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EFFECTIVENESS OF USING THE COMPLEX DRUG 'KARAFAND+OV,ZN' TO INCREASE THE REPRODUCTIVE CAPACITY OF MALES OF DOMESTIC ANIMALS

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Summary. Experimental research on male domestic animals (boars, bulls) proved the effectiveness of using the complex drug 'Karafand+OV,Zn' to increase their reproductive capacity. This preparation contains carotenoids, biologically active substances from the rhizome of marsh calamus and nanoparticles of orthovanadate gadolinium activated by europium and zinc carbonate. It was observed that the introduction of the drug increased the activity of antioxidant protection — catalase activity in the serum and erythrocytes in boars by 15.9% ($p < 0.01$) and 11.4% ($p < 0.05$), in bulls — by 7.3% ($p < 0.05$) and 12.4% ($p < 0.01$), respectively, and SOD in boars by 30.2% ($p < 0.01$), in bulls — by 16.5% ($P < 0.05$) — when reducing the intensity of lipoperoxidation processes (reducing the amount of TBA-AP in the serum and erythrocytes of males — in boars by 19.4% ($p < 0.05$) and 16.9% ($p < 0.001$), in bulls — by 25% ($p < 0.05$) and by 12.4% ($p < 0.01$), respectively. There was an improvement in sperm quality (especially in terms of motility and content of sperm with morphological abnormalities) and endocrine function of the testes — testosterone levels increased by 16.8% in boars ($p < 0.05$), and in bulls — by 44.3 % ($p < 0.001$). At the same time, there was an increase in protein-vitamin-mineral metabolism in the body: the amount of vitamin A reliably increased in boars by 14.5% ($p < 0.001$), in bulls — by 7.6% ($p < 0.05$), and the content of zinc — in boars by 36.5% ($p < 0.001$), in bulls — by 16.0% ($p < 0.01$), the amount of total protein, calcium and phosphorus also increased. Thus, the obtained results allow us to recommend the complex drug 'Karafand+OV,Zn' as an effective means for increasing the reproductive capacity of males with pronounced sperm-modeling and androgen-stimulating effects and powerful antioxidant properties

Keywords: reproductive system, lipoperoxidation, nanoparticles, biologically active substances, sperm quality, hormonal background

Introduction. The main cause of reduced reproductive capacity of males is gonadopathy of alimentary deficiency and toxic genesis (Koshevoi et al., 2015, 2016; Sabeti et al., 2016; Barik, Chaturvedula and Bobby, 2019). Their occurrence is most often caused by the use of poor quality feed and water, defective diets of breeders, uncontrolled use of chemical fertilizers, etc. (Hunchak et al., 2010; Chornozub, 2013; Koshevoi et al., 2015).

The pathogenetic chain of hypofertility of males (decreased reproductive capacity) includes changes in vitamin and mineral metabolism, imbalance of the prooxidant-antioxidant system, reduced sperm quality, and negative dynamics of the hormonal background. Increasing the synthesis of active forms of biogenic elements, mainly oxygen, causes oxidative stress, which is the result of the damaging effects of the above factors (Agarwal, Makker and Sharma, 2007; Agarwal et al., 2018; Dziekońska et al., 2017; Koshevoy and Naumenko, 2020; Otasevic et al., 2020; Ritchie and Ko, 2021).

For the treatment of animals with gonadal pathology, drugs based on nanobiomaterials have been proposed, which have pronounced antioxidant and androgen-stimulating effects and increase the body's overall resistance (Karpenko et al., 2020; Koshevoy et al., 2021).

Given the etiology and prevalence of gonadopathies among breeders, the urgent scientific task is to develop ways to increase the reproductive capacity of males (Piomboni et al., 2008; Yaremchuk et al., 2017; Ribas-Maynou and Yeste, 2020).

Our work aimed to establish the effectiveness of the complex drug 'Karafand+OV,Zn' to increase the reproductive capacity of male domestic animals.

Materials and methods. The research was conducted in the laboratories of the Department of Veterinary Reproductology of the Kharkiv State Zooveterinary Academy (since 01.09.2021 — Department of Veterinary Surgery and Reproductology of the State Biotechnological University) and the Nanostructured Materials Department named after Yu. V. Malyukin of the Institute for Scintillation Materials of the National Academy of Sciences of Ukraine (Kharkiv).

The material of the research was males of different species of animals, in particular groups of boars ($n = 10$) and bulls ($n = 10$), which belonged to farms of different forms of ownership. Groups of animals consisted of clinically healthy males, kept on a standard diet and had free access to water, with full reproductive capacity, as established by andrological examination according to our methodology (Koshevoi et al., 2015).

The method of increasing the reproductive capacity of male farm animals included the use of a complex preparation 'Karafand+OV,Zn' containing carotenoids, biologically active substances from the rhizome of marsh calamus and nanomaterials — nanoparticles of europium-activated gadolinium orthovanadate and zinc carbonate (Koshevoi et al., 2016). Animals of the experimental groups were administered the drug at a dose of 7.5 cm^3 per boar and 10 cm^3 per bull, orally, once a day for 10 days.

The effectiveness of the complex drug 'Karafand+OV,Zn' was determined by changes in total protein, carotene, vitamin A, zinc, total calcium and inorganic phosphorus, the dynamics of the prooxidant-antioxidant system (content of thiobarbiturate-active products (TB-AP), glutathione (VG), catalase and superoxide dismutase (SOD) activity), testosterone levels and sperm quality.

The studied indicators were established by commonly used methods (Vlizlo, 2012). The concentration of total testosterone in the serum was determined in the 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 performed by Student's *t*-test (Rebrova, 2006).

Results and discussion. Analyzing previous studies and data from literature sources found a positive effect of nanobiomaterials on the body of males with gonadopathy (Koshevoi et al., 2016; Koshvoy et al., 2021; Karpenko et al., 2020; Yaremchuk et al., 2017). The results of studies on their use to improve reproducibility, indicate a significant clinical and biochemical effect of the complex drug 'Karafand+OV,Zn".

The administration of the drug led to improved protein-vitamin-mineral metabolism — for example, the content of carotene in the serum of bulls increased by 19.8% (2.90 ± 0.03 μmol/l, $p < 0.001$), and the amount of vitamin A reliably increased in boars by 14.5% ($p < 0.001$), in bulls — by 7.6% ($p < 0.05$), while the zinc content increased by 36.5% ($p < 0.001$) in boars, and by 16.0% ($p < 0.01$) in bulls (Fig. 1).

Mineral metabolism was characterized by an increase in the content of total calcium in the serum of boars by 6.9% ($p < 0.05$), in bulls — by 3.5% ($p < 0.05$) and inorganic phosphorus in boars and bulls by 10% ($p < 0.05$) and 8.3% ($p < 0.05$), respectively (Fig. 2). There was an increase in total protein content in boars by 2.9% (73.8 ± 0.62 g/l, $p < 0.05$), in bulls — by 5.5% (78.7 ± 0.48 g/l, $p < 0.001$).

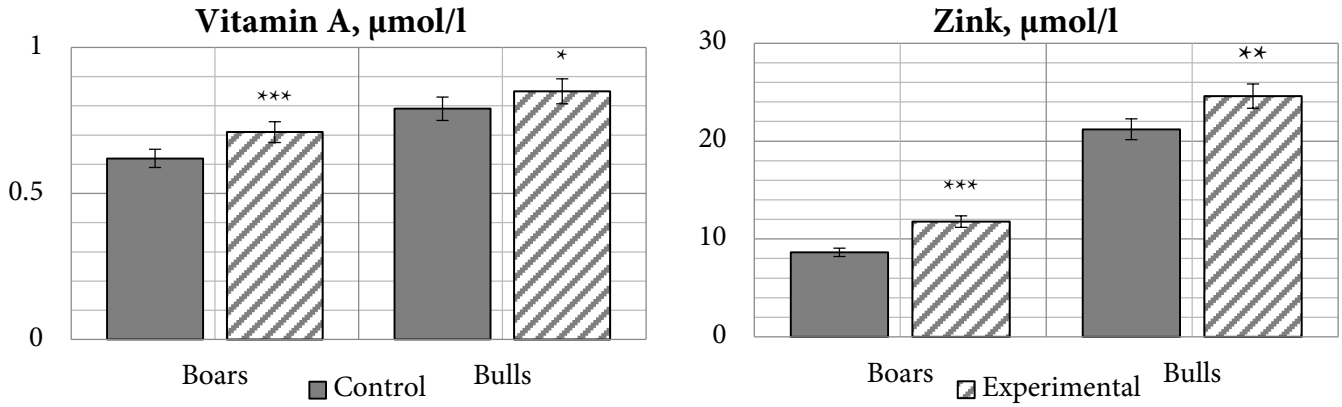

Figure 1. The effect of the complex drug 'Karafand+OV,Zn' on the content of vitamin A and zinc in the blood serum of males (* — $p < 0.05$; ** — $p < 0.01$; *** — $p < 0.001$ compared with the control group)

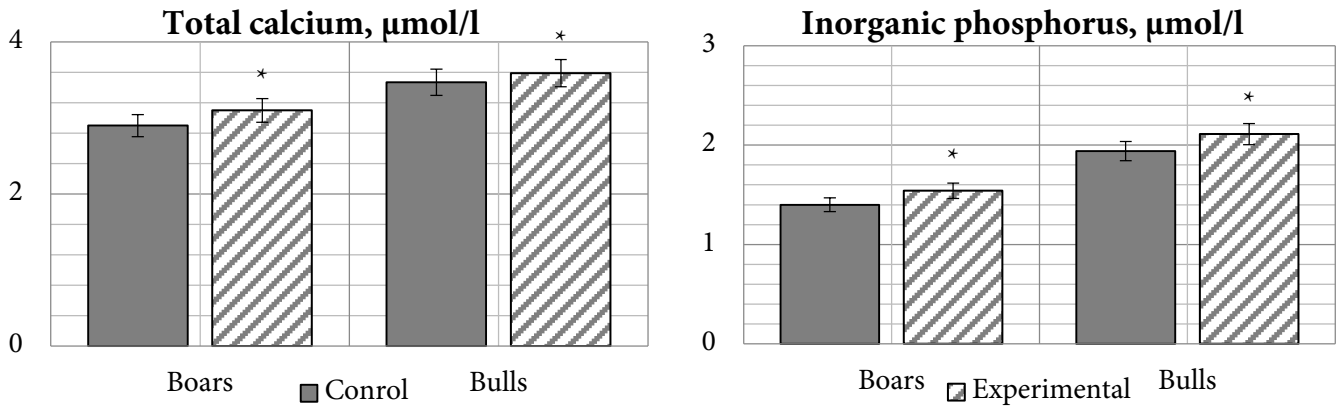

Figure 2. The effect of the complex drug 'Karafand+OV,Zn' on the content of calcium and phosphorus in the blood serum of males (* — $p < 0.05$ compared with the control group)

Activation of the antioxidant defense system and reducing the intensity of lipid peroxidation (LPO) processes have a significant impact on the body's resistance (Piomboni et al., 2008; Agarwal et al., 2018; Palani, 2018; Ritchie and Ko, 2021).

Figure 3 shows a decrease in the intensity of LPO processes after drug administration — the amount of TB-AP in erythrocytes was lower than in the control group — in boars by 16.9% ($p < 0.001$), in bulls — by 12.4% ($p < 0.01$).

The positive effect of the drug on the antioxidant status of males was noted (Fig. 4) — catalase activity in boar erythrocytes increased by 11.4% ($p < 0.05$), bulls — by 12.4% ($p < 0.01$). SOD activity increased in boars by 30.2% ($p < 0.01$) and in bulls by 16.5% ($p < 0.05$). At the same time, the content of GSH was reliably higher in boars by 3.2% ($p < 0.05$) of the control group, and in bulls tended to increase.

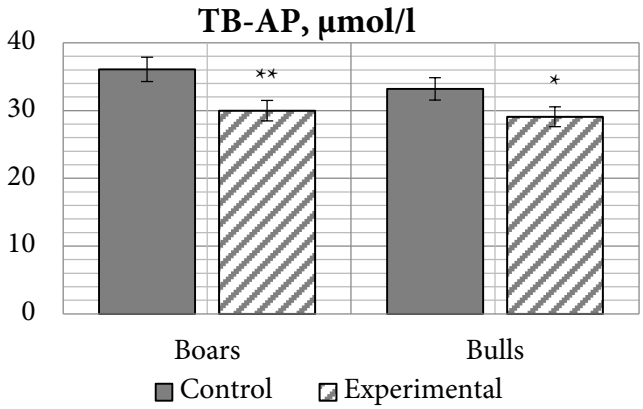

Figure 3. The effect of the complex drug 'Karafand+OV,Zn' on the intensity of lipoperoxidation in males (* — $p < 0.01$; ** — $p < 0.001$ compared with the control group)

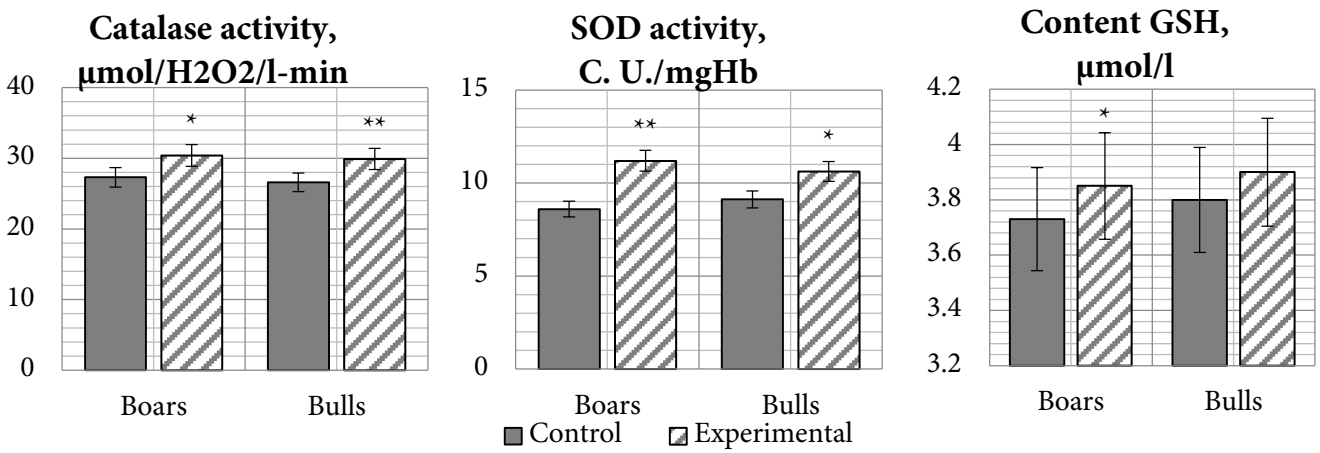

Figure 4. The effect of the complex drug 'Karafand+OV,Zn' on the antioxidant status of males (* — $p < 0.05$; ** — $p < 0.01$ compared with the control group)

Administration of the drug has a positive effect on the germinative and endocrine functions of the male gonads, which is probably due to increased resistance to oxidative stress, which is the main pathogenic mechanism of gonadopathies (Tvrda et al., 2017; Koshevoy et al., 2021).

The method of prevention had particularly effect on the motility of sperm in ejaculate, for example, it was reliably higher in boars by 28.1% ($p < 0.05$) of the control group, and in bulls tended to increase (Fig. 5).

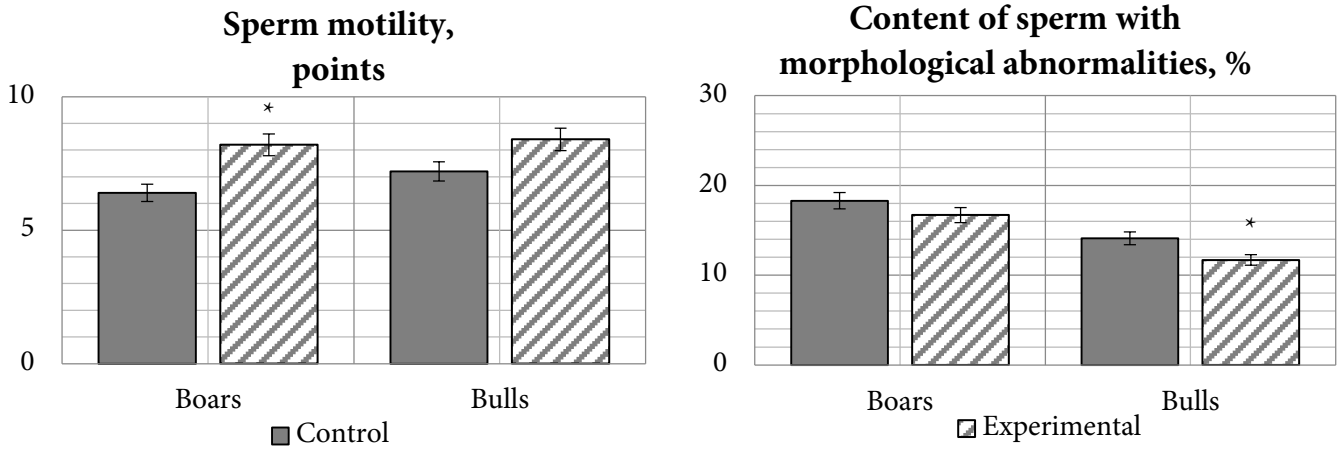

Figure 5. The effect of the complex drug 'Karafand+OV,Zn' on the quality of male sperm (* — $p < 0.05$ compared with the control group)

At the same time, there was a tendency to increase the volume of ejaculate in boars, and in bulls it reliably increased by 28.7% (4.39 ± 0.24 ml, $p < 0.05$).

The concentration of sperm in animals of the experimental groups did not change significantly, and the content of sperm with morphological anomalies was reduced by 17% in bulls ($p < 0.05$), and in boars it tended to decrease.

Testosterone levels increased by 16.8% in boars ($p < 0.05$), and in bulls — by 44.3% ($p < 0.001$), which confirms the androgen-stimulating effect of the drug (Fig. 6).

Conclusions. The obtained results allow us to recommend the complex preparation 'Karafand+OV,Zn' to prevent gonadal pathology of alimentary-deficient genesis in males. Thus, during the introduction of this drug activation of dynamics in the antioxidant defense system, improvement of protein-mineral metabolism with a decrease in the intensity of lipoperoxidation processes was observed.

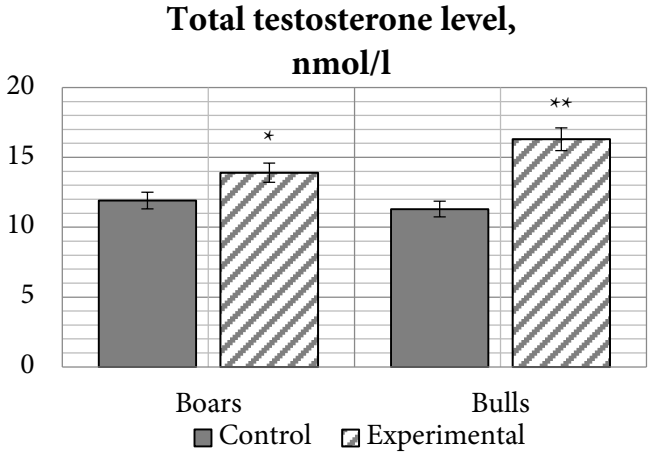

Figure 6. The effect of the complex drug 'Karafand+OV,Zn' on endocrine function of male testes (* — $p < 0.05$; ** — $p < 0.001$ compared with the control group)

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EFFECTIVENESS OF ALBENDAZOLE-BASED ANTI-PARASITIC DRUGS UNDER MODERN CONDITIONS

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Summary. An important problem in veterinary medicine is still the control of parasitic diseases of farm and domestic animals. The market of antiparasitic drugs is widely represented by anthelmintics based on albendozole, both imported and domestic, but data on the effectiveness of these drugs are currently insufficient. The aim of the research was to determine in the comparative aspect the effectiveness of the use of antiparasitic drugs in different forms with the content of albendazole in different species of animals. In the previous stage of the studies the experimental animals were diagnosed with invasions. The most common infections in cattle were dictyocaulosis and fasciolosis; *Trichostrongylus colubriformis, Cooperia oncophora, Oesophagostomum radiatum* were not detected. No causative agent of *Dictyocaulus vivaparus* infection was detected in horses. The most common infection in dogs and cats was toxacariasis, and in mink the most common was infection wih *Uncinaria* spp. Along with this, no *Taenia solium* and *Diphyllobothrium latum* eggs were detected in dogs, and *Opisthorchis felineus, Taenia solium, Diphyllobothrium latum* were not detected in cats. After the use of preparative forms of albendazole in sick animals for 2–5 days, the presence of dead sexually mature nematodes and helminth eggs were noted in the feces of animals. The efficacy of albendazole drugs regarding helminthiasis in farm and domestic animals was 100%

Keywords: ruminants, horses, dogs, cats, minks, helminths

Introduction. Parasitic diseases of farm animals cause significant economic loses to the livestock industry and reduce the quality of their products (Taylor, 2012; Fitzpatrick, 2013; Majeed et al., 2015; Paliy et al., 2018).

Prevention and control of parasitic animal diseases is a mandatory step in general economic and veterinary activities in any agricultural enterprise (Thamsborg et al., 2010; Takeuchi-Storm et al., 2019; Paliy et al., 2020a). These measures should be carried out in a scientifically sound approach with an understanding of both the existing epizootic situation and the use of certain preparative forms (Henrioud, 2011; Paliy et al., 2020b).

The most common helminthiases that cause the most significant loses to livestock farming are fasciolosis, dicroceliosis, strongylatosis of the gastrointestinal tract, dictyocaulosis, etc. (Khanjari et al., 2014; Ezatpour et al., 2015). Ruminants are most often affected by various types of helminths, the most common of which are gastrointestinal nematodes, pulmonary helminths and hepatic trematodes.

These helminths can cause serious diseases, adversely affect animal productivity (Almeida et al., 2010; Charlier et al., 2015). Invasive diseases pose a great danger to young animals and poultry, as they are more likely to have mixed infections (Bogach et al., 2021). The treatment of parasitic diseases of farm animals remains an important problem in veterinary medicine (Bahk et al., 2018; Paliy et al., 2020a, 2020b; Dantas-Torres et al., 2020).

Science and practice have accumulated considerable experience in the use of various antiparasitic drugs in animal husbandry (Matos et al., 2015; Bustnes et al., 2006; Paliy et al., 2021b, 2021c). Thus, in 1976 in the United States, 'Smith Kline & French Laboratory' synthesized a drug called albendazole by selective search among benzimidazole derivatives.

Chemical name of albendazole: [5-(Propylthio)-1H-benzimidazol-2-yl] carbamic acid methyl ester is a very fine powder with a particle size of less than 50 μm, matte white, stable for two years when stored at room temperature. Among the many benzimidazole derivatives, albendazole is essentially the only drug that, in addition to nematodes, is effective against cestodes and trematodes (Seifu et al., 2019; Horton, 2000).

Its effect on helminths is quite complex, based on interference in the energy metabolism of parasites and inhibition of fumarate reductase — an enzyme of the Krebs cycle, which leads to lower glycogen levels and death of parasites from depletion, because the process is 30 times more intense in parasite cells than in their host cells. In some helminth species, albendazole inhibits protein synthesis (John and Petri, 2006).

Albendazole is used in veterinary medicine to treat helminthiasis in cattle, sheep, goats, horses, pigs, dogs, cats, fur animals and poultry (Demeler et al., 2009). For veterinary use, albendazole is available in powder, tablet, suspension and gel form. The new dosage form 'gel' significantly increases the effectiveness and speed of the drug. In addition, the shape of the gel facilitates the feeding of the drug, especially for cats and dogs, which protects animals from excessive stress and provides confidence that they have received the full dose.

The aim of the research was to determine in the comparative aspect the effectiveness of the use of

antiparasitic drugs in different forms with the content of albendazole in different species of animals.

Materials and methods. In accordance with the objectives of the study visual and microscopic methods were used in accordance with the practical manual (Vasil'kova, 1955). Intravital diagnostics of helminthiasis, determination of the number of helminth eggs, identification of pathogens by microscopic method were conducted (Halat et al., 2009). The mean intensity (MI) was determined by counting the number of helminth eggs in the field of view of the microscope.

The following animals were selected for the experiment: cows (n = 30), goats (n = 30), sheep (n = 30), horses (n = 10), dogs (n = 18), cats (n = 20), minks (n = 15) of different breeds and ages. During the clinical examination, the main attention was paid to fatness, general state and keeping of animals. To establish a preliminary diagnosis, fecal samples were taken from the animals for laboratory testing.

Two groups were formed of the animals diagnosed with helminthiasis: the first group of animals was given the drug No. 1 (1 ml of the drug contains the active substance albendazole — 100 mg; excipients: propylene glycol, xanthan gum. Drug form — gel for oral use, once individually with food, without diet). Animals of the second experimental group were given drug No. 2 (1 ml of the drug contains the active substance albendazole — 100 mg; excipients — up to 1 ml. Drug form — suspension).

The dosage of drugs for the treatment of animals was:

— cattle: for nematodes and cestodes — 0.75 ml per 10 kg of animal weight once, for trematodes — 1.0 ml per 10 kg of weight twice;

— small cattle: for nematodes and cestodes — 0.5 ml per 10 kg of animal weight once, for trematodes — 1.0 ml per 10 kg of weight twice;

— horses: for nematodes and cestodes — 0.5 ml per 10 kg of animal weight, once;

— dogs, cats, minks: for nematodes and cestodes — 2.5 ml per 10 kg of animal weight, once.

Clinical examination of animals was performed during and after treatment. 5, 10, 15, and 30 days after treatment, the results of studies were recorded based on the clinical examination of treated animals, detection of helminth eggs in fecal samples. Prevalence of infection after treatment and effectiveness of drugs were determined. The animals of the first and second groups were cared identically.

Examination of fecal samples for the presence of helminth eggs was performed by the Fülleborn flotation method and the Baermann method (Kotel'nikov, 1984) using a light microscope at magnification × 100. For identification, the detected helminth eggs were examined at magnification × 400.

Prevalence (P) of infection was determined by the formula:

$$P = \frac{X}{Y} \times 100,$$

where: P — prevalence of infection, %;
X — number of fecal samples in which helminth eggs were detected;
Y — total number of fecal samples;
100 — conversion factor into percent.

Effectiveness (E) of the drugs was calculated by the number of treated animals in percent that were completely free of parasites.

Experiments on animals were conducted following the recommendations 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), and in accordance with Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017).

The research program was reviewed and approved by the Bioethics Commission of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' in the current order.

Results and discussions. During the clinical examination of farm animals (cattle, small cattle, horses), we observed in some of them weight loss, indigestion, dullness of the coat. It was found that with a balanced diet, adult animals and young animals did not gain weight. Fecal samples were taken from all animals for laboratory testing (Table 1).

Table 1 — Prevalence (P) and mean intensity (MI) of helminthic infection in cattle (n = 30), sheep (n = 30), goats (n = 30), and horses (n = 10)

Animal species	Species of helminths	P, %	MI, the number of eggs, larvae of helminths in the microscope, pcs.
Cattle	*Ostertagia* spp.	11.4	3.5 ± 1.5
	Trichostrongylus colubriformis	0.0	—
	Cooperia oncophora	0.0	—
	Oesophagostomum radiatum	0.0	—
	Nematodirus spathiger	10.0	1.5 ± 0.5
	Dictyocaulus vivaparus	28.6	15.0 ± 3.0
	Fasciola hepatica	23.3	2.0 ± 1.0
Sheep	*Haemonchus contortus*	11.4	1.5 ± 0.5
	Nematodirus spathiger	13.3	1.0 ± 0.5
	Dictyocaulus filaria	30.0	11.5 ± 3.5
	Moniezia expansa	21.7	10.5 ± 3.5
Goats	*Haemonchus contortus*	20.0	1.5 ± 0.5
	Nematodirus spathiger	13.3	1.0 ± 0.5
	Dictyocaulus filaria	50.0	10.5 ± 3.5
	Moniezia expansa	6.6	8.5 ± 2.5
Horses	*Anoplochephala magna*	6.6	5.5 ± 0.5
	Dictyocaulus vivaparus	0.0	—

According to the results of research (Table 1) it was found that dictyocaulosis with prevalence 28.6% and fasciolosis with prevalence of 23.3% were the most common infections in cattle. In goats and sheep, the prevalence for dictyocaulosis was 50% and 30%, respectively.

Along with this, we did not detect pathogens *Trichostrongylus colubriformis*, *Cooperia oncophora*, *Oesophagostomum radiatum* in cattle. No causative agent of *Dictyocaulus vivaparus* infection was detected in horses.

Examination of domestic animals (dogs, cats, minks) revealed animals with signs of indigestion, changes in their physiological state. To diagnose diseases in animals, samples were taken and studied (Table 2).

Table 2 — Prevalence (P) and mean intensity (MI) of helminthic infection in dogs (n = 18), cats (n = 20), and minks (n = 15)

Animal species	Species of helminths	P, %	MI, the number of eggs, larvae of helminths in the microscope, pcs.
Dogs	*Toxocara canis*	38.8	1.8 ± 0.2
	Toxascaris leonina	33.3	13.6 ± 1.4
	Dipylidium caninum	27.7	1.5 ± 0.5
	Uncinaria spp.	11.1	1.0 ± 0.5
	Ancylostoma spp.	5.5	1.0 ± 0.5
	Trichocephalus spp.	4.4	0.5 ± 0.5
	Taenia solium	0.0	—
	Diphyllobothrium latum	0.0	—
Cats	*Opisthorchis felineus*	0.0	—
	Toxascaris leonina	35.0	13.6 ± 1.4
	Dipylidium caninum	20.0	12.5 ± 0.5
	Uncinaria spp.	10.0	1.0 ± 0.5
	Taenia solium	0.0	—
	Diphyllobothrium latum	0.0	—
Minks	*Uncinaria* spp.	20.5	1.0 ± 0.5
	Ancylostoma spp.	6.6	0.5 ± 0.5
	Toxocara canis	13.3	1.0 ± 0.5
	Dipylidium caninum	6.6	0.5 ± 0.5

According to the results of the studies presented in Table 2, toxocariasis was the most common in dogs and cats, and the prevalence was 38.8% and 35.0%, respectively. In mink, the *Uncinaria* spp. infection was the most common, and the prevalence was 20.5%. Eggs of *Taenia solium* and *Diphyllobothrium latum* were not found in dogs. *Opisthorchis felineus*, *Taenia solium*, and *Diphyllobothrium latum* were not found in cats.

In order to treat animals diagnosed with parasitic diseases, they were given anthelmintics with the active substance albendazole. The results of the study of the effectiveness of experimental drugs in helminthic invasions of cattle, small cattle, and horses for their treatment are presented in Table 3.

Table 3 — Effectiveness of anthelmintic drugs in cases of helminthic infection of farm animals

Animal group	Before treatment		After treatment							
			5th day		10th day		15th day		30th day	
	P, %	Average MI, psc.	P, %	Average MI, psc.	P, %	Average MI, psc.	P, %	Average MI, psc.	P, %	Average MI, psc.
Cattle										
Group I (n = 7)	100.0	6.25	15.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0
Group II (n = 5)	100.0	6.50	15.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Sheep										
Group I (n = 5)	100.0	29.40	20.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0
Group II (n = 5)	100.0	26.80	20.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0
Goats										
Group I (n = 4)	100.0	27.50	25.0	2.1	0.0	0.0	0.0	0.0	0.0	0.0
Group II (n = 4)	100.0	28.00	25.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0
Horses										
Group I (n = 5)	100.0	6.50	20.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0
Group II (n = 5)	100.0	6.00	20.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0

After the use of experimental drugs in farm animals (cattle, small cattle, and horses) from the 2nd to the 5th day in the feces of animals the presence of dead adult nematodes was noted. In some animals in the feces we found a small number of eggs of other helminths up to 5th day. In animals, there was an improvement in appetite, normalization of the digestive process. In some animals, especially goats, thirst was observed on the first day after application of the drugs. From the 10th day after the use of anthelmintics, in the samples of feces from animals adult helminths and their eggs were not detected.

The results of the study of the effectiveness of experimental drugs in helminthic infections of dogs, cats, and minks before and after their treatment are presented in Table 4.

After the application of experimental drugs in dogs, cats, and minks, 100% effectiveness was observed after 5th day. Therefore, albendazole-based drugs are highly effective against nematodes, cestodes and trematodes of animals.

The etiological factors of parasitic animal diseases are a number of both endo- and ectoparasites (Atehmengo and Nnagbo, 2014; Paliy et al., 2021a).

Table 4 — Effectiveness of anthelmintic drugs in cases of helminthic infection of domestic animals

Animal group	Before treatment		After treatment							
			5th day		10th day		15th day		30th day	
	P, %	Average MI, psc.	P, %	Average MI, psc.	P, %	Average MI, psc.	P, %	Average MI, psc.	P, %	Average MI, psc.
Dogs										
Group I (n = 5)	100.0	16.80	20.0	7.5	0.0	0.0	0.0	0.0	0.0	0.0
Group II (n = 5)	100.0	16.75	20.0	7.0	0.0	0.0	0.0	0.0	0.0	0.0
Cats										
Group I (n = 5)	100.0	15.80	20.0	6.5	0.0	0.0	0.0	0.0	0.0	0.0
Group II (n = 5)	100.0	15.50	20.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0
Minks										
Group I (n = 3)	100.0	5.50	33.3	0.5	0.0	0.0	0.0	0.0	0.0	0.0
Group II (n = 3)	100.0	5.00	33.3	0.5	0.0	0.0	0.0	0.0	0.0	0.0

Changes in the conditions of their existence in the environment directly affect the manifestation of the invasion of the final host (Bogach et al., 2020). The spread of parasitic diseases is facilitated by the uncontrolled reproduction of stray animals, as well as the contamination of pasture with helminth eggs by sick animals (Paliy et al., 2019; Verheyden et al., 2020).

During diagnostic tests we found a number of helminths in farm and domestic animals. Cases of bovine diseases caused by *Trichostrongylus colubriformis* have been reported in many countries, including Iran, Japan, Thailand, South Korea, China, the United States, and Australia (Ghadirian, 1977; Shahbazi et al., 2012).

In Ukraine, cases are registered sporadically. *Cooperia oncophora* is a gastrointestinal nematode of the genus *Cooperia*, which belongs to the group of trichostrongylides parasitizing in ruminants (Amarante et al., 2014). Nematodes of the genus *Cooperia* parasitize in the small intestine of domestic and wild ruminants. In fact, *Cooperia* spp. considered less pathogenic than other species of bovine and ovine nematodes. A large number of these helminths can significantly reduce the productivity of host animals, as the invasion is associated with lack of appetite and insufficient growth of animals (Ramünke et al., 2018).

Generally, higher temperatures and humid environments are more favorable for these parasites (Gibbons, 1981). The parasite *Oesophagostomum radiatum* is a nematode that causes esophagostomosis. According to studies by other scientists, sheep are most often affected by this parasite in mountainous areas, sometimes even 100% (Magomedov et al., 2014).

The parasite *Taenia solium*, porcine tapeworm, is not detected in the feces of dogs and cats, as the dog is only an intermediate host (García et al., 2003).

The causative agent of diphyllobothrium is *Diphyllobothrium latum*, an intestinal parasitic zoonotic invasion. The most common cause of diphyllobothriosis in humans is the consumption of fish that contain invasive larvae of the genus *Diphyllobothrium*, which cause invasive disease (Ito and Budke, 2014; Le Bailly and Bouchet, 2013). *Diphyllobothrium latum* eggs were not found in the feces of dogs and cats, as they are not the definitive hosts of this helminth.

Opisthorchiasis is caused by *Opisthorchis felineus*. The main source of invasion is humans. Of the various carnivorous species (definitive hosts), the fur animal otter plays an important role in the spread of opisthorchiasis, which has close contact with the intermediate (freshwater mollusk *Bithinia leachi*) and additional hosts (carp) (Pakharukova and Mordvinov, 2016).

According to other researchers (Glamazdin et al., 2013), albendazole and its dosage forms are widely used to control animal helminthiasis. Our results are consistent with the results of other researchers regarding the high anthelmintic effect of drugs with the active substance albendazole.

Conclusions. Veterinary drugs based on albendazole in the form of gel and suspension are effective in the treatment of farm and domestic animals with helminthic infections, they are well tolerated and do not give side effects in the clinical state of animals.

Albendazole-based antiparasitic drugs (gel/suspension) are effective in the treatment of cattle, sheep, goats, horses, dogs, cats, and minks with nematode, cestode and trematode infections.

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PECULIARITIES OF FORMATION OF INTESTINAL BACTERIOCENOSES IN CALVES IN DIFFERENT TECHNOLOGICAL PERIODS OF RAISING

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Summary. The article presents data on the peculiarities of the bacteriocenoses formation in calves in different technological periods of raising and different animal housing systems. It has been shown that in calves up to three days of age the quantitative content of microorganisms is minimal. The amount of bifidobacteria and lactobacilli does not exceed 3 log CFU/g. In calves older than three days of age, their level increased, and depending on the method of housing, ranged from 5 log CFU/g (in calves with group housing) to 8 log CFU/g (in calves with individual housing). The microbiocenosis in calves with an individual housing system was characterized by a more constant and less variable composition of *Escherichia coli* ($4 \pm 0.8\times10^2$ CFU/g) and bacillary spore microflora ($3 \pm 1.3\times10^1$ CFU/g). In case of the group housing of calves, the content of bifidobacteria was lower ($20.9 \pm 5.5\times10^6$ CFU/g), and the content of *E. coli* and saprophytic microorganisms of the genus *Bacillus* was high and more variable ($30 \pm 20.4\times10^5$ CFU/g and $31 \pm 11.3\times10^2$ CFU/g respectively). In calves older than fifteen days of age, the amount of lactobacilli ranged from 6 to 8 log CFU/g, and the number of bifidobacteria ranged from 7 to 10 log CFU/g. Thus, to exclude dysbiotic disorders, it is necessary to maintain the optimal composition and quantitative level of the main microflora of the intestinal tract, in particular the number of lactobacilli should be at least 6 log CFU/g, bifidobacteria at least 7 log CFU/g, *E. coli* no more than 7 log CFU/g (except for calves under three days of age)

Keywords: dysbiosis, normal intestinal microflora

Introduction. The body of animals is exposed to a range of adverse factors that alter the normal functioning of main vital systems and cause the development of dysbiotic disorders, including the gastrointestinal tract. This and a number of other factors are associated with an increase in the incidence of enteritis in livestock farms (Batrakov et al., 2021; Trofimov, 2019; Pudovkin et al., 2019; Efimova and Udalova, 2011; Shakhov, Sashnina and Erina, 2016; Avdeeva et al., 2016; Usachev, 2010; Burova and Blokhin, 2017; Andreeva et al., 2015; Maslianko et al., 2013; Lapinska, 2013; Kalinichenko, Korotkykh and Tishchenko, 2016; Basova, et al., 2016).

In most farms, the disease of newborn calves reaches 90–120%, i. e. calves are mostly sick on the 2nd–3rd days of life and they get sick again on the 5th–7th days after birth (Batrakov et al., 2021). Complications after antibiotic therapy are equally important in the development of intestinal dysbiosis. During treatment, large doses of antibiotics are often prescribed, after which the occurrence or deepening of pre-existing dysbacteriosis occurs in almost each case (Burova and Blokhin, 2017).

Feeding calves with milk and colostrum containing antibiotics promotes the spread of resistant microorganisms and disrupts the formation of adequate specific and nonspecific response of the organism. Even microdoses of antimicrobial drugs disrupt the formation of a normal intestinal microbiocenosis (Pudovkin et al., 2019).

Normoflora competes for pathogens, and the mechanisms of inhibition of their growth are quite diverse: selective binding of surface receptors of cells, especially epithelial; pronounced antagonism against pathogenic species (Efimova and Udalova, 2011).

Thus, the in-depth study of bacteriocenoses in animals is a topical issue that will enable to recommend in practice more effective means and measures to combat and prevent infectious diseases.

Knowledge of the qualitative composition of the normoflora and the dynamics of quantitative changes in bacteriocenoses can predict the clinical manifestations of dysbiotic disorders, the development of enteritis, timely take measures to maintain a stable intestinal bacteriocenosis, or its correction.

Therefore, the **aim of the research** was to study and analyze the taxonomic composition of bacteriocenoses of the gastrointestinal tract in calves in different technological periods of raising.

Materials and methods. The research was conducted in the Laboratory for the Study of Bacterial Diseases of Animals of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine) in 2021. To determine the normocenosis, fecal samples and smears from the intestinal tract of calves were studied in different technological periods of raising.

Samples were taken from calves up to three days of age, in colostrum and milk periods under different housing systems. There were no differences in the parameters of the microclimate, rations, except for the system of housing (group and individual) for calves. Animal studies have been conducted taking into account the basic principles of bioethics. Housing, caring for animals and feeding them was carried out following the norms and rations. Quantitative and qualitative diet in animals with different systems of housing did not differ.

The material for studying the microbiocenosis of the colon cavity was its content. The contents of the intestine were collected from calves with sterile spatulas in sterile containers. The material was delivered to the laboratory and examined no later than 2 hours after selection. To determine the quantitative value of microorganisms we made serial ten-fold dilutions of homogenized material in sterile isotonic sodium chloride solution from 10^{-1} to 10^{-10}.

From each tube of the titration series, 1 cm^3 of homogenate was cultured on optimal nutrient media for each species of microorganisms and incubated under optimal temperature conditions and periods. Simple and selective nutrient media produced by Farmaktiv LLC (Ukraine) and HiMedia Laboratories Prv. Ltd. (India) were used. Endo, Ploskirev, Levin, MacConkey mediums, bismuth-sulfite agar, Olkenitsky medium, selenite broth (for the accumulation of salmonella), Simons medium (for the differentiation of enterobacteria by their property to use sodium citrate as the only source of carbon) were used to isolate enterobacteria. For staphylococci we used yolk-salt agar, Chistovich medium; for streptococci — media containing glucose (1%), blood (5–10%) and serum (10–20%); for fungi and yeast — Saburo and Suslo-agar; for anaerobes — Kitt-Tarotzi, Wilson-Blair agar, L.D. agar with esculin (for anaerobes), Voget-Fredett agar. Blaurock medium was used to isolate bifidobacteria, and LactoBacagar was used to isolate lactobacilli. To determine the hemolytic activity of microorganisms we used 5% blood agar, coagulase activity — dry citrate rabbit plasma produced by PJSC 'Pharmstandard-Biolik' (Ukraine).

After incubation of the cultures on the media under optimal conditions, the colonies grown from each dilution were counted. The population level of microorganisms was indicated in the decimal logarithm of the indicator — log CFU/g (colony forming units in 1 g of feces).

To determine the number of microorganisms we took into account the degree of dilution, the number of colonies that grew, the inoculation dose. The number of CFU/g was calculated by the formula:

$$M = \frac{N}{V} \times 10^{n+1},$$

where: M — the number of microorganisms in 1 g of feces;
N — the average number of colonies in 1 bacteriological cup;
V — the volume of suspension applied during plating on the agar surface;
10^{n+1} — dilution, from which the plating was carried out.

Bacteria were identified according to Bergey's Manual of Systematics Bacteriology (Goodfellow et al., 2012).

The obtained results were processed by methods of variation statistics using Microsoft Excel for Windows 2010. To compare mean values Student's *t*-test was used (Van Emden, 2019).

Research results. It was found that in calves up to three days of age the quantitative content of microorganisms is minimal. The number of bifidobacteria and lactobacilli did not exceed $3 \pm 1.8 \times 10^3$ CFU/g, *E. coli* and enterococci — $4 \pm 0.8 \times 10^2$ CFU/g, saprophytic microorganisms of the genus *Bacillus* and yeast-like fungi of the genus *Candida* — $7 \pm 2.1 \times 10^2$ CFU/g.

The level of lactobacilli on the 4th–14th days of age increased to $190 \pm 14.5 \times 10^6$ CFU/g under the group housing, and $11 \pm 2.8 \times 10^7$ CFU/g under the individual housing. The level of bifidobacteria on the 4th–14th days of life increased to $2 \pm 1.5 \times 10^7$ CFU/g under the group housing, and $4 \pm 0.7 \times 10^8$ CFU/g under the individual housing. In the feces of animals aged 15–30 days, the content of bifidobacteria increased to $77 \pm 3.2 \times 10^7$ CFU/g under the group housing, and $141 \pm 41.8 \times 10^8$ CFU/g under the individual housing.

E. coli was detected during the entire raising period in 100% of cases, from $2 \pm 1.0 \times 10^2$ CFU/g up to $19.6 \pm 1.5 \times 10^6$ CFU/g. The content of *E. coli* in animals older than three days of age increased from $2 \pm 1.0 \times 10^2$ CFU/g to $30 \pm 20.4 \times 10^5$ CFU/g. The level of enterococci in the feces of animals older than three days of age ranged from $6 \pm 0.3 \times 10^2$ CFU/g to $41 \pm 1.0 \times 10^2$ CFU/g.

The highest content of enterococci was in the body of calves of 15–30 days of age, their number was $129 \pm 43 \times 10^3$ CFU/g under the group housing, and $4 \pm 1.0 \times 10^5$ CFU/g under the individual housing. It was observed that under the group housing of animals, the number of enterococci was more variable.

Representatives of the genus *Bacillus* and yeast-like fungi of the genus *Candida*, in comparison with other bacteria, were present in the studied material in smaller quantities. During the colostrum period, the number of microorganisms of the genus *Bacillus* did not exceed $310 \pm 41.31 \times 10^1$ CFU/g, in animals of 15–30 days of age — $17 \pm 8.54 \times 10^3$ CFU/g. The content of yeast-like fungi of the genus *Candida* did not exceed $23 \pm 17.1 \times 10^2$ CFU/g.

Staphylococci were not isolated in all technological groups. Their content was minimal (from 0 to $6 \pm 2.1 \times 10^3$ CFU/g) in calves up to three days of age.

Sulfite-reducing clostridia (*Clostridium* spp.) were not detected in the intestinal contents in 100% of cases. Their minimum content (from 0 to $5 \pm 2.3 \times 10^3$ CFU/g) was in calves of the colostrum period. The maximum content of sulfite-reducing clostridia was in calves of 15–30 days of age, but their number did not exceed $30 \pm 12.3 \times 10^3$ CFU/g.

The content of bifidobacteria and lactobacilli was more stable in calves kept individually in boxes. Calves kept in groups had higher levels of saprophytic microorganisms of the genus *Bacillus*. Their content in calves under the group housing was from 2–4 log CFU/g, and under the individual housing did not exceed 3 log CFU/g. The concentration of yeast-like fungi of the genus *Candida* in both groups did not exceed 3 log CFU/g.

Thus, the microbiocenosis in calves under the individual system of their housing is characterized by a high level of bifidobacteria, lactobacilli and a more

constant and less variable composition of *E. coli* and bacillary spore microflora. Under the group housing of calves, the content of lactobacilli was lower and the content of *E. coli* was high. It was observed that the range of quantitative indicators of *E. coli*, clostridia, staphylococci, enterococci, and saprophytic spore bacteria was more variable. Under the group housing, low levels of bifidobacteria were observed, the concentration of which did not exceed 8 log CFU/g. According to the results of research, the dynamics is observed that the higher the number of anaerobic spore-forming microorganisms, the lower the number of lactobacilli, the lower the number of bifidobacteria, the higher the number of *E. coli*. Thus, there is a correlation between the quantitative indicators of bifidobacteria and *E. coli*, between the high content of *E. coli* and low content of lactobacilli, between the high content of clostridia and low content of lactobacilli.

The results of bacteriological studies on the peculiarities of the formation of bacteriocenoses in calves in different technological periods of raising and for different systems of housing are shown in Table 1.

Table 1 — Dynamics of the quantitative composition of the microflora of the large intestine of calves in different technological periods of raising

Indicators, CFU/g		**Age of calves, day**					
		1–3		**4–14**		**15–30**	
Form of housing		**Individual (n = 10)**	**Group (n = 10)**	**Individual (n = 10)**	**Group (n = 10)**	**Individual (n = 10)**	**Group (n = 10)**
Lactobacilli *Lactobacillus*	log	2	2	7–8	6–8	6–8	6–7
	M ± m	$5 \pm 1.2\times10^{2}$	$7 \pm 2.2\times10^{2}$	$11 \pm 2.8\times10^{7*}$	$190 \pm 14.5\times10^{6}$	$192 \pm 93.6\times10^{6*}$	$82 \pm 11.5\times10^{6}$
Bifidobacteria *Bifidobacterium*	log	3	3	8	7	8–10	7–8
	M ± m	$3 \pm 1.8\times10^{3}$	$1 \pm 0.8\times10^{3}$	$4 \pm 0.7\times10^{8*}$	$2 \pm 1.5\times10^{7}$	$141 \pm 41.8\times10^{8*}$	$77 \pm 3.2\times10^{7}$
E. coli	log	2	2	5	5–6	5–7	6–7
	M ± m	$4 \pm 0.8\times10^{2}$	$2 \pm 1.0\times10^{2}$	$6 \pm 1.2\times10^{5*}$	$30 \pm 20.4\times10^{5}$	$113 \pm 52\times10^{5*}$	$19.6 \pm 1.5\times10^{6}$
Staphylococci *Staphylococcus*	log	0–3	0–3	0–3	2–4	2–5	3–5
	M ± m	$2 \pm 1.4\times10^{3}$	$6 \pm 2.1\times10^{3}$	$6 \pm 2.2\times10^{3*}$	$15 \pm 4.6\times10^{3}$	$196 \pm 96.6\times10^{3}$	$191 \pm 36.7\times10^{3}$
Sulfite-reducing clostridia (*Clostridium*)	log	0–2	0–2	0–2	0–3	0–4	0–4
	M ± m	$3 \pm 1.0\times10^{2}$	$6 \pm 2.1\times10^{2}$	$6 \pm 1.8\times10^{2*}$	$5 \pm 2.3\times10^{3}$	$16 \pm 8.7\times10^{3*}$	$30 \pm 12.3\times10^{3}$
Enterococci (*Enterococcus*)	log	2	2	2	2–3	5	3–5
	M ± m	$4 \pm 0.7\times10^{2}$	$1 \pm 0.8\times10^{2}$	$6 \pm 0.3\times10^{2*}$	$41 \pm 1.0\times10^{2}$	$4 \pm 1.0\times10^{5*}$	$129 \pm 43\times10^{3}$
Saprophytic microorganisms of the genus *Bacillus*	log	0–1	0–1	1–2	1–3	2–3	3–4
	M ± m	$3 \pm 1.3\times10^{2}$	$7 \pm 2.1\times10^{2}$	$61 \pm 2.1\times10^{1*}$	$310 \pm 41.3\times10^{1}$	$17 \pm 6.7\times10^{2*}$	$17 \pm 8.54\times10^{3}$
Yeast-like fungi of the genus *Candida*	log	0–2	0–2	0–2	2–3	2	2–3
	M ± m	$1.6 \pm 0.5\times10^{2}$	$2.2 \pm 1.0\times10^{2}$	$2.3 \pm 1.3\times10^{2*}$	$24 \pm 15.7\times10^{2}$	$7 \pm 9.7\times10^{2*}$	$23 \pm 7.1\times10^{2}$

Note. * — $p \le 0.05$ in relation to the group method of animal housing.

Knowledge of the qualitative composition of the normoflora and the dynamics of quantitative changes in bacteriocenoses can predict the clinical manifestations of dysbiotic disorders, the development of enteritis, timely take measures to maintain a stable intestinal bacteriocenosis, or its correction.

According to the results of these studies it can be noted that to exclude dysbiotic disorders it is necessary to maintain the optimal composition and quantitative level of the main microflora of the intestinal tract, in particular the number of lactobacilli should be at least 6 log CFU/g, bifidobacteria at least 7 log CFU/g, the amount of *E. coli* not more than 7 log CFU/g (except for calves up to three days of age).

Discussion. According to the analysis of literature data, no uniform normative indicators on the composition of the normal flora of the intestinal tract in calves in different technological periods of raising and under different housing systems were found (Batrakov et al., 2021; Trofimov, 2019; Pudovkin et al., 2019; Efimova and Udalova, 2011; Shakhov, Sashnina and Erina, 2016; Avdeeva et al., 2016; Usachev, 2010; Burova and Blokhin, 2017; Andreeva et al., 2015; Maslianko et al., 2013; Lapinska, 2013; Kalinichenko, Korotkykh and Tishchenko, 2016; Basova, et al., 2016). Of course, individual housing of animals in all respects is better than group one, in particular the feed is better normalized and dosed, no competition and negative impact between weak and strong calves, minimal stress (Trofimov, 2019; Shakhov, Sashnina and Erina, 2016).

According to the results of our research, it was concluded that the content of bifidobacteria and

lactobacilli in calves kept individually in boxes was more stable and high. In calves kept in groups, higher and variable levels of saprophytic microorganisms of the genus *Bacillus*, yeast-like fungi of the genus *Candida*, enterococci, *E. coli* and clostridia were observed.

Batrakov et al. (2021) in their studies noted that a particularly important factor in the individual housing of calves is significant deterioration of conditions for bacterial and viral contamination of the body of calves and the environment. With this technology, calves have the best specific resistance, and each calf acquires a specific microflora, with which it functions and develops normally.

Data of Basova et al. (2016) show that in the early postnatal period intestinal microorganisms predominate, the content of which reached 9–10 log CFU/g, the content of enterococci and enterobacteria was compared with the number of symbiotic microorganisms (bifidobacteria and lactobacilli) and was within 3–4 log CFU/g. Fungi of the genus *Candida*, clostridia and hemolytic forms of bacteria were isolated from the feces of calves, the number of which ranged 1–2 log CFU/g. By the 30th day of life in the feces of calves of all groups there was a decrease in lactose-positive escherichia, increased number of lactobacilli to 5 log CFU/g and saprophytic staphylococci up to 4 log CFU/g. Clostridia were not isolated from feces of one-month-old calves.

In contrast, Efimova and Udalova (2011) in their monograph noted that the normal composition of bifidobacteria for animals is from 7 to 10 log CFU/g, lactobacilli — from 5 to 7 log CFU/g, clostridia — from 4 to 5 log CFU/g, escherichia — up to 7 log CFU/g, enterococci — from 6 to 7 log CFU/g, staphylococci — from 3 to 4 log CFU/g, microorganisms of the genus *Bacillus* — from 3 to 4 log CFU/g, fungi — up to 3 log CFU/g, enterobacteria — from 0 to 5 log CFU/g.

According to the results of our research, it was found that in calves up to three days of age the quantitative content of microorganisms is minimal. The number of bifidobacteria and lactobacilli did not exceed 3 log CFU/g, clostridia — from 0 to 2 log CFU/g, escherichia — 2 log CFU/g, enterococci — 2 log CFU/g, staphylococci — from 0 to 3 log CFU/g, microorganisms of the genus *Bacillus* — from 0 to 1 log CFU/g, fungi — from 0 to 2 log CFU/g.

Usachev (2010) in his work noted that the enteric microbiocenosis in lambs is formed by the 10th day of age. Thus, the composition of bifidobacteria for day-old animals was 2–4 log CFU/g, lactobacilli — 2–3 log CFU/g, escherichia — 2 log CFU/g, enterococci — 2–3 log CFU/g, microorganisms of the genus *Bacillus* — 1 log CFU/g, fungi — 1 log CFU/g. The composition of bifidobacteria for animals of 10 days of age was 9–10 log CFU/g, lactobacilli — 7–8 log CFU/g, *E. coli* — 7 log CFU/g, enterococci — 5–6 log CFU/g, microorganisms of the genus *Bacillus* — 5 log CFU/g, fungi — 3 log CFU/g.

Andreeva et al. (2015) in newborn calves isolated bifidobacteria in the amount from 5.8 ± 0.1 log CFU/g to 6.0 ± 0.1 log CFU/g, lactobacilli — from 3.6 ± 0.1 log CFU/g to 3.8 ± 0.05 log CFU/g. In calves, the number of bifidobacteria reached maximum values at three months of age (7.9 ± 0.1 log CFU/g); the number of lactobacilli — at 30 days of age (7.0 ± 0.1 log CFU/g).

There are scientific studies that show that the intestinal microflora in newborns is formed in 43% of mothers, including the vagina, and 28% of the environment, including air and floor units (Diao, Zhang and Fu, 2019; Bi et al., 2019). In addition, it is noted that feeding regimens affect the transmission of bacteria to newborns from mothers and the environment (Bi et al., 2019). It has been reported that microbial colonization of mammalian intestines begins to form before birth, but these observations are contradictory due to problems with reliable sampling and analysis of low-content microbiota (Alipour et al., 2018; Mayer et al., 2012). The microbial composition of the intestinal microflora resembles an oral microbiocenosis, not fecal or vaginal. During the first day after birth, microorganisms of the genus *Escherichia*, *Shigella*, and *Clostridum* settle in the rectum. The microflora changes within seven days, after that its composition already resembles the microbiocenosis of the rectum of adult animals (Alipour et al., 2018). In general, the intestinal microbiocenosis of calves undergoes dynamic changes during the first twelve weeks of life and this is due to the peculiarities of the digestive system of ruminants (Uyeno, Sekiguchi and Kamagata, 2010).

In our research, we studied changes in the normal flora of the intestine over 30 days. It was found that the composition of the microflora depended on the method of housing. *E. coli* was detected in 100% of cases during the entire period, starting from the first day of life. The content of *E. coli* in animals older than three days of age increased from 2 ± 1.0×10^2 CFU/g to 30 ± 20.4×10^5 CFU/g, clostridia — from 3 ± 1.4×10^2 CFU/g to 30 ± 12.3×10^3 CFU/g.

Conclusions. 1. It was found that in calves up to three days of age the quantitative content of microorganisms is minimal. The amount of bifidobacteria and lactobacilli did not exceed 3 log CFU/g. In calves older than three days of age, their level increased, and depending on the method of housing, ranged from 6–8 log CFU/g in calves under the group housing, 7–8 log CFU/g — in calves under the individual housing.

2. The microbiocenosis in calves of 4–14 days of age under the individual housing system was characterized by a high level of bifidobacteria (4.2 ± 0.7×10^8 CFU/g), lactobacilli (11.1 ± 2.8×10^7 CFU/g), the more constant and less variable composition of *E. coli* (6 ± 1.2×10^5 CFU/g) and bacillary spore microflora (61 ± 2.1×10^1 CFU/g).

3. Under the group housing of calves of 4–14 days of age, the content of bifidobacteria was lower (2 ± 1.5×10^7 CFU/g), and the content of *E. coli* and saprophytic microorganisms of the genus *Bacillus* was high and more variable (30 ± 20.4×10^5 CFU/g and 310 ± 41.3×10^1 CFU/g respectively) than in the individual housing system.

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Part 2. Biotechnology

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STUDY OF ISOLATE OF INFECTIOUS RYNOTRACHEITIS VIRUS IDENTIFIED IN THE ACUTE COURSE OF THE DISEASE

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Summary. Isolation of the virus from biological material from a two-month-old calf with pathology of the respiratory system from a herd with a morbidity rate of 48% was performed. After detection the presence of IRT antigens in the lungs of the dead animal, the pathogen was isolated on a continuous culture of calf kidney cells, where a characteristic cytopathic effect was observed. The genetic material of the bovine herpesvirus type 1 (Bovine herpesvirus-1, BHV-1) was identified by polymerase chain reaction in the test sample. The virus isolate was adapted to continuous cell cultures of calf kidneys, sheep kidney, cow embryo lung and calf trachea, and the most suitable biological system was determined, where adsorption and reproductive properties of the virus were more pronounced. It was found that the highest titer of infectious activity of BHV-1 isolate (6.1 lg TCD_{50}/cm^3) was obtained on continuous culture of lung embryonic cells of a cow embryo after its reproduction during three consecutive passages (observation period)

Keywords: virus isolation, herpesvirus type 1, infectious activity, infectious rhinotracheitis, cell culture, cytopathic effect

Introduction. Pathogens of bovine respiratory diseases, including infectious rhinotracheitis (IRT) virus, are known to have significant adverse effects on animal health and productivity (Headley et al., 2018), and to limit international trade in animals worldwide (Fernandes et al., 2018).

Due to the fact that according to domestic and foreign researchers (Headley et al., 2018; Kornieikov et al., 2019; Barrett et al., 2018) a complex of viral diseases of the respiratory group (infectious rhinotracheitis (IRT), viral diarrhea (VD), parainfluenza-3 (PI-3) and respiratory syncytial infection (RSI)) occurs both in the form of monoinfections and in their associations, their timely diagnosis is of paramount importance.

Thus, several methods are commonly used to detect IRT-infected animals, namely enzyme-linked immunosorbent assay (ELISA), neutralization reaction (NR), polymerase chain reaction (PCR) (OIE, 2018), and immunofluorescence assay (IFA).

It should be noted that modern diagnostic enzyme-linked immunosorbent assay systems are used in two ways — to assess the level of total antibodies and to differentiate animals immunized with marker vaccines from those infected with an epizootic strain of the virus (Van Oirschot et al., 1997).

Regarding PCR and IFA, these methods allow to effectively identify the etiological cause in sick animals and to differentiate from other diseases (Maidana et al., 2020; Hou et al., 2017). However, effective non-costly means for diagnostics and specific prevention of the disease are needed to implement large-scale IRT control measures in cattle herds. For this purpose it is necessary to use strains of the pathogen circulating on the territory of Ukraine. Knowledge of the biological features of this pathogen will allow to give epizootological and biotechnological answers.

Analysis of local strains allows to study the peculiarities of the course, to establish local associations of microorganisms and to conduct effective immunoprophylaxis (Headley et al., 2018; Barrett et al., 2018). Thus, Brock et al. (2020) studied the dependence of the prevalence of infectious rhinotracheitis of the first type in different age groups of vaccinated and unvaccinated livestock population.

The study by Barrett et al. (2018) in 161 meat farms established seroprevalence of the herd and the relationship in the associated course with viral diarrhea and neosporosis. Depending on the characteristics of the association of pathogens, an individual program of disease control in the herd, immunoprophylaxis schemes, risk assessment of the introduction of pneumoenteritis and measures to prevent the spread of disease are developed (Benavides et al., 2020). Knowledge of the etiological component of pneumoenteritis in cattle herds allows to develop individual effective eradication programs, including programs at the state level with the involvement of state and private manufacturers (Hostnik et al., 2021).

However, all these works are impossible without isolation of the pathogen from animals, preceded by indication and differentiation from other viruses and types (in the case of herpesvirus), isolation from biological material from animals, identification by molecular-genetic methods and subsequent adaptation and study of biological properties on continuous cell cultures (Dagalp et al., 2020; d'Offay, Mock and Fulton, 1993). In the future, this will allow the development of effective domestic vaccines and diagnostic systems, such as ELISA based on monoclonal antibodies (Teixeira et al., 2001), using the experience of previous developments of researchers of NSC 'IECVM'.

The aim of the study was to isolate the pathogen of bovine respiratory disease of viral etiology with further study of its biological properties in the laboratory.

Materials and methods. The analysis of the epizootic situation in the farm was carried out with the help of clinical and epizootological examination of the cattle herd, the results of which revealed animals with signs of respiratory disease. Samples of biological material were taken from laboratory animals for laboratory confirmation of the diagnosis, namely nasal washes and serum from calves up to 6 months of age, as well as pathological material (lungs, spleen, mesenteric and mediastinal lymph nodes) from dead animals with signs of respiratory disease (2–6 months of age calves).

Indication and identification of viruses in biological material. In the laboratory, biological material from animals was studied using serological (bovine serum), virological and molecular-genetic methods (pathological and clinical material from cattle).

Detection of specific antibodies to viruses of infectious rhinotracheitis (IRT), viral diarrhea (VD), parainfluenza-3 (PI-3), and respiratory syncytial infection (RSI) was performed using enzyme-linked immunosorbent assay (IRT, VD and RSI) using test-systems manufactured by IDEXX (France) and hemagglutination inhibition (HI) assay (PI-3) using a test system manufactured by SRE 'Veterinary Medicine' LLC (Ukraine). Indication and identification of antigens of IRT, VD, PI-3, and RSI viruses was performed by immunofluorescence assay (IFA) following the generally accepted method, which included preparing of touch smears from the organs of a dead animal (spleen, lungs and lymph nodes), as well as nasal washes of sick animals. After fixation they were stained with specific fluorescent immunoglobulins and washed in phosphate-buffered saline with the addition of 1% solution of Evans blue.

Subsequently, the smears were examined under a fluorescent microscope at a magnification of ×40 at the eyepiece ×10, in the blue-green spectrum of rays at a wavelength 490–510 nm. The reaction was recorded by the intensity of the fluorescent glow of the cells (Syurin, Belousova and Fomina, 1984). Under the conditions of obtaining a positive result of the study of biological material in IFA, virological studies on cell culture were conducted.

Cell cultures. Continuous cultures of cow embryonic lung cells (CELC), calf kidney (CK), sheep kidney (ShK-2) and calf trachea (CT) stored in a cryobank of the NSC 'IECVM' were used as sensitive biological test objects for virus isolation and study of biological properties of pathogens. DMEM and 199 mediums were used as growth medium in equal proportions with the addition of 10% native inactivated bovine serum and antibiotics (penicillin 100 IU/cm^3 and streptomycin 100 μg/cm^3) (Krasochko et al., 2016). DMEM and 199 nutrient medium in a 1:1 ratio without the addition of bovine serum was used as a supportive medium.

Isolation of viruses on cell culture. The essence of this method is to detect the cytopathogenic effect (CPE) of the virus isolate on cell culture with its subsequent identification. For this purpose, pieces of organs from a dead animal were homogenized to form a 20% suspension in phosphate-buffered saline and centrifuged at 3000 rpm for 20 min.

The supernatant was transferred to vials and 200 IU/cm^3 of benzylpenicillin sodium and 200 μg/cm^3 of streptomycin sulfate were added. After incubation for 2–4 h at 4.0 ± 0.1°C, the resulting suspension was used to infect sensitive cell culture.

The study was performed in test tubes. 0.2 cm^3 (1:10) of each test material was added to five tubes with a monolayer of continuous CK cell culture after removal of the nutrient medium. Tubes with material were incubated at 37.0 ± 0.5°C for one hour, after that the test fluid was removed from each tube and 2.0 cm^3 of supportive nutrient medium for cell culture was added.

As a control, five tubes with intact cell culture were used, in which the nutrient medium was similarly replaced with a supportive one. Experimental and control tubes were incubated at a temperature of 37.0 ± 0.5°C for 5–6 days. The cytopathic effect of field viral isolates was recorded daily using a light inverted microscope (Syurin, Belousova and Fomina, 1984).

In the absence or weak manifestation of cytopathic action in cell culture in the first passage, the second and third passages were carried out with an interval of 5–6 days. The material of each passage was stored at a temperature of minus 20.0 ± 2.0°C. The result of the study of pathological material was considered negative if after the third passage the CPE of virus was not detected.

In the presence of a stable CPE of the same type in cell culture in the last passage the accumulation of viral culture biomass was conducted for further identification of the viral field isolate by polymerase chain reaction.

Virological studies of the selected virus isolate were performed on cell cultures by determining the dose dependence of infection and susceptibility of continuous cell lines (CEL, CK, ShK-2, and CT), as well as by determination the level of infectious activity of pathogens and their rate of accumulation.

The susceptibility of cell cultures to the virus was determined by the degree of cytotopathic effect, which was assessed in crosses (from '++++' to '–'), where '–' — complete absence of CPE, '+' — violation of cell

morphology and integrity of the monolayer, not more than 25%, '++' — affected no more than 50% of monolayer cells, '+++' — no more than 75% of monolayer cells affected, and '++++' — complete cell degeneration and destruction of the monolayer.

In each experiment, four samples of continuous cell culture were used. The integrity of the cell membrane was determined using 0.2% trypan blue solution (in the absence of cell coloring) (Krasochko et al., 2016). In addition, the rate of virus accumulation and its infectious activity in the 3rd passage were taken into account. Infectious activity of viral isolates was determined by the level of cytopathic effect (CPE) by titration in one-day cell culture with ten-fold dilution of the virus. The titer of infectious activity was calculated by the Reed and Mench method (Syurin, Belousova and Fomina, 1984).

Identification of the genetic material of the IRT virus by PCR. Detection of genetic material of the virus was performed by polymerase chain reaction (PCR).

Extraction of nucleic acid from the material was performed using the sorbent method in our modification, which is based on the lysis of guanidine cells by thiocyanate in the presence of Triton X-100, sorption of DNA into silicon dioxide, final washing with a solution containing chloroform, and elution of DNA into a solution with low ionic strength (Boom et al., 1990; Stegniy and Gerilovych, 2014).

The amplification reaction to detect the genetic material of the IRT virus was performed using the polymerase chain reaction method using primer systems flanking a section of glycoprotein E-gene length of 325 bp herpesvirus type 1 (Weiss et al., 2015):

BoHV-1 gE_F GCCAGCATCGACTGGTACTT
BoHV-1 gE_R GCACAAAGACGTAAAGCCCG

PCR conditions provided for 40 cycles of initial denaturation at 95°C for 0.5 min; followed by annealing at 57°C for 0.5 min, elongation at 72°C for 0.5 min, and final elongation for 10 min at 72°C. Ten microliters of each reaction were subjected to 1.5% agarose gel electrophoresis and stained with ethidium bromide.

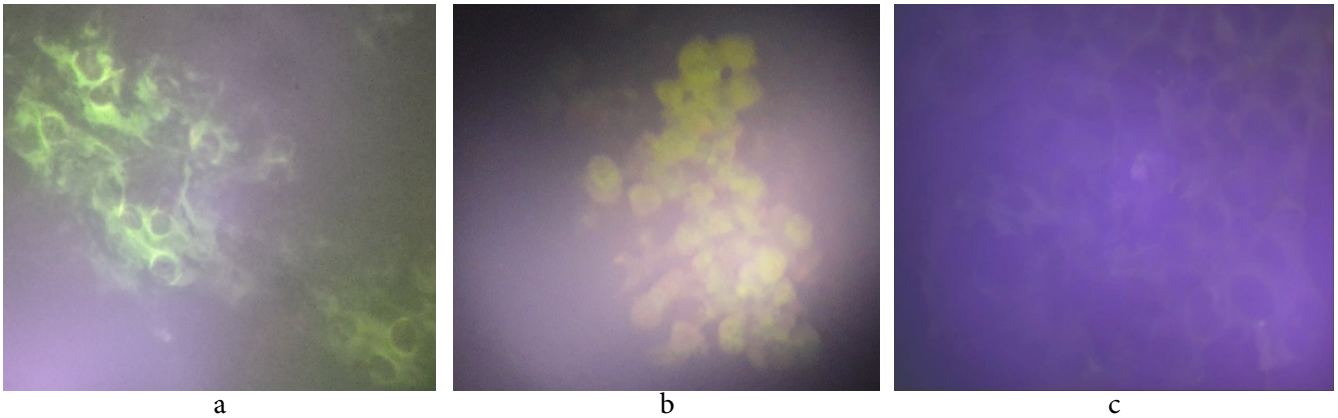
Amplification of nucleic acids was performed using a programmable amplifier T 3000 (Biometra, Germany). Agarose produced by Biozym (Germany) was used for electrophoretic analysis at a voltage of 10 V/cm for 45–50 min.

The obtained data were analyzed statistically using the Student's *t*-test and the Mann-Whitney-U-test (Lakin, 1990).

Results. According to the results of clinical and epizootiological examination regarding bovine viral pneumoenteritis in cattle of the Kirovohrad Region, it was found that animals 2–6 months of age showed respiratory disease, characterized by increased salivation, cough, redness of mucous membranes, including redness of the nasal mirror. The incidence among animals was 48% and the mortality rate was 12%. The analysis of plans of anti-epizootic measures established that the means of specific prevention have not been used in the farm for the last three years. Examination of serum from animals of different age groups (calves up to 2 months of age, calves 6 months of age, and cows) for the presence of specific antibodies to IRT, VD, and PI-3 viruses found that seropositivity of animals (n = 21) to IRT virus was 76.2%, to the VD virus — 38.1%, and to the pathogen PI-3 — 57.1%. Considering that serological diagnosis is an indirect method of identifying infected animals and requires the study of 'paired samples' of serum, which in the context of exacerbation of the epizootic situation was impractical, a preliminary diagnosis was made taking into account clinical signs of the disease. According to the previous diagnosis, the cause of respiratory disease in animals was the bovine infectious rhinotracheitis (IRT) virus.

For the purpose of laboratory confirmation of the previous diagnosis, samples of pathological material (lungs, spleen, mesenteric and mesenteric lymph nodes) were taken from the dead 2-month-old calf with signs of respiratory syndrome.

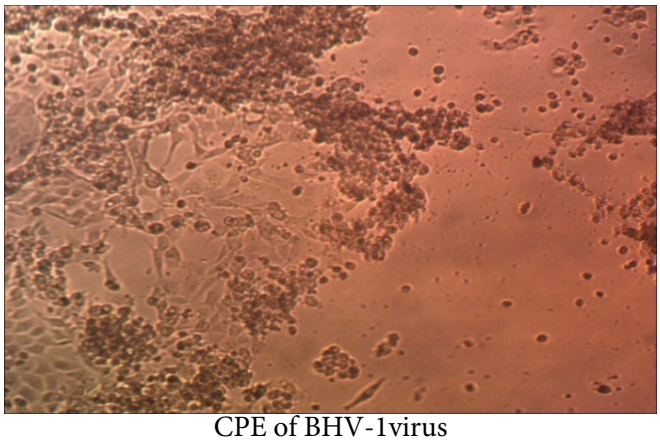
In addition, nasal washes (n = 7) were selected from calves with clinical signs of respiratory syndrome. This biological material was investigated by immunofluorescence assay (IFA) for the presence of antigens of IRT, VD, PI-3 and RSI viruses. In samples of pathological (lungs) and clinical material (nasal washes) from animals, antigens of the IRT virus were identified (Fig. 1).



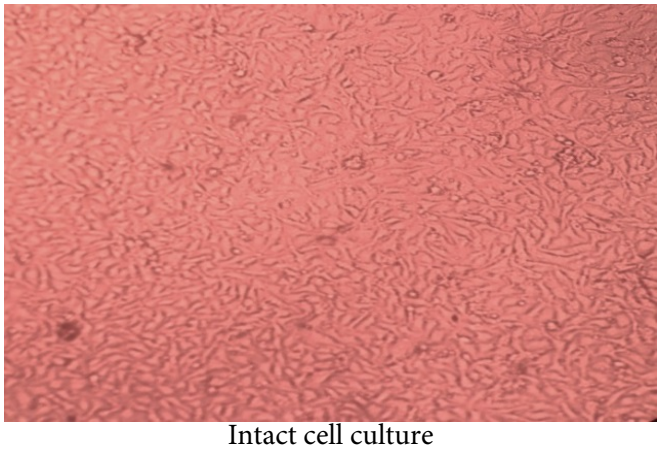
a b c

Figure 1. Results of indication and identification of the IRT pathogen in biological material (a — lungs; b — nasal washes; c — negative control) using IFA

According to the results of the identification of the IRT virus in the biological material, the specified pathogen was isolated from a suspension made from the lungs of a dead animal. After two days of incubation of the calf kidney cell culture, on the monolayer of which a virus-containing suspension of biological material was previously applied, the manifestation of the cytopathic effect of the virus was observed. CPE was characterized by the appearance of round cells in the monolayer, which accumulated in the form of conglomerates and resembled grape clusters, with subsequent detachment of individual cells and their conglomerates from glass, the formation of windows, the number of which increased rapidly and ended with complete destruction of the monolayer of cells of the continuous calf kidney line (Fig. 2).



CPE of BHV-1virus



Intact cell culture

Figure 2. Cytopathic effect of infectious rhinotracheitis virus isolate on continuous calf kidney cell culture (52 hours of incubation)

After adaptation of the virus isolate to the specified cell culture for three consecutive passages, it was determined that the infectious activity of the herpesvirus pathogen type 1 in the sensitive cell line was 4.7 lg TCD_{50}/cm^3. In order to further identify the viral isolate adapted to the biological object at the molecular-genetic level, the genetic material of the pathogen was detected by PCR. For this purpose, a sample of virus-containing fluid obtained from a continuous culture of CK cells after co-culturing it with a virus isolate was studied during three consecutive passages.

According to the results of molecular-genetic studies, it was found that the genetic material of herpesvirus type 1 (BHV-1) was identified in the studied sample (Fig. 3).

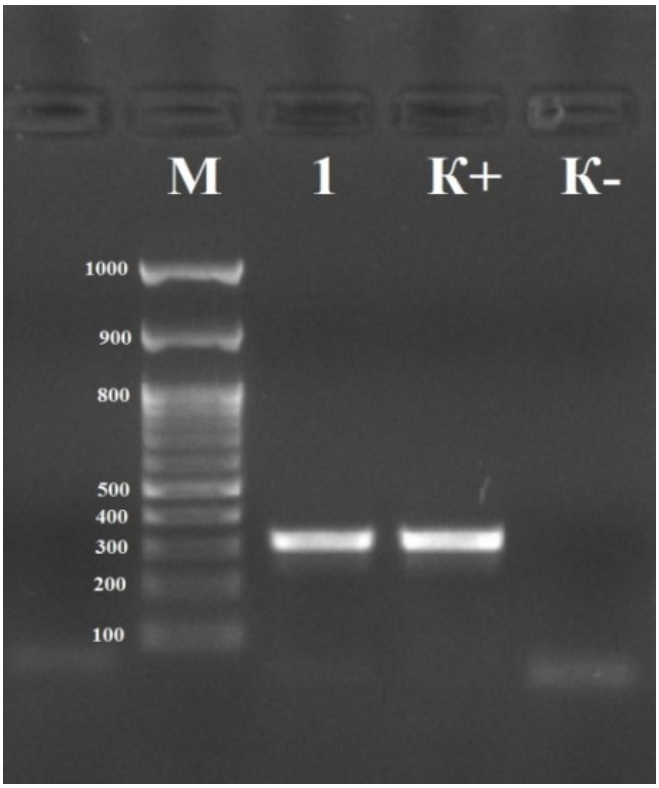


Figure 3. Results of molecular genetic studies of IRT virus isolate using polymerase chain reaction (1 — amplicon formation (325 bp) in the study of IRT virus isolate; K– — negative control; K+ — positive control; M — molecular mass marker with a step of 100 bp)

Thus, the results of molecular genetic studies confirmed the data of virological studies on the belonging of the field isolate of infectious rhinotracheitis virus to the family Herpesviridae and allowed to identify it as herpesvirus type 1 (BHV-1).

In order to determine the sensitivity of continuous cell cultures to the BHV-1 isolate, its cultivation was performed on the corresponding cultures of CK, ShK-2, CEL, and CT. The results of the research are presented in Table 1.

According to the results of studies on the adaptation of the obtained virus isolate to cultures of CK, ShK-2, CT and CEL cells, as well as determining their susceptibility to the pathogen, it was found that reproduction and adsorption properties of BHV-1 were more pronounced on continuous CK and CEL cultures — complete destruction of the monolayer was observed on the 2nd–3rd days of incubation, even under conditions of application of the pathogen at a dilution of 1:1000. The lowest activity of the virus was observed in the continuous culture of calf tracheal cells and was

characterized by the manifestation of the cytopathic effect of the pathogen from 25 to 100% after 4 days of incubation. The total number of affected cells in the corresponding repeats (n = 4) of each of the test modes did not differ statistically (U = 4.5, p < 0.05).

Table 1 — Determination of the sensitivity of continuous cell cultures to the isolate of BHV-1 virus

Cell culture	Dose of infection	Cytopathic effect of the virus, hours after infection, n = 4			
		24	48	72	96
Calf kidney	1:10	++++	++++	++++	++++
	1:100	++	++++	++++	++++
	1:1000	–	++	++++	++++
Sheep kidney	1:10	++	++++	++++	++++
	1:100	+	+++	++++	++++
	1:1000	–	+	+++	++++
Calf trachea	1:10	+	+++	++++	++++
	1:100	–	+	++	+++
	1:1000	–	–	+	++
Lungs of cow embryo	1:10	+++	++++	++++	++++
	1:100	+++	++++	++++	++++
	1:1000	–	+++	++++	++++

Notes: '–' — CPE of the virus is absent, '+' — CPE not more than 25%, '++' — CPE not more than 50%, '+++' — CPE not more than 75%, '++++' — CPE at the level of 100%.

Confirmation of the presence of genetic material BHV-1 after reproduction of the pathogen on these cell cultures was performed by PCR, examining the virus-containing fluid obtained after the third passage of the virus on them (Fig. 4).

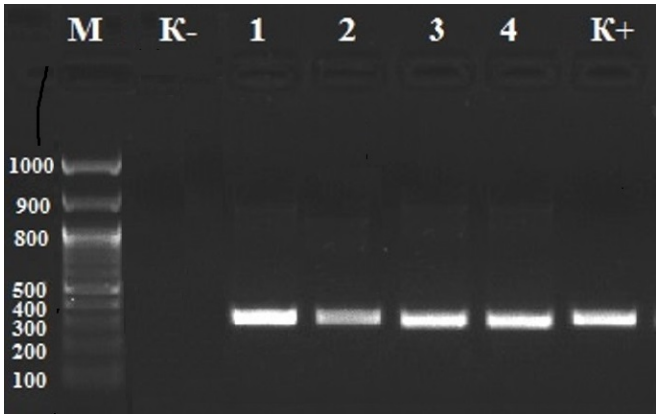


Figure 4. Formation of amplicon (325 bp) in the study of isolate BHV-1 after passage on continuous cell cultures: 1 — CK; 2 — CT; 3 — ShK-2; 4 — CEL; K– — negative control; K+ — positive control; M — molecular mass marker with a step of 100 bp.

According to the results of the PCR study, it was found that the genetic material of herpesvirus type 1 (BHV-1) was identified in the studied samples obtained from infected cell cultures. That is, the results of molecular genetic studies confirmed the reproduction of BHV-1 isolate on continuous cell cultures of CK, ShK-2, CEL, and CT during three consecutive passages. Further, in order to determine the infectivity of the obtained virus isolate, the titer of its infectious activity was determined (Fig. 5).

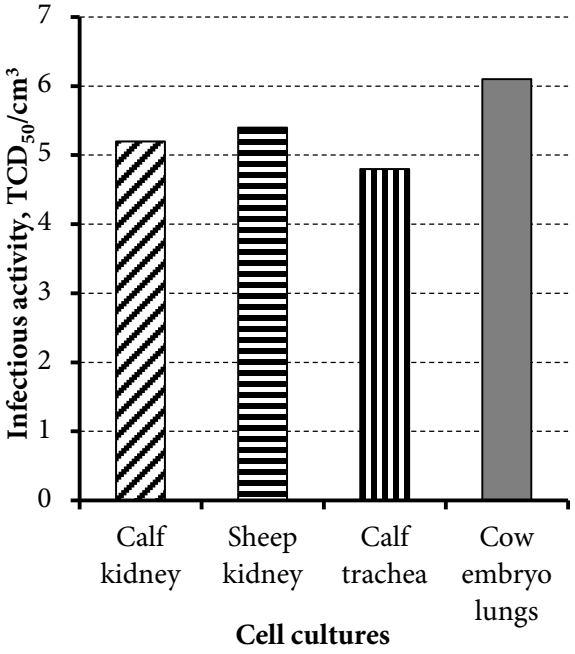


Figure 5. Infectious activity of BHV-1 isolate on continuous cell cultures at the level of the 3rd passage.

As can be seen in Fig. 5, the highest titer of infectious activity (6.1 lg TCD_{50}/cm^3) of BHV-1 isolate was obtained after its adaptation and reproduction during three consecutive passages on continuous cell culture of cow embryonic lungs. Whereas the lowest infectious activity was observed under the conditions of reproduction of the BHV-1 pathogen on the continuous culture of calf tracheal cells — 4.8 lg TCD_{50}/cm^3. With regard to the infectious activity of the IRT virus isolate obtained after reproduction on continuous cultures of calf kidney and sheep kidney cells, this indicator did not differ significantly and was at the level of 5.2–5.4 lg TCD_{50}/cm^3. It should be noted that the obtained level of infectious activity of the virus is mostly correlated with the intensity of its cytopathic effect on continuous cell cultures. In addition, the obtained titer of infectious activity is slightly lower than that of the IRT virus, which may be due to the small number of passages performed on the respective cell lines. According to the results of this work, the obtained isolate of infectious rhinotracheitis virus after certification, was deposited in the Depository of the NSC 'IECVM' under the name 'K-17' ('Kirovohrad-2017').

Conclusions. 1. As a result of virological studies, a field isolate of the virus was identified and adapted to continuous cultures of CEL, CK, SHk, and CT cells. The isolate was identified as a herpesvirus type 1 (BHV-1) using a molecular-genetic research method.

2. Reproduction and adsorption properties of BHV-1 isolate were more pronounced on continuous cultures of CK and CEL cells — complete destruction of the monolayer was observed on the 2nd–3rd days of incubation, even under conditions of application of the pathogen at a dilution of 1:1000.

3. The highest titer of infectious activity (6.1 lg TCD50/cm3) of BHV-1 isolate was observed after its adaptation and reproduction on continuous culture of cow embryonic lung cells.

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Part 3. Biosafety

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VIRUCIDAL ACTIVITY OF DISINFECTANT 'BIOLAID'

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Summary. The article presents the results of the study of toxic and virucidal action of the disinfectant 'Biolaid', which includes hydrogen peroxide, lactic acid, and supralactic acid. The research was conducted following the 'Methodical Approaches to the Control of Disinfectants for Veterinary Medicine' (Kovalenko and Nedosiekov, 2011). Toxicity of the disinfectant 'Biolaid' was characterized in SPEV and BHK-21/C13 cell cultures (ATCC CCL-10). Determination of virucidal activity of the disinfectant 'Biolaid' was performed on models of Aujeszky's disease virus (strain 'Arsky') and rabies virus (strain CVS-11, ATCC VR 959). The toxic effect of the drug 'Biolaid' was determined for concentrations of 2.0%, 1.5%, 1.0%, 0.5%, and 0.25% at exposures of 30 and 60 min in an incubator at 37°C. The virucidal effect of 'Biolaid' was determined for similar concentrations using working dilutions of viral suspensions: for Aujeszky's disease virus — 4.0 CPE_{50}/cm^3, for rabies virus — 4.0 $TCID_{50}/cm^3$. The results of the study showed that the disinfectant 'Biolaid' is not toxic to SPEV and BHK-21/C13 cells in all test concentrations (2.0%, 1.5%, 1.0%, 0.5%, and 0.25%) at exposures of 30 and 60 min. Disinfectant 'Biolaid' has 100% virucidal activity against Aujeszky's disease virus (strain 'Arsky') and rabies virus (strain CVS-11, ATCC VR 959) in all tested concentrations (2.0%, 1.5%, 1.0%, 0.5%, and 0.25%). The virucidal effect of these viruses was manifested at exposures of both 30 and 60 min. The obtained results give grounds to recommend disinfectant 'Biolaid' for disinfection of various livestock and poultry farms in case of detection of viral infections

Keywords: rabies virus, Aujeszky's disease virus, virucidal activity, cell culture

Introduction. The introduction of intensive technologies for the production of livestock products involves a significant concentration of livestock in a limited area, which creates conditions for a significant spread of opportunistic and pathogenic microflora and the emergence of infectious animal diseases. It is necessary to develop new effective methods and means to ensure stable epizootic welfare of livestock. One such method is the use of highly effective disinfectants at all stages of livestock production. The effectiveness of disinfectants should be studied at the stage of their development and selection of substances, because a significant number of disinfectants are toxic, immunosuppressive and cause long-term effects on animals and have high corrosive activity (Curran, Wilkinson and Bradley, 2019; Kovalenko et al., 2018; Paliy et al., 2018). Today, bactericidal preparations based on lactic acid are widely used. They are readily soluble in water, colorless, have high bactericidal and surface activity, combined with low toxicity and the absence of irritants and other side effects (Addie et al., 2015; Van Haute et al., 2015; Ríos-Castillo, González-Rivas and Rodríguez-Jerez, 2017; Cap et al., 2019). They do not form toxic products, are not inactivated by proteins, and are non-aggressive (Kovalenko et al., 2020).

Along with this, there is a need for multilevel testing, including *in vitro* systems. The generally accepted models for biotesting of bactericidal drugs are cultures of differentiated and undifferentiated animal cells. From an economic point of view, *in vitro* methods, reducing the time to obtain reproducible and reliable data, promote the introduction of new disinfectants.

Detection of toxicity of compounds in the early stages of testing reduces the financial cost of studying substances that will not be implemented in the future. In addition, it is possible to test disinfectants for virucidal activity against many viruses using cell cultures, which in experiments *in vivo* requires special biosafety conditions and significant financial costs (Ríos-Castillo, González-Rivas and Rodríguez-Jerez, 2017; Cupo and Beckstead, 2019).

Therefore, the development and implementation of new, environmentally friendly, effective, harmless to animals complex disinfectants is an important scientific field.

Aim of the study. The purpose of the work was to investigate the virucidal activity of disinfectant 'Biolaid' in cell cultures to Aujeszky's disease virus and rabies virus.

Materials and methods. Study of toxicity and virucidal activity of the disinfectant 'Biolaid' were performed following the 'Methodical Approaches to the Control of Disinfectants for Veterinary Medicine' (Kovalenko and Nedosiekov, 2011). 'Biolaid' contains active substances: hydrogen peroxide, lactic acid, and supralactic acid.

Determination of the level of toxic effects of disinfectant 'Biolaid' was performed in continuous cultures of SPEV (pig embryo kidney) and BHK-21/C13 (ATCC CCL-10) cells.

Determination of the virucidal action of the disinfectant 'Biolaid' was performed on models of Aujeszky's disease virus (strain 'Arsky') and rabies virus (strain CVS-11, ATCC VR 959). Infectious activity of Aujeszky's disease virus (strain 'Arsky') 7.31 ± 0.20 lg CPE_{50}/cm^3, rabies virus (strain CVS-11) with infectious activity 7.53 ± 0.11 lg $TCID_{50}/cm^3$.

The following reagents were used for the study: DMEM (Dulbecco's Modified Eagle Medium), Sigma (GB); Fetal Bovine Serum (FBS), Gibco (Brazil); Dulbecco's Phoshate Buffered Saline (DPBS), Sigma (GB); Trypsin-EDTA (0.5%), no phenol red, Gibco (GB); Plasmocin, InvivoGen (France); Antibiotic-Antimycotic, Sigma (Israel); culture microplates (96-well), Sarstedt (Germany); tissue culture flask (75 cm^2), Sarstedt (Germany); ACA acetone, 80%, (Ukraine); FITC Anti-Rabies Globulin Kit, Fujirebio (USA).

The studies were performed using the following equipment: CO_2-incubators Esco Cellulture and Jouan 150; microscope Zeiss — Aviovert 40CFL; inverted luminescent microscope Zeiss AXIOVERT 25CA; Eppendorf and Biohit variable volume dispensers for 20–200 µl and 100–1,000 µl; Jokan MSC9 biosafety cabinets; Holten SAFE-2010 and Hereus HS-18; Naibor's cell counting chamber.

Study of 'Biolaid' toxicity. Plating of SPEV and BHK-21/C13 cell cultures in 96-well microplates (seed concentration of 1.0–1.2×10^5 cells/well) was prepared. After 24 h, the medium was removed from the 96-well microplates (subject to availability of 80–90% monolayer) and appropriate dilutions of disinfectant 0.05 cm^3/well, previously prepared on DMEM medium with 10% FBS at a final concentration of 2.0%, 1.5%, 1.0%, 0.5%, and 0.25% were added.

Contact of SPEV and BHK-21/C13 cells with appropriate disinfectant dilutions was performed in an incubator at 37°C (for BHK-21/C13 cell culture also 5% CO_2) for 30 and 60 min. 32 wells were used for one concentration of 'Biolaid' disinfectant.

For control, SPEV and BHK-21/C13 cells, DMEM medium with the addition of 10% FBS was added to 32 wells of a 96-well microplate, 0.05 cm^3/well for a similar period of time of cell contact with the disinfectant.

At the end of the contact period, disinfectant solutions were removed from the 96-well microplates, they were washed three times with DPBS, and 0.20 cm^3 of maintenance medium containing 10% FBS was added to each well. Incubation of 96-well micropanels with SPEV and BHK-21/C13 cell cultures was performed for 72 h with daily microscopy of the cell monolayer in the wells for cytopathic effect (CPE).

'Biolaid' virucidal studies. The disinfectant effect of 'Biolaid' was determined for concentrations of 2.0%, 1.5%, 1.0%, 0.5%, and 0.25%. Test items: Aujeszky's disease virus (strain 'Arsky') and rabies virus (strain CVS-11, ATCC VR 959). Preliminarily, cultures of SPEV and BHK-21/C13 cells were plated in 96-well microplates (plating concentration of 1.0–1.2×10^5 cells/well).

In each experiment, the working dilution of viral suspensions was obtained on the basis of virus activity titers: for Aujeszky's disease virus — 4.0 CPE_{50}/cm^3, for rabies virus — 4.0 $TCID_{50}/cm^3$. A certain amount of disinfectant was added to the viral suspensions to obtain the appropriate final concentration: 2.0%, 1.5%, 1.0%, 0.5%, and 0.25%.

Contact of viral suspensions with appropriate dilutions of disinfectant was performed at room temperature (recommended by the manufacturer for disinfection) for 30 and 60 min. 32 wells with SPEV and BHK-21/C13 cell cultures were used for each concentration of 'Biolaid' disinfectant.

After that, appropriate dilutions of disinfectant with Aujeszky's disease virus (strain 'Arsky') were added to the daily monolayer of SPEV cell cultures and dilutions of disinfectant with rabies virus (strain CVS-11, ATCC VR 959) were added to the daily monolayer of BHK-21/C13 cell cultures. Adsorption of the mixture of virus and disinfectant in cell cultures for 30 and 60 min.

Dilution of virus disinfectant was then removed from the 96-well micropanels, washed three times with DPBS, and 0.20 cm^3 of maintenance medium containing 10% FBS was added to each well.

96-well micropanels with SPEV cell culture containing different concentrations of disinfectant and working dilution of Aujeszky's disease virus (strain 'Arsky') were incubated for 72 h with daily microscopy of the cell monolayer in the wells for the detection of cytopathic effect (CPE).

96-well micropanels with BHK-21/C13 cell culture containing various concentrations of disinfectant and working dilution of rabies virus (strain CVS-11, ATCC VR 959) were incubated for 72 h. At the end of the incubation period, the cells were fixed in the wells with 80% acetone and, after drying, stained with FITC Anti-Rabies Globulin Kit.

After washing the cells with DPBS, the presence of a specific rabies virus glow was assessed under a fluorescent microscope.

To control the cells we used DMEM medium with the addition of 10% FBS, which was introduced in 32 wells of a 96-well microplate for a similar period of time of adsorption of a mixture of virus and disinfectant. As positive controls, viral suspensions in working dilution (Aujeszky's disease virus — 4.0 lg CPE_{50}/0.2 cm^3, rabies virus — 4.0 lg $TCID_{50}$/0.2 cm^3) were used, which were added to 32 wells of a 96-well microplate.

The disinfecting effect of 'Biolaid' on experimental viruses was expressed in the absence of virus expression in cell cultures, namely: lack of CPE in SPEV cell culture for Aujeszky's disease virus and lack of specific glow of rabies virus in BHK-21/C13 cell culture.

Results and discussion. ***Toxicity study of the drug 'Biolaid'.*** In experiments to determine the toxicity of

'Biolaid' disinfectant, a certain difference in the effect of different concentrations of the drug in cell cultures SPEV and BHK-21/C13 has been determined. In cell cultures SPEV and BHK-21/C13, on the daily monolayer of which disinfectant 'Biolaid' was applied in concentrations of 1.5%, 1.0%, 0.5%, and 0.25%, both for 30 min and for 60 min contact, visually no adverse effects were detected.

The concentration of 2.0% 'Biolaid' disinfectant did not visually have a negative effect on SPEV cell culture. However, the use of this concentration of disinfectant in BHK-21/C13 cell culture reduced the rate of cell proliferation compared to control.

That is, during 24 h of incubation, the monolayer of BHK-21/C13 cells was unchanged at 80–90% at 100% monolayer in wells with cell control. During the next 48 h of cultivation, cell proliferation was restored. At the end of the cultivation period, the monolayer of BHK-21/C13 cells was 100% without signs of adverse effects of disinfectant (compared to control).

Study of virucidal action of the drug 'Biolaid'. Studies of the virucidal activity of the disinfectant 'Biolaid' in the model of Aujeszky's disease virus (strain 'Arsky') in SPEV cell culture showed that all applied concentrations of disinfectant (2.0%, 1.5%, 1.0%, 0.5%, and 0.25%) for 30 min (duration of exposure) showed 100% disinfecting effect (Table 1).

No CPE was detected in any wells containing mixtures of different concentrations of disinfectant and working dilution of Aujeszky's disease virus (strain 'Arsky').

Table 1 — Virulicidal activity of disinfectant 'Biolaid' against Aujeszky's disease virus (strain 'Arsky') in SPEV cell culture

'Biolaid' concentration, %	Exposition, min	Virus presence	Cell control	Virus control (presence of CPE in 24-48-72 h of cultivation)
2.0	30	–	#	+
	60	–	#	+
1.5	30	–	#	+
	60	–	#	+
1.0	30	–	#	+
	60	–	#	+
0.5	30	–	#	+
	60	–	#	+
0.25	30	–	#	+
	60	–	#	+

Notes: '–' — no CPE in cell culture; '+' — the presence of CPE in cell culture; '#' — the presence of 100% monolayer for 72 h of cultivation.

Control wells with SPEV cell culture (Fig. 1a) remained intact throughout the observation period (72 h). In virus-controlled wells (Fig. 1b), 100% CPE was observed as early as 24 h after infection.

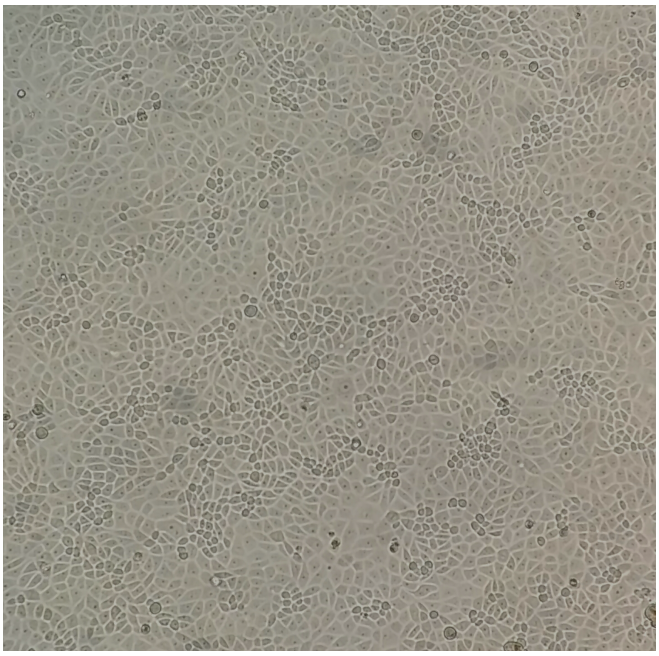

Figure 1a. SPEV cell culture

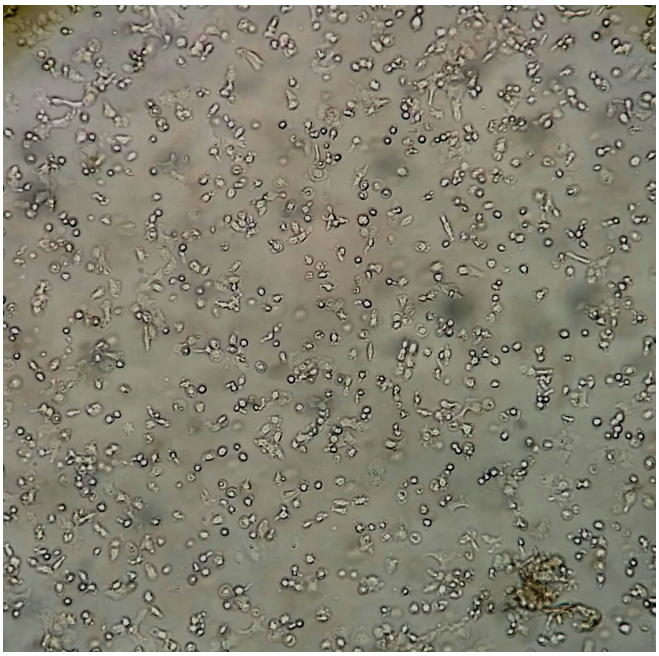

Figure. 1b. Virus control. CPE in SPEV cell culture 24 h after infection with Aujeszky's disease virus (strain 'Arsky')

The infectious titer of the working dose of Aujeszky's disease virus (strain 'Arsky') used in the experiments was 4.22 ± 0.15 lg $CPE_{50}/0.02$ cm^3 at 30 min exposure and 4.37 ± 0.15 lg $CPE_{50}/0.02$ cm^3 for 60 min of exposure.

Studies of the virucidal activity of the disinfectant 'Biolaid' on the model of rabies virus (strain CVS-11, ATCC VR 959) in the cell culture BHK-21/C13 similarly showed that all applied concentrations of disinfectant (2.0%, 1.5%, 1.0%, 0.5%, and 0.25%) for 30 min (duration of exposure) showed 100% disinfectant effect (Table 2).

Table 2 — Virulicidal activity of disinfectant 'Biolaid' against rabies virus (strain CVS-11, ATCC VR 959) in the cell culture BHK-21/C13

'Biolaid' concentration, %	Exposition, min	Virus presence	Cell control	Virus control (presence of a specific glow on the 72nd h of cultivation)
2.0	30	–	#	+
	60	–	#	+
1.5	30	–	#	+
	60	–	#	+
1.0	30	–	#	+
	60	–	#	+
0.5	30	–	#	+
	60	–	#	+
0.25	30	–	#	+
	60	–	#	+

Notes: '–' — the absence of specific glow in immunofluorescence microscopy of cell culture after 72 h of incubation; '+' — the presence of a specific glow of rabies virus in immunofluorescence microscopy of cell culture after 72 h of incubation; '#' — the presence of 100% monolayer after 72 h of cultivation.

No specific glow, characteristic of rabies virus, was detected by fluorescence microscopy in any of the wells, in which mixtures of different concentrations of disinfectant and working dilution of rabies virus were applied, after 72 h of cultivation. In control wells with BHK-21/C13 cell culture (Fig. 2a), the monolayer was 100% during the entire observation period (72 h). In the wells with virus control after 72 h, a specific glow of rabies virus was detected (Fig. 2b).

Titration of the working dose of rabies virus (strain CVS-11, ATCC VR 959) used in the experiments showed a value of 4.75 ± 0.22 lg CPE_{50}/0.02 cm^3 at exposure for 30 min and 4.82 ± 0.15 lg CPE_{50}/0.02 cm^3 at exposure for 60 min.

The results of our studies generally coincide with the information provided by other authors who studied and analyzed experimental data on the toxicity and disinfectant effects of drugs containing hydrogen peroxide, lactic acid and supralactic acid (Goyal et al., 2014; Thomas et al., 2020; Melo et al., 2020; Wlazlo et al., 2020).

Conclusions. 'Biolaid' disinfectant is not toxic to SPEV and BHK-21/C13 continuous cells in concentrations from 2.0% to 0.25%.

'Biolaid' disinfectant has high virucidal activity against Aujeszky's disease virus (strain 'Arsky') and rabies virus (strain CVS-11, ATCC VR 959) in concentrations from 2.0% to 0.25% at exposure for 30–60 min, which allows to recommend it for disinfection of various objects of livestock and poultry farms in case of detection of viral infections, disinfection of veterinary tools and equipment.

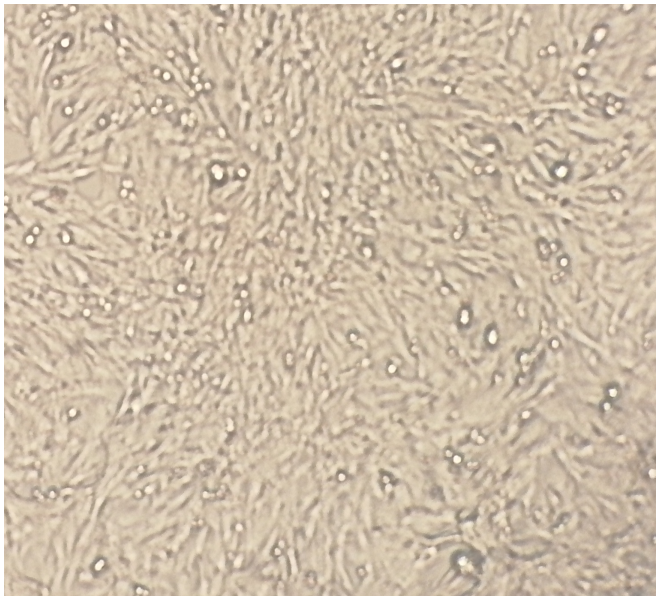

Figure 2a. BHK-21/C13 cell culture

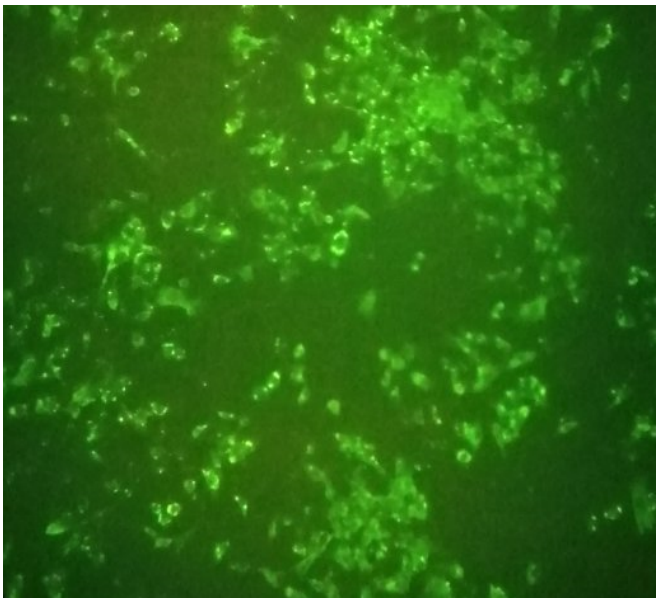

Figure 2b. Virus control. Luminescent microscopy of rabies virus (strain CVS-11, ATCC VR 959) in cell culture BHK-21/C13, 72 h after infection

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MICROBIAL BIOFILMS AND MICROBIAL CONTAMINATION OF FEED FOR LIVESTOCK ANIMALS: CHALLENGES AND WAYS TO OVERCOME THEM

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Summary. The article describes the problem of microbial contamination of feed in animal husbandry and the microflora that causes mastitis in lactating cows. The microbial contamination of 52 commercial batches of fodder from 5 farms of 3 regions of Ukraine (barley, corn silage, oat haylage, alfalfa hay, sunflower meal) has been determined. *Pasteurella multocida* in association with *Neisseria lactamica*, *Actinobacillus pleuropneumonia*, *Clostridium perfringens* was isolated from 61.5% of barley, 66.7% of corn silage, 60.0% of alfalfa hay, and 50.0% of sunflower meal. 262 samples of milk from cows with mastitis have been studied. *Aspergillus candidus*, *Aspergillus niger* were most often isolated in association with *Mycoplasma bovis*, *Streptococcus agalactiae*, *Candida albicans*, *Neisseria sicca*, *Clostridium perfringens*. High film-forming activity of microorganisms in feed was determined, by optical density: *Pasteurella multocida* + *Actinobacillus pleuropneumonia* D_{620} = 3.76 and *Pasteurella multocida*, *Actinobacillus pleuropneumonia*, *Neisseria lactamica* D_{620} = 3.62. While from the milk of cows with mastitis we isolated associations of microorganisms that were strong producers of biofilms by the optical densities D_{620} = 4.02 and 4.23

Keywords: cows, mastitis, bacteria, fungi

Introduction. It is known that microbial biofilms are a dynamic complex biological system for protecting microorganisms from adverse environmental factors (Flemming and Wingender, 2010; Bednarska et al., 2013).

Pathogenic bacteria in microbial biofilms increase resistance to antimicrobial drugs by 100–1,000 times compared to planktonic (free-floating) cells (Austin et al., 1998; Dewachter, Fauvart and Michiels, 2019; Pu et al., 2016) The ability of microorganisms to form biofilms (film-forming activity) is now considered as a factor in their pathogenicity (Roy et al., 2018; O'Loughlin et al., 2013; Gostev and Sidorenko, 2010; Uruén et al., 2020).

Therefore, the study of the composition of microorganisms in feed and their biofilm-forming activity is important for preventing the development of associated animal diseases and, consequently, microbial contamination of the human food chain. According to the European Union's strategy regarding the development of animal husbandry, the biosafety of feed production and animal feeding is one of the key factors in preventing epizootics and, consequently, microbial contamination of the human food chain (EC, 2007).

Therefore, in the European Economic Community (EEC), based on the doctrine of 'One Health', the requirements for the sanitary quality of feed are formulated almost stricter than for food products (EP and CEU, 2017). Unfortunately, feed production in Ukraine, in terms of control of microbial contamination of raw materials and final product, is still regulated by old standards (MAPFU, 2012), which do not take into account new scientific knowledge, including the existence of bacterial associations in the form of microbial biofilms.

The **study aimed** to determine the microbiological contamination of the feed chain in animal husbandry and milk from lactating cows with biofilm-forming bacteria.

Materials and methods. Microbiological studies of feed were conducted in the Laboratory for the Pig Diseases Study of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine) following modern methods.

Isolation, cultivation and study of cultural and morphological properties of feed microorganisms were performed on nutrient media: meat-peptone broth (MPB) with a pH of 7.2–7.4; Hottinger broth; Martin medium; Edward medium, 2.5% meat-peptone broth (MPB) with the addition of 2.0%, meat-peptone agar (MPA) with a pH of 7.2–7.4; Endo agar; modified Kitt–Tarozzi medium; Saburo agar; Olkenytsky medium; Simons citrate; acetate agar.

Biofilm formation was studied by determining the ability of consortium isolates and individual microorganisms to adhere to the surface of a 96-well polystyrene plate (O'Toole and Kolter, 1998). Microorganisms were cultured in meat peptone broth (MPB) at a temperature of 37 ± 0.5°C for 48 h. According to the standard protocol, planktonic cells were removed from the wells of the plate and the microbial biofilms were stained with crystalline violet.

To do this, 150.0 µl of distilled water and 20.0 µl of 1% crystal violet were added to the well and incubated for 45 min at room temperature. After washing three

times with distilled water, 200.0 µl of 96% ethanol was added to the wells to extract the paint from the biofilm, and the optical density of the solution was measured on an ELISA reader at an optical wavelength of 620 nm (D_{620}).

52 commercial batches of fodder from 5 farms of 3 regions of Ukraine (barley, corn silage, oat haylage, alfalfa hay, sunflower meal) were studied. Feed selection was carried out both in the feed shop and in the livestock facility where the animals are kept. 262 samples of milk from lactating cows with mastitis were taken.

Results and discussion. According to the results of microbiological studies, plant feeds for cattle, regardless of the place of sampling, had a high level of microbial contamination, and their species composition was very diverse (Table 1). 52 samples of 5 types of feed at a dilution of 1:100,000 (10^{-5}) and 10^{-2} formed stable associations.

Table 1 — Associations of bacteria isolated from commercial batches of feed for cattle (4 repetitions each, $p < 0.005$)

Type of feed	Total, batches	Isolated microorganisms		Contaminated batches***
		10^{-5}, n*	10^{-2}, n**	
Barley	13	*Clostridium perfringens*, n = 3	*Pasteurella multocida*, *Clostridium perfringens*, n = 5	8
Corn silage	9	*Pasteurella multocida*, n = 2	*Pasteurella multocida*, *Neisseria lactamica*, n = 4	6
Oat haylage	17	*Pasteurella multocida*, n = 5	*Pasteurella multocida*, *Actinobacillus pleuropneumonia*, n = 6	11
Alfalfa hay	5	*Clostridium perfringens*, n = 1	*Pasteurella multocida*, *Neisseria lactamica*, *Clostridium perfringens*, n = 2	3
Sunflower meal	8	*Actinobacillus pleuropneumonia*, n = 1	*Pasteurella multocida*, *Actinobacillus pleuropneumonia*, *Neisseria lactamica*, n = 3	4

Notes: * — according to the current Decree No. 131 (MAPFU, 2012); ** — by microbial biofilms (see Table 3); *** — actual bacterial contamination.

Pasteurella multocida was isolated in associations with *Neisseria lactamica*, *Actinobacillus pleuropneumonia*, *Clostridium perfringens* from 8 contaminated batches of barley (61.5%), 6 batches (66.7%) of corn silage, 11 batches (60.0%) of alfalfa hay, and 4 batches (50.0%) of sunflower meal.

Bacterial associations in feed affect the immune system of animals. Before, during and after calving, cows experience significant stress due to the many physiological changes associated with calving and the onset of lactation. The microflora in animal feed increases this stress due to suppression of immunity and reduced feed intake, increased negative energy balance and increased risk of metabolic disorders and the development of inflammation of the mammary gland (mastitis).

Therefore, the next stage of research was the isolation of pathogens that cause mastitis in lactating cows in autumn and spring (Table 2).

Table 2 — Microorganisms that cause mastitis in lactating cows (4 repetitions each, $p < 0.001$)

Sampling period	Number of samples	Isolated microorganisms
Spring	125	*Pasteurella multocida*, *Mycoplasma bovis*, *Streptococcus agalactiae*, *Candida albicans*
Autumn	137	*Mycoplasma bovis*, *Neisseria sicca*, *Clostridium perfringens*, *Candida albicans*, *Aspergillus niger*

The pathogenic microflora of *Aspergillus candidus* and *Aspergillus niger* was isolated from cows with clinical mastitis regardless of the season, due to the use of litter and feed contaminated with spores of these fungi, as well as high humidity (> 90%), which promotes the reproduction of fungi in livestock facilities.

Aspergillus forms stable associations with mycoplasmas, streptococci, clostridia, neisseria, which can enter the udder from dairy calves infected with these microorganisms.

At the next stage of research, the film-forming ability of bacterial associations isolated from feed and mastitis milk from lactating cows was studied (Table 3). High film-forming activity of microorganisms in feed was determined, by optical density, *Pasteurella multocida* + *Actinobacillus pleuropneumonia* $D_{620} = 3.76$ and *Pasteurella multocida*, *Actinobacillus pleuropneumonia*, *Neisseria lactamica* $D_{620} = 3.62$. While from the milk of cows with mastitis we isolated associations of microorganisms that were strong producers of biofilms by the optical densities $D_{620} = 4.02$ and 4.23.

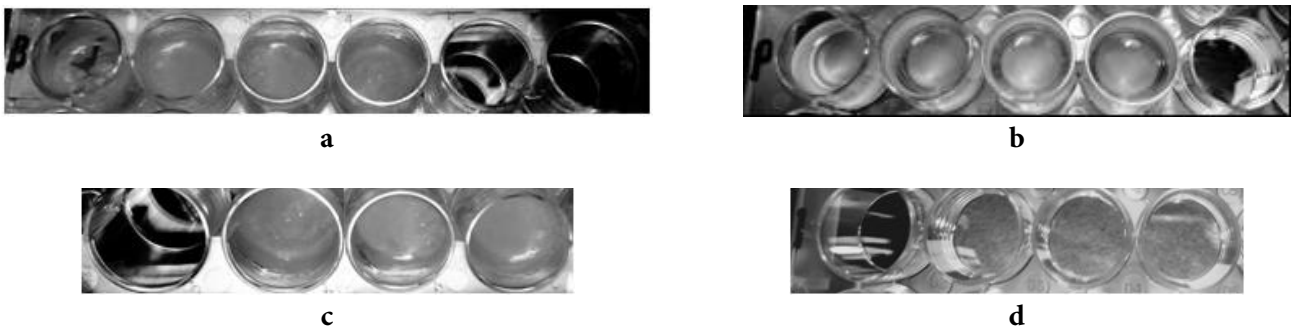
Table 3 — Estimation of the density of microorganism biofilms isolated from different types of feed and mastitis milk

Type of feed / Group of cows with mastitis	Bacterial associations	Biofilm growth time, h	Relative density of microbial biofilm (D_{620})
Bacteria associations from feed			
Barley	*Pasteurella multocida*, *Clostridium perfringens*	72	3.13 ± 0.57*
Corn silage	*Pasteurella multocida*, *Neisseria lactamica*	72	3.48 ± 0.46*
Oat haylage	*Pasteurella multocida*, *Actinobacillus pleuropneumonia*	72	3.76 ± 0.51*
Alfalfa hay	*Pasteurella multocida*, *Neisseria lactamica*, *Clostridium perfringens*	72	3.35 ± 0.49*
Sunflower meal	*Pasteurella multocida*, *Actinobacillus pleuropneumonia*, *Neisseria lactamica*	72	3.84 ± 0.52*
Control	Nutrient medium without biofilms	72	0.12 ± 0.04
Bacteria associations from mastitis milk			
Experimental 1	*Pasteurella multocida*, *Mycoplasma bovis*, *Streptococcus agalactiae*, *Aspergillus candidus*, *Candida albicans*	72	4.23 ± 0.61*
Experimental 2	*Mycoplasma bovis*, *Neisseria lactamica*, *Clostridium perfringens*, *Candida albicans*, *Aspergillus niger*	72	4.02 ± 0.58*
Control	Nutrient medium without biofilms	72	0.10 ± 0.03

Notes: * — $p \leq 0.05$ relative to the control. Scale for assessing film-forming activity: Optical density of biofilms up to ≤ 2×OD — low; from > 2×OD up to ≤ 4×OD — moderate; > 4×OD — expressed.

In this case, the yeast-like fungi *Candida albicans* and the fungi *Aspergillus candidus*, *Aspergillus niger* are a matrix that has a high degree of tolerance to antibacterial substances (Borgersen et al., 2018; Garrett, Bhakoo and Zhang, 2008; Karatan and Watnick, 2009; Vorobey, Voronkova and Vinnikov, 2012), thus protecting pasteurella, mycoplasmas, neisseria, clostridia from the action of antibiotics, which explains the multidrug resistance of these biofilms to 47 antibiotics (macrolides, fluoroquinolones, cephalosporins, aminoglycosides, lincosamides) and complicates the course of the disease and has a chronic course.

In Fig. 1 you can see the difference in shape and consistency between microbial biofilms from bacteriological cultures from different objects. Thus, Fig. 1a and Fig. 1b show the biofilms characteristic of mastitis cows' milk plated on Hottinger medium in dilutions of 1:50 and 1:1,000, respectively.



a **b** **c** **d**

Figure 1. Typical microbial biofilms in platings of milk from cows with mastitis (a and b) and hay (c) and straw (d) extracts. Wells without biofilms — control of the bacterial medium

Obviously, in the second case, the microbial biofilm is thinner and more fragile. According to the results of bacterioscopy, this is due to the absence of such a concentration of fungal microflora and most of its species, as well as higher volumes of anaerobic bacteria compared to the same sample studied at a dilution of 1:50.

Fig. 1c and Fig. 1d show examples of microbial biofilms of plating of samples (1:50), fresh hay from the feeder, and straw from the litter in the calf house, respectively. According to the results of bacterioscopy in the microbial biofilms of calf litter, the content and biodiversity of fungal microflora are much higher, compared to the platings of hay extracts. This may explain why these biofilms are more massive and colorful in appearance.

Thus, according to the results of our own research, it was found that various associations of bacteria that contaminate animal feed and milk from cows with mastitis have high film-forming activity. This indicates the probable formation of microbial biofilms of different composition and physical properties on the surface of grain, granules and other feed components, as well as on

the surfaces of containers, feeders, etc. These biofilms may contain and promote the survival of infectious agents dangerous to livestock (Lazăr and Chifiriuc, 2010; Santos-Lopez et al., 2019; Magana et al., 2018).

A contributing factor in the development of mastitis is the use of disinfectants for the treatment and prevention which kill bacteria. This provokes a violation of the microflora on the skin of the nipples. This fact contributes to the development of multidrug resistance of bacteria to antibiotics, disinfectants, the formation of bacterial biofilms in which the main role (matrix) is played by fungi *Aspergillus candidus*, *Aspergillus niger*, *Candida albicans*, due to which the bacteria in the biofilm remain viable in the environment for a long time (Zhang et al., 2020; Guzmán-Soto et al., 2021).

The results of research indicate a high probability that the current norms of feed contamination control in Ukraine do not provide an objective assessment, as they do not take into account the presence in feed of strong microbial biofilms, of which according to current procedures it is almost impossible to extract sufficient amounts of microflora, so that it is manifested in the dilution of extracts 10^{-5}.This is clearly beneficial to feed manufacturers, but it poses a real and great danger to livestock and the human food chain under the doctrine of 'One Health'.

Conclusions. 1. Microbial biofilms in cows with mastitis, as well as in animal feed, pose a serious biosafety threat when they are inhabited by opportunistic pathogens, and even more, pathogenic microflora. Therefore, their monitoring and study of properties should be given much more attention than is currently the case.

2. It is highly probable that this will lead to a revision of the current methods and standards of control of feed bacterial contamination, as well as the spectrum of antibiotic resistance of pathogenic bacteria — i. e. basic veterinary and sanitary indicators.

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