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ANTHELMINTIC-SALT MIXTURES – AN EFFECTIVE MEANS IN PREVENTION OF HELMINTHIASES IN SHEEP

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Abstract. *The article presents results of studying the effectiveness of the developed anthelmintic-salt mixture enriched with microelements (Co, I, Zn, Mn, Mg, and Cu) against sheep helminths and its effect on hematological parameters of animals. Experimental studies were performed under laboratory and farm conditions. The results of laboratory studies have shown that one animal consumed an average of 6.48 g of an anthelmintic-salt mixture per day. It was found that in sheep of the experimental group the extensiveness of infection by Marshallagia was 18.75%, Nematodirus – 6.25%, other gastrointestinal Strongyloides – 43.75%, Moniezia – 12.5%, Fasciola – 37.5%, Dictyocaulus – 25.0%, while in sheep of the control group, these figures were much higher and constituted 87.5%, 50.0, 50.0, 37.5, 62.5, and 50.0%, respectively. The results of hematological studies have shown that long-term feeding of an anthelmintic-salt mixture enriched with microelements does not have a negative effect on the body of sheep. When conducting studies directly under sheep farm conditions, in sheep fed an anthelmintic-salt mixture enriched with microelements for 8 months, the extensiveness of infection was 28.6%, while among sheep in the control group, this figure was 70.0%. Therefore, the results of studies indicate the feasibility of application the anthelmintic-salt mixture enriched with microelements for the treatment and prevention of helminthiases in sheep.*

Keywords: *anthelmintic-salt mixture, microelements, helminths, helminthiases, extensiveness of infection, intensity of infection*

Introduction

Sheep breeding is one of the important areas of animal husbandry. In the Republic of Uzbekistan, the sheep population amounts to more than 20 million, of which more than half are Karakul sheep (Aripov et al., 2020). The domestic sheep provide humans with milk, meat, wool, and sheepskins. Besides that, the by-products obtained from the sheep, like tallow, intestine, wool wax, horns, and hooves, are also valuable. The main challenges that the sheep industry faces with in desert and semi-desert areas are water shortage, complicated grazing problems, and a prevalence of diseases, including helminthiases. On the territory of the Republic of

Uzbekistan, the spread of helminthiases among sheep is facilitated by extensive sheep breeding, year-round grazing in case of insufficient compliance with the terms and frequency of deworming. The infection of sheep by helminths causes complex pathological processes in the body, which may decrease the growth rate in young animals, reduce the qualitative and quantitative performance indicators (wool, meat, and dairy), increase feed conversion ratio. Therefore, parasitic diseases cause significant economic damage to the sheep industry, and the development of modern methods and tools for the treatment and prevention of helminthiases in sheep is important.

Analysis of recent researches and publications

Among helminth infections in sheep, the lungworms and gastrointestinal parasites are dominated. The impacts of parasitism on livestock can be severe, arising from pathological changes to the gastrointestinal tract. However, many impacts are sub-clinical, acting through suppression of appetite and impaired assimilation of forage (Coulson et al., 2018).

The levels of infection by parasites is highly dependent on the season and have annual variations. For example, the incidence of the liver fluke, Fasciola hepatica, is inextricably linked to high rainfall and is particularly prevalent in high rainfall years. Even climate change may have profound effects on parasite epidemiology, especially for those parasitic diseases where the weather has a direct effect on the development of free-living stages (Taylor, 2012).

According to Karlsson & Greeff (2012), there is evidence of genetically determined host resistance mechanisms for most of the sheep parasites. These mechanisms vary; from no or reduced establishment, early expulsion, to suppression of parasites resulting in reduced size and fecundity.

There are different methods for identifying species of helminth parasites in ruminants. The development of molecular markers for the identification of helminths has given successful results as far as precision is concerned. The molecular methods include amplified fragment-length polymorphism analysis, a parasite-specific DNA probe, restriction fragment length polymorphism analysis, a polymerase chain reaction (Dallas et al., 2000; Pyziel et al., 2015; Biswal, 2016). The association of light and scanning electron microscopy allowed a detailed analysis of the morphology and ultrastructure of nematodes (Bashtar et al., 2011; Borji et al., 2011). However, the fecal egg count reduction test (FECRT) is the recommended method to monitor anthelmintic drug efficacy in ruminants. There is a large variation in fecal egg count methods applied to determine FECRT (Levecke et al., 2012).

Prophylactic treatment with anthelmintics is the primary control measure for internal parasite infection in sheep. Since their first introduction into the market in the late 1930s (phenothiazine) and advances in the 1960s (thiabendazole and levamisole), these chemicals have had a revolutionary effect on sheep husbandry practices, in particular facilitating single enterprise intensive sheep farming. Anthelmintic use is now so widespread and conventional that it is believed that without anthelmintic drugs the sheep industry could not exist in its current form (Sayers & Sweeney, 2005).

It is known that helminthiases of sheep, such as gastrointestinal strongylatoses, dictiocaulosis, anoplocephalatoses, especially monieziosis, are widespread in all climatic and geographical

zones all over the world and cause colossal damage to sheep breeding. Against the widespread occurrence of these helminthiases, in the 50–60s of the past century, a prophylactic drug was developed and widely introduced – an anthelmintic-salt mixture, consisting of 10% phenothiazine, 1% copper sulfate, and 89% sodium chloride (NaCl). However, with the cessation of phenothiazine production in the 90s, the use of an anthelmintic-salt mixture became impossible.

Due to the importance and effectiveness of this prophylactic drug in the Republic of Uzbekistan, studies were carried out to develop a new composition of the anthelmintic-salt mixture using modern anthelmintic drugs – albendazole, fenbendazole, tetramisole instead of phenothiazine (Oripov et al., 2007, 2007a, 2007b). These anthelmintic drugs have shown fairly high efficiency in the prevention of clinically manifested cases of the above-mentioned helminthiases in sheep.

However, in order to improve these means, as well as taking into account the enrichment of the composition of the anthelmintic-salt mixture with microelements, which are usually lacking in the soil and plants of desert and semi-desert regions – the main areas of sheep breeding, we conducted research on the development of an anthelmintic-salt mixture enriched with microelements. So, the purpose of the study was to test the developed anthelmintic-salt mixture enriched with microelements for its anthelminthic properties in laboratory and farm conditions.

Materials and methods of research

The experiments were carried out in laboratory conditions as well as directly under conditions of Karakul farms. The sheep of experimental groups were fed with anthelmintic-salt mixture containing 0.02% albendazole (by active substance), 1.0% copper sulfate (CuSO4), 0.1% zinc sulfate (ZnSO4), 0.05% magnesium sulfate (MgSO4), 0.05% manganese sulfate (MnSo4), 0.005% sodium iodine (NaI), 0.025% cobalt chloride (CoCl), and 98.5% sodium chloride (NaCl) as the filler. The experiments conducted on sheep in the laboratory conditions lasted for 630 days, and the experiments under farm conditions in accordance with the recommended terms of feeding the anthelmintic-salt mixture – 8 months, from October 1 to May 31.

Before the beginning of feeding sheep with anthelmintic-salt mixture and during the experiment, helminth-ovoscopic and larvoscopic examinations were carried out in order to determine the infection of animals by the causative agents of helminthiases – gastrointestinal and pulmonary nematodoses, anoplocephalatoses (monieziosis), fascioliasis, and other helminthiases.

Hematological examinations were carried out to determine the blood formed elements, the amount of hemoglobin, and erythrocyte sedimentation rate by conventional methods.

Results of the research and their discussion

The results of experiments carried out in the laboratory conditions have shown that the average amount of consumed anthelmintic-salt mixture per one animal was 6.476 g per day.

The level of infection in sheep of the 1st group, i.e. the experimental, fed with the anthelmintic-salt mixture enriched with microelements for 630 days, was significantly lower than in sheep of the control group (Table 1).

1. Results of laboratory testing of anthelmintic-salt mixture enriched with microelements on sheep

Group	Extensiveness of infection, %						
	Marshallagia	Nematodirus	Other gastrointestinal Strongyloides	Moniezia	Fasciola	Dictyocaulus	All helminths
1st Experimental, n=16	18.75	6.25	43.75	12.50	37.50	25.00	90.00
2nd Control, n=8	87.50	50.00	50.00	37.50	62.50	50.00	100.00

So, the sheep of the 1st (experimental) group were infected by different helminths by 90.0%, their extensiveness of infection by Marshallagia was 18.75%, Nematodirus – 6.25%, other gastrointestinal Strongyloides – 43.75%, Moniezia – 12.5%, Fasciola – 37.5%, and Dictyocaulus – 25.0%. These indicators in sheep of the 2nd (control) group were 100.0%, 87.5, 50.0, 50.0, 37.5, 62.5, and 50.0%, respectively, i.e. were significantly higher than indicators in sheep of the experimental group.

Our results have shown that long-term, i.e. within 630 days, feeding sheep with an anthelmintic-salt mixture enriched by microelements does not have a negative effect on the body, as evidenced by the results of hematological studies given in Table 2. So, the amount of hemoglobin in the blood of sheep of the experimental group was on average 10.82%, the number of erythrocytes – 5.9 million/mm3, leukocytes – 8.9 thousand/mm3, the erythrocyte sedimentation rate in 24 hours – 8.9 mm. These indicators in sheep of the control group were 11.82%, 5.8 million/mm3, 7.4 thousand/mm^3, and 11.9 mm/hour, respectively.

Consequently, the blood parameters in sheep of both the experimental and control groups did not undergo significant changes and were within the physiological norm during the experimental period.

The results of testing anthelmintic-salt mixture enriched with microelements directly in sheep breeding farms (Table 3) showed that the extensiveness of infection in sheep of the experimental flock that received anthelmintic-salt mixture for 8 months with all helminths, i.e. the total extensiveness of infection by helminths was 28.6%, and the extensiveness of infection by Marshallagia was 2.8%, by Nematodirus – 17.14%, by other gastrointestinal Strongyloides and Moniezia – 2.8%. The sheep of the control flock that did not receive anthelmintic-salt mixture were infected with all helminths by 70.0%, Marshallagi

2. Hematological parameters in sheep treated with anthelmintic-salt mixture and control animals

Group	Hemoglobin, g%	Erythrocyte sedimentation rate, mm/hour		Erythrocytes, million/mm^3	Leukocytes, thousand/mm^3
		1 hour	24 hours		
1st Experimental	10.8 ± 0.56	1.9	8.4	5.9 ± 0.38	8.9 ± 0.26
2nd Control	11.0 ± 0.71	1.5	11.9	6.2 ± 0.44	7.4 ± 0.41

3. Results on testing the anthelmintic effectiveness of anthelmintic-salt mixture enriched with microelements in sheep farms

Group	Content of active substance	Extensiveness of infection in relation to the control group, %				
		Marshallagia	*Nematodirus*	*Other gastrointestinal Strongyloides*	*Moniezia*	Total
1st Experimental, n = 961	Albendazole (0.02%), $CuSO_4$ (1%), $ZnSO_4$ (0.1%), $MgSO_4$ (0.05%), $MnSO_4$ (0.05%), NaI (0.005%), CoCl (0.025%)	2.8 16.8	17.14 57.1	2.8 16.8	2.8 16.8	28.6 40.8
2nd Control	None	16.6 100.0	30.0 100.0	16.6 100.0	26.7 100.0	70.0 100.0

and other Strongyloides – 16.6%, Nematodirus – 30.0%, Moniezia –26.7%.

It should be noted that despite the definitely high extensiveness of infection indices of sheep in the experimental group by some helminths (Nematodirus – 17.1%) and total infection (40.6%), the intensity of infection by helminths was significantly lower than those in sheep of the control group.

Consequently, the application of the anthelmintic-salt mixture enriched with microelements for sheep in farm conditions causes a significant (by 60–80%) decrease in the level of infection by helminths, which leads to the conclusion that it is advisable to use the developed anthelmintic-salt mixture in sheep farming.

The helminth-ovoscopic and larvoscopic examinations allowed determining the species of helminths that infected sheep. These results have shown that sheep in Karakul farms were infected with Marshallagia, Nematodirus, gastrointestinal Strongyloides, Moniezia, Fasciola, and Dictyocaulus.

The intestinal cestode Moniezia spp. is of global occurrence, and its infection leads to economic losses in livestock, especially in calves and lambs (Obanda et al., 2019). Cestodes are common gastrointestinal parasites of humans and livestock. They attach to the host gut and, without a mouth or intestinal system, absorb nutrients through their epidermis (Mair et al., 2020).

Species of Marshallagia are abomasal parasites in free-ranging and domesticated ungulates in temperate climatic zones throughout the world. The development of Marshallagia marshalli in the abomasal glands of ruminants causes pathophysiological changes, which include a reduced acidity of the abomasal contents, increased abomasal pH, and increased serum pepsinogen concentrations. The reduced acid secretion is explained by a replacement of functional parietal cells by undifferentiated cells (Moradpour et al., 2013).

The presence of Strongyloides spp. and in the host community is of public health interest because of the zoonotic nature of these nematodes (Obanda et al., 2019). The pathogenic impact of the parasites on the host derives from the pres-

ence of the larvae during migration and/or the adult forms in the intestines, which through their mechanical and secretory/excretory products are harmful to the host's tissues. The presence of Strongyloides papillosus causes disturbances in animals' health, quite frequently inducing the sudden death syndrome in lambs due to heart failure (Dimitrijević et al., 2012).

Fasciola hepatica and Fasciola gigantica are the major causative agents of liver fluke disease (fascioliasis) in domestic animals in regions with temperate and tropical climates, respectively. Although traditionally regarded as a disease of livestock, fascioliasis is now recognized as an important emerging zoonotic disease of humans. Transmission occurs where rural farming communities regularly share the same water source as their animals or consume water-based vegetation growing in endemic areas (Robinson & Dalton, 2009).

Nematodirus spp. are among the most common nematodes of ruminants, mainly inhabiting the small intestines in sheep. Nematodirosis is a major cause of severe diarrhea and mortality in young lambs (Melville et al., 2020). Acute disease is the consequence of very heavy larval challenge and the effects of the developing larvae (Morrison et al., 2014).

Dictyocaulus lungworms are the causative agents of parasitic bronchitis (dictyocaulosis) in ruminants. Clinical signs include coughing, nasal discharge, emphysema, and pneumonia, which may lead to death of heavily infected individuals (Pyziel et al., 2015). Lungworms cause the development of bacterial pneumonia due to secondary complications in the lungs, as well as a decrease in milk production and liveweight loss, especially in young animals. Dictyocaulus filaria is the most pathogenic lungworm in sheep. The adult worms live in the trachea, bronchi, and bronchioles of small ruminants (Sevimli et al., 2011).

Thus, the high prevalence of mixed parasitic infections among sheep in Karakul farms is a very serious problem for owners and requires urgent control measures. There are several ways for effective control and prevention of described helminth infections. Novel developments for the management of nematode parasites such as vaccines, biological anthelmintics, genetic markers, and selective breeding of sheep may, in the future, provide additional or alternative means of parasite control. However, such alternative control methods are likely to be more dependent on a sound understanding of the lifecycle and population dynamics of the parasites involved and the epidemiology of disease they cause than current methods that rely heavily on broad-spectrum anthelmintics (Vlassoff et al., 2001).

The application of anthelmintic treatment of sheep on farms and pasture management are the main tools to provide effective control measures of gastrointestinal and respiratory tract helminths. However, the emergence of anthelmintic-resistant strains of parasitic nematodes and the increasing reliance placed on anthelmintics for their control can exert profound changes on the epidemiology of these helminths (Taylor, 2012).

This proves the necessity to develop new effective anthelmintics for sheep husbandry. The application of anthelmintic-salt mixture enriched with microelements have shown sufficient anthelmintic efficacy in testing on sheep under laboratory and farm conditions, didn't have a negative impact on hematological parameters and might be recommended to use for control and prevention of parasitic infections among sheep.

Conclusions and future perspectives

The long-term ad libitum feeding of sheep with anthelmintic-salt mixture consisting of 0.02% albendazole (by active substance), 1.0% copper sulfate (CuSO4), 0.1% zinc sulfate (ZnSO4), 0.05% magnesium sulfate (MnSO4), 0.05% manganese sulfate (MnSo4), 0.005% sodium iodine (NaI), and 0.025% cobalt chloride (CoCl) does not have a negative effect on the body and significantly reduces the degree of infection by gastrointestinal Strongyloides and Moniezia in animals, and also prevents the clinical manifestation of the diseases caused by these helminths.

It is advisable to use an anthelmintic-salt mixture in sheep-breeding farms, which includes microelements missing in the soil and plants of a particular region.

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Анотація. *У статті представлені результати дослідження ефективності застосування розробленої антгельмінтно-сольової суміші збагаченої мікроелементами (Co, I, Zn, Mn, Mg і Cu) проти гельмінтів овець та її вплив на гематологічні показники тварин. Експериментальні дослідження проводили в лабораторних та виробничих умовах. За результатами досліджень проведених у лабораторних умовах встановлено, що одна*

тварина в середньому споживала 6,48 г антгельмінтно-сольової суміші на добу. З'ясовано, що в овець дослідної групи екстенсивність інвазії маршалагіями складала 18,75%, нематодірусами – 6,25%, іншими стронгілятами травного каналу – 43,75%, монієзіями – 12,5%, фасціолами – 37,5%, диктіокаулами – 25,0%, тоді як у овець контрольної групи ці показники були значно вищими і відповідно складали: 100,0%, 87,5, 50,0, 50,0, 37,5, 62,5 і 50,0%. Результати гематологічних досліджень показали, що тривале згодовування антгельмінтно-сольової суміші збагаченої мікроелементами не має негативного впливу на організм овець. Під час проведення досліджень безпосередньо у вівчарських господарствах у овець, яким упродовж 8 місяців згодовували антгельмінтно-сольову суміш збагачену мікроелементами, екстенсивність інвазії гельмінтами складала 28,6%, тоді як поміж овець контрольної групи цей показник становив 70,0%. Отже, результати досліджень вказують на доцільність застосування антгельмінтно-сольової суміші збагаченої мікроелементами для лікування та профілактики гельмінтозів у овець.

Ключові слова: *антгельмінтно-сольова суміш, мікроелементи, гельмінти, гельмінтози, екстенсивність інвазії, інтенсивність інвазії*

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TRYPANOSOMIASIS IN MICE OF THE CHERNOBYL ZONE OF RADIOACTIVE CONTAMINATION

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Abstract. *One of the most obvious and long-lasting consequences of the Chornobyl nuclear power plant accident is the creation of an exclusion zone and a zone of unconditional (compulsory) alienation. The effect of succession is manifested in the fact that a significant part of aquatic and terrestrial ecosystems as of 1986 was artificial or semi-artificial systems that were under regulatory control by humans. Parasitic systems are one of the informative indicators of processes occurring in ecosystems. Any changes in the host population lead to changes in the parasite population. The degree of imbalance of the "parasite-host" system*

depends on the nature and strength of the influence of external factors. In order to study the parasitic systems of the exclusion zone as informative indicators, rodents were selected. Among the rodents studied for the presence of causative agents of parasitic diseases were field (Apodemus agrarius) and yellow-necked (Apodemus flavicollis) mice. A study of them was conducted for the presence of causative agents of blood-borne parasitic diseases.

Examination of mice blood smears revealed the presence of causative agents of blood parasitic and infectious diseases in all of the investigated animals. Among the causative agents of these diseases, Trypanosoma spp., Babesia spp., Rickettsia spp. and others were identified. Trypanosoma spp. was identified for the first time on the territory of Ukraine in blood smears of 3 mice among investigated, which were 25 % (95 % confidence intervals 6.8–54.1). The number of parasites in 200 fields of view of the microscope from the studied mice varied from 2 to 25.

Keywords: *hemoparasitic diseases, mice, Apodemus flavicollis, Apodemus agrarius, Trypanosoma spp.*

Introduction

One of the most obvious and long-lasting consequences of the Chornobyl nuclear power plant accident is the creation of an exclusion zone and a zone of unconditional (compulsory) alienation. Here, the negative impact of radioactive contamination on humans and the environment has manifested itself to the maximum. This led to the evacuation of the population and the curtailment of economic activity in an area of 2.600 km^2. 90–95% of this area has no systematic human activity and the regime is equal to the reserve (Balashov et al., 1992). Ecosystems formed in the exclusion zone are influenced by several key factors. Among them – radioactive contamination, succession, and climate change.

A characteristic feature of radioactive contamination of Chornobyl origin is the spatial heterogeneity of density, radionuclide composition, and physicochemical forms of precipitation. Most of the radionuclides were part of the "hot particles". The largest "hot particles" fell in the near zone. Contamination of most of the zone territory was formed by the finely dispersed fraction of "hot particles". In the form of radioactive contamination outside the exclusion zone, the condensation component is dominated. Today, the radiation situation is formed by the following elements: ^{137}Cs, ^{90}Sr, Pu (isotopes 238, 239, 240), ^{241}Am.

The effect of succession is manifested in the fact that a significant part of aquatic and terrestrial ecosystems as of 1986 was artificial or semi-artificial systems that were under regulatory control by humans. These are agro-landscapes, monocultural forests, irrigation and drainage systems, inhabited localities. The removal of regulatory control has led to the inclusion of natural mechanisms of dynamics, including catastrophic (fires, outbreaks, floods, etc.) (Vishnevskiy et al., 2004). That is, a significant part of ecosystems is in a state far from equilibrium, where the processes of succession are active.

The testamentary effect is the result of a radical reduction in economic activity and the creation of a strict protection regime. The increase in the number of background species and the appearance of rare species of animals and plants has been recorded for several years after the accident. We should also take into account the high landscape diversity and a large area of the exclusion zone.

Climatic factors are external to natural complexes and do not depend on

them. In recent years, climate change has had a significant impact on the hydrological regime and vegetation.

Based on the above, we can say that the dynamics of ecosystems in the exclusion zone is unique. The need for its study is due to a number of reasons: management of water and forest resources, assessment of the significance of natural complexes, the study of parasitic systems and zoonoses, and so on.

Parasitic systems are one of the informative indicators of processes occurring in ecosystems. Any changes in the host population lead to changes in the parasite population. The degree of imbalance of the "parasite-host" system depends on the nature and strength of the influence of external factors (Semenko et al., 2017; Semenko et al., 2020).

In order to study the parasitic systems of the exclusion zone as informative indicators, rodents were selected. Among the rodents studied for the presence of causative agents of parasitic diseases were field (*Apodemus agrarius*) and yellow-necked (*Apodemus flavicollis*) mice.

Analysis of recent research and publications

Trypanosomoses are diseases, which are caused by parasites of the genus *Trypanosoma*. They could be extracellular (*Trypanosoma evansi*) and intracellular (*Trypanosoma cruzi*) parasites (Riedel, 1975; McGhee et al., 1980; Nagle et al., 1980; Rodriguez-Morales et al., 2011; Norman et al., 2013; O'Connell et al., 2015). Species of this genus may differ in their transmission types (Sato et al., 2003; Rocha et al., 2004; Morio et al., 2008; Rotureau et al., 2013). Species, which are independent of tsetse flies belong to non tsetse transmitted animal trypanosomoses (NTTAT) (Schaub et al., 1994; Sharma et al., 2009). Investigation of these parasites, their control measures are coordinated with OIE Non Tsetse Transmitted Animal Trypanosomoses Network according to support of a global strategy for the control of NTTAT. NTTAT are spread in Africa, Asia, Latin America, and Europe.

We haven`t found information about the spreading of these parasites on the territory of Ukraine. But on the territory of Russia, causative agents of breeding disease of one-hoofed animals (*Trypanosoma equiperdum*) and surra of horses and camels (*T. evansi* (Fig. 1)) have been registered (Georgiu, 2015). *T. evansi* may cause in experimentally infected mice splenomegaly and hepatomegaly (Ghaffar et al., 2016) and might be a reason for human disease (Joshi et al., 2005).

Trypanosome species that are transmitted without tsetse flies include *T. evansi*, *T. equiperdum,* and *T. vivax.* These species cause surra, dourine, and nagana in a wide range of domestic animals like cattle, buffalo, horse, camel, small ruminants, and others. All these diseases lead to economic losses in endemic areas (Hoare et al., 1972; Stevens et al., 1999; Powar et al., 2006; Otto et al., 2010; Schwan et al., 2016). And also, these species could parasitize in the body of rodents such as mice.

One of the species, which could parasitize in the body of rodents and is transmitted by fleas is *Trypanosoma (Herpetosoma) lewisi*. Animals are infected by the oral route, through contamination by flea feces or ingestion of fleas (Shaw et al., 1973; Guerrero et al., 1997; Desquesnes et al., 2002). *Trypanosoma musculi* is also transmitted between mice by ingestion of infected fleas. These trypanosomes are of similar morphology, making it difficult to distinguish them by microscopy. There are also few records of human infection cases caused by *T. lewisi* (Viens et al., 1972; Ponzi et al., 1984; Howie et al., 2006; Rayat et al., 2014; Hong et al., 2017).

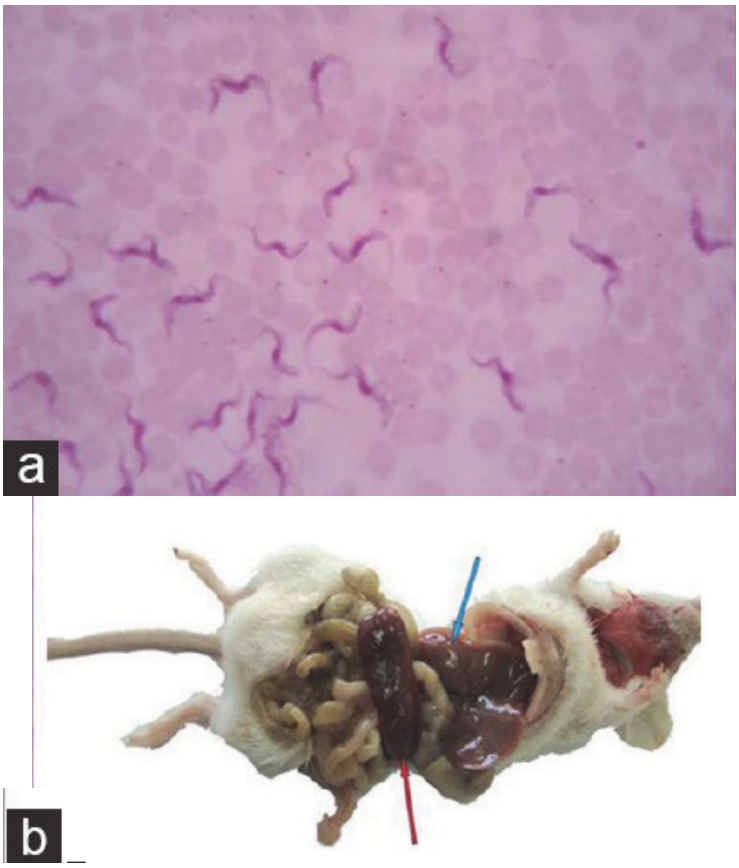

Fig. 1. (a) Giemsa stained blood smears from mice infected with T. evansi, ×1000; (b) Splenomegaly (red arrow) and hepatomegaly (blue arrow) in experimentally infected mice with T. evansi *(Ghaffar et al., 2016)*

3 morphologically indistinguishable subspecies of *Trypanosoma brucei* are involved in a complex transmission cycle between humans, tsetse, and reservoir hosts. These subspecies cause Gambian and Rhodesian sleeping sickness or coexist with the other trypanosomes in reservoir hosts and vectors (Dumas et al., 1999). And according to the results of investigations, *T. cruzi* DNA has been identified in mummified human beings in South America dating back 4000 years, which means that human beings were exposed to African trypanosomes concomitantly with their evolution (Guhl et al., 2000; Barrett et al., 2003; Otero et al., 2012).

Methods for identification of trypanosomes include rodent subinoculation, cryopreservation, *in vitro* methods, isoenzyme electrophoresis, molecular characterization, population genetics methods, true phylogenetic methods, and others (Dumas et al., 1999).

Pentamidine, suramin, melarsoprol (late-stage disease with central nervous system disorders), eflornithine are licensed for the treatment of trypanosomiases in humans. Nifurtimox, although only licensed for use against Chagas' disease. Diminazene (berenil), a veterinary medicament, has also been used occasionally without a license (Pepin

et al., 1994; Barrett et al., 2003; Pereira et al., 2009; Shikanai-Yasuda et al., 2012). But such species as *T. vivax,* according to the research, is resistant to diminazene aceturate but sensitive to isometamidium chloride (Desquesnes et al., 1995).

To the principles of control of trypanosomiases belong treatment of infected individuals, active case finding with mobile teams, vector control, personal prophylaxis, and serological control of blood banks (Barrett et al., 2003).

Materials and methods of research

The research was carried out on the 2nd landfill (51 ° 22'20.60 "N 30 ° 8'26.94" E) located on the drained areas at the bottom of the cooling reservoir of the Chornobyl nuclear power plant. The strength of the exposure dose of γ-radiation and the flux density of β-particles above the soil surface in places of catching animals were determined by γ-, β-radiometer "Pripyat" RKS 20.3.

Catching murine rodents of various species, which are widely used in radiobiological studies as indicators, were carried out in the second half of September 2020 using traps of the Sherman system. The duration of catches at the landfill was 3 days with daily inspection of traps. After capture, the animals were taken to the laboratory of the Institute for Nuclear Research of the National Academy of Sciences of Ukraine (Kyiv, Ukraine), examined, registered with individual numbers and values of the date and place of capture, species name, gender, body weight. Species affiliation of individuals was determined by morphological characteristics. Age was determined by a set of features: weight, body length, development of reproductive organs, and thymus. Thin blood smears were prepared from a drop of blood obtained from the heart. Staining of smears and their further examination was carried out on the basis of the Department of Pharmacology, Parasitology and Tropical Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine. A set of Leucodif 200 (LDF 200) kit in accordance with the manufacturer's instructions was used in order to stain the smears. Examination of stained blood smears was performed using a microscope with ×1000 and ×1150 magnification.

Results of the research and their discussion

12 mice were caught using traps of the Sherman system and then investigated. Among them, 6 mice were field (*Apodemus agrarius*) and 4 – yellow-necked (*Apodemus flavicollis*). For two mice, the species was not identified.

6 mice were females and 5 – males. For one mouse, age was not identified.

According to the blood smear examinations from mice of Chornobyl Radioecological Biosphere Reserve, *Trypanosoma spp*. agents were revealed for the first time in Ukraine (Fig. 2, 3). They were identified in blood smears from 3 mice, which accounted for 25% (95% confidence intervals 6.8–54.1). 2 of 3 positive mice were males *Apodemus agrarius* and 1 was unidentified gender *Apodemus flavicollis.*

The number of parasites in 200 fields of view of the microscope from the studied mice varied from 2 to 25.

Sizes of the *Trypanosoma spp*. in blood smears were 30–40 μm in length.

Also during blood smear microscopic examinations, *Babesia spp.* was identified in 8 mice (66.7%; 95% confidence intervals 37.7–88.4), *Rickettsia spp.* – in 9 mice (75%; 95% confidence intervals 45.9–93.2); anisocytosis, polychromasia, poikilocytosis, basophilic granularity, plasma cells, reactive lymphocytes, Jolly little bodies, and hypochromic erythrocytes were observed.

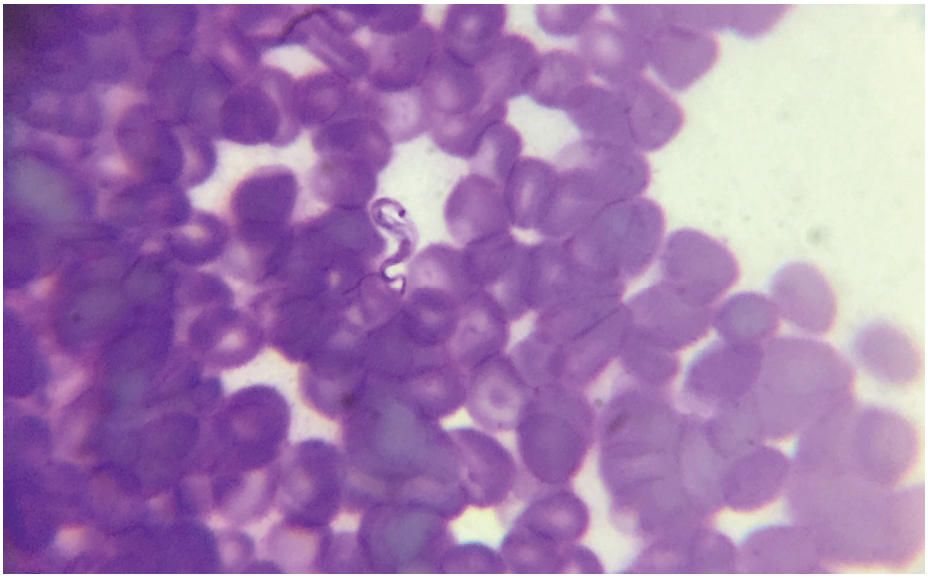

Fig. 2. Leucodif 200 (LDF 200) stained blood smears from mice infected with *Trypanosoma spp.*, ×1000

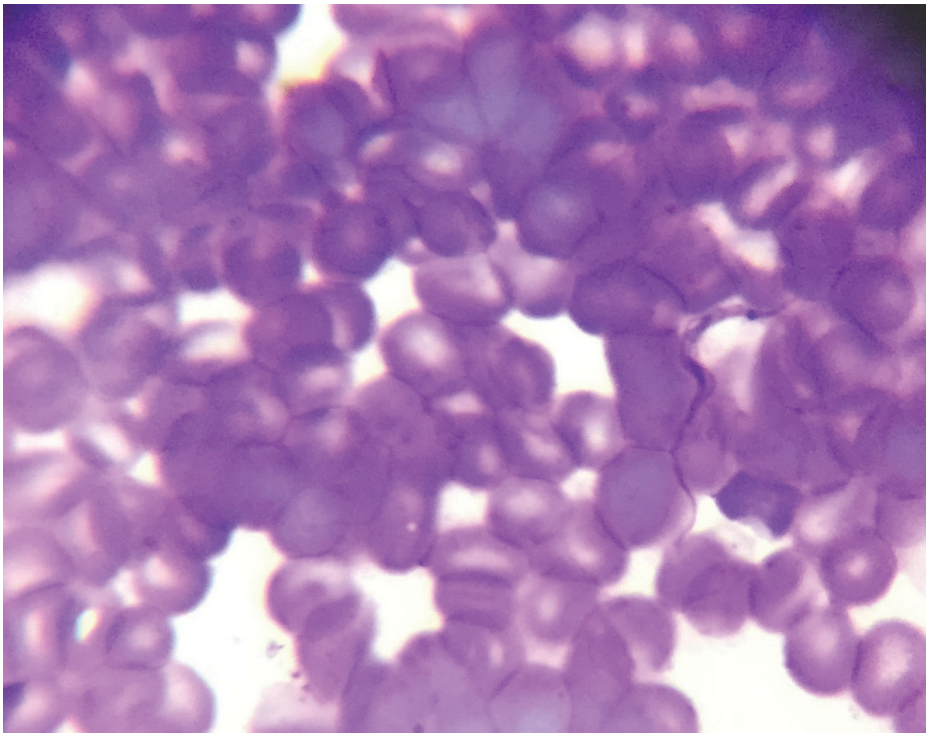

Fig. 3. Leucodif 200 (LDF 200) stained blood smears from mice infected with *Trypanosoma spp.*, ×1000

Conclusions and future perspectives

This is the first identification of the *Trypanosoma spp.* in Ukraine. Thus, murine rodents are hosts for a significant number of blood parasites, both infectious and parasitic. Some of them are also causative agents of zoonotic diseases. Further research, including PCR investigation, is essential to identify species of these agents, possible routes of transmission to murine rodents, and risks of transmission from animals to humans. In our future investigations we plan to conduct also genetic analysis to establish the registered species of *Trypanosoma spp.*, *Babesia spp*., and *Rickettsia spp.* not only morphologically in blood smears.

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Анотація. *Одним із найбільш очевидних і довготривалих наслідків аварії на Чорнобильській атомній електростанції стало створення зони відчуження та зони безумовного відчуження. Ефект сукцесії проявляється в тому, що значна частина водних і наземних екосистем станом на 1986 рік були штучними або напівштучними системами, які перебували під регуляторним контролем людини. Паразитоценози можуть бути інформативним фактором процесів, що відбуваються в екосистемах. Будь які зміни в популяції хазяїв призводять до змін у популяціях паразитичних організмів. Рівень співвідношення у паразито-хазяїнних системах залежить від сили і впливу зовнішніх факторів. З метою вивчення паразитарних систем зони відчуження як інформативних показників було відібрано гризунів. Серед досліджуваних мишей на наявність збудників паразитарних захворювань були польові (Apodemus agrarius) та жовтогорлі (Apodemus flavicollis). Було проведено їхнє дослідження на наявність збудників кровопаразитарних захворювань.*

Дослідження мазків крові мишей виявило наявність збудників паразитарних та інфекційних захворювань крові у всіх досліджуваних тварин. Серед збудників цих хвороб були виявлені Trypanosoma spp., Babesia spp., Rickettsia spp. та інші. Trypanosoma spp. було вперше виявлено на території України в мазках крові 3 мишей серед досліджуваних, які становили 25% (95% довірчі інтервали 6,8–54,1). Кількість паразитів у 200 полях зору мікроскопа досліджуваних мишей коливалася від 2 до 25. Трипаносомози тварин і людини є надзвичайно поширеними. Збудники-трипаносоми спричиняють багато різних хвороб великої рогатої худоби, коней, верблюдів, дрібної рогатої худоби, зокрема, сурру, дурину, нагану та інші. Крім того є причиною гамбійської й родезійської сонної хвороби людини.

Ключові слова: *кровопаразитарні хвороби, миші, Apodemus flavicollis, Apodemus agrarius, Trypanosoma spp*

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CLINICAL AND HAEMATOLOGICAL PARAMETERS IN LACTATING COWS UNDER THE INFLUENCE OF HIGH AIR TEMPERATURE AND VOLUNTARY AND FORCED MILKING

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Abstract. *The effect of high air temperature on clinical and hematological parameters in lactating cows during voluntary and forced milking when they are kept in frame cowsheds made of steel structures was studied. During forced (milking parlor) and voluntary (milking robot) milking the heart rate of cows increased by 5 and 15 beats per minute, respectively, and the number of respiratory movements increased from 50 to 77 at high air temperature in the cowshed that is 1.7 and 2.1 times higher than at optimal values of this microclimate indicator. It was identified that the body surface temperature of cows depended on the air temperature in the cowshed during forced milking and, at high air temperature, it increased to 35.8–37.9 °C, which was 6.9–7.5 °C above its optimal value, while this indicator of the udder increased from 31.6–33.3 °C up to 32.3–38.5 °C. Humidification and irrigation of body surface in lactating cows in combination with increasing the airspeed to 1.2 m/s in the storage reduced body surface temperature of lactating cows to 33.2–34.5 °C that an average turned out to be by 2.5–4.7 °C lower than similar indicators for animals in the cowshed. The hemoglobin concentration in the blood of lactating cows is lower by 15.6 g/L, the number of band neutrophils – by 3.9% and monocytes – by 2.6% under high air temperature compared with the optimal. During voluntary milking at high temperatures compared with the optimal, the hemoglobin content in the blood was lower by 47.2 g/L, the number of white blood cells – by 1.95 g/L, and monocytes – by 6.25%.*

Keywords: *lactating cows, milking parlor, milking robot, forced and voluntary milking, air temperature, clinical and hematological parameters*

Introduction

High-yielding cows differ from low-yielding animals by the high metabolic intensity in tissues and the ability to transform nutrients into milk components more efficiently (Chagas et al., 2007). As a result, most animal organs and systems, functioning at the limit of their capabilities, undergo a physiologic abnormality in the body, especially the ability to reproduce. Hot ambient conditions and low humidity in the cowsheds are critical for high-yielding lactating cows in summertime (Chomayev & Mityashova, 2009).

Analysis of recent researches and publications

According to research (Martynova, 2012), hot ambient conditions in the cowshed reduce the average daily milk production, and the cow lactation performance is not restored to its previous level. Under such conditions, the number of non-yielding cattle reaches 27–30% of the total number of high-yielding cows, and the average term of their productivity is reduced to an average of 2.3–2.7 lactations (Mishchenko, 2008). Intensive use of cows under conditions of constant exposure to stress factors on the body, including hot ambient conditions (Taranov, 1983; Andreev et al., 2012), adversely affects animal health, changes hematological parameters and metabolic state in the body (Sheyenkina et al., 2013). Thus, according to Koryakina (2010), in summer, the hemoglobin concentration in the blood of cows increases by 16.8–27.0%, the red blood cell count increases by 13.0–34.0% and the white blood cell count increases up to 10.2×10^9/L. The eosinophil count in the blood of cows increases by 2.94% in summer and the monocytes count increases by 29.42–32.5% compared to these figures in spring (Mukhamed'yarova, 2014). The thermoneutral temperature limits for lactating cows have been established, the value of their critical points is from -5 to 20 °C, and the comfort zone corresponds to the air temperature of 5–10 °C. In hot ambient stressful conditions, the feed consumption and milk yield decrease in lactating cows, lung ventilation increases, and the physical and chemical thermoregulation processes are compromised (Dibirov, 2013). Under the stress conditions induced by prolonged hot ambient temperature, lactating cows change their behavior, the animals spend most of the time standing in the cubicles, less resting lying down, these factors cause "high-stress" tendon and claudication (Petrusha & Dibirov, 2014). Under stress induced by hot ambient conditions, the lactating cows' reproductive function compromises, endometrium disfunction appears, embryonic mortality increases because of changes in the follicular ceruminous maturation and the hormone concentrations in the blood. Recently, a voluntary method for cow milking using modern milking robots has been introduced into the practice of dairy cattle operations. However, studies of clinical and hematological parameters in high-yielding lactating cows under stress induced by hot ambient conditions and milking using milking robots when they are kept in frame-type cowsheds made of steel structures are still out of consideration.

Purpose is to study clinical and hematological parameters in lactating cows under hot ambient stressful conditions in the cowshed in the terms of forced and voluntary milking.

Materials and methods of research

The experiment has been conducted in institution "Terezine" PLC, Bila Tserkva district of Kyiv oblast. For the

experiment, 2 groups of lactating cows of holsteinized black-spotted breed of the 2nd–3rd lactation with lactation performance of 8000–8500 kg of milk per lactation, 8 heads in each, were formed. Cows of the first experimental group were kept in the frame-type cowshed cubicles, designed for 400 heads and 100 animals were kept in each technological (production) group. The animals were fed from the feeding table with a high-energy feed mixture. The animals had free access to food and water during the day. Cows were milked three times a day in a milking parlor (forcibly), equipped with a De-Laval installation (parallel) designed for simultaneous milking of 32 cows.

The second experimental group of lactating cows in the amount of 8 heads was also formed from animal analogs, which were kept in the cubicle of another cowshed designed for 500 heads, 125 heads in the technological (production) group. The cows of the second experimental group were milked voluntarily using a milking robot (De-Laval). The animals were fed with a feed mixture from the feeding table and provided free access to feed and water during the day. The type of diet and diet density for cows during voluntary milking were the same as for the forced milking but with free-stall housing and the ability to rest in cubicles. Animal waste from the second cowshed was removed using a delta scraper. Lactating cows selected for the research were clinically healthy, and their average daily milk production averaged 26.2–28.0 kg. In the course of the experiment, clinical and hematological parameters were monitored in cows of the experimental groups, taking into account the method of milking – forced (milking parlor) and voluntary (milking robot) under optimal (10–18 °C) and hot (25–29 °C) ambient conditions in the cowsheds.

These clinical parameters in cows of the first experimental group were determined when they were both in the cowshed and in the storage (in front of the milking parlor), and cows of the second experimental group were in the cowshed, controlling body temperature, pulse, respiratory rate, udder and body surface temperature (torso) in the dynamics at 6:00, 9:00, 12:00, 15:00, 18:00 o'clock for two days in the period of optimal (April) and critically hot ambient conditions (July) (Tsvilikhovskyi et al., 2014). Blood samples in the experimental cows were taken from the coccygeal vein before morning feeding, which were used to determine the blood morphological composition, namely the red blood cell count and white blood cell count as well as the ratio of eosinophils, band forms and segmented neutrophils, monocytes, and lymphocytes. Erythrocyte sedimentation rate (ESR), hemoglobin content, and leukocyte analysis were also examined in animal blood (Karpishchenko, 2002).

The research results were processed statistically using Microsoft Excel software.

Results of the research and their discussion

Experiments have shown that during forced and voluntary milking, the rectal body temperature of lactating cows corresponded to the physiologically normal state established for animal species and the clinical state of animals. In hot ambient conditions in the cowshed, this indicator in lactating cows did not differ from its values under optimal air temperature during both voluntary and forced milking (Table 1). It should be noted that the difference in the pulse beat number and the respiratory movement frequency during forced (milking parlor) and volun-

tary (milking robot) milking hasn't been established. However, the heart rate in lactating cows has increased by 5 beats per minute during forced milking and hot ambient conditions in the cowshed compared to the optimal one, and at the same time, the respiratory movement number has increased to 50, which turned out to be 20 beats more. In such hot ambient conditions in the cowshed and voluntary milking of cows by a milking robot, the pulse rate in experimental animals has also turned out to be 15 beats per minute higher, and the respiratory movement number has increased to 65, which turned out to be 42 beats higher than similar figures at optimal air temperature. In hot ambient conditions, the number of respiratory movements in lactating cows during voluntary milking has turned out to be 15 higher compared to forced milking in the milking parlor. Thus, forced and voluntary milking had almost no effect on the clinical condition parameters in time of optimal air temperatures, which corresponded to their statutory figures (Tsvilikhovskyi et al., 2014).

Significant clinical changes in the lactating cows under hot ambient stressful conditions have been determined. It has turned out that the respiratory movement number in lactating cows under hot ambient conditions in the cowshed compared to the optimal temperature in the terms of forced milking (milking parlor) has increased in the morning at 6:00 by 20, at 9:00 by 28, at 12:00 by 29, at 15:00 by 41, at 18:00 by 35 respiratory movements per minute (Table 2).

In the course of the experiment, this figure in lactating cows during forced milking under hot ambient conditions on average has increased by 1.7 times compared to its optimal value. During the day, the udder temperature in lactating cows during forced milking under hot ambient stressful conditions has also become significantly higher than in animals under optimal air temperatures, e.g., at 6:00 by 5.0 °C higher, at 9:00 by 4.80 °C higher, at 15:00 by 4.68 °C higher, at 18:00 by 3.20 °C higher. In lactating cows under hot ambient conditions in the cowshed, the body surface temperature has increased compared to its values under optimal air temperature. Thus, in the morning at 6:00 o'clock, this figure in animals turned out to be 6.30 °C higher, at 9:00 by 7.6 °C higher, at 12:00 by 6.89 °C higher, at 15:00 by 6.75 °C higher, at 18:00 by 5.55 °C higher. Hot ambient conditions in the cowshed have also significantly affected the body surface temperature in lac-

1. Indicators of the clinical condition in lactating cows under optimal and high air temperature during forced and voluntary milking (M ± m)

Indicator	High air temperature		Optimal air temperature	
	milking cows		milking cows	
	forced	voluntary	forced	voluntary
Body temperature, °C	39.12 ± 0.37	39.0 ± 0.30	38.34 ± 0.26	38.07 ± 0.16
Puls, beats/min	77.0 ± 2.0 *	78.0 ± 5.0 *	72.0 ± 1.0	63.0 ± 3.0
Number of respiratory movemens/min	50.0 ± 6.0 **	65.0 ± 15.0 **	30.0 ± 6.0	23.0 ± 3.0

Note: * a significant difference ($P < 0.05$) between indicators in cows during forced and voluntary milking; ** under the exposure to optimal and high air temperature.

tating cows, which were moved to the milking parlor before milking. In hot ambient conditions, this lactating cows' factor almost did not differ from similar figures in animals at optimal air temperature, but it has turned out to be on average 2–3 °C higher in both the first and second cases than its figure in animals in the cowshed. A significant reduction in the body temperature of cows in the storage can be achieved by irrigating the animal body surface, humidifying the air with water, and increasing airspeed. Air humidification and irrigation of the body surface in lactating cows in combination with increasing airspeed to 1.2 m/s in the storage have reduced the body surface temperature of lactating cows to 33.2–34.5 °C, which has turned out to be on average 2.5–4.7 °C lower than similar figures in animals that were in the cowshed.

Thus, experiments have shown that the surface temperature of the body and udder, as well as the number of respiratory movements in lactating cows significantly depend on the air temperature in the cowshed, and the latter on the outside air, which often goes beyond the thermoneutral zone in summer (5–10 °C). The most significant hygienic effect on the clinical parameters of lactating cows was registered at 15:00 o'clock, when the air temperature in the cowshed reached its maximum and was 28–32 °C. Under such factor's figures, the body surface temperature in lactating cows increases to 35.8–37.9 °C, which reduces the process of heat transfer to the environment, and leads to heat stress in animals.

The cowshed's air temperature has significantly affected the hematological parameters in lactating cows, despite the fact that during forced milking the num-

2. Indicators of the clinical condition in lactating cows under optimal and high air temperature (forced milking) (M ± m, n = 8)

Air temperature in the cowshed	Time of day	Parameters			
		Number of respiratory movements/ min	Udder temperature, °C	Body surface temperature of cows, °C (cowshed)	Body surface temperature of cows, °C (storage)
High	6:00	50.0 ± 9.0*	37.33 ± 1.30*	35.80 ± 0.98*	34.52 ± 1.86
	9:00	59.0 ± 9.0*	36.62 ± 0.74*	36.60 ± 0.47*	
	12:00	61.0 ± 13.0*	32.27 ± 0.81	37.94 ± 0.81*	33.24 ± 1.21
	15:00	77.0 ± 5.0*	37.81 ± 0.88*	37.68 ± 0.82*	
	18:00	67.0 ± 12.0*	38.53 ± 0.78*	37.01 ± 0.88*	34.48 ± 0.72
	The average	63.0 ± 7.0*	36.51 ± 1.69	37.00 ± 0.64*	34.08 ± 0.56
Optimal	6:00	30.0 ± 5.0	32.33 ± 1.07	29.48 ± 1.88	32.13 ± 0.85
	9:00	31.0 ± 7.0	31.75 ± 1.62	28.94 ± 2.72	
	12:00	32.0 ± 6.0	31.63 ± 1.30	31.05 ± 1.32	31.95 ± 1.24
	15:00	36.0 ± 6.0	33.13 ± 0.88	30.93 ± 0.76	
	18:00	31.0 ± 6.0	33.29 ± 0.76	31.46 ± 1.06	33.20 ± 0.67
	The average	32.0 ± 2.0	32.42 ± 0.62	30.37 ± 0.92	32.42 ± 0.51

Note: * a significant difference ($P < 0.05$) between indicators under high air temperature compared to optimal.

ber of red blood cell count in the blood of animals under hot ambient conditions did not change compared to optimal air temperature and corresponded to the physiologically normal state (Table 3).

It has been found that the hemoglobin concentration in the blood of lactating cows during forced milking under hot ambient conditions in the cowshed was within the physiologically normal state and did not differ from its content in animals under optimal air temperature. The number of red blood cell count in the blood of lactating cows during voluntary and forced milking under hot ambient conditions, compared with the optimal temperature also did not change (Table 3). In addition, in the blood of lactating cows during both forced and voluntary milking under hot ambient conditions in the cowshed, no changes have been found in the total white blood cell count compared to optimal air temperature, this indicates no temperature effect on the functional status of hematopoietic glands.

However, control of the different white blood cell count ratio in the blood of lactating cows with different milking methods has shown that hot ambient conditions had an effect on some subpopulations of white blood cells. Thus, the eosinophil count in the blood of lactating cows under different methods of milking in hot ambient conditions has not changed compared to optimal air temperature. In the lactating cows under hot ambient conditions in the cowshed both during forced and voluntary milking, a small basophil count has been detected in the blood, while under optimal factors, these cells have not been detected (Table 3). In lactating cows under hot ambient conditions and voluntary milking, the band neutrophils have turned out to be 2.3 times higher, and in the case of forced milking, on the contrary, it has become 1.9 times lower than the figure under optimal air temperature. The experiment has also shown differences in the band neutrophils count in the blood of cows under optimal air temperature, which turned out to be 3.4 times less for voluntary milking than for forced one. However, no differences regarding this indicator have not been found in lactating cows during forced and voluntary milking under high temperatures in the cowshed. It has been found that under the optimal air conditions in the cowshed in the blood of lactating cows during voluntary milking, the segmented neutrophils count is 1.7 times higher compared to the forced milking, while at high temperature the difference in this parameter has not been established. There has been a significant decrease in the absolute monocyte count in the blood of cows under hot ambient conditions in both forced (7.9 times) and voluntary milking (9.3 times), compared with its content in animals at the optimal temperature (Table 3). In hot ambient conditions in the cowshed, the lymphocytes count in the blood of cows during voluntary milking compared to the optimal temperature has turned out to be 32.7% higher, and during forced milking has not changed at all. Hot ambient conditions in the cowshed reduced blood ESR in lactating cows during voluntary milking by 5.7 times compared to its figure at optimal temperature. There have also been revealed certain differences in blood ESR in lactating cows during voluntary and forced milking both under optimal and hot ambient conditions. Thus, at the optimal temperature, the blood ESR in lactating cows during voluntary milking has been 4.2 times higher, and in hot ambient conditions it has turned out to be 2.0 times higher compared to forced milking (Table 3). The revealed changes in the blood morphological com-

3. Influence of high air temperature on hematological parameters in lactating cows during voluntary and forced milking (M ± m, n = 8)

Parameter	High air temperature		Optimal air temperature	
	forced milking	voluntary milking	forced milking	voluntary milking
Red blood cells, 1012/L	5.50 ± 0.94	5.08 ± 0.69	6.61 ± 0.39	6.50 ± 0.50
Hemoglobin, g/L	95.19 ± 9.31**	79.38 ± 9.38**	110.85 ± 4.44	126.62 ± 7.87
White blood cells, 109/L	5.38 ± 1.10	5.17 ± 0.90**	7.12 ± 0.93	7.12 ± 2.15
Eosinophils, %	0.88 ± 0.66	2.0 ± 1.50	2.28 ± 1.55	1.87 ± 1.12
Basophils, %	0.75 ± 0.56	0.25 ± 0.38	0	0
Band neutrophils, %	4.50 ± 1.62**	5.88 ± 2.59	8.42 ± 2.65	2.50 ± 1.50*
Segmented neutrophils, %	29.50 ± 8.75	23.25 ± 4.06	30.42 ± 4.08	52.25 ± 8.37*
Lymphocytes, %	63.63 ± 6.13	69.13 ± 3.38	55.71 ± 5.38	36.37 ± 7.78*
Monocytes, %	0.38 ± 0.47**	0.75 ± 0.56**	3.00 ± 1.71	7.00 ± 1.75*
ESR, mm	1.25 ± 0.25	0.63 ± 0.28	0.85 ± 0.30	3.62 ± 1.28*

Note: * a significant difference between the indicators during forced and voluntary milking of cows, ** under high and optimal air temperature.

position parameters of lactating cows, which were kept in a cowshed under hot ambient conditions, are probably the consequences of a decrease in the feed consumption by animals and the motor activity reduction. The influence of hot ambient conditions on the hematopoietic glands' functional state by changing the intensity of redox processes in tissues has not been excluded.

Thus, based on the research, it can be concluded that hot ambient conditions in the cowshed, not only change the clinical state in lactating cows, but also the blood morphological composition to a much greater extent than during forced or voluntary milking of animals.

Conclusions and future perspectives

Experiments have shown that hot ambient conditions increase the number of respiratory movements and heart rate in lactating cows at constant animal body temperature compared to optimal air temperature. In hot ambient conditions in the cowshed, the udder and body surface temperature of cows increases, and humidification and irrigation of the animal body surface with water in the storage reduces the body surface temperature by 2.6–4.7 °C. In the blood of lactating cows under hot ambient conditions and voluntary milking, the hemoglobin concentration, absolute basophil count, absolute monocyte count, segmented neutrophil count, and erythrocyte sedimentation rate decreases, but the band neutrophil count and absolute lymphocyte count increases compared with optimal air temperature.

The blood morphological composition changes in lactating cows under hot ambient conditions and forced milking that is confirmed by an increase in absolute basophil count and erythrocyte sedimentation rate, in parallel with a decrease in the band neutrophil count as well as absolute monocyte count at constant figures of other parameters.

Prospects for further research may be the thermoregulation phenomenology in lactating cows in hot ambient conditions.

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Анотація. *Досліджено вплив високої температури повітря на клінічні та гематологічні показники в лактуючих корів під час добровільного і примусового доїння за їхнього утримання в корівниках каркасного типу із металевих конструкцій. У корів за високої температури повітря в корівнику, порівнюючи з оптимальною, частота серцевих скорочень під час примусового (доїльний зал) і добровільного (доїльний робот) доїння зростала відповідно на 5 і 15 ударів за хвилину, а кількість дихальних рухів збільшувалась із 50 до 77, що в 1,7 і 2,1 раза вище, ніж за оптимальних значень цього показника мікроклімату. Встановлено, що температура поверхні тіла корів під час примусового доїння залежала від температури повітря в корівнику й за високих значень зростала до 35,8–37,9 °С, що було вищим на 6,9–7,5 °С від її оптимальної величини, тоді, як цей показник молочної залози збільшувався з 31,6–33,3 °С до 32,3–38,5 °С. Зволоження повітря та зрошування поверхні тіла лактуючих корів у комплексі з підвищенням швидкості його руху до 1,2 м/с у накопичувачі знижувало температуру поверхні тіла лактуючих корів до 33,2–34,5 °С, що виявилось у середньому на 2,5–4,7 °С нижче, ніж аналогічні показники у тварин, які знаходилися у корівнику. У лактуючих корів за дії високої температури повітря, порівнюючи з оптимальною, в крові нижча концентрація гемоглобіну на 15,6 г/л, кількість паличкоядерних нейтрофілів – на 3,9% і моноцитів – на 2,6%. За високої температури повітря та добровільного доїння корів вміст гемоглобіну в крові виявився нижчим на 47,2 г/л, кількість лейкоцитів – на 1,95 г/л і моноцитів – на 6,25%, порівнюючи з оптимальною.*

Ключові слова: *лактуючі корови, доїльний зал, доїльний робот, примусове й добровільне доїння, температура повітря, клінічні та гематологічні показники*

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QUALITY OF NATURAL HONEY AFTER TREATMENT OF HONEY BEE COLONIES WITH NEOMYCIN

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Abstract. *Our country occupies first place in Europe and the third in the world in terms of honey production. However, there are problems with the honey export in Ukraine associated with the detection of antibiotics. In addition to compliance with safety indicators in accordance with the standards of Ukraine and the European Union, the quality control of honey is carried out by physicochemical parameters.*

The aim of the study was to determine the quality of linden honey after treatment of honey bee colonies with 1% solution of neomycin, an antibiotic that is often used by beekeepers in the treatment and prevention of infectious diseases in honey bees.

25 groups of honey bee colonies, which were kept under natural conditions in the apiary of the Laboratory of technological and special measures for honey bee diseases prevention at the National Science Centre "Institute of beekeeping named P. I. Prokopovich", were used in the experimental study.

It was found that most of the physical and chemical parameters met the requirements of DSTU 4497:2005 "Natural honey. Technical conditions", except for water and sucrose mass fractions when feeding bees by the syrup with a 0.1% neomycin solution.

Indicators of water and sucrose mass fractions when feeding syrup with 0.1% neomycin solution did not meet the requirements of the current national standard and constituted: water – 24.16 ± 0.08% on the 10th day, 24.13 ± 0.06% on the 30th day, 24.00 ± 0.11% on the 120th day; sucrose – 8.23 ± 0.09% on the 10th day, 6.08 ± 0.11% on the 30th day, 5.93 ± 0.07% on the 120th day.

Thus, the dynamics of physicochemical parameters of linden honey on the 10th day of the experiment and after 30 and 120 days of storage depended on the treatment of honey bee colonies with 1% neomycin solution.

Keywords: *linden honey, neomycin, indicators of honey quality, processing methods*

Introduction

Council Directive 2001/110/EC states that honey is a natural sweet substance produced by *Apis mellifera* bees from plant nectar or from the secretion of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature (Council Directive, 2002; Sohaimy et al., 2015).

It is known that honey is a unique product of beekeeping and is characterized by the content of active substances, valuable and necessary for the vital functions of the human body. Due to its beneficial properties, honey is used as a high-quality food product and an effective means for the treatment in human medicine. Consumer requirements have always been put forward for the quality of honey – taste, color, composition, the possibility for long-term storage without losing properties of the product. At the present stage of the advancement of science, new scientific methods for evaluating the organoleptic, physicochemical, and biological properties of honey have been developed, which have become the quality criteria. The most important of them, indicative, are included in state standards, which determine the suitability of honey for use as a natural quality product or sweets (Amin et al., 2018; Reybroeck, 2018).

Currently, the world community raises questions about the need for the approval of national rules due to the lack of requirements for the characteristics of monofloral honey, the declaration of the geographical origin of the product, the natural difference between types of honey. Also, there are differences between European legislation and Codex Alimentarius standards, mainly in terms of moisture content, hydroxymethylfurfural content, diastase activity, electrical conductivity, sugar content, and the provision of microscopic confirmation (Thrasyvoulou et al., 2018). These issues are relevant in Ukraine (Pambuk et al., 2019).

We followed the requirements of the current national standard (DSTU 4497:2005) to determine the physicochemical parameters in linden honey. The Council Directive 2001/110/EC states that the color of honey may vary from almost colorless to dark brown (Council Directive, 2002). Its consistency can be liquid, viscous, partially, or completely crystallized. The flavor and aroma may vary but are derived from the plant origin. At the same time, the dynamics of indicators of honey quality after treatment of honey bee colonies with antibiotics remains unresolved.

Analysis of recent researches and publications

It is known from the scientific literature that the formation of honey aroma ends by the third or fifth month of its storage. Calcium oxalate crystals are found in linden honey, the identification of which can serve as an additional sign of the botanical origin of honey. Linden honey does not have the amino acids lysine and histidine, contains 39.27% fructose and 34.96% glucose, pH = 3.7. It has well-defined nutritional and medicinal properties, has antibacterial action against gram-positive and gram-negative microorganisms, as well as against ciliates, Amoebae, and Trichomonas. Also, linden honey contains volatile and non-volatile antimicrobial substances. It has expectorant, mild laxative, and anti-inflammatory effects. It is customary to use linden honey for sore

throat, runny nose, laryngitis, bronchitis, bronchial asthma, as a heart tonic, for inflammation of the gastrointestinal tract, kidney, and biliary diseases. At the same time, linden honey has a local healing effect on purulent wounds and burns (Polishchuk et al., 2013).

Bogdanov (Swiss Confederation), speaking at the Apimondia Forum 2010 in Bulgaria, stressed that the main contaminants of honey are antibiotics used in various countries to control American and European rot: aminoglycosides, tetracyclines, amphenicols, macrolides, beta-lactams. They contaminate royal jelly, propolis, and especially wax (Ponomariv & Faramazian, 2010).

Aminoglycosides are antibiotics that are often used in animal husbandry. If antibiotics are used in violation of the instructions, their residues are determined in food products of animal origin and the environment. Effective detection of their residues is an important part of human health protection (Yi-Fang et al., 2015).

Thus, the group of first-generation aminoglycosides (neomycin A, neomycin B, neomycin C), formed during the functioning of the ray fungus actinomycete (*Streptomyces fradie*) or related microorganisms, is neomycin.

Purpose – to determine the quality of linden honey after treatment of honey bee colonies with 1% neomycin solution.

Materials and methods of research

For the experimental study, 25 groups of honey bee families were formed: 1 control and 24 experimental, which were divided according to the methods for the treatment: 1st control group – treatment was not carried out; 2nd group – aerosol treatment by spraying (0.1% neomycin solution) and 3rd group – feeding syrup (0.1 g/0.1 L of syrup corresponding to a concentration of 0.1% – 0.5 L of neomycin per honey bee colony).

During the experiment, a certified substance of neomycin was used under the condition of a single treatment of honey bee colonies. The choice of 0.1% antibiotic concentration was justified by the scientific literature, which shows the results of their inhibitory effect on pathogens of bee rot in the laboratory conditions and to determine the antiseptic effect in the treatment of honey bee colonies affected by European and American rot in the wild nature.

The probability of the experimental results was ensured by conducting them in triplicate. All obtained digital data were processed statistically using a computer software package "Microsoft Excel" with the calculation of the arithmetic mean and its error ($M \pm m$), the level of probability (P) according to the Student's table ($P < 0.05$, $P < 0.001$, $P < 0.0001$).

Results of the research and their discussion

To conduct this experimental study, we relied on the results of scientific research that in recent years the interest of consumers and researchers in honey as a food product, which a person consumes, and ways it could help to maintain good health. Honey production is now commercialized and may now run a high risk of being falsified not only with syrups from other plants, which are difficult to detect, but also contain antibiotic residues. The dynamics of physicochemical (qualitative) parameters in natural honey, in particular from linden, after application of various methods for the treatment of honey bee colonies with 1% neomycin solution, is of scientific interest.

Table 1 shows the physicochemical parameters of linden honey under different treatment methods of honey bee colonies with neomycin.

According to the indicators shown in Table 1, it follows that the water mass fraction in honey after aerosol treatment of honey bee colonies with neomycin was significantly lower by 0.5% ($P < 0.01$), and after feeding bees with syrup – significantly higher by 7.13% ($P < 0.001$) compared with the control. When feeding bees with neomycin syrup, this figure was significantly higher by 7.63% ($P < 0.001$) compared to aerosol treatment.

The diastase number both under aerosol treatment with neomycin and feeding bees with syrup was significantly lower by 15.9 and 38.6% ($P < 0.05$; $P < 0.001$) than in the control. Also, the diastase number of honey after feeding syrup with neomycin was significantly lower by 27.0% ($P < 0.01$) than after aerosol treatment.

There was no statistically significant difference in the hydroxymethylfurfural content. There was only a tendency to reduce the hydroxymethylfurfural content after feeding bees syrup with neomycin compared to the control and to increase – after aerosol treatment of honey bee colonies. The hydroxymethylfurfural content in linden honey when feeding bees syrup with neomycin was slightly lower compared to aerosol treatment.

The acidity of linden honey depends on the method for the treatment of bees with a neomycin solution. Thus, both after feeding syrup to bees and aerosol treatment with this antibiotic, the acidity was significantly lower by 37.4 ($P < 0.001$) and 29.8% ($P < 0.001$), respectively, compared with that in the control.

At the same time, the acidity of linden honey after feeding bees syrup with neomycin was significantly lower by 10.8% ($P < 0.05$) than after the aerosol treatment.

The mass fraction of reducing sugars after feeding syrup with neomycin was significantly higher by 2.6% ($P < 0.05$) than in the control. This indicator shows a tendency to increase the mass fraction of reducing sugars in linden honey after feeding syrup with neomycin compared

1. Physicochemical parameters of linden honey on 10th day after treatment of honey bee colonies with neomycin (M ± m, n = 3)

Parameter	1st (control)	Experimental groups	
		2nd (aerosol treatment)	3rd (syrup feeding)
Water mass fraction, %	17.03 ± 0.03	16.53 ± 0.08●●	24.16 ± 0.08**▲
Diastase number, Gothe units	12.97 ± 0.49	10.90 ± 0.20●●●	7.96 ± 0.37*▲▲
Hydroxymethylfurfural content, mg/kg	0.73 ± 0.13	1.03 ± 0.14	0.80 ± 0.11
Acidity, mEq NaOH per 1 kg	8.83 ± 0.17	6.20 ± 0.17●	5.53 ± 0.03**▲▲▲
Mass fraction of reducing sugars, %	86.63 ± 0.30	88.03 ± 1.19	89.23 ± 0.77***
Sucrose mass fraction, %	9.17 ± 0.13	8.00 ± 0.15●●	8.23 ± 0.09*

Note: * $P < 0.001$, ** $P < 0.01$, *** $P < 0.05$ – 3rd experimental group compared with the control; ▲ $P < 0.001$, ▲▲ $P < 0.01$, ▲▲▲ $P < 0.05$ – 3rd experimental group compared with the 2nd experimental; ● $P < 0.001$; ●● $P < 0.01$; ●●● $P < 0.05$ – 2nd experimental group compared with the control.

with the indicator after aerosol treatment of bees and in the control.

The sucrose mass fraction after feeding bees syrup with neomycin and aerosol treatment was significantly lower by 0.94 and 1.17% ($P < 0.01$), respectively, than in the control. Also, it remains just a trend of a slight increase in the sucrose mass fraction when feeding bees syrup with neomycin compared with aerosol treatment.

This percentage of sucrose (from 8.00 ± 0.15 to 9.17 ± 0.13%) in the studied samples of linden honey might be explained based on evidence from other authors that linden, apple, and some other types of honey may contain a significant amount of sucrose in the first period after pumping because the flower nectar from these plants contains it in a large quantity. The rate of sucrose hydrolysis in maturing honey is high but, at the time of pumping, the sucrose content may remain at 10 to 25%. During further storage, the sucrose content is set at the level of 0 to 1.0%. The same processes of sucrose hydrolysis occur in sugar honey (Polishchuk et al., 2017).

According to the indicators shown in Table 2, it follows that the water mass fraction in linden honey after aerosol treatment of bees with a neomycin solution was significantly lower by 0.77% ($P < 0.001$) than in the control. At the same time, when bees were fed syrup with neomycin, the water mass fraction in linden honey was significantly higher by 7.03 ($P < 0.001$) and 7.80% ($P < 0.001$) compared with aerosol treatment and the control, respectively.

The level of diastase number in linden honey both after the aerosol treatment of bees and feeding them syrup with neomycin was significantly lower by 9.9 ($P < 0.05$) and 35.3% ($P < 0.001$), respectively, compared to that in the control. When bees were fed syrup with neomycin, the diastase number was significantly lower by 28.2% ($P < 0.001$) than after the aerosol treatment of bees.

The hydroxymethylfurfural content in linden honey tended to increase when feeding bees syrup with neomycin compared with the control, as well as aerosol treatment.

The acidity of linden honey, both after aerosol treatment of bees and feeding them syrup with neomycin was significantly lower by 30.9 ($P < 0.01$) and by 38.2% ($P < 0.001$), respectively, compared with the control. When feeding syrup with neomycin, the acidity of

2. Physicochemical parameters of linden honey on 30th day of storage after treatment of honey bee colonies with neomycin (M ± m, n = 3)

Parameter	1st group (control)	Experimental groups	
		2nd (aerosol treatment)	3rd (syrup feeding)
Water mass fraction, %	17.10 ± 0.06	16.33 ± 0.06●	24.13 ± 0.06**▲
Diastase number, Gothe units	12.06 ± 0.13	10.86 ± 0.29●●●	7.80 ± 0.14**▲
Hydroxymethylfurfural content, mg/kg	0.53 ± 0.07	0.53 ± 0.17	0.80 ± 0.23
Acidity, mEq NaOH per 1 kg	9.16 ± 0.33	6.33 ±0.16●●	5.66 ± 0.16**▲▲▲
Mass fraction of reducing sugars, %	87.96 ± 0.46	87.26 ± 0.40	89.33 ± 0.57▲▲▲
Sucrose mass fraction, %	3.63 ± 0.12	4.77 ± 0.24●●●	6.08 ± 0.11**▲▲

Note: similar to Table 1.

honey was significantly lower by 10.6% ($P < 0.05$) compared with the aerosol treatment of bees.

The mass fraction of reducing sugars in linden honey when feeding syrup with neomycin was significantly higher by 2.07% ($P < 0.05$) compared to the aerosol treatment of bees with this antibiotic.

The sucrose mass fraction in linden honey was significantly higher by 2.45 ($P < 0.001$) and 1.14% ($P < 0.05$) compared with the control both after feeding syrup to bees and aerosol treatment of bees with a neomycin solution. At the same time, the sucrose mass fraction in honey was significantly higher by 1.31% ($P < 0.01$) after feeding syrup with neomycin compared with the value after aerosol treatment of bees with this solution.

After aerosol treatment of bees with neomycin (Table 3), the water mass fraction in linden honey was significantly lower by 0.67% ($P < 0.01$) compared with the control.

However, when bees were fed syrup with this antibiotic, the water mass fraction was significantly higher by 6.87 ($P < 0.001$) and 7.54% ($P < 0.001$) compared with the control and aerosol treatment, respectively.

The diastase number of honey when feeding syrup with neomycin was significantly lower by 36.4% ($P < 0.01$) compared with the control, and by 30.4% ($P < 0.01$), compared with the aerosol treatment of bees with this antibiotic. Also, there was only a tendency to reduce the diastase number of linden honey after aerosol treatment of bees with neomycin.

In terms of hydroxymethylfurfural content in linden honey, there is a tendency to increase under different methods for treatment of bees with neomycin and to decrease after feeding syrup with this antibiotic compared to aerosol treatment of bees.

The acidity of linden honey after aerosol treatment of bees with neomycin and feeding syrup was significantly lower by 25.0 ($P < 0.01$) and 12.5% ($P < 0.01$) compared with the control, respectively. However, when feeding syrup with neomycin, the acidity of linden honey was significantly higher by 16.5% ($P < 0.05$) compared with the aerosol treatment of bees. According to the mass fraction of reducing sugars, there is a tendency to decrease in their content in linden honey after aerosol

3. Physicochemical parameters of linden honey on the 120th day of storage after treatment of honey bee colonies with neomycin (M ± m, n = 3)

Parameter	1st group (control)	Experimental groups	
		2nd (aerosol treatment)	3rd (syrup feeding)
Water mass fraction, %	17.13 ± 0.07	16.46 ± 0.06●●	24.00 ± 0.11**▲
Diastase number, Gothe units	12.43 ± 0.55	11.36 ± 0.26	7.90 ± 0.45*▲▲
Hydroxymethylfurfural content, mg/kg	0.53 ± 0.13	1.06 ± 0.29	0.60 ± 0.11
Acidity, mEq NaOH per 1 kg	9.33 ± 0.16	7.00 ± 0.28●●	8.16 ± 0.16*▲▲▲
Mass fraction of reducing sugars, %	86.80 ± 0.72	88.33 ± 0.8	89.23 ± 0.49
Sucrose mass fraction, %	3.73 ± 0.09	4.05 ± 0.16	5.93 ± 0.07**▲

Note: similar to Table 1.

treatment of bees with neomycin, and increase after feeding syrup with this antibiotic compared to both the control and aerosol treatment of bees with a neomycin solution.

The sucrose mass fraction in linden honey when feeding bees syrup with this antibiotic was significantly higher by 2.20 ($P < 0.001$) and by 1.88% ($P < 0.001$) compared with the control and aerosol treatment of bees with a solution of neomycin, respectively.

The water mass fraction after feeding bees syrup with neomycin was higher than the requirements of the current DSTU 4497:2005. According to this indicator, such honey is prohibited for sale and is not amenable to long-term storage.

There is no sufficiently substantiated information in the scientific literature on the effect of antibiotic residues on the quality of honey. It is known that the water content below 20% is characteristic of good, mature honey, which can be stored for a long time. DSTU 4497:2005 allows the water content in commercial honey up to a maximum of 21%. This limit is applied to honey, which is intended for immediate consumption, for honey, which is sent for storage, such water content is unacceptable because there is a tendency to delamination and fermentation.

At the same time, it is known that there is a clear difference between the botanical varieties of honey by the average values of diastase number. Thus, according to some authors, the highest diastase number have buckwheat (48.12) and pine (33.15) honey, and the lowest – acacia (9.82) and sunflower (16.6). The linden, flower, and herb honey have diastase number at the level of 19–25 Gothe units (Adamchuk & Bilotserkivets, 2015). Other scientists prove that diastase activity is very low in some types of natural honey – white acacia, clover, linden, sunflower, fireweed (Samarghandian et al., 2017). However, the indicators of diastase number in our experiment met the requirements of DSTU 4497:2005.

It is known that hydroxymethylfurfural content in freshly pumped honey is minimal and constitute 1–5 mg/kg (Kovalskyi & Kyryliv, 2001). At the same time, linden honey is characterized by a higher value of active acidity. If all light-colored honey has a pH value from 3.5 to 4.1 units (for example, in sunflower honey, this figure does not exceed 4.15; heather – 4.14; acacia – 4.11; melilot – 3.95; sainfoin – 3.85; raspberry – 3.8; phacelia – 3.77), then aqueous solutions of linden honey have a pH from 4.5 to 7 units (Samarghandian et al., 2017).

Conclusions and future perspectives

The dynamics of physicochemical parameters in linden honey on the 10th day of the experiment and after 30 and 120 days of storage depended on the method for treatment of honey bee colonies with 1% neomycin solution.

At the same time, most of the physical and chemical parameters met the requirements of DSTU 4497:2005. "Natural honey. Technical conditions", except for the mass fractions of water and sucrose when feeding bees with syrup containing 0.1% neomycin solution.

After feeding syrup with 0.1% neomycin solution, the water mass fraction did not meet the requirements of the current national standard and constituted $24.16 \pm 0.08\%$ on the 10th day, $4.13 \pm 0.06\%$ on the 30th day, and $24.00 \pm 0.11\%$ on the 120th day.

The sucrose mass fraction in linden honey did not meet the current national standard when feeding syrup with the studied antibiotics. Thus, on the 10th day

after feeding the syrup with 0.1% neomycin solution, the mass fraction of sucrose in linden honey decreased compared to the control group (9.17 ± 0.13%) and amounted to 8.23 ± 0.09%; on the 30th day – was higher compared to the control group (3.63 ± 0.12%) and amounted to 6.08 ± 0.11%; on the 120th day of storage of linden honey – was higher compared to the control group (3.73 ± 0.09%) and amounted to 5.93 ± 0.07%.

On the 10th day after aerosol treatment of bees with 0.1% solution of neomycin, the mass fraction of sucrose in linden honey did not meet the requirements of the current national standard and was 8.00 ± 0.15%, although it was lower compared to the control group (9.17 ± 0.13%).

In the future, further research is needed to determine the residual amounts of antibiotics in honey by an improved method of enzyme-linked immunosorbent assay.

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Анотація. *Наша держава займає перше місце в Європі та третє у світі за показниками виробництва меду. Проте, з експортом меду в Україні виникають проблеми, які пов'язані з виявленням антибіотиків. Окрім відповідності показникам безпечності відповідно до стандартів України та Європейського Союзу, контроль якості меду проводять за фізико-хімічними показниками.*

Метою дослідження було визначити якісні показники меду з липи після обробки бджолиних сімей 1% розчином неоміцину, антибіотику, що часто використовується пасічниками в лікуванні та профілактиці інфекційних хвороб бджіл.

В експериментальному дослідженні використали 25 груп бджолиних сімей, які знаходилися за природних умов на пасіці лабораторії технологічних і спеціальних заходів профілактики хвороб бджіл ННЦ «Інститут бджільництва ім. П. І. Прокоповича».

У результаті проведеного дослідження встановили, що більшість фізико-хімічних показників відповідала вимогам ДСТУ 4497:2005 «Мед натуральний. Технічні умови», окрім масової частки води та масової частки сахарози за згодовування бджолам сиропу з 0,1% розчином неоміцину.

Показники масової частки води та сахарози за згодовування сиропу з 0,1% розчином неоміцину не відповідали вимогам чинного національного стандарту і становили: води – на 10 добу – 24,16 ± 0,08%; на 30 добу – 24,13 ± 0,06%; на 120 добу – 24,00 ± 0,11%; сахарози – на 10 добу – 8,23 ± 0,09%; на 30 добу – 6,08 ± 0,11%; на 120 добу – 5,93 ± 0,07%.

Таким чином, динаміка фізико-хімічних показників у меді з липи на 10 добу експерименту та після 30 і 120 доби зберігання залежала від обробки бджолиних сімей 1% розчином неоміцину.

Ключові слова: *мед із липи, неоміцин, показники якості меду, способи обробки*

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PATHOMORPHOLOGICAL CHANGES IN DOGS IN CASE OF CORONAVIRUS INFECTION

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Abstract. *In recent years, there is a tendency to increase the incidence of the disease in dogs with signs of diarrhea, not only in Ukraine but also in Europe.*

The article presents the results of a study on histological changes in organs and tissues of dogs with coronavirus infection. A histological study of pathological material sampled from 6 dogs of various breeds and gender, aged from 2 to 6 months, died from diarrheal syndrome was carried out. The presence of the coronavirus was confirmed in fecal samples by a polymerase chain reaction.

The most expressed injuries and typical changes in all dead dogs we recorded in the small intestine, namely in the jejunum and ileum, regional lymph nodes, and also the spleen.

Morphological manifestations of coronavirus infection in dogs at the macroscopic level are the following signs: the presence of exudative inflammation in the small intestine in form of serofibrinous jejunoileitis, hyperplasia and serohemorrhagic lymphadenitis of the mesenteric lymph nodes, multiple foci of hemorrhage in the parenchyma of the spleen and serous membrane of the small intestine, passive venous hyperemia of the liver and kidneys, dilatation of the right ventricle of the heart, pulmonary edema, cachexia and dehydration of the body.

At the microscopic level, we registered the following: serofibrinous jejunoileitis, hyperplasia of the lymphoid nodules of the spleen and lymph nodes, hemorrhagic infarctions in the spleen, hyperplasia of individual and aggregated lymphoid nodules in the small intestinal mucosa, degenerative processes in the parenchyma of the liver and kidneys.

Keywords: *coronavirus infection, dogs, histological changes, hemorrhages, serofibrinous jejunoileitis, serohemorrhagic lymphadenitis, hyperplasia, lymphoid nodules*

Introduction

The main threat to global health, economic stability, and national security is zoonotic viral diseases, both known and re-emerging (Graham et al., 2018). The emergence of zoonoses can be considered a logical consequence of the ecology and evolution of pathogens that use new niches and adapt to new hosts.

Due to the constant human interference with the animal habitat and our

close contact with domestic animals, the number of zoonoses will continue to increase, given the spread and density of population and livestock production over the next century.

In developed countries, the problem of infectious pathology of small domestic animals, as well as in ours, has its own stages of exacerbation, since pathogens evolve, adapt, occupy new niches, and, thus, do not allow to weaken our attention to them. At the same time, there is a lack of domestic literature devoted to the problems of infectious pathology of small animals, presented with consideration of modern data obtained by scientists on a global scale.

More than 60% of infectious diseases in humans are caused by pathogens inherent in wild or domestic animals. Microorganisms that cause zoonoses include those that are endemic in human populations or enzootic in animal populations with frequent interspecies transmission to humans (Karesh et al., 2012). The role of viruses in the emergence and development of human infectious diseases is growing steadily. Only at the beginning of the 21st century, many new viruses appeared, which were not previously known, and most of which are able to cause severe infectious diseases, often fatal.

Among them are new human coronaviruses (CoV), which are called SARS-CoV (Severe acute respiratory syndrome, SARS) and MERS-CoV (Middle East respiratory syndrome, MERS). Both of them cause acute respiratory distress syndrome (ARDS) and are associated with high mortality rates (Wong et al., 2016).

In Decaro and Buonavoglia (2008) review on the genetic evolution of canine CoV and the emergence of new ones, it is also noted that the emergence of severe acute respiratory syndrome in humans has provoked new interest, in animal coronaviruses as well, as potential agents of direct and indirect zoonoses.

Analysis of recent research and publications

Canine coronavirus (CCoV) has been known since 1970. In dogs, CoV is associated with mild enteritis. But CoV strains with different biological and genetic properties in relation to the classic CCoV strains have been found in dogs in recent years, leading to a complete reconsideration of the CoV-induced canine diseases.

The practice of specific prophylaxis allows us to state that in the etiological aspect, diseases emerging with this symptom can be caused not only by parvo-, but also corona- and rotavirus enteritis. Godsall et al. (2010) also noted that canine parvovirus (CPV) and canine enteric coronavirus (CECoV) usually cause diarrhea in dogs. Although the CECoV is less common, it still remains a potentially important pathogen, the authors noted (Godsall et al., 2010). These diseases are extremely dangerous for puppies and miniature breed dogs, due to rapid dehydration and, as a consequence, death.

The name "coronavirus" dates back to 1968 and derives from the Greek κορώνα, which means "corona" (corona-like structure observed in these viruses in an electron microscope). CoVs are enveloped viruses with spherical, and sometimes pleomorphic virions with a diameter of 80 to 120 nm. They have the largest genomes of all RNA-viruses known today with an average length of 27 to 31 thousand bp and replicate using a unique mechanism, which leads to a high frequency of recombinations (Wang et al., 2016).

Previously, coronaviruses were divided into three genera (I-III), which were usually called groups and based on serological cross-reactivity (Pratelli, 2011).

Phylogroup I CoV include human coronaviruses HCoV-229E and HCoVNL63 that cause respiratory infections, feline coronaviruses (FCoVs) type 1 and 2, transmissible gastroenteritis virus (TGEV) of pigs, porcine respiratory coronavirus (PRCoV), porcine epidemic diarrhea virus (PEDV), and canine coronaviruses (CCoVs). Ferret coronavirus has recently been identified as a member of phylogroup I.

Phylogroup II of the mammalian CoV is divided into two subgroups, bovine-like subgroup 2a and SARS-like subgroup 2b. Subgroup 2a includes bovine coronavirus (BCoV), murine hepatitis virus (MHV), sialodacryoadenitis virus of rats (SDAV), porcine hemagglutinating encephalomyelitis virus (PHEV), human coronaviruses (HCoV-OC43 and HCoV-HKU1), which cause respiratory infections, human enteric coronavirus (HECV-4408), the recently recognized equine coronavirus (ECoV) and canine respiratory coronavirus (CRCoV).

Phylogroup III includes only avian coronaviruses, such as infectious bronchitis virus (IBV), turkey coronavirus (TCoV), and pheasant coronavirus.

Canine coronavirus (CCoV) causes sporadic cases of enteritis in dogs. In general, CCoV is recognized as the etiological agent of self-limiting small intestine infections, which can lead to mild gastroenteritis. Mild illness or asymptomatic carrier state are likely to be the usual consequences of infection in most cases. Canine coronavirus enteritis is a common infection, usually in young dogs, especially those in large groups such as dog pounds, shelters, and kennels (Stavisky et al., 2012). However, several years ago a highly virulent strain (pantropic CCoV) that caused an outbreak of fatal, systemic diseases in puppies was isolated. This strain showed some genetic changes relative to the existing strains circulating in the dog population. By definition, 53 feline and canine coronaviruses are common pathogens in cats and dogs that can cause fatal infections such as feline infectious peritonitis (FIP) and canine pantropic coronavirus infection. Alphacoronavirus and Betacoronavirus respiratory coronavirus have been found in dogs (Takano at al., 2016).

The extent of the natural disease caused by canine coronavirus is not well understood, as the pathogenesis of CCoV is not yet well defined. It was previously reported that the infection was associated with moderate to severe gastroenteritis, but it now appears that CCoVs are predominantly mild or subclinical, with low mortality rates.

CCoV is acid resistant, so it passes through the stomach unchanged. Viremia and generalized infection are not observed. The superficial epithelium of the small intestine is the main target of CCoV but the large intestine is resistant to infection. In 2 days after oral infection, CCoV can be detected in the cytoplasm of mature epithelial cells of the villi in the upper two-thirds of the duodenum, compared to canine parvovirus type 2 (CPV-2), which destroys the epithelium of intestinal crypts.

Loss of villi in mature enterocytes causes atrophy of intestinal villi and, as a consequence, weakening of the integrity of the intestinal barrier (Wang et al., 2016). After the fourth day of infection, the small intestine usually has a heterogeneous distribution of infected villi (Gizzi et al., 2014). Together these changes in the morphology of the small

intestine result in a loss of normal digestive and absorption functions, as well as clinical signs of diarrhea and dehydration in sick dogs.

The factors regulating the course of natural diseases caused by intestinal CCoVs are not well understood. CCoVs are responsible for enteritis in dogs, and signs of infection can range from mild to moderate, but more severe in young puppies or combination with other pathogens. All data raise several questions and request deeper studies of the pathobiology of CCoVs types 1 and 2.

Materials and methods of research

The study was performed in the pathohistological laboratory of the Academician V. G. Kasianenko Department of Animal Anatomy, Histology and Pathomorphology of the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine.

The material of the study was the pathological material collected during the postmortem autopsy in dogs of various breeds and genders who died with the diarrheal syndrome at the age from 2 to 7 months (n = 6). The presence of coronavirus, with no other pathogens, was confirmed in fecal samples of dogs before death by a polymerase chain reaction. According to the anamnestic data, animals had pronounced clinical signs such as the disruption of the digestive system of an infectious origin (fever, lack of appetite, vomiting, diarrhea). The pathological and anatomical autopsy of the dogs' carcasses was performed in the supine position by the method of partial evisceration in the generally accepted sequence.

The main method used in the study was a histological examination, during which microstructural changes in organs and tissues were recorded and described. The pathological material was processed according to generally accepted histological methods.

Pieces of organs were fixed with a 10% Neutral Buffered Formalin according to Lilly's prescription, graded alcohols were used for dehydration and embedded in paraffin after chloroform. Using a sled microtome, sections with a thickness of 6-7 μm were obtained. The histological structure of organs and tissues was studied by sections' staining with Carazzi's hematoxylin and eosin. The histological preparations were examined and photographed using an Olympus BX-41 microscope (Goralskij et al., 2018).

Results of the research and their discussion

On external examination of the dead animals' carcasses in all cases we noted dull coat, the skin was dry, not elastic, the visible mucous membranes were dry, pale with a grayish color, not shiny. All animals showed signs of malnutrition as a result of dehydration and diarrhea.

The liver was enlarged, elastic, dark cherry color with insignificant areas of putrefactive color, which indicated the presence of dystrophic processes in its parenchyma. A red, bloody fluid flowed from the incision. Histological examination revealed the destruction of the radiant structure of the liver. Hepatocytes are placed randomly with signs of granular dystrophy, which was morphologically manifested by cell swelling, the disappearance of cytoplasm transparency, and appearance of protein origin grains. Hepatocytes showed signs of karyolysis. Sinusoidal capillaries were engorged with blood in the center of the lobules. In the lumen of the cen-

tral veins, there were formed elements of blood, mainly erythrocytes. In some cases, small areas of fatty degeneration were found with the formation of characteristic vacuoles of various sizes in the cell cytoplasm.

The gallbladder in all cases was filled with thick dark green bile.

The pancreas was of gray-pink color, slightly enlarged, edematous. The lobulated structure was well-defined.

The spleen was moderately enlarged, had a soft texture with pointed edges. Hemorrhagic infarcts were registered on the surface of the spleen.

Microscopically, the red pulp showed hemorrhages and clear contours, and a specific triangular structure of hemorrhagic infarcts. In the section, a pronounced granular structure of the organ was shown by hyperplastic lymphoid nodules due to the active proliferation of lymphoid cells.

The kidneys in all cases were dark cherry in color. On the section, the border between the cortical and medullary substances was smoothed, the section surface had increased moisture. It should be noted that venous hyperemia of the liver and kidneys was the result of hemodynamic disturbances in the systemic circulation due to heart failure. The main microscopic changes of a dystrophic nature were found in the epithelium of the convoluted tubules. The affected epithelium is massively desquamated into the lumen of the tubules.

The heart is enlarged in size, round in shape due to the right ventricle enlargement, less often the entire right side, and due to the displacement of the heart apex to the left. The heart muscle was soft, unevenly colored, a gray-pink color. The cut surface had increased moisture, the blood vessels of the heart were full of blood. Such pathological processes may indicate the development of compensated heart failure during the life of the animal, which developed as a result of the disease.

Lungs had even red color, pastry, elastic consistency, the pieces floated difficult in water. A foamy reddish liquid flowed down from the cut surface. The same fluid was in the trachea and in the lumen of large bronchi, what was a morphological sign of pulmonary edema due to venous hyperemia.

Examination of the abdominal organs in all cases showed enlarged mesenteric lymph nodes, vascular hyperemia, and hemorrhages in the serous membrane of the intestine. On the cut, the parenchyma of the lymph nodes was pink-red, juicy, and shiny. Signs of serohemorrhagic inflammation were noted in the form of serous impregnation of the lymph node parenchyma and the release of formed blood cells with exudate.

Microscopically the lymphoid nodular hyperplasia of lymph nodes was shown as a result of the active proliferation of reticulocytes and lymphoid cells. In some nodules, necrosis and devastation were observed.

Along the entire length of the small intestine, striped, less often spotted hemorrhages appeared on the serous membrane. The content of the small intestine, in particular the jejunum and ileum, looked like a cloudy gray-pink fluid. The surface of the mucous membrane of the jejunum and ileum was covered with a large amount of liquid cloudy mucus, under which small spotty hemorrhages were manifested. In the cut, the wall of the small intestine was edematous and gelatinous. In some cases, pityriasis with gray-yellow films was noted on the mucous membrane of the jejunum and ileum that were difficult to remove (Fig. 1).

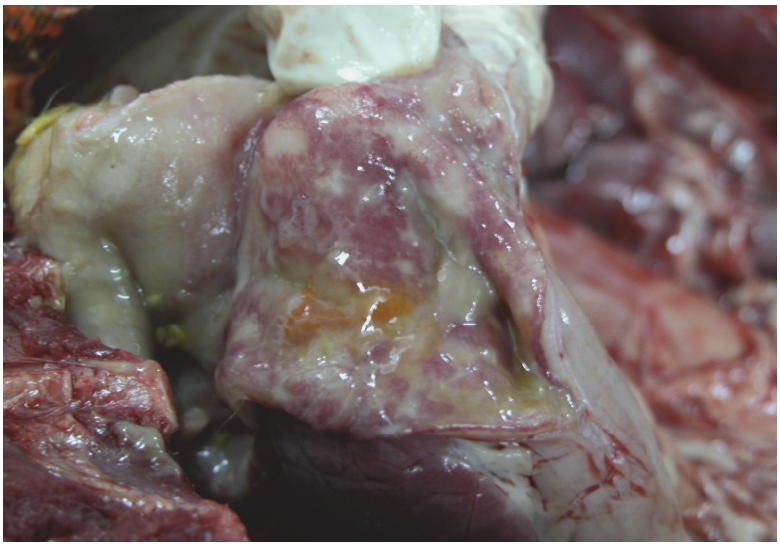

Fig. 1. Catarrhal hemorrhagic inflammation of the small intestine

In the jejunum, an increase in the lymphoid formations of the intestinal wall was observed: rare and aggregated lymphoid nodules due to the proliferation of reticulocytes and the accumulation of a large number of lymphocytes. They acquired a clear rounded shape and were intensively stained with hematoxylin. The main changes were localized mainly in the villi area. Effusion of a fibrinous exudate was observed on the surface of the mucous membrane, which, together with the desquamated epithelial cells, covered the villi in the form of lumps. In epithelial cells, there were karyopyknosis, karyorrhexis, and signs of cytolysis. Necrosis and loss of the epithelium of the villi in the small intestine were accompanied by their atrophy, deformation, shortening, and desquamation of the virus-infected enterocytes into the lumen (Fig. 2).

Along with expressive alterative changes in the epithelium, regeneration

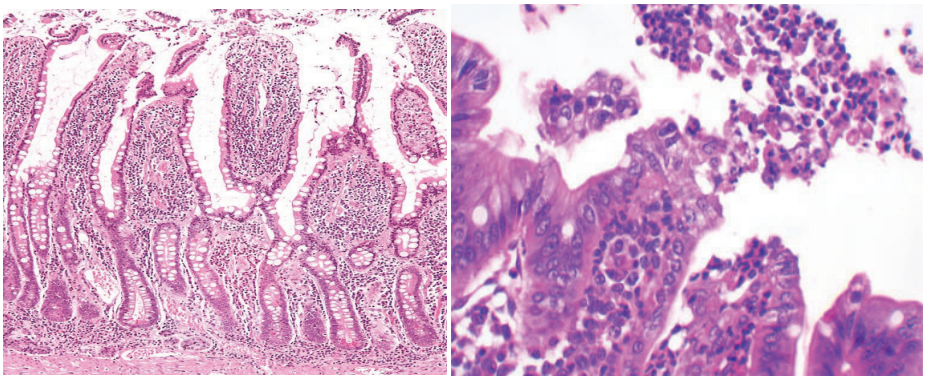

Fig. 2. Desquamation of the epithelium, deformation, and destruction of villi. Hematoxylin and eosin, x 400

processes were often clearly expressed, mainly in crypt areas. There were no significant histological changes. The structure of the crypts and the epithelium was preserved. Necrosis and loss of epithelium of the villi in the small intestine and the probable disruption of goblet cell secretion lead to the destruction of its protective barrier. Diarrheal syndrome, one of the main clinical manifestations and a key link in the pathogenesis of coronavirus infection, is due to the development of serofibrinous enteritis, since in the areas of inflammation there is a loss of mature epithelium, parietal digestion and cellular absorption are disturbed.

Conclusions and future perspectives

1. Morphological manifestations of coronavirus infection in dogs at the macroscopic level include serofibrinous jejunoileitis, hyperplasia and serohemorrhagic lymphadenitis of mesenteric lymph nodes, dilatation of the right ventricle of the heart, multiple hemorrhages in the spleen parenchyma and the serous membrane of the small intestine, cachexia, and dehydration of the body.

2. At the microscopic level, the following was noted: serofibrinous jejunoileitis, hyperplasia of the lymphoid nodules of the spleen and lymph nodes, hemorrhagic infarcts in the spleen, hyperplasia of individual and aggregated lymphoid nodules of the mucous membrane of the small intestine, passive venous hyperemia of the liver and kidneys, dystrophic processes in the parenchyma of the liver and kidneys.

Perspectives. For a complete study of the pathomorphological picture of coronavirus enteritis in dogs, as the next stage, it is advisable to investigate the characteristics of this disease using histochemical methods.

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Анотація. *В останні роки відмічається тенденція збільшення випадків захворювання собак з ознаками діареї не тільки в Україні, а й у Європі.*

У статті наведено результати вивчення гістологічних змін в органах і тканинах собак за коронавірусної інфекції. Проведено гістологічне дослідження патологічного матеріалу, відібраного від трупів 6 собак різних порід і статей віком від 2 до 6 місяців, що загинули з діарейним синдромом. Присутність коронавірусу була підтверджена полімеразною ланцюговою реакцією під час аналізу зразків фекалій.

Найбільш виразні пошкодження й типові зміни в усіх загиблих собак зафіксовані нами в тонкому відділі кишечнику, а саме в порожній і клубовій кишках, регіонарних лімфатичних вузлах, а також у селезінці.

Морфологічними проявами коронавірусної інфекції в собак на макроскопічному рівні є такі ознаки: наявність ексудативного запалення в тонкому відділі кишечнику у вигляді серозно-фібринозного єюно-ілеїту, гіперплазія й серозно-геморагічний лімфаденіт мезентеральних лімфовузлів, множинні фокуси геморагій у паренхімі селезінки й у серозній оболонці тонкого відділу кишечнику, пасивна венозна гіперемія печінки й нирок, дилатація правого шлуночка серця, набряк легень, кахексія й дегідратація організму.

На мікроскопічному рівні відмічали серозно-фібринозний єюно-ілеїт, гіперплазію лімфоїдних вузликів селезінки та лімфатичних вузлів, геморагічні інфаркти в селезінці, гіперплазію поодиноких і агрегованих лімфоїдних вузликів слизової оболонки тонкого відділу кишечнику, дистрофічні процеси в паренхімі печінки й нирок.

Ключові слова: *коронавірусна інфекція, собаки, гістологічні зміни, геморагії, серозно-фібринозний єюно-ілеїт, серозно-геморагічний лімфаденіт, гіперплазія, лімфоїдні вузлики*

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THE EFFECTIVENESS OF AMNIOTIC MEMBRANE DEPENDING ON THE CAUSE OF CORNEAL DAMAGE IN DOGS

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Abstract. *The results of studies on clinical cases of keratitis in dogs of different etiology using measuring instruments and visual methods after amniotic membrane transplantation, which is the inner of the three amniotic membranes, transparent, avascular, developing from the fetal ectoderm and consisting of the epithelial layer, located on the main membrane and connective tissue stroma. These results make it possible to analyze and further study the effect of allogeneic mesenchymal stem cells during amniotic membrane transplantation for various defects of the eye in general, as well as corneas with ulcers of various etiologies.*

Eye injuries and chemical burns are ophthalmic emergencies that require immediate diagnosis and treatment, as they can lead to deterioration and loss of vision. Clinical manifestations of such burns are caused by exacerbation of the innate immune response due to the infiltration of inflammatory cells and activation of stromal fibroblasts. New treatments are emerging that address restorative mechanisms that improve the surface of the eye after injury; for example, stem cell transplantation has been successfully reported for this purpose.

The main task of the study is to prove the effectiveness of the amniotic membrane to indicate activation of proliferation, stopping tissue apoptosis, reducing the activity of the inflammatory process, accelerating epithelialization and normalization of the corneal stroma, which leads to normal restoration of transparency without vascularization and turbidity.

Keywords: *clinical cases of keratoconjunctivitis, dogs of different breeds, mesenchymal stem cells, amniotic membrane transplantation, corneal condition*

Introduction

The use of the advances in biotechnology to increase the effectiveness of treatment of animals with different etiological pathogenicity is one of the most promising approaches to veterinary science and practice, especially the initiative results of experimental studies on the treatment of different animals through transplantation of mesenchymal stem cells, which have not been used before.

The use of mesenchymal (mesenchymal, stromal) stem cells in veterinary medicine for various eye pathologies in animals is based on a sufficient scientific and methodological basis. Developed methods for the selection of animal bone marrow and isolation from it of mononuclear cell fractions with high proliferative activity allow to obtain the required number of mesenchymal stem cells as soon as possible and use them to stimulate reparative processes in pathologically altered eye tissues.

The method of treatment is extremely relevant because it solves an urgent issue, which is extremely important at present, as eye diseases among animals occupy from 40 to 80% in all species of animals, namely in small ones.

Analysis of recent researches and publications

Among the pathologies of the organ of vision, the leading place is occupied by inflammatory diseases, traumatic eye injuries. According to the domestic literature, the share of eye injuries accounts for more than 10% in the structure of the entire pathology of the organ of vision, with burns accounting for 8%. Of all eye injuries, the cornea accounts for up to 80% (Bothra & Arumugam, 2018).

Burns caused by various physical (thermal and radiation) and chemical (alkalis and acids) factors differ significantly in the mechanisms of eye tissue damage but have a similar clinical picture, mainly due to the severity of the damage (Colombo & Melo, 2018).

Physiological regeneration of the anterior corneal epithelium is carried out by constant reproduction of cambial elements and their differentiation to the center by rapid migration from the periphery to the visual axis Complete renewal of the anterior epithelium occurs within 5–7 days. (Calitz, 2018; Gariani, 2017; Gryshchenko et al., 2019)

But only 2% of corneal epithelial basal cells have similar activity. Epithelial basal cells, being highly differentiated, are not capable of reproduction (Gryshchenko, 2018; Daniels et al., 2018).

There is also evidence that in some animals, corneal stem cells along with the limbus may be diffusely scattered throughout the corneal epithelium (Gula & Margitich, 2000). According to most researchers, normal corneal epithelization is possible if at least half of the limbal area is preserved (Melnychuk, 2019; Sang-Chul, 2020). With complete limbal insufficiency, the epithelialization of a cornea is possible only due to the conjunctival epithelium (Solís-Calero, 2015; Starr et al., 2019).

Many studies have been performed on laboratory animals in the experiment and very little is known about the use of the amniotic membrane in clinical cases for dogs with different causes of injury. Therefore, studies in the direction of nosology of various eye pathologies in animals are very relevant today and require more extensive research in this area.

After severe injuries with the total destruction of the germinal zone of the limbus, pannus develops, and in this case, the cornea is always permeated with blood vessels (Tomchuk, 2019). In addition, limbal cell failure contributes to the development of corneal defects after burns (Udut, 2013; Vlizlo et al., 2012).

The purpose of the study: to determine the effectiveness of amniotic membrane transplantation for various injuries and pathologies of the cornea in spontaneous clinical cases, with the study of the regeneration of entire corneal epithelium.

Materials and methods of research

The experimental study was carried out on the eyes of dogs of different breeds, with spontaneous cases of different injuries with different degrees and complexity, using the same transplant material by the same technique.

Control studies were performed on days 10, 20, and 30 of the experiment. Surgical interventions were performed on the basis of the veterinary clinic of the Nemishaevo Agricultural College. All procedures provided for in the study protocol were performed in accordance with the requirements of the European Convention for the Protection of Pets and Laboratory Animals (the Convention was ratified by the Law of Ukraine, No 578-VII (578-18) of 18/09/2013).

Fig. 1 shows dog eyes with different injuries:

1.1 Deep purulent keratitis (with white purulent infiltrate and vascularization).

1.2 Keratomalacia of the cornea (after chemical burns, with immune-mediated complications).

1.3 Subtotal corneal sequestration (congenital).

1.4 Pannus and plasma of the third eyelid (complicated by ehrlichiosis).

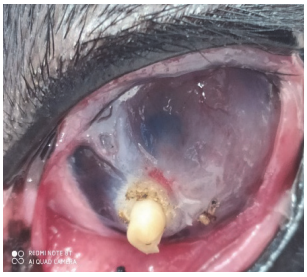


Fig. 1.1

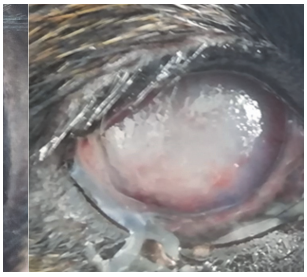


Fig. 1.2

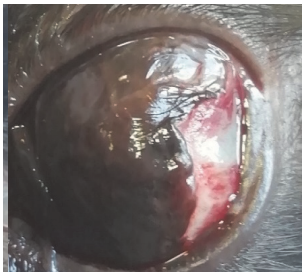


Fig. 1.3

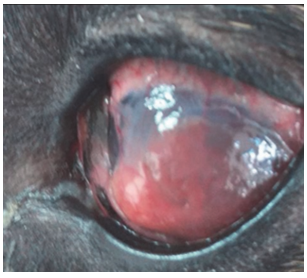


Fig. 1.4

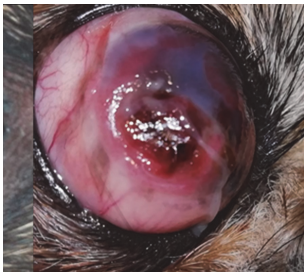


Fig. 1.5

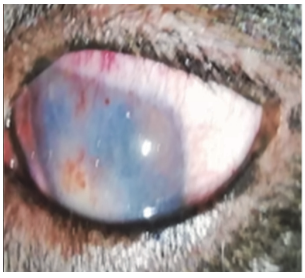


Fig. 1.6

1.5 Perforated septic corneal ulcer (as a result of mechanical trauma).

1.6 Herpesvirus epithelial dystrophy with pathological neovascularization of the cornea.

After surgical cleaning of the pathological tissues, the prepared amniotic membrane was sutured with a purse-string suture along the edge of the limbus (Polyamide 0007 thread), and the amniotic membrane extract was installed.

To assess the degree of inflammation and determine the extent of damage to the layers of the cornea, the following studies were used:

1. Fluorescein Seidel test (use of fluorescein dye, which indicates the degree of damage to the surface of the cornea). The degree of keratopathy was determined by the area and intensity of corneal staining with fluorescein. The cornea was divided into 5 segments (upper, lower, lateral, medial, and central), in each of which the degree of staining was assessed on a 3-point scale depending on the intensity of staining, where

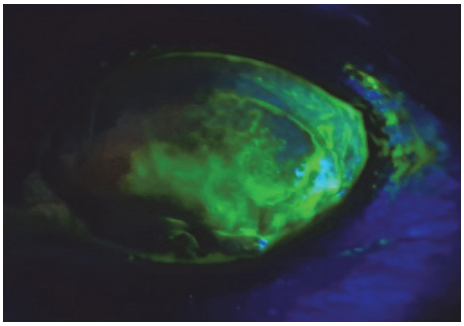


Fig. 2

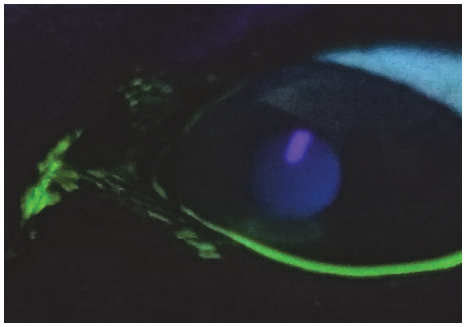


Fig. 3

0 points – no staining, 1 point – slight staining, 2 points – moderate color, 3 points – pronounced color (Fig. 2–3).

2. Tonometry in a non-contact way using a Tonovet veterinary tonometer, the norm was taken as an average of 15–23 mm Hg, where 0 points – norm, 1 point –hypotension, 2 points – hypertension.

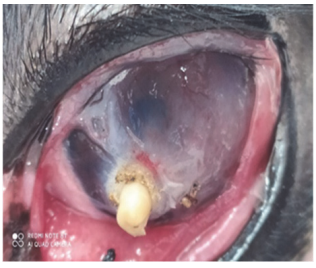
3. Evaluation by review according to the criteria (used the developed score scale). I. Isolation in the conjunctival cavity: 0 – absent, 1 – minor mucous, 2 – pronounced mucous; II. The degree of conjunctival hyperemia: 0 – pale pink, corresponds to the physiological norm, 1 – slight conjunctival hyperemia, 2 – moderate conjunctival hyperemia, 3 – severe conjunctival hyperemia; III. Corneal edema: 0 – no corneal edema, 1 – mild local corneal edema in the area of inflammation, 2 – moderate local corneal edema in the area of inflammation, 3 – diffuse edema in the area of inflammation with the transition to the surrounding cornea; IV. Inflammatory infiltration: 0 – absent, 1 – single (not more than three) subepithelial infiltrates, 2 – multiple (more than three) subepithelial infiltrates, 3 – local drain infiltration of the cornea; V. Vascularization of the cornea: 0 – absent, 1 – is within one square, 2 – is within two squares; VI. Condition of the surrounding cornea: 0 – transparent, no corneal edema, 1 – transparent, moderate corneal edema, 2 – decreased transparency, diffuse corneal edema; VII. The state of the amniotic membrane: 0 – the membrane is completely lysed, 1 – the membrane is partially lysed, 2 – the membrane is preserved; VIII. Degree of corneal opacity: 0 – absent, 1 – slight opacity, 2 – well-defined opacity, 3 – opaque over the entire plane.

4. Schirmer's test (estimation of the amount of tear fluid using a test strip). A stopwatch was used. When evaluating the results, ≥15 mm (0 points) was considered the norm; from 5 to 10 mm – moderate insufficiency (1 point); ≤5 mm – severe insufficiency of tear production (2 points), from 15 to 35 mm – pathological tearing (3 points).

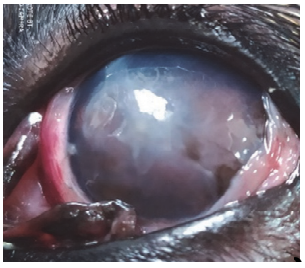
The obtained results (digital material) were processed statistically using the Student's t criterion.

Results of the research and their discussion

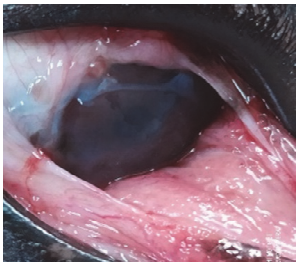
In dogs, as a result of corneal injury and a long inflammatory process, there was purulent keratitis with white purulent infiltrate and vascularization. Fig. 1 shows the pathology at the time of the patient's admission to the clinic, Fig. 1a – the result in 10 days after suturing the amniotic membrane and installation of the extract from its tissues. In Fig. 1b – after suturing of eyelids in 30 days, the restored transparent cornea with lobes of not completely lysed amnion tissues on edge of a limb is visible.



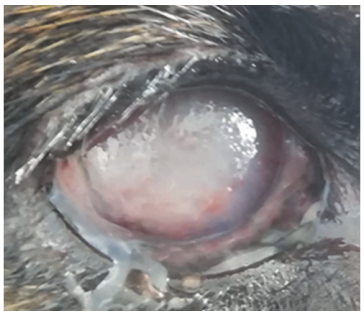
1



1a



1b



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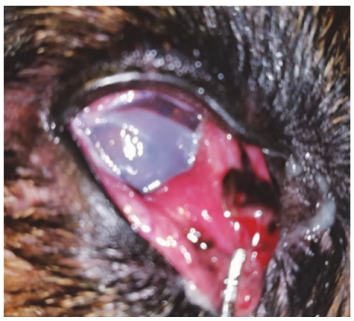
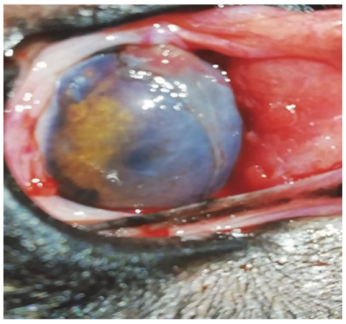
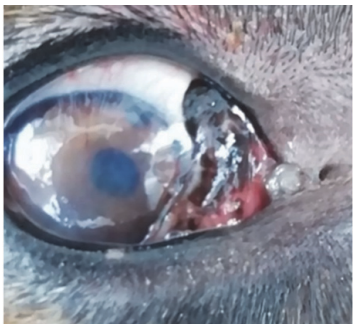


2a



2b



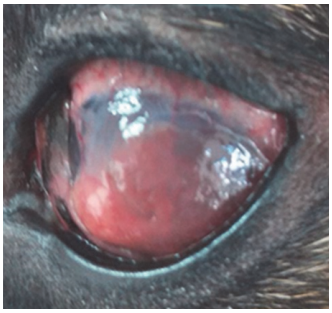
2c

In Fig. 2, a dog after chemical burns with immune-mediated complications, diagnosed with corneal keratomalacia. Surgical insertion was performed by the same method and in Fig. 2a and 2b you can see the processes that occur on the 10th and 20th day after suturing the amniotic membrane. And on day 30, a completely restored cornea and a fully lysed amniotic graft are clearly visible (Fig. 2c).

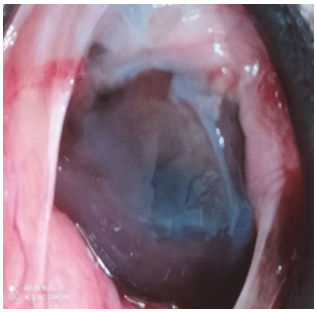
In Fig. 3, a dog with recurrent pannus and plasma of the third eyelid is complicated by ehrlichiosis. Having performed the operation with transplantation of the prepared amnion according to the developed method in 10 days, as can be seen from Fig. 3a, the area of corneal impression decreases and by 20 days it is completely restored, but with some corneal vascularization (Fig. 3c). And on the 30th day, the cornea was restored almost over the entire surface of the cornea, with parts of non-lysed amnion along the edge of the limbus (Fig. 3c).

In Fig. 4, the dog was diagnosed with progressive, congenital subtotal corneal sequestration, and after surgical examination, an amnion transplant was installed (Fig. 4a), and as can be seen from Photo 4b and 4c, there are areas with completely transparent and restored layers of the cornea, and other vascularized ones require re-scaring and transplantation.

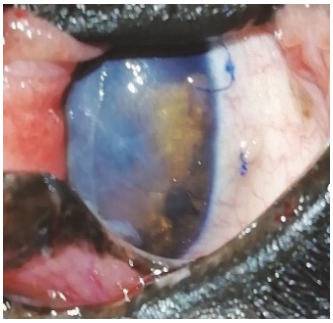
As a result of mechanical eye injury in Fig. 5, a perforated septic corneal ulcer was formed. After transplantation of the prepared amniotic membrane in 10 days, Photo 5a shows the active process of cleansing from the depth of the ulcer and non-lysed amnion, and within 20 days – its complete lysis with complete



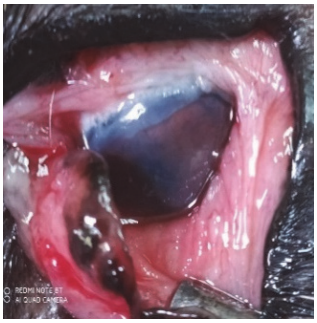
3



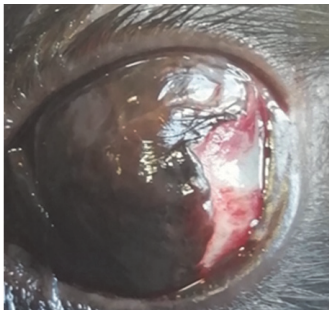
3a



3b



3c



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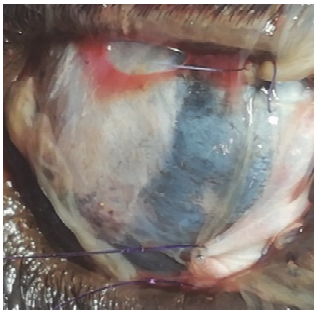
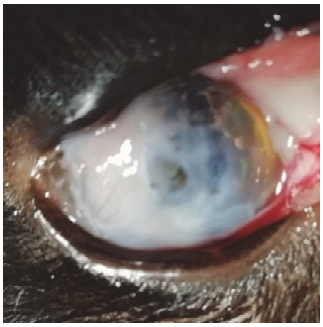
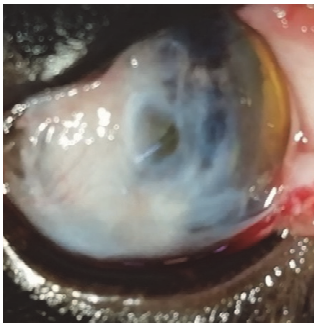


4a



4b



4c

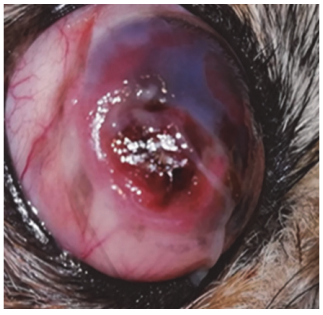
healing of the ulcer and slight vascularization. On the 30th day, you can see a completely restored cornea with a slight opacity in the area of damaged deep layers of the cornea.

In Fig. 6 – a patient with herpesvirus epithelial dystrophy that caused pathological neovascularization of the cornea. In this case, only installations of amniotic membrane tissue extract using eye films were used (Fig. 6a), and already on the 20th and 30th day the restored surface of the cornea is visible, with almost no visible signs of vascularization.

Table 1 shows the results of the assessment of the state of damage by the evaluation criteria according to the developed scale for each study and summarized in one numerical indicator for each patient with different clinical diagnoses and different etiology of corneal damage in dogs.

10 days after amniotic membrane suturing in five cases out of six criteria, there were improvements by 50%, inflammatory tissue infiltration decreased, stroma edema decreased, clinical manifestations decreased, and only in congenital pathology no significant changes occurred, only postoperative corneal stroma edema increased (Table 2).

On the 20th day after amniotic membrane transplantation, again in five clinical cases out of six, the results according to the criteria in total significantly improved, there was a significant reduction in the inflammatory process, both in the conjunctiva and in the stroma of the cornea (Table 3). Significant lysis of the amniotic membrane was visible in all cases, which was noticeable only in the limbus area. In all cases, there was a slight vascularization and opacity of the almost



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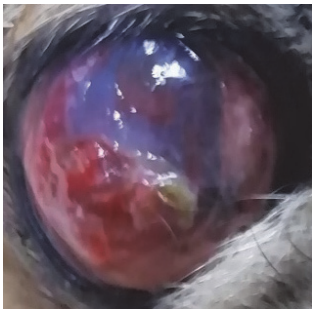
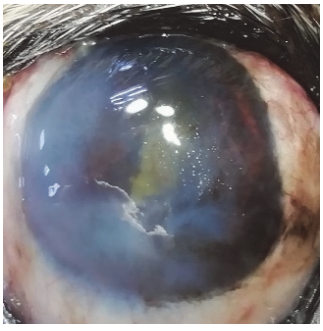
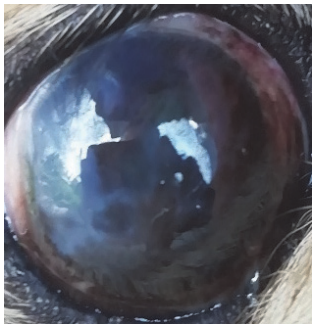


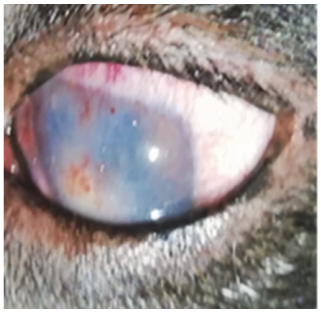
5a



5b



5c



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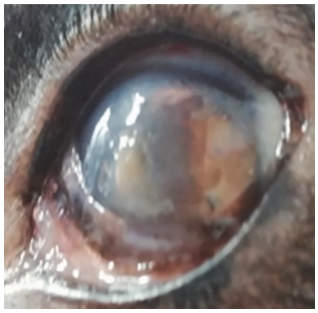
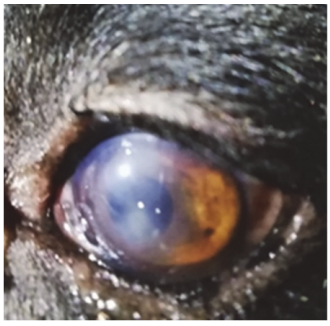
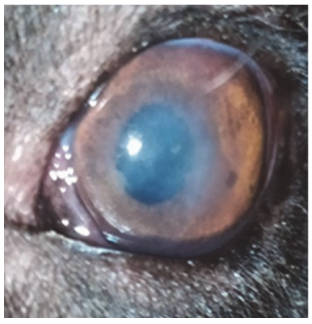


6a



6b



6c

1. Assessment of the state of damage according to the criteria (on a scale) in the intact eye

Dogs with various corneal injuries						
Evaluation criteria	1.1	1.2	1.3	1.4	1.5	1.6
Fluorescein test (FT)	2	3	3	0	3	1
Tonometry (T)	1	2	2	0	2	0
Discharge in the conjunctival cavity (TYPE)	2	2	2	0	2	2
Corneal stroma edema (NAB-C)	3	2	2	1	3	2
Inflammatory infiltration (INF)	3	2	3	0	2	2
Condition of the conjunctiva (CC)	2	3	2	0	1	1
Condition of the surrounding cornea (CORN)	2	2	2	0	2	2
Amniotic membrane (AM) condition	-	-	-	-	-	-
Corneal opacity (OPAC)	2	3	3	3	3	2
Corneal vascularization (VAS)	2	3	3	0	2	2
Schirmer's test (SHT)	3	3	2	0	3	2
The sum of evaluation criteria	22	25	24	4	23	15

restored corneal epithelial layer, but almost unchanged with the case of congenital pathology.

On day 30, all patients had no epiphora, the mucous membranes were pale pink (Table 4). The amniotic membrane

2. Assessment of the state of damage according to the criteria (on a scale) on day 10

Dogs with various corneal injuries						
Evaluation criteria	1.1	1.2	1.3	1.4	1.5	1.6
Fluorescein test (FT)	-	-	-	-	-	-
Tonometry (T)	0	2	2	0	2	0
Discharge in the conjunctival cavity (TYPE)	1	1	1	0	1	1
Corneal stroma edema (NAB-C)	2	1	1	1	2	1
Inflammatory infiltration (INF)	2	1	2	0	1	1
Condition of the conjunctiva (CC)	1	2	1	0	0	0
Condition of the surrounding cornea (CORN)	1	1	1	1	1	1
Amniotic membrane (AM) condition	2	2	2	2	2	0
Corneal opacity (OPAC)	1	2	2	1	2	1
Corneal vascularization (VAS)	1	2	2	0	1	1
Schirmer's test (SHT)	2	2	1	0	2	1
The sum of evaluation criteria	13	16	15	6	14	7

was completely lysed, the integumentary epithelium of the cornea was not stained according to Seidel's test, which indicates the restoration of the morphological structure without damage in six cases, but in two cases a slight residual opacity was observed, and in two others – he phenomenon of partial infiltration along the limb with minor nodules suture, and no changes were observed with the case of congenital pathology, but the place of transparency of the corneal epithelial layer was clearly expressed, in contrast to the initial acanthosis, corneal sequestration, which is well expressed in Photo 1.4 and Table 5 in the column under the same number.

3. Assessment of the state of damage according to the criteria (on a scale) on day 20

Dogs with various corneal injuries						
Evaluation criteria	1.1	1.2	1.3	1.4	1.5	1.6
Fluorescein test (FT)	-	-	-	-	-	-
Tonometry (T)	0	0	0	0	0	0
Discharge in the conjunctival cavity (TYPE)	0	1	1	0	0	0
Corneal stroma edema (NAB-C)	1	0	0	1	1	0
Inflammatory infiltration (INF)	1	0	1	0	0	0
Condition of the conjunctiva (CC)	1	2	1	0	0	0
Condition of the surrounding cornea (CORN)	1	1	1	1	1	1
Amniotic membrane (AM) condition	1	1	1	0	0	0
Corneal opacity (OPAC)	1	2	2	2	2	1
Corneal vascularization (VAS)	0	1	1	1	0	0
Schirmer's test (SHT)	1	1	1	0	2	0
The sum of evaluation criteria	7	9	9	5	6	2

4. Assessment of the state of damage according to the criteria (on a scale) on day 30

Dogs with various corneal injuries						
Evaluation criteria	1.1	1.2	1.3	1.4	1.5	1.6
Fluorescein test (FT)	0	0	0	0	0	0
Tonometry (T)	0	0	0	0	0	0
Discharge in the conjunctival cavity (TYPE)	0	0	1	0	0	0
Corneal stroma edema (NAB-C)	0	0	0	1	0	0
Inflammatory infiltration (INF)	0	0	0	0	0	0
Condition of the conjunctiva (CC)	0	1	1	0	0	0
Condition of the surrounding cornea (CORN)	0	0	0	1	0	0
Amniotic membrane (AM) condition	0	0	0	0	0	0
Corneal opacity (OPAC)	1	1	0	2	1	0
Corneal vascularization (VAS)	0	0	0	1	0	0
Schirmer's test (SHT)	0	0	1	0	0	0
The sum of evaluation criteria	1	2	3	5	1	0

5. Summary information on the sums of evaluation criteria

Dogs with various corneal injuries						
The sum of evaluation criteria	1.1	1.2	1.3	1.4	1.5	1.6
For the period of pathology	22	25	24	4	23	15
10 days after	13	16	15	6	14	7
20 days after	7	9	9	5	6	2
30 days after	1	2	3	5	1	0

Conclusions and future perspectives

It is established that after amniotic membrane transplantation for eye injuries of different genesis, proliferation processes are activated, tissue apoptosis processes are stopped, inflammatory process activity is reduced, which accelerates corneal stromal epithelialization and normalization, which causes a normal restoration of transparency without vascularization and opacity.

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Анотація. *Висвітлено результати досліджень кератиту в собак різної етіології в клінічних випадках за допомогою вимірювальних приладів та візуальних методів після трансплантації амніотичної мембрани. Вона є внутрішньою з трьох плодових оболонок, прозора, аваскулярна, розвивається з фетальної ектодерми і складається із шару епітеліальних клітин, розташованих на основній мембрані, і сполучнотканинної строми. Ці результати досліджень дають можливість аналізувати та в подальшому вивчати вплив алогенних мезенхімальних стовбурових клітин під час трансплантації амніотичної оболонки за різних дефектів ока загалом, а також рогівки з виразками різної етіології.*

Серед патологій органу зору провідне місце займають запальні захворювання та травматичні пошкодження очей. За даними вітчизняної літератури, на частку травм ока припадає понад 10% в структурі всіх патологій органу зору, водночас, опіки становлять 8%. З усіх пошкоджень очей на частку рогівки припадає до 80%. Опіки, спричинені різними фізичними (термічними й радіаційними) і хімічними (лугами й кислотами) факторами, значно різняться механізмами пошкодження тканин ока, але мають подібну клінічну картину, зумовлену в основному тяжкістю пошкодження.

Фізіологічна регенерація переднього епітелію рогівки здійснюється постійним розмноженням камбіальних елементів і їхнім диференціюванням до центру, стрімкою міграцією від периферії до зорової осі. Повне оновлення переднього епітелію відбувається впродовж 5–7 діб.

Основне завдання дослідження – довести ефективність застосування амніотичної оболонки, щоби вказувало на активізацію процесів проліферації, зупинку процесів апоптозу тканин, зменшення активності запального процесу, що прискорює епітелізацію і нормалізацію строми рогівки, що й зумовлює нормальне відновлення прозорості без васкуляризації та помутнінь.

Ключові слова: *клінічні випадки кератокон›юнктивіту, собаки різних порід, мезенхімальні стовбурові клітини, трансплантація амніотичної оболонки, стан рогівки*

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INDICATORS OF PROTEIN METABOLISM IN CALVES WITH BRONCHOPNEUMONIA UNDER INDIVIDUAL THERAPY

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Abstract. *Respiratory diseases of animals are a significant problem in veterinary medicine. They cause a lot of economic damage to farms, consisting of decreasing productivity and breeding qualities of animals and their deaths. The significant spread of respiratory diseases in animals is caused by decreasing natural resistance due to violation of the housing technology, as well as a high concentration of opportunistic and pathogenic microflora in the indoor air.*

The results of individual therapy in calves with nonspecific catarrhal bronchopneumonia with the use of the drug Calfmin, which is based on compounds of biogenic micro- and macroelements, and plant immunomodulators, are presented. The clinical parameters of calves, namely, habitus, condition of hair, skin, visible mucous membranes, superficial lymph nodes, body temperature, number of respiratory movements, and heart contractions were studied during bronchopneumonia and their changes under individual treatment of animals.

The indicators of protein metabolism in the blood serum of clinically healthy and calves with nonspecific bronchopneumonia were also studied. The content of total protein, albumin, α-, β- and γ-globulins was examined in the blood serum of calves by nephelometric method using phosphate buffers. The dynamics of the comparative analysis of total protein content and its fractions in the blood serum between calves of separate experimental groups is shown. The results of studies indicate a decrease in the body's natural resistance and suppression of immunity in sick calves and the rapid recovery under the conditions of the application of nanoaquachelate macronutrients in the form of the drug Calfmin in combination with Echinacea.

Keywords: *calves, nonspecific bronchopneumonia, clinical parameters, total protein, albumins, globulins, natural resistance*

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Introduction

Dairy farming in Ukraine has always been, and is now, a traditional and dominant branch of animal husbandry, which is the main part of providing the population with food. However, prolonged general economic instability and underfunding of this livestock industry provokes a violation of animal husbandry technology, which is often manifested by unsatisfactory housing conditions, insufficient provision of animals complete feed rations, etc. (Chumachenko et al., 2000; Ruda, 2000). As a result, the natural resistance is significantly reduced in the cattle, which is manifested by the low level of immunobiological reactivity in calves (Ruda, 2000; Chumachenko et al., 2000; Griffin et al., 2010). Such calves are usually prone to diseases of various etiologies. The diseases of the digestive tract (dyspepsia) and respiratory (bronchopneumonia) system are very often registered.

Respiratory diseases of animals are a significant problem in veterinary medicine. They cause a lot of economic damage to farms, consisting of decreasing productivity and breeding qualities of animals and their deaths (Demidchik, 2005; Antonenko, 2007; Bliznetsova et al., 2008).

Diseases of the respiratory system are widespread among animals of all species and age groups but are more commonly diagnosed in young, exhausted, and old animals (Ruda, 2000; Kondrakhin et al., 2012; Wolfger et al., 2015). In modern economic conditions, respiratory diseases of young animals are widespread in Ukraine (Tatarchyk, 2007) and abroad (Tuleva, 2007; Slupsky et al., 2009; Trofimov et al., 2009; Basoglu et al., 2016), and could be both in large high-tech farms of industrial type or small farms (Chumachenko et al., 2000; Slupsky, 2009).

The significant spread of respiratory diseases of animals is caused by decreasing the natural resistance due to violation of the housing technology, as well as a high concentration of opportunistic and pathogenic microflora in the indoor air (Tuleva, 2009; Basoglu et al., 2016; Braun et al., 2018;).

Approximately 20–30% of calves suffer from bronchopneumonia in Ukraine every year. The disease mainly occurs in winter and spring. Risk groups are calves at the age from 2 weeks to 2–3 months (Tatarchyk, 2007; Griffin et al., 2010; Kondrakhin et al., 2011).

In the etiology of nonspecific bronchopneumonia in calves in combination with reduced resistance, immunological reactivity of the newborn animals, and the influence of adverse environmental factors, the bacterial microflora of the upper respiratory tract plays an important role, which under certain conditions becomes pathogenic (Nicholas et al., 2008; Trofimov et al., 2009; Basoglu et al., 2016).

In recent years, the incidence of calves with bronchopneumonia has increased due to a decrease in their body's natural resistance, suppression of immunity, and lack of scientifically sound means and methods of prevention and treatment of animals.

During bronchopneumonia in calves, pathological processes develop not only in the respiratory system but also in the whole body, because all types of metabolism are impaired. This, in turn, leads to the suppression of the functions of all vital organs and systems in animals. Therefore, to achieve the desired result, it is important to take a comprehensive approach when choosing drugs for the treatment of patients with bronchopneumonia in calves (Tatarchyk, 2007; Wolfger et al., 2015; Zitzmann et al., 2019;).

The economic losses from bronchopneumonia consist of a significant percentage of calf deaths, a decrease in the average daily weight gain (sick calves have a lack of growth and development), the cost of veterinary care, and additional treatment for sick animals (Dariusz et al., 2003; Jodi et al., 2020).

The most effective is a comprehensive treatment of calves with bronchopneumonia, which should be aimed at eliminating violations of the keeping and feeding technology, increase the natural resistance, and includes the simultaneous use of various therapies: antimicrobial, pathogenetic, symptomatic, application of stimulants and vitamins.

That is why, nowadays, the development and introduction into the practice of veterinary medicine of new highly effective and cost-effective schemes for the prevention of nonspecific bronchopneumonia and treatment of calves with this pathology are especially relevant.

The purpose of this work is to study the metabolism of proteins and their fractions in the blood serum of calves with nonspecific bronchopneumonia under individual therapy using nanoaquachelates of macronutrients in the form of a drug Calfmin in combination with Echinacea.

Materials and methods of research

The research was conducted in the farm "Podilskyi Gospodar 2004", a village of Velyka Medvedivka, Shepetivka district, Khmelnytskyi region.

Four groups of black-spotted calves aged 2 months were formed for the study: one control (clinically healthy calves) and three experimental (calves with nonspecific acute catarrhal bronchopneumonia), seven animals in each group.

During the selection of calves into groups, the results of clinical trials obtained using the methods of examination, thermometry, percussion, and auscultation were taken into account, and the infectious nature of bronchopneumonia was excluded.

Sick calves of the 1st experimental group were treated due to the treatment regimen used in the farm (basic treatment): calves were injected intramuscularly with a combined antibiotic Combikel at a dose of 1 ml per 10 kg of body weight, anti-inflammatory drug Dexakelum at a dose of 1 ml per 100 kg of body weight body, and hepatoprotector HEPAVI-kel at a dose of 1 ml per 10 kg of body weight.

Calves of the 2nd experimental group received basic treatment according to the scheme used in the farm and, in addition, they were given the immunomodulator N (D-8) with milk.

Calves of the 3rd experimental group were treated using only nanoaquachelates of micronutrients in the form of the drug Calfmin and plant immunomodulator Echinacea. The drugs were used dissolved in 1 liter of milk at a dose per animal of 3.5 ml of Calfmin and 1.25 ml of Echinacea, the frequency of application – twice a day.

Monitoring of the clinical condition in calves and the course of the disease was carried out daily throughout the treatment period, and biochemical examinations were performed on the 1st day of the disease, and the 3rd and 7th day of treatment.

Blood samples were taken from the jugular vein in the morning before feeding. The content of total protein and its fractions was determined. The results of the research were processed statistically using the program named Statistica.

Results of the research and their discussion

It is established that in the farm "Podilskyi Gospodar 2004" of Khmelnytskyi region, the winter-spring outbreak of bronchopneumonia in calves begins in February with the maximum number of sick animals and their further deaths in March and April. The results of our research indicate that the farm has susceptible to disease calves at the age of 1–3 months, and our long-term monitoring of the incidence of calves with nonspecific acute catarrhal bronchopneumonia showed that on average during 2006–2018 in this farm, nearly 23.1–24.7% of calves become sick and nearly 7.2–9.4% of them died from this pathology at age of 1–2 months and 3.4–6.3% – at age of 23 months.

During the research, we found that the rate of survived 1–2 months old calves with bronchopneumonia is up to 90.6%, which indicates the low efficiency of treatment schemes. The number of 2–3 months old calves suffering from bronchopneumonia after treatment according to the scheme of the farm was slightly higher and amounted to 93.2%.

The study of etiopathogenetic relationships under nonspecific acute catarrhal bronchopneumonia in calves on the farm made it possible to identify several groups of etiological factors that affect the body of calves of different ages in different combinations.

The first group includes anthropogenic factors, disorders of biogeocenosis, and adverse factors of fetal development. They lead to the birth of malnourished calves with weakened resistance, which is confirmed by such criteria as the body temperature immediately after birth. Thus, the temperature of some calves was not 39.1–39.3 °C but reduced by 1–1.5 °C.

These calves showed bradycardia, myopathy, and malnutrition. The sucking reflex in many newborn calves was weak with a delay. Most of these calves had dyspepsia and often developed bronchopneumonia in some time after recovering from dyspepsia. This indicates that the diseases of young animals in the neonatal period are associated with their anatomical and physiological characteristics.

According to our data, the prevalence of diseases in newborn calves under the scheme "dyspepsia – bronchopneumonia" is due to the violation of the conditions for keeping and feeding cows of breeding stock under their different physiological conditions. It is necessary to highlight the insufficient feed base, violation of the diet structure, especially in the dry season, insufficient feed quality and deficiency of macro-, micronutrients, and vitamins.Nonspecific catarrhal bronchopneumonia occurs in calves on the farm also due to a violation of the diet structure and housing conditions of animals in the postnatal period. Among this group of etiological factors, we distinguish the following: congestion, accumulation of harmful gases, colds (cold, high humidity). Analysis of the diet showed that the feed base for 1.5–2 months old calves does not provide the physiological need for protein, vitamins, especially A, C, as well as macro- and microelements, especially iodine and calcium.

In a clinical study, sick calves showed general weakness, loss of appetite, redness of the conjunctiva and mucous membranes of the nasal cavity, the appearance of catarrhal discharge, and dry painful cough. Respiration in animals was accelerated, difficult and shallow, abdominal type (Table 1). Sick calves laid down most of the time, when they were forced to get up, there was an unconfident gait, muscle tremor. The hair was disheveled, wet.

1. Clinical parameters in calves with bronchopneumonia under individual therapy (M ± m, n = 7)

Period	Clinically healthy calves (control group)	Sick calves		
		1st experimental group (basic treatment)	2nd experimental group (basic treatment + D-8)	3rd experimental group (Calfmin + Echinacea)
Body temperature, °C				
1st day	38.80 ± 0.16	40.84 ± 0.21***	40.95 ± 0.21***	40.87 ± 0.23***
3rd day	38.99 ± 0.06	39.50 ± 0.17*	39.30 ± 0.18	39.11 ± 0.09
7th day	38.90 ± 0.07	39.01 ± 0.12	38.94 ± 0.09	38.87 ± 0.10
Pulse rate, beats/min				
1st day	78.14 ± 0.96	97.29 ± 1.14***	96.14 ± 1.41***	97.57 ± 1.05***
3rd day	78.86 ± 1.32	88.86 ± 1.13***	86.86 ± 1.43***	85.29 ± 1.53**
7th day	76.86 ± 1.48	78.14 ± 2.32	77.71 ± 1.88	76.14 ± 1.43
The number of respiratory movements per 1 min				
1st day	29.43 ± 0.60	46.57 ± 0.94***	46.43 ± 1.34***	46.71 ± 1.41***
3rd day	28.43 ± 0.75	37.29 ± 1.51***	35.29 ± 1.48***	34.43 ± 1.52**
7th day	28.14 ± 0.47	29.57 ± 1.19	29.29 ± 0.63	28.57 ± 0.57

Note: hereinafter * P < 0.05, ** P < 0.01, *** P < 0.001 compared to clinically healthy calves.

On the 1st day of researches, the body temperature in calves of all experimental groups was 40.84–40.95 °C, which is significantly higher by 1.04–1.15 °C (P < 0.001) compared with clinically healthy animals. The number of heart contractions in calves with bronchopneumonia was in the range of 96–97 beats per minute, which is also significantly higher than in clinically healthy animals in 1.25 times (P < 0.001). The conjunctiva and nasal mirror in sick calves had a cyanotic shade, indicating a violation of hemostasis in the small circulation. The number of respiratory movements in calves of the experimental groups was in the range of 46.4–46.7, which is 1.58 times significantly higher (P < 0.001) than in clinically healthy calves (Table 1). The mixed-type shortness of breath, a sharp, dry, intermittent, painful cough, a discharge of catarrhal exudate from nasal passages were also observed. Wet rales were detected during auscultation of the heart, which indicates a violation of the elastic properties of the interalveolar septa due to the accumulation of exudate in them. Also, muffled heart sounds and weakening of the pulse wave were noted during auscultation of the heart. Chest percussion revealed a focus of dullness in the apical and cardiac parts of the lungs.

Our data on protein metabolism in calves with bronchopneumonia indicate the presence of hypoproteinemia under this pathology.

Thus, the total protein content in the blood serum of calves with bronchopneumonia in the experimental groups is significantly lower by 12–13% (P < 0.05) compared with clinically healthy calves (Table 2). According to our data, this is mainly due to proteins of the albumin fraction.

2. Indicators of protein metabolism in the blood serum of calves with catarrhal bronchopneumonia under individual therapy (M ± m, n = 7)

Period	Clinically healthy calves (control group)	Sick calves		
		1st experimental (basic treatment)	2nd experimental group (basic treatment + D-8)	3rd experimental group (Calfmin + Echinacea)
Total protein, g/L:				
1st day	65.43 ± 2.27	58.37 ± 2.36*	56.93 ± 2.53*	57.93 ± 2.19*
3rd day	62.35 ± 2.39	59.56 ± 2.89	58.48 ± 2.80	60.68 ± 2.99
7th day	62.23 ± 4.33	60.13 ± 4.45	59.05 ± 3.64	63.38 ± 4.60
Albumins, g/L:				
1st day	27.57 ± 2.12	21.60 ± 1.62*	20.30 ± 1.67*	19.79 ± 1.68**
3rd day	25.59 ± 1.45	23.32 ± 1.15	22.13 ± 1.66	22.12 ± 1.43
7th day	26.55 ± 2.44	24.54 ± 2.08	23.36 ± 3.05	26.07 ± 2.35
Globulins, g/L:				
1st day	37.86 ± 2.34	36.77 ± 3.15	36.63 ± 2.76	38.14 ± 2.22
3rd day	36.76 ± 3.55	36.24 ± 4.05	36.35 ± 3.33	38.56 ± 3.98
7th day	35.68 ± 2.67	35.59 ± 2.78	35.69 ± 2.45	37.31 ± 3.74
α-Globulins, g/L:				
1st day	7.87 ± 1.12	8.70 ± 1.11	9.47 ± 2.23	8.83 ± 2.43
3rd day	8.15 ± 1.78	8.17 ± 2.45	8.35 ± 1.76	8.49 ± 1.55
7th day	8.05 ± 1.56	7.63 ± 1.54	7.85 ± 1.76	8.04 ± 1.83
β-Globulins, g/L:				
1st day	7.41 ± 1.34	11.59 ± 1.45*	11.11 ± 1.95*	10.89 ± 2.55*
3rd day	8.07 ± 1.67	10.44 ± 2.04	9.23 ± 1.82	9.91 ± 1.44
7th day	8.82 ± 1.87	8.95 ± 2.65	8.27 ± 1.55	8.24 ± 2.56
γ-Globulins, g/L:				
1st day	20.61 ± 2.91	12.33 ± 2.22	11.43 ± 1.11	12.25 ± 2.16
3rd day	19.48 ± 1.44	13.84 ± 3.34	12.08 ± 2.05	13.08 ± 3.33
7th day	19.21 ± 1.86	14.62 ± 2.64	12.74 ± 1.77	15.30 ± 1.17

Note: similar to Table 1.

The results of studies have shown that at the beginning of the treatment (1st day) the albumin content in the blood serum was 22–28%, which is significantly lower ($P < 0.05$) compared to clinically healthy animals.

The indicators of the globulin content in the blood serum of calves of all experimental groups at the beginning of the treatment did not differ significantly from those in clinically healthy animals. However, the albumin/globulin ratio was in the range of 0.51–0.59 in calves of the experimental groups, while in clinically healthy animals – 0.73–0.84.

It should be noted that the content of individual globulin fractions in the blood serum of calves with broncho-

pneumonia differs from their content in the blood serum of clinically healthy calves. Thus, in the blood serum of calves with bronchopneumonia at the beginning of the treatment, there was a pronounced tendency to increase the content of α- and ß-globulins and a significant decrease in the content of γ-globulins by 40–44% ($P < 0.05$) compared with clinically healthy calves.

Our treatment of calves helped to improve the metabolism of proteins in their organism.

Thus, on the 3rd day of our research, the albumin content in the blood serum of sick calves of the experimental groups was 22–23.3 g/L, but this figure did not reach the values of clinically healthy calves (25.59 ± 1.45). That is, during this period of the treatment in calves with bronchopneumonia, there was already a pronounced tendency to increase the globulin content in their blood and the gradual approximation of this indicator to the values of clinically healthy calves (Table 2).

The total content of globulins in the blood serum of calves with bronchopneumonia on the 3rd day of the treatment, as at the beginning, was at the level of clinically healthy calves. However, the albumin to globulin ratio in these animals on the 3rd day of the research was in the range of 0.57–0.61, while in clinically healthy calves – 0.70. That is, on the 3rd day of the treatment, well-pronounced dysproteinemia was still observed in the body of sick calves.

Analyzing the content of individual globulin fractions in the blood of calves of the experimental groups, it should be noted that on the 3rd day of the treatment of calves with bronchopneumonia compared with the 1st day, the content of α- and ß-globulins tended to decrease, while γ-globulins – to increase. In relation to clinically healthy calves, the content of α-globulins in the blood serum of calves with bronchopneumonia of all experimental groups during this period was at the same level, ß-globulins – had a slight tendency to increase, and γ-globulins – significantly decreased in calves of the 1st experimental group by 28% ($P < 0.05$), the 2nd – by 36% ($P < 0.05$), and the 3rd – by 33% ($P < 0.05$).

On the 7th day of the therapy, the content of total protein in the blood serum of calves of the experimental groups had a pronounced tendency to increase compared to the 1st day in animals of all groups. At the same time, the content of total protein in the blood serum of calves of the 3rd experimental group, for the treatment of which was used the drug Calfmin in combination with Echinacea, on the 7th day of the experiment did not differ from that in clinically healthy calves. During this period, the albumin content in the blood serum of calves of the experimental groups was within the range of clinically healthy calves, and compared with the 1st day it increased significantly: in calves of the 1st group – by 14% ($P < 0.05$), the 2nd – by 15% ($P < 0.05$), and the 3rd – by 32% ($P < 0.01$). As a result, the albumin to globulins ratio significantly increased in calves of the 1st group to 0.69, the 2nd – 0.65, the 3rd – 0.70. In the blood serum of clinically healthy calves, the albumin/globulin ratio at this time was 0.74. The content of individual globulin fractions in the blood serum of calves of the experimental groups on the 7th day of the experiment was characterized by the normalization of α- and ß-globulins. At the same time, it should be noted that the blood serum protein content of the γ-globulin fraction in calves of all experimental groups decreased compared with clinically healthy calves.

Conclusions and future perspectives

It is known that the state of hypoxia under bronchopneumonia causes changes not only in the lungs but also in other organs. Thus, a significant decrease in the total protein content in the blood serum of calves with nonspecific catarrhal bronchopneumonia is apparently due to pathological changes in the liver and other organs due to hypoxia. According to Usha (2018), in calves with bronchopneumonia due to impaired gas exchange and the development of hypoxia, there are deep structural disorders of the liver, changes in the structure of hepatocytes, they undergo degeneration, the necrosis occurs. These changes start from the center of the hepatocyte and spread to the periphery. In addition, the liver cells that are furthest from the vessels that supply them do not receive enough oxygen and other nutrients. This causes their degeneration and necrosis. Changes in the structure of separate liver cells lead to changes in the whole organ and disruption of its physiological functions. Therefore, the protein-forming function of the liver is disrupted, the albumin content decreases by 1.5 times ($P < 0.05$) in the blood serum against the background of an increase in the globulin content, which leads to a decrease in albumin/globulin ratio in 1.5 times ($P < 0.001$), that is, to the manifestation of dysproteinemia. According to the research of Usha and other authors (Usha et al., 2018), in the blood serum of calves with catarrhal bronchopneumonia, there is a tendency to decrease the content of α- and β-globulins, while the content of γ-globulins on the contrary – tends to increase. According to our data, at the beginning of the disease in the blood serum of calves with bronchopneumonia, the content of α-globulin fraction significantly increases, the protein content of β-globulin fraction is less pronounced, and the content of γ-globulin fraction decreases, which is consistent with other studies.

The total duration of the treatment of calves of the 3rd experimental group, which was treated with the drug Calfmin in combination with Echinacea, averaged 7–8 days. The treatment period of calves of the 1st experimental group lasted more than 12 days, which implies a high probability of transition of the disease to a chronic form. It should be noted that during the treatment of calves of the 1st experimental group, one animal died.

Our results indicate a significantly higher therapeutic efficacy of the treatment of calves with catarrhal bronchopneumonia using nanoaquachelates of microlements in the form of Calfmin in combination with Echinacea, compared with the treatment of calves including the combined antibiotic Combikel, anti-inflammatory drug Dexakelum, and hepatorotector HEPAVI-kel.

The research results provide for the continuation of scientific experiments to study the effect of nanoaquachelates of microelements in combination with Echinacea on other indicators of the blood of calves with bronchopneumonia.

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Анотація. *Респіраторні хвороби тварин є значною проблемою ветеринарної медицини. Вони завдають значних економічних збитків господарствам, що складаються зі зниження продуктивних та племінних якостей тварин і їхньої загибелі. Значне поширення респіраторних хвороб тварин зумовлене зниженням природної резистентності їхнього організму внаслідок порушення технології утримання, а також високою концентрацією умовно-патогенної й патогенної мікрофлори в повітрі приміщень.*

Представлені результати індивідуальної терапії хворих на неспецифічну катаральну бронхопневмонію телят за застосування їм препарату Кальфмін, що створений на основі сполук біогенних мікро- і макроелементів та рослинних імуномодуляторів. Досліджені клінічні показники телят, а саме, габітус, стан волосяного покриву, шкіри, видимих слизових оболонок, поверхневих лімфатичних вузлів, температура тіла, кількість дихальних рухів та скорочень серця за бронхопневмонії та їхні зміни за індивідуальної терапії тварин.

Досліджені показники обміну білків у сироватці крові клінічно здорових і хворих на неспецифічну бронхопневмонію телят. Вміст загального білку, альбумінів, α-, в- і γ-глобулінів досліджували в сироватці крові телят нефелометричним методом із використанням фосфатних буферів. Показано динаміку порівняльного аналізу вмісту загального білка та його фракцій у сироватці крові між телятами окремих дослідних груп. Результати досліджень вказують на зниження природної резистентності організму і пригнічення імунітету у хворих телят та швидке відновлення цих показників за умов застосування тваринам наноаквахелатів мікроелементів у формі препарату Кальфмін у комплексі з ехінацеєю.

Ключові слова: *телята, неспецифічна бронхопневмонія, клінічні показники, білок загальний, альбуміни, глобуліни, природна резистентність*

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BLOOD BIOCHEMICAL PARAMETERS IN TRANSFER FACTOR DONOR COWS DEPENDING ON SENSITIZATION SCHEME

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Abstract. *Research and development of means for effective prevention and treatment of diseases in animals are one of the priorities for modern veterinary science. Means based on the transfer factor are quite promising to solve these problems. One of the stages of obtaining a qualitative transfer factor specific to a particular disease is the sensitization of the body of donor animals. The purpose of this work was to investigate the blood biochemical parameters of donor cows after sensitization according to different schemes. The experiments were performed on cows of the Ukrainian black-spotted dairy breed, aged 4–5 years. Sensitization of pregnant cows was performed 1–1.5 months before calving with a concentrated formol-alum vaccine against salmonellosis of calves manufactured by the Kherson Biofactory. The vaccine was administered to the animals of the first experimental group one month before calving, one-time in a dose of 10 ml. Animals of the second experimental group 1.5 months before calving were two-time vaccine administered with an interval between injections of 10 days in doses of 10 and 15 ml. Studies have shown that in donor cows, which were two-time vaccine administered, there was an increase in hemoglobin content by 13% ($P<0.05$). There was also a decrease in glucose and creatinine content by 13–28% ($P<0.05$–0.01) in the blood serum of pregnant cows, which did not depend on the sensitization scheme, and a tendency to a decrease in total protein content. Regardless of the sensitization scheme of cows, an increase in serum aminotransferase activity was observed by 1.3–1.5 times ($P<0.05$–0.001), and if alanine aminotransferase activity increased mainly with a single injection of the vaccine, then aspartate aminotransferase activity was*

more intensively increased after a two-time vaccine administration. There was a slight decrease in calcium (by 5–9%) and phosphorus (by 2–3%) content and an increase in potassium content (by 2–5%) in the blood serum of pregnant cows two weeks after vaccine administration regardless of the sensitization scheme.

Keywords: *transfer factor, sensitization, cows, blood*

Introduction

Research and development of means for effective prevention and treatment of diseases in animals are one of the priorities for modern veterinary science. Given the low effectiveness of existing means of specific prevention of most infectious diseases of animals, in particular salmonellosis, the use of drugs based on transfer factor is relevant.

Analysis of recent researches and publications

The term "transfer factor" was proposed by H. Lawrence, who for the first time in 1955 established the possibility of transferring delayed-type hypersensitivity to tuberculin and streptococcal M-antigen in almost healthy people using donor blood lysate, sensitized to these substances (Lawrence, 1955). Transfer factors are essentially small immune messenger molecules that are produced in all higher organisms (Sell et al., 1996). Transmission factors were originally described as immune molecules that are derived from blood or spleen cells and cause delayed-type hypersensitivity and lymphokine synthesis, as well as antigen binding. They have a molecular weight of approximately 5,000 Daltons and consist exclusively of amino acids (Kirkpatrick, 1993).

The immune system helps in recognizing, fighting, remembering invading pathogens. Each pathogen can bring out a transfer factor, at least one transfer factor is created for every piece of the pathogen that the immune system faces. Transfer factors influence the activities of various immune components and also regulate cytokines. The time taken for complete development of immature immune response/delayed hypersensitivity is 10–14 days, but transfer factor induces an immune response within 24 hours (Krishnaveni, 2013). Transfer factor have many therapeutic and prophylactic applications, especially in diseases where cell-mediated immunity plays a major role (García-Hernández et al., 2014). Transfer factor has been used as a therapeutic agent in the treatment of viral, parasitic, fungal, and some bacterial infections, as well as immunodeficiencies, neoplasias, allergies, and autoimmune diseases (Lara et al., 2010; Viza et al., 2013). The main advantages of transfer factor application are efficacy for treating and also for preventing infections, low manufacturing cost, and absence of toxicity (Viza et al., 2013).

One of the stages of obtaining a qualitative transfer factor specific to a particular disease is the sensitization of the body of donor animals. The choice of donors is carried out taking into account the specific purpose of transfer factor application. Donors can be different species of animals with physiological maturity of the immune system (Fudenberg & Pizza, 1994). If the drug is prepared to prevent disease in a particular farm, it is important that the donor animals were also from this farm, herds (Petrov et al.,

1994). As an antigen to sensitize donors, standard vaccines or inactivated strains of the pathogen (viruses, bacteria, protozoa, fungi, bacterial toxins) are used. After immunization, the donor must have a high degree of sensitization of the body, which is determined by the skin reaction to the antigen to which the hypersensitivity is transferred (Galbraith & Fudenberg, 1985). The material for the preparation of transfer factor is lymphocytes obtained from lymph nodes, spleen, thoracic lymphatic duct, peripheral blood, and colostrum of sensitized donors (Rozzo & Kirkpatrick, 1992).

The purpose of the study was to investigate the blood biochemical parameters in transfer factor donor cows depending on the scheme of sensitization of their body.

Materials and methods of research

Ukrainian black-spotted dairy breed aged 4–5 years (second or third lactation) were used as donor animals to obtain the transfer factor. For the experiment, 2 groups of analogous animals were selected (10 cows per group). Sensitization of pregnant cows was carried out 1–1.5 months before calving with a concentrated formol-alum vaccine against salmonellosis (paratyphoid) of calves manufactured by the Kherson Biofactory (suspension of a formalin-inactivated culture of *Salmonella dublin* strain 373 (4 × 107 CFU/ml). The vaccine was administered to the animals of the first experimental group one month before calving, once in a dose of 10 ml. Animals of the second experimental group in 1.5 months before calving were immunized twice with an interval between injections of10 days in doses of 10 and 15 ml.

Blood samples (5 animals from each group) were taken from the jugular vein before and two weeks after the vaccine administration. The hemoglobin content was determined in the whole blood, while the activity of aspartate and alanine aminotransferases, total protein, glucose, creatinine, calcium, phosphorus, potassium, and sodium content – in blood serum.

The hemoglobin content in the whole blood was determined by the hemoglobin cyanide method – by its interaction with potassium ferricyanide, where it is oxidized to methemoglobin, which forms with acetone cyanohydrin colored hemiglobincyanide, the intensity of which is proportional to the content of hemoglobin.

Determination of aminotransferase activity (alanine aminotransferase and aspartate aminotransferase) was performed by the Reitman-Frankel method. The principle of the method is based on the fact that as a result of reamination, which takes place under the action of alanine aminotransferase and aspartate aminotransferase, oxalacetic acid and pyruvic acids are formed, which when 2.4-dinitrophenylhydrazine is added form alkaline hydrazones with an absorption maximum at wavelengths of 500–560 nm.

Determination of total protein content was performed by the Lowry method. The method combines a reaction of copper ions with the peptide bonds under alkaline conditions and a Folin–Ciocalteu reaction. The Folin-Ciocalteu reagent interacts with the cuprous ions and the side chains of tyrosine, tryptophan, and cysteine to produce a blue-green color that can be detected between 650 nm and 750 nm. The protein detection range is 5–100 μg.

Glucose content was determined by the glucose oxidase method. The principle of the method is that the oxidation

of glucose by glucose oxidase produces hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase oxidizes O-dianisidine, turning it into a compound colored blue. The color intensity is determined photometrically at a wavelength of 530 nm.

Determination of serum creatinine was performed by the Jaffe's color reaction (Popper method), where creatinine produces quantitatively an orange color with picric acid in an alkaline medium. The intensity of the color is proportional to the concentration of creatinine.

Determination of total serum calcium was performed with arsenazo III complexone. Arsenazo III is a metal complex from the group of diazo complexes that have different affinities with different metal ions. When mixing a solution of arsenazo III and serum, a calcium complex with arsenazo III is formed, which is characterized by absorption with a maximum of 650–670 nm. The absorption intensity is directly proportional to the calcium concentration in the range of 0.5–3.5 mmol/L.

Determination of inorganic phosphorus in blood serum was performed by reduction of phosphomolybdic acid. The principle of the method is based on the fact that after precipitation of proteins in the centrifuge remains inorganic phosphorus, which with molybdic acid forms phosphomolybdic acid. The latter is reduced by eiconogen to the blue phosphomolybdenum complex. The intensity of the color is directly proportional to the concentration of inorganic phosphorus in the serum.

Serum potassium concentration was determined by the turbidimetric method without deproteinization. The principle of the method is that the interaction of potassium with tetraphenylborate ions in an alkaline medium forms a stable suspension. The turbidity of the suspension, measured at a wavelength of 578 nm, is proportional to the concentration of potassium ions in the serum.

The obtained results were processed statistically. The arithmetic mean (M) and the arithmetic mean error (m) were calculated (Vlizlo et al., 2012). The probability of differences was evaluated by the probability coefficient of the Student's table (P) and the difference between the indicators was considered probable at $P < 0.05$.

Results of the research and their discussion

It is known that the sensitization of a living organism is accompanied by a restructuring of metabolism with a reorientation towards the protective systems. The conducted researches have shown a significant difference in the blood biochemical parameters in animals under different sensitization scheme (Table 1). However, it should be noted that despite the significant difference in the content of individual metabolites in the blood, the indicators did not exceed the norm, taking into account the physiological condition of pregnant cows.

Regardless of the sensitization scheme, there was a decrease in the intensity of protein metabolism in pregnant cows, resulting from a decrease in total serum protein by 2–3% and creatinine content by 13–19% ($P<0.05$–0.01) in two weeks after vaccine administration. And the simultaneous increase in the urea content in the blood serum of animals by 9–13% (although within the trend) indicates an increase in protein catabolism in the body of these animals.

The hemoglobin content in pregnant cows increased by 13% ($P<0.05$) in two weeks after re-administration

1. Blood biochemical parameters in pregnant cows under the different scheme of sensitization (M ± m, n = 5)

Period	Parameter				
	Total protein, g/L	Hemoglobin, g/L	Glucose, mmol/L	Creatinine, μmol/L	Urea, mmol/L
One-time vaccine administration					
Before sensitization	80.7 ± 1.0	91.5 ± 2.6	2 9 ± 0.1	106.0 ± 5.4	6.0 ± 0.4
14 days after sensitization	78.6 ± 4.4	93.0 ± 7.8	2.0 ± 0.2**	92.0 ± 4.7*	6.5 ± 0.2
Two-time vaccine administration					
Before sensitization	80.6 ± 1.7	91.0 ± 1.9	2.4 ± 0.1	106.5 ± 4.0	6.1 ± 0.3
14 days after the second vaccine administration	79.8 ± 1.6	102.0 ± 3.6*	2.0 ± 0.1*	86.5 ± 4.7**	6.9 ± 0.4

Note: The difference is significant at * P<0.05; ** P<0.01.

of the vaccine in comparison with the basal level. In contrast, a one-time vaccine administration did not significantly affect the hemoglobin content in the blood of animals.

Studies have shown that, regardless of the sensitization scheme, in two weeks after administration of the vaccine to pregnant cows, the blood glucose content was significantly reduced. Thus, in animals that were administered the vaccine once, the glucose content in the blood serum was reduced by 28% (P<0.01), while in pregnant cows that were vaccinated twice – by 17% (P<0.05).

The results of studies of transaminase activity in the blood serum of pregnant cows are shown in Figure 1. It was

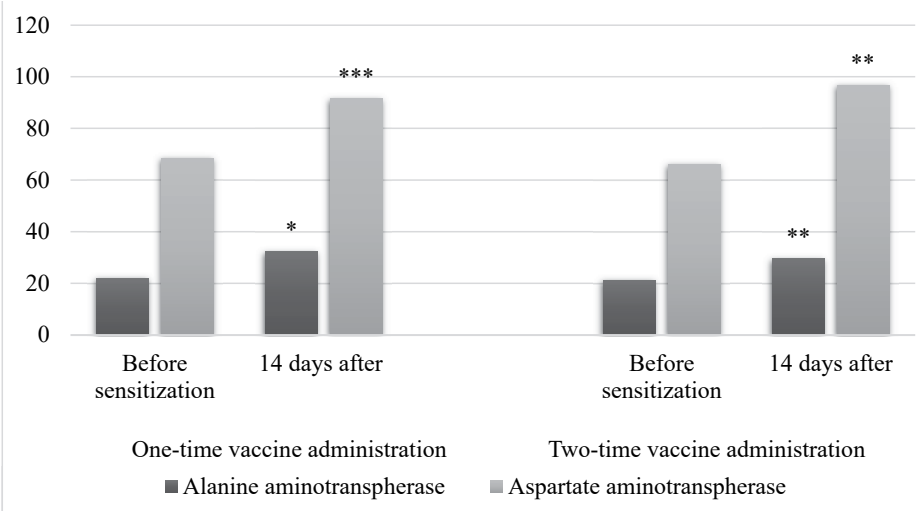

Fig. 1. The aminotransferase activity in the blood serum of cows under different sensitization scheme, units/L (M ± m, n = 5)

2. The content of macroelements in the blood serum of pregnant cows under different sensitization scheme (M ± m, n = 5)

Period	Parameter			
	Calcium, mmol/L	Phosphorus, mmol/L	Potassium, mmol/L	Sodium, mmol/L
One-time vaccine administration				
Before sensitization	2.14 ± 0.06	2.03 ± 0.03	4.05 ± 0.13	141 ± 1.29
14 days after sensitization	1.94 ± 0.11	1.99 ± 0.06	4.25 ± 0.13	142 ± 1.83
Two-time vaccine administration				
Before sensitization	2.15 ± 0.04	1.94 ± 0.04	4.03 ± 0.06	141 ± 1.29
14 days after the second vaccine administration	2.05 ± 0.04	1.89 ± 0.07	4.13 ± 0.05	140 ± 0.82

found that the activity of alanine aminotransferase and aspartate aminotransferase increased by 34–47% (P<0.05–0.001) comparing with the basal level in two weeks after sensitization depending on the vaccine administration scheme.

It should be noted that if in two weeks after one-time vaccine administration in animals, the activity of alanine aminotransferase increased more (by 46.5%; P<0.001) than aspartate aminotransferase (by 33.6%; P<0.05), then in animals which were administered vaccine twice – on the contrary, the activity of aspartate aminotransferase increased more intensively.

According to the data from Table 2, the content of individual macroelements in the blood serum of pregnant cows under different sensitization schemes did not change significantly. It is only necessary to note the insignificant decrease in the content of calcium (by 5–9%) and phosphorus (by 2–3%) and increase in the content of potassium (by 2–5%) in the blood serum of pregnant cows in two weeks after vaccine administration regardless of the sensitization scheme.

The property of most vaccines to show a suppressive effect is known, as evidenced by changes in the immunological and biochemical parameters of animals (Chen et al., 2012). Our results have shown that regardless of the sensitization scheme, there was a significant decrease in glucose and creatinine content in the blood serum of pregnant cows by 13–28% (P<0.05–0.01), a significant increase in activity of alanine aminotransferase and aspartate aminotransferase (by 34–47%; P<0.05–0.001) and a tendency to reduce the total protein content, which indicates a violation of metabolism in animals.

Conclusions and future perspectives

Thus, the sensitization of animals is accompanied by changes in metabolism that depend on the scheme of vaccine administration. An increase in the level of destructive processes in the body of pregnant cows after sensitization, accompanied by a decrease in the intensity of protein metabolism and blood glucose, an increase in the content of end products of its metabolism (urea), which may indicate an intensification of protein catabolism. However, the content of individual macro elements in the blood of pregnant cows after sensitization did not change significantly. The

suppressive effect of two-time administration of vaccine against salmonellosis of calves did not differ significantly, and in some cases was less pronounced than under one-time vaccine administration to pregnant cows.

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Анотація. *Дослідження та розроблення засобів для ефективної профілактики й терапії захворювань у тварин є одним із пріоритетних напрямів сучасної ветеринарної науки. Засоби на основі трансфер-фактора є досить перспективними для вирішення цих*

проблем. Одним з етапів отримання якісного трансфер-фактора специфічного щодо певного захворювання є сенсибілізація організму тварин-донорів. Метою даної роботи було дослідження біохімічних показників крові корів-донорів після проведення сенсибілізації за різними схемами. У дослідах використовували корів української чорно-рябої молочної породи, віком 4–5 років. Сенсибілізацію тільних корів здійснювали за 1–1,5 місяці до отелення концентрованою формол-галуневою вакциною проти сальмонельозу телят виготовленою Херсонською біофабрикою. Тваринам І дослідної групи вакцину вводили за місяць до отелення, одноразово в дозі 10 мл. Тваринам ІІ дослідної групи за 1,5 місяці до отелення вводили вакцину дворазово з інтервалом між ін'єкціями 10 діб у дозах 10 та 15 мл. Проведені дослідження показали, що в корів-донорів, яким вакцину вводили дворазово, відмічали зростання вмісту гемоглобіну на 13% (P < 0,05). Також встановлено зниження вмісту глюкози та креатиніну на 13–28% (P < 0,05–0,01) у сироватці крові тільних корів, що не залежало від схеми сенсибілізації, та тенденцію щодо зниження вмісту загального білка. Незалежно від схеми сенсибілізації корів, спостерігали підвищення активності амінотрансфераз у сироватці крові в 1,3–1,5 раза (P < 0,05–0,001), причому якщо за одноразового введення вакцини переважно зростала активність аланінамінотрансферази, то за дворазового – активність аспартатамінотрансферази. Встановлено незначне зниження вмісту Кальцію (на 5–9%) та Фосфору (на 2–3%) і збільшення вмісту Калію (на 2–5%) у сироватці крові тільних корів через два тижні після введення вакцини незалежно від схеми сенсибілізації.

Ключові слова: *трансфер-фактор, сенсибілізація, корови, кров*

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ESTABLISHMENT AND ORGANIZATION OF VETERINARY AND SANITARY SUPERVISION OF KYIV IN THE LATE 19th AND EARLY 20th CENTURY

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Анотація. *Information on the formation of veterinary and sanitary supervision in Kyiv since the end of the 19th century is given. By 1888, there were 17 small private abattoirs in Kyiv, scattered throughout the city. The abattoirs were located in yards and dirty barns, where cattle were primitively slaughtered and no importance was attached to veterinary and sanitary supervision. These abattoirs caused great dissatisfaction among the urban population and the Kyiv City Duma decided to close the existing abattoirs and open city abattoirs, i.e. to form a regulated institution that would adhere to highly humane tasks, the responsibility for which is assigned on veterinarians.*

To clarify the issues of veterinary and sanitary supervision at city abattoirs in 1899, a special subcommittee was appointed, the chairman of which was elected a member of the Sanitary Commission A. K. Stolpchevskyi. The subcommittee was set a task to develop instructions for veterinarians responsible for the veterinary and sanitary situation at Kyiv city abattoirs, which was carried out by the Sanitary Commission. It was only in 1899 that purely veterinary supervision was separated from sanitary supervision. For the proper organization of veterinary and sanitary supervision in 1909, mandatory resolutions were introduced, which included the rules of arrangement and maintenance of abattoirs. These resolutions introduced a uniform procedure in the abattoir business.

In 1900, the city veterinarian M. K. Kobylyanskyi proposed to expand the veterinary staff in Kyiv and introduce mandatory resolutions on the keeping of farms and dairy cattle. For the first time, in 1901, according to the resolution of the Kyiv Governor, a koumiss and kefir establishment began to function in the village of Pushcha Vodytsia, which was under the subordination of veterinary and sanitary supervision. However, the resolution on

the supervision of cowsheds, dairy farms, koumiss establishments, and individual farms engaged in the sale of milk and dairy products in Kyiv was issued only in 1916.

Ключові слова: *city abattoirs, veterinary and sanitary condition, abattoir business, control and trichinosis station, dairy business*

Introduction

Currently, the state veterinary and sanitary supervision and control over the activities of economic entities for the slaughter of animals, processing, storage, transportation, and sale of products of animal origin (raw materials, food raw materials, by-products, including food) are carried out in accordance with the laws of Ukraine "On the liability of enterprises, institutions and organizations for violations of the legislation on veterinary medicine", "On the quality and safety of food and food raw materials", "On withdrawal from circulation, processing, utilization, destruction or further use of low-quality and dangerous products", rules, instructions, orders, and directives of the Department of Food Safety and Veterinary Medicine of Ukraine, other regulations. According to the legislation of Ukraine, the state veterinary and sanitary supervision and control are carried out by the authorities of public administration, namely: the Department of Food Safety and Veterinary Medicine, and its territorial bodies – the Department of State Food and Consumer Services in the city of Kyiv, cities of regional importance, districts and specially appointed veterinarians who have the powers of the State Food and Consumer Services of Ukraine. Veterinary and sanitary control is also carried out by specialists of veterinary medicine of the economic entities under the direct supervision of official veterinarians. Veterinary measures and veterinary expertise in the procurement and slaughter of animals, trade in meat, meat products, milk, dairy products, etc., as well as supervision over the sanitary condition of trade places in the markets, are under the responsibility of veterinary specialists. Under the Soviet Union, the activities of veterinary specialists were regulated by Veterinary legislation, which included the Veterinary Statute and other legislative acts. In independent Ukraine, in 1999, the State Department of Veterinary Medicine of the Ministry of Agriculture of Ukraine issued "Legislation of Ukraine on veterinary medicine" (Dostoievskyi, 1999). This law includes the general, legal, organizational, and financial foundations of veterinary medicine. It regulates activities in the field of veterinary medicine in accordance with international requirements, recognizes the legal status of veterinary medicine structures, establishes the necessary veterinary and sanitary requirements and the basics of veterinary control.

Results of the research and their discussion

Until 1885, there was a Sanitary Commission in Kyiv, which consisted of medical doctors and there were no veterinarians. Only in 1885, the Kyiv Governor proposed to include a veterinarian into the Sanitary Commission. The first appointed veterinarian was V. K. Ponomarev (since 1886). With the introduction of this position, the City Sanitary Commission did not determine the scope of activities of the veterinarian. After the dismissal of V. K. Ponomarev, P. M. Genevskyi, who

previously worked as a senior veterinarian of slaughterhousesabattoirs, was appointed to this position. By 1888, there were 17 small private slaughterhousesabattoirs in Kyiv, scattered throughout the city and in an unsanitary condition. The slaughterhousesabattoirs were located in yards and dirty barns, where cattle were primitively slaughtered and no importance was attached to veterinary inspection. It was difficult for the veterinary service to deal with the unsanitary conditions of slaughterhousesabattoirs. These slaughterhousesabattoirs caused great dissatisfaction among the urban population and the Kyiv City Duma decided to close the existing slaughterhousesabattoirs and open city slaughterhousesabattoirs, i.e. to form a regulated institution that would adhere to highly human tasks, the responsibility for which is assigned on veterinarians. Veterinarian P. M. Genevskyi, being a member of the Sanitary Commission, noted: "I think it is unnecessary to talk here about the veterinary and sanitary significance of public abattoirs for the population: thousands of annually destroyed diseased organs and carcasses, previously sold and ignorantly eaten, eloquently prove the benefits of this institution, protecting the health of locals" (Otchet, 1894).

Thus, on October 20, 1888, the "Mandatory Resolution on City SlaughterhousesAbattoirs and Their Use" was issued. The Kyiv city slaughterhousesabattoirs were opened on December 17, 1888, located on the street of Velyka Vasylkivska, 137 (Dubrova, 1911), they were built on the model of the St. Petersburg and Odesa slaughterhousesabattoirs, but over time these slaughterhousesabattoirs did not meet the expectations of the Kyiv City Duma and the veterinary service, which was due to non-compliance with veterinary and sanitary requirements and restrictions. Stopakevich K. E. in the article "Five years (1887–1894) of the Kyiv public abattoirs in the veterinary-sanitary relation" noted that "... city abattoirs are veterinary and sanitary institutions which should serve as a guarantee of good quality and safety for the used edible meat and meat products, to be a bulwark against the spread of epizootics in the city and to be a regulator of the meat trade in the city" (Stopakevych, 1894).

According to the literature (Stopakevych, 1894; Stehney, 2008), the city slaughterhousesabattoirs of the Western European states of Berlin, Vienna, Paris, Strasbourg, Munich, and Leipzig followed the veterinary and sanitary rules. The position of director or inspector of these slaughterhousesabattoirs was held by a veterinarian who dealt with special veterinary and economic issues and was the chief veterinary consultant in the city. In Kyiv, the veterinary staff of the slaughterhousesabattoirs was subordinated to the abattoir manager (not the veterinarian) or the head of the Sanitary Commission (medical doctor). Veterinarians were not members of the Sanitary Commission or Council. Almost all meetings of various commissions were held without the participation of veterinarians. In the case of slaughterhousesabattoirs, the degree of meat quality and the age of the animal were determined not by the Council of different veterinarians, but by the supervisor of the slaughterhousesabattoir activities, who resolved veterinary issues without having a legal right to do so. Accordingly, the veterinarians of the slaughterhousesabattoirs did not perform their professional duties. The Kyiv city slaughterhousesabattoirs from the time of their opening were subordinated to the Public Administration of the city. To improve veterinary affairs in Kyiv city slaughterhousesabattoirs, veterinarians

advocated subordinating slaughterhousesabattoirs to the Ministry of Internal Affairs: "... city veterinary staff will act more independently…, and public health would be less damaged by the anomalies of sad reality" (Tomylyn, 1892).

This attitude towards veterinary specialists led to the fact that in the summer of 1890, the Kyiv city slaughterhousesabattoirs were left without a single veterinarian (Novyi Kyivskyi yntsydent, 1894).

The premises of the Kyiv city SlaughterhousesaAbattoirs were built of stone, well lit, and spacious. Since the moment of opening (1888), the slaughterhousesabattoirs were divided into four sections: chamber abattoir; pavilion abattoir, or kosher; abattoir for small animals; swine fever and central intestinal tract.

The territory of the Kyiv city Slaughterhousesabattoirs was divided into five sites (yards) where animals were placed. In the animal yards, there were almost 200,000 head of cattle to be slaughtered per year. There was a room for a veterinarian in the № 1 cattle yard. In 1902, this position was held by K. E. Stopakevich. The working day lasted for 10–16 hours, depending on the number of animals delivered for slaughter. The owner of each animal that came in for slaughter was charged a percentage fee. The veterinarian of the abattoir undertook to take into account the receipts of the state interest fee. For the use of the yard at the city abattoirs, the owners of animals were charged: 10 kopecks for a bull, a cow, and a heifer, and 5 kopecks for a calf. If the cattle stayed overnight, the fee was 5 and 3 kopecks, respectively (Uvedomlenye, 1906).

On the territory of the Kyiv city Slaughterhousesabattoirs, there was a trading platform or yard № 2, where cattle were bought and sold. The marketplace of the slaughterhousesabattoirs was not popular with the meat industry, as cattle were brought here for slaughter very exhausted. The best groups of animals were sent to other points of sale, and the Ministry of Internal Affairs decided in 1894 to provide one-time courts for the purchase of livestock through agents directly in the owners' yards in order to support the meat traders.

In the message to the Tambov City Council (dated 23/02/1899), it was noted that there are 5 cattle yards in the Kyiev City Council (Uvedomlenye, 1902).

In yard № 1, the reception of various cattle is carried out. This yard is common for different types of livestock (cattle, cattle, and pigs). Cattle sorting, veterinary inspection, and branding were carried out here. After sorting, cattle entered the yard № 2, calves and small cattle – № 3, pigs – № 4. Yard № 5 was allocated for animals suffering from infectious diseases or suspected forof the disease.

Stopakevych K. E., a veterinarian of the Kyiv Cattle Farm, informed the Kyiv Mayor (1902) that there was a stone room in the Kyiv Abattoir Infectious Disease Yard with two departments: the first one was for animals obviously suffering from infectious diseases; the second – for animals with a plague. A veterinary and police abattoir for slaughtering animals was located near these yards to confirm the diagnosis of the disease.

In 1899, 5 veterinarians worked at the Kyiv city Slaughterhousesabattoirs: S. P. Dubrova, M. D. Burlak, F. M. Benevskyi, M. I. Tarakanov, and I. A. Liubimirskyi. This number of veterinarians lasted until 1909. In 1909, 2 more positions of veterinarians were allocated, who replaced paramedics. Dubrov S. P. performed the duties of a senior veterinarian and L. N. Liapunov was in charge of the cattle yard. The remaining 5 doctors (M. D. Burlak, M. I. Tarakanov, M. D. Korotkov, O. I. Kandyba, and

L. P. Kozhin) headed the abattoirs for cattle and cattle, pig, and horse breeding (Dubrova, 1911). The senior veterinarian, in turn, was in charge of the trichinoscopic station. Almost three years after its opening, in 1891, 125,379 cattle were slaughtered at the Kyiv city abattoirs, of which 14,292 (14%) were bulls and oxen, 13,698 (10.9%) – cows, and 13,196 (10.5%) – calves under two years of age, 13281 (10.6%) – dairy calves, 46542 (37.1%) – sheep, 24315 (19.4%) – pigs, 55 (0.04%) – horses (Tomylyn, 1892). Cattle was delivered to the Kyiv city abattoirs for slaughter from Poltava (44.3%), Kyiv (43.5%), Kherson (5.5%), Chernihiv (4.5%), Katerynoslav (0.9%), Volyn (0.7%), and Podolsk (0.6%) provinces.

Veterinarians in urban areas worked in poor conditions. Over the 10 years of its existence, city slaughterhousesabattoirs became famous for their unsanitary condition from which "stench" extended. The newspaper "Kievlianin" noted: "Along the bridge going from the bridge at the entrance to the village of Demievka from the side of Kyiv and to the manor of the state purification wine warehouse is issued such a stench, which can be observed in the most unsanitary landfills. The air contaminated with miasms spreads from here over a long distance around but, above all, such air reaches the estates of the sugar factory and the state wine warehouse, which is known to employ several hundred people" (Ochah zarazy, 1898). When creatingof the Kyiv city slaughterhousesabattoirs, in addition to preventing the pollution of water, soil, and air with slaughterhouse waste, first of all, the task was to provide people with good quality meat. According to archival data (Kyevskye skotoboyny, 1892; Raport, 1899), the wastewater of the Kyiv city slaughterhousesabattoirs descended into the extremely shallow Lybid river, which was saturated with blood until it was red. The sandy bottom of the river was also saturated with blood clots. As noted by veterinarian Vyshnytskyi V. I., the blood flowing into the Lybid river was disinfected with lime but this did not reduce the spread of unpleasant odors beyond the slaughterhousesabattoirs. Containing a scar and excrement entered special pits in the intestinal tract and were taken out in barrels of a sewage convoy outside the city, where they were buried. The city spent up to 6,000 rubles per year on such a procedure.

With the openining of city slaughterhousesabattoirs (according to §1 of the Mandatory Resolutions of the Duma on Urban SlaughterhousesAbattoirs), a slaughter of cattle was carried out exclusively at city slaughterhousesabattoirs. At the same time, it was not allowed to slaughter even sheep, which was carried out for religious purposes by Muslims living in Kyiv (Korolev, 1914). To do this, a special room was set aside in the city, where sheep were slaughtered for Muslims. Despite the fact that the city slaughterhousesabattoirs were regulated institutions, according to §11 of these regulations, the owner was allowed to take away the skin, head, and offal no later than 3 hours after the slaughter of livestock. Otherwise, the remains of the slaughter were managed by the administration of slaughterhousesabattoirs. To clarify the issues of veterinary and sanitary affairs on city slaughterhousesabattoirs in 1899, a special subcommittee was allocated, the chairman of which was elected a member of the Sanitary Commission A. K. Stolpchevskyi. The subcommittee was set a task to develope instructions for veterinarians responsible for the veterinary and sanitary situation in Kyiv city SlaughterhousesAabattoirs,

which was carried out by the Sanitary Commission. It was only in 1899 that purely veterinary supervision was separated from sanitary supervision.

The meeting of the subcommittee raised the question of the need for microscopic examination of meat for trichinosis (Kyevskye skotoboyn, 1899). One of the five veterinarians slaughterhousesabattoirswas appointed to city abattoirs to run the station. With the opening of the microscopic station, the Kyiv city slaughterhousesabattoirs were functionally divided into four departments: trichinosis and microscopic station; cattle and pig abattoir; inspection and branding of imported meat; cattle yard. Independent instructions were developed for the heads of each of these departments. For the proper organization of veterinary and sanitary supervision in 1909, mandatory regulations were introduced, including rules for the arrangement and maintenance of slaughterhousesabattoirs. These resolutions introduced a uniform procedure in the abattoir business. The significance of this case is evident from the activities of the Kyiv city SlaughterhousesAabattoirs, where in 1909 16277 cattle, 40043 calves, 264 pigs, 14594 sheep, and 472 horses were slaughtered at the abattoirs in the city's cattle yard. There was a control trichinosis station at the city slaughterhousesabattoirs, the management of which was entrusted to the head of the pig farm. The head was assisted by a senior microscopist, who was appointed by the city council from among the microscopists at the station. All microscopists, including the senior, were subordinated to the head of the slaughterhousesabattoirs. When microscopists detect substandard meat, a senior slaughterhouse veterinarian has been notified for a more detailed examination and decision. A separate microscope and a tool storage box were attached to each microscope. At the same time, the microscopists undertook to keep the microscope properly clean. Butchers brought animals to the Kyiv city slaughterhousesabattoirs. According to the Vilnius City Council, the list of the Kyiv city slaughterhousesabattoirs included 160 Orthodox butchers and 133 Jewish Jews. In order to distinguish imported meat from local meat (slaughtered animals in the Kyiv city abattoirs), two varieties of stamps of different colors were used. Meat from the city was sold better and more expensive than imported meat.

Dr. Rohrbeck's apparatus was used to disinfect finnose, tuberculous, or any other meat. The finnose meat was sterilized for 1 hour, after which it was branded with a special stamp and sent for sale on the market. Carcasses from sick animals were subjected to sterilization against foot and mouth disease, actinomycosis, hemorrhagic septicemia (pasteurellosis), etc. Thus, in 1901–1902, 16 carcasses of cattle, 5 carcasses of calves, 2 carcasses of pig affected by tuberculosis, 99 – foot and mouth disease, 15 – actinomycosis were sterilized. In 1911, a Hennicke system was purchased to sterilize meat. The lard, which was used to prepare soap and ointment, was also subject to sterilization, and the condensed glue obtained from the sterilization was sold to printing houses and mechanical weaving.

With the reappointment of V. K. Ponomarev for the position of a veterinarian in Kyiv (1888), the leading place belongs to veterinary and sanitary supervision, which has been expanding every year. At the same time, considerable attention was paid to the dairy business, supervision of milk and dairy products (Protokol, 1914). At the congress of veterinarians, which took place in Kyiv (1900), there was a report of M. K. Kobylyanskyi "On the importance of veterinary and sanitary super-

vision of dairy cattle". In his report, the author emphasized the ability of milk and its products to transmit infectious diseases from cows to humans. It should be noted that the Kyiv farms were in the unsanitary state and without the supervision of the veterinarian. Kobylyanskyi M. K. proposed to expand the veterinary staff of Kyiv and introduce a mandatory regulation on the keeping of farms and dairy cattle. To combat tuberculosis, he suggested using tuberculin as a valuable diagnostic tool. The congress decided to recommend the City Administration organize a demonstration farm in the city, where all cows should be tuberculized. Such a demonstration cattle farm was later organized by the city veterinarian M. P. Slesarevskyi. Milk from his farm went to children's institutions.

It should be noted that in Kyiv, a significant percentage of tuberculous cows, whose milk was sold, was diagnosed. During the period from 1885 to 1892, 4,306 people died from tuberculosis in Kyiv, i.e. almost 539 people a year.

The significant spread of tuberculosis among the population of Kyiv forced the veterinarian V. K. Ponomarev in 1888 to conduct the first census of cattle in Kyiv. Based on this census, the draft "Mandatory Rules for Persons Keeping Cows for Industrial Purposes" was submitted to the Sanitary CommissionIn the same year, for the first time, an examination of a koumiss medical establishment was conducted. Due to lack of funds, dairy farms were inspected 1–2 times a year. The need for such supervision is evidenced by the fact that in 1892 the Sanitary Commission found the funds to pay extra for the inspection of farms for 25 rubles.

Milk in the Kyiv markets was only partially controlled. For sale, milk came extremely contaminated not only with bacteria but also with mechanical impurities. Measures to control the milk and dairy products were introduced only in 1913. Permanent and regularly functioning control of milk in Kyiv could not be carried out due to the lack of Mandatory Resolutions on the trade of milk and dairy products in Kyiv, although the need for such regulations was repeatedly discussed at the meetings of the Sanitary Executive Commission and the City Duma in 1902. Veterinarian F. M. Benevskyi even proposed to create a special dairy market in Kyiv, where it was planned to open new positions of veterinarians. Until 1914, the control of milk in the markets was carried out by sanitary doctors.

On January 17, 1914, a meeting of the City Sanitary and Executive Commission on the control of milk entering the markets of Kyiv took place. At this meeting, veterinarian V. K. Korolev raised the issue of milk control from the benches in the chemical department of the sanitary station, equipped with everything necessary for research. In this department, it was proposed to conduct an examination of milk for the percentage of fat, milk falsification, preservatives, contamination, and the presence of leukocytes. The average sample was 1 liter, of which 0.5 liters went to these studies, and 0.5 liters was sent to one of the institutions: Sanitary station for bacteriological examination; Bacteriological Institute of the Society for the Control of Infectious Diseases; District veterinary bacteriological laboratory for research on a microbial count. The cost of milk on the market ranged from 2.5 to 5 kopecks per glass, a "quarter" (2.5–3 liters), depending on the season, cost 18–35 kopecks, a bucket of milk – from 1 ruble 20 kopecks up to 1 ruble 50 kopecks.

During the veterinary and sanitary supervision of milk and dairy products, considerable attention was paid to cowsheds, dairy farms, koumiss establishments. For

the first time, in 1901, according to the resolution of the Kyiv Governor, in the village of Pushcha Vodytsya, a koumiss and kefir establishment began to function, which was under the subordination of veterinary and sanitary supervision. It should be noted that the sale of these products was allowed by the Sanitary Commission only after examination of mares by a veterinarian.

Most "farmers" in Kyiv kept cows in rented premises, for which they paid high rents. In 1913, a survey of cowsheds was carried out in Kyiv, where 29% of cowsheds were found to be heavily contaminated, 49% were of medium purity and only 21% were clean. Most of the cowsheds were old barns made of planks with slots, without filling, etc. The productivity of cows was not high. They gave from 3 to 10 liters of milk per day. In 1916, they issued "Mandatory regulations on the supervision of cowsheds, dairy farms, koumiss establishments and individual farms selling milk and dairy products in Kyiv" (The project of these resolutions was issued in 1914). At that time, cowsheds, dairy farms,and etc. were farms, in which two or more cows were kept for the purpose of selling milk and dairy products. In Kyiv, most cowsheds and farms kept 2 to 8 cows. There were 73% of such cowsheds. There were only twenty large farms with more than 10 cows. The opening of the cowsheds required the permission of the City Council, which appointed a special commission to inspect the cowshed, which included the city district veterinarian, the city sanitary doctor, and a police officer. Upon obtaining permission to open a cowshed, the entrepreneur had the right to make a sign and publication about the sale of milk and cows from this cowshed. It should be noted that persons wishing to have their own cowshed or dairy farm should report to the City Council. The cows on the dairy farms were examined by the city veterinarian, who entered the results of the inspection in the sanitary book, the form of which was established by the city administration. The book included the number of cattle and their content, cows, feed quality, vaccinations, tuberculin testing, diagnostic tests, and more. Cows in which an infectious disease was detected after a veterinary examination were not allowed to be kept in cowsheds. The owner recorded the number of cows in the cowshed in the register and periodically submitted a copy to the district veterinarian. All changes that occurred when moving animals in the cowshed were reported to the city veterinarian, who noted in his lists. Considerable attention was paid to the rules of building cowsheds. According to these rules, the building of cowsheds was not allowed in the yards of veterinary dispensaries and hospitals, factories, plants, on trading platforms, and cattle burial grounds, i.e. in places where there was a high probability of being animals with infectious diseases. When choosing places, it was allowed to build cowsheds at a distance of at least 5 yards (10.7 m) from them. The premises were built of any material, provided that it was stable. The walls of the cowsheds must be whitewashed and the rooms well ventilated. A stall of 13/4 arshins (1.12 m) and 4 arshins long was allotted for one cow (2.84 m). The light area of the premises was not less than 1:12. The wooden floor on asphalt or cement base. It should be noted that keeping any other animals in the barn, except for goats, was not allowed. Such requirements for milk and dairy products in Kyiv were approved by the city administration for the prevention of tuberculosis among humans, the causative agent of which was transmitted during the consumption of milk from tuberculous cows. Veterinary and sanitary supervision of milk and dairy products

was important and significantly complemented the veterinary and sanitary affairs of the Kyiv province in the late nineteenth–early twentieth century.

Conclusions and future perspectives

Veterinary and sanitary supervision in Kyiv began to develop only at the end of the 19th century when city abattoirs were created, that is, a regulated institution was organized, where, in addition to preventing the pollution of water, soil, and air by slaughter waste, the task was to provide people with good quality meat and slaughter products. Initially, the responsibility for the observance and performance of these tasks rested with medical doctors, and later with veterinarians. In this regard, the veterinarians of Kyiv SlaughterhousesAcity abattoirs advocated that the slaughterhousesabattoirs should be subordinated to the Ministry of Internal Affairs. The separation of purely veterinary supervision from sanitary supervision (1899) forced the City Council to allocate a special subcommittee chaired by a member of the Sanitary Commission A. K. Stolpchevsky, which developed instructions for veterinarians responsible for veterinary and sanitary supervision at the Kyiv city slaughterhousesabattoirs. In addition to the slaughterhousesabattoirs, much attention was paid to veterinary and sanitary supervision of milk and dairy products, which significantly complemented the veterinary and sanitary activities in Kyiv in the late nineteenth and early twentieth centuries, after all, until 1914, the control of milk in the markets was carried out by sanitary doctors.

The prospect of further research is an in-depth study of the formation and development of veterinary medicine in Kyiv and Ukraine as a whole.

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Стегней М. М. (2020). СТАНОВЛЕННЯ І ОРГАНІЗАЦІЯ ВЕТЕРИНАРНО-САНІТАРНОГО НАГЛЯДУ КИЄВА КІНЦЯ XIX – ПОЧАТКУ XX СТ. *Ukrainian Journal of Veterinary Sciences, 11(4): 79–88, https://doi.org/10.31548/ujvs2020.04.008*

Анотація. *Подано відомості щодо становлення ветеринарно-санітарного нагляду в м. Київ, починаючи з кінця XIX століття. До 1888 р. в Києві функціонувало 17 невеликих приватних різниць, розкиданих по всьому місту. Різниці розміщувалися при дворах і брудних коморах, де примітивно забивали худобу та не надавали значення ветеринарно-санітарному нагляду. Ці різниці викликали велике незадоволення в міського населення й Київська міська дума вирішила закрити існуючі різниці і відкрити міські різниці, тобто сформувати регламентований заклад, який дотримувався б високогуманних завдань, відповідальність за виконання яких покладалася на ветеринарних лікарів.*

Для з'ясування питань ветеринарно-санітарного нагляду на міських різницях у 1899 році виділено спеціальну підкомісію, головою якої обраний член Санітарної комісії А. К. Столпчевський. Підкомісією ставилося завдання розробити інструкцію для ветеринарних лікарів, що відповідали за ветеринарно-санітарний стан у Київських міських різницях, який проводила Санітарна комісія. Лише в 1899 року розмежовується нагляд суто ветеринарний від нагляду санітарного. Для правильної організації ветеринарно-санітарного нагляду, в 1909 р. введено обов'язкові постанови, що включали правила влаштування й утримання різниць. Цими постановами було введено одноманітний порядок у різничу справу.

У 1900 році міський ветеринарний лікар М. К. Кобилянський запропонував розширити ветеринарний персонал у м. Києві і ввести обов'язкову постанову щодо утримання ферм і молочної худоби. Уперше в 1901 р., за резолюцією Київського губернатора, у селищі Пуща Водиця почав функціонувати кумисно-кефірний заклад, що був у підпорядкуванні ветеринарно-санітарного нагляду. Проте, постанова за наглядом за корівниками, молочними фермами, кумисними закладами й окремими господарствами, що займаються продажем молока й молочних продуктів у м. Києві, вийшла лише в 1916 році.

Ключові слова: *міські різниці, ветеринарно-санітарний стан, різнича справа, контрольно-трихінельозна станція, молочна справа*