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Effectiveness of group therapy of calves for nonspecific bronchopneumonia by aerosol method

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Abstract. Nonspecific bronchopneumonia of calves causes significant economic damage to farms, and therefore, it is important to develop effective therapeutic drugs, especially for group use. Aerosol-based medicines deserve attention. The purpose of the study was to investigate the effectiveness of the group aerosol method of administering the drug “Calfmin” to calves with nonspecific bronchopneumonia. Analogue calves of two to three months of age, both clinically healthy and patients with nonspecific bronchopneumonia, were involved in the experiment. Standard clinical and haematological research methods were used to determine the diagnosis and monitor the functional state of the calves’ bodies. It was found that in the blood of calves with nonspecific bronchopneumonia, an increase in the number of white blood cells was observed by 1.3 times ($P<0.05$) compared to clinically healthy animals. Their blood also showed an increase in the number of stab and segmented neutrophils by 2.0 times ($P<0.05$) and 1.6 times ($P<0.01$), respectively, and a decrease in the number of lymphocytes by 1.3 times ($P<0.001$) and eosinophils by 1.4 times ($P<0.001$), respectively. In sick calves, there was a 1.2-fold decrease in the number of red blood cells in the blood ($P<0.001$) and a 1.2-fold decrease in the haemoglobin content ($P<0.001$) compared to the control. An improvement in the clinical condition and haematological parameters in sick animals was recorded within 3-5 days, a reduction in the duration of therapy by 1.9-2.0 times, and 100% preservation of all sick calves

Keywords: nanoaquachelates; silver; copper; iodine; cobalt; Echinacea purpurea; blood morphology

Introduction

Respiratory diseases are among the most common diseases among different age groups of animals. Most often, such pathologies are identified in young, emaciated, and old animals. Respiratory diseases cause economic damage to the livestock industry, including the death of animals, reduced productivity of sick animals, and low-quality products (Buczinski & Pardon, 2020).

The most common respiratory disease is bronchopneumonia. Bronchopneumonia of young cattle is detected throughout Ukraine, and among the registered cases it ranks second after diseases of the gastrointestinal tract. Every year, up to 20-30% of calves with catarrhal bronchopneumonia are registered in Ukrainian farms. The incidence rate increases in the winter-spring period. The disease is registered in calves, starting from two weeks of age to 2-3 months (Sharandak *et al.*, 2022).

The causes of nonspecific bronchopneumonia in young cattle are a decrease in resistance,

immunological reactivity of newborn calves, and the influence of adverse environmental factors. Bacterial opportunistic microflora of the anterior respiratory tract plays a leading role in the development of this pathology. Reduced natural resistance of calves, immunity of young cattle, unjustified means and methods of therapy and prevention are factors contributing to the spread of this disease (Van Driessche *et al.*, 2020). Bronchopneumonia in calves is characterised by the development of pathology not only in the respiratory system, but also throughout the body, since there is a violation of all types of metabolism. This leads to pathological changes in many organs of calves. In order to overcome bronchopneumonia in calves, it is necessary to apply a comprehensive approach to the selection of therapeutic agents and measures (Leenen *et al.*, 2020).

Analysis of literature and research results indicate that nonspecific bronchopneumonia is

the subject of constant attention of researchers and veterinary medicine specialists, since this pathology is widespread among calves – from 15 to 25% with 90% preservation. Economic losses from bronchopneumonia consist of a significant percentage of calf deaths, a decrease in the average daily weight gain of the animal (sick calves lag behind in growth and development), the cost of veterinary examination, maintenance, and other care for sick animals (Sharandak *et al.*, 2022).

In addition, the effects of bronchopneumonia on the subsequent performance of calves are usually underestimated. It is proved that repair heifers who have suffered subacute or chronic lung diseases gain the necessary body weight more slowly and have disorders in the development of reproductive organs. In other words, it will not be possible to get high milk yields from cows in the future. If the farm creates proper conditions for keeping animals and diseases of young animals (dyspepsia and bronchopneumonia) can be avoided, then reliable promising prerequisites are provided for the optimal age of the first calving of heifers and high milk productivity of cows (Nishi *et al.*, 2019).

Nonspecific bronchopneumonia is characterised by inflammation of the bronchi and lungs, accompanied by their filling with catarrhal exudate, a disorder of external and internal gas exchange, the development of respiratory and cardiovascular insufficiency, and a violation of the functions of various organs and systems. The most effective treatment for bronchopneumonia is complex treatment of animals, which is aimed at eliminating violations of the technology of their maintenance and feeding, increasing the body's resistance with the simultaneous use of various means of therapy: antimicrobial, pathogenetic, symptomatic, stimulating, and vitamin (Sharandak *et al.*, 2022).

Treatment of calves for bronchopneumonia is most effective in the initial stages of the

disease, when the inflammatory process mainly affects the upper respiratory tract. In the case of a prolonged course of the disease, as oedema, cell infiltration, and sclerosis increases around the inflammatory focus, capillaries are squeezed and desolated in the lungs, as a result of which the penetration of drugs into the area of inflammation becomes more difficult (McGill & Sacco, 2020). Nowadays, the issue of developing and practical use of new highly effective and economically justified treatment and prevention schemes for nonspecific bronchopneumonia of calves is relevant. The advantage of aerosol therapy is that it can be incorporated into existing meat and milk production technologies. It is this approach to the treatment of animals that will allow producing organic livestock products that meet the highest quality standards.

The purpose of the study was to investigate the effectiveness of group therapy of calves with non-specific bronchopneumonia using the aerosol method.

Materials and Methods

The study was conducted in 2017-2021 at the Department of Therapy and Clinical Diagnosis, and Academician Volodymyr Kasyanenko Department of Animal Anatomy, Histology and Pathomorphology at the National University of Life and Environmental Sciences of Ukraine (Kyiv). Experimental trials were conducted on the farm "Podolsky hospodar 2004" of the Khmelnytskyi Oblast, Shepetivka district. Analogue calves of 2-3 months of age, black-and-white breed, were used as the object of research. Animals of the control and experimental groups were kept in the same conditions of keeping, feeding, and care. The study complied with ARRIVE's recommendations and was conducted without violating the Directive 2010/63/EU guidelines on the protection of animals used for scientific purposes (Percie du Sert *et al.*, 2020).

Before starting the research, the spread and etiopathogenetic relationships of nonspecific bronchopneumonia of calves in farm conditions were studied. Clinically healthy black-and-white calves and animals suspicious and sick of nonspecific bronchopneumonia were selected for the dispensary examination. Studies were conducted on the clinical condition of young cattle, in particular, determining the habitus of animals, measuring body temperature, calculating the frequency of respiratory movements and heartbeats in one minute, examining the condition of hair, skin, visible mucous membranes, superficial lymph nodes, cardiovascular, respiratory, digestive, urinary, and sexual systems. Standard clinical research methods (examination, palpation, percussion, auscultation, thermometry) were used to study the clinical condition of animals.

Heparin-stabilised blood obtained from the jugular vein (*vena jugularis*) in the morning, before feeding, was used for morphological studies, along with clinical examination of the animals. During morphological studies of blood, the number of red blood cells was determined manually in a hemocytometer with a Goryaev grid; the number of leukocytes – by counting with a small magnification of the microscope in 100 large squares of a hemocytometer with a Goryaev grid; platelets were counted manually in a hemocytometer with a Goryaev grid; the leukogram was derived by counting 200 cells in blood smears stained according to Romanowsky-Giemsa; the hematocrit value – by microcentrifugation according to Shklyar; haemoglobin – by hemiglobincyanide method; the content of haemoglobin in one erythrocyte (CHE) was calculated by mathematical calculations according to the equation.

To conduct experimental studies, three groups of calves were established – analogues of 2-3 months of age, 50 animals each, namely: group 1 – clinically healthy calves; experimental

group 1 and group 2 – calves with nonspecific bronchopneumonia. Monitoring of the clinical condition of animals and the effectiveness of group therapy was monitored on the first, third, seventh, ninth, and fifteenth days of treatment. The experiment lasted 30 days. Calves of the control group were not treated with aerosol. Aerosol treatment of calves of the experimental group 1 was carried out by using turpentine at the rate of 0.5 mL/m³, lactic acid – 0.04 g/m³, and chlorinated lime – 2 g/m³. The frequency of aerosol treatment of animals is 1 time per day, the exposure is 45 minutes according to the therapy scheme adopted on the farm. The treatment was carried out every second day, 12 consecutive days. For the prevention of nonspecific bronchopneumonia in calves of experimental group 2, the method of group aerosol treatment was used using the developed experimental preparation “Calfmin” containing silver and copper nanoaquachelates, iodine solution, cobalt lactate (Drobot *et al.*, 2013), and echinacea tincture. The drug was applied at a dose of 7 mL per animal with an exposure of 45 minutes. Treatment with this drug was carried out on the first, third, and seventh days of research. To prevent rapid evaporation of aerosol particles and exposure to harmful gases, as well as their long-term retention in a suspended state, a stabiliser was added to the experimental preparation – a 20% glucose solution at the rate of 1 mL/m³ of the room. Preventive aerosol treatment of calves was carried out in a separate room with carefully closed doors and windows with ventilation turned off (Nedosekov *et al.*, 2010).

The obtained results were recorded, and their digital indicators were processed statistically using StatSoft Statistica (2016) software suite, considering the specifics of statistical methods in biomedical research (Horalskyi *et al.*, 2015). The data is presented in tables as $x \pm SD$ (standard deviation). Differences between

the values were determined using the Tukey range test, where the differences were considered reliable at $P < 0.05$, $P < 0.01$, and $P < 0.001$ (considering the Bonferroni correction).

Results and Discussion

According to the conducted propaedeutical studies to investigate the causes of nonspecific bronchopneumonia in calves in the experimental farm “Podilskyi hospodar 2004”, it was established that several groups of aetiological factors that act on the body of calves of different age groups in different combinations and combinations lead to the occurrence of this pathology. The first group includes anthropogenic factors, biogeocenosis disorders, and unfavourable factors of intrauterine development that lead to the birth of calves with weakened resistance. Thus, the body temperature decreased by 1–1.5°C compared to the norm. These calves were found to have bradycardia, myopathy, and hypotrophy. The sucking reflex in many newborn calves is weakened with a delay. Most of these calves had dyspepsia, and after some time after getting over dyspepsia, bronchopneumonia developed. This indicates that diseases of young animals in the neonatal period are associated with the anatomical and physiological characteristics of the calves’ body.

According to the results obtained, the prevalence of the disease in the “dyspepsia – bronchopneumonia” scheme in newborn calves is due to a violation of the conditions of keeping structural analysis of the diet of breeding cows, it was found that the farm does not always maintain and feeding the breeding stock of cows in different physiological conditions. According to the structure, quality, and completeness of the diet. This is especially relevant for the diet of cows during the interlactation period, where there is a violation of feed quality and a deficiency in the content of macro- and microelements and vitamins.

Nonspecific bronchopneumonia of calves on the farm is caused by the accumulation and accumulation of harmful gases (ammonia, hydrogen sulphide, which were studied organoleptically), low temperature and high humidity in the room. The feed base for calves aged 1.5–2.0 months does not provide a physiological need for protein and vitamins, especially A and C, as well as macro- and microelements, especially iodine and calcium. The increased level of bronchopneumonia in calves is conditioned by the presence of opportunistic microflora in the upper respiratory tract, which under these conditions becomes pathogenic and is the causative agent of nonspecific bronchopneumonia. Drugs were used by the aerosol method, since this method is the most effective for respiratory diseases of animals, since the ways of getting the drug and the causative agent of the disease into the body of sick animals are the same (Sharandak *et al.*, 2022).

According to the conducted study, it was found that mainly calves of 1–3 months of age were ill on the farm, which is 23.1–24.7%; and 7.2–9.4% of calves of 1–2 months of age and 3.4–6.3% of calves of 2–3 months of age died from this pathology. The preservation of calves of 1–3 months of age in this pathology is 90.6%, which indicates a low effectiveness of treatment of animals in the experimental farm.

In a clinical study of calves with nonspecific bronchopneumonia, it is important not to miss the first symptoms of the disease. At the beginning of the disease, attention was paid to animals with symptoms of general depression, decreased appetite, and the appearance of a jerky, sharp, dry, and painful cough. Liquid serous-mucous discharge from the nasal passages of calves and lacrimation were often detected, which on the second or third day changed to catarrhal (they became milky white, became thicker) or even catarrhal-purulent. The presence of liquid serous-mucosal

secretions indicated that the role of viral load is not excluded in the aetiology of nonspecific bronchopneumonia of calves, and the change of secretions to catarrhal (or catarrhal-purulent) – that bacterial microflora plays a leading role in the pathogenesis of bronchopneumonia. In all patients with nonspecific bronchopneumonia of animals (100%), at the beginning of the disease, an increase in the number of respiratory movements, the appearance of inspiratory dyspnea and the abdominal type of breathing were observed.

During the clinical examination, cyanosis of the mucous membranes of the oral cavity, conjunctiva and nasal mirror was detected in 100% of calves with nonspecific bronchopneumonia. This indicates a violation of blood circulation and the accumulation in the blood of animals of an excessive amount of reduced haemoglobin, which has a dark, red-blue colour. The accumulation of excessive amounts of reduced haemoglobin in the blood of calves with nonspecific bronchopneumonia is obviously associated with insufficient oxygen saturation of the blood due to inflammatory processes in the lungs. During auscultation of the lungs at the beginning of the disease, most calves showed increased broncho-vesicular respiration. This is due to difficulty in air intake due to the narrowing of airways, a decrease in the elasticity of the alveoli due to infiltration of their walls, and a decrease in the number of alveoli involved in

respiratory movements. On the second or third day, wet wheezing was detected, which indicated an accumulation of exudate in the respiratory tract. Crepitation was also noted, which was best listened to at the peak of inspiration.

At the same time, wet wheezing was detected during auscultation of the lungs, which indicates a violation of the elastic properties of the inter-alveolar membranes, due to the accumulation of exudate in the bronchi. Auscultation of the heart noted a muffling of heart tones and a weakening of the pulse wave. In addition, an increase in the second tone was noted, which is obviously associated with a “shock” of blood during the closure of the semilunar valves of the pulmonary artery. The intensity of the second heart tone in sick calves had a correlation with the speed of blood flow in the small circle of blood circulation, and, consequently, the value of blood pressure, the elasticity of the alveoli and the pressure in the interalveolar membranes.

Chest percussion revealed areas of blunting in the apical and cardiac areas of the lungs. When measuring body temperature in animals, intermittent fever was noted. Almost 90% of calves had a body temperature of 39.5-39.7°C in the morning and 40.7-41.4°C in the evening (Table 1). This can be explained as a protective reaction of the animal’s body to the influence of pathological factors, in which leukocytosis, nonspecific and specific protection factors are activated.

Table 1. Clinical parameters of healthy and sick calves with nonspecific bronchopneumonia, $M \pm m$, $n=7$

Indicator	Calf groups	
	clinically healthy	with nonspecific bronchopneumonia
Body temperature, °C	38.9±0.1	40.4±0.1
Pulse rate, beats/min	81.9±1.4	97.1±1.4***
Respiratory rate, movements/min	28.8±0.6	41.8±1.9***

Note: *** $P < 0.001$ compared to data from clinically healthy calves

Source: developed by the author

During the study of the clinical condition of calves, general animal depression, loss of appetite, sharp, dry and jerky cough, and catarrhal exudate discharge from the nasal passages were observed. Sick calves lay down most of the time of the study, and when forced to get up, they showed unsteady gait and muscle tremors. In sick animals, tousled and wet hair was noted. The conjunctiva and nasal mirror in sick calves had a cyanotic tint, which is a sign of impaired hemostasis in the small circle of blood circulation. Shortness of breath in calves was of a mixed type. Hyperthermia was observed in sick calves, and the pulse rate and respiration significantly increased by 1.2 times ($P<0.001$) and 1.5 times ($P<0.001$), respectively, compared to

data from clinically healthy animals. Thus, the results of clinical studies indicate the polyaetiology and acute course of bronchopneumonia in calves.

The blood of calves with nonspecific bronchopneumonia is characterised by a significant decrease in haemoglobin content by 1.2 times ($P<0.001$), the number of red blood cells by 1.2 times ($P<0.001$) and a tendency to reduce CHE, hematocrit and a significant increase in platelet count by 1.3 times ($P<0.001$) (Table 2). At the beginning of group therapy in calves with non-specific bronchopneumonia, the number of red blood cells in the blood significantly decreased by 1.3-1.4 times ($P<0.001$) compared to the indicators of clinically healthy animals.

Table 2. Blood parameters of clinically healthy and sick calves with nonspecific bronchopneumonia, $M\pm m$, $n=7$

Indicator	Group of calves	
	clinically healthy	with nonspecific bronchopneumonia
Hematocrit, %	36.42 ± 1.70	33.43 ± 0.53
Haemoglobin, g/L	114.3 ± 1.8	$92.3\pm 1.4^{***}$
Platelets, $10^9/L$	256.0 ± 10.3	$331.7\pm 4.7^{***}$
Red blood cells, $10^{12}/L$	6.8 ± 0.1	$5.6\pm 0.2^{***}$
Haemoglobin content in one erythrocyte (CHE), pg	16.8	16.5

Note: $***P<0.001$ compared to data from clinically healthy calves

Source: developed by the author

Morphological examination of blood showed that the number of white blood cells in the blood of calves suffering from nonspecific bronchopneumonia is 1.3 times significantly higher ($P<0.05$) compared to the data of clinically healthy calves (Table 3). The leukogram of sick calves was

characterised by an increase in the number of stab and segmented neutrophils by half ($P<0.05$) and 1.6 times ($P<0.01$), respectively, and a decrease in the number of lymphocytes by 1.3 times ($P<0.001$) and eosinophils by 1.4 times ($P<0.001$) compared to clinically healthy animals (Table 3).

Table 3. Leukocyte count and leukogram parameters of clinically healthy and sick calves with nonspecific bronchopneumonia, $M\pm m$, $n=7$

Indicator	Group of calves	
	clinically healthy	with nonspecific bronchopneumonia
White blood cell count, $10^9/L$	6.1 ± 0.1	$8.2\pm 0.2^*$

Table 3. Continued

Indicator	Group of calves	
	clinically healthy	with nonspecific bronchopneumonia
Basophils, %	1	1
Eosinophils, %	2.2±0.2	1.6±0.3*
Stab neutrophils, %	2.1±0.1	4.2±0.4*
Segmented neutrophils, %	25.3±0.6	39.6±1.3**
Lymphocytes, %	62.7±1.2	47.7±0.7***
Monocytes, %	6.7±0.3	5.9±0.4

Note: * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to data from clinically healthy calves

Source: developed by the author

Neutrophilic leukocytosis, eosinopenia, and lymphocytopenia were observed in the blood of calves with bronchopneumonia. As a result of clinical and morphological studies on the dispensary examination of animals, not only aetiological factors and the spread of the disease in the farm were determined, but also its specificity

and the diagnosis was clarified – nonspecific bronchopneumonia of calves. Based on the results of clinical studies on the effectiveness of the drug “Calfmin” and the use of aerosol therapy (Drobot *et al.*, 2013), a significant improvement in the clinical condition of calves with nonspecific bronchopneumonia was established (Table 4).

Table 4. Indicators of body temperature, pulse, and respiratory rate in calves with nonspecific bronchopneumonia when using aerosol therapy, $M\pm m$, $n=7$

Day of experiment	Control group (clinically healthy calves):	Sick calves	
		experimental group 1	experimental group 2
Animal body temperature (day 1)	38.8±0.2	41.0±0.2***	40.9±0.2**
Animal body temperature (day 3)	38.8±0.1	39.5±0.1*	39.1±0.1
Animal body temperature (day 7)	38.9±0.1	39.0±0.1	38.4±0.1
Pulse rate, beats/min (day 1)	78.1±0.3	98.2±1.1***	97.7±1.0***
Pulse rate, beats/min (day 3)	78.9±1.1	87.9±1.1***	84.5±1.1**
Pulse rate, beats/min (day 7)	76.9±1.5	78.2±1.2	76.1±1.2
Respirations per minute (day 1)	29.1±0.1	47.2±0.6***	46.7±1.1***
Respirations per minute (day 3)	29.0±0.8	35.3±1.5***	33.2±1.0**
Respirations per minute (day 7)	28.2±0.5	29.1±1.2	28.3±0.4

Note: * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to data from clinically healthy calves

Source: developed by the author

Thus, cough in calves with bronchopneumonia of experimental group 2, which were treated with “Calfmin”, stopped on the third day. During the treatment of calves with bronchopneumonia, an improvement in their general clinical

condition was observed. Thus, on the third day, the animals showed a decrease in body temperature, pulse rate, and respiration compared to the beginning of treatment (Table 4). Table 4 showed that on the seventh day of the study,

body temperature, pulse and respiration rates in calves with nonspecific bronchopneumonia of the experimental groups were within the limits of indicators of clinically healthy animals. During the treatment period, the body temperature of the calves of the experimental groups decreased by 1.2 times relative to the data on the first day of the experiment, the heart rate – by 1.3 times, and the respiratory rate – by 1.6 times.

Already on the third day from the beginning of the experiment, the number of red blood cells in the blood of calves that were treated with “Calfmin” during group aerosol therapy significantly increased by 1.3 times ($P<0.01$) and differed from that in clinically healthy calves. The number of red blood cells in the blood of calves during basic treatment

also tended to increase, but on the third day of the experiment it remained significantly lower by 1.1 times ($P<0.05$) compared to clinically healthy calves. On the seventh day of application of group aerosol therapy, the blood parameters of animals of both experimental groups in terms of the number of red blood cells did not differ from those in clinically healthy calves. At the same time, the number of red blood cells in the blood of calves of experimental group 2 with aerosol use of “Calfmin” increased by 7% compared to animals of experimental group 1 that used basic treatment. The number of platelets in the blood of calves with bronchopneumonia at the beginning of the experiment increased by 1.3–1.4 times ($P<0.01$) compared to clinically healthy calves (Table 5).

Table 5. Morphological parameters of blood of calves with nonspecific bronchopneumonia using group aerosol therapy, $M\pm m$, $n=7$

Day of experiment	Control group (clinically healthy calves)	Sick calves:	
		experimental group 1	experimental group 2
Number of red blood cells, $10^{12}/L$ (day 1)	6.7 ± 0.1	$5.2\pm 0.4^{***}$	$5.6\pm 0.2^{***}$
Number of red blood cells, $10^{12}/L$ (day 3)	7.2 ± 0.2	6.3 ± 0.4	7.0 ± 0.2
Number of red blood cells, $10^{12}/L$ (day 7)	7.6 ± 0.2	7.2 ± 0.2	7.7 ± 0.2
Haemoglobin content, g/L (day 1)	115.2 ± 2.0	$98.5\pm 4.9^{**}$	$98.7\pm 2.9^{**}$
Haemoglobin content, g/L (day 3)	121.7 ± 2.0	$113.8\pm 2.8^*$	125.0 ± 1.3
Haemoglobin content, g/L (day 7)	128.0 ± 1.9	$118.0\pm 2.8^*$	132.6 ± 1.1
Hematocrit value, % (day 1)	39.44 ± 1.70	$33.43\pm 0.93^{***}$	$35.14\pm 1.06^{***}$
Hematocrit value, % (day 3)	39.67 ± 1.58	$37.53\pm 0.99^*$	$39.57\pm 1.05^*$
Hematocrit value, % (day 7)	40.63 ± 1.60	39.62 ± 1.89	40.43 ± 0.65
Platelet count, $10^9/L$ (day 1)	256.0 ± 10.3	$332.6\pm 20.6^{**}$	$339.6\pm 14.0^{***}$
Platelet count, $10^9/L$ (day 3)	297.7 ± 6.4	316.7 ± 6.1	305.1 ± 6.9
Platelet count, $10^9/L$ (day 7)	276.3 ± 12.7	289.4 ± 11.4	$273.6\pm 8.9^*$

Note: $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to data from clinically healthy calves

Source: developed by the author

On the third day of aerosol group therapy, compared to the first day of the experiment, the number of platelets in the blood of calves treated with basic treatment had a pronounced tendency

to decrease, and in the blood of calves treated with “Calfmin” decreased by 10% ($P<0.05$). At the same time, this indicator in calves of experimental group 1 remained another 7% ($P<0.05$)

higher compared to clinically healthy calves. On the seventh day of application of group aerosol therapy, the blood parameters of calves of both experimental groups in terms of platelet count did not differ from the blood parameters of the control group of animals.

The haemoglobin content in the blood of calves of the experimental groups at the beginning of group aerosol therapy decreased by 1.15-1.17 times ($P<0.01$) compared to the data of clinically healthy animals. Thus, on the third day, in the blood of calves with nonspecific bronchopneumonia who received basic treatment on the farm, the haemoglobin content significantly increased by 1.2 times ($P<0.01$), and in comparison with clinically healthy calves decreased by 7% ($P<0.05$). In the blood of calves of experimental group 1, who were treated with "Calfmin", this indicator also significantly increased by 1.3 times compared to the first day and became as close as possible to the indicators of clinically healthy animals, and in relation to calves of experimental group 1, it increased by 10% ($P<0.01$) (Table 5). On the seventh day of the study, the concentration of haemoglobin in the blood of calves of both experimental groups did not

change in comparison with the indicators of clinically healthy calves. However, the haemoglobin content in the blood of calves of experimental group 2, who were treated with group aerosol therapy using "Calfmin", increased by 11%, compared to calves of experimental group 1, who received basic therapy.

At the beginning of group therapy in calves with nonspecific bronchopneumonia, the hematocrit value decreased by 1.2-1.1 times ($P<0.01$) compared to that in clinically healthy animals. On the third day of aerosol therapy, the hematocrit value in the blood of calves of both experimental groups significantly increased by 12% ($P<0.05$), compared to the first day of the experiment. However, on the third day, there was no significant difference in the hematocrit value in the blood of calves of the experimental groups. Group aerosol therapy in calves with bronchopneumonia had a significant effect on the leukogram parameters of animals (Table 6). Before starting group aerosol therapy in the blood of patients with bronchopneumonia calves, the number of white blood cells in the blood was 1.4 times higher ($P<0.01$), compared to the indicators of clinically healthy calves (Table 6).

Table 6. Leukogram of calves with nonspecific bronchopneumonia based on the results of group aerosol therapy, $M\pm m$, $n=7$

Day of experiment	Control group (clinically healthy calves):	Sick calves	
		experimental group 1	experimental group 2
White blood cell count, $10^9/L$ (day 1)	6.2 ± 0.1	$8.4\pm 0.2^{***}$	$8.5\pm 0.4^{***}$
White blood cell count, $10^9/L$ (day 3)	5.8 ± 0.2	$6.8\pm 0.4^*$	6.1 ± 0.3
White blood cell count, $10^9/L$ (day 7)	5.9 ± 0.3	6.3 ± 0.3	6.2 ± 0.1
Basophils, % (day 1)	0.7 ± 0.1	0	0.7 ± 0.1
Basophils, % (day 3)	0	0	0
Basophils, % (day 7)	0	0	0
Eosinophils, % (day 1)	2.2 ± 0.2	$1.2\pm 0.1^{***}$	$1.5\pm 0.1^{***}$
Eosinophils, % (day 3)	3.4 ± 1.0	1.4 ± 0.9	3.1 ± 0.3
Eosinophils, % (day 7)	4.5 ± 0.7	1.4 ± 0.8	3.5 ± 0.6

Table 6. Continued

Day of experiment	Control group (clinically healthy calves):	Sick calves	
		experimental group 1	experimental group 2
Stab neutrophils, % (day 1)	2.1±0.4	6.8±1.3	7.2±1.2**
Stab neutrophils, % (day 3)	2.3±0.4	4.3±1.2	3.1±0.6
Stab neutrophils, % (day 7)	2.9±0.4	4.1±0.4	3.4±0.4
Segmented neutrophils, % (day 1)	25.3±0.6	38.1±3.8**	37.3±2.1***
Segmented neutrophils, % (day 3)	25.8±0.9	35.1±0.9***	25.7±2.7
Segmented neutrophils, % (day 7)	26.4±1.2	32.1±1.8*	27.4±1.4
Lymphocytes, % (day 1)	62.7±2.2	48.8±1.9**	48.3±2.1**
Lymphocytes, % (day 3)	63.7±3.1	53.0±2.8*	61.1±2.4
Lymphocytes, % (day 7)	62.4±2.7	55.9±2.5	58.5±1.9
Monocytes, % (day 1)	6.7±0.3	5.2±0.7	5.0±0.5
Monocytes, % (day 3)	7.6 ±0.6	5.2±0.3	7.0±0.8
Monocytes, % (day 7)	7.3±0.8	6.3±0.7	7.2±0.8

Note: * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to data from clinically healthy calves

Source: developed by the author

On the third day from the beginning of the experiment, the number of white blood cells in the blood of calves of experimental group 1 significantly decreased by 1.2 times ($P<0.01$), while in calves of experimental group 2, this indicator increased by 1.4 times ($P<0.001$). There was no significant difference in the number of white blood cells between calves of the experimental groups during this period. On the seventh day of group aerosol therapy, the number of white blood cells in the blood of calves of both experimental groups was as close as possible to the indicators of clinically healthy animals. In relation to the first day of the experiment, the number of white blood cells in the blood of calves of experimental group 1 decreased by 25% ($P<0.001$), and in the blood of calves of experimental group 2 – by 28% ($P<0.001$).

Neutrophilic leukocytosis was noted in the leucogram of calves, which was accompanied by an increase in the number of neutrophils by 1.5 times ($P<0.001$) with a simultaneous decrease in the number of lymphocytes and monocytes by 1.3 times ($P<0.01$), eosinophils – by 1.9 times

($P<0.001$) in calves of experimental group 1, and – by 1.5 times ($P<0.001$) in calves of experimental group 2. Group aerosol therapy of calves of the second experimental group using the drug “Calfmin” on the third day caused a decrease in the number of stab and segmented neutrophils in the blood by 2.4 times ($P<0.01$) and 1.5 times ($P<0.01$), respectively, compared with the first day of the experiment. In addition, there was an increase in the number of eosinophils by more than 2.0 times ($P<0.001$) and lymphocytes by 1.3 times ($P<0.01$). That is, the leukograms of calves with nonspecific bronchopneumonia who used the “Calfmin” by aerosol method were already as close as possible to the indicators of clinically healthy calves during this period.

Calves of the first experimental group were treated with group aerosol therapy using a farming scheme. The leukogram on the third day was characterised by a tendency to reduce the number of stab and segmented neutrophils by 1.5 times ($P<0.001$) and by 1.1 times, respectively. An increase in the number of eosinophils by 1.2 times and lymphocytes by 1.1 times

($P < 0.05$) was also noted compared to the first day of the experiment. In comparison with the leukogram indicators of calves of the control group (clinically healthy animals), calves of the first experimental group did not show significant changes, with the exception of the number of segmented neutrophils, which increased by 36% ($P < 0.001$). This indicates the development of a pathological process of an inflammatory nature and the painful state of their body.

On the seventh day of the experiment, the leukogram of calves that were treated with group aerosol therapy using the drug “Calfmin” almost did not differ from the indicators of clinically healthy animals. The leukogram of calves of experimental group 1, which used the basic method of therapy, was characterised by blood parameters that were as close as possible to those in clinically healthy animals. However, calves of experimental group 1 showed a tendency to reduce the number of eosinophils and lymphocytes and an increase in the number of stab neutrophils. The number of segmented neutrophils in the blood of calves of experimental group 1 exceeded the indicators of clinically healthy animals by 22% ($P < 0.05$), which is a sign of the transition of the inflammatory process to a chronic form.

The effectiveness of using the drug “Calfmin” for the prevention of nonspecific bronchopneumonia in calves has also been proven (Sharandak *et al.*, 2022). At the end of the experiment, when using basic aerosol treatment according to the farm scheme, there was a significant increase in the body weight of calves of experimental group 1 by 28% ($P < 0.001$) compared to the initial data, which is 12% ($P < 0.05$) higher than in calves of the control group. The body weight of calves of experimental group 2, which were treated with the drug “Calfmin” in the form of an aerosol, increased by 35% ($P < 0.001$), which is 13.7% ($P < 0.01$) more than the corresponding indicators in animals of the

control group. The lower growth energy of the calves of the control group was probably conditioned by the presence of diseases of the gastrointestinal and respiratory tracts in them.

At the beginning of aerosol prevention, the number of red blood cells, haemoglobin content, hematocrit value, and platelet count in the blood of calves in all experimental groups were within the standard indicators. At the end of the experiment, the blood parameters of calves of experimental group 1, which were used for aerosol prevention of nonspecific bronchopneumonia according to the farm scheme, did not differ in their values from the blood indicators of animals in the control. On the third day of the experiment, a significant increase in haemoglobin content and an increase in platelet count by 1.05 times ($P < 0.05$) and 1.10 times ($P < 0.01$) was noted in the blood of calves of experimental group 2, respectively, compared with the indicators in animals of the control group. On the seventh day of the study, the haemoglobin content in the blood of calves of experimental group 2 increased by 1.1 times ($P < 0.01$) compared to the control group. Other blood parameters of calves of experimental group 2 tended to improve at the end of the experiment, but did not have a significant difference compared to the corresponding blood parameters of calves of the control group.

At the beginning of group aerosol prophylaxis, the number of white blood cells in the blood of calves of all groups did not differ and this trend continued throughout the entire period of experimental studies. According to the results of the analysis of the blood leukogram of calves of the experimental groups, an increase in the number of neutrophils by 1.3 times ($P < 0.001$) was established on the first day of the experiment, while a decrease in the number of lymphocytes by 1.1 times ($P < 0.01$) compared to the control group. On the third day of aerosol prophylaxis of calves of experimental

group 2 using the drug “Calfmin”, the number of neutrophils in the blood decreased by 1.3 times ($P < 0.01$) compared to the first day of the experiment, and the number of lymphocytes increased by 1.1 times ($P < 0.01$). On the third day of the experiment, the leukogram of calves of experimental group 1, which were treated with aerosol prophylaxis using a farm scheme, was characterised by a tendency to reduce the number of stab and segmented neutrophils. The leukogram of calves in experimental group 1 did not differ from that in the animals of the control group. On the seventh day of group aerosol prophylaxis, the leukogram indicators of calves in both experimental groups did not have significant differences compared to the corresponding indicators in the control.

M.M. Koptev (2011) notes that in healthy animals, the lungs are free of microflora. K. Suzuki *et al.* (2012) and B.T.S. Pantoja *et al.* (2020) reported that this feature is conditioned by the action of protective mechanisms, among which an important role belongs to the ciliated epithelium of the upper respiratory tract, the mucous layer that covers them, surfactant, interferon, lysozyme, micro- and macrophages. Researchers J.B.A. Polanco *et al.* (2020) note that it is this factor that contributes to the occurrence of the disease, when moist cold air irritates trophic nerves. E.R. Schachner (2017) and E. Oliveira *et al.* (2020) report that when an animal overheats, biochemical and biophysical changes occur in its body, manifested by a violation of the cardiovascular system and a decrease in metabolic processes. The bactericidal activity of blood serum and phagocytic activity of white blood cells decreases, which helps to reduce the resistance of lung tissue. This, in turn, contributes to the development of opportunistic microflora and the occurrence of toxico- sis, which leads to pneumonia.

B. Wolfger *et al.* (2015) indicate that in the acute course of bronchopneumonia,

superficially located lobes of the lungs are first affected. In the initial stages of the disease, interparticle connective tissue is a barrier to the transition of inflammation from the affected areas of the lungs to healthy ones, but in the future, the barrier function of connective tissue is lost. J. Glodek *et al.* (2016) and P. Sharandak *et al.* (2022) note that nonspecific bronchopneumonia is characterised by inflammation of the bronchi and lungs, accompanied by their filling with catarrhal exudate, a disorder of external and internal gas exchange, the development of respiratory and cardiovascular insufficiency, and impaired functions of various organs and systems. This classical picture of the pathogenesis of this pathology was naturally manifested in the study described in this paper.

Researchers expressed that in the affected lobes of the lungs, exudate covers the alveolar epithelium and thereby reduces the lumen of the alveoli and bronchioles (Jodi & Randy, 2020). Therefore, according to K. Hermeyer *et al.* (2012) and N.C. Gaeta *et al.* (2018), part of the respiratory surface of the lungs is excluded from gas exchange and shortness of breath and hypoxia occur, which leads to disruption of tissue and cellular respiration, weakening of oxidative processes and the formation of energy for the vital activity of the body. K. Hermeyer *et al.* (2012) and N.C. Gaeta *et al.* (2018) indicate that cardiac activity accelerates, the speed of blood flow increases, as a result of which tissues receive more blood, and with it oxygen. According to K. Dudek *et al.* (2020), all this compensates for impaired breathing, but with the further development of pneumonia and the damage to large areas of the lungs, the compensatory activity of the cardiovascular system weakens, which was also observed in this study.

Many researchers and practitioners of veterinary medicine have always paid attention to the causes of bronchopneumonia and its course. P. Sharandak *et al.* (2022) are unanimous

in their opinion that pathogenic microflora and unfavourable environmental conditions play a leading role in the development of the disease. However, the opinion of researchers on the issue of primacy is far from ambiguous. In a clinical study of calves with nonspecific bronchopneumonia, it is important not to miss the first symptoms of the disease. The presence of liquid serous-mucosal secretions suggests that the role of viral load is not excluded in the aetiology of nonspecific bronchopneumonia of calves, and the change of secretions to catarrhal (or catarrhal-purulent) indicates that bacterial microflora plays a leading role in the pathogenesis of bronchopneumonia. All animals with nonspecific bronchopneumonia (100%) at the beginning of the disease showed an increase in the number of respiratory movements, the appearance of inspiratory dyspnea and abdominal breathing, which coincides with the results of studies by C. Senthilkumaran *et al.* (2013).

The respiratory rate is a very valuable indicator, since tachypnea always occurs when gas exchange is disrupted due to hypoxemia. Recording of the respiratory rate is an indirect method of analysing the gas composition of blood. Healthy calves older than two weeks should normally have no more than 40 breathing movements per 1 minute. As an exception, respiratory rates of more than 40 per 1 minute are allowed only in animals that are kept indoors at high ambient temperatures. In addition, it is necessary to determine the respiratory rate at a distance, from the passage of the calf house, because this is the only way to get reliable data on the respiratory rate of the animal at rest.

During the conducted studies, the respiratory rate in calves with bronchopneumonia averaged 43 respiratory movements per 1 minute, and in some animals, it reached 50 or even 60 respiratory movements per 1 minute. This makes it possible to diagnose gas exchange disorders in

the body of calves due to hypoxemia. The depth (intensity) of breathing in healthy calves is so low that chest movements at a distance are sometimes impossible to distinguish. This is always a favourable prognostic symptom. On the other hand, the easier it is to visually determine the respiratory rate, the more effort the animal spends on breathing movements, which is an unfavourable symptom.

When determining the clinical condition of calves with nonspecific bronchopneumonia, the rhythm of breathing was also evaluated. In the initial phase of the disease for nonspecific bronchopneumonia, the act of inspiration in calves was prolonged and the coefficient was 1:1, or even 1.2:1. This characterised inspiratory dyspnea and indicated a predominance of inflammatory processes in the respiratory tract, rather than in the lung parenchyma. Signs of expiratory dyspnea, which significantly extended the duration of exhalation relative to inhalation (1:1.6-1.8), were found in calves with a prolonged course of bronchopneumonia, which indicated an increase in the volume of intrathoracic gases (the appearance of areas of the lungs with emphysema).

During a clinical examination of calves with nonspecific bronchopneumonia, cyanosis of the mucous membranes of the oral cavity, conjunctiva, and nasal mirror was detected in all (100%) animals. This indicated circulatory disorders and the accumulation of excessive amounts of reduced haemoglobin in the blood of animals, which has a dark colour. The accumulation of excessive amounts of reduced haemoglobin in the blood of patients with nonspecific bronchopneumonia in calves is obviously associated with insufficient oxygen saturation of the blood due to inflammatory processes in the lungs (Amat *et al.*, 2019; Buczinski & Pardon, 2020; Di Teodoro *et al.*, 2020).

Y. Yamamoto *et al.* (2003) reported that auscultation of the lungs, at the beginning

of the disease in most calves, showed broncho-vesicular respiration, which was noted in this study. This is conditioned by difficulty in air intake due to narrowing of the airways, a decrease in the elasticity of the alveoli due to infiltration of their walls, and a decrease in the number of alveoli involved in respiratory movements (Van Driessche *et al.*, 2020). On the second or third days, wet wheezing was detected, which indicated an accumulation of exudate in the respiratory tract. Crepitation was also observed, which was best listened to at the peak of inspiration.

The results of morphological studies of the blood of calves with nonspecific bronchopneumonia confirmed the development of an inflammatory process in their body. Neutrophils, especially segmented ones, are known to have the highest phagocytic activity. Pronounced leukocytosis, with a significant increase in the relative and absolute number of these cells in the leukogram of sick calves, indicates activation of defence mechanisms against bacterial infection. A 2.0-fold increase ($P < 0.05$) in the relative and absolute number of stab neutrophils in the blood of these animals indicates an acute course of the inflammatory process. Along with the development of neutrophilic leukocytosis (considered as a positive protective reaction of the body), eosinopenia and lymphocytopenia were recorded in sick calves. A decrease in the number of lymphocytes – the main immunocompetent cells in the blood of animals, is interpreted as a decrease in the immune status of the body of calves (Basoglu *et al.*, 2016; Braun *et al.*, 2018). Thus, lymphocytes (namely B-lymphocytes) are the main producers of plasma cells, which are transformed into immunoglobulins (antibodies) and perform antibacterial protection in the body of animals and humans. Due to intoxication of the animal's body with inflammatory products due to bronchopneumonia, deep violations of the receptor

apparatus of lymphocytes occur. A decrease in the immune status of calves' bodies with bronchopneumonia is indicated by a decrease in the number, and sometimes complete absence, of eosinophils in the blood, which were recorded in animals of experimental groups.

The use of aerosol medicines is the most effective in the treatment of respiratory diseases in animals. In this case, direct contact of the drug with the pathogenic microflora of the respiratory tract and the site of the lesion is detected. As a result, the concentration of drugs on the mucous membrane of the respiratory tract is higher compared to intramuscular or oral administration. In this regard, the absorption of the drug was more efficient and faster, which also reduced its consumption by 4 times.

D. Bednarek *et al.* (2005) indicate that complex therapy is the most effective for bronchopneumonia, which is aimed at eliminating violations of the technology of maintenance and feeding, increasing the body's resistance, and includes the simultaneous use of various means of therapy: antimicrobial, pathogenetic, symptomatic, stimulating, and vitamin. The authors recommend using nonsteroidal anti-inflammatory drugs as the main medications. The study investigated the drug "Calfmin", which is a complex with the plant immunomodulator echinacea and silver and copper nanoaquachelates, iodine solution, and cobalt lactate (Drobot *et al.*, 2013).

Therefore, the developed method is rational for modern high-tech livestock complexes with a large number of animals and is focused on obtaining high-quality and safe livestock products, the consumption of which in human nutrition should ensure high standards of quality of life. The method of group aerosoletherapy and aerosoleprophylaxis of diseases of young animals in preventive veterinary technologies using a natural remedy in combination with the plant immunomodulator echinacea, will allow

agricultural enterprises to obtain organic products that meet the highest quality standards.

Conclusions

In calves with nonspecific bronchopneumonia, which are raised at a modern high-tech enterprise for the production of milk, the following clinical symptoms are observed: significant inhibition of animals (mainly lying down, low appetite, a shaky gait and muscle tremor were noted when forced to get up), visible mucous membranes were cyanotic, which is a sign of impaired gas exchange in the small circle of blood circulation. When examining the respiratory system, mixed shortness of breath, sharp, dry, jerky and painful cough were identified. Exudate discharge was detected in the nasal passages, which is a typical symptom of the disease. Auscultation of the lungs diagnosed the presence of wet wheezing, and chest percussion revealed areas of blunting of the apical and cardiac areas of the lungs. During auscultation of the heart, muffling of heart tones and an increase in pulse wave were noted when examining the arteries. Hyperthermia by 1.0-1.5°C higher than normal, as well as tachycardia and tachypnea, which corresponded to an acceleration of 1.2 and 1.5 times ($P<0.001$), respectively, compared to clinically healthy animals, were noted in sick animals. According to the results of morphological examination of the blood of

sick calves, a 1.2-fold decrease in the number of red blood cells ($P<0.001$) and a 1.3-fold increase ($P<0.005$) in the number of white blood cells were noted. Specific changes in the leukogram for bronchopneumonia were established: an increase in the number of stab and segmented neutrophils by 2.0 times ($P<0.005$) and 1.6 times ($P<0.01$), respectively, and a decrease in the level of lymphocytes by 1.3 times ($P<0.001$) and eosinophils by 1.4 times ($P<0.001$) compared to the control group of animals. Characteristic signs of the disease were oligocytopenia, hypochromia, neutrophilic leukocytosis, lymphocytopenia, and eosinopenia. The use of the test agent at a dose of 7.0 mL per 1 animal and exposure of 45 minutes in group therapy of calves with nonspecific bronchopneumonia by aerosol method improved the clinical condition and blood parameters of calves for 3-5 days and reduced the duration of their treatment by 1.9-2.0 times. In the future, it is planned to consider in more detail the immunological parameters of calves' blood for using the proposed method of their treatment in the case of nonspecific bronchopneumonia.

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Conflict of Interest

None.

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Ефективність групової терапії телят за неспецифічної бронхопневмонії методом аерозолію

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Анотація. Неспецифічна бронхопневмонія телят завдає значних економічних збитків фермерським господарствам, а тому актуальним є розробка ефективних терапевтичних препаратів, особливо для групового застосування. Заслужують на увагу лікарські засоби, що застосовуються аерозольно. Метою роботи було дослідити ефективність групового аерозольного методу застосування телятам препарату «Кальфмін» за неспецифічної бронхопневмонії. У дослід залучали телят-аналогів двох-трьох місячного віку як клінічно здорових, так і хворих на неспецифічну бронхопневмонію. Для визначення діагнозу і контролю за функціональним станом організму телят використовували стандартні клінічні та гематологічні методи дослідження. Встановлено, що у крові телят, хворих на неспецифічну

бронхопневмонію, відзначалося підвищення кількості лейкоцитів в 1,3 раза ($P<0,05$) порівняно з клінічно здоровими тваринами. В їх крові також виявляли збільшення кількості паличкоядерних і сегментоядерних нейтрофілів в 2,0 рази ($P<0,05$) і 1,6 раза ($P<0,01$), відповідно, та зменшення кількості лімфоцитів в 1,3 раза ($P<0,001$) і еозинофілів в 1,4 раза ($P<0,001$), відповідно. В хворих телят відмічали зменшення в крові кількості еритроцитів в 1,2 раза ($P<0,001$) і вмісту гемоглобіну в 1,2 раза ($P<0,001$) порівняно з контролем. Зафіксували покращення клінічного стану і гематологічних показників у хворих тварин упродовж 3-5 діб, скорочення тривалості терапії в 1,9-2,0 рази та 100% збереженість усіх хворих телят

Ключові слова: наноаквахелати; срібло; мідь; йод; кобальт; ехінацея пурпурова; морфологія крові