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New journal 'Journal for Veterinary Medicine, Biotechnology and Biosafety", discovered in 2015, aimed to consolidate and share the new developments and achievements in the area of biological science. This was recognized as the profile edition for veterinary medicine doctors and biologists in Ukraine. Our journal promotes the research of Ukrainian institutions, publishing their achievements in English, and sharing it among the scientific community. It includes cooperative veterinary and medical aspects, fitting to One Health Approach declared by WHO, OIE, and FAO. It was included in Index Copernicus and eLibrary scientific databases.

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Sincerely yours,
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MODULATION OF INNATE IMMUNITY OF CALVES IN THE EARLY NEONATAL PERIOD WITH PROBIOTIC NANOMETAL GLOBULIN DRUG

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Summary. The article highlights the results of studying the effect of a new probiotic nanometal globulin drug (PNMGD) on biomarkers of innate immunity of newborn calves. The experiment was performed on two groups of calves. Animals of the experimental group from the 2nd day of life were fed the drug for 5 days at a dose of 0.25 g/kg of body weight with milk, calves of the control group received milk without its addition. Before the experiment, and on the 10th, 20th, 35th day of the experiment, blood was taken from calves for clinical and biochemical studies. The obtained results show that the use of PNMGD causes an increase in the natural resistance of calves. This is indicated by an increase in the expression of such markers of innate immunity as globulins, circulating immune complexes and nitrogen metabolites by an average of 17–25%, as well as inhibition of seromucoid synthesis by 16.9%. Signs of anti-stress effect of the drug on the calves in the early postnatal period have been found. The positive effect of PNMGD on the state of innate immunity of calves can be regarded as one of the factors increasing the average daily weight gain by 32.2% in the first 36 days of life

Keywords: immune resistance, clinical and biochemical parameters, anti-stress effect

Introduction. Health protection of young farm animals and poultry at an early age is one of the most topical problems of animal husbandry in Ukraine. The economic losses from diseases and deaths of productive livestock, especially young animals, are quite significant. This is due to their low level of resistance caused by insufficient and unbalanced feeding, unsatisfactory maintenance of cows during pregnancy, which leads to impaired embryonic development, reduced content of immune globulins, immune-competent cells, vitamins, macro-and micronutrients in colostrum and milk (Van de Perre, 2003). The polyetiological nature of diseases of newborn animals necessitates the development of effective complex drugs that would have diverse stimulating and immune modulatory properties (Lytvyn V. P. et al., 2002; Chornyi et al., 2016; Cuttance and Laven, 2019). Today, there is a trend towards the use of drugs of endogenous origin, which can activate the immune system by enhancing the proliferation and function of immune competent cells, as well as stimulating innate and adaptive immunity (Lavelle and McLachlan, 2018; Noh et al., 2019).

In this regard, the development of effective complex drugs, including the use of nanoparticles of macro- and micronutrients, the study of the biological effect of these drugs on the state of natural resistance to markers of humoral factors of the immune response remains a problem (Smith, Simon and Baker, 2013; Lee et al., 2010).

Previous studies have found a positive effect of developed probiotic nanometal globulin drug (PNMGD) on the state of nonspecific resistance of chickens (Kovalenko et al., 2017). The study of its effect on the body of young cattle is also of considerable scientific and practical interest.

The aim of the study was to determine the direction and levels of the effect of PNMGD on the functional state of innate immunity and health status, indicator of which is the weight gain in calves up to 36 days of age.

Materials and methods. PNMGD, which was used in the experiment, contains serum globulins, aqueous solutions of nano-iron (Fe) aquachelates and metal salts ($CoSO_4$, $CuSO_4$, $MnCl_2$, and $ZnSO_4$), as well as a mixture of cultures of lactobacteria (*Lactobacillus plantarum* No. 7) and bifidobacteria (*Bifidobacterium adolescentis* No. 17).

The research was conducted in the conditions of cattle breeding farm on newborn calves. Thus two groups of five animals in each were formed. Calves of the 1st group from the 2nd day of life for 5 days were fed the drug at a dose of 0.25 g/kg body weight with milk (optimal dose has been selected in previous experiments on laboratory animals), calves of the 2nd (control) group were fed milk without the drug.

Each animal was weighed before and at the end of the experiment. Before the administration of the drug and on the 10th, 20th, and 35th day of the experiment, the blood

from calves was examined by clinical and biochemical methods.

The level of leukocytes was determined in the blood, the level of total protein, protein fractions (Kondrakhin et al., 1985), circulating immune complexes of average molecular weight and seromucoids (Men'shikov, 1987), nitric oxide were determined in blood plasma (Lee et al., 2009), lysozyme activity (Labinskaya, 1978) and the state of the oxidant-antioxidant system by the level of diene conjugates, malonic dialdehyde and catalase activity (Stegniy et al., 2007).

Experiments on animals were carried out in accordance with the rules of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 86/609/EEC (CEC, 1986).

The obtained results were processed by methods of variation statistics using Microsoft Excel for Windows 2007. Student's *t*-test was used to compare mean values (Van Emden, 2019).

Results and discussions. Analysis of the results obtained when feeding PNMGD to calves, shows that the drug has a positive effect on the weight gain of animals, the dynamics of which is shown in Fig. 1. According to calculations, the average daily weight gain of calves in the control group was 0.62 ± 0.08 kg, and in calves of the experimental group — 0.94 ± 0.07 kg, which is higher by 32.2% ($p \leq 0.05$). Exceeding the average body weight of calves in the experimental group on the 10th, 20th, and 35th day of the experiment reached 14.2%, 17.8% and 27.8%, respectively. The level of safety of newborn calves in both groups was 100%.

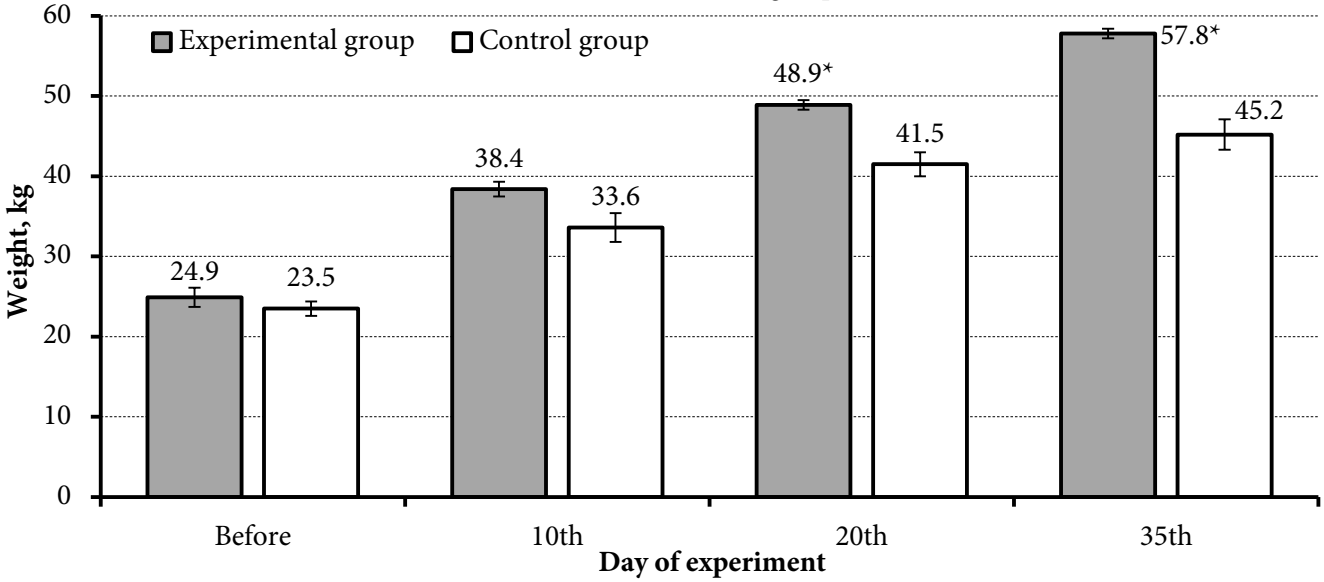

Figure 1. Dynamics of live weight of calves with the use of PNMGD (* — the difference is statistically significant relative to the indicators of the control group at $p \leq 0.05$)

During the experiment, a dynamic increase in the level of leukocytes in the blood of calves of both groups was observed (Table 1), which, according to Koryakina and Borisov (2015), is the evidence of the body's response to stressors in the early postnatal period. However, if in animals of the control group this index increased by 54.5%, in calves of the experimental group — only by 16.3%. The established differences between the indicators can be explained by the increased efficiency of adaptive reactions of the body of newborn calves under the action of the drug.

Analysis of the dynamics of the plasma protein profile presented in Table 1 shows an increase in the level of total protein in the plasma of calves of the experimental group by 17.6% on the 10th day of the experiment and by 13.8% on the 20th day. The concentration of globulins in the 2nd and 3rd experiments increased by 17.9% and 24.6%, respectively, and on the 35th day, this index remained increased by 14.4% relative to the level of the control.

It was also found that the use of PNMGD causes an increase in concentration of a number of mediators of innate immunity in the plasma. Thus, the level of circulating immune complexes on the 10th and 20th days of the experiment increased by 17.1% ($p \leq 0.05$) and 9.5%, respectively, compared with the control. A similar direction of changes was established with respect to the level of nitric oxide metabolites — their concentration in calves of the experimental group exceeded the control values by 23.9% and 14.2% ($p \leq 0.05$), respectively. Considering the biological role of the circulating immune complexes of average molecular weight and NO metabolites, it can be stated that the developed drug promotes the induction of mediators of cellular immunity and increase the body's natural resistance (Bogdan, Röllinghoff and Diefenbach, 2000; Weigert et al., 2018).

The level of seromucoids, which are considered inhibitors of the humoral part of specific immunity, in the blood plasma of calves of the experimental group on the

10th day was lower by 16.9% ($p \le 0.05$) relative to the values of the control group, in the following terms a gradual approximation of values to the control level was observed.

At the same time, it was found that PNMGD does not significantly affect the activity of lysozyme in the blood plasma of experimental calves, which, in addition to direct antimicrobial action, is one of the modulators of the body's immune response to infection (Ragland and Criss, 2017).

The most pronounced effect on the lipoperoxidation process was recorded on the 10th day of the experiment, when the level of diene conjugates was 28.1 ± 0.1 mmol/l and malonic dialdehyde — 5.1 ΔD, which is lower by 15.9% and 18.6% ($p \le 0.05$) relative to the control values, respectively. In subsequent studies, significant differences in lipid peroxidation activity were not observed. At the same time, an increase in catalase activity was found, up to the 10th day of the experiment, this indicator was reliably increased by 24.2% in animals of the experimental group relative to the control group (Fig. 2).

Considering the biological role of the lipid peroxidation system and antioxidant protection, the established signs of its slight activation also indicate an increase in innate immunity under the action of PNGMD (Weigert et al., 2018; Maldonado Galdeano et al, 2019).

Table 1 — Dynamics of markers of innate immunity of calves when using PNMGD (M ± m; n = 5)

Indexes	Before experiment		10th day of experiment		20th day of experiment		35th day of experiment	
	Experimental group	Control group	Experimental group	Control group	Experimental group	Control group	Experimental group	Control group
The number of leukocytes, $\times 10^9$/l	7.6 ± 0.2	6.6 ± 0.4	6.8 ± 0.5	7.5 ± 0.7	7.6 ± 0.4*	8.9 ± 0.7	9.1 ± 0.5	10.2 ± 0.8
Total protein, g/l	54.6 ± 2.6	53.8 ± 1.5	64.7 ± 1.7*	55.0 ± 0.9	69.3 ± 2.7	60.9 ± 2.4	63.6 ± 1.1	62.5 ± 1.7
Globulin, g/l	26.8 ± 0.8	24.6 ± 1.2	29.9 ± 1.5	25.4 ± 0.9	32.3 ± 1.6*	25.9 ± 1.5	31.6 ± 1.2*	27.6 ± 0.5
Circulating immune complexes, mg/ml	0.110 ± 0.003	0.110 ± 0.005	0.128 ± 0.006*	0.112 ± 0.006	0.110 ± 0.010	0.100 ± 0.005	0.110 ± 0.002	0.110 ± 0.005
Seromucoids, mg/ml	0.13 ± 0.002	0.12 ± 0.005	0.13 ± 0.005	0.16 ± 0.005	0.15 ± 0.002	0.14 ± 0.002	0.13 ± 0.006	0.12 ± 0.005*
Nitric oxide, μMol/l	378.5 ± 3.5	367.8 ± 4.2	342.1 ± 9.8*	260.4 ± 5.6	239.8 ± 5.5*	205.7 ± 2.1	206.3 ± 2.1	196.8 ± 1.9

Note. * — the difference is statistically significant relative to the indicators of the control group at $p \le 0.05$.

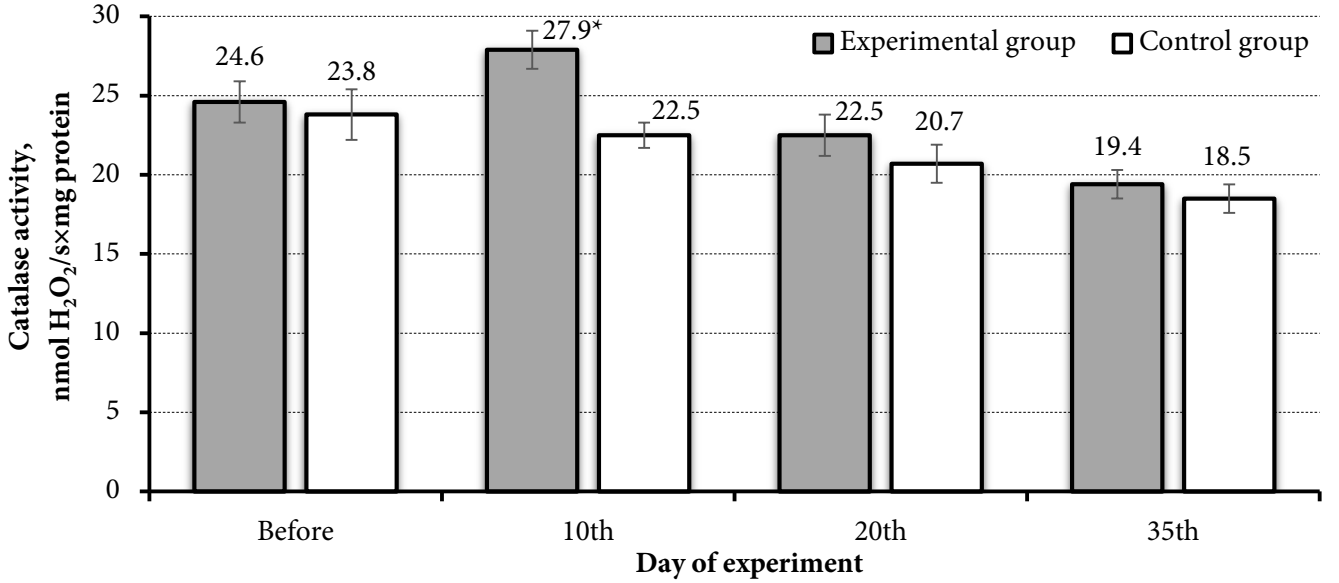

Figure 2. Dynamics of catalase activity in the blood plasma of calves when feeding PNMGD (M ± m; n = 5; * — the difference is statistically significant relative to the indicators of the control group at $p \le 0.05$)

Conclusions. 1. The use of PNMGD helps to increase the level of natural resistance of newborn calves, as evidenced by the increase in expression, on average by 17–25%, its markers such as globulins, circulating immune complexes and nitrogen metabolites, as well as inhibition of seromucoid synthesis by 16.9%.

2. Signs of anti-stress effect of the test drug on the body of calves in the early postnatal period have been established, which is manifested in a decrease in the manifestation of leukocyte adaptive-compensatory response, as well as a decrease in lipoperoxidation, one of the factors of which may be a compensatory increase in catalase activity, which on the 10th day of the experiment was 24.2%.

3. The revealed positive effect of PNMGD on the state of innate immunity of calves can be regarded as one of the factors increasing the average daily weight gain of calves by 32.2% in the first 36 days of life.

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MONITORING OF CYSTICERCOSIS OF RABBITS IN FARMS OF DIFFERENT FORMS OF OWNERSHIP

Bogach M. V. [1], Horobei O. O. [2], Ivanchenko O. M. [3], Vovk D. V. [2]

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Summary. The aim of the study was to determine the spread of pathogens of parasitic diseases in the digestive organs of rabbits in farms of various forms of ownership in Odesa Region. One thousand and two hundred rabbits of different age groups in specialized farms (which they use cage keeping of animals in compliance with all zoohygienic requirements and a balanced feeding ration), as well as 582 rabbits in private farms (which the type of feeding was mixed) were examined. Prevalence of parasitic infections of rabbits in specialized farms is 52.3%, in household farms — 85.1%. In specialized farms, prevalence of cysticercosis is 2.6% (with intensity of 3–7 cysticerci), eimeriosis — 14.0%, trichostrongylosis — 13.6%, and passalurosis — 20.6%, two-component (eimeriosis + cysticercosis) infection —1.0%, three-component (eimeriosis + cysticercosis + passalurosis) infection — 0.5%, total infestation (both mono- and mixed infections) with cysticerci — 4.1%. In homestead farms, prevalence of cysticercosis is 27.7% (with intensity of 21–64 cysticerci), eimeriosis — 15.6%, trichostrongylosis — 3.6%, and passalurosis — 6.7%, two-component (eimeriosis + cysticercosis) infection —24.2%, three-component (eimeriosis + cysticercosis + passalurosis) infection — 7.2%, total infestation (both mono- and mixed infections) with cysticerci — 59.1%. Cysticercosis is a common infection of rabbits in Odesa Region, which occurs often as part of mixed infections with pathogens eimeriosis and pasalurosis. The total infestation of rabbits with cysticerci in homestead farms was 55.0% higher than in specialized ones. One-component infestations in specialized farms is 97.1% of sick rabbits, and in homestead farms — 63.0%; two-component (eimeriosis + cysticercosis) infection — 1.9% and 28.5%, respectively; and three-component (eimeriosis + cysticercosis + passalurosis) infection — 1.0% and 8.5%, respectively

Keywords: pathogens, spread, Odesa Region, Ukraine, eimeriosis, passalurosis, trichostrongylosis

Introduction. Rabbits are prone to various diseases. Among the many pathogens, parasites play a major role in the emergence of a number of diseases in rabbits with increased morbidity and mortality, leading to economic losses. Some of the parasites are helminths, such as round worms, tapeworms and eimeria (Hajipour and Zavarshani, 2020; Bogach and Franchuk, 2018; Szkucik et al., 2014).

A significant number of reports, based on studies by both domestic and foreign authors, indicate that in the body of an animal of this species, several species of parasites can be localized simultaneously, which form a parasitocenosis (Yatusevich et al., 1990; Bogach and Trofimov, 2007; Strohlein and Christensen, 1983).

Along with protozoa, helminths and mites, various types of bacteria, viruses and fungi can be synchronously included in its composition (Youn, 2009).

The spread of the infestation depends on the age of the animals, the housing system, as well as the preventive and therapeutic measures used (Drouet-Viard and Fortun-Lamothe, 2010; Kosenko et al., 2004; Jeklova et al., 2007; Pakandl et al., 2008).

Scientists have found that 41.6% of rabbits and 21.7% of hares are affected by pisiform cysticercosis. The intensity in rabbits ranges from 3 to 121, in hares — from 7 to 48, and even up to 600 bubbles (Dubina, 2002; Duda et al., 2018; Melillo, 2007). In terms of industrial production, cysticercosis was registered in 4.27% of rabbits (Sołtysiak, Bednarski and Piekarska, 2007).

The study of parasitic fauna of rabbits in homestead and specialized farms is of current scientific and practical importance, as it allows for timely diagnosis and development of effective schemes for the treatment and prevention of mixed infections in rabbits.

The aim of the study was to determine the spread of pathogens of parasitic diseases in the digestive organs of rabbits in farms of various forms of ownership in Odesa Region.

Materials and methods. The material for the study were rabbits of different age groups, which belonged to specialized farms of LLC 'BBPROM' (Shemetove, Berezivka District), SG LLC 'Southern' (Ruskoivanivka, Bilhorod-Dnistrovskyi District), and PSP 'Druzhba' (Izmail District) in Odesa Region in which they use cage keeping of animals in compliance with all zoohygienic requirements and a balanced feeding ration (the main feeding ration was granulated feed), as well as private farms in Odesa, Berezivka, and Rozdilna districts of Odesa Region in which the type of feeding was mixed (hay, grain, and roots were additionally added to the granulated feed).

The diagnosis was established taking into account epizootological data, clinical signs, laboratory tests and data of autopsy, which were performed in the Laboratory of Parasitology of the Odesa Research Station of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' and in slaughterhouses of the enterprises.

To diagnose rabbit eimeriosis, the selected material (feces) was examined by Darling and Fülleborn method according to GOST 25383-82 (Gosstandart, 1982). The number of oocysts was counted under a small magnification microscope (10×10) in 20 fields of view, followed by calculation of the average. In order to determine the level of infestation with *Passalurus ambiguus* and *Trichostrongylus instabilis*, the feces of rabbits were examined by McMaster method for the presence and number of eggs of the pathogen. The level of spontaneous cysticercosis in rabbits was determined visually after slaughter and at autopsy by the number of bubbles on the internal organs.

The prevalence was determined by statistical processing. The intensity was determined by counting the number of helminth eggs and cysticerci in the implementation of incomplete helminthological autopsies of intestines of slaughtered rabbits according to Scrjabin (1928).

Results and discussions. In specialized farms, 1,200 rabbits were examined, of which 627 (52.3%) animals were infested with parasitic pathogens. In household farms, 495 (85.1%) from 582 examined rabbits were infected with parasites (Table 1).

According to the data of postmortem examination in specialized farms, cysticercosis of rabbits was registered in 31 (2.6%) animals with intensity of 3–7 cysticerci. Eimeriosis was registered in 168 (14.0%) animals, trichostrongylosis — in 163 (13.6%), and passalurosis — 247 (20.6%). Two-component (eimeriosis + cysticercosis) infection was present in 1.0% of rabbits, three-component (eimeriosis + cysticercosis + passalurosis) infection — in 0.5%. The total infestation (both mono- and mixed infections) of rabbits with cysticerci in specialized farms was 4.1% (Table 1).

In homestead farms, cysticercosis was registered in 161 (27.7%) animals with intensity of 21–64 cysticerci. Eimeriosis was registered in 91 (15.6%) animals, trichostrongylosis — in 21 (3.6%), and passalurosis — 39 (6.7%). Two-component (eimeriosis + cysticercosis) infection was present in 24.2% of rabbits, three-component (eimeriosis + cysticercosis + passalurosis) infection — in 7.2%. The total infestation (both mono- and mixed infections) of rabbits with cysticerci in homestead farms was 59.1% (Table 1).

Table 1 — Measures of parasitic diseases in digestive organs of rabbits in farms of various forms of ownership in the Odesa Region (according to the results of autopsies)

Farm type / feeding type / total number of examined rabbits	Disease / Pathogen	Disease cases	Prevalence, %	Percentage of cases, %	Intensity, specimens
Specialized / granulated feed / 1,200	Eimeriosis / *Eimeria* spp.	168	14.0	26.8	1,060.0 ± 112.5*
	Passalurosis / *Passalurus ambiguus*	247	20.6	39.4	22–36**
	Trichostrongylosis / *Trichostrongylus instabilis*	163	13.6	26.0	11–13**
	Cysticercosis / *Cysticercus pisiformis*	31	2.6	4.9	3–7***
	Eimeriosis + Cysticercosis	12	1.0	1.9	—
	Eimeriosis + Cysticercosis + Passalurosis	6	0.5	1.0	—
	Total	627	52.3	100	—
Household / mixed feed / 582	Eimeriosis / *Eimeria* spp.	91	15.6	18.4	1,210.0 ± 105.2*
	Passalurosis / *Passalurus ambiguus*	39	6.7	7.9	35–111**
	Trichostrongylosis / *Trichostrongylus instabilis*	21	3.6	4.2	21–35**
	Cysticercosis / *Cysticercus pisiformis*	161	27.7	32.5	21–64***
	Eimeriosis + Cysticercosis	141	24.2	28.5	—
	Eimeriosis + Cysticercosis + Passalurosis	42	7.2	8.5	—
	Total	495	85.1	100	—

Notes: * — number of oocysts in 1 g of feces; ** — number of helminths in the intestinal cavity; *** — number of cysticerci on the mesentery and omentum.

The total infestation of rabbits with cysticerci in homestead farms was 55.0% higher than in specialized ones.

One-component infestations in specialized farms were registered in 97.1% of sick rabbits, and in homestead farms — in 63.0%; two-component (eimeriosis +

cysticercosis) infection was present in 1.9% and 28.5%, respectively; and three-component (eimeriosis + cysticercosis + passalurosis) infection — in 1.0% and 8.5%, respectively (Figs. 1–2).

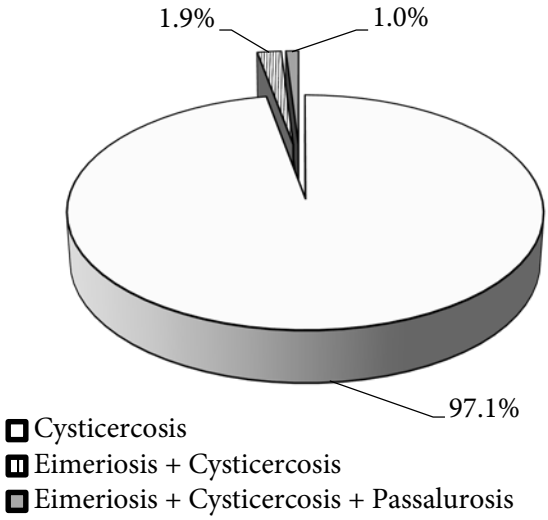

Figure 1. Mono- and mixed infections of rabbits in specialized farms

Thus, cysticercosis is a common infection of rabbits in Odesa Region, which occurs often as part of mixed infections with pathogens eimeriosis and pasalurosis.

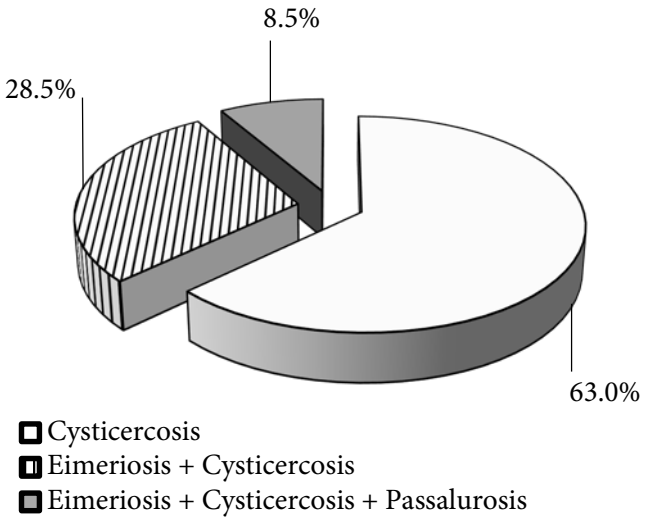

Figure 2. Mono- and mixed infections of rabbits in homestead farms

Conclusions. 1. Prevalence of parasitic infections of rabbits in specialized farms in Odesa Region is 52.3%, in household farms — 85.1%.

2. In specialized farms, prevalence of cysticercosis is 2.6% (with intensity of 3–7 cysticerci), eimeriosis — 14.0%, trichostrongylosis — 13.6%, and passalurosis — 20.6%, two-component (eimeriosis + cysticercosis) infection —1.0%, three-component (eimeriosis + cysticercosis + passalurosis) infection — 0.5%, total infestation (both mono- and mixed infections) with cysticerci — 4.1%.

3. In homestead farms, prevalence of cysticercosis is 27.7% (with intensity of 21–64 cysticerci), eimeriosis — 15.6%, trichostrongylosis — 3.6%, and passalurosis — 6.7%, two-component (eimeriosis + cysticercosis) infection —24.2%, three-component (eimeriosis + cysticercosis + passalurosis) infection — 7.2%, total infestation (both mono- and mixed infections) with cysticerci — 59.1%.

4. Cysticercosis is a common infection of rabbits in Odesa Region, which occurs often as part of mixed infections with pathogens eimeriosis and pasalurosis. The total infestation of rabbits with cysticerci in homestead farms was 55.0% higher than in specialized ones. One-component infestations in specialized farms is 97.1% of sick rabbits, and in homestead farms — 63.0%; two-component (eimeriosis + cysticercosis) infection — 1.9% and 28.5%, respectively; and three-component (eimeriosis + cysticercosis + passalurosis) infection — 1.0% and 8.5%, respectively.

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METHOD OF BIOCHEMICAL CHANGE CORRECTIONS IN THE BOAR ORGANISMS WITH TOXICANT-INDUCED REPRODUCTIVE DYSFUNCTIONS

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Summary. The pathogenetic mechanism of reproductive diseases is oxidative stress, which is manifested by an increase in lipid peroxidation and a decrease in antioxidant potential. The aim of the study was to develop a method for the correction of biochemical changes in the body of boars with toxicant-induced reproductive dysfunctions using drugs based on nanobiomaterials, based on reducing lipoperoxidation, neutralization of toxic substances by antioxidant protection increasing of animals and endocrine activity stimulating of their gonads. The article presents the results of research on the effectiveness of the complex drug 'Karafand+OV,Zn', which contains carotenoids, phytoandrogens and nanomaterials — nanoparticles of gadolinium orthovanadate, activated by europium, and zinc carbonate. Experimental toxicant-induced reproductive dysfunctions were caused by feeding sodium nitrate at a dose of 0.3 g NO_3^-/kg body weight. The drug was administered in a dose of 15 ml per male, orally, once a day for 14 days. Blood samples for test were taken before and on the 20th day after drug administration. Conventional biochemical methods were used, as well as chemiluminometry and enzyme-linked immunosorbent assay. There was a positive effect of the developed drug on the hormonal state (testosterone concentration increased by 91.8% (20.6 ± 0.32 nmol/l, $p < 0.001$), the content of vitamin A increased 1.3 times (0.65 ± 0.02 μmol/l, $p < 0.001$) and zinc by 47.6% (24.8 ± 0.86 μmol/l, $p < 0.001$), the dynamics of lipoperoxidation processes (the concentration of malonic dialdehyde in the serum was reduced by 53.2% (0.394 ± 0.01 μmol/l, $p < 0.001$)) and the system of antioxidant protection of boars (increased activity of catalase and superoxide dismutase in serum by 71.5% (41.4 ± 1.03 μmol/H_2O_2/l-min, $p < 0.001$) and 54.8% (8.98 ± 0.09 st. un./mgHb, $p < 0.001$), respectively), increased the content of reduced glutathione by 23.2%, indicators of the oxygen metabolism system (concentration 2,3-diphosphoglycerate increased 1.3 times (1.4 ± 0.03 mmol/l, $p < 0.001$), the activation of which reduces the hypoxic state. The total antioxidant activity of boars increased, as evidenced by a decrease in the light sum of chemiluminescence of serum by 47.6% (4.4 ± 0.15 un., $p < 0.001$). The results of research convincingly testify to the high efficiency of the use of the complex drug 'Karafand+OV,Zn' as a means of correction of toxicant-induced reproductive dysfunctions in boars and proves the possibility of its use in practical veterinary andrology

Keywords: complex drug, Karafand+OV,Zn, nanobiomaterials, lipoperoxidation, antioxidant protection, oxygen metabolism

Introduction. The negative impact of environmental factors on the body of males causes the development of a complex of pathological processes — reproductive diseases, characterized by biochemical, hormonal and structural changes and lead to dysfunction of the testes, in particular hypofertility (Koshevoi et al., 2015).

The pathogenetic mechanism of reproductive disorders is oxidative stress (Agarwal et al., 2014). Oxidative stress is manifested by an increase in lipid peroxidation and a decrease in antioxidant potential (Danchuk, Karpovskyi and Danchuk, 2016; Khariv et al., 2016). It is known that the high content of thiobarbituric acid reactants leads to a deterioration in the quality of sperm and its fertilizing ability (Chornozub, 2013).

One of the leading causes of such conditions is the action of toxic factors on the body of animals through food, for example, the receipt of toxic doses of nitrates from water and feed (De Celis, Pedrón-Nuevo and Feria-Velasco, 1996; Hunchak et al., 2010). In an experimental model of chronic nitrate-nitrite toxicosis, we showed its effect on the dynamics of the prooxidant-antioxidant system, oxygen metabolism and sperm quality (Koshevoi et al., 2016; Naumenko, 2020).

The development of modern means of reproductive disorder corrections is based on the interdependent action on the processes of lipoperoxidation, neutralization of toxic substances by increasing the antioxidant protection of animals and stimulating the endocrine activity of their gonads (Koshevoi et al., 2015, 2016).

The aim of the study was to develop a method for the correction of biochemical changes in the body of boars with toxicant-induced reproductive dysfunctions using drugs based on nanobiomaterials.

Materials and methods. The research was conducted on boars belonging to the FP 'Vlada' (Yurivka, Pavlohrad District, Dnipropetrovsk Region). The method of correction of toxicant-induced reproductive dysfunctions includes the use of a complex drug 'Karafand+OV,Zn', synthesized in the laboratories of the Department of Veterinary Reproductology of the Kharkiv State Zooveterinary Academy and the Nanostructured Materials Department of Institute for Scintillation Materials of the National Academy of Sciences of Ukraine under the agreement on scientific and practical cooperation. The developed preparation in 1.0 cm^3 contains carotenoids (10.0 ± 0.75 mg), biologically active

substances from the rhizome of marsh calamus (1.0 ± 0.05 mg) and nanomaterials — nanoparticles of gadolinium orthovanadate activated by europium (0.00015 ± 0.00001 mg) and zinc carbonate (2.0 ± 0.1 mg), the basis of the pharmaceutical composition was refined oil.

The group of animals for production testing consisted of clinically healthy males (n = 5), live weight 291.0 ± 5.3 kg aged 4–6 years, kept on a standard diet and had free access to water. Experimental toxicant-induced reproductive dysfunctions were induced by feeding sodium nitrate at a dose of 0.3 g NO_3^-/kg body weight of the male. The drug was administered in a dose of 15 ml per male, orally, once a day for 14 days. Blood samples for test were taken before and on the 20th day after drug administration.

The effectiveness of the developed drug was determined by changes in the content of vitamin A, zinc, the dynamics of the prooxidant-antioxidant system, oxygen metabolism and hormonal state. In the laboratory of the Department of Veterinary Reproductology, the content of vitamin A was determined by the Bessey method in the modification by Levchenko et al. (Vlizlo, 2012). Indexes of oxygen metabolism were determined: the number of erythrocytes by photocolorimetric registration of the studied samples optical density on KFK-3 at a wavelength of 670 nm, the hemoglobin concentration was investigated by hemoglobin cyanide method followed by photocolorimetry at an optical path of 540 nm, the content of 2,3-diphosphoglycerate in the erythrocyte suspension was determined spectrophotometrically (Dyce method modified by Apukhovska). The content of zinc in the serum was determined by atomic adsorption spectrophotometry at the Department of Animal Internal Medicine of the Kharkiv State Zooveterinary Academy.

In boars the following parameters were spectrophotometrically determined: the dynamics of the prooxidant-antioxidant system was determined by the content of the final product of lipoperoxidation — malonic dialdehyde by reaction with thiobarbituric acid (Fedorova, Korshunova and Larsky, 1983), and the activity of superoxide dismutase by the degree of inhibition of the reaction by the enzyme to reduce nitroblue tetrazolium in the presence of NADH and phenazine methosulfate (Dubinina et al., 1990), catalase activity on the ability of hydrogen peroxide to form a stable complex with ammonium molybdate, the color intensity of which was measured at λ = 410 nm (Korolyuk et al., 1988), the amount of reduced glutathione was determined by the Butler method using Ellman's reagent (Vlizlo, 2012) at the Central Research Laboratory of the National University of Pharmacy.

The general antioxidant activity of boars was studied by chemiluminescent analysis in the laboratory of Institute for Scintillation Materials of the National Academy of Sciences of Ukraine.

The concentration of testosterone in the serum was determined in the State Institution 'V. Danilevsky Institute for Endocrine Pathology Problems of the National Academy of Medical Sciences of Ukraine' using the method of enzyme-linked immunosorbent assay. Statistical processing of the results was conducted by Student's *t*-test (Rebrova, 2002).

Results. In the correction of toxicant-induced reproductive dysfunctions in boars, we found high efficiency of the developed drug. In particular, there was a positive effect on the hormonal state, the content of vitamin A and zinc (Table 1).

Table 1 — The effect of the complex drug 'Karafand+OV,Zn' on the content of vitamin A, zinc and testosterone concentration in boars (M ± m)

Indexes	Before administration	After administration
Vitamin A, μmol/l	0.28 ± 0.012	0.65 ± 0.02*
Zinc, μmol/l	16.8 ± 0.374	24.8 ± 0.86*
Testosterone concentration, nmol/l	10.74 ± 0.214	20.6 ± 0.32*

Note. * — $p < 0.001$ compared to pre-introduction.

The content of vitamin A in boars increased almost 1.3 times (0.65 ± 0.02 μmol/l, $p < 0.001$), while the amount of zinc in the serum increased by 47.6% (24.8 ± 0, 86 μmol/l, $p < 0.001$). Normalization of the hormonal state was noted — the concentration of testosterone was higher by 91.8% (20.6 ± 0.32 nmol/l, $p < 0.001$) compared to the indicators before administration.

The effect of the drug on the dynamics of lipoperoxidation processes and the system of antioxidant protection of boars was effective (Table 2).

Table 2 — Dynamics of the prooxidant-antioxidant system of boars under the action of a complex drug 'Karafand+OV,Zn' (M ± m)

Indexes	Before administration	After administration
Erythrocyte content:		
Malon dialdehyde, μmol/l	46.4 ± 1.21	37.6 ± 0.81**
Catalase, μmol/H_2O_2/l-min	14.6 ± 0.68	26.5 ± 0.52**
Reduced glutathione, μmol/l	3.02 ± 0.16	3.72 ± 0.15*
Serum content:		
Malon dialdehyde, μmol/l	0.842 ± 0.02	0.394 ± 0.01**
Catalase, μmol/H_2O_2/l-min	24.14 ± 1.02	41.4 ± 1.03**
Superoxide dismutase, st. un./mgHb	5.8 ± 0.14	8.98 ± 0.09**
Light-sum of chemiluminescence, un.	8.4 ± 0.14	4.4 ± 0.15**

Notes: * — $p < 0.05$, ** — $p < 0.001$ compared to pre-introduction.

It was noted that the significant effect of the drug 'Karafand+OV,Zn' — was effectively reduced the amount of malonic dialdehyde in serum by 53.2% (0.394 ± 0.01 μmol/l, $p < 0.001$) and erythrocytes by 19% (37.6 ± 0.81 μmol/l, $p < 0.001$). Under the action of the drug revealed a significant increase in catalase activity in serum by 71.5% (41.4 ± 1.03 μmol/H_2O_2/l-min, $p < 0.001$) and in erythrocytes by 81.9% (26.5 ± 0.52 μmol/H_2O_2/l-min, $p < 0.001$). There was an increase in superoxide dismutase activity by 54.8% (8.98 ± 0.09 st. un./mgHb, $p < 0.001$) compared with pre-administration. This is marked in the body of boars by a decrease in the activity of catalase and superoxide dismutase activity (Shostya et al., 2020). The content of reduced glutathione in erythrocytes was probably higher by 23.2% (3.72 ± 0.15 μmol/l, $p < 0.05$) of the group of animals before drug administration. Similar results of the research of a decrease in the activity of antioxidant enzymes in boars in the testes and epididymis and increasing their values after correction (Tang et al., 2019). The total antioxidant activity of boars increased, as evidenced by a decrease in the light sum of chemiluminescence of serum by 47.6% (4.4 ± 0.15 un., $p < 0.001$).

There was also a positive effect of this drug on the indicators of the oxygen metabolism system, the activation of which reduces the hypoxic state observed in the development of toxicosis (Table 3).

An increase in the number of erythrocytes by 44.9% (7.42 ± 0.14×10^{12}/l, $p < 0.001$), hemoglobin content by 25.65% (106.8 ± 1.93 g/l, $p < 0.001$) and probable increase in the concentration of 2,3-diphosphoglycerate in erythrocytes by 1.3 times (1.4 ± 0.03 mmol/l, $p < 0.001$).

Table 3 — The state of the system of oxygen metabolism of boars under the action of the complex drug 'Karafand+OV,Zn' (M ± m)

Indexes	Before administration	After administration
Erythrocytes, ×10^{12}/l	5.12 ± 0.09*	7.42 ± 0.14*
Hemoglobin concentration, g/l	85 ± 1.84	106.8 ± 1.93*
2,3-diphosphoglycerate concentration, mmol/l	0.6 ± 0.04	1.4 ± 0.03*

Note. * — $p < 0.001$ compared to pre-introduction.

Our results coincide with the use of antioxidant enzymes in the liver of wild boars against the background of selenium deficiency occurred during the winter (Jankowiak et al., 2015).

Conclusions. The results of research convincingly show the high efficiency of the complex drug 'Karafand+OV,Zn' as a means of correction of toxicant-induced reproductive dysfunctions in boars, in particular, its positive effect on the prooxidant-antioxidant system (reduction of malonic dialdehyde by 53.2% ($p < 0.001$) and increase in the activity of catalase by 81.9% ($p < 0.001$) and superoxide dismutase by 54.8% ($p < 0.001$), increase in the content of reduced glutathione by 23.2% ($p < 0.05$), oxygen metabolism (increase in concentration of 2,3-diphosphoglycerate by 1.3 times ($p < 0.001$)), homeostasis (increase in vitamin A and zinc by 1.3 times and 47.6%, respectively) and hormonal state (increase in testosterone concentration by 91.8% ($p < 0.001$)).

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Part 2. Biosafety

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COMPARATIVE ASSESSMENT OF THE EFFECT OF DISINFECTANTS ON THE LEVEL OF BIOTIC CONTAMINATION AND HATCHABILITY OF CHICKEN EGGS

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Summary. The widespread presence of modern high-productive egg crosses of chickens in poultry farms causes some negative consequences, one of which is a decrease in hatchability and survival of young chickens. The worsening of the quality of hatching eggs is associated with a violation of the morphological and biochemical parameters of the shell and shell membranes, which leads to the egg breakage, increased hatchery waste, contamination of young birds with infectious agents, and reduced immune resistance. Modern poultry farming uses a fairly large arsenal of disinfectants of various chemical origins and mechanisms of action. The introduction into practice of disinfectants is not possible without prior laboratory evaluation of their effectiveness as to the object of intended use. The aim of our work was to determine the bactericidal properties of disinfectants with different active substances in relation to the microbiota of hatching eggs from chickens of different productivity directions. The research was conducted following the guidelines 'Methods for determining and evaluating the safety and quality of disinfectants, detergents and detergent-sanitizers used in the production, storage, transportation and sale of animal products' (Kotsiumbas et al., 2010). According to the results of the research, it was found that for the purpose of pre-incubation treatment and during the incubation period for chickens it is effective to use the drug 'Polydez' in 0.1% concentration and the drug 'Virosan' in a concentration of 0.1%. These disinfectants can be used for sanitation of chicken hatching eggs and hatcheries

Keywords: incubation, microorganisms, Polydez, Virosan, Sterylii AB, concentration

Introduction. Incubation of eggs is a complex process that requires special knowledge and equipment. The incubation conditions determine the process of embryonic development of birds. Even highly qualified specialists are sometimes unable to control this process, despite the fact that it is possible to control the development of the embryo and its membranes, to monitor the timeliness of changes in their size and position, and to predict qualitative and quantitative results of incubation long before its completion, and, if necessary, adjust the incubation conditions (Van de Ven et al., 2011; Damaziak et al., 2018).

Achievements of world science and advanced production can significantly increase the hatchability of eggs and the quality of day-old birds (Almeida et al., 2008). To obtain positive results it is necessary to meet a number of conditions, which include the presence of modern incubators, main and auxiliary facilities, biologically complete eggs, qualified personnel, strict adherence to the sequence of the technological process (Boleli et al., 2016; Palii et al., 2020; Nasri et al., 2020).

Today, it is important to develop measures to prevent the deterioration of the quality of hatching eggs and, as a consequence, reduce their hatchability, which is inherent in modern poultry egg crosses (Kutsira, Nwulu and Dogo, 2019). Deterioration of quality indicators is associated primarily with a violation of the parameters of the protective bioceramic structures of eggs, which include shells and shell membranes (Svobodová and Tůmová, 2015; Hincke et al., 2019). Violation of the structural formations of the hatching eggs leads to a noticeable egg breakage, contamination by pathogenic microflora of young birds and a decrease in their immune resistance (Cook et al., 2005a). A necessary condition for the production of high quality and safe poultry products is effective sanitation of eggs (Sander and Wilson, 1999).

Microbial contamination of incubating and hatching cabinets depends on the degree of microbial contamination of eggs entering the incubator (Cook et al., 2005b). Increased content of pathogenic microflora on the surface of the shell, in the air pool of the incubator, on the surface of its equipment and ventilation ducts leads to a

decrease in hatchability of eggs (Furuta and Maruyama, 1981; Grizard et al., 2014).

There is also a mass infection of embryos, and subsequently a significant lag in the growth and development of hatched young, reducing its resistance and safety during growing (Wang, Firestone and Beissinger, 2011). The level of contamination of the egg surface depends on the degree of microbial contamination of containers, equipment, indoor air (poultry house, egg storage, hatchery, etc.) (Grizard et al., 2014).

Pathogenic microorganisms spread among susceptible poultry in case of non-compliance with veterinary and sanitary norms and rules (Zavgorodniy et al., 2013; Paliy and Paliy, 2019), which in turn requires a scientifically sound application of highly effective disinfectants with a wide range of biocidal action (Paliy et al., 2015; Paliy, 2018; Stegniy et al., 2019; Kovalenko et al., 2020).

In connection with the above, the task of veterinary science includes the development and production testing of new detergents and disinfectants (Paliy et al., 2016; Bondarchuk, Paliy and Blazheyevskiy, 2019), as well as egg processing technologies from the moment of laying eggs to the hatching of young poultry (Samiullah et al., 2013). It is necessary to keep in mind not only the filling of the shortage of drugs, but also to take into account the increasing requirements for labor safety and the environment protection from pollution (Buhr et al., 2015; Jiang et al., 2018; Paliy et al., 2020d).

Currently, the main method of disinfection is chemical. It is based on the application of disinfectants from different chemical groups, which must meet a number of modern requirements (Paliy et al., 2020c). Treatment of hatching eggs by chemical methods involves the use of substances having bactericidal, bacteriostatic and fungicidal activity (Kalidari et al., 2009; Olsen et al., 2017). Measures to control and prevent diseases of poultry of various etiologies should be based on a comprehensive approach to the technological process of decontamination of both environmental objects and eggs from the moment of their laying to hatching (Kusstatscher et al., 2017; Paliy et al., 2018a).

Existing egg disinfectants used before placing eggs in the incubator and during the incubation period need to be revised to take into account new approaches to assessing their effectiveness.

The aim of the study was to determine the bactericidal properties of disinfectants with different active substances in relation to the microbiota of hatching eggs from chickens of different productivity directions.

Materials and methods. For research we have chosen disinfectants with various active substances:

'Polydez' — a disinfectant that contains hydrogen peroxide (12.5 ± 2.5%) and benzalkonium chloride (QACs) (15 ± 1%) as the active substance. In addition, the composition includes cocamidopropyl betaine, neonol and other components.

'Sterylii AB' — a disinfectant containing: derivatives of salts of guanidine and alkylamine; sodium bicarbonate; excipients; drinking water — up to 100%.

'Virosan' — a disinfectant containing benzalkonium chloride (QACs) — 25.0%, glutar aldehyde — 11.0%, excipients.

Study of bactericidal properties of disinfectants was carried out following the guidelines 'Methods for determining and evaluating safety and quality of disinfectants, detergents-disinfectants used in the production, storage, transportation and sale of animal products' (Kotsiumbas et al., 2010) and other current methods (Kovalenko et al., 2014).

Eggs from layers and broilers were used for incubation.

Results and discussions. At the first stage of research we determined the possibility of incubation of eggs from chickens of different directions of productivity in case of treatment with various disinfectants. The results of bacteriological studies of washes showed that in the case of incubation of eggs of different groups in one cabinet, regardless of the drug used, the level of microbiological contamination of the egg shell surface during incubation of eggs is almost equally stable (Table 1). The results presented in Table 1 indicate that in one incubating cabinet the level of microbiological contamination of the egg surface does not depend on the use of disinfectants with different active substances, as the microbial background is aligned with the circulating air.

In the next experiment, mycological studies showed that on the 7th day of incubation, the number of micromycete spores on the surface of the egg shell in the group in which the drug 'Polydez' was used, was 0.200×10^4, and in the group where the drug 'Virosan' was used — the growth of microscopic fungi was not found (Table 2). On the 12th day of incubation, almost the same level of contamination of eggs with micromycetes was observed: $(0.875 \pm 0.012) \times 10^4$ — 'Polydez' and $(0.075 \pm 0.001) \times 10^4$ — 'Virosan'. Additional treatment of eggs on the 14th day of incubation significantly reduced the mycological load — $(0.075 \pm 0.002) \times 10^4$ ('Polydez') and $(0.050 \pm 0.006) \times 10^4$ ('Virosan') spores on the surface of the shell of 18-day-old embryos. In the groups without additional treatment with disinfectants, the number of microscopic fungi was almost twice higher — $(0.125 \pm 0.003) \times 10^4$ ('Polydez') and $(0.150 \pm 0.002) \times 10^4$ ('Virosan') spores on the egg surface.

A comparative analysis of the effect of disinfectants 'Polydez' and 'Virosan' on the bacterial contamination of the surface of the egg shell is presented in Table 3. The level of contamination of the egg shell surface with microorganisms before incubation was > 10^4 (*Escherichia coli, Corynebacterium* spp., *Staphylococcus* spp., *Streptococcus* spp.). After disinfection with drugs and placing eggs for incubation, the total bacterial contamination significantly decreased (7th day of incubation — 1.0 ± 0.1 CFU by 'Polydez' and

2.0 ± 0.1 CFU by'Virosan'). On the 12th day of incubation, the total bacterial contamination had already increased several times and amounted to 10.0 ± 0.5 CFU by'Polydez' and 30.0 ± 0.4 CFU by 'Virosan'. Additional treatment of eggs on the 14th day of incubation significantly reduced the microbiological load in both groups (10 times by 'Polydez' and 30 times by 'Virosan'). The surface of the egg shell without additional treatment on the 14th day of incubation remained at the level of 12-day-old embryos (10.0 ± 0.2 CFU by 'Polydez' and 30.0 ± 0.3 CFU by 'Virosan').

The results of tests of the effect of disinfectants 'Polydez' and 'Virosan' on the hatchability of eggs are given in Table 4.

Table 1 — The results of bacteriological studies of washes from the surface of the shell of eggs that were incubated in one cabinet

Day of incubation	Eggs from layers (n = 120)	Broiler eggs (n = 100)
	'Polydez', 0.1%	**'Sterylii AB', 1.0%**
Before incubation	> 10^4 *Escherichia coli*, *Corynebacterium* spp., *Staphylococcus* spp., *Streptococcus* spp.	
7th day	> 10^4 *Escherichia coli*, *Staphylococcus* spp.	> 10^4 *Escherichia coli*, *Corynebacterium* spp., *Staphylococcus* spp., fungal microflora
11th day	> 10^4 *Staphylococcus* spp., fungal microflora	
17th day	> 10^4 *Escherichia coli,* fungal microflora	

Table 2 —The results of mycological studies of washes from the surface of the shell of eggs treated with disinfectants

Drug	Day of incubation	The level of contamination by micromycetes	
'Polydez', 0.1 %	7th day	(0.200 ± 0.005) × 10^4 spores on the egg surface ($S_{average}$ = 65.0 ± 2.0 sm^2)	
	12th day	(0.875 ± 0.012) × 10^4 * spores on the egg surface ($S_{average}$ = 62.0 ± 2.0 sm^2)	
	14th day	Additional treatment of eggs	Without additional treatment
	18th day	(0.075 ± 0.002) × 10^4 spores on the egg surface ($S_{average}$ = 64.0 ± 2.0 sm^2)	(0.125 ± 0.003) × 10^4 spores on the egg surface ($S_{average}$ = 60.0 ± 2.0 sm^2)
'Virosan', 0.1 %	7th day	growth of microscopic fungi has not been established ($S_{average}$ = 65.0 ± 2.0 sm^2)	
	12th day	(0.075 ± 0.001) × 10^4 * spores on the egg surface ($S_{average}$ = 68.0 ± 2.0 sm^2)	
	14th day	Additional treatment of eggs	Without additional treatment
	18th day	(0.050 ± 0.006) × 10^4 spores on the egg surface ($S_{average}$ = 66.0 ± 2.0 $см^2$)	(0.150 ± 0.002) × 10^4 spores on the egg surface ($S_{average}$ = 70.0 ± 2.0 sm^2)

Note. * — $p < 0.05$ in relation to the 7th day of incubation.

Table 3 — The effect of disinfectants 'Polydez' and 'Virosan' on bacterial contamination of the egg shell surface (n = 3)

Day of incubation	'Polydez', 0.1 %		'Virosan', 0.1 %	
Before incubation	> 10^4 *Escherichia coli*, *Corynebacterium* spp., *Staphylococcus* spp., *Streptococcus* spp.			
7th day	1.0 ± 0.1 CFU		2.0 ± 0.1 CFU	
12th day	10.0 ± 0.5 CFU *		30,0 ± 0,4 CFU * *Acinetobacter* spp.	
14th day	Additional treatment of eggs	Without additional treatment	Additional treatment of eggs	Without additional treatment
18th day	1.0 ± 0.2 CFU	10.0 ± 0.2 CFU	1.0 ± 0.1 CFU	30.0 ± 0.3 CFU

Notes: CFU — colony-forming units; * — $p < 0.05$ in relation to the 7th day of incubation.

Table 4 — Hatchability of eggs treated with various disinfectants

Placed eggs, pcs.	Not fertilized		Blood ring		Dead-in-shell		Addled eggs		Weak and crippled		Hatched chickens		Hatch-ability
	pcs.	%	pcs.	%	pcs.	%	pcs.	%	pcs.	%	heads	%	%
'Polydez'													
61	14	23.0	6	9.8	2	3.3	2	3.3	—	—	37	60.7	78.7
'Virosan'													
61	14	23.0	9	14.8	4	6.6	2	3.3	—	—	32	52.5	68.0
Total													
122	28	23.0	15	12.3	6	4.9	4	3.3	—	—	69	56.6	70.2

From the results shown in Table 4 it is seen that the hatchability of eggs in the group, the surface of the shell of which before incubation was treated with 0.1% solution of the drug 'Polydez' was 78.7%, and in the group where the surface of the shell before incubation was treated with 0.1% solution of the drug 'Virosan' was 68.0%. The main losses during the incubation period were due to the presence of an increased number of unfertilized eggs (23.0%).

Autopsy of the dead embryos did not show a negative effect of disinfectants on the hatching egg quality. It should be noted that in the group of eggs, the surface of the shell of which before incubation was treated with 0.1% solution of the drug 'Virosan', there was a slightly increased rate of embryo death in the middle of incubation (blood ring, dead-in shell) compared to the group where the surface of the shell before incubation was treated with 0.1% solution of the drug 'Polydez'. However, in our opinion, this is more due to the excessive period (about 10 days for collecting eggs to form a batch) of egg storage before incubation.

Summarizing the results of the study, it was found that the drugs 'Polydez', 'Virosan', and 'Sterylii AB' do not have a negative impact on the development of birds in the embryonic and postnatal periods. However, due to the lack of fungicidal properties, 'Sterylii AB' should not be used to disinfect egg surfaces before incubation. For the purpose of both pre-incubation treatment and during the incubation period, it is recommended to use for chicken eggs the drug 'Polydez' in 0.1% concentration and the drug 'Virosan' in a concentration of 0.1%.

The large-scale application of disinfectants in animal husbandry is caused by the widespread of pathogens of animal diseases regardless of the facility and areas (Paliy et al., 2018c, 2019). The intensification of production has led to the concentration of a large number of birds of different ages in relatively limited areas. Violation of basic zooveterinary requirements in egg and meat production very often leads to diseases of various etiologies in poultry (Paliy et al., 2018b; Bogach et al., 2020; Paliy et al., 2020a). This is due to the fact that the air basin at such poultry farms is heavily polluted by microflora due to violations in various technological operations for clean and dirty air flows (Palii et al., 2019).

The possibility of re-infection of poultry increases due to the lack of cleaning of the supply and exhaust air in the adjacent premises, as well as due to the placing of eggs for incubation from both healthy and sick birds. In this regard, almost every world leader in incubator construction has its own technological approach to the treatment of premises, equipment, hatching eggs, disinfection and supply of air to the incubator, the scheme of its distribution and the level of air exchange in rooms, cabinets, etc. (Jiang et al., 2018).

One of the key stages of egg incubation is the genetic potential and age of the parent flock (Nangsuay et al., 2013; Ipek and Sozcu, 2015), the shelf life of eggs before incubation (Fasenko, 2007; Goliomytis, Tsipouzian and Hager-Theodorides, 2015), egg size (Iqbal et al., 2016), timely and careful overturning (Elibol and Braket, 2003), quality of ventilation (Okur, Eleroğlu and Türkoğlu, 2018; Ishchenko et al., 2019). For sanitary treatment of eggs before incubation it is necessary to use only those antimicrobials, the effectiveness of which is proven in the laboratory, and their properties meet modern requirements for high quality and safe production (Gehan et al., 2009; Banach et al., 2016; Paliy et al., 2020b; Orobchenko et al., 2020).

In this regard, the urgent issue today is to determine the effect of new disinfectants on the egg microbiota, embryogenesis of poultry, growth and development of hatched young birds, the level of their resistance and safety during growing.

Conclusions. In the course of research the bactericidal properties of disinfectants with different active substances on the microbiota of hatching eggs from chickens of different directions of productivity were determined.

For the purpose of pre-incubation and during the incubation period treatment of chicken eggs, it is effective to use the drug 'Polydez' in 0.1% concentration and the drug 'Virosan' in a concentration of 0.1%.

These disinfectants can be used for sanitation of chicken hatching eggs.

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FEED MICROBIAL CONTAMINATION IN PIG-BREEDING: MODERN THREATS AND WAYS TO OVERCOME THEM

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Summary. The paper presents the results on the species and percentage composition of the microflora in biofilms of pig feed, which varies depending on the seasonal factor. Bacteria *Streptococcus* spp., *Pasteurella multocida*, *Neisseria* spp., and *Clostridium perfringens* in biofilms were found much more often (by 25% or more) in the warm period of the year, while listeria in silage and haylage — in the autumn–winter period. This property of feed biofilms is also significantly influenced by the conditions of cultivation, harvesting and storage of agricultural products. In the study of biofilms of microflora of barley, corn and wheat, it was found that their structural basis are aerobic fungi of the mold *Aspergillus* spp. Bacteria *Streptococcus* spp., *Pasteurella multocida*, *Neisseria* spp., and *Clostridium perfringens* without mold form much looser biofilms *in vitro* and these biofilms are much more sensitive to a wide range of commercial antibiotics. The structural basis of polymicrobial biofilms of barley, corn and wheat microflora is highly likely to be aerobic fungi of *Aspergillus* spp.

Keywords: bacterial biofilms, fodder crops, veterinary and sanitary quality of fodder, listeria, pasteurellosis, clostridiosis

Introduction. Problems of microbial (fungal-bacterial-viral) contamination of feed and equipment critically affect the productivity of industrial livestock. Therefore, in the EU livestock industry, in accordance with the requirements of the 'GMP+' standard, fodder is inspected at all stages of its production — and even more strictly than food. After all, it is believed that the cost of maintaining fodder biosafety is disproportionately lower than the diagnosis and treatment of animals, and then people as consumers of livestock products (Roy et al., 2018). In Ukraine, this attitude to fodder production at the state level, unfortunately, is only beginning to emerge.

It is scientifically proven that the veterinary and sanitary condition of feed is determined by the level of microbial contamination of raw materials (and hence fodder-producing areas!), as well as its contamination in the process of fodder production and during storage of finished feed. It is no secret that today for the production of feed 'residual grain' (practically, grain waste) is often used, and even raw materials from technical ('energy') crops, containing a special microflora — primarily clostridia, which synthesize biofuels (Bajracharya et al., 2016).

In addition, under conditions of heat treatment (extrusion, etc.), crops such as vetch and some others emit hydrocyanic acid (Rybachenko, 2011). Feeds contaminated with microflora not only quickly lose their nutritional value due to bacterial and fungal activity, but they pose a serious threat to pig health. A special threat is the entry into the feed of pathogens of devastation diseases and anthropozoonoses: during transportation, storage or even at the stage of growing forage crops.

Virulent variants of *Clostridium perfringens*, *Neisseria* spp., *Pasteurella multocida*, and *Actinobacillus pleuropneumonia*, which cause acute pneumoenteritis in piglets and chronic infections in older pigs, pose a significant risk to pig health (Pace, Rupp and Finch, 2005; Dalili et al., 2015; Meena and Kanwar, 2015).

These microorganisms are not included in the updated 'List of maximum permissible levels of undesirable substances in feed and feed materials for animals' (MAPFU, 2012), but according to our own research since 2010 we have noted the presence of bacteria associations in feed for pigs, and in 2016–2019, using laboratory methods for the study of bacterial biofilms, we have concluded that their presence in feed for pigs is regular.

This paper is an attempt to experimentally and theoretically substantiate this pattern, which, in our opinion, is insufficiently studied and is essential for the biosafety of pig breeding in Ukraine.

The aim is to study the species composition of microflora in biofilms of pig feed depending on the seasonal factor.

Materials and methods. Bacteriological studies of feeds were performed according to generally accepted methods in Ukraine (MAPFU, 2012), as well as by experimental methods for the study of bacterial biofilms (Oggioni et al., 2006). Sampling and delivery of feed samples (barley, oats, corn, wheat, grits, prestarter, etc.) from 18 pig farms in 5 regions of Ukraine and their veterinary and sanitary assessment was carried out according to the order of the Ministry of Agrarian Policy and Food of Ukraine No. 131 of 19.03.2012, and, simultaneously, following the developed experimental approaches and the obtained experimental-analytical data. In particular, for the destruction of forage biofilms and the subsequent isolation of target bacteria (*Clostridium perfringens*, *Neisseria* spp., *Pasteurella*

multocida, and *Actinobacillus pleuropneumonia*) we used sterilized by filtration eluent, the composition of which is being patented.

Isolation, cultivation, and study of cultural and morphological properties of feed microorganisms were performed on nutrient media: meat peptone broth (MPB, pH 7.2–7.4), Hottinger broth, Martin's medium, 2.5% MPB with the addition of 2% glucose or selective Fraser additive (for isolation of listeria), meat peptone agar (MPA, pH 7.2–7.4), Endo agar, modified Kita-Tarotzi medium, MGM-4 medium, Blauroca, Saburo agar, Olkenitsky medium, Simons citrate, acetate agar, PALCAM agar (for identification of listeria), Mueller-Hinton agar for disco-diffusion test (DDT). The ability of bacterial isolates to form biofilms was studied by the micromethod (O'Toole, 2011). Antibiotic resistance and the ability of isolated bacterial isolates to form biofilms were studied by modern experimental methods (MHU, 2007; Oggioni et al., 2006).

Pathogenicity of isolated field isolates of bacteria was tested on white mice (weighing 16–18 g) by intra-abdominal infection at a dose of 0.5×10^9 bacterial cells in accordance with the requirements of the Law of Ukraine No. 3447-IV from 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006), the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986), and Council Directive 86/609/EEC (CEC, 1986). Ethyl ether was used for anesthesia.

Results and discussions. In the period 2016–2019, 220 samples of animal feed were examined for the presence of film-forming and planktonic forms (free-floating cells) of opportunistic microflora (including 34 samples of silage and haylage for dairy cattle regarding the risk of listeriosis in pig breeding through skim for piglets), and the rest — feeds and their grain ingredients for pig breeding) in 18 farms (crop with livestock including pig breeding or pig breeding with livestock, both cattle and small cattle) in 5 regions: Kharkiv (n = 4), Sumy (n = 4), Kherson (n = 4), Poltava (n = 4), and Vinnytsia (n = 2). These areas were selected because of their eco-geographical distribution in terms of soil moisture — one of the most important factors in the survival of opportunistic pathogens in the environment, in particular on forage lands.

Common features of the surveyed farms, in addition to the multidisciplinary nature typical of modern agriculture in Ukraine, were also the conditions of their location in the range of soil moisture from 125 to 175 mm, the share of cereals of their own origin at least 75% in the diet of pigs and use of pig manure for growing their own fodder crops.

Table 1 shows that opportunistic pathogens, as a component of bacterial and fungal biofilms, were found in almost all samples of the surveyed farms. The bioassay with the obtained isolates (n = 75) was negative (none of them caused the death of mice within 10 days after infection, although there were some signs of deterioration in the health of experimental mice during the first 3–4 days).

At the same time, in the nasal smears of pigs (n = 49), almost all examined, we found pasteurella of those serotypes that were found in the feed in these farms, and 32 of their isolates (65.3%, $P < 0.01$; isolates both from clinically sick and healthy pigs) were virulent in mice. Clostridia isolated from rectal smears (n = 27) of clinically sick piglets (n = 12) were virulent for mice, and clostridia isolated from sows with healthy nests (n = 15) were avirulent for mice.

In our opinion, the obtained data indicate a direct connection between the epizootic process of pasteurellosis and anaerobic enterotoxemia in pig farming with contamination of feed (and hence the environment) with biofilms with the corresponding opportunistic microflora. In turn, this may be a consequence of the widespread use in crop production of the surveyed farms insufficiently decontaminated pig manure.

As shown in Table 1, the composition of biofilms containing the studied opportunistic microflora differed significantly between samples of cereals of different species. Its greatest diversity (4 or more studied species of microorganisms) in all farms was registered in samples of barley grain and, accordingly, in bran and feed mixtures with its content (*Streptococcus* spp., *Pasteurella multocida*, *Aspergillus niger*, *Neisseria* spp., *Clostridium perfringens*), the least — in wheat samples *(Pasteurella multocida, Aspergillus niger).*

In the sample of litter from straw waste for cattle in one of the pig farms in Sumy Region we detected by laboratory methods biofilm of 8 species of opportunistic pathogens, including spirochete: at the time of the survey at a fattening site 7 km away from the pig farm, cattle leptospirosis was registered with abortions and stillbirths of calves.

Mycoplasmas were often sown (39–58% of the studied samples from different farms) together with *Pasteurella multocida*, *Clostridia*, and *Neisseria* from biofilms of prestarter feed samples from different producers (n = 27). A characteristic feature of the circulation of industrially produced prestarter and other feed for pigs in these farms was virtually free access of synanthropic birds and rodents to places of their storage.

The results of a survey of two farms in Poltava and Vinnytsia regions regarding listeriosis need special consideration.

In Poltava Region, the diet of pigs and cattle for a number of years included grain and haylage from energy crops, which contain a specific 'biofuel' microflora of the so-called 'Clostridium complex of biofuels No. 1'. In Vinnytsia Region, the diet of pigs and cattle included potatoes with a high content of gossypol.

Table 1 — Geographical and seasonal dynamics of dangerous for pigs microorganisms, which were found in the grain in surveyed farms and fodder of plant origin in 2016–2019

Type of feed, fodder ingredients	Species of isolated potentially dangerous microflora*	Percentage of positive samples from the farms (n) in spring–summer (A) and autumn–winter (B) seasons									
		Kharkiv, n = 4		Sumy, n = 4		Kherson, n = 4		Poltava, n = 4		Vinnytsia, n = 2	
		A	B	A	B	A	B	A	B	A	B
Barley (n = 28)	*Streptococcus* spp.	62	38	57	43	65	35	52	48	69	31
	Pasteurella multocida serotype D	54	46	55	45	58	42	46	50	51	40
	Aspergillus niger	73	27	78	22	66	34	75	25	64	36
	Neisseria spp.	52	48	50	50	57	43	55	45	49	51
	Clostridium perfringens	23	77	26	74	29	71	32	68	21	79
Corn (n = 21)	*Aspergillus niger*	65	35	68	32	72	28	67	33	74	26
	Streptococcus spp.	51	49	56	44	53	47	48	52	59	41
	Candida albicans	48	52	46	54	57	43	62	38	65	35
Oat (n = 26)	*Pasteurella multocida* serotypes A and D	41	59	47	53	42	58	56	44	54	46
	Staphylococcus aureus	73	27	71	29	68	32	65	35	62	38
	Clostridium perfringens	33	67	28	72	31	69	25	75	38	62
Wheat (n = 24)	*Pasteurella multocida* serotype A	45	55	48	52	53	47	56	44	59	41
	Aspergillus niger	70	30	74	26	78	22	67	33	77	23
Bran (n = 25)	*Mycoplasma* spp.	63	37	65	35	56	44	62	38	58	42
	Pasteurella multocida serotype D	43	57	46	54	51	49	55	45	57	43
	Aspergillus niger	69	31	73	27	77	23	79	21	66	34
	Neisseria spp	57	43	54	46	55	45	51	49	48	52
	Clostridium perfringens	14	86	11	89	22	78	17	83	23	77
Bran compound feed mixtures (n = 35)	*Pasteurella multocida* serotype D	56	44	61	39	58	42	48	52	63	37
	Mycoplasma hyopneumonia	25	75	20	80	22	78	19	81	23	77
	Clostridium difficile	31	69	36	64	33	67	28	72	26	74
	Clostridium botulinum	17	83	15	85	13	87	19	81	12	88
Prestarter (n = 27)	*Pasteurella multocida* serotypes A and D	57	43	51	49	45	55	58	42	61	39
	Neisseria spp.	64	36	58	42	56	44	61	39	60	40
Silage and haylage** (n = 34)	*Neisseria* spp.	47	53	42	58	49	51	46	54	54	46
	Pasteurella multocida serotype D	52	48	56	44	51	49	47	53	55	45
	Listeria monocytogenes	0	16	0	12	0	0	0	45	0	73

Notes: * —isolated microflora was avirulent for mice-albino; ** — pigs + cattle, etc.

Therefore, a certain period of time 'humpback' and paresis in pigs, sow agalactia and subclinical mastitis of cows, as well as swine neuroinfection and reproductive disorders in cattle in Vinnytsia Region were associated with feed and genetic factors. However, the isolation of *Listeria monocytogenes* from silage samples in both farms, as well as from the spinal cord and intestinal lymph nodes of young pigs, respectively, indicates the leading role of listeria in combination with clostridia in taking root enzootic associated infections in pigs in these farms.

Also from the data in Table 1 it is seen that the species and percentage composition of the microflora in the biofilms of feed for pigs varies depending on the seasonal factor. Bacteria *Streptococcus* spp., *Pasteurella multocida*, *Neisseria* spp., and *Clostridium perfringens* in these biofilms were found much more often (by 25% or more) in the warm period of the year, while listeria in the silage and haylage — in the autumn–winter period.

In our opinion, this property of feed biofilms is also significantly influenced by the conditions of cultivation, harvesting and storage of agricultural products. In the study of biofilms of the microflora of barley, corn and wheat, it was found that their structural basis ('framework') are aerobic fungi of the mold *Aspergillus* spp.

Without mold bacteria *Streptococcus* spp., *Pasteurella multocida*, *Neisseria* spp., and *Clostridium perfringens* form much looser biofilms *in vitro*, which disintegrate rapidly even with light shaking and are much more sensitive to a wide range of commercial antibiotics.

At the same time, according to the literature for the growth of mold in the soil and feed, especially in grain

processing products, higher humidity required: at humidity below 13% and at 27–40°C fungi do not grow (Abraskova, Shashko and Shashko, 2013). That is, according to soil moisture indicators, the surveyed farms were in the risk zone of fungal contamination, and, accordingly, the formation of fungal-bacterial biofilms dangerous for pig breeding.

Conclusions. 1. According to the results of studies of 220 feed samples in the period 2016–2019 for the presence of film-forming and planktonic forms of opportunistic pathogenic microflora, a direct connection has been established between the epizootic situation in pig farming and contamination of feed with microorganisms capable of forming bacterial biofilms containing pathogens of pasteurellosis and anaerobic enterotoxemia.

2. The greatest diversity of opportunistic microflora (4 or more studied species of microorganisms) was found in samples of barley grain and, accordingly, in the bran and feed mixtures with its content (*Streptococcus* spp., *Pasteurella multocida*, *Aspergillus niger*, *Neisseria* spp., *Clostridium perfringens*), the least — in samples of wheat grain *(Pasteurella multocida*, *Aspergillus niger).*

3. Species and percentage composition of microflora in biofilms of pig feed varies depending on the seasonal factor. Bacteria *Streptococcus* spp., *Pasteurella multocida*, *Neisseria* spp., and *Clostridium perfringens* in these biofilms were found much more often (by 25% or more) in the warm period of the year, while listeria in silage and haylage — in the autumn–winter period.

4. Contamination of silage and haylage by listeria from 16% to 72% in four regions of Ukraine was registered in the autumn–winter season, which poses a direct danger to of multidisciplinary pig farms and human health.

5. The structural basis of polymicrobial biofilms of microflora of barley, corn and wheat with high probability are aerobic fungi of *Aspergillus* spp.

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SALMONELLOSIS AND DETECTION RATE OF *SALMONELLA* SPP. IN FOODSTUFFS

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Summary. Today, salmonellosis remains one of the leaders in zoonoses and the cause of toxic infections in humans, which are common throughout the world. The epidemiological feature of salmonellosis is suddenness and mass character. Salmonella is very stable in the environment, and can multiply intensively at 20–37°C in various foods: meat and dairy products, sausages, especially blood and liver, jellies, pates, cream confectionery, salads and other dishes. The organization and conduct of laboratory tests for the diagnosis and prevention of salmonellosis is an important component of the epidemiological surveillance system. The aim of the study was to analyze the detection rate of bacteria of the genus *Salmonella* in food products on the territory of Ukraine in 2019 and to establish the serovars of *Salmonella*, dangerous to human health. The study and analysis of statistical data was carried out based on the results of research and reports from the regional laboratories of the State Food and Consumer Service of 24 regions of Ukraine, as well as own research conducted in the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise. According to the results of the research, it was established that 72 isolates of *Salmonella* spp. were isolated out of 184,951 food samples studied in 2019. Compared to 2018, the number of isolated salmonella from 189,517 samples was 121 isolates, of which dangerous strains of *S.* Enteritidis (group D) were detected in 25 cases, which is 20.6%, and *Salmonella* spp. — 32.2% of all isolated salmonella. These variants of salmonella were isolated from meat of various species of animals, poultry co-products, meat semi-finished products, minced meat and mechanically deboned meat, sauce, eggs and feed. In 2017, only 32 isolates of *Salmonella* were isolated from 142,977 tested samples, mainly *Salmonella* spp. The products from which this pathogen was isolated differed slightly from the following years, namely: meat, co-products, meat semi-finished products, animal oil, salted fish, fish semi-finished products and cookies. That is, the largest number of isolated salmonella is observed in 2018, although the number of samples in 2019 was slightly lower than in 2018. Thus, the obtained data indicate that it is necessary to follow strictly the sanitary and hygienic rules during the preparation, processing and consumption of food

Keywords: bacteriological studies, Ukraine, serovars

Introduction. Salmonellosis is an infectious disease of animals, birds and humans, which is characterized by acute course, fever, diarrhea and lesions of the small and large intestine, and in chronic course — pneumonia.

The source of the pathogen is sick and diseased animals-carriers and their feces, and the route of transmitting the pathogen is the fecal-oral route. Animals can secrete the pathogen for months, sick humans — from 3 days to 3 weeks.

For humans and pets, the source of infection can be meat and meat products from animals-carriers, fish and seafood, eggs and egg products, milk and dairy products, vegetables and fruit (Afshari, 2018; Crump et al., 2015; Eng et al., 2015).

In 96–98% of cases, infections are related to the consumption of salmonella-contaminated food. In food, especially in semi-finished products, salmonella are not only stored, but they also multiply rapidly, without changing the taste of dishes (Percival and Williams, 2014; Antunes et al., 2016).

In the last 20 years, the largest outbreak of salmonellosis has been recorded in the United States, which was caused by infected ice cream, which in turn was made from contaminated eggs. The outbreak affected 224,000 people (Seladi-Schulman and Brazier, 2020; Beuchat and Mann, 2015).

The most common form of salmonellosis is gastrointestinal. It begins with intoxication, and is characterized by a temperature of up to 39°C, headache, chills, weakness, aches and dizziness. At the same time, the symptoms of gastrointestinal dysfunction increase — stomach pain, vomiting, diarrhea, etc.

Separately, we can distinguish salmonellosis in subclinical form, as well as carriage of bacteria with a chronic state that does not manifest itself clinically and is detected exclusively by laboratory methods (Allaoui and Filali, 2016; Galatiuk et al., 2016).

Salmonella are highly resistant to drying, high temperatures and other adverse environmental conditions, and can be stored at its facilities for 160 days; in manure — 420 days, in salted and smoked meat — 2.5–3 months; in cheese and butter — up to 6 months. They withstand freezing for 4–5 months, and are stored in sunlight for 150 days. When heated to 70–75°C they are inactivated during 15–30 min and some can withstand temperatures up to 85°C for 45 min (Crump et al., 2015; Eng et al., 2015). Today, the problem of antibiotic resistance of microorganisms released from biological material and food is relevant (Hadzevych et al., 2019).

The aim of the study was to analyze the frequency of detection *Salmonella* in food products in 2019 in Ukraine and to establish dangerous to human health serovars.

Materials and methods. In order to establish the spread of bacteria of the genus *Salmonella* in food products the results of research and reports from the regional laboratories of the State Food and Consumer Service of 24 regions of Ukraine, as well as the results of studies of the Laboratory for Microbiological Researches of Foodstuffs the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (Kyiv) were analyzed. The following foodstuffs were studied: pork and beef, minced meat of poultry and other animals, meat and fish semi-finished products, eggs, cheese, fresh chilled fish, seafood. A total of 184,951 food samples were studied in 2019. Bacteriological studies were performed by conventional methods following ISO 6579-1:2017 'Microbiology of the Food Chain — Horizontal Method for the Detection, Enumeration and Serotyping of *Salmonella* — Part 1: Detection of *Salmonella* spp.' (ISO, 2017) respectively to each test product.

Prepared food samples were added to non-selective enrichment media with subsequent enrichment on selective media and subcultured on solid differential diagnostic media. After incubation, the enriched material was transplanted into selective enrichment media (Mueller-Kaufmann and Rappaport-Vasiliadis), and then into differential diagnostic media, respectively.

Identification of isolated cultures was performed on the basis of their cultural-morphological and biochemical properties. Subsequently, serotyping of salmonella isolates was performed to detect O- and H-antigens. Determination of antigenic belonging was performed in the agglutination test on glass with polyvalent serum ABCDE and determination of the O-group of salmonella using monovalent sera.

Results and discussion. Studies have identified 72 positive cases of *Salmonella* spp. These data were obtained from 12 regions of Ukraine: Volyn, Dnipropetrovsk, Donetsk, Zhytomyr, Zaporizhzhia, Mykolaiv, Odesa, Poltava, Rivne, Khmelnytsky, and Cherkasy. The percentage of indication of the pathogen in food in different areas is presented in Fig. 1.

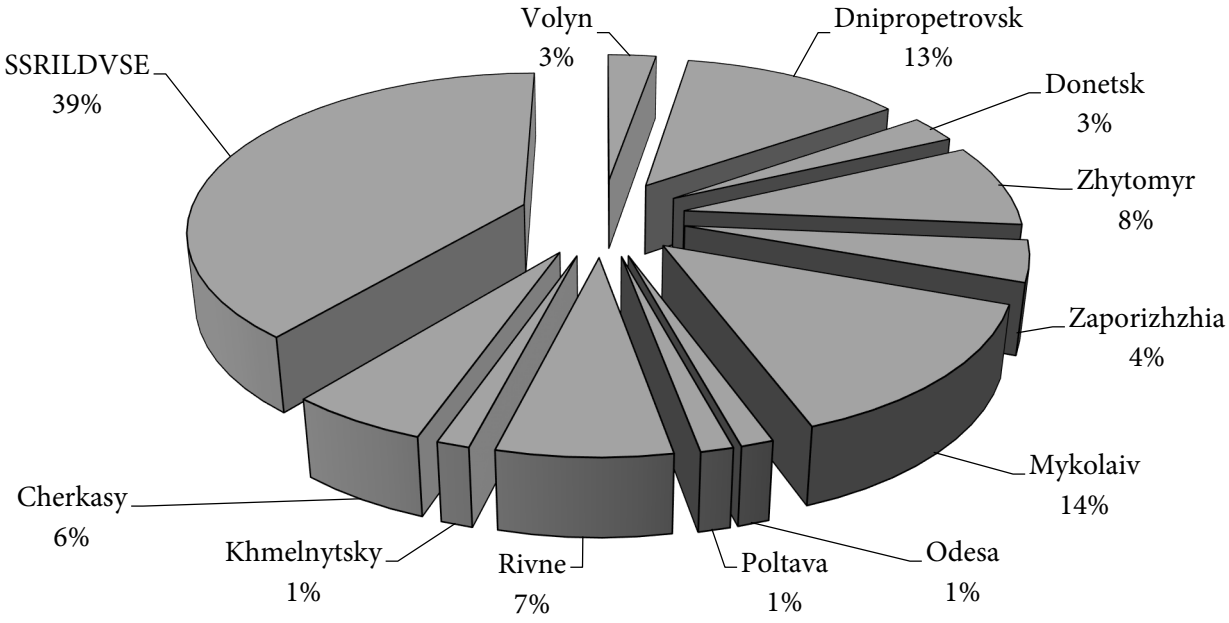

Figure 1. Percentage of cases of *Salmonella* detection in the regions of Ukraine

The highest number of salmonella was observed in Zhytomyr Region — 6 (8.3%), Mykolaiv Region — 10 (13.8%), Dnipropetrovsk Region — 9 (12.5%), and in samples received for research at SSRILDVSE in Kyiv — 28 (38.8%) isolates.

The highest amount of salmonella was found in meat products, which was 40.3% (Fig. 2). In semi-finished products, this index reached 28%, finished products contained 13.9% and 4.2% of the pathogen accounted for eggs and egg products, respectively.

Thus, in the meat of different species of animals — 4 positive samples (of the total number of positives), poultry meat — 11 (15.2%), in minced meat and mechanically deboning meat — 9 (12.5%). Quite a large number of positive results was obtained in the study of meat semi-finished products, which was 18 samples (25%). In the finished meat products there was a significant amount of *Salmonella*, which was 4 positive samples (5.5%). In 2019, in the study of feed for microbiological indicators, 9 (12.5%) samples with Salmonella were isolated.

By serotyping (Table 1), it was determined that *Salmonella* group D and *Salmonella* group C composed 19 and 16 isolates, respectively, of all tested products. *S.* Typhimurium and *S.* Haifa composed two cases, and *Salmonella* spp. — nine. This indicates a high probability of infection of pets with salmonella or their subsequent bacteria carriage.

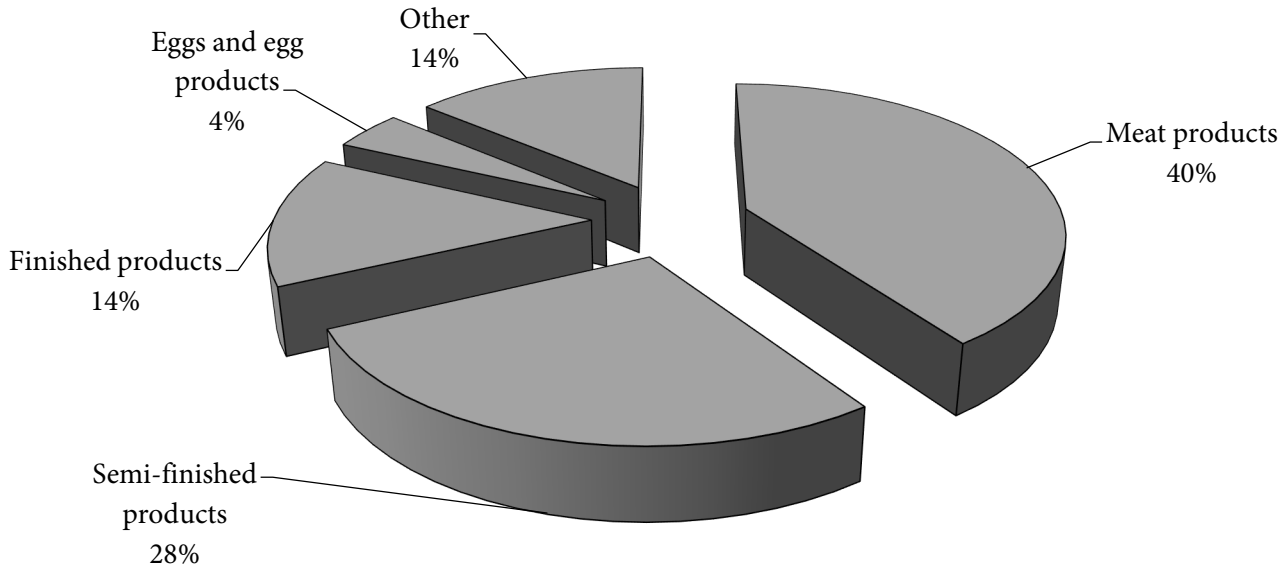

Figure 2. Percentage of cases of *Salmonella* detection in different food products

Table 1 — Serological structure of *Salmonella* isolated from different products

Type of product or raw material		*Salmonella* serovars/serogrups									
		Group D	**Group E**	**Group C**	**Group B**	***S.* spp.**	**Enteritidis**	**Typhimurium**	**Gallinarum**	**Haifa**	**Total**
Meat products	poultry meat	5	—	3	2	—	3	—	—	—	13
	meat from other animal species	1	—	—	—	—	—	—	—	—	1
	minced meat and mechanically deboning meat	4	1	—	—	4	—	—	—	—	9
	by-products	—	—	4	—	—	1	—	—	—	5
Semi-finished products	from meat	3	—	7	4	—	2	—	1	—	17
	from fish	—	—	—	—	—	1	—	—	—	1
	without meat	—	—	—	—	—	1	—	—	—	1
Finished products	meat	3	—	—	—	—	1	—	—	—	4
	fish	1	—	—	—	—	—	—	—	—	1
	cheese	—	—	—	—	—	1	—	—	—	1
	soups, porridges and other	—	—	—	—	—	1	—	—	—	1
	confectionery	—	—	—	—	—	1	—	—	—	1
	caviar, mollusks	1	—	—	—	—	—	—	—	—	1
	sauces	—	—	—	—	—	—	1	—	—	1
Eggs and egg products		1	—	1	—	—	1	1	—	—	4
Casein, gelatin		—	—	1	—	—	—	—	—	—	2
Animal feed		—	—	—	—	5	2	—	—	2	9
Total		19	1	16	6	9	15	2	1	2	72

Most salmonella are pathogenic for both humans and animals, but the most dangerous in epidemiological terms for humans were isolated *S.* Enteritidis in 15 cases (poultry meat, by-products from farm animals and poultry, semi-finished fish, eggs, dairy products) and *S.* Typhimurium in one case from the sauce and in one case — from eggs. It should be noted that *S.* Enteritidis has become widespread in the last 20 years. Representatives of this serovar cause outbreaks of salmonellosis at low doses of contamination of the product, and diseases are usually characterized by more expressed clinical manifestations.

Summarizing the results, we can say that the largest number of salmonella cases were registered in food products that require heat treatment (meat, minced meat, semi-finished products), and a slightly lower percentage — in ready-to-eat food products.

Therefore, due to the fact that salmonellosis has severe courses and consequences, and the pathogen is very resistant and can adapt to the macroorganism for symbiosis, preventive measures and control measures must be strict and effective. In veterinary medicine, the prevention of salmonellosis should be aimed at increasing the organism resistance of the broodstock and newborns. Feeding with contaminated with salmonella feed is not allowed. Feed for animals must be tested bacteriologically. Timely deratization of the premises is required.

It is necessary to adhere more strictly to sanitary and hygienic rules during cooking, to use only sufficiently heat-treated meat, milk, eggs, or products from them. Strictly separate storage places for raw and finished products. It is forbidden to buy dairy and meat products, eggs at spontaneous markets, and it is necessary to pay attention to the temperature of storage of these products in shopping facilities. Do not consume expired products. It is necessary to maintain personal hygiene to prevent the getting the pathogen into the body: to wash hands with soap after the toilet, before eating and cooking. Be sure to wash vegetables and fruit thoroughly under running water. Timely deratization and disinsection of food storage facilities are required.

It should be noted that salmonella of any serovars can cause a wide variety of clinical forms of the disease — from severe generalized forms to asymptomatic carriage.

Conclusions. 1. During 2019, 184,951 food samples were studied, from which 72 isolates of *Salmonella* in 12 regions of Ukraine were identified. The largest number of *Salmonella* cases was registered in Zhytomyr Region — 6 (8.3%), Mykolaiv Region — 10 (13.8%), Dnipropetrovsk Region — 9 (12.5%), and in samples received for research at SSRILDVSE in Kyiv — 28 (38.8%).

2. Products contaminated with salmonella, that require heat treatment accounted for 73% of the total amount of salmonella isolated, 26% — are ready to eat products.

3. The most dangerous *Salmonella* serovars group D (*S.* Enteritidis) were isolated from poultry meat, by-products from farm animals and poultry, semi-finished fish, eggs, and dairy products; group B (*S.* Typhimurium) — from sauce and eggs.

4. Compared to 2018, the number of isolated salmonella from 189,517 samples was 121 isolates, of which dangerous strains of *S.* Enteritidis (group D) were detected in 25 cases, which is 20.6%, and *S.* spp. — 32.2% of all isolated salmonella. In 2017, only 32 isolates of *Salmonella* were identified from 142,977 tested samples, mainly *Salmonella* spp.

Prospects for further research. Salmonellosis in animals and humans is an acute medical and veterinary problem, which justifies its permanent monitoring.

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Contents

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Part 2. Biosafety