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Control of Chilled Meat of Broiler Chickens by Bacterioscopic Method

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Abstract. The relevance of this study is conditioned upon ensuring proper risk-based control over the safety and quality of chilled broiler chicken meat at its production facilities, where a system of hazard analysis and control at critical points should be implemented. In this regard, this study was aimed at identifying the issue of control of chilled broiler chicken meat for conducting research to establish the freshness of broiler chicken meat at sales facilities – agri-food markets and supermarkets. The leading approach to the study of this issue was the developed patented bacterioscopic method, which allows comprehensively establishing the freshness of chilled broiler chicken meat. The presented method is simple to perform, with obtaining quantitative indicators for establishing the freshness of chilled meat of broiler chickens for 5 days, 6-7 and 8 days at a temperature of 0-4 °C, as well as with establishing the number of microorganisms in the field of view of a microscope and by the degree of muscle tissue decay, by staining one smear-imprint according to Gram in Hooker's modification, and by counting the number of microorganisms in 10 fields of view, followed by deriving the average value per field of view, as well as determining the shape of the cells. The reliability of the results in tests using this method is 99.9%. It was found that the highest content of microorganisms was in stale chilled carcasses of broiler chickens on Day 8: in the chest muscles – 45 ± 3 ($P<0.001$), in the thigh muscles – 52 ± 5 ($P<0.001$) compared to fresh meat. Rod-shaped gram-positive microorganisms dominated stale meat, in some places single cocci were recorded, including a considerable breakdown of muscle tissue. The content of volatile fatty acids in the chilled meat of broiler chickens and the acid value of fat increased significantly ($P<0.001$) on the 8th day of sale at a temperature of 0-4 °C, respectively – 11.05 ± 0.37 – 10.97 ± 0.33 and 2.83 ± 0.33 mg of NaOH. During the examination of the freshness of the meat of broiler chicken carcasses, the highest percentage of fresh meat of broiler chickens during its sale in agri-food markets was 79.3%, in supermarkets – 75.0%, stale meat, respectively – 3.8% and 5.4%. The materials of this paper are of practical value for the work of state inspectors of veterinary medicine when using the bacterioscopic method to determine the freshness of chilled meat of broiler chickens

Keywords: microbial count, organoleptic assessment, pectoral muscles, thigh muscles, meat freshness

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Introduction

Violation of the temperature regimes of cooling broiler chicken carcasses (the norm of the temperature regime of cooling poultry carcasses – from 0 to 4 °C – no more than 5 days, increasing the temperature above 4 °C and extending the period for more than 5 days) during sale or storage is important a critical control point for the effectiveness of the hazard analysis and control system at critical HACCP points (Hazard Analysis and Critical Control Point). Market operators should implement this safety system throughout the food chain of production and circulation of broiler chicken meat [1].

The traceability system, which is a component of the HACCP system, provides identification and analysis of hazardous risks, management of critical control points, and improvement of sanitary and hygienic requirements for the production and turnover of broiler chickens. To ensure the quality and safety of meat and meat products, constant sanitary, microbiological, and hygienic monitoring of the cold chain is required [2].

The safety and quality of broiler chicken meat depends on the poultry feeding, cultivation and slaughter technology, temperature and shelf life. Thus, during the breeding of poultry in private farms, the indicators of meat safety and quality were better than in farms, namely: an increase in protein content by 1.24%, and fat by 3.61% [3]. The consumer prefers chilled poultry meat since it retains all the nutrients necessary for a full-fledged diet [4].

For the effectiveness of the HACCP system, it is necessary to follow European and national regulations [5; 6] on the implementation of risk-based control of chemical, biological and physical hazards, hygiene of production, storage, and sale of broiler chicken meat. However, deviations from the temperature regime above 4°C and delay in sale and storage (more than 5 days) of broiler chicken meat is a daily issue, which leads to spoilage of products, deterioration of organoleptic properties and consumer characteristics of meat [7].

The temperature and duration of refrigerated storage (4 °C) of broiler chicken meat depends on its quality and safety in terms of microbial insemination and changes in physical and chemical parameters [8]. The cooling temperature of broiler chicken meat is one of the key factors in the entire food chain to ensure high-quality and safe products [9]. The quality of poultry meat with an extended shelf life in a chilled state correlates with freshness indicators [10].

Poultry meat is a perishable product, a considerable number of microorganisms multiply in it, organoleptic changes occur, namely the discolouration of muscle tissue, the formation of an unpleasant smell. One of the most effective methods is the use of infrared spectroscopy for the identification and characterisation of bacterial microorganisms that cause spoilage of poultry meat during refrigerated storage, considering the level of mesophilic microorganisms: 102–103 CFU/g – the initial content of microorganisms, and after 107 CFU/g – changes organoleptic indicators (doubtful freshness of meat) and more than 108 CFU/g – an unpleasant rotten smell appears (stale meat) [11].

Spoilage of broiler chicken meat leads to an increase in microorganisms, deterioration of organoleptic and physico-chemical parameters, and therefore it is necessary to develop more effective methods for controlling the freshness of meat products [12]. Changes in the quality of chicken fillet meat due to hypothermia at temperatures from 0 to 4 °C for 8-10 days during storage are expressed in the deterioration of organoleptic indicators, fat oxidation [13].

Antioxidants of plant origin are often used in the meat industry to increase the amount of meat raw materials and extend the shelf life of refrigerated storage up to 14 days, to reduce spoilage of meat and meat products in the absence of oxidation of lipids and proteins [14].

Scientists claim that the addition of various biological additives to poultry feed, such as probiotics and prebiotics, improves the quality of broiler chicken meat and extends its shelf life and improves freshness indicators [15].

Implementation of risk-oriented control of food market operators for the production and circulation of broiler chicken meat by veterinary medicine specialists requires effective and reliable methods of controlling its safety and quality [16].

The scientific originality of this study lies in the development of a bacterioscopic method for determining the freshness of meat of broiler chickens using the Gram staining method in Hooker's modification and counting the number of microorganisms in 10 fields of view, which ensured the reliability of the indicators in the tests, reduced the cost of reagents and reduced the time spent on tests for one sample. Currently, the development of new optimised methods in the system of laboratory control of the safety and quality of chilled broiler chicken meat is important and relevant for veterinary medicine inspectors using simple tests.

The purpose of this study was to develop, test and approbate a method for bacterioscopic assessment of the freshness of meat from chilled broiler chicken carcasses to identify the number of microorganisms.

Materials and Methods

After slaughter, broiler chickens were cooled in cold storage rooms at a temperature of 0-4 °C for further sale in supermarkets (ATB chain) and agri-food markets (city of Bila Tserkva) of the Kyiv Oblast within 5 days according to the current regulatory document DSTU 3143:2013 [17]. The study was conducted on 36 samples of broiler chickens. Later, on the 6-7th and 8th day of sale, spoilage of chilled meat of broiler chickens was determined with obtaining indicators for different degrees of freshness: questionable and stale.

To determine the different degrees of freshness of chilled meat of broiler chickens, generally accepted methods were used: organoleptic examination, determination of the content of volatile fatty acids and the acid number of fat, ammonia, and ammonium salts with Nessler's reagent, reaction with copper sulphate, according to the requirements of DSTU 8253:2015 [18].

During the organoleptic assessment, the appearance of broiler chicken carcasses was determined – the degree of exsanguination, the condition of the surface of the carcasses (surface cleanliness); degree of plumage removal (state of plumage removal); skin condition (clean, dry, unweathered, without scratches, tears, and bruises); the state of the bone system (without fractures and deformations);

the consistency of the chilled meat of broiler chickens (the muscles are dense, elastic, when pressed with a spatula, the pit is quickly levelled); muscle tissue colour (pale pink or with changes); skin colour (pale yellow or with changes); colour of subcutaneous and internal adipose tissue (pale yellow and yellow or with changes); the smell on the surface of the carcass (characteristic of good-quality fresh poultry meat, without extraneous odours or with changes); cooking test (broth with a pleasant smell, transparent, a significant number of fat balls on the surface of the broth, without extraneous odours or with changes).

The content of volatile fatty acids in poultry meat was determined by their separation by distillation and titration with a solution of potassium hydroxide with a mass fraction of 0.1 mol/dm^3 and subsequent calculation according to the formula in milligrams of potassium hydroxide per 100 g of meat.

The acid number of fat was determined by dissolving a melted sample of fat with a mixture of diethyl ether and ethyl alcohol with a mass fraction of 96% and titrating free fatty acids with a solution of potassium hydroxide with a mass fraction of 0.1 mol/dm^3, and expressing it in cubic centimetres (cm^3) of potassium hydroxide, spent on neutralisation of free fatty acids contained in 1 g of fat, and calculated according to the formula in milligrams of potassium hydroxide.

The method for determining ammonia and ammonium salts was based on the ability of ammonia and ammonium salts formed as a result of autolysis of meat to form yellow-brown mercurammonium iodide with Nessler's reagent (10 drops of Nessler's reagent were added to 1 cm^3 of aqueous extract from meat, the contents were shaken tubes and observed the colour: greenish-yellow – fresh meat; intense yellow colour – meat of questionable freshness; yellowish-orange – stale meat).

According to the reaction with copper sulphate, 60 cm^3 of distilled water was added to 20 g of minced meat in the broth to determine the products of the primary breakdown of proteins. The contents were mixed, covered with glass, and put in a boiling water bath for 10 minutes. The broth was then filtered through a layer of cotton wool. If the broth is cloudy and after filtering, protein flakes remain in it, then the broth was additionally filtered through filter paper. 2 cm^3 of filtrate and 3 drops of 5% copper sulphate solution were poured into the test tube. The test tube was shaken three times. The reaction was read after 5 min. The meat is fresh – the broth remains transparent; of questionable freshness – slight cloudiness of the broth; stale – significant turbidity of the broth, the formation of flakes in it or the loss of a jelly-like clot.

The developed bacterioscopic method of determining the insemination of poultry meat by microorganisms and determining the freshness of chilled broiler chicken carcasses was based on the change in the number of smears-imprints from poultry meat, their Gram staining in the Hooker's modification [19], as well as the change in the number of fields of view of the microscope under time of counting microorganisms. Ukrainian utility model patent 147996 was obtained for this technique.

Based on the results of own research, a bacterioscopic method for establishing the insemination of poultry meat with microorganisms and determining the degree of freshness of chilled meat of broiler chickens was developed, which is based on determining the number of microorganisms by counting in smears-imprints from meat samples and their Gram staining in the Hooker's modification.

According to the bacterioscopic method of establishing the insemination of poultry meat by microorganisms and determining the degree of its freshness, a piece of poultry meat with an area of 2.0 x 2.1 cm^2 was used, cut to a depth of 1.0-1.5 cm with a sterile scalpel or scissors, and which was applied the cut surface to the surface of a sterile slide to obtain 1 smear-imprint. Later, this smear-imprint was fixed over the flame of an alcohol still, by passing it three times through the flame for no more than 1-2 seconds, and was stained by Gram in Hooker's modification, and a microscopic study was carried out using immersion oil with a magnification of x90 and an eyepiece – with x10 magnification to count the number of microorganisms in 10 fields of view of the microscope. Next, the average value per field of view of the microscope was calculated, as well as the shape of the cells (cocci, micrococci, rod-shaped bacteria), sporulation was determined, and the emergence of the corresponding Gram colouring was observed (gram-positive microorganisms acquire a purple colour, gram-negative – red), which allows determining the degree of freshness of poultry meat.

An essence of this method is the staining of a smear-imprint according to Gram in Hooker's modification. The following steps can be described:

– application of a strip of filter paper, then a few drops of Hooker's basic staining solution to the paper for 0.5-1.0 min so that the filter paper is completely wet;

– washing with a jet of distilled water the slide with the painted smear-imprint from the meat;

– application of Burke's iodine solution for 0.5-1.0 min.

– washing the smear-print with ethyl alcohol with a mass fraction of 96%, then immersing the slide in a chemical beaker with a capacity of 100 cm^3 with ethyl alcohol with a mass fraction of 96% for 0.5-1.0 min;

– washing with distilled water a smear-imprint from meat;

– application of an alcoholic solution of fuchsin with a mass fraction of 0.5% for 2-3 min on a washed smear-imprint from meat and washing with distilled water and drying with filter paper.

The reliability of the obtained research results is confirmed using certified equipment, modern test methods, and the use of statistical processing. Variational and statistical processing of the results was carried out using computer software packages "Microsoft Excel", "Maple-12" (Maplesoft, 2008). The probability of the obtained results was determined by Student's test considering the significance criteria: $P<0.05$; $P<0.01$, $P<0.001$.

Results and Discussion

Research has established that the organoleptic indicators of fresh chilled carcasses of broiler chickens at 0-4 °C for 5 days for sale in agri-food markets and supermarkets were as follows: the carcasses were well bled, their surface was clean, dry, without damage and haemorrhages, specific smell, the muscles were well bled, dense, elastic, the pit was quickly levelled when pressed with a spatula; the plumage was completely removed from the carcasses; bone system

without fractures and deformations; the colour of muscle tissue was pale pink; skin colour – pale yellow; the colour of the subcutaneous adipose tissue was light yellow, the internal adipose tissue was white; the smell on the surface of the carcass was characteristic of good-quality poultry meat, without extraneous odours; the broth had a pleasant smell, was transparent, on the surface of the broth there were many fat balls, without extraneous odours.

At the same time, it was established that the organoleptic indicators of questionable freshness of chilled carcasses of broiler chickens at 0-4 °C on the 6th-7th day of sale in agri-food markets and supermarkets were as follows: bleeding of the carcass was satisfactory, a slight sliminess of the surface of the carcasses and a sour smell on the surface of the carcasses were observed; the consistency of the muscles was less elastic – the pit slowly flattened out when pressed with a spatula, the colour of the meat was pinkish-grey; the colour of the subcutaneous and internal adipose tissue was pale yellow with a grey tint, with a slight sour smell; the broth had an unpleasant smell, with an extraneous sour smell, cloudy, a few fat balls on the surface of the broth.

Therewith, it was established that the organoleptic indicators of stale chilled carcasses of broiler chickens at 0-4 °C on the 8th day of sale in agri-food markets and supermarkets were as follows: the carcasses were poorly bled, their surface was slimy, and there was an acidic unpleasant smell on the surface carcass; the consistency of the muscles was not elastic – the pit did not align when pressed with a spatula, the colour of the meat was greyish; the colour of the subcutaneous and internal adipose tissue was yellow-grey with a smell of oxidation; the broth had an unpleasant smell, with an extraneous musty smell, was cloudy, no fat balls were found on the surface of the broth.

The prototype of the developed bacterioscopic method for determining the insemination of poultry meat with microorganisms and determining the freshness of meat of broiler chickens is a method of determining the freshness of meat of slaughter animals (beef, pork, lamb, goat) by the bacterioscopic method [20], which is based on determining the number of microorganisms and the degree of breakdown of muscle tissue by Gram staining and subsequent microscopic examination in 25 fields of view in three smears-prints on two slides. The disadvantage of this method is that it is cumbersome and gives an error of 10-15%.

A comparative evaluation of the test results of the above-mentioned methods of determining the freshness of poultry meat by the bacterioscopic method of establishing the insemination of chilled poultry meat with microorganisms and determining its freshness to the prototype are presented in Table 1.

Table 1. Comparison of the indicators of the prototype and the invention of determining the freshness of broiler chicken meat using the developed bacterioscopic method

Indicator for comparison	Conventional method	Newly created method
Method components: Meat cutting depth, cm: Area of a piece of meat, cm^2 Number of smears-prints from meat	 1.0-1.5 2.0x2.5 6	 1.0-1.5 2.0x2.1 1
Time for fixing smears-prints from meat, s	2-3	1-2
The method of staining smears-imprints of meat	According to Gram	According to Gram in the Hooker's modification
Exposure of staining, min	10.0-11.0	4.0-5.0
Microscopy of smears-impressions using immersion oil	90^x magnification; eyepiece with 10^x magnification	90^x magnification; eyepiece with 10^x magnification
Number of visual fields studied	25	10
Experiment detection rate, min	50-60	20-22
Stability of indicators for determining the number of microorganisms in broiler chicken meat, %	80.2	99.9
% Ratio of research results to indicators of the content of volatile fatty acids in the meat of broiler chickens	81.5-85.3	98.5-99.6
% Ratio of research results to quantitative indicators of the acid number of fat of broiler chickens	80.5-83.1	99.2-99.7

The data in Table 1 suggests that the developed bacterioscopic method of establishing the insemination of poultry meat with microorganisms and determining the freshness of chilled meat of broiler chickens has the statistical significance of 99.9%. The results of determining the freshness of broiler chicken meat according to the developed method and generally accepted methods are presented in Table 2.

Table 2. Safety and quality indicators of chilled broiler chicken meat at sales facilities using the developed bacterioscopic method and generally accepted methods, M ± m, n = 36

Indicator	Quality of broiler chicken meat					
	On day 5 at 0-4 °C		On days 6-7 at 0-4 °C		On day 8 at 0-4 °C	
	Fresh broiler chicken meat, n = 12		Broiler chicken meat of questionable freshness, n = 12		Stale broiler chicken meat, n = 12	
	Pectoral muscles	Muscles thighs	Pectoral muscles	Thigh muscles	Pectoral muscles	Thigh muscles
Number of microorganisms by meat microscopy per 1 average field of view	5 ± 1	7 ± 1	17 ± 2***	23 ± 2***	45 ± 3***	52 ± 5***
Determination of ammonia and ammonium salts with Nessler's reagent	The extract from the meat is greenish yellow in colour, transparent		Meat extract of intense yellow colour, considerable turbidity with the formation of a thin layer of sediment		Meat extract is yellow-orange, rapid formation of large flakes falling into the sediment	
Content of volatile fatty acids, mg KOH	2.61 ± 0.24	2.70 ± 0.22	6.62 ± 0.43***	6.70 ± 0.34***	11.05 ± 0.37***	10.97 ± 0.32***
Acid number of broiler chicken fat, mg NaOH	0.72 ± 0.04		1.76 ± 0.12***		2.83 ± 0.07***	
Reaction with copper sulphate (determination of the products of the primary breakdown of proteins in the broth)	Meat broth is transparent, blue green in colour		Meat broth is transparent, blue with a slight turbidity		Meat broth has considerable turbidity, the formation of flakes or the loss of a jelly-like clot, blue in colour	

Note: *** – *P<0.001, compared to fresh meat indicators*

It was established that the content of microorganisms in the chilled meat of broiler chickens (breast muscles and thigh muscles) with questionable freshness was, respectively, 3.4 and 3.3 times higher (P<0.001) and in stale meat, respectively, 9.0 and 7.4 times higher (P<0.001), compared to the indicators of fresh meat. Single cocci were mainly detected in fresh meat, muscle tissue decay was not detected; in meat of questionable freshness – cocci, diplococci, a small amount of gram-positive and gram-negative rod-shaped microorganisms, slight decay of muscle tissue; in stale meat, rod-shaped gram-positive microorganisms predominated, isolated cocci were found in some places, significant decay of muscle tissue.

The content of volatile fatty acids in the chilled meat of broiler chickens of questionable freshness (breast muscles and thigh muscles) was 2.5 times higher (P<0.001), compared to the parameters of fresh meat. At the same time, in stale chilled meat, the content of volatile fatty acids in pectoral muscles and thigh muscles, respectively, was 4.2 times (P<0.001) and 4.1 times higher (P<0.001) compared to fresh meat.

The acid number of fat in chilled meat of questionable freshness was 2.4 times higher (P<0.001), compared to the indicators of the acid number of fat in fresh meat; in stale chilled meat, the acid number of fat was 3.9 times higher (P<0.001), compared to the indicators of the acid number of fat in fresh meat.

The results of qualitative reactions for determining the content of ammonia and ammonium salts with Nessler's reagent and determining the products of primary breakdown of proteins in the broth using copper sulphate were corrected with indicators for determining fresh, questionable freshness and stale meat of broiler chickens.

The developed method of bacterioscopic study of insemination of poultry meat with microorganisms is easy to perform, and based on quantitative results, reliable indicators can be obtained for establishing the degree of freshness of chilled meat of broiler chickens by counting the number of microorganisms in one smear-imprint from the deep layers of poultry meat and in 10 fields of view of the microscope, and establishing the breakdown of muscle tissue. The proposed quantitative bacterioscopic method for establishing the insemination of poultry meat with microorganisms can be used in the control system for determining the safety and quality of broiler chicken meat in production laboratories at meat and meat processing facilities, wholesale bases during storage, in supermarkets, in state laboratories of the State Production and Consumer Service of Ukraine and in state laboratories of veterinary and sanitary examination in agri-food markets. This method is proposed for establishing the freshness of chilled meat of broiler chickens along with the generally accepted methods of laboratory testing of meat – qualitative reactions for the detection of ammonia and ammonium salts and the determination of the products of the primary breakdown of proteins in the broth using copper sulphate, the content of volatile fatty acids, the establishment of acid number of fat of broiler chickens and organoleptic indicators.

The study was carried out to identify the freshness of broiler chicken meat at sales facilities: agri-food markets and supermarkets. The results are presented in Table 3.

Table 3. Microclimate indicators in poultry rearing shops, M ± m, n = 5

Total samples of broiler chicken meat	Quality of broiler chicken meat					
	Fresh broiler chicken meat		Broiler chicken meat of questionable freshness		Stale broiler chicken meat	
	Amount samples	%	Amount samples	%	Amount samples	%
	Agri-food markets					
n = 53	42	79.3	9	17.0	2	3.8
	Supermarkets					
n = 56	42	75.0	11	19.6	3	5.4

According to the data in Table 3, the highest percentage of fresh meat of broiler chickens according to generally accepted methods and the developed bacterioscopic method of establishing the insemination of poultry meat with microorganisms was 79.3% for the sale of products in agri-food markets and 75.0% in supermarkets; of questionable freshness, respectively – 17.0% and 19.6%. And the lowest percentage of stale meat of broiler chickens was found during sale in agricultural food markets – 3.8% and in supermarkets – 5.4%.

This bacterioscopic method of establishing the insemination of poultry meat with microorganisms to determine the freshness of chilled meat of broiler chickens has been tested and implemented at the facilities for its production and circulation and is recommended for implementation by state veterinary medicine specialists during the implementation of the state risk-oriented safety and quality control system chilled meat of broiler chickens.

The author's patented bacterioscopic method for establishing the insemination of poultry meat with microorganisms is simple to perform, and its results provide particular quantitative indicators for determining the freshness of chilled meat of broiler chickens for 5, 6-7, and 8 days at 0-4 °C with calculation of the number of microorganisms and the degree of breakdown of muscle tissue: fresh meat – up to 10 microorganisms, no breakdown of muscle tissue is observed; meat of questionable freshness – 11-30 microorganisms, traces of decay of muscle tissue; stale meat – more than 30 microorganisms (rod-shaped gram-positive ones prevailed), considerable breakdown of muscle tissue. To facilitate the work of veterinary medicine specialists, it is necessary to apply simple laboratory tests (organoleptic, chemical, physical) to establish the freshness of meat of broiler chickens during storage and sale when it is cooled under different temperature regimes and terms [21]. In the system of risk-based control of the storage of chilled meat of broiler chickens, research should be directed towards the identification of dangerous food products, which can lead to the development of food poisoning in consumers [22]. For the cooling of broiler chicken carcasses, it is necessary to assess the control of pathogens of food infections, namely *Campylobacter spp.,* in which their number will subsequently decrease under the control of compliance with the cold chain, which will enable the facilities for the production and circulation of poultry meat to comply with the hygiene criteria of the technological process and reduction of meat contamination of broiler chickens [23].

Furthermore, scientists have developed an optimised 99.8% statistically significant method for determining the contamination of fresh, questionable freshness and stale poultry meat with microorganisms by counting the number of microorganisms by preparing two smears-imprints and staining them according to Gram in Hooker's modification and counting the number of microorganisms in 15 fields of view [24].

Due to non-compliance with the temperature regimes of meat cooling of broiler chickens, fat in which aldehydes and peroxides accumulate is primarily subject to spoilage, and therefore it is necessary to control the quality of poultry fat [25]. And in the results of this study, the acid number of fat corresponded to fresh, questionable, and stale meat of broiler chickens. In their studies, scientists demonstrate the positive effect of chlorine-containing oxide on the quality of meat of broiler chickens by cooling the carcasses of broiler chickens at 4 °C on the 4th and 7th day of sale due to the reduction of the contamination of the meat with microorganisms [26]. Deterioration of meat due to non-compliance with temperature regimes is one of the microbiological risks that must be promptly detected and prevented to provide consumers with high-quality and safe meat products [27].

In general, the policy of the Ukrainian national strategy is aimed at implementing control from the farm to the table by introducing new methods in the system of laboratory control of fresh meat and meat products, applying compliance with sanitary and hygienic requirements at the facilities for their production and circulation [28].

Conclusions

The developed bacterioscopic method of detecting contamination of poultry meat with microorganisms can be used in the laboratory control system to establish the freshness of chilled meat of broiler chickens during production, storage, and sale. This method has 99.9% confidence. At the same time, more reliable data – 98.5-99.6% were obtained according to the results of studies of indicators of the content of volatile fatty acids, and 99.2-99.7% – according to the results of studies of indicators of the acid number of fat of broiler chickens.

According to the developed bacterioscopic method and several generally accepted methods, on the 5th, 6th, 7th, and 8th day, the conformity of the chilled meat of broiler chickens to different degrees of freshness was established: fresh, questionable freshness, and stale. At the same time, the indicators of the number of microorganisms, the

content of volatile fatty acids in the meat of broiler chickens and the acid number of fat of questionable freshness and staleness had a high degree of probability (P<0.001) compared to the indicators of fresh meat and fat of broiler chickens.

At meat sales facilities: agri-food markets and supermarkets, the largest percentage is fresh meat from broiler chickens, respectively – 79.3% and 75.0%; of questionable freshness, respectively – 17.0% and 19.6%; the lowest percentage is stale meat of broiler chickens, respectively – 3.8% and 5.4%.

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Контроль охолодженого м'яса курчат-бройлерів за бактеріоскопічним методом

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Анотація. Актуальність дослідження зумовлена забезпеченням належного ризик-орієнтованого контролю за безпечністю та якістю охолодженого м'яса курчат-бройлерів на потужностях з його виробництва, де має бути впроваджена система аналізу небезпек і контролю у критичних точках. У зв'язку з цим дана стаття спрямована на розкриття питання контролю охолодженого м'яса курчат-бройлерів за проведення досліджень на встановлення свіжості м'яса курчат-бройлерів на потужностях з реалізації – агропродовольчих ринках і супермаркетах. Провідним підходом до дослідження цієї проблеми є розроблений запатентований бактеріоскопічний метод, що дозволяє комплексно виявити свіжість охолодженого м'яса курчат-бройлерів. Представлений метод є простим у виконанні, з отриманням кількісних показників щодо встановлення свіжості охолодженого м'яса курчат-бройлерів на 5 добу, 6–7 і 8 добу за температури 0–4 °С, а також зі встановленням кількості мікроорганізмів в полі зору мікроскопа і ступенем розпаду м'язової тканини, шляхом фарбування одного мазка-відбитка за Грамом у модифікації Хукера, та підрахунком кількості мікроорганізмів в 10 полях зору з подальшим виведенням середнього значення на одне поле зору, а також визначення форми клітин. Достовірність результатів у випробуваннях за даним методом складає 99,9 %. Встановлено, що найвищий вміст мікроорганізмів становив у несвіжих охолоджених тушок курчат-бройлерів на 8 добу: у грудних м'язах – 45 ± 3 ($P<0,001$), у м'язах стегна – 52 ± 5 ($P<0,001$) порівняно з показниками свіжого м'яса. В несвіжому м'ясі – переважали паличкоподібні грам-позитивні мікроорганізми, подекуди фіксували поодинокі коки, значний розпад м'язової тканини. Уміст летких жирних кислот в охолодженому м'ясі курчат-бройлерів і кислотне число жиру достовірно ($P<0,001$) зростали на 8 добу реалізації за температури 0–4 °С, відповідно – 11,05 ± 0,37 – 10,97 ± 0,33 і 2,83 ± 0,33 мг NaOH. За проведення обстеження свіжості м'яса тушок курчат-бройлерів найбільший відсоток свіжого м'яса курчат-бройлерів становив за його реалізації на агропродовольчих ринках – 79,3 %, у супермаркетах – 75,0 %, несвіжого, відповідно – 3,8 і 5,4 %. Матеріали статті становлять практичну цінність для роботи державних інспекторів ветеринарної медицини під час використання бактеріоскопічного методу щодо встановлення свіжості охолодженого м'яса курчат-бройлерів

Ключові слова: кількість мікроорганізмів, органолептична оцінка, грудні м'язи, м'язи стегна, свіжість м'яса

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Features of Biofilm Formation of some Pathogenic and Commensal *Escherichia coli* Isolated from the Body of Dogs and Cats

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Abstract. The relevance of this study is conditioned upon epidemic growth of nosocomial infections, which include *Escherichia Coli (E. coli)*. One of the factors of pathogenicity of such microorganisms is the ability to form a biofilm – a complex community, within which bacteria acquire increased resistance to environmental factors, primarily to antibacterial drugs, which considerably complicates the course of the infectious process. In this regard, the purpose of this study was to determine the features of the formation and dependence of the density of the formed biofilm on the antibiotic resistance of pathogenic and commensal *E. coli* strains isolated from dogs and cats. The resistance of *E. coli* isolates to antibacterial drugs was established according to the disk diffusion method, according to EUCAST recommendations. The ability of microorganisms to form biofilms and determine their density was investigated in sterile plastic 96-well plates. The ability to form biofilms was assessed visually and microscopically, the density of biofilms was determined in units, spectrophotometrically, by the optical density of the washing solution. The paper presents the results of a study of 63 samples of pathological (wound infections) and biological material. From them, 10 *E. coli* isolates were obtained (6 from dogs and 4 from cats), which were selected for further research. It was established that all *E. coli* isolates had the ability to form phenotypic biofilm. The study investigated the interdependence of antibiotic resistance of *E. coli* isolates and their ability to form biofilms. Thus, isolates that were parted from pathological material and had a positive reaction on the CHROMagar™ ESBL medium for the determination of extended-spectrum beta-lactamases had greater resistance to various groups of antibacterial drugs and formed high- and medium-density biofilms, while *E. coli* isolates parted from pathological and biological materials with a negative reaction on CHROMagar™ ESBL medium formed a low-density biofilm and had less resistance to different groups of antibacterial drugs. The results obtained allow searching for innovative, sometimes alternative, methods of treatment and prevention of pathologies caused by them

Keywords: microorganism, biofilm, antibiotic resistance, antibacterial drugs, wound infection

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Introduction

The constant evolution of pathogens of infectious diseases requires a more thorough study of their biological properties, and sometimes their mutual conditionality. Research in this area helps find innovative, and sometimes alternative, ways to treat and prevent the diseases that they cause.

Some microorganisms, apart from showcasing adhesive activity, have the property of producing an extracellular polymer substance that envelops the bacteria in a thin layer. This structural formation is known as a biofilm. The ability to form a biofilm can be evaluated as a manifestation of the powerful pathogenetic effect of microorganisms on the macroorganism. Therewith, the biofilm performs a protective function – it restricts direct contact of the microorganism with body defence factors and antibacterial drugs, which factually transforms the pathogen into an invulnerable target.

The category of bacteria with high variability includes *Escherichia coli* (*E. coli*), the modification of which is caused by the action of several factors, including the influence of antibiotics. Polyresistance to antibiotics of distinct groups acquired by individual strains of *E. coli* has become the cause of a global problem – the formation of nosocomial infections (ESKAPE). The results of earlier studies [1] of similar properties of other types of bacteria indicate the manifestation of a special level of resistance to factors of negative influence due to the formation of a specific form of protection in them – a biofilm. Regarding *E. coli*, the study of biofilm formation is particularly relevant, which is due to the rapid acquisition of a pathogenic state by commensal forms.

E. coli belongs to the group of bacteria that can show adhesive properties. The need for its detailed study became clear when this bacterium was included in the list of pathogens of nosocomial purulent-inflammatory infections. *Escherichia coli* is one of those microorganisms that can form biofilms, both in the animal body and outside it – on various surfaces. Cultivation of *E. coli* does not require special conditions and specific nutrient media, which makes it an indicative model for investigating the morphological and physiological features of biofilm formation [2].

Recently, in Ukraine, as in the entire world, a clear trend has been established regarding the increase in the level of resistance of *E. coli* strains to antibacterial drugs [3; 4]. At the same time, there are considerable fluctuations in the antibiotic sensitivity of these bacteria to certain groups and classes of antibacterial drugs, which causes increasing concern among the world community [5]. This situation has developed because up to 95% of cases of severe infections are treated without proper bacteriological examination [6].

Therefore, understanding the specific features of biofilm formation and the development of resistance to antibacterial drugs in *E. coli* strains will help open new areas in the diagnostics, treatment, and prevention of infectious diseases associated with biofilm-forming *E. coli* strains.

Biofilms are specific microbial communities formed on biotic and abiotic surfaces by secreting extracellular polymer substances that increase the level of adhesion to surfaces [7].

Bacteria inside the biofilm become more tolerant to the effects of various exogenous factors, such as antibacterial drug [2]. Increased stability of biofilms is explained by several factors: 1) different rate of diffusion of substances; 2) the accumulation in the matrix of extracellular enzymes that destroy antibacterial drugs; 3) unavailability of bacteria due to clumping; 4) stable properties of the cells themselves, which are involved in the formation of a biofilm [8-10]. Thanks to these properties of the biofilm, pathogenic bacteria, which are the causative agents of many chronic infections, necessitate increased requirements for the disinfection of medical equipment and medical instruments [11]. Biofilm formation is an intra- and interspecific phenomenon that requires dynamic interactions between bacteria in mixed biofilm communities [12]. Bacterial species from biofilms interact through cell-to-cell communication, metabolic interaction, or spatial organisation [1].

Since its discovery in 1885, the status of *E. coli* has changed many times. Among the commensal *E. coli* strains, it was mostly shown that they do not have specialised virulence determinants and are useful for their host [13]. While among the pathogenic strains of *E. coli*, enterotoxigenic *Escherichia coli* (ETEC) and enteropathogenic *Escherichia coli* (EPEC) are recognised as the most common cause of bacterial putrefactive infections, especially in low-income countries with unsatisfactory sanitary conditions [14]. *E. coli* is a common inhabitant of the intestines of animals and humans, but can also occur in environmental objects, namely in water, soil, and vegetation. It is the leading causative agent of urinary tract infections [15] and is one of the most common pathogens that cause blood flow infections, wound infections, otitis media, and other complications in both animals and humans [5]. Unlike commensals and enteropathogenic strains, extraintestinal pathogenic *E. coli* cause infections of the urinary tract, bloodstream, cerebrospinal fluid, respiratory tract, and peritoneum. With such infections, the pathology can occur in the form of cholecystitis, bacteraemia, cholangitis, urinary tract infection, or neonatal meningitis. Infections caused by such pathogenic strains are widely reported in public places, as well as in veterinary clinics and long-term animal care facilities, which causes a considerable burden on medical and economic resources around the world [16; 17].

Although the current knowledge of bacterial biology is largely based on work carried out on planktonic cultures of *E. coli* laboratory strains, many isolates have the ability to form biofilms *in vivo* and *in vitro* [18].

Bacteria of the *Enterobacteriaceae* family are the most common pathogens of nosocomial infections that occur in veterinary clinics and medical institutions of Ukraine [19]. *E. coli* strains are polyresistant to antibacterial drugs of various groups and therefore belong to one of the most problematic pathogens of nosocomial infectionsx – ESKAPE. The researchers found that 52.8% of enterobacteria strains isolated from surgical wounds were beta-lactamase producers. *E. coli* ranks second among beta-lactamase producers after *Klebsiella pneumoniae* and makes up 41.2% of the total number of strains [19].

Thus, M.D. Kukhtin et al. (2014) indicate that *E. coli* strains isolated from milking equipment in farms with good and satisfactory sanitary conditions, wherein antimicrobial drugs were widely used, formed biofilms of

high and medium density in 80% or more, which is 1.6-2.4 times more compared to farms with unsatisfactory sanitary conditions [20]. Furthermore, the results of the study by M.M. Mishyna et al. (2020) testify that the formation of biofilms in strains of microorganisms isolated from chronically ill patients who were treated with antibacterial drugs for a long time was more pronounced compared to patients with acute forms of infections [21]. According to Yu.Yu. Vishovan et al. (2021), all microorganisms isolated from sick and healthy animals can form biofilms [10]. Therewith, the highest density biofilm is observed in microorganisms described by a higher number of phenotypic and genotypic pathogenicity factors.

Therefore, *the purpose of this study* was to investigate the phenotypic features of biofilm formation, as well as to establish the interdependence between the density of the formed biofilm and resistance to antibacterial drugs of different groups of *E. coli* strains that were parted from pathological and biological materials isolated from cats and dogs.

Materials and Methods

The study was performed based on the Ukrainian Laboratory of Quality and Safety of Agricultural Products of the National University of Life and Environmental Sciences of Ukraine (Kyiv, Ukraine) and at the Department of Epizootology, Microbiology, and Virology of the National University of Life and Environmental Sciences of Ukraine (Kyiv, Ukraine) during 2020-2021.

For the study, 10 cultures of *E. coli* were selected among parted isolates from various species of sick and clinically healthy animals that were patients at the "Multivet" veterinary clinic (Kyiv Oblast, Ukraine). Cultures of *E. coli* were isolated as a result of bacteriological examination of pathological and biological materials from 36 dogs, of which 27 were clinically healthy, and 9 had purulent wound complications, and 27 cats, of which 18 were clinically healthy, and 6 had purulent wound complications.

Biological material was seeded on selective and differential diagnostic nutrient media: 5% blood agar produced by Graco (Poland), Endo medium produced by HiMedia (India), yolk-salt Agar produced by HiMedia (India), meat-peptone broth produced by HiMedia (India) with the addition of 5% glucose. Sowing was carried out by the quantitative method of sector seeding according to the Gold's method. Microorganisms were identified using a Vitek 2 compact bacteriological analyser (bioMérieux, France).

Sensitivity to antibacterial drugs of *E. coli* cultures was determined according to the disco-diffusion method using discs manufactured by HiMedia (India) and Muller-Hinton Agar manufactured by HiMedia (India) according to the EUCAST recommendations (European Committee for Antimicrobial Sensitivity Testing) [22]. Reference strains *E. coli* ATCC 25922 and *E. coli* ATCC 35218 were used for quality control of antimicrobial susceptibility tests.

Phenotypic determination of resistance factors was performed using CHROMagar™ ESBL manufactured by Graco (Poland).

The ability to form a biofilm in isolates and the interpretation of the results obtained were performed according to the modified method of M. D. Kukhtin [20]. This study was performed using sterile polystyrene tablets (Greiner Bio-One GmbH, Germany) n=96, in each well of which 100 µL of cardiovascular broth produced by HiMedia (India) was added, and 10 µL of inoculant containing 0.5 McFarland daily culture of the *E. coli* isolates under study. Each individual isolate was cultured in 1 row with 12 wells in two different plates. Planchettes were cultured in a thermostat at 37°C for 24 hours. The remaining nutrient medium was carefully removed with a pipette dispenser. Planchette wells were washed three times from planktonic forms of *E. coli* isolates with a sterile phosphate buffer solution (KH_2PO_4•Na_2PO_4•H_2O), pH 7.2-7.4. The planchettes were air-dried and 100 µL of 96% ethanol was added to fix the resulting biofilms. The fixation exposure was 10 minutes. Then the fixing liquid was drained. Subsequently, the planchettes were divided into two groups. The first plate was stained with a 0.1% alcohol solution of crystal violet for 10 minutes; the second was dyed with a saturated aqueous Congo Red solution for 15 minutes. Subsequently, the planchettes were washed three times with a sterile phosphate buffer solution (pH 7.2) and dried. Then 100 µL of 96% ethanol was added and placed on a shaker to shake and then repel the biofilm from the well walls for 30 minutes. Finally, the contents of the planchette wells were pipetted and the amount of dye absorption by the biofilm was measured on an Evolution 300 spectrophotometer (Thermo Fisher Scientific, USA) at a wavelength of 570 nm for a tablet with a solution of crystal violet and 495 nm for a tablet with a saturated aqueous Congo Red solution. The density of the resulting biofilm was determined by measuring the level of dye adsorption with ethanol, which was expressed in units of optical density (OD) using a spectrophotometer.

When the optical density value is less than 0.10, it was assumed that the strains do not form biofilms, from 0.10 to 0.49 – the ability to form a film was considered low. If the optical density is from 0.50 to 1.0, it is the average density of the biofilm and its ability to form it. At values above 1.0 – high ability to form a biofilm and its high density.

Statistical analysis was performed in a Microsoft Excel 2010 spreadsheet. The Online Epitools epidemiological calculator was used to estimate the 95% confidence interval (CI) [23].

Results and Discussion

This paper presents the results of the study of 10 *E. coli* cultures, which were selected among isolates parted from various species of sick and clinically healthy animals. This study investigated 6 strains of *E. coli* isolated from sick animals and 4 strains from clinically healthy individuals. Samples of the material from which the cultures under study were isolated were selected from the "Multivet" Veterinary Clinic (Kyiv Oblast, Ukraine) for the 2nd quarter of 2020.

E. coli cultures were isolated as a result of bacteriological examination of pathological and biological materials from dogs and cats: 4 isolates (40%) from dogs with purulent wound complications; 2 isolates (20%) from clinically healthy dogs, collected from the intestine and oral cavity; 2 isolates (20%) – from cats with purulent wound complications; 2 isolates (20%) from the intestines of clinically healthy cats (Table 1).

Table 1. The results of determining the presence of extended-spectrum beta-lactamases in *E. coli* cultures, which were selected among isolates parted from different animal species

No. Seq. No.	Strain	Isolation object	Locus	Presence of extended-spectrum beta-lactamases
1	EC1/21	Dogs	Wound	–
2	EC2/21	Dogs	Wound	+
3	EC3/21	Cats	Wound	+
4	EC4/21	Cats	Wound	–
5	EC5/21	Dogs	Wound	+
6	EC6/21	Dogs	Wound	+
7	EC7/21	Cats	Intestines	–
8	EC8/21	Cats	Intestines	–
9	EC9/21	Dogs	Intestines	–
10	EC10/21	Dogs	Oral cavity	–

The presence of extended-spectrum beta-lactamases for E. coli cultures was determined on the CHROMagar™ ESBL medium (Table 1, Fig. 1). Therewith, after cultivation for 24 hours, colonies of EC1/21, EC4/21, EC7/21, EC8/21, EC9/21, EC10/21 strains on chromium agar had weak growth of small colourless colonies. Strains EC2/21, EC3/21, EC5/21, and EC6/21 formed lilac-coloured colonies.

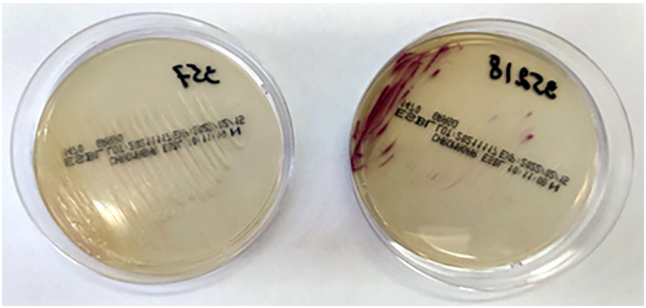

Figure 1. Phenotypic determination of resistance factors on CHROMagar™ ESBL

Note: *EC4/21 culture dish on the left (negative), EC5/21 culture dish on the right (positive)*

During the study of isolates for sensitivity to antibacterial drugs, the following data were obtained (Table 2): 60% of isolates were resistant to the penicillin group; 80% of isolates – to the cephalosporin group; 60% of isolates – to the fluoroquinolone group; 10% of isolates – to the carbapenem group; 30% of isolates – to the aminoglycoside group. Therewith, all isolates were sensitive to tobramycin and ceftazidime/avibactam. Isolates selected from biomaterial from clinically healthy animals showed resistance to fluoroquinolones, while isolates from pathological material showed a higher percentage of sensitivity.

Table 2. Sensitivity of *E. coli* isolates to antibiotics of different groups according to EUCAST recommendations (M±m, n=6, P<0.01)

Anti-bacterial preparation	EUCAST (mm)		EC1/21	EC2/21	EC3/21	EC4/21	EC5/21	EC6/21	EC7/21	EC8/21	EC9/21	EC10/21
	S≥	R<										
Penicillins												
Piperacillin	20	20	6.1R	10.2R	9.1R	20.2S	9.6R	10.4R	6.1R	21.6S	24.7S	21.1S
Piperacillin / tazobactam	20	20	6.2R	22.4S	9.5R	24.9S	12.6R	21.4S	13.3R	25.3S	26.2S	22.1S
Ticarcillin / clavulanate	23	20	6.7R	25.3S	21.2I	9.3R	16.2R	15.9R	22.1I	15.5R	26.8S	10.4R
Ampicillin / sulbactam	14	14	16.2S	19.5S	9.5R	6.2R	14.5S	6.9R	19.2S	22.5S	24.7S	20.8S

Table 2, Continued

Amoxicillin / clavulanate	19	19	12.1R	21.1S	15.5R	14.2R	16.8R	20.6S	16.1R	14.2R	16.7R	12.2R
Fluoroquinolones												
Levofloxacin	23	19	6.2R	12.3R	16.1R	24.5S	26.1S	16.1R	28.1S	12.1R	10.2R	12.8R
Ciprofloxacin	25	22	6.5R	6.1R	9.2R	27.1S	20.1I	10.2R	27.2S	15.3R	16.2R	20.0R
Moxifloxacin	22	22	12.2R	22.1S	19.1R	26.2S	20.1R	13.8R	24.2S	19.1R	22.1S	17.4R
Ofloxacin	24	22	8.2R	26.8S	16.8R	28.9S	26.7S	14.2R	16.8R	18.9R	16.9R	15.2R
Cephalosporins												
Cefepime	27	24	26.4I	19.2R	11.8R	28.1S	14.4R	13.4R	18.8R	12.2R	19.8R	13.2R
Cefotaxime	20	17	12.9R	10.8R	9.1R	22.7S	13.1R	7.1R	11.7R	20.8S	22.8S	16.8R
Cefuroxime	50	19	16.8R	15.9R	10.7R	29.6I	16.9R	12.6R	14.5R	13.8R	16.3R	14.1R
Ceftazidime	22	19	10.1R	13.1R	13.2R	25.2S	17.0R	9.2R	12.1R	14.4R	21.2I	20.6I
Ceftazidime / avibactam	13	13	16.2S	18.3S	20.0S	16.9S	23.1S	16.4S	19.1S	22.1S	20.9S	25.3S
Carbapenems												
Meropenem	22	16	25.2S	23.3S	28.1S	26.2S	10.2R	29.1S	24.3S	23.2S	20.2I	29.1S
Imipenem	22	19	30.3S	14.6R	13.2R	29.1S	16.1R	20.1I	28.7S	13.1R	21.6I	10.9R
Aminoglycosides												
Amikacin	18	18	20.2S	21.9S	23.6S	22.9S	20.4S	25.3S	10.3R	9.2R	12.3R	14.4R
Tobramycin	16	16	20.1S	26.2S	19.2S	23.3S	25.2S	22.1S	17.2S	18.3S	17.6S	22.0S
Gentamicin	17	17	19.2S	15.1R	20.1S	23.8S	10.2R	13.9R	11.9R	16.1R	19.9S	8.2R
Other												
Aztreonam	26	21	10.8R	24.6I	11.3R	13.6R	19.0R	24.3I	27.1S	25.2I	29.2S	26.3S
Fosfomycin	21	21	13.0R	10.6R	18.2R	20.0R	11.3R	26.8S	13.8R	24.5S	26.7S	22.2S
Tigecycline	18	18	20.2S	23.9S	26.8S	25.1S	26.1S	20.1S	19.7S	6.8R	20.2S	9.8R

Based on the results of the study of the phenotypic formation of biofilms (Table 3, Fig. 2) upon using the crystal violet staining method, EC2/21, EC3/21 strains formed a high-density biofilm; EC5/21, EC6/21 strains formed a medium-density biofilm; and EC1/21, EC4/21, EC7/21, EC8/21, EC9/21, EC10/21 strains formed a low-density biofilm.

Table 3. Results of phenotypic determination of *E. coli* biofilm formation (crystal violet and Congo Red staining)

No. Seq. No.	Strain	Density of the formed biofilm (n=12)	
		Upon crystal violet staining	Upon Congo Red staining
		λ 570	λ 495
1	EC1/21	0.421	0.462
2	EC2/21	1.006	1.063
3	EC3/21	1.144	1.178
4	EC4/21	0.524	0.518
5	EC5/21	0.635	0.696
6	EC6/21	0.596	0.554
7	EC7/21	0.298	0.356
8	EC8/21	0.293	0.334
9	EC9/21	0.427	0.498
10	EC10/21	0.436	0.496

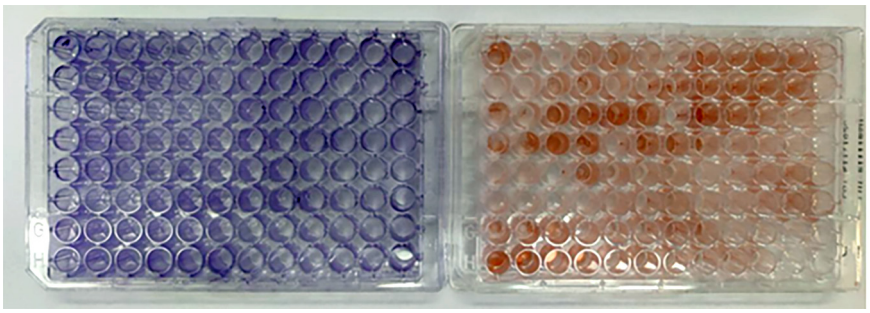

Figure 2. *E. coli* biofilm formation stained with crystal violet (left panel) and Congo Red (right panel)

In turn, for the study of the phenotypic formation of biofilms (Table 3, Fig. 2) upon using the Congo Red staining method, EC2/21, EC3/21 strains formed a high-density biofilm; EC/21, EC6/21 strains formed a medium-density biofilm; and EC1/21, EC4/21, EC7/21, EC8/21, EC9/21, EC10/21 strains formed a low-density biofilm.

In 4 *E. coli* strains isolated from dog wounds, the optical density of biofilm *in vitro* ranged from λ 0.421-1.006 for crystal violet and λ 0.462-1.063 for Congo Red. In 2 *E. coli* strains isolated from clinically healthy dogs, the optical density of biofilms was λ 0.427 (EC9/21) and 0.436 (EC10/21) for crystal violet and λ 0.354 (EC9/21) and 1.063 (EC10/21) for Congo Red. In 2 *E. coli* strains isolated from wounds in cats, the optical densities were λ 1.144 (EC3/21) and 0.524 (EC4/21) for crystal violet and λ 1.178 (EC3/21) and 0.518 (EC4/21) for Congo Red. In 2 E. coli strains isolated from the intestines of cats, the optical density was λ 0.298 (EC7/21) and 0.293 (EC8/21) for crystal violet and λ 0.356 (EC7/21) and 0.334 (EC8/21) for Congo Red.

According to the above results, there is an interdependence of antibiotic resistance of *E. coli* isolates and their ability to form a biofilm. The ability of isolates to form a higher-density biofilm was accompanied by better resistance to antibacterial drugs. Thus, EC2/21 and EC3/21 isolates parted from pathological material that had a positive reaction to CHROMagar™ ESBL medium for the determination of extended-spectrum beta-lactamases and greater resistance to antibacterial drugs (Table 2) formed a high-density biofilm with λ 1.006 (EC2/21) and λ 1.144 (EC3/21). Isolates from the pathological material isolated from dogs, EC5/21, EC6/21, which had a positive reaction to CHROMagar™ ESBL medium and high resistance to antibacterial drugs (Table 2) formed a medium-density biofilm with λ 0.635 (EC5/21) and λ 0.596 (EC6/21). Therewith, isolates from pathological and biological material that had a negative reaction to the CHROMagar™ ESBL medium formed a low-density biofilm. This indicates a positive correlation between the level of antibiotic resistance of isolates and the intensity of biofilm formation.

The results obtained regarding the biofilm formation of *E. coli* coincide with the results of the study by M.D. Kukhtin et al. [20], who indicated an increase in the density of the biofilms formed with the intensive use of antibacterial drugs. In the case under study, *E. coli*, which had less resistance to antibacterial drugs, formed a lower-density biofilm than bacteria that had higher resistance to these drugs, indicating an increase in the biofilm-forming capacity of bacteria in response to the use of antibacterial drugs. Furthermore, the regularities established in this paper confirm the results of studies by M.M. Mishyna [21] and Yu.Yu. Vishovan [10], who note that an increase in both phenotypic and genotypic antibiotic resistance of microorganisms increases their ability to form biofilms, which is an essential factor in the mechanism of formation of resistance to antibacterial drugs.

Thus, upon developing an effective regimen for the treatment of purulent lesions with antibacterial drugs, it is necessary to conduct a bacteriological study for each individual isolate. Equally important is the monitoring of the antibiotic sensitivity of opportunistic and pathogenic bacteria to adjust the already available antibiotic therapy regimens. Furthermore, upon identifying isolates with a high level of antibiotic resistance, their increased ability to form biofilms should be considered. This may require added measures to disinfect medical equipment and instruments, as well as the need to introduce drugs that prevent the formation of biofilms into the treatment regimen.

Notably, an in-depth study of the correlation of antibiotic resistance among biofilm-forming strains of opportunistic and pathogenic bacteria will be the subject of the author's further research in this area.

Conclusions

As a result of the study of 63 samples of pathological and biological material, 10 *E. coli* strains were isolated – 6 from dogs and 4 from cats. Isolates parted from pathological material had higher resistance to antibacterial drugs than strains isolated from clinically healthy animals.

When investigating the results of phenotypic biofilm formation in a comparative aspect using the Congo Red and crystal violet staining method, no substantial differences were found. *E. coli* isolates with high resistance to antibacterial drugs were found to have the ability to form higher-density biofilms compared to more sensitive isolates. *E. coli* isolates that had the ability to form beta-lactamase formed a higher-density biofilm than isolates in which this enzyme was not synthesised.

To screen the ability of microorganisms to form extended-spectrum beta-lactamases, it is advisable to use chromium-agar, which will substantially speed up obtaining informative results.

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Особливості біоплівкоутворення деяких патогенних та комменсальних *Escherichia coli*, ізольованих з організму собак і котів

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Анотація. Актуальність дослідження зумовлена процесом епідемічного росту внутрішньолікарняних інфекцій, до яких відноситься *Escherichia Coli (E. coli)*. Одним із факторів патогенності таких мікроорганізмів є здатність до утворення біоплівки – складноорганізованого співтовариства, всередині якої бактерії набувають підвищеної стійкості до факторів довкілля, насамперед, до антибактеріальних препаратів, чим значно ускладнюють перебіг інфекційного процесу. У зв'язку з цим, метою роботи було визначити особливості формування та залежності щільності сформованої біоплівки від антибіотикорезистентності патогенних та комменсальних штамів *E. coli*, виділених від собак і котів. Диско-дифузійним методом, згідно з рекомендаціями EUCAST, встановлено стійкість ізолятів *E. coli* до антибактеріальних препаратів. Вивчення здатності мікроорганізмів формувати біоплівки та визначення їх щільності проводили у стерильних пластикових 96-лункових планшетках. Здатність формувати біоплівки оцінювали візуально та мікроскопічно, щільність біоплівок визначали в одиницях, спектрофотометрично, за оптичною густиною промивного розчину. Наведено результати дослідження 63 проб патологічного (ранові інфекції) та біологічного матеріалу. З них отримано 10 ізолятів *E. coli* (6 від собак і 4 від котів), які було обрано для подальшого дослідження. Встановлено, що всі ізоляти *E. coli* мали здатність до фенотипового утворення біоплівки. Досліджена взаємозалежність антибіотикорезистентності ізолятів *E. coli* та їх здатність утворювати біоплівку. Так ізоляти, які були виділені з патологічного матеріалу та мали позитивну реакцію на середовищі CHROMagar™ ESBL для визначення бета-лактамаз розширеного спектру, мали більшу резистентність до різних груп антибактеріальних препаратів і утворювали біоплівку високої та середньої щільності, тоді, як ізоляти *E. coli*, виділені з патологічного та біологічного матеріалів з негативною реакцією на середовищі CHROMagar™ ESBL утворювали біоплівку низької щільності та мали меншу резистентність до різних груп антибактеріальних препаратів. Отримані результати дають можливість пошуку нових, інколи альтернативних, методів лікування та профілактики викликаних ними патологій

Ключові слова: мікроорганізм, біоплівка, антибіотикорезистентність, антибактеріальні препарати, ранова інфекція

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Morphological Features and Morphometric Parameters of the Lungs of Sexually Mature Horses (*Equus Ferus Caballus* L., 1758)

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Abstract. The lungs, which form part of the respiratory apparatus, provide gas exchange between the environment and the tissues of the human and animal body. Therefore, lung diseases are one of the most pressing issues for modern humane and veterinary medicine. This problem is caused by the progressive growth of diseases in mammals such as bronchial asthma, bronchitis, bronchopneumonia, pneumonia, coronavirus infections, etc. Therefore, to effectively solve this issue in terms of prevention, effective treatment, and prompt differential diagnosis of respiratory diseases, an essential area in morphology is the study of the respiratory apparatus, namely the lungs, in clinically healthy animals, to develop marker test criteria that will serve as indicators of the norm in the differential diagnosis of these diseases. The purpose of this study was to investigate the macro- and microscopic structure of the lungs, conduct a morphometric assessment of their morphological structures in domestic sexually mature horses (class Mammals, species – domestic horse (*Equus ferus caballus* L., 1758). The object of this study was the lungs of clinically healthy sexually mature horses (n = 5). Fresh lungs of the animals under study were subjected to anatomical preparation. For histological studies, generally accepted methods of fixing pieces of material and making histological sections were used, which were then stained with haematoxylin and eosin and according to Van Gieson's method. The basis of the lungs in horses are pyramidal or cone-shaped lobules. Part of the structure of the lobes are acini covered with a thin layer of connective tissue. The microscopic structure of acini is formed by alveolar ducts, alveolar sacs, and alveoli. According to the results of research, the alveolar tree in horses is shortened and wide and has a bubble shape. The results of morphometric studies showed that the average volume of pulmonary alveoli in clinically healthy horses is 699.80 ± 106.42 thous. μm^3. The respiratory part of the lungs in horses occupies 54.8 ± 7.4% of the total area of the lung parenchyma, the connective tissue base – 45.2 ± 7.4%. Such studies of morphological features and morphometric parameters of equine lungs are of practical importance in veterinary medicine since they are markers and criteria for pathomorphological diagnosis of diseases associated with the respiratory system

Keywords: morphology, asymmetry of the lungs, bronchi, terminal bronchioles, alveolar tree

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Introduction

The human and animal bodies are a complex biological system built from interconnected structural elements – organs and tissues that interact with each other [1]. The body's response to environmental influences, transforming all organs and systems into a single whole, occurs only with the normal morphofunctional activity of all its systems, including the respiratory apparatus, which includes the lungs [2].

The scientific literature currently holds a considerable number of studies on the morphology of lungs in animals [3]. According to the results of morphological studies of the lungs [4], their structure is described in detail in laboratory animals, rabbits, dogs, ruminants, and pigs. Thus, according to scientists [5; 6], cattle, sheep, and pigs have a tracheal bronchus in the branching of the bronchial tree, which is absent in horses. Furthermore, in most domestic mammals, each lung in its structure has cranial, middle, and caudal lobes, and on the right lung there is an added lobe, which causes their asymmetry.

Therewith, the features of lung morphology in domestic animals, including horses, with the use of complex research methods – anatomical, histological, organo- and histometric, statistical – are not described at all, despite the importance of this knowledge for the veterinary clinic (morphology, clinical diagnostics, pathological anatomy, surgery, etc.) and experimental physiology. In addition, the macroscopic structure, organometric characteristics of the lung lobes, their absolute and relative masses, branching of the bronchial tree, histometric parameters of the lungs in horses in the scientific literature are fragmentary.

Therefore, the priority area for the prevention of diseases, differential diagnosis [7], detection, and clarification of the mechanisms of occurrence of the development of the disease and its individual manifestations at different levels of the body (cellular, tissue, organ, systemic, organisational) should be to conduct an in-depth study of the lungs in clinically healthy animals, primarily at the macro- and microscopic levels [8; 9]. Furthermore, the establishment of data on macro- and organometric parameters of lung lobes in horses, features of branching of the bronchial tree, histo- and cytometric characteristics at the tissue level will contribute to the development of comparative morphology of the respiratory apparatus in domestic animals. This allows, based on the conducted studies, obtaining new data and establishing quantitative organo- and histometric characteristics of the lungs of horses in normal conditions, as tests for differential diagnosis of diseases associated with the respiratory system.

That is why the study of the lungs of domestic animals is of urgent importance in morphology [10; 11], which ensure gas exchange in the body and whose regulatory activity takes place involving the nervous system, which coordinates and regulates their work, uniting the body into a single entity [12].

The purpose of this study is to investigate the features of lung morphology, assess their morphological structures at the organo- and histometric level in domestic sexually mature horses.

Materials and Methods

The research was carried out at the Department of Anatomy and Histology, the Laboratory of Pathomorphology of the Faculty of Veterinary Medicine of Polissia National University (city of Zhytomyr) during 2019-2022. The entire experimental part of the study was conducted according to the requirements of the international principles of the "European Convention for the prote ction of vertebrates used in experiments and other scientific purposes" [13], Law of Ukraine "On the protection of animals from ill-treatment" (No. 3447-IV of 02/21/2006, Kyiv) [14].

Macro- and microscopic, morphometric and statistical research methods were used in this study. The object of the study was lungs taken from five clinically healthy sexually mature horses (domestic horse – *Equus ferus caballus* L., 1758).

Lungs from freshly slaughtered research animals at a meat processing plant were subjected to anatomical dissection. For histological studies, pieces of material were fixed in a 12% cooled solution of neutral formalin for 48 hours, followed by pouring it into paraffin according to the schemes proposed in the manual [15]. Paraffin sections were made on a sled Microtome MS-2, their thickness did not exceed 10-12 microns. To investigate cell and tissue morphology, histological sections were stained with haematoxylin (Diapath, Italy, 2020) and eosin (Leica Geosystems, Germany, 2020) after their deparaffinisation. Stained histological sections were used to obtain survey preparations and conduct histometric studies.

Histometric studies of the structural elements of the lung tissue: determination of the respiratory part and the connective tissue base of the lungs (per unit area equal to 5.0 mm^2), the average volume of the alveoli, was carried out using light microscopy with microscopes "Micros" (Austria, 2012) and MBS-10 (Micromed, Russia, 1998) with a constant tube length, according to the recommendations outlined in the manual [15]. Histological sections were photographed with a CAM V–200 video camera (Inter Med, China, 2017) mounted in a microscope with an image output system with histological sections. Anatomical and histological terms of structural parts of the lungs were presented according to the International Veterinary Anatomical Nomenclature [16]. Mathematical processing of research results was performed statistically using the Statistica 7.0 software package (StatSoft, Tulsa, USA). The reliability of the results obtained was determined according to the Student's t-test, considering the significance criteria: $P<0.01$, $P<0.001$.

Results and Discussion

The lungs of horses are contained in the chest cavity and according to morphotopography relative to the body of animals are divided into left and right lungs. They have a pale pink colour. On the lungs of horses, the dorsal and ventral edges are clearly differentiated. The dorsal edge of the lungs is blunt and adjacent to the spine. The ventral edge of the lungs is sharp and directed ventrally. On the lungs, their surfaces are contoured – costal and diaphragmatic. The costal (lateral) surface of the lungs in horses is adjacent to the ribs, the diaphragmatic surface is adjacent to the diaphragm, it is directed caudally.

Between the cranial and caudal lobes of the right and left lungs are interparticle surfaces, and between the right and left lungs are mediastinal surfaces that are adjacent to the mediastinum, and they are located on each lung on the medial side. On the same surface, there is indentation from

the aorta, oesophagus, and vena cava. Each lung in most mammalian animals is divided into three lobes: cranial, middle, and caudal. Therewith, the right lung also has an added lobe [17]. Consequently, the interstitial heart notch divides the right and left lungs in horses into only two lobes – cranial (much smaller) and caudal (large), which are separated from each other (Fig. 1). The average proportion of lungs that other domestic animals have is absent in horses. Furthermore, in the animals under study, in the caudal part of the lungs (craniodorsally), a small part of the lung tissue was separated – a dorsal added part, which had a pyramidal shape and was characteristic of one or both (right and left) lungs. On the right lung of the horse, there is also an added lobe on the medial side (Fig. 1).

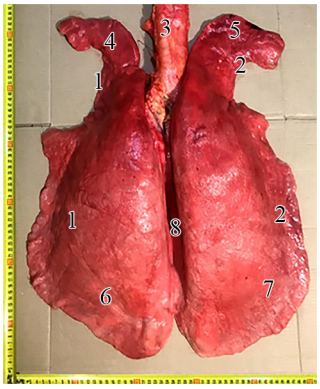

Figure 1. Anatomical structure of the lungs of a sexually mature horse (costal surface): 1 – left lung; 2 – right lung; 3 – trachea; 4 – left cranial lobe; 5 – right cranial lobe; 6 – left caudal lobe; 7 – right caudal lobe; 8 – added lobe of the right lung. Macropreparation

The particulate pattern of the surface of the lungs in horses, formed by the stroma of the lungs, is noticeable, but compared to other animals, their pattern is smoothed. Therefore, the surface of the lungs is smooth. On each lung, on their medial surface, there is a lung gate through which the main bronchus enters.

The lungs of horses, as in other domestic mammals, are formed by branches of the bronchi of various calibres, forming the bronchial tree and branches of histological structures of the respiratory department, forming the alveolar tree. They are accompanied by blood vessels, nerves, and layers of loose connective tissue. The bronchi, in turn, differentiate into extrapulmonary and pulmonary. Extrapulmonary bronchi are main and interlobular, and pulmonary bronchi are structures that form part of the parenchyma of the lungs and, branching there, form the bronchial tree.

A distinctive feature of the bronchial tree in horses is the absence of a tracheal bronchus, which is present in ruminants – cattle, sheep, pigs, and other animals [5]. Branching of the bronchi of the bronchial tree of the lungs in horses occurs according to the main type. In each lung, the main bronchi, at the base of their blunt edges, are divided into large, medium, and small – terminal bronchioles, forming a bronchial tree.

At the initial stage of the formation of the bronchial tree, the trachea of horses forms a considerably large bifurcation, where it branches into two main bronchi, which immediately (at the bifurcation of the trachea) in each lung, form their bifurcation and divide into two large bronchi – cranial and caudal. The cranial bronchus (slightly smaller) is located closer to the cranial lobe and is directed in the cranial direction at an angle of 30-35° relative to the tracheal axis, in the cranial lobe and is described by retrograde relative to the direction of the main movement. The caudal bronchus (slightly larger) is directed caudally, towards the caudal lobe (Fig. 2).

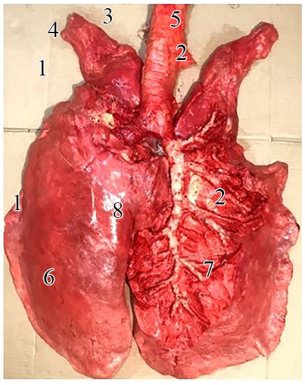

Figure 2. Anatomical structure of the lungs of a sexually mature horse (mediastinal surface): 1 – left lung; 2 – right lung; 3 – trachea; 4 – left cranial lobe; 5 – right cranial lobe; 5 – left caudal lobe; 6 – added lobe; 7 – tracheal bifurcation; 8 – main bronchus; 9 – cranial bronchus; 10 – caudal bronchus; 11 – branches of the caudal bronchus. Macropreparation

The main bronchus, which goes to the cranial lobe, after a certain interval divide into two branches, which, branching out, give rise to segmental bronchi of varied sizes. The main bronchi, which go to the caudal lobes of the lungs, branch into four dorsal and four ventral branches in the parenchyma of each lung. The smallest intralobular bronchi branch into the lung lobules, where they divide into terminal bronchioles, which divide into respiratory bronchioles, alveolar ducts, and then alveolar sacs, forming the alveolar tree.

The right lung in mammals is slightly larger than the left because the heart is shifted to the left. Therefore, an inherent feature of the structure of mammalian lungs is their pronounced asymmetry, which is manifested by varied sizes, ambiguous absolute mass of the right and left lungs, their position and ambiguous shape of their lobes, depending on the functional load [18]. The ratio of their size (left to right lung) is 1.21:1 in horses, 1.38:1 in cattle, 1.35:1 in pigs, and 1.32:1 in dogs [19].

Some scientists [20] consider the manifestation of lung asymmetry in domestic mammals to be a genetic trait. Others argue [21] that the asymmetry is associated with an asymmetric position of the heart and other organs in the chest cavity, depending on the intensity of their gas exchange function, which manifested itself during the evolutionary development of animals. The most pronounced asymmetry of all mammals is inherent in small rodents (rat, guinea pig, hamster), in which the left lung is not divided into lobes, and the right lung has four lobes [22].

According to the results of morphological studies, the coefficient of asymmetry of the left lung to the right in horses is 1:1.2 and this is due to the displacement of the heart and aorta to the left half of the chest cavity. Such data coincide with the results of other scientists, who indicate that the volume of the left lung in mammals, compared with the right lung, decreases due to the heart by two-thirds on the left side [23].

At the same time, the study of the lungs of various animal species showed the presence of individual morphological features in the lobular structure of the lungs [21]. Thus, in bats, the left lung is not divided into lobes, and in mink and sable, the left lung is divided into only two lobes – cranial and caudal [6].

According to the results of research, the distribution of lungs in domestic mammals into separate pronounced lobes directly depends on the very structure of the chest cavity and the characteristics of animal maintenance and individual physiological characteristics of animals, and, accordingly, the physiological load on the corresponding organ. Thus, in the left lung of horses there are only two lobes – cranial and caudal, in the right lung there are three lobes – cranial, caudal, and added. According to N.V. Zelenevsky, the caudal lobe of the lungs in horses is formed by the fusion of the caudal and middle lobes into one, and therefore is called the cardiorespiratory lobe [24]. According to the data obtained, such a lobe is caudal (phrenic), since there are no interlobe notches between the middle and caudal lobes in the right and left lungs, and their surface is adjacent to the diaphragm, and therefore the authors of this paper propose to call the cardiopulmonary lobe – phrenic (caudal).

An essential criterion for the development of an organ is its absolute mass, which directly indicates its morphofunctional maturity. At the same time, the relative lung mass in the animals under study depends directly on the animal's body weight and the absolute mass of the organ.

According to the organometry conducted by the authors of this paper, the absolute lung mass of sexually mature horses is 3,318.10 ± 364.40 g (Table 1). However, the relative lung mass in horses, which according to classical textbooks on pet anatomy [25] is 1.43% of the animal's body weight, does not coincide with the results of this study. Thus, according to studies, the relative lung mass in horses is much smaller and is equal to 0.60 ± 0.052%. Accordingly, the absolute mass of the left lung is 1,506.20 ± ± 60.48 g, and the right lung is 1,811.90±72.92 g (Table 1).

Table 1. Absolute and relative mass of horse lung lobules, M ± m, n = 5

Lobe Lungs	Left lung		Right lung		Left + right lungs	
	AM (g)	VM (%)	AM (g)	VM (%)	AM (g)	VM (%)
Cranial	197.43 ± 19.24	5.95 ± 0.51	214.02 ± 24.04	6.45 ± 0.62	411.45 ± 39.62	12.40 ± 0.94
Caudal diaphragmatic	1308.66 ± 8.75	39.44 ± 3.57▲	1423.80 ± 102.71	42.91 ± 4.06▲	2732.46 ± 209.97	82.35 ± 7.56
Added	–	–	174.20 ± 16.02•	5.25 ± 0.68	174.20 ± 16.02	5.25 ± 0.67
Total:	1506.10 ± 60.48*	45.39 ± 4.08	1812.0 ± 62.92*	54.61 ± 5.02	3318.10 ± 364.40	100

Note: ** – P<0.01, in comparison with the absolute mass (AM) of the left and right lungs; ▲ – P<0.001, compared to the cranial lobe; • – P<0.001, in comparison with the absolute mass of the caudal diaphragmatic lobe of the right lung; RM is the relative mass*

At the same time, the absolute mass of the cranial lobe of the left lung in horses is 197.43 ± 19.24 g. This indicator in the right lung is 214.02 ± 24.04 g, respectively. The caudal lobes of the lungs have the greatest absolute mass: in the left lung, this indicator averages 1,308.66 ± 98.75 g, and in the right lung – 1,423.80 ± 102.71 g, respectively. The smallest is the absolute mass of the added lobe of the right lung, which, accordingly, is 174.20 ± 16.02 g in horses (Table 1).

In terms of absolute mass, the left lung is 2.20 times smaller (P<0.01), while the right lung is 1.83 times smaller (P<0.01) compared to the absolute total mass of the horse's left and right lungs. At the same time, the relative mass of the caudal phrenic lobe of the left and right lungs is 6.63 times higher (P<0.001) and 6.65 times higher (P<0.001), respectively, compared to the cranial lobe. Therewith, there is a tendency to increase the relative mass of the caudal phrenic lobe of the right lung to the left.

The absolute mass of the added lobe of the right lung is 8.17 times less (P<0.001) compared to the absolute mass of the caudal diaphragmatic lobe (Table 1). Determination of morphometric parameters of organs and tissues, including lungs in domestic animals, is not only of cognitive importance, but also forms the basis for determining the shape, establishing comparative anatomical types of certain organs [9]. According to the results of organometry, the total length of the lungs in horses is 61.50±6.32 cm, width – 48.44±4.14, thickness – 9.60±1.10 cm. Therewith, the ratio of the total length of the lungs to their width in horses is 1.27:1. Therefore, the lungs of horses belong to the narrowed-elongated type.

Microscopically, the horse's lungs are formed by branches of the bronchi, the stroma of the lungs, and branches of the respiratory department of the lungs (Fig. 3), which form the alveolar tree. The basis of the lungs of horses are pyramidal or cone-shaped lobes that form the stroma of the lungs. Part of the structure of the lobes are acini covered with a thin layer of connective tissue.

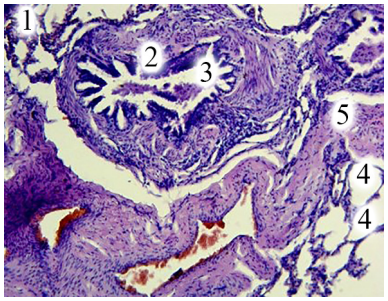

Figure 3. A fragment of the microscopic structure of a horse's lungs: 1 – respiratory part; 2 – small bronchus; 3 – lumen of the bronchus; 4 – alveoli; 5 – connective tissue stroma. Haematoxylin and eosin. x 280

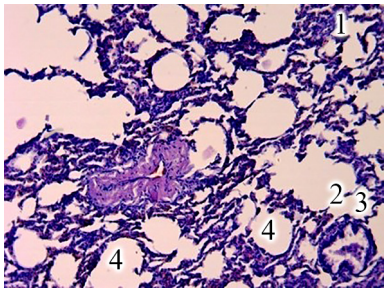

Figure 4. Fragment of the microscopic structure of the horse's lungs: 1 – respiratory part; 2 – alveolar passage; 3 – alveolar sac; 4 – alveoli. Haematoxylin and eosin. x 280

The bronchi of the lungs have varied sizes, which, according to their size, are divided into extrapulmonary (head and interlobe) and pulmonary (part of the lung parenchyma), where they branch out and form the bronchial tree. The bronchi in their composition have three membranes – mucous, fibrous-cartilaginous, and adventitia. The main bronchi of the lungs have the largest diameter. Compared to the middle and small bronchi, their membranes are clearly defined and have a microscopic structure similar to that of the trachea. Thus, their mucous membrane is formed by the epithelial, own, muscle plate and submucosal base (Fig. 5).

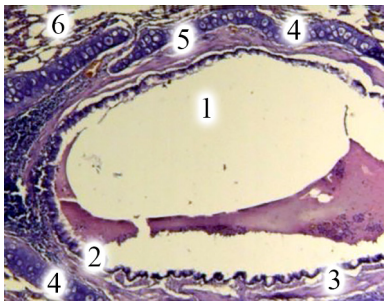

Figure 5. A fragment of the microscopic structure of the main bronchus of the lung of a horse: 1 – lumen of the bronchus; 2 – epithelial plate; 3 – muscle plate; 4 – fibrous-cartilaginous shell; 5 – lymphoid tissue; 6 – alveoli. Haematoxylin and eosin. x 280

The microscopic structure of the epithelial plate is represented by a single-layer multi-row ciliated epithelium, the epitheliocytes of which are located on its basal membrane. In the mucous membrane of its plate, formed mainly by loose fibrous connective tissue, lymphoid tissue appears in the form of clusters (Fig. 5). The muscle plate of the mucous membrane is formed by bundles of smooth muscle cells that form the circular and longitudinal layers. Consequently, the muscle plate of the shell of such bronchi does not form internal folds as in large, medium, and small bronchi (Fig. 5).

The submucosal base of such bronchi is formed by loose connective tissue, where the terminal parts of the bronchial glands are located. However, in comparison with other species of the animals under study, bronchial glands are found in them in small numbers [5]. Collagen fibres are also present in the submucosa. The microscopic structure of the fibrous-cartilaginous membrane of the main bronchi has certain features – their cartilage tissue is continuous, in the form of rings along the entire perimeter of the fibrous-cartilaginous membrane (Fig. 5).

The adventitious membrane of the main bronchi is formed by a thin layer of loose fibrous connective tissue. The wall of the large bronchi has a similar structure to that of the main bronchi. However, the cartilage rings of the fibrous-cartilaginous membrane do not have a continuous structure, but are formed by separate, pronounced, large cartilage plates (Fig. 6).

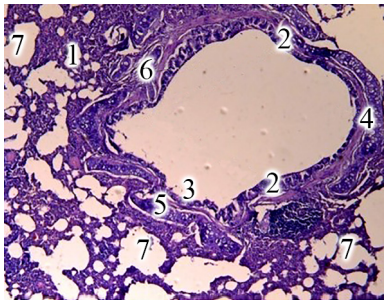

Figure 6. Fragment of the microscopic structure of the horse's lungs: 1 – respiratory part; 2 – large bronchus; 3 – epithelial plate; 4 – muscle plate; 5 – cartilage plates; 6 – lymphoid tissue; 7 – alveoli. Haematoxylin and eosin. x 280

The mucous membrane of the wall of the middle bronchi is covered with a single-layer multi-row respiratory epithelium, and the muscle plate of the mucous membrane forms well-defined folds. The fibrous-cartilaginous shell of the middle bronchi contains only individual cartilage islands of small size, which are formed by hyaline cartilage tissue (Fig. 7).

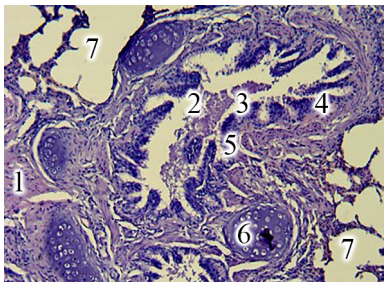

Figure 7. Fragment of the microscopic structure of the horse's lungs: 1 – respiratory part; 2 – middle bronchus; 3 – epithelial plate; 4 – muscle plate; 5 – cartilaginous plates; 6 – cartilage islands; 7 – alveoli. Haematoxylin and eosin. x 280

The lung wall of the small bronchi, as in other experimental animals, is formed only by the mucous membrane and adventitia. The muscle plate of such bronchi is clearly defined, so that the inner wall of the mucous membrane forms pronounced folds. Furthermore, cartilaginous islands in the walls of the small bronchi are not detected (Fig. 8).

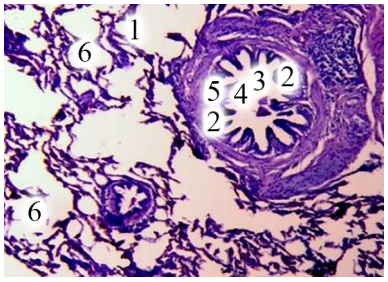

Figure 8. Fragment of the microscopic structure of the horse's lungs: 1 – respiratory part; 2 – small bronchus; 3 – bronchial lumen; 4 – epithelial plate; 5 – muscle plate; 6 – alveoli. Haematoxylin and eosin. x 280

The terminal bronchioles of the lung parenchyma are formed by a thin wall, similar to small bronchi, and its muscular plate is formed by smooth myocytes, which are in the form of a grid and do not form folds (Fig. 9). Bronchial arteries of varied sizes are found around the bronchi.

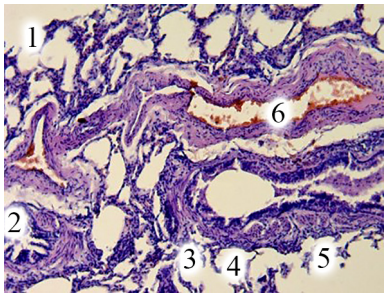

Figure 9. Fragment of the microscopic structure of the horse's lungs: 1 – respiratory part; 2 – small bronchus; 3 – terminal bronchiole; 4 – alveolar sacs; 5 – alveoli; 6 – vessel. Haematoxylin and eosin. x 120

The microscopic structure of the respiratory part of the lungs of horses is represented by the alveolar tree (respiratory bronchioles, alveolar ducts, alveolar sacs), in the walls of which there are alveoli (Fig. 10). Such histostructures form the structural and functional unit of the lungs – the pulmonary acinus. The microscopic structure of the respiratory bronchiole wall is similar to that of terminal bronchioles. At the same time, ciliated cells are absent in the epithelial cells of the lamina.

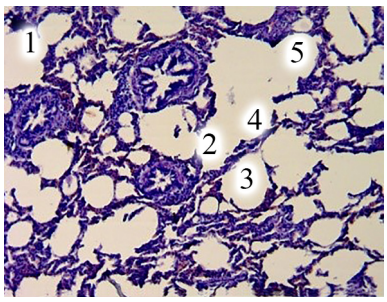

Figure 10. Fragment of the microscopic structure of the horse's lungs: 1 – respiratory part; 2 – terminal bronchiole; 3 – alveolar passages; 4 – alveolar sacs; 5 – alveoli. Haematoxylin and eosin. x 120

Alveolar passages formed because of branching of the second- and third-order bronchioles have two or three times the diameter of the respiratory bronchioles. Alveolar passages have many alveoli in their microscopic structure. Alveolar sacs (blind endings of alveolar passages) are formed by alveoli located next to each other.

Alveoli in the form of bubbles are connected to each other by interalveolar membranes, which are formed by delicate layers of loose connective tissue, which contains numerous elastic fibres. The inner wall of the alveoli is made up of alveocytes located on the basal membrane. The alveoli of the lungs have varied sizes – small, medium, and large (Fig. 11).

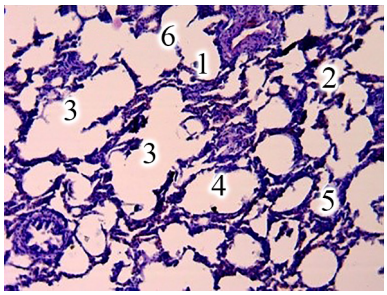

Figure 11. Fragment of the microscopic structure of the horse's lungs: 1 – respiratory part; 2 – alveolar sacs; 3 – large alveolus; 4 – middle alveolus; 5 – small alveolus; 6 – interalveolar membranes. Haematoxylin and eosin. x 280

According to the results of this study, the alveolar tree in horses is shortened and wide and has a bubble shape. Alveolar bronchioles are poorly differentiated. Due to expansion, the alveolar sacs are wide with smoothed alveoli. According to the results of morphometric studies, the average volume of pulmonary alveoli in clinically healthy horses is 699.80 ± 106.42 thous. μm^3. The respiratory part of the lungs in horses occupies 54.8 ± 7.4% of the total area of the lung parenchyma, the connective tissue base – 45.2 ± 7.4% (Fig. 12).

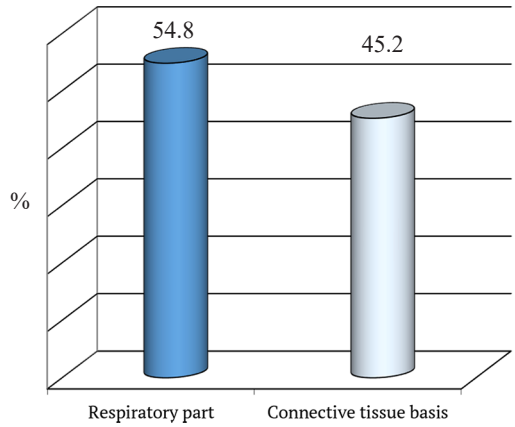

Figure 12. Histometric parameters of equine lungs

Conclusions

The lungs of horses have a partial structure: the left lung – cranial and caudal, the right – cranial, caudal, and added. The coefficient of lung asymmetry (left to right) is 1:1.2. The ratio of the total length of the lungs to their width is 1.27:1, so the lungs of horses are of a narrowed-elongated type.

The absolute lung mass of sexually mature horses is 3,318.10±364.40 g, the relative weight is 0.60±0.052%. Therewith, the absolute mass of the left lung is 1,506.20±60.48 g, the right lung – 1,811.90±72.92 g. The relative mass of the diaphragmatic lobes of the left and right lungs is 6.63 times ($P<0.001$) and 6.65 times ($P<0.001$) higher compared to similar cranial lobes.

The internal histoarchitectonics of the lung tissue is formed by cone-shaped or pyramidal lung lobes, which are separated by connective tissue partitions that form their connective tissue stroma. The connective tissue stroma (45.2±7.4%) is formed by loose connective tissue and contains elastic fibres, blood, and lymphatic vessels.

The respiratory parenchyma of the lungs (54.8±7.4%) is formed by respiratory bronchioles, alveolar passages, and alveolar sacs, in the walls of which alveoli are located, the average volume of which is 699.80±106.42 thous. μm^3.

In the future, the research will be aimed at ultramicroscopic examination of the respiratory part of the lungs of domestic animals.

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Морфологічні особливості та морфометричні показники легень статевозрілих коней (*Equus ferus caballus* L., 1758)

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Анотація. Легені, які входять до складу апарату дихання, забезпечують газообмін між навколишнім середовищем і тканинами організму людини й тварин. Тому, хвороби легень – одна з актуальних проблем для сучасної гуманної та ветеринарної медицини. Ця проблематика зумовлена прогресивним зростанням у ссавців таких захворювань, як бронхіальна астма, бронхіти, бронхопневмонії, пневмонії, коронавірусні інфекції тощо. Тому, для ефективного вирішення зазначеної проблеми щодо профілактики, ефективного лікування та своєчасної диференційної діагностики захворювань органів дихання, важливим напрямком у морфології є дослідження апарату дихання, зокрема легень, у клінічно здорових тварин, задля розробки маркерних тест-критеріїв, які будуть слугувати показниками норми у диференційній діагностиці цих захворювань. Метою роботи було дослідження макро- та мікроскопічної будови легень, проведення морфометричної оцінки їх морфологічних структур у свійських статевозрілих коней (клас Ссавці, вид – кінь свійський (*Equus ferus caballus* L., 1758). Об'єктом дослідження були легені клінічно здорових статевозрілих коней (n = 5). Анатомічному препаруванню піддавали свіжі легені досліджуваних тварин. Для проведення гістологічних досліджень застосовували загальноприйняті методи фіксації шматочків матеріалу та виготовлення гістологічних зрізів, які у подальшому фарбували гематоксиліном та еозином і за методом Ван-Гізон. Основою легень у коней є пірамідальної або ж конусоподібної форми часточки. Частиною будови часток є ацинуси, що покриті тонким шаром сполучної тканини. Мікроскопічна будова ацинусів сформована альвеолярними ходами, альвеолярними мішечками та альвеолами. За результатами досліджень альвеолярне дерево у коней, укорочене та широке і має пухирчасту форму. Результатами морфометричних досліджень встановлено, що середній об'єм легеневих альвеол у клінічно здорових коней становить 699,80 ± 106,42 тис. мкм^3. Дихальна (респіраторна) частина легень у коней займає 54,8 ± 7,4 % від загальної площі паренхіми легень, сполучнотканинна основа – 45,2 ± 7,4 %. Такі дослідження морфологічних особливостей та морфометричних показників легень коней мають практичне значення у ветеринарній медицині, оскільки є маркерними ознаками та критеріями патоморфологічної діагностики захворювань пов'язаних з органами дихання

Ключові слова: морфологія, асиметрія легень, бронхи, термінальні бронхіоли, альвеолярне дерево

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Cardiogenic and Non-Cardiogenic Pulmonary Oedema in a Domestic Cat: Pathological Mechanisms, Differential Diagnosis, and Treatment

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Abstract. The relevance of this study is conditioned upon the prevalence of pulmonary oedema in cats and special approaches to their therapy. In this regard, this paper is aimed at identifying aetiological factors, breed predisposition, clinical manifestations, haematological parameters, and radiological signs. Leading in the study of this issue is an integrated approach, which includes consideration of the aetiology, pathogenesis, diagnosis of cardiogenic and non-cardiogenic pulmonary oedema and treatment of cats. It was found that in the conditions of the "Vet House" Veterinary Centre (Vinnytsia), cats of the British Shorthair, Sphinx, Maine Coon breeds and their hybrids were most often registered with this pathology. 68 cats were diagnosed with cardiogenic pulmonary oedema due to hypertrophic, restrictive, and unclassified cardiomyopathy. 42 animals were found to have non-cardiogenic pulmonary oedema due to traumatic brain injury, toxic substance poisoning, anaphylactic reaction, airway obstruction, and electric shock. It was found that the most informative during the differential diagnosis of pulmonary oedema in cats are the results of echocardiography and X-ray examination. It was established that for the differential diagnosis of cardiogenic and non-cardiogenic pulmonary oedema, clinical indicators and results of haematological examination of animals are low in information. It was discovered that therapeutic measures for pulmonary oedema are effective in considering aetiological factors, although at the first stage, for both forms of pulmonary oedema, they are aimed at reducing and eliminating the manifestations of acute respiratory failure. The materials of this paper are of practical value for veterinary doctors of general practice and intensive care on differential diagnosis, prediction of the development of the disease and effective treatment of cats

Keywords: biochemical and morphological parameters of blood, shortness of breath, interstitial pattern, pathogenesis, radiography

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Introduction

Pulmonary oedema in a domestic cat develops quickly and threatens the animal's life. For cardiomyopathy complicated by pulmonary oedema, mortality among cats is 23%, regardless of the age of the animal at the time of diagnosis [1]. Different forms of the disease can have various clinical manifestations and course. In the first stages of the development of the disease, cats may not show any symptoms at all and appear absolutely healthy. Therefore, it is exceedingly difficult to detect and prevent this disease [2]. However, during a clinical examination, a veterinary doctor may detect some early symptoms of cardiovascular diseases. A range of factors can lead to acute pulmonary oedema, but the most common among them are cardiovascular diseases, electric shock, poisoning, asthma, injuries, sepsis, and shock. The physiological movement of fluid through the vascular membrane into the surrounding tissues depends on three factors – membrane permeability, oncotic pressure gradient, and hydrostatic pressure [3-5]. As an added factor, the state of the lymphatic drainage system counteracts extravascular fluid. Oedema develops if one of these factors is disturbed so much that it cannot be compensated. There are two main types of pulmonary oedema: cardiogenic and non-cardiogenic [3; 4]. Accurate differential diagnosis is based on data from clinical and radiological examinations.

Pulmonary oedema is the accumulation of extravascular fluid inside the pulmonary parenchyma or alveoli. The two main forms of the disease are high-pressure oedema (due to increased hydrostatic pressure in the capillaries of the lungs) and high-permeability oedema (due to damage to the microvascular barrier and alveolar epithelium in more severe cases). According to various scientific sources, for the development of pulmonary oedema, it is always necessary to increase intravascular hydrostatic pressure or disrupt vascular permeability [2-4].

Pulmonary oedema leads to a decrease in oxygenation, usually due to a mismatch between ventilation and perfusion; and therefore, 98% of animals have symptoms of respiratory failure. Some of these patients are extremely vulnerable to stress. Therefore, before even conducting a clinical examination, it is worth considering evaluating the risk-benefit ratio. Oxygen therapy should be given to all patients with respiratory failure. Initial diagnostic assessment should be aimed at identifying the severity of the respiratory disease and the underlying cause [5].

As with many critical conditions, the severity of the course of the disease is often inversely proportional to the duration of clinical symptoms. In animals with symptoms of pulmonary oedema, during auscultation of the lungs, "crackling" is not heard in all cases. However, 80% of patients will hear either loud lung sounds or "crackling" sounds. It is especially difficult to hear the sounds of "crackling" and "crunching" in the lungs in patients with rapid breathing and small respiratory volume. Careful auscultation can help localise abnormal lung sounds in one area, and it can help in diagnostics, for example, differentiate between traumatic brain injury and aspiration pneumonia [6].

Differentiation between cardiogenic and non-cardiogenic oedema is critical for patient survival, as the treatment of these conditions varies. It is even more important to determine the particular cause of non-cardiogenic oedema, not only from a therapeutic, but especially from a prognostic standpoint. Depending on the cause, the prognosis can range from unfavourable to favourable [7]. According to foreign scientists, pulmonary oedema causes life-threatening respiratory disorders in cats, which requires their artificial ventilation for 50-60% of animals [6; 8]. Therewith, cardiogenic pulmonary oedema is diagnosed in 68.5% of cats, non-cardiogenic – in 31.5% of animals and is a common pathology [5; 7; 8].

Finding the factors of occurrence of pulmonary oedema is the leading point affecting the success of treatment of an animal. In 45% of cats, the medical history of the disease is non-specific, and changes in clinical parameters are atypical. A critical situation for the animal is also severe shortness of breath, which can lead to threatening decompensation due to a stress reaction in response to conducting differential diagnostics. Heart failure can be suspected based on the animal's anamnesis data on the presence of previously diagnosed cardiomyopathy or cardiomyopathy of genetic origin. A history of coughing or wheezing in cats with heart failure is infrequent and, in 87% of cases, is a manifestation of respiratory tract pathology [9].

With cardiogenic pulmonary oedema, the life expectancy of an animal is affected by the degree of damage to their parenchyma, visualised by radiography, the presence of systolic dysfunction, and changes in the size of the left atrium during heart sonography. It was proved that spontaneous contrast and the size (thickness) of the left ventricular myocardium do not affect the patient's life expectancy at the beginning of hospitalisation [7; 10; 11].

Acute respiratory distress syndrome (ARDS) can provoke the development of non-cardiogenic pulmonary oedema [12; 13]. It is based on severe diffuse damage to the pulmonary parenchyma, which leads to a violation of the permeability of the endothelium and epithelium and the appearance of oedema with a high-protein content. This syndrome is often the result of primary lung damage, such as inhalation of toxic gases (smoke poisoning) and hyperbaric oxygen (oxygen poisoning), aspiration of stomach contents, and pneumonia. It can also occur as a complication of serious systemic diseases such as sepsis, burns, and acute pancreatitis. At the same time, the prognosis is unfavourable even in the case of overly complex maintenance therapy [5; 8]. The pathogenesis of ARDS is understudied.

Another important group of causes of non-cardiogenic pulmonary oedema is neurogenic oedema. Excessive sympathetic adrenergic activation leads to a redistribution of blood from the large to small circulatory system, an increase in hydrostatic pressure, and, finally, the development of oedema. Causes include head trauma, epileptic seizures, and electric shock [7; 14].

Thus, due to the low attention of Ukrainian scientists to the aetiology, mechanism of development, treatment, and prognosis of pulmonary oedema in a domestic cat, due to the lack of publications on the clinical practice of Ukrainian veterinary doctors, this issue requires research.

The purpose of this study was to determine the features of the aetiology, pathogenesis, diagnosis of cardiogenic and non-cardiogenic pulmonary oedema and treatment of cats in conditions of Ukrainian veterinary clinics.

To achieve this purpose, it was necessary to perform the following tasks: to identify the most common aetiological factors, breed predisposition, clinical symptoms of this pathology, radiological and haematological indicators, the mechanism of its development in a domestic cat, to investigate

and justify the protocol of differential diagnosis and treatment of cats, depending on the form of the disease.

Materials and Methods

The study was performed based on the Veterinary Centre "Vet House" (Vinnytsia) in 2018-2022, considering the requirements of the European Convention for the protection of vertebrates that are used for experimental and other purposes (Strasbourg, 1986), the European Convention for the protection of domestic animals (2013) and the Law of Ukraine "On the Protection of Animals from Ill-treatment" (2006) [15-17]. The registration forms of the electronic database of animal registration, the owners of which applied to the "Vet House" Veterinary Centre, were evaluated. For this study, cats with pulmonary oedema who were admitted to a veterinary centre were selected to stabilise their serious condition. The diagnosis was established based on data from a clinical examination of animals, the results of chest X-ray examination, echocardiography (EchoCG) and laboratory analysis of blood samples. During 2018-2022, 110 cats were examined with a diagnosis of "pulmonary oedema". The average body weight of the animals was 4.0±0.4 kg, and their average age was 7 years (from 2 to 12 years). Sick animals were divided into two experimental groups according to the form of pulmonary oedema. The first study group (cardiogenic oedema) included 68 cats, the second study group (non-cardiogenic oedema) – 42 animals. In cats of both groups, differential diagnosis was evaluated based on the results of clinical examination, echocardiography, radiography, and laboratory analysis of blood parameters. The control group included ten clinically healthy cats who underwent a preventive examination at the "Vet House" Veterinary Centre.

Special studies of the clinical condition of animals were carried out using an ultrasound machine (GE Logiq E9, USA) and an X-ray machine (MicroCC-20Plus, USA). During the ultrasound examination, basic measurements of the size of the heart were made. X-rays of the chest cavity were performed in direct and right lateral projections.

Laboratory blood tests were carried out based on the diagnostic laboratory of the veterinary centre using a semi-automatic biochemical analyser BS-3000M (SINNOWA, China) and reagents from the companies SpinLab (Ukraine) and High Technology Inc. (USA). Blood collection from cats was performed from the lateral or medial vein of the pelvic extremities (*v. safena*).

In blood serum, the concentration of total protein, glucose, albumin, total bilirubin, direct bilirubin, total calcium and inorganic phosphorus was determined by generally accepted colorimetric methods; activity of enzymes – alkaline phosphatase (ALP, EC 3.1.3.1), aspartate aminotransferase (AST, EC 2.6.1.1), alanine aminotransferase (ALT, EC 2.6.1.2) and gamma-glutamyl transferase (GGT, EC 2.3.2.2) – by kinetic methods.

The general clinical analysis of whole blood included counting erythrocytes and leukocytes in a chamber with a Goryaev grid using an Axioskop 40 microscope (ZEISS, Germany), determining the haemoglobin content according to the haemoglobin cyanide method with acetone cyanhydrin, deriving a leukogram according to the Schilling calculation method. Blood smears were stained with a set of paints "Leucodif 200" (LDF 200) (Erba Lachema s.r.o., Czech Republic). A puncture was performed, and blood was taken into test tubes with heparin. Potassium concentration was determined using a semi-automatic analyser Genrui GE-1, reagent – Pak GE500 (Genrui, China). The sample was measured using ion-selective electrodes for 1 min. 100 µL of blood was used to analyse the sample. The results were displayed and printed automatically.

For non-invasive monitoring, random verification, display, storage, and transmission of blood oxygen saturation information (SpO_2) the UT1OOV pulse oximeter (China) was used. The device works with particular sensors that provide SpO_2 measurements and the pulse rate in animals. The sensor was placed on an unpigmented area, usually the mucous membranes of the tongue and lips, as well as the prepuce, vulva, ear, or membranes between the fingers of the extremities.

A Pettrust automatic tonometer (BioCare, Taiwan), a battery-type device that measures blood pressure (systolic, diastolic, and mean) and heart rate in cats based on the principle of plethysmography, was used for non-invasive automated monitoring of blood pressure. The cuff of the device was applied to the pelvic limb or to the base of the animal's tail. The average value was calculated from 3-4 measurements of these indicators.

The resulting digital data was processed using Statistica 6.0 (StatSoft Inc., USA). The M±m format was used to represent the data in the table. Differences in $P<0.05$, $P<0.01$, and $P<0.001$ were considered statistically probable.

Results and Discussion

Among the cats with symptoms of pulmonary oedema under study, breed characteristics were established. These were cats of different breeds and mixed breeds, namely British Shorthair – 44 animals, Sphinx – 24, Maine Coon – 22, mixed breeds – 20 animals. 61.8% of cats with symptoms of pulmonary oedema were diagnosed with cardiomyopathy, among them: 47.0% were diagnosed with hypertrophic cardiomyopathy (HCMP), 26.5% – with restrictive cardiomyopathy (RCMP), 26.5% – with non-classified cardiomyopathy (NCMP) (Table 1). 38.2% of the animals were diagnosed with non-cardiogenic pulmonary oedema. The factors that caused non-cardiogenic pulmonary oedema included traumatic brain injury – in 35.7%, electric shock – in 23.8%, toxic substance poisoning – in 19.1%, anaphylactic reaction – in 11.9%, airway obstruction – in 9.5% of animals.

Table 1. Causes of pulmonary oedema in a domestic cat

Cardiogenic pulmonary oedema, n = 68		**Non-cardiogenic pulmonary oedema, n = 42**	
Hypertrophic cardiomyopathy	32	Traumatic brain injury	15
Restrictive cardiomyopathy	18	Electric shock	10
Non-classified cardiomyopathy	18	Poisoning with toxic substances	8
		Anaphylactic reaction	5
		Airway obstruction	4

In cats, cardiogenic pulmonary oedema was more likely to have an abrupt and severe course. The occurrence of symptoms in most cases was caused by corresponding stressful situations (visit to the clinic, fixation, ovariohysterectomy). The release of catecholamines into the blood causes vasoconstriction and increases blood volume during cardiac output, which leads to the sudden occurrence of clinical symptoms. As a result, the pressure in the cavity of the left ventricle, left atrium, and pulmonary veins increases (over 12 mm Hg and sometimes above 20, if the process is chronic). This causes an increase in hydrostatic pressure in the pulmonary veins and effusion of plasma into the interstitium of the lungs and pleural cavity [10; 18].

In 30 animals with symptoms of heart failure, tachypnoea (30-110 respiratory movements/min) and shortness of breath were observed, which develop due to pulmonary oedema and effusion of fluid into the pleural cavity. Paradoxical breathing was observed in 15 cats due to effusion into the pleural cavity. No changes (noise, gallop rhythm, or arrhythmia) were observed during cardiac auscultation in 13 animals. During the examination, 10 cats showed pallor of the visible mucous membranes (conjunctiva and gums), weak pulse, sometimes a decrease in body temperature (36.5-37.5°C), arrhythmia, noise, weak/filamentous pulse, and prolonged capillary filling time with blood.

Among all animals with symptoms of heart failure, changes in the lungs and pleural cavity were detected during X-ray examination. The most characteristic of them were visualisation of the interstitial pattern in the lung parenchyma of varying severity in 38 animals, free fluid in the chest cavity was visualised in 14 animals, and a more pronounced venous pattern of the lungs was noted in 16 animals.

The development of non-cardiogenic oedema occurs by various pathological mechanisms: a decrease in alveolar pressure (rapid removal of fluid or air from the pleural cavity, obstruction of the upper respiratory tract), an increase in vascular permeability, an increase in hydrostatic intravascular pressure, and a combination of these mechanisms [10; 11].

Pulmonary oedema due to obstruction of the cranial (upper) airway in cats is more often recorded than diagnosed. Many cases of post-obstructive oedema are misinterpreted as cardiogenic oedema, since shortness of breath and oedema occur due to physical exertion or a stressful situation, e.g., oedema due to laryngeal paralysis or oedema during or after anaesthesia, or because the animal has two pathologies at once [19].

Another important group of causes of non-cardiogenic pulmonary oedema is neurogenic oedema. Excessive sympathetic adrenergic activation leads to a redistribution of blood from the large to small circulatory system, an increase in hydrostatic pressure, and, finally, the development of oedema. Potential causes include head trauma, epileptic seizures, and electric shock [14; 20]. Acute respiratory distress syndrome (ARDS) is a substantial provoking factor in the development of non-cardiogenic pulmonary oedema. It is based on severe diffuse damage to the pulmonary parenchyma, which leads to a violation of the permeability of the endothelium and epithelium and the occurrence of oedema with a considerable protein content. Despite the insufficient study of the pathogenesis of ARDS, the main pathogenetic links of its development are as follows [21]:

1. The accumulation of numerous activated white blood cells and platelets, which excessively secrete biologically active substances (proteinases, lipid peroxidation products, leukotrienes, etc.) damages the epithelium of alveoli and blood vessels and changes the tone and reactivity of blood vessels. White blood cells enter the reactive process zone, and the lung parenchyma infiltrates.

2. Due to the altered vascular permeability, plasma, and red blood cells enter the alveoli and interstitium and develop pulmonary oedema and atelectasis.

3. With a considerable surfactant deficiency, hypoventilation of the alveoli occurs, the elasticity of their wall decreases, disc-shaped atelectasis, venous blood shunts into the arterial bed are formed, the process of ventilation-perfusion, oxygen and carbon dioxide diffusion is disrupted, hypoxia and hypercapnia develop.

4. In the absence of circulatory pathology, increased pressure in the pulmonary artery is a characteristic manifestation of pulmonary oedema.

Acute respiratory distress syndrome is often the result of primary lung damage, such as inhalation of toxic gases (smoke poisoning), aspiration of stomach contents, inhalation of hyperbaric oxygen (oxygen poisoning), and pneumonia. It can also occur as a complication of serious systemic diseases such as sepsis, burns, and acute pancreatitis. This syndrome is a known complication of the aforementioned diseases [14; 22], and the prognosis is unfavourable, even with very complex maintenance therapy.

A decrease in alveolar pressure develops after rapid removal of a pleural effusion, pneumothorax, or lung lobes, called re-dilation oedema. Mortality from this rare complication in humans is possible in 20%. In veterinary medicine, 2 cases were described among cats that died [5].

A decrease in alveolar pressure is also the result of obstruction of the cranial (upper) airways, called post-obstructive oedema, e.g., laryngeal paralysis, tracheal collapse, during intubation and bronchoscopy [19].

Oncotic pressure primarily depends on the concentration of albumin in the blood plasma and is one of the essential factors of fluid retention inside the vessels. The interstitial space of the lungs usually has a higher concentration of albumin than other interstitial tissue, and a small oncotic gradient, since the permeability of the pulmonary capillaries is higher than in other capillaries. When the plasma albumin content decreases, the concentration of albumin in the interstitium also decreases, and this substantially affects the oncotic gradient. Thus, it is unusual to detect pulmonary oedema when hypoalbuminemia is the only detected anomaly [18; 21].

Animals diagnosed with pulmonary oedema showed changes during auscultation of the lungs and heart (noise, gallop rhythm, arrhythmia). Cats in both groups were most likely to have tachypnoea or orthopnoea, respiratory distress, and cough. Patients with primary heart disease were diagnosed with arrhythmia, murmur, weak/filamentous pulse, and slow capillary refill rate (CRR). These changes were observed very unevenly in animals of both groups, and therefore it is impossible to diagnose pulmonary oedema only by symptoms in cats. Special research methods (chest X-ray, echocardiography) were used to confirm the diagnosis. Consequently, respiratory distress was the primary clinical sign for all examined animals with pulmonary oedema.

Radiologically, pulmonary oedema was described by a decrease in the transparency of the lung area and image instability (Figs. 1, 2). Radiographs of all the cats under study showed signs of a reticulated or granular interstitial pattern (Table 2). According to the features of changes in the pulmonary pattern for pulmonary oedema, radiographs were divided into groups: with diffuse/uneven pattern in 35 animals (31.8%); diffuse/uniform in 23 (20.9%); pronounced alveolar pattern in 52 (47.3%). Among the radiographs that visualised a pronounced alveolar pattern, there were air bronchograms in 66 animals (60%) and with an increased diameter of pulmonary vessels and a bronchial pattern in 44 animals (40%). According to the localisation of the process in the lungs, changes were visualised in the following areas: regionally in 37 animals (33.6%), ventrally in 33 animals (30%), caudally in 30 animals (27.3%), and in 10 animals (9.1%) – ventrally with subsequent spread. X-ray symmetry of lung structure damage, which was assessed by studying dorsoventral or ventrodorsal projections, could be determined for 33 animals (30%), in the remaining 77 animals (70%), X-ray lesions of the lung structure were asymmetrical in these projections. Areas of reduced lung transparency were bilaterally symmetrical in 42 animals (38.2%). In the remaining 52 (47.3%) of the animals under study, the lesion of the lung structure on radiographs was asymmetric, and in 16 (14.5%) of them – mainly right-sided.

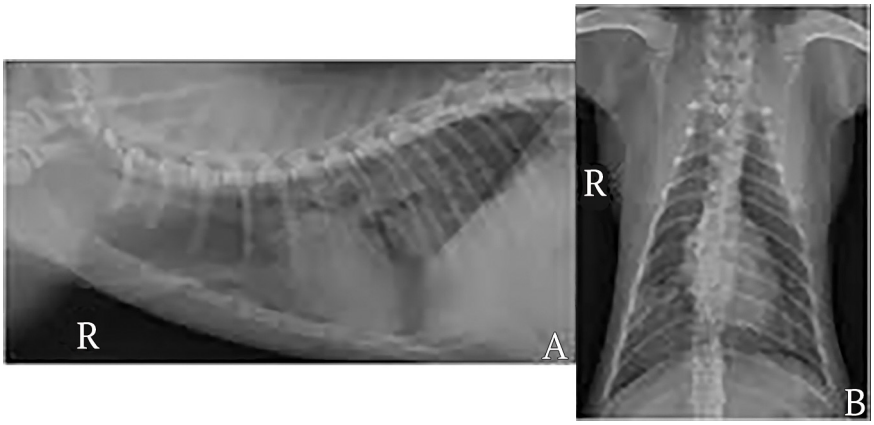

Figure 1. Mediolateral (A) and ventrodorsal (B) X-ray of the chest cavity of cats with signs of noncardiogenic pulmonary oedema; (R) right lung

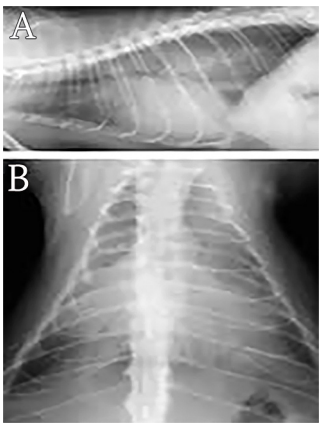

Figure 2. Mediolateral (A) and ventrodorsal (B) chest X-ray of cats with signs of cardiogenic oedema of lungs

Table 2. The most characteristic changes on radiographs of the chest cavity in cats with pulmonary oedema

Cardiogenic pulmonary oedema, n=68		**Non-cardiogenic pulmonary oedema, n=42**	
Description of pattern changes	**Quantity, (%)**	**Description of pattern changes**	**Quantity, (%)**
Pronounced mesh interstitial pattern with alveolar pattern and an increased diameter of pulmonary vessels	27 (39.7%)	Pronounced granular interstitial pattern with alveolar pattern and pronounced bronchial pattern	25 (59.5%)
Diffuse, inhomogeneous, asymmetrical interstitial pattern	22 (32.4%)	Diffuse, inhomogeneous, asymmetrical interstitial pattern	13 (31.0%)
Diffuse, uniform, symmetrical interstitial pattern	19 (27.9%)	Diffuse, uniform, symmetrical interstitial pattern	4 (9.5%)

Lateral radiographic projections mainly involved the caudodorsal quadrant of the pulmonary field. As a rule, animals with non-cardiogenic pulmonary oedema due to airway obstruction had the greatest degree of radiographic damage to the structure of the lungs. After them, the decrease was followed by animals with pulmonary oedema caused by craniocerebral trauma and electric shock.

To assess the severity of lung damage, the area of lesions was calculated on radiographs. Thus, local or diffuse lesions of the interstitial lung tissue that did not exceed 25% of the lung area on the radiograph were found in 30 animals; parenchymal lesions covering up to 50% of the lung area on the radiograph – in 45 animals; lung parenchymal lesions over 75% of the lung area on radiographs – in 35 animals.

Most of the values of the blood parameters of experimental cats for pulmonary oedema did not go beyond the physiological limits, and there was no statistically significant difference between them, depending on the form of oedema (Table 3).

Table 3. Haematological parameters in experimental cats, M±m, n=10

Indicator			Reference limits	Clinically healthy	Cardiogenic pulmonary oedema	Non-cardiogenic pulmonary oedema
Haemoglobin, g/L Lim $_{min-max}$			100-150	126.4 ± 2.8 115-142	139.7 ± 5.2* 122-159	139.2 ± 5.2* 112-167
Red blood cells, x10^{12}/L Lim $_{min-max}$			5.8-10.7	7.4 ± 0.4 5.9-9.3	8.2 ± 0.5 5.8-10.4	7.6 ± 0.5 5.9-10.0
White blood cells, x10^{9}/ L Lim $_{min-max}$			5-14	8.0 ± 0.8 5.4-14.0	15.8 ± 1.4*** 7.9-21	20.7 ± 2.5*** 12.4-34.4
Haematocrit value, % Lim $_{min-max}$			30-47	36.7 ± 0.9 32.6-41.8	41.9 ± 2.1* 32.6-51.0	39.3 ± 1.4 34.4-47.8
Platelets, x10^{9}/L Lim $_{min-max}$			200-600	373.3 ± 37.1 238-567	413.3 ± 55.8 212-634	428.4 ± 48.2 224-664
Thrombocrit, % Lim $_{min-max}$			0.1-0.5	0.32 ± 0.04 0.2-0.5	0.37 ± 0.05 0.2-0.6	0.37 ± 0.05 0.2-0.6
Leukogram, %	Basophils Lim $_{min-max}$		0-1	0.2 ± 0.1 0-1	0.7 ± 0.3 0-2	0.6 ± 0.3 0-2
	Eosinophils Lim $_{min-max}$		0-8	2.3 ± 0.7 0-6	5.9 ± 0.9** 1-10	5.1 ± 0.9* 1-10
	Neutrophils:	rod-shaped Lim $_{min-max}$	1-6	3.2 ± 0.4 1-6	5.2 ± 0.5** 2-8	5.5 ± 0.8* 1-9
		segmented Lim $_{min-max}$	40-68	52.3 ± 1.9 44.5-60.5	79.5 ± 3.2*** 69.4-93.5	75.2 ± 4.8*** 45.6-94.6
	Lymphocytes Lim $_{min-max}$		25-40	31.6 ± 1.8 25.0-37.7	43.2 ± 2.8** 39.5-55.0	39.0 ± 3.0* 26.4-58.5
	Monocytes Lim $_{min-max}$		0-5	1.7 ± 0.6 0-4	5.2 ± 0.7** 2-9	3.8 ± 0.5* 1-6
Total protein, g/L Lim $_{min-max}$			55-76	62.9 ± 2.4 56.1-75.4	63.7 ± 2.3 56.7-76.3	65.9 ± 2.2 56.3-75.6
Albumin, g/L Lim $_{min-max}$			25-40	32.4 ± 1.3 26.4-35.9	47.1 ± 3.4** 30.4-65.0	40.9 ± 3.4 26.9-65.4
Glucose, mmol/L Lim $_{min-max}$			1.6-6.5	5.2 ± 0.3 3.8-6.4	6.84 ± 0.50* 3.7-9.4	6.05 ± 0.72 3.0-9.3
Urea, mmol/L Lim $_{min-max}$			5.5-11.0	7.4 ± 0.6 5.5-10.6	15.9 ± 1.0*** 12.0-23.5	17.8 ± 2.7** 3.0-35.0
Creatinine, µmol/L Lim $_{min-max}$			50-140	83.4 ± 9.3 52.0-120.4	134.9 ± 15.2** 69.4-220.0	205.1 ± 35.1** 78.0-437.0
Total calcium, mmol/L Lim $_{min-max}$			2.0-2.7	2.36 ± 0.08 2.1-2.7	2.28 ± 0.08 2.0-2.5	2.31 ± 0.09 2.0-2.6

Table 3, Continued

Phosphorus inorg., mmol/L Lim $_{min-max}$	0.9-1.7	1.32 ± 0.09 0.9-1.7	1.86 ± 0.09** 1.1-2.2	1.96 ± 0.23* 0.9-3.2
Potassium, mmol/L Lim $_{min-max}$	3.5-5.3	4.49 ± 0.19 3.7-5.3	5.51 ± 0.21** 4.3-6.3	5.12 ± 0.41 3.2-6.5
Total bilirubin, μmol/L Lim $_{min-max}$	0.5-10.0	6.3 ± 0.8 2.7-3.5	6.0 ± 0.9 2.1-10.0	4.9 ± 0.7 2.4-9.5
Direct bilirubin, μmol/L Lim $_{min-max}$	0.5-8.0	5.4 ± 0.6 2.4-8.4	5.39 ± 0.45 3.0-7.4	4.96 ± 0.62 3.0-8.0
Alkaline phosphatase, U/L Lim $_{min-max}$	12.0-100.0	47.2 ± 7.3 11.5-68.5	96.4 ± 16.9* 12.0-165.4	93.3 ± 9.7** 54.6-156.0
Alanine aminotransferase, U/L Lim $_{min-max}$	5.0-75.0	42.7 ± 7.7 14.7-69	145.3 ± 9.4*** 98.6-189.0	109.2 ± 7.4*** 84.7-154.0
Aspartate aminotransferase, U/L Lim $_{min-max}$	5.0-50.0	40.9 ± 6.2 13.9-94.8	108.8 ± 11.6*** 65.3-176.9	89.5 ± 10.1$_{***}$ 45.6-134.0
Gamma-glutamyltransferase, U/L Lim $_{min-max}$	0-10.0	5.8 ± 0.9 1.0-8.5	8.7 ± 1.3 4.9-16.4	8.3 ± 0.7$_{*}$ 5.6-12.5

Note: * *P<0.05,* ** *P<0.01,* *** *P<0.001 relative to the indicators of clinically healthy cats*

At the same time, in the blood of animals with cardiogenic pulmonary oedema, a probable increase was found: the number of white blood cells by 2.0 times, neutrophils by 29%, lymphocytes by 11.6%, eosinophils by 3.6%, monocytes by 3.5%, haematocrit value by 5.2%; the content of haemoglobin by 9.5%, albumin by 1.3 times, glucose by 1.2 times, urea by 2.1 times, creatinine by 1.6 times, phosphorus inorganic by 1.3 times, potassium by 1.2 times; ALT activity by 3.4 times, AST by 2.7 times, and ALP by 2.0 times, compared to clinically healthy cats (Table 3). In the blood of cats with non-cardiogenic pulmonary oedema, a probable increase was found: the number of white blood cells by 2.6 times, neutrophils by 25.2%, lymphocytes by 7.4%, eosinophils by 2.8%, monocytes by 2.1%; haemoglobin content by 9.0%, urea by 2.4 times, creatinine by 2.5 times, inorganic phosphorus by 1.3 times; ALT activity by 2.6 times, AST by 2.2 times and GGT by 1.3 times compared with the indicators of clinically healthy cats (Table 3). However, the results of morphological and biochemical studies of pulmonary oedema in cats constitute an added diagnostic method and allow determining the severity of the disease.

In case of lung pathology complicated by the development of acute renal failure (ARF), an increased concentration of creatinine and urea was recorded in the blood serum of cats. Upon the occurrence of pulmonary oedema of non-cardiogenic origin, the manifestation of the clinical picture of the disease in patients was determined by changes caused by the underlying disease. With the simultaneous development of acute hepatic insufficiency syndrome in the blood serum of cats, the activity of aminotransferases and GGT increased.

Arterial blood sampling is invasive and requires high qualification for rapid execution. This does not always depend on the severity of the patient's shortness of breath. But if the patient's condition allows for this manipulation, it was an ideal method for determining the clinical condition of the animal. More information can be obtained by calculating the alveolar-arterial oxygen gradient (Aa gradient). Then the $AaDO_2$ gradient is a comparison of the concentration of oxygen in the alveoli with the concentration of arterial blood. Values over 20 mm Hg. Art. may indicate a ventilation/perfusion mismatch.

In case of failure to collect arterial blood from the patient to assess the concentration of oxygen-rich haemoglobin, pulse oximetry (SpO_2) was used. Pulse oximetry is fast, non-invasive, and easily tolerated by most patients. Normal SpO_2 values for a patient who breathes room air are 95-99%. Patients with SpO_2 under 93% are considered hypoxemic and require added oxygenation.

Pathogenetic treatment used in animals for pulmonary oedema differed in several features depending on aetiological factors. At the initial stage of development of the disease, treatment tactics were aimed at reducing the clinical manifestation of acute respiratory failure.

To stimulate respiratory function, patients were given an oxygen mask or placed in a special oxygen chamber. Liquid therapy was developed for each animal individually.

At the beginning of treatment, most sick animals were unable to eat food and drink water on their own. Isotonic crystalloids, namely Ringer's solution and Ringer's lactate, were administered to maintain proper hydration and replace body losses. However, a sharp increase in intravascular volume can provoke an increase in hydrostatic pressure in the lungs and lead to a deterioration in the patient's health. Therefore, colloidal therapy is recommended for patients who do not have haemodynamic stability. Hetastarch® and Vetstarch® are large molecular weight hydroxyethyl starches that help maintain colloidal oncotic pressure and maintain blood pressure. Administration of these solutions to patients was avoided if substantial damage to the pulmonary endothelium was suspected, since these large molecules seep into the alveolar spaces. This can lead to a sharp deterioration in the patient's clinical condition [8; 20].

There is some debate about whether furosemide can be effective in treating cats with non-cardiogenic pulmonary oedema. Furosemide is a loop diuretic that is used to promote the free elimination of water and salt by the kidneys and is extremely effective in treating cardiogenic pulmonary oedema. However, it is not useful for patients with non-cardiogenic pulmonary oedema due to alveolar epithelial disorders and exudative effusion. Some studies have shown that low-dose, constant-rate furosemide infusion can be beneficial for patients by reducing capillary pressure in the lungs and reducing the amount of fluid "filling" lung tissue in patients with changes in permeability [22].

β_2-Adrenoceptor agonists such as terbutaline and albuterol have also been used to treat patients with non-cardiogenic pulmonary oedema. Although the effectiveness of these drugs is unknown, it is believed that by triggering receptors lining the alveolar epithelium, it can increase lung fluid clearance.

β_2-Adrenoceptor agonists provide temporary relief to the patient by reducing bronchospasm caused by non-cardiogenic pulmonary oedema. β_2-adrenoceptor agonists were used with caution, as they also have a cardiogenic effect: they increase the heart rate and blood pressure. These effects increase the hydrostatic pressure of the lungs, which increases the patient's pulmonary oedema. More research is required to determine the urgent need for the use of these drugs because these drugs should be used as a last resort [9].

Nutrition maintenance was considered at the beginning of the patient's treatment, especially if the patient suffers from burns to the oral cavity caused by electric shock. These patients are more likely to be reluctant to consume food due to elevated levels of shortness of breath and/or discomfort. A nasoaesophageal or nasogastric feeding tube was placed quickly and without much stress for the animal. Patients were fed liquid diets to speed up their recovery. Oxygen therapy and rest were used (Fig. 3).

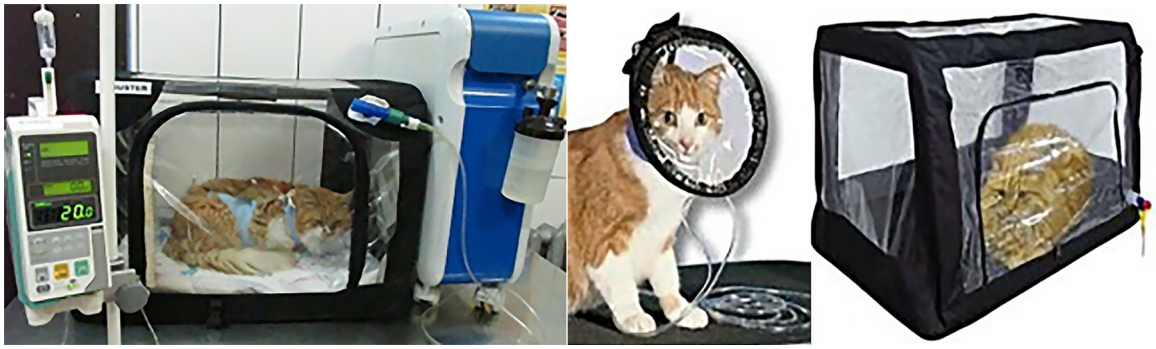

Figure 3. Oxygen therapy

Opioid painkillers (butorphanol 0.2-0.25 mg/kg) were used for sedation. In cardiogenic pulmonary oedema, the main therapeutic effect is to reduce the previous load due to aggressive diuresis with loop diuretics. Furosemide was used as a multiple bolus from 1 to 2 mg/kg or as an infusion at a constant rate of 0.66-1 mg/kg/h. Thoracocentesis was performed in the presence of pleural effusion. In cats with low cardiac output (bradycardia), treatment with pimobendane was considered. Patients, in the absence of an effect, were given dobutamine by infusion and at a constant rate (2.5-10.0 mcg/kg/min). Nitroglycerides and angiotensin-converting enzyme (ACE inhibitors) inhibitors were not indicated during the acute decompensation phase. Given the fact that diuresis does not affect the pathogenesis of non-cardiogenic oedema, fluid therapy is recommended for the supportive treatment of the underlying disease in various pathologies, rather than diuretics, as, e.g., in the case of sepsis or pancreatitis. However, in these cases, infusion therapy should be used with caution. Oxygenation is still the main means of maintaining the patient's respiratory function, and it is enough for the animal to be in a calm state in an environment saturated with oxygen. Furthermore, artificial ventilation may become necessary. The usefulness of corticosteroids is controversial [12].

Treatment of sick animals with corticosteroids and bronchodilators was not effective in patients with non-cardiogenic pulmonary oedema. Bronchodilators are prescribed for patients with diseases of the small respiratory tract, such as asthma, but do not work at the level of the alveoli. Corticosteroids may also be beneficial in patients with asthma but have not been shown to reduce or prevent lung endothelial damage [6; 11].

After the patient's condition stabilised, the focus was on maintenance treatment during the treatment of the underlying disease. To stop the progression of pulmonary oedema, aggressive therapy was used to eliminate the underlying disease. As a rule, maintenance therapy is required for 24-72 hours, until the symptoms of pulmonary oedema disappear.

The condition of cats with cardiogenic pulmonary oedema was stabilised depending on blood pressure indicators: for tonometry and detection of hypertension, angiotensin-converting enzyme (ACE) inhibitors (vasotop, enap, kapoten, hartil) were used; in case of hypotension, dobutamine 5 μg/kg/min, dopamine 3-5 μg/kg/min were administered. Nitroglycerides and ACE inhibitors were not used during the acute decompensation phase.

After stabilisation of the animal's clinical condition, it is recommended to transfer it to outpatient treatment as soon as possible. Repeated assessment of the condition is carried out 3-7 days after discharge (assessment of chronic heart failure, kidney function, and determination of the state of electrolyte metabolism). Owners should control the frequency of the cat's respiratory movements (during its sleep) at the rate of up to 30 breathing movements/min.

Among the examined 110 animals with symptoms of pulmonary oedema, 68 animals were transferred to outpatient treatment, 42 animals died.

Conclusions

Thus, according to the results of the study in the conditions of the "Vet House" Veterinary Centre, it was found that the differential diagnosis of pulmonary oedema in a domestic cat has an essential tactical and prognostic value. This involves the use of various therapeutic schemes. To confirm the diagnosis of this disease, it is necessary to comprehensively consider the indicators of the clinical examination of the animal, the results of X-ray examination of the chest cavity and laboratory analysis of blood parameters.

According to the results of the conducted research, the predisposition of certain breeds of cats and their hybrids was established: British Shorthair, Sphynx and Maine Coon. It was found that the most common aetiological factors for cardiogenic pulmonary oedema are hypertrophic, restrictive, and non-classified cardiomyopathies, and for non-cardiogenic oedema – traumatic brain injury, electric shock, toxic substance poisoning, anaphylactic reaction, and airway obstruction. Thus, cardiogenic and non-cardiogenic causes are responsible for the development of pulmonary oedema. Accurate determination of the underlying cause of this pathology is of paramount importance for therapy and prognosis.

The most probable indicators for differential diagnosis were X-rays of the chest cavity (the degree of lung damage and the degree of congestive phenomena), data on the size of the left atrium, and indicators of echocardiography to exclude or confirm cardiomyopathy. The least significant were the results of the clinical picture and haematological studies, which are not specific tests and reveal many common features for both forms of pulmonary oedema.

The main principle of animal therapy at the initial stage is to reduce the clinical manifestations of acute respiratory failure. Treatment of cats with pulmonary oedema has a pathogenetic justification and differs in several features depending on aetiological factors and the patient's condition. According to the results of treatment, 68 animals were transferred to outpatient treatment, 42 died.

It is promising to develop diagnostic algorithms and treatment protocols for pulmonary oedema in a domestic cat, depending on its form (cardiogenic or non-cardiogenic) and the results of blood pressure measurement.

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Кардіогенний та некардіогенний набряк легень у свійського кота: патологічні механізми, диференційна діагностика та лікування

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Анотація. Актуальність дослідження зумовлена поширеністю набряку легень у котів і особливими підходами до їх терапії. У зв'язку з цим, дана стаття спрямована на виявлення етіологічних факторів, породної схильності, клінічних проявів, гематологічних показників та рентгенологічних ознак. Провідним у дослідженні цієї проблеми є комплексний підхід, який включає розгляд етіології, патогенезу, діагностики кардіогенного і некардіогенного набряку легень та лікування котів. З'ясовано, що в умовах Ветеринарного центру «Vet House» (м. Вінниця) найчастіше за цієї патології реєстрували котів порід британська короткошерста, сфінкс, мейнкун і їх метисів. У 68 котів встановлено кардіогенний набряк легень за гіпертрофічної, рестриктивної і некласифікованої кардіоміопатії. В 42 тварин виявлено некардіогенний набряк легень за черепно-мозкової травми, отруєння токсичними речовинами, анафілактичної реакції, обструкції дихальних шляхів та ураження електричним струмом. З'ясовано, що найбільш інформативними під час диференційної діагностики набряку легень у котів є результати ехокардіографії та рентгенологічного дослідження. Встановлено, що для диференційної діагностики кардіогенного і некардіогенного набряку легень клінічні показники і результати гематологічного дослідження тварин мають низьку інформативність. Виявлено, що лікувальні заходи за набряку легень є ефективними за врахування етіологічних факторів, хоча на першому етапі за обох форм набряку легень спрямовані на зменшення і усунення проявів гострої дихальної недостатності. Матеріали статті становлять практичну цінність для лікарів ветеринарної медицини загальної практики та інтенсивної терапії з питань диференційної діагностики, прогнозування розвитку хвороби та ефективного лікування котів

Ключові слова: біохімічні і морфологічні показники крові, задишка, інтерстиціальний рисунок, патогенез, рентгенографія

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Effect of the Biosapin Probiotic and the Biolide Disinfectant on the Microclimate of Poultry Houses

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Abstract. Currently, in the field of poultry farming, the issue of using disinfectants for surface treatment of poultry houses, incubators and hatching eggs is particularly acute. Preparations must be both effective for the destruction of pathogenic microorganisms resistant to antibacterial substances, and safe for the environment, productive poultry and poultry goods. The purpose of this study was to test the Biosapin probiotic and the Biolide disinfectant in industrial conditions of a poultry house and incubator room. These preparations were used by aerosol spraying in the incubator and output cabinet, as well as in the poultry house in the presence of poultry. They were tested both individually and in combination, compared to the control group, where no preparations were used. The parameters of the microclimate of the premises were determined: temperature – with a weekly thermograph M-21, relative humidity – with an Augusta psychrometer and hygrograph M-16, speed of air movement – with an ASO-13 vane anemometer, illumination – with a luxmeter, concentration of carbon dioxide, ammonia, hydrogen sulphide – using gas analyser UG-2, dust pollution – according to weight method, microbial pollution – according to sedimentation method. For the first time, a production test of the use of the Biosapin probiotic and the Biolide preparation in the complex was carried out. The obtained research results indicate an improvement in the microclimate of poultry houses. It was found that when spraying a disinfectant, microbial contamination in poultry premises decreases by 48.1% ($P<0.001$), and when spraying a probiotic – by 62.4% ($P<0.001$), while with a complex alternating action of both drugs – by 84.1% ($P<0.01$). At the same time, the bird's body is sanitised and enriched with probiotics. The positive effect of drugs on the microclimate in both the incubator room and the output hall sections has been experimentally confirmed. In particular, the concentration of ammonia decreases by 7.6% ($P<0.01$), 12.3, 27.8% ($P<0.001$) and hydrogen sulphide – by 16.6% ($P<0.001$), 18.5 and 34% ($P<0.001$). At the same time, the carbon dioxide content also decreases by 2.8, 3.3, and 5.0 times, respectively. Spraying the Biosapin probiotic and the Biolide disinfectant both separately and in combination stimulate embryogenesis, contribute to the production of more fertilised hatching eggs and the hatching of conditioned young chickens. Thus, the breeding rate of young animals increased by 5.5% ($P<0.01$), by 7.0% ($P<0.01$) and by 11.0% ($P<0.01$). The use of the preparations under study lies in the optimisation of the microclimate in poultry premises through such indicators as relative humidity, concentration of harmful gases (ammonia, hydrogen sulphide, carbon dioxide), dust, and microbial air pollution

Keywords: broiler chickens, microorganisms, sanitation, incubation, hatchery, eggs, poultry house

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Introduction

Hygiene and disinfection form an integral part of the technological process in poultry farming. Biocides protect poultry from the pathogenic effects of microorganisms and contribute to improving the safety of animal food production [1].

However, most known disinfectants, e.g., based on polymers of phenols – isothiazolinones, have toxic effects on the human body and the environment. Furthermore, most of the active substances of these drugs are of synthetic origin. Considering these factors, the demand for solutions with a biological basis is increasingly growing [2].

Conventionally, various disinfectants are used to disinfect poultry premises, which do not affect the further operation of incubation, hatching rooms, and equipment. However, it should be considered that the effectiveness of disinfection depends on such factors as exposure, temperature, concentration, pH, compatibility with detergents.

Eco-friendly alternatives to synthetic drugs are relatively safe biologics. Such preparations can include probiotics, their metabolites, organic compounds, for example, lactic acid obtained by the natural fermentation process. The use of biological preparations solves the problem with these disadvantages, which is explained by an effective combination of their natural origin, efficiency, safe handling, and environmental friendliness [3]. Since there is an urgent problem of antibiotic-resistant microorganisms in industrial animal husbandry and poultry farming [4], the search for an alternative to conventional disinfectants is relevant and important.

The effectiveness of poultry rearing substantially depends on sanitary and hygienic and zootechnical indicators, such as the method of keeping, the size of groups, the density, the microclimate of premises, as well as the regime and balance of feeding. For each hygienic parameter, there are certain ranges of normal values at which animals spend the minimum amount of energy to maintain physiological processes at an optimal level [1]. The key indicators of the microclimate for the body of adult and young poultry are the ambient air temperature, its relative humidity, air velocity, concentration of carbon dioxide, ammonia, dust and microbial pollution, and air. The use of safe disinfectants in the presence of poultry has a positive effect on the state of the air environment of poultry premises [5].

It is necessary to monitor hygiene and sanitation on the farm and in the incubator, since the air quality inside the poultry house is an essential factor affecting the health of chickens and the epizootic situation on the farm. Due to the violation of sanitary and hygienic requirements in production and as a result of keeping a lot of livestock in a limited area, the concentration of microorganisms in the air of poultry houses can considerably increase with the circulation of opportunistic and pathogenic microorganisms [4].

The accumulation of microorganisms in the poultry house can occur in different ways: due to poor-quality disinfection after the previous cycle of raising poultry, non-compliance with the time of biological rest of the room (the principle "everything is occupied – everything is empty"), through the personnel servicing the room, through poor-quality water or contaminated water supply system, with biofilm, contaminated feed, infected hatching eggs, with air entering the room, contaminated ventilation system, etc. A necessary element for achieving the best disinfection results is also the correct selection of effective detergents and disinfectants [6]. Therefore, it is necessary to observe technological processes in production and perform timely sanitary measures, especially disinfection.

Probiotics are quite effectively used in veterinary medicine for the prevention of various diseases, in namely dysbacteriosis with digestive disorders and deterioration of the assimilation of feed nutrients, as well as in the form of means that increase the immune status and effectiveness of vaccination. Interest in probiotic drugs is growing every year, as they can be used as an alternative to antibiotics in poultry farming, including in organic production [7].

Probiotics contain live microorganisms and components of normal intestinal microflora, which normalise the qualitative and quantitative composition and biological activity of avian digestive canal automicroflora [8].

The advantage of products based on lactic, supramolecular acids and hydrogen peroxide is environmental friendliness, biodegradability and safety, since the main components of the product decompose into water, oxygen and lactic acid at the time of disinfection. The latter is a product of the vital activity of living organisms and is absolutely safe for the environment [9].

Especially important is the use of ecological, biological, effective, and safe disinfectants in organic production. Their use is strictly controlled by the certification authority, and any violation may lead to the loss of the certificate by the organic market operator [2; 3]. There is a close interrelation between the state of the microflora of hatching incubators and the formation of the own intestinal microflora of chickens. The formation of a stable microbiocenosis of the digestive canal takes several weeks [9].

By their mechanism of action, probiotics can actively colonise the gastrointestinal tract of animals with microorganisms that produce biologically active metabolites and have a detrimental effect on pathogens [10]. Symbiotic microflora uses its enzymatic ability to process organic substances, synthesises proteins, amino acids, polypeptides, vitamins and other metabolites that are useful for the macroorganism [6; 11]. Microorganisms affect the absorption of calcium, iron, vitamins, as well as the synthesis of amino acids and other biologically active compounds [12; 13].

The mechanism of biological action of bacteriocins produced by lactobacilli and other microorganisms is based on the destruction of the cytoplasmic membranes of pathogenic bacteria sensitive to them. Bacteriocins act selectively and affect antibiotic-resistant microorganisms, followed by their splitting and final removal from the body [14]. The microflora of incubator and hatching rooms also depends on high-quality disinfection and maintaining an appropriate microclimate [15].

Complex use of disinfectants and rotation in production is an essential link of well-being and ensuring productivity in poultry farming [16]. The use of preparations based on lactic and supramolecular acids, hydrogen peroxide, in combination with a probiotic, allows reducing the microbial background in the room in the presence of poultry and improve its microclimate, partially modifying the gas composition of the air [17; 18].

The purpose of this study was to investigate the effectiveness of the complex and separate use of the Biosapin

probiotic and the Biolide disinfectant to improve the microclimate by aerosol spraying in the incubator and hatching cabinet, as well as in the poultry room in the presence of poultry.

Materials and Methods

The study was conducted in one of the farms of the Lviv region on broiler chickens of the Ross-308 Cross, starting from the 1-day age in February 2022. The experiment consisted of three stages. On the first stage, the main parameters of the indoor microclimate were investigated: temperature – with a weekly thermograph M-21, relative humidity – with an Augusta psychrometer and hygrograph M-16, air speed – with an ASO-13 vane anemometer, illumination – with a luxmeter, concentration of carbon dioxide, ammonia, hydrogen sulphide – using the UG-2 gas analyser, dust pollution – according to the weight method, microbial pollution – according to the sedimentation method against the background of irrigation with the Biosapin probiotic, as well as separately with the Biolide disinfectant and together with a disinfectant [19]. These drugs were developed by employees of the State Research Institute for Laboratory Diagnostics and Veterinary and Sanitary Expertise (city of Kyiv, SRILDVSE).

At the second stage, the effectiveness of treatment with the studied preparations of the incubator and the extraction cabinet was determined. Three experimental and one control groups of 200 hatching eggs each were formed. The third stage of research was a production experiment on broiler chickens of the ROSS-308 cross. Three experimental and one control groups of 100 heads each were formed.

Aerosol treatment of premises with Biolide was carried out using a cold mist generator. The Biolide disinfection preparation (TU U 24.2-00699690–001:2022), which includes lactic acid, perlactic acid, hydrogen peroxide, water, and according to preliminary laboratory studies, exhibits bactericidal and virulicidal activity with a prolonged effect.

The Biosapin probiotic preparation (TU U 24.2-00699690–004:2022) includes: mixtures of probiotic bacteria *Bacillus sybtilis, Bacillus amyloliquefaciens* and aluminium silicate. Treatment with Biosapin was performed with a dry aerosol generator at the rate of 10-30 g/m^2, once every two weeks.

For the second stage of the experiment, eggs were taken from 180-day-old, 260-, 350-day-old chickens, which met the basic requirements of the standard for hatching eggs: in terms of weight, size, shell hardness, etc. During the incubation process, constant monitoring was carried out, and after incubation was completed, the survival rate of chickens, their viability on the first day and the corresponding care were visually determined.

In the first group, to establish the effect of the Biosapin probiotic preparation on the microclimate of the hatchery and hatching rooms, a dry aerosol generator was used, which was evenly sprayed at the rate of 10-30 g/m^3, twice a week for exposure of 120 minutes for 19 days, with 200 hatching eggs.

In the second experimental group, the incubator and hatching rooms were disinfected with a 0.1% solution of Biolide disinfectant once a week, for 19 days, where there were 200 pieces of hatching eggs.

In the third experimental group, a complex of the Biosapin probiotic and the Biolide disinfectant was sprayed using the same method as in the first two groups. Therewith, a disinfectant was used on the first day, and a probiotic was used on the next day. This procedure was not performed in the control rooms. After completing the studies, the percentage of hatchability of chickens was calculated.

Production experiment (the third stage of this study) on chickens was carried out as follows: the farm carried out preliminary disinfection of premises without poultry with Biolide disinfectant. According to the principle of analogues, four groups (100 heads each) of broiler chickens of the ROSS-308 cross, equal in weight, were formed – control and three experimental. Standard mixed feed was used in the control. The term of rearing chickens was 41 days.

After placing one-day-old chicks, a thorough inspection of the entire flock was conducted. The state of feeding and care of a healthy number of chickens was monitored, eliminating adverse factors. Temperature, humidity, air movement speed were measured with an aspiration psychrometer MV-41-L, weekly thermograph, hygrometer M-16 and hygrograph M-21, vane anemometer ASO-13, layer catathermometer, concentration of harmful gases – with universal gas analyser UG-2, dioxide level carbon – according to the Hess method, illumination – with a Yu-16 luxmeter [20].

Once a week, the premises were disinfected in the presence of poultry with Biolide disinfectant. Then, for effective use of the method of increasing productivity and preserving the poultry population, the complex Biosapin preparation was evenly sprayed in the room at the rate of 10-30 g/m^2. The preparation was used once in two weeks. Chickens of the control group were fed standard mixed feed according to the current feeding standards from the manufacturer of ROSS-308 cross (Aviagen), and no preventive preparations were used [21].

Chickens of the first experimental group were fed standard mixed feed, evenly sprayed with the complex Biosapin preparation in the room at the rate of 10-30 g/m^2, once every two weeks. Chickens of the second experimental group were fed standard mixed feed, according to the manufacturer's instructions and recommendations for growing this cross. Once a week, the premises were disinfected in the presence of poultry with 0.1% solution of the Biolide disinfectant. Chickens of the third experimental group were fed standard mixed feed. Once a week, the premises were disinfected in the presence of poultry with a Biolide disinfectant, after two days, once in two weeks, the complex Biosapin preparation was evenly sprayed in the room at the rate of 10-30 g/m^2.

Digital data was processed using the biometric method of variational non-parametric analysis using the Microsoft Excel software of the Microsoft Office Professional XP table editor package and the Origin 6.1 programme. The difference between the values of indicators was considered statistically probable: $P<0.05$, $P<0.01$, and $P<0.001$.

Results and Discussion

The conducted studies have established the effect of the Biosapin probiotic and the Biolide disinfectant on the parameters of the microclimate in the incubator room. Air temperature, relative humidity, air velocity, concentration

of ammonia, hydrogen sulphide, carbon dioxide, dust content, and microbial contamination were monitored.

The results presented in Table 1 show that the spraying of the Biosapin probiotic and the Biolide preparation had a positive effect on the optimisation of the main parameters of the microclimate in the premises of the incubator. Thus, the relative humidity of the air decreased improbably in the experimental section of the incubation hall, where the probiotic was sprayed without the use of a disinfectant, in comparison with the control group. At the same time, in another section where the Biolide preparation was sprayed, this indicator decreased by 6.09% (P<0.01), and in the group where complex treatment with the Biosapin probiotic and the Biolide preparation was used – by 8.32% (P<0.001), respectively, compared to the control group.

Table 1. Microclimate of industrial premises against the background of the use of the Biosapin probiotic and the Biolide preparation, M ± m, n = 5

Indicator	Group			
	Control	1st experimental, Biosapin preparation	2nd experimental, Biolide preparation	3rd experimental, preparations Biozapin + Biolide
	Incubator room			
Air temperature, 0 °C	21.10 ± 0.11	21.13 ± 0.03	22.02 ± 0.08	22.15 ± 0.15
Relative humidity, %	72.42 ± 0.21	69.12 ± 0.23	66.33 ± 0.33*	64.10 ± 0.12**
Air velocity, m/s	0.21 ± 0.02	0.22 ± 0.03	0.21 ± 0.05	0.24 ± 0.02
Ammonia concentration, mg/m³	7.85 ± 0.02	7.31 ± 0.03*	6.32 ± 0.04*	6.12 ± 0.05**
Hydrogen sulphide concentration, mg/m³	3.31 ± 0.05	2.61 ± 0.09**	2.49 ± 0.04**	2.31 ± 0.03**
Carbon dioxide concentration, %	0.17 ± 0.01	0.11 ± 0.01	0.10 ± 0.03	0.09 ± 0.03**
Dust concentration, mg/m³	8.42 ± 0.13	6.08 ± 0.10*	4.85 ± 0.11*	4.07 ± 0.12**
Microbial contamination, thous. t/m³	71.35 ± 0.54	41.32 ± 0.34**	31.56 ± 0.14**	9.89 ± 0.44**
	Hatching room			
Air temperature, °C	22.10 ± 0.14	22.12 ± 0.02	22.45 ± 0.14	22.40 ± 0.09
Relative humidity, %	73.60 ± 0.24	68.95 ± 0.17*	67.17 ± 0.22*	63.41 ± 0.21*
Air velocity, m/s	0.24 ± 0.04	0.26 ± 0.02	0.29 ± 0.03	0.29 ± 0.05
Ammonia concentration, mg/m³	6.98 ± 0.12	6.45 ± 0.10*	6.12 ± 0.11**	5.04 ± 0.11**
Hydrogen sulphide concentration, mg/m³	3.08 ± 0.02	2.57 ± 0.02**	2.51 ± 0.11**	2.03 ± 0.01**
Carbon dioxide concentration, %	0.6 ± 0.01	0.21 ± 0.03	0.18 ± 0.02*	0.12 ± 0.04**
Dust concentration, mg/m³	8.80 ± 0.12	5.12 ± 0.13**	4.01 ± 0.11**	3.45 ± 0.02**
Microbial contamination, thous. t/m³	73.60 ± 0.22	42.08 ± 0.11**	35.62 ± 0.02**	12.06 ± 0.12**

Note: **P<0.01; **P<0.001, probable compared to the control group*

Therewith, spraying the Bioapin probiotic both alone and in combination with the specified disinfectant did not have a noticeable effect on the air temperature, which in all experimental sections of the incubation room was at the level of 21-22 °C and its movement speed, which varied from 0.21 to 0.24 m/s, i.e., within the limits of permissible physical fluctuations without a probable difference between the indicators of the control and experimental sections of the incubation hall.

Under the influence of these measures, a decrease in the concentration of harmful gases was noted in all experimental sections of the incubation room. Thus, a probable decrease in the concentration of ammonia by 6.9% (P<0.01), respectively, occurred in the air of the room of the first experimental section, where a separate spray of disinfectant was used, and in the second (spray with probiotic) – by 6.8% (P<0.01), in the third section (spray with disinfectant and probiotic – by 22.0% (P<0.001), respectively, compared to the control group.

A probable decrease in the concentration of hydrogen sulphide by 21.1% (P<0.001) occurred in the air of the first experimental section, where Biolide disinfectant was used, and in the second (probiotic spraying) – by 24.8% (P<0.001), in the third section (disinfectant and probiotic spraying) – by 30.2% (P<0.001), respectively, compared to the control group.

In the air of the room of the third experimental section, where the complex spraying of the Biosapin probiotic and the Biolide disinfectant was carried out, a probable decrease in the concentration of carbon dioxide was observed by 1.9 times or by 0.08% (P<0.01) compared to the control group. A slight decrease in the value of this indicator in the first and second experimental sections of the room turned out to be statically improbable.

As a result of using the Biosapin probiotic, the dust content in the air of the first experimental section decreased by 1.4 times or by 2.34 mg/m³ (P<0.001) compared to the control group. In other experimental sections, the decrease in dust concentration in the air occurred to a greater extent – by 1.7 times or by 3.57 mg/m³ (P<0.01) and with the combined use of spraying, this indicator was 2.07 times higher or 4.35 mg/m³ (P<0.001) compared to the control group.

The use of spray preparations had a positive effect on reducing microbial contamination of the air in the incubation room. In the first experimental section, as a result of

spraying with the Biosapin probiotic, the value of microbial contamination decreased by 42.0% (P<0.001) compared to the control group. In other sections of this room, with the combined use of preparations spraying, the level of microbial contamination of the air decreased to a greater extent than in the control section. Thus, this indicator decreased in the second experimental section by 56.0% (P<0.001), in the third – by 86.0% (P<0.001) compared to the control group.

Similar changes in the microclimate were recorded in the sections of the hatching hall. Thus, the probable decrease in the concentration of ammonia in the first, second, and third groups compared to the control group occurred, respectively, by 7.6% (P<0.01), 12.3 and 27.8% (P<0.001).

A probable decrease in the concentration of hydrogen sulphide in the first, second, and third groups compared to the control group occurred by 16.6% (P<0.001), 18.5%, and 34.0% (P<0.001), respectively. The content of carbon dioxide in the air of the experimental groups also decreased: the first – by 2.8 times (by 0.39%), the second – by 3.3 times (by 0.42%), the third – by 5.0 times (by 0.48%) compared to the control.

According to the indicators presented in Table 2, of the eggs laid for incubation, 191 birds of the control group were fertilised, which is 95.5%, and in the three experimental groups this indicator was 97.5%, 96.5%, and 97%, respectively.

Table 2. Incubation of chicken eggs during the use of Biosapin and Biolide preparations

Indicator	Group			
	Control	1st experimental, Biosapin preparation	2nd experimental, Biolide preparation	3rd experimental, preparations Biozapin + Biolide
Laid eggs, pcs.	200	200	200	200
including fertilised eggs, pcs	191	195	193	194
Moved to output, pcs	185	191	192	193
Received chickens, heads	162	173	176	184
Breeding of young animals, %	81	86.5	88	92
Egg hatching, %	84.5	88.7	91.2	94.8

Table 2 shows that a larger number of chickens were obtained in the experimental poultry groups. Thus, in the first experimental group, more chickens were obtained by 6.79%, in the second – by 8.64% and in the third – by 13.6% compared to the control group.

In the first experimental group, the rate of hatching eggs was higher by 5.5% (P<0.01), in the second – by 6.7% (P<0.01), and in the third – by 10.3% (P<0.01) compared to the control group.

Hatching of chickens was characterised by the number of healthy chickens bred, expressed as a percentage of the number of eggs laid in the incubator. Thus, the rate of hatching of young in the first experimental group increased by 5.5% (P<0.01), in the second – by 7.0% (P<0.01), and in the third – by 11% (P<0.01) compared to the control group.

Thus, the conducted studies showed that spraying the Biozapin probiotic and the Biolide disinfectant both individually and in combination stimulate embryogenesis, contribute to obtaining a larger number of fertilised hatching eggs and the hatching of conditioned young chickens.

According to the indicators presented in Tables 1 and 3, the optimisation of the main parameters of the microclimate in the hatching hall and in the workshop of raising young chickens under the influence of complex use of drugs occurred similarly.

Table 3. Microclimate indicators in poultry rearing shops, M±m, n=5

Indicator	Research day	Group			
		Control	1st experimental, Biosapin preparation	2nd experimental, preparation Biolide	3rd experimental, preparations Biosapin + Biolide
Temperature (20-25 cm above floor), °C	1-5	33.20 ± 0.12	33.60 ± 0.13	34.10 ± 0.11	34.20 ± 0.12
Temperature (20-25 cm above floor), °C	6-10	32.54 ± 0.33	32.12 ± 0.13	32.56 ± 0.12	32.45 ± 0.11
Temperature (20-25 cm above floor), °C	11-20	30.10 ± 0.3	30.20 ± 0.12	30.12 ± 0.13	30.34 ± 0.13
Temperature (20-25 cm above floor), °C	21-30	25.13 ± 0.19	25.50 ± 0.18	24.90 ± 0.11	24.89 ± 0.13
Temperature (20-25 cm above floor), °C	31-40	22.40 ± 0.19	22.78 ± 0.14	22.56 ± 0.12	22.89 ± 0.13
Indoor air temperature, °C	41-60	20.01 ± 0.01	19.87 ± 0.11	20.24 ± 0.14	19.58 ± 0.11
Indoor air temperature, °C	>60	18.31 ± 0.12	18.60 ± 0.12	18.14 ± 0.13	18.34 ± 0.11
Relative humidity, %	1-110	71.85 ± 0.12	66.80 ± 0.12**	65.56 ± 0.11***	64.34 ± 0.11***
Air velocity, m/s	1-110	0.23 ± 0.01	0.22 ± 0.02	0.23 ± 0.02	0.21 ± 0.05
Ammonia concentration, mg/m^3	1-110	7.34 ± 0.12	6.45 ± 0.11*	6.08 ± 0.11**	5.90 ± 0.12***
Hydrogen sulphide concentration, mg/m^3	1-110	5.02 ± 0.11	4.24 ± 0.12*	3.64 ± 0.10***	2.90 ± 0.11***

Table 3, Continued

Carbon dioxide concentration, %	1-110	0.25 ± 0.02	0.24 ± 0.01	0.23 ± 0.01	0.22 ± 0.04
Dust concentration, mg/m^3	1-110	9.04	6.40 ± 0.15**	5.50 ± 0.14***	4.06 ± 0.14***
Microbial contamination, thous. t/m^3	1-110	81.46 ± 1.56	42.31 ± 0.36***	30.61 ± 0.34***	12.99 ± 0.18**

Note: **P<0.05; **P<0.01; ***P<0.001, probable compared to the control group*

This procedure did not affect the temperature and speed of indoor air movement. In the hatching hall, these indicators varied within acceptable values. As the age of the chickens increased, the temperature in these rooms gradually decreased, according to the technology of their rearing. By the 40-day age of young animals, it was reduced to 19°C. At the same time, under the influence of the measures taken, a gradual decrease in air humidity was observed, which was recorded in all experimental sections of these premises: the first – by 5.05%, the second – by 6.29%, the third – by 7.50%.

The concentration of ammonia in the air of the workshop for growing young chickens of the first experimental group decreased by 12.13% (P<0.05), of the second – by 17.16% (P<0.01), and of the third – by 19.62% (P<0.001) compared to the control group. The concentration of hydrogen sulphide in the air of the shop for growing young chickens of the first experimental group decreased by 16.0% (P<0.05), the second – by 27.0% (P<0.001), and the third – by 42.2% (P<0.001) compared to the control group. The concentration of carbon dioxide in the air of these rooms under the influence of procedures decreased by 0.01-0.03%, but these values turned out to be statically incorrect.

During the spraying of the preparations, the content of solid aerosols (dust) in the air of the premises of the removal hall and the workshop for growing young chickens of experimental groups of poultry decreased in comparison with the control group. Thus, in the indoor air of the first experimental group, a probable decrease in dust concentration was established by 29.2% (P<0.01), the second – by 39.2%, and the third – by 55.1% (P<0.001) compared to the control group.

Spraying of the Biosapin probiotic both separately and in combination with the Biolide preparation had a certain positive effect on reducing microbial contamination in the air of the hatching hall and the workshop for rearing young poultry. Thus, the number of microorganisms in the air of the workshops where poultry of the first experimental group was kept decreased by 48.1% (P<0.001), the second – by 62.4% (P<0.001), and the third – by 84.1% (P<0.01) compared to the control.

Evidently, the main indicators of the microclimate, such as humidity, temperature, dust, and microbial air pollution, depend on the number of birds kept in the room. Recommendations for the use of probiotic as a spray in the room and for changes in ventilation, air exchange multiplicity, and temperature regime of poultry rearing are still absent [20; 21].

The effectiveness of complex use of drugs was higher in this indicator than with their separate use in the first two experimental groups. The obtained data are consistent with the results of other scientists regarding the improvement of air quality in poultry houses by carrying out disinfection in the presence of birds. According to Boleli et al. [14], ongoing disinfection in incubators increases and optimises the hatchability of healthy young chieckens.

In the technological process of rearing poultry, it is impossible to completely abandon preventive vaccinations, disinfection, the use of antibiotics, anthelmintics, and other preparations. After their use, or together with pro- and prebiotics, there is a need to restore the normal intestinal microflora of the chicken, which is necessary to increase the assimilation of feed nutrients. And this, in turn, as is known from [4; 10], affects the improvement of the nutritional and biological value of poultry slaughter products and contributes to a more rational use of feed.

Thus, the use of the Biosapin probiotic in the incubator premises helped optimise the microclimate in them in terms of such indicators as relative humidity, the concentration of harmful gases (ammonia, hydrogen sulphide, hydrogen dioxide), dust and microbial air pollution. The use of the Biosapin probiotic and the Biolide preparation in the spray complex increased the effectiveness of the procedure in the incubator rooms, where the best microclimatic indicators were obtained.

Conclusions

The use of modern eco-friendly and safe disinfectants for poultry and service personnel in industrial poultry farming is a promising solution to the issues of normalising the microclimate of poultry premises, namely microbial air pollution.

1. The complex use of spraying Biosapin probiotic and Biolide disinfectant based on lactic acid, hydrogen peroxide, and supramolecular acid in working concentrations increases the efficiency of the procedure in the incubator, hatching hall and poultry room, helps optimise the microclimate, especially in terms of relative humidity, concentration of harmful gases, dust and microbial air pollution.

2. Spraying the Biosapin probiotic and the Biolide disinfectant both separately and in combination stimulates embryogenesis, contributes to the production of more fertilised hatching eggs and the hatching of conditioned young chickens by 5.5-10.0%.

3. To reduce microbial air pollution in poultry premises, it is appropriate to use the Biosapin probiotic at the rate of 10-30 g/m^2, once every two weeks. Thus, the number of microorganisms in the air of the workshops where the birds of the first research group were kept decreased by 48.1% (P<0.001), the second – by 62.4% (P<0.001), and the third – by 84.1% (P<0.01) compared to the control group.

4. To disinfect poultry houses in the presence of poultry, it is advisable to use a 0.1% solution of the Biolide preparation using a cold mist generator. Therewith, the consumption of the working solution of the disinfectant is 30 ml/m^3 of the room.

Prospects for further research: development of modes and schemes for the use of the Biosapin probiotic and the Biolide disinfectant in production conditions.

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Вплив пробіотика «Біозапін» і дезінфікуючого препарату «Біолайд» на мікроклімат птахівничих приміщень

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Анотація. Нині у сфері птахівництва особливо гостро постає питання застосування дезінфікуючих засобів для обробки поверхонь пташників, інкубаторів та інкубаційних яєць. Препарати мають бути одночасно ефективними для знищення патогенних мікроорганізмів, резистентних до антибактеріальних речовин, і безпечними для довкілля, продуктивної птиці та продукції птахівництва. Мета роботи полягала у випробуванні пробіотика «Біозапін» і дезінфікуючого препарату «Біолайд» у промислових умовах пташнику та інкубаторного приміщення. Вказані препарати застосовували методом аерозольного розпилення у приміщенні інкубаторію та вивідної шафи, а також у птахівничому приміщенні в присутності птиці. Їх випробовували як окремо, так і в комплексі, порівняно з контрольною групою, де не застосовувалися жодні препарати. Визначали параметри мікроклімату приміщень: температуру – тижневим термографом М-21, відносну вологість – психрометром Августа і гігрографом М-16, швидкість руху повітря – анемометром крильчатим АСО-13, освітленість – за допомогою люксметру, концентрацію вуглекислого газу, аміаку, сірководню – з використанням газоаналізатору УГ-2, пилову забрудненість – ваговим методом, мікробну забрудненість – седиментаційним методом. Уперше було здійснено виробниче випробування застосування в комплексі пробіотика «Біозапін» і препарату «Біолайд». Отримані результати досліджень свідчать про покращення мікроклімату птахівничих приміщень. Встановлено, що за розпилення дезінфектанту зменшується мікробна забрудненість у птахівничих приміщеннях на 48,1 % ($P<0,001$), а за розпилення пробіотику – на 62,4 % ($P<0,001$) і за комплексної почергової дії обох препаратів – на 84,1 % ($P<0,01$). Водночас санується і збагачується пробіотиками організм птиці. Експериментально підтверджено позитивний вплив препаратів на мікроклімат як у секціях інкубаторного приміщення, так і вивідної зали. Зокрема, знижується концентрація аміаку на 7,6 % ($P<0,01$), 12,3, 27,8 % ($P<0,001$) і сірководню – на 16,6 % ($P<0,001$), 18,5 і 34 % ($P<0,001$). Водночас зменшується і вміст діоксиду вуглецю у 2,8 раза, 3,3 і 5,0 разів. Розпилення пробіотика «Біозапін» і дезінфектанту «Біолайд» як окремо, так і в поєднанні стимулюють ембріогенез, сприяють отриманню більшої кількості запліднених інкубаційних яєць та виведенню кондиційного молодняку курей. Так, показник виведення молодняку підвищився на 5,5 % ($P<0,01$), на 7,0 % ($P<0,01$) і на 11,0 % ($P<0,01$). Застосування досліджуваних препаратів полягає в оптимізації мікроклімату в птахівничих приміщеннях за такими показниками, як відносна вологість, концентрація шкідливих газів (аміак, сірководень, діоксид вуглецю), пилова та мікробна забрудненість повітря

Ключові слова: курчата-бройлери, мікроорганізми, санація, інкубація, вивідне відділення, яйця, пташник

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Epizootological Features of Coronavirus Infection in Cats

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Abstract. The relevance of the study of coronavirus infection in animals is conditioned upon the lack of sufficient information about the mechanisms of development of this disease, imperfect methods of diagnosis and treatment, and, most importantly, almost 100% of their mortality. The purpose of this study was to identify the age, breed, seasonal, and sexual characteristics of cats' predisposition to coronavirus infection and the development of infectious peritonitis. The paper presents the results of epizoothogolic features of the spread of coronavirus infection among animals of this species and feline infectious peritonitis during 2020-2022 based on the veterinary clinic "White Wolf" (city of Kyiv). During this period, 483 samples were examined from cats with symptoms of coronavirus infection. From these samples, using immunochromatographic analysis, a virus of the *Coronaviridae* family was detected in 399 animals, and infectious peritonitis was established in 63 animals. This paper highlights the results of a study of the age, breed, and sexual predisposition of cats to coronavirus infection and the development of infectious peritonitis. It was found that cats of any age are susceptible to coronavirus infection, while infectious peritonitis develops in animals aged from 2 months to 3 years. Coronavirus infection was most frequently found in mixed breed cats and British shorthair cats, infectious peritonitis – in Burmese and Bengal breeds. The study also summarised data on the seasonal manifestation of coronavirus infection and infectious peritonitis of cats. Based on the results of epizootological analysis, a nosological profile of infectious diseases was formed in animals of this species that had similar clinical symptoms of coronavirus infection and infectious peritonitis. Eight infections are presented, of which the most frequently recorded diseases were caused by viruses of the *Herpesviridae, Caliciviridae, and Parvoviridae* families. The results of this study provide new information about the epizootological features of the manifestation and development of coronavirus infection in cats, which allows not only improving the available diagnostic methods, but also developing new ones

Keywords: breed, sensitivity, seasonality, epizootic process, real-time PCR, test systems

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Introduction

Coronavirus infection in cats is a contagious disease of wild and domestic animals and is widespread all over the world. Thus, according to various sources, from 73% to 90% of cats are infected with coronavirus. Only 2-13% of these animals, which are carriers of the intestinal type of coronavirus, can develop infectious peritonitis. The latter is manifested clinically by fibrinous polyserositis and accumulation of a significant volume of exudate and is also characterised by high mortality [1; 2].

The feline coronavirus has two different genotypes, called feline intestinal coronavirus (FCoV) and feline infectious peritonitis virus (FIPV), which show different clinical symptoms. Feline intestinal coronavirus mainly infects cats by fecal-oral route and causes mild and transient gastroenteritis, frequently leading to the development of asymptomatic infections. The feline infectious peritonitis virus, on the contrary, occurs due to a mutation of the feline intestinal coronavirus and is observed in a small percentage of infected animals [1].

Feline infectious peritonitis today is still one of the most studied infectious diseases. This is because the mechanism of development of the disease, diagnosis, and treatment are understudied, and almost 100% mortality encourages further study of this infection [1]. Coronavirus is one of the most common pathogens of cat diseases. Infection of cats with coronavirus is quite common, usually characterised by the development of mild intestinal infections, the main sign of which is diarrhoea. Feline coronavirus (FCoV) is a contagious disease of this animal species and occurs worldwide [2].

Notably, the epizootological characteristics of coronavirus infection in cats, including infectious peritonitis of cats, are understudied, which creates the need to analyse statistical data on the results of clinical and laboratory studies of these animals.

The relevance of this subject is indisputable, and therefore this study is primarily aimed at clarifying and generalising data on the epizootological features of the spread of coronavirus infection and infectious peritonitis among cats. This will provide an epizootological insight into the situation regarding these diseases and possible solutions to prevent the development of these diseases.

The purpose of this study was to identify the predisposition of cats to coronavirus infection and the development of infectious peritonitis depending on age, breed, time of year, and sex.

Literature Review

Coronaviruses are RNA-positive single-stranded viruses belonging to the *Coronaviridae* family of the *Nidovirales* order. They are divided into four genera: Alpha-, Beta, Gamma-, and Delta-coronaviruses. Gamma and Delta coronaviruses mainly affect birds. Alpha- and Beta- coronaviruses affect only mammals. Coronaviruses have the largest genome among RNA viruses, the size of which ranges from 26 to 32 kb [2].

Upon study, coronaviruses show a high mutation rate during replication and therefore exist as clusters of genetically diverse populations. Feline coronavirus infection (FCoV) has been found to be widespread all over the world, with a few isolated islands [3].

There are two genotypes of cat coronavirus: type 1, which represents the vast majority of field isolates, and type 2, which occurs due to recombination between the feline coronavirus type 1 and the canine coronavirus. The two serotypes differ primarily in their transmembrane adhesive (S) glycoprotein [3-5].

Infection of cats with the intestinal type of coronavirus reaches about 35% of the population of these pets. Antibodies to FCoV were detected, which indicates virus transmission in cats. Thus, in households with one cat, the prevalence of intestinal coronavirus type decreases to 21%, but, accordingly, in households with many cats, the prevalence can exceed 90%. Most cases of intestinal coronavirus in cats are transient infections, and only a small percentage of animals become resistant “carriers” of coronavirus [4].

The solitary lifestyle of the ancestors of domestic cats led to the emergence of a variety of virus transmission pathways and stimulated the appearance of latent, chronic and/or asymptomatic infections, which contributed to a decrease in the level of immune response of the sick animal's body, and eventually led to an increase in the population of the pathogen carriers [5].

In their studies, C. Li et al. (2019) established the prevalence of feline intestinal coronavirus [6]. According to their data, FCoV is common in most cat populations. Moreover, the infection rate exceeds 20%: in northern China – 80%; southern China – 73.1%; southwest China – 80.4%; eastern China – 74.6%; Portugal – 47.5%; Germany – 76.5%; Malaysia – 84%; southern Italy – 80%; Japan – 37%.

L.A. McKay et al. (2020) found that feline coronavirus type 1 was the only serotype found in fecal and tissue samples in North American and European cat populations. It has already been established that 46% of cats are carriers of feline coronavirus [7].

W. Zhang et al. (2014) examined the feces of 25 cats from the shelter for the presence of various pathogens of infectious diseases [8]. Therewith, eight viral families were identified: *Astroviridae, Coronaviridae, Parvoviridae, Circoviridae, Herpesviridae, Anelloviridae, Caliciviridae* and *Picobirnaviridae*. Among the coronaviruses, feline type 1 coronavirus was identified in 15 fecal samples under study.

There are two forms of manifestation of coronavirus infection in cats: feline infectious peritonitis and the actual coronavirus infection of cats, which affects the intestines of animals [9].

Infectious feline peritonitis is an infection that is common among wild and domestic cats and is described by a high degree of mortality. It is manifested by the development of fibrinous and granulomatous polyserositis, the accumulation of protein-rich serous effusion in body cavities, and/or granulomatous inflammatory lesions of organs. The causative agent of feline infectious peritonitis is the feline coronavirus, which is widespread all over the world. The proportion of FCoV-infected cats that can develop feline infectious peritonitis is estimated at 5-12% [1; 9].

The feline infectious peritonitis virus, unlike the intestinal coronavirus, is not contagious and is not transmitted by oral-fecal route, but occurs as a result of a mutation from avirulent FCoV in 11-13% of infected cats, which leads to the development of a serious disease – infectious feline peritonitis, which is lethal [10].

Feline infectious peritonitis (FIP) can occur in animals of any age but is more common in cats younger than three years and especially between the ages of 4 and 16 weeks. Feline infectious peritonitis is observed in kennels (breeding cats), animal shelters, and places with a high concentration of animals per 1 m^2. Typically for an enzootic infection, the incidence of feline infectious peritonitis can vary widely over time. Mortality from infectious peritonitis in cats is very high, especially after the onset of clinical symptoms, although some animals can live with this disease for several weeks, months, or, rarely, years [11; 12].

Risk factors contributing to the development of infectious peritonitis in cats include those that affect the rate of transmission and replication of the virus in the animal's body, including young age, reduced immune response of the body to the causative agent of the disease, physiological stress, viral load in the environment, group habitat of animals and genetic predisposition [9].

Y. Yin et al. (2021) found that most cats suspected of infectious peritonitis belonged to young animals (under 3 years of age) and intact males [1]. They also determined that the effusive form of infectious peritonitis was observed more frequently, i.e., in 85.8% of animals with diagnosed feline infectious peritonitis.

According to S.J. Yen and H.W. Chen (2021) infectious peritonitis was detected in 47.7% of purebred animals and 47.1% in the mongrel cat group [13]. Purebred cats included the following breeds: American Shorthair, British Shorthair, Scottish Fold, Russian Blue, Chinchilla, Munchkin, and Ragdoll. The age-related predisposition to feline infectious peritonitis was also established. Therewith, the authors of this study found that the highest positive result for feline infectious peritonitis was obtained in the group of animals aged from 0 to 24 months – 67.4%, and the lowest – in the group of cats older than 73 months – 15.6%.

Q. Zhou et al. (2021) found that infectious feline peritonitis is more common in males (48.4%) and 44.2% among females. The author also determined the prevalence of feline infectious peritonitis relative to the density of animal habitat. Thus, among cats raised in groups, the incidence of infectious peritonitis was 58.1%, while among cats raised alone – 37.2% [14].

Based on the results of the study by Y. Yin et al. (2021), of the 59 cats with infectious peritonitis under study, 26% of the sick cats were female (11 animals) and 74% were male (32 animals). Of the 59 animals under study, 47 belonged to the group of young animals – from 2 months to 2 years [15].

Materials and Methods

The material for the research was cats with coronavirus infection during 2020-2022. The study was carried out based on the Department of Epizootology, Microbiology and Virology of the National University of Life and Environmental Sciences of Ukraine and the veterinary clinic "White Wolf" (Kyiv). The analysis of the epizootic situation of coronavirus infection in cats was carried out by analysing the data of the journal of registration of sick animals for the above period, considering the data of anamnesis and clinical and laboratory studies. The specific features of the spread of coronavirus infection in cats and infectious peritonitis in relation to the age, time of year, sex, and breed of sick animals were determined. The prevalence of various forms of infectious peritonitis in cats was determined. The nosological profile of infectious pathology circulating among cats that were admitted to the above-mentioned clinic for comprehensive studies was established.

To identify the prevalence of various feline infectious diseases, rapid blood tests of animals were performed using test systems based on three-phase immunochromatographic analysis (ICA). The authors of this paper used test systems from VetExpert.

Laboratory tests of blood samples and effusion from the pleural and abdominal cavities were carried out based on the laboratory of the veterinary clinic "White Wolf" and the veterinary laboratory "BioSoft" (Kyiv). The sampling of exudate from the chest and abdominal cavities of cats with an effusive form of infectious peritonitis was carried out under the control of an ultrasound diagnostic device (USD device).

Feline Corona Virus Antigen (FCoV Ag) Test of the Asan Pharm company was used to study fecal samples of sick cats. To confirm the diagnosis of infectious peritonitis in cats, polymerase chain reaction (PCR) was performed with samples of exudate from the thoracic or abdominal cavities in the case of an effusive form of infectious peritonitis in cats. As an auxiliary diagnostic method, an enzyme-linked immunosorbent assay (Elisa) was used to identify virus-specific antibodies.

In the dry form of infectious peritonitis in cats, the antibody titre in the blood serum was also identified using Elisa. For this, venous blood was taken and centrifuged for 10 minutes at 3,000 rpm. Quantitative PCR (RealPCR Test) was used to establish the diagnosis. The principle of operation of this test is that specific short fluorescent hydrolysis probes either detect two separate nucleoside mutations in feline coronavirus (FeCV) and FIPV or set the nucleotide sequence in wild-type FeCV at a different fluorescence wavelength.

For the study, a maximum of 1 cm^3 of native EDTA-stabilised blood was taken, centrifuged for 10 minutes at 2,500 rpm. Plasma was separated from blood cells and stored at -20°C. One volume of PBS was added to the blood cells. Total RNA was isolated according to the Total Quick RNA Blood Kit Protocol (Talent) [16].

For real-time PCR, oligonucleoside primers that respond to the M gene sequence (primer 212) of the FeCV genome were used with a primer targeting the FCoV genome leader sequence (primer 1179).

The reaction mixture was placed in a thermal cycler (Biozym). The temperature cycle protocol consisted of 10 minutes of incubation at 95 °C, followed by 30 cycles of 1 minute of denaturation at 95 °C, 1 minute of primer annealing at 62 °C, and 1 minute of primer elongation at 72 °C. After 30 cycles, the reaction mixture was kept for 10 minutes at 72 °C, followed by cooling to a temperature of 4 °C.

N.C. Pedersen [11] et al. [7; 13] report that a definitive diagnosis of feline infectious peritonitis can be made by immunohistochemical examination. However, the authors of the paper did not use this method due to the lack of proper equipment in Ukraine.

Digital data were processed biometrically, applying methods of statistical analysis using computer programs Statistica 6.0 and Microsoft Excel 2019.

Results and Discussion

The main clinical symptoms observed in feline coronavirus infection and infectious peritonitis include vomiting, diarrhoea, weight loss, lethargy, refusal of food, accumulation of exudate in the chest and abdominal cavities.

Of all the samples under study using test systems, the feline coronavirus antigen was detected in 399 samples, which is a percentage of 82.6% of animals (399/483). Therewith, feline infectious peritonitis was detected only in 63 animals out of 483, which is 13%.

It was found that among cats registered in the veterinary clinic "White Wolf" there were seven infectious diseases of cats, namely panleukopenia, coronavirus infection, herpesvirus infection, calicivirus infection, feline infectious peritonitis, feline immunodeficiency virus and feline leukaemia virus (Table 1).

Table 1. Nosological profile of feline infectious diseases detected in 2020-2022, n = 483

Diseases	% of sick animals	Number of sick animals
Panleukopenia	23.0	111
Coronavirus infection	82.6	399
Herpesvirus infection	31.0	150
Calicivirus infection	14.2	69
Feline infectious peritonitis	13.0	63
Feline immunodeficiency virus	2.9	14
Feline leukaemia virus	1.2	6

Laboratory tests revealed the pathogens of the above infections: panleukopenia – 23.0%; feline coronavirus infection – 82.6%; herpesvirus infection – 31.0%; feline calicivirus infection – 14.2%; feline infectious peritonitis – 13.0%; feline immunodeficiency virus – 2.9%; feline leukemia virus – 1.2%.

Infectious diseases such as panleukopenia, coronavirus infection, and herpesvirus infection were more frequently reported. Notably, the feline coronavirus was registered simultaneously with other diseases. Thus, out of 111 cats with panleukopenia, 57 were found to have feline coronavirus. This indicates that coronavirus infection in cats can occur with other diseases with a decrease in immunity.

As a result of epizootological examination of sick cats with clinical symptoms of infectious peritonitis (anorexia, weight loss, accumulation of exudate in the chest/abdominal cavity, uveitis, nervous system damage), 13.0% were found, of which males – 54.0% (34/63), females – 46.0% (29/63) (Fig. 1).

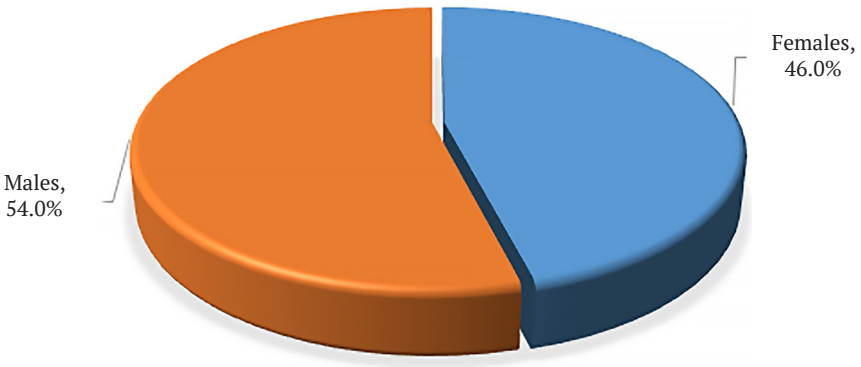

Figure 1. Predisposition to feline infectious peritonitis depending on gender

From this, it can be concluded that both male and female cats are equally susceptible to feline infectious peritonitis. Authors N.C Pedersen, S. Felten claim that intact males are more susceptible to feline infectious peritonitis [10; 11]. N.C. Pedersen (2014) [11] found that male cats, especially intact males, are not directly at risk of developing feline infectious peritonitis, as other authors claim [13].

Age-related features of the spread of feline coronavirus infection and feline infectious peritonitis were established. Among the 399 cats under study, the largest proportion of patients with feline coronavirus infection was observed in the group of animals from 1 to 2 years (26%) and in the group of 2-5 years (28%) (Fig. 2).

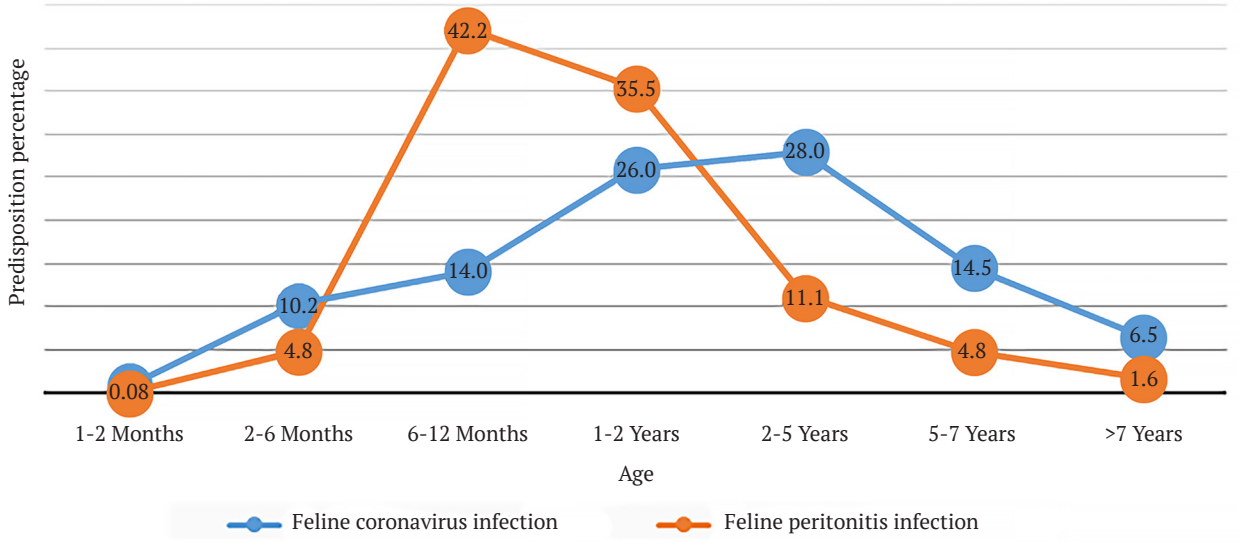

Figure 2. Predisposition to feline infectious peritonitis depending on gender

It was found that animals of geriatric age (older than 7 years) also get sick. 25 such cases were registered, which was 6.5%. Among kittens of the first two months of life, only 3 cases of coronavirus infection were detected, which may be due to infection from the mother. The above results are consistent with the literature data [7; 13].

Among the 63 cats studied with infectious peritonitis, it was determined that the disease is most often registered in young animals (Fig. 2). Thus, the largest proportion of sick cats was found at the age of 6 to 12 months (41.2%) and in the group of 1-2 years (35.5%). There was also 1 case of feline infectious peritonitis in the geriatric group of animals (older than 7 years), which is 1.6%.

Analysis of data from the journal of registration of sick animals for 2020-2022 allowed establishing and determining the seasonal manifestation of feline coronavirus infection (Fig. 3). According to the results obtained, the largest number of cases of feline coronavirus infection was registered in summer (26.8%) and spring (32%), which is confirmed by literature data [12].

Figure 3. Features of seasonal manifestation of feline coronavirus infection

Analysis of the seasonal spread of feline infectious peritonitis showed that at each time of the year, the number of patients with infectious peritonitis in cats had approximately the same number – 20-30%. According to the research of E.N. Barker and S. Tasker (2020) [3] and N.C. Pedersen et al. (2015) [12] feline infectious peritonitis is also seasonally absent.

The breed predisposition to feline coronavirus infection and feline infectious peritonitis was determined. Among 399 positive samples for cat coronavirus infection, mongrel cats (31.3%), Bengal cats (14.8%) and British shorthair cats (17.0%) were found to be the most susceptible to infection (Fig. 4).

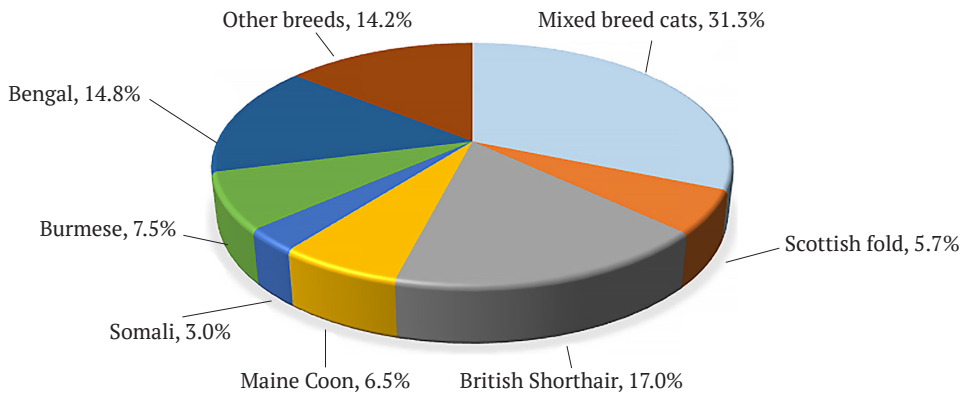

Figure 4. Percentage of breed predisposition of cats to feline coronavirus infection

According to research by S. Felten and K. Hartmann (2019) [10] and N.C. Pedersen (2014) [11] thought that mixed breed cats were more resistant to coronavirus infection, but most authors (Y. Yin, T. Li, C. Wang) [1] are against such a definition. Feline infectious peritonitis was diagnosed in 63 animals. When determining the breed predisposition to this disease, it was found that the largest percentage of sick animals is observed among Burmese cats (33.3%) and mixed breed cats (28.5%) (Fig. 5). Notably, many cases of feline infectious peritonitis were detected in the Bengal breed (13 out of 63).

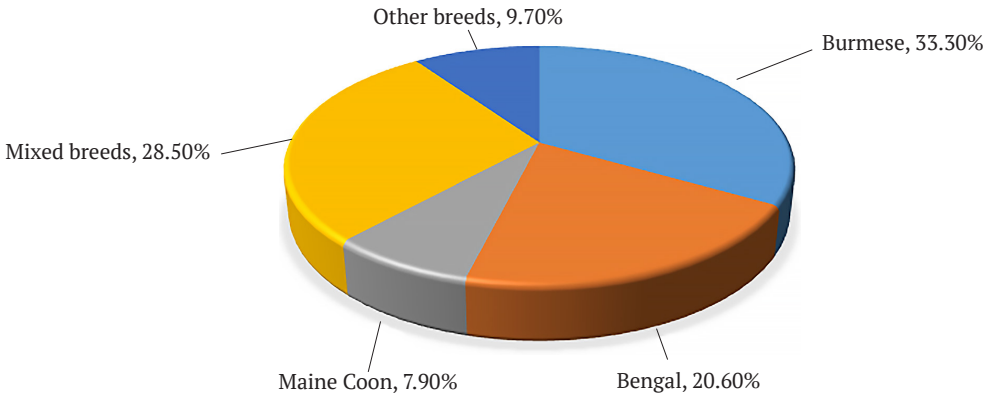

Figure 5. Percentage of breed predisposition of cats to feline infectious peritonitis

Among the analysed literature data, there is no reliable information about the breed predisposition to feline infectious peritonitis. The authors consider these data to be relative due to the diversity of populations in different cities and countries. However, to date, the predisposition to feline infectious peritonitis in Burmese cats has been determined, and the genetic component has been determined by GWAS (genome-wide association studies) [17].

Furthermore, data on the prevalence of forms of feline infectious peritonitis were analysed (Fig. 6). It is known that feline infectious peritonitis occurs in three forms – eufused (wet), dry, and mixed.

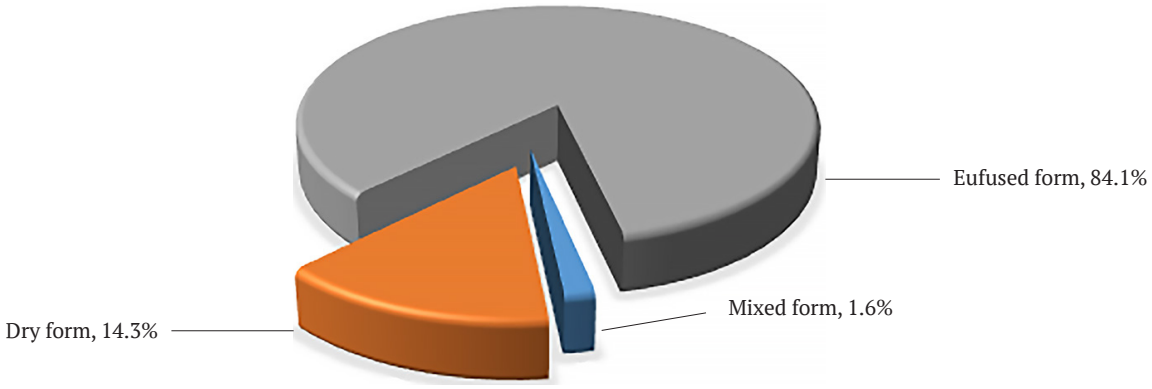

Figure 6. Percentage of breed predisposition of cats to feline infectious peritonitis

Of the 63 reported cases of feline infectious peritonitis, eufusion was detected in 84.1% (53 out of 63), dry – in 14.3% (9 out of 63). One cat had a mixed form of infectious feline peritonitis (1.6%). In this case, the accumulation of exudate in the abdominal cavity, the development of uveitis and keratitis were observed. Y. Yin (2021) et al. found that the eufusion form of feline infectious peritonitis is diagnosed more frequently, i.e., in 85.8% of animals with suspected feline infectious peritonitis [1].

In cats with a dry form of infectious peritonitis, the titre of antibodies to coronavirus was determined. Therewith, an enzyme-linked immunosorbent assay was used to determine the titre of Ig G antibodies. Nine blood sera from cats with a dry form of feline infectious peritonitis were examined in the laboratory.

Thus, it was found that in seven cats with a dry form of feline infectious peritonitis, the antibody titre corresponded to >1:160, in one animal – 1:50, and in another animal <1:20 (Fig. 7). This means that in cats with feline infectious peritonitis, the antibody titre to infection may be low. Since the titre of antibodies based on the clinical symptoms of the disease and a positive PCR test can give a negative result, it is inappropriate to use only ELISA for its diagnosis. N.C. Pedersen (2014) found that the antibody titre in cats with an effusive or dry form of feline infectious peritonitis in the late stages of the disease may be low, but this does not exclude the presence of the pathogen in the animal's body [11].

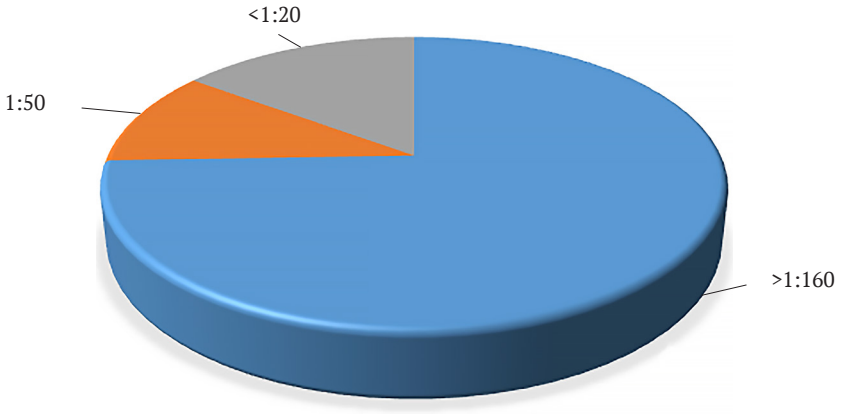

Figure 7. Titre of Ig G antibodies to coronavirus in cats with a dry form of feline infectious peritonitis

It was found that of the three animals under study who had symptoms of rhinitis, one cat was diagnosed with infectious peritonitis by PCR. Therewith, catarrhal discharge from the nasal passages and loss of the sense of smell were noted. E.N. Barker and S. Tasker (2020) [3] proved the possibility of developing rhinitis and conjunctivitis, which are associated with the development of feline infectious peritonitis, which confirms the reported case.

Conclusions

Studies indicate that the most sensitive breeds to feline coronavirus infection are British Shorthair and Bengal, as well as mestizo cats. Burmese and Bengal cat breeds are most susceptible to feline infectious peritonitis.

Along with this, it was found that gender and age do not affect the prevalence of feline coronavirus infection. The risk of feline infectious peritonitis is higher in kittens and animals aged 2 months to 3 years.

It was established that feline coronavirus infection can occur combined with other feline infectious diseases, especially with a complication of feline panleukopenia. At the same time, the coronavirus can be detected in clinically healthy animals that are carriers of the virus.

It was found that 84.1% of cats have a euphusial form of feline infectious peritonitis. One animal showed symptoms of rhinitis and conjunctivitis, which is a possible manifestation of the dry form of feline infectious peritonitis. This disease is non-infectious and occurs because of a mutation of the intestinal type of coronavirus in susceptible animals. Therefore, not all individuals with feline coronavirus infection are patients with feline infectious peritonitis.

Further studies should be aimed at investigating the features of the course, the main clinical symptoms of feline infectious peritonitis. Furthermore, it is necessary to identify the key diagnostic deviations in standard studies (general blood test, biochemical blood test), to establish new areas in the complex diagnosis of feline infectious peritonitis. A prominent issue is the determination of effective ways to diagnose feline infectious peritonitis, as well as the improvement of treatment measures for this disease.

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Епізоотологічні особливості коронавірусної інфекції у котів

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Анотація. Актуальність дослідження коронавірусної інфекції в тварин зумовлена відсутністю достатньої інформації щодо механізмів розвитку цього захворювання, недосконалістю методів діагностики та лікування і, найголовніше, майже 100 % їх летальністю. Метою цієї роботи було визначення вікових, породних, сезонних та статевих особливостей схильності котів до коронавірусної інфекції та розвитку інфекційного перитоніту. У статті наведені результати епізоотоголічних особливостей поширення коронавірусної інфекції серед тварин цього виду та інфекційного перитоніту котів упродовж 2020–2022 років на базі ветеринарної клініки «Білий Вовк» (м. Київ). За цей період було досліджено 483 проби від котів з симптомами коронавірусної інфекції. З цих проб за допомогою імунохроматографічного аналізу у 399 тварин було виявлено вірус родини *Coronaviridae*, а у 63 – встановлено інфекційний перитоніт. У роботі висвітлено результати дослідження вікової, породної та статевої схильності котів до коронавірусної інфекції та розвитку інфекційного перитоніту. Встановлено, що до коронавірусної інфекції є схильними коти будь-якого віку, тоді ж як інфекційний перитоніт розвивається у тварин віком від 2 місяців до 3 років. Коронавірусну інфекцію найчастіше реєстрували у котів-метисів та британських короткошерстних, інфекційний перитоніт – у бурманської та бенгальської їх порід. У статті також узагальнено дані сезонного прояву коронавірусної інфекції та інфекційного перитоніту котів. За результатами епізоотологічного аналізу було сформовано нозологічний профіль інфекційних захворювань у тварин цього виду, які мали схожі клінічні симптоми коронавірусної інфекції та інфекційного перитоніту. Представлено вісім інфекцій, з яких найчастіше реєстрували захворювання, спричинені вірусами родин *Herpesviridae, Caliciviridae* і *Parvoviridae*. Результати цієї роботи вносять нові відомості щодо епізоотологічних особливостей прояву та розвитку коронавірусної інфекції в котів, що дасть можливість не тільки удосконалювати існуючі методи діагностики, а й розробляти нові

Ключові слова: порода, чутливість, сезонність, епізоотичний процес, ПЛР в реальному часі, тест-системи

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The History of the Discovery and Research of Lyme Borreliosis in Animals and Humans

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Abstract. The relevance of this study is conditioned upon the substantial importance of the history of the discovery and research of Lyme borreliosis for medical science and veterinary medicine, since new theories, experiments, conclusions are built on the research of scientists who have proved the existence of this disease in animals and humans, and positive practices are accumulated regarding methods and schemes of treatment and prevention. The purpose of this study was to analyse the literature primary sources covering the history of the discovery, the results of the study of Lyme borreliosis disease to understand the features of its course and search for effective diagnostic and treatment methods. The chronology of the main results confirming the existence of Lyme borreliosis caused by Ixodidae ticks is summarised. Scientific sources covering the history of the discovery and research of Lyme borreliosis were analysed to understand the features of its course and search for effective methods of laboratory diagnostics and treatment. The main projects of the European Union programme aimed at financing research and innovative development "Horizon 2020" were determined. It was found that the projects in the field of Lyme borreliosis research also relate to certain topics, namely the creation of tests for the detection of Lyme disease, the study of the vectors of the causative agents of this disease, the development of an appropriate vaccine, and the assessment of the risks of consequences for human and animal health. It was concluded that the main areas of research are as follows: persistence of diseases and their causative agents transmitted by Ixodidae ticks; natural control of Ixodidae ticks; detection of pathogens in Ixodidae ticks; level of infection of vertebrates; habitats and natural foci of vectors and their hosts; testing and medical trials; effect of magnetic field on Borrelia; genome mapping of Borrelia species, visualisation of the human brain for disease research; research by polymerase chain reaction; endocrine studies; immune complexes, diagnosis of neuropsychiatric complications in children and adults. The practical value of this study lies in the awareness of modern scientists about the chronology of the study of Lyme borreliosis, the main ways of distribution and methods of its diagnosis in animals and humans, determining the prospects for further research according to the topical issues covered in this paper, which are solved within the framework of international projects

Keywords: Ixodidae ticks, Borrelia, spirochaetes, Ixodidae tick bites

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Introduction

Lyme borreliosis is a transmissible, naturally occurring disease that is transmitted by Ixodidae ticks and caused by borrelia (spirochetes). Most often, *Borrelia burgdorferi* is detected, which is transmitted between Ixodidae ticks and their hosts – animals and humans [1]. According to A. Afzelius (1910) [2], A.C. Steere (1977) [3], W. Burgdorfer (1984) [4], N.M. Magpies, V.V. Nedosekov et al. (2020) [5], this disease is described by a variety of clinical symptoms, as well as a tendency to a chronic and latent course in both humans and animals. The natural reservoir of Borrelia (spirochetes) is most often mouse-like rodents, birds, and deer, less so – cattle, dogs, and other animal species [2; 3; 4]. However, the disease had a long history of its discovery, justification, and proof of existence [5].

This paper is devoted to a retrospective of the development and analysis of studies of Lyme borreliosis in animals and humans. The study highlighted the key issues that were investigated by scientists during studies of various diseases in mammals, which gave them the opportunity to draw conclusions about the existence of Lyme borreliosis disease and ways of infection with the pathogen. The analysis of various scientific sources covering the history of the discovery and the results of the study of Lyme borreliosis disease provided an opportunity for scientists to identify the features of the course of this disease and search for effective diagnostic methods and treatment methods.

The historical aspect of the study of Lyme borreliosis was investigated by Ukrainian scientists: V.Yu. Klius (2018) during the study of clinical and instrumental features of chronic forms of Lyme disease with a predominant lesion of the nervous system and improvement of their treatment tactics in humans [6]; I.I. Ben (2019) [7] during working on the definition of clinical and epidemiological characteristics of human granulocytic anaplasmosis and creating an algorithm for diagnosing this disease; V.A. Levytska et al. (2021) in her research on the animal body protection system on transmissible diseases [1]; foreign scientists: M.P. Littman et al. (2018), provided recommendations for the diagnosis, treatment, and prevention of Lyme borreliosis in dogs and cats [8], A.G. Barbour and R.S. Gupta (2021) investigated spirochaetes of the Borrelia genus, especially their morphology, biology, as well as vector and pathogenicity for vertebrates to identify phylogenetic data confirming their conservation by reptiles, monotremes, and birds [9]. Researchers J.R. Bobe et al. also analysed Lyme disease prevention and control methods and considered other issues in this area that have not yet been studied (2021) [10]. X. Zhuang, X. Yang and others (2018) investigated one of the determinants of *Borrelia burgdorferi* virulence, designated in the study as Lmp1 and is a surface, conserved and potential multi-domain protein that takes part in various functions during the invasiveness (infectivity) of Borrelia (spirochaetes) [11; 12].

The purpose of this study was to analyse the literature sources that cover the history of the discovery of Lyme borreliosis, methods for diagnosing this disease and the search for effective treatment.

The objectives of this study were to analyse the state of the issue, its research in scientific publications; to summarise Ukrainian and foreign practices in the historical aspect of the occurrence of Lyme borreliosis, to describe the chronology of research in this area and introduced methods for detecting pathogenic pathogens in Ixodidae ticks.

Results and Discussion

Retrospective analysis of Lyme borreliosis studies

The history of the discovery of Lyme borreliosis begins in 1909, when the Swedish botanist Adam Afzelius described a case of chronic migratory erythema in a person, which occurred at the site of a tick biteRetrospective analysis of Lyme borreliosis studies

The history of the discovery of Lyme borreliosis begins in 1909, when the Swedish botanist Adam Afzelius described a case of chronic migratory erythema in a person, which occurred at the site of a tick bite [5; 9]. A few years later, the Austrian dermatologist B. Lipschutz [9] described similar clinical manifestations in another patient that lasted 7 months and proposed to call them "chronic migratory erythema" (erythema chroniicum migrans, ECM) due to the long-term course.

In further studies, scientists described headaches and neurological disorders, cases of chronic lymphocytic meningitis [13; 14] in humans that occurred after attacks of Ixodidae ticks. Thus, in 1922, two French neurologists, Ch. Garin and B.A. Paralysis [13] from Lyon provided the first description of neurological complications in humans after an attack of Ixodidae ticks and skin lesions. They reported a 58-year-old man who developed reddened, painful, and swollen skin lesions accompanied by inguinal lymphadenopathy a few weeks after being attacked by Ixodidae ticks. This was followed by bilateral gluteal pain, intercostal pain, and finally painful unilateral right shoulder plexopathy with deltoid amyotrophy. The serological test for syphilis (Wasserman test) was weakly positive. The researchers ruled out syphilis as the cause of the clinical manifestations and provided a medical history as an example of "paralysie par les tiques" (tick paralysis), or disorders that can be caused by a tick-borne virus, but the cause was attributed to ticks of the Dermacentor genus, while the patient was found to have *Ixodes hexagonus* [5].

In 1930, S. Hellerstrom [14] recognised a causal relationship between attacks and bites of Ixodidae ticks described by dermatoses and neurological manifestations [15]. In 1941-1944 A. Bannwarth [12] in Germany reported 14 patients who suffered from severe body, leg, and arm pain after being attacked by Ixodidae ticks, facial paralysis, and lymphocytic meningitis. However, the researcher at that time did not establish a link between a previous Ixodidae tick bite, migrating erythema, and neurological disorders, and instead preferred allergies and rheumatism.

The cause of migrating erythema remained unclear and unfounded. At the same time, S. Hellerstrom [14] and W. Burgdorfer et al. [16] testified to the role of spirochetes in the development of erythema migrans, which at that time was investigated by C. Lenhoff [17], a member of the Dermatology Clinic at the Karolinska Institute. He claimed to have developed a staining technique based on the interaction of mercuric chloride with spirochaetes, which enabled him to identify "spirochaete elements" in many human skin lesions of unknown aetiology, including psoriasis, chickenpox, lymphadenosis (benigna cutis), and "erythema-migrant" among other diseases. It turned

out that his staining method is suitable for demonstrating spirochaetes in culture but is not suitable for identifying them in smears or in areas of tissue damage with migrating erythema.

In 1943, B. Bäfverstedt [18] reported on benign lymphadenosis in humans, which developed as a result of an attack of Ixodidae ticks. According to D. Lipsker and B. Jaulhac (2009) [19], Ch. Brunnemann (2010) [20] at that time, researchers observed skin lesions, namely swelling and red spots. At the same time, S. Hellerstrom (1951) [21], E. Binder et al. [22], H. Götz [23], reported that the bite of *I. ricinus* causes oedema and erythema in humans. However, the true aetiology remained unclear until arthritis and erythema were discovered and investigated by scientists in the northeastern United States [24].

More than six decades after the first report by A. Afzelius, in the 1970s [2], W.E. Mast, W.M. Burrows (1976) investigated an unusual epidemic of oligoarticular rheumatoid arthritis in children, adolescents, and adults in Lyme, Connecticut (USA) [25]. At the same time, professor of rheumatology at Harvard University in Lyme, Connecticut (USA) A.C. Steere [3], studying 39 children and 20 adults, found an extraordinary incidence of juvenile idiopathic arthritis, which occurred after an attack of Ixodidae ticks and was often combined with migratory ring-shaped erythema.

Thus, in the 1970s, the number of cases of spotted fever has increased in people who visited or lived in Rocky Mountain Cities [4] (in the southern and north-eastern United States; in Long Island and other coastal areas of New England, and the Massachusetts offshore islands). This disease was called Rocky Mountain spotted fever, (RMSF). RMSF cases were documented by microbiologists W. Burgdorfer and A. Barbaur(1975, 1979, 1982, 1983, 1984) [4; 26].

In 1979, L. Reik et al. [27] identified neurological manifestations among a group of sick people affected by Ixodidae tick attacks. At the same time, neurological and cardiac disorders were added to the already known manifestations on the skin and affected joints, and, in general, a person developed a complex systemic disease [8; 28].

It is also worth paying attention to the research and development of the "Western blot" diagnostic technique, aimed at determining IgM and IgG to specific Borrelia antigens, which was carried out in the 1970s [5].

Most researchers attest that the Western blot evolved from Southern blotting developed by Edwin Southern at the University of Edinburgh in 1975 [5], then Northern blotting [29] developed by George Stark's group at Stanford in 1977 [30]. The results of these studies contributed to further improvement of methods for detecting proteins using antibodies, which improved the quality of diagnosis of Lyme borreliosis in both humans and animals [31].

Following the observations made in Old Lyme [32], a correlation was noted between skin rashes in sick people who suffered from Ixodidae tick attacks and arthritis. For epidemiological reasons, further studies have shown that erythema migrans and arthritis are tick-borne diseases and are associated with a carrier *Ixodes scapularis* [32].

Isolation of spirochetes from the intestines of *I. scapularis* ticks by W. Burgdorfer et al. [16] for the first time testified to the presence of an aetiological factor causing this disease, since these pathogenic microorganisms demonstrated a reaction with immune sera from sick people (USA) who suffered tick attacks and Lyme disease [28; 33].

Thus, the causative agent and carrier of Lyme disease were identified simultaneously. Just a few months later, similar spirochaetes were also isolated in Europe from *I. ricinus*, which is closely related to *I. scapularis* [8]. Extra studies conducted in the same and subsequent years confirmed Burgdorfer's conclusions [3; 9; 34]. In 1984, spirochaetes were named after their discoverer as *Borrelia burgdorferi* [35]. Furthermore, the disease was named after the discovery site as "Lyme borreliosis".

The existence of Lyme borreliosis caused by Ixodidae ticks is evidenced by historical data summarised in Table 1.

Table 1. Chronology of the main results confirming the existence of Lyme borreliosis caused by Ixodidae ticks

Year scientific research	Scientist, country	Results of studies confirming Lyme borreliosis
1910	Arvid Azfelius, Sweden [2]	Erythema migrans in humans are described
1913	Lipschütz, Austria [34]	The term "chronic migratory erythema" (erythema chroniicum migrans, ECM) is proposed
1922	Garin and Bujadoux, France [13]	Meningopolyneuritis in a person after an attack of Ixodidae ticks and its probable connection with erythema migrans are described
1930	Hellerström, Sweden [21]	Meningitis is described and a cause-and-effect relationship between Ixodidae tick attacks and neurological manifestations and characteristic dermatoses in humans is established
1943	Bo Bäfverstedt, Sweden [18]	Lymphadenosis (benigna cutis) is described
1944	Bannwarth, Germany [12]	Further descriptions of meningopolineuritis caused by Ixodidae tick attacks
1948	Carl Lennhoff, Germany [17]	Spirochaetes are reported in human tissue samples from biopsies (however, these were artefacts)

Table 1, Continued

1949	Hellerström, Sweden [21]	A suggestion appeared that spirochaetes may be the cause of human disease
1949	Thyresson, Sweden [37]	Successful treatment of sick people with penicillin for chronic acrodermatitis
1951	Hellerström, Sweden [21]	Successful treatment of people after attacks of Ixodidae ticks
1954	Gotz, Germany [23]	It was established that Ixodidae ticks are the carrier of chronic human acrodermatitis (Acrodermatitis chronica atrophicans)
1955	Binder et al., Germany [16]	A link was found between arthritis in humans and attacks of Ixodidae ticks
1974	K. Weber, Germany [36]	Successful treatment of people with ECM with antibiotics
1975-1979	E.M. Southern (1975) [37]; J.C. Alwine, D.J. Kemp, G.R. Stark (1977) [38]	The "Western blot" diagnostic method was developed
1983	W. Burgdorfer, USA [26]	Spirochetes were first isolated from the intestines of ticks *I. scapularis*, which confirms the aetiological factor that causes Lyme borreliosis
1985	V. Preac-Mursic [32]	Damage to the central nervous system in humans after an attack of Ixodidae ticks was described
1984-1985	F.W. Hyde, R.C. Johnson [33]	Cultivation of *B. burgdor*feri in skin samples and in fluids was developed

According to the literature, occurrence of Lyme borreliosis is geographically associated with mountainous and wet areas in Sweden, Germany, and the United States [39-41]. At the same time, these studies are becoming global in the 21st century due to environmental changes, the development of microbiology, epizootology, modern technologies, and the latest methods for diagnosing transmissible infections and infestations [5; 42].

In 1991, the Lyme Disease Association was established in the United States, [40] and in 2000 it became an official national organisation. Its mission was to promote awareness and control of the spread of Lyme disease and other tick-borne diseases; to identify and investigate complications using modern diagnostic methods; to raise and allocate funds for research and other issues related to Lyme and tuberculosis; to provide monetary support to seriously ill people with transmissible diseases and conduct educational activities. The Association's website [40] covers the following issues:

– conducting scientific conferences (archives, videos, data on requirements for scientific research, conditions of participation, etc.);

– regarding Lyme and other transmissible diseases (diagnosis, statistics, etc.);

– maps of registered transmissible diseases and their spread in the United States and other countries (https://lymediseaseassociation.org/LDA_Apps/content/Maps/index.html);

– reports on cases of Lyme borreliosis in humans;

– description of grants and requirements for scientific research submitted for grants;

– extra information for sick people (advanced methods of treatment of patients with Lyme borreliosis, methods of diagnosis, testing, characteristic and uncharacteristic clinical manifestations of the disease, treatment regimens, complications, genomics, vector transmission, certain aspects of pathogenesis, resistance of pathogens to medications, prevention measures, financing, long-term course of the disease and its impact on the general condition of the patient, care centres, etc.);

– on preventive measures for Lyme borreliosis in pets (problems that pet owners face when detecting the disease; measures and means of prevention; a list of acaricidal drugs to prevent attacks of Ixodidae ticks; diagnostic methods and approaches in the treatment of animals with transmissible diseases, etc.).

Lyme Disease Association Inc. Columbia University and the Vagelos College of Physicians and surgeons have been jointly hosting the Annual Scientific Conference "Lyme and other tick-borne diseases: research for treatment" since 2000 [41]. Each conference presents reports from more than a dozen national and international scientists and physicians who speak on a wide variety of topics, including epidemiology, information on pathogens that cause tick-borne diseases, diagnostic methods, treatments, cognitive difficulties, vaccines, veterinary problems, the spread of Ixodidae ticks, and genome mapping.

Notably, the Association awarded 122 grants for research on Lyme disease and diseases transmitted by Ixodidae ticks. In the United States, and in partnership with a branch, the Association established the world's first Centre for the study of the chronic course of Lyme disease, which was opened at Columbia University in 2007. The centre brings together researchers from various fields of medicine and veterinary medicine from all over the United States [42].

Since 1999, the Association has sponsored 20 scientific and medical conferences and 18 conferences held jointly with the Columbia University College of Physicians and Surgeons. It also funds cutting-edge research projects with more than 36 different researchers and institutions across the country, including Columbia University College of Physicians and Surgeons, Medical School of New Jersey, Fox Chase Cancer Centre, UC Davis, University of Pennsylvania, Bringham & Women's Hospital, New York College of Medicine, Rockefeller University, Tulane Regional Primate Center, University of North Florida, NIH/NASA and UDSA, etc. [42].

The results of the Association's research projects are published in 56 peer-reviewed scientific journals, such as the American Medical Association (JAMA), The Proceedings of the National Academy of Science, The Psychiatric Clinics of North America, Infection; Psychiatric Clinic of North America; Neurology, JSTBD, Clinical Microbiology, International Neuropsychological Society and Infection and Immunology; New, Infectious Diseases (CDC); Bacteriology, Entomology; Neuropsychiatry and Clinical Neurosciences; International Journal; Neuropsychological Society; Infection and immunity; Gene, Genetics and PLOS 1 [42].

According to the results of the project of the "LymeAid 4 Kids" Association, the data were used to apply for an NIH grant in the amount of 4.7 million US dollars. Considerable genome mapping, initially funded by the Association, has shown that different Borrelia species can exchange genetic material with each other, which is beneficial for their survival and probably interferes with the ability of an infected human or animal body to fight pathogens [43; 44].

Current state of diagnosis, prevention, and treatment of Lyme borreliosis in humans and animals

From 2012 to 2021, many projects related to research in the field of Lyme borreliosis are being implemented under the program of the European Union aimed at financing research and innovative development "Horizon 2020" [45]. 68 results were found for the keywords "Lyme borreliosis" on the EU website "Horizon2020" [45].

The analysed projects allowed identifying the main topics of research on this disease, the which relevance is timeless.

Thus, in projects that have won the competition in the EU Horizon 2020 programme, scientists pay attention to [45]:

– creating tests to detect Lyme disease, for example:

"DualDur: A Disruptive Diagnostic Technology that Enables for the First Time an Early and Accurate Diagnosis of the tick-borne Lyme Disease", 2018-2021, 2024, Hungary [46];

"Development of a prophylactic vaccine and diagnostic markers to prevent and diagnose Lyme borreliosis specific to Europe and North America", 2004-2007, Austria [47];

"A novel immunity-based test for early diagnosis of Lyme disease", 2016-2020, Netherlands, Germany, Austria [48];

"Highly sensitive and specific low-cost lab-on-a-chip system for Lyme disease diagnosis", 2010-2013, Spain, Germany, Portugal, Italy, Finland, Belgium [49];

"Demonstration Activities for the clinical validation of the prototype HILYSENS Lab-on-a-Chip", 2014-2016, Portugal, Spain, Germany, Italy, Sweden [50];

– research of Lyme borreliosis carriers, for example:

"Exploring the salivary transcriptome of Ixodes ricinus, the Lyme disease vector in Europe, and the potential role of its cystatins in pathogen transmission", 2010-2014, Czech Republic [50];

"Co-evolution and implementations of vector adaptation: a case study on seabird ticks and Borrelia", 2008-2010, France [51];

"Effects of co-infections on the emergence of an avian disease Mycoplasma gallisepticum", 2018-2022, Belgium [52];

"Phylogeny of borrelia genus", 1996-1999, France, Spain [53];

"Co-evolution and implementations of vector adaptation: a case study on seabird ticks and Borrelia", 2008-2010, France [54];

– development of a vaccine against Lyme borreliosis, for example:

"Development of a prophylactic vaccine and diagnostic markers to prevent and diagnose Lyme borreliosis specific to Europe and North America", 2004-2007, Austria, Czech Republic, Germany, Sweden [55];

"Functional genomics study of lysyl-trna synthesis as a target for the diagnosis and treatment of microbial infections and mitochondrial myopathies", 2000-2003, France, Ireland, Germany [56];

"Anti-tick Vaccines to Prevent Tick-borne Diseases in Europe", 2013-2018, Netherlands, Slovakia, Czech Republic, Spain, Germany [57];

– assessment of the risks of Lyme borreliosis consequences in relation to human and animal health for example:

"Lyme borreliosis in north Africa: risk assessment and implementations for tick management and for control of the human disease", 2000-2004, France, Argentina, Tunisia, Morocco, Portugal, Belgium, Switzerland [58];

"Complexity and predictability of epidemics: towards a computational infrastructure for epidemic forecasts", 2008-2013, Italy [59].

Notably, all projects that take place under the EU programme Horizon2020 have substantial results and are published in journals indexed by international databases such as Scopus and Web of Science. For instance, according to the project "A novel immunity-based test for early diagnosis of Lyme disease" [60], the following articles were published in 2020: "Allergenomics of the tick *Ixodes ricinus* reveals important α-Gal–carrying IgE-binding proteins in red meat allergy" [61].

Within the framework of the new research and prospects for studying Lyme disease, scientists propose to continue investigating the system of antigenic variations encoded in the vls system, which allows tracking genetic changes in *B. Burgdorferi* using the next-generation DNA sequencing method developed by Pacific Biosciences [62]. As evidenced by C. Winslow, J. Coburn, the mechanisms of this segmental system of gene conversion and the system of antigenic variations that enable *B. burgdorferi* to maintain invasions (infections) deserve additional study. Scientists also consider it important to identify and investigate more genes necessary for invasiveness (infectivity) and virulence of *B. burgdorferi*, which will expand the understanding of pathogen biology and contribute to the improvement of the proposed tests and vaccines [63].

Within the framework of the new EU programme Horizon Europe [64], conducted since 2021, research on Lyme borreliosis continues.

Under such conditions, researchers pay special attention to the study of the causative agent *Borrelia burgdorferi*. Thus, for example, researchers T. Casselli, A. Divan, E.E. Vomhof-DeKrey, H.L. Pecoraro, C.A. Brissette [65] conducted experiments on the infection of white mice with Lyme disease pathogens and *Borrelia burgdorferi*. A murine model of Lyme disease demonstrates that *Borrelia burgdorferi* colonizes the dura mater and induces inflammation in the central nervous system The researchers concluded that the results of such studies provide insight into the potential mechanisms of pathologies of the central nervous system (CNS) in humans and animals associated with Lyme disease, and also describe a model system that will allow future research to assess bacterial and host and environmental factors that may contribute to the severity of CNS damage by the *B. burgdorferi* pathogen [65].

Lyme borreliosis research in Ukraine

In Ukraine, the first cases of the disease in humans have been known since 1994. However, official registration of the disease began in 2000 [66]. The Order of the Ministry of Healthcare of Ukraine [66] states that 562 cases of this disease were detected in 2000-2004. According to the "List of enzootic territories of Ukraine with tularaemia, leptospirosis, other particularly dangerous infections and their prevention measures for 1999-2003" [66], among all regions of Ukraine, only in the territory of Kirovohrad and Luhansk regions, no cases of the disease were registered.

In 1995-2004, the Danylo Halytskyi Scientific Research Institute of Epidemiology and Hygiene of Lviv National Medical University [7] at the request of medical and preventive institutions conducted laboratory examinations of 1,907 sick people from 20 regions of Ukraine. At the same time, 1108 (58.1%) seropositive individuals were identified, of which the diagnosis was confirmed in 412 (37.2%) cases. It is important to note that the role of pathogens in the infectious pathology of the population was confirmed in the studies; various clinical manifestations of the disease (Afcelius erythema, ring-shaped erythema mite, acrodermatitis, chronic atrophic acrodermatitis, skin lymphadenosis, serous meningitis, radiculoneuritis, Bannwart's lymphocytic meningoradiculoneuritis (Bannwart's syndrome), chronic arthritis, etc.).

It is also important to pay attention to pharmaceutical research for the treatment of Lyme borreliosis in humans and animals. Initially, researchers and veterinary doctors chose specific therapeutic paints that proved effective for some protozoal diseases in animals caused by arthropods and Ixodidae ticks [1]. Thus, among the pharmaceutical products used in the world veterinary practices for the treatment of animals with piroplasmidosis, drugs with an active basis have received the widest recognition – Ionic associate of 4,4-(diazoamino)-dibenzimidine with N-acetyl glycinate (diminazene aceturate) [1; 67; 68]. This active substance is active against protozoan pathogens from the genera *Babezia, Trypanosoma* and *Thelleria*, and has an antibacterial and fungistatic effect. In pharmaceuticals and preparations, this active substance is combined with an antipyretic and an analgesic – phenazone (azidine, azidine-vet, batrizan, berenil, bbveriben, diamidine, neosidine, pyrosan, trypanol, etc.). In the 1970s, the above-mentioned manufacturers in their guidelines recommended using this drug in the form of injections only for productive animals (large and small cattle) in a 7% aqueous solution at the rate of 3.5 mg of diminazene per 1 kg of body weight, and for horses – 5 mg/kg. For these species of animals, it was recommended to re-introduce the drug solution after a day in the same dose [8]. Over time, Ukrainian scientists and practitioners began to introduce azidine to sick dogs in the form of a 7% solution at the rate of 3.5 mg/kg [61]. In Ukraine, the production of a drug called azidine-vet based on diminazene was first started at Brovapharma LLC in 2000 [61]. However, it was already clear at that time that not all animals could easily tolerate the above suggested doses of the drug. The animals also did not always recover. They frequently had complications from both the disease and these drugs. Therefore, veterinary doctors conducted more studies in practice and determined the effectiveness of new drugs for Lyme borreliosis.

K. Stiasny et al. conducted an experiment, which allowed determining that the most successful is the treatment of animals and humans within 24-48 h after the diagnosis of the disease [69]. They also noted that treating animals and humans with arthritis without intensive and prolonged administration of antibiotics does not contribute to recovery and restoration of limb function.

For Lyme borreliosis, according to V.F. Galat [70], the most effective are antibiotics of the tetracycline series – tetracycline, doxycycline hydrochloride, oxytetracycline hydrochloride, etc. However, other groups of antibiotics are also used, namely penicillin – benzylpenicillin sodium salt, Bicillin-1, 3, 5; ampicillin, ampiox, etc.; groups of cephalosporins – claforan, cefuroxime, cefazolin, etc. [70].

For erythema migrans, azithromycin (sumamed) is prescribed – 1 g on the first day, and then 500 mg for 4 days (children – 10 mg/kg). If a person was not treated at an early stage, then drugs are used depending on the lesions [71]:

– neuroborreliosis – ceftriaxone (or cefotaxime), 2 g intravenously for 2 weeks (sometimes up to 1 month);

– arthritis and cardioborreliosis – doxycycline 100 mg 2 times a day for 2 weeks (sometimes up to 1 month);

– chronic atrophic acrodermatitis – ceftriaxone, 2 g for 3 weeks (from 14 to 30 days) or doxycycline, amoxicillin, etc.

Currently, according to N.M. Soroka and V.V. Nedosekov [5], a vaccine has been developed for humans and animals (artificially synthesised surface protein OspA), which is considered an effective means of preventing Lyme borreliosis.

Conclusions

An analysis of historical data on the discovery and research of Lyme borreliosis allowed describing the chronology of the main results supporting the existence of Lyme borreliosis caused by Ixodidae ticks, which included the following events: the recording and description of erythema migrans in humans; definition of the term "chronic migratory erythema"; description of meningopolyneuritis in a person after an attack of Ixodidae ticks and putting forward a version regarding its connection with erythema migrans; establishment of a cause-and-effect relationship between attacks of Ixodidae ticks and neurological manifestations

and characteristic dermatoses in humans and further research of this problem; detection of Borrelia (spirochaetes) in biomaterials of people with migratory erythema and without erythema, but with nervous disorders; successful treatment of sick people with penicillin for chronic acrodermatitis and after attacks of Ixodidae ticks; establishing that the carrier of human chronic acrodermatitis is Ixodidae ticks; successful treatment of people with chronic erythema migrans with antibiotics; development of the "Western blot" diagnostic method; isolation of spirochaetes from the intestines of *I. scapularis* ticks, which confirms the aetiological factor that causes Lyme borreliosis; development of methods of cultivation of *B. burgdorferi* in skin samples and in fluids. According to these discoveries, research projects are being continued in the world practice in such areas as identifying the characteristics of the kinetics of *B. burgdorferi* colonisation and related immune reactions in the central nervous system of animals during the early and subacute stages of invasion; development and testing of immune-based tests for early diagnosis of Lyme disease; study of the influence of ecology on the genome of *B. burgdorferi*, etc.

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Історія відкриття та досліджень Лайм-бореліозу в тварини і людини

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Анотація. Актуальність статті зумовлена суттєвим значенням історії відкриття та досліджень Лайм-бореліозу для медичної науки і, зокрема, ветеринарної медицини, оскільки на дослідженнях учених, які довели існування цієї хвороби у тварин і людини, будуються нові теорії, експерименти, висновки, накопичується позитивний досвід щодо методів і схем лікування та профілактики. Метою дослідження був аналіз літературних першоджерел із висвітлення історії відкриття, результатів дослідження хвороби Лайм-бореліозу для розуміння особливостей її перебігу та пошуку ефективних методів діагностики та засобів лікування. Узагальнено хронологію основних результатів, що підтверджують існування Лайм-бореліозу, спричиненого іксодовими кліщами. Проаналізовано наукові джерела з висвітлення історії відкриття та досліджень Лайм-бореліозу для розуміння особливостей її перебігу та пошуку ефективних методів лабораторної діагностики та засобів лікування. Визначено основні проєкти програми Європейського Союзу, які спрямовані на фінансування досліджень та інноваційних розробок «Горизонт 2020». З'ясовано, що проєкти з напряму дослідження Лайм-бореліозу також стосуються певних тем, зокрема, створення тестів для виявлення хвороби Лайма, дослідження переносників збудників цієї хвороби, розроблення відповідної вакцини, оцінювання ризиків наслідків щодо здоров'я людини та тварин. Зроблені висновки, що основними напрямами роботи є: стійкість хвороб і їхніх збудників, які передаються іксодовими кліщами; природний контроль іксодових кліщів; виявлення патогенних збудників в іксодових кліщах; рівень зараження хребетних тварин; ареали та природні вогнища переносників і їх хазяїв; тестування та лікувальні випробування; вплив магнітного поля на борелій; картографування геному видів Borrelia, візуалізація мозку людини за дослідження хвороби; дослідження за полімеразною ланцюговою реакцією; ендокринні дослідження; імунні комплекси, діагностика нейропсихіатричних ускладнень у дітей і дорослих. Практична цінність наукового дослідження полягає у поінформованості сучасних науковців щодо хронології вивчення захворювання на Лайм-бореліоз, основних шляхів розповсюдження і методів його діагностики у тварини і людини, визначення перспектив подальших досліджень згідно з висвітленими у статті актуальними проблемами, що вирішуються у межах міжнародних проєктів

Ключові слова: іксодові кліщі, борелії, спірохети, укуси іксодових кліщів

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Distribution of *Toxoplasma Gondii* among Cattle in Certain Regions of Ukraine

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Abstract. *Toxoplasma gondii* is the agent of toxoplasmosis, which is common to both humans and cattle. That is why the goal of work was to study the spreading of the agent of toxoplasmosis among cattle in certain regions of Ukraine, which in turn will make it possible to predict the appearance of the disease among people and prevent the development of its clinical signs. Whole blood or serum samples of cattle of different ages and sexes from farms in Kyiv, Zhytomyr, Khmelnytskyi and Lviv regions of Ukraine were sent to the laboratory for research. The presence of total antibodies to *T. gondii* was determined in the serum of the animals. To do this, we used the method of enzyme-linked immunosorbent assay using test kits from different manufacturers in accordance with the guidelines. In general, the prevalence of *T. gondii* among cattle in Kyiv, Zhytomyr, Khmelnytskyi and Lviv regions of Ukraine according to the results of studies was 10.3% (95% confidence interval: 6.7-15.1). At the same time, the study of animals was carried out in two stages, in 2014 and 2018, and the prevalence did not differ significantly. Thus, in 2014, according to the results of research, a positive reaction to the agent of toxoplasmosis was detected in 10 samples or 13.9% (95% confidence interval: 7.3-23.4), while in 2018 this figure was 11 samples or 8.4% (95% confidence interval: 4.5-14.1) of the studied animals. Also, in 2014 and 2018 studies found that the highest level of seroprevalence of the agent of toxoplasmosis in both years of research was registered in cattle from farms of Zhytomyr and Kyiv regions was 11.9% (95% confidence interval: 4.5-24.4) and 11% (95% confidence interval: 5.9-18.3), respectively. During the conducted research, an increase in the prevalence of *T. gondii* with the age of animals was statistically significant. Among animals under six months of age, of the 65 serum samples tested, 3 tested positively to the agent (4.6; 95% confidence interval: 1.2-12.1), while in the group of animals older than three years (49 cows) 10 samples were positively responded (20.4; 95% confidence interval: 10.9-33.4) compared to the previous group of animals, the P-value was 0.0114. So, the data obtained indicate the presence of the agent of toxoplasmosis among cattle in Ukraine, which requires further study and development of approaches to prevent human infection when eating meat and dairy products of positively reacting animals

Keywords: toxoplasmosis, serology, enzyme-linked immunosorbent assay, Kyiv, Zhytomyr, Khmelnytskyi, Lviv regions

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Introduction

Toxoplasmosis is a common protozoan disease of various animal and human species in the world, which is caused by parasitisation of a single-celled *Toxoplasma gondii* [1; 2; 3]. It is recorded both in cattle and among other animal species. Infection with human toxoplasmosis is possible in utero, if invasive oocysts will be swallowed, or when eating insufficiently heat-treated meat and dairy products of productive animals. The definitive hosts of the agent of toxoplasmosis are representatives of the Felidae family, most often – pets, namely cats. Intermediate hosts include vertebrates, as well as humans.

The percentage of people affected by the agent of toxoplasmosis in different countries of the world is considerable. Thus, according to the results of serological studies using the indirect immunofluorescence method for the presence of antibodies against *T. gondii*, the preva- lence rate among pregnant women in Brazil was 26.8% [4]. According to other Brazilian scientists [5], the presence of antibodies against T. gondii with a profile indicating an acute stage of invasion, accounted for 12.3% of positive cases among people. In [5], the authors also conducted a study of possible sources of human infection – grain cheese, water, and vegetables. According to the results of the conducted studies, fragments of the DNA of the agent of toxoplasmosis were found in two of the samples of grain cheese under study (p=0.01).

Infection with *T. gondii* is possible both in utero, and when invasive oocysts of the agent enter the body, or when eating insufficiently heat-treated meat and dairy products. Thus, according to research results in the Netherlands, beef causes 84% of the total number of suspected (probable) toxoplasmosis infestations among the population, followed by pork (12.0%) and lamb (3.7%) [6]. Therefore, it is important to consider meat processing methods to minimise the risk of toxoplasmosis.

Due to the presence of diverse ways of infection of animals and humans with toxoplasmosis researchers are investigating the issue of prevalence of *T. gondii*, which is quite different depending on many factors. For example, in Brazil, out of 2970 cows, the prevalence of *T. gondii* was found, on average, at the level of 8.5% (the 95% confidence interval was 7.48 to 9.49) [7]. In the Cerrado (Brazilian savanna) of Brazil the prevalence of the agent was 8.9% (137/1533) [8]. Other studies [9] from this country show that the overall prevalence was already 30.9% (332/1073) and covered 93.6% of the farms under study (44/47). All subpopulations investigated in Amazonas had herds of cattle and reacted positively to the *T. gondii* in animals, and in some areas, there was a higher level of prevalence [9]. In general, the frequency of detection of antibodies against *T. gondii* in Brazil's cattle range from 1% to 89.1%, depending on the region, based on data from 1978 to 2018 [10]. In 70% of cases, ruminant studies in Brazil were carried out using the indirect fluorescent method [10]. Based on these data, schemes were constructed to minimise the risks of toxoplasmosis infection and awareness trainings were carried out among the population [10; 11].

Currently, there are no effective specific treatments for toxoplasmosis. Thus, to prevent symptoms, including abortion in the case of human toxoplasmosis, it is necessary to develop new drugs and vaccines. A promising new method for molecular characterisation of organisms is mass spectrometric imaging (MSI) with high resolution [12], ezetimibe, which blocks the replication of tachyzoites of *T. gondii*, *Besnoitia besnoiti*, and *Neospora caninum* in primary bovine host endothelial cells, is being tested in hu- man medicine [13], bovine bone marrow-derived macrophages (BMDM), pre-stimulated with interferon gamma (IFN-γ), limiting the growth of intracellular toxoplasmas independently of nitric oxide [14]. Currently, measures to combat *T. gondii* are insufficient [15] due to the lack of an effective vaccine or effective treatment methods. At the same time, according to scientists, successful candidate vaccines should have the ability to induce a cellular immune response and the production of interferon gamma IFN-γ, but their search and development are still ongoing [15].

Data on the spread of the agent of toxoplasmosis on the territory of Ukraine are quite limited. Distribution has not been investigated among all animal species, but only among a few, and there is no official statistical information. Preliminary studies have developed methods [16; 17] and established the seroprevalence among various animal species, namely among horses [18; 19].

The purpose of this study is to investigate the prevalence of the causative agent of toxoplasmosis among cattle in the Kyiv, Zhytomyr, Khmelnytskyi, and Lviv regions of Ukraine.

Literature Review

According to scientists [20; 21] the presence of antibodies against *T. gondii* in women can pose a threat to the foetus, in case of pregnancy, and the prevalence of the agent in different regions of the world is considerable and ranges from 7% to 51%. According to a meta-analysis of the PubMed, WoS, Scopus, Science Direct, Google Scholar, and ISC databases, a quarter of livestock and poultry were infected with *T. gondii* between 2000 and 2019 [22]. However, data on prevalence vary depending on the types of animals, their age, and the country of study. Thus, in Italy, the prevalence of *T. gondii* was recorded at 10.2%, with differences between regions and values from 5.3% in Liguria to 18.6% in the Piedmont region (p=0.0001) [23]. Animals of different age groups and genders responded positively without substantial differences (age and gender: P value <0.05). Lower values of the prevalence of toxoplasmosis agent were recorded in cattle born in Italy (8.7%), compared with animals imported from abroad (13.4%) (p=0.046) [23]. Prevalence of *T. gondii* according to the results of serological studies in Iran, accounted for 13.0% of cattle [24]. 5.4% of animal tissue samples from dairy farms in Iran were found to be positive when tested using molecular methods for the presence of *T. gondii* [25]. The prevalence in Algeria was 29.8% (37/124) for *C. abortus* and *T. gondii* [26].

According to the meta-analysis, the prevalence of *T. gondii* in cattle in China from 2010 to 2019 was 10.1% (4217/39 274). At the same time, it was highest in southwest China (21.6%, 727/3117) and lowest in northern China (4.5%, 185/1966) [27].

The method of immunoenzymatic analysis based on the surface antigen of *T. gondii* (TgSAG2t) for the detection of both specific IgM and IgG revealed 1.9% (IgM) and 3.1% (IgG) of positively reacting animals among cattle in the Menoufiya province of Egypt [28]. Among the studied buffaloes, the prevalence of *T. gondii* was 9.0% (IgM) and

8.2% (IgG). The mixed invasion rate was 1.5% among cattle and 4.9% among buffaloes. No significant differences were found by age or gender. Statistically significant changes in the prevalence of parasites relative to the period of the year were demonstrated [28]. Using the same method, other researchers in Egypt identified 38.9% of positively responding animals [29].

In general, the prevalence of *T. gondii* during the study of cattle carcasses (n = 2912) was 17.4% [30]. Most of the buffaloes that reacted positively to the agent of toxoplasmosis detected by the modified agglutination method had antibodies in low serum dilutions, while the highest dilution rate was 1:768 in females aged 30 months [31].

When studying the consistency of the modified agglutination method and enzyme immunoassay, the prevalence of *T. gondii* was 8.1 and 6.6%, respectively [31]. These two methods are most widely used in the world for monitoring studies among different animal species for toxoplasmosis.

B1 and P30-specific polymerase chain reaction can detect *T. gondii* according to the authors of the study, blood samples are more accurate than enzyme-linked immunosorbent assay [29; 32]. At the same time, the researchers found no correlation between the direct and indirect method of detecting tthe agent of toxoplasmosis. Thus, *T. gondii* was isolated from the tissues of the diaphragm in a 30-month-old male buffalo using real-time quantitative polymerase chain reaction (PCR) and from the tissues of a mesenteric lymph node in a female buffalo. The results of studies of both animals were negative using modified agglutination and enzyme-linked immunosorbent assay methods. At the same time, infection of laboratory animals did not occur, which may indicate the non-viability of the agent [31].

Since toxoplasmosis is the cause of abortions among animals [33; 34; 35], thanks to the use of the PCR method in the territory of north-western Tunisia, 5% of positive samples were found among the uterine tissues tested for the presence of *T. gondii* DNA. At the same time, simultaneous involvement of the last two pathogens was recorded – *T. gondii* and *Neospora caninum* at the level of 2.85% [36].

In the Mazandaran province of northern Iran, *T. gondii* was verified using a specific polymerase chain reaction in 13 (18.6%) animal foetal tissue samples [37]. Another group of researchers found that the prevalence of *T. gondii* DNA in cattle was 56%. The most infected tissue with the agent was the diaphragm (54.4%), followed by the heart (48.8%) and tongue (43.2%). The most common genotype (70%) among *T. gondii* isolates was type II [38]. This is the same type of agent strain that other researchers have discovered. But at the same time, they pointed out the probability of difficulties in isolating parasites from beef [30].

Viable strain of *T. gondii* was isolated from the blood of 1 out of 60 pregnant cows in Brazil, as well as from the tissues of one foetus. At the same time, researchers believe that the role of beef in the epidemiology of *T. gondii* invasion is still not fully understood [39].

During experimental *T. gondii* infection of buffaloes inoculated with *T. gondii* strain GT1, five animals developed moderate anorexia, weakness, and shortness of breath, and one animal died 11 days after inoculation of the agent. The researchers note that the development of an immunological response occurred 21 days after inoculation of the agent, and the antibodies persisted for 63 days, and then fell below the limit value. Using a mouse bioassay, *T. gondii* was isolated from the brain, lungs, liver, kidneys, lymph nodes, and spleen of a calf that died of acute toxoplasmosis [40].

Materials and Methods

The study was carried out based on the Department of Pharmacology, Parasitology, and Tropical Veterinary Medicine of the Faculty of Veterinary Medicine, the Interdepartmental Educational and Scientific Laboratory of Veterinary and Diagnostic Research and the Ukrainian Laboratory of Quality and Safety of Products of the Agro-Industrial Complex of the National University of Life and Environmental Sciences of Ukraine, as well as in the Institute of Veterinary Medicine of the National Academy of Agrarian Sciences of Ukraine (Research Training Centre for Diagnostics of Animal Diseases).

Blood serum samples from 203 cattle animals were examined. Samples for the study were selected in 2014 and 2018. 72 bovine blood serum samples were analysed in 2014. In 2018, serum samples from 131 animals were examined.

For research, samples of blood or blood serum of cattle of different ages and genders selected by veterinary doctors were sent to the laboratory. The animals were from farms in Kyiv, Zhytomyr, Khmelnytskyi, and Lviv regions. All samples were numbered and had accompanying animal information.

If whole blood was sent to the laboratory, serum was prepared from it. For this, blood samples were placed first in a room temperature for 30 minutes, and then in the refrigerator for 12-24 hours, after which the serum was separated using a dispenser and transferred to a clean test tube. If necessary (in the presence of red blood cells), the samples were centrifuged at 3000 rpm for 3 minutes. After that, the blood serum was transferred to a clean test tube. Before the study, the samples were stored in the refrigerator at +2–(+4) °C. The presence of total antibodies to *T. gondii* was determined in the blood serum of animals. For this, the authors of this study used the method of enzyme-linked immunosorbent assay using a test kit to detect total antibodies to *T. gondii* according to the manufacturer's instructions. For this purpose, 100 µL of control positive, negative, and weakly positive samples were introduced in the repeat, as well as 100 µL of blood serum samples that were to be analysed. Incubated for 30 minutes at 37°C. Washed with a washing solution, filling each well with 400 µL, repeated 5 times. 100 µL of conjugate was added to each well. Incubated for 30 minutes at 37 °C. Washed with a washing solution, filling each well with 400 µL, repeated 5 times. 100 µL of tetramethylbenzidine solution was added to each well. Incubated for 25 minutes at 18-25 °C in the dark. 100 µL of stop reagent solution was added to each well. The optical density was measured at 450 nm at a reference wavelength of 620-655 nm. The obtained numerical results were processed statistically using the OpenEpi software [41].

Results and Discussion

For the first time in Ukraine, the authors conducted monitoring studies to detect antibodies to the agent of toxoplasmosis in bovine blood serum samples.

According to the literature data [23; 24; 25] and the instructions of the manufacturer of the test kit used in the studies, the degree of prevalence of the agent of toxoplasmosis among cattle ranged from 4 to 25%. The number of cattle in Ukraine as of 2014 was 4,534 thousand heads. It gradually decreased and, by 2018, amounted to 3530.8 thousand heads according to the published data of the Department of Agriculture and Environment Statistics of the State Statistics Service of Ukraine [42]. Therewith, the sample size ranged from 60 to 289 animals in the 95% confidence interval and up to 124 animals in the 80% con- fidence interval.

Of the 203 animals studied, 21 animals, or 10.3% (95% confidence interval: 6.7-15.1), responded positively to *Toxoplasma gondii* based on the results of research in 2014 and 2018.

Since the study of animals was carried out in two stages, in 2014 and 2018, Figure 1 presents the results of the study of the first 72 samples.

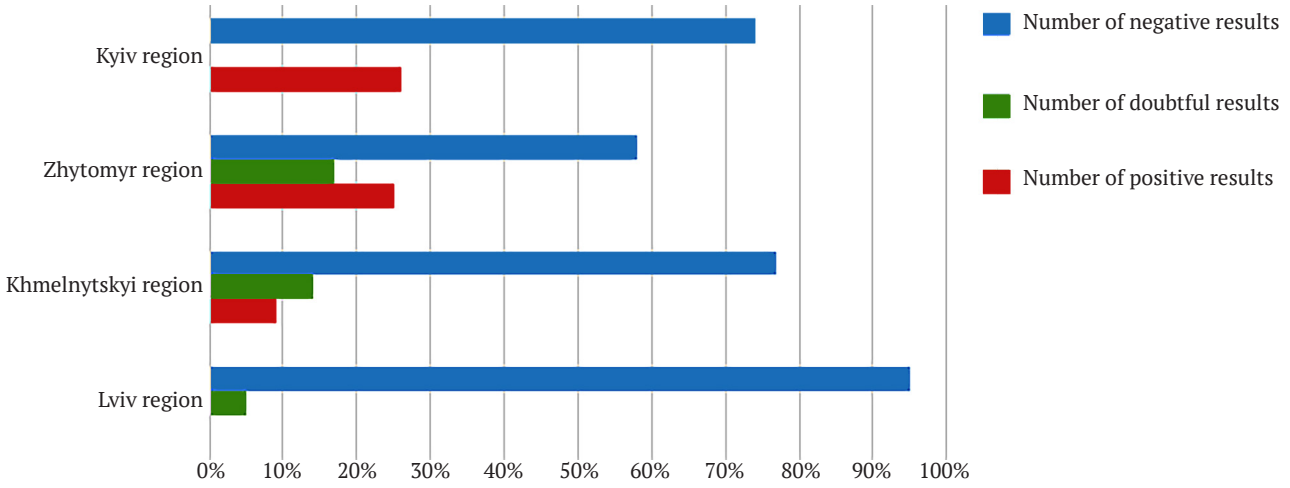

Figure 1. Seroprevalence of the causative agent of toxoplasmosis among cattle in Ukraine according to enzyme-linked immunosorbent assay (2014)

Among them, a positive reaction to the agent of toxoplasmosis was found in 10 samples or 13.9% (95% confidence interval: 7.3-23.4) from among 72 examined. 6 samples were questionable (8.3; 95% confidence interval: 3.4-16.5), and 56 samples were negative (77.8; 95% confidence interval: 67.1-86.3). Among the 61 cow serum samples studied, a positive reaction was observed in 9 samples (14.8; 95% confidence interval: 7.4-25.4), doubtful – in 6 samples (9.8; 95% confidence interval: 4.1-19.3), and negative – in 46 samples (75.4; 95% confidence interval: 63.5-85.0). At the same time, among 11 samples of bovine blood serum, only one sample was positive (9.1; 95% confidence interval: 0.5-37.3), the remaining 10 (90.9; 95% confidence interval: 62.7-99.6) were negative. Thus, no substantial difference was identified between the results of blood serum samples of cows and bulls regarding the detection of antibodies to *T. gondii* in them (P=0.6921). The highest prevalence rate was found among animals from farms in the Kyiv and Zhytomyr regions – 26.3% and 25%, respectively (Fig. 1).

In 2018, 131 samples of animal blood serum were examined. The results were summarised according to the year of the study, regions of Ukraine, age groups of animals and sex, and are presented in Table 1.

Table 1. Seroprevalence of *Toxoplasma gondii* among cattle in some regions of Ukraine according to the year of the study, age groups and sex of animals (using the enzyme immunoassay method, n = 203)

Dependence of *Toxoplasma gondii* prevalence	***N***	***n* of ELISA-positive animals**	**% of ELISA-positive animals**	**95% confidence interval**
By year of research conducted				
2014	72	10	13.9	7.3-23.4
2018	131	11*	8.4	4.5-14.1
By regions where animals were kept (for 2014 and 2018 together)				
Kyiv	100	11	11	5.9-18.3
Zhytomyr	42	5**	11.9	4.5-24.4
Khmelnytskyi	22	2***	9.1	1.6-26.9
Lviv	39	3****	7.7	2.0-9.5

Table 1, Continued

By age group of the studied animals (for 2014 and 2018 together)				
⩽ 6 months	65	3	4.6	1.2-12.1
6-18 months (inclusive)	41	2*****	4.9	0.8-15.2
18 months-3 years	48	6******	12.5	5.2-24.2
⩾ 3 years	49	10*******	20.4	10.9-33.4
By animal gender (for 2014 and 2018 together)				
Cows	184	20	10.9	7.0-16.0
Bulls	19	1********	5.3	0.3-23.3
Total	203	21	10.3	6.7-15.1

Note: **P=0.2341 – animal research results of 2018 in relation to the results of 2014: **P=0.8603 – the ratio of the results in cattle of Kyiv and Zhytomyr regions; ***P=0.8482 – the ratio of the results in cattle of Khmelnytskyi and Kyiv regions; ****P=0.5964 – the ratio of the results in cattle of Lviv and Kyiv regions; *****P=0.9357 – ratio of research results of cattle from 6 to 18 months (inclusive) and animals ⩽ 6 months; ******P=0.1499 – the ratio of research results of cattle from 18 months to 3 years and cattle ⩽ 6 months; *******P=0.0114 – the ratio of the results in cattle ⩾ 3 years and cattle ⩽ 6 months; ********P= 0.5017 – the results of the study of bulls compared to cows*

In general, among the 184 cow serum samples under study, a positive reaction was recorded in 20 (10.9; 95% confidence interval: 7-16), and a negative reaction was recorded in 164 samples (89.1; 95% confidence interval: 84-93). At the same time, out of 19 samples of bovine blood serum, only one was positive (5.3; 95% confidence interval: 0.3-23.3), the remaining 18 (94.7%) were negative.

Based on the results of the research, a probable increase in the seroprevalence of *T. gondii* was established with the age of the cattle. Among the examined 65 samples of a group of animals under the age of 6 months, 3 reacted positively to *T. gondii* (4.6; 95% confidence interval: 1.2-12.1). Therewith, in the group of 49 animals older than 3 years, the prevalence increased by 15.8% (10 positively responding samples) (20.4; 95% confidence interval: 10.9-33.4), compared to the previous group of animals, the P value was 0.0114.

Among farm animals of Kyiv and Zhytomyr regions, the prevalence of *T. gondii* was the highest (11% (95% confidence interval: 5.9-18.3) and 11.9% (95% confidence interval: 4.5-24.4), respectively). Prevalence of *T. gondii* among the animals of farms in Lviv and Khmelnytskyi regions, it was 7.7% and 9.1%, respectively. During the conducted studies, an increase in the prevalence of the agent of toxoplasmosis with the age of animals was statistically probable.

The level of prevalence of the agent in the world, both among different animal species and among humans, varies due to differences in climate, culture, eating habits, behaviour, personal hygiene, and cooking habits. The resulting indicator is higher than the data of Chinese researchers (5.0% (27/535) and 9.4% according to other researchers [43; 44], the average values for metadata processing results (7.0%) [45] and lower than the results of studies conducted in Poland (13.0%) [46], Nigeria (16.3%) [47], conducted by another group of Chinese researchers (19.9%; 144/723) [48], meta-analysis results (20.0%) [49], research results in the Democratic Republic of São Tomé and Príncipe (27.1%) [50], and data from researchers in Brazil (34.27%; 1307/3814 and 60.29%; 369/612) [51; 52]. At the same time, various risk factors were identified, namely the use of raw or poorly heat-treated meat, physical contact with cats or cat toilets, the use of unwashed raw vegetables and fruits, the use of contaminated water and milk [20; 7]. Thus, according to the researchers, the prevalence of *T. gondii* was the highest (11.5±3.1) among cows older than 8 years, which coincides with the results of this paper, and no substantial differences were found regarding the dependence of the spread of the agent of toxoplasmosis on the breed of cows and the area [36]. Similarly, the prevalence was considerably higher in adult buffaloes (12.5%) compared to calves and young animals (1.9%) [31]. This is confirmed by the results of the dependence of animal lesions on age (P<0.0001) with a prevalence of 5.3% for calves (<8 months) and 23.1% for adult animals (> 8 months) [30], as well as the results of studies by scientists in China, where the prevalence was higher among animals aged ⩾ 12 months (9.6%, 1248/12438) than among animals aged <12 months (6.7%, 226/3132). Female cattle had a slightly higher prevalence (12.5%, 793/6670) than males (11.7%, 418/3856). Comparable results were obtained in this study, but they were not statistically significant. The prevalence in summer (11.8%, 517/4744) was the highest of all seasons [27; 29; 53].

Researchers include the number of animals on the farm (OR=4.43) and the presence of domestic cats in the territory where the animals are kept (OR=1.98) [8; 9; 25]. Thus, at the herd level, the risk factors associated with a higher risk of *T. gondii* infection were the drilling of certain wells as sources of drinking water (OR=7.50; 95% confidence interval: 2.11-26.69) [26].

Another risk factor for toxoplasmosis infection is the presence of rodents on farms (P=0.006) [24]. Eating raw or insufficiently heat-treated meat is known to increase the risk of *T. gondii* infection. Freezing meat products can eliminate this risk, but buyers very often prefer unfrozen meat due to its better nutritional quality [54; 55].

Another risk of toxoplasmosis infection is the consumption of unpasteurised milk and certain dairy products [20]. Thus, out of 164 selected raw milk samples from 33 buffalo herds in Iran, 4.3% were recognised as positive by Vero cell culture, and 3.7 and 3.0% were positive by enzyme immunoassay and polymerase chain reaction methods, respectively. The researchers also used bioassays on cats and fed the animals 50 cm^3 buffalo milk for 3 days. All infected cats excreted oocysts with faeces, demonstrating the presence of viable tachyzoites in the animals, which were

in the milk of productive animals and may pose a threat to further infection with toxoplasmosis [40; 56].

Conclusions

According to the results of the research, the prevalence of *T. gondii* among cattle in Kyiv, Zhytomyr, Khmelnytskyi, and Lviv regions of Ukraine is 10.3% (95% confidence interval: 6.7-15.1). Therewith, according to data for 2014 and 2018, it differed slightly. Thus, in 2014, *T. gondii* was found in 13.9% of animals, and in 2018 this figure was 8.4%. The obtained prevalence rates in 2014 in some regions of Ukraine are close to the results of studies conducted in Poland. Moreover, an increase in the prevalence of *T. gondii* was recorded in this species of animal with age. Thus, in the group of 49 animals older than 3 years, the prevalence increased by 15.8% compared to the group of cattle under 6 months of age, which coincides with the results of other researchers. Therefore, eating insufficiently heat-treated meat and unpasteurised milk and certain dairy products can cause human toxoplasmosis infection.

Given the considerable percentage of positively responding animals in some regions of Ukraine, at the next stage of research, it is necessary to analyse the risks of infection with the agent of human toxoplasmosis when eating meat and milk of cattle.

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Поширення *Toxoplasma gondii* серед великої рогатої худоби в окремих регіонах України

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Анотація. *Toxoplasma gondii* як збудник токсоплазмозу, хвороби яка є спільною як для людини, так і для великої рогатої худоби, є надзвичайно поширеним у світі і завдає як економічних збитків так і становить загрозу здоров'ю людини, зокрема при вживанні у їжу продуктів тваринного походження. Саме тому мета роботи полягала у дослідженні поширеності збудника токсоплазмозу у великої рогатої худоби окремих областей України. Для проведення досліджень до лабораторії надсилали зразки цільної крові або сироватки великої рогатої худоби різного віку і статі з господарств Київської, Житомирської, Хмельницької та Львівської областей України. У сироватці крові тварин визначали наявність сумарних антитіл до *Toxoplasma gondii*. Для цього використовували метод імуноферментного аналізу. Загалом поширеність *T. gondii* серед великої рогатої худоби Київської, Житомирської, Хмельницької і Львівської областей України за результатами проведених досліджень становила 10,3 % (95 % довірчий інтервал: 6,7–15,1). Водночас дослідження тварин здійснювалось у два етапи, у 2014 і 2018 роках, а поширеність відрізнялася незначно. Так, у 2014 році за результатами досліджень зареєстровано позитивну реакцію до *T. gondii* в 10 зразках або 13,9 % (95 % довірчий інтервал: 7,3–23,4), у той час, як у 2018 році цей показник становив 11 зразків або 8,4 % (95 % довірчий інтервал: 4,5–14,1) досліджених тварин. Водночас за 2014 і 2018 роки досліджень встановлено, що найвищий рівень поширеності збудника токсоплазмозу відзначався серед тварин господарств Житомирської та Київської областей, відповідно 11,9 % (95 % довірчий інтервал: 4,5–24,4) й 11,0 % (95 % довірчий інтервал: 5,9–18,3). Причому вірогідне підвищення рівня поширеності *T. gondii* відмічали у тварин з віком. Зокрема, серед тварин віком до шести місяців, із 65 досліджених зразків сироватки крові позитивно прореагували до збудника хвороби три (4,6; 95 % довірчий інтервал: 1,2–12,1). У той час, як у групі тварин старших за три роки (49 корів), які позитивно прореагували, виявилися 10 зразків (20,4; 95 % довірчий інтервал: 10,9–33,4), у порівнянні з попередньою групою тварин P-значення становило 0,0114. Отже, отримані дані дають можливість зрозуміти рівень поширеності збудника токсоплазмозу серед великої рогатої худоби на території України, що своєю чергою дозволить попередити захворювання людей при вживанні в їжу м'ясних і молочних продуктів позитивно реагуючих тварин

Ключові слова: токсоплазмоз, серологічні дослідження, імуноферментний аналіз, Київська, Житомирська, Хмельницька, Львівська області

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