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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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1. Papers must be submitted in an electronic variant and should be sent directly to the editorial board at nsc.iecvm.kharkov@gmail.com or inform@vet.kharkov.ua with subject 'Article in JVMBBS'

2. Papers must be written in English

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

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NSC 'Institute of Experimental and Clinical Veterinary Medicine'
83 Pushkinska Str., Kharkiv, Ukraine, 61023
tel. +38 (057) 707-20-53, 704-10-90
E-mail: nsc.iecvm.kharkov@gmail.com, inform@vet.kharkov.ua

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PHYLOGENETIC ANALYSIS OF UKRAINIAN *BACILLUS ANTHRACIS* STRAINS

Biloivan O. V. [1], Stegniy B. T. [1], Arefiev V. L. [1], Solodiankin O. S. [1], Gerilovych A. P. [1], Duerr A. [2], Schwarz J. [2], Grass G. [2], Napnenko O. O. [3], Deryabin O. M. [3]

[1] National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: silverscreen91@gmail.com
[2] Bundeswehr Institute of Microbiology, Munich, Germany
[3] State Scientific Control Institute of Biotechnology and Strains of Microorganisms, Kyiv, Ukraine

Summary. In many countries anthrax is a common zoonotic disease which poses a serious threat to human and animal health. Sporadic cases of anthrax occur each year both among farm animals and humans in Ukraine. The cutaneous form of anthrax is the most widespread in Ukraine. The capability of *Bacillus anthracis* spores to remain viable in soil for decades, as well as the possibility to use this pathogen as biological terror agent make effective diagnostic and research capabilities extremely important. This comprises molecular methods including state-of-the-art methods for accurate genotyping of *B. anthracis* strains. A total of 12 *B. anthracis* DNA samples from a Ukrainian strain collection were studied by qPCR to confirm chromosomal and plasmid markers. To characterize regional and global phylogeographic relationships of these strains, canonical Single Nucleotide Polymorphism analysis (canSNP) and Multiple-Locus Variable-number of tandem repeat Analysis (MLVA-25) were conducted. *B. anthracis* chromosomal DNA-markers (dhp61 and gyrA) as well as those of the pXO1 plasmid could be detected in all 12 DNA samples. However, only 5 out of 12 tested strains contained the pXO2 plasmid-marker. All pXO2 positive strains group into the A.Br.008/009 SNP-clade, which belongs to the major 'A' branch of *B. anthracis*. MLVA-25 analysis suggested that Ukrainian *B. anthracis* genotypes are related to strains from Southern Europe (in particular, to Bulgarian, Greek, and Italian isolates). In contrast, the pXO2- negative strains might be related to the Russian vaccine strain STI as they grouped to A.Br.008/011 canSNP group. The infrequent occurrence of anthrax in the country of Ukraine is likely caused by a heterogeneous population of *B. anthracis*. This population is phylogenetically composed of at least two different canSNP groups of the world-wide dominating A-branch of the pathogen. While one group might stem from environmental recovery of live vaccine strains used in Ukraine (or the former Soviet Union in the past) the other one, the A.Br.008/009 group, could be enzootic as indicated by the presence of related strains in countries of southeastern Europe in relatively close geographical vicinity to Ukraine.

Keywords: anthrax, genotyping, PCR, HRM, canonical SNP, MLVA, Ukraine

Introduction. Anthrax, caused by bacterium *Bacillus anthracis,* is a zoonotic disease with a natural transmission cycle involving wildlife, livestock and humans (Van Ert et al., 2007). Infection with the disease poses a serious threat to human and animal health due to its mortality especially for animals (Purcell, Worsham and Freidlander, 2007). In addition, world-wide vigilance for anthrax is high due to the agent's potential to be used for nefarious purposes including bioterrorism which provides evidenced by the letter attacks in the United States in 2001 (Hoffmaster et al., 2002). In addition, anthrax was developed as potential biological weapon by several countries in the past, including the United States, the United Kingdom and the former Soviet Union (Keim et al., 2004). The ability of *B. anthracis* spores to remain viable in soils for decades simplifies both their isolation from the environment and dissemination as a biological weapon (Martin, Christopher and Eitzen, 2007). Importantly, the capability to form dormant and highly persistent spores in the environment plays major role in the ecology and evolution of the anthrax pathogen (Keim et al., 2000). Notably, during the spore phase, evolution rate is greatly reduced limiting the degree of genetic diversity found among isolates of this species (Van Ert et al., 2007). Therefore, not only reliable anthrax diagnosis is needed but also bio forensic capabilities including state-of-the-art methods for accurate genotyping of *B. anthracis* strains.

Two molecular approaches, namely Multiple-Locus Variable-number tandem repeat Analysis (MLVA) and whole genome Single Nucleotide Polymorphism (SNP) discovery and analysis, have greatly enhanced the identification of genetic markers for analysis of phylogenetic relationships among *B. anthracis* isolates (Keim et al., 2000; Pearson et al., 2004; Smith et al., 2000).

The *B. anthracis* global phylogeny is divided into three major lineages: A, B, and C. The A clade is by far the largest group and geographically the most wide-spread

genotype (causing 89.6% of all cases) (Van Ert et al., 2007). The A sub-linage radiates into multiple closely related and widely dispersed subgroups (Van Ert et al., 2007; Keim et al., 1997, 2000). The topological complexity among and within these groups, can be reliably resolved by using whole genome sequences to discover a relatively small number of SNPs (a few hundred). Once identified, these SNPs are highly discriminatory characters with high consistency in phylogenetic reconstructions (Pearson et al., 2004, 2009).

Ukraine is a large agrarian country in Eastern Europe. It has reported livestock outbreaks for more than a century (Gierczyński et al., 2004). At the turn of the 20th century, anthrax was a major animal and public health concern in the Russian Empire (which included a large part of Ukraine) (Korotich and Pogrebnyak, 1976), throughout the Soviet Period and since independence in 1991 (Bezymennyi et al., 2014). According to statistical data, 24,954 outbreaks of anthrax were detected in Ukraine throughout the period from 1920 to 2014 (Bobyliova et al., 2001; Bobyliova and Mukharska, 2002; Maly et al., 2013; data from OIE WAHIS). More than 4,500 anthrax burial sites are known in the Ukraine, and 60% of these are classified as old (grave sites from 1954 and older) (Zaviriuha, Yanenko and Zaviriuha, 2015). Moreover, sporadic infrequent cases of anthrax in Ukraine might be caused by naturally and anthropogenic re-release of spores from soil at old burial sites posing a constant risk of re-emergence of anthrax disease.

Currently, there is still a complete lack of information on the phylogenetic diversity of Ukrainian *B. anthracis* strains.

The aim of the study was genotyping of 12 *B. anthracis* DNA samples by canonical Single Nucleotide Polymorphisms analysis (canSNP) and Multiple-Locus Variable-number of tandem repeat Analysis (MLVA-25) for better phylogenetic placement of Ukrainian *B. anthracis* strains within the global diversity of the pathogen.

Materials and methods. A total of 12 *B. anthracis* DNA samples from Ukrainian strain collection of the State Scientific Control Institute of Biotechnology and Strains of Microorganisms (SSCIBSM, Kyiv, Ukraine) were isolated from live strains cultivated on soft agar and then re-inoculated on Hottinger broth. DNAs were prepared using 'Tissue DNA Isolation Kit' (MO BIO Laboratories, Inc., USA). Work was done at the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE, Kyiv, Ukraine).

DNAs were further analyzed at the Bundeswehr Institute of Microbiology (Munich, Germany) by qPCR to confirm the presence of anthrax chromosomal and plasmid markers. Metadata for the samples' origins is shown in Table 1 and Fig. 1.

To confirm the presence of specific DNA of *B. anthracis* chromosomal and plasmid markers, samples were analyzed by real-time qPCR using specific primers and probes for *dhp61* (chromosome), *pagA* (plasmid pXO1) and *capC* (plasmid pXO2) (Antwerpen et al., 2008; Rume et al., 2016). Canonical Single Nucleotide Polymorphism analysis (canSNP) was conducted as published (Derzelle et al., 2011; Derzelle, 2015). Primers and working concentrations are summarized in Table 2.

For VNTR typing of the DNA samples, we employed the MLVA-25 scheme as previously described by Lista et al. (2006). This collection of 25 loci represents all VNTR loci in the MLVA-8, MLVA-15 schemes (Keim et al., 2000; Beyer et al., 2012; Thierry et al., 2014; Leski et al., 2009) (Table 3).

From the MLVA data a UMPGA-tree was built using BioNumerics software (version 6.6).

Figure 1. Map of Ukraine with locations where studied samples originate from: 1 — BA-C-10-Cher (Khotyn district, Chernivtsi region); 2 — BA-D-12-Mel (Voznesenka village, Melitopol district, Zaporizhia region, Ukraine); 3 — BA-C-12-Sm (Makiivka village, Smila district, Cherkasy region); 4 — State Scientific Control Institute of Biotechnology and Strains of Microorganisms

Table 1 — Metadata of *B. anthracis* DNA samples from the SSCIBSM Strain Depositary

#	ID	Originating institution	Country	Source	Year
1	BA-C-10-Cher	SSRILDVSE	Khotyn district, Chernivtsi region, Ukraine	cattle	2010
2	BA-D-12-Mel		Voznesenka village, Melitopol district, Zaporizhia region, Ukraine		2012
3	BA-C-12-Sm		Makiivka village, Smila district, Cherkasy region, Ukraine		2012
4	55 VNIIVViM	Federal Research Center of Virology and Microbiology	Russia	vaccine live stock	2007
5	M-71	SSCIBSM	Ukraine		2001
6	K-79-Z	Institute of Veterinary Medicine of the NAAS			1997
7	B. anthr. SB	SSCIBSM			1997
8	B. anthr. 55	missing data	Russia		2012
9	Tsenkovskii-II				1997
10	STI				2007
11	Sterne 34F2	SSCIBSM	Ukraine		2015
12	UA-07		Ukraine		2007

Table 2 — Sequences and concentration of primers used for canSNP assay

canSNP	Name	Forward primer (5′→3′)	Reverse primer (5′→3′)	Concentration in duplex, μM
A.Br.001	BA1A	GTGGTAAGGCAAGCGGAAC	ACGGTTTCCCTTTATCATCG	0.20
A.Br.002	BA2	GCAGAAGGAGCAAGTAATGTTATAGGT	CCTAAAATCGATAAAGCGACTGC	0.15
A.Br.003	BA3	AAAGGAATTTAGATTTTCGTGTCG	ATAAAAACCTCCTTTTTCTACCTCA	0.20
A.Br.004	BA4	ATCGCCGTCATACTTTGGAA	GGAATTGGTGGAGCTATGGA	0.15
A.Br.006	BA5	GCGTTTTTAAGTTCATCATACCC	ATGTTGTTGATCATTCCATCG	0.20
A.Br.007	BA6	TTACAAGGTGGTAGTATTCGAGCTG	TTGGTAACGAGACGATAAACTGAA	0.20
A.Br.008	BA7	CCAAACGGTGAAAAAGTTACAAA	GCAACTACGCTATACGTTTTAGATGG	0.20
A.Br.009	BA8	AATCGGCCACTGTTTTTGAAC	AGGTATATTAACTGCGGATGATGC	0.25

Table 3 — The list of 25 published *B. anthracis* VNTR markers

Locus	Forward primer (5′→3′)	Reverse primer (5′→3′)
vrrA[a]	CACAACTACCACCGATGGCACA	GCGCGTTTCGTTTGATTCATAC
vrrB1[a]	ATAGGTGGTTTTCCGCAAGTTATTC	GATGAGTTTGATAAAGAATAGCCTGTG
vrrB2[a]	CACAGGCTATTCTTTATCAAACTCATC	CCCAAGGTGAAGATTGTTGTTGA
vrrC1[a]	GAAGCAAGAAAGTGATGTAGTGGAC	CATTTCCTCAAGTGCTACAGGTTC
vrrC2[a]	CCAGAAGAAGTGGAACCTGTAGCAC	GTCTTTCCATTAATCGCGCTCTATC
CG3[a]	TGTCGTTTTACTTCTCTCTCCAATAC	AGTCATTGTTCTGTATAAAGGGCAT
pXO1[a]	CAATTTATTAACGATCAGATTAAGTTCA	TCTAGAATTAGTTGCTTCATAATGGC
pXO2[a]	TCATCCTCTTTTAAGTCTTGGGT	GTGTGATGAACTCCGACGACA
bams01[b]	GTTGAGCATGAGAGGTACCTTGTCCTTTTT	AGTTCAAGCGCCAGAAGGTTATGAGTTATC
bams03[b]	GCAGCAACAGAAAACTTCTCTCCAATAACA	TCCTCCCTGAGAACTGCTATCACCTTTAAC
bams05[b]	GCAGGAAGAACAAAAGAAACTAGAAGAGCA	ATTATTAGCAGGGGCCTCTCCTGCATTACC
bams07[b]	GAATATTCGTGCCACCTAACAAAACAGAAA	TGTCAGATCTAGTTGGCCCTACTTTTCCTC
bams13[b]	AATTGAGAAATTGCTGTACCAAACT	CTAGTGCATTTGACCCTAATCTTGT
bams15[b]	GTATTTCCCCCAGATACAGTAATCC	GTGTACATGTTGATTCATGCTGTTT
bams21[b]	TGTAGTGCCAGATTTGTCTTCTGTA	CAAATTTTGAGATGGGAGTTTTACT
bams22[b]	ATCAAAAATTCTTGGCAGACTGA	ACCGTTAATTCACGTTTAGCAGA
bams23[b]	CGGTCTGTCTCTATTATTCAGTGGT	CCTGTTGCTCCTAGTGATTTCTTAC

Locus	Forward primer (5′→3′)	Reverse primer (5′→3′)
bams24[b]	CTTCTACTTCCGTACTTGAAATTGG	CGTCACGTACCATTTAATGTTGTTA
bams25[b]	CCGAATACGTAAGAAATAAATCCAC	TGAAAGATCTTGAAAAACAAGCATT
bams28[b]	CTCTGTTGTAACAAAATTTCCGTCT	TATTAAACCAGGCGTTACTTACAGC
bams30[b]	GCATAATCACCTACAACACCTGGTA	CAGAAAATATTGGACCTACCTTCC
bams31[b]	GCTGTATTTATCGAGCTTCAAAATCT	GGAGTACTGTTTGTTGAATGTTGTTT
bams34[c]	CAGCAAAATCAATCGAATCAAA	TGTGCTAAATCATCTTGCTTGG
bams44[c]	GCGAATTAATTGCTCCTCAAAT	GCACTTGAATATTTGGCGGTAT
bams51[c]	ATTTCCTGAAGCAGGTTGTGTT	TGCATCTAACAATGCAGAACAA
bams53[c]	GAGGTGTGTTAGGTGGGCTTAC	CATATTTTCACCTTAATTTTGGAAG

Notes: [a] Keim et al. 2000; [b] Le Flèche et al. 2001; [c] Lista et al. 2006

Results. ***Chromosomal and plasmid marker analysis and canonical SNP typing.*** *B. anthracis* chromosomal and pXO1 plasmid markers were detected in all 12 DNA samples. However, only 5 out of 12 tested strains were positive for the pXO2 plasmid marker (Table 4).

Table 4 — The list of 25 published *B. anthracis* VNTR markers

#	Sample	Chromosome *dhp61*	pXO1 *pagA*	pXO2 *capC*
1	BA-C-10-Cher	+	+	+
2	BA-D-12-Mel	+	+	+
3	BA-C-12-Sm	+	+	+
4	55 VNIIVViM	+	+	-
5	M-71	+	+	+
6	K-79-Z	+	+	-
7	SB	+	+	-
8	55	+	+	-
9	Tsenkovski-II	+	+	+
10	STI	+	+	-
11	Sterne 34F2	+	+	-
12	UA-07	+	+	-

Next, canSNP assays were performed to examine the phylogenetic position and genetic diversity of Ukrainian *B. anthracis* isolates. Allelic states denoted as 'ancestral' or 'derived' were scored in Table 5. No allelic discrepancies (contradictions) were observed.

Table 5 — Results of canSNP typing for samples from Ukrainian strains

#	Sample	A.Br.001	A.Br.002	A.Br.003	A.Br.004	A.Br.005	A.Br.006	A.Br.008	A.Br.007
1	BA-C-10-Cher	Ancestral	Ancestral	Ancestral	Ancestral	Derived	Ancestral	Derived	Ancestral
2	BA-D-12-Mel								
3	BA-C-12-Sm								
4	55 VNIIVViM								
5	M-71								
6	K-79-Z								
7	SB								
8	55								
9	Tsenkovski II								
10	STI								
11	Sterne 34F2								
12	UA-07								

All strains grouped into the A.Br.008/009 SNP-clade, which belongs to the major 'A' branch of *B. anthracis*. This clade is also known as Trans-Eurasian subgroup, which is spread across Europe, the Middle East, and part of Asia, including China.

MLVA-typing. For more detailed phylogenetic characterization of Ukrainian samples, MLVA-25 analysis was conducted (Fig. 2). The majority of the Ukrainian *B. anthracis* strains, namely BA-C-10-Cher, BA-D-12-Mel, BA-C-12-Sm, 55 VNIIVViM, K-79-Z, SB, 55, STI, Sterne 34F2, and UA-07, were similar to strains from Bulgaria, described by Antwerpen et al. (2011). These strains form a unique sub-cluster within the A1.a lineage which is different from clusters of strains from geographically neighboring regions, such as Turkey, Georgia, Albany or Italy. Conversely, two samples had different genotypes which were similar to Italian (M-71) and Spanish strains (Tsenkovski-II). The preliminary results indicate that the pXO2-negative strains (55 VNIIVViM, K-79-Z, SB, 55, STI, Sterne 34F2 and UA-07) might be related to Russian vaccine strains. Based on these results, MLVA-tree was built (Fig. 2). Thus, the Ukrainian strain designated Sterne 34F2 is not a Sterne vaccine derivative (belonging to SNP group A.Br.001/002) but is most likely a relative to the STI-vaccine strain (A.Br.008/009).

Discussion. The analysis of a small set of canonical SNPs is a fast way to determine the major clonal sublineages of *B. anthracis* when assay costs are essential issues. The original setup described by Van Ert et al. (2007) required the use of 26 sequence-specific TaqMan minor groove binder (MGB) probes. This setup is quite expensive for laboratories where only a few strains have to be typed per year.

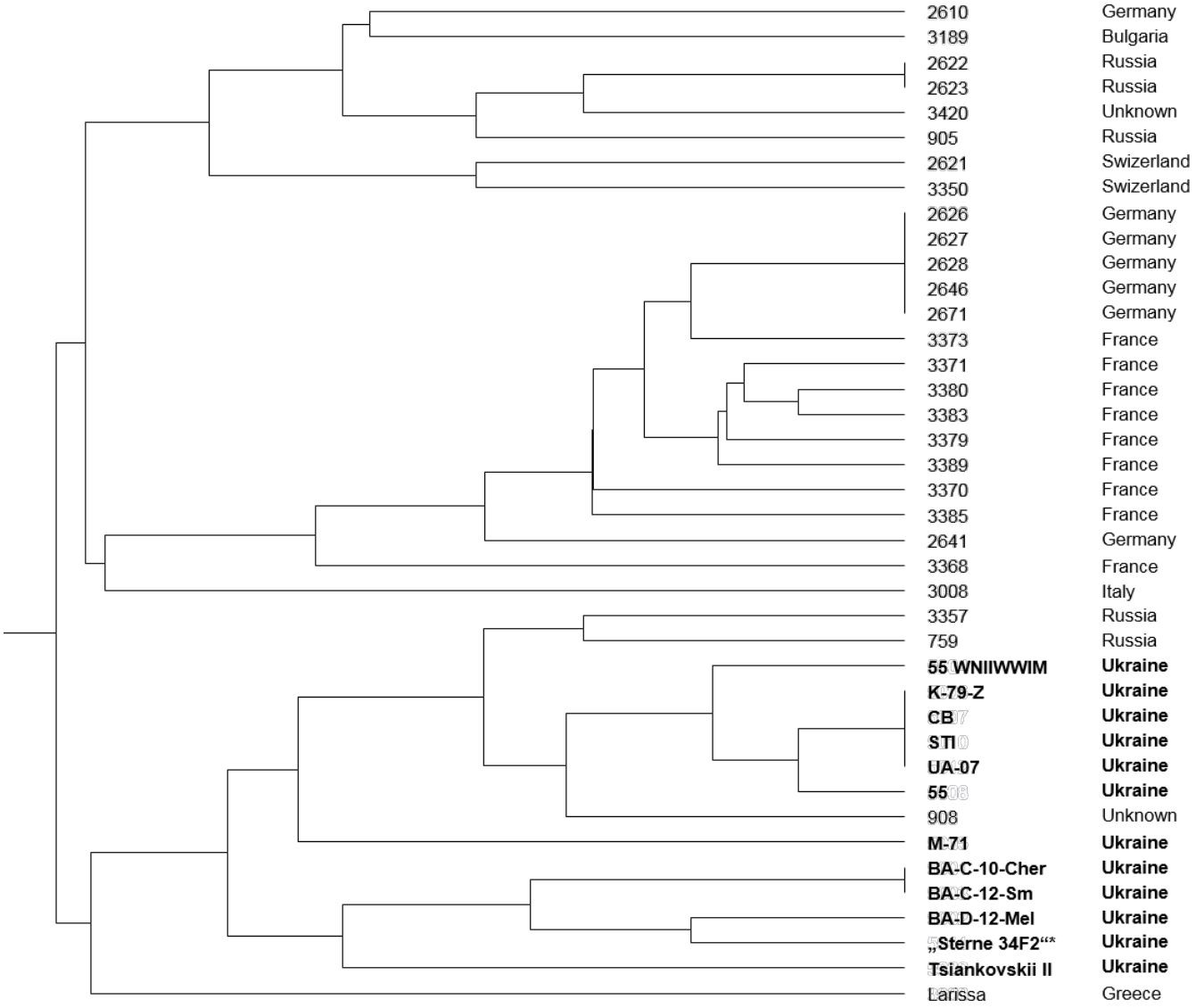

Figure 2. Dendrogram based on multi-locus variable-number tandem repeat analysis (MLVA-25) of Ukrainian and related isolates (numbers and letters) with countries of origin. A categorical coefficient was used for cluster analysis by the unweighted pair group method with arithmetic mean (UPGMA); * — incorrect name designation

The development of cheaper alternatives for interrogating canSNPs would increase access to these important phylogenetic markers for a larger number of laboratories. HRM is an attractive method, as it is simpler and cheaper than alternative approaches. HRM is a two-step, closed-tube assay that has high discriminatory power. The HRM assay requires only a few hours for each run, including follow-up data analysis, on a suitable PCR-instrument and can be performed in reaction volumes of less than 10 μl, reducing de facto the cost per analysis.

Over the recent years, significant research efforts have been undertaken to develop appropriate genotyping methods for differentiation of diverse *B. anthracis* strains. The currently available methods take advantage of tandem repeat polymorphisms or single base variations. A typing strategy relying on a combination of genetic markers that are progressively less stable but have increasing resolving power (SNP, VNTRs, including SNRs) has been recommended (Keim, 2004). In this system canSNPs typing is used to establish strain placement within broad phylogenetic groups followed by genotyping using MLVA. SNPs are evolutionary stable DNA signatures with low mutation rates (10^{-10} changes per nucleotide per generation) and only two allelic states. In addition, canSNPs typing can be applied to very low amounts of DNA, and/or degraded DNA, which can be essential in a forensic context (Birdsell et al., 2012). VNTR loci are genomic regions with higher mutational rate (ranging from $<10^{-5}$ to $>10^{-4}$ insertion-deletion mutations per generation) and typically higher numbers of possible allelic states (Keim, 2004). Notably, in contrast to canSNP-states, the number of VNTR repeats is not an indicator of

phylogenetic distance. Instead, these are just categorical differences useful for additional differentiation of strains harboring the same canSNP type.

The infrequent occurrence of anthrax in the country of Ukraine is likely caused by a heterogeneous population of *B. anthracis*. This population is phylogenetically composed of at least two different canSNP groups of the world-wide dominating A-branch of the pathogen. While one group might stem from environmental recovery of live vaccine strains used in Ukraine (or the former Soviet Union in the past) the other one, A.Br.008/009 is likely the autochthonous one that could be enzootic as indicated by the presence of related strains in countries of southeastern Europe in relatively close geographical vicinity to Ukraine.

Conclusions. The present work establishes the first preliminary picture of the genetic diversity of *B. anthracis* in Ukraine and provides valuable data sets for future epidemiological or forensic studies. This work might be a primer for a more detailed database describing the genetic landscape of *B. anthracis* diversity in Ukraine. It will then be possible to conduct future epidemiological and epizootological studies as well as for deriving bioforensic hypotheses on the origin of strains with unclear origin.

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GENOTYPING OF *FRANCISELLA TULARENSIS* ISOLATES FROM UKRAINE

Zlenko O. B. [1], Durr A. [2], Schwarz J. [2], Vydaiko N. B. [3], Gerilovych A. P. [1]

[1] National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: oksana.ceratium@gmail.com
[2] Bundeswehr Institute of Microbiology, Munich, Germany
[3] State Institution 'Public Health Center of the Ministry of Health of Ukraine', Kyiv, Ukraine

Summary. The aim of the work is to provide fingerprinting of 20 tularemia isolates collected at the territory of Ukraine and find their relationships with other typed tularemia isolates. The 20 randomly sampled *Francisella tularensis* thermolysates were collected during 1997–2016 on the territory of Ukraine. The panel included samples from 11 regions of Ukraine. Samples were collected from patients (3 samples), rodents (8 samples) and lagomorphs (1 sample), ticks (6 samples) and environment (2 samples). The genotyping and processing of data was carried out using the MLVA 12+1 method. Data were statistically processed using the method of unweighted pair group method with arithmetic mean-clustering (UPGMA). It was found that all specimens belong to the same subgenus *F. tularensis* subsp. *holarctica* and belong to three genotypes: Russian-Azerbaijan (5 samples), Czechoslovakia (1 sample) and European (14 samples) one. The European genotype is inherent in the 70% of studied samples almost in every studied year and can be named as the leading genotype that distributed on the territory of all Ukraine and is related to European genotypes of *F. tularensis*.

Keywords: MLVA, tularemia, Ukraine, UPGMA, *Francisella tularensis* subsp. *holarctica*

Introduction. Tularemia agent — *Francisella tularensis* — is a Gram-negative, facultative, bacterial pathogen that occurs naturally in lagomorphs and rodents, especially microtine rodents such as voles, vole rats and muskrats, as well as in beavers. In addition, the infection has been reported in a wide variety of other mammals, birds, amphibians and arthropods. There is a high risk of human infection with *F. tularensis*, as the infective dose is extremely low (beginning from 10 bacteria) and infected animals excrete bacteria with urine and feces. Humans can get infection through the simple contact with animals (WHO, 2007; Oyston, Sjöstedt and Titball, 2004). Thus, *Francisella tularensis* is a bacterium that could be potentially used for biological weapon development (Oyston, Sjöstedt and Titball, 2004). Tularemia is an endemic disease in most European countries. Natural foci of tularemia may exist for centuries, showing themselves as periodic disease outbreaks. That is why tularemia often becomes a public health problem, especially on the territory of former Soviet Union and USA (WHO, 2007; Oyston, Sjöstedt and Titball, 2004; Farlow et al., 2001).

The epidemiological and epizootological situation in Ukraine varied over the years and depends on the level of diagnosis and quality of the implementation of appropriate response measures. The first confirmed cases of tularemia were documented in the 1940's and were associated with occupational exposure among furriers. Then, in 1960's, tularemia was registered in the south of Ukraine where several arthropods and small mammals were recognized as vectors and hosts of the disease. The Ministry of Health of Ukraine had been reporting data on 3,086 positive isolates of *F. tularensis* from 1941–2008 collected at 1,084 locations form all regions of Ukraine. The distribution of *F. tularensis* in Ukraine is primarily connected to croplands followed by forests, grasslands (steppe), and water (Hightower et al., 2014). According to this, outbreaks occur the most often in north and south regions of Ukraine, and nearby river channels. Nowadays, the problem of tularemia infection is particularly important for uncontrolled territories in the east of the country, because of increasing density of rodent's population, how it was in Kosovo (Reintjes et al., 2002).

MLVA (Multi-locus variable number tandem repeat)-analysis of tularemia agent can determine its genotypic characteristics, as well as the frequency and distribution of individual genotypes. It is also called 'fingerprinting', and it may differentiate suspected, fast-evolving bacterial strains from an outbreak even though those strains might look the same using other methods of genotyping. This can be used for tracing back the pathways of pathogen circulation at the territory of one country and between countries and continents (Vogler et al., 2009; Johansson et al., 2004).

The aim of our work was to provide fingerprinting of 20 *F. tularensis* isolates collected at the territory of Ukraine and find their relationships with other typed tularemia isolates.

Materials and methods. The thermolysates from 20 *F. tularensis* cultures were kindly provided for MLVA-genotyping by the State Institution 'Public Health Center of the Ministry of Health of Ukraine'. All samples were collected during 1997–2016 on the territory of Ukraine. The panel included samples from 11 regions of Ukraine (Sumy, Chernihiv, Rivne, Volyn, Lviv, Poltava, Odesa, Crimea, Mykolaiv, Zaporizhia, Vinnytsia). Samples were collected by Regional Laboratory Centers from patients (3 samples), rodents (8 samples) and

lagomorphs (1 sample), ticks (6 samples) and environment (2 samples) (Table 1).

Table 1 — The list of collected samples from the territory of Ukraine

#	Strain number	Source	Date of sample collection	Region
1	222	*Mus musculus*	18.05.1999	Crimea
2	86	*Dermacentor reticulatus*	26.05.2011	Chernihiv
3	317	*Apodemus uralensis*	16.11.2010	Chernihiv
4	138/15	*Microtus arvalis* nest	28.04.1998	Mykolaiv
5	201	*Ixodes ricinus*	17.07.2006	Rivne
6	201	*Micromys minutus*	13.04.2005	Lviv
7	205/15	Bubo punctate	29.11.2000	Sumy
8	103	*Apodemus agrarius*	31.03.1999	Poltava
9	456	*Mus spicilegus*	01.02.2000	Crimea
10	21	Water from the Uday River	04.03.2003	Poltava
11	128	*Lepus europaeus* brain	05.05.1998	Odessa
12	523	Hay	29.12.1999	Chernihiv
13	562/278o	*Sorex araneus*	05.11.2008	Sumy
14	60	Unknown ticks	23.07.1997	Zaporizhia
15	351/278o	Inguinal node punctate	13.05.2005	Sumy
16	37	*Hyalomma plumbeum*	13.05.1999	Crimea
17	359/278o	Inguinal node punctate	16.09.2005	Sumy
18	493	*Myodes glareolus*	12.09.1996	Vinnytsia
19	132	*Dermacentor reticulatus*	28.06.2016	Volyn
20	66		10.06.2008	Rivne

The genotyping and processing of data was carried out at the Bundeswehr Institute of Microbiology (Munich, Germany) within the Ukrainian-German Biosafety Program using the MLVA 12+1 method (Vogler et al., 2009; Svensson et al., 2009). Genotyping was performed using the Genetic Analyzer 3130 (Applied Biosystems, USA) device. Data were statistically processed in Genemapper (Thermoficher Scientific, USA) and Bionumerics (Applied Maths, USA) programs using the method of unweighted pair group method with arithmetic mean-clustering.

Results. It was found that all specimens belong to the same subgenus *Francisella tularensis* subsp. *holarctica*, which coincides with the OIE and WHO data for the distribution of various subtypes of *F. tularensis* throughout the globe. Within this subtype, three distinct genotypes were conventionally named according to the genotypes of other related countries: Russian-Azerbaijan (5 samples), Czechoslovakia (1 sample) and European (14 samples) cluster. These clusters are emphasized with red circles (Fig. 1).

The European cluster included samples from all studied regions: Sumy, Chernihiv, Rivne, Volyn, Lviv, Poltava, Odesa, Crimea, Mykolaiv, Zaporizhia, Vinnytsia (strain numbers 222, 86, 317, 138/15, 201 (2 samples), 205/15, 103, 21, 128, 351/278o, 359/278o, 132, 66). The Russian-Azerbaijan cluster included samples from Crimea, Zaporizhia, Sumy, and Vinnytsia regions (strain numbers 456, 60, 37, 562/278o, 493). The Czechoslovakia cluster was detected in Chernihiv region and included strain number 523 (Fig. 2).

The most commonly encountered genotype related to the European cluster. This genotype was registered during the study in 1996, 1998–2000, 2003, 2005, 2006, 2008, 2010, 2011, and 2016 in all studied regions of Ukraine (Fig. 3).

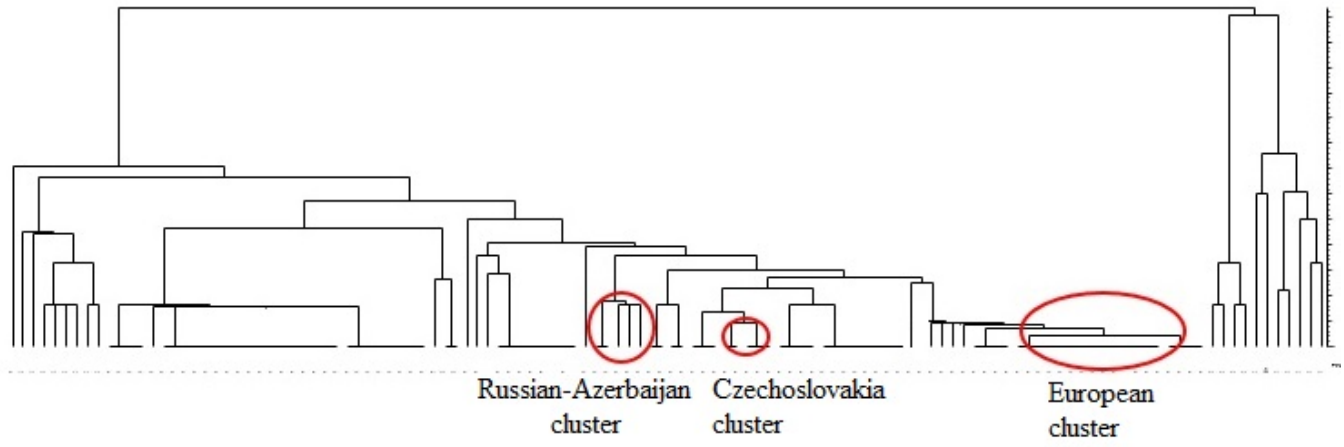

Figure 1. Clustering of the studied MLVA samples using the unweighted pair group method with arithmetic mean-clustering

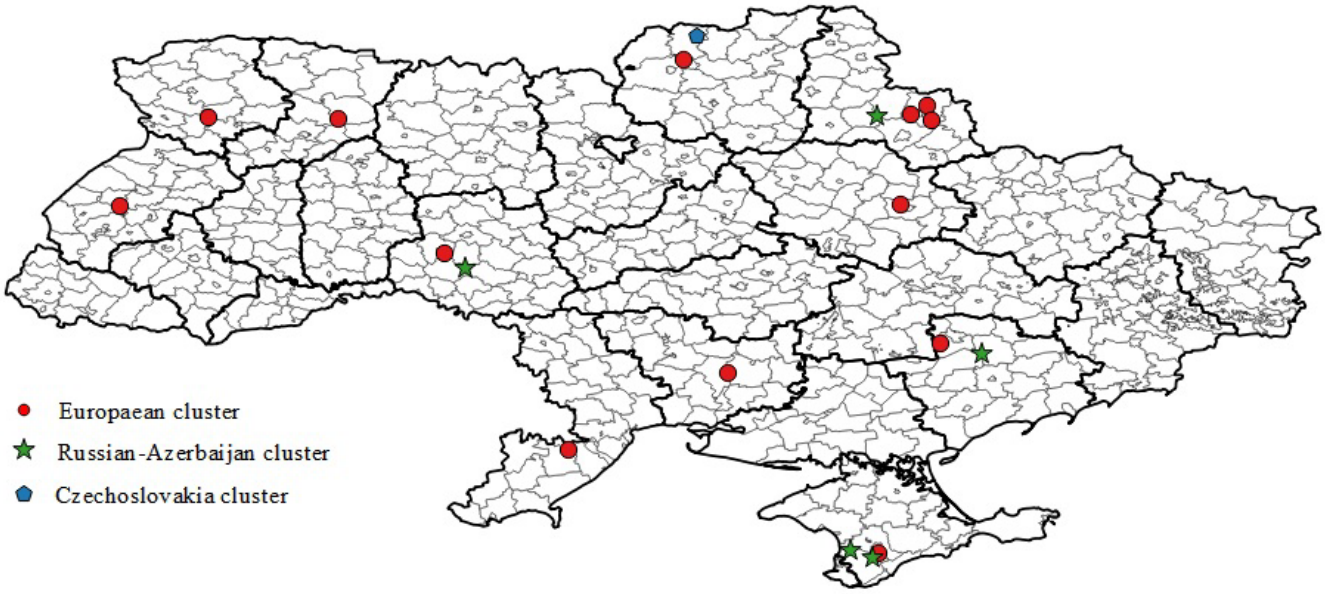

Figure 2. The geographical distribution of studied samples

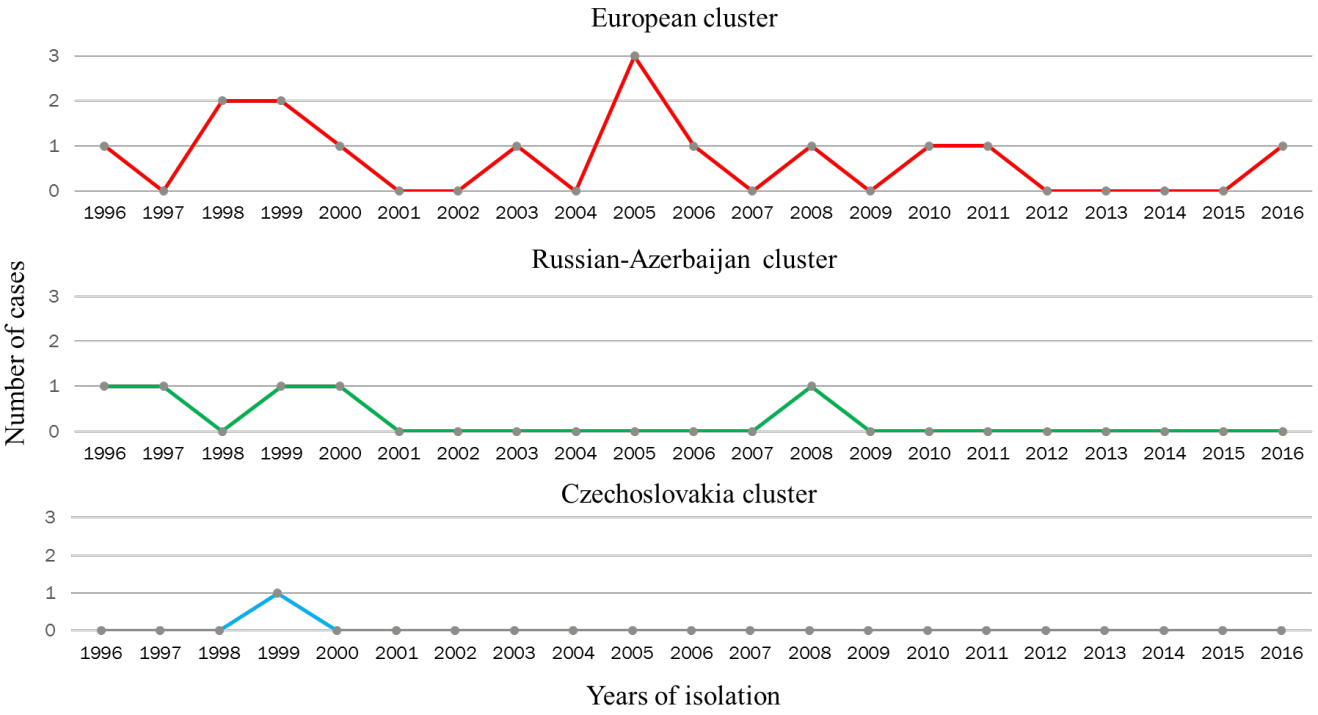

Figure 3. The cluster-distribution of studied samples according to years of isolation

It can be assumed that this European genotype is typical for both the territory of Ukraine and many European countries. The sample, which was attributed to the Czechoslovak genotype was isolated from hay in Chernihiv region and may have been accidentally brought to the country.

Conclusions. The studied panel included 20 samples that were randomly chosen for MLVA-analysis. This panel is quite small, but it includes samples collected during different years from different regions of Ukraine that makes possible to proceed data in time and space. Considering these output conditions, singling out the European genotype that inherent in the 70% of studied samples almost in every studied year can say that we found the leading genotype that distributed on the territory of all Ukraine and is related to European genotypes of *Francisella tularensis*. Other genotypes need studies in larger panels for more correct traceability and conclusions.

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OCCURRENCE OF DERMATOPHYTOSES AMONG STRAY DOGS AND CATS IN THE CITY OF KHARKIV

Ponomarenko G. V. [1], Kovalenko V. L. [2], Ponomarenko O. V. [1], Severyn R. V. [1], Bulavina V. S. [1], Shapovalova Yu. G. [3], Laptiy O. P. [3]

[1] Kharkiv State Zooveterinary Academy, Kharkiv, Ukraine, e-mail: gpkh1966@gmail.com
[2] State Scientific Control Institute of Biotechnology and Strains of Microorganisms, Kyiv, Ukraine, e-mail: kovalenkodoktor@gmail.com
[3] Municipal Enterprise 'Animal Care Center', Kharkiv, Ukraine, e-mail: pochta@animals.kharkov.ua

Summary. Spreading of zooanthroponoses in big cities of Ukraine and other countries is connected with the existence of certain group of animals which are sensitive to these diseases. It concerns, first of all, stray dogs and cats which might both be infected and carry infectious agents common for animals and humans. Dermatophytoses are one of the most common zooanthoponotic diseases among stray animals. The goal of this work is to monitor the occurrence of dermatophytoses among stray dogs and cats in the city of Kharkiv. We took into account clinical and epizootic data, as well as results of common microscopic and mycological laboratory studies when diagnosing the dermatophytoses in homeless animals.

Studying 723 dogs and 85 cats with clinical signs of skin and fur lesions, we isolated cultures of dermatophytic fungi from 96 dogs (13.3% of animals with skin lesions) and 33 cats (38.8%). The share of pathogenic dermatophytic fungi made 33.4% among dogs with skin and fur lesions and 45.4% among sick cats.

The obtained data proves the prevalence of dermatophytoses among stray dogs in Kharkiv and encourages the need of further improvement of eradicative and preventive measures against animal infectious skin diseases.

Keywords: dermatophytoses, infectious agents, stray animals, dogs and cats

Introduction. Spreading of infectious diseases, primarily zooanthroponoses, in big cities of Ukraine and other countries connected with the existence of certain group of animals which are sensitive to these diseases. It concerns, first of all, stray dogs and cats which might both be infected and carry infectious agents common for animals and humans (Capelli et al., 2006; Ponomarenko, Fedorova and Bulavina, 2009; Jittapalapong et al., 2009; Awadallah and Salem, 2015; Ponomarenko, 2017b).

According to veterinary specialists and researchers which carry out monitoring of animal contagious diseases in human settlements in Ukraine, the highest level of infectious and parasitic diseases is registered among homeless (Ponomarenko et al., 2008; Soroka and Dakhno, 2010; Ponomarenko et al., 2013; Kornyushin, Malyshko, and Malega, 2013; Ponomarenko, 2017a). It is connected with insufficient control of animals' population, the absence of proper veterinary service for them, namely vaccination against infectious diseases and appropriate antiparasitic treatment and the prevalence of asymptomatic or latent forms of diseases et al.

Dermatophytoses take a special place among skin disorders of small domestic animals. These diseases are the most common mycotic infections in the world (Kovalenko et al., 2017). They do not cause significant mortality but they cause high level of infection both in animals and in humans.

The presence of clinically healthy animals which carry pathogenic dermatophytic fungi is the important epidemiological hazard. Fungi carriers pose a constant threat of human and animal infection and contaminate environmental objects with dermatophytic spores (Manoyan, Ovchinnikov and Panin, 2012; Kovalenko et al., 2015; Morozova, Severin and Ponomarenko, 2015).

The aim of the study. Relying on the relevance of this issue, the goal of our studies was to monitor the occurrence of dermatophytoses among stray dogs and cats in the city of Kharkiv

Materials and methods. Studies, their analysis and generalization were conducted in 2012–2017 at Municipal Enterprise 'Animal Care Center' as well as at the P. I. Verbytskyi Educational and Research Laboratory of Molecular and Genetic Studies of the Department of Epizootology and Veterinary Management in the Kharkiv State Zooveterinary Academy.

We took into account clinical, epizootic, microscopic and mycological laboratory studies when diagnosing the dermatophytoses. Clinically, we took into account typical lesions on the surface of skin and fur cover both in positive and in doubtful or negative clinical results.

To study cultural and morphological characteristics of isolated cultures, we used selective media, such as Saburo agar, Wort agar, Chapek's agar, meat peptone glycerol and nutrient agar. Seedings were cultivated in incubator at 28–30 °C throughout 20–30 days. Isolated cultures of dermatophytic fungi were identified using common techniques (Kovalenko et al., 2017; Sutton et al., 1998).

Results. In 2012–2017, we clinically studied 17,138 dogs and 2,820 cats from Municipal Enterprise 'Animal Care Center' (Kharkiv, Ukraine) in frames of

city's program for treatment of stray animals and control of their population. As a result, we detected 723 dogs (4.2% out of all studied animals) and 85 cats (3.0%) with clinical signs of skin and fur lesions (Table 1).

Table 1 — Dermatophytosis morbidity dynamics of stray animals in the city of Kharkiv

Year	Number of studied animals		Number of animals with skin lesions, %		Number of sick animals confirmed, %	
	dogs	cats	dogs	cats	dogs	cats
2012	3,622	217	152 (4.2)	7 (3.2)	24 (15.8)	3 (42.9)
2013	3,580	324	167 (4.7)	12 (3.7)	16 (9.6)	5 (41.7)
2014	3,124	178	123 (3.9)	6 (3.4)	14 (11.4)	2 (33.3)
2015	2,760	237	111 (4.0)	9 (3.8)	21 (18.9)	3 (33.3)
2016	2,138	985	98 (4.6)	27 (2.7)	10 (10.2)	11 (40.7)
2017	1,914	879	72 (3.8)	24 (2.7)	11 (15.3)	9 (37.5)
Total	17,138	2,820	723 (4.2)	85 (3.0)	96 (13.3)	33 (38.8)

As a result of microscopic and mycologic studies of biological samples from these animals, dermatophytosis was laboratory confirmed in 96 dogs, or 13.3% of all animals with skin lesions. Cultures of dermatophytic fungi were isolated from samples from 33 cats (38.8% of animals with lesion of skin surface). The level incidence rate in dogs explicitly valued from 9.6% in 2013 to 18.9% in 2015. Fluctuations in the morbidity rate in cats were less obvious — from 33.3% in 2014–2015 to 42.9% in 2012.

Both pathogenic dermatophytes and mold yeast-like fungi cultures were isolated from stray dogs and cats (Table 2).

Table 2 — Results of cultural and morphological identification of isolated dermatophytic fungi cultures

Culture	Dogs		Cats	
	Total number	%	Total number	%
Microsporum canis	18	18.8	15	45.4
Trichophyton mentagrophites	14	14.6	—	—
Malassezia pachydermatis	8	8.3	3	9.1
Candida albicans	7	7.3	3	9.1
Alternaria alternata	27	28.1	8	24.2
Aspergillus fumiqatus	10	10.4	2	6.1
Mucor	12	12.5	2	6.1
Total	96	100.0	33	100.0

As a result, the share of pathogenic dermatophytic cultures isolated from stray animals was 33.4% from total number of fungal cultures. Significant difference was noted between percentage of *Microsporum canis* cultures isolated from sick dogs (18.8%) and cultures from infected cats (45.4%).

The share of yeast-like fungi isolated from dogs was 15.6%, and the share of similar cultures from cats was 18.2%.

At the same time, the share of mold fungi was 51.0% with 28.1% of *Alternaria alternata* cultures in dogs. In 24.2% of cases *Alternaria alternata* were isolated from cats. The percentage of other mold fungi cultures was two times less — 12.2%.

Conclusions. Studying 723 dogs and 85 cats with clinical signs of skin and fur lesions, we isolated cultures of dermatophytic fungi from 96 dogs (13.3% of animals with skin lesions) and 33 cats (38.8%).

Cultures of pathogenic dermatophytic fungi were isolated in 33.4% of cases when studying dogs with skin and fur lesions and 45.4% among sick cats.

The share of pathogenic dermatophytic fungi made 33.4% among dogs with skin and fur lesions and 45.4% among sick cats.

The obtained data proves the prevalence of dermatophytoses among stray dogs in Kharkiv and encourages the need of further improvement of eradicative and preventive measures against animal infectious skin diseases.

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Part 2. Biology and biotechnology

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BIOPHYSICAL MODEL OF BIOLOGICAL CELL CONDUCTIVITY BASED ON THE MEMBRANE ELECTROPORATION PROBABILITY

Shigimaga V. O. [1], Paliy And. P. [1], Pankova O. V. [1], Paliy Anat. P. [2]

[1] Kharkiv Petro Vasylenko National Technical University of Agriculture, Kharkiv, Ukraine, e-mail: biovidoc@gmail.com
[2] National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: paliy.dok@gmail.com

Summary. The membrane electroporation of a biological cell was well known as a convenient, multipurpose and universal way of temporarily increasing its permeability in a pulsed electric field (PEF) with certain parameters. The process and result of the membrane interaction with the PEF is greatly influenced by its heterogeneous biological structure, which has both native pores of various sizes and various protein inclusions. This leads to heterogeneity of the electrophysical properties. All this ultimately affects the cellular conduction in the PEF, which is both an indicator and an integral characteristic of the electroporation process of the membrane as a whole. This process can be modelled, considering the physical properties of the membrane and the cells, as conductors of the pulsed current. However, to consider in modelling all the features of the native structure of the membrane pores, as well as newly formed electropores as a result of interaction with the external PEF is impossible. However, if we apply a probabilistic approach to the formation of electropores, it becomes possible to construct an adequate model of electroporation.

In this article is presented the developed biophysical (BP) model of cell conductivity, constructed on the basis of the electropores formation probability in a membrane under the influence of a pulse electric field (PEF). The model assumes that in membrane are formed electropores of different calibers, the distribution of which submits to normal Gauss's law. The integral for the total conductivity of the electroporated membrane is obtained using the integral equation for the total current through the electropore membrane and the equation for its conductivity, including the formation of the electropore probability function. The general view of the electropore formation probability function is received by solution of Fokker-Planck's differential equation. Substitution of this equation solution to conductivity integral gave the general view of the conductivity function connecting it with electropore caliber. A comparison of the constructed probability electroporation BP model with experimental data on mice oocyte conductivity showed that the main reason for exponential increase of cell conductivity in increasing electrical field strength is similar nature of conductivity increase with increasing electropore caliber up to membrane breakdown. The constructed probability BP model of cell conductivity at membrane electroporation in increasing PEF agrees with the experimental data.

Keywords: pulse electric field, increasing strength, electropore caliber, cell membrane, Gauss's law, conductivity integral

Introduction. The pulsed electric field (PEF) is widely used in the latest biotechnologies for electromanipulation with cells, which underlies modern methods of cellular, genetic engineering and biomedicine (Miklavčič, 2017; Rems and Miklavčič, 2016; Yarmush et al., 2014; Shigimaga, 2014, 2015). The main method of electromanipulation is electroporation — an adequate effect on the transport function of the cell membrane by temporarily increasing its permeability due to the formation of an electropores in the PEF. The PEF is formed by special equipment in a liquid medium with cells (Dermol-Cerne and Miklavčič, 2018; Hoiles, Krishnamurthy and Cornell, 2018; Shigimaga, 2013a,b, 2017). From the point of view on physical impact, the events occurring in the membrane during and after application of the external field initially develop on the basis of strong electrical interaction with the membrane, and then, probably, on the basis of the pressure. In addition, this pressure arises as a result of electrodiffusion under the field forces (Miklavčič, 2017; Chang et al., 1991; Smith and Weaver, 2012). However, despite the generalizing models of this process based on artificial membranes are modeled, the mechanism of the biological membrane electroporation is still not completely clear. It is supposed that the following fact is established: an external PEF, affecting the cell, changes the electrochemical potential on both sides of the membrane, and as a result it disrupts the phospholipid double layer. (Gurtovenko and Lyulina, 2014; Fernández, Risk and Vernier, 2018; Neu and Neu, 2009; Luitel, Schroeter and Powell, 2007; Mahnič-Kalamiza, Miklavčič and Vorobiev, 2014; Kotnik et al., 2012). This leads to the separation and

accumulation of charges on the membrane between the cytoplasm and the external medium. It is expressed in an increase in the transmembrane potential (TMP). Thereby it induces a temporary instability in the polarized lipid bilayer (Fernández, Risk and Vernier, 2018; Fuertes et al., 2011; Towhidi et al., 2008; Kotnik, Pucihar and Miklavčič, 2010; Kotnik et al., 2012; Morshed, Shams and Mussivand, 2013; Polak et al., 2014). Consequently, the unstable state of the membrane starts up a gradual change in its shape to a more energetic advantageous shape by forming perforating channels of different sizes. They are realized by nanoscale pores across the membrane (Böckmann et al., 2008; Kotnik, Pucihar and Miklavčič, 2010; Kotnik et al., 2012; Wang et al., 2010). This phenomenon is defined by the term 'electroporation'. Thus, the main process that characterizes electroporation as a biophysical phenomenon is the formation of electropores in the membrane.

In view of the complexity of the biological systems structure at any level, including the cellular system, an attempt to unambiguously describe them within the bounds of one or even several BP models to explain the effects of membrane electroporation in the PEF is doomed to failure in advance (Shigimaga, 2014). Great number of such models were made (Son et al., 2014; Neu and Neu, 2009; Miklavčič and Towhidi, 2010; Mahnič-Kalamiza, Miklavčič and Vorobiev, 2014; Kotnik et al., 2012; Miklavčič, 2012; Polak et al., 2014; Shigimaga and Megel', 2012; Shigimaga, 2013a,b; Morshed, Shams and Mussivand, 2013; Smith and Weaver, 2012) and all of them in a varying degree have described the effects of PEF on the membrane of a real biological cell. To model the electroporation of membranes in the PEF of simple biological cells, as an example are usually preferred the denuclearized erythrocytes. They are very simple in structure, a huge number of researches is devoted to them, and therefore artificial bilipid membranes (BLM) are predictable by physical properties or abstract spherical cells (Hoiles, Krishnamurthy and Cornell, 2018; Smith and Weaver, 2012; Hoiles et al., 2014; Polak et al., 2014; Pavlin et al., 2008; Miklavčič, 2012, 2017; Morshed, Shams and Mussivand, 2013). Reproductive cells of animals and, especially, multicellular embryos, which are much more complex in structure and composition, are very difficult as bioobjects, for BP modeling of electrical conductivity. Therefore, the modeling of the conductive properties of these bioobjects is practically unknown, except several recent works (Miklavčič, 2017; Shigimaga, 2013a,b, 2014; Shigimaga et al., 2017).

The application of the electroporation method requires the justification of certain electrical treatment regimes in the PEF of living biological cells so that they keep their functional for further use, in particular, in animal reproduction biotechnology. The value parameters of these regimes are determined, first of all, by the electrical characteristics of the membrane, the cell, and the liquid medium. These characteristics can be obtained experimentally during measurements of the conductivity of a biological cell using the method and equipment of pulsed conductometry in a variable PEF (Smolyaninova, Shigimaga, and Kolesnikova, 2009; Smolyaninova et al., 2014; Kolesnikova, Shigimaga and Smolyaninova, 2013; Shigimaga, Levkin and Megel, 2011; Shigimaga and Megel', 2011a,b, 2012; Shigimaga, 2013a,b, 2014, 2017). The variable field strength provides a different degree of the membrane electroporation and therefore makes it possible to justify and calculate all the necessary modes of exposure on the varying of the cell rely conductivity. This allows not only to realize well-known applications but also to develop new applications of electroporation in the biotechnology of animal reproduction and biomedicine within the framework of a single hardware -methodical process of pulsed conductometry (Shigimaga, 2015). On the other hand, the justification of the PEF parameters during the process of pulsed conductometry requires a comprehension of the physical mechanism of the electropores formation (which are available in many calibers) with increasing field strength. Disclosure of this mechanism is a bit of possible with the help of the BP modeling of the membrane electroporation process which was considered below, and using the known physical laws of the electric current flow through it and also various mathematical methods and approaches.

Material and methods. Electroporation BP modeling should be started with consideration of integrated characteristic of electropore formation — the free energy describing mechanical and electric contribution of forces at electroporation (den Otter, 2009; Chang et al., 1991; Böckmann et al., 2008). The electropore is thus approximated by the round cylinder of radius (*r*) and the height (*h*) equal to membrane thickness (Fig. 1).

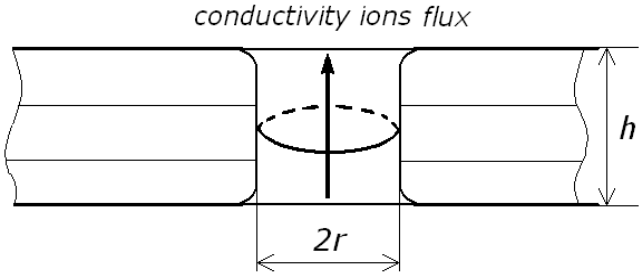

Figure 1. Electropore approximation by the cylinder on a membrane surface

Free electropore energy represents generally by the sum:

$$\Delta E = \Delta E_m + \Delta E_e + E_o \quad (1)$$

where ΔE_m — mechanical component, ΔE_e — electric component, E_o — constant.

The mechanical component of electropore energy is defined by the formula (Böckmann et al. 2008):

$$\Delta E_m = \pi\left(2 E_p r - E_{mw} r^2\right) \quad (2)$$

where E_p — linear energy density of pore edge, E_{mw} — surface energy density of interaction at membrane-water interface.

It is known that mechanical breakdown of flat membrane is reached, when one or several electropores exceeds critical radius $r > r_c$, where $r_c = E_p / E_{mw}$ (Chang et al., 1991; Böckmann et al., 2008). Considering that cell radius exceeds electropore radius by 4–5 orders of magnitude, it is possible to neglect curvature of membrane surface in a pore vicinity and consider it as flat surface.

The electric component of energy (1) represents electropore as the condenser with some conductivity leak (Chang et al., 1991):

$$\Delta E_e = -\frac{\pi(\varepsilon_w - \varepsilon_l)U^2}{h^2}\int_{r_{min}}^{r} g^2 r\, dr \qquad (3)$$

where ε_w and ε_l — dielectric permeability of water and membrane lipids respectively, $_U$ — transmembrane potential (TMP), $g(r)$ — the function considering effect of external voltage distribution, connected with resistance distribution on the boundary (with solution) and within a pore. This function can be written as follows (Chang et al., 1991):

$$g(r) = \left[\frac{2hG_s}{2hG_s + \pi r G_p(r)}\right] \qquad (4)$$

where G_p and G_s — conductivity within a pore and solution at pore entrance respectively.

The effect of voltage distribution has following physical explanation. There is a non-uniform electric field in the solution around pore entrance. The related voltage fall is estimated by introduction of resistor with the resistance R_s which value is set by formula (Chang et al., 1991):

$$R_s \approx \frac{1}{2G_s r} \qquad (5)$$

Respectively, the internal resistance of a pore R_p is estimated through its conductivity G_p (Chang et al., 1991):

$$R_p = \frac{h}{\pi r^2 G_p} \qquad (6)$$

These resistances R_s and R_p are included in series, forming a voltage divider, leading to TMP reduction by the value of R_s. Considering these resistances, it is possible to write down a current through an electropore, according to the Ohm's law as:

$$I_p = \frac{U}{R_s(r) + R_p(r)} \qquad (7)$$

For calculation of the total current through a surface of the membrane, which electroporated by PEF force, we will introduce the concept of probability formation density of electropore $n(r,t)$, as a function depending on radius (caliber) of electropore and time. The process of the electropore evolution represents a combination of various physical forces (1), therefore electropores of different radii are present in a membrane (Talele and Gaynor, 2010). Radii distribution can be described by density function $p(r)$. It is supposed that the number of electropores $w(t)$ changes in time due to their appearance and reparation having radius r_{min}. From these assumptions it follows that radii and time distribution are two independent functions. Therefore, it is possible to write down a total density of an electropore formation probability as a function of their product (Shigimaga, 2014):

$$n(r,t) = p(r)w(t) \qquad (8)$$

Having defined electropore formation probability density, it is possible to write down the equation for total current through membrane, using (Chang et al., 1991):

$$I(r,t) = U\int_{r_{min}}^{r}\left[\frac{p(r)w(t)}{R_s(r) + R_p(r)}\right] dr \qquad (9)$$

From equation (9), using ratios (5) and (6), the equation for total conductivity of electropoated membrane becomes (conductivity integral):

$$G(r,t) = \int_{r_{min}}^{r}\left[\frac{p(r)w(t)}{R_s(r) + R_p(r)}\right] dr = \\ = 2\pi\int_{r_{min}}^{r}\left[\frac{G_s G_p r^2}{2hG_s + \pi r G_p} p(r)w(t)\right] dr \qquad (10)$$

Thus, the solution of the equation (10), including finding of function type $n(r,t)$, will results in probability BP model of cell conductivity.

Results. For defining the general view of electropore formation probability density function $n(r,t)$ it is possible to use the differential equation, deduced on the basis of the Fokker-Planck's equation (Kamenshchikov, 2014; Risken, 1996):

$$\frac{\partial n}{\partial t} = D_p\left[\frac{\partial^2 n}{\partial r^2} + \frac{1}{kT}\frac{\partial}{\partial r}\left(n\frac{\partial \Delta E}{\partial r}\right)\right] \qquad (11)$$

where D_p — an effective diffusion constant for a pore of radius *r*.

The value ΔE was taken in the form of (1), and in the equation (11) has crucial importance as $(\partial \Delta E/\partial r)_U$ is the effective force changing of electropore radius.

Further, the volume conductivity of solution is a function of concentration and mobility of the ions (Chang et al., 1991):

$$G_s = \sum_{i=1}^{n}(z_i e)^2 \alpha_i C_i \qquad (12)$$

where z_i — charge, α_i — mobility and C_i — concentration of ions, $e = 1{,}6 \cdot 10^{-19}$ K (electron charge).

It is assumed that the transport of ions through the membrane occurs by passing them through a pore which is large enough to contain hydrated ions, for example Na^+ and Cl^-. However, the presence of ions in small pores

requires considering the effect that inhibits their movement (Smith and Weaver, 2012; Ziegler and Vernier, 2008; Chang et al., 1991).

Thus, opposite to the solution, the volume conductivity in a pore is reduced:

$$G_p = \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i H_i \exp\left(\frac{\mu_i^o}{kT}\right) \qquad (13)$$

where μ_i^o — standard chemical potential of the i-th ion within pore, $\mu_i^o = \frac{(z_i e)^2}{\varepsilon_l} P\left(\frac{\varepsilon_l}{\varepsilon_w}\right)$ moreover, the value has a maximum value of 0.25, H_i – a hindrance factor of ion movement in a pore — the Renkin function (Chang et al., 1991):

$$H_i = H(r, r_i) = \left[1 - \left(\frac{r_i}{r}\right)\right]^2 \left[1 - 2{,}1\left(\frac{r_i}{r}\right) + 2{,}09\left(\frac{r_i}{r}\right)^3 - 0{,}95\left(\frac{r_i}{r}\right)^5\right] \qquad (14)$$

Simplifying further cumbersome calculations with the expression (14), by the way of restricting the summands of summits by a small value, we will assume that the Renkin function can be written in the form:

$$H(r, r_i) = 1 - 4{,}1\left(\frac{r_i}{r}\right) + \overline{O}\left[\left(\frac{r_i}{r}\right)^2\right] \qquad (15)$$

Substituting function (15) in the equation (13), we receive the expression for conductivity within a pore:

$$G_p = \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i \left\{1 - 4{,}1\left(\frac{r_i}{r}\right) + \overline{O}\left[\left(\frac{r_i}{r}\right)^2\right]\right\} \exp\left(\frac{\mu_i^o}{kT}\right) = \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i \left[1 - 4{,}1\left(\frac{r_i}{r}\right)\right] \exp\left(\frac{\mu_i^o}{kT}\right) + \overline{O}\left[\left(\frac{r_i}{r}\right)^2\right] \qquad (16)$$

Substituting (4), (12) and (13) in equation (3) we will get the electric component of the energy:

$$\Delta E_e = -\frac{\pi(\varepsilon_w - \varepsilon_l) U^2}{h^2} \int_{r_{min}}^{r} \left[\frac{2h G_s}{2h G_s + \pi r G_p(r)}\right]^2 r\, dr = -\frac{\pi(\varepsilon_w - \varepsilon_l) U^2}{h^2} \int_{r_{min}}^{r} \left[\frac{2h \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i}{2h \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i + \pi r \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i \left[1 - 4{,}1\left(\frac{r_i}{r}\right)\right] b_i}\right]^2 r dr =$$

$$= -\frac{\pi(\varepsilon_w - \varepsilon_l) U^2}{h^2} \int_{r_{min}}^{r} \left[\frac{2h}{2h + \pi r \sum_{i=1}^{n} \left[1 - 4{,}1\left(\frac{r_i}{r}\right)\right] b_i}\right]^2 r dr \qquad (17)$$

Taking the constants behind the integral sign (17) and introducing the symbol:

$$K = -4\pi(\varepsilon_w - \varepsilon_l) U^2 \qquad (18)$$

we obtain an integral with a variable upper limit, which is calculated as follows:

$$K \int_{r_{min}}^{r} \left[\frac{1}{2h + \pi r \sum_{i=1}^{n} \left[1 - 4{,}1\left(\frac{r_i}{r}\right)\right] b_i}\right]^2 r dr =$$

$$= K \int_{r_{min}}^{r} \left[\frac{r}{\sum_{i=1}^{n} \frac{4h^2}{(\pi b_i)^2} + \sum_{i=1}^{n} \left[16{,}8 (r_i)^2 - 16{,}4\left(\frac{h r_i}{\pi b_i}\right)\right] + r^2 + \sum_{i=1}^{n} \left(\frac{4h}{\pi b_i} - 8{,}2 r_i\right) r}\right]^2 dr =$$

$$= K \int_{r_{min}}^{r} \frac{\frac{1}{2} d(r^2) + d\left[r \sum_{i=1}^{n} \left(\frac{4h}{\pi b_i} - 8{,}2 r_i\right)\right] - d\left[r \sum_{i=1}^{n} \left(\frac{4h}{\pi b_i} - 8{,}2 r_i\right)\right]}{r^2 + r \sum_{i=1}^{n} \left(\frac{4h}{\pi b_i} - 8{,}2 r_i\right) + \sum_{i=1}^{n} \left[\left(\frac{2h}{\pi b_i}\right)^2 + 16{,}8 (r_i)^2 - 16{,}4\left(\frac{r_i}{\pi b_i}\right)\right]} =$$

$$= K \left[ln\left(\frac{r + \sum_{i=1}^{n} \left(\frac{2h}{\pi b_i} - 4{,}1 r_i\right)}{r_{min} + \sum_{i=1}^{n} \left(\frac{2h}{\pi b_i} - 4{,}1 r_i\right)}\right)^2 - \sum_{i=1}^{n} \left(\frac{4h}{\pi b_i} - 8{,}2 r_i\right) ln\left(\frac{r + \sum_{i=1}^{n} \left(\frac{2h}{\pi b_i} - 4{,}1 r_i\right)}{r_{min} + \sum_{i=1}^{n} \left(\frac{2h}{\pi b_i} - 4{,}1 r_i\right)}\right)^2\right] \qquad (19)$$

Considering expression (19), we obtain the electric component of the energy formation of the electric pore:

$$\Delta E_e = -4\pi(\varepsilon_w - \varepsilon_l)U^2 ln\left(\frac{r + \frac{2h}{\pi b_i} - 4{,}1r_i}{r_{min} + \frac{2h}{\pi b_i} - 4{,}1r_i}\right)^2 \cdot \left[1 - \left(\frac{4h}{\pi b_i} - 8{,}2r_i\right)\right] \quad (20)$$

where $b_i := exp\left(\frac{\mu_i^o}{kT}\right)$.

Considering electropore formation probability density again, we will notice that at reversible electroporation of membrane in PEF the number of formed electropores is of an order $\sim 10^4$–10^5 (Krassowska and Filev, 2007). Therefore, it is possible to argue safely that the radii distribution of electropore at any moment of their evolution follows the Gauss's law. Therefore, it is possible to introduce the corresponding probability density for $p(r)$:

$$n(r,t) = p(r)w(t) = \frac{1}{\sqrt{2\pi}} exp\left(-\frac{r^2}{2}\right) w(t) \quad (21)$$

where $p(r) = \frac{1}{\sqrt{2\pi}} exp\left(-\frac{r^2}{2}\right)$ — density by Gauss's law.

Substituting ratios (1), (2), (20), (21) in the equation (11), we receive the differential equation with divided variables:

$$\frac{\partial n}{\partial t} = A(r)w(t) \quad (22)$$

where $A(r)$ — designation of the function part depending on pore radius:

$$A(r) = D_p \left\langle \frac{1}{\sqrt{2\pi}} exp\left(-\frac{r^2}{2}\right)\left(r^2 - 1\right) + \frac{1}{kT}\left\{\frac{-r}{\sqrt{2\pi}} exp\left(-\frac{r^2}{2}\right) \cdot \right.\right.$$

$$\cdot 2\pi \left(\left(E_p - E_{mw} r\right) - 2(\varepsilon_w - \varepsilon_l)U^2 \left[\frac{1}{2h + \pi r \sum_{i=1}^{n}\left[1 - 4{,}1\left(\frac{r_i}{r}\right)\right]b_i}\right]^2 r \right) +$$

$$+ \frac{1}{\sqrt{2\pi}} exp\left(-\frac{r^2}{2}\right) \cdot \left[-2\pi E_{mw} - 4\pi(\varepsilon_w - \varepsilon_l)U^2 \cdot \left(\frac{-2\pi r}{\left(2h + \pi r \sum_{i=1}^{n}\left[1 - 4{,}1\left(\frac{r_i}{r}\right)\right]b_i\right)^3} \sum_{i=1}^{n} b_i + \right.\right.$$

$$\left.\left.\left. + \left[\frac{1}{2h + \pi r \sum_{i=1}^{n}\left[1 - 4{,}1\left(\frac{r_i}{r}\right)\right]b_i}\right]^2 \right) \right] \right\} \Bigg\rangle \quad (23)$$

The solution of the equation (22) looks like:

$$w(t) = exp\left[A(r)t\right] \quad (24)$$

Thus, the function $n(r,t)$ from expression (21) combined with the solution of (24) will become:

$$n(r,t) = \frac{1}{\sqrt{2\pi}} exp\left[A(r)t - \frac{r^2}{2}\right] \quad (25)$$

To get the total membrane conductivity final function type, we will substitute the obtained probability (25) to the conductivity integral (10), consistently considering expressions (5), (6), (12), (13), (20).

Afterward the integration is performed:

$$G(r,t) = \int_{r_{min}}^{r} \left[\frac{\frac{1}{\sqrt{2\pi}} exp\left[A(r)t - \frac{r^2}{2}\right]}{\frac{1}{2G_s r} + \frac{h}{\pi r^2 G_p}}\right] dr = \sqrt{2\pi} \int_{r_{min}}^{r} \left[\frac{G_s G_p \, exp\left[A(r)t - \frac{r^2}{2}\right] r^2}{\pi r G_p + 2G_s h}\right] dr =$$

$$= \sqrt{2\pi} \int_{r_{min}}^{r} \frac{\sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i \cdot \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i \left[1 - 4{,}1\left(\frac{r_i}{r}\right)\right] b_i \left\{ exp\left[A(r)t - \frac{r^2}{2} \right] r^2 \right\}}{\pi r \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i \left[1 - 4{,}1\left(\frac{r_i}{r}\right)\right] b_i + 2h \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i} dr =$$

$$= \sqrt{2\pi} \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i b_i \int_{r_{min}}^{r} \frac{\sum_{i=1}^{n} \left[1 - 4{,}1\left(\frac{r_i}{r}\right)\right] \cdot \left\{ exp\left[A(r)t - \frac{r^2}{2} \right] r^2 \right\}}{\pi r \sum_{i=1}^{n} \left[1 - 4{,}1\left(\frac{r_i}{r}\right)\right] b_i + 2h} dr =$$

$$= 2\pi h \left(exp\left(A(r) - \frac{r^2}{2} \right) exp\left(\frac{2\pi E_p D_p}{kT} t \right) \right) \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i +$$

$$+ \left(\exp\left(A(r) - \frac{r^2}{2} \right) \right) \frac{1}{t} \left(\frac{\frac{1}{\pi} - \frac{2{,}05}{h} \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i r_i b_i - 8{,}2\pi \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i r_i b_i}{2\sqrt{\sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i (2h - 4{,}1\pi)}} \right) \cdot$$

$$\cdot \left[\frac{ln\left| r - \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i (2h - 4{,}1\pi) \right|}{ln\left| r + \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i (2h - 4{,}1\pi) \right|} - \frac{ln\left| r_{min} - \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i (2h - 4{,}1\pi) \right|}{ln\left| r_{min} + \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i (2h - 4{,}1\pi) \right|} \right] \quad (26)$$

In the formula (26) the following estimate was used:

$$exp\left[A(r) - \frac{r^2}{2} \right] \le exp\left(- \frac{2\pi E_p D_p}{kT} \right) \quad (27)$$

The resulting formula (26) can be considerably simplified by replacing complex algebraic expressions, that do not contain variables, by some constants K_i, where i = 1, 2, 3, 4, to the following form:

$$G(r,t) = exp\left(A(r) - \frac{r^2}{2} \right) \left\{ exp\left(\frac{2\pi E_p D_p}{kT} t \right) \cdot K_1 + \frac{K_2}{2t\sqrt{K_3}} \cdot \left[\frac{ln|r - K_3|}{ln|r + K_3|} - K_4 \right] \right\} \quad (28)$$

Discussion. To find the characteristic parameters that determine the state of the membrane and its integrity, it is possible to make several theoretical models based on the approximation of the experimental dependencies on different functions. These parameters are chosen due to the technological necessity to influence the cells by the PEF for the implementation of electrofusion, stimulation, electrical breakdown, etc. (Smolyaninova, Shigimaga, and Kolesnikova, 2009; Smolyaninova et al., 2014; Saulis et al., 2013; Kotnik et al., 2012; Gowrishankar, Smith and Weaver, 2013; Krassowska and Filev, 2007; Miklavčič, 2012; Kolesnikova, Shigimaga and Smolyaninova, 2013; Shigimaga, Levkin and Megel, 2011; Shigimaga and Megel', 2011a,b, 2012; Shigimaga, 2014, 2015). However, it should be specially noted that in known models, the conductivity of cells, as a natural indicator of the membrane electroporation process, is rarely used as a parameter (Rems et al., 2016; Suzuki et al., 2011; Morshed, Shams and Mussivand, 2013; Schmeer et al., 2004; Dehez et al., 2014; Pucihar et al., 2011; Gowrishankar, Smith and Weaver, 2013), and the continuous effect on the membrane and the cell of the PEF increasing intensity is hardly considered at all, except a small number of works by Prof. D. Miklavčič, as well as our recent work about BP modeling (Kramar, Miklavčič and Maček Lebar, 2007; Kramar et al., 2012; Pucihar et al., 2011; Shigimaga, 2013a,b, 2014; Shigimaga et al., 2017). It is interesting to note that the probabilistic or statistical approaches have not been used recently in BP modeling. Nevertheless, in the productivity of one of these approaches we can convince, for example, the alternative electroporation model which has been proposed in the work (Golberg and Rubinsky, 2010). However, this work is rather of an applied nature, whereas it considers only irreversible electroporation in connection with the application of this effect in the clinical practice of biomedicine for the lysis of certain cells in the tissue. The advantages of using continuous influence on the cell by IEP of increasing intensity become into the open in the context of the above-findings of BP conductivity modelling based on the electroporation representation of the membrane, as a probabilistic process which is represented by the formula (28).

Analyzing the formula (28) derived on the basis of the probability approach, it is possible to notice that dependence of membrane conductivity on electropore radius has as exponential character. If assumed that electropores radius is the mean value at given PEF strength, then according to equation (28) we will get the

mean value of conductivity at mean radius $\bar{r}$ at the moment of time *t*. With the increase of PEF strength, the mean electropores radius $\bar{r}$ and conductivity will also increase, and equation (28) can be considered as a function, describing this process (Fig. 2).

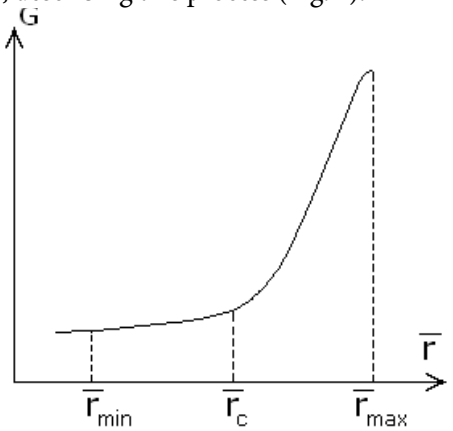

Figure. 2. Theoretical dependence of membrane conductivity on mean electropore radius (probability BP model)

Such suggestion is legitimate, as the mean electropore size depends almost linearly on the field strength in the reversible electroporation mode, and exponentially — in irreversible (Golberg and Rubinsky, 2010; Pavlin et al., 2008; Kotnik et al., 2012). Additionally, the theoretical function (28) is limited by the value $r_{max} = 2r_c$ as electropore radius cannot infinitely increase but only to membrane breakdown (Shigimaga, 2014). Time dependence of conductivity in this case can be neglected, since time of electropore reparation (in reversible electroporation mode) is much less than the selected period of PEF strength variation. Function (28) qualitatively describes our experimental data on cells conductivity in PEF with increasing intensity (Fig. 3).

The minimum radius of the electroporator and the conductivity are defined by the membrane properties and the physicochemical properties of the solution, and also small PEF strength. With PEF strength increases the radius of the electroporator also slowly increases, reaching a critical radius. Above a certain field strength (individual for cells with all the solution factors), the membrane reaches r_{max} and undergoes an irreversible breakdown. This corresponds to the critical PEF strength for irreversible breakdown, which is defined at a point of the maximum conductivity curvature (Shigimaga, 2014, 2015) (Fig. 3).

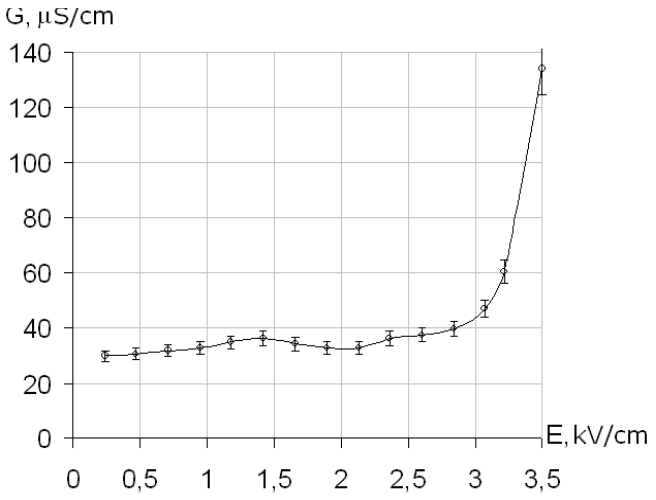

Figure. 3. Experimental dependence of mice oocyte condutivity from PEF strength

The $r_{min} < r < r_c$ interval (Fig. 2), corresponds to reversible membrane electroporation phase with various extents of damage and reparation.

Thus, it is possible to suggest that the main reason for exponential increase in the conductivity of cells in PEF with increasing strength (in experiment) is similar in nature to an conductivity increase with electropore radius up to membrane breakdown. In addition, the constructed probability BP model of cell conductivity at membrane electroporation in increasing PEF agrees with the experimental data.

Conclusions. A probabilistic BP model is made on the basis of the physical characteristics of the cell conductivity during the membrane electroporation in the PEF of increasing intensity. The exponential characteristic of the cell conductivity rise with the average radius increase of the electropores (up to the rupture of the membrane in the case of increase of the critical field intensity) is obtained based on the BP model. It is adequate to the experimental data. The constructed BP model allows theoretical justification and calculation of the PEF parameters. The variable field intensity provides a different degree of the membrane electroporation and therefore makes it possible to justify and calculate all of its necessary safe and critical regimes for pulsed conductometry of living animal cells according to their varying conductivity. Within the scope of an integrated hardware-methodical process of pulsed conductometry it is possible not only to realize the known applications, but to develop new applications of electroporation in the biotechnology of animal reproduction and biomedicine. It is prospective approach.

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Part 3. Biosafety

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RESEARCH OF TOXICITY PARAMETERS OF DISINFECTANT 'SUN STIM'

Kovalenko I. V. [1], Fotina T. I. [2], Fotina H. A. [2], Berezovskyi A. V. [2], Kovalenko V. L. [3], Fotin A. I. [2], Fotin O. V. [2]

[1] National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: subotairen@gmail.com
[2] Sumy National Agrarian University, Sumy, Ukraine, e-mail: tif_ua@meta.ua
[3] State Scientific Control Institute of Biotechnology and Strains of Microorganisms, Kyiv, Ukraine, e-mail: kovalenkodoktor@gmail.com

Summary. Acute toxicity of set 'Sun Stim' was determined on laboratory animals in an experimental laboratory research. The disinfectant has high biological activity and low toxicity due to the active substance which is able to show a broad spectrum of activity against microorganisms. Based on the results, DL_{50} of the set for sanitation 'Sun Stim' for female rats 2,000.0 ± 35.0 mg/kg body weight, male — 2,033.0 ± 34.3 mg/kg. Thus, this drug, when injected into the stomach, according to the classification of toxicity in accordance with state standard (GOST 12.1.007-76), is allowed to be classified as III hazard class, low-hazard compounds. The set for sanitation 'Sun Stim' and the substances from which it was made do not have carcinogenic, mutagenic or genotoxic effects.

Keywords: 'Sun Stim', PGMG (polyhexamethylene guanidine hydrochloride), acute toxicity, laboratory animals, disinfectant

Introduction. Sodium hydroxide, formalin, chloramine, etc. are used for pre-incubation eggs processing in Ukraine. These disinfectants are highly effective, easy to use and economical. So they are widely used in poultry farming.

These agents are not expensive, exhibit bactericidal properties, but are toxic and have corrosive and carcinogenic action. For example, disinfectant 'Virkon C' has a high bactericidal effect but if we will use it before incubation of eggs it can causes the death of a part of the embryos from hyperemia and hemorrhages to allantois (Kalyn, 2009).

Currently, the using of disinfectants which based on polyhexamethylene guanidine hydrochloride and quaternary ammonium compounds is very relevant. They can well dissolve, they are colorless, almost odorless, and have highly bactericidal and surface activity, have low toxicity. These disinfectants do not have irritating action and other side effects (Mandygra et al., 2008; Kovalenko, 2011; Kovalenko and Nedosiekov, 2011; Kovalenko et al., 2013; Souza et al., 2015; Addie et al, 2015; Kim et al., 2016).

Disinfectants which based on polyhexamethylene guanidine hydrochloride and quaternary ammonium compounds do not form toxic products. These disinfectants do not inactivate by proteins, stable, nonaggressive. The bactericidal effect of PGMG (polyhexamethylene guanidine hydrochloride) is due to the ability of derivatives of guanidine to bind with cell walls and bacterial membranes.

Derivatives of guanidine can penetrate inside the cell and have the ability to inhibit cellular enzymes. Bactericidal agents with high biological activity and low toxicity, the active substance of which is able to exhibit a wide spectrum of action (bactericidal, antiviral and antifungal), can be offered as an alternative to traditional disinfectant and antibacterial agents (Lysytsya et al., 2015; Kovalenko et al., 2009, 2018).

The aim of our research was to examine the acute toxicity of bactericidal drug based on guanidine groups (PGMG) which is the active substance of set for sanitation 'Sun Stim'.

Materials and methods. We have used the set for sanitation 'Sun Stim' in our experiment. 'Sun Stim' consists of two parts. The first part is an aqueous solution of PGMG which contains 15% active substance. The second part is a mixture of organic (citric, succinic, malic) acid salts. To determine acute toxicity we have used clinically healthy white male rats (5 groups, 6 in each group, n = 30) and white female rats (5 groups, 6 in each group, n = 30), body weight 180–200 g, 6 months of age. We have determined the average lethal dose (DL_{50}) of the set for sanitation 'Sun Stim' depending on the amount of the drug, and we have determined the main parameters of acute toxicity, by using Kerber's and Pershin's methods (Kotsiumbas et al., 2006; Buckmaster, 2012).

Animals had food and water every 4 hours after the administration of the drug. At the same time, we observed the appearance and behavior of animals, the state of wool and mucous membranes, feed using, mobility, rhythm and respiration rate, the occurrence and nature of intoxication. To study the acute toxicity of the set for sanitation 'Sun Stim' laboratory animals were observed daily. Classification of substances by toxicity was made according to the table of toxicity levels with state standard (GOST 12.1.007-76).

To study the cumulative effect of the set for sanitation 'Sun Stim', experimental and control groups of rats (n = 10) were formed. Researches of skin-resorptive action were performed on two experimental and one control groups of rats. Within 15 days, two hours per day, the tails of the rats from the first experimental group were being submerged in a test tube with 0.5%, and the tails of the rats from the second group — in 5% of the 'Sun Stim' solution. Tails of animals from control group were being placed in test tubes with water. The irritating and sensitizing effects were determined on guinea pigs according to generally accepted methods in accordance with valid methodological recommendations 'Toxicological control of new means of animal protection' (Kosenko et al., 1997). Experiments on laboratory animals were conducted in accordance with the requirements of the European Convention for the Protection of Experimental Animals (EC 86/609/EEC) (CEC, 1986).

The results and discussion. The analysis of the indicators in Tables 1 and 2 shows that the toxic effect of the set for sanitation 'Sun Stim' clinically manifested almost equally both in males and females.

In 1–3 hours after oral administration of the drug in a subtoxic dose to laboratory animals, dyspnea and suppression of the central nervous system were observed. Most of them have died during the first day.

Based on the results, DL_{50} of 'Sun Stim' for female rats is 2,000.0 ± 35.0 mg/kg body weight, for male is 2,033.0 ± 34.3 mg/kg. Thus, this drug, when injected into the stomach, according to the classification of toxicity in accordance with state standard (GOST 12.1.007-76), is allowed to be classified as III hazard class, moderate hazardous substance. Further survived animals observation showed that their moving reaction was suppressed over the next 24–72 hours (Table 3). In addition, the experimental rats showed a marked reduction in moving activity, anxiety, reactivity and aggressive, moving disorders, reduced reaction to the touch and pain, irritation, and a decrease in the respiration rate.

In addition, the experimental rats showed a marked reduction in moving activity, anxiety, reactivity and aggressive, moving disorders, reduced reaction to the touch and pain, irritation, and a decrease in the respiration rate.

Table 1 — Expression of acute toxicity of set for sanitation 'Sun Stim' in male rats

Indexes	Dose of the drug, mg/kg				
	1800	1900	2000	2100	2200
Number of animals:					
total	6	6	6	6	6
survived	6	5	4	2	0
died	0	1	2	4	6
Z		0.5	1.5	3.0	5.0
D		100	100	100	100
DZ		50	150	300	500

Table 2 — Expression of acute toxicity of the set for sanitation 'Sun Stim' in female rats

Indexes	Dose of the drug, mg/kg				
	1800	1900	2000	2100	2200
Number of animals:					
total	6	6	6	6	6
survived	6	4	4	1	0
died	0	2	2	5	6
Z		1.0	2.0	3.5	5.5
D		100	100	100	100
DZ		100	200	350	550

Table 3 — Influence of subtoxic dose of the set for sanitation 'Sun Stim' at oral administration on the general functional characteristics of experimental rats

Indexes	Time of observation, hours		
	6	24	72
Reactions in behavior:			
anxiety	–3	–2	–1
reactivity	–3	–2	–1
aggressive	–3	–3	–2
Neuro muscular reactions:			
tremor	0	0	0
cramps while walking	–3	–3	–1
reaction to pain stimuli	–2	–1	–1
power of catch	–2	–1	0
Vegetative reactions:	unchanged		
pupil size	slow		
conditions of wool cover	disheveled		
color of the mucous membranes	cyanotic		
number of faecal masses	slight increased		
consistency of fecal masses	semi liquid		
frequency of urination	slight increased		
the color of urine	unchanged		
heart rate	unchanged		

Notes: 0 — the effect is absent; '–' — braking effect.

Daily, within 30 days, submerging of rats' tails in a 5% 'Sun Stim' solution caused an increase of tails' volume and

an increase in the amount of leukocytes in the blood. Significant changes in biochemical parameters in serum were not detected. (Table 4).

Table 4 — Hematological indices of peripheral blood of white rats at 30-days application of 5% solution of the set for sanitation 'Sun Stim' on the tails' skin

Hematologic indices	Experimental group			Control group
	back-ground	on the 15th day	on the 30th day	
Number of erythrocytes, 10^{12}/l	7.1 ± 0.3	6.9 ± 0.12	6.8 ± 0.2	6.8 ± 0.2
Hemoglobin content, g/l	156.3 ± 4.0	148.0 ± 4.6	157.3 ± 9.3	153.3 ± 2.6
Color index, conditional units	0.63 ± 0.01	0.6 ± 0.01	0.63 ± 0.03	0.63 ± 0.03
Number of white blood cells, 10^9/l	9.6 ± 0.4	10.3 ± 0.6	8.9 ± 1.8	9.1 ± 1.0
Segment-nuclear neutrophils, %	21.6 ± 1.4	18.5 ± 0.5	20.6 ± 2.3	15.6 ± 4.1
Palatine-neutrophils, %	0.3 ± 0.3	0.5 ± 0.5	0.33 ± 0.33	0.3 ± 0.3
Lymphocytes, %	71.0 ± 1.5	73.0 ± 1.0	71.3 ± 3.2	77.3 ± 5.7
Monocytes, %	4.6 ± 0.8	3.3 ± 0.3	3.3 ± 0.8	3.9 ± 1.0
Eosinophils, %	2.3 ± 0.3	5.3 ± 1.7	4.6 ± 0.3	1.6 ± 0.6

At studying possible irritating or damaging effects of the skin and the causing contact non-allergic dermatitis, it was found that a single application of 'Sun Stim' on undamaged skin of the back of white rats in most significant recommended concentration of working solutions (2%), have not caused signs of skin irritation.

Non-diluted concentrate of the 'Sun Stim' caused irritation from insignificant to moderate (2–3 points). A single application of 'Sun Stim' on 2/3 of the skin surface of the tails of white rats did not lead to the development of irritative skin reactions.

At determining the teratogenic effect in experiments with rats and rabbits at feeding disinfectant in doses of 2.5 and 1.5 mg/kg of body weight it was found that the drug does not have teratogenic effect.

At determining carcinogenic, mutagenic or genotoxic effects of the set for sanitation 'Sun Stim' it was found that the set and the substances from which it was made do not have carcinogenic, mutagenic or genotoxic effects.

Conclusion. Based on the results, DL_{50} of the set for sanitation 'Sun Stim' for female rats 2,000.0 ± 35.0 mg/kg body weight, male — 2,033.0 ± 34.3 mg/kg.

Thus, this drug, when injected into the stomach, according to the classification of toxicity in accordance with state standard (GOST 12.1.007-76), is allowed to be classified as III hazard class, low-hazard compounds.

The set for sanitation 'Sun Stim' and the substances from which it was made do not have carcinogenic, mutagenic or genotoxic effects.

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PROVIDING OF SANITARY AND HYGIENIC MEASURES AT FARMS AS AN IMPORTANT FACTOR IN INCREASING OF RESISTANCE AND PROPHYLAXIS AGAINST DISEASES IN PIGS

Chernyi N. V., Mitrofanov A. V., Machula O. S., Shchepetilnikov Yu. A.

Kharkiv State Zooveterinary Academy, Kharkiv, Ukraine, e-mail: dmchorn@ukr.net

Summary. The article summarizes our own research and publications on the importance of sanitary hygiene and technological factors to ensure disease prevention, to increase the resistance and productivity of pigs. The emphasis has been done not on the treatment but on the prevention of swine diseases due to the optimization of hygienic parameters' standards, tolerated permissible concentrations of substances in the environment, their influence not only on the processes of thermoregulation in the body and body adaptation but also to the increase of body resistance to infectious agents. Due to non-observance of hygiene and sanitation rules, feeding and drinking regimes in the pig-breeding enterprises, the annual death of animals exceeds 25%, digestive tract diseases are registered in 40–50% and respiratory diseases — in 25–30% of cases. Growth retardation is registered in young animals and the durability does not exceed 83.2–85.6%. High indoor air humidity should be considered as a factor of importance in the etiopathogenesis of respiratory diseases and temperature changes — as a factor to prevent hyperthermia and hypoglycemia. The increase in the efficiency of the work in pig-breeding complexes can be achieved: first, due to the decrease in the influence of environmental factors having the negative impact on the development of swine immunity; second, due to the elimination of negatively acting agents from the external environment and the strengthening of factors that increase the body resistance; third, due to the development of nonspecific immunostimulants and their rational use in the pig-breeding practice.

Keywords: swine, resistance, health, hygiene, sanitation, abiotic and biotic factors

Introduction. One of the main tasks of veterinary science and practice is to provide the effective protection of swine from diseases (Smirnov, 2012). Growth intensity retardation and the manifestation of non-contagious diseases are caused by such factors as non-compliance with microclimate and sanitary intervals for each age group of pigs, their feeding and drinking regime, low level of qualification of service personnel and zootechnical and veterinary specialists. As a result, the annual death of pigs exceeds 25%, respiratory diseases are registered in 25–32% of cases, the diseases of the digestive tract are revealed in 40–50% (Karelin, 1979; Chernyy et al., 2004). The nonspecific resistance of the body is weakened in the pig-breeding enterprises with a capacity of 12–24–36–54 thousand of heads and with 2–2.2 farrows, with the weight gains that are not less than 400 g/day in the pigs on rearing and 500 g/day in the pigs on fattening (Plyashchenko and Sidorov, 1979; Urban and Naymanov, 1984; Schulman, 1985)

The problem of swine disease prevention, the increase in their productivity and resistance to stressful abiotic and biotic factors is one of the most urgent since it is directly connected with the quality of the products. Therefore, special attention should be paid to the issue of sanitary and hygienic provision on pig-breeding farms (Dostoievskyi, 2012).

The aim of our work is to analyze the publications on the role of sanitary and hygienic and technological factors in the prevention of swine diseases and in the increase in swine productivity.

Materials and methods. The review of the publications has been done and the results of our own studies on swine resistance, productivity, conservation and incidence have been analyzed to obtain the necessary information to reveal the factors that affect the efficiency of the pig-breeding industry.

Results. The importance of the above mentioned problem and the necessity of its solution can be explained, on the one hand, by the biosphere pollution with emissions from the complex and, on the other hand, by the prevention of swine diseases. According to the reports (Volkov and Danilov, 2004; Tyurin et al., 2013) a significant amount of ammonia, hydrogen sulfide, carbon dioxide, dust, microflora is emitted into the atmosphere (Table 1). The above mentioned emissions cause respiratory diseases and growth retardation in pigs.

Table 1 — Air contamination at pig-breeding complex

Indicator, kg/h	Power, thousands of pigs			
	12	24	30	108
Ammonia, kg/h	8.9	17.2	20.1	54.3
Hydrogen sulfide, kg/h	6.8	12.1	14.2	25.8
Dust, kg/h	9.9	12.6	24.2	43.2
Microflora, $\times 10^9$ CFU	19.8	36.6	40.2	71.8

Each 10% of animals are observed with affected lung tissue after slaughtering, which means a loss of 30 to 40 g of daily live weight gain. A swine that loses 30 g of average daily weight gain needs 0.1 kg more feeds and if the animal

puts on 75 kg (25 to 100 kg) weight, it leads to a surplus of 7.5 kg of feeds/animal, it is 1.3–1.5 €/animal in European prices and as a result it leads to the shortfall in the production (Table 2).

Table 2 — Shortfall in livestock production per 1,000 animals due to poor microclimate

Quantity, animals	Livestock production			
	pork, t	beef, t	milk, t	eggs, thousands
1,000	10	15	400	25

The live weight gain in the young animals occurs due to muscle tissue and in the adult animals — mainly due to fat deposition. Therefore, to obtain high live weight gains at an early stage of rearing is economically and physiologically beneficial. The production of 1 kg of meat requires 30–35 MJ of exchange energy and 1 kg of fat — twice more.

Researchers and practical specialists pay much attention to the provision of optimal microclimate parameters, in particular, the temperature and humidity regime (Genev et al., 1974; Torpakov, 1980; Prange and Bergfeld, 1975; Schulman, 1985; Demchuk and Pavliuk, 1994).

The experience of pork production proves that 'keeping pigs in cold conditions is expensive'. That is, the optimal microclimate, even if it is not cheap, can provide high productivity, conservation of animals and the production of high-quality products that makes it possible not only to recoup all the costs but also to make profit.

Modern genotypes of swine are like a Mercedes, they require proper feeding, care and maintenance in order to get the maximum out of them. Such conditions are: temperature and humidity regime, full and balanced feeding with amino acid composition, especially lysine, which is essential for protein digestion in animal and human body. In addition, if 25 to 30 years ago pigs were treated 'pig-like', that time has irretrievably gone and therefore animals require careful attention (Demchuk and Chornyi, 2011; Medvedskyi, Brylo and Sadomov, 2014).

In our opinion, the reports on the advisability of keeping pigs at low temperatures are debatable. In the conditions of the economy of energy resources to ensure the required temperature and humidity regime, first of all, it is necessary to increase the resistance to heat transfer of the enclosing constructions and then, if necessary, to use a heating system.

The studies have shown that at the outdoor temperatures below – 10–15 °C (with daily fluctuations from 0 to 20 °C), the biological heat of animals is insufficient to provide the optimum temperature and humidity regime in the pigsties. The coefficient of walls' resistance to heat transfer (CRH) of walls is below 0.86, ceilings — below 1.34 W/m^2 °C but it is necessary to have not less than 1.5 W/m °C and 1.8 m^2 K/W are needed (Plyashchenko and Sidorov, 1987; Chernyy et al., 2004).

This is particularly true for the construction of the floor since the pigs contact with the floor for 70% of daily time. And if the coefficient of resistance to heat transfer is less than 2 W/m^2 °C it leads to hypothermia and, as a result, to respiratory disease (Kuznetsov et al., 2001).

An important factor that ensures a high level of sanitary conditions on the farms is the compliance with the principle 'everything is empty–everything is occupied'. In our experiments the pigs of the control group were grown in the pen (without sanitary interval), the pigs in the experimental group 2 were kept in accordance with the principle 'everything is empty–everything is occupied' and the pigs of the experimental group 1 were kept in accordance with the above principle when the pens were free (wet disinfection, current repair). Under these conditions, the level of air contamination with the microflora was: in the control group — $486.4 \pm 20.1 \times 10^3$ CFU/m^3 before setting and $892.0 \pm 17.1 \times 10^3$ CFU/m^3 after 60 days, in the experimental group 1 — from 390.3 ± 18.3 to 792.6×10^3 CFU/m^3, in the experimental group 2 the air contamination by microflora did not exceed from 115.3 ± 0.54 to 211.6×10^3 CFU/m^3, respectively. The young animals in sections 0–1 and control one were grown under the influence of biological stress — microflora, especially *E. coli*, α-β-hemolytic streptococci. The results are given in Table 3.

Table 3 — Productivity, morbidity and preservation of the young depending on the principle of 'everything is empty–everything is occupied'

Group	Average daily weight gain, g live weight, kg/2 months	Disease with symptoms, %		Preservation, %
		gastro-intestinal disorders	respiratory disorders	
Control	11.64/177	25.8	18.5	83.2
Experimental 1	12.35/189	17.6	11.2	85.6
Experimental 2	16.35/225	3.4	2.3	96.1

It can be seen from Table 3 that the use of the premises without sanitary intervals (control) or partially (0–1) did not give a positive effect. The average daily gain did not exceed 177 and 189 g, the preservation was 83.2 and 85.6%, gastro intestinal disorders were registered in 25.8–17.6%, bronchopneumonia — in 18.5–11.2%, respectively.

A factor to prevent hypothermia and hypoglycemia is the provision of temperature of 30–32 °C with a decrease to 20–24 °C for the suckling pigs in the area of their housing and high humidity should be considered as a

factor of importance in the etiopathogenesis of respiratory diseases (Prange and Bergfeld, 1975; Vachev, 1979).

The duration of sows' use for rearing is also an important factor in the effectiveness of pig breeding. The importation of animals from outside, especially from abroad, can hardly be considered expedient and economically justified. From our point of view, the recognition of such an approach is not unambiguous and justified. Because of the weak selection, non-compliance with hygiene and full-value feeding, a threat to high productivity appears, and therefore it is necessary to recognize that the importation from other states is forced. Forcibly transported animals of import breeding are in a stressful situation in the absence of similar conditions and technologies that were in the 'homeland'. The practice shows that many highly productive imported animals have to be prematurely culled (Baranikov, Mikhaylov and Kolosov, 2012). The genetic potential of domestic swine genotypes is as good as that of imported ones and to a large extent they exceed imported animals. According to Akselgaard, the founder of the Danish firm 'Danosha' in Ukraine the selection, proper feeding, temperature regime and lighting for every period of animal growing, strict discipline, knowledge and skills of the staff are the solution of the problem (Koval'chuk, 2013).

The experience of the work of the farms shows that the metabolic pathology (avitaminosis, alimentary anemia, microelementosis, hepatodystrophy, ketosis), limb diseases: arthritis is registered in 3.8% of the pigs at the beginning of fattening, in 25.8% — at the end of the fattening period. Of the 200 surveyed 6 month aged pigs on fattening 36% of pigs of the Landrace, Large White breed kept without bedding on the slatted floors had the posture of a sedentary dog and immobility.

The animals of meat breeds are more susceptible to diseases. Morphofunctional disorders of the extremities were recorded in 72–80% of boars, deformation of the tarsal bones of the hind limbs was revealed in 35–43% of boars, in 7–10% of boars — paralysis of the forelegs and in 1.5–3% — paralysis of the hind limbs was registered.

In some European countries due to the achievement of genetics and quality of feeds, the costs for pork production were 2.0-3.1 kg, in Denmark — 2.66 kg, in Germany — 2.92 kg, in Ukraine — 4 kg, that is, the organization of proper feeding is a problem. Every 4th pig does not reach the commodity conditions because of the improper feeding, antisanitary conditions and respiratory diseases. Mycotoxins cause great damage to the pig-breeding enterprises that are protein factories. Data analysis of toxicological laboratories proved high contamination of grain and fodder with mycotoxins. Thus, desoxynivalenol (DON) was detected in 94%, T2 — in 39%, zearalenol — in 81% of the examined samples. Mycotoxins that contaminate fodder and food (meat, milk, eggs, cheese, cottage cheese) are of great danger. The impact of 200 °C temperature and higher on the above mentioned mycotoxins is ineffective and aflatoxin is inactivated at 320 °C. Because of the low molecular weight of toxicogenic fungi, animals and humans do not produce antibodies to mycotoxins, that is, the body is not protected from their effects during their lifetime (Smirnov, 2012). The content of mycotoxins in feeds in any concentration has a negative impact on the productive parameters of pigs (Table 4).

Table 4 — Effect of desoxynivalenol DON on the efficiency of weaner rearing

Indicators	DON content in feeds, mg/kg			
	0	4	8	10
Feeds consumption, kg/day	2.05	1.76	1.47	1.28
Feeds conversion/gains, kg/k. ed.	2.32	2.22	2.63	2.94
Average daily weight gain, g	890	800	580	450
Price of wheat, $/t	155	143	126	59

Despite the numerous studies, the requirements of pigs of different ages to temperatures, combination of temperature and humidity in the environment, duration of light, bacterial composition of the air, its permissible species and quantitative characteristics and the geometric parameters of the pigsties are not studies properly. In the early 1980s, a sensation was reported by the Irish farmer about the effectiveness of keeping pigs on fattening at the temperature of + 30 °C and humidity — 100%, when the ventilation went out of work. Nothing negative happened: no cases of animal death or diseases were registered, the average daily gain remained at the same level (Stasenko, 1974). It is worth mentioning because we have to state that according to the literature sources, very few people are engaged in studying the combination of microclimate parameters (temperature and humidity) and their influence on pigs. During the experiment (1965–1966) it was proved that the pigs weighing up to 60–80 kg grew better at 21 °C (the average daily gain was 200 g more than at 16 °C). It was found that when keeping pigs at temperatures up to 15 °C, the synthesis of nitrogenous compounds in pigs on fattening decreased by 2–3 times but it was enhanced and the above compounds were used as the energy material at low temperatures (Heyitman, Kelli and Bond, 1965 cited by Stasenko, 1974)

The main components of the productive potential are feeding (65%), breed features (15%), veterinary and zootechnical support (10%), conditions of maintenance (10%) (Plyashchenko and Sidorov, 1979; Kavardakov et al. 2012). According to the reports, the piglet of 60 kg live weight and an average daily gain of 850 g should consume 2.5 kg of dry matter of feeds that contain 435 g of crude protein and the body synthesizes 127.5 g of protein out of the above amount to maintain the productive potential.

This protein contains only 22 g of lysine, without lysine there is no assimilation of protein in the body of animals and humans.

There is no data on the health status of the long-living boars, sows, 6–8 farrows of different genotypes used in the industrial pig-breeding enterprises.

In the pigsties with 2-row-location of pens and imperfect ventilation the share of aerostases was 25–30% and with 4-row-location of pens — 35–40% of the floor space. Aerostases are characterized by high temperature — 28–30 °C, humidity — 86–92%, the concentration of ammonia — 20–30 mg/m^3, carbon dioxide — 2.5–3.5 l/m^3 (0.25–0.35%) which is the reason for the decrease in resistance and, hence, the safety and productivity of pigs (Sokolov, 1998). Until now, the size of technological groups of pigs on fattening has not been substantiated. The researchers who carried out the experiments with variants of 10, 20, 40, 50, 60, 100, 150, 200 animals in the pen without giving a reason came to the trend: 'The less animals in the group, the higher the live weight gain and the higher the fodder payment for the best indicators of the quality of pork' (Stasenko, 1974). The standards of water consumption have not been substantiated. The above-mentioned standards of water for drinking 6–8 l/kg of dry fodder adopted more than 100 years ago (Klimmer, 1912) did not have changes for pigs of different age groups (Skorokhod'ko, 1930; Onegov, Khrabustovskiy and Chernykh, 1984; Medvedskyi, Brylo and Sadomov, 2014). And only Sas (2008) clarified the drinking regimes and provided data on the norms of water consumption for pigs. Since the ratio and mixed fodders' composition has been changed, a technologist should know not only how to calculate but also what norms to introduce into the project with given rations, as well as fodder preparation methods. The problem of wastes utilization and the norms of manure and urine output has not been solved. For example, an error in calculations per 100 g/animal at farms with a population of 5,000 pigs requires additional daily capacities (manure stores) or machines for its processing by 5 t. In our opinion, the existing standards for pigs, for water supply and manure output have the errors of up to 50% of daily excretion. (Stasenko, 1974)

The parameters of some factors such as the front and the frequency of feeding, the temperature of feeds prepared for eating have not been substantiated, there is not enough research on the study of swine ethology. The maximum permissible concentration of ammonia, carbon dioxide is required to be refined and revised since the above parameters have been provided for extensive pig breeding (Tyurin, 2004; Voronin, Kochish and Naydenskiy, 2006).

Discussion and conclusion. Data analysis of literature sources (Yurkov, 1985; Plyashchenko and Sidorov, 1987; Smirnov, 2004; Kirillov, Petryankin and Semenov, 2006; Baranikov, Mikhaylov and Kolosov, 2008; Chornyi et al., 2015) has shown that high level of morbidity in animals, premature culling of the breeding stock, low resistance of young animals and their growth retardation at pig-breeding enterprises are caused by the complex of abiotic and biotic factors: non-observance of the optimal microclimate, the maintenance of pigs under conditions of adynamia and hypoxia, high contamination of the air with microflora, using of premises without sanitary intervals; ecologically poor habitat, weak adaptation of the imported from outside pigs to the production technology, feeding type with commercial feeds, lack of insolation; metabolic disorders caused by the using of feeds contaminated with fungi; the presence of conditionally pathogenic microflora, causative agents of cutaneous and parasitic diseases (Urban and Naymanov, 1984; Kirillov, Petryankin and Semenov, 2006). The problem of non-contagious diseases is the most important pathology in general, including infectious pathology. That is why, in conditions of intensive animal use, the activity of veterinary specialists should be directed not to treatment of diseases but to their prevention. We keep in mind: first of all, prevention of non-contagious and infectious diseases and stress, mycotoxicosis, development of methods for stimulation of natural resistance mechanisms; second, microclimate optimization in accordance with age characteristics of animals providing their life ability and production of ecologically safe pork; third, the quality of feeds, the ecologization of the biosphere and minimization of drugs' use, especially antibiotics; fourth, studying the impact of environmental factors on body resistance and immunological reactivity of pigs.

The solution of the posed tasks also refers to infectious pathology since abiotic stress factors can have depressing effect on immunogenesis and lead to decreasing in immunity intensity. We think that the problem of disease prevention can be solved by joint work of infectionists and hygienists (Sokolov, 1998; Smirnov, 2012). For today, hygienic and ecological standards of environmental conditions and sanitation cannot be considered acceptable without their physiological substantiation although immunological and physiological indicators have not been widely used in the hygienic research.

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News

5TH EUROPEAN MEETING ON ANIMAL CHLAMYDIOSIS (EMAC-5)

NSC IECVM in cooperation with the Zurich University under Swiss National Foundation support organized the 5th European Meeting on Animal Chlamydiosis (EMAC-5) which took place in Odesa (Ukraine) on October 3–5, 2018.

The Organization Committee Chairman was B. T. Stegny, the Director of the NSC IECVM, NAAS academician. It included Prof. A. P. Gerilovych, Dr. V. I. Bolotin and a number of scientists from both institutions. The Scientific Committee was headed by Prof. Nicole Borel, the Head of the OIE reference laboratory for Enzootic Abortion in Ewes.

More than 60 delegates from 18 countries were attended the EMAC-5.

Invited Keynote Lectures at EMAC were caused by lectures by prominent scientists. Barbara Susanne Sixt (Umeå, Sweden), Robert Schoborg (Johnson City, TN, USA) and Servaas A. Morré, (Amsterdam, The Netherlands). An important event was the foundation of the European Society for the Animal Chlamydiosis and Related Zoonoses, and Prof. Mooredan David Longbotom (Great Britain) became its President. The Charter of the Society was adopted and the program of work for the next five years was approved.

This event also included a Poster Session regarding the problems of Chlamydiosis and related animal infections, as well as presentations by industry representatives about innovative systems and diagnostic tools for chlamydia detection.

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