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The development and globalization of the modern World creates a lot of threats and challenges for consumer safety and quality of food. These risks are associated with different factors, and the infectious diseases are the most relevant of them. Emergent infectious diseases are the reasons of multiple economical, social, and other losses. They cause threats and problems in international trade and other types of collaboration within countries. This is the reason why the emergent and zoonotic diseases became to be one of the most significant baselines of joint WHO-OIE-FAO strategy One health.

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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 aria of biological science. This was recognized as the profile edition for veterinary medicine doctors and biologists in Ukraine. This promote the research of Ukrainian institutions, publishing their achievements in English, and sharing it among the scientific community.

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Sincerely yours,
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SNP L127V OF GROWTH HORMONE GENE IN BREEDING HERD OF ABERDEEN ANGUS IN KHARKIV REGION, EASTERN UKRAINE

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Summary. Bovine growth hormone (*bGH*) effects on animal growth and metabolism. Therefore, it plays a vital role in regulation of body weight, fertility and lactation performance of cattle. This enables *bGH* gene to be used as a candidate marker for improving growth, meat or milk production and for marked-assisted selection programs of cattle too. The aim of this study is analyzing the polymorphic variant L127V of *bGH* gene in Aberdeen-Angus bred in Kharkiv region and comparing of obtained result with commercial herds from other countries.

PCR-RFLP methods were set up for the SNP genotyping. Testing deviation from the Hardy-Weinberg equilibrium was performed using Pearson's chi-squared test. Pearson's correlation coefficient *r* was used to measure the strength of association between two characteristics.

The allele and genotype frequencies of SNP *L127V* (rs41923484; *g.2141C>G*) are: *L* — 0.319 and *V* — 0.681; *LL* — 8.6%, *LV* — 46.6% and *VV* — 44.8% (n=58), population is in Hardy-Weinberg equilibrium. The studied population is close to beef herds of Russian selection. It was found a positive correlation of *L-allele* with birth weight within the moderate climatic zone (*r*=0.93).

Keywords: Aberdeen-Angus breed, growth hormone gene, SNP L127V

Introduction. Bovine growth hormone (*bGH*) belongs to a family of somatolactogenic hormones. It is a single polypeptide chain with 190 or 191 amino acids and molecular weight 22 kDa (Salces et al., 2011). Growth hormone has an effects on growth and metabolism by interacting with specific receptor on the surface of target cells. *bGH* gene is located in 19 BTA and consists of five exons separated by four introns (Hadi et al., 2015). *bGH* plays a vital role in regulation of body weight, fertility and lactation performance of cattle. Therefore, SNPs in *bGH* gene can be used as predictors of growth, meat or milk production traits for supporting marked-assisted selection programs in cattle.

To date there are following SNPs of bGH gene investigated in cattle: four (*253 C>T*, *303 C>T*, *502 C>T*, and *559 G>A*) in the promoter, one (*679 C>T*) in exon 1, one (*1,692 T>C*) in intron 3 four (2141 C>G, 2258 C>T, 2277 C>T, and 2291 A>C) in exon 5 (Lee et al., 2013) and one (C/T in 40.5 Mb locus) in intron 4, which could be used to differentiate humped (*B. indicus*) from humpless (*B. taurus*) cattle (Thomas et al., 2007).

Considering a multiple effect of *bGH* on growth and lactation processes SNPs of *bGH* gene was studied both in beef and dairy breeds: Aberdeen Angus — in Japan, USA, Brazil (Chikuni et al., 1991; Ge et al., 2003; Vasconcellos et al., 2003), Simmental and Hereford — in Lithuania (Krasnopiorova et al., 2012), Charolais — in Lithuania, Brazil (Krasnopiorova et al., 2012; Kemenes et al., 1999; Regitano et al., 1999), Limousin — in Lithuania, Indonesia (Krasnopiorova et al., Hartatik, 2013), Holstein — in Japan, Germany, Hungary, Poland, Lithuania, Iran (Chikuni et al., 1991; Hradecka et al., 2008; Balogh et al., 2009; Krasnopiorova et al., 2012; Hadi et al., 2015), Jersey — in Italy, Poland (Dario et al., 2008; Komisarek et al., 2011), Brangus — in Mexico (Thomas et al., 2007), Nellore — in Brazil (Kemenes et al., 1999), as well as endemic cattle breeds — in Philippines (Salces et al., 2011) and Korea (Lee et al., 2013). These *bGH* SNPs are associated with birth weight, weight gain, constitution, milk yield, fat, protein, fertility.

Studies of *bGH* SNP *L127V* in Ukrainian breeds were carried out in small populations of Aberdeen-Angus, Southern Beef, Polissian Beef (Kopylova et al., 2009; Kostenko, Starodub, 2011), Ukrainian Grey (Podoba et al., 2009; Mokhnachova et al., 2016), Volinian Beef (Bochkov et al., 2009), Holstein (Kopylova et al., 2009;

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Hyl et al., 2011; Nekrasov et al., 2016), Ukrainian Black-and-White Dairy (Nekrasov et al., 2016), Ukrainian Red-and-White Dairy and Ukrainian Red Dairy (Kopylova et al., 2009).

The purpose of the study was to conduct a comparative analysis of the alleles and genotypes frequencies distribution for SNP *L127V* in the Aberdeen-Angus breeding herd in Kharkiv region and to compare obtained result with similar parameters of commercial herds from other countries.

Material and methods. The study object was Aberdeen-Angus breeding herd (n=58; cows: n=52, bulls: n=6) bred at PE 'Agrofirma Svitanok' (Kharkiv region, Ukraine). Evaluation of growth dynamics was conducted via the control weighing at birth and at the age of 8, 12, 15, 18 months, and 2, 3, 4, 5 years.

DNA was extracted from blood samples using DNA extraction kits 'Diatom DNA Prep 100' ('Isogene', Russian Federation). PCR-RFLP methods were set up for the SNP genotyping using primer pairs (Lee et al., 2013) and restriction endonuclease AluI ('Fermentas', Lithuania). The digested fragments were electrophoresed on 2.0% agarose gel.

The genetic distance between the studied population and data known from the literature was determined according to Nei (1972). The deviation of allele frequencies from Hardy-Weinberg equilibrium was tested using Pearson's chi-squared test. Normality test assessment was used for data distribution. Pearson's correlation coefficient *r* was used to measure the strength of association between two characteristics. Means for more than two groups were compared by ANOVA. The statistical hypotheses were tested on the significance level of 0.05 (Atramentova, Utevskaya, 2008).

Results. According to the literature the preferred allele is *L-allele*, as it is associated with higher birth weight (Lee et al., 2013; Thomas et al., 2007) and mature weight, marbling (Gill et al., 2009).

Allele and genotype frequencies of SNP *L127V* and its deviation from the Hardy-Weinberg equilibrium for studied Aberdeen-Angus herd are given in Table 1.

Table 1 – Allele and genotype frequencies of SNP *L127V* in the Aberdeen-Angus herd in Kharkiv region

Parameter	***L127V***		
Allele frequencies	***L***	***V***	
Cows (n=52)	0.333	0.667	
Bulls (n=6)	0.167	0.833	
Total (n=58)	0.319	0.681	
Genotype frequencies	***LL***	***LV***	***VV***
Cows (n=52)			
$n_{act.}$	5	25	22
%	9.6	48.1	42.3
$n_{exp.}$	5.9	23.2	22.9
%	11.3	44.6	44.1
Statistics	$\chi^2_{act.}$=0.16; p>0.05		
Bulls (n=6)			
$n_{act.}$	0	2	4
%	0.0	33.3	66.7
$n_{exp.}$	0.2	1.7	4.1
%	2.8	27.8	69.4
Statistics	$\chi^2_{act.}$=0.20; p>0.05		
Total (n=58)			
$n_{act.}$	5	27	26
%	8.6	46.6	44.8
$n_{exp.}$	5.9	25.2	26.9
%	10.2	43.4	46.4
Statistics	$\chi^2_{act.}$=0.15; p>0.05		

Note: df=2, $\Sigma\chi^2_{st.0.05}$=5.99.

Data given in Table 1 suggest that studied population was found to be in equilibrium state. Consequently, there are no evidence of increasing preferred allele frequency without targeted selection.

Three genotype groups separated by SNP L127V were compared via body weight dynamics from birth to the age of five years (Table 2).

Table 2 – Body weight dynamics in Aberdeen-Angus by SNP *L127V*, $x \pm s_x$

Age	Weight, kg		
	L127V		
	LL	*LV*	*VV*
Birth	35.2±1.3*	30.4±1.0*	29.9±0.9*
Average daily gain, kg/day	0.763±0.54	0.771±0.15	774±19
8 months	220.8±15.3	213.3±4.7	208.7±4.1
12 months	288.8±18.6	280.4±4.4	272.8±6.0
15 months	342.0±19.8	324.0±4.9	318.9±4.4
18 months	380.8±24.9	369.1±6.2	362.6±4.1
2 years	421.4±19.0	416.2±6.6	413.1±7.1
3 years	460.3±22.8	446.9±8.5	446.8±10.3
4 years	505.0±18.1	488.7±9.8	479.9±10.9
5 years	588.0±40.0	557.0±19.8	579.8±21.8

Note: $x \pm s_x$ — mean ± standard error; * — differences are significant at $p<0.05$

Table 3 – Genotype and allele frequencies at SNP *L127V* in the beef cattle populations by climate zones (longitudinal length)

Breed	Breed characteristics	Country	Longitudinal length	n	Genotypes, %			Alleles		HWE	Reference	D
					LL	*LV*	*VV*	*L*	*V*			
Beef or beef-dairy breeds												
Aberdeen-Angus	CLM=500–700 kg BLM=750–1000 kg BW=16–25 kg	Ukraine	49° N	58	8.6	46.6	44.8	0.319	0.681	E	Present study	—
		Ukraine	49° N	9	11.1	55.6	33.3	0.389	0.611	E	Kostenko et al., 2011	0.005
		Ukraine	49° N	10	60.0	40.0	0.0	0.800	0.200	E	Kopylova et al., 2009	0.428
		USA	40° N	468	34.4	55.1	10.5	0.620	0.380	DE	Geet al., 2003	0.163
		Japan	35° N	6	33.3	50.0	16.7	0.590	0.410	E	Chikuniet al., 1991	0.130
		Brazil	11° S	52	—	—	—	0.770	0.230	—	Vasconcellos, 2003	0.378
Auliekol	CLM=540–560 kg BLM=950–1050 kg	Kazakhstan	48° N	50	—	—	—	0.640	0.360	—	Beyshova et al., 2016	0.188
Charolais	CLM=600–700 kg BLM=1000–1200 kg BW=40–45 kg	Lithuania	55° N	—	75.0	20.0	5.0	0.850	0.150	E	Krasnopiorova et al., 2012	0.516
		Brazil	11° S	32	—	—	—	0.720	0.280	—	Kemenes et al., 1999	0.299
		Brazil	11° S	36	—	—	—	0.736	0.264	—	Regitanoet al., 1999	0.323
Hanwoo	CLM=550–600 kg	South Korea	36° N	231	84.8	11.7	3.5	0.907	0.093	E	Lee et al.,2013	0.622
Hereford	CLM=650–850 kg BLM=900–1350 kg BW=28–35 kg	Lithuania	55° N	—	90.0	0.0	10.0	0.900	0.100	DE	Krasnopiorova et al., 2012	0.608
		Japan	35° N	10	70.0	20.0	10.0	0.800	0.200	E	Chikuni et al., 1991	0.428
Indigenous Philippine Cattle	CLM=300–400 kg	Phillipines	13° N	55	20.0	45.0	35.0	0.430	0.570	E	Salces et al., 2011	0.017
Kalmyk	CLM=420–480 kg BLM=750–950 kg BW=20–25 kg	Russia	60° N	60	1.7	20.0	78.3	0.117	0.883	E	Sulimova et al., 2011	0.057
Kazakh White-headed	CLM=540–580 kg BLM=850–950 kg BW=27–30 kg	Russia	60° N	79	5.1	31.6	63.3	0.209	0.791	E	Sulimova et al., 2011	0.021
Limousin	CLM=550–600 kg BLM=1000–1100 kg BW=34–42 kg	Lithuania	55° N	—	58.3	11.1	30.6	0.639	0.361	DE	Krasnopiorova et al., 2012	0.186
		Indonesia	5° S	6	66.67	33.33	0.0	0.830	0.170	E	Hartatik et al., 2013	0.480
		Indonesia	5° S	81	81.5	18.5	0.0	0.910	0.090	E	Hartatik et al., 2013	0.627

Limousin-Ongole crossbred	—	Indonesia	5° S	56	78.6	21.4	0.0	0.890	0.110	E	Hartatik et al., 2013	0.590
Madura	BLM=250–300 kg	Indonesia	5° S	65	100.0	0.0	0.0	1.000	0.000	—	Hartatik et al., 2013	0.802
Mongolian cattle (beef-dairy)	—	Russia	60° N	47	0.0	25.0	75.0	0.125	0.875	E	Sulimova et al., 2011	0.053
Polissian Beef	CLM=550–600 kg BLM=900–1000 kg BW=28–32 kg	Ukraine	49° N	13	69.2	23.1	7.7	0.807	0.193	E	Kostenko et al., 2011	0.440
Simmental	CLM=550–900 kg BLM=850–1300 kg BW=34–36 kg	Lithuania	55° N	—	50.0	12.5	37.5	0.563	0.438	DE	Krasnopiorova et al., 2012	0.102
Southern Beef	CLM=550–650 kg BLM=950–1100 kg BW=25–30 kg	Ukraine	49° N	8	87.5	12.5	0.0	0.937	0.063	E	Kostenko et al., 2011	0.679
		Ukraine	49° N	21	61.9	38.1	0.0	0.810	0.190	E	Kopylova et al., 2009	0.445
Ukrainian Grey	CLM=450–500 kg BLM=800–850 kg BW=27–30 kg	Ukraine	49° N	84	98.0	2.0	0	0.980	0.020	E	Mokhnachova et al., 2016	0.763
		Ukraine	49° N	51	—	—	—	0.706	0.294	—	Podoba et al., 2009	0.278
Volinian Beef (breeding herd)	CLM=500–550 kg BLM=950–1050 kg BW=28–32 kg	Ukraine	49° N	27	56.0	44.0	0.0	0.778	0.222	E	Bochkov et al., 2009	0.391
Volinian Beef (production herd)		Ukraine	49° N	41	34.0	44.0	22.0	0.561	0.439	E	Bochkov et al., 2009	0.101
Wagyu	CLM=450–500 kg	Japan	35° N	25	24.0	40.0	36	0.440	0.560	E	Chikuni et al., 1991	0.020
Zeboid hybrid	—	Russia	60° N	95	3.2	37.9	58.9	0.222	0.778	E	Sulimova et al., 2011	0.018

Note: CLW — cow live weight, BLW — bull live weight, BW — birth weight; HWE — Hardy-Weinberg equilibrium: E — equilibrium, DE — disequilibrium; D — Nei's genetic distance.

The differences between the groups in birth weight were 4.8–5.2 kg or 14.6–16.1% ($p<0.05$). Group *LL* was superior to *LV* or *VV* growth at each time point, although the growth dynamics between groups was comparable. The differences in the live weight decreased over time and were 3–25 kg or 1–5% between *LL* and *LV* groups, and 10–25 kg, or 2–7% between *LL* and *VV* groups.

Our findings are consistent with the results obtained by Lee et al. (2013) and Thomas et al. (2007): birth weight of *LL*-animals was eventually greater. In beef cattle *L-allele* was associated with a higher body weight and marbling score (Gill et al., 2009).

Literature data on beef cattle populations in the world are summarized in Table 3.

Mostly, literature data suggest that analyzed populations were in a state of equilibrium, except Simmental, Limousine, Hereford in Lithuania and Aberdeen-Angus in USA. Observed disequilibrium may be a consequence of an unbalanced number of heterozygous animals.

The different breeds in Russia show a low frequency of allele *L* (Sulimova et al., 2011), presumably, to be determined by long-term selection in indigenous isolated populations. Alternatively, the *L-allele* frequency in different breeds within equatorial zone is high (Hartatik et al., 2013, Lee et al., 2013). Smaller animal size, being characteristic for this climate zone according to the Bergman's rule, can therefore support a size increasing due to long-term selection, resulting to *L-allele* frequency increase.

Since the *L-allele* is associated with a higher body weight, correlation analysis for this allele frequency vs. different species birth and mature weight within a single zone climate was carried out. A positive correlation of *L-allele* with birth weight ($r=0.93$, $p<0.05$) was established for different breeds within the moderate climatic zone. In general, Ukrainian breeds are similar for mature weight (the differences do not exceed 50–100 kg) and the *L-allele* frequency. The last observation is supported by our data on growth dynamics, where effect of *bGH* is mostly pronounced before till two-year age.

There was a trend to increasing *L-allele* frequency with decreasing latitude for Limousine and Aberdeen-Angus breeds, while the reverse trend was observed for Charolais and Hereford breeds. That is the last larger breeds (average CLW=650 kg, BLW=1200 kg) in the warmer climate show decrease in *L-allele* frequency being associated with decrease of animal body size in warm climate. Smaller breeds, Limousine and Aberdeen-Angus, (up to CLW=600 kg, BLW=800–1000 kg) demonstrate more stable allele frequencies with a tendency to a slight increase. Regardless of the region, the breed (being introduced worldwide) demonstrates stable *L-allele* frequency to be typical for the breed.

Nei's genetic distances showed that the closest to the studied population was the Ukrainian Aberdeen-Angus population (Kostenko et al., 2011) and some breeds of Russian selection (Sulimova et al. 2011). The highest differences, over 0.65, are observed for breeds of Ukrainian selection — Southern Beef and Ukrainian Grey, as well as for Madura (Hartatik et al., 2013).

Conclusion. The preferred allele frequency of SNP *L127V* was 0.319. The studied population is close to beef herds of Russian selection. It was found a positive correlation of *L-allele* with birth weight within the moderate climatic zone ($r=0.93$).

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PHYSIOLOGICAL AND BIOCHEMICAL MECHANISMS OF CONTACT INTERACTION OF NANOPARTICLES OF GOLD WITH *BACILLUS ANTHRACIS* VACCINE STRAIN *STERNE* 34F2 CELLS

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Summary. The article presents the results of experimental studies of mechanisms of Aurum nanoparticles contact interaction on *Bacillus anthracis* vaccine strain.

This research was aimed to study the physiological and biochemical mechanisms of influence of Aurum nanoparticles (AuNP) on *B. anthracis* cells (strain *Sterne* 34F2 — productive vaccine strain).

The cell cultures biomass of *B. anthracis* (strain *Sterne* 34F2) was used as a model for our experiments. The sterile aqueous dispersion of Aurum nanoparticles with average size 19.0±0.9 nm and initial concentration of 19.3 μg/cm^3 for the metal was used for accumulation of *B. anthracis* (strain *Sterne* 34F2) biomass and with average size of 30.0±0.6 nm in initial concentration of 38.6 μg/cm^3 and 77.2 μg/cm^3 by the metal — in experiments on the study of physiological and biochemical mechanisms of interaction of cells. The effectiveness of the interaction of *B. anthracis* cells (strain *Sterne* 34F2) with AuNP was evaluated by a membrane filter method with a followed by determination of optical density changes of mixture on a spectrophotometer. The value of H^+-ATPase activity (KF 3.6.3.6) in total membrane fraction (TMF) bacterial cells was recorded by the accumulation of inorganic phosphorus (P_i). Respiratory activity (RA) of *B. anthracis* was measured by oxygen of electrode Clarks type. Measured parameter was the maximum speed of reducing the concentration of oxygen in the environment measurement, reduced to a unit of bacterial biomass.

According to results of the impact of nanoparticles of Aurum (gold, AuNP) for basic the physiological and biochemical indicators of *B. anthracis* cells (strain *Sterne* 34F2) appeared promising metal nanoparticles in a concentration range 2.90–8.69 μg/cm^3 for metal, as indicated by the presence of ATPase and respiratory activity stimulation and is consistent with the highest accumulation of cells research AuNP along with the intensification of proliferative activity of *B. anthracis* cells.

The mechanisms of gold accumulation in *B. anthracis* cells have the metabolism-dependent nature which is characteristic only active metabolized cells, and desplayed within our experiment involving certain assets metabolism (determining role of the transmembrane potential and its generators — ATPase, respiratory activity (RA)) in the overall regulation of the metabolic system and functional organization (proliferation activity) of the bacterial cells.

Keywords: *Bacillus anthracis*, bacterial cell, Aurum (gold) nanoparticles, contact interaction, proliferative activity of H^+-ATPase activity, respiratory activity

Introduction. The problem of epidemic and epizootic wealth regarding Anthrax in Ukraine belongs to the list of national priorities in order to provide state measures for of animal and people health protection from the especially dangerous diseases.

Anthrax is the multispecies zoonotic disease of the agricultural, domestic and wild animals and humans. Once it has been emerged in a particular area it can take root, keeping the threat of outbreaks in decades (Ipatenko, 1996).

The causative agent of Anthrax — *Bacillus anthracis* — is one of the aerobic spore-forming bacteria. Under favorable conditions (ambient temperature not below 12°C) pathogen can form spores. In spore form it may survive in the soil, remaining viable and maintaining pathogenicity long time. The soil that contaminated by Anthrax spores, could be the possible source of infection for susceptible animals or humans for 70 years and more.

The main source of Anthrax agent are the sick animals that spread the pathogen to the environment with urine, faeces, milk, bloody discharge even before the clinical signs of the disease.

There are thousands of unsatisfactory points for Anthrax where over the years there were outbreaks of the disease today in Ukraine.

Current scientific views on the issue of anthrax changed its vector – the causative agent of Anthrax

ranks the first in the list of agents applied to bioterrorism (Barth et al., 2004; Turnbull, 2008).

The leading role in the prevention of Anthrax occupies vaccination, that should be applied in all susceptible animals. Only in Ukraine, according to current statistics, the annual amount of using vaccines is 30–40 million doses. Currently, national manufacturers of veterinary immunological means not fully satisfy the existing effectively level of vaccine against Anthrax in animals, that encourages to the search for ways to improve existing preparations, particularly through the using of nanoparticles of metals in vaccine production biotechnology.

Among the variety of the existing nanoparticles of metals a special attention attracted nanoparticles of Aurum (gold) (Chen, Mwakwari and Oyelere, 2008). It causes a great interest perspectives for usage of the metal nanoparticles in technology of design of highly efficient diagnostic tools and targeted to prevention and treatment means and measures (Fu et al., 2005; Hainfeld et al., 2006; Cai et al., 2007), the attention of researchers mainly focused on the study of the biological effects of exposure nanogold at the cellular level (Feng, Tianjun and Li, 2008; Zhang et al., 2009).

Study of the impact of Aurum nanoparticles on proliferative activity and immunogenicity of bacterial cells of different taxonomic classes, including *B. anthracis*, made earlier (Dibkova et al., 2009; Ushkalov et al., 2011a, 2011b; Roman'ko, 2012; Roman'ko, Machusskiy and Ushkalov, 2013) indicates the possibility of controlling and managing the intensity of physiological and biochemical responses of bacterial cells as a result of metal nanoparticles of a certain size and concentration, their safety for microbial cells that introduction vaccine preparation of nanoparticles gold can contribute significantly increase its immunogenicity and economic efficiency of the production process.

However, the identified high biological activity of Aurum nanoparticles demonstrates the need for a detailed study of the mechanisms of their effects on *B. anthracis* cells from the *Sterne* 34F2 strain. The study of the interaction of bacterial cells with colloidal particles of gold demonstrates the significant role of them in colloid-chemical aspects of this process (De Roe, Courtoy and Baudhuin, 1987; Olsen and Bernstein, 1989). It has been proved the dependence of cytotoxic, immunotoxic, genotoxic, mutagenic and other negative effects of Aurum nanoparticles from the form, concentration and particle size. The a correlation between the established nature of the listed effects and biological compatibility, reduction of toxic membrane-alternating products of lipid and protein oxidation in biomembranes of prokaryotic cells (lipid peroxidation products and nitrites derivatives OMP), lack of inhibition of inflammatory cytokines TNF-α and IL1-β where described. They convincingly indicate the possibility of nanosized colloidal gold using to create the therapeutic and immunological means (Paciotti et al., 2004; Shukla et al., 2005; Dybkova et al., 2009; Dybkova, 2010; Roman'ko et al., 2010; Roman'ko, 2010). The consensus on the mechanisms of action of biocompatibility and bioavailability of nanoparticles of gold on the microbial cell was not formed today. So the safety or toxicity of nanometals for certain groups of organisms, and a prerequisite for the development and implementation of any biotechnological processes were not determined.

The purpose of this work was to study the physiological and biochemical mechanisms of influence of Aurum nanoparticles on *B. anthracis* cells (strain *Sterne* 34F2 – productive vaccine strain).

Materials and methods. The research were carried out on the model experiments using cell cultures biomass of *B. anthracis* (strain *Sterne* 34F2) from the collection of the National Center of Microorganism Strains in State Scientific Control Institute of Biotechnology and Strains of Microorganisms (Kyiv, Ukraine).

The sterile aqueous dispersion of gold nanoparticles weve synthesized by chemical condensation in an water solution, using the original protocol, developed at the F. D. Ovcharenko Institute of Biocolloidal Chemistry of NAS of Ukraine (Kyiv, Ukraine). Notation conventions of Aurum nanoparticles used in the work is AuNP.

Nanoparticles were synthesized by restoring the Aurum hydrochloric acid ($HAuCl_4$) by sodium citrate in the presence of potassium carbonate. Used nanoparticles of gold had spherical geometry.

Study of the size and form of gold nanoparticles were performed by laser-correlation spectrometry (LCS) and transmission electron microscopy (TEM). Measurements were performed on laser correlation spectrometer 'Zetasizer-3' (Malvern Instruments Ltd., UK). Visualization of prototypes nanoparticle metal was performed by transmission electron microscopy with an electron microscope 'JEOL JEM-1230' (Tokyo Boeki Ltd., Japan). Monodisperse LCS method allows to determine accurately the constant rate of particle diffusion and calculate their hydrodynamic diameter, based on the assumption of spherical geometry of the particles (Rawle, 1994).

Were used (accumulation of biomass of *B. anthracis* (strain *Sterne* 34F2)) AuNP water dispersion medium size 19.0±0.9 nm with initial concentration of 19.3 μg/cm^3 for the metal. In experiments on the study of physiological and biochemical mechanisms of interaction of cells strain *Sterne* 34F2 *B. anthracis* – were used AuNP with average size of 30.0±0.6 nm in initial concentration of 38.6 μg/cm^3 and 77.2 μg/cm^3 by the metal, respectively (Fig. 1, 2A, 2B).

For culturing of the cells and *B. anthracis* biomass accumulation standard commercial environment hydrolyzate medium of fish meal and Hottynheris medium (pH 7.2–7.6) was used (as a source of nitrogen). Amino nitrogen 100–120 mg%, with added by aseptic conditions gold dispersion of nanoparticles average size of ~19 nm in the ratio 1:2, 1:4, 1:6, 1:8, 1:10, 1:12 and 1:20 respectively has been used. The sterilization of prepared culture medium was performed by autoclaving at 0.7 atm. Later it was maintained at a temperature 37±1°C for 48 hours for the purpose of controlling sterility.

Prepared medium groming capacity was studied by adding to 1 cm^3 of medium the culture of *B. anthracis* with a known concentration. It was cultivated at a temperature 37±1°C for 20–24 hours.

As a 'control' standard environment-commercial hydrolyzate medium and fish meal Hottynhes's medium (pH 7.2–7.6) and amino nitrogen (100–120 mg%) without adding AuNP were used.

The effectiveness of the interaction of bacterial *B. anthracis* cells (strain *Sterne* 34F2) with AuNP average size of ~30 nm membrane filter method was evaluated by determining of optical density changes of mixture, containing metal nanoparticles and bacterial cells filtrates, received using membrane filters with pore diameter 0.15–0.17 mm. Optical density values were recorded for λ 520–550 nm (absorption maximum AuNP different sizes) spectrophotometer ('SHIMADZU UV-1800', Japan).

Defining features of the interaction was conducted under conditions of constant final concentration of bacterial cells in a range of dispersion AuNP dilution in the culture medium — 1.1, 2.8, 5.5, 8.3, 11.0, 16.5, 22.0 μg/cm^3 for the metal, respectively.

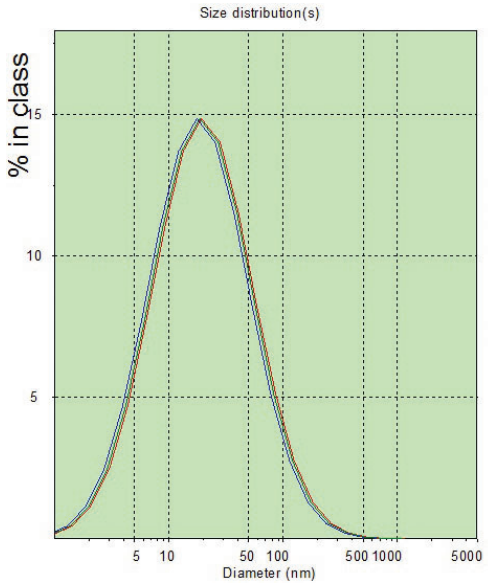
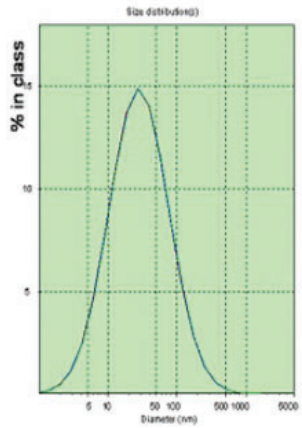


Figure 1. The average size AuNP, (19.0±0.9 nm) (according LCS)



Run	Angle	KCps.	ZAve	Poly	Fit	Time
1	90.0	219.0	30.1	1.000	0.000928	16:29:28
2	90.0	218.9	29.2	1.000	0.000754	16:33:45
3	90.0	220.5	30.3	1.000	0.000826	16:38:01
Average		219.5	**29.9**	1.000		
+/-		0.9	**0.6**	0.000		

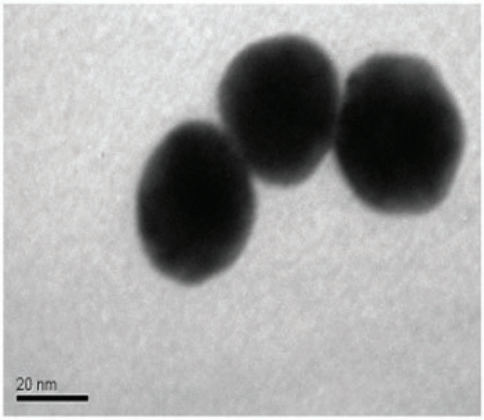


Figure 2. AuNP, (30.0±0.6 nm) properties (form and size): A — according to LCS, B — according to TEM

The quantyty of linked gold (%) was calculated using the formula $(1–D/D_0)\times100\%$, where D — optical density of AuNP filtrate after contact with bacterial cells, D_0 — density filtrate 'control' preparations AuNP. Cultivation of 'control' preparations AuNP was performed with distilled water, the amount of dispersion AuNP, which added to the contact mixture remained unchanged. Contact interaction time was 5 min.

The value of H^+-ATPase activity (KF 3.6.3.6) total membrane fraction (TMF) bacterial cells was recorded by the accumulation of inorganic phosphorus (P_i) concentrations in the environment is determined by Fiske-Subbarou method (Fiske and Subbarow, 1925). Testing has been performed by the incubation in medium with the following composition: 10 mM Tris-HCl, 3 mM $MgCl_2$, 3 mM ATP (pH 7.5). The duration of incubation was 10 min. Quantity of the membrane protein — 60–70 μg/cm^3. The reaction is initiated by the introduction of conditions for the incubation environment TMF aliquots of cells and stopped by adding 1 cm^3 10% solution TCAA.

AuNP dispersion was made in TMF medium and incubated for 3 min. The range of concentrations in the contact mixture AuNP — 1.10–23.16 μg/cm^3. The 'control' sample AuNP instead of adding 10 mM Tris-HCl buffer.

In all experiments, 'control' for non-enzymatic hydrolysis of ATP served incubation environment without it TMF. As the 'control' on endogenous phosphorus in the environment was used TMF, which included only the TMF in aqueous solution.

Respiratory activity (RA) of *B. anthracis* was measured by oxygen of electrode Clarks type (MO128, Mettler Toledo, Switzerland). Measured parameter was the maximum speed of reducing the concentration of oxygen in the environment measurement, reduced to a unit of biomass used bacterial strains (specific RA).

The speed of reducing the concentration of oxygen in the environment measurement AuNP dispersion have been tested in with incubating bacterial cells. The range of concentrations in the contact mixture AuNP — 0.39–7.70 μg/cm^3 for the metal. The 'control' sample was added instead AuNP 5 mM Tris-HCl buffer.

ATP-hydroxymethyl aminometan has been used in the study manufactured by 'Gibco RBL', Scotland, other reagents — by domestic production qualification 'analytical grade'.

The calculation the average values has been done in at least 3 repeats in a series of 5 independent experiments. Statistical analysis of the results was carried out in accordance with common requirements (Lakin, 1990) using Student's test ($P<0.05$).

Results and discussion. Previous research found, that the adding of dispersions AuNP (average size ~19 nm) to cultivation nutrient medium for *B. anthracis* (strain *Sterne* 34F2) increased the cells proliferative activity and the level of biomass accumulation of germs.

Thus, when cultured in the commercial standard environment ('control') increment of biomass *B. anthracis* experimental strain accounted for no more than 10^9 colony forming units (CFU/cm^3), whereas for terms of adding to the environment AuNP level biomass accumulation anthrax increased and reached by value in 10^{15} CFU/cm^3, respectively.

It was established that the maximum increasing of the level of biomass growth of bacterial cells has been observed in the culture medium containing AuNP in dilution of 1:8 (concentration gold $1{,}90\times10^{-3}$ g/l) and 1:10 (concentration gold $2{,}38\times10^{-3}$ g/l), recorded to a dilution of culture *B. anthracis* 10^{-15} inclusive. Increasing of biomass of anthrax meaningfully CFU at 2 and 6 logarithms exceeded in a 'control'.

When cultured microbial mass ranging from 10^{-10} dilution in the medium containing AuNP range of concentrations (1:2–1:6), 1:12, and 1:20, as well as in the standard nutrient medium ('control') recorded growth retardation of *B. anthracis* by studied strain.

The research results have been protected by a declarative patent of Ukraine for a utility model (Holovko et al., 2011).

The Figure 3 shows the concentration curves of AuNP medium size ~30 nm in intact (Fig. 3, 1) and thermo inactivated (Fig. 3, 2) cells of *B. anthracis* (strain *Sterne* 34F2).

The data show that in the range of low concentrations AuNP (1.1–5.5 μg/cm^3 for the metal) weak accumulation of metal nanoparticles bacterial cells: the number of linked gold does not average exceed 50% observed (Fig. 3, 1).

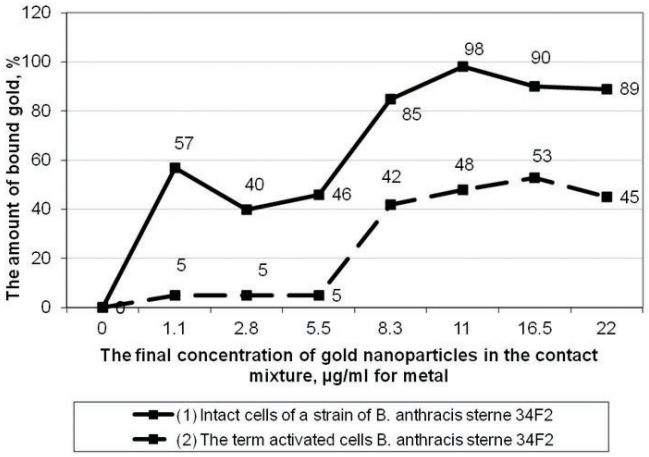


Figure 3. The concentration of binding curves AuNP (average size of ~30 nm) intact (Fig. 3, 1) and thermoinactivated (Fig. 3, 2) cells *B. anthracis* (strain of *Sterne* 34F2)

Efficiency of the AuNP interactions with *B. anthracis* cells significantly increased in concentrations of nanoparticles range (8.3–22.0 μg/cm^3 for the metal). Thus, the quantity number of Aurum bound by of Anthrax cells in the concentration range was averaged 90%.

Maximum accumulation of the AuNP by bacterial cells (98%) has been observed in the final concentration of nanoparticles 11.0 μg/cm^3 (initial concentration dispersions AuNP — 38.6 μg/cm^3 of the metal).

Inactivation of bacterial cells *B. anthracis* (strain *Sterne* 34F2) led to a drop in the efficiency of AuNP binding on average 40–50% (Fig. 3, 2). Thus, accumulation AuNP thermoinactivated cells in the range of low concentrations (1.1–5.5 μg/cm^3 for the metal) was virtually absent. The level of binding of AuNP by anthrax cells in a concentration range 8.3–22.0 μg/cm^3 for the metal was not exceed 50%.

Consequently, studies indicate that the mechanisms of contact interaction AuNP with average size ~30 nm

with of bacterial cells of *B. anthracis* strain research can be defined as passive localization by electrostatic, coordination and other types of connection (especially the structural organization of the cell surface), so and metabolism-dependent accumulation that is characteristic only active metabolized cells (determining role transmembrane potential and its generators).

The hypothesis about way do determine the basic mechanisms of the interaction of Aurum nanoparticles of *B. anthracis*, agreement with the literature (Ipatenko, 1996). So, at prompting the typical properties of *B. anthracis* and *B. cereus*, that was studied by the methods of electron microscopy indicated that *B. anthracis* cells had a thick cell membrane and more advanced membrane structure, due to increased activity of oxidation-reduction enzymes.

The presence of the folded structure of the membrane, increasing of the membrane enzymes, including H^+-ATPase, and the earlier data demonstrated stimulation of proliferative activity of *B. anthracis* (*Sterne* 34F2 strain) under the AuNP influence, suggest that the predominant mechanism of interaction with nanoparticles of tested strain is metabolism-dependent accumulation. It plays a decisive role for functioning transmembrane potential bacterial cell.

H^+-ATPase is the main generator of transmembrane potential bacterial cells. The H^+-ATPase activity of TMF cells and respiratory activity of intact of *B. anthracis* (*Sterne* 34F2 strain) cells under the influence AuNP were studied.

Figure 4 demonstrates the graph changes H^+-ATPase activity in TMF cells under the influence of AuNP average size ~30 nm to *B. anthracis* cells in the range of concentrations, compared with control values.

The most pronounced effect on the H^+-ATPase activity (by increasing its activity to 30%) TMF-growed bacterial cells comparing to control, was observed under the influence of AuNP in concentration 8.69 μg/cm^3 for the metal.

The gold nanoparticles the range of concentrations 2.90–23.16 μg/cm^3 in contact interaction with TMF-growed cells of strain *B. anthracis* has been commitment inhibition with of ATPase. Its values in overall significantly increased relative to the 'control', which indicated no deceleration rate of ATP hydrolysis in the membranes of bacterial cells.

In addition, high level of bacterial cells energy of has been the regulatory factor in their ability for active accumulation of metals nanoparticles (Danilovich et al., 2007), and also consistent with the hypothesis put forward by us regarding the mechanisms of metabolism-dependent accumulation of nanoparticles gold in *B. anthracis* cells.

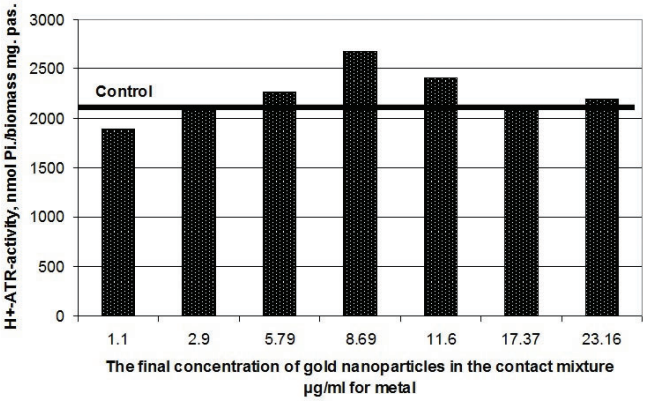


Figure 4. Changes in the level of H^+-ATPase activity TMF of bacterial cells *B. anthracis* (strain of *Sterne* 34F2) under the influence of AuNP (average size of ~ 30 nm) in the range of concentrations

Since respiratory activity is the major physiological reaction of microorganisms (Basnak'yan, Borovkova and Kuz'min, 1981), it could be used as an integral criteria for assessing the stability of the biological potential of the bacterial cell.

However, AuNP addition to the incubation medium led to a significant stimulation of the respiratory activity of the *B. anthracis* cells (Fig. 5).

It was proved that as a result of adding AuNP (average size ~30 nm) in the concentration range 0.39–7.70 μg/cm^3 for the metal to the incubation medium bacterial cells of *B. anthracis* (strain *Sterne* 34F2) led the increasion of RA on average by 74% compared with 'control'.

This concentration dependence for AuNP impact on the value of RA is not installed.

As a result of the RA study during incubation of concentration range of *B. anthracis* cells with AuNP demonstrated the safety of nanoparticles for the physiological status of Anthrax bacterial cells.

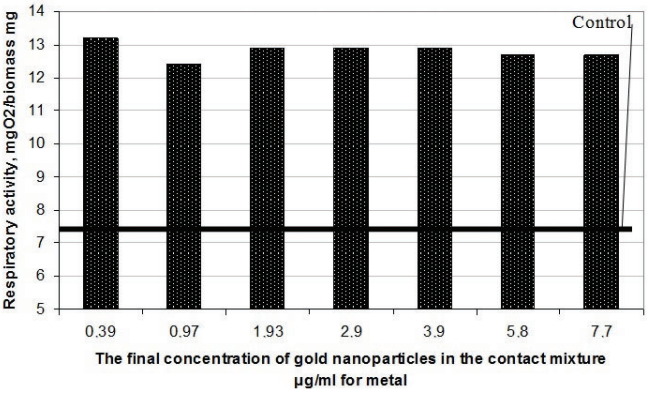


Figure 5. Changes in the level of respiratory activity of *B. anthracis* cells (strain of *Sterne* 34F2) under the influence of AuNP (average size of ~30 nm) in the range of concentrations

Conclusion. According to results of the study impact of sold nanoparticles of basic physiological and biochemical indicators of *B. anthracis* cells (strain of *Sterne* 34F2) it was appeared promising metal nanoparticles in a concentration range 2.90–8.69 μg/cm^3 for metal, as indicated by the presence of ATPase stimulation and respiratory with activity. This is also consistent with the highest accumulation of cells research AuNP along with the intensification of cell proliferative activity of agent cells.

Thus, the results indicate AuNP strong biological activity, their biocompatibility and bioavailability of the conditions of contact interaction of bacterial cells of *B. anthracis* (strain *Sterne* 34F2). On the other hand — along with the possible presence of a passive localization, mechanisms of gold accumulation in *B. anthracis* cells with metabolism-dependent nature has been deserited. It demonstrates peculiar only active metabolized cells, and reflectes within our experiment involving certain assets metabolism (determining role transmembrane potential and its generators — ATPase, RA) in the overall regulation of metabolic and functional organization systems (proliferation activity) of bacterial cells.

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Part 2. Veterinary medicine

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HACCP SYSTEM AS PASS FOR QUALITY AND SAFETY OF POULTRY PRODUCTS

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Summary. The article provides data about monitor of harmful agents of food toxicoses and toxicoinfections in the poultry farms in Ukraine for the first Principle of HACCP performance. Microbiological surveillance was conducted by test systems of R-Biopharm Company: RIDA® COUNT, RIDA CHECK. LumitesterPD-20; LuciPacPen, RIDASCREEN Verotoxin, RIDASCREEN SETA, B, C, D, RIDACREEN Salmonella AFNOR (ENISO 16140), RIDACREEN Listeria, RIDASCREEN Campylobacter, SureFoodBAC. These tests allow quick and efficient rapid diagnosis and determination of microorganisms presence, and also the number of these microorganisms.

Analysis of critical points in the poultry farms of various technological direction was conducted according to HACCP principles. Microbiological testing demonstrated: *S. aureus, S. faecalis, C. fetus, S. pneumoniae, C. perfringens, E. coli, K. pneumoniae, P. aeruginosa, P. mirabilis, P. vulgaris, E. agglomerans, S. enteritidis, S. pullorum-gallinarum, M. gallisepticum, P. multocida, Y. Enterocolitica, A. fumigatus* presense in tested materials associated with poultry breeding. *E. coli* was presented by O2, O4, O8, O78, O157. Similar microflora was isolated from all chains of poultry production. The most dangerous among them are *S. enteritidis* and *C. jeuni*. It was proved that it is necessary to provide HACCP for prevention the identification and control of potential food safety hazards that may occur in a food production.

Keywords: harmful agents HACCP, food markets, food safety, legislation, disinfectants, fertilizers

Introduction. The globalization of food markets is led for the needing to decide the problem of food safety risks and it is necessary to reduce the risks of their negative impact on human health (Arvanitoyanuis, 2009). Foodborne diseases are the significant and growing problems. The control of foodborne disease is the responsibility of food handlers, food production operators and agencies developing and implementing national food control systems. This problem has very serious and large scale character and that is why governments and leading association of food manufacturers are discussing the question of food safety and looking for ways how to support and control this problem. The main effective decision is introduction of common international standards and requirements for food safety (Bauman, 1990; Bunčić, 2009). Ukrainian production industry has to test products the effects of the international requirements of World Trade Organization (WTO). Members states of the WTO restrict access to its market for Ukrainian goods that are not fit the requirements of these countries for safety. Our companies for receiving competitive advantage in the market should introduce high development according to providing food safety. Ignoring of international normatives could negatively impact on competitiveness of Ukrainian food products and could cause damage the internal producers, both on the internal and foreign markets. Currently, the system of food safety management are using almost everywhere as the consumers protection from the dangers that can affect food. Legislation of the European Union, USA, Canada, Japan, New Zealand and of many other countries is required the introduction of food safety management. (Perović and Krivokapić, 2007). Therefore, the Ukrainian effort to access to the European Union, declares that all companies involved in food production and distribution should implement and apply the principles of HACCP, which are considered the foundation of GHP Good Hygiene Practice and Good Manufacturing Practice GMP (Curcic, Milunovic and Djuric, 2009). The HACCP system is recognized as the most effective tool to ensure that food is not contaminated or polluted, and it is safe for the consumes. Since July 1, 2003 in Ukraine the national standard ISO 4161-2003 'Systems of food safety. Requirements' and since the August 1, 2007 has entered into force the national standard of ISO 22000:2007 (identical to ISO 22000:2005) are implemented. There are some difficulties and that is why it is not easy for Ukrainian enterprises to perform requirements

of ISO 22000 (for example leased, but not their own production facilities) (Al-Kandari and Jukes, 2009) The implementation process of ISO 22000 for enterprises, where the system of food safety is functioning with ISO 4161-2003 will be easier if they will be taken into account in the beginning of manufacturing design. Standard ISO 22000:2007 combines generally recognized elements: interactive communication; system of management; and necessary conditions for program — HACCP principles. (Počuča and Radovanović, 2004; Al-Qassemi et al., 2011). Requirements of the standard can be used to create a food safety management system by all organizations, that directly or indirectly involved in the food chain, for example: feed manufacturers, farmers, producers of ingredients and additives manufacturers and suppliers of food, retail and wholesale trade, catering and organizations that provide services for the transportation, storage and distribution services for cleaning and disinfection, etc. Although manufacturers and suppliers of equipment for the food industry, detergents and disinfectants, fertilizers, pesticides and veterinary drugs, packaging and other materials could be in contact with food, etc. We should note, that the introduction of food safety management in the company — a lengthy process that applies to all services and all staff. It is not just the development of documents. It is very necessary to do training for professionals working groups and for persons that are responsible for the operational control, correction process of documentation, equipment replacement and alterations to the premises, to implement the system of food safety (Mortimore and Wallace, 2013). The HACCP — is scientifically — grounded system to ensure the production of product safety by identifying and controlling of the hazards. HACCP — Hazard Analysis and Critical Control Point system is scientifically based, rational and systematic approach for identification, assess and risks control in the process of production, processing, preparation and use of food, in order to ensure that food is safe for consumers, that it does not represent an unacceptable risk to human health (Vucinic and Milanov, 2006). The main goal of the HACCP concept is to produce a safe product. The microorganisms, causing various diseases, as well as a number of harmful chemicals, are the examples of the hazards that the HACCP concept can reduce or eliminate completely. There are no processes, that have 100% safety, but constant effort for avoiding errors must always be present (Hall et al., 2003). HACCP system provides: — identification and assessment of any physical, chemical or biological risk at all stages of food production, including all mid-processes and distribution; — it also is responsible for determination of the necessary measures for their prevention and control; — ensuring that these measures will be successfully and effectively implemented. HACCP concept as a preventive system ensures food safety in every step of the production process. Food producers and processors have implemented the HACCP program in their operation to reduce the possibility of food-borne pathogens. The Hazard Analysis Critical Control Point (HACCP) program is used to monitor and control the production process by identifying food safety hazards. Additionally, critical control points in production, processing and marketing are identified. Critical limits for each of these points are determined and surveilled for food quality and safety achievement. It is applied to the meat, egg and poultry reproduction industries. It is developed specifically for each product, product group or process and must be defined to fit the specific conditions of production and distribution of each product separately. This concept attempts to reduce the need for testing of the final product. HACCP system is the only system to ensure food safety, which proved effective and accepted by international organizations. HACCP concept as a preventive system ensures food safety in every step of the production process. It is developed specifically for each product/product group or process and must be defined to fit the specific conditions of production and distribution of each product separately. This concept attempts to reduce the need for testing of the final product. Before this system was developed, many manufacturers could find out whether their product meets certain standards only after the testing of the final product. The testing of final product can be extremely time-consuming, and can lead to the loss of a portion of the product, since some forms of the testing are extremely destructive (Bauman, 1990). HACCP concept is trying to reduce the need for testing of the final product by conducting a series of checks during the process. With the current pace range of food products are expanding, new technologies in the production and storage of food are improving or implementing, nutrition is changing, most of food is changing and spoiling. This phenomen has set before veterinary sanitary examination very responsible and challenging tasks that require strict adherence to quality control and food safety during their production, transportation and storage (Kosar and Raseta, 2005). Animal products contaminated by microorganisms such as *E. coli* O157:H7, *Listeria monocytogenes*, *Campylobacter jejuni*, *Campylobacter fetus*, *Vibrio vulnificus*, *Vibrio parahaemolyticus*, genus *Salmonella*, etc., has biological danger for the health of consumers. And they had antibiotic resistance as well (Mayes and Mortimore, 2001). Resistance to antibiotics is a problem for most countries of the world. The infections

are caused by resistant strains of *Salmonella, E. coli and Campylobacter* cause serious diseases and can be lethal for animals and humans.

The aim of our research was to monitor harmful agents of food toxicosis and food toxicoinfection for the first Principle of HACCP performance (Swanson and Anderson, 2000).

Materials and methods. Research were carried out in lab of Sumy National Agrarian University and Ukrainian poultry production farms. Microbiological surveillance was carried out with using R-biopharm's test systems, such as: RIDA® COUNT, RIDA CHECK. LumitesterPD-20; LuciPacPen, RIDASCREEN Verotoxin, RIDASCREEN SETA, B, C, D, RIDACREEN Salmonella AFNOR (ENISO 16140), RIDACREEN Listeria, RIDASCREEN Campylobacter, SureFoodBAC, that allow to conduct quickly and efficiently express diagnostics and to determine not only the presence of microorganisms, but also to estimate their quantity. Express check of surface's and liquid's cleanliness through a set RIDA®ATP was used for determining conditional-pathogenic microorganisms in poultry farms. RIDA®COUNT cards were used for express control of sanitary indicator and conditional-pathogenic microorganisms. The unique characteristics of the patented test card RIDA®COUNT make them indispensable for testing of sanitation effectiveness to any company (within HACCP) or for inspection (Stevenson, 1990).

Results. A wide range of Gram-positive and Gram-negative bacteria were detected during microbiological surveillance of the poultry associated objects. The data of quantitative and microbiological microflora's composition, isolated in farms of various technological direction, is shown in Figure 1.

Analyzing the data, we can note that the highest percentage of microorganisms which were isolated from farms in various technological areas belonged to *Escherichia genus*. Their percentage was 62.3%. Coccal microflora was isolated in 24.2% of cases. A significant numbers of *Proteus, Pseudomonas aeruginosa, Klebsiella, Yersinia, Campylobacter, Enterobacteria, Clostridia* and *Tsytrobakter* were isolated — 13.5% (Fig. 1).

Analysis of critical points in the poultry farms of various technological directions was conducted according to the HACCP principles. It was set that the same microflora was isolated from the poultry slaughter plants. It is important to know that most of the microorganisms' types are pathogens of food poisoning and toxicosis in humans and consumption of poultry products contaminated by these microorganisms dangerous for us.

The most dangerous among circulating in poultry farms pathogenic bacteria are *S. enteritidis* and *C. jeuni*. According to their serotyping salmonella were identified as: *S. enteritidis* — 46.9%, *S. typhimurium* — 14.1%, *S. pullorum* — 10.1%, *S. gallinarum* — 10.0%, *S. virchow* — 6.3%, *S. infantis* — 2.1%, *S. arizona* — 1.2%, *S. jawa* — 0.6%, *S. montevideo* — 0.4%, *S. copenhagen* — 0.4%. We can see that serovar *S. enteritidis* is dominating.

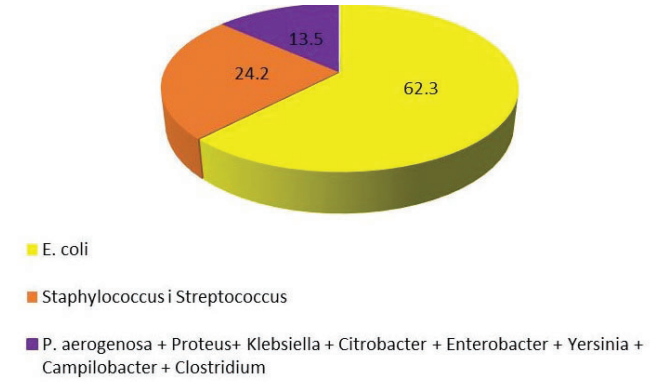

Figure 1. Comparison selection's frequency of different groups conditionally pathogenic bacteria in the poultry farms which was examined (average indicators)

It was proved that respiratory syndrome of poultry is caused most off all by *S. aureus, S. pneumoniae, C. perfringens, E. coli, K. pneumoniae, P. aeruginosa, P. mirabilis, P. vulgaris, S. enteritidis, M. gallisepticum, P. multocida, A. fumigatus.*

S. aureus, S. faecalis, C. fetus, C. jejuni, C. perfringens, E. agglomerans, E. coli, P. aeruginosa, P. mirabilis, P. vulgaris, S. enteritidis, S. pullorum-gallinarum, Y. enterocolitica were isolated at intestinal syndrome. *E. coli* were represented by O2; O4, O8; O78, O157 serovars.

Conclusion. The microbiological surveillance in poultry farms of Ukraine demonstrated that the most part of circulating in poultry farms zoonotic bacteria were represented by *S. enteritidis* and *C. jeuni*. These bacteria are the potential agents of food toxicosis and food toxicoinfection. These bacteria are strongly adopted to poultry. That is why it is necessary to provide strict detection control of infections outbreaks, which caused by bacterial etiology at all critical points of poultry production. And that is why it is necessary to conduct a hazard analysis, determine the Critical Control Points, establish Critical Limits, establish a system to monitor the control of CCPs, and to provide HACCP for prevention the identification and control of potential food safety hazards that may occur in a food production environment.

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IMMUNOSENSORS FOR THE EXPRESS DETECTION OF ANTIBIOTIC RESISTANT BACTERIAL PATHOGENS

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Summary. Resistant microorganisms can spread rapidly over countries, regions and the world, facilitated by global trade, travel and tourism. This problem concerns all countries.

The article is devoted to the analysis of methods for the indication of bacterial pathogens. The authors compare the characteristics of the immune biosensors based on the SPR, TIRE, photoluminescence and on the ISFETs with CeO_x gate surface and conclude that they have similar sensitivity and may provide to achieve low cost of analysis.

Keywords: immunosensor, bacteria, antibiotic resistant microorganisms, determination, antibody, antigen

Introduction. The antibiotic resistance phenomenon has become a global concern as geographic borders among countries and continents have become less distinct due to increasing global trade, expanding human and animal populations, societal advances and technological developments. Because of this increasing global connectivity, now we see rapid transport of infectious agents and their antibiotic-resistant genes. Thus, the use of antimicrobials in one area, such as aquaculture, can have an impact on the resistance situation in another area, such as in human medicine, and resistance problems in one country can spread to another (GAO, 2004; FAO, 2011, 2015).

Many expert panels, including WHO consultations, national committees and independent organizations, have examined the association between use of antimicrobial agents in food animals and antimicrobial resistance among bacteria isolated from humans. WHO organized two consultations, in Berlin in 1997 and in Geneva in 1998, to qualitatively assess the risk of human health consequences associated with the use of antimicrobial agents in food-producing animals (Angulo, Nargund and Chiller, 2004; WHO, 1997, 1998, 2011a, 2011b). The WHO Consultation in Geneva focused on the human health importance of fluoroquinolones and public health concern of increasing resistance to such substance, particularly among *Salmonella* and *Campylobacter.* There is the risk of human health consequences associated with the use of fluoroquinolones in food animals too. This meeting was entitled 'Use of Quinolones in Food Animals and Potential Impact on Human Health'. It was concluded at this meeting that the use of fluoroquinolones in food animals has led to the emergence to *Campylobacter* and of *Salmonella* with reduced susceptibility to them. (WHO, 1998). Similar conclusions have been presented to two committees of the Codex Alimentarius Commission: The Codex Committee on Food Hygiene (CCFH) and Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF). A risk profile on anti-microbial-resistant bacteria in food presented to the 34th of CCFH in October 2001, stated that: Antimicrobials are used in food animals for growth promotion, prophylaxis, metaphylaxis and therapy. This use is the principle contributing factor to the emergence and dissemination of Consequences of the Use of Anti-Microbial Agents in Food Animals 377 anti-microbial resistance among bacterial pathogens and commensals that have food animal reservoirs (Codex Committee on Food Hygiene, 2001; Codex Committee on Residues of Veterinary Drugs in Foods, 2001).

Bacteria can be resisted to antibiotics as a result of the chromosomal mutation or inductive expression of a latent chromosomal gene or by exchange of the genetic material through transformation (the exchange of DNA), transduction (bacteriophage), or conjugation with plasmids (extrachromosomal DNA). The last is particularly common among the Enterobacteriaceae, *Pseudomonas* and anaerobic species. In addition to conjugative plasmids, bacteria may possess transposons, the so-called jumping genes, that have the ability to enter transmissible plasmids or chromosomes (Bryan, 1988). Resistance can be transferred horizontally by plasmids or by chromosomally located conjugative transposons that spread the resistance to other species. It has been postulated that *E. coli* transferred the ability to produce P-lactamase enzymes that destroy compounds with an I-lactam nucleus into *Haemophilus influenzae* by initially infecting *Haemophilus parainfluenzae.* Intergenus spread of resistance can occur between Gram-positive species such as staphylococci and enterococci and between

Enterobacteriaceae and *Pseudononas* or anaerobes such as *Bacteroides* (Neu, 1992).

Poor infection control in any setting can greatly increase the spread of drug-resistant infections, especially during outbreaks of disease. Rapid diagnostic and infection prevention should be very essential to curb the movement of antimicrobial-resistant organisms, starting with good basic hygiene, which limits the spread of all infections, including those that are resistant to antimicrobial medicines (WHO, 2015). The antimicrobial resistance among bacteria isolated from humans could be the result of using antimicrobial agents in food animals and is leading to human health consequences (Angulo, Nargund and Chiller, 2004).

A wide range methods are available for the antibiotic resistant bacteria identification and detection, in connection with these programs, for the prevention and identification of problems related to health and safety. The choice of the method is a key factor for the detection of pathogens and the intended use of the method, for instance whether for a qualitative or semi-quantitative screening, quantitative and/or confirmatory analysis, must be clearly defined (Stead, 2014; Pividori et al., 2016).

Identification and detection of bacteria is in general required for routine surveillance and monitoring. The conventional analytical techniques for the quality and safety analyses are very tedious, time consuming and require trained personal, therefore there is a need to develop quick, sensitive and reliable techniques for quick monitoring of food quality and safety (OIE, 2016; Buchanan, 2004). In this connection biosensor is an appropriate alternative to the conventional techniques. Biosensor/immune biosensor devices are emerging as one of the foremost relevant diagnostic techniques for food, clinical and environmental monitoring due to their rapidity, specificity, ease of mass fabrication, economics and field applicability. They obtain the specificity from biological binding reaction, which is derived from a range of interactions that include antigen/antibody, enzyme/substrate/cofactor, receptor/ligand, chemical interactions and nucleic acid hybridization in combination with a range of transducers (Thakur, Ragavan, 2013).

Aim. To compare different methods for the detection of bacterial pathogens and present different types of immune biosensors which are involved into bacteria detection and compare their efficiency.

Materials and methods. The main method was analysis of the existed literature data about the efficiency of the biosensors at the reveal of different types of microorganisms.

Results. The highest sensitivity in species identification of bacteria has been achieved with molecular methods based both on polymerase chain reaction (real-time PCR, digital PCR) and isothermal amplification methods, like rolling circle amplification (RCA), recombinase polymerase amplification (RPA) and loop-mediated isothermal amplification (LAMP). In addition to the quantitative evaluation of the amplified DNA by quantitative real-time PCR, other methods have been developed, which are based on the hybridization of target DNA with highly selective probes bound to a surface. The detection limit of DNA methods range between 10 to 100 colony forming units/ml of sample (Poltronieri, de Blasi and D'Urso, 2009; D'Urso et al., 2009; Pividori et al., 2003).

Nowadays, the developed immunoassays are based upon bacterial species-specific antibodies, aptamers (Amaya-González et al., 2013; Paniel et al., 2013) and immuno-recognition of bacterial antigens (such as bacteriophage tailspike protein) (Dutt et al., 2013). These methods require standard conditions for the optimum binding of proteins or other highly-specific affinity compounds on: beads (Luminex, Austin, TX, USA), glass slides, gold surfaces, microplates and membranes suitable for chromatographic separation of antigen-antibody complexes, combined with dipsticks, microfluidic channels on paper (μPADs) (Liana et al., 2012) or lateral flow immuno-assays (LFIA), in which the capture antibody is conjugated with a detection molecule exploiting colorimetric methods, chemiluminescence or gold nanoparticles (Cimaglia et al., 2012). We found that the sensitivity of this last method applied in *Salmonella spp*. detection was approximately 10^5 CFU/ml, thus making it unsuitable for detection in pre-enrichment broth at early stages of growth (18–24 h). Presently many biosensor-based methods are still labor-intensive, expensive, and not easily implementable in-field applications.

There are now a large number of immune biosensors available for detection of target microorganisms in a variety of food, water, clinical, and industrial samples. Ivnitski et al. (1999) provided a comprehensive overview of different physicochemical instrumental techniques for direct and indirect identification of bacteria, including infrared and fluorescence spectroscopy, flow cytometry, chromatography, and chemiluminescence techniques, as a basis for biosensor construction (Ivnitski et al., 1999). Biosensor/immune biosensor development and application are exciting fields in applied microbiology. Basically, a biosensor is a molecule or a group of molecules of biological origin attached to the detector surface. When an analyte comes in contact with this surface, the interaction will initiate a recognition signal that can be registered in an instrument. An ideal biosensor should detect target molecules directly without the use of labelled ligands

or multiple washing steps. Many types of immune biosensors have been developed, including a large variety of enzymes, polyclonal and monoclonal antibodies, nucleic acids, and cellular materials. In some applications, whole cells can also be used as a biosensor. Detected analytes include toxins (e.g., staphylococcal enterotoxins, tetrodotoxins, saxitoxin, and botulinum toxin); specific pathogens (e.g., *Salmonella, Staphylococcus*, *Pseudomonas* and *Escherichia coli* O157:H7); carbohydrates (e.g., fructose, lactose, and galactose); insecticides and herbicides; ATP; antibiotics (e.g., penicillins); and others. The used recognition signals include electrochemical (e.g., potentiometry, voltage changes, conductance and impedance, and light addressable); optical (e.g., ultraviolet, bioluminescence, chemiluminescence, fluorescence, laser scattering, reflection and refraction of light, surface plasmon resonance, and polarized light); and miscellaneous transducers (e.g., piezoelectric crystals, thermistor, acoustic waves, and quartz crystal) (Shah and Wilkins, 2003).

Optical immuno-sensing technologies can be split into two categories, namely luminescence (fluorescence) sensors and label-free sensors. In the first case sensitive elements, such as proteins, antibodies, enzymes, nano-particles are conjugated with the fluorescent labels; binding analyte molecules to such receptors causes luminescence (fluorescence) or it's quenching. As result, the response can be easily visualized either by naked eye or with a suitable photodetector. The example could be the method of ELISA, which was established as a standard bio-sensing method in analytical laboratories, and other bio-sensing methods commonly compared with it. Label-free optical methods based on the phenomenon of evanescent field or wave which appear as electromagnetic wave propagating along the interface between two materials with the different refractive indices when the light enter the material with lower refractive index at total internal reflection condition (Nabok, 2016; Qi, Gao and Jin, 2011).

Starodub with group (Starodub and Ogorodnijchuk, 2012a, 2012b; Starodub et al., 1986, 2001, 2005; Starodub, Ogorodniichuk and Novgorodova, 2016; Starodub, Ogorodnijchuk and Romanov, 2011) also studied the possibility of different substances detection using SPR based onbiosensors and *S. typhimurium* was among them. Two SPR biosensors/immune biosensors were used for the study: one was based on commercial Spreeta module and the other one 'Plasmonotest' was designed V. M. Glushkov Institute of Cybernetics of National Academy of Sciences of Ukraine. As reactive part antigen-antibody interactions were used and previous working surface preparation was occurred which included several sequential steps: a) covering of surface by polyalylamine hydrochloride (PAA); b) immobilization of protein A from *St. aureus*; c) the oriented binding of the specific antibodies; d) bovine serum albumin immobilization (BSA) for blocking free non-specific binding centers on the gold surface. Polyelectrolyts are widely used for biological material immobilization on the gold surface (Starodub et al., 2005). Thin obtained films using small charged organic molecules are common since this molecules form insoluble polymer which electrostatically sorbs molecules with opposite charge (Starodub et al., 2001).

SPR can be used for the setup of immune biosensors applied to the detection of food pathogens in enrichment broth, in liquids or in food dilutions. The SPR technique for biosensing allows real-time monitoring of chemical and bio-chemical interactions occurring at the interface between a thin gold film and a dielectric interface or transparent material, such as the liquid analyte (Sun, 2014).

One of the promising optical methods for biosensors creating is TIRE. Ellipsometry is an optical method for studying of surfaces and environments, which is based on analysis of amplitude and phase changes of a light wave during its interaction with the investigated object. Later it was studied the possibility of using phase change of reflected light in terms of total internal reflection. This method was called total internal reflection ellipsometry and was firstly described in 1976 (Abelès, 1976). Getting ellipsometry parameters in terms of total internal reflection significantly increased sensitivity and detection level comparing with conventional ellipsometry and SPR technology. Thus, the method of TIRE provides a level of determination within 5×10^{-7} RIU while for ellipsometric measurements this index is 10^{-5} RIU (Iwata and Maeda, 2007). The reflected wave is formed on the edge of optically contrasting environments therefore ellipsometric measurements provide the information about optical structure of surface area and these processes that affect to its optical properties (Arwin, Poksinski and Johansen, 2004; Baleviciute et al., 2013).

A very successful commercial immunosensing instrument was based on dual polarization interferometer (DPI). The idea of this instrument is based on interference of two waves propagating in adjacent slabs and the formation of the interference pattern. Since the upper waveguiding slab is exposed to the environment, the molecular adsorption affecting the propagating wave causes the shift of the interference pattern which can be quantified in the concentrations of adsorbed molecules. The sensitivity of this method is claimed to be of 10^{-7} RIU which is comparable with the best SPR achievements. The interpretation of the results is not model-dependent (as compared to SPR

or ellipsometry), so the outcomes can be easily interpreted as changes in refractive index or thickness, or optical density of molecular layer. The instrument is however quite expensive, bulky, and obviously laboratory based (Nabok et al., 2009; Nabok, 2016).

Recently, there is a growing interest in the studying and obtaining photoluminescent nanomaterials such as nanoscale structures and quantum dots of metal oxides. This is because nanoparticles of metal oxides acquire new qualitative changes of physical and chemical properties, catalytic ability and reactivity, which are not observed in microscopic bodies of the same chemical nature. One of these properties is PhL. It is a powerful technology for development of optical biosensors since it does not require any procedures of bioreceptors preparation, complex electrical circuits and expensive equipment. The principle of PhL based biosensor operating is to measure changes of PhL spectra of nanoparticles (intensity and peak position) caused by interaction of biological components. Many different substances were successfully detected by means of nanoparticle PhL such as ions, DNA molecules, dopamine, carbohydrate antigen, bovine leukemia virus and *S. typhimurium* etc. (Guo et al., 2012; Liang et al., 2014; Qian et al., 2014; Gu et al., 2011; Viter et al., 2014).

Graphene nanostructures with their microscale area, sensitive electrical properties, and modifiable chemical functionality are excellent candidates for such biodevices at both biocellular and biomolecular scale. Graphene has already been successfully applied in immunosensors creating for numerous substances detection such as DNA and proteins (Xue et al., 2014; Zhang et al., 2013) and pathogenic bacteria are among them. Abdelhamid et al. described graphene magnetic nanosheet decorated with chitosan as a promising biosensor/immunosensor for fluorescence spectroscopy and it can be also applied for matrix assisted laser desorption/ionization mass spectrometry (MALDI) for sensitive pathogenic bacteria detection. *P. aeruginosa* and *St. aureus* were detected in cell suspension and in blood. Limit of detection of *P. aeruginosa* and *St. aureus* in suspension using fluorescence was 5.0×10^2, 4.5×10^2 CFU/ml and for blood it was 4.0×10^2, 1.0×10^2 CFU/ml for each bacteria respectively. MALDI provided sensitivity on the level of 5.0×10^2, 4.5×10^2 CFU/ml in suspension and 6.0×10^2, 5.0×10^2 CFU/ml in blood samples (Abdelhamid and Wu, 2013).

ISFETs represent the group of semiconductor potentiometric devices and are widely applied in biosensors. The interest to these devices only grows since 70s of past century when P. Bergvald and T. Matsuo have proposed pH-FET as promising transducers for biosensors (Starodub and Starodub, 2000; Caras and Janata, 1980; Van der Schoot and Bergveld, 1987) and reported that these structures are suitable for highly sensitive detection of protons generated during the biochemical reaction and as elements for integrated multifunctional and multiparametric on-chip biosensors. Starodub and Ogorodnijchuk (2012a, 2012b) developed new type of immune biosensor based on ISFETs with CeO_x instead of Si_3N_4 gate surface, which provides high sensitivity and stability of the analysis. The biosensor was used for *S. typhimurium* detection in model solutions using immune reaction. The surface was activated twice by water solution of glutaraldehyde (GA). The sensitivity of the analysis of *Salmonella* was about 2–3 cells/ml with the maximal response up to 5×10^5 cells/ml.

Discussion and conclusion. The antibiotic abuse may lead to serious disorders in living organisms. To prevent non-desirable effect from bacterial diseases there is necessary to fulfill the constant and effective control of all types of bacteria, in particular, of pathogenic ones among of different environmental objects and a special in water, foods and feed. Today medical controlling organs use a huge set of methods but, unfortunately, as a rule they are routine, expensive and demand a lot of time for accomplishing. As alternative to them, there is a new generation development of instrumental analytical approaches based on the principles of biosensorics.

Immune biosensors are the effective tools for the express detection of microorganisms in the real time. They have shown tremendous promise to overcome the limitations of the traditional methods and to provide rapid, reliable and sensitive detection of bacteria. A special attention in this article is given to the results obtained by authors at the control of antibiotic resistant bacteria as *Pseudomonas aeruginosa* and *Salmonella typhimurium* with the different types of biosensors in particular based on the surface plasmon resonance (SPR), total internal reflectance ellipsometry (TIRE), photoluminescence (PhL) and on the ion-selective field effect transistors (IsFETs) with CeO_x gate surface. Also is described the last tendencies of biosensors creation with the application of the nanostructures.

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Part 3. Emergent diseases and biosafety

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THE INTERLABORATORY TESTING OF THE TEST-SYSTEM FOR DETECTION OF THE AFRICAN SWINE FEVER VIRUS DNA USING REAL-TIME PCR 'SUIDNATESTASF'

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Summary. African swine fever (ASF) is a highly contagious viral disease of pigs that produces a wide range of clinical signs and lesions that closely resemble those of classical swine fever, and associated with the hemorrhagic lesions in pigs of different species.

Keywords: African swine fever, virus, PCR

ASF is enzootic in many African countries and the Mediterranean island, Sardinia. In June 2007, ASF was confirmed for the first time in Georgia in the Caucasus region. Since its introduction into Caucasus region, African swine fever virus (ASFV) has spread rapidly into vast areas of western and southern Russia, where it is currently (2013) circulating out of control in domestic and wild pig populations. The virus has spread to the edges of Europe, with outbreaks reported in both the Ukraine and Belarus in 2013 (OIE, 2015). Since 2014–2015 it has been widely distributed in Ukraine (both wild and domestic cases, almost 111 cases were reported by CPS of Ukraine) (CPSU news-line at www.consumer.gov.ua). Also the disease has been distributed in Poland, Latvia, and Lithuania.

Disease surveillance both in wildlife and the domestic animals is one of the most significant ways for the disease control. Multiple measures were development using serological and molecular techniques, sufficient for the virus detection (OIE, 2012).

PCR, as the direct virus detection mean is appropriate for surveillance and diagnostics of the disease. Several PCR techniques were developed and described using conventional and real-time PCR protocols. PCR allows to detect ASF agent, using primers from a highly conserved region of the genome, to detect and identify a wide range of isolates belonging to all the known virus genotypes, including both non-haemadsorbing viruses and isolates of low virulence.

Two validated PCR procedures are described and consist of a sample preparation followed by the test procedure in OIE Terrestrial Manual (OIE, 2012). These procedures serve as a general guideline and a starting point for the PCR protocol. Optimal reaction conditions (incubation times and temperatures, models and suppliers of equipment, concentrations of assay reagents such as the primers and dNTPs) may vary so the described conditions should be evaluated first.

Application of the real-time PCR allows providing an effective detection of the virus in multiple type and ranges of samples with parallel reduction of the cross-contamination risks in laboratory conditions (Stegniy and Gerilovych, 2014).

National Scientific Center 'Institute for Experimental and Clinical Veterinary Medicine' and State Scientific and Research Institute for Laboratory Diagnostics and Veterinary-Sanitary Expertise were developed *Test-system for detection of the African swine fever virus DNA using real-time PCR 'SuiDNATestASF'* due the reason of enhancement of ASF control measures in Ukraine.

The aim of the presented research is to study sensitivity, specificity, repeatability and reproducibility of this diagnostics kit.

Materials and methods. During commission trials evaluated appearance, activity, specificity, sensitivity of components of *Test-system for detection of the African swine fever virus DNA using real-time PCR 'SuiDNATestASF'* and reproducibility.

5 positive samples derived from infected pigs from Sumy and Chernigiv regions were used (blood and

spleen). Their DNA extracts were diluted 1:100–1:10000 (samples # 1–20).

For the study of the taxonomic specificity the DNA extracts from PCV2 (isolate Poltava), PPV (isolate Sumy), PRV, Aujeszky's disease virus UNDIEV18V and *M. hyopmeumonia* (SB1 isolate) were used from the collection of NSC 'IECVM' (samples # 21–25).

Also the eukaryotic porcine DNA (# 26–28) and PK15 cells DNA (# 29–30) were used.

The polymerase chain reaction was managed under the recommended conditions described in the manual to *Test-system for detection of the African swine fever virus DNA using real-time PCR 'SuiDNATestASF'*. All testing was done without changes of the recommended features of reaction. To estimate the repeatability of testing this has been performed tree times.

Table 1 – Amplification cycle for ASF virus detection

Stage	Temperature	Length	Step	Number of cycles
1	95°C	3 min	Activation of the DNA-polymerase	1
2	95°C	10 sec	DNA denaturation	45
3	58°C	30 sec	Primer annealing/ elongation	

Results. Tests were conducted to verify the commission sensitivity and specificity of the test system and reproducibility obtained when using the results.

At the beginning of the commission were checked for completeness PCR test systems. All components necessary for operation and instruction were available.

In assessing the appearance found that the test system consists of the following components:

DNA extraction kit (kit # 1):

- 'extraction buffer' (lysis buffer) — 1 flack — 15 (30) ml, transparent non-colored liquid;
- sorbent solution — 1 (2) tube — 1.5 ml, opalescentic fluid with white color;
- 'washing buffer' — 1 flack — 50 (100) ml, transparent non-colored liquid;
- 'washing buffer ethanol' — 1 flack — 50 (100) ml, transparent non-colored liquid;
- 'solution № 4' for the final washing — 1 flack — 30 (60) ml, transparent non-colored liquid;
- TEbuffer — 1 flack — 20 (40) ml, transparent non-colored liquid.

Kit for PCR amplification (kit # 2):

- 'RTPCR MasterMix' — 1 (2) tubes — 0.5 ml — transparent non-colored liquid;
- probe solution (10 pM/μl) — 1 tube — 0.02 or 0.04 ml — transparent rose liquid;
- primer solution (20 pM/μl) — 1 tube of each — 0.125 (0.25) ml — transparent non-colored liquid;
- deionized water — 1 (2) tubes — 1.25 ml — transparent non-colored liquid;
- positive control template (for 5 or 10 reactions) — 1 tube — 0.05–0.1 ml — transparent non-colored liquid.

The PCR master mix was prepared using number of samples amount plus one under proportion per sample:

№	Component	Final concentration	1× for reaction (μl)
1	Water for PCR		6.7
2	RT-PCR Master Mix-Path ID	1×	10
3	Probe ASF probe (10 pM/μl)	250 nM	0.5
4	Primer ASVF1 (20 pM/μl)	400 nM	0.4
5	Primer ASFV2 (20 pM/μl)	400 nM	0.4
	DNA (template or control)		2

The results of the sensitivity testing is the ability to identify all encrypted obviously positive samples, it was found that the test system is able to detect DNA of the African swine fever virus in both positive clinical samples, including their dilution 1:100–1:10000 with Ct value 19–28.4. This is equal to the minimum titer of virus in the pork organism, we recorded an infected animals (in the nasal swabs and blood samples) in our early works.

The specificity of the test system proved amplicon in the absence of any size of Ct value or Ct value over 38 in samples of pig's tissues from non-infected animals and culture intact cells of PK15 line. In addition, primers designed did not hybrydizated with DNA samples from other viruses, including DNA extracts from PCV2 (isolate Poltava), PPV (isolate Sumy), PRV, Aujeszky's disease virus UNDIEV18V and *M. hyopmeumonia* (SB1 isolate) (Table 2).

It was also marked by complete coincidence test results using *Test-system for detection of the African swine fever virus DNA using real-time PCR 'SuiDNATestASF'* in three repetitions under similar conditions and using different thermocyclers same type.

Table 2 – Testing results for the *Test-system for detection of the African swine fever virus DNA using real-time PCR 'SuiDNATestASF'* with the panels of positive, negative and heterogenic samples

№	Material	1st repeat result	2nd repeat result	3rd repeat result
1	*Sample 1*	Ct 18	Ct 18.2	Ct 18
2	*Sample 1 1:100*	Ct 22	Ct 22.1	Ct 22.2
3	*Sample 1 1:1000*	Ct 26.3	Ct 26.4	Ct 27.2
4	*Sample 1 1:10000*	Ct 28.2	Ct 27.4	Ct 27.6
5	*Sample 2*	Ct 19.2	Ct 19.6	Ct 19.8
6	*Sample 2 1:100*	Ct 21.2	Ct 22.4	Ct 22.2
7	*Sample 2 1:1000*	Ct 24.5	Ct 24.6	Ct 24.5
8	*Sample 2 1:10000*	Ct 28.2	Ct 27.8	Ct 27.7
9	*Sample3*	Ct 19.6	Ct 18.8	Ct 18.9
10	*Sample 3 1:100*	Ct 22.4	Ct 22.3	Ct 22.6
11	*Sample 3 1:1000*	Ct 27.3	Ct 27.2	Ct 27.2
12	*Sample 3 1:10000*	Ct 29.2	Ct 29.7	Ct 29.7
13	*Sample 4*	Ct 22.2	Ct 22.4	Ct 22.4
14	*Sample 4 1:100*	Ct 24.5	Ct 24.6	Ct 24.3
15	*Sample 4 1:1000*	Ct 27.2	Ct 27.3	Ct 28.2
16	*Sample 4 1:10000*	Ct 31.4	Ct 31.2	Ct 30.9
17	*Sample 5*	Ct 19.4	Ct 19.6	Ct 19.4
18	*Sample 5 1:100*	Ct 21.8	Ct 22.1	Ct 22.3
19	*Sample 5 1:1000*	Ct 25.5	Ct 25.6	Ct 25.5
20	*Sample 5 1:10000*	Ct 29.2	Ct 29.8	Ct 29.7
21	PCV-2 (isolate Poltava)	N/d	N/d	N/d
22	PPV (isolate Sumy)	N/d	N/d	N/d
23	PRV	N/d	N/d	N/d
24	Aujeszky's disease virus UNDIEV-18V	N/d	N/d	N/d
25	*M. hyopmeumonia* (SB1 isolate)	N/d	N/d	N/d
26	Pig intact 1	N/d	N/d	N/d
27	Pig intact 2	N/d	N/d	N/d
28	Pig intact 3	N/d	N/d	N/d
29	PK15 cells DNA	N/d	N/d	N/d
30	PK15 cells DNA	N/d	N/d	N/d

Conclusions: 1. Specificity of the *Test-system for detection of the African swine fever virus DNA using real-time PCR 'SuiDNATestASF'* developed by National Scientific Center 'Institute for Experimental and Clinical Veterinary Medicine' and State Scientific-Research Institute for Laboratory Diagnostics and Veterinary-Sanitary Expertise has the appropriate level of sensitivity and specificity, aligned with the detection of ASF virus DNA in clinical samples and their 1:100–1:10000 dilutions, in Ct values of 18–31.4, with its absence of Ct value over 38 in the 'negative samples'. The developed kit doesn't demonstrate the false positive reactions with heterogenic DNA samples of DNA extracts from PCV2 (isolate Poltava), PPV (isolate Sumy), PRV, Aujeszky's disease virus UNDIEV18V and M.hyopmeumonia (SB-1 isolate) were used from the collection of NSC 'IECVM'.

2. The sensitivity of the system can be considered satisfactory, since positive results were positive for samples with low concentrations of specific DNA.

3. Primers, probe and other components of the proposed test kits are highly sensitive, specific and comply with TU.

4. Reproducibility and repeatability of African swine fever virus DNA detection method using the ASFV target gene amplification for that is the basis of the PCR test system *Test-system for detection of the African swine fever virus DNA using real-time PCR 'SuiDNATestASF'* has the appropriate level, in all three repetitions differences were observed results.

The perspectives for further application of the results. As the commission test *Test-system for detection of the African swine fever virus DNA using real-time PCR 'SuiDNATestASF'* developed by National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' and State Scientific-Research Institute for Laboratory Diagnostics and Veterinary-Sanitary Expertise indicators of quality, such as sensitivity, specificity and reproducibility fit the requirements. In this regard, this test system can be recommended for interestablishmental testing and using in practice of the State veterinary laboratories for the detection of DNA of African swine fever virus.

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LUMPY SKIN DISEASE: CHARACTERIZATION AND POSSIBLE RISKS FOR CENTRAL AND EASTERN EUROPE

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Summary. Lumpy skin disease (LSD, nodular dermatitis of cattle) — is the contagious poxviral disease of cattle. It is characterized by severe losses and different ranges of mortality and morbidity. The disease is endemic in many Asian and African countries.

The article is devoted to explanation of LSD history, epizoothology, and distribution, risks associated with the disease, diagnostics, differential diagnostics and prevention. The situation regarding LSDV introduction to Ukraine is likely to be non-optimistic. Russia, Caucasian countries, and Bulgaria high LSD-associated risks put our territory on high range of risk regarding LSDV introduction. Disease introduction probabilities could be estimated as extremely high, and high from the side of Russia.

NSC 'IECVM' in collaboration with SSRILDVSE developed the in house PCR-based protocol for LSDV detection that requires fast implementation. Joint collaboration in area of development regional LSDV distribution control policy and contingency plan are required.

Keywords: lumpy skin disease, risk analysis, epidemiology, diagnostics, animals

Lumpy skin disease (LSD, nodular dermatitis of cattle) — is the contagious poxviral disease of cattle. Severe losses and different ranges of mortality and morbidity characterize LSD. It is endemic in many Asian and African countries, and it is rapidly spreading throughout the Middle East. Turkey, Bulgaria, Russia and Caucasian countries are affected with the disease (AHAW, 2015; OIE, 2016).

LSD clinical picture is supported by following symptoms: fever, nodules on the skin, mucous membranes and internal organs, emaciation, enlarged lymph nodes, oedema in the skin. The epicrisis of the disease is sometimes committed with death of infected animals.

The disease has the economic importance because of temporary reduction in milk production, temporary or permanent sterility in bulls and fertility of cows. LSD could be increased in the damage level in association with secondary bacterial infections (Coetzer, 2004).

Historically, the first cases of LSD were described in 1929 in Zambia. In the beginning, LSD signs were considered to be the consequence either of poisoning or a hypersensitivity to insect bites. Same clinical signs were occurred in Botswana, Zimbabwe and the Republic of South Africa between 1943 and 1945, where the infectious nature of the disease was recognized in these outbreaks (Al-Salihi, 2014).

In South Africa, LSD occurred as a panzootic, which affected eight million cattle. The disease continuous until 1949, and generate massive economic losses. In 1957, LSD was identified in East Africa in Kenya. In 1972, the disease was reported in Sudan and West Africa in 1974. Nowadays, LSD occurs in most countries in Africa (except Libya, Algeria, Morocco and Tunisia) (Tuppurainen and Oura, 2011), Asia and Mideast (Ali and Amina, 2013). One of the recent outbreaks of LSD in African continent was occurred in central Ethiopia in 2007 to 2011.

The disease has been reported in Turkey in October 2013, Iran and Iraq in 2014. The expectation of the travelling and invasion of the LSD to free neighbour countries is possible. LSD may invade north and west from Turkey into Europe and the Caucasus and East to Central and South Asia.

LSD causative agent is the virus from family Poxviridae, genus *Capripoxvirus*, called lumpy skin disease virus (LSDV). The prototype strain is the Neethling strain. These are antigenic and genetic homology in high rates with the sheep pox and goat pox viruses. LSD has a partially different geographical distribution from sheep and goat pox, suggesting that cattle strains of capripoxvirus do not infect and transmit between sheep and goats (Woods, 1988).

LSDV is susceptible to 55°C for 2 hours and 65°C up to 30 minutes. It can be recovered from skin nodules and kept at –80°C for 10 years. The infected tissue culture fluid can be stored at 4°C for 6 months. The virus is susceptible to high rates of alkaline or acid pH. However, there is no significant.

It demonstrates the susceptibility to ether, chloroform, formalin, and some detergents, e.g. sodium dodecyl sulfate. In addition, it is also susceptible to phenol, sodium hypochlorite, Virkon® (2%) and quaternary ammonium compounds. LSDV has remarkably stable

surviving for long periods at ambient temperature, especially in dried scabs. LSDV is very resistant to inactivation.

It also can remain viable for long periods in the environment. Meanwhile, the virus is susceptible to sunlight and detergents containing lipid solvents, while, in dark environmental conditions, such as contaminated animal sheds, it can persist for many months.

The LSDV genome is presented by 151 kbp dsDNA. It includes the central coding region bounded by identical 2.4 kbp-inverted terminal repeats. Viral genome contains 156 putative genes. LSDV genes share a high degree of colinearity and amino acid identity (average of 65%) of its genomic region with genes of other known mammalian poxviruses, particularly suipoxvirus, yatapoxvirus, and leporipoxviruses. LSDV is closely related to other members of the Chordopoxvirinae, it contains a unique complement of genes responsible for viral host range and virulence. The complete genome sequences of several capripoxviruses, including LSDV, sheep poxvirus and goat poxvirus, have been published (Tulman et al., 2001, 2002).

Epidemiology of LSD. The significant variation in the morbidity and mortality rates of LSD outbreaks has been observed. It depends on different factors, such as geographic location and climate, the conditions of livestock management, nutritional and keeping factors, general condition of the animal, breed of affected animals, and their immune status. Also the population levels and dissemination of putative insect vectors in the various habitats create a great level of influence. Virus virulence rate also play the significant role. The morbidity rate for LSD is ranging from 5 to 45%. However, the morbidity rates 1–5% are considered more usual.

The significant morbidity and mortality rates were described in 2009 in Holstein cattle in Asian countries (Wainwright, 2013).

Disease has been observed in various Middle East countries, where it could be recognized as the endemic. Turkey, Bulgaria, Russia and Caucasian countries are affected with the disease (Fig. 1).

The risk of introduction of LSD into the EU via the illegal movement of animals was modeled. The number of animals that need to be moved to have a probability of introduction of LSD into Europe greater than 0.95 or lower than 0.05 would be above 1'300 and below 25, respectively (seroprevalence equal to 30%), or above 7'800 and below 140, respectively (seroprevalence equal to 5%).

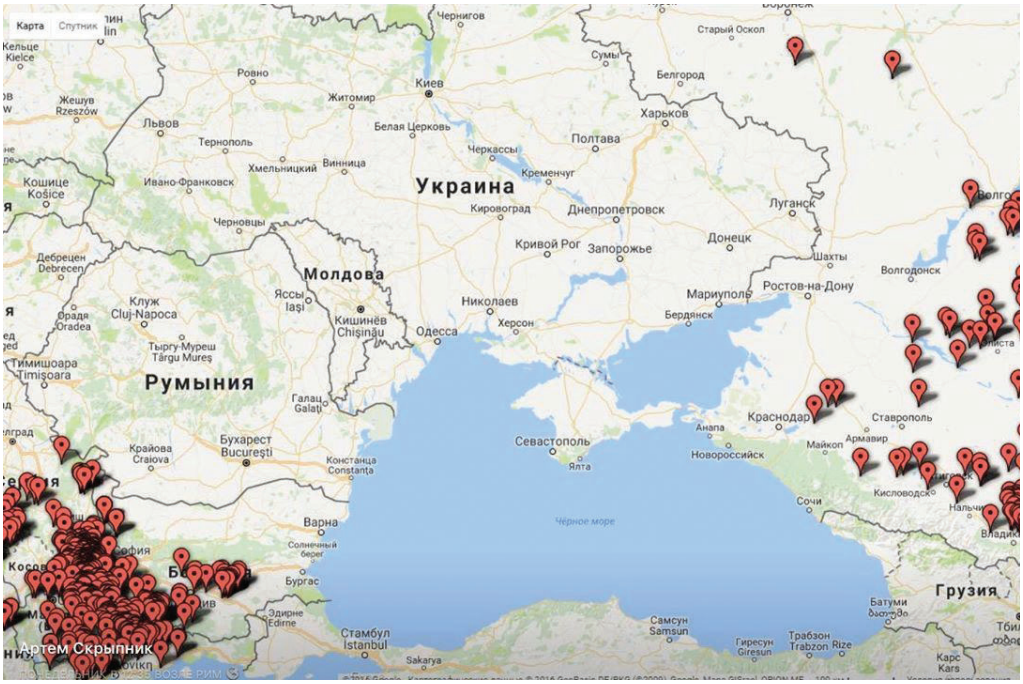


Figure 1. LSD outbreaks map (2nd September, 2016, OIE)

Based on the transmission patterns of LSD as investigated in Israel, a mathematical model was developed to simulate LSD spread between farms over space after an incursion in Greece. When the control measures entail the removal of animals showing generalized clinical signs, approximately 90% of epidemics remain confined to the region around the initial site of incursion. However, the remaining 10% of simulated epidemics are more extensive, with the virus spreading up to approximately 300–400 km from the

site of introduction by six months after the incursion. This identifies the potential for disease outbreaks to spread in Bulgaria and Greece.

Regarding the risk of LSD becoming endemic in animal populations in the EU, owing to a lack of data regarding the ability of potential European vectors of disease transmission, the international data cannot be extrapolated directly to the European situation. Nevertheless, under the current EU policy and according to the scenarios produced using the spread model, if the situation and ability of vectors was the same as in Israel, LSD would most likely not become endemic in the EU.

Situation regarding LSDV introduction to Ukraine is likely to be non-optimistic. Russia, Caucasian countries, and Bulgaria high LSD-associated risks put our territory on high range of risk regarding LSDV introduction. Disease introduction probabilities could be estimated as extremely high, and high from the side of Russia. The first way for possible introduction could be potentially associated with warm and wet summer-spring period, sufficient for growing of the population of different insects, potentially could be LSDV transmission factors in the wildlife and farming animals, especially backyards kept on free pastures.

Also the Eastern way of introduction is actual because of absence of proper antiepizootic control measures in occupied territories of antiterrorist operation in Lugansk and Donetsk regions of Ukraine.

Table 1 – Transboundary risks estimation for LSD introduction from Central Europe in Ukraine

Risk factor	High level	Midlevel	Low level
Existing of the disease in the wildlife	+		
Existing of the disease in domestic animals	+		
Existing of the transmission vectors	+		
Existing of the transboundary transmission ways (migratory wildlife)	+		
Transport communications (migration of people and trade)	+		3+3+3+3+3=15 Extreme risk (confirmed)

The transmission of LSD virus (LSDV) could be potentially executed via insects, or by the natural contact transmission in the absence of insect vectors.

Lesions and signs of the disease. LSD gross lesions include the skin nodules that usually may fuse into large irregular and circumscribed plaques; they have different sizes and ranges. The cut surface of the nodules is reddish-gray. They contain serous fluid and edema in the subcutis layer, after induration they may form deep ulcers. The typical circular necrotic alimentary lesions may also be seen on the muzzle, nasal cavity, larynx, trachea, bronchi, inside of lips, gingiva, dental pad, forestomach, abomasum, uterus, vagina, teats, udder and testes (Fig. 2) (Ali et al., 1990).

Table 2 – Transboundary risks estimation for LSD introduction from Russia in Ukraine

Risk factor	High level	Midlevel	Low level
Existing of the disease in the wildlife		+	
Existing of the disease in domestic animals	+		
Existing of the transmission vectors	+		
Existing of the transboundary transmission ways (migratory wildlife)	+		
Transport communications (migration of people and trade)		+	3+2+3+3+2=13 High risk (confirmed)

The regional lymph nodes are grossly enlarged in 3–5 times from their usual size. The oedematous and pyaemia changes are occurred. Muscle tissue and the fascia over limb muscle may show nodular lesion that are grey-white surrounded by red inflammatory tissue. The same nodules are distributed throughout the carcass. It is about 10–30 mm diameters in the kidney. Interstitial or bronchopneumonia associated with 10–20 mm diameter lesions are also scattered in the lungs.

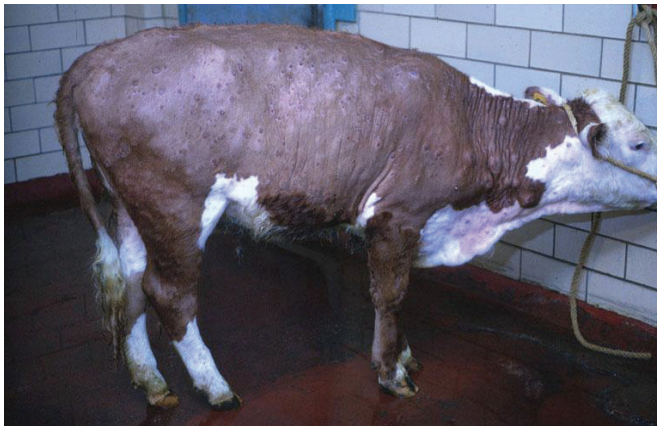
The lesions are separated from the necrotic epithelium far from the healthy tissue. The necrotic tissue sloughs away to leave an ulcer that slowly heals by granulation. Severely infected animals show secondary bacterial pneumonia, tracheal stenosis, acute and chronic orchitis, mastitis with secondary bacterial infection, and similar lesions in the female reproductive tract (Al-Salihi, 2014).

Pathological lesions of the LSD disease in microlevel vary considerably depending on the stage of development. In the acute stage of the disease,

it is mostly characterized by lesions of vasculitis, thrombosis, infarction, and perivascular fibroplasia. Inflammatory cell are infiltrated the infected areas, which includes macrophages, lymphocytes and eosinophils.

A

B



C

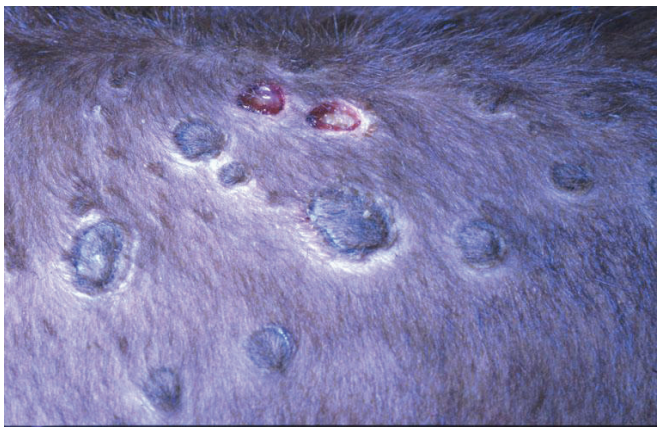


Figure 2. A LSD lesions (A — photo by Orap Zenzele from http://orapzimbabwe.blogspot.com/2014/06/wh-t-is-lump-y-skin-disease-lsd-it-is.html; B and C — photos by Noah's Arkive, PIADC from http://www.cfsph.iastate.edu/DiseaseInfo/disease-images.php?name=lumpy-skin-disease).

The oedema and infiltration of the epidermis and dermis with large epithelioid macrophage type cells, presented in LSV-affected animals, are well described for sheep pox. They are found with plasma cells and lymphocytes in early lesions, and in older lesions, fibroblasts and polymorphonuclear leucocytes with some red cells predominate (Al-Salihi, 2014; Ali and Amina, 2013).

Diagnostics. The diagnostics of LSD should be managed in the complex way, including data of epidemiological investigation, pathological examination (on macroscopic and microscopic levels), and confirmatory laboratory diagnostics, using different tests and tools.

The laboratory testing and LSDV identification are based on OIE Terrestrial Manual (OIE, 2016). Confirmation of lumpy skin disease in a new area requires virus isolation and identification. Samples for virus isolation should be collected within the first week of the occurrence of clinical signs, before the development of virus-neutralising antibodies (Davies, 1991). Skin biopsies of early lesions could be used for the virus isolation. In addition, LSD virus can be isolated from buffy coat from the blood sample collected into EDTA or heparin during the viraemic stage of LSD. Samples should be taken at least from three animals belonging to affected herd. Samples aspirated from enlarged lymph nodes can be also used for virus isolation. LSD virus grows in tissue culture of bovine, ovine or caprine origin. Bovine dermis cells or lamb testis (LT) cells (in primary or continuous culture), are considered to be the most susceptible cells. LSD capripoxvirus have been also adapted to grow on the chorioallantoic membrane of embryonated chicken eggs and Vero cells. But these are not recommended for primary isolation (OIE, 2016; Al-Salihi, 2014).

Transmission electron microscopy (TEM) can be used for confirmatory diagnosis of LSD. Skin specimens or mucosa swabs are used. Mature capripox virions have an average size 320×260 nm and are a more oval profile and larger lateral bodies than orthopox virions (OIE, 2016).

The *Capripoxvirus* antigen can also be identified on the infected cover slips or tissue culture slides using fluorescent antibody tests. An agar gel immunodiffusion (AGID) test has been used for detecting the precipitating antigen of capripoxvirus, but has the disadvantage that this antigen is common with parapoxvirus.

The recombinant antigen for the production of P32 monospecific polyclonal antiserum and monoclonal antibodies (MAbs) was developed for virus antigen detection using ELISA (Carn et al., 1994).

Polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP), as very fast tools for agent's identification are widely used and recommended by OIE. The assay have been used for detection of capripoxviruses with higher sensitivity (Bowden et al.,

2009). In house PCR test has been development in NSC 'IECVM' and SSRILDVSE based on FAO protocol (Stegniy B. T., Nevolko O. M. et al., pers. comm., APHL, 2014).

Multiple serological examination tools are also developed for surveillance and control of LSD: ELISA, VN, Western blot analysis (Tuppurainen and Oura, 2011).

There are many diseases causing similar signs of LSD. It is important to obtain a definite ***differential diagnosis*** to ensure the best preventative and control measures for susceptible herds.

LSD can be confused with the following diseases:

- Pseudo-lumpy-skin disease,
- Bovine virus diarrhoea/mucosal disease,
- Demodicosis (Demodex),
- Bovine malignant catarrhal fever (Snotsiekte),
- Rinderpest,
- Besnoitiosis,
- Oncocercariasis,
- Insect bite allergies.

Prevention of LSD. As far as LSD vaccines are concerned, only live attenuated vaccines against LSD are currently commercially available. RM-65 attenuated sheep pox vaccine at the recommended dose for sheep has limited effectiveness in protecting animals from LSD. There is field evidence that 10 times the dose of RM-65 is more effective in terms of protection, although is less effective than vaccination with a homologous strain. The Neethling attenuated lumpy skin disease virus vaccine is highly effective in the prevention of morbidity, thus confirming the need to use homologous vaccines for the control of *Capripoxvirus* infections. Nevertheless, some safety issues have been reported that are linked to generalize clinical reactions due to vaccination with LSD strains that can be observed.

Concerning the effectiveness of control measures, according to Israeli experience, while using the attenuated RM-65 vaccine at the recommended dose for sheep; culling only those animals with generalized skin lesions has controlled epidemics of limited extent. Large epidemics can be controlled by the use of effective vaccination. Epidemics are not self-limiting when effective vaccination or culling are not applied. Although insecticides are frequently used to control LSD outbreaks, there is no evidence to date to prove their effectiveness in controlling LSD spread.

The AHAW Panel recommends further investigation into the potential relevant vector species for LSD transmission in controlled environments and the mode of transmission, besides the ecology of different blood feeding and biting arthropod species in the cattle farming setting. In relation to this, the effectiveness of insecticides for LSD control should also be investigated.

Owing to the risk of LSD spreading from the Middle East to the rest of Asia or to Europe, the development of safe, efficient and non-replicating 'differentiating infected from vaccinated animals' (DIVA) vaccines against LSDV is required, as well as an associated diagnostic test. Furthermore, the efficacy of currently available live vaccines in cattle against LSDV should be evaluated using challenge experiments in controlled environments (AHAW, 2015).

Conclusion. LSD is the high risks associated emergent disease of cattle. It demonstrated high trends of transboundary distribution in Central and Eastern Europe and requires development of new strategies of surveillance and control. NSC 'IECVM' in collaboration with SSRILDVSE developed the in house PCR-based protocol for LSDV detection that requires fast implementation. Joint collaboration in area of development regional LSDV distribution control policy and contingency plan are required. It needs development of the multi-authorities collaborative effort.

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News

ABOUT THE REGIONAL SCIENTIFIC WORKSHOP 'IMMEDIATE MEASURES FOR THE SPREAD COUNTERMEASURES OF AFRICAN SWINE FEVER IN PIG FARMS AND LUMPY SKIN DISEASE PREVENTION IN THE CATTLE FARMS OF KHARKIV REGION'

The regional scientific workshop 'Immediate Measures for the Spread Countermeasures of African Swine Fever in Pig Farms and Lumpy Skin Disease Prevention in the Cattle Farms of Kharkiv Region' was held October 12, 2016 in the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'.

Kharkiv Region State Administration organized the workshop with the participation of Main Department of State Service on Food Safety and Consumer Protection in Kharkiv Region and NSC 'Institute of Experimental and Clinical Veterinary Medicine'.

Deputy heads of district administrations, representatives of of rural councils, local authorities of State Service on Food Safety and Consumer Protection, veterinary doctors and scientists attended the seminar.

With a foreword by the audience turned deputy head of regional state administrations Bekker M.L. He noted that the management of districts, towns, villages and cities where no outbreaks of ASF shall ensure that all safety measures for non-proliferation diseases.

Epizootic situation in the Kharkiv region, Ukraine and European countries on the African swine fever, spread of the lumpy skin disease of cattle in Europe and the risk of disease entering the territory of Ukraine were disclosed in the speech of the Acting Chief of State Service on Food Safety and Consumer Protection in Kharkiv Region Tertyshnyk A.V.

Director of NSC 'IECVM', Academician of NAAS Stegniy B.T. in his lecture revealed the role of the NSC 'Institute of Experimental and Clinical Veterinary Medicine' in scientific support veterinary support livestock and proposed a system of urgent measures to prevent the spread of the pathogen of African swine fever in pig farms and directions scientific support problems in Ukraine.

Risk analysis and emergency measures to prevent the lumpy skin disease of cattle herds in the Kharkiv region presented the deputy director of NSC 'IECVM' for Research, Professor Gerilovych A.P.

Director of the Institute of Animal-breeding of NAAS, Corresponding Member of NAAS Rudenko E.V. has been disclosed the question of monitoring the quality of raw milk in the context of early diagnosis of diseases of cattle and feed quality as a prerequisite for Animal Health.

At the end of the seminar there was the discussion on the above named issues, which was attended by heads of district administrations and experts of State Service on Food Safety and Consumer Protection. In particular, experts discussed measures to eliminate the causative agent of African swine fever in the territory Dergachi, Kharkiv and Chuguevsky districts of oblast also raised problem of compensation for stamped-out piglets.

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