC from the surrounding loose fibrous connective tissue.

In the blood vessels (arteries and veins) of the mesentery, necrosis, and destruction of their walls, as well as destruction of lymphatic endothelial cells were detected in all the studied cases (Fig. 12). A. Kipar & M. Meli *et al*. (2004)

found that coronavirus infection in cats leads to monocyte-associated viremia and the formation of circulating immune complexes. Foreign researchers have also found that circulating immune complexes can be deposited in the subendothelial region and damage endothelial cells, causing autoimmune vasculitis (Wang & Law, 2019), and endothelial cell damage occurs as a result of the interaction of immune complexes with Fcgamma receptors (FcγR) expressed on inflammatory cells. At the same time, nitric oxide (NO) induced by immune complexes is one of the main mediators of inflammation that contributes to vascular inflammation and endothelial cell death in immune complex vasculitis (Mula *et al.*, 2016). However, according to other authors (Xu *et al.*, 2022), endothelial cell damage is associated not only with the deposition of immune complexes, but also with complement activation, inflammatory factors and chemokines, oxidative stress, haemodynamic, and coagulation factors.

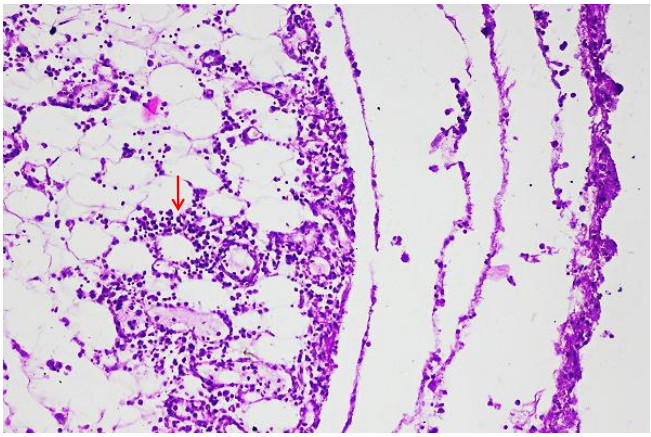

Figure 11. Cat's small intestine mesentery (dry form of infectious peritonitis)
Note: *infiltration of mesenteric adipose tissue by lymphocytes and monocytes (arrow). Hematoxylin and eosin, ×50*
Source*: developed by the author*

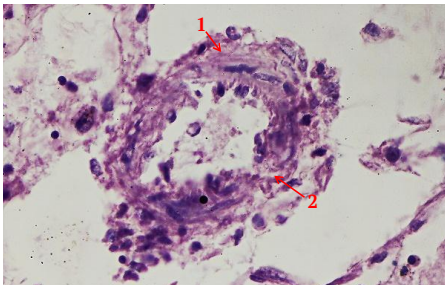

Figure 12. Cat's small intestine mesentery (dry form of infectious peritonitis)
Note: *1 – necrosis of the artery wall; 2 – destruction of the artery wall. Karatsi haematoxylin and eosin, ×400*
Source*: developed by the author*

In addition to other tissues, perivascular lymphoid nodules were found in the mesentery of cats outside the mesenteric lymph nodes (Fig. 8). These nodules in the mesentery of the small intestine of cats are localised around venules and postcapillaries and consist mainly of lymphocytes and a much smaller number of monocytes. Such microscopic structure of perivascular lymphoid nodules in the mesentery of the small intestine of cats corresponds to the microscopic picture of similar nodules in the mesentery and other mammalian organs previously described by researchers (Sorenson & Brelje, 2014; Evtushenko *et al.*, 2019).

In cats that died from infectious peritonitis, a distinct increase in perivascular lymphoid nodules of the small intestine was found. This increase is due to both swelling of these nodules and an increase in the number of cells in them. In the perivascular lymph nodes, lymphatic vessels were also found to be dilated and some of the endothelial cells of blood and lymphatic vessels were destroyed. Some of the lymphatic vessels of the small intestine mesentery, which ran both in the loose fibrous connective tissue and in the peripheral parts of adipose tissue foci and around which there were no perivascular lymph nodes, were distinctly dilated and filled with lymph, which contained a significant number of lymphocytes, monocytes, and single neutrophils (Fig. 13). Hypertrophic perivascular nodules together with distinctly dilated and lymphatic vessels filled with lymph formed the above-described macroscopically visible white spots on the intestinal mucosa of cats that died from infectious peritonitis.

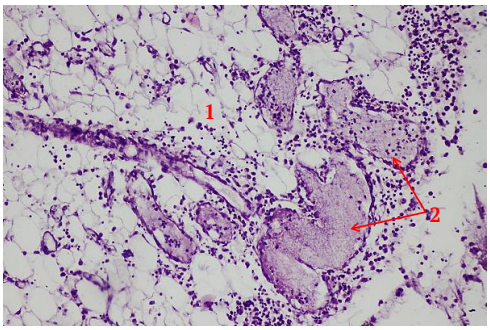

Figure 13. Cat's small intestine mesentery (a mixed form of infectious peritonitis)

Note: *1 – adipose tissue infiltrated with inflammatory cells; 2 – distinctly dilated lymphatic vessels. Karatsi haematoxylin and eosin, ×100*

Source*: developed by the author*

The changes in the small intestine mucosa of cats with infectious peritonitis established by this study significantly complement the previously described results on pathomorphological changes in cats with this disease, published in different chronological periods: from 1960 to 1979 (Wolfe & Griesemer, 1966; Ward & Pederson, 1969; Hayashi *et al*., 1977), at the beginning of 80s (Hayashi *et al*., 1980; Weiss *et al*., 1981; Weiss & Scott, 1981), from end of 80s to end of 90s (Boudreaux *et al*., 1989; Pedersen, 1995) and in different years of the XXI century (Pedersen, 2009; Kipar & Meli, 2014). The data obtained in this study are the basis for a deeper understanding of the processes that occur in the abdominal cavity of cats with UTI and lead to the accumulation of fluid in it.

Conclusions

For the first time, the peculiarities of the microscopic structure of the mesentery of the small intestine of cats were clarified and morphological changes in this mesentery were described in animals that died from dry and mixed forms of infectious peritonitis. Mesentery of the small intestine of cats was found to be covered with mesothelium of typical microscopic structure. The mesothelium layer was supported by a layer of thick collagen fibre bundles, which ensured the strength of the small intestinal mesentery. Under the layer of thick collagen fibre bundles was a loose fibrous connective tissue, which constituted the bulk of all tissues of the small intestine mesentery. This tissue contained nests of adipose tissue and perivascular lymphoid nodules. These nodules were formed around venules and postcapillaries and consisted of lymphocytes and monocytes. In dry and mixed forms of infectious peritonitis, necrosis and destruction of mesothelial cells and the layer of thick collagen fibre bundles located under them were microscopically detected in the mesentery of the small intestine of cats. The loose fibrous connective tissue of the small intestine mesentery was distinctly swollen and infiltrated with lymphocytes and monocytes.

Some monocytes transformed macrophages. Eosinophilic inclusion bodies were detected in the cytoplasm of some monocytes and macrophages, indicating replication of the virus in the cells. The adipose tissue of the mesentery of the small intestine was also unevenly infiltrated with lymphocytes and monocytes. The largest number of these cells was localised in the peripheral parts of the adipose tissue nests. There was no transformation of monocytes into macrophages in adipose tissue. Some lymphatic vessels of the mesentery, around which there were no perivascular lymphoid nodules, were distinctly dilated and filled with lymph, which contained a significant number of lymphocytes, monocytes, and single neutrophils. The perivascular lymphoid nodules were markedly enlarged due to their significant swelling and an increase in the number of lymphocytes and monocytes. Such enlarged perivascular nodules, together with severely dilated lymphatic vessels, looked like mesenteric granulomas at pathological autopsy.

In the future, it is necessary to establish macroscopic and microscopic changes in the colonic mucosa of cats with infectious peritonitis.

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Conflict of Interest

None.

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Морфологічні зміни в брижі тонкої кишки котів за інфекційного перитоніту

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Анотація. Актуальність дослідження зумовлена тим, що морфологічні зміни в брижі котів за інфекційного перитоніту до цього часу не вивчені, але їх розуміння необхідне для пояснення механізму розвитку основного симптому хвороби – випоту рідини в черевну порожнину. Мета роботи – встановити макроскопічні і мікроскопічні зміни в брижі тонкої кишки котів за інфекційного перитоніту. Методами роботи є макроскопічне вивчення та гістологічні дослідження брижі тонкої кишки котів за інфекційного перитоніту. Зрізи брижі фарбували гематоксиліном та еозином. З'ясовано, що за сухої і змішаної форм інфекційного перитоніту котів макроскопічні та мікроскопічні зміни в брижі тонкої кишки подібні. У брижі макроскопічно виявлялися невеликі білі плями, які випинали над загальною поверхнею і мали однорідний вигляд на розрізі. Мікроскопічні зміни в брижі тонкої кишки котів за сухої і змішаної форм інфекційного перитоніту не відрізнялися. Під час гістологічних досліджень відмічали, що мезотелій на поверхні брижі некротизований чи відсутній. Субмезотеліальний шар колагенових волокон некротизований або містив частково лізовані чи фрагментовані волокна. Пухка волокниста сполучна тканина брижі була набрякла, місцями некротизована та інфільтрована лімфоцитами, моноцитами і макрофагами. В цитоплазмі частини моноцитів і макрофагів виявляли еозинофільні тільця-включення. Скупчення жирової тканини в брижі тонкої кишки виглядали інфільтрованими лімфоцитами й моноцитами. У кровоносних судинах виявляли некроз і руйнування їх стінок, а в лімфатичних судинах – руйнування клітин

ендотелію. Периваскулярні лімфоїдні вузлики також відзначалися збільшенням розмірів внаслідок їх набряку і збільшення в них кількості клітин. У периваскулярних лімфоїдних утвореннях встановлено розширення лімфатичних судин і руйнування частини клітин їх ендотелію. Частина лімфатичних судин брижі виразно розширена й переповнені лімфою, яка містила значну кількість лімфоцитів, моноцитів та поодинокі нейтрофіли. Представлені результати становлять практичну цінність для анатомів, гістологів і патоморфологів, а також для науковців, які вивчають патогенез інфекційного перитоніту котів

Ключові слова: коронавіруси котів; периваскулярні лімфоїдні вузлики; еозинофільні тільця-включення; макроскопічні зміни; мікроскопічні зміни

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Peculiarities of organometry and morphoarchitectonics of the heart of the Domestic ram (*Ovis aries L.*, 1758)

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Abstract. Today, cardiovascular diseases are the dominant diseases of animals, which most often lead to their death both in Ukraine and worldwide. Therefore, morphological studies of the heart at the cellular, tissue, and organ levels are essential in clinical cardiology for in vivo ultrasound diagnosis of functional and organic heart lesions. The aim of the study was to perform a histological and cytomorphometric assessment of the morphological structures of the

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sheep heart using macroscopic, histological, and morphometric methods. Using morphological methods, the hearts of sexually mature clinically healthy animals (n=5) belonging to the class *Mammalia* – Mammals, species *Ovis aries L.*, 1758 – domestic sheep (ram) were studied. The absolute and relative heart weights of mature sheep were determined, which are, respectively, 208.4±9.82 g and 0.44±0.007%, and the weight without epicardial fat is 175.0±8.17 g. The heart of sheep, according to the development index (145.5±4.02%), belongs to the expanded-shortened anatomical type. The most developed components are the ventricles. At the same time, the ratio of the mass of the heart ventricles to its net mass is 1:0.78, respectively, the ratio of the mass of the atria is 1:0.22, and the ratio of the mass of the atrial myocardium to the mass of the ventricular myocardium is 1:0.29. The largest volume was found in the cardiomyocytes of the left ventricle – 3982.99±423.96 μm^3, the smaller – in the right ventricle – 2463.02±318.04 μm^3 and the smallest – in the atrial cardiomyocytes – 1215.93±176.94 μm^3. The volumes of cardiomyocyte nuclei in the left (53.42±5.18 μm^3), right (52.85±4.33 μm^3) ventricles and atria (50.16±4.57 μm^3) were almost identical. Nuclear-cytoplasmic ratio was the lowest in cardiomyocytes of the left ventricle – 0.0136±0.0062, slightly higher in cardiomyocytes of the right ventricle – 0.0219±0.0079 and the highest – 0.0430±0.0096 in atrial cardiomyocytes. The obtained results on the macro- and microscopic structure of the heart of bighorn sheep (*Ovis aries L.,* 1758) complement the information on the morphology of the sheep heart in the relevant sections of histology and comparative anatomy and are necessary for clinical cardiology

Keywords: morphology of organs; domestic animals; histostructure; development index; myocardium; cardiomyocytes

Introduction

The cardiovascular system is one of the most important systems of a living organism, which includes the heart, blood and lymphatic vessels that are systemically interconnected (Raiola *et al.*, 2023). It is a system that performs a number of vital functions, including regulating blood supply to organs, ensuring blood pressure in the body and the outflow of lymph from organs and its transport to veins, and contributing to the functions of the immune defence, nervous and endocrine systems (Khomych *et al.*, 2020). Thus, it plays an important role and participates in the regulation of homeostasis in the animal body and is one of the integrating systems of all living organisms. The heart is the central organ of the cardiovascular system in humans and animals. Due to the constant contraction of myocardial cardiomyocytes, the heart drives blood flow in a closed system of blood vessels (Somberg, 2020). The study of the structural features of the internal structures of the heart is considered to be a relevant and essential link for the development of national morphology (Rykiel *et al.*, 2020). This explains why veterinary cardiology, which studies diseases of the heart and blood vessels of animals, as well as cardiovascular surgery, are currently among the priority areas that are actively developing in veterinary medicine (Frąk *et al.*, 2022; Pilz *et al.*, 2022).

The sheep, as an experimental animal in this area of research, is often used as a model used in biomedical research and is perhaps one of the most influential models of human organ systems. At the same time, morphometric studies of organs and systems in clinically healthy

animals are a priority for timely and reliable diagnosis of diseases of various geneses, which are diagnostic criteria as indicators of normalcy for the diagnosis of diseases of contagious and non-contagious pathology (Hryhorieva & Cherniavskyi, 2018a; Shevchenko, 2018). The mathematical analysis of the structures of morphological objects has been recognized as a modern method that is distinguished by objectivity and reliability, which allows for a deeper understanding of the development of the pathological process and logical interpretation of the results of scientific research D. Baldridge *et al*. (2021) and I. Chala *et al*. (2021). This area is widely used in modern veterinary cardiology and provides objective information on the course of various physiological and pathological processes that occur in the organs and systems of the body due to damage to the cardiovascular system.

Currently, research of this nature is extremely relevant and is fundamental for the development, improvement, and application of new methods of prevention and treatment of cardiac diseases, which will be aimed at improving the quality of life of animals, including humans. The aim of the study was to investigate the morphological parameters of the heart of domestic sheep (*Ovis aries L.*, 1758) at the organ, tissue and cellular levels.

Literature Review

Over the past decades, many fundamental works have been published summarizing modern concepts and achievements of morphological and functional regularities in the structure and development of the heart in domestic animals of the mammalian and bird classes, structural components of its wall, etc. (Shevchenko, 2018; Hryhorieva & Cherniavskyi, 2018b), which is important for clinical cardiology to establish diagnoses in pets of certain species, including humans.

The study of the structure of the heart and its components in the comparative species and age aspects in different animals is devoted to the work of D. Solc (2007) and O.B. Slabyi *et al*. (2017). However, the literature often contains information on the structure of the heart mainly during the period of intrauterine development of animals or in laboratory, domestic animals and humans, less in newborn domestic maturational animals (Lelovas *et al*., 2014; Slabyi, 2017). Thus, recent studies on the cardiovascular system of vertebrates, including domestic animals, have led to the discovery of new, previously unknown facts that require further in-depth study of the heart and its structures in comparative anatomical, species, breed, and age aspects (Stakhurska & Pryshliak, 2014).

Despite the fact that in domestic animals, morphological and haemodynamic parameters of the normal heart have a fairly wide range of fluctuations and constitute a certain number of studies of the cardiovascular system (Granger, 1998; Duncker & Bache, 2008; Hnatiuk & Slabyi, 2016), there are currently no clear objective morphological characteristics of structural and functional transformations obtained by morphological methods in the species aspect. There are numerous studies on the study of certain morphological aspects of the heart structure and structural features of its structure in domestic animals that have been studied by previous researchers (Anderson & Ho, 2003; Vansiatskaia & Kyrpaneva, 2014). At the same time, as the analysis of domestic and foreign scientific literature shows, the comparative and age-related morphology of the inner surface, its components at the macro- and microscopic level remain extremely poorly understood, so a significant number of issues remain unresolved and debatable in the problem of sheep heart morphology. Particularly poorly understood issues are the study of morphometric parameters of contractile muscle cells of the

heart myocardium (volume of cardiomyocytes, volume of their nuclei and nuclear-cytoplasmic ratio (NCR) of the ventricles and atria, which perform different functions: the atria receive blood returning to the heart from the body of animals; ventricles pump blood from the heart to the body of animals, while performing the greatest load.

Some authors (Solc, 2007; Sahni *et al*., 2008) have shown a certain dynamism of the heart structure in the prenatal and early stages of postnatal ontogeny in domestic animals. For example, in mammals, as in other vertebrates, the heart has a dilated base and a narrowed apex. In large Mammals, the heart is located in the ventral part of the mediastinum and in most of them, the heart tends to have an elongated apex, except for sheep, in which the heart may have a blunted apex (DiVincenti *et al*., 2014). In such animals, three types of hearts are distinguished: elongated-narrowed, cone-shaped, and dilated-shortened. As for the structure of the myocardium of domestic animals, there is a characteristic sharply expressed reticulation, which is due to the arrangement of fibres of the myocardium formed by cardiomyocytes, where thick layers of connective tissue are visible between the intermuscular fibres. The transverse striation of cardiomyocytes and insertion discs between myocardial contractile myocytes in cattle are poorly visible (Emam & Abugherin, 2020). Such characteristics of the heart structure, according to the literature analysis, are ambiguous, especially the dynamics of external parameters of the heart in sheep, its mass, internal architecture in the species age, etc. need to be clarified.

Therefore, the studies conducted by the authors on the structure of the heart and its morphometric assessment of morphological structures in sheep are important indicators of accurate morphological criteria at the macro- and microscopic levels, which is of exceptional interest for biology, medical and veterinary practice. In particular, such data on the regularities of the structure and formation of the structural components of the heart in the normal state can be used in the diagnosis of diseases of various geneses in domestic animals.

Materials and Methods

In this study, sexually mature clinically healthy animals belonging to the class *Mammalia* – Mammals, species *Ovis aries L.*, 1758 – domestic sheep (ram) were investigated. The experimental animals were selected according to the principle of analogues, weight, breed, and age. Based on the objectives of the research, the following stages were performed: anatomical dissection of the hearts of the studied animals, description of the shape, structure and topography, determination of the absolute and relative weight of the heart, its components, microscopic examination, organ and cytometric parameters of the organ. 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 Research and other Scientific Purposes (1986, March) and the Law of Ukraine No. 3447-IV "On Protection of Animals from Cruelty" (2006, February).

The sheep were dissected from the thorax and the hearts (n=5) were taken together with the pericardium for further macroscopic examination and organometric analysis, which included determination of the heart shape, location in the thoracic cavity, absolute and relative mass, height, width, and circumference. The absolute weight of the heart and its ventricles and atria was determined by weighing it on a laboratory balance, RADWAG PS 6000/C/2 (Poland). Relative mass of heart (RM) was calculated by the formula (1):

$$RH = \frac{\text{AM}}{\text{MA}} \cdot 100, \qquad (1)$$

where: *AM* is the absolute mass of the heart; *MA* is the mass of the animal.

Determination of the linear parameters of the organ (height, width, and circumference) was carried out by direct measurement. The heart development index (HDI) was determined by the ratio of its total height to its width using the following formula (2):

$$HDI = \frac{HO}{WO} \cdot 100, \quad (2)$$

where: *HO* is the height of the organ; *WO* is the width of the organ.

For histological studies of the heart, general methods of fixation and preparation of histological sections were used (Horalskyi *et al*., 2019). For this purpose, the corresponding pieces of longitudinal and transverse sections of the heart were fixed in a 12% chilled aqueous solution of neutral formalin for 24 hours or more, after which they were embedded in paraffin according to the schemes proposed by L. Horalskyi *et al*. (2019). Paraffin sections of the studied material (10-12 μm thick) were made on a sled microtome MS-2. To study the histological structure of the heart, such sections, after deparaffinisation, were stained with the classical staining method – haematoxylin and eosin (Diapath, Italy, 2020) and the Heidenhain method. The latter staining method allowed for better and clearer differentiation of cardiomyocytes, as well as detection of cardiomyocyte junctions (insertion discs).

Stained histological sections were used to perform histometric studies of the structural elements of the heart wall, including measurements of cardiomyocyte length and width, volume of cardiac cells and their nuclei, and, as a rule, the nuclear-cytoplasmic ratio. These studies were performed by light microscopy using Micros (Austria, 2012) and MBS-10 (Micromed, 1998) microscopes, according to the recommendations set out in L. Horalskyi *et al*. (2019).

The nuclear-cytoplasmic ratio (NCR) of cardiomyocytes was determined by formula (3):

$$NCR = \frac{V(n)}{V(c)-V(n)}, \quad (3)$$

where: *NCR* — nuclear-cytoplasmic ratio; $V(n)$ is the volume of the nucleus; $V(c)$ – cardiomyocyte volume.

The following formula (4) was used to determine the volume of cardiomyocytes:

$$Vc = P \times (B/2)^2 \times A, \quad (4)$$

where: *Vc* – cardiomyocyte volume; *P* – 3.14; B – width (diameter) of the cardiomyocyte; B/2 – cardiomyocyte radius; A is the length of the cardiomyocyte.

Determination of volume of nuclei of cardiomyocytes were determined by formula (5):

$$V(i) = P/6 \times A \times B^2, \quad (5)$$

where: $V(n)$ is the volume of nuclei of cardiomyocyte; *P* – 3.14; *A* is the length of the nuclei of cardiomyocyte; *B* is the width of the nuclei of cardiomyocyte.

The length and width of cardiomyocytes and their nuclei were measured using a microscope eyepiece ruler. Histological terms for structural parts of the organ are given according to the International Veterinary Histological Nomenclature and the International Veterinary Anatomical Nomenclature (Khomych *et al*., 2020).

Histological sections were photographed using a 5.0 megapixel CAM V-200 video camera (InterMed, China, 2017) mounted in a microscope using a ×10; ×40 objective and a ×10 eyepiece. The digital material was processed using variational and statistical methods on a personal computer using Microsoft Excel. The arithmetic mean (M) and the statistical error of the arithmetic mean (m) were determined, the probability of the difference between the arithmetic means of two variation series was

determined by the probability criterion (P) and Student's tables. The difference between two values was considered significant at $P<0.05$, $P<0.01$, $P<0.001$.

Results and Discussion

The sheep heart is a hollow fibro-muscular organ that ensures continuous blood flow through a closed system of vessels. The heart has a conical shape, with a widened base and a narrowed apex (Figs. 1, 2). The organ is located in the mediastinum of the thoracic cavity between both lungs, in the area from the third to the sixth rib cranially of the diaphragm: cranially it reaches the third rib, caudally – the sixth rib. In relation to the median sagittal plane, the heart is displaced to the left by 5/7, adjacent to the left chest wall between the third and fourth ribs. The base of the heart is craniodorsal and is located at the height of the middle of the first or second rib. Accordingly, the apex of the heart is directed caudoventrally and is located opposite the fifth rib cartilage, or caudally from it, not reaching the sternum by two cm, and cranially from the diaphragm – from two to five cm.

The heart is contained in a thin but dense sac – the heart jacket (pericardium). The latter surrounds the organ from all sides, thus forming a closed serous sac, which is attached to the breastbone by two ligaments in the area of the sixth rib cartilage. Externally, the heart of mature sheep is divided into left and right ventricular outer grooves and a septum inside, which are not connected to each other. From the outside, each half of the heart is divided (left and right) into atria and ventricles by a transverse coronary furrow that runs across the heart closer to its base. The atria and ventricles of the same name (right and left) are connected to each other by the atrial-ventricular orifices.

The right and left atria in experimental animals are located at the very base of the heart, where they form bag-like protrusions – the right and left heart ears, which are directed in the cranial direction and are located to the right and left, respectively, of the pulmonary arteries and aorta. The ventricles of the heart occupy the main part of the heart, externally they are separated from each other by the interventricular subaxillary and circumcondylar grooves, which are combined on the cranial surface of the heart, not reaching its apex, separating the right ventricle from the left. The apex of the heart in sheep refers to the left ventricle, which is located to the left in the caudal direction. The right ventricle of the heart, respectively, is located to the right in the cranial direction. The interventricular furrows have a similar location (subaxillary – in the caudal direction, nearcone – in the cranial direction) (Figs. 1, 2).

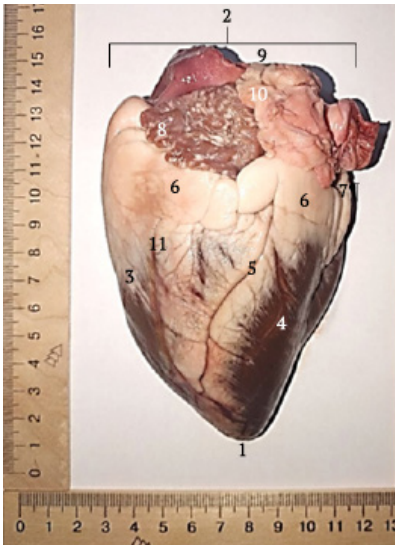

Figure 1. Macroscopic structure of a sheep's heart (left side)

Notes: *Symbols: 1 – apex of the heart; 2 – the base of the heart; 3 – right ventricle; 4 – left ventricle; 5 – semi-conical interventricular groove; 6 – subepicardial fat; 7 – left atrium; 8 – left auricle; 9 – right auricle; 10 – pulmonary trunk; 11 – blood vessels. Photo from macropreparation*

Sourse*: author's development*

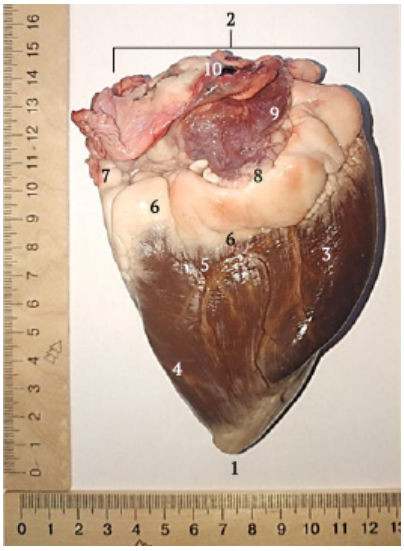

Figure 2. Macroscopic structure of a sheep's heart (right side)

Notes: *1 – apex of the heart; 2 – the base of the heart; 3 – right ventricle; 4 – left ventricle; 5 – axillary interventricular groove; 6 – subepicardial fat; 7 – left atrium; 8 – right atrium; 9 – right auricle; 10 – pulmonary trunk. Photo from macropreparation*

Sourse*: author's development*

The criteria of organ development, which directly indicate its morphological and functional maturity, are absolute and relative weight, its linear parameters, etc. Indicators of morphometric parameters not only indicate the development and morphological and functional maturity of the organ, but also have cognitive value and are the basis for determining the shape, establishing comparative anatomical types of certain organs (Linask, 2003; Belimenko *et al.*, 2021). According to the current studies, the absolute heart weight of mature sheep was 208.4±9.82 g, and the relative weight was 0.44±0.007%. The net heart weight of the experimental animals (without epicardial fat) was 175.0±8.17 g. Moreover, the height of the heart was 13.1±0.4 cm, the width was 9.0±0.3 cm, and the circumference was 22.2±0.6 cm (Table 1).

Depending on the species, breed, and age of the animal, the following heart shapes can be distinguished in domestic mammals: narrowed-elongated (cattle), narrowed-shortened (rabbit), expanded-shortened (horse), and round-oval (dog). In dogs, it can be ellipsoidal (43%), cone-ellipsoidal (24%), ellipsoidal-spherical (26%) and spherical (7%), in cattle it can be elongated-narrowed, cone-shaped and expanded-shortened. Pigs have three main types of hearts: elongated-narrowed, conical; shortened, relatively narrowed; expanded-shortened, triangular (Rudyk, 2004; Demus, 2015). According to the analysis of the morphometry, in terms of linear parameters, the index of sheep heart development was 145.5±4.02%, so the heart of this species of animals is of the expanded-shortened type (Table 1).

Table 1. Linear parameters of the heart of sheep, M±m, n=5

Indicator	Numeric values
The height of the heart, cm	13.1±0.4
The width of the heart, cm	9.0±0.3
Circumference of the heart, see	22.2±0.6
Heart development index, %	145.5±4.02
The average value of the thickness of the wall of the ventricles, mm	12.42±0.17
The thickness of the wall of the left ventricle, mm	16.2±0.22
The thickness of the wall of the right ventricle, mm	8.04±0.11
The average value of the thickness of the atrial wall, mm	6.62±0.43

Table 1. Continued

Indicator	Numeric values
The thickness of the wall of the left atrium, mm	7.05±0.09
The thickness of the wall of the right atrium, mm	5.06±0.07

Source: *author's development*

The most developed anatomical structures of the heart are its left and right ventricles, then the left and right atrium, which correlates with linear indicators of the thickness of their walls and their absolute and relative mass, in relation to the net mass of the heart (without epicardial fat) (Table 1, 2).

Table 2. Morphometry of the heart, ventricles, and atria of sheep, M±m, n=5

Indicator	Absolute mass, g	Relative mass, %
Left atrium	27.9±3.31	15.94±1.49
Right atrium	11.2±2.02	6.4±0.82
Right and left atrium (together)	39.1±4.64	22.34±2.02
Left ventricle	90.3±5.21	51.6±3.06
Right ventricle	45.6±3.04	26.06±1.32
Left and right ventricles (together)	135.9±7.16	77.66±4.36
Heart weight (without apical fat)	175.0±8.17	100
The coefficient of the ratio of the mass of the ventricles to the mass of the heart	1:0.78	
The coefficient of the ratio of the mass of the atria to the mass of the heart	1:0.22	
The coefficient of the ratio of the mass of the myocardium of the atria to the mass of the myocardium of the ventricles	1:0.29	

Source: *author's development*

Thus, the wall thickness of the left ventricle was significantly greater than that of the right ventricle, respectively, by 2.01 times (P<0.01) and was equal to 16.2±0.22 mm, and the right ventricle was 8.04±0.11 mm. At the same time, the thickness of the atrial wall was 6.62±0.43 mm, respectively, of the left atrium – 7.05±0.09 mm, and of the right atrium – 5.06±0.07 mm (Table 1). With such linear parameters of the heart components, the average mass of the left atrium was 27.9±3.31 g (15.94±1.49%), the average mass of the right atrium relative to the left atrium was significantly (P<0.01) 2.5 times less and equalled 11.2±2.02 g (6.4±0.82%). The average weight of the atria of the sheep heart is 39.1±4.64 g (22.34±2.02%). The mass of the left ventricle is the largest and was 90.3±5.21 g (51.6±3.06%), the weight of the right ventricle was intermediate and equalled 45.6±3.04 g (26.06±1.32%), the average weight of both ventricles was 135.9±7.16 g (77.66±4.36%). Consequently, the mass of the ventricles of the sheep heart was significantly (P<0.001) 3.5 times higher than the mass of the atria. Accordingly, the ratio of the mass of the ventricles of the heart of a mature sheep to its net mass (without epicardial fat) was 1:0.78, the ratio of the mass of the atria to its net mass was 1:0.22, and the ratio of the mass of the atrial myocardium to the mass of the ventricular myocardium was 1:0.29 (Table 2).

The wall of the sheep heart is formed by the respective membranes: endocardium – inner, myocardium – middle and epicardium – outer. The main structural component of the ventricular and atrial heart wall is the myocardium, a muscular

membrane consisting of cardiac myocytes (cardiomyocytes). Histological analysis of myocardial sections of the ventricular wall of the heart (left and right) stained with haematoxylin and eosin reveals five gradual layers: the outer and inner layers (their muscle fibres have an oblique longitudinal direction), then the outer and inner deeper layers and the deepest layer, whose fibres have a figure-eight direction (Fig. 3).

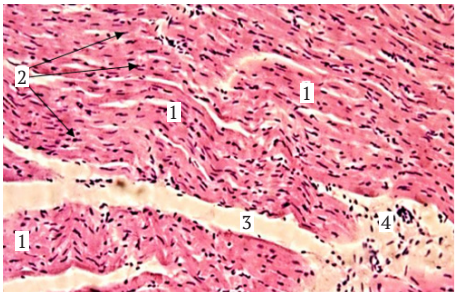

Figure 3. Microscopic structure of the myocardium of the left ventricle of a heart of a sheep

Notes: *1 – muscle fibres (longitudinal section); 2 – nuclei; 3 – intermuscular connective tissue; 4 – microcirculatory vessel. Haematoxylin and eosin. ×120*

Source*: author's development*

The myocardium of the atrial wall is formed by only two layers of muscle membrane – the outer (common to both atria) and the deep. The muscle fibres of the outer layer of the myocardium are located in a transverse direction from the right to the left ear. The muscle fibres of the deep myocardial layer of the right and left atria are located in the longitudinal direction. However, in the area of the myocardial venous orifices, circular bundles of muscle fibres are found. Due to the more intensive development of the ventricular myocardium relative to the atria, the ventricular walls are much thicker relative to the atrial wall, which is associated with their morphological and functional activity. The histoarchitecture of the ventricular and atrial myocardial walls is formed by cardiac striated muscle tissue, which is represented by cardiomyocytes that form muscle fibres and intermuscular layers of loose fibrous connective tissue with the presence of blood and lymphatic vessels and nerves (Figs. 3, 4).

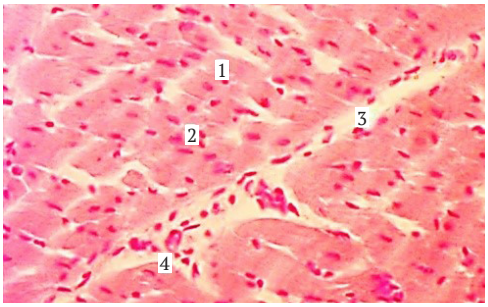

Figure 4. Microscopic structure of the myocardium of the left ventricle of a heart of a sheep

Notes: *1 – muscle fibres (transverse section); 2 – nuclei; 3 – intermuscular connective tissue; 4 – microcirculatory vessel. Haematoxylin and eosin. ×280*

Source: *author's development*

Cross-striated muscle fibres, built from cardiac myocytes (cardiomyocytes), which perceive colour in different ways (Fig. 5).

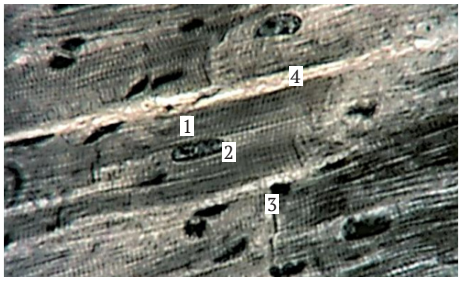

Figure 5. Microscopic structure of the myocardium of the left ventricle of a heart of a sheep

Notes: *1 – cardiomyocytes; 2 – nuclei of cardiomyocytes; 3 – insert disks; 4 – intermuscular connective tissue. Dyeing according to the Heidenhain method. ×600*

Source: *author's development*

Cardiac myocytes in the myocardium form a network of thin and thicker, porosely striated muscle fibres, between which there is a gap space filled with intermuscular connective tissue. The parallel myocardial muscle fibres, formed by cardiomyocytes, are interconnected by anastomoses and form a mesh-like structure, forming a single contractile system of the heart. The central part of the cardiomyocyte sarcoplasm contains one, rarely two nuclei, which are oval or elongated and unevenly spaced. Their nuclear chromatin in the form of small or large grains is located around the perimeter of the caryoplasm (Fig. 5). Cardiomyocytes in the fibre structure, when staining histological specimens using the Heidenhain method, are arranged in a chain, interconnected by insertion discs (Fig. 6). When histological sections are stained with hematoxylin and eosin, cardiomyocytes in the cardiac muscle tissue form histological structures similar to the muscle fibres of somatic muscle tissue.

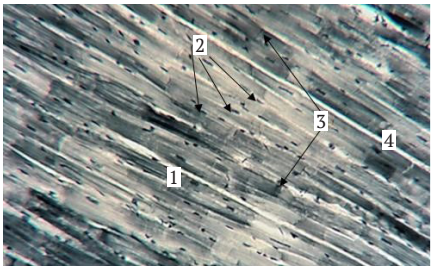

Figure 6. Microscopic structure of the myocardium of the left ventricle of a heart of a sheep

Notes: *1 – cardiomyocytes; 2 – nuclei of cardiomyocytes; 3 – insert discs; 4 – intermuscular connective tissue. Dyeing according to the Heidenhain method. ×280*

Source: *author's development*

This connection of cardiomyocytes with each other by insertion discs, forming muscle fibres, provides a support function for the contractile elements of cardiac cells (myofilaments) and ensures a single contraction of the myocardium and thus forms a functional syncytium. In light microscopy of histological specimens stained by the Heidenhain method, cardiomyocytes in the longitudinal section appear as dark stripes, rectangular (Fig. 7), and in the transverse section – rounded (Fig. 8), so their shape is cylindrical.

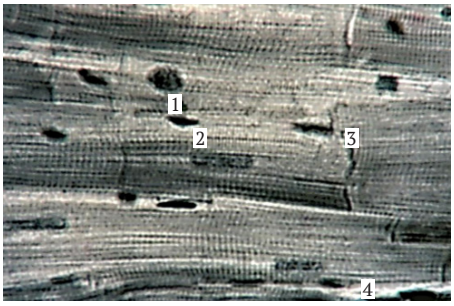

Figure 7. Microscopic structure of the myocardium of the left ventricle of a heart of a sheep

Notes*: 1 – cardiomyocytes; 2 – nuclei of cardiomyocytes; 3 – insert discs; 4 – intermuscular connective tissue. Dyeing according to the Heidenhain method. ×600*

Source: *author's development*

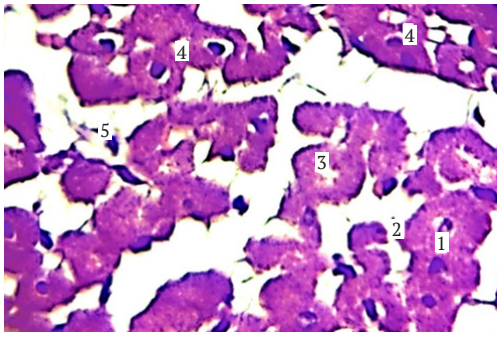

Figure 8. Microscopic structure of the myocardium of the right ventricle of a heart of a sheep

Notes: *1 – cardiomyocytes (transverse section); 2 – sarcolemma; 3 – sarcoplasm; 4 – nuclei of cardiomyocytes; 5 – intermuscular connective tissue. Hematoxylin and eosin. ×400*

Source: *author's development*

In cardiomyocytes with such a histological structure, their sarcolemma, sarcoplasm, nuclei, and myofibrils are distinctly differentiated. They have clearly defined transverse (due to the presence of myofibrils) and longitudinal (due to the presence of actin and myosin proteins) striations (Fig. 9).

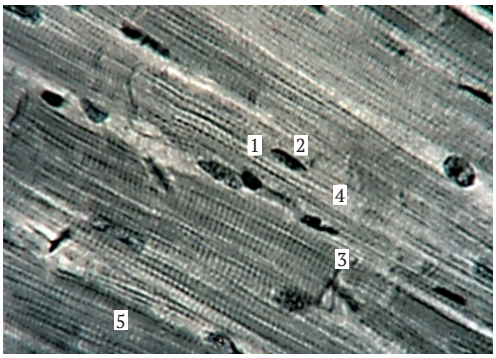

Figure 9. Microscopic structure of the myocardium of the left ventricle of a heart of a sheep

Notes: *1 – cardiomyocytes; 2 – nuclei of cardiomyocytes; 3 – insert disks; 4 – transverse striation; 5 – longitudinal striation. Dyeing according to the Heidenhain method. ×600*

Source: *author's development*

Myofibrils (special-purpose organelles) on the longitudinal section of cardiomyocytes, under a light microscope, look like thin filaments running longitudinally, parallel to each other, and having the length of the cardiomyocytes themselves (muscle fibres) (Fig. 9). In most cases, myofibrils are located along the entire perimeter of the sarcoplasm, which is visible in a cross-section of cardiomyocytes, where they appear as dots of several dozen in one cardiomyocyte (Fig. 10). Special-purpose organelles, often via anastomoses, pass from one fibre to another, thus ensuring the joint contractile function of the cardiac myocardium.

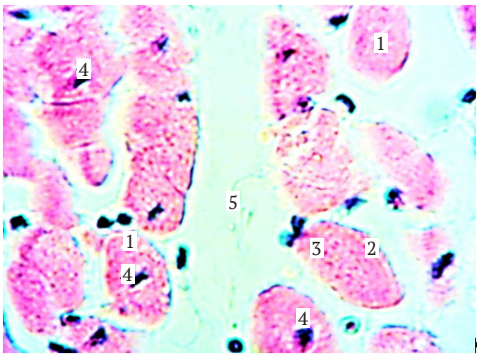

Figure 10. Microscopic structure of the myocardium of the left ventricle of a heart of a sheep

Notes: *1 – cardiomyocytes (transverse section); 2 – sarcolemma; 3 – sarcoplasm; 4 – nuclei of cardiomyocytes; 5 – intermuscular connective tissue. Haematoxylin and eosin. ×600*

Source: *author's development*

Myofibrils that are densely packed in the fibre structure and are located closer to its periphery are connected to other fibres by anastomoses. With a low density of myofibrils, the longitudinal striation of muscle fibres is clearly expressed, and the transverse striation is relatively weak. Thicker muscle fibres are much less susceptible to colour, so their transverse striation is weakly expressed, and myofibrils have a more elegant appearance. In muscle fibres of small thickness, myofibrils are more densely arranged.

In modern morphology, morphometric research methods are widely used, which make it possible to establish the relationship and interdependence of quantitative changes in individual structures of the animal body, quantitative and relative characteristics of certain morphological components at different stages of ontological and phylogenetic development of animals and different functional states of a particular animal body system, depending

on their species characteristics, etc. According to the analysis of literature sources (Demus, 2015; Slabyi *et al*., 2017) and the results of the author's research, the microscopic structure of the sheep heart, its histoarchitectonics of the atria and ventricles have a similar structure, but differ in histo- and cytometric parameters, which make it possible to establish even minor changes in the microscopic structure at the tissue and cellular levels, depending on their functional load and haemodynamics. Thus, according to the results of histometry, cardiomyocytes forming layered muscle fibres, depending on their morphotopography (left, right ventricles, atria), are characterized by ambiguous cytometric parameters (Table 3).

Table 3. Histometric indicators of cardiomyocytes sheep, M±m, n=5

Indexes	Length of cardiomyocytes (μm)	Width of cardiomyocytes (μm)	Volume of cardiomyocytes (μm³)	Volume of nuclei of cardiomyocytes (μm³)	Nuclear-cytoplasmic ratio
Left ventricle	62.92±1.84	8.98±0.64	3982.99±423.96	53.42±5.18	0.0136±0.0062
Right ventricle	49.52±1.62*	7.96±0.56*	2463.02±318.04*	52.85±4.33	0.0219±0.0079**
Auricle	42.04±1.27**	6. 07±0.38*	1215.93±176.94**	50.16±4.57	0.0430±0.0096***

Note: **P<0.05; **P<0.01; ***P<0.001 compared to the left ventricle*

Source*: author's development*

At the same time, the quantitative indicators of contractile myocytes of the left ventricle of the sheep heart myocardium are greater than those in the right: the average length of cardiomyocytes of the left ventricle is significantly (P<0.05) is 1.27 times greater than that of the right ventricle and is equal to 62.92±1.84 μm, the width of cardiomyocytes, respectively (P<0.05) is 1.13 times greater and is 8.98±0.64 μm (Table 3). Similar morphometric characteristics were found in the calculation of cardiomyocyte volumes: the largest volume of cardiomyocytes is characteristic of the left ventricle (3982.99±423.96 μm³), the volume of cardiomyocytes of the right ventricle, compared with the left, is significantly (P<0.05) less by 1.62 times and is equal to 2463.02±318.04 μm³. Similar changes in cytometric parameters were found in determining the volume of cardiomyocyte nuclei: a larger volume of cardiomyocyte nuclei was characteristic of the left ventricle (53.42±5.18 μm³), slightly smaller for the right ventricle (52.85±4.33 μm³) (Table 3; Fig. 11).

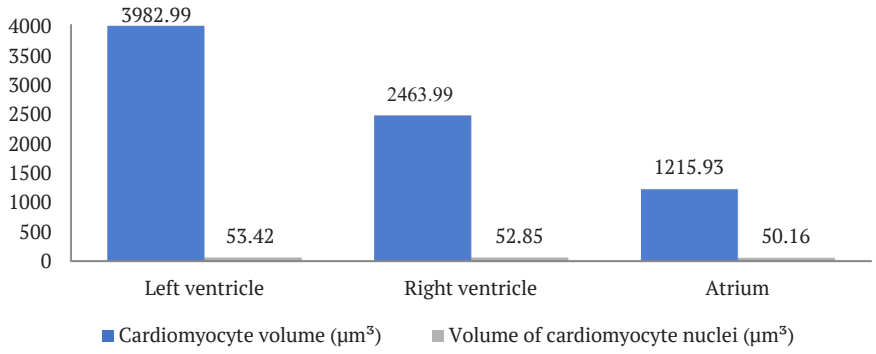

Figure 11. Histometric parameters of cardiomyocytes of heart myocardium of sheep

Source: *author's development*

The revealed ambiguous morphometric parameters of the volumes of cardiomyocytes and their nuclei in the right and left ventricles of the heart lead to different nuclear-cytoplasmic ratios: the lowest nuclear-cytoplasmic ratio is characteristic of cardiomyocytes of the left ventricle (0.0136±0.0062) and significantly (P<0.01) 1.61 times higher for cardiomyocytes of the right ventricle (0.0219±0.0079), which indicates the morphological and functional activity of cardiomyocytes of the left ventricle (Fig. 12).

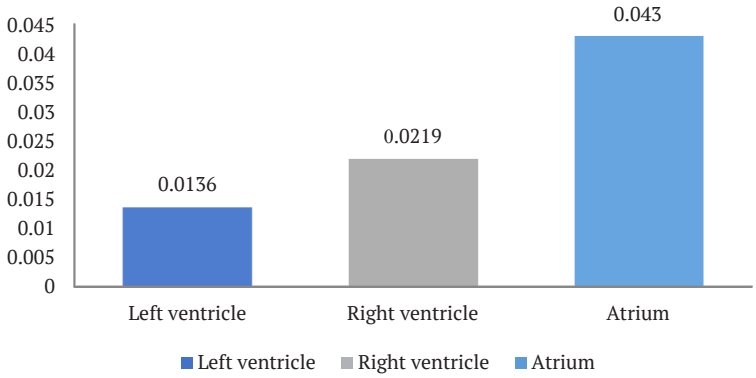

Figure 12. Nuclear-cytoplasmic ratio of cardiomyocytes of heart myocardium of sheep
Source: *author's development*

This should not be considered a coincidence, but an objective and realistic characteristic associated with differences in the activity of the heart ventricles, since the left ventricle functions mainly as a pump, and the right ventricle – as a volume ventricle (Halıgür & Dursun, 2009). Therefore, an increase in cytometric parameters (length, width, volume) and a decrease in the nuclear-cytoplasmic ratio of cardiac cardiomyocytes in the left ventricle myocardium, compared to the right ventricle, are associated with the functional characteristics of the myocardial muscle tissue, which is capable of spontaneous rhythmic contractions, facilitating the movement of blood through the vessels. Accordingly, the cardiac contractile myocytes of the left ventricle of the heart myocardium provide a much higher load, thus promoting blood flow in the vessels of the large (somatic) circulation, and, accordingly, the cardiomyocytes of the right ventricle provide a lower load, supporting blood flow in the vessels of the small (pulmonary) circulation.

The smallest cytometric parameters of the heart structure (length, width, volume) were characteristic of atrial cardiomyocytes, in which the nuclear-cytoplasmic ratio relative to that of the left and right ventricles was significantly (P<0.001) 3.16 and 1.96 times (P<0.01) higher and equalled 0.0430±0.0096 (Table 3; Fig. 12). These cytometric parameters of cardiomyocytes indicated a significantly lower functional load of atrial contractile myocytes compared with ventricular cardiomyocytes. It is known that the most morphologically and functionally active and mature somatic cells in humans and animals are those characterized by a low nuclear-cytoplasmic ratio index and, conversely, cells with a high nuclear-cytoplasmic ratio are less functionally active (Anderson & Ho, 2002). Different cytometric and karyometric parameters of ventricular and atrial cardiomyocytes are

primarily associated with the functional activity of the heart: the atria receive blood returning to the heart from the body of animals, and the ventricles, respectively, pump blood from the heart to the body, performing a significant load.

At the same time, certain characteristics of cardiomyocytes and their nuclei have different meanings, depending on the morphological and functional activity of the myocardial tissue of the ventricles and atria. For example, morphometric parameters (thickness, length, average volume of sarcoplasm and cardiomyocyte nuclei) are larger in the left ventricle than in the right ventricle. Such ambiguous cytometric parameters of the left ventricular myocardium in experimental animals, relative to the right ventricle, are probably related to the activity of the ventricles themselves: the left ventricle functions, in most cases, as a pump, and the right ventricle functions as a volumetric compartment with morphological and functional features of myocardial tissue capable of spontaneous rhythmic contractions, which result in blood flow in the vessels. At the same time, the cardiomyocytes of the left ventricle provide a greater load, promoting blood flow through the vessels of the corporal (large) circulation, and the cardiomyocytes of the right ventricle, respectively, have a much lower load, ensuring blood flow through the vessels of the small circulation (Halıgür *et al*., 2009; Braile, 2013). Thus, the studies have shown that the morphological structure of the sheep heart myocardium has similar macro- and histoarchitectonics to other species of mammals, but differs in morphometric parameters.

Conclusions

A comprehensive morphological study of the heart of the domestic sheep was carried out, and new data on the morphological features and structural organization of the heart in the normal state were obtained. The morphological analysis allowed systematizing, deepening and significantly supplementing the information about the microscopic structure and morphometric parameters of the heart structures of the normal bovine heart and can serve as a basis for further experimental studies of the heart in sheep and humans with various pathological conditions.

The absolute weight of the sheep heart was 208.4±9.82 g, the relative weight was 0.44±0.007%, and the net weight of the heart (without epicardial fat) was 175.0±8.17 g. According to the linear parameters of the heart (height – 13.1±0.4 cm, width – 9.0±0.3 cm, circumference – 22.2±0.6 cm), the index of its development is 145.5±4.02%, so the heart is of the dilated-shortened type. The more developed components of the heart are the ventricles (left and right), then the atria (left and right):

- The mass of the left ventricle is the largest and was equal to 90.3±5.21 g (51.6±3.06%), the mass of the right ventricle was 45.6±3.04 g (26.06±1.32%), the average mass of both ventricles was 135.9±7.16 g (77.66±4.36%);
- The average weight of the left atrium was 27.9±3.31 g (15.94±1.49%), the average weight of the right atrium relative to the left atrium was significantly ($P<0.01$) 2.5 times less and amounted to 11.2±2.02 g (6.4±0.82%), the average weight of both atria was 39.1±4.64 g (22.34±2.02%).
- The ratio of the mass of the ventricles of the sheep heart to its net mass was 1:0.78, the ratio of the mass of the atria was 1:0.22, the ratio of the mass of the atrial myocardium to the mass of the ventricular myocardium was 1:0.29.

The cardiomyocytes of the left ventricle had a larger volume (3982.99±423.96 μm^3), the right ventricle – 2463.02±318.04 μm^3, and the smallest – atrial cardiomyocytes (1215.93±176.94 μm^3). The volumes of cardiomyocyte nuclei in the left (53.42±5.18 μm^3), right (52.85±4.33 μm^3) ventricles and atria

(50.16±4.57 μm³) of the heart were characterized by almost the same values. The nuclear-cytoplasmic ratio was the lowest in cardiomyocytes of the left ventricle – 0.0136±0.0062, significantly (P<0.01) higher in cardiomyocytes of the right ventricle – 0.0219±0.0079) and significantly (P<0.001) the highest in atrial cardiomyocytes – 0.0430±0.0096, which is associated with the physiological characteristics of myocardial muscle tissue capable of spontaneous rhythmic contractions. In the future, it is planned to conduct comprehensive morphological studies of the heart of domestic animals of the class Mammalia – Mammals in a comparative aspect.

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None.

Conflict of Interest

None.

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Особливості органометрії та морфоархітектоніки серця барана свійського (*Ovis aries L.*, 1758)

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Анотація. На сьогодні домінуючими хворобами тварин є серцево-судинні захворювання, які найчастіше призводять до їх загибелі як в Україні, так і в усьому світі. Тому морфологічні дослідження серця на клітинному, тканинному та органному рівнях є необхідними у клінічній кардіології для прижиттєвої ультразвукової діагностики функціональних та органічних уражень серця. Мета роботи – за допомогою макроскопічних, гістологічних, морфометричних методів досліджень здійснити гісто- та цитоморфометричну оцінку морфологічних структур серця овець. З використанням морфологічних методів досліджено серце статевозрілих клінічно здорових тварин (n=5), які належать до класу *Mammalia* – Ссавці, виду *Ovis aries L.*, 1758 – баран (вівця) свійський. Обґрунтовано абсолютну та відносну масу серця статевозрілих овець, що становить, відповідно, 208,4±9,82 г і 0,44±0,007 %, масу без епікардіального жиру – 175,0±8,17 г. Серце овець відповідно за індексом розвитку (145,5±4,02 %) відноситься до розширено-вкороченого анатомічного типу. Розвинутішими складовими є шлуночки. При цьому, коефіцієнт відношення маси шлуночків серця до його чистої маси дорівнює 1:0,78, відповідно коефіцієнт відношення маси передсердь становить – 1:0,22, а коефіцієнт відношення маси міокарду передсердь до маси міокарду шлуночків дорівнює 1:0,29. Найбільший об'єм встановлено у кардіоміоцитів лівого шлуночка – 3982,99±423,96 мкм^3, менший – правого 2463,02±318,04 мкм^3 і найменший – у кардіоміоцитів передсердь – 1215,93±176,94 мкм^3. Об'єми ядер кардіоміоцитів у лівому (53,42±5,18 мкм^3),

правому (52,85±4,33 мкм^3) шлуночках серця та його передсердях (50,16±4,57 мкм^3) майже однакові. Ядерно-цитоплазматичне відношення найменше у кардіоміоцитів лівого шлуночка – 0,0136±0,0062, дещо більше у кардіоміоцитів правого – 0,0219±0,0079 і найбільше – 0,0430±0,0096 у кардіоміоцитах передсердь. Отримані результати щодо макро- та мікроскопічної будови серця барана свійського (*Ovis aries L.*, 1758) доповнюють відомості з морфології серця овець у відповідних розділах гістології та порівняльної анатомії і є необхідними для клінічної кардіології

Ключові слова: морфологія органів; свійські тварини; гістоструктура; індекс розвитку; міокард; кардіоміоцити

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Dogs' general response to babesiosis infection of various severities

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Abstract. Global climate change in recent decades has led to an increase in the activity and expansion of the range of many diseases. One of them is canine babesiosis. Therefore, it is becoming increasingly important to monitor the functional state of the organism in sick dogs and timely detection of kidney, liver, spleen, cardiovascular system, anaemia, and other disorders that complicate the course of babesiosis. The research aims to determine the peculiarities of metabolic and functional changes in dogs with different intensities of babesiosis infection. During the laboratory diagnostics, general and special research methods were used: light microscopy, centrifugation, spectrophotometry, and mathematical statistics. Based on the results of comprehensive microscopic, morphological, and biochemical studies of blood in dogs with different intensities of babesiosis infection, the most characteristic functional and metabolic changes in their body were identified. Thus, at different intensities of babesiosis infection in diseased animals, the features of the haematological profile are leukocytopenia against the background of eosinopenia (at a mild degree of infection) and

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lymphocytopenia with a simultaneous compensatory increase in the number of monocytes and neutrophils, as well as erythrocytopenia, hypochromemia, thrombocytopenia with a decrease in thrombocrit and haematocrit. In addition, these patients developed hyperfermentemia of aspartate and alanine aminotransferases, alkaline phosphatase, and γ-glutamyl transpeptidase, indicating structural and functional changes, primarily in the myocardium, liver, skeletal muscle, brain and kidneys due to their toxic damage by babesia waste products. At the same time, hypoproteinaemia, hypoalbuminemia and hyperazotemia were observed in the affected animals, indicating an increase in the intensity of catabolic processes in functional cells of organs and tissues, especially the liver. The most pronounced changes in the studied parameters were observed at high intensity of babesiosis infection. The established regularities allow the use of these haematological parameters as markers of functional and metabolic changes in the body of dogs at different intensities of babesiosis infection

Keywords: morphological blood parameters; haematocrit; thrombocrit; biochemical blood parameters; leukogram; enzymes

Introduction

Babesiosis is a blood-borne parasitic disease of domestic and wild carnivores and fur-bearing animals caused by protozoa of the genus *Babesia*, spread by ixodid ticks, and is recorded on all continents. In dogs, the disease is most often acute with a high mortality rate. The pathogenesis of severe babesiosis in dogs is primarily based on generalised, multiorgan changes, which require timely diagnosis and constant monitoring of the functional state of both individual organs and their systems, as well as the organism as a whole. Therefore, the research relevance lies in the need to determine marker indicators, in particular blood, for effective and timely laboratory diagnosis of functional disorders in dogs with babesiosis infection.

According to the researchers, due to significant climate change on the planet, there is an intensive increase in the period of activity of babesiosis vectors, and therefore this disease is increasingly recorded in dogs in winter. As a result, babesiosis, which used to be a localised disease, is now becoming widespread and should be subject to constant monitoring. V.A. Levytska *et al.* (2020) reported that the territory of Ukraine is considered an epizootic focus of babesiosis in dogs. It should also be noted that infected animals develop non-sterile immunity for 1-2 years, and therefore J.A. Panti-May & R.I. Rodríguez-Vivas (2020) focus on the prospects for a comprehensive study of this infection in mammals. According to A. Hildebrandt *et al.* (2021), more than 400 such cases have already been recorded among the US population and 21 in Europe. According to V.A. Levytska *et al.* (2020), the most common causative agent of the disease in European countries is *B. canis*, but species such as *B. vogeli, and B. gibsoni* are also common. I.I. Torianyk (2021) also notes defects in the walls of blood vessels with dissection, haemorrhage, microvesiculation and endothelial desquamation, the development of intravascular coagulation and thrombosis, microcirculatory disorders, and ischaemia. As noted by W. Zygner *et al.* (2023), protozoa of the genus *Babesia* parasitise primarily erythrocytes, although they can be found in blood plasma and the cytosol of cells of the reticuloendothelial system. According to K. Rajamanickam & V. Leela (2023), *Babesia*

canis destroys red blood cells in diseased animals directly in the vascular bed, which is complicated by the development of hypotensive shock and generalised damage to functional cells of organs and tissues due to oxygen deficiency.

Among the most characteristic changes in red blood cells in the peripheral bloodstream, M. Liu *et al.* (2022) focus on the development of oligocythemia with anisocytosis and poikilocytosis, as well as the occurrence of oligochromemia, manifested by macrocytosis and a decrease in hematocrit. At the same time, significant changes in the morphological and functional state of erythrocytes are diagnosed in affected dogs. In particular, they affect the population composition of erythrocytes, namely the number of mature red blood cells that undergo rapid destruction predominates, and the number of young forms significantly decreases. D. Sojka *et al.* (2022) describe an increase in the content of free iron in the blood and a simultaneous decrease in the level of saturation of transferrin with this trace element. As a result of the described processes, haemoglobin synthesis at the level of red bone marrow is impaired, which leads to the development of anaemia, and subsequently jaundiced skin, visible mucous membranes, and haemoglobinuria. Among the established patterns, normochromic, normocytic, and non-regenerative anaemia is observed during the first day of clinical manifestation of the disease, and then, on the second or third day, hypochromic, macrocytic, with reticulocytosis.

During the active phase of the disease in dogs, according to V.A. Gryshchenko & D.S. Bilokur (2021), functional disorders of the cardiovascular, digestive, and urinary systems are most often detected, and in severe clinical forms, even cerebral pathology. Therefore, a general clinical blood test for babesiosis infection allows for determining the degree and extent of damage to internal organs in such patients. In particular, J. Kuleš *et al.* (2023) note the need for an in-depth study of the features of the disease manifestation at the cellular level and the identification of new markers for assessing the functional state of both the whole organism and its internal organs. Modern laboratory diagnostics of animal diseases is based on the use of sensitive biochemical tests and methods for diagnosing most animal diseases, including babesiosis, which also allows for the control of the course of the pathological process, the occurrence of possible complications and determining treatment tactics.

Based on the aforementioned, the research aims to determine the general reaction of the Labrador dog to different intensities of babesiosis infection caused by *B. canis*. To achieve the aim of the study, the following tasks were set: to identify and differentiate the causative agent of canine babesiosis microscopically; to select animals with low and high intensity babesiosis infection; to study the effect of the blood parasite on the functional state of the organism and to determine marker changes in blood parameters in sick dogs with different intensities of babesiosis infection.

Literature Review

The clinical manifestation of Babesiosis infection in dogs has its characteristics depending on the type of *Babesia* species involved. As noted by V.A. Levytska *et al.* (2020), different strains of *Babesia canis* differ in pathogenicity. In some regions, the disease may be moderately expressed, while in others it is highly pathogenic. Dogs of all ages are susceptible, but puppies are more often affected. The incubation period for *B. canis* is 10-21 days, and in the case of *B. gibsoni*, it is 14-28 days. V. Djokic *et al.* (2021) described that the mortality rate from *Babesia spp.* reaches more than 12%, and in the case of *B. rossi*, up to 1%. There is no cross-immunity between *Babesia canis* and *Babesia gibsoni*.

According to B.K. Atkinson *et al.* (2022), in acute cases, the first clinical symptom of the disease is a significant increase in body temperature (up to 42°C). According to M. Torti *et al.* (2020), the most common symptom of babesiosis in dogs is the development of haemolytic anaemia caused, in particular, by the destruction of red blood cells due to increased osmotic fragility, extra- and intravascular haemolysis, erythrophagocytosis and immune-mediated destruction of red blood cells by parasitic antigens, parasite-induced cell membrane damage, and anaemia. Haemoglobin dysfunction, oxidative damage, contamination, and sequestration of red blood cells are also possible. O. Mazanny *et al.* (2020) noted an increase in haematocrit values as a result of relative haemoconcentration, which manifested itself simultaneously with increased haemolysis against the background of fluid movement from the intravascular to the extravascular component. Such animals were at risk due to the high threat of functional disorders of internal organs.

V. Djokic *et al.* (2021) note that the severity of the clinical course of babesiosis in dogs can vary significantly, from subclinical to acute or even chronic. Typical symptoms of this pathology include fever, depression, anorexia, anaemia of the mucous membranes and skin, splenomegaly, tachypnoea, and tachycardia. Clinical symptoms are caused by the development of anaemia and, as a result, tissue hypoxia in affected dogs, as well as the active release of cytokines, which provokes the manifestation of a systemic inflammatory response syndrome. Patients have no appetite, but thirst and general weakness are noted. Frequent manifestations include haemoglobinuria. M. Torti *et al.* (2020) point out that in chronic cases, body temperature is not too high and rarely lasts more than a few days. Anaemia and jaundice are less severe. In general, dogs become noticeably weaker and lose weight. Damage to the circulatory system is manifested by vasodilation with a tendency to disseminate intravascular coagulopathy at the capillary level, increased permeability and may progress to hyperaemia and oedema. In case of central nervous system damage, movement disorders, paresis, epileptic seizures, etc. occur.

T. Rawangchue & S. Sungpradit (2020) indicate that *Babesia vogeli* in adult dogs causes an asymptomatic infection. Parasitaemia is extremely low, and babesiosis infection may not be diagnosed by blood smear examination. However, puppies show severe anaemia, and the subclinical course is mainly observed in adult dogs. B.L. Penzhorn (2020) reports the clinical manifestation of infection caused by *Babesia canis* shows greater variability in pathogenicity, intermediate between *B. vogeli* and *B. Rossi*, with most dogs showing anaemia. According to O. Teodorowski *et al.* (2022), *Babesia gibsoni* infection has a subacute, acute, or chronic course. As noted by the researchers, the most common is the acute course, which is manifested by the development of fever, haemolytic anaemia, severe weakness, splenomegaly, lymphadenopathy, and thrombocytopenia. The superacute state is rare. American Pit Bull and Staffordshire Bull Terrier disease is mainly transmitted through dog bites. PCR-positive subclinical infections with *B. gibsoni* have also been reported.

Materials and Methods

Haematological studies were conducted in 2022-2023 at the interdepartmental educational and scientific laboratory of veterinary diagnostic research at the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine and the laboratory of the Zoolux Veterinary Clinic (39 Dmytrivska Str., Kyiv).

The research methodology included a general clinical blood test and assessment of the biochemical profile of blood in dogs with acute babesiosis with different intensities of infection.

For scientific purposes, three groups of Labrador dogs aged 2 to 7 years and weighing 23.6-35.2 kg were formed. Experimental group 1 included dogs with a low-intensity babesiosis infection (4 animals with the names: Akita, Bruno, Jess, and Malysh), experimental group 2 included animals with a high-intensity infection (4 animals with the names: Danya, Bonia, Peta, and Charlie). The control group included 6 clinically healthy dogs (nicknames: Lisa, Fly, Boy, Day, Alma, Bars) aged 2 to 7 years, which underwent preventive examination at the Zoolux veterinary clinic (Kyiv). The results of a general clinical blood test in animals of this group corresponded to the reference range of physiological values typical for healthy dogs.

Blood samples for haematological studies were taken from the anterior saphenous vein of the forearm. The study design included the following steps:

- creation of smears of heparin-stabilised blood and determining the presence of babesia;
- identification of the pathogen and determination of the intensity of the babesiosis infection;
- blood centrifugation of stabilised ethylenediaminetetraacetic acid (EDTA) for biochemical tests at 3000-3500 rpm for 5 min;
- conducting a general clinical blood test using a Dymind DF51 haematological analyser (China) (examination of the number of formed elements, leukogram, haematocrit and thrombocrit, as well as haemoglobin content in whole blood);
- biochemical analysis of blood parameters on analysers "StatFax 4500" (USA), "Mindray BS-230" (China) (study of the activity of the following enzymes in blood plasma: alanine aminotransferase (ALT, ES 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1), alkaline phosphatase (ALP, EC 3.1.3.1), γ-glutamyl transpeptidase (γ-GTP, EC 2.3.2.2);
- a complex of biochemical parameters: total protein, albumin, creatinine, urea, cholesterol, glucose, total bilirubin, direct bilirubin, potassium, calcium, inorganic phosphorus, and magnesium);
- research result summarisation and their analysis.

Laboratory diagnostic studies were performed using reagents from Global Scientific (USA). Microscopic assessment of the detection of intraerythrocytic parasites in stained blood smears was performed using the Romanowsky-Giemsa method. Light microscopy was used using a Micros microscope (Austria, 2012). Leukodif 200 stains (LDF 200, Czech Republic) were used to stain blood smears.

The intensity of erythrocyte damage by protozoa was determined per 100 cells and marked as "+":

"+" – up to 1-2 erythrocytes affected by *Babesia* were assessed as low intensity of infection;

"++" – up to 3-4 erythrocytes affected by Babesia were assessed as medium intensity of infection;

"+++" – up to 1-4 erythrocytes affected by *Babesia* were assessed as severe infection.

At low intensity of infection, 1-2 *Babesia* were detected in one erythrocyte, and at high intensity – 4-6 or more protozoa in one cell. Experimental groups were formed from animals with only low and high-intensity of infection.

The results of the conducted research were processed using generally accepted methods of variation statistics with the involvement of computer programs. In this case, the values of arithmetic means (M) were calculated considering n – the size of a particular sample. The Student's t-test was used to finally determine the existence of significant differences between the mean values for each indicator. Differences were considered significant at $P<0.05$.

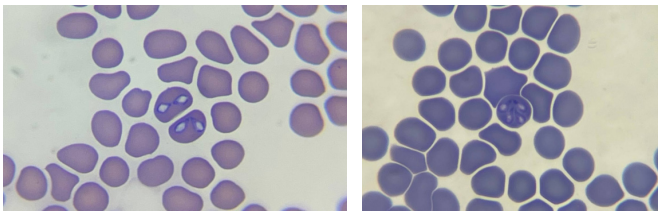
Experimental studies complied with the requirements of ISO/IEC 17025:2005 (2006). The animals were kept, and all manipulations were carried out following the provisions of the

Procedure for conducting experiments and experiments on animals by scientific institutions (Law of Ukraine No. 249, 2012), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European convention..., 1986).

Results

According to the results of clinical examination of dogs with babesiosis, the presence of hyperthermia (increase in body temperature to 40-41°C), anaemia of non-pigmented skin and visible mucous membranes was noted, which later in some animals with a high intensity of infection was replaced by icterus, loss of appetite, depression, exhaustion and the appearance of brown urine colour (in three animals named Danya, Peta, Charlie). The latter may indicate the occurrence of a symptom characteristic of high-intensity infestation – haemoglobinuria. Generalised changes in most internal organs and their systems during this infection significantly affect the severity of its course, which requires regular monitoring of the functional state of the organism of sick dogs. Eight cases – 4 of low and 4 of high-intensity babesiosis infection in dogs caused by *Babesia canis* were described.

Microscopic picture of blood in dogs with different intensities of Babesiosis infection. Microscopic examination revealed *Babesia canis* in the blood smear. In four dogs – Akita, Bruno, Jesse, and Malysh – the infection was assessed as "+", these dogs were included in the group with a low intensity of infection (Fig. 1).



a b

Figure 1. Blood sample analysis for babesiosis in dogs with low intensity of *B. canis*

Note: *photos of blood samples: a and b, magnification ×400*

Source: *compiled by the authors*

In four dogs - Danya, Bonya, Peta and Charlie – the infection corresponded to "+++", which was assessed as a high intensity of the lesion (Fig. 2).

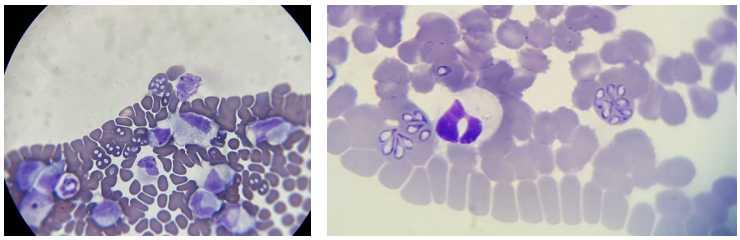


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Figure 2. Blood sample analysis for babesiosis in dogs with high intensity of *B. canis*

Note: *photos of blood sample preparations: a – ×200 magnification, b – ×360 magnification*

Source: *compiled by the authors*

General clinical blood test in dogs with different intensities of babesiosis infection. Morphological studies of the blood of Labrador dogs with babesiosis showed the presence of leukocytopenia, which was characterised by a decrease in the number of leukocytes by 31.0% at low and 60.6% at high intensity of infection. Lymphocytopenia was diagnosed in dogs of both experimental groups, in particular, a decrease in the relative number of lymphocytes by 2.2 times at low and 2.3 times at high intensity of babesiosis infection. The fact indicates a decrease in the resistant state of the organism in diseased animals, which may be a consequence of impaired functional capacity of leukocyte poiesis organs (bone marrow, lymph nodes, spleen) due to infection with the pathogen of blood cells and progression of intoxication. At the same time, neutrophilia was observed in the blood of the diseased animals compared to the control, i.e., an increase in the relative number of segmented neutrophils by 1.1 times in both experimental groups and monocytosis, i.e., an increase in the relative number of monocytes both at low (2.0 times) and high (2.3 times) intensity of invasion, which reflects the body's defence response and indicates the phenomenon of active phagocytosis of protozoa, erythrocyte decay products, bacteria and endotoxins (Table 1). Eosinopenia was diagnosed only in animals with a low intensity of infection. In this case, the relative number of eosinophils was 3.0 times lower compared to the control.

Table 1. General clinical analysis of blood parameters in dogs with different intensities of babesiosis infection (M±m, n=4-6)

Indicator	Reference limits	Control	Dogs with low infection severity	Dogs with high infection severity
White blood cells, 10^9/L	4.5-15.5	7.1±0.5	4.9±0.4*	2.8±0.3*
Stick-nucleated neutrophils, %	0-3	1.7±0.2	0	0
Segmented neutrophils, %	60-77	67.5±2.4	75.6±2.1*	72.3±1.9
Eosinophils, %	2-10	2.4±0.3	0.8±0.2*	2.0±0.1
Basophils, %	0-1	0.5±0.1	0	0
Monocytes, %	0-10	7.3±0.8	14.3±1.0*	16.9±0.9*
Lymphocytes, %	12-30	20.6±1.8	9.3±0.8*	8.8±0.7*
Red blood cells, 10^{12}/L	4.8-9.3	6.1±0.8	5.1±0.4	3.9±0.2*
Haemoglobin, g/L	120-180	127.0±4.2	113.2±3.3*	83.8±4.1*
Haematocrit, %	36-55	43.5±1.2	32.0±1.4*	23.0±1.2*
Platelets, 10^9/L	170-500	339.3±17.1	32.8±2.1*	25.8±2.4*
Thrombocrit, %	0.14-0.46	0.30±0.1	0.03±0.01*	0.02±0.01*

Note: **P<0.05, statistically significant differences compared to the control*
Source: *compiled by the authors*

The analysis of the results of morphological blood examination proved the presence of anaemia in diseased dogs. Thus, in the blood of animals with babesiosis at a high intensity of infection, a decrease in the number of red blood cells by 36.1% (erythrocytopenia) and haemoglobin content (hypochromemia) by 10.9% (at low intensity of infection) and 34.0% (at high intensity of infection) was observed with a simultaneous decrease in hematocrit value, respectively, by 1.4 and 1.9 times (Table 1). Significant changes were also noted in the number of platelets and thrombocrit in the blood of diseased animals. Thus, in

diseased animals, the number of platelets was reduced (thrombocytopenia) by 10.3 times (at low intensity of infection) and 13.2 times (at a high intensity of infection), and the value of thrombocrit decreased according to the intensity of infection by 10 and 15 times compared to the control.

Thus, in dogs with babesiosis, characteristic changes in the morphological profile of the blood are leukocytopenia against the background of eosinopenia (with a mild degree of infection) and lymphocytopenia, along with adaptive manifestations of monocytosis and neutrophilia. In addition, these animals were diagnosed with hypochromemia, erythrocytopenia (with high intensity of invasion), and thrombocytopenia with a decrease in thrombocrit and haematocrit. The intensive development of anaemia in sick dogs, especially pronounced at high intensity of infection, provokes the development of circulatory and tissue hypoxia, which causes shifts in the homeostasis system and corresponding disorders of functional activity on the part of organs and their systems in the body, which significantly affect the biochemical profile of blood plasma.

Biochemical profile of blood plasma in dogs with different intensities of babesiosis infection. In dogs with babesiosis, in addition to changes in the morphological parameters of the blood, a significant increase in the activity of several enzymes was noted (Table 2). Thus, in dogs with babesiosis, hyperenzymemia was found: aspartate aminotransferase 1.7 times (at low intensity of infection) and 4.0 times (at high intensity of infection), alanine aminotransferase 1.1 times (at low intensity of infection) and 2.8 times (at high intensity of infestation); γ-glutamyl transpeptidase by 1.3 times (at mild intensity of infestation) and 1.5 times (at high intensity of infestation), alkaline phosphatase by 2.8 times (at mild intensity of infestation) and 4.6 times (at high intensity of infestation) compared to the control.

Table 2. Plasma enzyme activity in dogs with different intensities of babesiosis infection (M±m, n=4-6)

Indicator	Reference limits	Control	Dogs with low infection severity	Dogs with high infection severity
Aspartate aminotransferase, U/L	5-55	30.3±0.8	50.9±1.4*	120.3±3.7*
Alanine aminotransferase, U/L	5-107	56.1±1.9	62.6±1.3*	156.8±4.1*
γ-Glutamyl transpeptidase, U/L	0-14	6.7±0.6	9.0±1.1*	9.8±1.3*
Alkaline phosphatase, U/L	10-150	80.5±2.6	228.1±6.4*	366.1±7.1*

Note: **P<0.05, statistically significant differences compared to the control*
Source: *compiled by the authors*

In addition, in the context of the biochemical profile of blood plasma, a significant decrease in the content of total protein by 15% (at low intensity of invasion) and 29.9% (at high intensity of invasion) was noted compared to the control, against the background of hypoalbuminemia (according to the degree of invasion, a decrease in albumin level by 26.2 and 35.0%). This resulted in a significant increase in blood plasma urea concentration by 70.5% (with severe infection), which reflected an increase in the intensity of catabolic processes, especially in liver cells (Table 3).

At the same time, the concentration of bile pigments – total and conjugated bilirubin, as well as other biochemical parameters – remained unchanged in the blood plasma of the affected dogs.

Table 3. Biochemical profile of dog blood plasma with different intensity of babesiosis infection (M±m, n=4-6)

Indicator	Reference limits	Control	Dogs with low infection severity	Dogs with high infection severity
Total protein, g/L	51-78	64.5±1.4	54.8±1.3*	45.2±1.1*
Albumin, g/L	24-44	32.1±1.1	23.7±0.8*	20.9±0.6*
Creatinine, μmol/L	44-138	79.2±3.1	74.6±2.3	73.1±3.4
Urea, mmol/L	2.5-9.6	6.1±0.2	4.9±0.6	10.4±0.9*
Cholesterol, mmol/L	2.4-8.4	4.8±0.3	5.2±0.3	4.3±0.8
Glucose, mmol/L	3.9-8.3	6.1±0.3	6.0±0.8	6.3±0.5
General bilirubin, μmol/L	0-5	2.5±0.4	1.8±0.3	2.2±0.4
Direct bilirubin, μmol/L	0-3	1.5±0.3	1.3±0.1	1.6±0.2
Potassium, mmol/L	3.6-5.5	4.6±0.2	4.0±0.3	4.3±0.4
Calcium, mmol/L	2.2-3.0	2.6±0.1	2.2±0.3	2.1±0.3
Inorganic phosphorus, mmol/L	0.68-2.00	1.3±0.2	1.0±0.2	1.5±0.4
Mangesium, mmol/L	0.7-1.1	0.9±0.1	0.7±0.2	0.9±0.2

Note: **P<0.05, statistically significant differences compared to the control*

Source: *compiled by the authors*

The results obtained are summarised and presented schematically in Figure 3, which shows the differences in the qualitative and quantitative characteristics of morphological and biochemical parameters of blood in dogs with different intensities of babesiosis infection.

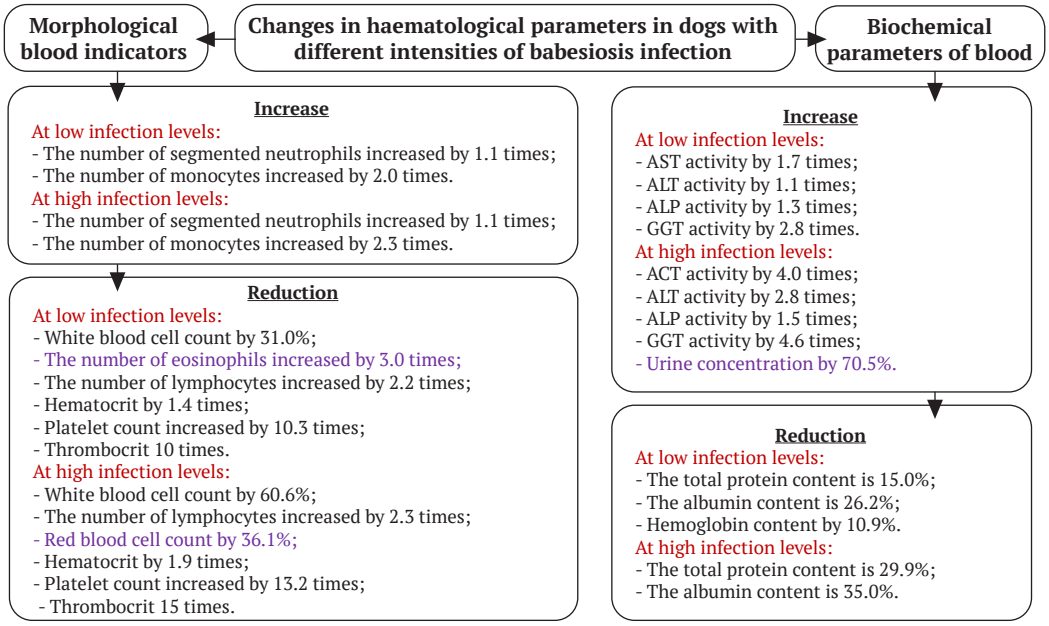

Figure 3. Peculiarities of changes in morphological and biochemical parameters at different intensities of babesiosis infection in Labrador dogs

Note: *Changes in indicators that are specific to a particular group of sick dogs are highlighted in purple*

Source: *compiled by the authors*

As such, marker changes among the indicators of the biochemical profile of blood plasma in dogs with different intensities of babesiosis infection were observed: the activity of aminotransferases – aspartate and alanine aminotransferase, γ-glutamyl transpeptidase and alkaline phosphatase, the content of total protein, albumin, urea (at high intensity of infection), which confirms the development of destructive changes and functional disorders in the cells of the liver, kidneys and other internal organs. This may be not only the result of the toxic effects of the blood parasite's waste products on functional cells of internal organs but also the consequence of the use of potent medicinal products in sick dogs.

Discussion

The results of observation of dogs with babesiosis indicate the development of a symptom complex typical of this blood-borne disease. According to O. Mazannyi *et al.* (2020), almost 60-70% of affected animals showed the following clinical changes: characteristic of visible mucous membranes, haemoglobinuria, and hyperthermia, which confirms our study results. In most animals, the researchers also recorded other symptoms (weakness, loss of appetite, shortness of breath, thirst, etc.), the appearance of which depended on the intensity of the babesiosis infection and the severity of the disease. A similar clinical picture of the course of babesiosis in dogs is described by J.A. Panti-May & R.I. Rodríguez-Vivas (2020) and W. Zygner *et al.* (2023), which may indicate the identity of the mechanisms of pathological effects of babesia on the body of dogs. It can be assumed that differences in the general manifestation of the disease in most cases correlate with the degree of pathogenicity of the pathogen, the intensity of its invasion and the individual characteristics of the resistant state of the dog's body. The results of comprehensive haematological studies in patients with babesiosis infection of Labrador dogs indicate pathological changes in the structural and functional state of most internal organs, which generally proves the generalised form of the disease course at both low and high intensity of infection. This indicates the need for continuous monitoring of the functional state of the body, which is also mentioned by O. Dubova & A. Duboviy (2018), A. Henning *et al.* (2020), M. Karasová *et al.* (2022) and other researchers.

In particular, according to the results of haematological studies of blood smears from sick dogs with varying degrees of babesiosis infection, a decrease in the body's resistance state was noted, which may be due to a dysfunction of the functional capacity of leukocyte poiesis organs (bone marrow, lymph nodes, spleen) during infection with the pathogen of blood cells and progression of intoxication. At the same time, similarly to a study by F. Dantas-Torres *et al.* (2021), neutrophilia and monocytosis were observed in the blood of diseased animals compared to controls, the degree of which was determined by the intensity of babesiosis infection. The established patterns reflect the organism's defence reaction and are closely related to the phenomenon of active phagocytosis of protozoa, erythrocyte decay products, bacteria and endotoxins. According to the study, eosinopenia was diagnosed only in animals with a low intensity of infection.

Furthermore, during the course of babesiosis infection in dogs, all four enzymes studied were sensitive: aspartate and alanine aminotransferases, γ-glutamyl transpeptidase and alkaline phosphatase. This was particularly pronounced in animals with a high intensity of infection and confirms the results of similar haematological studies described by O. Shchebentovska *et al.* (2022). In particular, they noted the development of hyperfermentation of aspartate aminotransferase and aspartate aminotransferase, respectively, by 5 and

3 times compared to the control. O. Mazannyi *et al.* (2020) reported a more than 3-fold increase in aspartate aminotransferase activity in the blood plasma of sick dogs, with no abnormalities in alanine aminotransferase activity, creatinine, and urea concentrations. These facts indicate similar trends, and the differences relate only to numerical values, which are explained by different approaches to research. The study by V.A. Levytska *et al.* (2020) found that in the group of dogs with azotemia, a higher ratio of aspartate aminotransferase/alanine aminotransferase activity was recorded. This fact suggests that kidney damage causes an increase in the activity of aspartate aminotransferase in affected dogs.

The diagnosed changes in the activity of enzymes in the blood indicate a generalised nature of functional and structural disorders in the internal organs of sick dogs. In particular, V. Djokic *et al.* (2021) reported disorders of the structural organisation of specialised cells and the dependent functional state of several internal organs (heart, liver, brain, kidneys). These changes are also confirmed by A.S. Ubah *et al.* (2019), who added skeletal muscle and brain to the previous list. This suggests that the dysfunction of these organs is primarily associated with intravascular haemolysis of red blood cells affected by blood parasites, progressive hypoxia, and intoxication of the body with babesia waste products, as well as severe hepatotoxicity of potent therapeutic agents.

The development of circulatory and tissue hypoxia in dogs with babesiosis occurs against the background of the progression of haemolytic anaemia and pathological changes in the blood vessel wall. As noted by M. Torti *et al.* (2020), with babesiosis infection in dogs, due to a decrease in the partial pressure of oxygen in the tissues, anaerobic glycolysis, the main end product of which is lactate, becomes important in maintaining the energy balance in cells. An increase in its concentration in the blood causes the development of lactate acidosis. According to these authors, acidification of the internal environment of the body in sick dogs provokes an increase in vascular wall porosity, vasodilation, blood stasis and a decrease in blood pressure, which are noted in the pathogenesis of this blood-parasitic disease. The development of lactate acidosis also causes a decrease in sensitivity to circulate catecholamines, an increase in the intensity of peroxidation of cell membrane lipids, disruption of oxidative phosphorylation, a sharp decrease in the production of adenosine triphosphoric acid, and, in general, affects enzymatic activity. As a result, according to T. Ullal *et al.* (2018), there are changes in the intensity of metabolic reactions and their direction, as well as disorganisation and disorders of the functional state of cell membranes of organs and tissues.

Thus, in the case of complex haematological studies in dogs with babesiosis with different intensities of *B. canis* infection, marker changes in the morphological and biochemical parameters of blood were determined. The obtained results are important for elucidating the pathogenesis of this disease in dogs both at the level of the whole organism and individual organs and their systems, as well as for determining the features of functional changes at low and high intensity of infection, which will contribute to the development of new approaches in their laboratory diagnosis, prevention of complications and increase the effectiveness of animal treatment.

Conclusions

Based on the results of comprehensive microscopic, morphological, and biochemical studies of blood samples taken from dogs with different intensities of babesiosis infection (mild and high) caused by *B. canis*, the most characteristic metabolic and functional changes in the

diseased organism were identified. Thus, depending on the level of babesiosis infection, the development of leukocytopenia was diagnosed in sick dogs, namely: a decrease in the absolute number of leukocytes in the blood by 31.0% at low and 60.6% at high intensity, which manifested itself against the background of eosinopenia (only at low intensity of infection) and lymphocytopenia with simultaneous monocytosis and neutrophilia and indicates suppression of leukocytopoiesis. In addition, these animals were characterised by erythrocytopenia, hypochromemia, and thrombocytopenia with a decrease in thrombocrit and hematocrit, indicating the development of anaemia and coagulopathy, which were more pronounced at a high intensity of infection.

The most sensitive biochemical parameters of blood plasma in sick dogs, especially at high intensity of babesiosis infection, were enzymes: aspartate and alanine aminotransferase, γ-glutamyl transpeptidase and alkaline phosphatase, indicating destructive changes in internal organs, in particular, liver, heart, skeletal muscle and kidneys. Sick dogs with varying intensity of infection, especially those with a high degree of its manifestation, are characterised by hypoproteinemia, hypoalbuminemia and hyperazotemia, which proves the presence of homeostasis disorders and the prevalence of catabolic processes in the cells of internal organs, in particular hepatocytes. The observed patterns of changes in the morphological and biochemical parameters of blood in dogs with different intensities of babesiosis infection are important for clarifying the pathogenesis of this disease, determining the characteristic functional changes in the diseased organism, and developing effective methods of their laboratory diagnosis, effective prevention of complications and clarifying the strategy in the treatment of such patients.

A promising area for future research is to determine the peculiarities of the rehabilitation period for the restoration of functional activity of internal organs and metabolism in dogs suffering from various degrees of babesiosis infection caused by *B. canis*.

Acknowledgements

None.

Conflict of Interest

None.

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Загальна реакція організму собак за різної інтенсивності бабезіозної інвазії

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Анотація. Глобальні зміни клімату, що відбуваються в останні десятиліття, призводять до збільшення активності та розширення ареалу розповсюдження багатьох хвороб. Однією з них

є бабезіоз собак. А тому, все актуальнішим стає здійснення моніторингу за функціональним станом організму в хворих собак та своєчасне виявлення уражень нирок, печінки, селезінки, серцево-судинної системи, розвиток анемії й інших порушень, що ускладнюють перебіг бабезіозу. Метою роботи було з'ясування особливостей метаболічних і функціональних змін в організмі собак за різної інтенсивності бабезіозної інвазії. Під час проведення лабораторної діагностики використовували загальні та спеціальні методи дослідження: світлову мікроскопію, центрифугування, спектрофотометрію та математичної статистики. За результатами комплексних мікроскопічних, морфологічних і біохімічних досліджень крові в собак за різної інтенсивності бабезіозної інвазії встановлено найхарактерніші функціональні та метаболічні зміни в їхньому організмі. Так, за різної інтенсивності бабезіозної інвазії в хворих тварин особливостями гематологічного профілю є лейкоцитопенія на тлі еозинопенії (за легкого ступеня інвазії) та лімфоцитопенії за одночасного компенсаторного збільшення кількості моноцитів і нейтрофілів, а також еритроцитопенія, гіпохромемія, тромбоцитопенія зі зменшенням величини тромбокриту і гематокриту. Крім того, у таких хворих фіксували розвиток гіперферментемії аспартат- і аланінамінотрансферази, лужної фосфатази, γ-глутамілтранспептидази, що вказує на структурно-функціональні зміни, насамперед, у міокарді, печінці, скелетних м'язах, головному мозку і нирках внаслідок їх токсичного ураження продуктами життєдіяльності бабезій. Водночас у хворих тварин відмічали гіпопротеїнемію, гіпоальбуміненію і гіперазотемію, що свідчить про зростання інтенсивності катаболічних процесів у функціональних клітинах органів і тканин, насамперед, печінки. Найвираженіші зміни досліджуваних показників встановлено за високої інтенсивності бабезіозної інвазії. Визначені закономірності дозволяють використовувати зазначені гематологічні показники в якості маркерів функціональних та метаболічних змін в організмі собак за різної інтенсивності бабезіозної інвазії

Ключові слова: морфологічні показники крові; гематокрит; тромбокрит; біохімічні показники крові; лейкограма; ензими

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Problems of motor activity in cows with orthopaedic pathology

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Abstract. The relevance of the study is conditioned by the fact that diseases that manifest various degrees of lameness in cows are widespread (50-70%) among livestock. Milk productivity and body weight gain decrease in sick animals, which causes their culling. It is important to establish nosological forms of such pathologies and determine the effectiveness of their treatment. The purpose of the study is to analyse the problems of motor activity in cows with orthopaedic pathology. The study included an assessment of herd mobility before entering the milking parlour, which allowed comprehensively considering the rhythm and length of steps and the load on the cows' limbs. It was found that the development of purulent-necrotic processes is accompanied by severe lameness in 66.6% of animals. A smaller number of animals (16.6%) were diagnosed with mild lameness. It was found that in 100% of cases, pathological processes were localised in the pelvic extremities. In the vast majority of animals, more than 3/4 of the lateral hooves were affected. There were no differences in the development of pathological processes on the right or left pelvic limb. The results of the examination of cows proved that the surgical pathology was accompanied by the development of local purulent inflammation. The most common pathology of the distal limbs in cows was purulent pododermatitis, which accounted for 66.6% of surgical diseases. Phlegmon in the area of the corolla and interdigital ulcers were diagnosed less frequently, with their respective shares in the pathology structure being 16.6%. It is proved that for the treatment of lame cows

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with the use of Levomekol ointment, clinical recovery was established with the disappearance of symptoms of lameness for purulent pododermatitis up to 24 days, and for phlegmonic processes in the corolla area and lesions of the finger arch – up to 22 days. The proposed therapy regimens will help to reduce the duration of treatment of purulent pododermatitis, phlegmon in the corolla area and lesions of the finger arch compared to conventional methods

Keywords: hoof; limb damage; purulent pododermatitis; corolla phlegmon; interdigital ulcer; lameness

Introduction

Pathologies in the finger area in cows have a great impact on both animal productivity and health. As noted by A. Stotskiy *et al.* (2020) the number of cows with finger tissue lesions is 50-70% in dairy complexes. N.M. Khomyn *et al.* (2019) found that violation of the conditions of keeping animals and the lack of an appropriate level of veterinary and sanitary culture on farms does not allow achieving a balance between the body of livestock and its habitat. The uneven distribution of body weight on the sole surface caused by keeping animals on uneven floors contributes to the appearance of bruises, sprains and ligaments, slippery floors lead to falls and various injuries, including limb fractures, ligament tears, joint capsulitis, and excessively hard floors lead to excessive abrasion of the heel horn, etc. In such herds, it is difficult to maintain the health of animals at the proper level and prevent the occurrence, in particular, of aseptic pododermatitis. In the hoof of the thoracic and pelvic limbs of cows with aseptic pododermatitis, the amount of moisture decreases by 8.2 and 9.3%, respectively, and the concentration of SH-groups increases by 10.6 and 15.8% with a likely decrease in the content of calcium, sulphur, copper and zinc. The density of the hoof horn decreases and the resistance to abrasion of the hooves of the pelvic limbs decreases by 1.6 and 18.0%.

Lameness is a symptom of many diseases in the extremities (muscles, joints, tendons, ligaments, hooves) and one of the most common causes of culling of cows from the herd. In dairy cows, lameness has serious negative consequences for the economy of production due to harmful effects on herd productivity. Violation of the function of movement of animals is manifested in a change in activity, which is associated with damage to the hooves, especially in the pelvic extremities. Hoof diseases are severe, which affects the well-being of dairy cows. Important factors that affect the health of the limbs are nutrition, animal hygiene, maintenance techniques, genetic and breeding predisposition. Nutrition is one of the main preventive factors that determine the quality and growth of the heel horn and the associated prevalence of diseases. The strength and structure of the heel horn are affected by the composition of the feed diet (amino acids, minerals, vitamins, toxic substances that pollute the feed or occur in them in the form of fungal metabolites) (Langova *et al.,* 2020). The main aetiological factors of hoof diseases are improper maintenance of animals, reduced feeding levels and unbalanced feed rations, lack of regular active exercise, sun exposure and regular orthopaedic cleaning (Khomyn *et al.,* 2017).

Observation and logistic regressions are used to obtain data and evaluate factors associated with lameness. As a result of research by M. Sadiq *et al.* (2020), hoof damage was reported in 470 cows out of 1,001 cows studied. Lameness was increased on farms with a high livestock density and in the presence of paths with

dirty concrete floors. There is also other data available, established by P. Kriz *et al.* (2021), on the prevalence of lameness and severe tarsal lesions and contamination of cows. Based on the results of collecting anamnesis and observations on 54 farms, cows were assessed for hock disease and the relationship with hygiene. Thus, it was found that the condition of the resting surface of cows is largely associated with the prevalence of lameness and the degree of lameness. The researchers found that on farms with loose keeping (17.4%), animals had a significantly lower prevalence of lameness (score ⩾3 on a scale of 1 to 5, where 1 = normal movement) than on farms with loose keeping, installs and rubber bedding (30.5%).

Lameness is causing more and more problems for the dairy industry around the world. However, little is known about lameness and its causes in grazing cattle, especially in tropical climates. Researchers have established the prevalence of hoof lesions and lameness in dairy herds of cattle grazing year-round in tropical conditions, and also identified the main lesions associated with lameness. In particular, 48 farms located in the state of Minas Gerais, Brazil, were studied. It was found that among the 2,267 cows evaluated during lactation, 16.0% were rated as lame and 7.0% as severely lame. The researchers noted that cows had at least one type of hoof lesion, of which heel horn erosion (90.0%), white line fissure (50.0%), and finger dermatitis (33.0%) were the most common. Heel horn erosion was present on all farms, and finger dermatitis was diagnosed in 96.0% of farms. A sole ulcer was observed in one animal. The results of the study show that finger dermatitis and white line cracking are the main problem and the main cause of lameness in cows (Moreira *et al.,* 2018).

Cattle lameness is an important welfare issue that also has an economic impact on the dairy industry. Factors associated with lameness were found to be poor assessment of body condition, long time spent in pens, confinement in pens during periods of drought, and poor hygiene. For hoof lesions, floor features were the most significant factor in determining the probability of heel horn erosion, white line cracks, and sole haemorrhage – more than 3 times. Results of the study by M. Kibar & T. Caglayan (2016) show that improving the hygiene conditions and floor conditions of rooms where animals are kept is the first step to planning measures aimed at reducing lameness. Lameness, according to M. Huyssteen *et al*. (2020), is a major concern for the health and productivity of dairy cattle, which is extremely common in North American herds. The researchers provided information on the prevalence of lameness and hoof damage in loose herds in the province of Alberta. It remains high, which indicates a low level of adoption by manufacturers of mitigation strategies.

The purpose of the study was to investigate the motor activity of cattle in pathologies of the motor system and test a new treatment regimen for purulent pododermatitis and phlegmon in the finger area in animals.

Literature Review

B.E. Griffiths *et al.* (2018) found that the mean prevalence of lameness on a farm was 31.6% with a standard deviation of 13.9% and a range of 5.8-65.4%. A total of 14,700 cows were evaluated for mobility, of which 4,145 cows were found to be lame (28.19%); 536 cows received a score of 3 (which is 3.65% of the cows evaluated). The repeatability of mobility assessment was investigated by evaluating one herd (189 dairy cows) twice on the same day. In particular, it was found that the prevalence of lameness in the herd was 27.5 and 28.0% (morning and afternoon milking, respectively). Researchers cite summary data estimates of the frequency of lameness that indicate a high level of the

disease. It is estimated that approximately 30% of British dairy cattle suffer from this disease during the year. This statement is reinforced in the paper by O. Atkinson (2022), who investigated the prevalence of lameness in dairy herds in the UK. The latter is estimated at about 30-32%. The researcher notes that most farmers constantly underestimate this indicator (Afonso *et al.*, 2020).

J. Somers *et al.* (2019) note that herd-level risk factors are associated with the cow's environment and lameness. The uncomfortable surface of the stall, insufficient depth of the litter, and the abrasive surface of the floor are factors that contribute to an increase in the level of lameness. It was found that dairy cattle that are grazed are exposed to a different set of lameness risk factors, mainly related to the animal's motor activity. Researchers have established the risk of lameness in the first 150 days of lactation. Lameness data were collected from 10 dairy herds in pastures. A total of 1,715 cows were examined, of which 1,675 cows were available for analysis. The association between lameness status and potential risk factors at the cow level was determined using multivariate logistic regression. The researchers note that data collected as part of herd health monitoring can be used in conjunction with lameness records to identify deficiencies in controlling it. J. Tunstall *et al.* (2021) suggest that animal lameness in beef production is not given enough attention in the UK, despite the fact that it is a generally recognised problem in the dairy industry. The researchers found that British beef farmers underestimated the prevalence of lameness on their farms. It is considered that its indicator is 7% with a range from 5 to 9%. L. Randall *et al.* (2019) note that obtaining accurate estimates of lameness levels in a dairy herd is challenging due to the difficulty of selecting truly random samples. Accurately quantifying the incidence of lameness in herds is problematic because most of the available information is based on non-random and potentially biased data sets.

The researchers determined the prevalence of lameness and examined potential risk factors in cows in Canada's Maritime provinces. Cows were also evaluated in 46 free-range herds and 33 tethered herds in Nova Scotia, New Brunswick, and Prince Edward Island. Thus, the prevalence of lameness was 21.0% for cattle that were kept loose, and 15% for cattle in premises. Of the 1,488 cows kept in stalls, 68.0% showed no behavioural changes, while 15, 15, 2, and <1% showed 1, 2, 3, or 4 changes, respectively. With the loose method of keeping, the chances of lameness were higher when cows spent ⩾3 hours/day in the milking area compared to cows in this area <3 hours/day. In animal herds, a higher probability of lameness was observed when the litter was wet than when it was dry. For both types of rooms for lactating cows, keeping dead cows and heifers in deep bedding compared to a tethered or loose method was associated with a reduced chance of lameness (Zhao *et al.*, 2015).

D. Beggs *et al.* (2019) note that on Australian pasture farms, where cows can often walk several kilometres and stand for several hours a day in a crowded concrete yard while they wait to be milked, the potential for the negative impact of lameness on animal welfare is a constant concern. Several studies have shown that farmers tend to underestimate cases of lameness. The researchers evaluated 19,154 cows on 50 farms for lameness in herd groups of about 100 to 1,000 cows after milking. The researchers compared these results with farmers' data on lameness of the same day. The following rating system was used: 0 – moves normally; 1 – moves unevenly; 2 – limps; 3 – severely limps. All very lame cows were identified by the farmer, but overall they identified only 24% of the cows identified by the lameness assessment. Analysis

of the position of lame cows in the milking order showed that an assessment of the lameness of the entire herd was necessary to identify all lame cows. However, the lameness score in only the last 200 cows that were milked can be used as a screening test to determine the prevalence of lameness below a given threshold.

J. Kofler *et al.* (2021) note that the effect of lameness on milk yield in dairy cows has already been investigated in many countries by many, but most often mobility indicators ⩾3 were considered almost exclusively. Overall, data from 4,005 cows from 144 dairy farms across Austria were evaluated using two statistical models. The farm, year and time of calving were considered, and the number of lactation days were included in the analysis of milk, fat, and protein yield indicators. The researchers found that the average cumulative prevalence of lameness during the follow-up period was 51.0%, and 8.1% of cows had adverse motor activity indicators. During the first 100 days of lactation, 34.7% of all cows were lame.

M. Garvey (2022) noted that lameness is one of the three main problems of dairy cattle worldwide after mastitis and infertility, leading to reduced productivity, economic losses, and animal welfare problems. Lameness is associated with reduced milk yield, insufficient weight gain, low fertility, and frequent culling of animals. Environmental risk factors (temperature, humidity) and surrounding animals contribute to the severity of the disease, making this multi-faceted disease difficult to eradicate and control. Thus, worldwide, the prevalence of lameness in dairy herds ranges from 17 to 35%.

D. Kucevic *et al.* (2022) investigated the effect of the system of keeping (tethered and free-stall cows) on the prevalence of hoof diseases, and also established the percentage of culling in 6,348 dairy cows of Holstein breed from 5 farms. Over three months of hoof examination, it was found that both retention systems were equally affected by the same diseases. In this regard, pathology in the area of the white line was diagnosed in both systems with a prevalence of 0.5-1%, finger ulcer 5% and necrosis in 3-6% of animals, Rusterholz ulcer in 20-23%, finger dermatitis in 18-20%, interdigital thyloma in 10-12%, interdigital phlegmon in 0.7-0.77%, while the prevalence of mechanical damage was insignificant and in similar quantities – 0.2-0.5%. According to the researchers, cows with untethered keeping were more likely to suffer from interdigital dermatitis (39.11%) compared to cows in the tethered keeping system (20.40%). In contrast, diagnosed acute, chronic, and haemorrhagic laminitis was more pronounced in cowsheds with a tethered stall (18.61%) than in cowsheds with a free stall (0.88%). In the statistical analysis performed, statistically significantly more diseases were registered in the tethered keeping system than in the untethered one ($P<0.01$). For three months of the study, the average percentage of culling with untethered keeping was 5.4%, and with tethered keeping – 4.9%. This difference was not statistically significant ($P<0.05$).

Materials and Methods

The research was conducted in the period from October 2022 to March 2023 at the private enterprise "Agroecology" of the Myrhorod district, Poltava Oblast. At the beginning of the work, limb diseases in cows were monitored. Digital colour photographs available in the literature in accordance with the international classification system adopted during the 15th International Lameness Committee (2008) were used to identify each hoof lesion. Animal conditions and experimental research methods were conducted in compliance with the requirements of the "general ethical principles for conducting animal experiments" approved by the 1st National Congress on Bioethics (Law of Ukraine…, 2006) and the provisions of the "European Conven-

tion for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (1986). A total of 12 cows with lameness were examined and treated. All the animals had hoof lesions in the pelvic limbs. The cows of the dairy herd were evaluated for mobility when they left the parlour after morning, afternoon, or evening milking. The mobility of each herd was evaluated at the entrance to the milking parlour. The proportion of clinically lame cows was calculated for each farm. 0 points – the animals have a gait with uniform support and rhythm on all hooves with a flat back, long smooth steps; 1 point – steps are uneven (rhythm or length of steps is shortened, the affected limb or limbs do not immediately move to the stage of support on all four hooves with a flat back, long smooth steps during movement; 2 points – uneven load on the limb, the affected limb immediately after removal tries to return to a static position (accelerated rhythm of movement of the affected limb, shortened steps, the affected limb or limbs not immediately identified as diseased, clearly curved towards the centre of the back; 3 points – the animal is unable to move as fast as a healthy one, does not keep up with a healthy herd. The movement of the cow was observed when the animal walked at a normal pace and moved along a straight route through the pen.

Diagnosis of hoof lesions in the studies was carried out during trimming based on visual analysis, the presence of a specific smell in the pathological focus, and local pain reactions of cows based on the international standardised diagnosis described by J. Espinasse *et al.* (1982). Lesions were marked as absent or present on the pelvic extremities. Additionally, the cleanliness of the shin, flanks and udder was also assessed on a 4-point scale based on the amount and freshness of the manure present (Militaru *et al.,* 2019). The evaluated limb area was located between the corolla and the lower half of the tarsal joint on the lateral surface of the right limb. The state of contamination on the right side of the animal was assessed. The surface was considered clean at 0 (fresh manure spatter on <50% of the area); 1 point (fresh manure spatter on ⩾50% of the area). Dirty at 2 (dry manure on ⩾50% of the area); 3 (dry manure on the entire area). Given that the accumulation of contamination occurs mainly due to the spatter of manure on the lower part of the limb during movement, it is advisable to have a balanced distribution for the cleanliness of the lower leg. It is well known that cows do not choose which side they lie on. Thus, it can be expected that manure contamination will be equally distributed on the right and left sides. Therefore, for consistency between cows and to reduce the amount of time spent on animal measurements, only the right side score was chosen. The obtained data on the spread of pathology and its types allowed planning further procedures aimed at recovering animals from lameness. It also made it possible to calculate the resources needed to overcome the disease. In addition, the survey data provided an opportunity to set priorities for initiatives in the treatment of animals, considering the time aspect. After performing orthopaedic hoof cleaning in sick animals and primary surgical treatment of the pathological area, a Chemi-spray aerosol drug was applied to the affected area. Levomekol ointment was applied to the affected surface of the tissues using cotton-gauze swabs on top of the "chemi-spray". The therapeutic substance was fixed with a dressing material (bandage). Manipulations were performed once every 48 hours. At the end of the experiments, the material was processed statistically and presented in the form of tables. Statistical data processing was performed in Excel.

Results and Discussion

During the monitoring studies, the analysis of reporting data on the frequency of hoof lesions

was carried out. It was established that in 2022-2023, the diagnosis of pathology in the distal part of the limb was made 12 times. The findings are presented in Table. 1.

Table 1. Assessment of motor activity of sick cows using the AHDB system

Scores	Indicator	Animal units (%)
0	Gait with uniform support and rhythm on all hooves with a flat back. Long smooth steps are possible	-
1	The steps are uneven (the rhythm or length of the steps is shortened, the affected limb or limbs do not immediately go into the stage of support on all four hooves with a flat back, when moving long smooth steps	2 (16.7)
2	Uneven load on the limb, the affected limb immediately after removal tries to return to a static position (accelerated rhythm of movement of the affected limb, steps are shortened, the affected limb or limbs are not immediately identified as patients, clearly curved to the centre of the back)	8 (66.6)
3	The animal is not able to move as fast as a healthy one, does not keep up with a healthy herd	2 (16.7)
Total		12 (100)

***Source**: developed by the author based on research by AHDB (2020)*

The established results prove that the vast majority of cows were diagnosed with severe lameness, the steps were different in size, significantly shortened compared to the norm. The animals held the limb in the air. In dynamics, the animal has long smooth steps. In the herd under study, both mild motor activity disorders and severe motor activity disorders were recorded in the same number of percentages. The results obtained are consistent with data by E. Flor & N. Tadich (2008), who noted that the prevalence of lameness was 33.12% in LDH and 28.7% in SDH ($P<0.02$). Of all lame cows, 50.85% and 44.05% had a lameness score ⩾2 for LDH and SDH, respectively. The three main lesions on large farms were white line lesions (54.9%), sole bleeding (52.7%), and heel erosion (48.4%), while on small farms they were white line lesions (82.5%), heel erosion (53.3%), and haemorrhages (24.6%). 92% of lesions were found in the pelvic limb area in both types of herds. The authors concluded that lameness is highly common on dairy farms.

4 behavioural changes were evaluated in animals at rest: standing on the edge of the stall, supporting one pelvic limb, moving weight between the pelvic limbs, and uneven load when moving from side to side. Before starting the assessment, the cows had to stand for at least 3 minutes, the cow was observed from behind for 30 seconds, and behavioural changes were evaluated from different angles. The cow was then urged to step from side to side 2-4 times to assess the condition of the pelvic limbs. The cow was monitored after moving for another 30 seconds for behavioural changes. A cow with 2 or more behavioural changes was considered lame. A cow that rested on one limb and held its weight unevenly while moving from side to side was more likely to suffer damage to the hooves of the pelvic limbs than a cow that did not exhibit this behaviour. When considering specific hoof lesions in a cow whose one limb was at rest and carried uneven weight, it had a higher chance of developing pathology compared to those that did not exhibit this behaviour. Behavioural indicators of weight resistance and limb position helped identify cows with pelvic limb hoof damage.

The expediency of conducting such an assessment is reflected in the papers by

P.T. Thomsen *et al.* (2012), who evaluated the motor activity of cows, recorded the behaviour of animals in a supine position and collected data on hoof damage seen during trimming. The results were analysed using logistic regression with hoof damage as a result and motor activity assessment (1-5), which found that the probability of all hoof and skin lesions increased with increasing locomotion assessment and an increase in the average duration of supine seizures. It was concluded that motor activity assessment can be used as a tool for managing healthy hoof health in dairy herds.

The researchers evaluated the state of animals in static dynamics and local changes. During a clinical examination, it was found that animals in which motor activity was estimated at 1 point showed uneven gait when moving, they limped, but not constantly, and lameness increased with intense movements. In a static position, the animals transferred their body weight as much as possible to the opposite healthy limb, the diseased limb was periodically removed from the support and supported only on the hooked part of the hoof. In animals that were given 2 points for lameness, a load transfer to the cervical part was noted clinically during movements. When the animal took a step, it raised its head up as much as possible and quickly carried the diseased limb out and returned it to a static position after carrying it out. That is, the animal took quick shortened steps with a sick limb. Cows that were rated limp at 3 points did not move much and often lay down. In the state of movement, they practically did not lean on the affected limb, jumping on three limbs. In a static position, the affected limb was in a suspended state and, only occasionally, the animal rested on the hooked hooves of the affected hooves for a short time. The obtained data matches the data of R.C. Ebling *et al.* (2019), who note that hoof damage is one of the main causes of reduced productivity in the dairy industry and is conditioned by the degree of motor activity of cows and the frequency of horn trimming in the area of the animal's finger.

N. Browne *et al.* (2022) identified lesions associated with a higher lameness score, established a relationship between the lesions, and identified risk factors. On 98 farms during the grazing period and on 74 of the same farms during the housing period, each cow was assessed for lameness (0-3 lameness score), including examination of the pelvic hooves of lame cows (scores 2 and 3) (maximum 20 cows per visit), and the prevalence of each type of lesion was recorded. The most common types of lesions during both grazing and keeping were white lines, sole haemorrhages, and pointed hooves; all other lesions had a prevalence of less than 15%. The prevalence of lesions at the cow level was 19% during the grazing period and 25% during the housing period; the most common lesion was sole ulcers in both periods. The researchers found significantly more foreign bodies in the sole of the hoof (pasture = 14%, indoor = 7%) and sharp-angled hooves (pasture = 71%, indoor = 55%) during the grazing period compared to the housing period. Cows with hoof damage, sole ulceration, pododermatitis, finger necrosis on the limb, or amputated hoof were more likely to develop severe lameness compared to mild lameness. According to the researchers, the results of such assessment will help understand the causes of lameness in partially pasture-raised dairy cows and can be used to develop prevention and treatment protocols.

Evaluating the localisation of pathological processes, the authors found that they were 100% localised on the pelvic extremities. In the vast majority of cows, more than 3/4 of the lateral hooves were affected. There was no difference in the development of pathological processes on the right or left pelvic limb. M. Rodriguez *et al.* (2021) indicate that to some extent, this effect on the pelvic limbs is equally conditioned by the

distribution of weight between paired pelvic limbs and the presence of hoof horn destruction. This apparently affected differences in local blood circulation in the affected and unaffected contralateral pelvic limbs in lame cows. According to the results of clinical studies, it can be concluded that the surgical pathology of cows was accompanied by the development of local purulent inflammation, according to the pathology of the finger, the development of lameness was recorded in the vast majority of moderate degrees (2 points). Nosological forms of lesions in the distal part of the limbs of cows were also established (Table 2).

Table 2. Nosological forms of hoof lesions in cows

Type of pathology	Animal units (%)
Purulent pododermatitis	8 (66.6)
Corolla phlegmon	2 (16.7)
Interdigital ulcer	2 (16.7)
Total	12 (100)

***Source**: compiled by the authors*

The results obtained indicate that purulent pododermatitis was the most common pathology of the hoof area, and phlegmon in the corolla area and interdigital ulcers were less diagnosed. Investigating the causes of the development of purulent pododermatitis, it was found that its appearance was provoked by mechanical damage that animals received when moving on a concrete floor. A contributing factor was that the concrete floor was rough and significantly erased the sole when the animal moved. Thus, the sole was thin and did not fulfil its protective properties. This fact was confirmed by the fact that when trimming, the soles of some cows had dark blue spots, which is evidence of the development of pododermatitis. The reason for the development of phlegmonous processes in the corolla area is also seen in the constant injury of the corolla area. Thus, the simultaneous influence of mechanical force and constant microtrauma caused the development of a pathological process in this area.

Symtomatics for corolla phlegmon in cows had a classic manifestation. Thus, with the corolla phlegmon, clinically the process was accompanied by swelling of the inflamed area, soreness when pressing on the hoof, pulsation of the digital arteries, an increase in local temperature, the release of purulent exudate from the fistula and a slight increase in the total body temperature in the range of 0.2-0.4°C, a decrease in milk yield in the range of 10-15%. Sick animals were lying down for a long time and lost their appetite. When the animal moved, lameness of the operated limb of a moderate degree was noted. In cases of diagnosis of purulent pododermatitis, the symptoms had some differences. Thus, with superficial purulent pododermatitis, similar symptoms were noted in sick cows as with a phlegmonous process in the corolla area and fistulas were detected, from which purulent exudate was secreted. When diagnosing deep purulent pododermatitis in sick animals, there were signs of a more pronounced effect on the animal body. The increase in the total body temperature in such cows has already reached 0.4-0.5°C.

A. Stotskiy *et al.* (2020) indicate that while in the superficial course of the process, the lameness was of a moderate degree, in the deeply severe course, the animals held the limb in a bent position and stood only on the hooked part of the hoof. In a clinical study of animals, pathological processes in the finger

area were characterised by the following symptoms. In purulent pododermatitis, swelling in the sole area was noted, and pressing it with fingers could cause a strong painful reaction in the animal. The cow tried to free the limb from fixation. When palpating the digital arteries, their strumming (an increase in the amplitude of vibrations) was observed. When performing a funnel-shaped autopsy in the sole area, purulent exudate leakage was observed. Evaluating the exudate, it was found that it was of a liquid consistency, the colour ranged from pink to dirty grey. In more than half of the animals under study, purulent exudate had a sharp, unpleasant, sweet smell. In phlegmon, local formation of a crescentic painful swelling of a doughy consistency in the corolla area was noted. The fingers were slightly apart. When performing oblique autopsies, the release of thick white purulent exudate without foreign impurities and smell was observed (Fig. 1).

Figure 1. Corolla phlegmon of cattle
***Source**: developed by the author*

Symptoms of an interdigital ulcer were the presence of a skin defect in the area between the fingers, in which signs of connective tissue development and weakly expressed skin epithelialisation processes were noted. The processes were localised in the middle third of the interdigital arch. High humidity in the premises in winter causes softening and maceration of the skin in the area of the interdigital gap. In addition, given that cattle were cloven-hoofed animals, foreign objects often got between the fingers in addition to manure, which injured the area, and the bacteria complicated the pathological process (Fig.2)

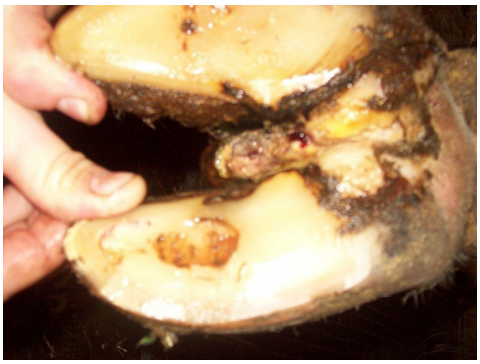

Figure 2. Ulceration of tissues in the area of the interdigital arch
***Source**: developed by the author*

After conducting studies, it was found that the predominant cause of finger pathology in cows was injuries, a low percentage of lameness reflects a high level of farm management. As noted by J. Kofler (2017), three theories for the development of finger lesions of the limb are currently proposed: traumatic hoof injury; disruption of blood vessels in the finger area due to both external and internal factors; and, as a result, the development of laminitis. To understand the influence of the environment on the pathological process, the contamination of the skin surface was assessed in the area of the limb, flanks, and udder on a 4-point scale based on the amount and freshness of the manure present (Table 3).

The results prove that the body surface of sick cows, despite the pathological process, remained clean (Fig. 3).

Table 3. Assessment of skin surface contamination of lame cows

Scores	Indicator	Animal units (%)
0	Fresh manure spatter on <50% of the area	2 (16.6)
1	Fresh manure spatter on ⩾50% of the area	10 (83.4)
2	Dry manure on ⩾50% of the area	-
3	Dry manure on the entire area	-
Total		12 (100)

Source: *compiled by the authors*

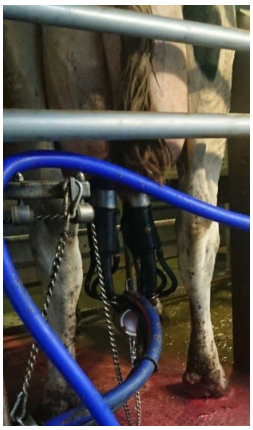

Figure 3. Assessment of skin surface contamination of cows with lameness

Source*: developed by the author*

Thus, B.E. Griffiths *et al.* (2018) note that improving the sanitary and hygienic conditions of keeping animals and the quality of the stable floor condition is the first step towards planning measures aimed at reducing lameness and hoof damage. The effectiveness of Levomekol ointment was established for the pathology diagnosed above in cows (Table 4).

Table 4. Effectiveness of the treatment performed

Type of pathology	Recovery period, days
Purulent pododermatitis, n=8	24.0
Corolla phlegmon, n=2	22.0
Skin ulcer of the interdigital arch, n=2	22.0

When using Levomekol ointment in animals with phlegmonous and ulcerative processes of the skin of the interdigital arch, the wound surface was released from necrotic detritus. However, a small amount of thick purulent exudate was simultaneously observed in the wound. After the first treatment, improvements in the general condition were recorded in the animals. They were less depressed, tried to lean on the affected limb, and their lameness decreased. After the 10th day of drug use, most animals showed no lameness and only some animals showed it during movement. In the lesion, the wound surface was released from the purulent exudate and the defect was filled with granulation tissue. In addition, in animals with phlegmonous and

ulcerative processes, the absence of inflammatory oedema and pus leaks was noted.

The use of Levomekol ointment helped accelerate the recovery of animals with pathologies in the finger area. Signs of lameness disappeared with purulent pododermatitis in 24 days. With phlegmonic processes in the corolla area and lesions in the finger arch area – in 22 days. The results obtained are consistent with the results of I. Bondarenko & S. Rublenko (2020), who note that Levomekol ointment in the treatment of pododermatitis promotes faster recovery compared to the use of other drugs of similar action. Thus, it was found that Levomekol ointment and Chemi-spray helped accelerate the elimination of clinical symptoms such as lameness, swelling, soreness of the sole and in comparison with conventional treatment with Chemi-spray and iodoform powder provided a reduction in the duration of treatment by 1.4 times.

Research of research by M. Ninkovic *et al.* (2021) confirm the findings, indicating that regular hoof trimming (twice a year) and adequate conditions of maintenance are crucial for improving hoof health. J. Afonso *et al.* (2020) note that monthly hoof examinations significantly reduce the prevalence of finger pododermatitis in the herd. After hoof trimming, removal of destroyed tissues and first treatment, animals with purulent pododermatitis showed a decrease in exudative phenomena and the release of the wound surface from avitalised tissues (Fig. 4).

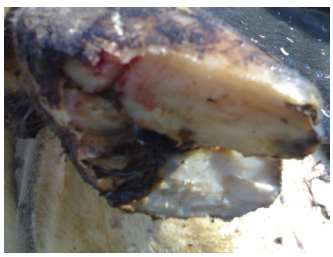

Figure 4. Trimming of the hoof horn in the area of the hoof sole in a cow with purulent pododermatitis

Confirmation of the effectiveness of maximum removal of necrotic tissues is found in the paper by R. Rinnovati *et al.* (2019), where a clinical study found out the effectiveness of various methods of treating pathologies in the finger area. The effectiveness of thorough and aggressive surgical removal of lesions of destroyed tissues for pathology in the white line area was established by treating 236 cows with lameness in combination with a therapeutic dressing. This contributed to rapid clinical healing (a marker of which was the growth of new tissue), followed by improved milk production. M. Alvergnas *et al.* (2019) note that the most effective strategy is early detection (regular checking of hooves with mirrors, pressure plates or infrared thermometers) and diagnosis (by veterinarians or trained farmers), regular trimming of hooves (no more than twice a year), bedding with sand and a clean and dry floor, a balanced diet to avoid any scarring acidosis.

Hoof baths are important for the prevention of infectious diseases of cattle hooves. Their use on farms, according to N.B. Cook (2017), helps maintain high milk yields by reducing the occurrence of musculoskeletal diseases. These treatments help reduce the risk of these disorders, especially finger dermatitis. According to M. Alvergnas *et al.* (2019), weekly use of 5% $CuSO_4$ solution for hoof baths has a therapeutic effect on bovine toe dermatitis, as this solution has antibacterial properties. However, according to N.B. Cook (2017), since 2006 in the European Union due to the toxicity of this compound and its insufficient biodegradability, the use of $CuSO_4$ was forbidden.

Thus, diseases such as purulent pododermatitis and interdigital ulcers are pathologies that were most often registered in cows during the study. When monitoring the cleanliness of the skin surface in the area of the affected limb, it was noted that cows with lameness remained clean. The treatment regimen using Levomekol

ointment and Chemi-spray aerosol has proven its effectiveness and can be proposed for use by practitioners in the treatment of cows with pathologies in the finger area accompanied by lameness.

Conclusions

Using a systematic review of prevalence and morbidity as a methodology in the field of evidence synthesis, it was proved that the vast majority of cows (66.6%) were diagnosed with severe lameness. The dominant pathology in the hoof area was purulent pododermatitis, the share of which in the structure of pathology was 66.6%. The spatter of fresh manure on ⩾50% of the area was observed in 83.4% of the animals under study. There was no difference whether inflammation occurred on the right or left limb. The reason for the development of purulent pododermatitis was the mechanical damage that animals received when walking on a concrete floor. The main aetiological factor was that the concrete floor had a rough surface, and therefore, cows significantly erased the sole during movement on it. This was confirmed by trimming the horn in the sole area. In some cows, the base of the skin had dark blue spots, which was evidence of the development of pododermatitis. The reason for the appearance of phlegmonous processes in the corolla area is a constant injury to its area. According to the clinical examination of cows, pathological processes in the hoof area were characterised by the development of local purulent inflammation. When palpating the digital arteries, their strumming (an increase in the amplitude of vibrations) was observed. When performing a funnel-shaped autopsy in the sole area, purulent exudate leakage was observed. As a result of its evaluation, it was found that the purulent exudate had a liquid consistency, and the colour ranged from pink to dirty grey. In more than half of the animals, exudate had a sharp, unpleasant, sweet smell. Topical application of Levomekol ointment for the treatment of sick animals already on the 10th day contributed to the removal of purulent exudate from the wound surface and filling the defect with granulation tissue. The use of Levomekol ointment provided clinical recovery with the disappearance of symptoms of lameness for purulent pododermatitis in 24 days, for phlegmonous processes in the corolla and lesions of the finger arch in 22 days, and in the treatment of wounds and abscesses, respectively, on 27 and 29 days.

In the future, it is planned to continue studying the nature of lameness in cows on farms and the effectiveness of using various therapeutic schemes for sick animals, considering the features of the influence of numerous environmental factors on the development of the pathological process.

Acknowledgements

None.

Conflict of Interest

None.

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Проблеми рухової активності в корів з ортопедичною патологією

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Анотація. Актуальність дослідження зумовлена тим, що хвороби, які проявляються різним ступенем кульгавості в корів, мають значне розповсюдження (50-70 %) серед поголів'я. У хворих тварин знижується молочна продуктивність і прирости маси тіла, що стає причиною їх вибракування. Важливим є встановлення нозологічних форм таких патологій та визначення ефективності їх лікування. Мета проведеного дослідження – проведення аналізу проблем рухової активності в корів з ортопедичною патологією. Наукове дослідження включало проведення оцінки мобільності стада перед входом у доїльну залу, що дозволяло комплексно розглянути ритм і довжину кроків та навантаження на кінцівки корів. Встановлено, що розвиток гнійно-некротичних процесів супроводжується у 66,6 % тварин вираженою кульгавістю. У меншої кількості тварин (16,6 %) діагностовано кульгавість легкого ступеня. З'ясовано, що в 100 % випадків патологічні процеси локалізувалися на тазових кінцівках.

У переважної більшості тварин, більш ніж 3/4, вражалися латеральні ратиці. При цьому, не відмічали різниці щодо розвитку патологічних процесів на правій чи лівій тазовій кінцівці. Результатами обстеження корів доведено, що хірургічна патологія супроводжувалася формуванням локального гнійного запалення. Із патології в дистальному відділі кінцівок у корів найпоширенішою патологією ділянки пальця були гнійні пододерматити, частка яких в структурі хірургічних хвороб становила 66,6 %. Менше діагностовано флегмон в ділянці вінчика та міжпальцевої виразки, їх частки відповідно в структурі патології становили по 16,6 %. Обґрунтовано, що за лікування кульгаючих корів із застосуванням мазі Левомеколь встановлено клінічне одужання із зникненням симптомів кульгавості за гнійних пододерматитів до 24 доби, а за флемононозних процесів у ділянці вінчика та ураження пальцевого склепіння – до 22 доби. Запропоновані схеми терапії сприятимуть скороченню термінів лікування гнійних пододерматитів, флегмон у ділянці вінчика та ураження пальцевого склепіння порівняно з традиційними методами

Ключові слова: ратиця; ураження кінцівок; гнійний пододерматит; флегмона вінчика; міжпальцева виразка; кульгавість

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Bioecology and pathogenicity of *Proteus* bacteria: A literature review

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Abstract. The role of *Proteus* bacteria in human and animal pathology has increased significantly in recent years, causing acute intestinal diseases, respiratory, hearing, nervous and urinary systems, as well as contributing to the formation of kidney and bladder stones, postoperative complications, and nosocomial infections. The persistence of some issues, such as their properties and interaction with the microbiocenosis, remains a subject of debate even after a long study of *Proteus* bacteria. The research aims to identify promising areas for further study of *Proteus* microorganisms. The information from scientific primary sources on the results of studying microorganisms of the genus *Proteus* was used for the analysis. The study results of *Proteus* bacteria performed by domestic and foreign scientists on the knowledge of their bioecology and potential pathogenicity factors (adhesins, toxins, haemolysins, etc.), characterisation of the positive role of proteins as biodegraders of harmful substances – bioremediators of proper environmental ecology; substantiation of promising areas for further research of bacteria of the genus *Proteus*, which will contribute to the development of an effective methodology for the prevention and treatment of diseases caused by them, the development of rational technologies for the use of their strains – bioremediators of the environment contaminated with harmful substances – are presented in the study. Further study of the genomic properties of *Proteus* bacteria will contribute to a clear understanding of the mechanisms of their potential pathogenicity factors and help to identify and understand the essence of the processes that contribute to the acquisition of new pathogenicity factors and drug resistance. The study of their interaction with representatives of the intestinal microbiocenosis of humans and animals will help to establish the nature of such interaction, determine the feasibility, prospects and rational directions in the creation of effective probiotics

Keywords: properties; swarming; pathogen; symbiont; biodegradation; promising areas of research

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Introduction

Bacteria of the genus *Proteus* are widespread prokaryotes in nature. They are often isolated from the intestines of clinically healthy humans and animals without any symptoms of disease, from various environmental objects such as soil, water, etc. (Al-Kubaisi & Al-Deri, 2022). The isolation of proteins from the intestines of clinically healthy mammals prompts some researchers to consider them representatives of the normal microflora of the macroorganism, but the arguments in this regard are insufficient. Currently, it would be more appropriate to consider such cases as bacterial carriers. However, it would be incorrect to categorically deny their possible belonging to the representatives of the intestinal microbiocenosis of macroorganisms in the future – the distinct plasticity of the biological properties of these prokaryotes, the presence of naturally circulating avirulent strains of them create prerequisites for the formation of the most rational variant of symbiosis with macroorganisms. At this stage of the phylogeny of proteins, the opinion of experts who consider them pathogens, causing a range of diseases in humans and animals, should be accepted. In particular, it concerns the naturally occurring *Proteus mirabilis*, which causes gastroenteritis and extraintestinal infections in humans, such as tympanitis, meningitis, urethritis, pyelonephritis, infectious cystitis, urolithiasis, etc. (Girlich *et al.,* 2020). In animals, proteins are also an etiological factor in gastrointestinal diseases, urinary tract, urolithiasis, etc. In humans and animals, they often cause postoperative complications, colonise medical instruments (mainly catheters) (Al-Sudani & Abdul-Kareem, 2023), and are also involved in foodborne toxicity (Gong *et al.,* 2019).

Potential pathogenicity factors of different proteus species have been studied in detail (Beltrão *et al.,* 2022; Liu *et al.,* 2023). Their nature and mechanism of pathogenic action have been characterised at an acceptable methodological level. This applies, in particular, to adhesins, toxins, haemolysins, and other factors (Girlich *et al.,* 2020; Gahlot *et al.,* 2022). At the same time, the circumstances (conditions) that affect the intensity of expression of genetic determinants of pathogenicity factors and the possible translocation of the latter within a species remain insufficiently characterised.

Effective prevention of infectious diseases, including those of protean aetiology, is possible only if the biology and ecology of their pathogens are fully comprehended. The basic biological properties of proteins have been thoroughly studied for a long time. Recently, molecular genetic mechanisms of protein film formation and drug resistance have been intensively studied (Akhter *et al.,* 2019). In this regard, it is promising to identify the facts and circumstances of the translocation of antibiotic resistance factors. Studies on the ecology of *Proteus* bacteria are also important. In this regard, their ecological niches have been identified and characterised both within populations of living beings and in various objects of the avital environment (Al-Kubaisi *et al.,* 2022). The results of the identification of protein strains capable of utilising harmful substances in a polluted environment are considered extremely encouraging. The creation of bioreanimator drugs based on them will allow us to address pressing environmental issues more effectively.

The data obtained by domestic and foreign researchers on the biology, ecology and pathogenicity factors of *Proteus* bacteria are not systematised, which hinders their effective use in the organisation of anti-epidemiological and anti-epizootic measures.

The research involved a comprehensive analysis of scientific sources aimed at studying the biological characteristics and pathogenic potential of *Proteus* bacteria. The search

and selection of literature included a thorough search in scientific databases such as Web of Science, Scopus, and PubMed and a selection of sources that best reflected the current state of research in this area. The sources were then systematised to create a coherent review. This included a thorough analysis and synthesis of information on the biology of Proteus bacteria, their role in natural and macrobiotic systems, and mechanisms of pathogenicity. The study will review both well-known and up-to-date articles to ensure that the research on this topic is complete and up to date. This approach allowed us to systematise and summarise the available knowledge and discoveries in this area, revealing key aspects of the bioecology and pathogenicity of bacteria of the genus *Proteus*. The research aims to analyse the literature reports on the bioecology and pathogenicity of bacteria of the genus Proteus and to identify promising areas for further research.

Systematics, morphology, culture, and enzymatic properties of *Proteus spp.*

According to the modern taxonomy of microorganisms, proteins are defined: Domain – *Bacteria*, Type – *Proteobacteria*, Class – *Gammaproteobacteria*, Order – *Enterobacteriales*, Family – *Enterobacteriaceae*, Genus – *Proteus*. Latter includes *P. vulgaris, P. mirabilis, P. penneri, and P. myxofaciens* (Brenner *et al.,* 2005).

Morphology. The *Proteus* bacteria are extremely polymorphic, as reported by the German microbiologist Hauser in 1885, who first identified and characterised the main morphological and cultural properties of *Proteus vulgaris* and *Proteus mirabilis* (Brenner *et al.,* 2005). Later, due to the improvement of microbiological research methodology, the emergence of more advanced equipment, in particular electron microscopes, the introduction of molecular genetic studies, etc., the ultrastructure and all other properties of proteins were studied in detail, revealing their biology, ecology and pathogenicity in great detail.

Proteus are gram-negative rods 0.4-0.6 μm by 1-3 μm in size, motile (peritrichs), do not form spores, and no typical capsules are detected. In the process of growth and proliferation, cells of different shapes, sizes and ultrastructure are also observed. The membrane of bacteria of the genus *Proteus* is virtually indistinguishable in its ultrastructural organisation from the cell membranes of other microorganisms of the family *Enterobacteriaceae*. Electron microscopy using cryotechnology reveals the outer and cytoplasmic membranes with a transparent periplasm 10.6-14.3 nm thick between them. The outer membrane is wavy, with a thin, 2-3 nm peptidoglycan layer intertwined. The cytoplasmic membrane is a thin bilayer structure closely adjacent to the periplasm. The proteins do not form typical capsules, but in *P. mirabilis* a capsular polysaccharide of fibrous structure with fibres located perpendicular to the cell surface was found. Lipopolysaccharide of the outer membrane (O-antigen) in *P. mirabilis* and *P. vulgaris*, in addition to monosaccharides typical for enterobacteria – glucose, glucosamine, D-glycero-D-manno-heptose, also contains galacturonic acid and, often, lysine (Brenner *et al.,* 2005). The peptidoglycan of *P. mirabilis*, unlike the peptidoglycan of other enterobacteria, is O-acetylated, which makes it resistant to lysozyme. The surface structures of bacteria of the genus *Proteus* are represented by flagella and fimbriae. Other structures (spines, curls) are also found (Gahlot *et al.,* 2022).

Culture and enzymatic properties. Proteins are facultative anaerobes, chemo-organotrophs, with both respiratory and fermentative metabolic types. They grow in the temperature range of 10-43°C. The temperature optimum for populations that exist as part of microbiocenoses in human or animal habitats is about

37°C. Proteins are undemanding to cultivation conditions, growing on simple nutrient media (pH 7.2-7.4). In meat and peptone broth, MPBs cause diffuse turbidity; in old cultures, sediment and a surface film are detected. On dense media, O-forms of the proteus form round, compact colonies, and H-forms are characterised by "creeping" growth, the medium is gradually covered with a kind of "veil" of smoky blue colour, which is called "swarming". During the latter process, the primary colony undergoes cell differentiation. Short rod-shaped bacteria (vegetative cells) differentiate into unsegregated multinucleated schwerm cells, ranging in length from 20-30 to 80-100 μm, with 50-500 times more flagella than their predecessors. The cytoplasm of the swarmer contains about 20 nucleoids. Compared to vegetative cells, swarmer cells are dominated by lipopolysaccharides (LPS) with long O-antigenic side chains, differences in the expression of some proteins and enzymes, and a markedly higher fluidity of the outer membrane (Allison *et al.,* 1994). The transformation of vegetative cells into swarmer cells is only the first phase of three observed during the swarming process. The next phases are "bacterial mass migration" and "consolidation" (Allison *et al.,* 1993). The migration of proteins on the surface of a dense medium is strictly multicellular. A group of swarmer cells rapidly migrates radially away from the colony on the surface of the dense medium. Gradually, their movement stops, and the swarmer cells differentiate into vegetative cells. The latter is called the consolidation phase. These phases are repeated, which is manifested by the formation of concentric rings on the surface of the medium. When swarmer cells are sown in a liquid medium, they differentiate into vegetative cells. The translocation of *P. mirabilis* cells on a dense surface is facilitated by a cell surface polysaccharide enriched with galacturonic acid and N-acetylgalactosamine, capsular polysaccharide (CPS), apparently due to a decrease in surface friction. The latter is substantiated by the fact that mutations in the 1112 bp gene encoding the enzymes necessary for the synthesis of LPS and the assembly of the above polysaccharide significantly inhibit cell migration (Gygi *et al.,* 1995). Based on the analysis of cell differentiation and group motility, a unique kinetic model of *P. mirabilis* swarming was developed, the key element of which is the proven dependence of the swarming process on cell age (Esipov & Shapiro, 1998). The differentiation of vegetative cells of uropathogenic *P. mirabilis* strains into schwerm cells is accompanied by an increase in the activity of some of their pathogenicity factors, in particular, extracellular haemolysin and metalloproteases, and intensification of invasion of urogenital epithelial cells (Allison *et al.,* 1993). Genetic analysis of the bases of *P. mirabilis* swarming shows that this process is regulated by at least 40-60 genes. Signalling molecules were found to initiate cell differentiation and migration. When studying the effect of 20 amino acids on the swarming process in proteins, it was proved that only glutamine can initiate this process on a minimal growth medium (Allison *et al.,* 1993). The authors consider glutamine to be a specific chemoattractant for swarming cells. Other factors, such as the viscosity of the medium and the presence of "anti-tagging" antibodies, are also likely to be involved in cell differentiation. It was found that with increased medium viscosity and the presence of antibodies, the rotational movement of flagella slows down, and abnormal differentiation of swarm cells is observed. It is believed that flagella function as tactile sensors of external growth conditions (Massad *et al.,* 1996).

Swarming is observed not only on solid media but also on the surface of many dense avital substrates, as well as in the macroorganisms contaminated with proteins – on

mucous membranes and other tissues (Brenner *et al.,* 2005). Proteins are characterised by a distinct enzymatic activity, in particular, they liquefy gelatin, peptidise milk, reduce nitrates to nitrites, decompose urea with the release of ammonia, etc. Based on the results of the enzymatic activity study, the genus of proteins within the Enterobacteriaceae family is determined and their species are identified.

Antigenic structure and pathogenicity factors of *Proteus spp*.

The antigenic structure of *Proteus* bacteria is complex, with O-, H- and, in some strains, K-antigens. The somatic (O-antigen) and flagellar (H-antigen) antigens of *P. mirabilis* and *P. vulgaris* have been characterised in detail. The chemical nature of the O-antigen is lipopolysaccharide. About 50 varieties of O- and about 20 H-antigens have been identified in proteins. Based on O-antigens, a methodology for determining the serogroup affiliation of clinical strains of proteins and serological diagnosis of diseases caused by them has been developed (Knirel *et al.,* 2011). However, this area requires further research because antigenically distinct strains are often isolated, especially among *P. penneri* (Palusiak, 2016).

The pathogenicity factors of bacteria of the genus *Proteus* are generally typical for members of the family *Enterobacteriaceae*. They ensure the ability of certain species (strains) to parasitise living organisms and cause pathological phenomena. Since the latter are most often observed under circumstances that lead to a weakening of the defence reactions of the human or animal body, proteins are classified as opportunistic microorganisms, and the diseases caused by them are called opportunistic infections. In the presence of pathogenicity factors, proteins adhere to the cell surface, colonising mucous membranes or the wound surface of the skin, form a biofilm, sometimes penetrate cells, and exert toxic effects directly on cells, tissues, organs and indirectly, causing a cascade of pathological phenomena in the infected macroorganism.

Regarding the representatives of the genus *Proteus*, it is worth noting their extremely pronounced biological plasticity, which contributes to the survival of bacteria in various environmental conditions and parasitism in humans and animals (Prystupa *et al.,* 2017). The latter is possible only if a particular strain has certain pathogenicity factors that provide it with virulence – the ability to penetrate the body and stay there, to resist the factors that maintain the latter's homeostasis, and to reproduce.

Adhesins. The primary phenomenon in the development of any infectious process caused by a pathogen is its adhesion to the surface of the epithelium or other cells in the macroorganism. It is ensured by physicochemical processes that occur when molecules located on the surface of the pathogen and molecules of the infected cells (tissues) come into contact. Only pathogens that synthesise adhesins, substances of protein or glycoprotein nature that are part of certain surface structures of their microbial cells, can attach to the cells of the latter reliably. In bacteria of the *Enterobacteriaceae* family, including Proteus, adhesins are concentrated mainly in the fimbriae (Massad *et al.,* 1996) and are characterised by a certain specificity – the ability to attach to the surface of certain cells (tissues) in an infected organism. An illustration of the latter can be found in strains of bacteria of the genus *Proteus*, which adhere intensively to urogenital epithelial cells. In this regard, it would be appropriate to assert that bacterial adhesins have a "targeted function", which is manifested by the pathogen's distinct tropism to certain tissues of the microorganism. Several types of fimbriae have been identified in representatives of the genus *Proteus*, which differ, in particular, in structure:

thick (7 nm in diameter) and thin (4 nm in diameter) (Armbruster *et al.,* 2012). The former is called MR/P *(Proteus-like fimbriae)*, and the latter are called MR/K (Klebsiella-like fimbriae). It was found that MR/P fimbriae provide more efficient adhesion of bacteria to epithelial cells compared to MR/K fimbriae (Silverblatt & Ofek, 1978). A 21 kDa protein isolated from MR/P fimbriae from *P. mirabilis* strain 3087 reacted intensively with urethral epithelial cells of the urinary tract *in vitro*. It was found that in the infected human body, it causes intensive colonisation of the kidneys and, as a result, pyelonephritis. MR/K fimbriae (MR/K haemagglutinins) differ from MR/P fimbriae not only in structure but also in their ability to bind to the tissues of the infected organism. They cause intensive adhesion of cells in the Bowman's capsule and basement membranes of the renal tubules and do not adhere to other epithelial cells of the urinary system. MR/K fimbriae are more common in *P. penneri* strains (Yakubu *et al.,* 1989).

In *P. mirabilis*, in addition to those described above, the following are also described: *"PMF fimbriae"*, "ambient temperature fimbriae" *(ATF)* and "uroepithelial cell adhesins". *PMF* fimbriae contain a polypeptide of 184 amino acids. It is believed that by recognising certain cell receptors in the infected organism, they ensure the adhesion of *P. mirabilis* to the bladder epithelium. A mutant of *P. mirabilis* that did not synthesise the above polypeptide colonised the bladder of experimentally infected mice to a much lesser extent compared to the field strain (Massad *et al.,* 1996). Ambient temperature fimbriae *(ATF)* are described by G. Massad *et al.* (1996). Electron microscopy revealed that they look like rod-like structures located on the surface of microbial cells. The main subunit of *ATF* fimbriae is a protein with a molecular weight of 24 kDa. The synthesis of ATF fimbriae depends on the culture conditions, in particular the temperature of the medium. Intensive expression of fimbriae in Luria broth occurred at a temperature of 23°C during the stationary phase of microbial culture growth. The pathogenetic significance of *ATF*-fimbriae has not been reported.

S.K. Wray *et al.* (1986) isolated a protein from the uropathogenic isolate *P. mirabilis HU 1069* and, having studied its properties, named it uroepithelial cell adhesin *(UCA)*. The researchers found that *UCA* adhesin is a polypeptide with a molecular weight of 17.5 kDa and causes bacterial adhesion to uroepithelial cells. I.G.W. Bijlsma *et al.* (1995), studying UCA adhesin synthesised by *P. mirabilis* strains isolated from dogs using electron microscopy, found that it has the appearance of thin fimbriae with a diameter of 4 nm. R. Pellegrino *et al.* (2013), using a wild type uropathogenic *P. mirabilis* strain that synthesised UCA adhesin and a *P. mirabilis* mutant that was unable to express UCA, confirmed in experiments on various biological models that UCA adhesin plays an important role in the colonisation of the urinary tract by *P. mirabilis*.

Proteins adhered to the cell surface can penetrate intracellularly under certain conditions. There is no convincing evidence that they possess the invasion factors characteristic of enterobacteria. However, the very fact of the ability of *Proteus* bacteria to penetrate macroorganism cells is well established. It has been proven that *P. mirabilis* and *P. vulgaris* invade *Vero* and *Hela* cells, mouse fibroblasts L-929 and human blood lymphocytes. The intensity of *P. mirabilis* invasion both *in vivo* and *in vitro* is stimulated by urea and correlates with the haemolytic activity of the strains. The ability of *P. mirabilis* to multiply in invaded cells of the permanent cell line L-929 and human blood lymphocytes has been experimentally confirmed (Peerbooms *et al.,* 1984).

Toxins. Bacteria of the genus *Proteus* synthesise endotoxins and exotoxins. The endotoxin is an outer membrane lipopolysaccharide

(LPS) (described previously as an O-antigen). Its pathogenic effect in an infected organism is similar to that of endotoxins of other gram-negative bacteria, characterised by pyrogenicity, the ability to cause hypotension, disseminated intravascular coagulation and lethal shock (Rózalski *et al.,* 2007). Its toxic effect on various cell types, including macrophages and lymphocytes, is realised through the lipid complex A, which is part of LPS. When it binds to the LPS-binding protein in the blood, it activates the CD14 receptor on macrophages, which leads to the intensive synthesis of biologically active mediators (TNF-α, IL-1, IL-6, IL-8 and IL-10). Depending on the level of expression of the latter, the effect in an infected organism can vary from beneficial adjuvant to toxic (Rietschel & Brade, 1992). Lipid A in *P. mirabilis* is considered to be an important factor of pathogenicity, characterised by mitogenic activity, lethal toxicity, and the ability to induce a local Schwartzman reaction (Sidorczyk *et al.,* 1983). A study has been published that proved the negative effect of LPS from *Proteus* bacteria on the activity of cytochrome P-450 enzymes in mouse liver (Kaca *et al.,* 1996). It is known that the destabilising effect on the latter, which is represented by a whole complex of enzymes and plays an important role in the metabolism of steroids, bile acids, unsaturated fatty acids, phenolic metabolites, as well as in the neutralisation of xenobiotics (poisons, drugs, etc.), leads to a disruption of physiological processes, which is manifested by a wide variety of pathophysiological phenomena in the infected organism.

Proteinases, urease, lecithinase. Other potential pathogenicity factors of proteins are their proteinases, hyaluronidase, urease, lecithinase, DNA and RNA nucleases. Protein strains isolated from patient materials are usually characterised by high proteolytic activity with a clear specificity for muscle and connective tissue proteins. They can hydrolyse gelatin, fibrin, albumin, and casein (Brenner *et al.,* 2005). The most pronounced biodestructive effect of these enzymes is observed when they are combined with microbial hyaluronidase. The latter, by destroying hyaluronic acid, causes increased tissue permeability in the infected organism, thus facilitating the migration of the pathogen.

The urease enzyme in *Proteus mirabilis* (PMU) consists of three subunits: PmUreα, PmUreβ and PmUreγ, formed into a quaternary structure. In the course of studying urease as a potential pathogenicity factor in bacteria of the genus *Proteus*, its significant role in the pathology of the kidneys and urinary organs was proved. In particular, it was found that *P. mirabilis* and *P. penneri* are involved in the formation of kidney and bladder stones due to the presence of urease (Rózalski *et al.,* 2007). As a result of urea hydrolysis caused by bacterial urease, the pH of urine increases, which leads to precipitation of its components – Mg^{2+} and Ca^{2+}. As a result, stones are formed, in particular struvite ($MgNH_4PO_4 \cdot 6H_2O$) (Broll *et al.,* 2021).

Immunoglobulin proteases. IgA protease has been found in *P. mirabilis, P. vulgaris* and *P. renneri* strains of different origin (Senior *et al.,* 1991). It has also been reported that a strain of *P. mirabilis* isolated from a patient with a chronic disease secreted a proteolytic enzyme that cleaved two classes of antibodies – IgA and IgG, as well as gelatin, casein, and bovine serum albumin. It turned out that proteases are metalloenzymes, the action of which is manifested in an alkaline environment, at a pH of 8 (Loomes *et al.,* 1992).

Lecithinase. The role of lecithinase as a pathogenicity factor in many bacteria is well known. *P. vulgaris* and *P. mirabilis* strains with pronounced lecithinase activity are often isolated from patients with signs of purulent inflammatory processes. Their O-forms produce the enzyme more intensively, especially during the period of dissociation of strains from the H-form to the O-form (Bozhko, 2012).

Haemolysins. Hemolysins are considered to be one of the leading factors in the pathogenicity of microorganisms. In bacteria of the genus *Proteus*, haemolytic activity correlates with the invasiveness of their strains (Peerbooms *et al.,* 1984; Rozalski *et al.,* 1993). Haemolysins of the *Proteus* genus belong to the family of pore-forming toxins (Braun & Focareta, 1991). There is enough information on the study of the haemolytic properties of bacteria of the genus *Proteus* to demonstrate the importance of this indicator, which has long been used as a marker of the pathogenicity of clinical strains. In a study of 84 strains of *P. mirabilis* and *P. vulgaris* isolated from patients with signs of urinary tract infection (UTI), it was found that the vast majority of them caused signs of *α-haemolysis* (greenish colouration around colonies) on dense medium (Kotełko *et al.,* 1983). The results of a study of the haemolytic properties of 126 strains of *P. mirabilis* and *P. vulgaris* isolated from various materials (from patients with signs of UTI, soil, etc.) are also reported. All studied strains haemolyse human and sheep erythrocytes during incubation in nutrient broth. The 10 strains of *P. mirabilis* studied under similar conditions also haemolyzed erythrocytes of guinea pigs, rabbits, mice, horses, cattle, and chicken (Kotełko *et al.,* 1983). A study of 45 strains of P. penneri revealed that they synthesised extracellular and/or cellular haemolysins (Senior *et al.,* 1991). The synthesis of extracellular haemolysin by *P. penneri* strains correlated with their cytotoxic activity determined *in vitro* on *Vero* cell lines and mouse fibroblasts L-929 (Rozalski *et al.,* 1993).

Siderophores are extremely important elements in the body of living things, including microorganisms. Prokaryotes, in the event of a deficiency of soluble iron in their environment, in particular in the human or animal body, synthesise and excrete their siderophores. The latter binds to iron and transports it into the microbial cell using transport mechanisms, thus causing iron deficiency in the infected microorganism, and weakening its resistance. In *P. mirabilis*, α-hydroxyisovaleric acid has been described as a siderophore (Rozalski *et al.,* 1993).

Peptidoglycan (murein) and a "capsular" acetylated highly hydrated polymer are also factors in the pathogenicity of the proteins. *P. mirabilis* peptidoglycan, unlike other enterobacteria, is glycosylated, which provides its resistance to lysozymes. It has been established that in the body of people infected with the protein, fragments of O-glycated peptidoglycan cause several pathological phenomena, including rheumatoid arthritis. The pathogenicity of *Proteus* bacteria also includes motility, film formation (Akhter *et al.,* 2019), and drug resistance.

Mobility. In the process of swarming, the proteins intensively colonise the epithelial membranes in the infected organism, forming biofilms that significantly protect them from immune defence factors and antibacterial drugs, in particular antibiotics. In addition, during this period, the activity of several other pathogenicity factors may increase. It was found that the differentiation of vegetative cells of uropathogenic *P. mirabilis* into schwerm cells is accompanied by an increase in the activity of extracellular haemolysin, urease, and metalloproteases (Allison *et al.,* 1994). It has also been reported that *P. mirabilis* swarmer cells, compared to vegetative cells, invade urogenital epithelial cells more intensively both *in vitro* and *in vivo* (Allison *et al.,* 1994). Recently, researchers devoted much attention to the study of virulence genes of pathogenic microorganisms, including the genus *Proteus*. It has been reported that the genetic profile of 36 clinical isolates of *Proteus mirabilis* isolated from patients with urinary tract infections in Brazil revealed the following genetic determinants of pathogenicity factors: mrpG, pmfA, ucaA, nrpG and pbtA (Beltrão *et al.,* 2022).

The pathogenicity factors described above are capable of providing certain strains with a parasitic mode of existence, usually in an insufficiently protected (immunologically weakened) human or animal organism. The design of pathogenicity factors in proteins is not stable within their species (strains). Some of them may be lost or, on the contrary, appear in the process of circulation of their strains in nature. The translocation of pathogenicity factors, as well as many other traits, occurs by mechanisms known in bacterial populations: transduction, conjugation, and transformation. Transmission of pathogenicity factors is possible both vertically – from parental individuals, and horizontally – to other strains and species. In the latter case, it is mainly due to plasmids. The above argues for the importance of continuous monitoring of the pathogenicity of clinical strains of proteins that cause diseases in humans and animals. Reliable information in this regard can be obtained both by using the modern methodology of molecular genetic testing of pathogenicity factors and the classical methodology based on the detection of phenotypic signs of virulence in clinical strains.

Protein-driven infection can be triggered by the pathogenic action of bacteria in the body's microbiocenosis or by exogenous infection. Autoinfection occurs mainly against the background of various other phenomena that weaken the immune system, including local (tissue) immunity, for example, in entero-, oral-, coronavirus, Escherichia coli or other infections, or under the influence of various toxic substances of avital nature, etc. Depending on the design of the strain in terms of the content of its virulence factors, the dose and site of penetration into the macroorganism, and finally, the immunoresistance of the latter and the nature of its exposure to environmental conditions, the pathogenesis and clinical manifestation of the disease may be different. This is clearly illustrated by the peculiarities of pathogenesis in urolithiasis and gastrointestinal disorders in humans and animals (Kroemer *et al.,* 2014; Vozianov *et al.,* 2016).

J.N. Schaffer *et al.* (2016) analysed the pathogenesis of urolithiasis and showed that *Proteus mirabilis* forms urease- and mannose-resistant clusters in the bladder of infected dogs and cats, which accumulate minerals, which is the starting point for the formation of urinary stones. The role of microbial urease in the formation of struvite stones in the kidneys and bladder of patients has already been reported. In addition to the above factors, glycocalyx substances (highly hydrated polymers on the surface of microbial cells) are also involved in the stone formation mechanism (Beynon *et al.,* 1992).

In the case of localisation of a protein-driven pathological process in the gastrointestinal tract, microbial toxins, in particular exotoxin, play a particularly important role in the pathogenesis of the disease. The thermolabile exotoxin synthesised by the protein penetrates intestinal epithelial cells via receptor-dependent endocytosis and causes several phenomena that activate adenylate cyclase, which begins to synthesise cyclic adenosine monophosphate (cAMP). The latter triggers a signalling pathway that leads to the outflow of chloride ions and other ions from the cell through CFTR channels and stops the entry of sodium ions into the cell. The latter are transported with water molecules, so their content in the cell is significantly reduced. Disruption of the water-salt balance leads to diarrhoea, intoxication, and related phenomena. In experiments using white mouse intestinal explants (Skibytsky, 1993), the toxic effect of *P. mirabilis* and *P. vulgaris* strains isolated from the intestine of a person with signs of gastrointestinal tract damage was manifested by the destruction of mucosal epithelial microvilli. A significant pathogenic role in the

gastrointestinal localisation of the process is also played by amino acid decarboxylation products: histamine, putrescine and cadaverine, which accumulate intensively as a result of the action of decarboxylases synthesised by proteins. Their pathogenic effect can also be intensified by the antagonism of proteins to representatives of the microbiocenosis of the contaminated biotope. In the case of mixed infection, in particular viral-bacterial infection, the pathogenesis of the disease can be much more complex, due to the nature of the direct interaction of proteins and other pathogens, or indirectly through the infected macroorganism.

Distribution of bacteria of the genus *Proteus* in the natural environment

To assess the epidemiological (epizootological) aspects of diseases caused by bacteria of the genus *Proteus*, in particular, to identify sources and factors of pathogen transmission, it is necessary to consider their ecological niches. First of all, the detection of *Proteus* in mammals. Bacteria of the genus *Proteus* are often isolated from the intestinal contents of humans and animals without any symptoms of disease. In particular, it has been reported that 4% of faecal samples from clinically healthy people contained bacteria of the genus *Proteus* (Bartges *et al.,* 1995). There have also been many reports on the isolation of *Proteus* from clinically healthy animals. Bacteria of the genus *Proteus* have been isolated, mainly from the intestinal contents, from gorillas (Bittar *et al.,* 2014), horses (Meyer *et al.,* 2010), cattle (Sadovsky, 1997), pigs (Kozlovska *et al.,* 2022), dogs (Liu *et al.,* 2023) and other animals.

Analysing the literature on the isolation of bacteria of the genus *Proteus* from various sources, D. Drzewiecka (2016) reports their isolation from various species of wild birds, including sparrows, blackbirds, white storks, wild turkey vultures, as well as from synanthropic rodents, snakes, bees, flies, cockroaches, aquatic animals – turtles and various fish species, including mackerel, freshwater Nile tilapia, tilapia from Lake Victoria, as well as from oysters and shrimps. *Proteus spp.* found in sea turtles near the Canary Islands in Spain have been identified as one of the causes of their deaths. *P. renneri* and *P. vulgaris*, isolated on Jekyll Island (USA), were found to be involved in embryonic mortality in turtles. The etiological role of *Proteus sp.* in the disease of alligators, which led to their death, was also proved. *P. hauseri* has also been reported to be involved in carp *(Cyprinus carpio)* disease (Kumar *et al.,* 2015).

Proteins are often found in food products (Al-Kubaisi *et al.,* 2022). In this regard, V.A. Bezugla (1975) carried out significant research in Ukraine. In the study of 1457 samples taken from slaughtered animals, she isolated 130 cultures of Proteus, which is 8.9% of the total number of samples examined. More than 70% of the isolates were identified as *P. mirabilis*, the rest were identified as *P. vulgaris*. Among the 365 *P. mirabilis* strains isolated, 105 (29.5%) were pathogenic to white mice, both by parenteral and enteral administration of the microbial culture. More than 60% of the isolated proteus cultures synthesised hyaluronidase, and more than 80% caused haemolysis of erythrocytes.

In a bacteriological study of 14 samples of minced meat and 38 samples of sausages (frankfurters) collected in the city's retail network, proteins were detected in seven cases (3.6%) – 2 strains of *Proteus vulgaris* from minced meat and 5 strains (4 strains of *Proteus vulgaris*, 1 strain of *Proteus mirabilis*) from the surface of sausages and frankfurters (Kozlovska *et al.,* 2022). It is also important to mention the positive role of *Proteus* species in the environment. Strains of *Proteus spp.* that protected legumes from the pathogenic *Fusarium moniliformae* and strains that produced volatile organic compounds that had a detrimental effect on nematodes, such as the soil-borne *Caenorhabditis*

elegans and the plant-pathogenic *Meloidogyne incognita*, have been identified (Lu *et al.,* 2014).

Many reports have been published on the important role of proteins in environmental bioremediation. In particular, the participation of *Proteus spp.* in the destruction of petroleum hydrocarbons in soil has been proven (Ibrahim *et al.,* 2013). The strains of *P. mirabilis* and *P. vulgaris* were found to be effective destructors of crude oil. A strain of *P. vulgaris* has been reported to be effective in utilising crude oil and diesel fuel (Olajide & Ogbeifun, 2010). A strain of *P. vulgaris* capable of reducing dichlorodiphenyltrichloroethane (DDT) to dichlorodiphenyldichloroethane (DDD) was isolated from the mouse intestine (Foght *et al.,* 2001). The ability of *Proteus sp.* to degrade dyes used in the textile industry has also been reported (Drzewiecka, 2016). Proteins are also related to the utilisation of heavy metals. In particular, a strain of *Proteus sp.* was found to be able to significantly reduce the concentration of the toxic metal Cr (VI) in seawater (Ge *et al.,* 2013).

Resistance to physical and chemical factors and drugs of bacteria of the genus *Proteus*

The global spread of proteins in nature is facilitated by their pronounced resistance to physical and chemical factors and drugs. Bacteria of the *Proteus* genus can adapt to survive in seawater. It has been reported that osmophilic (halotolerant) strains of *Proteus spp.* were isolated from the water of a Salt Lake in the Algerian Sahara (Hačene *et al.,* 2004). The resistance of bacteria of the genus Proteus to copper and other heavy metals has been reported (Ge *et al.,* 2013).

N. Rau *et al*. (2009) found *P. mirabilis* to be resistant to arsenic, copper, chromium, cobalt, cadmium, zinc, mercury, nickel, and lead. The sensitivity of proteins to drugs has been determined by many researchers. When antibiotic susceptibility of 100 strains of *Proteus mirabilis* isolated from urine was determined, 16 (16%) were found to be multidrug-resistant to cephalosporins. High resistance was observed concerning co-trimoxazole (97%), nalidixic acid (93%), cefotaxime (77%) and amoxicillin (62%). The highest susceptibility was observed for aminoglycosides, cephalosporins, glycopeptides, polypeptides, and macrolides in a study of antibiotic susceptibility of 20 strains isolated from animals in Ukraine (Aishpur *et al.,* 2016). They were less sensitive to penicillin. The genetic profile of 36 clinical isolates of *Proteus mirabilis* isolated from patients with urinary tract infections in Brazil revealed the presence of the following drug resistance determinants ($bla_{VIM,}$ bla_{IMP}, bla_{SPM}, $bla_{GES,}$ $bla_{OXA-23-like}$, $bla_{OXA-48-like}$, $bla_{OXA-58-like}$ and $bla_{OXA-10-like}$) (Beltrão *et al.,* 2022).

It has been proven that natural resistance to antibiotics is due to the ability of proteins to synthesise enzymes that inactivate antibiotics, in particular, β-lactamases: extracellular penicillinase, carbenicillin hydrolysing penicillinase, ampicillinase, etc. Resistance of naturally circulating strains is not a stable indicator and may change under certain conditions. Mechanisms of resistance modification may be associated with mutations in structural genes, recombination, and the acquisition or loss of R-plasmids (Luzzaro *et al.,* 2006).

An analysis of literature reports on the study of *Proteus* bacteria shows their widespread distribution in the environment and their involvement in diseases in humans, animals, and other living creatures, including amphibians and fish (Kumar *et al.,* 2015; Drzewiecka, 2016; Al-Kubaisi & Al-Deri, 2022). In humans, bacteria of the genus Proteus affect the digestive and respiratory organs, urinary tract, nervous system, etc. (Hamilton *et al.,* 2018). In animals, proteins are etiological factors of gastrointestinal diseases and the urinary tract, and often cause secondary infection in viral enteritis in newborn animals, etc. Proteins are also implicated

in foodborne toxicity (Gong *et al.,* 2019). Scientists have carried out many important studies concerning, in particular, the biology of bacteria of the genus *Proteus* (Esipov & Shapiro, 1998), factors of their pathogenicity (Armbruster *et al.,* 2012; Broll *et al.,* 2021; Gahlot *et al.,* 2022), in particular, film formation and drug resistance (Akhter *et al.,* 2019), pathogenesis of diseases caused by them in humans and animals (Schaffer *et al.,* 2016; Prystupa, 2017), and involvement in foodborne toxicity (Gong *et al.,* 2019).

Studies on the ecology of bacteria of the genus *Proteus* are also important. In this regard, their ecological niches have been identified and characterised both within populations of living beings (Drzewiecka, 2016) and in various objects of the avital environment (Lu *et al.,* 2014; Drzewiecka, 2016). The results of the identification of protein strains capable of utilising harmful substances in a polluted environment are extremely encouraging (Ibrahim *et al.,* 2013; Ge *et al.,* 2013). The development of bioremediation drugs based on them will allow us to solve urgent environmental problems more effectively.

The literature review shows that molecular genetic methods are widely used in the study of *Proteus* bacteria (Allison *et al.,* 1994; Beltrão *et al.,* 2022). The results obtained have allowed us to more clearly interpret important elements related to both the biology of proteins and the phenomena in nature caused by them, including diseases in humans and animals.

Conclusions

The study highlights the significant growth of the role of bacteria of the genus *Proteus* in the pathology of both humans and animals. They are known for their ability to cause a variety of diseases, including acute intestinal diseases, respiratory, hearing, nervous and urinary systems, and contribute to the formation of kidney and bladder stones. It is noted that bacteria of the genus *Proteus* have become active participants in nosocomial infections, which indicates their prevalence and potential threat to public health. The results of the literature review also reflect the positive role of Proteus bacteria in environmental bioremediation and the fight against harmful substances and emphasise the importance of further research in this area. It is determined that the study of their genomic properties and interaction with the intestinal microbiota of humans and animals is crucial for understanding the mechanisms of pathogenicity and the development of drug resistance. This will also contribute to the development of effective probiotics and rational technologies for the use of *Proteus* bacterial strains as bioremediators in the environment.

The presented analysis of literature reports shows the relevance of scientific research on bacteria of the genus *Proteus*. However, despite the long period of study of Proteus, a significant number of studies conducted at different methodological levels, several important phenomena related to both their biology and pathogenic potential remain insufficiently deciphered. The issues of understanding the mechanisms of possible antagonistic action of representatives of the ubiquitous gastrointestinal microflora against Proteus and the prospects for developing effective means of preventing excessive Proteus proliferation in human and animal habitats on their basis remain relevant. The methodology for diagnosing diseases of protean aetiology also needs to be improved. An important aspect of further research on proteins is to detail the conditions that affect the intensity of toxin formation in various products and animal feed.

The natural adaptive capacity of proteins argues for the importance of researching to further analyse the known and likely ecological niches for them. The latter concerns, first of all, various water bodies, in particular, the determination of conditions that affect the intensity of inter-population and interspecies

translocation of genetic determinants of pathogenicity and drug resistance. Intensification of research on interspecies communication, detailing the factors and circumstances that regulate its nature and consequences would certainly contribute to the identification of rational directions in the development of an optimal methodology for controlling the pathogenicity of naturally occurring bacteria of the genus *Proteus*, minimising the growth of their resistance to antimicrobial agents. Further study of proteins as potential utilisers of harmful substances in terms of developing effective environmental bioremediators remains extremely important.

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Conflict of Interest

None.

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Біоекологія та патогенність бактерій роду *Proteus*: літературний огляд

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Анотація. В останні роки значно зросла роль бактерій роду *Proteus* у патології людини і тварин, спричинюючи гострі кишкові захворювання, ураження органів дихання, слуху, нервової та сечовивідної систем, а також сприяючи утворенню каменів у нирках і сечовому міхурі, післяопераційним ускладненням та нозокоміальним інфекціям. Втриманість деяких питань, таких як їхні властивості, взаємодія з мікробіоценозом, залишається предметом дискусій навіть при тривалому вивченні бактерій роду *Proteus*. Мета роботи – визначення перспективних напрямів подальшого вивчення мікроорганізмів роду *Proteus*. Було використано для аналізу інформацію в наукових першоджерелах про результати вивчення мікроорганізмів роду *Proteus*. У статті викладені результати досліджень бактерій роду *Proteus*, виконаних вітчизняними та зарубіжними науковцями, щодо пізнання їх біоекології та потенційних факторів патогенності (адгезинів, токсинів, гемолізинів та ін.), охарактеризовано позитивну роль протеїв як біодеградантів шкідливих речовин – біореаніматорів належної екології довкілля; обґрунтовано перспективні напрями подальшого дослідження бактерій роду *Proteus*, які сприятимуть розробці ефективної методології профілактики та лікування обумовлюваних ними захворювань, розробці раціональних технологій використання їх штамів – біореаніматорів забрудненого шкідливими речовинами довкілля. Подальше вивчення геномних властивостей бактерій роду *Proteus* сприятиме чіткому розумінню механізмів реалізації потенційних їх факторів патогенності, допоможе виявляти та розуміти суть процесів, що сприяють набуттю ними нових факторів патогенності, резистентності до лікарських засобів. Вивчення взаємодії їх із представниками кишкового мікробіоценозу людини і тварин дозволить встановити характер такого взаємовпливу, визначить доцільність, перспективи та раціональні напрями у створенні ефективних пробіотиків

Ключові слова: властивості; роїння; патоген; симбіонт; біодеградація; перспективні напрями досліджень

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Indicators of protein exchange in dogs with different types of higher nervous activity

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Abstract. The relevance of the study is due to the current lack of data on cortical mechanisms of protein metabolism regulation in dogs, which is important to consider when developing methods of corrective action on metabolism. The aim of the study was to determine the peculiarities of protein metabolism in dogs with different types of higher nervous activity, and to establish the relationship between the main characteristics of nervous processes and indicators of protein metabolism in the blood. The leading method in the study of this issue was a new method for determining the typological features of the nervous system in dogs, and the obtained scores of strength, balance, and mobility of nervous processes helped to identify the degree of their influence on protein metabolism. A significant effect of short-term nutritional deprivation on the content of certain indicators of protein metabolism in the blood of dogs, depending on their temperament, was established. Under the action of the stimulus during the day, a decrease in the level of albumin and changes in the ratio of globulins were observed in the blood of dogs, in particular, an increase in the relative content of α- and β-globulins and a decrease in γ-globulins. It was determined that the type of higher nervous activity has a significant effect on the content of total protein, albumin, globulins, β-globulins, γ-globulins and the value of the albumin-globulin ratio ($F=3.02\text{-}14.6>FU=2.90$; $P<0.05\text{-}0.001$) in the blood plasma of dogs. A direct relationship between the strength of nervous processes and the content of total protein and albumin, γ-globulins ($r=0.62\text{-}0.73$; $P<0.01$) and an inverse relationship between the content of α- and β-globulins ($r=-0.51\text{-}0.56$; $P<0.01$) was found three days after the onset of short-term nutritional deprivation. The balance of nervous processes was inversely related to the content of β-globulins ($r=-0.44$; $P<0.05$). The fundamental knowledge gained is of practical value for the development of new, modern methods of metabolic correction taking into account the temperament of animals

Keywords: albumins; globulins; total protein; albumin-globulin ratio; blood plasma; temperament

Introduction

The dog (*Canis familiaris*) was the first domesticated animal, and today there are hundreds of breeds in the world. During domestication, dogs were primarily selected for temperament, behaviour and cognitive abilities. However, the genetic basis of these abilities is not fully understood (Tonoike *et al.*, 2022). Like many other mammals, they have a complex nervous system that allows them to perceive, process, and respond to a variety of stimuli. Research on higher nervous activity (HNA) in dogs focuses on understanding their cognitive abilities, perception, learning and social behaviour.

While studying the physiological basis of temperament, I.P. Pavlov developed a classification of HNA types that is still relevant today. This classification is based on the ratio of the main characteristics of nervous processes (strength, balance, and mobility), which together define four types of HNA. In modelling the HNA, Pavlov highlighted the dynamic and largely plastic nature of brain functioning, as well as the functional nature of some long-term behavioural disorders in dogs (Derouesné, 2021). Despite the fact that in 1934, in an article in Imago, Schilder sharply criticized Pavlov's HNA theory, his teachings have been widely used both in scientific research and in cynology (Morris, 2022). A link has been established between the type of HNA, certain biochemical parameters, and drug responsiveness (Netter, 2018).

Temperament directly determines how an animal copes and reacts to the current physical and social environment, including during

stressful situations. For example, when therapy dogs interact with strangers in new medical facilities. Despite the fact that temperament is to some extent genetically determined, its formation depends on the environment. Dogs are characterized by a high rate of adaptation to changes in environmental conditions, which determines their high adaptive capacity. Dogs are an integral part of society, performing a wide range of work, from assistance (guide dogs), therapy (canine therapy), service (search and rescue dogs, explosive detection dogs), etc. However, success in these roles, which require dogs to meet complex behavioural criteria and undergo extensive training, is far from guaranteed (Bray *et al*., 2021).

The parameters of the main characteristics of nervous processes determine the speed and quality of training of working and domestic dogs. Dogs with strong and balanced nervous processes are easy to train and are used for a wide range of tasks (Liczner *et al*., 2021). Dogs with strong, balanced inert nervous processes are good handlers and are used in social programmes, including canine therapy. Dogs with unbalanced nervous processes are excellent bodyguards. Thus, taking into account the typological features of the dog's nervous system allows for the optimal use of their working qualities.

The study of the state of protein metabolism in dogs with different types of nervous system under the influence of a food stimulus is an important task for the development of new methods of metabolic correction in veterinary medicine. The aim of the study is to investigate protein metabolism in dogs with different characteristics of the nervous system and to establish their relationship with the main features of this system under the influence of short-term nutritional deprivation. It is planned to compare these values and determine how the properties of the nervous system affect protein metabolism. The study is based on fundamental knowledge of the cortical mechanisms of protein metabolism regulation in dogs, and it will help to develop new methods of metabolic correction, taking into account individual characteristics of the types of nervous activity in animals.

Literature Review

There are currently two main approaches to optimizing the use of working dogs: developing tests and selection criteria that can efficiently and effectively identify ideal candidates from the general pool of candidate dogs, and developing approaches to improve performance, both at the individual and population level, through improvements in rearing, training and breeding (Bray *et al*., 2021). Improvements in the selection process are critical to the effectiveness of working dog programmes and have the potential to optimize how resources are invested in these programmes, increase the number of available working dogs and improve their welfare (Lazarowski *et al*., 2020).

There is a wide range of methods for determining the temperament, higher nervous activity or working qualities of dogs, which vary considerably and are used for different purposes. In particular, based on behavioural observations, it is possible to reliably assess the temperament of dogs (introvert or extrovert) (Karpiński *et al*., 2022). Quantifying behaviour and temperament with sensor-based dog toys can provide a way to predict which dogs will be suitable for guide dog work (Byrne *et al*., 2022). The method of determining the type of behavioural profiles of dogs using LCA (Latent class analysis) based on the C-BARQ (Canine Behavioural Assessment and Research Questionnaire) has proven to be an effective and flexible tool for determining temperament (Zapata *et al*., 2022). The influence of the dog-owner relationship on the emotional reactivity of dogs, behavioural changes, and physical activity has been proven to have an impact on the animal's temperament (Somppi *et al*., 2022).

Behavioural traits, such as aggression, anxiety, and learning ability, differ significantly between dog breeds and are heritable (Hecht *et al*., 2021). However, the neural basis for these differences is unknown. Morphology and age are important factors that influence temperament and emotional reactivity in dogs. Studies have shown that large dogs with long muzzles are less sensitive to negative stimuli and punishment than dogs with short muzzles, while the opposite is true for small dogs. Even the body weight of the dogs showed an influence on their temperament. At the same time, heavier dogs were less sensitive to negative stimuli (Ayrosa *et al*., 2022). The established link between dog body size and behaviour, which is associated with a disproportionate increase in areas in dogs with larger brains, explains the greater reactivity with decreasing body size in dogs (Hecht *et al*., 2021). Today, it is already known that the type of HNA of dogs is formed during ontogeny, but its general features are inherited. Recent studies have shown that puppies born to bitches with more childbirth experience and better maternal behaviour had higher basal cortisol concentrations and better adaptability, a more balanced nervous system, and higher levels of resistance (Nagasawa *et al*., 2021). Thus, the laying of the temperament of dogs begins immediately after birth, ends after puberty, and, in the absence of critical environmental changes and severe stress, does not change throughout life.

Proteins are known to perform a number of vital functions, including cell reproduction, enzyme synthesis, and the production of antibodies and hormones. The state of protein metabolism determines and regulates most chemical transformations in the body (Oltjen, 2013). The majority of plasma proteins are synthesized in the liver – albumin, α_1-, α_2- and some β-globulins, fibrinogen, some clotting factors, and the rest of β- and γ-globulins are synthesized in the cells of the immune system. The metabolism of amino acids in dogs has its own differences. Domesticated dogs, which evolved after domestication by humans, have slightly altered their protein metabolism compared to their wild relatives (Humbert *et al*., 2002).

The effect of nutritional deprivation on biochemical changes in dogs has been well studied, in particular, changes in metabolism at different durations of fasting. Metabolism in dogs changes after just one day without feeding (Duckett *et al*., 2021). The highest priority of a dog's metabolic processes is the need to maintain blood glucose concentration at a normal level. In this case, both lipid and protein metabolism are affected (Khoo *et al*., 2019).

To date, a number of studies have been conducted that indicate cortical mechanisms of metabolic regulation in cows and pigs (Danchuk *et al*., 2020) and other animal species. However, the influence of the main characteristics of the nervous system on protein metabolism in dogs has remained unexplored.

Materials and Methods

The study was conducted during 2020-2023 at the Multidisciplinary Laboratory of Veterinary Medicine of Odesa State Agrarian University (Odesa), VITAVET and Bravo Vet veterinary clinics (Kamianets-Podilskyi). To conduct the experiment, 20 dogs (5 of each type of HNA) of the Beagle breed were selected. The strength, balance, and mobility of nervous processes in dogs were determined by the author's modified method. On the basis of the experiment, 4 groups of animals were formed, 5 animals in each group: Group I – strong balanced mobile type (SBM); Group II – strong balanced inert type (SBI); Group III – strong unbalanced type of HNA (SU); Group IV – weak type of higher nervous activity (W). Nutritional deprivation was performed for 36 hours. Blood samples obtained before food deprivation, one and three days after it were used as material for the study. The

content of total protein, albumin, globulins, and their fractions was determined in the blood plasma of dogs and the value of the albumin-globulin ratio was calculated (Vlizlo *et al*., 2012).

The experiment was carried out in compliance with the requirements of the European Convention (1986, March) and the Law of Ukraine No. 3447-IV "On Protection of Animals from Cruelty" (2006, February). Three tests were conducted to determine the typological features of nervous activity in dogs. It is important that all manipulations with animals are carried out playfully to prevent stress.

Test 1. The animals were kept on a starvation diet 24 hours before the experiment. After that, the experimenter (a stranger to the dog) took the animal to the exercise area with a plate with a small amount of food, about 20 g (it is important that the food is traditional for these animals). Based on the results of this experiment, the strength of the dogs' nervous processes was assessed, and the following scores were awarded: 3 points – the animal reacts normally to the experimenter and immediately after the approach begins to eat food from the plate; 2 points – the dog reacts with caution to the experimenter, sometimes resists, eats food immediately or after a certain period of time (1-2 minutes) when approaching the plate, constantly looks around and is distracted while eating; 1 point – the dog reacts with caution to the experimenter, resists, does not eat the food at all or can only partially taste it when approaching the plate. During the experiment, it looks frightened. Based on the results of this test, a conclusion was made about the strength of the animals' nervous processes. Animals that scored 2 or 3 points were classified as dogs with strong nervous processes, and those that scored 1 point were classified as dogs with weak nervous processes.

Test 2. Mobility of nervous processes. The test was performed with animals with strong nervous processes immediately after the first test in the same place. To do this, the animal was brought to two bowls (placed at a distance of 1-1.5 m from each other), one of which contained a small amount of food. It was given the opportunity to eat this food. Then we took it aside and filled the same bowl again (without the animal seeing it). The animal was allowed to eat the food. The test was carried out until the animal made five consecutive approaches to the food bowl. Each time, the animal was led to an empty bowl. After that, the bowls were swapped and the animal was allowed to go in. The approaches were performed until the conditioned reflex to a different location of the food bowl was relearned. The number of approaches for the formation and processing of the reflex was recorded. Based on the test results, the corresponding number of points was assigned: 3 points – conditioned food reflexes were formed within 3-5 approaches and reworked in 3-7 approaches; 2 points – conditioned food reflexes were formed within 5-10 approaches and were not reworked or fixed in more than 10 approaches; 1 point – during the experiment it was not possible to form or rework conditioned food reflexes.

Test 3. Conducted immediately after the second test. To do this, a significant amount of food was poured into a bowl and the dog was allowed to eat it freely. After 10-15 s after the start of feed intake, an unexpected sound stimulus was applied (a starting horn in a compressed air canister of 400 Hz, 112 dB – at a distance of 1 m, a ratchet and other means can be used). After that, the animal's reaction was assessed: 3 points – the dog does not react at all to the sound stimulus, or leans back more often, gets scared, but continues to eat food from the plate after a short period of time; 2 points – the dog reacts strongly to the sound stimulus, leans back, becomes frightened, and continues to eat food from the plate after a long period of time

(3-5 minutes); 1 point – the dog reacts strongly to the sound stimulus, or the reaction to the stimulus is inadequate and does not return to eat food from the plate within 5 minutes.

Having conducted the experiment according to the above methodology, the values of strength, balance, and mobility of excitation and inhibition processes in the cerebral cortex were obtained, which allowed establishing the affiliation of an individual animal to the corresponding type of HNA. Biometric data processing was performed on a PC using MS Excel software with built-in statistical functions. The arithmetic mean (M) and its error (m) were determined, and two-factor analysis of variance and correlation were performed. The probability of differences in mean values was determined by the Student's criterion. Changes in indicators were considered significant at $P<0.05$ (including $P<0.01$ and $P<0.001$).

Results and Discussion

Today, there are a number of methods for determining the typological characteristics of the nervous system in dogs. However, most of them are difficult to perform and require time and resources. On the other hand, these methods are only able to establish the temperament of the animal, without characterizing the parameters of the main cortical processes. Improving and standardizing assessment technology that allows for the identification and improvement of behavioural characteristics will be key to the development of dog detection technology in general (Lazarowski *et al.*, 2020). Therefore, the authors set out to develop an express method for determining the type of HNA in dogs with a scoring system for assessing the strength, balance, and mobility of cortical processes.

The parameters of the main characteristics of nervous processes determine the speed and quality of training of working and domestic dogs. Dogs with strong and balanced nervous processes are easy to train and are used for a wide range of tasks (Liczner *et al.*, 2021). After conducting the experiment according to the above-mentioned author's methodology, the values of indicators of strength, balance, and mobility of the nervous processes of dogs were obtained (Table 1). Animals with strong processes of excitation and inhibition in the cerebral cortex.

Table 1. Indicators of cortical processes in dogs with different types of higher nervous activity (M±m, n=5; conventional unit)

Type of higher nervous activity	Index of cortical processes			
	power	balance	mobility	average score
SBM	3.00±0.00	2.80±0.22	2.80±0.22	2.87±0.15
SBI	2.80±0.22	2.60±0.27	1.00±0.00***	2.13±0.09***
SU	2.80±0.22	1.00±0.00***	2.40±0.27	2.07±0.08***
W	1.00±0.00***	1.00±0.00***	1.00±0.00***	1.00±0.00***

Note: *A significant difference with a strong balanced mobile type of higher nervous activity: ** $P<0.01$; *** $P<0.001$*
Source: *author's development*

The determination of the typological features of the HNA in dogs using this methodology made it possible to form experimental groups of dogs, the Beagle breed, with different types of HNA for further research. The obtained results of temperament tests in dogs are comparable in terms of reliability to similar studies on cattle and pigs (Danchuk *et al.*, 2020). Higher nervous activity, including the processes of thinking, perception, emotions, decision-making, and

other cognitive processes, affects the body's metabolism. This interaction occurs through a complex system of signals and reactions between the nervous, humoral system and organs involved in the regulation of metabolism (Niijima, 1989; Berthoud & Neuhuber, 2019), including protein metabolism. It was found that the content of total protein and its fractions before the effect of food deprivation in dogs with different types of HNA did not differ significantly and did not exceed physiological limits (Figs. 1-4).

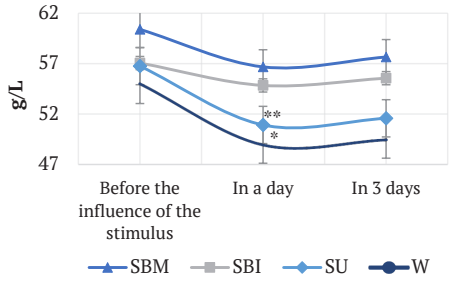

Figure 1. Content of total protein in the blood plasma of dogs with different types of HNA, g/L (M±m, n=5)

Note: *Significant difference with the previous period of research: *P<0.05; **P<0.01*

Source*: author's development*

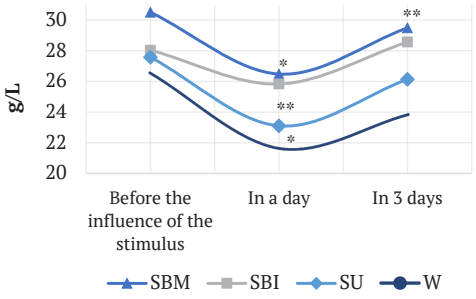

Figure 2. Albumin content in the blood plasma of dogs with different types of HNA, g/L (M±m, n=5)

Note: *Significant difference with the previous period of research: *P<0.05; **P<0.01*

Source*: author's development*

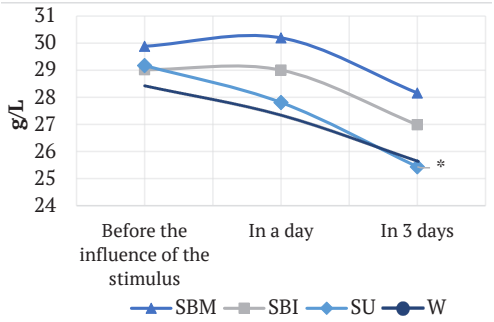

Figure 3. The content of globulins in the blood plasma of dogs with different types of HNA, g/L (M±m, n=5)

Note: *Significant difference with the previous period of research: *P<0.05*

Source*: author's development*

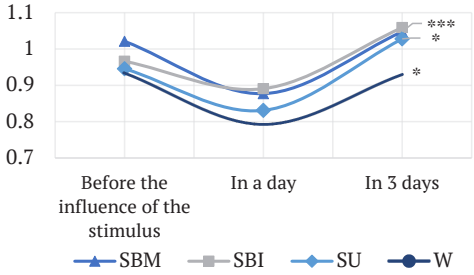

Figure 4. The value of albumin-globulin ratio in the blood plasma of dogs with different types of HNA, units (M±m, n=5)

Note: *Significant difference with the previous period of research: *P<0.05; **P<0.01; ***P<0.001*

Source*: author's development*

According to M.E. Duckett *et al.* (2021), signs of anxiety in dogs occur as early as four hours after a missed feeding, which leads to the gradual development of stress syndrome. The highest priority of a dog's metabolic processes is the need to maintain blood glucose concentration at a physiological level. At the same time, both lipid and protein metabolism change (Khoo *et al.*, 2019). Under the influence of nutritional deprivation, the content of total protein in the blood plasma of dogs with SBM and SBI of

the HNA type decreased within the trend, while in dogs with SU and W type it was significantly reduced by 10.3% (P<0.01) and 10.8% (P<0.05), respectively (Figs. 5-7). A decrease in the content of total protein in the blood plasma of dogs was also recorded by C.G. Vecchiato *et al.* (2023), which, in their opinion, is explained by the development of stress in animals.

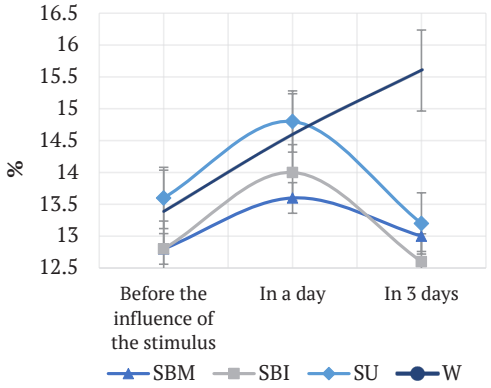

Figure 5. The content of α-globulins in the blood plasma of dogs with different types of HNA, % (M±m, n=5)

Source: *author's development*

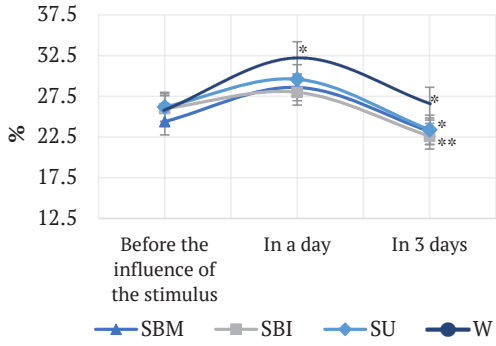

Figure 6. The content of β-globulins in the blood plasma of dogs with different types of HNA, % (M±m, n=5)

Note: *Significant difference with the previous period of research: *P<0.05; **P<0.01*

Source: *author's development*

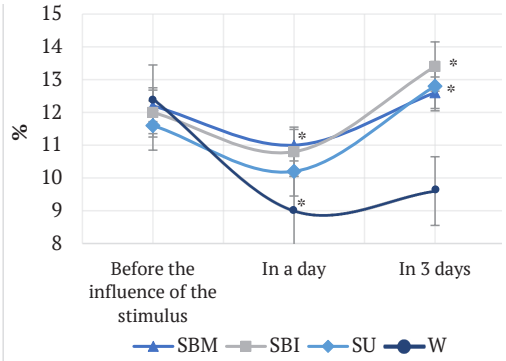

Figure 7. The content of γ-globulins in the blood plasma of dogs with different types of HNA, % (M±m, n=5)

Note: *Significant difference with the previous period of research: *P<0.05*

Source: *author's development*

The decrease in the content of total protein in the blood plasma of dogs under short-term food deprivation was due to albumin. The content of globulin fractions in the blood plasma of dogs did not change significantly. At the same time, the content of albumin in the blood plasma of dogs of SBM, SBI, SU and W type of HNA decreased by 13.2% (P<0.05), 7.9% (P<0.001), 16.2% (P<0.001) and 18.3% (P<0.05), respectively, during the day from the beginning of the experiment. The researchers note that the reasons for the decrease in plasma albumin levels may be a lack of protein in the diet, starvation, and stress (Van De Wouw & Joles, 2022). As for the decrease in albumin levels due to short-term nutritional deprivation, its mechanism is apparently not so much related to its deficiency (as it will not appear in a day), but rather to stress and adaptive metabolic changes. Differences in the content of this protein fraction in blood plasma are probably due to the different reactivity of dogs with different temperaments. Protein metabolism, according to M.A. Tarnopolsky (2000), serves as an auxiliary source of energy during exhaustive

exercise. However, as noted by M.J. Rennie *et al.* (2006), under certain conditions, the percentage of energy metabolism from protein sources can reach 20%. Obviously, the decrease in the content of total protein and albumin in the blood plasma of dogs under the influence of food deprivation may be associated with the use of blood proteins as an additional source of energy during short-term food deprivation.

Some researchers have proposed a number of potential markers of stress, among which the albumin-globulin ratio is mentioned (Ijiri *et al*., 2022). This is justified by the inflammatory and acute-phase response that is known to occur under stress. As a result of these changes in various protein fractions of blood plasma, within a day of the onset of food deprivation, the value of the albumin-globulin ratio in the blood plasma of dogs decreased by 7.8-15.1%, which confirms the development of a stressful state in animals.

Three days after the effect of food deprivation, the content of total protein in the blood plasma of dogs with different types of HNA did not change significantly, while the content of albumin in dogs with SBM type of HNA increased by 11.4% ($P<0.01$). This pattern was also found in animals with other types of HNA. However, these differences had the character of a trend (6.0-13.1%). It should be noted that the lower content of albumin in the blood plasma of dogs with SU and W types of HNA, respectively, by 11.4% ($P<0.05$) and 19.1% ($P<0.001$) of the indicators of animals with SBM type. The content of globulins in the blood plasma of dogs from the first to the third day from the beginning of the study decreased in dogs depending on the type of HNA by 6.7-8.5%. The content of globulins in the blood plasma of dogs with HF and mild type of HNA three days after the start of the study was 9.7% ($P<0.05$) and 8.9% less than in animals with SBM type, respectively. Due to the dynamic changes in the content of albumin and globulins in the blood plasma of dogs, there was a significant increase in the value of the albumin-globulin ratio in the blood plasma of dogs depending on their type of HNA by 17.2-23.7% ($P<0.05$-0.001).

J.W. Oltjen (2013) reports that the state of protein metabolism determines and regulates most chemical transformations in the mammalian body. Most proteins found in the blood plasma are formed in the liver. This includes albumin, as well as α_1, α_2 globulins, some β globulins, fibrinogen, and some clotting factors. On the other hand, the rest of the β- and γ-globulins are produced by cells of the immune system (Humbert *et al.*, 2002). Therefore, the dynamic changes in protein metabolism metabolites in the blood of dogs under the influence of short-term nutritional deprivation indicate the corresponding functional changes in liver tissue and the state of the immune system. Despite the lower variability of total globulins in the blood plasma of dogs under the influence of food deprivation compared to the content of albumin, the level of different globulin fractions underwent significant changes (Figs. 5-7). It should be noted that the proportion of α- and β-globulins increased in the day after the onset of food deprivation, and the proportion of γ-globulins decreased in the blood plasma of dogs of all types of HNA. Although these changes were mostly in the nature of trends. The most reactive were dogs with type W of HNA, in particular, in these animals, the proportion of α- and β-globulins increased by 1.2% and 6.4%, respectively, one day after the onset of food deprivation ($P<0.05$), and the content of γ-globulins decreased by 3.4% ($P<0.05$). It is known that a decrease in the level of immunoglobulins in the blood plasma of dogs under stress negatively affects their resistance to infectious diseases (Lensen *et al.*, 2019).

It should be noted that as of 2023, cortisol and secretory immunoglobulin A are widely used as an indicator of acute stress in dogs of

all ages. H. Zeier *et al*. (1996) attributed the increase in secretory immunoglobulins after short-term stressors to the action of cortisol. At the same time, there is evidence of a decrease in the level of immunoglobulins A under stress in dogs (Fahlman *et al*., 2001). In children, higher cortisol concentrations were associated with lower immunoglobulin concentrations, and this was associated with a higher incidence of disease (Watamura *et al*., 2010). Thus, the available data on the reactivity of the humoral immune system under stress in dogs is rather contradictory. It is obvious that the content of immunoglobulins in the blood plasma of dogs during short-term food deprivation decreases during the day due to the secretion of hormones and mediators of the stress system, which suppresses immunoreactivity. Later, by the third day of the study, corticosteroids, in particular cortisol, stimulate the function of cells that produce antibodies, resulting in an increase in the content of immunoglobulins in the blood plasma. Thus, three days after the start of the study, the content of various globulin fractions in the blood plasma of dogs with SBM, SBI and SU type of HNA returned to the values that were before the start of the study. Whereas, in dogs with W type of the HNA, the content of α-globulins continued to increase and was 2.2% higher than before the experiment, while the level of γ-globulins became lower by 2.8% ($P<0.05$). Thus, three days after the start of the study, the content of β-globulins in the blood plasma of dogs with type W of the HNA was 3.4% higher ($P<0.05$) and γ-globulins was 3.0% lower ($P<0.01$) compared to animals with type SBM.

Functional connections between the strength, balance, and mobility of cortical processes and the intensity of protein metabolism, as well as the degree of influence of individual characteristics of the nervous system on the indicators of their metabolism in pigs, have already been established (Karpovskyi *et al*., 2017). The results of our studies indicate that before the effect of food deprivation, the balance of nervous processes had a direct correlation with the albumin content in the blood plasma of dogs ($r=0.47$; $P<0.05$), while other indicators of protein metabolism did not significantly correlate with the main characteristics of nervous processes (Table 2). However, already a day after the effect of food deprivation, a direct relationship between the strength and balance of nervous processes in dogs and the content of total protein and albumin appeared ($r=0.55$-0.72; $P<0.05$-0.001).

Table 2. Relationships of the main characteristics of nervous processes with indicators of protein metabolism in the blood plasma of dogs (n=20)

Study period	Characteristics of nervous processes					
	power		balance		mobility	
	r	D	r	D	r	D
Total protein						
Before the stimulus	0.36	0.13	0.24	0.06	0.24	0.06
A day later	0.55*	0.30*	0.64**	0.41**	0.23	0.05
In 3 days	0.67**	0.44**	0.67**	0.45**	0.33	0.11
Albumins						
Before the stimulus	0.33	0.11	0.47*	0.22*	0.38	0.15
A day later	0.55*	0.30*	0.72***	0.52***	0.30	0.09
In 3 days	0.73***	0.53***	0.71***	0.50***	0.36	0.13
Globulins						
Before the stimulus	0.25	0.06	-0.04	0.00	0.04	0.00
A day later	0.39	0.15	0.39	0.15	0.10	0.01

Table 2. Continued

Study period	Characteristics of nervous processes					
	power		balance		mobility	
	r	D	r	D	r	D
In 3 days	0.37	0.14	0.41	0.17	0.18	0.03
Albumin-globulin ratio						
Before the stimulus	0.00	0.00	0.39	0.15	0.24	0.06
A day later	0.21	0.04	0.38	0.14	0.22	0.05
In 3 days	0.55*	0.31*	0.50*	0.25*	0.27	0.07
α-Globulins						
Before the stimulus	-0.19	0.04	-0.19	0.04	0.02	0.00
A day later	-0.18	0.03	-0.25	0.06	0.00	0.00
In 3 days	-0.51**	0.26**	-0.34	0.12	0.26	0.07
β-Globulins						
Before the stimulus	0.10	0.01	-0.27	0.07	-0.19	0.04
A day later	-0.29	0.08	-0.37	0.14	-0.30	0.09
In 3 days	-0.56**	0.31**	-0.44*	0.19*	-0.29	0.08
γ-Globulins						
Before the stimulus	-0.10	0.01	0.09	0.01	-0.02	0.00
A day later	0.39	0.15	0.36	0.13	0.26	0.07
In 3 days	0.62 **	0.39 **	0.35	0.12	0.32	0.10

Notes: *1. r is the correlation coefficient, D is the coefficient of determination. 2. Indicators are reliable for: *P<0.05; **P<0.01; ***P<0.001*

Source*: author's development*

Three days after the start of the study, the relationship between the strength of nervous processes and the content of total protein and albumin only increased (r=0.67-0.73; P<0.01-0.001), while the relationship with balance did not change significantly. In addition, there was an inverse relationship between α- and β-globulins (r=-0.51-0.56; P<0.01) and a direct relationship with the content of γ-globulins (r=0.62; P<0.01) and the strength of nervous processes. At the same time, the balance of nervous processes was inversely related to the content of β-globulins (r=-0.44; P<0.05). There were no significant correlations between the mobility of nervous processes and the studied indicators of protein metabolism in the blood plasma of dogs during the experiment.

The effect of nutritional deprivation on biochemical changes in the body of dogs is a sufficiently studied issue, in particular, changes in metabolism at different duration of fasting have been established. For example, M.E. Duckett *et al*. (2021) noted that metabolism in dogs changes significantly after a day without food consumption. A two-factor analysis of variance determined that short-term nutritional deprivation had a significant effect on the content of albumin, globulins, β-globulins, γ-globulins, and the value of the albumin-globulin ratio in the blood plasma of dogs. At the same time, the effect of the irritant on the content of total protein and α-globulins in the blood plasma of these animals was not observed (Table 3).

Table 3. Multivariate variance analysis of indicators of protein metabolism in the blood of dogs with different types of HNA under the effects of food deprivation

Source of variation	SS	df	MS	F	P-value	F critical
Total protein						
HNA type	390.1	3	130.0	11.2	<0.001	2.90

Table 3. Continued

Source of variation	SS	df	MS	F	P-value	F critical
Action of the stimulus	5.11	1	5.11	0.439	0.512	4.15
Interconnection	0.299	3	0.100	0.009	0.999	2.90
Internal	372.8	32	11.7	–	–	–
In total	768.3	39	–	–	–	–
Albumins						
HNA type	172.2	3	57.4	14.6	<0.001	2.90
Action of the stimulus	75.1	1	75.1	19.1	<0.001	4.15
Interconnection	1.20	3	0.402	0.102	0.958	2.90
Internal	126.0	32	3.94	–	–	–
In total	374.5	39	–	–	–	–
Globulins						
HNA type	48.2	3	16.1	3.02	0.044	2.90
Action of the stimulus	41.0	1	41.0	7.71	0.009	4.15
Interconnection	0.614	3	0.205	0.039	0.990	2.90
Internal	170.0	32	5.31	–	–	–
In total	259.8	39	–	–	–	–
Albumin-globulin ratio						
HNA type	0,078	3	0.026	3,12	0.040	2.90
Action of the stimulus	0.274	1	0.274	32.7	<0.001	4.15
Interconnection	0.004	3	0.001	0.176	0.912	2.90
Internal	0.268	32	0.008	–	–	–
In total	0.624	39	–	–	–	–
α-Globulins						
HNA type	21.7	3	7.23	1.497	0.234	2.90
Action of the stimulus	4.23	1	4.23	0.876	0.356	4.15
Interconnection	10.5	3	3.49	0.724	0.545	2.90
Internal	154.4	32	4.825	–	–	–
In total	190.8	39	–	–	–	–
β-Globulins						
HNA type	99.1	3	33.0	4.07	0.015	2.90
Action of the stimulus	319.2	1	319.2	39.3	<0.001	4.15
Interconnection	1.08	3	0.358	0.04	0.9871	2.90
Internal	259.6	32	8.11	–	–	–
In total	679.0	39	–	–	–	–
γ-Globulins						
HNA type	48.7	3	16.2	5.52	0.004	2.90
Action of the stimulus	34.2	1	34.2	11.7	<0.001	4.15
Interconnection	6.88	3	2.29	0.780	0.514	2.90
Internal	94	32	2.94	–	–	–
In total	183.8	39	–	–	–	–

Notes: *df – the number of factor levels (-1); F is the criterion for evaluating the influencing factor on the dependent variable; F critical – critical value of the impact factor; MS – mean square; SS – sum of squares; P is reliability*
Source*: author's development*

The role of HNA in the regulation of metabolism in mammals is undoubted, but the mechanisms of regulatory influence of the nervous system and the main characteristics of nervous processes on metabolism, in particular, proteins, remain poorly defined (Niijima, 1989;

Myers Jr & Olson, 2012). According to Table 3, the type of HNA had a significant effect on the content of total protein, albumin, globulins, β-globulins, γ-globulins and the value of the albumin-globulin ratio in the blood plasma of dogs. The influence of dog temperament on the content of α-globulins was not observed. Thus, taking into account the obtained patterns of protein metabolism in dogs with different types of HNA and establishing the relationship between the main characteristics of nervous processes and indicators of their metabolism in the blood can be used in the development of new methods of metabolic correction depending on the temperament of animals.

Conclusions

The new method of determining the typological features of the nervous system in dogs allows reliably establishing their type of higher nervous activity within 30-60 minutes of the experiment. A significant effect of short-term nutritional deprivation on the content of certain indicators of protein metabolism in the blood plasma of dogs with different types of HNA was determined. Under the action of the stimulus during the day, the albumin content in the blood of dogs decreased and the ratio of globulins changed, in particular, the proportion of α- and β-globulins increased and the proportion of γ-globulins decreased. The most pronounced changes in protein metabolism under the influence of short-term food deprivation were found in animals with a weak type of HNA.

Under the influence of nutritional deprivation, changes in plasma protein metabolism are closely related to the main characteristics of nervous processes in dogs. Three days after the onset of short-term nutritional deprivation, a direct correlation between the strength of nervous processes and the content of total protein and albumin ($r=0.67-0.73$; $P<0.01-0.001$), γ-globulins ($r=0.62$; $P<0.01$) and an inverse correlation with the content of α- and β-globulins ($r=-0.51-0.56$; $P<0.01$) in the blood plasma of dogs was established. The balance of nervous processes was inversely related to the content of β-globulins ($r=-0.44$; $P<0.05$) in the blood plasma of dogs. No significant correlations between the mobility of nervous processes and the studied indicators of protein metabolism in the blood plasma of dogs were observed during the experiment.

The two-factor analysis of variance revealed a significant effect of short-term food deprivation on the content of albumin, globulins, β-globulins, γ-globulins and the value of the albumin-globulin ratio ($F=3.02-14.6>FU=2.90$; $P<0.05-0.001$) in the blood plasma of dogs. In the future, it will be useful to study the development of methods for correcting protein metabolism with the help of nanoaquahelates feed additives, taking into account the characteristics of the nervous system of dogs.

Acknowledgements

None.

Conflict of Interest

None.

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Показники обміну білків у собак з різними типами вищої нервової діяльності

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Анотація. Актуальність дослідження зумовлена відсутністю на сьогодні даних щодо кортикальних механізмів регуляції обміну білків в організмі собак, на що важливо зважати у розробці методів коригувального впливу на метаболізм. Мета роботи полягала у визначенні особливостей обміну білків у собак із різними типами вищої нервової діяльності та встановлення взаємозв'язків основних характеристик нервових процесів із показниками обміну білків в крові. Провідним методом у дослідженні цього питання була нова методика визначення типологічних особливостей нервової системи у собак, а отримані бали сили, врівноваженості і рухливості нервових процесів сприяли виявленню ступеня їх впливу на показники обміну білків. Встановлено достовірний вплив короткотривалої харчової депривації на вміст окремих показників обміну білків в крові собак залежно від їх темпераменту. За дії подразника впродовж доби в крові собак відмічали зменшення рівня альбумінів та зміни у співвідношенні глобулінів, зокрема, зростання відносного вмісту

α- і β-глобулінів та зменшення – γ-глобулінів. Визначено, що тип вищої нервової діяльності чинить достовірний вплив на вміст загального протеїну, альбумінів, глобулінів, β-глобулінів, γ-глобулінів та величину альбуміно-глобулінового співвідношення (F=3,02-14,6>FU=2,90; P<0,05-0,001) у плазмі крові собак. Виявлено прямий зв'язок сили нервових процесів із умістом загального протеїну та альбумінів, γ-глобулінів (r=0,62-0,73; P<0,01) та обернений взаємозв'язок між умістом α- і β-глобулінів (r=-0,51-0,56; P<0,01) через три доби після початку короткотривалої харчової депривації. Врівноваженість нервових процесів була обернено взаємозв'язана з умістом β-глобулінів (r=-0,44; P<0,05). Отримані фундаментальні знання становлять практичну цінність для розробки нових, сучасних методів корекції обміну речовин із урахуванням темпераменту тварин

Ключові слова: альбуміни; глобуліни; загальний протеїн; альбуміно-глобулінове співвідношення; плазма крові; темперамент

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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. Muguiro *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 Haemaophysalis 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. Ebelt *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.

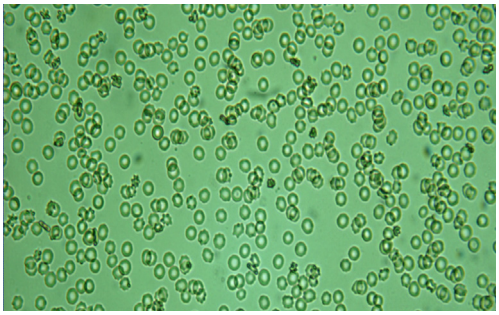
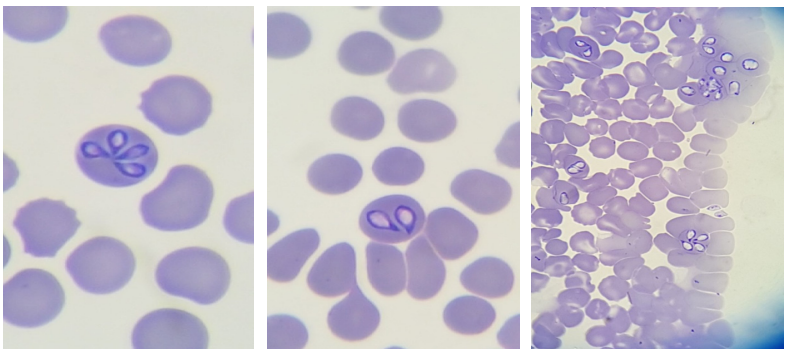


Figure 1. Large crossover sample. The agglutination reaction is negative (magnification ×40)
Source: *author's development*

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).



a b c

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

Notes: *a, b – magnification ×1000; c in – ×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).

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

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°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. Muguiro *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. Zygner *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. Zygner *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).

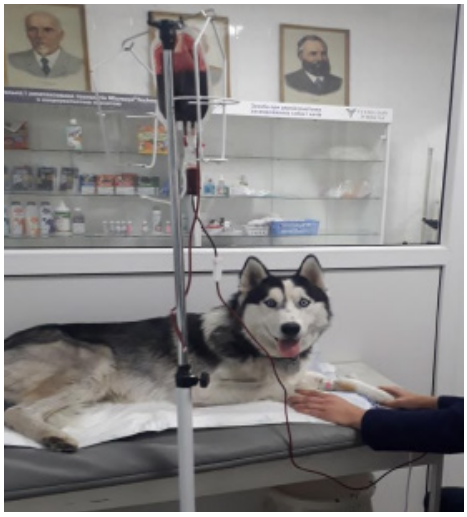


Figure 3. Transfusion of erythrocyte mass to a recipient dog with hemolytic anaemia caused by babesiosis

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	the second	the fifth	the tenth
		before transfusion	after transfusion		
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 m^2), 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. Zygner *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.

Acknowledgements

None.

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 °С) 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 десяту доби лікування. Впродовж другої та п'ятої діб відмічали поступове збільшення в крові вмісту гемоглобіну та величини гематокриту. На десяту 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

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