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The National Scientific Center 'Institute of the Experimental and Clinical Veterinary Medicine' is the leading specialized research institution in Ukraine created for solving scientific and practical tasks of veterinary animal. NSC IECVM's basic research are focused on: immunogenesis and disease pathogenesis, indications, authentications, isolations and studies of biological features of their causative agents, developments of facilities and systems of monitoring, diagnostics, prophylaxis and prognostication of infectious diseases of animals, monitoring of quality and unconcern of agricultural produce and development of the normative basis for animal diseases control and biosafety. NSC IECVM coordinates implementation of scientific researches on questions veterinary medicine, that conduct scientific establishments of NAAS, State Service of Ukraine for Food Safety and Consumer Protection, and Higher educational establishments of Ukraine of agrarian profile.

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2. Papers must be written in English

3. Authors make sure there are no typographical errors in the manuscript

4. Papers must be presented in Word format, in an A4 layout, using Times New Roman 14 point font, which should be single-spaced with 25 mm margins

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INFLUENCE OF BOVINE LEUKEMIA VIRUS ASSOCIATED WITH OTHER VIRAL INFECTIONS ON CATTLE IMMUNITY

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Summary. Comparative hematological and biochemical studies in heifers at the age of 6–8 months with different epizootic background revealed that the persistence of the association of viruses (causative agents of bovine leukemia, infectious bovine rhinotracheitis, bovine parainfluenza-3, and bovine viral diarrhea) negatively affect the status of immunocompetent cells. As the cell number of the macrophage-neutrophil group increases by 25–37%, their functional activity decreases. Significant shifts in the state of protein metabolism, the development of immunosuppression, and intensification of lipid peroxidation processes occur in the bodies of the infected animals, indicating a decrease in the natural resistance of these animals. Significant difference in the intensity of the specific immune response in the vaccinated against pneumoenteritis of the viral etiology animals from BLV-free and BLV-positive farms has not been established.

Keywords: bovine leukemia, infectious bovine rhinotracheitis, bovine parainfluenza-3, bovine viral diarrhea, vaccines, immunity, Ukraine

Bovine leukemia virus (BLV) belongs to the family Retroviridae and closely related to the primate T-lymphotropic viruses types 1–5. It is an oncogenic pathogen that is widespread in cattle globally especially in dairy herds (Polat, Takeshima and Aida, 2017; OIE, 2018). It is known that the persistence of herpes-, paramyxo- and pestiviruses in the herd especially under the conditions of livestock infirmity in relation to bovine leukemia, causes a decrease in the overall resistance of the animal and increases the incidence of infestation (Scott et al., 2006; VanLeeuwen et al., 2001; Konnai, Murata and Ohashi, 2017). It has been proved that the BLV causes an immunosuppression, thereby activating infectious pathology, which in the normal state of the immune system may not manifest itself (Erskine et al., 2012; Frie et al., 2016). It is noted that the increase of the level of infection in young animals with BLV leads to a significant increase of morbidity and mortality due to the activation of respiratory-enteric diseases caused by conditionally pathogenic pathogens (Hopkins and DiGiacomo, 1997; Kobayashi et al., 2015; Buehring, Kramme and Schultz, 1994).

The purpose of this work was to study immunosuppressive changes in the immune system of cattle under herpes-, paramyxo-, pest-, and retroviruses association.

Material and methods. Four groups of animals were formed for the experiments. The number of animals in each group was 10 heads at the age of 6–8 month. The heifers of the first group were selected from the herd, where the circulation of the herpes virus of the first serotype (infectious bovine rhinotracheitis virus, IBRV) was previously confirmed. The clinical manifestation of the disease in the herd was restrained by the preventive vaccination. The second group of heifers was selected from the livestock where the bovine parainfluenza-3 virus (BPIV-3) and IBRV were confirmed by serological study and typical clinical signs of respiratory disorders were observed. Moreover, these farms have had BLV-free status for the last four years. The third group included BLV-positive animals. At the same time, frequent outbreaks of respiratory diseases were observed in the animals of the latter farm, and antibodies against three viruses: BPIV-3, IBRV, and bovine viral diarrhea virus (BVDV), were detected by serological studies of serum samples. The fourth group of animals has been formed from intact individuals.

Serum samples were taken from the animals of each groups for comparative hematological, immunological and biochemical studies.

Number of T- and B-lymphocytes was estimated by hematological studies using the reactions of spontaneous indirect globulin and complement rosette formation, phagocytosis activity, content of large granular lymphocytes, dynamics of cellular changes in the reaction of blast transfer transformations.

Using the serological methods, the presence and titers of antibodies against retro-, herpes-, paramyxo-, and pestiviruses were determined, and the titer of heterohemagglutinins in serum was also determined.

Biochemical studies were performed to determine a total protein, protein fractions, circulating immune complexes of average molecular weight, seromucoids, and rate of lipid peroxidation in the serum.

In order to determine the effect of the cattle leukemia agent circulation on the effectiveness of vaccination against the agents of cattle pneumoenteritis, the level of specific immune response in animals after their inoculation with an inactivated drug containing the agents of IBR, BVD, and BPI-3 has been conducted.

The titer of specific antibodies to the IBRV and BVDV was studied by neutralization reaction, and the titer of specific antibodies to the BPIV-3 by hemagglutination inhibition test. The study of the level of specific antibodies was performed before the inoculation of the drug (start indicators) in 14 days after vaccination of animals, as well as in 30 days, 3 months, and 5 months after administration of the drug. Blood from cows and heifers older than 6 months of age was studied. The indication of IBRV, BVDV, and BPIV-3 was performed by immunofluorescence.

Analysis of the obtained research results was performed using variational methods (Lakin, 1990).

Results. Most animals of the experimental groups had active leukocytosis, which is a significant reaction of the host immune system during the development of acute infectious process. Moreover, in the group of vaccinated animals, which were inoculated with the vaccine against IBRV (the first group), the average leukocyte count exceeds by 8% the same data of the control group (8.75×10^3 and 7.8×10^3 cells/μl respectively). In the third group of calves, where in the chronic course of leukemia, antibodies against BLV to three types of respiratory-intestinal category were detected, the level of leukocytosis in the group average values, on the contrary, was 4% less than in animals control groups (6.73×10^3 and 7.8×10^3 cells/μl respectively).

A similar situation was observed concerning the neutrophil cells. It was found that the quantitative blood counts of the animals from the first two groups were significantly higher than in the control group, whereas in the animals of the third group they decreased.

It should be noted that there is a significant increasing of the number of large granular lymphocytes in the peripheral blood of experimental animals, and a significant decreasing in the number of erythrocytes, as well as the low level of hemoglobin (22% less in animals of the third group and 9% less in animals of the first group) in comparison with the control.

An important indicator of the level of animal resistance is the functional capacity of phagocytes. During the clinical observation of the peripheral blood samples, a significant twofold increasing of the number of monocytes in the first group, moderate in the second group, and a slight decreasing in the third group in comparison with the intact animals were observed.

The most significant phagocytic activity was demonstrated by blood testing in the animals from the first group. In the second and third groups, the phagocytic activity of neutrophils was reduced by 37 and 25% respectively, relative to the control. It was noted that the neutrophils of the animals of the two experimental groups mentioned above had a delayed phagocytosis with a sufficient ratio (25–30%) in peripheral blood samples. The lowest phagocytic and enzymatic activity of neutrophils was observed in animals of the third experimental group against the background of deficiency of these cells and low functional activity.

In addition to the level of neutrophil phagocytosis, the functional activity of immunocompetent cells of the experimental animals was studied using an evaluation of the lymphocyte blast transformation reaction. It is proved that there is a decrease in the functional activity of lymphocytes in all experimental groups, compared to the control animals. It was found that in the animals of the first and second groups the population of T-cells is more active to the immunological loads, while in the animals of the third group the B-lymphocytes are preferred. The animals of the third group had the lowest T-lymphocyte activity and the highest proliferation rate. Generalization of the information obtained as a result of the analysis of the materials of hematological studies, shows that the presence of the association of infectious diseases of different nature, in our case, viruses, provides a significant decrease in the functional activity of immunocompetent cells, resulting in the development of immunodeficiency even in lymphocytosis.

The biochemical study of the serum samples from animals of the first group showed that the level of protein metabolism and the intensity of lipid peroxidation is almost indistinguishable from similar indicators of calves in the control group (Table 1).

This applies primarily to indicators of total protein, protein fractions, average molecular circulating immune complexes and suppressor proteins (seromucoids). Within the first experimental group, it was found that the level of the lipid peroxidation — conjugated dienes increased by an average of 69.3% compared to the limit of their level in the control animals which might be explained by vaccination as a compensatory response.

Analysis of the biochemical parameters in serum of animals from the second group, where two pathogens (IBRV and BPIV-3) circulated, with moderate manifestation of the infectious process, shows that there is no significant deviation of their level from the control one.

The study of serum indicators in animals of the third group showed a decreasing of the total proteins level and albumins by an average of 14.4 and 23.8% ($p \le 0.05$) compared to the indicators of the control group. It might be caused by inhibition of protein-synthetic function of the liver.

Table 1 — Biochemical parameters in the serum samples from calves of experimental and control groups (M ± m, n = 10)

Variables		Groups			
		I	II	III	IV
Total proteins, g/l		71.8 ± 2.1	75.8 ± 1.9	67.6 ± 2.8*	79.0 ± 6.0
Albumins, g/l		32.2 ± 1.1	32.9 ± 1.2	25.5 ± 1.5*	33.3 ± 3.4
Globulins, g/l		46.8 ± 0.05	42.6 ± 2.0	42.1 ± 2.4	38.8 ± 2.6
Albumins/globulins ratio		0.84 ± 0.05	0.71 ± 0.06	0.67 ± 0.12*	0.90 ± 0.03
Circulating immune complexes, mg/ml		0.110 ± 0.005	0.10 ± 0.04	0.12 ± 0.01	0.16 ± 0.04
Seromucoids, mg/ml		0.20 ± 0.005	0.19 ± 0.01	0.25 ± 0.01*	0.15 ± 0.03
Lipid peroxidation process activity	Conjugated dienes, μmol/l	50.8 ± 0.4*	39.5 ± 0.92	56.1 ± 1.05*	30.0 ± 4.0
	Malondialdehyde, ΔD	9.2 ± 0.1	8.1 ± 0.24	10.0 ± 0.27*	7.5 ± 0.5

Note: * — $p \leq 0.05$ compared with healthy cows.

On the other hand, there was a significant increasing of the seromucoids concentration by 66.7% in the blood of these animals compared to the control level, which is a sign of pronounced development of immunosuppression in the experimental calves. At the same time, excessive accumulation of membrane-altering toxic products such as conjugated dienes and malondialdehyde were registered in the animals of this group, which in averages is 187.0 and 133.3% ($p \leq 0.05$) respectively in compare to the control values. This indicates the intensification of the lipid peroxidation processes and is a sign of impaired functional and structural state of the host cells, including immune system. The established nature of changes in biochemical parameters in the blood of the test calves is consistent with the serological monitoring of the disease of this stock from such viruses as IBRV, BPIV-3, BVDV, and BLV and it illustrated the pathogenetic shifts that accompany the development mentioned above.

It was determined that in the groups of animals in which the above association of viruses circulates, within 2 weeks after the second inoculation of the inactivated virus vaccine against IBRV, BVDV, BPIV-3 both in the BLV-free farms and in those where cattle leukemia is registered, an increase in the level of specific antibodies to identified pathogens was noted.

It should be noted that it was exactly the period of the maximum raise of the level of specific antibodies, which was for IBRV 4.2 ± 0.4 $\log_2$ for the BLV-free farm (Fig. 1) and 3.8 ± 0.6 $\log_2$ for the BLV-positive farm (Fig. 2); for BVDV virus — 3.0 ± 1.0 and 3.2 ± 0.8 $\log_2$ respectively, for the pathogen of BPIV-3 — 5.2 ± 1.2 and 5.0 ± 1.0 $\log_2$ respectively.

Analysis of clinical and epizootic examination of animals from both farms revealed that no new cases of animal disease were detected in both farms. In general, observing the vaccinated animals for five months, it was noted that the use of inactivated drugs for specific prevention of pneumoenteritis, both in farms where the circulation of BLV was detected and in free from the causative agent farms, had a positive effect on epizootic situation concerning respiratory and intestinal diseases. Regarding the dynamics of the level of specific antibodies in vaccinated animals, it was noted that in both farms the use of the vaccine provided a stable high level of specific immune response to the administration of the drug. Thus, after the maximum rise in the level of specific antibodies two weeks after the vaccination of animals from both farms, we recorded a consistently high level of them, ranging from one month to 5 months (observation period) after vaccination.

It should be noted that in free from BLV farm, the level of specific antibodies to IBRV was 4.6–3.2 $\log_2$, and in the retrovirus-positive farm — 4.4–3.6 $\log_2$; to BVDV — 3.0–3.4 $\log_2$ (in both farms); and to the causative agent of BPIV-3 — 5.2–6.0 $\log_2$ (in BLV-free farm) and 5.0–5.4 $\log_2$ (BLV-positive farm) (Fig. 2).

Due to the fact that serological diagnostics is an indirect method of indicating pathogens, we determined the presence of infectious pneumoenteritis pathogens circulation in biological material from animals kept in farms with different epizootic BLV-status.

It was found that, three months after vaccination of the animals in BLV-free farm, the antigens of the IBRV, BVDV, and BPIV-3 were detected in individual animals. Conducting similar studies in animals from BLV-positive farm we also identified the circulation of IBRV and BPIV-3 in individual animals.

Analyzing the epizootic situation in both farms, it was found that manifestation of the disease of animals with respiratory syndrome was not observed, the mortality of animals due to infectious pathology was not detected. Examining samples of biological material selected from vaccinated animals 5 months after vaccination, it was found that both in the animals kept in the BLV-free farm, and in the BLV-positive herd the IBRV, BVDV, and BPIV-3 were not detected.

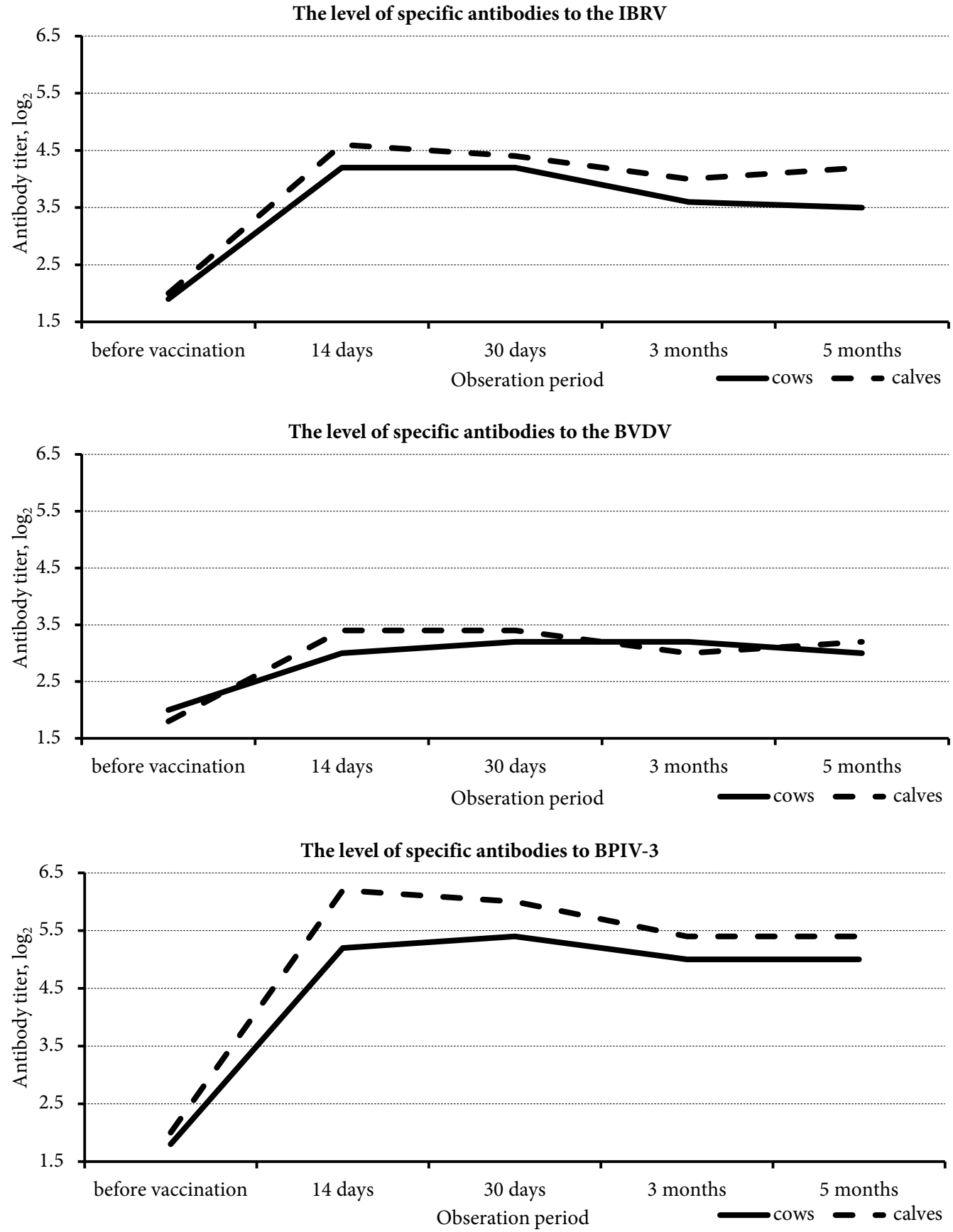

Figure 1. Dynamics of the intensity of specific immune response to the agents of IBRV, BVDV, and BPIV-3 long time after vaccination in the BLV-free farm.

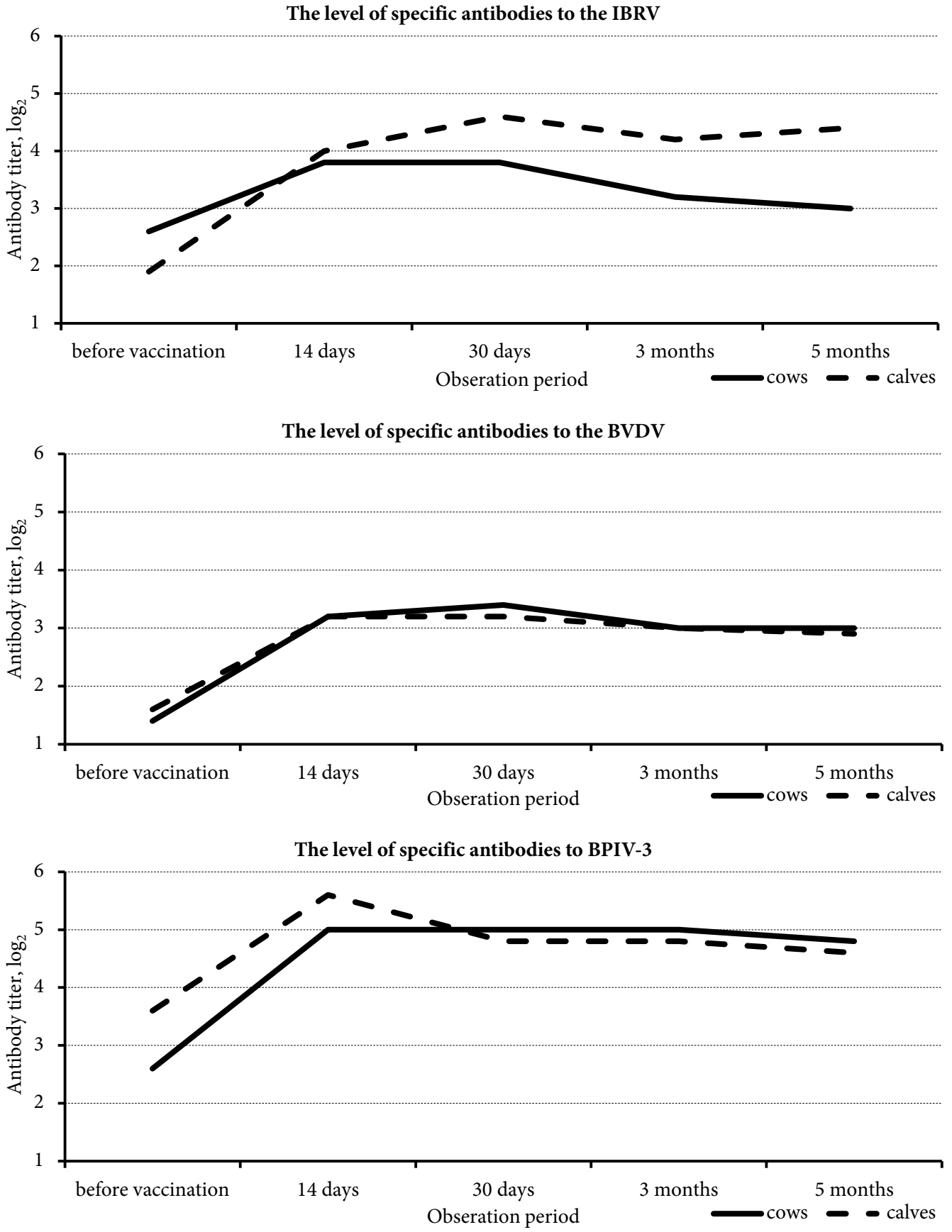

Figure 2. Dynamics of the intensity of specific immune response to the IBRV, BVDV, and BPIV-3 long time after vaccination in the BLV-positive farm.

Conclusions. 1. It is established that in experimental animals on the background of increasing cell number of macrophage-neutrophil group there is a significant (25–37%) decreasing in their functional and enzymatic activity. The decreasing of the functional activity of immunocompetent cells is significant during the BLV, IBRV, BVDV, and BPIV-3 persistence in animals.

2. In infected animals (BLV, IBRV, BVDV, and BPIV-3) there are significant changes in the state of protein metabolism, development of immunosuppression and intensification of the processes of lipid peroxidation, which indicates a decreasing of the natural resistance in these animals.

3. According to the results of the comparative determination of the effectiveness of specific prevention of cattle pneumoenteritis using inactivated vaccine, which was introduced in BLV-negative, and BLV-positive herds, there was found that epizootic status of farms regarding retrovirus did not have a significant effect on the intensity of specific immunity and the effectiveness of ensuring sustainable well-being regarding IBRV, BVDV, and BPIV-3.

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ZOONOTIC INTESTINAL FLUKE DISEASES OF FOXES (*VULPES VULPES*) IN UKRAINE

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Summary. The aim of the work was to study the epizootic situation on the occurrence of intestinal fluke diseases in foxes in Eastern Ukraine. The epizootic situation regarding fox fluke diseases was studied in vivo according to the results of feces coproscopy and posthumously by a partial helminthological autopsy according to K. I. Scrjabin. In total, 213 samples of feces from foxes were collected and examined by helminthologic methods. As a result of the research, the wide spread of intestinal fluke diseases was found in foxes of Eastern Ukraine. Two trematode species, *Alaria alata* and *Nanophyetus salmincola,* were found in foxes of the forest-steppe zone. In the Eastern Ukraine, the invasion of foxes with *A. alata* reaches 67.13%, mean intensity — from 2 to 38 eggs per microscopic field, median intensity — 12.7 ± 1.7. The invasion of foxes with *N. salmincola* is insignificant: prevalence — 0.93% with mean intensity — 1–8 eggs per microscopic field (median intensity — 4.5 ± 2.5). In the forest-steppe geographical zone of Eastern Ukraine, alariasis was found in 91.24% of foxes with low, medium, and high levels of mean intensity — from 3 to 47 eggs per microscopic field (median intensity — 16.5 ±1.0). Nanophyetiasis was detected in two foxes (prevalence — 1.45%) with median intensity — 4.5 ± 2.5 eggs per microscopic field.

Keywords: foxes, alariasis, nanophyetiasis, Ukraine

Introduction. Throughout the last decade, the increasing number of specially protected natural zones, ecological, faunistic, entomological, hydrological and other nature reserves is observed in Ukraine. There are 242 objects of the natural reserve fund with a total area of 76,434 ha (Klimov, Klimov and Gaidrich, 2015).

The increase in natural protected areas caused the restoration and a significant increase in the representatives of wild fauna, various predators (Carnivora) and, in particular, the population of foxes (*Vulpes vulpes*). There are approximately 57,000 wild foxes in Ukraine. The population density of foxes exceeds by 2–4 times in some regions. It is worth mentioning that, a scientifically based standard of population density in biological ecosystems is 0.5–1.0 individuals per 1,000 ha (Banik, Skorobogatov and Atemasov, 2009; KhRDFH, 2017; Tokarsky, Grubnik and Tokarskaya, 2016). Moreover, foxes have become more socialized. They are increasingly being noticed near settlements and even in large cities.

At the same time, foxes can carry helminths and act as a source of invasions, including trematodes. Some of them are dangerous not only for animals but also for humans. Foxes (*Vulpes vulpes*) are directly involved in the formation of natural foci, steadily circulating biocenoses and ecosystems, and causative agents of fluke diseases.

Such intestinal helminthiases as alariasis and nanophyetiasis of foxes occupy a special place among the fluke diseases of carnivorous. These helminthiases have significant epidemiological, veterinary and social importance due to their ability to cause incidence, disability and even fatal cases in humans.

Researchers from Europe, America, Asia, Africa, Japan, Australia, Russia, and other countries have done a great deal of research on the diversity of intestinal helminths of foxes (*Vulpes vulpes*). The majority of researchers found that the intestinal trematodes dominate of the total number of helminths (Andreyanov et al., 2016; Criado-Fornelio et al., 2000; Davidova and Shemyakov, 2015; Eira et al., 2006; Gicik et al., 2009; Loos-Frank and Zeyhle, 1982).

Alariasis of foxes (pathogen *Alaria alata* (Goeze, 1782)) is widespread and its cases have been detected worldwide (Malysheva et al., 2013; Borecka et al., 2009; Bružinskaitė-Schmidhalter et al., 2012; Papadopoulos et al., 1997; Esīte, Deksne and Bagrade, 2012). It is explained by agent's biological characteristics, namely, be the presence of a wide range of definitive hosts — members of the Canidae family; intermediate hosts — freshwater *Planorbis* mollusks, as well as by wide range of reservoir hosts — tadpoles, adult *Anura* amphibians, rodents, insectivores, reptiles, birds, artiodactyls, and humans.

The invasion of foxes by *Alaria* in European countries (Germany, Ireland, Poland) and others reaches 6–15–97% (Trusova, 2009; Murphy et al., 2012; Esīte, Deksne and Bagrade, 2012), up to 96% in Lithuania, up to 30% in Turkey and 10.9–33.3 % in Netherlands (Romashov, Nikulin and Lesnikov, 2009; Trusova, 2009), in Denmark — up to 87.1 %.

The *Alaria* invasion of foxes reaches from 25.0 to 59.3% in Kursk, Voronezh, Oryol, Tula, and other regions of the European part of Russia (Romashov, Nikulin and Lesnikov, 2009; Trusova, 2009). The invasion of foxes with *Alaria* ranges from 50 to 100% in the North of Russia (Yakutia, Omsk, Kamchatka) and the Far East.

Six species of trematodes are pathogenic for humans and carnivorous, three of them are intestinal parasites, which were detected in foxes in the Far East. In the Ivanovo, Smolensk, Vladimir, Kostroma, Yaroslavl, and Moscow regions, foxes were infected with two types of trematodes — *A. alata* and *Nanophyetus salmincola* (Chapin, 1926). In Novosibirsk and in Tyumen, foxes and carnivores were infected by three species of trematodes, including *A. alata*.

Two species of trematodes were identified in foxes in Krasnoyarsk: *A. alata* and *Echinochasmus perfoliatus* (Rats, 1908).

Nanophyetiasis of foxes (Salmon Poising Disease) is caused by *N. salmincola*. Due to pathogen's biological characteristics and established food links, it is less commonly spread, although it has a fairly wide range of definitive hosts — domestic and wild carnivorous, rodents, some species of birds, as well as humans. The intermediate hosts of this parasite are freshwater mollusks and additional hosts are fishes (mainly salmon), flathead grey mullet, cyprinids, silver carp, bream, sablefish, and other freshwater fish.

The occurrence of *Nanophyetus* in foxes is mostly registered in the United States and Canada.

In Russia, nanophyetiasis was registered among foxes of the Far East (prevalence reached 3.5–7.8%), as well as in numerous regions of the Central Non-Black Earth Region with prevalence 5.1% and mean intensity 38–52 *N. salmincola* worms in intestine.

Only a few works devoted to the study of alariasis have been published in Ukraine, and there is a lack of scientific data on the distribution of nanophyetosis of foxes over the past 10 years. Therefore, the study of epizootology and spread of zoonotic intestinal fluke diseases (alariasis and nanophyetiasis) in a single ecosystem of different regions of Ukraine has a great medical, veterinary, social, and economic importance.

The aim of the work was to study the epizootic situation on the distribution of intestinal fluke disease of foxes in Eastern Ukraine.

Material and methods. Studies of the epizootic situation on the occurrence of intestinal fluke disease of foxes (*Vulpes vulpes*) in the East of Ukraine were carried out during 2016–2018 *in vivo* with coproscopic methods and posthumously using partial helminthological autopsy method described by K. I. Scrjabin.

Feces of foxes were used for the research. Samples of feces (n = 213) were collected by random sampling on movement ways and in the places of foxes' locations. Selected samples were labeled and placed in plastic bags. Feces of foxes were identified together with the zoologists of O. S. Tertyshny Department of Applied Biology, Aquatic Bioresources and Hunting Sector in the Kharkiv State Zooveterinary Academy.

Collected biological material was studied at the laboratory of the Parasitology Department in Kharkiv State Zooveterinary Academy using serial irrigation and Shcherbovich techniques.

The species of ovoscopic elements detected with microscopy under a small (10×15) and medium (10×40) magnification was carried out according to the results of our own morphology studies (length, width, color of the shell and its formations — caps, tubercles), comparing them with the data atlases and keys (Kozlov, 1977; Cherepanov et al., 2001; Thienpont, Rochette and Vanparijs, 1986).

The intensity of foxes' invasion by intestinal trematodes was determined using such indices as prevalence (%), mean intensity (number of eggs, number of helminths in the intestine), and abundance index (AI).

Results. We carried out parasitological studies of foxes' population in the East of Ukraine in the natural conditions of the forest-steppe and steppe zones in Kharkiv and partially in Luhansk and Donetsk regions.

The epizootic situation concerning the invasion of intestinal trematodes in foxes (*A. alata* and *N. salmincola*) was studied *in vivo* using the results of helminthology and ovoscopy of feces.

Material for research (feces) was randomly collected at foxes' habitats and movement paths.

In total, 213 samples of feces from foxes were collected and tested using helminthological and ovoscopic methods.

The results of coproscopy of foxes' fecal samples are presented in Table 1.

Table 1 — The level of infection of foxes (*Vulpes vulpes*) with intestinal trematodes

Geographic zone	Species of helminths	Studied samples	Number of positive samples	Prevalence, %	Intensity, number of eggs
Forest-steppe	*A. alata*	137	125	91.24	3–47 (16.5±1.0)
	N. salmincola	137	2	1.45	1–8 (4.5±2.5)
Steppe	*A. alata*	76	18	23.68	1–29 (9.3±1.8)
	N. salmincola	76	—	—	—
Total	*A. alata*	213	143	67.13	2–38 (12.7±1.7)
	N. salmincola	213	2	0.93	1–8 (4.5±2.5)

As can be seen from Table 1, intestinal fluke diseases of foxes are widespread.

In Eastern Ukraine, the invasion of foxes with *A. alata* reaches 67.13% with mean intensity from 2 to 38 eggs per microscopic field; median intensity was 12.7 ± 1.7. The invasion of foxes with *N. salmincola* is insignificant: prevalence — 0.93% with mean intensity 1–8 eggs per microscopic field (median intensity — 4.5 ± 2.5).

In forest-steppe zone of Eastern Ukraine, alariasis was found in 91.24% of foxes with low, medium, and high levels of mean intensity (from 3 to 47 eggs per microscopic field); median intensity was 16.5 ± 1.0. Nanophyetiasis was detected in two foxes (prevalence — 1.45%) with median intensity 4.5 ± 2.5 eggs per microscopic field.

In the steppe zone, only the trematode species of foxes, namely *A. alata*, is represented. Its prevalence was 23.68% with median intensity 9.3 ± 1.8 eggs per microscopic field.

Dead foxes' carcasses were previously investigated for rabies (n = 6) and partially parasitological autopsied according to K. I. Scrjabin technique. At the same time, intestines and their contents were examined for the presence of trematodes. As a result of a thorough study of the intestinal tract, we identified *A. alata* and *N. salmincola*.

The results of parasitological study of foxes' intestines are presented in Table 2.

As it can be seen, AI of alariasis was 9.66 ± 3.7, and AI of nanophyetiasis — 5.0.

Table 2 — Invasiveness of foxes' intestinal tract with trematodes

Species of helminths	Number of intestines, samples		Helminths detected	AI, number of helminths
	investigated	positive		
A. alata	6	6	58	9.66±3,7
N. salmincola	6	1	5	5.0

Conclusions. Zoonotic intestinal fluke diseases of foxes (alariasis and nanophyetiasis) are widespread in Ukraine.

Two species of trematodes were found in foxes in forest-steppe zone — *A. alata* and *N. salmincola* with prevalence up to 91.24% and 1.45% respectively.

Prospects for further research. Further research suggest the study of the degree of invasion of intermediate, additional, and reservoir hosts by the larval stages of zoonotic trematodes.

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Part 2. Biotechnology and genetics

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VALIDATION OF ANTHRAX SPECIFIC *pagA* QUANTITATIVE PCR FOR DETECTION OF *BACILLUS ANTHRACIS* pXO1 PLASMID

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Summary. This paper represents qPCR validation results for the detection of *Bacillus anthracis pagA* pXO1 plasmid marker. The aim of the work was to transfer, implement and validate anthrax specific *pagA* qPCR assay for the detection of *pagA*, the genetic marker of the pXO1 plasmid of *Bacillus anthracis*. qPCR was conducted using the Applied Biosystems Fast 7500 Real-time PCR system including Applied Biosystem specific reagents (AmpliTaq Gold). Anthrax pXO1 *pagA* primers (pagA_forward, pagA_reverse) and TaqMan *pagA* probe. Data analysis and statistical calculations were performed using Microsoft Excel. The limit of detection (probit analysis) was calculated using the Statgraphics software. Robustness of qPCR was adjusted by optimization of amplification parameters (annealing temperature) and concentration of reaction components ($MgCl_2$, primers, probe and Taq polymerase). In order to test the repeatability and precision of the qPCR assay after optimization, the variation within the experiment (Intra-assay variability) and between several independent experiments (Inter-assay variability) was evaluated. Probit analysis with serial dilutions of positive control with five replicates per dilution was carried out to define the 95% limit of detection (LOD). To determine if the CT value correlates with the amount of template DNA, the linearity of qPCR was analyzed. The standard curve was generated and the linear regression line and the coefficient of correlation (R^2) were calculated. To define the ability to detect sequence of interest (sensitivity), we tested mixed panel of *Bacillus anthracis* DNAs. As the result, *pagA* marker could be detected in all tested strains . To find out the specificity of our assay, we also tested DNA of various strains of *B. cereus*, *B. thuringiensis*, *B. mycoides*, and *B. globigii* (potential cross-reacting organisms) as well as DNA samples of various pathogenic bacteria and viruses which cause similar clinical symptoms as anthrax (differential diagnosis relevant organisms).

Keywords: anthrax, plasmid, validation, quantitative PCR

Introduction. Anthrax is particularly dangerous zoonotic disease caused by bacterium *Bacillus anthracis* — Gram-positive, spore-forming facultative anaerobic rod (Purcell, Worsham and Freidlander, 2007; Hoffmaster et al., 2002; Keim et al., 2004). Depending on the way of transmission, it can cause cutaneous, gastro-enteritic of pulmonary forms of anthrax (WHO, FAO and OIE, 2008). Spores of this pathogen are able to remain viable in soil for decades (Martin, Christopher and Eitzen, 2007). When spores penetrate to host organism, they turn to vegetative form, reproduce and therefore cause the disease. The ability to produce toxins and form capsule in host organism, which protects bacterial cell from phagocytosis, is key virulence factor of *B. anthracis*. Genes responsible for capsule formation are located on pXO2 plasmid, while pXO1 plasmid genes encode synthesis of toxins. Both these plasmids together with chromosome form *B. anthracis* genome (Mock and Fouet, 2001).

Together with classical bacteriological and serological methods, classical polymerase chain reaction and real-time PCR (quantitative PCR, qPCR) are commonly used for express diagnostics of anthrax. Herewith, it is necessary to take into account that, according to ISO/IEC 17025, prior to the implementation of any analytic method to laboratory practice, is has to pass the validation procedure — the set of studies for evaluation of specificity, sensitivity, accuracy and repeatability of any method (ISO, 2017; OIE, 2013; Antonov, 2002).

The **goal of this study** was to carry out validation of qPCR method for the detection of *pagA*, specific pXO1 plasmid marker of *Bacillus anthracis*.

Materials and methods. To carry out these studies, we used *pagA* TZ57 R/T recombinant positive control, which had been prepared before using TA-cloning method (Biloivan et al, 2018). To reduce the risk of contamination in the laboratory when working with plasmid DNA, we obtained *pagA* insert from it using classical PCR with *M13 forward* and *M13 reverse* primers (Chandra and Wikel, 2005). This insert was purified using Monarch™ commercial kit (New England, USA). Concentration of DNA was measured with Nanodrop DS-11 spectrophotometer by DeNovix. Taking into account the measured concentration, we prepared serial dilutions of purified product (from 10^4 to 1 copies of DNA per 1 µl)

and studied them via qPCR. Anthrax specific pXO1 *pagA* primers (*pagA_forward* GTACAAGTGCTGGACCTACG, *pagA_reverse* CACTGTACGGATCAGAAGCC), and *pagA* probe (FAM-ACCGTGACAATGATGGAATCCCTGA-BBQ) by MolBiol (Germany), as well as reagents by Applied Biosystems (AmpliTaq Gold) were used. The reaction was conducted using the Applied Biosystems Fast 7500 Real-time PCR system. Following parameters were used for amplification: activation of HotStart *AmpliTaq Gold* DNA polymerase (Applied Biosystems, 5 U/μl, 5′–3′) — 95 °C, 5 min; denaturation — 95 °C, 15 s; annealing — 60 °C, 20 s; elongation — 72 °C, 40 s; final elongation — 72 °C, 1 min; number of cycles — 40.

The robustness of the method was adjusted by the optimization of primer annealing temperature (several experiments were carried out with 57, 59, 60, and 62 °C annealing temperatures), as well as concentrations of reaction mix components ($MgCl_2$, primers, probe and *Taq*-polymerase).

For this purpose, we conducted titration of these components (several different concentrations of each component were analyzed and the most optimal results were chosen).

Thus, $MgCl_2$ was titrated in concentrations of 0.5–5 μM; primers — 0.1FW/0.1RV–0.6FW/0.6RV μM; probe — 0.1–1 μM and polymerase — 0.13–0.35 μl. Data was statistically analyzed using Microsoft Excel.

In order to test the repeatability and precision of qPCR method after optimization, we determined coefficients of variation (CV) within single and several independent experiments. Herewith, CV values should not be higher than 3% for single and 15% for several experiments (Moens et al., 2009).

Analytical sensitivity was determined using probit analysis, as well as measuring of linearity. We carried out probit analysis with serial dilutions in five replicates in order to determine 95% limit of detection (LOD), which was calculated using Statgraphics software.

To determine if the CT value correlates with the amount of template DNA, the linearity of qPCR was analyzed. Therefore, the fluorescence response to a standard dilution series was evaluated. The standard curve was generated and the linear regression line and the coefficient of correlation ($R^2 \geq 0.98$) were calculated (Rodríguez-Lázaro and Hernández, 2013).

To determine diagnostical sensitivity of the method (the ability to detect sequence of interest), we studied DNA mixed panel of various *Bacillus anthracis* strains (Table 1).

To determine the specificity, we also analyzed DNA of various strains of closely related bacteria: *B. cereus, B. thuringiensis, B. mycoides*, and *B. globigii* (potentially cross-reactive microorganisms), as well as DNA samples of other pathogens causing diseases with similar to anthrax clinical signs (differential diagnostics relevant organisms, Table 2).

Table 1 — Mixed DNA panel of *Bacillus anthracis* strains used for the determination of method's sensitivity

Strain	Strain number	Dilution
6282 Tirol	916	1:100
Wien A5	2610	undiluted (10 ng/μl)
BGA Nr. 2 A7	2612	1:100
2844-9IZSVE	3007	undiluted (10 ng/μl)
4-IZSLT	3008	1:10
3-IZSLT	3009	1:10
CARBOSAP	3010	undiluted (10 ng/μl)
FARMER CUTE	3011	undiluted (10 ng/μl)
FERARRA	3012	undiluted (10 ng/μl)
CEB95-002	3013	1:15
CEB94-033	3015	undiluted (10 ng/μl)
Vollum	3017	1:10
Ames	3018	undiluted (10 ng/μl)
BUL 16	3165	undiluted (10 ng/μl)
BUL 17	3166	undiluted (10 ng/μl)
BUL 28	3177	undiluted (10 ng/μl)
BUL 39	3187	1:10
BUL 41	3189	undiluted (10 ng/μl)

Table 2 — Heterological DNA panel of infectious pathogens causing diseases with similar to anthrax clinical signs

No.	Pathogen	Strain number
1	*Acinetobacter baumanii*	B431
2	*Brucella* spp.	03-0391
3	*Burkholderia cepacia*	P112
4	*Burkholderia mallei*	05-0580
5	*Burkholderia pseudomallei*	Jun 88
6	*Burkholderia thailandensis*	P412
7	*Campylobacter jejuni*	B1229
8	*Candida albicans*	B885
9	*Chlamydophila pneumoniae*	N/A
10	*Citrobacter freundii*	B22
11	*Clostridium perfringens*	B888
12	*Coxiella burnetii*	Nine Mile
13	*Eikenella corrodens*	N/A
14	*Enterobacter aerogenes*	B16
15	*Enterococcus faecalis*	B871
16	*Escherichia coli*	B893
17	*Francisella tularensis holarctica*	F49
18	*Haemophilus influenzae*	B895
19	*Klebsiella pneumoniae*	B896
20	*Legionella pneumophila*	IMB 072813
21	*Listeria monozytogenes*	B435
22	*Moraxella catarrhalis*	B433
23	*Neisseria meningitidis*	B1232
24	*Propionibacterium acnes*	B438
25	*Proteus mirabilis*	B23
26	*Pseudomonas aeruginosa*	N/A

No.	Pathogen	Strain number
27	*Salmonella typhi*	20-3267
28	*Serratia marcescens*	B14
29	*Shigella dysenteriae*	B476
30	*Staphylococcus aureus/SEB*	B946
31	*Staphylococcus epidermidis*	B26
32	*Stenotrophomonas maltophilia*	B918
33	*Streptococcus pneumoniae*	B847
34	*Streptococcus pyogenes*	N/A
35	*Streptococcus pyogenes*	B846
36	*Vibrio cholerae*	B962
37	*Yersinia enterocolitica*	Y105
38	*Yersinia pestis*	02. Apr
39	*Clostridium sporogenes*	DSMZ795
40	Affenpocken Virus	MSF-6
41	Vaccinia Virus	VACV-0273/2004
42	Varicella-zoster-Virus	N/A

Samples of DNA used for these studies were given by Bundeswehr Institute of Microbiology (Munich, Germany) in frames of German Partnership Programme for Excellence in Biological and Health Security.

In order to visualize obtained results, gel-electrophoresis of amplified in 1.5% agarose gel was conducted after qPCR.

Results. Optimization of qPCR parameters was the first step of validation process. PCR product which we obtained after purification of *pagA* TZ57 R/T recombinant plasmid using M13 primers, was diluted from 10^4 down to 1 copy of DNA in 1 µl. The dilution which contained 10^3 copied of DNA and had Ct value of 27 cycles was used for further experiments as template DNA (Fig. 1).

As a result of primer annealing temperature optimization, we chose the temperature of 62 °C for further experiments (Fig. 2 and Fig. 3).

Titration of $MgCl_2$ has shown that the most optimal reaction results can be obtained with its concentration of 3 µM. The best concentration of primers and probe (*pagA*_forward, *pagA*_reverse and *pagA*_probe) is 0.3 µM. As a result of *AmpliTaq Gold* DNA polymerase, we found that it works the most specifically with the concentration of 0.2 µl per reaction.

As a result of repeatability evaluation, we obtained coefficients of variability of 1.89% for the Intra-assay (Table 3) and 5.23% for the Inter-assay (Table 4), indicating that the variability within the *pagA* qPCR assay is low resulting in repeatable results.

As a result of probit analysis, we obtained a LOD of 4.26 copies, indicating that in 95% cases 4 copies can be detected, demonstrating that our qPCR assay is highly sensitive (Fig. 4).

Based on the linearity results, the standard curve was generated, and the linear regression line and the coefficient of correlation (R^2) were calculated. The obtained R^2 values of 0.9995 for *Bacillus anthracis* sample 3014 and 0.9994 for *B. anthracis* sample 3189 show that fluorescence signal is direct proportional to the administrated templated DNA and that the efficiency of the qPCR is high and consistent at varying concentrations (Fig. 5).

The results of *Bacillus anthracis* mixed panel (Table 2), have shown that genetic marker *pagA* is present in all tested samples (Table 5, Fig. 6).

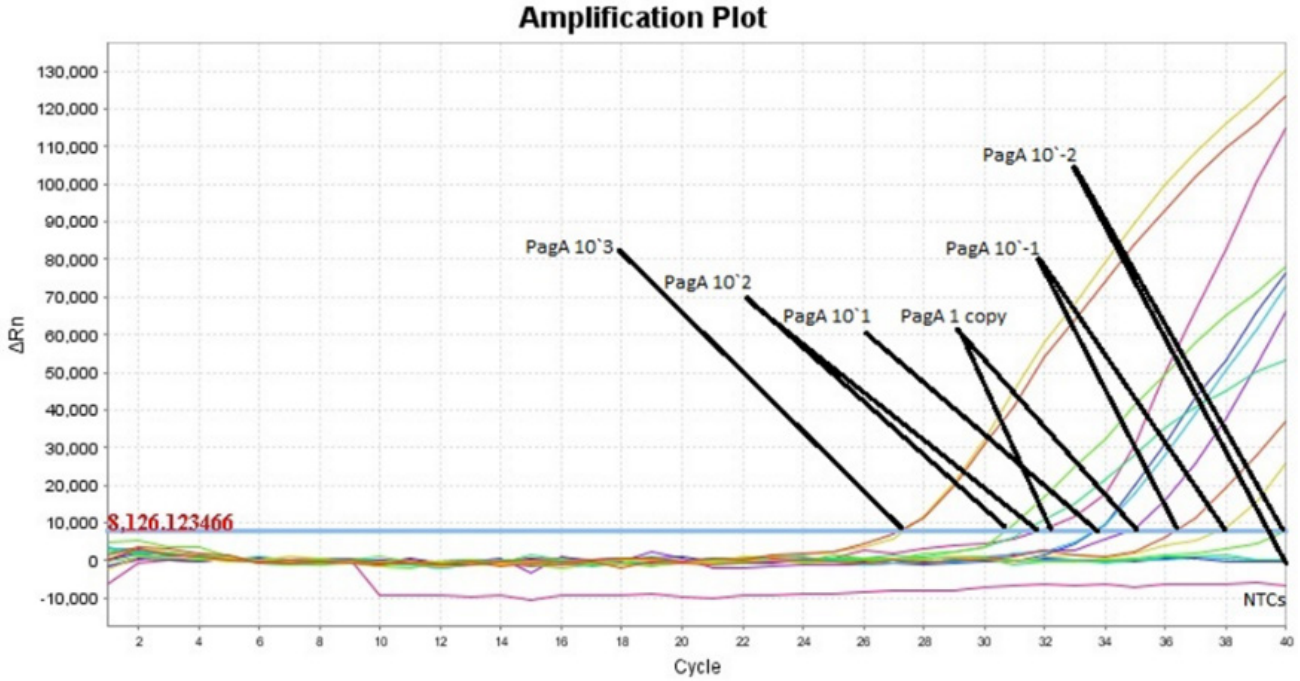

Figure 1. Results of qPCR with *pagA* DNA dilution series obtained from *pagA* TZ57 R/T plasmid

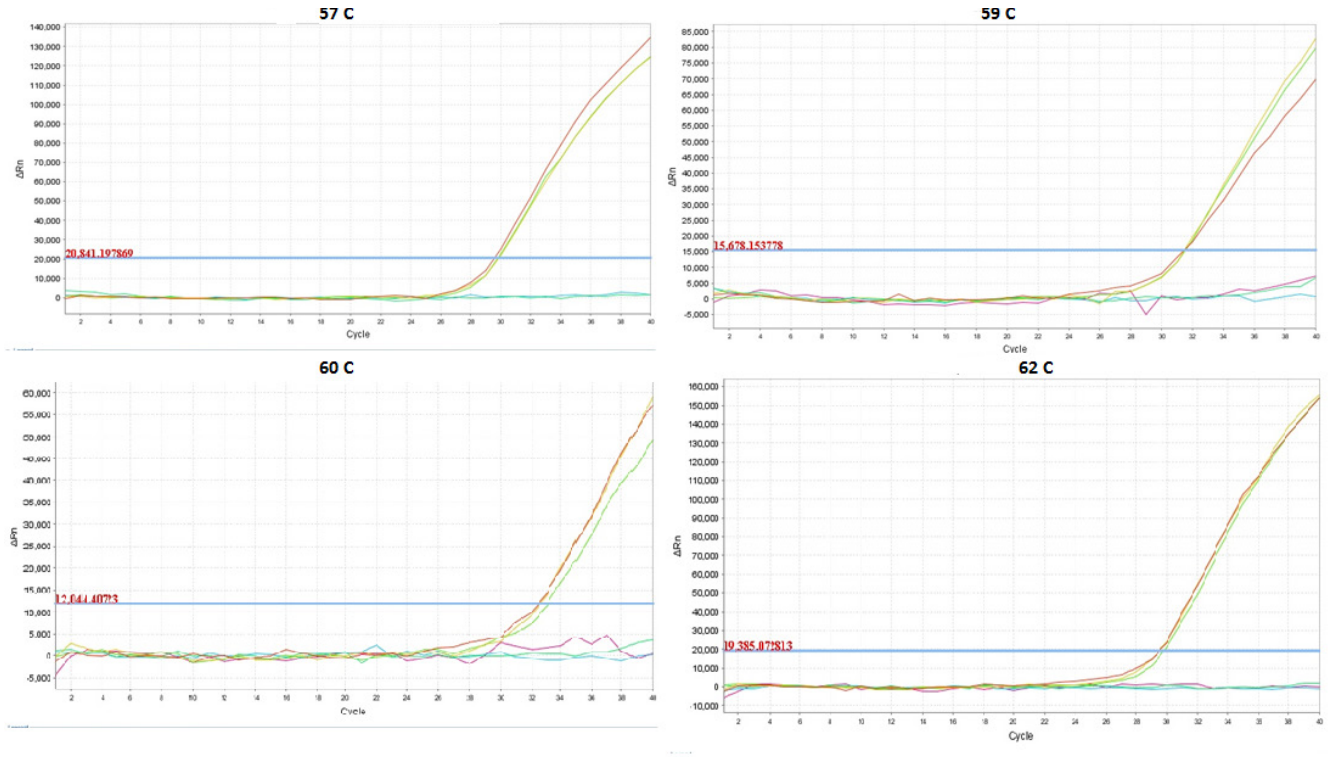

Figure 2. Results of qPCR with *pagA* DNA obtained using different primer annealing temperatures

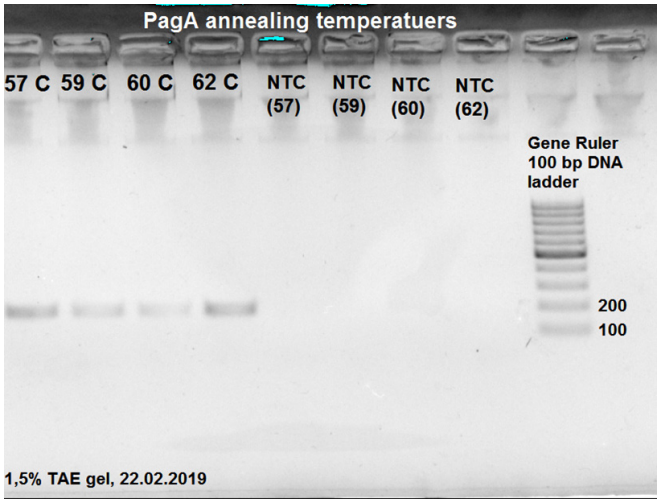

Figure 3. Gel picture of *pagA* qPCR amplification products obtained with different primer annealing temperatures. Negative controls are marked as 'NTC'

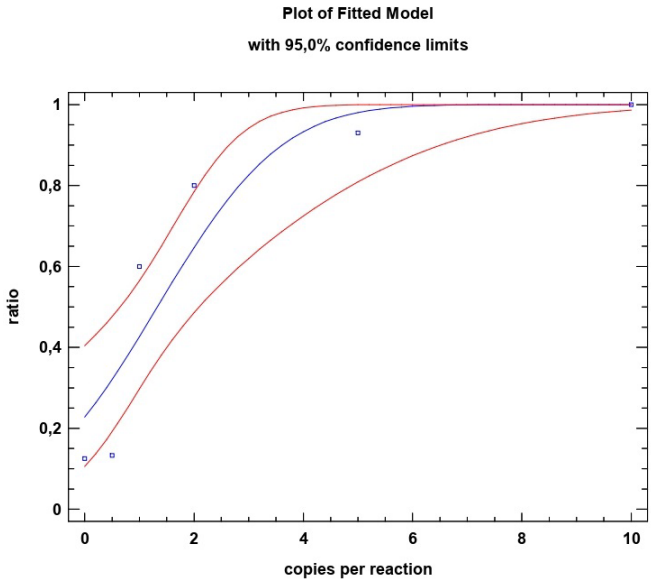

Figure 4. Graphic model based on the results of probit analysis

Table 3 — Ct values obtained after Intra-assay variability test, as well as calculated mean values, standard deviations and CVs

Sample	Ct value			Mean	Standard deviation	CV, %
	Replicate 1	Replicate 2	Replicate 3			
B. anthracis 3012 (1:4) (positive sample)	18.94	18.32	18.35	18.54	0.35	1.89
B. anthracis 3189 (1:50000) (weakly positive sample)	30.00	30.06	29.99	30.02	0.04	0.14
Negative sample	Negative	Negative	Negative	—	—	0

Table 4 — Mean Ct values, standard deviations and CVs obtained after several independent experiments (Inter-assay variability)

Sample	Day 1 mean	Day 2 mean	Day 3 mean	Mean (days 1–3)	Standard deviation (days 1–3)	CV, %
B. anthracis 3012 (1:4)	18.54	20.45	20.17	19.72	1.03	5.23
B. anthracis 3189 (1:50000)	30.02	30.27	30.09	30.13	0.13	0.44
Negative sample	0	0	0	0	0	0

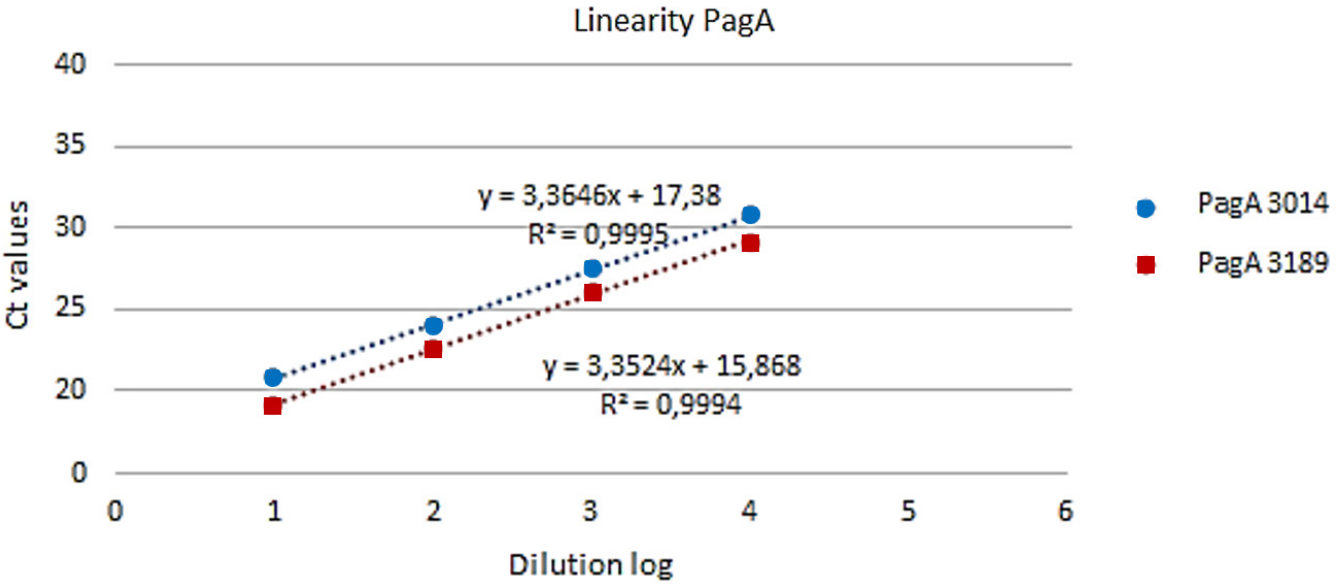

Figure 5. Linearity of *pagA* qPCR: linear regression lines and coefficients of correlation for tested $\log_{10}$ dilution series of samples 3014 and 3189. Samples were diluted from 101 to 103 (3014) and from 101 to 104 (3189)

Table 5 — *pagA* qPCR results of mixed DNA panel of *Bacillus anthracis* for evaluation of method's sensitivity. Positive control (*pagA* template DNA) is marked as 'PC *pagA* 10^3'

No.	Sample	Strain number	Ct values
1	6282 Tirol	916	29.68
2	Wien A5	2610	23.43
3	BGA Nr. 2 A7	2612	27.08
4	2844-9IZSVE	3007	17.37
5	4-IZSLT	3008	27.56
6	3-IZSLT	3009	26.63
7	FARMER CUTE	3011	20.10
8	FERARRA	3012	20.37
9	CEB95-002	3013	28.97
10	CEB94-033	3015	20.15
11	Vollum	3017	30.47
12	Ames	3018	26.68
13	BUL 16	3165	22.35
14	BUL 17	3166	20.01
15	BUL 28	3177	22.08
16	BUL 37	3185	34.75
17	BUL 39	3187	27.96
18	BUL 41	3189	22.06
19	PC *pagA* 10^3	—	32.02

Testing of homological DNA panel of closely-related *Bacillus* bacteria has shown that only one out of six *Bacillus cereus* strains was positive for the presence of *pagA* pXO1 genetic marker (Table 6, Fig. 7). As expected, this sample had pXO1 plasmid in its genome, and therefore *pagA* genetic marker located on it.

Table 6 — Homological DNA panel of closely related *Bacillus* bacteria used for evaluation of specificity (cross-reactivity) of the method

Microorganism	Ct values	Expected result
B. cereus E33L (diluted 1:15)	negative	negative
B. cereus 03BB102	19.60	positive
B. cereus B792	32.62	positive
B. cereus ATCC10987 (BCE-0696)	33.03	positive
B. thuringiensis spp. israeliensis WIS493 (diluted 1:15)	35.10	positive
B. mycoides B298 (diluted 1:15)	35.60	positive
B. globigii WIS399	36.44	positive
B. weihenstephanensis B498 (diluted 1:15)	38.24	positive
B. cereus var. anthracis CI (3265)	19.35	positive
B. cereus var. anthracis CA (3266)	20.52	positive

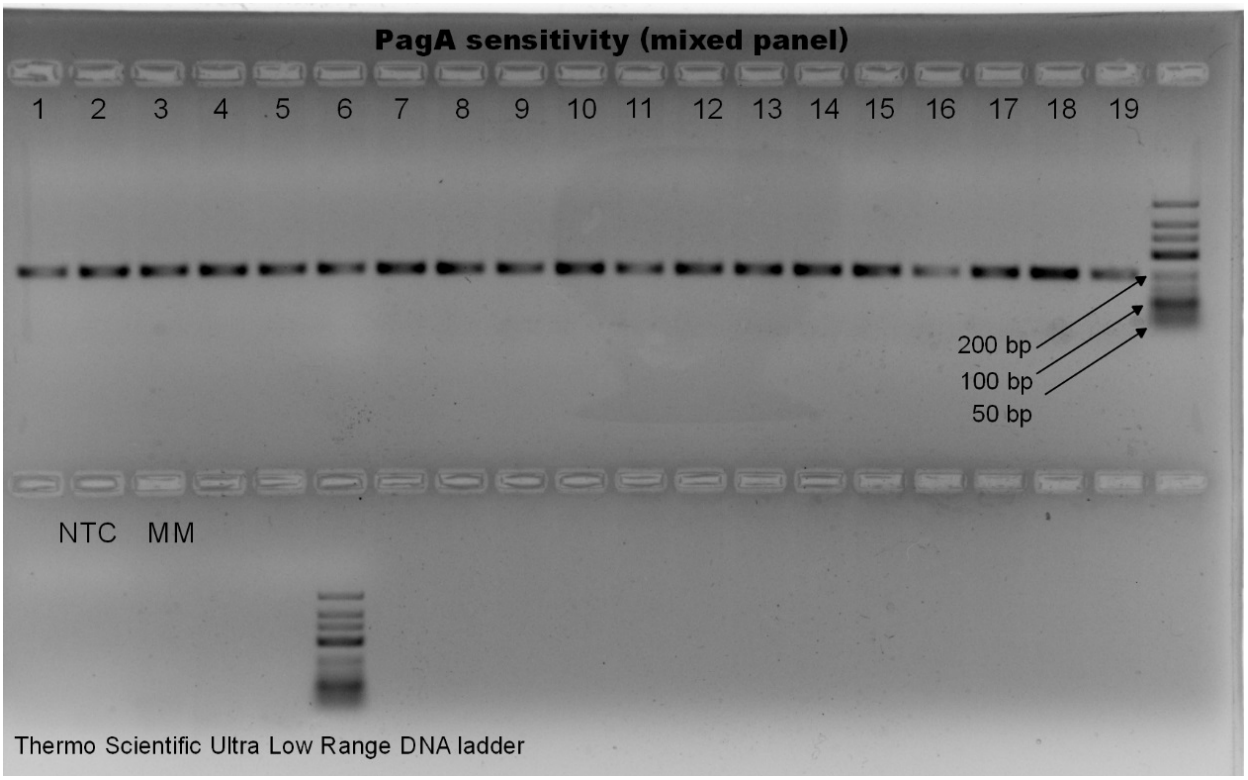

Figure 6. Gel picture of *pagA* qPCR results with mixed DNA panel of *Bacillus anthracis* samples for evaluation of method's sensitivity

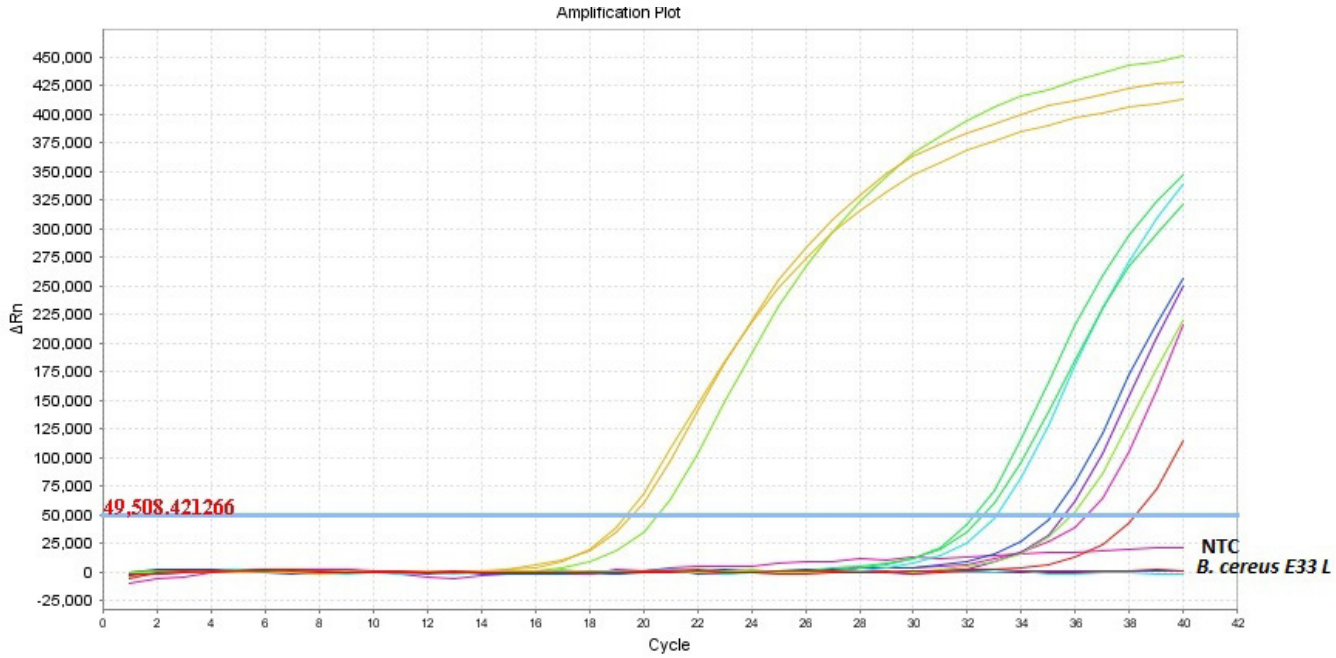

Figure 7. *pagA* qPCR results of homological DNA panel of closely related *Bacillus* bacteria used for evaluation of specificity (cross-reactivity) of the method

The result of qPCR with heterological panel did not show any samples containing *pagA* genetic marker, which is specific only for *Bacillus anthracis* and some bacteria of *Bacillus* group.

Conclusions. All conducted assays proved that qPCR test which we used for detection of *pagA*, *Bacillus anthracis* pXO1 genetic marker, is specific and trustable. In addition, this validation procedure allowed to make this test more robust, repeatable and sensitive. This method was adjusted in the laboratory of molecular diagnostics at NSC 'IECVM' for diagnostical detection of anthrax in environmental samples.

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STUDY OF IMMUNOGENIC PROPERTIES OF EXPERIMENTAL SERIES OF BIVALENT VACCINE AGAINST CARNIVOROUS LEPTOSPIROSIS IN LABORATORY CONDITIONS

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Summary. Leptospirosis is an infectious natural-focal disease of many species of animals and human with a wide range of clinical signs. The most common serovars causing leptospirosis in dogs are Icterohaemorrhagiae and Canicola. Analyzing the epizootic situation concerning leptospirosis of carnivores in Ukraine, employees of the Laboratory of Leptospirosis of Institute of Veterinary Medicine of the National Academy of Agrarian Sciences have developed and produced successively three series of bivalent inactivated vaccine against leptospirosis of carnivores, taking into account the etiological structure of the disease of this animal species. The paper presents the results of the study of three experimental series of inactivated polyvalent vaccine against leptospirosis of carnivores regarding: pH, sterility, residual amounts of inactivant, completeness of inactivation, harmlessness and immunogenic activity. It has been established that according to the indicators of sterility, completeness of inactivation, residual amount of inactivant, harmlessness and concentration of hydrogen ions, all three series of vaccine meet the requirements and norms of normative documentation. Intravenous administration of all three experimental series of the vaccine to the experimental rabbits provided the formation of specific anti-leptospirosis antibodies in the titers corresponding to the parameters of the immunogenicity norms established in the technical conditions of the drug.

Keywords: vaccine, leptospirosis, Icterohaemorrhagiae, Canicola, dogs, rabbits, microscopic agglutination test

Introduction. Leptospirosis is a zoo-anthroponotic natural-focal infection characterized by short-term fever, anemia, jaundice, necrosis of the mucous membranes and skin, hemoglobinuria, atony of the gastrointestinal tract and weight loss, abortions and the birth of an unviable offspring (Nedosiekov, Ukhovskyi and Kucheriavenko, 2011). One of the main measures to control leptospirosis is vaccination. The vaccine against leptospirosis is used for the active immunization of animals. It prevents the acute course of the disease, the death of animals, abortions, carriage of *Leptospira* (Adler, 2015; Balks et al., 2013; Walker and Srinivas, 2013).

The biological industry of Ukraine does not produce domestic vaccine against leptospirosis of carnivores, and uses only foreign vaccines for the prevention of carnivorous leptospirosis, therefore we have carried out scientific researches on perfection and development of a new technology for production of immunoprophylactic agent against leptospirosis of carnivores.

The employees of the Laboratory of Leptospirosis of Institute of Veterinary Medicine of the National Academy of Agrarian Sciences have developed and produced sequentially three series of bivalent inactivated vaccine against leptospirosis of carnivores taking into account the etiological structure of the disease of this species of animals.

The technical result of the designed vaccine is: increased immunogenic and antigenic activity of selective strains (registration numbers provided by the Depository of the State Scientific and Control Institute of Biotechnology and Strains of Microorganisms 354 and 360) used for the production of the vaccine; reduced immunization dose of the vaccine due to the modern methods of concentration with polyethylene glycol.

Leptospirosis is one of the most prevalent antropozoonotic infections in many countries of the world and in particular in Ukraine (Ukhovskyi et al., 2018). To date, there are over 250 leptospira serovars in 26 serogroups (Walker and Srinivas, 2013). Animals of different species suffer from leptospirosis: cattle, pigs, horses, sheep, goats, deer, foxes, arctic foxes, mink, dogs, etc. (Pyskun et al., 2018; Stepna et al., 2016; Ukhovskyi et al., 2014). The most common serovars causing leptospirosis in dogs before the introduction of leptospirosis vaccine 30 years ago were Canicola and Icterohaemorrhagiae. After the production of the bivalent vaccine, other strains have become more common, including Grippotyphosa, Pomona, Bratislava, and Autumnalis (Alton et al., 2009). This may be the result of an increasing number of contacts between dogs and reservoir pathogen hosts (Martin et al., 2014). The selection of leptospira serovars for vaccine development is a very important step. For the most effective prevention, you need to use only those serovars that circulate in this region.

At present, the most common leptospira, which cause leptospirosis disease among carnivores in Ukraine, are leptospira of the following serogroups: Canicola and Icterohaemorrhagiae (Babyuk et al., 2009).

The aim of the work was to study the properties of three, produced sequentially, experimental series of polyvalent vaccines in laboratory animals, to determine the following parameters: pH, sterility, residual amount of inactivant, completeness of inactivation, harmlessness and immunogenic activity.

Materials and methods. Two leptospira strains, Icterohaemorrhagiae and Canicola, were used in the production of three series of experimental bivalent vaccines. The list of these strains is shown in the Table 1. All the series of these vaccines were made from the same leptospira strains, and by the same technology.

Table 1 — List of strains used in the manufacture of the vaccine

No.	Serogroup	Serovar	Strain	Registration number
1	Ictero-haemorrhagiae	Ictero-haemorrhagiae	VGNKI-2	354
2	Canicola	Canicola	VGNKI-3	360

The leptospira strains used to make the vaccine, were cultured on a Kortgof medium with the addition of 10% of sheep blood serum at 27–28 °C in a dark room.

For the manufacture of vaccines there were used cultures with accumulation of at least 75 million leptospira in one centimeter cubic, that is, not less than 60 leptospira in the field of view of the microscope.

Each leptospira serogroup was cultured separately, then they were poured into one container and inactivated.

Inactivation of bacteria was carried out with a solution of phenol, it was added to the culture in the amount of 0.5% to the volume of the vaccine. The preserved culture was maintained at 27–28 °C for 12 hours, this was followed by microscopy of the culture in a dark field of the microscope. To concentrate the vaccine, a mixture of leptospira cultures in a bottle was precipitated by the addition of a solution of polyethylene glycol (PEG 6000) of 10–12% to the volume of the preparation. It was previously prepared sterile mother liquor of PEG — 70%. After that, the vaccine was mixed vigorously with a stirrer for 10–15 minutes. Additionally, the preparation was concentrated by removing 50% of the supernatant from a mixture of cultures that were inactivated and precipitated.

After adding the PEG from the bottle, samples were taken and checked for sterility. Sterility of the vaccine was determined according to DSTU 4483:2005 (DSSU, 2005).

The concentration of hydrogen ions was determined by a pH meter in accordance with the instructions for its use. Determination of the residual amount of inactivant was carried out in accordance with the Guidelines 4.1/4.2.588-96.

To determine the completeness of inactivation of the vaccine, we conducted three consecutive passages of it on the Kortgof medium with addition of 10% rabbit serum. In this case, three test tubes were used for each passage for samples from each vial taken for control. The presence of live leptospira in the field of view of the microscope was determined by microscopy in a dark field (an increase of 20×10×1.5 or 20×15).

The harmlessness of the experimental series of vaccines was determined on white mice with body weight from 18 to 20 g, 10 mice for testing of each series.

Vaccinations were carried out by subcutaneous administration of vaccines at a dose of 0.3 cm^3. The injection site was treated with 70% ethyl alcohol. Experimental animals were observed for 10 days to determine their general state. Each animal was used once.

The antigenic properties of the vaccine were determined on rabbits with body weight of 3.0–3.5 kg, five animals for testing of each vaccine series.

After shaking, 10 cm^3 of vaccine were taken with the sterile pipette from each of five vials with one series of vaccine, and transferred to a sterile vial of 100 cm^3. The resulting average sample of the vaccine was shaken and administered intravenously at a dose of 0.75 cm^3 to five rabbits. 25 days later, blood samples were taken from vaccinated rabbits and examined in the microscopic agglutination test. Similar studies have been conducted with all three series of vaccines.

Blood serum of rabbits was investigated in the microscopic agglutination test with the strains of the corresponding leptospira serovars, which were part of the tested series of vaccines.

The antibody titers in the indicated test were determined in 6 dilutions from 1:50 to 1:1,600 (multiplicity of 2). Solution of serum, in which half and more agglutination of leptospira was observed, was considered as a titer of antigen.

Experimental data was calculated statistically (Lakin, 1990).

Results. All series of the drug in appearance and by color were homogeneous grayish-white liquid with a slight precipitate, which, when shaking, easily broke down to the formation of a homogeneous suspension.

The results of the study of vaccine series by the parameters of sterility, completeness of inactivation, residual amount of inactivant (phenol), harmlessness and concentration of hydrogen ions are presented in the Table 2. According to the requirements of the regulatory documents, the concentration of hydrogen ions in the finished preparation should be within the range of 7.2–7.4. According to the data of the Table 2, the concentration of hydrogen ions in the experiment varied in the range 7.24–7.36, which corresponds to the norm. Testing the preparation for sterility, according to DSTU 4483:2005 (DSSU, 2005), showed that all series of vaccines were sterile.

During the three times passage of the vaccine on a nutrient medium for the growth of leptospira, no growth of live spirochaeta was observed, indicating complete inactivation of the antigen in the tested vaccine.

The residual amount of inactivant in the vaccine ranged from 0.41 to 0.48, that is, within the permissible concentrations, which should not exceed 0.5% according to the norm.

Table 2 — Physical and biological characteristics of experimental series of vaccine against leptospirosis of carnivores

Characteristics	Series 1	Series 2	Series 3
pH of vaccine	7.24	7.36	7.28
Sterility	sterile		
Completeness of inactivation	At three-time passage of the vaccine on the nutrient medium there was no growth of leptospira		
Harmlessness	harmless		
Residual amount of inactivator (phenol), %	0.48	0.41	0.47

When determining the harmlessness of the vaccine, by administering the drug to laboratory animals, it has been established that during the observation period all the animals remained alive without any local and symptomatic manifestations caused by the vaccine. The vaccine was considered harmless.

Thus, by the characteristics of sterility, completeness of inactivation, residual amount of inactivant, harmlessness and concentration of hydrogen ions, all three series of vaccine met the requirements and norms of normative documentation.

When determining the indicators of the immunogenicity of the produced drug, we have, based on the principle of analogues, formed three groups of rabbits (5 animals in the group) which were given a single intravenous administration of the vaccine at a dose of 0.75 cm^3. On the 25th day, the blood was taken from the vein of vaccinated rabbits. The blood was examined in the microscopic agglutination test. The results obtained are presented in the Table 3.

Before conducting a study of the vaccine immunogenicity, blood was collected from all experimental rabbits, and tested in the microscopic agglutination test for the presence of antibodies to pathogenic leptospira. Eight diagnostic strains of leptospira were used for the test, they belong to the eight serological groups Icterohaemorrhagiae, Australis, Pomona, Canicola, Sejroe, Hebdomadis, Grippotiphosa, Tarassovi. For all rabbits, the test was negative.

According to the requirements of normative and technological documentation, the vaccine is considered to be active, if not less than in four of the five vaccinated rabbits, the titer of antibodies in blood serum to Icterohaemorrhagiae and Canicola serogroups will be no less than 1:100.

The results of the studies indicate that all series of experimental vaccines were immunogenic and consistent with the indicators of immunogenicity standards set out in the technical specifications for this preparation.

As can be seen from the Table 3, the administration of all three experimental series of the vaccine into experimental rabbits provided the formation of specific anti-leptospirosis antibodies.

Table 3 — Titer of antibodies in the microscopic agglutination test for different leptospira serogroups in blood serum of vaccinated rabbits on the 25th day after vaccination

Series	Serial numbers of rabbits	Titers of antibodies to leptospira serogroups	
		Ictero-haemorrhagiae	Canicola
Series 1	Rabbit 1	1:1,600	1:1,600
	Rabbit 2	1:800	1:200
	Rabbit 3	1:800	1:800
	Rabbit 4	1:1,600	1:800
	Rabbit 5	1:800	1:800
	Average titer	1:1,120 ± 384	1:840 ± 304
Series 2	Rabbit 1	1:600	1:800
	Rabbit 2	1:200	1:800
	Rabbit 3	1:600	1:600
	Rabbit 4	1:800	1:200
	Rabbit 5	1:600	1:200
	Average titer	1:580 ± 206	1:520 ± 190
Series 3	Rabbit 1	1:1,600	1:1,600
	Rabbit 2	1:800	1:200
	Rabbit 3	1:200	1:800
	Rabbit 4	1:1,600	1:200
	Rabbit 5	1:1,600	1:200
	Average titer	1:1,160 ± 390	1:600 ± 195

Thus, the manufactured preparations are immunogenic and meet the requirements of the technical conditions for this immunobiological preparation.

According to the results of the conducted research, the 'Certificate of analysis of successively produced 3 series of vaccine' was drawn up and submitted to the State Research and Control Institute of Biotechnology and Strains of Microorganisms.

Conclusions. 1. When studying three experimental series of bivalent inactivated vaccine against leptospirosis in animals, it has been found that all of them were harmless to mice at a single subcutaneous administration at a dose of 0.3 cm^3.

2. The titers of antibodies in vaccinated with the experimental series of vaccine animals met the requirements of the technical conditions for this product.

3. According to the results of the research, it has been found that all three vaccine series met the necessary requirements, and this vaccine could be tested on carnivores.

Further research will be continued to study the effectiveness of the created bivalent vaccine in carnivorous animals.

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Part 3. Biosafety

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DETERMINATION OF ACUTE TOXICITY OF THE 'BONDARMIN' DISINFECTANT

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Summary. In the article the results of the study of toxic effect of the designed disinfectant (active ingredient potassium peroxomonosulfate) on laboratory animals (mice) are presented. For the recent years a variety of scientific works both by domestic and by foreign scientists has been devoted to the study of different disinfectants' toxicity. However today there is a number of issues that require more detailed studying and scientific justification. Among them the problem of toxic effects of disinfectants on the animal organism occupies a special place. The aim of our work was to study the toxic effect on the laboratory animals and to assess the acute toxicity (LD_{50}) of the designed 'Bondarmin' disinfectant. Tests were carried out at the Laboratory of pharmacology and toxicology of the National University of Pharmacy (Kharkiv) and on the base of Educational and scientific laboratory of genetic and molecular research methods named after P. I. Verbitskiy in the Kharkiv State Zooveterinary Academy. Acute toxicity assessment (LD_{50}) was carried out with intragastrointestinal administration of the designed drug to laboratory animals (mice). Changes in the internal organs of animals that were removed from the experiment for humane reasons and those who died after the experiment were detected by macroscopic examination. The lethality of laboratory animals after the intragastric administration of disinfectant was determined by the Prozorovskiy method The dynamic of changes in body weight of mice after the administration of disinfectant in high doses (from 1,500 to 3,500 mg/kg) was found out. The influence of the disinfectant on the mass coefficients of the internal organs of male mice after intragastric administration was evaluated. Toxic effect of the designed disinfectant 'Bondarmin', when using intragastric method of administration to laboratory animals (mice), according to the age and sexual index (LD_{50} = 2,702.40 ± 156.32 mg/kg), was established. Disinfectant 'Bondarmin' refers to IV toxicity class (low toxic substances).

Keywords: disinfectant, laboratory animals, toxic effect, lethal dose

Introduction. It is important to create scientifically grounded and comfortable animal housing conditions to ensure the high resistance of animals to diseases of different etiology (Chumachenko, Chumachenko and Pavlenko, 2004).

Timely and effective disinfection of premises and equipments has fundamental importance in the system of measures for the prevention and control of infectious diseases of farm animals (Kondratjuk, 2009; Holovko, Kochmarskyi and Tupozlieiev, 2011; Paliy, Paliy and Naumenko, 2015).

Today during disinfection when animal tuberculosis is declared, disinfectants, the active substance of which is glutaraldehyde, which interacts with the protein components of the membrane structures of bacterial cells, disrupting the processes of their synthesis and ultimately causing their death, are widely used (Chupakhin et al., 1987; Gutyj et al., 2017; Paliy et al., 2018).

At the same time, traditional means, based on chlorinated compounds, have high antimicrobial activity against mycobacteria. The bactericidal action of these means lies in the denaturation of protein and nucleic acids (Dychdala, 2001; Chapman, 2003; Zavgorodniy et al., 2013).

Taking into consideration the risk of infectious diseases, the improvement of the existing, finding new and developing more effective, environmentally safe, relatively cheap, technological, simple and accessible for use disinfectants with high bactericidal properties against mycobacteria remains the task of great importance (Paliy et al., 2015; Kozlovskaya, 2016).

Alongside, one of the main requirements for disinfectants is their low toxicity to animals and humans, which allows more extensive use of these drugs in the agriculture (Zavgorodniy et al., 2013).

It is necessary to start determination of the toxicity of the developed new veterinary medicines with an acute test, that is, with obtaining information about the dangers of the main experimental active substance for animal health after a short-term presence, as a result of which data on the lethal dose and concentration are expected (Kotsiumbas et al., 2006; Todoriuk et al., 2018).

'Bondarmin', a modern, powdered form disinfectant, well soluble in water, odorless, the active ingredient of which is potassium peroxomonosulfate, has been developed.

The aim of the study was the determination of the toxic effect of the 'Bondarmin' disinfectant on laboratory animals.

Material and methods. The toxicological properties of the 'Bondarmin' disinfectant, which is a domestic import-substituting development, have been identified. Studies were carried out using a rapid method to study moderately lethal doses of chemical compounds (Pastushenko et al., 1985).

The least squares method was used to analyze the lethality curves (Prozorovskiy, 1962).

For experimental studies 40 CD-1 mice were used. Mice were grown in the vivarium of the Kharkiv State Zooveterinary Academy and prior to the experiment they had undergone acclimatization under the conditions of the testing room for 7–10 days. The animal keeping conditions complied with the current rules for vivarium devices, equipment and maintenance. In accordance with the code of practice, animals received standard nutrition (CEC, 2010).

Animals were treated in accordance with the requirements of the Commission on Bioethics and the General Ethical Principles of Experiments on Animals, consistent with the provisions of the 'Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes' (CEC, 2010).

The acute toxicity of 'Bondarmin' was studied on laboratory animals (mice) trough intragastric injection to reproduce the acute poisoning clinic and to determine the LD_{50}.

Results and discussions. The determination of the 'Bondarmin' acute toxicity (LD_{50}) trough intragastric injection to laboratory animals (mice) was the primary stage of our research. For this purpose, laboratory animals (mice) were divided into groups of three. A dose range from 1,580 to 4,470 mg/kg was chosen. The tested disinfectant was injected intragastrically to the animals in the form of an aqueous mixture through a metal probe at a rate of 0.2 ml/10 g of body weight. The results of the experiment are presented in the Table 1.

According to the study results, the death of mice was noted when they were administered a dose of 2,000 mg/kg and higher. The required dose range for calculating LD_{50} was highlighted. For this purpose a sequence of animal death 1–3–3 was used. In doses for mice it is 2,000, 2,820, and 3,160 mg/kg. With the help of the table express method by Pastushenko et al. (1985) the LD_{50} was determined. According to this method, lower doses of the selected interval in the table find the LD_{50} value and its confidence limits for mice — 2,580 (1,930–3,220) mg/kg.

In order to confirm the above mentioned results of LD_{50} and to study other toxicity parameters, the acute disinfectant toxicity studies with intragastric administration using the least squares method for probit analysis of mortality curves according to Prozorovskiy (1962) was conducted (Table 2).

Table 1 — Lethality rate of laboratory animals after intragastric administration of the 'Bondarmin' disinfectant (n = 3)

Animal species, sex	Dose, mg/kg	Received effect, animals that died/overall number
Mice (male)	1,580	0/3
	2,000	1/3
	2,500	1/3
	2,820	3/3
	3,160	3/3
	3,550	3/3
	3,980	3/3
	4,470	3/3

Table 2 — Mortality of laboratory animals after intragastric injection of the 'Bondarmin' disinfectant according to Prozorovskiy method (n = 6)

Animal species, sex	Dose, mg/kg	Received effect, animals that died/overall number
Mice (male)	1,500	0/6
	2,000	1/6
	2,500	2/6
	2,750	3/6
	3,000	5/6
	3,500	6/6

To determine the average median lethal dose, experimental groups of six animals were formed. The animals were observed for 14 days after the injection of the disinfectant, registering manifestations of violations in the physiological state of the animals and mortality. From the data presented in Table 2, it was established that the death of mice occurs in the dose range of 2,000–3,500 mg/kg.

At the next stage calculation of the disinfectants toxic effect parameters for mice using Prozorovskiy method were carried out (Table 3).

According to the data presented in the Table 3, the calculated LD_{50} parameters for disinfectant after intragastric injection to mice are: A0 = 2.19; A1 = 0.87; LD_{16} = 2,087.71 mg/kg; LD_{50} = 2,702.40 mg/kg; LD_{84} = 3,298.53 mg/kg; m = 156.32 mg/kg.

Disinfectant 'Bondarmin' trough the intragastric injection to laboratory animals (mice) belongs to the IV toxicity class (low-toxic substances) with LD_{50} = 2,702.40 ± 156.32 mg/kg or 2,702.40 (2,378.82–3,025.97) mg/kg.

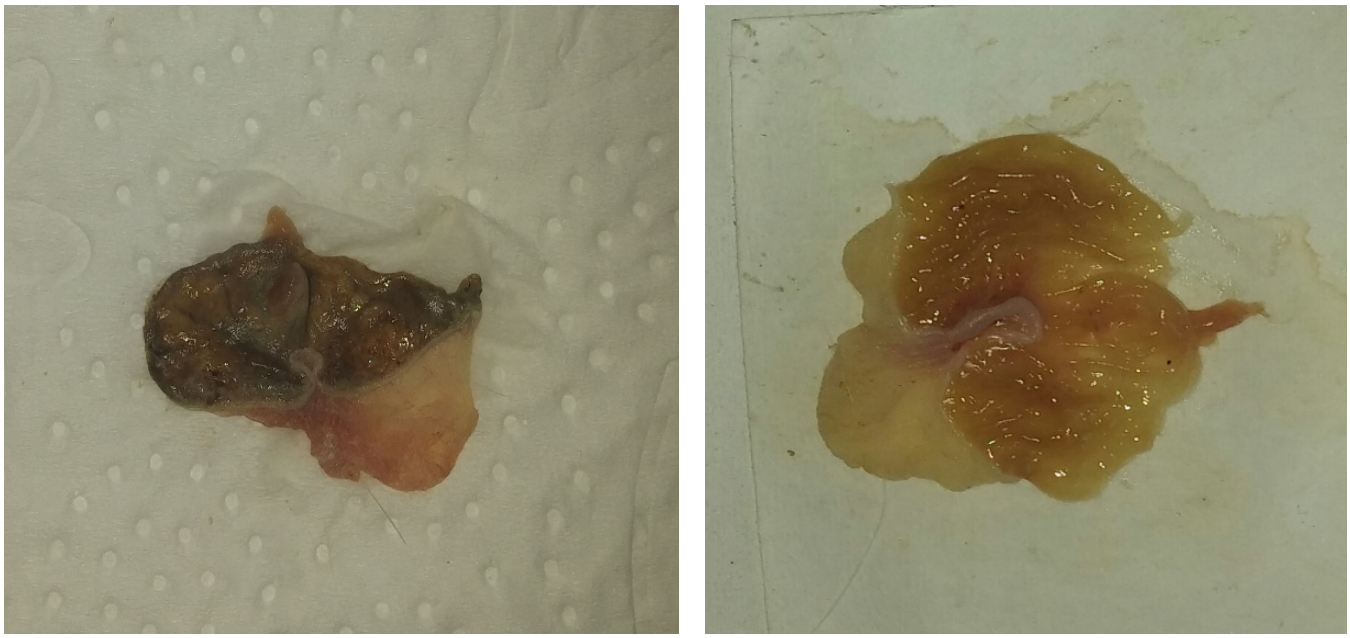
Table 3 — Calculation data for the determination of the LD_{50} of disinfectant in mice after intragastric injection by Prozorovskiy method

Dose, mg/kg	Lethality, %	Dose place (X)	Probit (Y)	Weighting coefficient (B)	xB	x^2в	yB	xyB
1,500	0	1	3.27	1.6	1.60	1.60	5.23	5.23
2,000	16.67	2	4.05	3.7	7.40	14.80	14.99	29.97
2,500	33.33	3	4.56	4.6	13.80	41.40	20.98	62.93
2,750	50.00	3.5	5.00	5	17.50	61.25	25.00	87.50
3,000	83.33	4	5.95	3.5	14.00	56.00	20.83	83.30
3,500	100	5	6.73	1.6	8.00	40.00	10.77	53.84
Σ				20	62.30	215.05	97.79	322.77

After administration of toxic doses of disinfectant to animals such signs of intoxication were observed: reduced motor activity, heavy breathing, cold limbs and tail, lack of appetite, developed stupor, and then death occurred. The death of animals was observed 2–3 hours after administration of doses 2,750–3,500 mg/kg and the death was delayed from 1 to 8 days after the injection of smaller doses of the disinfectant 'Bondarmin'.

A macroscopic examination of the internal organs of animals, which were removed from the experiment according to humane motives and those who died, showed that the gastric mucosa had a broken relief of folds and was full of blood, as well as numerous point and linear ulcers were observed (Fig. 1).

As was shown by the results of survived animals' observation, they were active, had a satisfactory appetite, responded to sound and light irritators, urination and defecation were normal, respiratory failure and convulsions were not observed. All animals maintained the reflex excitability. The water and food consumption in all experimental animals did not differ from the animals of the intact control group.



A **B**

Figure 1. Mice stomach: A — after injection of a disinfectant in a dose of 2,750 mg/kg, B — intact control

The study of mice body weight showed (Table 4) that the mass of survived animals was not statistically different from the values of the control group during the study period.

As to the other indicators of the internal organs of the survived animals, the use of disinfectant did not lead to their change compared with animals of the intact control group. Indicators were within the physiological range (Table 5).

Calculation and statistical analysis of the mass coefficient indicators of the mice internals showed that intragastric injection of the 'Bondarmin' disinfectant in high doses resulted in decrease of the thymus mass coefficient.

Table 4 — Dynamics of the mice body weight after the injection of the 'Bondarmin' disinfectant in high doses (n = 6)

Group	Dose, mg/kg	Animal body weight, g			
		Data line	For the 3rd day	For the 7th day	For the 14th day
Intact control	—	26.7 ± 1.4	27.1 ± 1.3	26.9 ± 1.2	27.1 ± 1.1
Experimental	1,500	27.6 ± 1.1	26.2 ± 1.3	26.4 ± 1.1	27.7 ± 1.1
	2,000	25.0 ± 0.8	23.9 ± 0.8	24.9 ± 0.9	25.1 ± 0.9
	2,500	25.9 ± 1.0	24.5 ± 0.2	24.9 ± 0.3	25.5 ± 0.2
	2,750	25.5 ± 1.5	24.5 ± 0.2	24.9 ± 0.4	25.1 ± 0.4
	3,000	26.3 ± 1.7	22.2	22.0	23.2
	3,500	25.1 ± 0.9	—	—	—

Table 5 — Mass coefficient of the male mice internals after intragastric injection of a disinfectant (n = 6)

Internals	Intact control (n = 6)	Disinfectant, mg/kg				
		1,500 (n = 6)	2,000 (n = 5)	2,500 (n = 4)	2,750 (n = 3)	3,000 (n = 1)
Liver	5.20 ± 0.21	5.59 ± 0.30	5.43 ± 0.37	5.16 ± 0.36	5.05 ± 0.33	6.03
Right kidney	0.62 ± 0.05	0.69 ± 0.05	0.79 ± 0.07	0.67 ± 0.08	0.66 ± 0.07	0.95
Left kidney	0.61 ± 0.04	0.66 ± 0.04	0.75 ± 0.07	0.69 ± 0.09	0.67 ± 0.08	0.82
Heart	0.57 ± 0.03	0.54 ± 0.06	0.66 ± 0.04	0.54 ± 0.04	0.53 ± 0.03	0.66
Lungs	0.70 ± 0.03	0.81 ± 0.06	0.88 ± 0.06	0.72 ± 0.04	0.71 ± 0.04	0.76
Spleen	0.79 ± 0.06	0.98 ± 0.31	1.00 ± 0.04	0.90 ± 0.04	0.88 ± 0.03	0.56
Adrenal	0.061 ± 0.005	0.052 ± 0.006	0.061 ± 0.008	0.052 ± 0.002	0.051 ± 0.002	0.056
Thymus	0.298 ± 0.009	0.203 ± 0.022*	0.205 ± 0.017*	0.200 ± 0.004*	0.196 ± 0.035*	0.320
Right spermary	0.38 ± 0.04	0.34 ± 0.03	0.48 ± 0.05	0.40 ± 0.03	0.39 ± 0.03	0.42
Left spermary	0.37 ± 0.03	0.34 ± 0.03	0.44 ± 0.03	0.38 ± 0.02	0.38 ± 0.03	0.37

Note: * — the deviation of the indicator is statistically significant relative to the values of the intact control group (Man-Whitney test with Bonferoni correction), $p < 0.01$.

Conclusions. The toxic effect of the newly designed disinfectant 'Bondarmin' was established during the intragastric injection to laboratory animals (mice).

The median lethal dose of the 'Bondarmin' disinfectant by Prozorovskiy method is LD_{50} = 2,702.40 ± 156.32 mg/kg. The disinfectant belongs to the IV toxicity class (low toxic substances).

The results of toxicological studies allow us to recommend the use of 'Bondarmin' for disinfecting livestock facilities.

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ANALYSIS OF CONTEMPORARY MEAT AND MEAT PRODUCTS' PROCESSING METHODS

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Summary. For today, the issue of maximum preservation of meat and meat products at all stages of their production, storage, transportation and realization does not lose its relevance. Existing ways of food products' preservation for prevention of their microbiological damage are divided into three groups: physical, chemical and biological. The article gives an analysis of modern methods of meat and meat products' processing. Physical (chill, freezing, super freezing, ionizing irradiation, ultraviolet irradiation, pressure processing, laser irradiation) and chemical (with the use of organic acids, trisodium phosphate, extracts of plants with bacteriostatic properties etc.) methods of meat and meat products' preservation are characterized. The data on the methods for extension of realization term due to the use of modern packaging materials (modified atmosphere, system of 'active packaging' and aseptic packaging) is given. The advantages and disadvantages of each of these methods are indicated.

Keywords: meat, meat products, method, processing, canning, analysis

Introduction. Meat and meat products remain one of the most consumed products on the world market. From the chemical point of view, the lean muscle tissue of farm animals and poultry consists of water (71–76%), proteins (20–22%), fats (lipids, 3–8%), carbohydrates (1.2%), soluble non-protein organic and inorganic substances (2.3%) as well as vitamins. From a microbiological point of view, these compounds are nutrients for microorganisms' living activity. That is why the terms of their storage, especially chilled meat and offal, are only 2–4 days. Only a few days are enough for starting of microorganism growth and reproduction under the influence of oxygen, light and temperature.

For today, physical and chemical methods of meat and meat products' preservation, as well as various methods for the extension of realization period using modern packaging materials (modified atmosphere, 'active packages' and aseptic packaging systems) are exist. However, the search of novel and safe method that would ensure the effective and reliable long-time storage of the product (together with keeping of product's quality and safety) is still an essential issue for veterinary and sanitary experts, controllers of ready-to-use products, technologists and commodity researchers.

The aim of this work is to analyze modern meat and meat products' processing methods in order to extend the realization term and to determine their advantages and disadvantages.

Material and methods. The analysis was carried out in the Department of Infectology, Quality and Safety of AIC Production of the Luhansk National Agrarian University. The objects of the research were processing methods of meat and meat products in order to extend the term of their realization.

Results. At the current stage of the meat processing industry development, the entire row of methods which are used for the extension of the term of realization of ready-to-use products, includes the following groups:

Physical methods:

1. Chill and freezing. Processing of meat and meat products with frost is commonly used and it is one of the best preserving methods. The widespread use of cold is associated with the following positive characteristics:

— ability for rapid preservation of a large quantity of raw materials;

— prolonged storage of meat with maintenance of its properties and nutritional value;

— minimal loss of product's weight during storage;

— the universal use of freezing technology for all kinds of meat raw materials and ready-to-use products;

— lower energy consumption of refrigeration in comparison with other preservation methods;

— possibility to use the frost not only as the main preserving factor, but also in combination with other preserving methods (salt, production of sausages etc.).

However, it is necessary to take into account the disadvantages of this method. First of all, microorganisms are resistant to low temperatures and the majority of bacteria remain viable after freezing. Moreover, some species are even capable for reproduction. Bacterial toxins are not destroyed even after slow or several repeated freezing acts. In addition, the action of low temperatures does not destroy the micellar fungi and yeast, but only reduces their activity (Nastasijevic, Lakicevic and Petrovic, 2018).

2. Super freezing is a refrigeration process that provides a reduction of meat temperature below the

cryoscopic temperature by 1–2 °C with partial ice formation (Mok et al., 2017).

The main parameter, which defines the quality of product's super freezing, is the degree of water transition into ice. Generally, the ice content from 5 to 30% in the product does not lead to a noticeable decrease in product's quality, but might increase the shelf life. The amount of ice in the product over 30% leads to a large loss of liquid (Stonehouse and Evans, 2015; Wu et al., 2014).

Storage of food products at super freezing temperature has three main advantages:

1) saving of product's freshness;

2) guarantee of product's high quality;

3) inhibition of microorganism's growth.

In addition, it can reduce the impact of freezing/defrosting, and thus increase output, decrease energy, labor and transportation costs. However, it should be kept in mind that during the storage of meat, which has been frozen using this method, dehydration of the outer layer of muscle tissue is up to 5 mm and product's weight loss rises up to 8% and, therefore, meat loses its commercial state (Kaale et al., 2011).

3. Processing with ionizing radiation. Radiation processing of food is a process in which food products (packaged or unpackaged) are exposed with carefully controlled ionizing radiation during a fixed period of time to improve their safety and quality.

Radionuclides, which are approved to irradiate food products, include ^{137}Cs and ^{60}Co. Radioactive cobalt (^{60}Co) decomposes into non-radioactive nickel by separating of high-energy particles and X-rays. X-ray radiography destroys fast-growing cells (microbes), but the product still stays radioactive.

Due to its ability to penetrate deeply into the tissue, it can be used to irradiate packaged products (Brewer, 2009).

This type of processing is aimed to be used only for products, which are produced with GMP principles.

Irradiation of food products is used for various purposes (Stefanova, Vasilev and Spassov, 2010):

1) to eradicate from pathogenic microorganisms and parasites;

2) to reduce the level of bacterial contamination which cause product's damage;

3) to prolong storage periods;

4) to make phytosanitary treatment.

Wherever it is possible, food irradiation procedure should be included as a part of the Food Safety Management System ISO 22000.

The highest allowable dose of food irradiation is different in various countries of the world. It is 30 kGy in the United States, in Belgium and Netherlands — 10 kGy, and in France — 11 kGy (Ehlermann, 2016). However, in 1981 the United Expert Committee of FAO, IAEA and WHO concluded that irradiation of any food stuff with doses, which do not exceed 10 kGy, does not cause the toxic effects in processed products. The use of higher doses (10–50 kGy) of ionizing radiation should be approved by the decision of the United Committee FAO/IAEA/WHO only to process certain types of food products based on their impact on nutritional value, microbiological and toxicological status. (Donskova and Zueva, 2016). In 2011, European Food Safety Authority Commission (EFSA) confirmed the effectiveness of radiation processing to make sure that the microbiological safety of food products and the perspectives of using of ionizing radiation in agricultural biotechnology (Sanzharova, 2016).

More than 220 specialized research centers have been created to keep recording of irradiated agricultural and food products. Each year, the number of irradiated products in the world is estimated at 700–800 thousand tons. The market of irradiation services is about 2 billion dollars and it has a steady upward trend. It is expected that it will reach up to 4.8 billion dollars by 2020, and to 10.9 billion dollars by 2030. (Belyaev, 2017a, 2017b).

For today, irradiation of food products with ionizing radiation is allowed in 69 countries.

However, processing with ionizing radiation may cause the appearance of extraneous (unwanted) taste and/or odor, as well as bleaching of meat products. Moreover, it also increases the rate of lipid and vitamin oxidation (Timakova et al., 2018).

4. Processing meat under pressure is a new method of preservation with great perspectives for the meat processing industry, despite the still high cost of this technology.

The established pressure level is 300 MPa. Under the lower pressure, cells are not destroyed, and the increase of pressure negatively affects the vital activity of microorganisms (Cheftel and Culioli, 1997). Inactivation of the microbial culture of *Escherichia coli* occurs under the pressure from 500 to 700 MPa. At the same time, the studies have shown that non thermic processing of *Escherichia coli* with 700 MPa pressure with exposure of 30–60 s allows to obtain a completely ready-to-use product with an output readiness of about 100%. At the same time, the product processed with pressure has high organoleptic parameters (Vinnikova and Prokopenko, 2015). It is possible to reduce the action of pressure with additional increasing of temperature: during processing with the pressure of 60 MPa and increasing of temperature from 25 to 93.6 °C, there is a significant decrease in microbial contamination. Pressure of 200 MPa and low temperatures (less than 0 °C) prolong the shelf life of the foodstuffs without freezing of liquid part (Okamoto and Suzuki, 2002).

The inhibiting effect on microorganisms while processing with high pressure is achieved with the damage of cell membrane, the conformation of protein molecules, cellular proteins and enzymes due to the breakage of hydrophobic bond and with increasing of pressure it the breakdown of hydrogen bonds occurs.

In addition, high pressure is one of the promising methods of processing food raw materials; it almost excludes the loss of nutrient components and extractives that are observed in any traditional heat processing. High bacteriological and bacteriostatic pressure affects the vital functions of microorganisms (Shelikhov, Gladkaya and Sasina, 2010).

Thus, foodstuffs, which are processed with high pressure, have unique composition and properties. Specificity of ready-to-use products' properties is associated with changes in some special structural elements contained in the raw material. For today, the effect of non-thermal processing of meat is insufficiently studied; therefore, it poses a significant interest for scientists worldwide. The entire group of researches is needed to be involved for the establishing of rational pressure regimes for this raw material, as well as to study the quality characteristic of ready-to-use products obtained using the high pressure technology.

5. Processing of meat and meat products by laser radiation. Biological action of laser radiation causes numerous structural, functional and biochemical changes occurring in a living body and animal raw material.

Partial absorption of energy that falls on the tissue and reflection of its residual part occurs when laser radiation interacts with biological tissues. At the same time, the biological effect of laser radiation on living organism is determined by the structural and physiological properties of its particular tissues and characteristics of laser radiation (Vyayzenen et al., 2010).

The optimal irradiation dose for the effective inhibition of pathogenic microorganisms in the meat raw material is 1.5–2 Mrad. In order to inactivate *Clostridium botulinum* the dose of radiation should be increased up to 3–5 Mrad (Murashov and Zhuravleva, 2014).

Laser processing has following advantages in comparison with traditional methods:

1) completely automatic process, where the lack of contact with the irradiated object is the most important feature;

2) stability in work,

3) special storage conditions of the product after treatment are excluded.

However, it is necessary to provide technological lines of industrial manipulators for accurately control the laser radiation impact on the human body.

6. Processing of meat by ultraviolet irradiation. According to our previous studies of the use of UV radiation on the meat surface, it has been noted that the highest bactericidal efficiency has UV radiation with a wavelength of 253.7 nm. Similarly, it was observed that the effectiveness of UV radiation depends on the radiation exposure and intensity of radiation on mold spores. UV rays in small doses stimulate the growth of fungi colonies. However, large doses of UV rays reduce the germination and growth of sowed spores, and much stronger doses have damaging impact on all mold spore cells.

It should be kept in mind that some types of bacteria form spores, which germinate in the presence of favorable conditions. Moreover, spores are much more resistant to UV rays than bacteria: an average dose for elimination of spores is 10 times greater, than for destruction of vegetative forms (Sokolenko et al., 2015).

However, ultraviolet radiation has numerous disadvantages (Degala et al., 2018), such as:

1) UV rays affect only microorganisms on the surface of the product;

2) The efficiency against large colonies of microorganisms is insufficient since significant part of cells continues to develop, especially after cessation of irradiation;

3) vitamins are destructed (for example, pyridoxine) when UV irradiation is applied;

4) meat surface becomes darker as a result of myoglobin destruction as well as transition of MbO_2 into metmyoglobin;

5) oxidative processes in fats intensify significantly as a result of ozone formation;

6) it is very difficult to achieve equal irradiation of carcasses, because of the inevitable presence of shaded areas on meat carcasses.

7. Ozonation of meat. Ozone is a very strong natural disinfectant. The application of ozone technologies is based on characteristics of gaseous ozone at certain concentrations and exposures to destroy microorganisms, bacteria, spores and viruses (Rodionova, 2017).

Regarding the mechanism of ozone's action on a microbial cell, it is known, that under the influence of the destruction of plasmatic membrane's phospholipids, cell and associated fragmented proteins, the integrity of its germs is disturbed and they are deformed, separated and exposed to lysis. The products of ozone lysis, which were formed at the same time, become an instrument of additional damage of intracellular structures that provide metabolic processes. The cytolysis is completed by the release of the stock of lithium enzymes (Belykh et al., 2010).

Studying the impact of ozone on the surface microflora of chilled meat, we have found that the resistance of bacterium to ozone action varies both against the type of bacteria and the concentration of ozone as well as environmental conditions (Rodionova, 2017).

Ozone technology in meat production has following advantages (Rezgo, 2011):

1) high efficiency of disinfection (ozone has a higher oxidation potential than chlorine and its derivatives);

2) the possibility to obtain ozone directly from the air in a place of oxygen consumption;

3) environmental safety and the absence of harmful side-effects due to the rapid decomposition of ozone to oxygen;

4) environmental compatibility (from all known oxidants only oxygen and limited number of peroxide compounds exist in nature and participate in biological processes);

5) the application of ozone can significantly increase the shelf life of products without losing their freshness and high nutritional qualities;

6) high economic efficiency in comparison to other disinfectants (ozone is 2 times cheaper than with the use of other disinfectants).

The disadvantage of the use of ozone is that it is a strong oxidant of fats and blood pigments. Due to this adipose tissue turns faster and muscle tissue darkens (Brewer, 2009).

In our opinion, ozone should be used to disinfect empty chamber before loading the product or to apply it at low concentrations during meat processing.

Chemical methods:

1. Chemical preservatives. Various microorganisms produce organic acids and alcohols by anaerobic fermentation of food substrates. These substances can act in food's preservation by inhibiting of other organisms that are concomitantly present and which may spoil food or make it toxic. For example, is a frequently effective inhibitory agent used in fresh meat preservation; however, other organic acids have also been found to be responsible for discoloration and production of pungent odors (Zhou, Xu and Liu, 2010).

Salts, such as sodium lactate have been used in the meat industry because of their ability to increase flavor, prolong shelf life, and improve the microbiological safety of products (Diez et al., 2009).

The antimicrobial effects of lactates are due to their ability to lower water activity and the direct inhibitory effect of the lactate ion (Houtsma, Wit and Rombouts, 1993; Koos and Jansener, 1995).

Several researchers have successfully extended the shelf life of fresh meat products (Vasavada et al., 2003) by adding sodium lactate. Nadeem et al. (2003) extended shelf life of freshly slaughtered sheep and goat carcasses stored at 5–7 °C for 3 and 2 days respectively, after spraying the carcasses with solution 'B' containing potassium sorbate, sodium acetate, sodium citrate, sodium lactate each at 2.5% and sodium chloride at 5%, when compared with solution 'A' (without potassium sorbate) and control.

2. Biopreservation and natural antimicrobials. Recently, the chemical food preservatives that were conventionally used in food industry to prevent the spoilage caused by pathogenic microorganisms, were analyzed due to their adverse effects and environmental concerns. Biological preservation is the use of naturally derived antimicrobial agents endowed with high antimicrobial potential to preserve food and beverages and thus extend their shelf lives (Yusuf, 2018).

Natural antimicrobial agents for foodstuffs which are processed under minimal thermal conditions (including meat and poultry meat products) are very attractive alternative against chemical preserve agents. The use of natural antimicrobial agents together with innovative packaging food technologies is ideal for elimination of food spoilage (Pisoschi et al., 2018). Natural antimicrobial agents include organic acids and their salts, nisin and other bacteriocins, various active components of microbiological or animal origin, vegetable essential oils, phenolic compounds and components of smoke (Yusuf, 2018). Organic acids and their salts are effective in soluble form for spraying or their immersion into the meat package processed for realization. Besides, they are effective in the form of additives to minced meat. Salts of organic acids are also characterized by antibacterial effects on raw cattle and poultry meat, but differ from acids that reduce the pH of muscle tissue only within limited measures. This is also true for salts of weak lipophilic acids — benzoin and sorbic (Sokolenko et al., 2015).

Potassium sorbate is well known as a substance that suppresses bacterial growth causing beef damage.

An increase interest in the use of bacteriogenic lactic acid bacteria (LAB) as protective cultures for the biological conservation of meat and meat products, has been observed in two last decades. Numerous forms and strains of LAB are capable to produce a significant amount of bacteriocin which suppresses pathogenic microorganisms in fresh meat. Regarding the control of spoilage microorganism reproduction and prolongation of shelf life, the use of bacteriogenic LAB for fresh meat and poultry has its limitations (Zhou, Xu and Liu, 2010).

The direct additive agents with bacteriosinogenic properties such as nizin, enterocyn and lacticin into row meat and meat is an alternative of protective cultures of microorganisms. It is known that these drugs are effective against a wide range of pathogenic bacteria and damage microorganisms, but their use is often limited by low volumes of their production, high prices, and thus low availability to be used at meat processing enterprises (Pisoschi et al., 2018).

The vital activity of damage microorganisms in meat raw materials and meat can be controlled with the help of antimicrobial agents of microbiological or animal origin, which are differ from bacteriocin, produced by LAB.

Rutterin and diacetyl are attributed to powerful antimicrobial agents, which are used to control harmful microorganisms of meat raw materials. The last one can be used to inhibit the growth of spoilage microorganisms only together with carbon dioxide, which allows to maintain red meat color and to prevent its darkening (McMillin, 2017).

Antimicrobial agents of plant origin include spices and herbs, essential oils, natural phenolic compounds of plants and smoke. They have antimicrobial and antioxidant properties and can be used to prolong the shelf life and

increase the safety of rapidly spoiled foods, including meat raw materials. It should be emphasized that the use of essential oils and other extracts of spices and herbs as natural fresh preservatives of row meat has its limitations. First of all, effective antimicrobial dosages might exceed the level of organoleptic perception due to the possible changes in taste (Yusuf, 2018; Pisoschi et al., 2018).

Except of the physical and chemical methods of preserving meat, the packaging of meat and meat raw materials in modified atmosphere, the system of 'active packaging' and the system of aseptic packaging are becoming increasingly popular and widespread.

1. Systems connected with using of 'active packaging'. The purpose of the 'active packaging' is the direct effect on the product to insure its higher quality, as well as to extend the period of stability and suitability for consumption (McMillin, 2017).

Packing technologies with using of 'active packaging' may include (Zhou, Xu and Liu, 2010):

— addition of chemicals into the packaging or in packaging materials that adsorb and remove oxygen from the atmosphere inside the package;

— the use of preservatives, bactericidal substances and antioxidants that are released from the packaging material;

— the use of humidity regulators;

— the use of technology, which allows to control the smell and taste;

— the use of light absorbers;

— the use of package that emits mineral substances retaining product's color;

— improvement of package surface to change its permeability.

The product is sterilized separately during aseptic packaging, in contrast with the traditional system, where the packed product is sterilized.

Systems with aseptic packaging are characterized with following useful advantages:

a) shorter term effect of high temperature allows to retain nutritional properties, taste;

b) packages are made under the sterile conditions of packaging machine with excess pressure and sterile air;

c) sterilization of product occurs as a result of the its thermal stabilization over a short period of time, through the high temperature and cooling;

d) long shelf life of products without freezing;

e) additional protection of products by addition of inert gas into the package;

f) high decontamination level of packaging material or package.

Other different ways of preserving food quality are also used, apart from package. (Tyaglova and Makarova, 2018).

2. Vacuum packaging. Vacuum packaging materials for primal cuts are usually three layered co-extrusions of ethyl vinyl acetate / polyvinylidene chloride / ethyl vinyl acetate, which generally have an O_2 permeability of less than 15.5 ml m^{-2} $(24\ h)^{-1}$ at 1 atmosphere under the polyvinylidene chloride layer (Lonergan, Topel and Marple, 2019). The lack of O_2 in packages may minimize the oxidative deteriorative reactions and reduce growth of aerobic bacteria. Vacuum skin packaging (VSP) systems for placing of retail cut in a barrier styrene or polypropylene tray and vacuum sealing barrier films compressed under the heat for conforming to the product form are usually used for low O_2 packaging of retail meat cuts (VSP) (Belcher, 2006). VSP packaging equipment removes atmospheric air or flushes the air from the package with gaseous compounds such as N_2, CO_2 or mixtures of N_2 and CO_2 before heat sealing the film layers. Nylon barrier polymer of polyvinylidene chloride or ethylene vinyl alcohol are common construction for the top and bottom package webs, tie layer and ionomer. Nylon provides bulk, toughness and low melting point, while the barrier layer prevents vapor permeation and the ionomer gives necessary sealing characteristics (Pisoschi et al., 2018). A variation of VSP is for the lidding film to have outer barrier and inner air-permeable layers so that before retail display, the outer barrier film layer is peeled away from the permeable layer so that air can then contact the meat product and result in a bloomed color (Wang et al., 2019; Kameník et al., 2014; Li et al., 2012).

3. Modified Atmosphere Packaging (MAP). The peculiarities of MAP are to replace the air in the package with a mixture of gases (oxygen, carbon dioxide and nitrogen). The ratio of gases, especially O_2, depends on the type of packaged product. Low levels of oxygen prevent the growth of fungi, bacteria and other microorganisms. MAP is a natural and environmentally friendly product preservation technology. The use a modified gas space allows you to maintain the quality, taste and appearance of the product, increase the shelf life (Rodionova and Paliy, 2018).

MAP technology allows to:

— increase significantly product's shelf life;

— reduce or completely exclude the use of preservatives;

— expand the geography of sales;

— reduce moisture exchange with the environment;

— produce a fundamentally new product with preservation of its original color;

— pack up products in attractive package without damage (crush) of the packaged product.

However, when meat and meat products are packaged in modified atmosphere, the following factors have to be taken into account:

— packaging material, its composition and barrier properties;

— choice of gas and the percentage of gases in the mixture;

— preparation of the product for packaging;

— the correct ratio product/gas;

— control of the packaging quality and storage of the packaged product.

Product which is intended for packaging, should have temperature inside the thickness of the product not lower than 0 °C and not higher than 4 °C. Depending on the product, The time from the end of the manufacturing process to the beginning of the packaging process in the conditions and storage regimes, established by the normative documents, should not exceed from 2 to 72 hours (Wang et al., 2019).

It should be noted that now this technology of food packaging is the most promising and widely used all over the world since it prolongs the shelf life of the finished product in 3–5 times, and the success of the marketing and realization of products depends mostly on it (McMillin, 2017).

Conclusions. Continuous scientific and technological progress in recent years has contributed to the development and implementation of new, modern technologies that help to increase the shelf life of meat raw materials. Today, the use of physical and chemical methods of preserving meat and meat products, modern packaging systems, reduces production risks, prolongs shelf life, and protects against secondary contamination, but each of them has its own disadvantages, which in one or another way affect the way on product quality indicators during storage. Therefore, the search of modern and ecological methods of preservation, with the aim to prolong the period of their realization and transportation, in condition to maintain the quality and safety indicators, remains a topical issue of food industry.

Prospects for further research. To develop complex contemporary and ecological methods of meat and meat products' processing using 'active packaging' system and physical methods of preservation (such as laser and ionizing radiation) as well as to develop a method of meat preserving using aseptic packaging and nanotechnology systems.

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