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The modern trends of biological threats growing, emergent diseases (Lumpy skin disease, Foot-and-mouth disease, African swine fever, Avian influenza and other in Europe and Asia) determine the necessarily to pay the extremely high attention to the biosafety issues and biological hazards control.

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

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Sincerely yours,
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FIRST REPORT OF CANINE BRUCELLOSIS IN UKRAINE: PATHOGEN ISOLATION AND CHARACTERIZATION

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Summary. For the first time in Ukraine we confirmed canine brucellosis caused by *Brucella canis*. The bacterium was isolated from testicles of three-year-old male Labrador retriever with orchitis and epididymitis. Initially blood serum sample was positive in cCFT, AGID and LFIA. In addition to the pathogen isolation and identification by biochemical test and PCR, the antimicrobial susceptibility test was performed that showed sensitive of *B. canis* to the commonly used antibiotics, which should be taken into account for the further therapy

Keywords: antibiotics, Bruce-ladder PCR, *Brucella canis*, orchitis, epididymitis, serology

Introduction. Canine brucellosis is a zoonotic disease mainly caused by *Brucella* (*B.*) *canis*, and sporadically by *B. melitensis*, *B. suis*, and *B. abortus* (Hensel, Negron and Arenas-Gamboa, 2018). *B. canis* can be transmitted through urogenital secretions of infected animals (Kang et al., 2011), and is particularly associated with the reproductive disorders (abortion in females, epididymitis and prostatitis in male dogs), discospondylitis and uveitis (Gyuranecz et al., 2013), leading to significant economic losses for breeding dogs in infected kennels. It is known that *B. canis* can persist in an animal even after long-term antibiotic treatment. Humans are susceptible to *B. canis* infection (Krueger et al., 2014).

Routine diagnostics of the disease based on the serological investigations, such as rapid slide agglutination test (RSAT) with and without 2-mercaptoethanol, tube agglutination test (TAT), complement fixation test (CFT), agar gel immunodiffusion (AGID) and ELISA with rough antigens (*B. canis* or *B. ovis*) (Hollet et al., 2006). As the definitive diagnosis of the infection pathogen isolation and the polymerase chain reaction (PCR) are recommended (Kang et al., 2014).

Canine brucellosis remains endemic in many regions of the world, with predominance in Central and South America (Lucero et al., 2008), in Asia and southern USA (Hubbard, Wang and Smith, 2018; Whitten et al., 2019; Jamil et al., 2019). Various cases have been also described in Europe (Holst et al., 2012; Egloff et al., 2018; Buhmann et al., 2019), but no data are available regarding Ukraine, where *B. canis* infection may be frequent due to the big population of stray dogs.

Aim of the study. In this article we report the first confirmed case of canine brucellosis in Ukraine.

Materials and methods. *Sampling.* In July 2020, three-year-old male Labrador retriever with obviously enlarged testicle was observed in the veterinary clinic in Volnovakha (Donetsk Region). Due to suspicion of brucellosis blood, serum blood, and urine samples were taken for the further studies. After primary samples studies surgically removed testicles were sent to the laboratory for the pathogen isolation.

Serological tests. The cold modification of complement fixation test (cCFT), agar gel immunodiffusion (AGID) with *B. ovis*-antigen and Rose Bengal test (RBT) were performed to detect *Brucella* antibodies in blood samples (Alton et al., 1988). The reference serum against *B. canis* was obtained from ANSES and was used as the positive sample. Additionally, samples were studied by lateral flow immunoassay (LFIA) using commercial kit 'Antigen Rapid C. Brucella Ab Test Kit' (BioNote Inc., South Korea).

Bacteriological studies. Blood, urine samples and testicles were plated on defibrinated sheep blood agar (5%) and tryptic soy agar. Plates were incubated at 37°C up to 10 days. Colonies of isolate were tested by agglutination with acriflavine, crystal violet staining, agglutination with monospecific sera against A and M antigens, hydrolysis of urea, oxidase test, H_2S production, and growth in the presence of CO_2. Growth on tryptic soya agar containing basic fuchsin (20 μg/mL) and thionin (20 μg/mL). A bacterial suspension was prepared from pure and fresh colonies and the tube turbidity adjusted to the 0.5 McFarland turbidity standards. The suspensions were spread onto *Brucella* agar plates and incubated at 37°C. Disk diffusion susceptibility tests were performed for 13 antibiotics: streptomycin (30 μg per disk), gentamicin

(10 μg per disk), rifampicin (5 μg per disk), tetracycline (30 μg per disk), doxycycline (30 μg per disk), ceftazidime (30 μg per disk), ampicillin (10 μg per disk), kanamycin (30 μg per disk), ciprofloxacin (5 μg per disk), gatifloxacin (5 μg per disk), azithromycin (15 μg per disk), sulfadiazine (300 μg per disk), and meropenem (10 μg per disk). The results of antimicrobial test were assessed within 48 h of incubation.

DNA extraction and PCR conditions. Obtained colonies were boiled for 10 min at 90°C. DNA extraction from blood, urea and testicles was realized using Qiagen DNA extraction kit (Germany) according to the manufacturer's instructions. A genus detection protocol based on *IS711* gene amplification by Real-Time TaqMan PCR assay (Hinić et al., 2008) and species identification protocols the Bruce-ladder PCR (López-Goñi et al. 2008) were performed. As the positive control *B. abortus* 99, *B. ovis* 63/290, *B. melitensis* REV-1, and *B. suis* 1330 were applied.

Results. As the first step, suspected dog was tested serologically. The reason for these investigations was an enlarged left testicle and epididymis. However, the dog was in an overall good general condition. As owner mentioned the dog was not imported from abroad, never bred, kept alone in the family with and regularly vaccination and deworming. RBT was negative whereas AGID with R-antigen (*B. ovis*) was clearly positive. cCFT results demonstrated positive reaction at the serum dilution of 1:40. LFIA also was positive. According to the bacteriological studies no *Brucella* colonies were grown from the urine and blood specimens.

After one week a serum sample was taken for additional testing. It was found increasing of antibody titer to 1:160 in cCFT. It was recommended to provide a surgical castration with the further bacteriological investigation of the normal and affected testicles. Castration executed and both testicles were cultured on defibrinated sheep blood agar (5%) and *Brucella* agar, in aerobic conditions.

In parallel homogenized testicular tissue samples were tested by real-time PCR with the aim to amplify *IS711* specific region for *Brucella* spp. Both samples were positive with the Ct value 25 and 27 (Fig. 1).

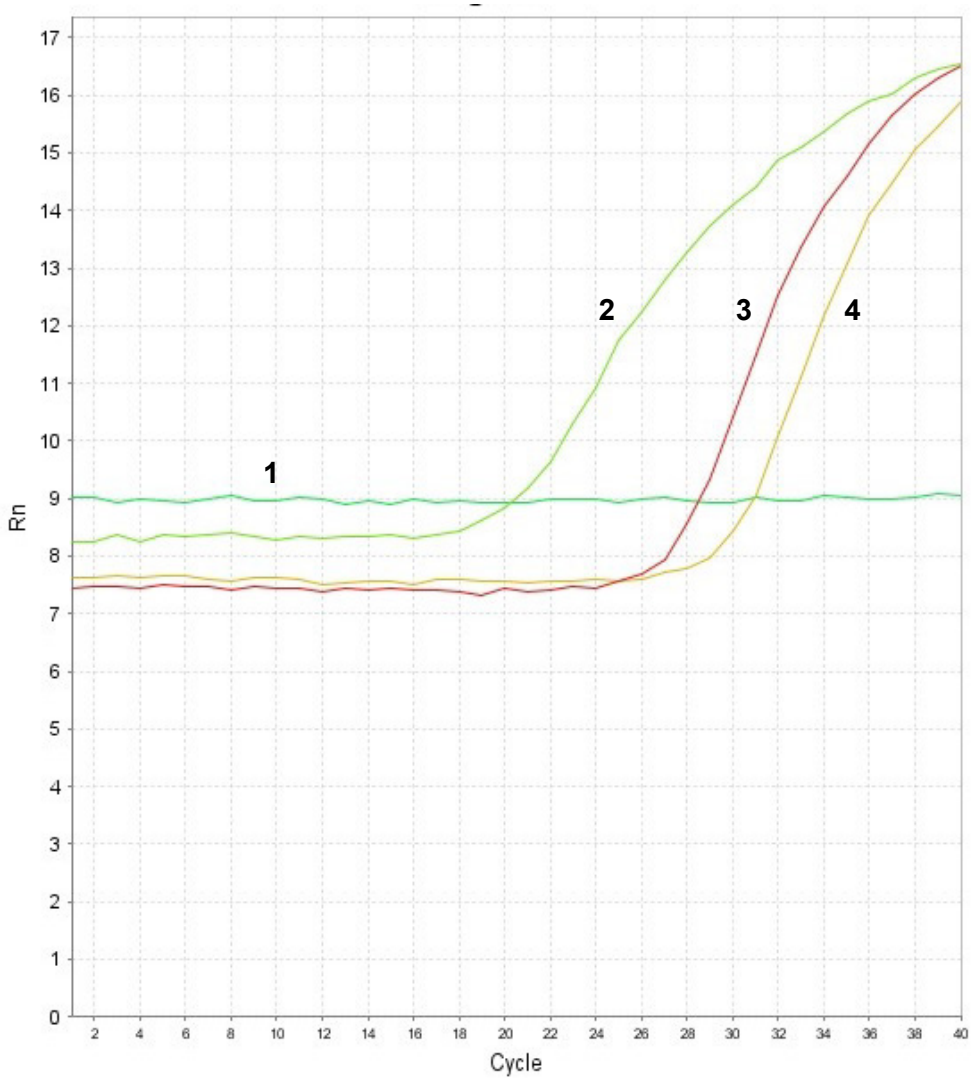

Figure 1. Amplification curves for *IS711* real-time PCR: 1 — negative sample, 2 — positive sample (*B. abortus* 99), 3 — homogenized tissue from the left testicle, 4 — homogenized tissue from the right testicle

After 48 h of incubation the colonies of Gram-negative coccobacilli were appeared in all plates. Obtained culture was characterized as *B. canis* by the following tests: oxidase and urease positive, not produced H_2S, resistant to thionin and basic fuchsin. Autoagglutination with acriflavine and crystal violet staining were positive. No agglutination with monospecific sera against A and M antigens were observed. Growth in the presence of CO_2 was moderate. *In vitro* antimicrobial susceptibility testing of the isolate was provided (Table 1).

Most of the tested drugs, except ceftazidime and sulfadiazine, are effective against obtained *B. canis* isolate. However, treatment should include combination of the different antibiotics with long period of their administration. Finally, obtained *Brucella* isolate was used for identification by providing Bruce-ladder assay (Fig. 2). In the sample positive PCR reaction was obtained with seven visible amplicons, corresponds to both *B. canis* and *B. suis* profiles.

Discussion. *B. canis* causes canine brucellosis that is zoonotic disease responsible mainly for abortions in bitches and orchitis in male dogs. The prevalence of the disease is variable according to the region and diagnostic methods used. In the present study, initially infection was found serologically and lately confirmed by culture isolation following PCR identification. However, the source of the infection for this pet dog is unknown. *B. canis* transmission realizes usually through natural mating, during oronasal contact with infected dogs, and inhalation of aerosolized material or ingestion of contaminated tissue or fluid (Greene and Carmichael, 2012; Hollett, 2006). Other authors reported vertical transmission of the pathogen from infected bitches to puppies and the disease development can occur some period of time later (Carmichael and Kenney, 1970; Gyuranecz et al., 2011; Holst et al., 2012). On the other hand, the high density of stray dog population in Ukraine and particular in Donetsk Region seems as potential harbor of *B. canis* infection for pet dogs. Infected animal can shed pathogen by urine, vaginal discharges and semen up to two years (Greene and Carmichael, 2012). Thus, control of stray dog population is an important measure which may minimize not only infection spreading in kennels but also a risk of possible zoonotic impact.

There is no totally effective antibiotic for the eradication of canine brucellosis therefore we provided *in vitro* antibiotic sensitivity of the obtained *B. canis* isolate by disk diffusion test with the aim to select optimal drug for therapy. It was shown high activity of doxycycline that is agreed with previously study (Mateu-de-Antonio and Martín, 1995) and it was recommended a long-term administration in combination with other antibiotics. A successful treatment regimen of therapy with tetracycline and streptomycin was also previously described (Greene and Carmichael, 2012).

The isolation of *B. canis* was correlated with serological positive results in cCFT, AGID, and LFIA. Due to molecular identification of the pathogen, we provided Bruce-ladder multiplex PCR as recommended by the OIE. The result showed the same profile as *B. suis,* that was previously reported (López-Goñi et al., 2011). However, differentiations in colony morphology and biochemical tests gave possibility to identify the isolate as *B. canis*. For the deep characterization and comparing with other strains, a full genome sequencing of the isolate needs to be provided with the identification of different markers.

Table 1 — Antibiotic sensitivity of *B. canis* isolate obtained by disk diffusion test

Antibiotic	Concentration, µg/disk	Range, mm	Antimicrobial sensitivity		
			Resistant	Intermediate	Sensitive
Streptomycin	30	35	≤ 20	21–24	≥ 25
Gentamicin	10	19	≤ 14	15–19	≥ 20
Rifampicin	5	19	≤ 16	17–19	≥ 20
Tetracycline	30	35	≤ 20	21–25	≥ 26
Doxycycline	30	38	≤ 14	15–18	≥ 19
Ceftazidime	30	0	≤ 20	21–24	≥ 25
Ampicillin	10	30	≤ 19	—	≥ 20
Kanamycin	30	26	≤ 15	16–19	≥ 20
Ciprofloxacin	5	32	≤ 19	20–24	≥ 25
Gatifloxacin	5	34	—	—	—
Sulfadiazine	300	0	—	—	—
Azithromycin	15	32	—	—	—
Meropenem	10	32	—	—	—

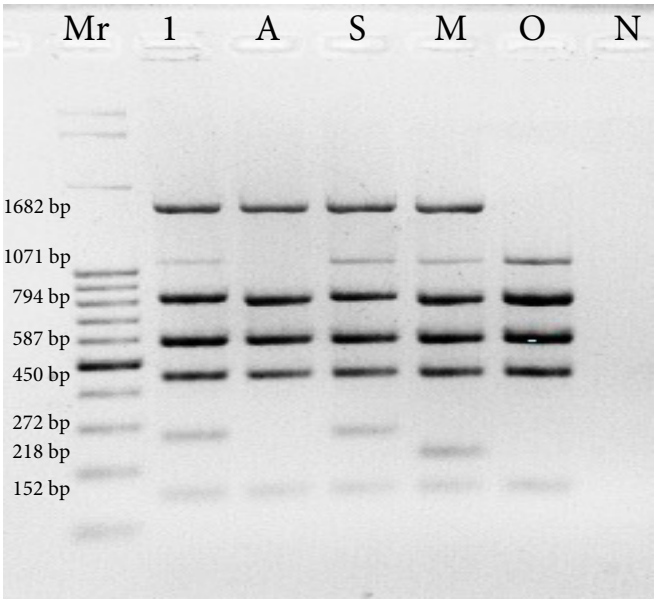

Figure 2. The Bruce-ladder PCR result of the obtained *Brucella* isolate: 1 — *Brucella* isolate, Mr — marker (Thermo Scientific, USA); Controls: A — *B. abortus* 99, S — *B. suis* 1330, M — *B. melitensis* REV-1, O — *B. ovis* 63/290, N — negative control (distilled water)

Conclusions. Our findings confirmed the circulation of *B. canis* in Ukraine, which could lead to significant economic losses also in commercial kennels. More investigations including a higher number of samples and other geographical locations of the country are needed to elaborate an effective measure for controlling of canine brucellosis among stray dogs and disease outbreak in kennels.

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EPIDEMIOLOGICAL FEATURES OF LUMPY SKIN DISEASE OF THE LARGE RUMINANTS: REVIEW OF LITERATURE

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Summary. Lumpy skin disease (LSD) is an infectious disease in cattle, characterized by nodules on the surface of the skin and which can have serious economic consequences. Starting from 2014, new outbreaks of LSD in the world and its spread to Central Asia and the Middle East are noted. Due to the huge economic impact on the economy, the World Organisation for Animal Health (OIE) has classified LSD as a particularly dangerous disease that needs to be notified. The analysis of the literature on LSD shows that many issues remain unstudied and require appropriate research to be carried out. In connection with the difficult epizootic situation and the threat of further spread of the virus, the urgent task is the study of biological properties of the causative agent with a view of development the specific prophylactic agents that would allow to prevent the spread of infection in a short time

Keywords: *Capripoxvirus*, nodular dermatitis, Neethling virus, outbreaks, Azerbaijan

Introduction. Lumpy skin disease virus (LSDV) belongs to the genus *Capripoxvirus* from the family Poxviridae and is the causative agent of transmissible disease of cattle with significant economic consequences (Mishchenko et al., 2017; Semakina et al., 2017). The disease is characterized by large skin nodules covering the entire body of the animal, exhaustion, poor milk production, and abortions. Degree of manifestation of clinical symptoms varies from acute to subclinical forms. Due to the huge economic impact on the global cattle industry, the World Organisation for Animal Health (OIE) has classified LSD as a notifiable disease. LSD is a vector-borne disease that is transmitted through blood-sucking arthropods, such as *Aedes aegypti* (Diptera: Culicidae). Direct and indirect contact between infected and susceptible animals is not considered as way of virus transmission (Mishchenko et al., 2017). The virus can infect other small ruminants, such as sheep and goats, but does not cause clinical diseases (Balinsky et al., 2008; Tuppurainen et al., 2017). However, contact of cattle herds with sheep and goats in pasture/watering areas is considered a potential risk factor for mechanical transmission of LSD (Chihota et al., 2001; Kitching and Mellor, 1986).

History of virus distribution. LSD was first found in Northern Zambia in 1929 (Kumar, 2011). In the 1940s, the disease distributed quickly among cattle in other countries of southern Africa. In 1989, the transcontinental spreading of LSD from African and Asian countries to European countries was first confirmed when the disease was reported in Israel. In the same year, suspicions for LSD cases in a herd of Arabian oryx (*Oryx leucoryx*) were also recorded in Saudi Arabia (Greth et al., 1992). The disease was imported into Egypt with cattle imported from Africa and kept in a local quarantine station. In the summer of 1988, the virus distributed locally, but before that there were no signs of a clinical disease. This epizootic showed a low morbidity rate (2%) due to the vaccination, which included almost two million head of cattle with a vaccine against smallpox sheep. On the new outbreaks have also been reported in Kuwait, Bahrain, Yemen, the United Arab Emirates, and Sudan (Tuppurainen and Oura, 2012; Vorster and Mapham, 2008). Between 2012 and 2013, LSD were first registered in Greece, Bulgaria, and Turkey. In 2014–2015, new cases of LSD were observed in Iran, Azerbaijan, Iraq, Greece, and Cyprus (Semakina et al., 2017; Tuppurainen and Oura, 2012).

Outbreaks of LSD in Azerbaijan. Following the outbreak in neighboring Iran in 2014, monitoring was carried out among livestock in the border areas. Animals having clinical signs consistent with LSD infection were first detected in the Bilasuvar District, and subsequently more cases were detected in the Jalilabad, Ujar, and Agdash districts (Fig. 1). Samples were taken from blood and/or lesions of suspicious infected animals and internal organs of cattle. Using real-time polymerase chain reaction (PCR), the presence of the causative agent was confirmed. From June to November 2014, 2,762 cattles in Azerbaijan had clinical signs and lesions at autopsy, corresponding to LSD. Of the 269 samples tested for LSD virus by PCR, 199 (74%) were positive. A total of 33 animals died, which amounted to 1.2% of those who had clinical signs of the disease. Preventive measures were taken for retardation the spread of the disease, including restrictions on animal movement, vector control, and vaccination (Zeynalova et al., 2016).

Epidemiology of the disease. There are large differences in the indicators of morbidity and mortality rates during the outbreak of LSD. These differences depend on the following factors: geographical location, climate, farm management conditions, nutritional status and general condition of the animal, cattle breed, immune status, population levels and distribution of insect vectors in various habitats, viral virulence.

Figure 1. Spread of disease in the Republic of Azerbaijan during 2014 (Zeynalova et. al., 2016)

The morbidity rate of LSD is from 5 to 45%. Higher rates are found in epizootics in South, West and East Africa and Sudan, although so far much lower rates can be observed during one epizootic episode. In addition, high morbidity and mortality rates of 30–45% and 12%, respectively, were also recorded in Oman in 2009 among the farm livestock population of Holstein breed. LSD has a narrow range of mammalian hosts. Cattle and buffalo are species that become naturally infected during field outbreaks. Five clinical cases of LSD have been reported in Asian water buffalos (*Bubalus bubalis*) (Semakina et al., 2017; Vorster and Mapham, 2008). Although the virus bears resemblance with sheep pox, other domestic ruminants do not naturally become infected during field outbreaks. All cattle breeds are equally susceptible to this disease. However, some other researchers have found that imported thin-skinned breeds, such as *Bos taurus*, cattle of Friesland and the Channel Islands, were much more susceptible than native thicker-skinned breeds such as African breeds. Young calves are more susceptible to the disease and can develop a characteristic lesion within 24–48 hours, although all age groups of animals are susceptible.

A severe form of the disease develops when a secondary (bacterial) infection is stratified and is characterized by damage of the oral cavity, respiratory and digestive organs. In sick animals, prolonged fever, depression, and a decrease in appetite are noted. Intradermal nodules with a flat surface (diameter 5–50 mm) are formed throughout the body of the animal, on the limbs and abdomen, the number of nodules varies from 1–10 to several hundred (Fig. 2). In 1–3 weeks, after the appearance of the tubercles, the necrotic tissue falls away (Chernykh et al., 2017).

Unsequestrated nodules are hardened and remain for a long time. Often, the disease is complicated by a secondary bacterial infection, while edema appears in the deeper layers of the skin and subcutaneous tissue. In lactating cows, the udder is affected, and sometimes it falls away. The temperature of infected animals rises to 40.0–41.5°C, which can persist for 6–72 hours or more and rarely can reach 10 days. Infected animals also have lacrimation, increased secretion of the nose and throat, anorexia, general depression and reluctance to move. Fallen animals exhibit signs of enteritis and hemorrhage on the intestinal mucosa, most often the small intestines.

Joint injuries and edema of the dewlap are recorded in individual animals. Under a visceral pleura the hemorrhage with a diameter of up to 1 cm, sometimes the same hemorrhages are found on the nasal turbinate, in the capsule of the spleen, liver, and in the mucous membrane of the rumen. Sometimes nodular lesions are found in the lungs. Typical round necrotic lesions can also be seen on the muzzle, nasal cavity, larynx, trachea, bronchi, inside the lips, gums, dental pillow, anterior abdomen, abomasum, uterus, vagina, nipples, udder, and testicles (Chernykh et al., 2017; Chevelev, 1984; Abutarbush et al., 2015; Tuppurainen and Oura, 2012).

Figure 2. Clinical picture of LSD in cattle

Diagnosis. Laboratory studies and identification of virus are carried out in accordance with OIE Terrestrial Manual (OIE, 2018).

Samples for virus isolation should be collected within the first week after the onset of clinical signs, before the production of neutralizing antibodies. A skin biopsy of early lesions (those where necrosis did not occur) gives samples that can be used to identify the virus by PCR (Orlova et al., 2006). Using this method, it is possible not only to identify the genome of the causative agent of LSD in cows, but also to differentiate it from the related viruses of sheep pox and goat pox. Electron microscopy is an express method for detecting the virus and its differentiation from other pathogens. LSD virus grows in tissue culture of bovine and sheep origin. In retrospective diagnosis for determination of antibodies to LSDV the neutralization reaction is used, which is the most specific serological test, but the test is not sensitive enough to identify animals that have been in contact with the virus and which have developed low levels of neutralizing antibodies (Balinsky et al., 2008; Zeynalova et al., 2016).

Control. LSD control with the help of quarantine and control over the movement is not very effective, as biting flies and some types of insects are the most important method of transmitting the disease. In prevention the spreading of LSD, using of insecticides with repellents can help in prevention the spread of the virus. Outbreaks can be eliminated by means of quarantine, depopulation of infected and affected animals, proper disposal of carcasses, cleaning and disinfection of premises, and insect control. Control can be through vaccination or immunoprophylaxis (Chernykh et al., 2016; Krivonos et al., 2017). In turn, live vaccines, help to control disease in endemic areas.

The following vaccines have been developed:

(1) Homologous live attenuated viral vaccine (Neethling strain: immunity granted lasts up to 3 years);

(2) Heterologous live attenuated viral vaccine (vaccine against sheep pox or goat pox, but can cause local, sometimes severe, reactions). This vaccine is not recommended in countries, free of sheep pox and goat pox, so long as otherwise live vaccines could be a source of infection for susceptible sheep and goat populations;

(3) New generation recombinant vaccines are not commercially available.

Conclusion. The analysis of the literature on LSD shows that many issues remain unstudied and require appropriate research to be carried out. In connection with the difficult epizootic situation and the threat of further spread of the virus, the urgent task is the study of biological properties of the causative agent with a view of development the specific prophylactic agents that would allow to prevent the spread of infection in a short time.

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Part 2. Biotechnology

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PROLONGED HYPOXIA INDUCED MELANOTIC PSEUDOTUMORS IN THE LARVAE OF BLOOD-SUCKING MOSQUITOES

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Summary. It was found that the presence of mosquito eggs in artificially created conditions of prolonged hypoxia causes the appearance of numerous melanotic pseudotumors in the larvae hatching from such eggs. In the cells of melanotic pseudotumors multilayer concentric membrane-like structures were found in the cytoplasm. In the immediate vicinity of such membranes, small spherical virus-like particles (VLP) with a diameter of about 30 nm were observed. The possible role of hypoxia in the development of melanotic pseudotumors of mosquito larvae is discussed

Keywords: electron microscopy, virus-like particles, *Aedes*, Culicidae, Diptera, Ukraine

Introduction. There are a large number of both benign and malignant insect tumors (Harshbarger and Taylor, 1968; Tascedda and Ottavifni, 2014). These tumors have many features in common with human and animal tumors. Modern molecular genetic research methods have established, for example, that more than 50% of the proteins that are involved in the processes of tumor formation in humans and animals have analogues in the fruit fly *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae) (Gonzalez, 2013).

The oncology of insects is mainly based on the results of studies of melanotic tumors of the fruit fly *D. melanogaster.* This fly has a phenomenon called 'melanotic encapsulation', which is formed as a result of the deposition of melanin grains in the form of pigmented masses on or near the surface of an embedded pathogen. In addition to melanin grains, these pigmented masses, as a rule, consist of clusters of adherent hemocytes, or various endogenous tissues encapsulated by these cells (Christensen et al., 2005). Since there is no full correspondence in the definitions between oncology of vertebrate and invertebrate animals, some researchers call such insect tumors 'pseudotumors' (Harshbarger and Taylor, 1968).

In Europe, some mosquitoes can transmit several diseases and parasites that dogs and horses are very susceptible (Hubálek, Rudolf and Nowotny, 2014; Pagès and Cohnstaedt, 2018). Despite the important role of mosquitoes in the transmission of pathogens of infectious diseases of humans and animals, neoplasms of blood-sucking mosquitoes have not been studied previously.

The aim of the work was describes the method of inducing pseudotumors in mosquito larvae by hypoxia in the laboratory conditions, and also presents the results of electron microscopic studies of affected cells obtained from this melanotic pseudotumors.

Materials and methods. Field studies of mosquito larvae were carried out in Kyiv Region (the villages of Kruhlyk, Vita-Poshtova, Feofaniia, and Zahaltsi).

To study the effect of hypoxia, soil samples collected in the habitats of mosquito larvae (family Culicidae) along with egg laying mosquitoes were stored for 6 months in dense plastic bags without air with temperature fluctuations from 0°C in the winter months to 25°C in the spring. Control soil samples with egg laying were kept at the same temperatures, but with air access.

Hatching after 6 months, mosquito larvae with melanotic pseudotumors were examined under a light microscope and then processed for electron microscopy. For this, the larva was cut into several fragments, fixed in 2.5% solution of glutaraldehyde on cacodylate buffer, and then in 1.5% solution of osmium tetroxide on phosphate buffer. Further, the material was dehydrated in alcohols of increasing concentration, acetone and placed into EPON-812. Ultrathin sections were made on LKB microtome, contrasted with 2% solution of uranyl acetate and 0.5% solution of lead citrate, and then examined under JEM-7A electron microscope.

Results and discussion. Numerous small melanotic pseudotumors of a dark color were revealed in the larvae of blood-sucking mosquitoes spawning from ovipositors kept under prolonged hypoxia, which were visible through a transparent cuticle (Fig. 1).

After storing of mosquitoes ovipositor under artificially created hypoxic conditions, melanotic pseudotumors formed in most mosquito larvae (Table 1). In control larvae, such tumors were very rare. The formation of melanotic pseudotumors and the accompanying symptoms of the disease in larvae of various mosquito species were similar in all cases. Most often, symptoms were manifested in stage III–IV larvae, less often in younger stage larvae and pupae.

Figure 1. Larva of the blood-sucking mosquito *Aedes communis* (De Geer, 1776) with numerous melanotic pseudotumors in the form of dark spots throughout the body

Table 1 — Manifestation of melanotic pseudotumors in blood-sucking mosquito larvae after the maintenance of their ovipositions under prolonged hypoxia

Indexes	Package No.						Mean
	1	2	3	4	5	6	
Total number of larvae, sp.	68	56	68	67	186	42	81.17 ± 21.37
Number of larvae with pseudotumors, sp.	44	32	56	34	132	36	55.67 ± 15.68
Percentage of larvae with pseudotumors, %	64.7	57.1	82.4	50.7	71.0	85.7	68.58 ± 5.65*
Percentage of larvae with pseudotumors in control groups (without hypoxia), %	0	0	0	0	0.53	0	0.09 ± 0.09

Note. * — $p < 0.001$ compared to control.

Affected larvae became inactive, convulsively twitching when irritated. Sometimes their body was deformed, distorted, the segments contracted, because of which the larva became like an accordion. At the first stages of the disease, the fatty body and the dorsal parts of the segments of the chest and abdomen were weakly pigmented, then separate spots of irregular shape appeared. They compacted, darkened and subsequently acquired a spherical or oval shape, reaching sizes of 50–80 μm.

An electron microscopic study of ultrathin sections of adipose body cells of affected larvae showed that a large number of peculiar multilayer concentric membrane-like structures were found in the cytoplasm (Fig. 2), which were never found in healthy mosquito larvae. In the immediate vicinity of such membranes, small spherical virus-like particles (VLP) with a diameter of about 30 nm were observed.

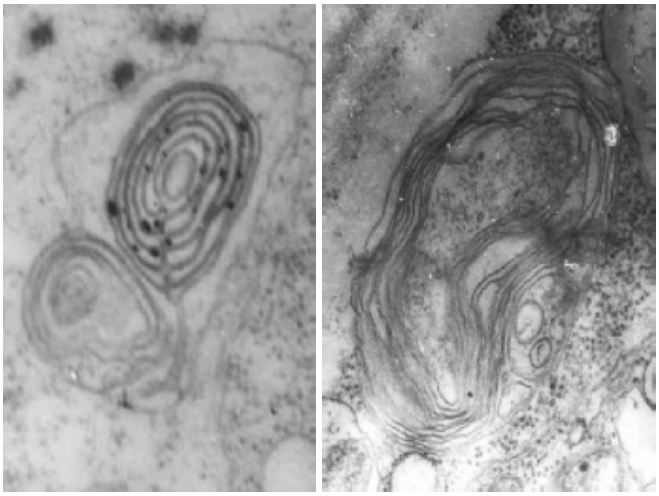

Figure 2. Concentric membrane structures in the cytoplasm of cells of the blood-sucking mosquito *Aedes cantans* (Meigen, 1818) larvae affected by a melanotic pseudotumors

In natural habitats, larvae of blood-sucking mosquitoes with melanotic pseudotumors are very rare, the extent of damage in water pools is only a fraction of a percent. As a result of many years of field studies, we found such melanotic pseudotumors in the larvae of 11 species of blood-sucking mosquitoes from natural populations: *Aedes dorsalis* (Meigen, 1830), *Ae. cantans* (Meigen, 1818), *Ae. annulipes* (Meigen, 1830), *Ae. excrucians* (Walker, 1856), *Ae. cyprius* Ludlow, 1919, *Ae. communis* (De Geer, 1776), *Ae. cataphylla* Dyar, 1916, *Ae. leucomelas* (Meigen, 1804), *Ae. vexans* (Meigen, 1830), *Ae. cinereus* Wiedemann, 1818, *Culex territans* Walker, 1856 (Buchatskyi and Sheremet, 1978).

As a result of the studies in laboratory conditions, it was found that the presence of mosquito eggs in artificially created conditions of prolonged hypoxia causes the appearance of numerous melanotic pseudotumors in the larvae hatching from such eggs. A characteristic feature of these pseudotumors is the presence of a large number of membrane structures in the affected cells. In *Drosophila*, similar melanotic pseudotumors occur during experimental infection with Rous sarcoma virus (Burdette and Yoon, 1967).

It is known that in cancer cells of humans and animals, as well as during viral reproduction, often observed are complexes of membrane structures formed by a modified endoplasmic reticulum, as well as a variety of concentric

membrane formations, which were called 'X-structures' (Solov'ev, Khesin and Bykovskiy, 1979). In kalyptorhynch flatworm *Gyratrix hermaphroditus* Ehrenberg, 1831 (Turbellaria: Polycystididae), similar membrane structures were seen in the cytoplasm of cells along with small virus-like particles. Sometimes these concentric membrane formations were surrounded by several VLP, but more often they were empty. Researchers called them 'concentric multilayer membrane structures' and believe that their appearance is associated with the reproduction of the virus (Reuter, 1975). Recently, Norwegian researchers (Bjørgen et al., 2015) found that in melanotic deposits, in a large number of Atlantic salmon (*Salmo salar* Linnaeus, 1758) present in skeletal and cardiac muscles, there are a large number of spherical virus-like particles with a diameter of about 30 nm. The authors suggest that these melanin deposits are produced by the fish organism in response to various viral infections often found in salmon.

As a result of our research, small spherical VLP of the same diameter as in salmon muscles (about 30 nm) were found in the cytoplasm of cells obtained from melanotic pseudotumors of blood-sucking mosquitoes of various species. In our opinion, all these small VLP under conditions of prolonged hypoxia can be induced by the transcription factor HIF-1α.

The role of hypoxia in the malignant transformation of cells is well known (Osinsky, Zavelevich and Vaupel, 2009; Minchenko et al., 2016; Zhang et al., 2020). The main factor of hypoxia is the transcription factor HIF-1α, the content of which is regulated at the level of protein synthesis (Centanin et al., 2008; Minchenko et al., 2015). In insects, the HIF-1α factor plays a large role in the process of postembryonic development, since a sharp increase in the body size of larvae during metamorphosis requires an increased level of oxygen supply (Lundquist, 2016). Under hypoxic conditions, this factor is able to activate the expression of many genes that affect the ontogenesis of insects and activate the LTR-retrotransposons of the *Tu-1-copia* family located in their genomes (Rachidi et al., 2005). The latter, as we know, form spherical VLP with a diameter of 30–40 nm in the cells (Yoshioka et al., 1990; Semin and Il'in, 1994). Such VLP by means of metagenomics method have recently been detected in flies and mosquitoes. All of them are united in the genus *Hemivirus* within the Pseudoviridae family (Buchatskyi, 2020). Thus, the induction of melanotic pseudotumors in blood-sucking mosquito larvae and the excessive formation of intracellular membranes under prolonged hypoxia can be the result of activation of endogenous viral elements. These endogenous viral elements, close to viruses of the families Retroviridae, Parvoviridae, Filoviridae, Bornaviridae, and Circoviridae are found in the genomes of various domestic and wild animals (Yudin, Aitnazarov and Ermolaev, 2011). According to some estimates, endogenous retroviruses, for example, can account for 7 to 9% of the vertebrate genome (Yu et al., 2019). Therefore, the study of the role of hypoxia in the activation of endogenous viral elements is an important task.

Currently, in many scientific laboratories, mosquitoes and flies are used to test on them insecticides, viral and bacterial preparations, as well as for various biotechnological experiments. The facts obtained in our experiments indicate that when breeding insects, the oxygen regime of their maintenance should be taken into account.

Conclusions. 1. In natural habitats, larvae of blood-sucking mosquitoes with melanotic pseudotumors are very rare, their incendence in water pools is only a fraction of a percent.

2. It was found that the presence of mosquito eggs in artificially created conditions of prolonged hypoxia causes the appearance of numerous melanotic pseudotumors in the larvae hatching from such eggs.

3. It is established that in the cells of melanotic pseudotumors multilayer concentric membrane-like structures were found in the cytoplasm. In the immediate vicinity of such membranes, small spherical virus-like particles with a diameter of about 30 nm were observed.

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Part 3. Biosafety

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ASSESSMENT OF BIOSECURITY POLICIES AND PRACTICES FOR THE CONTROL OF AFRICAN SWINE FEVER VIRUS ON UKRAINIAN PIG FARMS

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Summary. With monetary support from the United States Department of Agriculture Foreign Agricultural Service (USDA FAS), an investigative effort was undertaken to document the biosecurity practices employed by commercial pig producers in Ukraine to prevent the introduction of African swine fever virus (ASFV) on their farms. The cohort of farms selected and evaluated were owned by producers who were active members of the Association of Ukrainian Pig Breeders (AUPB). The assessment of biosecurity policies and practices consisted of an interview and in-person completion of a questionnaire that evaluated various aspects of biosecurity practices used on pig farms in Ukraine. The results of the interviews and completion of survey questionnaires support the conclusion that Ukrainian pig producers recognize the importance of farm biosecurity as it relates to preventing ASFV introduction on their farms and all the participating farms had biosecurity policies that were in force at the time of completion of the questionnaire. However, the results also support the conclusion that significant gaps in understanding about biosecurity exists and that there is a need for more education of Ukrainian pig producers about this critical aspect of health management and disease control. The broad impact of the project detailed that prospective, more comprehensive work on Ukrainian pig farms is required to adequately assist producers with ASFV control and effective applications of biosecurity

Keywords: African swine fever virus, Association of Ukrainian Pig Breeders, questionnaire, preventive measures

Introduction. African swine fever (ASF) is a longstanding disease that was first recognized as a viral disease of pigs on the continent of Africa in the early 1900s (Sánchez-Cordón et al., 2018). After introduction of African swine fever virus (ASFV) into the Caucasus region in 2007 via the Black Sea Port of Poti (Brown and Bevins, 2018), ASFV was later diagnosed in Ukraine in 2012 after circulating for several years in Eastern Europe and Russia (Arias et al., 2018). A diagnosis of ASF is disastrous for numerous reasons and not limited to the virulent and lethal nature of the virus or the lack of a vaccine to promote immunity among susceptible pigs (FAO, 2012). Due to its severity as a transboundary pathogen and its associated mandatory reporting requirements, an ASF diagnosis has devastating impacts on international trade and the national and agricultural economies of affected territories (Gallardo et al., 2015). ASF epidemiology in Eastern Europe has elevated the importance of comprehensive biosecurity as the primary means of controlling or mitigating virus introduction on commercial pig farms (Jurado et al., 2018). Like other infectious agents that cause disease in pigs, ASFV gains access to pig farms through various routes (Olesen et al., 2018; Sánchez-Cordón et al., 2018). Therefore, if effective control of ASFV is to be achieved, it is necessary to attempt to identify deficiencies in farm biosecurity and subsequently develop effective protocols to counter the identified deficiencies.

The purpose of the effort described herein was to evaluate the biosecurity policies and practices used on commercial pig farms in Ukraine with a focus on preventing the introduction of ASFV. A questionnaire was developed and used to assess the biosecurity of farms participating in the investigative effort. In addition, the questionnaire was also developed with the specific purpose of making it available to pig producers in an electronic format so that they may perform periodic self-evaluations of their farms' biosecurity in the future and make adjustments to their biosecurity protocols as needed.

Materials and methods. In 2018, a competitive grant proposal was funded by the United States Department of Agriculture Foreign Agricultural Service (USDA FAS) for the purpose of assisting the Ukrainian Ministry of Agriculture and its nations' pig producers with their efforts to control the spread of ASFV. The diagnosis of ASF has curtailed Ukraine's ability to engage in significant

trade of pork and pork products internationally and inspired the Ukrainian Ministry of Agriculture to develop a working relationship with USDA FAS. In collaboration with the Association of Ukrainian Pig Breeders (AUPB), a cooperative of commercial pig operations throughout Ukraine, farms were identified for assessment of their biosecurity policies and practices to prevent the introduction of ASFV. Assessment of farm biosecurity consisted of interviews with farm owners or their managers and completion of a questionnaire to document farm policies and practices. The questionnaire was a comprehensive document that evaluated various aspects of the farm biosecurity plan and can be reviewed at www.pigua.info. The participating farms varied in size and scope and included farrow-to-finish and wean-to-finish operations. The time required to complete the interviews and questionnaire varied and typically exceeded 90 minutes in length from start to finish.

Results. For preservation of privacy, the identity and location of the farms participating in the investigative effort are not disclosed. The participating farms are identified by the sequence in which they were visited and the type of swine operation. Areas of concern with farm biosecurity were identified based on responses from interviews of farm representatives and completion of the questionnaires.

Farm 1, Farrow-to-Finish. The presence of wild boars has been documented in areas adjacent to the farm. The farm is located within 2 km of residences where backyard pigs may be maintained. Truck drivers are permitted to exit and re-enter their vehicles after entering the production area without consideration for biosecurity. Barn personnel participates in the loading of pigs for transport and the loading dock is located within the production area, which requires entry of transport vehicles into the production area for access. Personnel and vehicles that are used to retrieve and transport mortalities are permitted to enter the production area without consideration for biosecurity or hygiene status of the vehicle. Barn personnel moved freely between clean and dirty sides of the shower entry area without consideration for biosecurity. After entry into the production area, personnel that exited the main unit to enter the quarantine facility were not required to shower to re-enter the main unit. Shower facilities were not provided for this purpose. The farm management team did not appear to have sufficient knowledge of the disease status of their genetic supplier. Multiple-use cloth towels are used in common areas instead of single use paper towels. The number of towels provided to barn personnel for entry and exit of the shower area was considered inadequate. The farm lacked a water purification system. Post-disinfection assessments of facility hygiene are not performed. Building air intakes lacked coverings to restrict entry by unwanted pests. Vegetation was overgrown adjacent to the barrier fence. Fruit trees were present and may act as an attractant for birds and other unwanted animals.

Farm 2, Wean-to-Finish. The presence of wild boars has been documented in areas adjacent to the farm. The farm was located within 2 km of residences where backyard pigs may be maintained. Weaned pigs are not obtained from a single source and some of the sow farms supplying weaned pigs do not have comprehensive biosecurity policies. A dedicated site for cleaning and disinfection of work vehicles does not exist and policies regulating vehicle movements within the production area have not been developed. Corridors used for moving pigs are not properly cleaned and disinfected after internal movements or load-outs for marketing. The farm lacked a water purification system. Barn personnel are permitted to take cellphones, cigarettes, and other personal items through the shower entry area and into production areas. The number of towels provided to barn personnel for entry and exit of the shower area was considered inadequate. Guard dogs are not confined to a specific area to prevent roaming off the premises. Fruit trees were present and may act as an attractant for birds and other unwanted animals.

Farm 3, Farrow-to-Finish. The presence of wild boars has been documented in areas adjacent to the farm. The farm is located within 2 km of residences where backyard pigs may be maintained and the farm is present in an area where there are other swine farms in close proximity with the nearest farm estimated to be within 500 m. Feed prepared on the farm is not subjected to heat treatment. Items are allowed to enter the farm without being subjected to decontamination. The farm does not have a current rendering agreement with the State, as required by law. Containers for storing mortalities under proper temperature conditions were not available. Shower facilities were poorly designed and would permit cross contamination of barn personnel entering and exiting the production area. Post-disinfection assessments of facility hygiene are not performed. Fruit trees were present and may act as an attractant for birds and other unwanted animals.

Farm 4, Farrow-to-Finish. The farm did not have a dedicated isolation facility for receiving pigs from outside sources. Vehicles are required to enter the production area for feed deliveries. Mortalities are placed in storage containers, but the containers are not temperature controlled. Barn personnel are permitted to take cellphones, cigarettes, and other personal items through the shower entry area and into production areas. Multiple-use cloth towels are used in common areas instead of single use paper towels. The farm lacked a water filtration system. The farm had a barrier fence in place but the rear area of the fence was cluttered and poorly maintained.

Farm 5, Farrow-to-Finish. Homes are within 2 km of the farm where backyard pigs may be maintained. The

farm does not have a current rendering agreement with the State, as required by law. Containers for storing mortalities under proper temperature conditions were not available. Heat treated feeds are not utilized. Feed delivery vehicles are required to enter the production area for unloading. A barrier fence was intact but appeared to be poorly maintained. A designated shower facility was not available for entry and exit from the quarantine area. A policy regulating movements of farm personnel around the premises has not been developed. Multiple-use cloth towels are used in common areas instead of single use paper towels. Guard dogs are not confined to a specific area to prevent roaming off the premises. Post-disinfection assessments of facility hygiene are not performed. Note: The farm management used formalin as one of its disinfectants, which while effective, raised safety concerns for the farm personnel and the environment. It was strongly recommended that commercial disinfectant products that are viewed as safe and approved be used for purposes of farm disinfection.

Farm 6, Farrow-to-Finish. Homes are within 2 km of the farm where backyard pigs may be maintained and the farm is present in an area where there are other swine farms in close proximity. The farm does not have a current rendering agreement with the State, as required by law. Containers for storing mortalities under proper temperature conditions were not available. Designated uniforms were not provided to farm personnel for handling mortalities. Truck drivers and vehicles were not monitored for compliance with biosecurity policies. There was a lack of knowledge about whether feed is heat treated by the feed supplier. The production area has to be accessed for feed deliveries. Birds are able to enter and occupy feed storage areas. The farm lacked a water filtration system. Supplies and materials entering the production area are not decontaminated. Biosecurity policies have not been developed for the different production areas on the farm or for movement of farm personnel around the premises. Barn personnel are permitted to take cellphones, cigarettes, and other personal items into production areas. Multiple-use cloth towels are used in common areas instead of single use paper towels. The number of towels provided to barn personnel to enter and exit the shower area was considered inadequate. A barrier fence was intact but did not restrict small rodents and there was no plan for rodent control. Post-disinfection assessments of facility hygiene are not performed.

Farm 7, Farrow-to-Finish. The farm was located within 2 km of residences where backyard pigs may be maintained. Farm personnel are permitted to own pigs. Another swine operation is located within 3 km of the farm. Vehicles entering the farm are required to pass through a wheel dip but effectiveness of the disinfection process is not assessed. A biosecurity policy has not been developed for truck drivers after arrival to the farm. There was a lack of knowledge about whether feed is heat treated by the feed supplier. The production area has to be accessed for feed deliveries. Barn personnel are permitted to take cellphones, cigarettes, and other personal items into production areas and have avoided showering before entering the production area. Barn personnel wear the same footwear into and out of production areas. Barn personnel are permitted to bring food items onto the farm. Farm personnel are not required to wear different coveralls to identify their work assignments. Farm personnel share work equipment between buildings that is not disinfected and supplies are permitted to enter the production area without decontamination. Animal loading areas are not cleaned after pigs are handled at load out. Multiple use cloth towels are used in common areas instead of single use paper towels. A barrier fence is intact but not adequately monitored. Fruit trees were present on the site and may act as an attractant for birds and other unwanted animals.

Farm 7a, Wean-to-Finish. The farm was located within 2 km of residences where backyard pigs may be maintained. Farm personnel are permitted to own pigs. Another swine operation was located within 3 km of the farm. Farm management did not have sufficient knowledge of the biosecurity plan used by its weaned pig supplier. Vehicles entering the farm pass through a wheel dip but disinfectant is not applied to the undercarriage and vehicles are permitted into the production area. A biosecurity policy has not been developed for truck drivers after arrival to the farm. Truck drivers participate in load outs and are permitted to enter the production area without showering and changing clothes. The site lacks a dedicated loading ramp and does not have a designated area for cleaning and disinfection of vehicles. Vehicles are not properly cleaned and disinfected after transporting pigs and records of vehicle disinfection are not maintained. Policies regulating vehicle movements within the production area does not exist. The vehicle used to transport mortalities is permitted in the production area and is operated in open areas around the premises. Dedicated employees are not assigned to handle mortalities and do not change clothing after handling mortalities. Containers for properly storing mortalities on the farm are not available. The farm does not use heat-treated feeds and trucks have to enter the production area for feed deliveries. Materials entering the farm are not decontaminated. The farm entry shower area is not monitored and is used for reasons other than entering and exiting the farm. Barn personnel are allowed to move freely between clean and dirty areas. Barn personnel are allowed to use the same footwear throughout multiple locations in the production area. The number of towels provided to barn personnel to enter and exit the production area was considered inadequate. Barn personnel are permitted to take cellphones, cigarettes, and other personal items into production areas. Farm

personnel are permitted to bring food items onto the farm. A biosecurity plan regulating the movements of barn personnel within the production area has not been developed. The barrier fence is incomplete and areas around the fence are overgrown and cluttered.

Farm 8, Farrow-to-Finish. The presence of wild boars has been documented in areas adjacent to the farm. The farm is located within 2 km of residences where backyard pigs may be maintained. The farm lacks an isolation facility for receiving pigs from outside sources. The load-out area is not cleaned and disinfected after use. Materials entering the farm are not decontaminated. Vehicles entering the farm are permitted into the production area without application of disinfectant to the undercarriage. A dedicated site for cleaning and disinfection of vehicles does not exist, records of vehicle disinfection are not maintained, Vehicles are not properly cleaned and disinfected after transporting live pigs. Vehicles used for transport of mortalities are not cleaned and disinfected after use. Feed is prepared on the farm but is not heat treated. Containers for mortalities are available but they are not temperature controlled. Farm personnel that handle mortalities are not required to change clothing after completing such tasks. The shower area is not monitored and barn personnel are permitted to take cellphones, cigarettes, and other personal items into production areas. Barn personnel are permitted to use their own undergarments and do not have access to a laundry service.

Farm 9, Farrow-to-Finish. The presence of wild boars has been documented in areas adjacent to the farm and have been documented to enter the farm premises and enter areas where farm vehicles routinely operate. The farm is located within 2 km of residences where backyard pigs may be maintained. Internal vehicles leave the production area to access other areas of the farm and a designated area for cleaning and disinfection of internal vehicles does not exist. The farm did not have an established policy for decontaminating supplies and materials entering the farm. The isolation facility lacked a designated shower for farm personnel working in the area. The farm has containers for mortalities but they are not temperature controlled. Moreover, the farm does have a contract with a renderer, but does not want the renderer entering the premises due to biosecurity concerns. The farm removed the incinerator that was used for disposing of mortalities. The health status of farm personnel is not assessed periodically. The shower area is not monitored and barn personnel are permitted to take cellphones, cigarettes, and other personal items into production areas. Biosecurity policies regulating the movement of farm personnel has not been developed. A barrier fence was intact but was overgrown with vegetation.

Discussion. In the absence of conducting extensive on-site assessments of farms, completion of the questionnaire with representatives of the different swine operations permitted a comprehensive assessment of the biosecurity policies and practices used by the respective farms. A rigorous approach to farm biosecurity is necessary for Ukrainian swine producers to successfully control ASFV. The transmission of ASFV by ticks does not appear to be associated with a high probability in Ukraine, unlike other areas in Europe (Frant et al., 2017; Chenais et al., 2019). Therefore, biosecurity efforts can and should be focused on more relevant possibilities of ASFV introduction. While all the farms had established biosecurity policies and practices for purposes of preventing pathogen introduction, the responses to some questions raised concerns about the risk of ASFV introduction into all of the assessed farms.

Wild boars appear to be a reservoir for ASFV (Chenais et al., 2019; Brown and Bevins, 2018; Oļševskis et al., 2016) and farms located in regions where wild boar habitats are in close proximity carries a heightened risk of ASFV introduction. When considered in the context of farms that lack barrier fencing or other physical means of preventing wild boars from accessing the premises, this finding should be viewed as a serious deficiency in farm biosecurity. Wild boars infected with ASFV shed copious amounts of virus in urine, feces, and oral secretions and this material is infectious to domestic pigs (Guinat et al., 2016). As such, pig production sites that are visited by wild boars are at risk because of the possibility of tracking infectious material left by wild boars into pig housing facilities. Studies have confirmed that ASFV can be detected in the blood of pigs for up to 90 days post-infection, but the risk of virus transmission by chronically infected pigs requires further research and clarification (Petrov et al., 2018). Conversely, statistical modeling suggests that ASFV movements by wild boars is often impacted by strain virulence, severity of the disease outbreak and the potential of infected pigs to transmit virus to naïve pigs in close proximity (Podgórski and Śmietanka, 2018). Therefore, the spread of more virulent strains of ASFV may be reduced because infected pigs often die soon after infection and do not have the opportunity-shed virus over wide geographic areas. However, decomposing carcasses will pose an infection risk for a period of time after the death of ASFV infected pigs (Petrov et al., 2018; Chenais et al., 2019). Although wild boars have resided in forested habitats for extended periods of time and would be difficult to impossible to evict completely, a proactive policy of killing and carefully removing these pigs where possible represents one approach in mitigating the threat posed by this population of pigs (Cwynar, Stojkov and Wlazlak, 2019). Recommendations for safely hunting and processing wild boar that recognizes the risks to regional biosecurity have been proposed (Chenais et al., 2019; Bellini, Rutili and Guberti, 2016).

The presence of home-raised or backyard pigs injects a significant degree of risk for ASFV introduction to farms and is problematic for several reasons. In areas where ASFV is endemic and wild boars are active, it is possible for backyard pigs to have contact with wild boars because they are often housed with little to no consideration for biosecurity (Jurado et al., 2018). As a result, producers that permit their employees to own backyard pigs are at risk because farm personnel may become a source of ASFV originating from their home-raised pigs. Practices like swill feeding of backyard pigs further increases the risk of ASFV introduction because of contaminated table waste and is considered to be counterproductive to efforts to effectively control ASFV (Bellini, Rutili and Guberti, 2016). Backyard pigs are often unregulated by state regulatory officials and it is possible for them to become infected with ASFV and go undetected and unreported, thereby putting commercial farms in a given region at risk. Due to the inherent risk to commercial farms from backyard pigs, producers should discourage their employees from owning and maintaining such pigs (Bojkovski, 2015; Jurado et al., 2018). It is also reasonable to remove backyard pigs from locations in close proximity to commercial production sites whenever possible. The interviews revealed that several producers allowed their employees to own backyard pigs and this finding should be viewed as a serious deficiency in farm biosecurity. To address this concern, some producers have opted to provide pork to their employees free of charge or at a discount to discourage ownership of backyard pigs. This should be recognized as a worthwhile investment of resources to enhance farm biosecurity. In addition, several producers provided meals to their employees during the workday to prevent introduction of ASFV into their farms through contaminated meat products. This should also be recognized as a worthwhile biosecurity practice since ASFV can survive in cured and processed meat products for extended periods of time (Petrini et al., 2019; Bellini, Rutili and Guberti, 2016).

Vehicle movements represent a critical activity in the daily operation of swine farms. Transport of pigs, feed, supplies, and mortalities all require vehicle movements on and off pig production sites. It is in this context that the importance of a rigorous biosecurity policy for vehicles cannot be overlooked. Vehicles operating in and around pig production sites should be cleaned and disinfected on a regular basis, incorporating the necessary downtime to ensure that the process is done correctly (Jurado et al., 2018). A noteworthy example of failed vehicle biosecurity is the spread of Porcine Epidemic Diarrhea Virus (PEDV) in the United States after it was introduced in 2013 (Lowe, 2014). The spread of PEDV was exacerbated due to inadequate cleaning and disinfection of transport vehicles that delivered finishing pigs to slaughter facilities. Transport vehicles were contaminated at slaughter facilities during delivery of slaughter hogs and subjected to inadequate cleaning and disinfection after departure from those facilities. Subsequent travel back to commercial pig farms with contaminated vehicles permitted transmission of PEDV, resulting in enteric disease outbreaks and an estimated 10 million neonatal pig deaths. Up to that time, it was assumed that existing practices for cleaning and disinfection of pig transport vehicles was adequate for effective pathogen control and in compliance with accepted biosecurity standards. Therefore, it is crucial that a heightened level of attention be placed on vehicles because of their potential role in pathogen introduction. Wheel baths or wheel dips are in use on many commercial pig farms in Ukraine for the purpose of sanitizing the tires of vehicles entering the farm. The premise for installation of such structures is fully appreciated since it is strongly recommended that vehicles entering production areas be subjected to proper cleaning and disinfection. However, there are concerns that wheel baths do not adequately sanitize the tires of vehicles entering and leaving production areas and should not be relied upon solely for acceptable vehicle biosecurity (Ford, 1995). A comprehensive approach to vehicle disinfection should include application of disinfectant to the tires and undercarriage of vehicles entering the production area, including animal transport vehicles. Maintenance of a record of cleaning and disinfection of transport vehicles is recommended to track compliance with this critical aspect of biosecurity (Bellini, Rutili and Guberti, 2016). Vehicles that regularly travel off production sites and those that are used to transport mortalities should receive added attention because of the potential for extensive contamination of such work vehicles (Ford, 1995). Contaminated footwear or clothing worn by transport personnel presents a serious risk of virus introduction into production sites (Štukelj and Plut, 2018) and farms should have policies and practices that acknowledge this reality. It is reasonable to insist that drivers carry disposable coveralls, boots, and gloves if they are to exit the cab of their vehicle after arrival to the farm. However, upon exiting their vehicle, they should not be permitted to assist with activities that require them to enter the production facility and have contact with pigs, such as unloading pigs after transport or loading pigs for transport (Bellini, Rutili and Guberti, 2016). Drivers should place their disposable items in garbage bags after use, making every attempt to avoid contaminating the cab of the truck or transport vehicle upon re-entry.

The wholesomeness and safety of feed is a major consideration for swine producers since the pigs that they market enter the food chain for human consumption. The introduction and circulation of ASFV in Europe has elevated the importance of feed and water quality on swine farms. Research conducted utilizing transboundary shipping models has demonstrated that contaminated feed ingredients can harbor infectious titers of ASFV and put farms at risk if feed ingredients are sourced from

territories that are endemic for ASFV (Dee et al., 2018). In addition to contaminated complete feed, it has been demonstrated that contaminated water sources can transmit ASFV, with transmission via the water requiring lower viral titers than those required for feed transmission (Neiderwerder et al., 2019). The potential for feed ingredients to introduce ASFV into swine farms is raising concerns around the world due to global sourcing of feed ingredients and sourcing of many critical feed additives from China, who is experiencing a major swine health crisis due to ASFV (Zhou et al., 2018). As such, the use of heat-treated feeds as an adjunct to other biosecurity practices would have considerable value in reducing the risk of ASFV introduction. Temperatures approaching 85°C are achieved during the feed pelleting process, which is in excess of temperatures that will inactivate ASFV (Thomas, Van Zuilichem and Van der Poel, 1997). A temperature of 60°C for a minimum of 20 minutes is considered necessary for effective inactivation of ASFV (Penrith and Vosloo, 2009). Most of the assessed farms did not have the necessary feed mill infrastructure to produce pelleted feeds. This reality is not restricted to Ukrainian farms and is best viewed as a significant global challenge as it relates to addressing the risk of ASFV introduction into ASFV-free and endemic territories. Moreover, the preparation of pelleted diets may have limitations with regards to ASFV inactivation and the potential for failure of this process should be recognized. As a result of investigations into Seneca Valley Virus (SVV) outbreaks in Brazil, Leme et al. (2019) demonstrated that SVV can be recovered from pelleted swine diets. Two conclusions are possible, this may be indicative of post-pelleting contamination or an indication that the pelleting process failed to completely inactivate infectious agents. Relative to farm water sources, most of the assessed farms did not utilize water purification systems. The risk of ASFV transmission via the water has been demonstrated under laboratory conditions by Niederwerder et al. (2019), so there is reason for concern about this potential route of virus introduction. An example of this risk is the assumption that contaminated water from the Danube River may have resulted in ASFV infection of a large swine farm in Romania (Mazur-Panasiuk, Żmudzki and Woźniakowski, 2019). Therefore, biosecurity of water sources represents another factor that must be considered in the formulation of comprehensive farm biosecurity plans.

A recognized principle of a viable biosecurity program for swine farms is controlled access to production areas. Pigs entering farms should be sourced from ASFV-free territories with approved movement permits to reduce the risk of virus introduction (Jurado et al., 2018). The potential for pathogen transmission from human-to-pig has been established in the literature (Amass and Clark, 1999) and farm biosecurity policies should be reflective of this major risk. Visitors should be restricted and farm entry for employees and veterinary staff should consist of an orderly process where street clothing and footwear that is worn to the farm is changed at a designated location and not allowed to enter areas where pigs are housed (Jurado et al., 2018; Bellini, Rutili and Guberti, 2016). All the farms were consistent in their requirements for farm personnel and visitors to shower upon arrival and enter the production area only after dressing in clothing maintained at the farm. However, the shower area was not adequately supervised on some farms and farm personnel were permitted to carry personal items through the shower and into the production area, thereby putting the farm at risk for pathogen introduction. A properly supervised shower area utilizing Danish entry protocols would help in reducing the likelihood of pathogen introduction into the farm (Reicks, 2019; Jurado et al., 2018). Transport of personal items from the dirty side of the entry area to the clean side should not be permitted because of the inherent risk with such practices. In addition, equipment that is used by farm personnel inside buildings where pigs are housed should not be shared between different units (Jurado et al., 2018; Bellini, Rutili and Guberti, 2016). If equipment sharing becomes necessary, all equipment should be cleaned and disinfected as best as possible. Farm personnel responsible for removal and disposal of mortalities should utilize an orderly process for exiting and re-entering production areas that is consistent with acceptable farm entry practices.

Management of mortalities is a critical aspect of biosecurity on swine farms. Mortalities present a particular challenge to swine farms in Ukraine because producers are required to have a relationship with a rendering service to handle pigs that die on their premises. In ASFV endemic areas, this raises concerns with farm biosecurity because it requires that vehicles with unknown hygiene status have contact with farms (Bellini, Rutili and Guberti, 2016). In addition, holding and storing carcasses in ASFV endemic areas raises biosecurity concerns as well. Utilizing video recordings, Probst et al. (2019) documented the activity of scavengers on wild boar carcasses in Germany and determined that birds (raven, common buzzard) and small mammals (red fox, raccoon dog) will consume wild boar carcasses when located. They also documented that the observed scavengers will remove pieces of tissue from carcasses and transport it away from the original location of the carcass. This raises the importance of securely storing mortalities on farms to prevent visits by birds and small mammals that may have contact with ASFV infected material in endemic areas. Containers for storing mortalities were lacking on some farms or the containers were not temperature controlled, thereby limiting the ability to influence the rate of decomposition of carcasses. Proper storage of mortalities is also critical because of ASFV risks from insects. This is consequential because stable flies infected with ASFV from a blood meal and later ingested by pigs resulted in infections and demonstrated that biting flies can acquire

an infectious viral titer sufficient to cause disease (Olesen et al., 2018). Therefore, actions that reduce attraction of flies to production areas would have value to the overall biosecurity of the farm.

Cleaning and disinfection of housing areas, vehicles, and equipment are major considerations when formulating biosecurity plans for pig farms. The goal of cleaning and disinfection is to decontaminate surfaces or objects, such that contact with those surfaces or objects does not result in pathogen transmission and disease. Effective cleaning involves mechanical removal of organic matter from surfaces with the aid of detergents, followed by application of disinfectants (Juszkiewicz, Walczak and Woźniakowski, 2019). An equally important part of cleaning and disinfection is drying (Amass and Clark, 1999). Drying promotes desiccation of microorganisms and creates a hostile environment that limits the survival of viruses and bacteria. Ideally, facilities should be allowed to dry completely before and after application of disinfectants. Numerous disinfectant classes are effective at inactivating ASFV and compared to viruses like PCV2, ASFV is very susceptible to disinfection because it is an enveloped virus (Juszkiewicz, Walczak and Woźniakowski, 2019). Caustic soda (sodium hydroxide), aldehydes (formalin), phenolics, hypochlorites, and iodine compounds are considered to be effective at inactivating ASFV (Jurado et al., 2018). The most effective ASFV disinfectants, formalin and caustic soda, present safety concerns with their use so disinfectant selection should be based on overall effectiveness of ASFV inactivation and safety for employees handling the chemicals. Proper cleaning and disinfection of housing spaces is critical to maintaining acceptable standards of health on swine farms. Therefore, this critical aspect of biosecurity should be assessed frequently to insure the quality of the cleaning and disinfection process. The farm veterinarian should serve as a resource for the development and implementation of protocols to assess the quality of hygiene on the farm due to their knowledge of laboratory methods used to accomplish this important task. Research completed by Luyckx et al. (2015) on poultry farms provides some insight into considerations related to sampling procedures and microbiological and non-microbiological criteria that can be utilized to evaluate the quality of cleaning and disinfection on swine farms.

Conclusions. African swine fever virus has captured the attention of government officials, regulatory agencies, veterinarians, researchers, and swine producers worldwide. Implementation of biosecurity practices that reduce the likelihood of introduction of ASFV should be the overarching goal for territories that are currently free of this devastating pathogen. For ASFV endemic territories, the ultimate goal should be eradication of the virus with subsequent recognition by the World Organisation for Animal Health (OIE) for such an accomplishment. Eradication of ASFV from endemic areas has proven to be very difficult and when eradication in a timely manner is not a realistic option, establishment of compartments, or ASFV-free zones within endemic areas, as recognized by OIE, is the most viable alternative. Implementation of stringent biosecurity standards and establishment of compartments in Ukraine will permit engagement in regional and international trade of pork and pork products. The described assessment of biosecurity practices on Ukrainian pig farms revealed that producers are aware of the importance of this aspect of health and farm management. As intended, the assessments uncovered numerous deficiencies in farm biosecurity that if adequately addressed, would improve the overall health status on Ukrainian pig farms, permit establishment of regional compartments and stimulate more lucrative trade in pork and pork products for the nation.

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DETERMINATION OF ACUTE TOXICITY OF THE 'BONDARMIN' DISINFECTANT WHEN ADMINISTERED INTRAPERITONEALLY TO LABORATORY ANIMALS

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Summary. The article presents the results of the study of the acute toxic effect of the innovative disinfectant 'Bondarmin' (active substance — potassium peroxomonosulfate) on laboratory animals (mice, rats) are presented. Many scientific works of scientists in recent years have been devoted to the study of the toxicity of various disinfectants both in our country and abroad. However, today there are many topical issues regarding the toxicity and safety of some antimicrobials. Our work aimed to study the toxic effect on the laboratory animals and to establish the acute toxicity (LD_{50}) of the developed disinfectant 'Bondarmin' when administered intraperitoneally. Experiments were carried out in the Laboratory of Pharmacology and Toxicology of the National University of Pharmacy (Kharkiv) and in the 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 intraperitoneal administration of the designed disinfectant to laboratory animals (mice, rats). The toxic effect of the newly developed disinfectant 'Bondarmin' for the intraperitoneal method of administration to laboratory animals (mice, rats) has been determined. For the intraperitoneal administration of the 'Bondarmin' disinfectant, the LD_{50} by Prozorovskiy method is 316.85 ± 19.26 mg/kg for mice, and 279.33 ± 19.80 mg/kg for rats. 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

Keywords: lethal dose, potassium peroxomonosulfate, mice, rats, disinfection

Introduction. The sanitary-epidemiological situation in animal husbandry today is characterized by a tendency to the emergence and spread of infectious diseases with respiratory and fecal-oral routes of transmission. This is facilitated by the irrational use of antibiotics, violation of the sanitary regime, untimely implementation of measures for non-specific prevention of infectious diseases, which include disinfection of livestock facilities. Disinfection is an effective measure for the prevention and elimination of all known diseases in both human (Al-Sayah, 2020; Takagi and Yagishita, 2020; Cimolai, 2020) and veterinary (Stegniy et al., 2019; Shkromada et al., 2019; Paliy et al., 2020c) medicine.

Extensive use of antiseptics and disinfectants has led to some assumptions about the development of microbial resistance, including cross-resistance to antibiotics (McDonnell and Russell, 1999).

At present, in the field of domestic disinfection there is significant information on the resistance of microorganisms to different groups of disinfectants. The range of effective drugs has been expanded, disinfection techniques have been developed and implemented (Rutala and Weber, 2016; Saccucci et al., 2018; Paliy and Paliy, 2019).

The range of disinfectants is represented by a wide variety of chemical compounds of various origins and is quite extensive. However, the existing disinfectants have a significant difference from each other, have different antimicrobial action, toxicity, corrosion (Zavgorodniy et al., 2013; Lin et al., 2020). Aldehyde compounds (Paliy et al., 2016, 2018, 2020a; Gedge, Hollingsworth and Suchmann, 2019), organochlorine agents (Mustapha et al., 2018; Paliy et al., 2020b) have been shown to have a broad spectrum of antimicrobial activity. Along with that, in experimental studies, alcohol disinfection was not effective for 11 from 30 (36.7%) and for 12 from 62 (19.4%) subjects, respectively (Ribeiro et al., 2015).

The study of the toxicity of any drug for further testing and production is a necessary step, which is provided by the current instructional requirements (Kotsiumbas et al., 2006; Nechyporenko et al., 2019; Kovalenko et al., 2020; Orobchenko et al., 2020). Previously, data were published on the results of study of acute toxicity of the disinfectant 'Bondarmin' when administered intragastrically to laboratory mice (Bondarchuk, Paliy and Blazheyevskiy, 2019).

The aim of the study was to investigate the toxic effects of the disinfectant 'Bondarmin' on laboratory animals (mice, rats) when administered intraperitoneally.

Material and methods. We determined the toxicological properties of the 'Bondarmin' disinfectant, which is a domestic import-substituting development. Laboratory studies were performed using the rapid method to study moderately lethal doses of chemical

compounds (Pastushenko et al., 1985). The least squares method was used to analyze mortality curves (Prozorovskiy, 1962).

For experimental studies outbred white mice and Wistar rats 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, rats) trough intraperitoneal administration to reproduce the acute poisoning clinic and to determine the LD_{50}.

Results and discussion. Acute toxicity studies were performed in two stages. To determine the toxicity parameters at the preliminary stage, the express method of Pastushenko et al. (1985), and finally, the least-squares method for probit analysis of mortality curves according to Prozorovskiy (1962) ware used. The study of acute toxicity was performed with a single intraperitoneal route of administration.

For the preliminary stage of the study, all animals, mice and rats, respectively, were divided into seven groups of four in each. A dose interval ranging from 158 to 398 mg/kg was selected. Disinfectant was administered to animals in the form of an aqueous solution intraperitoneally using a syringe with a needle. The animals were observed for 14 days (Table 1).

Table 1 — Mortality of laboratory animals after intraperitoneal administration of the 'Bondarmin' disinfectant (n = 4)

Caused effect	Dose, mg/kg						
	158	200	250	282	316	355	398
Mice, males							
Dead animals/ total number	—	0/4	0/4	2/4	2/4	4/4	4/4
Rats, males							
Dead animals/ total number	0/4	0/4	2/4	2/4	4/4	4/4	4/4

According to the results of studies, the death of mice was observed with the introduction of disinfectant at a dose of 282 mg/kg and higher, and rats — 250 mg/kg and higher. The required dose range was selected to calculate the LD_{50}. The sequence of animal deaths 2-2-4 was used for this purpose. In doses for mice it is 282, 316, and 355 mg/kg, for rats — 250, 282, and 316 mg/kg. Using the table of the express method by Pastushenko et al. (1985) LD_{50} was determined. According to this method, the LD_{50} of the 'Bondarmin' disinfectant for mice is 330 (272–387) mg/kg, and for rats — 294 (242–346) mg/kg.

In order to confirm the above results and to study other toxicity parameters, we studied the acute toxicity of the disinfectant with intraperitoneal administration using the least-squares method for probit analysis of mortality curves by Prozorovsky (1962). Experimental groups were formed to determine the mean lethal dose. The animals were observed for 14 days after the introduction of disinfectant, recording the manifestations of disorders of the physiological state of the animals and mortality (Table 2).

Table 2 — Mortality of adult male mice after intraperitoneal administration of the 'Bondarmin' disinfectant (n = 6)

Animal species, sex	Dose, mg/kg	Caused effect, dead animals/total number
Mice, males	200	0/6
	250	1/6
	300	3/6
	350	4/6
	400	6/6
Rats, males	200	0/6
	250	2/6
	300	3/6
	350	5/6
	400	6/6

The results presented in Table 2 were the basis for calculating the parameters of the toxic action of the 'Bondarmin' disinfectant for mice using the method by Prozorovsky (1962) (Table 3).

The calculated parameters of LD for the 'Bondarmin' disinfectant when administered intraperitoneally to mice are: $A_0 = 2.49$; $A_1 = 0.79$; $LD_{16} = 238.42$ mg/kg; $LD_{50} = 316.85$ mg/kg; $LD_{84} = 387.59$ mg/kg; $m = 19.26$ mg/kg (the value of the average error obtained by calculating the LD_{50} of the experimental groups in accordance with this method). Therefore, $LD_{50} = 316.85 \pm 19.26$ mg/kg or 316.85 (276.99–356.71) mg/kg.

In addition, the results presented in Table 2 were the basis for calculating the parameters of the toxic action of the 'Bondarmin' disinfectant for rats using the method by Prozorovsky (1962) (Table 4).

The calculated parameters of LD for the 'Bondarmin' disinfectant when administered intraperitoneally to rats are: $A_0 = 2.70$; $A_1 = 0.82$; $LD_{16} = 197.5$ mg/kg; $LD_{50} = 279.33$ mg/kg; $LD_{84} = 350.6$ mg/kg; $m = 19.8$ mg/kg (the value of the average error obtained by calculating the LD_{50}

of the experimental groups in accordance with this method). Therefore, LD_{50} = 279.33 ± 19.80 mg/kg or 279.3 (238.00–320.27) mg/kg.

When administered toxic doses in laboratory animals (mice, rats) the following signs of intoxication were observed: decrease in motor activity, lack of appetite, impaired coordination of movements, stupor developed, and then there was a death. Deterioration of the animals was recorded on the first or third day after administration of the disinfectant. The severity of these signs increased with increasing dose. At autopsy of dead animals we observed pathological changes in the abdominal cavity.

Thus, the parameters of acute toxicity of the 'Bondarmin' disinfectant on laboratory animals (mice, rats) have been determined. The research results are shown in Table 5.

Table 3 — Calculation data for the determination of the LD_{50} of the 'Bondarmin' disinfectant in mice after intraperitoneal administration by Prozorovskiy method

Dose, mg/kg	Mortality, %	Dose place (X)	Probit (Y)	Weighting coefficient (B)	xB	x^2в	yB	xyB
200	0	1	3.27	1.6	1.60	1.60	5.23	5.23
250	16.67	2	4.05	3.7	7.40	14.80	14.99	29.97
300	50.00	3	5.00	5.0	15.00	45.00	25.00	75.00
350	66.67	4	5.44	4.6	18.40	73.60	25.02	100.10
400	100	5	6.73	1.6	8.00	40.00	10.77	53.84
Σ				16.5	50.40	175.00	81.01	264.14

Table 4 — Calculation data for the determination of the LD_{50} of the 'Bondarmin' disinfectant in rats after intraperitoneal administration by Prozorovskiy method

Dose, mg/kg	Mortality, %	Dose place (X)	Probit (Y)	Weighting coefficient (B)	xB	x^2в	yB	xyB
200	0	1	3.27	1.6	1.60	1.60	5.23	5.23
250	33	2	4.56	4.5	9.00	18.00	20.52	41.04
300	50	3	5.00	5.0	15.00	45.00	25.00	75.00
350	87	4	6.13	3.2	18.40	73.60	24.89	99.54
400	100	5	6.73	1.6	8.00	40.00	10.77	53.84
Σ				17.3	52.00	178.20	86.41	274.66

Table 5 — The degree of toxicity of the 'Bondarmin' disinfectant for different routes of administration to laboratory animals

Route of administration	Animal species, sex		LD_{50}, mg/kg		Toxicity class
			by Pastushenko et al. (1985)	by Prozorovsky (1962)	
Intragastric (Bondarchuk, Paliy and Blazheyevskiy, 2019)	mice	males	2,580 (1,930–3,220)	2,702 (2,379–3,026)	IV (low toxic substances)
	rats	males	2,940 (2,420–3,460)	3,014 (2,483–3,544)	
Intraperitoneal	mice	males	330 (272–387)	317 (277–357)	
	rats	males	330 (272–387)	279 (238–320)	

The 'Bondarmin' disinfectant for intragastric and intraperitoneal routes of administration to laboratory animals (mice, rats) belongs to the IV toxicity class (low toxic substances).

Conclusions. The toxic effect of the newly developed disinfectant 'Bondarmin' for the intraperitoneal method of administration to laboratory animals (mice, rats) has been determined. For the intraperitoneal administration of the 'Bondarmin' disinfectant, the median lethal dose by Prozorovskiy method is 316.85 ± 19.26 mg/kg for mice, and 279.33 ± 19.80 mg/kg for rats. 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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MICROBIOLOGICAL MONITORING OF POULTRY PRODUCTS IN DNIPROPETROVSK REGION (UKRAINE)

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Summary. The aim of the work was to analyze the microbial status of poultry products in Dnipropetrovsk Region in 2019. The author summarizes the results of studies of three groups of potentially dangerous microbiological factors. It was found that 0.9–2.9% of the studied samples had higher quantity of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM), while in 0.5–2.9% of cases coliform bacteria were isolated, in 13.23% of samples — *Salmonella* spp., in 0.37–0.70% — cocci, in 0.18–0.28% — *Proteus* spp., in 0.18–0.55% — *Listeria monocytogenes*. During the work, violations of the safety criteria for raw materials and poultry products were found, as evidenced by the isolation of pathogens *Salmonella* spp. and *L. monocytogenes*. Geographical serotype predisposition is shown in the occurrence and development of salmonellosis infection in the region, which is caused by the dominance of *Salmonella* group D among poultry in Dnipropetrovsk Region. The most intensive contamination with microorganisms (in 2.9% of samples) was observed in minced meat and meat of mechanical deboning from poultry

Keywords: MAFAnM, coliform bacteria, *Salmonella*, *Proteus*, *Staphylococcus aureus*, *Listeria monocytogenes*

Introduction. For industrial enterprises in many branches, microbiological parameters are the important quality indicators of the products. Their control allows the manufacturer to avoid economic (unusable products, penalties), image losses, and, ultimately, the loss of the market. First of all, they control the microbiological parameters that are standardized. Thus, products that contain a lot of moisture — food products, are affected by microorganisms especially quickly and severely (Kachan, 2020).

According to Regulation No 178/2002 of the European Parliament and the EU Council (EP and CEU, 2002), ensuring a high level of protection of human life and health is one of the main objectives of EU food law. Poultry production is of particular note, due to the volume of its production and widespread use due to the relatively low cost, as compared with the products of other livestock industries. To date, more than 100 infectious diseases are known to be transmitted to humans through products of animal origin (Seryogin, Nikitchenko and Abdullaeva, 2015). Thus, only in Dnipropetrovsk Region in 2019, 20 nosological forms of infectious diseases were registered, the sources of which were animals. Analysis of the epidemic situation in the region during this period, according to the State Service of Ukraine on Food Safety and Consumer Protection, shows that the etiological structure of acute intestinal infections was represented by norovirus (30% outbreaks), bacterial pathogens (30%), hepatitis A virus (10%) or had an unknown etiology (30 %). Violations of the requirements of the legislation on food safety and quality, prove their social significance and the need for careful study.

The aim of the work was to analyze the microbial status of poultry products in Dnipropetrovsk Region in 2019.

Materials and methods. The compliance of microbiological indicators with the requirements of normative documents in seven types of poultry products was determined. We analyzed the results of veterinary statistical reporting of Dnipropetrovsk Region for 2019.

A total of 2,076 samples were examined in real time, of which 94.0% were products of domestic Ukrainian production, 3.2% — imports, 2.1% — exports, 0.6% — state control (Table 1).

Table 1 — List of researched poultry products in Dnipropetrovsk Region during 2019

Type of poultry products	Number of samples
Semi-finished and culinary products from meat, in particular poultry	715
Sausages, in particular poultry	543
Poultry meat	362
Eggs	302
Minced meat and mechanically deboned poultry meat	68
Egg products	55
Poultry co-products	31
Total	2,076

Preparation of the tested samples was performed in accordance with DSTU ISO 6887-2:2005 (ISO 6887-2:2003, IDT) (DSSU, 2005a).

The methods provided by DSTU ISO 4833:2006 (ISO 4833:2003, IDT) (DSSU, 2008), DSTU ISO 4832:2015 (ISO 4832:2006, IDT) (SE 'UkrNDNC', 2018), DSTU EN 12824:2004 (EN 12824:1997, IDT) (DSSU, 2005d), DSTU ISO 11290-1:2003 (ISO 11290-1:1996, IDT) (DSSU, 2005c), DSTU 7444:2013 (MEDTU, 2014), and DSTU ISO

6888-2:2003 (ISO 6888-2:1999, IDT) (DSSU, 2005b) were used for microbiological control of poultry production (Table 2).

Results and discussion. Non-compliance of microbiological indicators with the requirements of regulatory documents were found in four types of poultry products (Table 3). It is found that the number of high-risk products includes poultry meat; semi-finished products and culinary products from it; minced meat and mechanically deboned poultry, as well as ready-to-eat meat products (those that have undergone heat treatment) — sausages, in particular from poultry.

The results of sowing samples showed that QMAFAnM exceeded the permissible norms in 2.8% of samples from semi-finished and culinary products from poultry meat, in 0.92% — in sausages, in 2.94% — in minced meat and mechanically deboned poultry, in 1.38% — in poultry meat.

Coliform bacteria were detected in 0.50–2.94% of samples, including in samples of semi-finished products — 2.94%, in sausages — 2.39%, in minced meat and mechanically deboned poultry meat — 2.94%, in poultry meat — 0.55%.

Salmonella group D bacteria were isolated in 13.23% of samples of minced meat from poultry and mechanically deboned poultry meat (Table 4).

The presence of staphylococci in semi-finished products is not standardized, but studies have shown that they are present in 0.70% of samples and in 0.37% of sausages.

Proteus bacteria were found in 0.28% of semi-finished products and in 0.18% of sausages.

Table 2 — List of investigated potentially dangerous microbiological factors in poultry products

Groups (names) of dangerous factors	Range	Control method
Sanitary-indicative:		
Mesophilic-aerobic and facultative-anaerobic microorganisms (MAFAnM)	Not more than 5.0×10^5 CFU/g	DSTU ISO 4833:2006 (ISO 4833:2003, IDT) (DSSU, 2008)
Coliform bacteria	Not allowed in 0.0001 g	DSTU ISO 4832:2015 (ISO 4832:2006, IDT) (SE 'UkrNDNC', 2018)
Pathogenic microorganisms:		
Salmonella spp.	Not allowed in 25 g	DSTU EN 12824:2004 (EN 12824:1997, IDT) (DSSU, 2005d)
Listeria monocytogenes	Not allowed in 25 g	DSTU ISO 11290-1:2003 (ISO 11290-1:1996, IDT) (DSSU, 2005c)
Conditionally pathogenic microorganisms:		
Proteus spp.	Not allowed in 0.1 g	DSTU 7444:2013 (MEDTU, 2014)
Staphylococcus aureus	Not standardized	DSTU ISO 6888-2:2003 (ISO 6888-2:1999, IDT) (DSSU, 2005b)

Table 3 — Non-compliance of microbiological indicators in poultry products in Dnipropetrovsk Region

Type of poultry products	Groups of dangerous factors, samples/%					
	MAFAnM	Coliform bacteria	*Salmonella* spp.	*S. aureus*	*Proteus* spp.	*L. monocytogenes*
Semi-finished and culinary products from meat, in particular from poultry	20/2.80	21/2.94	—	5/0.70	2/0.28	—
Sausages, in particular from poultry	5/0.92	13/2.39	—	2/0.37	1/0.18	1/0.18
Minced meat and mechanically deboned poultry meat	2/2.94	2/2.94	9/13.23	—	—	—
Poultry meat	5/1.38	2/0.55	—	—	—	2/0.55

Note. '—' — not found.

Table 4 — The results of serological identification of *Salmonella* (n = 9)

Serological group	Serological variant	Poultry products and their origin	
		Frozen mechanically deboned poultry meat (domestic), %	Chicken meat — backs (import),%
D	0:9, Vi	44.4	55.6

L. monocytogenes bacteria were isolated from 0.18% of sausage samples and 0.55% of poultry meat, indicating a presence of listeriosis in poultry farming areas.

Research data show that raw materials and poultry food products in 0.9–2.9% of cases had increased microbial contamination. The most intense contamination by microorganisms (2.9%) was observed in minced meat and mechanically deboned poultry, compared with other types of samples, as evidenced by reports of other researchers (Abdullaeva, Seryogin and Nikitchenko, 2017). The increase in the contact surface of the structural particles of minced meat and mechanically deboned poultry meat with air and the surface of the equipment, as well as the high degree of grinding of various tissues and bone marrow, the presence of excess meat juice and high pH make this raw material a good breeding ground for microorganisms. In this regard, minced meat and mechanically deboned meat belong to the category of high-risk, perishable raw materials. EU Commission Regulation No 2073/2005 (CEC, 2005) also states that minced meat and semi-finished meat products belong to high-risk products.

Detection of *Salmonella* spp. and *L. monocytogenes* in poultry meat and mechanically deboned meat proves that the specified pathogenic microflora can penetrate into finished products and semi-finished products.

Thus, salmonella can be stored for a long time in the environment: in water for up to 5 months, in meat and sausages — 2–4 months, in frozen meat — about 6 months (in poultry carcasses — more than a year). In some meat products, salmonella can not only be stored but also reproduced without changing the appearance and taste of the products. Salting and smoking have a very weak effect on them, and freezing even increases the survival time of microorganisms in food. Today, according to the results of epidemiological investigations, it is established that sick poultry is one of the leading factors in the transmission of this disease (FBHI 'CHERSY', 2020). Detection of *Salmonella* group D in mechanically deboned meat samples indicates a geographical serotype predisposition, which is due to the dominance of *Salmonella* group D among poultry in Dnipropetrovsk Region (Martynenko, 2019). The prevalence of *Salmonella* group D pathogens in the etiological structure of human salmonellosis (more than 80%) is reported by Pimenov, Laishevtcev and Pimenova (2017). Current trends in the development of poultry farming in Ukraine draw attention to the products of this industry, which are imported into the country, as indicated by the identified inconsistencies DSTU EN 12824:2004 (EN 12824:1997, IDT) (DSSU, 2005d). The analysis of outbreaks of *Salmonella* infections caused by poultry products shows that they are quite common in importing countries (*The Guardian*, 2019; Whitworth, 2019; Kalisz, 2019). Thus, today five countries (Great Britain, Denmark, Poland, Hungary, and the Czech Republic) have international veterinary certificates for the import of minced meat and/or mechanically deboned meat into the customs territory of Ukraine.

Detection of *L. monocytogenes* and its increased ability to grow or survive in a refrigerated environment compared to most other microorganisms confirms that this pathogen is a significant risk factor in food production. This is especially true for ready-to-eat foods that do not undergo heat treatment during production, as well as foods that may be contaminated during production.

Detection *Proteus* bacteria in semi-finished products and sausages allowed to establish that the degree of contamination of the tested samples with organic residues did not exceed 0.28%. Therefore, the source of pathogenic bacteria are infected poultry products. The obtained results confirm that meat products are at risk, as reported in other sources (FSBI 'ORCFSVPS', 2016).

Conclusions. Violations of food safety criteria in poultry products and raw materials have been established, as evidenced by the isolation of *Salmonella* spp. and *L. monocytogenes*. The geographical serotype predisposition in the occurrence and development of *Salmonella* infection in the region is shown. Violation of hygiene criteria of technological processes is proved, as evidenced by exceeding the permissible limits of QMAFAnM in 0.9–2.9% of the studied samples.

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