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ANALYSIS OF QUALITY AND SAFETY INDICATORS OF POULTRY MEAT DURING PRIMARY PROCESSING

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Summary. The article presents the analysis results of quality and safety indicators as to the poultry meat during primary processing. Based on bacteriological studies the microbial contamination of the poultry carcasses during the slaughter process was investigated.

It was set that the highest contamination of the poultry meat takes place in the cooling chamber. The quantity of MAFAnM 5.6 times exceeds the norm.

At the analysis of factors affecting microbial contamination of broiler chickens carcasses it was set, that air and equipment in the production premises, hands and staff overalls could be the reason that does not correspond the quantity index of MAFAnM in 1 cm^2 of final product and be an additional source of meat contamination.

Keywords: meat, poultry carcasses, microorganisms, contamination, air, equipment

Introduction. Contaminated meat becomes growing medium for activity and reproduction of the microorganisms during the primary animal slaughter processing. Contamination of raw meat by microorganisms starts during slaughter process, when the microorganisms from skin, gastrointestinal tract and lymph nodes of the animals and equipment surfaces contaminate the carcass. The main sources of carcass contamination are faeces, animal skin and bird feathers, that contain a large number of microorganisms (CAC, 1993; EP and CEU).

Additional bacterial contamination source for carcass could be: air, water, hands and stuff overalls, slaughter and butchering instruments, etc. The most significant meat contamination occurs while technological processes of evisceration, when skin removing, removing internal organs and dry or wet cleaning carcasses (Oliynyk, 2004; Yakubchak et al., 2003; Anderson, Marshall and Dickson, 1991; Antic et al., 2010).

Due to the diversity of contamination sources, seasonal differences and different conditions in slaughterhouses, the number and types of micro-organisms that could be found in meat at animals are widely varied (CAC, 1993; EP and CEU).

It should be noted that to avoid meat contamination completely is nearly impossible, but it could be controlled at slaughter and animal processing. This control is the most important system element of 'correct manufacturing and sanitary-hygienic practices' (GMP and GSP) and HACCP programs that are aimed to obtain the safe meat products with high quality (Minaev, Bataeva and Krasnova, 2008; Rodionova, 2016). It is necessary provide sanitary-microbiological control of the water which is used in technological process (Rodionova and Paliy, 2017), the staff hygiene (Paliy, Rodionova and Paliy, 2016), organization of supervision veterinary objects as well (Rodionova and Paliy, 2016).

The aim of the work. To carry out micro-biological testing at the meat processing enterprises, where slaughtering and poultry primary processing take place, to determine the safety degree of the products.

Materials and methods. The experimental part of the work was carried out in the poultry farm of Volynska oblast and Laboratory of veterinary sanitation and disinfectology of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv).

The objects of study were the bird carcasses. Before swab sampling, quality of carcass processing were visually determined: carcass and its parts contamination by filth after defeathering etc.

Non-destructive swab sampling method, was used to study according to ISO 17604 requirements. Samples were selected from different technological points of the poultry slaughter process and carcass processing at slaughterhouse enterprises.

The sampling area was 100 cm^2 at each section. Before the swab sampling, the swab was moistened in 10 cm^3 peptone-salt solution. For each space, selected for sampling, the stencil frame was placed. The total area inside frame stencil was wiped by swab in the horizontal direction with a little pushing and turning for better use the whole swab area. Then the swab was placed into transport media. The dry swab and wipe were collected in the same area again, as mentioned above, and placed it into the same flask with solution.

After sampling, the specimens were transported at temperature of 4–5 °C (in a fridge-bag) to the laboratory. The samples were examined within 2 hours.

Afterwards the numbers of serial dilutions according to the standard technique were prepared. Determination of total number of microorganism was carried out of all prepared dilutions. For this purpose the volume of 1.0 cm^3 of each dilution was transferred into sterile Petri dishes and poured by molten and cooled to a temperature of 55 °C MPA. Plating incubation was produced at 37 °C for 48 hours. Total microorganism number per 1.0 cm^2 of area were determined.

Coli-titer indicators were determined by primary inoculation and swab dilutions 1:10 in test flask with the Code medium (MacConkey Broth), with further thermostat incubation at 37 °C within 24 hours. Accounting test was held according to the standard technique after cultivation.

Sanitary conditions and bacteriological air testing were carried out by air sampling sedimentation method using meat-peptone agar (MPA) and medium Sabouraud according to standard technique.

Results. The slaughter and poultry processing shop were brought into service in 2015. Nowadays, enterprise capacity is 110 tons per day. The enterprise assortment includes more than 30 positions in chilled and frozen form. The main product is broiler chicken carcasses, semi-finished products and by-products made from broiler chickens.

The enterprise collects the birds for slaughtering after the presentation of veterinary reference or veterinary certificate and lading bill and only then they are taken out. The veterinary inspector examines veterinary-sanitary status and makes the thermometry of the poultry.

Chicken carcasses have series of sequential operations during the production process. The speed and duration of each stage should be focused on maximizing freshness, palatability and attractive meat presentation.

Control of sanitary days, exact mechanical purification, tools and equipment disinfection are carried out at the enterprises. Current mechanical purification proceeds continuously throughout the day to prevent product contamination. According to the data from our study floor during the day, was not disinfected, but if necessary, washed by water. Tools, which are used, are purified by hot water and disinfected.

Series of microbiological studies were conducted with the aim to evaluate the carcasses contamination by pathogenic and conditionally pathogenic microflora because the primary poultry processing significantly influences on the quality of meat produced.

On the first stage of our research, we determined the general number mesophilic-aerobic and facultative-anaerobic microfloras while the total slaughtering process line and processing poultry (Table 1). Samples were taken during work time in equal period of time.

It was established that the main contamination occurs in evisceration and half-evisceration process when intestine, gall bladder and egg follicles are damaged. Examination of carcasses swaps at this area show constant growth MAFAnM during the work shift, from $5.3\pm0.03\times10^2$ CFU/cm^2 at the beginning of the shift to $11.3\pm0.02\times10^2$ CFU/cm^2 at the end of the shift. The most contaminated carcass part at evisceration bay was the outer part of the skin $10.7\pm0.5\times10^2$ CFU/cm^2. Some decrease of MAFAnM was observed in the middle of work shift due to the fact cleaning floors, walls and equipment with water under pressure after each slaughter process.

The following technological process was chilling. Poultry meat was chilled to prevent microbial contaminate. Cooling of the eviscerated carcasses processed by the cold water baths or value drip chilling chamber at temperature from 0 to 2 °C. Cooling process continues until the muscles temperature inside is lowered to 4 °C. Such chilling does not kill the bacteria, but only inhibits their reproduction. Part of microorganisms is washed off by immersion of poultry carcasses into the cooling baths, but it increased the risk of cross-contamination of poultry bodies.

Analyses of swab samples from broiler chickens carcasses during transport from screw bath No 1 to screw bath No 2 determined that the quantity of MAFAnM on the studied objects surface during working time increases in 4.4 times and at the end of the work shift is 1.9×10^2 CFU/cm^2. The most contaminated part was the neck skin $7.6\pm0.4\times10^2$ CFU/cm^2.

During the study, swab samples from poultry carcasses after cooling bath, the quantity of MAFAnM varied in the range of $0.6\pm0.04\times10^2$ CFU/cm^2 to complete growth on the nutrient media.

According to the study of quantity MAFAnM on the broiler chickens carcasses surface in the cooling chamber was determined that on this bay there is the greatest contamination of raw meat. The quantity of MAFAnM exceeds the norm in 5.6 times (Fig. 1).

Swab samples contained *Escherichia coli* group (ECBG) that proved violations of sanitary-hygienic control in workplace at all bays of primary poultry meat processing.

To identify the factors, causing the influence on microbial contamination of broiler carcasses, we carried out a number of additional microbiological testing. The results of the study are shown in Table 2.

Table 1 — The results of bacteriological analyses of swab samples from chicken broiler carcasses during the slaughtering technological process

Technological stage	Sampling area	*E. coli* Group Bacteria (presence/ absence)	Quantity of MAFAnM, $\times 10^2$ CFU/cm^2 (Standard till 1000 CFU/cm^2)		
			Time taking samples		
			6.00-8.00	9.00-12.00	14.00-17.00
Evisceration bay	Outer skin surface	revealed	10.7±0.5	4.9±0.2	16.0±0.5
	Neck skin	revealed	4.1±0.15	1.7±0.02	9.9±0.4
	Carcass	revealed	1.1±0.04	1.8±0.02	7.9±0.2
Transfer from screw bath No 1 to screw bath No 2	Outer skin surface	revealed	4.6±0.2	2.2±0.02	19.0±1.2
	Neck skin	revealed	7.6±0.4	2.3±0.04	21.1±0.15
	Carcass	revealed	2.8±0.15	1.8±0.07	17.3±0.2
After chilled bath	Outer skin surface	revealed	0.7±0.1	general increase	general increase
	Neck skin	revealed	0.6±0.04	2.1±0.07	10.7±0.3
	Carcass	revealed	0.9±0.03	1.0±0.01	15.7±0.1
After air chilled camera	Outer skin surface	revealed	80.3±0.3	general increase	general increase
	Neck skin	revealed	6.9±0.1	22.1±0.15	34.3±0.3
	Carcass	revealed	6.5±0.03	11.7±0.2	42.1±0.1

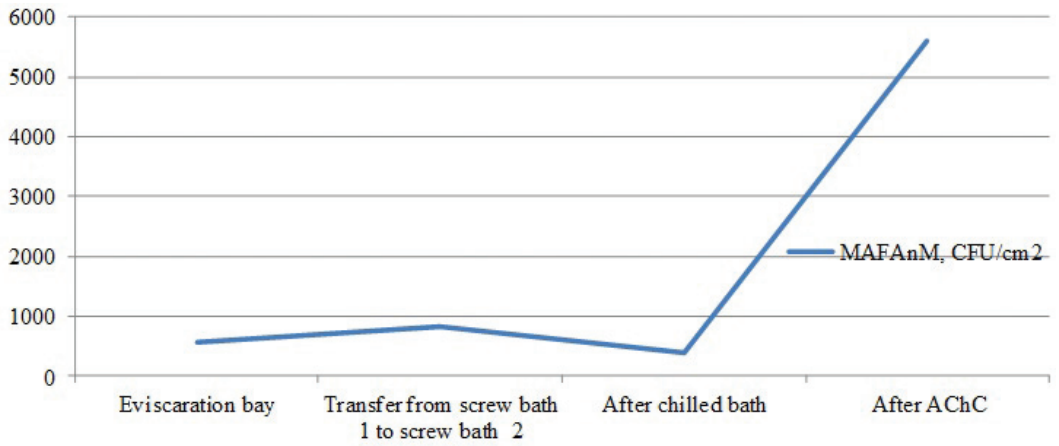

Figure 1. Broiler chicken carcasses microbial contamination in primary processing

According to the results of sanitary-microbiological research it was determined that the total microorganisms number in the air at the slaughter bay and poultry processing line was exceeded standard norms in 3.3 times, and did not correspond to established norms in cooling chamber. Thus, it could be concluded that internal industrial premise air might be the discordance reason of quantity MAFAnM in 1 cm^2 of readymade product.

In addition, we investigated sanitary condition of equipment, tools, hands and stuff overalls (Table 3).

Table 2 — Analysis of sanitary-microbiological air state

Bay name	Total microorganisms quantity, CFU/cm³		Fungi, CFU/cm³	
	Fact	Standard	Fact	Standard
Poultry slaughtering bay	3.3×10^4	1.0×10^4	8	20
Chilling chamber	1.1×10^4	1.0×10^4	6	20
Poultry evisceration bay	7.4×10^3	1.0×10^4	3	20
Poultry weighting, sorting, evisceration and packaging bay	2.7×10^3	1.0×10^4	2	20
Frozen products weighting, sorting, evisceration and packaging bay	7.4×10^3	1.0×10^4	2	20

Table 3 — Analysis of equipment sanitary-microbiological condition

Bay name	Total microorganisms quantity, CFU/cm³		E. coli Bacteria Group (presence/absence)
	Fact	Standard	
Suspended conveyor (bleeding line)	2.2×10^3	1.0×10^3	revealed
Suspended conveyor (defeathering line)	2.7×10^3	1.0×10^3	revealed
Suspended conveyor (Evisceration bay)	1.0×10^3	1.0×10^3	revealed
Suspended conveyor (camera AChC)	3.4×10^3	1.0×10^3	revealed
Knives	2.1×10^3	1.0×10^3	revealed
Stuff hands	1.8×10^3	1.0×10^3	revealed
Stuff overalls	2.3×10^3	1.0×10^3	revealed

From the presented results in Table 3 results, it was determined that the sanitary condition of equipment, tools, clothing and stuff hands were do not correspond to microbiological standards. Investigation of equipment sanitary state detected that the quantity of MAFAnM in general is 2.3×10^3 CFU/cm² and relatively exceeded established standard in 2.3 times. Swab samples analysis standard on the knives were exceeded in 2.1 times. The MAFAnM quantity on stuff hands and overalls is 1.8×10^3 CFU/cm² and 2.3×10^3 CFU/cm² relatively and also does not correspond to the standard requirements. In addition, it should be noted that all of swab samples that are taken from the investigated objects secured *E. coli* Bacteria Group.

Conclusions. Control of the meat contamination during primary processing is one of the most important elements of appropriate manufacturing and hygienic practices (GMP/GHP) during the safe and high quality meat production.

On the basis of bacteriologic examinations the steady height of MAFAnM was set on the surface of carcasses of chickens of broilers during the technological process of slaughter and processing of bird from $5.3\pm0.03\times10^2$ CFU/cm² to $42.1\pm0.1\times10^3$ CFU/cm².

The most severe contamination is in the cooling chamber. The quantity of MAFAnM on the carcass surfaces on this site 5.6 times (5.6×10^3 CFU/cm²) exceeds the norm.

The air and equipment in the premises, hands and overall of workers can be the reason of correspondence lack as to the index of MAFAnM quantity in 1 cm² of the final product and become another source of meat contamination.

Further research prospects: the development of effective disinfection modes of technological equipment at meat processing enterprises by modern integrated disinfection means.

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CLINICAL AND BIOCHEMICAL ASPECTS OF DIAGNOSTICS OF OSTEODYSTROPHY OF GOATS

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Summary. The purpose of this study was to carry out the comparative analysis of data of the clinical examination of goats and biochemical testing of bone tissue metabolism of the metabolites level in blood serum and urine for determination of criteria at diagnostics of osteodystrophy. The study was conducted on 20 adult goats. Diagnosis for osteodystrophy was made according to physical examination of goats and analysis of biochemical tests of bone tissue metabolism using blood serum and urine. Activity of aminotransferase (alanine aminotransferase, aspartate aminotransferase) and alkaline phosphatase and also concentration of glycoproteins (GP), chondroitin sulphate and fractional content of glucosaminoglicans content of total and ionized calcium were determined in blood serum. The content of hydroxyproline and total uronic acid were defined in urine. Comparative analysis of total clinical status of goats and level of biochemical components in blood serum and urine that characterize the connective tissue status at diagnostics of osteodystrophy was conducted. The intergroup significant differences of some biochemical parameters (aspartate aminotransferase, alkaline phisphatase, chondroitin sulphate in blood serum and fraction II mucopolysaccharides, sum of fractions glucosaminoglicans in urine) were observed. Further testing must be conducted to determine the biochemical parameters in goat with subclinical signs of osteodystrophy.

Keywords: osteodystrophy, biochemical parameters, goats, blood serum, urine

Introduction. The problem of diagnostics of different stages of osteodystrophy is the relevant issue of modern veterinary science. One of the main directions of research in this field is working out and implementation of non-complicated methods of diagnostics, for including to laboratory practice (Kondrakhin, 1989).

Conducting of biochemical analysis of blood serum is limited by facilities of regional veterinary laboratories in the farms, taking into account that for diagnostics of osteodystrophy they mainly determine only content of total calcium and inorganic phosphorus in the blood serum. These indexes characterize in a certain measure the state of mineral homeostasis that changes substantially only in the perspectives of abnormalities metabolism and considerable impairment of structure of the bone tissue. It is not always possible to define such pathology as osteodystrophy, especially at the early stage. Methods for determination of content and resulting metabolites of organic matrix of connective tissue in blood serum and urine which reflect the state of bone tissue are more informative for diagnostics of early stage of osteodystrophy (Liesegang, Risteli and Wanner, 2007; Cruz, Lima and Peleteiro, 2002; Carstanjen et al., 2004). Information about the status of organic content of bone tissue of goats in the normal range and with osteodystrophy is quite limited (Timoshenko and Maslak, 2008).

Analysis of the level of biochemical indicators in blood serum of goats is a relevant problem these days.

The purpose of the present study was to conduct comparative analysis of data that have been obtained after the clinical examination of goats and analysis of bone tissue metabolism markers level in blood serum and urine for determination of criteria at diagnostics of osteodystrophy.

Materials and methods. Twenty 2–3-year-old goats were examined in this. They were bred in 'Study and Scientific Center of Plant Growing and Animal Husbandry of Kharkiv State Zooveterinary academy'. Samples of blood serum and urine were collected for biochemical parameter analysis.

Activity of aminotransferases (alanine aminotransferase, aspartate aminotransferase) and alkaline phosphatase (Kamyshnikov, 2000) and also concentration of glycoproteins (GP), chondroitin sulphate and fractional content of glucosaminoglicans (Shteynberg, and Dotsenko, 1962; Shtern et al., 1982) were determined in goat blood serum. Content of total and ionized calcium in blood serum were defined using the analyzer of electrolytes (AEK-01). The content of hydroxyproline and total uronic acid in urine were determined using the techniques by Krel' and Furtseva (1968) and Di Ferrante and Rich (1956) respectively.

The results of the research were processed with biometric statistics techniques using Microsoft Excel.

Results and discussion. It was established that the average temperature was 39.2±0.1 °C (Lim 38.5–40.0) and pulse rate was 89.0±6.0 (Lim 70.0–110.0) on the basis of clinical examination. Fourteen animals (70%) had dullness of heart tones. The number of respiratory movements per minute was 15.3±0.9 (Lim 12.0–20.0).

Three of the examined goats (15%) had pain of liver on palpation. So biochemical investigations of blood serum and urine were conducted for objective assessment of clinical data (Table 1).

More detailed information about the state of bone-articular system was collected by the determination of metabolites that characterize the state of components of organic matrix of connective tissue (Table 2).

Table 1 — Biochemical indicators of blood serum of goats with clinical signs of osteodystrophy

Indicator		Clinically healthy	Osteodystrophy
Alanine aminotransferase, U/L	M±m	28.50±1.04	33.80±2.71
	Confidence interval	25.7–31.3	27.8–39.8
Aspartate aminotransferase, U/L	M±m	26.00±1.08	38.90±1.56 ***
	Confidence interval	23.1–28.9	34.8–41.7
Alkaline phosphatase, m/L	M±m	3.78±0.63	8.31±1.09 *
	Confidence interval	2.08–5.48	5.85–10.77
Total calcium, m/L	M±m	2.67±0.08	2.78±0.05
	Confidence interval	2.45–2.89	2.68–2.88
Ionized calcium, m/L	M±m	1.28±0.04	1.32±0.04
	Confidence interval	1.18–1.38	1.24–1.40
Phosphorus, m/L	M±m	1.24±0.02	1.24±0.02
	Confidence interval	1.19–1.29	1.20–1.28
Chondroitin sulphate, g/L	M±m	0.11±0.01	0.24±0.04 *
	Confidence interval	0.09–0.13	0.16–0.32
Glycoprotein, m/L	M±m	0.60±0.01	0.67±0.05
	Confidence interval	0.58–0.62	0.44–0.70

Notes: * — $p<0.05$, *** — $p<0.001$

Table 2 — Biochemical indicators of the state of connective tissue at osteodystrophy of goats (according to the analysis of blood serum)

Indicator		Clinically healthy	Osteodystrophy
Fraction I mucopolysaccharides, U/L	M±m	5.9±0.55	8.1± 0.52
	Confidence interval	4.4–7.4	6.9–9.27
Fraction II mucopolysaccharides, U/L	M±m	2.63±0.08	3.42±0.17 *
	Confidence interval	2.41–2.85	3.04–3.8
Fraction III glucosaminoglicans, U/L	M± m	2.0±0.17	2.6±0.31
	Confidence interval	1.53–2.47	1.92–3.28
Sum of fractions glucosaminoglicans, U/L	M± m	10.2±0.62	14.2±0.84 *
	Confidence interval	8.5–11.9	12.4–16.0

Notes: * — $p<0.05$

Other clinical signs of osteodystrophy were the following: sixteen goats (80%) had thinning and tuberosity of ribs, the incisor teeth of fourteen animals (70%) were loose, four goats had partial lysis of the last pair of ribs. After clinical examination twenty goats were divided into two groups (four animals — clinically healthy, sixteen animals — with clinical signs of osteodystrophy).

In terms of obtained data we can conclude that the affected goats had clinical signs of osteodystrophy. It is known that acquired clinical signs could remain for the whole period of animals' life of the animal (tuberosity of ribs) and clinical signs not always reflect the state of bone tissue at the moment of examination.

Indicators of the mineral metabolism (total and ionized calcium, inorganic phosphorus) of affected goats were almost the same with another group of control that confirms low level of informational content. At the same time affected goats demonstrated enlarged level of chondroitin sulphate (by 54%) and increased activity of alkaline phosphatase that can designate the pathology of support-locomotion system. 50% of animals had increased activity of alanine amino transferase in blood serum that made up 41.2±1.9 U/L. Increased activity of aspartate aminotransferase of goats with clinical signs of osteodystrophy 38.90±1.08 U/L on comparison with 26.00±1.08 U/L in the normal range might be the result of abnormity of myocardium and liver as it is known that functions of these organs decay at the postprimary osteodystrophy. In particular, it might be abnormality of cardiac muscle that coincides with such clinical sign as dullness of heart tones.

Analysis of Table 2 shows that goats with osteodystrophy had larger concentration of fraction II glucosaminoglicans, (by 23%) that led to increase of total glucosaminoglicans, in blood serum. Increase of level of fraction II that contains mostly chondroitin-4-sulphate is evidence of pathology of bone system in particular, as fraction chondroitin-4-sulphate dominates quantitatively in bones (Borovkov, 2006). If indicators that were analyzed before cannot be considered as specific only for diagnostics of osteodystrophy of animals, determination of excretion of hydroxyproline with urine is undoubtedly a marker of bone resorption. It reflects its metabolism that with definition of level of excretion of glucosaminoglicans, and calcium with urine can give detailed information about direction of metabolism of bone tissue (Liesegang, Risteli and Wanner, 2007).

In terms of Table 3 it can be concluded that animals with osteodystrophy had larger excretion of hydroxyproline (by 33%), uronic acid (by 39%) and calcium (by 78%). From our point of view it is caused by different correlation of osteosynthesis and resorption of bone tissue during examination.

Table 3 — Biochemical indicators of connective tissue at osteodystrophy of goats (according to the analysis of urine)

Indicator		Clinically healthy	Osteodystrophy
Hydroxyproline, mg/l	M±m	50.6±4.04	75.1±4.82 *
	Confidence interval	39.5–61.7	64.1–86.1
Uronic acid, mg/l	M±m	3.17±0.34	8.08±1.35 *
	Confidence interval	2.09–4.25	5.09–11.07
Calcium, mg/l	M±m	92.5±12.4	420.6±60.43 **
	Confidence interval	59.0–126.0	284.1–557.1
Phosphorus, g/l	M±m	0.17±0.04	0.13±0.02
	Confidence interval	0.06–0.28	0.09–0.17

Notes: * — $p<0.05$, ** — $p<0.01$

Conclusions. On the basis of clinical examination of goats and taking into account data from biochemical analysis of blood serum and urine it was established that level of clinical appearance of osteodystrophy in goats coincide with changes in some of biochemical indicators in blood serum and urine.

Among biochemical components of blood serum the most informative for diagnostics of osteodystrophy is determination of content of chondroitin sulphate, fraction II glucosaminoglicans, and activity of alkaline phosphatase, and in urine — level of excretion of hydroxyproline, uronic acid and calcium.

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ANALYSIS OF THE SEROLOGICAL INVESTIGATION RESULTS OF AUJESZKY'S DISEASE AMONG SWINE IN UKRAINE DURING 2011–2016

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Summary. This study was aimed to analyze the serological diagnostics of Aujeszky's disease among swine in Ukraine. The article presents data on serological studies of blood sera samples from swine for the presence of specific humoral antibodies against the Aujeszky's disease virus during six years (2011–2016).

The visual mapping and statistical analysis were conducted by using GIS technologies through software 'ESRI ArcGIS 10.1'. The research for the presence of specific humoral antibodies against Aujeszky's disease virus in blood sera from domestic swine was performed by enzyme-linked immunosorbent assay (ELISA) using test system IDEXX Herd®PRV gI Antibody Test Kit.

During the period 2011–2016, 9,026 blood sera samples from swine were studied and in 2,277 were received positive reactions against Aujeszky's disease (antibodies were detected in 25.2% animals from the total number of investigated swine). The monitoring investigations have covered all regions of Ukraine. For this period, 331 farms were examined and 103 of them turned disadvantage to Aujeszky's disease, which amounted to 31.1%.

During the analyzed period, the largest number of blood sera samples from swine were investigated in three regions: Dnipropetrovsk (1,789 samples), Kyiv (1,647 samples) and Donetsk (1,215 samples) oblasts. At the results of serological monitoring, it was established that the highest seroprevalence to Aujeszky's disease was registered in four oblasts: Kirovohrad — 57.1%, Kherson — 52.2%, Kharkiv — 49.3% and Sumy — 47.6%. Antibodies to the virus were not detected in this species of animals from Ivano-Frankivsk, Luhansk, Rivne and Khmelnytsk oblasts.

The obtained data of serological research allow us to approve that the causative agent of Aujeszky's disease circulates among the swine herds in Ukraine.

Keywords: Aujeszky's disease, swine, monitoring, mapping, antibody

Introduction. Aujeszky's disease usually called pseudorabies in USA is a highly contagious viral disease in swine that occurs in the form of epizootic and sporadic cases. It is an economically important disease that may cause significant losses for livestock farms, especially in countries with intensive development of swine and fur animals breeding (Pomeranz, Reynolds and Hengartner, 2005; Tong et al., 2015). It is caused by Aujeszky's disease virus (Suid Herpesvirus 1), that belong to the family Herpesviridae, subfamily Alphaherpesvirinae, genus Varicellovirus (Verpoest, Cay and De Regge, 2014; Moreno et al., 2015).

Scientists from different countries pays a great attention to the pathology of pseudorabies from the perspective of a separate disease or associated with other different forms of manifestations in the industrial swine breeding (Maes et al., 2000).

The lethal cases of this disease occur mainly in piglets. In mature swine, Aujeszky's disease is accompanied by establishing a lasting or lifelong virus carrying. With increasing the age of animal, manifestation of clinical signs is decreasing. In fattening and breeding swine, pseudorabies often characterized by respiratory syndrome (cough, dyspnea, fever, upper respiratory tract and lungs lesions). Swine that have recovered, are latently infected and the main reservoir of the virus and the source of infection (Verpoest et al., 2016).

Because of the variety of Aujeszky's disease forms, the laboratory confirmation of the diagnosis is a prerequisite for suspected in all cases (Vrublevskaya et al., 2016).

Nowadays, thanks to numerous scientific research of this disease, become clear issues of the pathogenesis, clinical and pathological-anatomical features, specificity of immunity and common complex of preventive measures. But at the same time in opinion of most leading experts on the study of Aujeszky's disease, to effective control it's need to constantly conduct monitoring investigation farm animals and wild boars, synanthropic rodents and natural foci residents (Sytyuk, 2011; Pannwitz et al., 2012; Elbers et al., 2000; Ruiz-Fons et al., 2007).

At the modern stage, one of the main directions of epizootic monitoring of natural focal infections is to define enzootic territories with pathogens circulation, including Aujeszky's disease. It should be emphasized, that the monitoring research of Aujeszky's disease in swine are an integral part of the strategy to combat and eliminate the causative agent of this disease.

Materials and methods. The goal of the work was to conduct analysis of the serological investigation

results of Aujeszky's disease among swine on the territory of Ukraine for the period 2011–2016.

Laboratory studies of blood sera samples from swine were carried on basis of serological laboratory 'Center of Veterinary Diagnostics' (Kyiv). The analysis of the serological investigation results was conducted during 2011–2016. Blood sera samples were collected from all regions of Ukraine.

The visual mapping and statistical analysis were conducted by using GIS technologies through software 'ESRI ArcGIS 10.1'.

The research for the presence of specific humoral antibodies against Aujeszky's disease virus in blood sera from domestic swine was performed by enzyme-linked immunosorbent assay (ELISA) using test system IDEXX Herd®PRV gI Antibody Test Kit. This test system is discriminatory. It helps to differentiate between infected and latently infected animals from vaccinated livestock after using marked gE-negative vaccine against Aujeszky's disease.

Results and discussions. During the period 2011–2016, 9,026 blood sera samples of swine were studied and in 2,277 were received positive reactions against Aujeszky's disease (antibodies were detected in 25.2% animals from the total number of investigated swine). The obtained results of serological investigation of blood sera samples from swine are summarized and shown in Table 1 and on Fig. 1.

Table 1 — The results of serological investigation of blood sera samples from swine on the presence of specific humoral antibodies against Aujeszky's disease

Indicator	The years of research					
	2011	2012	2013	2014	2015	2016
The number of investigated regions	23	22	17	20	20	15
The number of identified disadvantaged regions	16	12	8	8	7	2
The number of investigated farms	80	77	49	54	46	25
The number of identified disadvantaged farms	35	28	15	11	10	4
The number of investigated animals	2270	2255	1510	1176	1180	635
The number of positively reacting swine	882	782	255	132	154	72
Percentage of positive reactions from investigated swine, %	38.8	34.7	16.9	11.2	13.0	11.3

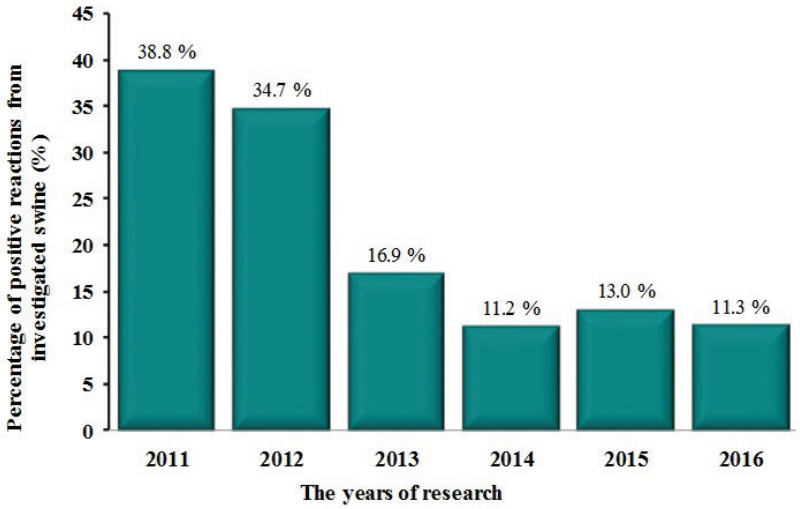

Figure 1. The dynamic of seroprevalence among swine to virus of Aujeszky's disease on the territory of Ukraine during 2011–2016

As shown on Fig. 1, seropositivity in swine herds to this disease for the analyzed period was the highest in 2011 and 2012 — respectively, 38.8% and 34.7% and the lowest in 2014 and 2016 — 11.2% and 11.3%, respectively. In 2013 there was a slight decrease in the number of positively reacting swine. Their number in comparison with the previous year decreased by 17.8%. In general, for the analyzed period from 2011 to 2016, there had observed a steady trend to reduce the incidence of seropositivity in swine to Aujeszky's disease.

The blood sera samples from swine for serological investigation were collected from all regions of Ukraine. For the analyzed period during 2011–2016, the largest number of regions were surveyed in 2011, 2012, 2014, and 2015 — 23, 22, 20, and 20 regions, respectively.

The obtained data volumes of swine serological diagnostic, in detecting specific humoral antibodies against Aujeszky's disease in the context of Ukraine regions, are shown on Fig. 2.

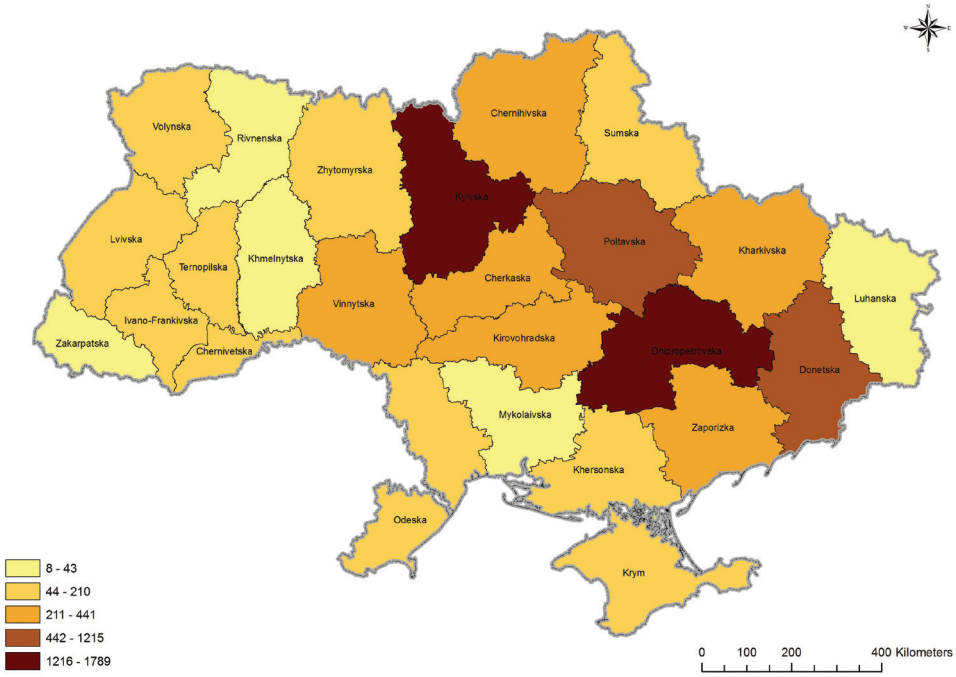

Figure 2. The number of investigated blood sera samples from swine on antibodies presence against Aujeszky's disease virus during 2011–2016

Analysis of obtained data on Fig. 2 shows, that monitoring investigations have covered all regions of Ukraine. For the period 2011–2016, the largest number of blood sera samples from swine were investigated in three oblasts: Dnipropetrovsk (1,789 samples), Kyiv (1,647 samples) and Donetsk (1,215 samples). The smallest amount of samples were investigated from the following oblasts: Volynsk (91 samples), Ternopil (88 samples), Ivano-Frankivsk (78 samples), Mykolaiv (43 samples), Luhansk (30 samples), Zakarpattia (24 samples), Khmelnytsk (22 samples) and Rivne (8 samples).

During the analyzed period the volumes of serological research were constantly decreasing: 2011 — 2,270 blood sera samples from swine, 2012 — 2,255 samples, 2013 — 1,510 samples, 2014 — 1,176 samples, 2015 — 1,180 samples, 2016 — 635 samples. For these years, 331 farms were examined and 103 of them turned disadvantage to Aujeszky's disease, which amounted to 31.1%. The percentage of identified disadvantaged farms for analyzed period was varied within the limits from 16.0% in 2016 to 43.7% in 2011.

The results of serological monitoring during 2011–2016 for the presence of specific humoral antibodies against Aujeszky's disease virus in blood sera from domestic swine in the context of Ukraine regions, are shown in absolute indicators (number of seropositive swine) on Fig. 3.

According to data on Fig. 3, the largest number of positive blood sera samples from swine to Aujeszky's disease in Ukraine were detected in Dnipropetrovsk oblast — 643. The significant number of positive samples were registered in five oblasts: Donetsk — 270 samples, Kirovohrad — 252, Kyiv — 231, Kharkiv — 178, Zaporizhia — 130. In oblasts such as the following: Sumy, Chernihiv, Cherkasy, Kherson, Lviv, AR Crimea, Vinnytsia, Poltava, Ternopil, Volyn, Mykolaiv, and Odesa the number of identified positive blood sera samples from swine were 100, 90, 78, 59, 49, 47 37, 31, 30, 24, 16, and 9, respectively. In Zhytomyr, Zakarpattia

and Chernivtsi oblasts were detected only one positive sample in each. Antibodies against the disease were not detected in Ivano-Frankivsk, Luhansk, Rivne, and Khmelnytskyi oblasts.

The indicators of swine seroprevalence to Aujeszky's disease virus in the context of Ukraine regions for the period 2011–2016 are presented as map (Fig. 4).

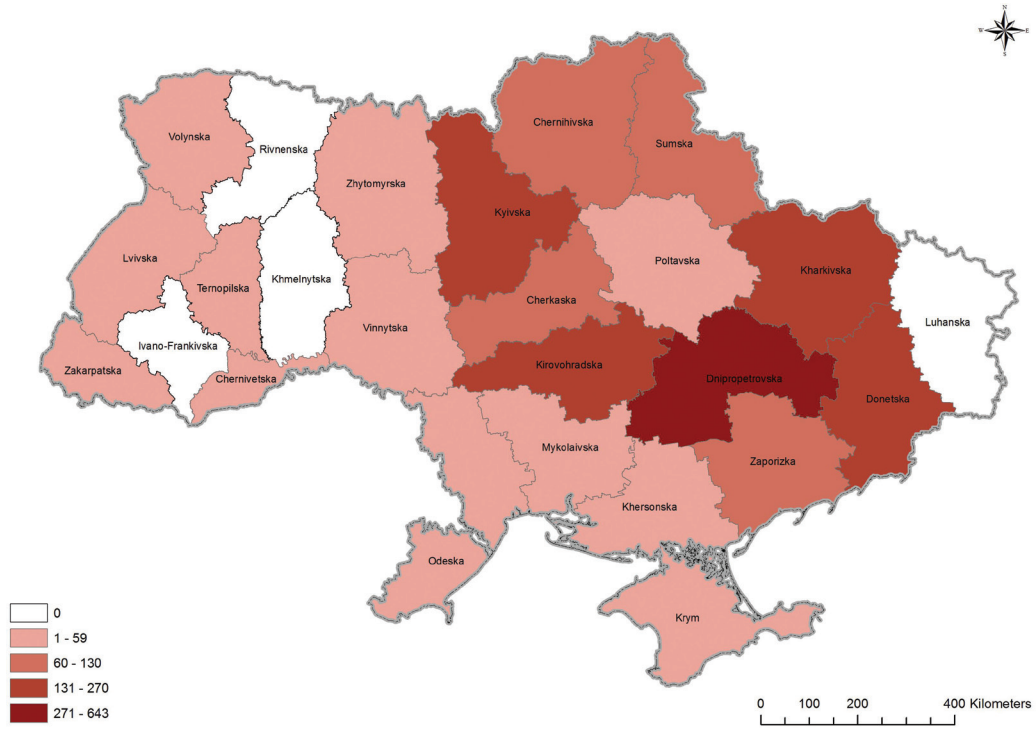

Figure 3. Map of density the number of seropositive swine to Aujeszky's disease virus on the territory of Ukraine during 2011–2016

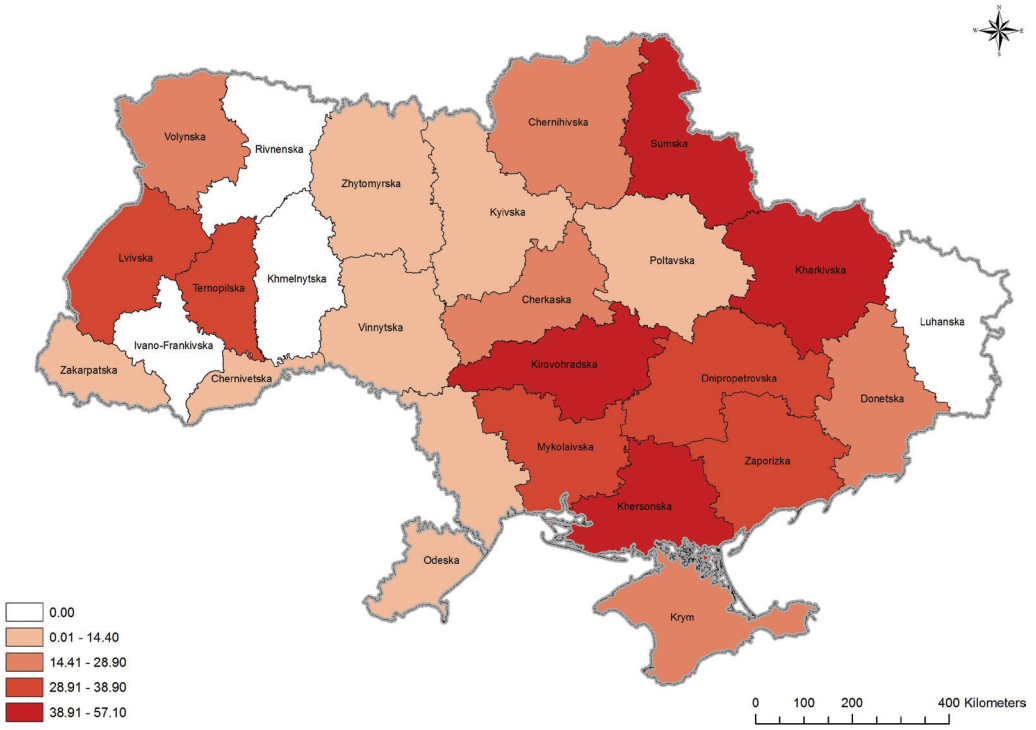

Figure 4. The cartographic analysis of swine seroprevalence to Aujeszky's disease virus on the territory of Ukraine by results of serological monitoring during 2011–2016

As shown on Figure 4, the highest indicators of swine seroprevalence to Aujeszky's disease were registered in four oblasts: Kirovohrad — 57.1%, Kherson — 52.2%, Kharkiv — 49.3% and Sumy — 47.6%. In Lviv, Mykolaiv, Dnipropetrovsk, Ternopil, Zaporizhia, Chernihiv, Volyn, Cherkasy, Donetsk, Vinnytsia, Kyiv oblasts and AR Crimea the percentages of positive blood sera from the total number of investigated swine were 38.9, 37.2, 35.9, 34.1, 34.0, 28.9, 26.4, 25.5, 25.0, 22.2, 14.4 and 14.0%, respectively. Insignificant percentages of the positively reacting swine were detected in Odesa (7.2%), Zakarpattia (4.2%), Poltava (3.5%), Chernivtsi (1.0%), and Zhytomyr (0.6%) oblasts. Specific antibodies to Aujeszky's disease were not diagnosed in animals from Ivano-Frankivsk, Luhansk, Rivne, and Khmelnytsk oblasts.

Conclusions. According to the results of serological monitoring of Aujeszky's disease among swine in Ukraine during six years, it was established that the overall rate of seroprevalence — 25.2% from the total number of investigated animals. In the context of years it was 38.8% in 2011, in 2012 — 34.7% in 2013 — 16.9%, in 2014 — 11.2%, in 2015 — 13.0% and in 2016 — 11.3%.

The obtained data of serological research allowus to approve that the causative agent of Aujeszky's disease circulates among the swine herds in Ukraine.

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POULTRY REOVIRUS INFECTION AND DEVELOPMENT OF ITS SPECIFIC PREVENTION IN HENS

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Summary. The article analyzes the epizootic situation on poultry farms in Ukraine to reovirus infection among different bird species (chickens, broilers, ostriches, geese, perching ducks), molecular-biological properties of isolates based on the selected reovirus strain with the aim to create the inactivated emulsion vaccine against AVRI (tenosynovitis) chickens.

Epizootological, serological and virological studies have demonstrated the circulation of the causative agent of AVRI among poultry in 7 oblasts of Ukraine. Serological studies carried out among different bird species for observation period 2006–2014. It was found four field isolates of reovirus and studied their biological properties. Technological parameters of manufacture of inactivated emulsion vaccine against AVRI chickens of local strain Br-06 were developed and substantiated as well as studied formation characteristics, dynamics, level and duration of specific immunity in birds vaccinated in the laboratory and found high against epizootic effectiveness in production.

It is proved the opportunity of using inactivated emulsion-vaccine against AVRI of strain Br-06 to immunize parental poultry flock. It is also established its use as import-substitute biological products for specific prevention of parental poultry flock. State vaccine does not surrender in immunogen ratio to the imported vaccine of 'Merial' firm (France).

Keywords: chicken reovirus infection (tenosynovitis), epizootiology, reovirus biological properties and specific prevention

Introduction. Scientists paid great attention to the study of chickens viral arthritis (tenosynovitis), the pathogen which belongs to the family Reoviridae in 80–90th of the last century. The infection has been registered for the first time among broiler chickens in the United States and England in 1954. Today it is observed among poultry in many countries in the world. The most susceptible are the young growth of the first three weeks of life (turkeys, ducklings, goslings, ostriches and chicks) and adult bird meat and egg breeds and crosses (Aliev, 2002; Jones, 2013; Parkhomenko, Ihnatov, and Zemlianska, 2006; Herman, I. V., Nevolko and Khamko, 2007).

Reovirus persistence in healthy bird organisms may be the cause of latent infections, lesions of the lymphatic system and immune suppression.

The disease significantly influences on economic profitable of poultry farming due to the birds death (from 5 to 20%), delayed growth and development (to 40%) and deficiency of poultry products (Malkinson, Perk and Weisman, 1981; Jones, 2000; Dzhavadov, 2008; Trefilov and Pruglo, 2003; Nalyvaiko, 2013; Nikolaenko, 2015).

In recent time, Ukraine imports a large number of different species and breeds of poultry from countries with different epizootic situation and so, it is not excluded the importation of new pathogens of infectious diseases, which include reovirus infection. The messages about the circulation and dissemination of the pathogen on the territory of Ukraine for the last 10 years remain limited.

Countries with developed poultry farming use alive and inactivated virus-vaccines to the control and prevent of this infection.

Today in Ukrainian market has registered a significant quantity of vaccine different companies against infectious diseases of poultry. Vaccine against Newcastle disease, chicken infectious bronchitis, Borsalino infectious disease and adenovirus infection plays the most important role (Hassan et al., 1993; Borisov et al., 2005; Trefilov, 2000; Bezrukava et al., 2005).

Vaccines of avireovirus infection (ARVI) of state production on Ukrainian market do not exist. Therefore, vaccination remains one of the most effective measures to provide stable prosperity concerning the disease and prevention of the occurrence this infection.

Therefore, the aim of our work was to study biological properties of isolated pathogens and development on their base state inactivated emulsion-vaccine against chicken reovirus tenosynovitis.

Materials and methods. The experimental part of the work was carried out in 2006–2014 at the Institute

of Poultry of NAAS and on poultry farms in the Kharkiv, Ivano-Frankivsk, Donetsk, Dnipropetrovsk, Luhansk, Odesa and Cherkasy oblasts of Ukraine. Industrial reovirus strains of (Br-06, isolated from a clinically sick chickens on farms in Kharkiv oblast) and epizootic (Str-07, isolated from sick ostriches on farms in Kharkiv oblast; G-07 — from sick geese on poultry farming in Ivano-Frankivsk oblast; K-14 — from sick ducks of the private farming in Odessa oblast) were used at investigation.

Culture of cells. Primary tripsinized culture of cells fibroblast, free of pathogen flora (SPF) of chicken embryos, aimed for reproduction and virus titration, were prepared of skin and muscle tissue of 10 days SPF embryos (Syurin et al., 1998; German, 2007). In addition, were used vaccinated cell cultures: Vero (was received from State Scientific-Control Institute of Biotechnology and Strains of Microorganisms, c Kyiv), LC, KC, BGM, BHK-21 (was received from Federal State Institution 'All-Russian Scientific Research Institute of Animal Protection' (FSI 'RSRIAP'), Vladimir, RF).

Adjuvant. It was used oil adjuvant 'Montanide ISA 70 VG' production 'Seppic' company (France).

Serological methods. Sampling, sample preparation and study of blood serum was carried out from recuperated and vaccinated against poultry reovirus infection. Antibody titers were determined in the indirect haemagglutination reaction (IHR) by erythrocyte diagnostic (National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv) and by enzyme-linked immunosorbent assay (ELISA) using the 'Kit for detection of antibodies to the pathogen poultry reovirus infection by enzyme immunoassay method' (FSI 'RSRIAP', Vladimir, RF) and the test-system 'Avian Reovirus Antibody Test Kit' ('BioChek', the Netherlands).

Indication of pathogen in the pathological material was carried out by PCR (polymerase chain reaction) with inverse transcription. *Identification* of the isolated viral agents were carried out by using PH with positive to strain 1133 blood serum (FSI 'RSRIAP', Vladimir, RF).

Virological investigations were carried out according to standard methods on 9–10 daily chicken and 11-days-old duck embryos. The infectious activity of the isolated strains was determined by titration and calculated for Reed-Muench method (Syurin et al., 1998).

Biological properties of field isolates reovirus were studied on chicken embryos, chicken embryo fibroblasts (CEF), duck embryo fibroblasts and revaccined cell cultures Vero, LC, KC, BGM, BHK-21 in comparison with the reference strain 1733.

Study of virulent properties strain Br-06 were carried out in 2 experiments, before the experimental procedure the live weight of day-old chicks was, on average, 41.0–46.5 g. In the first experiment 3 day-old intact chickens were infected with field strain Br-06 and control pathogenic strain 1733 reovirus in soft tissue of paws in volume of 0.2 cm^3 per os — 0.5 cm^3. In the second experiment the first chicken group was infected with a field strain Br-06 in the soft tissue of paws in a volume of 0.2 $см^3$, the second — per os — 0.5 $см^3$, the third in soft tissue of paws and per os in the same volume. Control chicken group received saline in the appropriate volumes in both experiments.

Virus inactivation was carried out by using ethylenimine production of the Institute of Chemical Physic named after M. M. Semenova of the Russian Academy of Sciences (Moscow, RF) in three final concentrations of (0.05, 0.1 and 0.15%) at two temperature conditions (25±0.5 °C i 37±0.5 °C) in period 12, 24, 30, and 42 hours. Control of inactivation completeness was carried out by conducting four consecutive 'blind' passages on cell culture of chicken embryos fibroblasts (CEF).

The lack of contamination by bacterial and fungal microflora was determined according to DSTU 4483:2005 and DSTU 4517:2006

Antigenic activity and immunogenicity of vaccine was tested at the repair young growth hens at 90–120 days age, which were once immunized intramuscularly at dose 0.5 cm^3, on harmlessness 1.5 cm^3. Antigenic activity of research series was studied in comparison with inactivated against infectious Borsalino disease vaccine and viral bird arthritis 'Galimun 201' of strain 1133 ('Merial', France).

Test of inactivated emulsion vaccine. Commission interdepartmental check '*Inactivated emulsion vaccine* against reovirus infection of chickens (AVRI) of strain Br-06' was carried out at the Institute of Poultry of National Academy of Agrarian Sciences in quality factor, with this aim the repair young growth of hens at 90–120 days age were immunized, that was obtained on free-problem infectious diseases farms. Antigenic activity and immunogenicity was tested monthly using ELISA for 360 days. Industrial testing was carried out on two poultry farms in Kharkiv and one farm in Donetsk oblasts.

Results. The data about infection of reovirus diseases among poultry according to the reports of Kharkiv branch of the State Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Inspection and the State Veterinary and Phytosanitary Service of Ukraine to nowadays are not available. On this point during 2006–2014 epizootology monitoring of reovirus infection was carried out on 11 poultry farms

of different ownership forms in 7 oblasts of Ukraine (Kharkiv, Donetsk, Luhansk, Dnipropetrovsk, Ivano–Frankivsk, Cherkasy, Odesa), where the poultry were not vaccinated against reovirus infections. It was studied 820 samples of blood serum from clinically sick birds of different species. Among broiler chickens by serological studies was found 79.3 % positively reacting to reovirus infection with antibody titers from 1:8 to 1:128 (IHR) and 1:400–1:800 (ELISA). On poultry farms of Dnipropetrovsk and Luhansk oblasts in blood serum of investigated poultry positive titers of antibodies to reovirus is not detected.

Sick birds had clinical signs of disease that were characterized by feed refusal, retarded growth and development, edema and erosion of cartilage joint limbs, arthritis, lameness, diarrhoea. On post mortal were noted hemorrhages under skin and chest muscles, spleen involution, flabby liver with ocher-green colored insulas, kidney marbling and hemorrhagic enteritis.

Economic losses from the disease at the period of study among birds of different age groups, species, breeds and crosses ranged from 2 to 36 %. Poultry was imported from abroad to the farms, where the research was conducted.

Molecular genetic studies. By polymerase chain reaction (PCR), with inverse transcription in postmortem material from diseased broiler-chickens and ostriches was found the plot of RNA genome poultry reovirus and sequenced of segment S3 order with the length of 985 b.p., carried out its comparative analysis with sequences of the strains presented in GenBank. It is defined that the selected isolates belong to the group vaccine strain 1133 of Reoviridae family.

Virological tests. For 3 serial passages in chicken embryos from pathological specimens (synovial fluid) selected from clinically sick birds, secured 4 isolates (Br-06, Str-07, G-07 and K-14), 3 of which led to the death of 10 daily chicken and duck embryos with specific reovirus infection modifications: Br-06 and K-14 in 4 days, Str-07 — on the 9th day of the 2nd serial. Tissue swelling and torso hyperemia, injection of the blood vessels in the chorioallantoic membrane, edema and hemorrhage at occiput and increase liver blood circulation with parts of necrosis, the blood filling of the kidneys, brain deformation with hemorrhages were observed from dead embryos. Consequently, it is established that isolates isolated by PH identification of isolates, secured from broiler-chickens, ostriches, geese and ducks belong to reovirus.

Biological properties of field isolates Br-06, Str-07 and K-14. According to the results carried out on chicken embryos, (CEF), duck embryos fibroblasts and revaccinated cell cultures Vero, LC, KC, BGM, BHK-21 studies, the highest infectious titers were determined in Br-06 and 1733 strain on the chicken embryos fibroblasts and 7.55 and 6.5 lg TCD_{50}/cm^3, accordingly. Other isolates were characterized by lower infectious titers, therefore, they have not been used in further researches.

While determining of level accumulation strain Br-06 reovirus chickens in stationary cell cultures was secured probable difference between the infectious titers on different cultivation systems. The greatest activity was observed when it was cultured on the chicken embryos fibroblasts (CEF) (from 6.5±0.14 to 7.5±0.07 lg TCD_{50}/cm^3) ($p \leq 0.01$) compared to cell cultures NT and LEK (from 5.4±0.18 to 4.1±0.19 lg TCD_{50}/cm^3) ($p \leq 0{,}001$). It is established that the sowing concentration of 600–800 ths cells/cm^3 with the dose of infection of 0.1 lg TCD_{50}/cell provided optimal accumulation of viral biomass in the titers from 6.00±0.14 to 7.37±0.11 lg TCD_{50}/cm^3 for 3 serials.

Study of virulent properties of the strain Br-06 on chickens. It is determined that the incubation period of infection with Br-06 strain is 9 days and strain 1733 — 3 days, with specific reovirus infection modifications at necropsy. Causative agent was taken from the dead birds.

In 20 days after infection with the reaction of indirect hemagglutination specific to reovirus antibodies in diagnostic titers (3.0–3.6 $\log_2$) with their further increase to 40th day (3.9–4.5 $\log_2$) were secured. In chicken control group, that were treated with saline, specific to reovirus antibodies were absent throughout the whole study period.

During the growth period (from 7th to 42nd days) average chicken live body weight in experimental groups, ranges from 156.9±6.88 to 232.8±43.86 g, and the control group was within the normal range (2,400 g). At the end of the study, the difference in weight between broiler chickens of control and test groups probable ($p \leq 0{,}001$) was 2,117.2 g.

Thus, confirmed etiological role of field strain Br-06 reovirus for chicks and found that the incubation period of the disease depends on the strain that poultry has been infected.

Developing procedures for the inactivation of Br-06 and 1733 strain reovirus and determining the ratio of adjuvant to inactivated antigen. The research result was found that ethylenimine at final concentration of 0.1% within 24 h at temperature of 37.5 °C completely inactivates the infectivity of strains of Br-06 and 1733 reovirus, while this retaining antigenic features. Poultry injection by inactivated ethylenimine antigen determines the formation of specific to reovirus antibodies in the titers from 1:5909±812 to 1:8304±327 in ELISA.

It is determined that combination of inactivated antigen with adjuvant 'Montanide ISA 70 VG'

in ratio 30:70 allows to obtain the stable emulsion for the manufacture of inactivated experimental samples vaccines against AVRI as well as from Br-06 and as 1733 strains.

Making inactivated emulsion vaccine against reovirus infection using state Br-06 strain reovirus. It was made 6 lab series *inactivated emulsion vaccine* against AVRI in total number of 2,400 doses. After checking experimental vaccine samples of Br-06 strain (C-1) and the control strain 1733 (C-3) as to the inactivation completeness, emulsion stability, sterility it was immunized reparing young chickens at the age of 90 days. Induction of specific antibodies to reovirus and duration of humoral immunity in vaccinated by two samples of the vaccine birds were checked by ELISA monthly over 390 days. It is established that the prepared of Br-06 and 1733 strains samples of inactivated emulsion vaccine against of ARVI provided persistent protective antibody levels of to the causative agent of the disease in vaccinated chickens. Birds immunized by experimental vaccine of strain 1733 (C-3), saved protective antibody titers within 360 days at 1:1,200 level, but in 390 days they were down to 1:881. Whereas the chickens vaccinated by the experimental sample made of strain Br-06 (C-1), had protective antibody titers higher and remained them during 390 days of the productive period (observation period) at 1:1,207 level. The control was unvaccinated poultry with the antibody titer in ELISA not higher than 1:400 (Fig. 1).

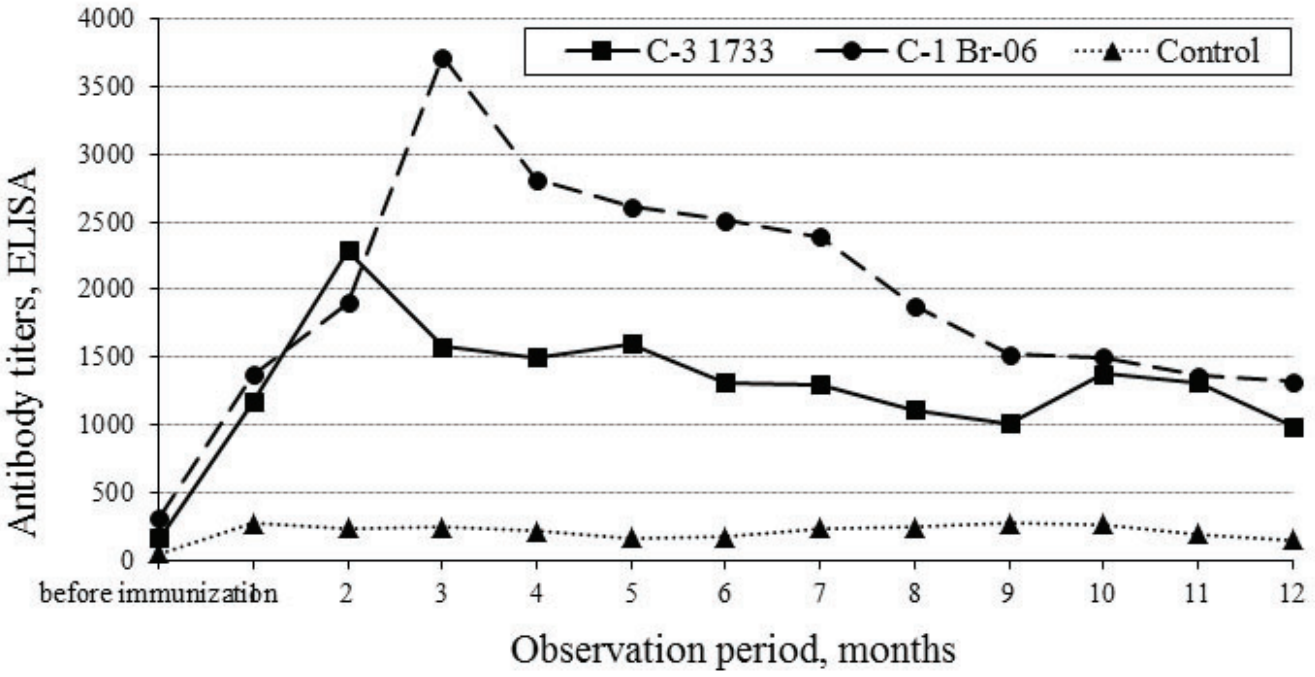

Figure 1. Dynamics of humoral immunity level of chicken after immunization by experimental samples of inactivated emulsion vaccine made of Br-06 (C-1) and 1733 (C-3) strains

The repair young growth hens at the age of 110 days were immunized by research samples of inactivated monovalent vaccine of strains Br-06 (C-2) and 1733 (C-1) reovirus to study immunity tension The dynamics of specific antibodies level to poultry vaccination was studied by ELISA, in 30 days after immunization and monthly during 330 days. Antibodies to reovirus were not found at experimental poultry before vaccination. Control infection of immunized chickens (experimental-infected and control-uninfected) was performed intramuscularly in a volume of 3.0 cm^3 by control pathogenic strain 1733 with infectious titer 6.6 lg TCD_{50}/cm^3. The birds were observed for 390 days. Vaccinated chickens before infection had antibodies titer to reovirus 1:3085, in 30 days after infection, it was reduced to 1:1,651, and in 60 days was increased to the previous level (1:3,221). However, the infected birds were decreased in egg production from 48.6 to 42.8%, that was rebounded to 56.6% in 30 days after infection.

Thus, use of the experimental sample of developed 'Emulsion vaccine inactivated against of chicken reovirus infection (AVRI) of strain Br-06' in 100%

of cases prevented the disease development after infection by the virulent control 1733 strain.

Comparative assessment of immunity tension in vaccinated by inactivated vaccines against AVRI of 1733 and Br-06 strains reovirus and 1133 of the company 'Merial (France) poultry. It is defined that protective antibody titers in ELISA for chickens vaccinated by three biological products were almost identical and remained their properties for 11 months of the productive period (observation period) at enough high protection level. But it should be noted that the average titer of antibodies in the case of the use of vaccines, made of Br-06 antigen, was higher (1:1,885±188) compared with these indicators according to vaccines of 1733 strains (1:1,324±164) and 1133 (1:1,435±222).

Therefore, developed by us sample of state inactivated emulsion-vaccine against reovirus infection made of state strain Br-06 did not surrender to inactivate vaccine of 'Merial' company (France) in immunologic. Antibody titers in chicken blood serum, vaccinated by state vaccine samples were higher in 1.2 times.

Detection of expire date of laboratory series vaccine against AVRI. Expire date of inactivated emulsion vaccine against *AVRI* was determined on three samples made of strains 1733 (C-2, C-3) and Br-06 (C-1), which had been stored respectively for 7 and 12 months at a temperature 4–8 °C. It is defined that the experimental vaccine samples after the deadline has remained the original antigenic activity to reovirus. In 30 days after vaccination, the antibody titers in ELISA were, in average, for C-1 — 1:2,873, C-2 — 1:1,880 and C-3 — 1:1,282. So, the obtained results indicate that the experimental vaccine samples of state vaccine were characterized by high immunogenicity. However, the sample made from antigen strain Br-06 (C-1), induced the formation of specific antibodies in 1.5–2.2 times at the highest titres.

Study of chick transovarial immunity formation made of vaccinated hens. With the aim of study transovarial immunity formation of hens parental flock, immunized with inactivated vaccine against AVRI of strain Br-06, it was received the chicks for 6 months while the productive period. In 30 days after hen vaccination the antibody titers in ELISA were 1:2,081±263, and in 180 days — 1:2,520±440. Antibody titers in decedents blood serum obtained of these poultry, were examined in the first day and daily for 21 days. On the basis of received results it was defined that the average protective titer of maternal antibodies in ELISA of young growth chickens at 14 days age was 1:424±7,22, that is confirmed by their control infection by pathogenic 1733 strain reovirus at 7 days age. Specific to reovirus antibodies were detected on the 21st day, however, their level did not reach the protective titers (1:21–1:170 ELISA). So, the young growth chickens of experimental groups, obtained from vaccinated progenitors, despite of low antibody titers, remained resistant to infection by pathogenic 1733 strain (clinical signs of reovirus infection were not observed). Infected in 1 day age chickens of the control groups were sensitive to the disease in 100% of cases.

Chickens, obtained of immnized against reovirus infection parental herd, were resistant to infection within 14 days despite of the low protect antibody titers ELISA, that were in the range of 1:400–1:450 (Fig. 2)

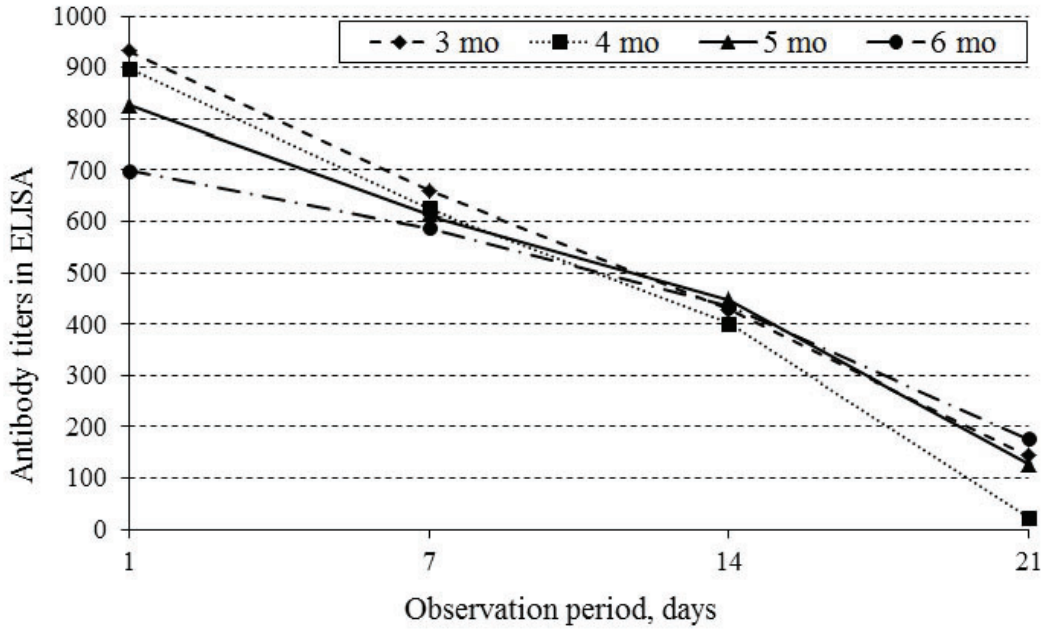

Figure 2. Transovarial dynamics of chicks immunity obtained from vaccinated against AVRI hens

Thus, the results indicate that the experimental sample of 'Inactivated emulsion vaccine against chicken reovirus infection (AVRI) of Br-06 strain' provided the tension immunity in 100% vaccinated birds for 6 months (observation period).

Commission used test 'Inactivated emulsion vaccine against chickens reovirus infection (AVRI) of strain Br-06'. Testing of series experimental vaccine was performed in the following parameters: appearance, color, presence of impurities, emulsion stability, bacterial and fungal contamination, viscosity, pH, immunogenicity, harmlessness.

It is defined, that experimental range inactivated emulsion vaccine, in 30 days after immunization of rearing chickens provided induction of antibodies to reovirus in protective titers that were persisted for 360 days (observation period). Harmlessness of the vaccine was tested within 30 days (observation period) on chickens, which were injected by triple dose of a biological product (1.5 cm^3). Cases of illness or death were not noted among the vaccinated chickens, as well as the remnants of emulsion and inflammation while autopsy at injection site. Conducted commission test of antigenic activity and immunogenicity showed that using of prototypes state 'Inactivated emulsion-vaccine against chicken reovirus infection (AVRI) of strain Br-06' provided 100 % immunity tension of vaccinated birds with antibody titers ELISA from 1:1,453±301.01 to 1:2,333±561.05 for 10–12 months of productive period (observation period).

Testing of native developed 'Inactivated emulsion vaccine against chicken reovirus infection (AVRI) of strain Br-06' in production conditions. Experimental samples inactivated emulsion vaccine against AVRI made of strain Br-06 of reovirus, passed the industrial test on two poultry farms in Kharkiv and one poultry farm in Donetsk oblasts at the repair young growth hens at the age of 90–120 days. The vaccine was injected intramuscularly once at 0.5 cm^3 dose. The tension immunity against AVRI in vaccinated chickens was controlled by ELISA method. before vaccination Experimental poultry did not have antibodies to reovirus before vaccination.

According to immune monitoring test among chickens on two poultry farms of Kharkiv oblast were found the antibody titers from 1:2,081±264 to 1:4,087±568 and from 1:4,916±452 to 1:7,539±599, respectively. On poultry farm in Donetsk oblast it is from 1:1,056±167 to 1:2,193±127. Results showed tension immunity to AVRI in vaccinated chickens was 100% for 10–12 months of the productive period (observation period).

Conclusions. Epizootological, serological and virological studies defined the pathogen circulation and spread of poultry reovirus infections with the fluctuation of sickness from 2 to 36% on 11 poultry farms of different ownership forms in 7 oblasts of Ukraine. Biological properties of four isolates, observed from broiler chickens, ostriches, geese and, firstly in Ukraine, from musk ducks were studied. Strains that are isolated from clinically ill poultry (Br-06, Str-07, G-07, K-14), were identified with PH and defined their relationship with the standard strain 1133 reovirus.

State 'Inactivated emulsion-vaccine against chicken reovirus infection (AVRI) of strain Br-06' is antigen active, immunogenic, harmless and provides 100% tension of immunity to causative disease agent. The vaccine could be used as import-substitute of biological product for the specific prevention of parental chicken flock, and does not surrender in immunogenic ratio to the activated vaccine of company 'Merial' (France).

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Part 2. Biosafety and emergent diseases

UDC 619:616.98:579.873.21:615.28

BACTERICIDAL PROPERTIES OF MYCOBACTERIA DISINFECTANTS

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Summary. Bactericide properties of disinfectant preparation 'Virosan' have been determined on *M. fortuitum* depending on the solution concentration, exposure. Also tuberculocidal effect on *M. bovis* was determined at different test-objects. Preparation 'Virosan' has the bactericide effect on tuberculosis agent of bovine species *M. bovis* in a 2.0% concentration with 24 hours' exposure and 3.0–4.0% concentrations with 5–24 hours' exposure.

Keywords: bactericidal properties, disinfectants, *Mycobacterium bovis*, *Mycobacterium fortuitum,* test objects, tuberculosis

Introduction. Tuberculosis — is an infectious disease of animals, humans, birds and sometimes cold-blooded animals which occurs in many cases in the chronic form and characterized by formation of non-vascular nodes (tubercules) in various organs and tissues subjected to the serous disintegration. *Mycobacterium bovis*, *Mycobacterium tuberculosis, Mycobacterium avium* have been the causative agents of tuberculosis in humans and farm animals. The basic source of tuberculosis agents are the sick animals which release mycobacteria with their secrets and excretes and infect the susceptible animals through the factors of transmission (manure, soil, water, feed-stuff, equipment, contaminated buildings for animals, personnel uniforms) making the sources of re-infection (Zavhorodnii et al., 2007; Arkhipova and Bessonova, 2007).

Tuberculosis is one of the most distributed zoonotic diseases among farm animals that causes great economic losses for the animal husbandries consisted from the losses by the productivity decreasing, planned or unplanned animal slaughter, carcasses utility, losses of reproduction value and also putting into practice additional diagnostic testing and veterinary sanitation measures. Economic losses caused by tuberculosis depend on the spread of disease in groups and efficiency of health-improving method and antiepizootic measures (Oshchepkov and Arzhakov, 2002; Eremeeva, 2009).

Tuberculosis agents are resistant to the various environmental factors and chemical compounds used for the prophylactic and forced disinfection. So, mycobacteria *M. bovis* have been remained viable for 21 months, and in the non-sterile soil — 1–1.5 months in the sterile soil (Kolychev, 1984), on the pastures they survive during the summer period (Kolychev, 1984; Kolychev and Karimova, 1984; Kadochkin, 1990; Vysotskiy, 2000), in the manure — 475 days, in the river water — 2 months, in the swamp water — 12 months, in the sea water — 200 days (Kislenko and Shepmov, 1972), in frozen meat — 45 days, in butter — to 300 days, in cheese — 260 days (Kirilova and Merkulova, 1990) and in milk — 14–18 days (Klebanova, 1931; Abdulin et al., 1969). In addition there have been some reports to certify that *M. bovis* preserved virulent properties for 24–26 months on the soil surface in depth 5–10 cm (Zvozchyk, 1966; Kislenko and Shepmov, 1972), and in the permafrost soils of Yakut for 27–30 months. These mycobacteria species have been viable in the barley grain — 1,358 days, in peas — 976 days, in wheat — 972 days, in the mixed feed — 793 days and preserved pathogenic properties 1,388, 883, 879 and 700, and in the unchanged bedding — 9.5 years (Kaplun and Kolyvanova, 1988; Dzyombak, 2011).

Disinfection of animal buildings is one of the methods for tuberculosis-prevention campaign in the complex of veterinary activities tended to one of the units in epizootology — factors of agent transmission from the sick to the susceptible (healthy) animal and the agent destruction in the environment (Bezrukava, Nalyvaiko and Nalyvaiko, 2008).

Various methods of disinfection are described: physical (high temperature, exposure, ultrasound, and high frequency current), chemical (chemical disinfectants in liquid, aerosol and gas states to be used

both for the immediate treatment and in the special devices) and biological (microorganisms or their metabolic products to be used for biologic manure desinfection and arthropods and rodents control) have been used (Bublii, 2000).

Chemical disinfectants (chlorine containing preparations, alkaline, aldehyde, derivatives of guanidine series etc.) have been widely used for the prophylactic and forced disinfection as the solutions for the surface irrigation or as aerosols in veterinary practice. However, the quantity of preparations has not been fully satisfied with modern requirements the market. Studies by Oshchepkov and Arzhakov (2002) revealed that out of 54 strains 7 mycobacteria species from which 9 museum and 45 epizootic demonstrated the high resistance to the preparations widely used in veterinary medicine. All the tested strains have been resistant to nirtan, amfolan and soda lye; only the strains *M.B.5, M. intracellularae, M. smegmatis, M. bovis 8, M. bovis* 14 have been sensitive to chlorine containing preparations (chloramine-b, neutral hypochlorite calcium); the rest ones have been even resistant to 5.0% solutions of these preparations. All the strains have been also resistant to a 3.0% formaldehyde solution and 1.0% glutaraldehyde solution, and only *M. phlei*, *M. fortinum* and field strains (epizootic)of *M. bovis* have been resistant to a 4.0% formaldehyde solution. The alkaline formaldehyde solution (3.0%) concentration had no effect on any tested strains and a 3.5% solution showed a harmful effect on such strains as *M.B-5, M. smegmatis, M. bovis, M. bovis* 14; 4.0% solution — on all the strains. The field culture isolates *M. phlei, M. fortuitum* and *M. bovis* have been resistant to 1.0% — solution of alkaline glutaraldehyde and all sensible tested cultures of mycobacteria have been resistant to a 4.0% solution (Dekanosidze, 1986).

The efficiency of disinfectants against the on causative agent depends on many reasons and foremost on the bactericidal properties of these, concentration of solution and its temperature, characteristics and environment temperature in which the agent contacts with the disinfectant. The disinfectant has to be used in a proper way (Dekanosidze, 1986).

It has been obvious that the knowledge about agent's resistance in the environment is necessary for the correct using of various disinfectants to annihilate them (Subbotina, 1991). So, the data testify that the tuberculosis agent can be preserved in the environment with its virulent properties and stipulate tuberculosis in the susceptible animals. Continuous preservation of mycobacteria in the environment is always a threat for the new recurrence of tuberculosis infection. Hence, it has been necessary to carry on thorough disinfection of animal facilities and objects to control the tuberculosis agent (Oshchepkov and Arzhakov, 2002; Haverkort, 2003).

The concentration and exposure of disinfectant effect are the basic conditions to influence on disinfection efficiency. The determination of its minimal concentration specifies microorganisms' devitalisation and exposure directly depends on its concentration and bactericidal properties (Skrypnyk, 2007). The final result of bacterium cell contact with disinfectant agents depends not only from the structure of microorganisms and their resistance to the chemical factors but also from the capability of chemical preparation to reveal bactericidal effect from the one hand and bacteriostatic properties in another case from the other hand (Shishkov and Urban, 1991).

Disinfection conditions provided by instructions and manuals are not always effective (Arzharkov and Arzharkov, 2009; Katoch, 2004) that's why the goal of our research to determine bactericidal properties of the new preparation 'Virosan' on *M. fortuitum* according to the solution concentration, exposure effect and tuberculocydic effect on *M. bovis* at different test-objects.

The aim of the work. Was to determine the bactericide properties of new disinfectant 'Virosan' on *M. fortuitum* depending on the solution concentration, time exposure, and anti-tuberculosis effect on *M. bovis* at different test objects.

Materials and methods. The bactericide properties of disinfectant and test-culture of atypical mycobacteria species *M. fortuitum* were studied by suspension method using 0.5, 1.0, 3.0% aqueous solutions under exposure for 3, 5 and 24 hours. Tuberculocide properties of disinfectant and test-culture of atypical mycobacteria species and *M. bovis* were tested on contaminated test-objects (glass, ceramic tile and wood) under bioburden conditions and by using 1.0, 2.0, 3.0, and 4.0% concentrations of disinfectant under 5–24 hours of exposure. Bactericidal effect of disinfectant 'Virosan' was conducted by presence of mycobacteria colonies growth on egg cultural media in control tubes and by absence of *M. fortuitum* and *M. bovis* growth in tested treated tubes (Zavhorodnii et al., 2007).

Results. The results of conducted experiments for the determination of bactericide properties of disinfectant 'Virosan' on *M. fortuitum* with the suspension method have been presented in Table 1.

The primary growth of 3–5 colonies *M. fortuitum* on the culture medium has been noticed on the 5th day of cultivation with after treatment with disinfectant 'Virosan' in 0.5 and 1.0% concentrations and 3 hours'

exposure. 30–50 colonies have been noticed on the 8th day in the test tubes on the surface of medium.

The primary growth of colonies from 3 to 8 has been found on the 8th day of cultivation, and the growth intensity increased from 25 to 50 colonies on the 21st day after contact with 'Virosan' in 0.5 and 1.0% solutions and 5 hours' exposure. The growth of colonies on the culture medium surface increased and calculated more than 50 colonies in 30 days after cultivation of bacteria material treated with the preparation. The growth of mycobacteria colonies has not been found in any cases on the surface of culture medium with the preparation use in 0.5 and 1.0% concentrations and 24 hours' exposure. The growth of 12–15 colonies *M. fortuitum* has been noticed on the 8th day after cultivation and their intensity increased to 50 colonies with a 3.0% solution 'Virosan' and 3 hours' exposure.

Table 1 — Bactericide properties of disinfectant 'Virosan' on *M. fortuitum*

Regime of use		Growth of colonies, days									
Solution concentration, %	Exposure, hours	Experiment					Control				
		5	8	21	28	30	5	8	21	28	30
0.5	3	+	+++	#	#	#	++	#	#	#	#
	5	–	+	+++	+++	#	++	#	#	#	#
	24	–	–	–	–	–	++	#	#	#	#
1.0	3	+	+++	#	#	#	+++	#	#	#	#
	5	–	+	+++	#	#	++	#	#	#	#
	24	–	–	–	–	–	++	#	#	#	#
3.0	3	–	++	#	#	#	++	#	#	#	#
	5	–	–	–	–	–	+++	#	#	#	#
	24	–	–	–	–	–	++	#	#	#	#

Notes: '–' — the growth of colonies is absent, '+' — the growth to 10 colonies, '++' — the growth from 11 to 20 colonies, '+++' — the growth from 21 to 50 colonies, '#' — the growth more than 50 colonies of mycobacteria

The growth of colonies *M. fortuitum* hasn't been recorded in the result of 'Virosan' interaction in concentration of 3.0%, with 5 and 24 hours' exposure. The growth from 15 to 20 colonies *M. fortuitum* has been noticed in control test tubes on the culture medium on the 5th day and their amount counted from 50 colonies and more in 8 days after cultivation.

The results of conducted experiments testify that preparation 'Virosan' in 0.5 and 1.0% concentrations with 3–5 hours' exposure and in a 3.0% concentration with 3 hours' exposure has only bacteriostatic properties against atypical mycobacteria species (*M. fortuitum*). The bactericidal properties of disinfectant 'Virosan' on *M. fortuitum* have been shown in 0.5 and 1.0% concentration with 24 hours' exposure and in a 3.0% solution with 5–24 hours' exposure.

Further, the final determination of bactericidal properties of disinfectant 'Virosan' on tuberculosis agent *M. bovis* (*Vallee* strain) in the experiments at the test objects in 1.0, 2.0, 3.0, and 4.0% concentration with 5 and 24 hours' exposure has been carried out.

The results to determine the bactericidal action of desinfectant 'Virosan' on *M. bovis* at the test objects have been presented in Table 2.

Disinfecting preparation 'Virosan' in a 1.0% concentration with 5–24 hours' exposure destroyed *M. bovis* on the contaminated test-objects: glass and ceramic tile, whereas the growth of mycobacteria colonies on the culture medium has been noted from the scrapes on wooden test-objects. The growth of colonies has not been noticed on the test-objects under the effect of 2.0% preparation with 24 hours' exposure.

In the control samples taken from the scrapers of glass, ceramic tile and wood the growth of colonies has been observed on the 13th–15th days after cultivation, and on the 30th day their intensity calculated more than 50 colonies; in the microscopy of smears taken from grown cultures stained by Ziehl-Neelsen's method there have been visible short red rods with turned edges.

Table 2 — Bactericidal action of desinfectant 'Virosan' on *M.bovis* at the test objects

The regime of use		The name of test object			Control
Solution concentration, %	Exposure, hours	Glass	Ceramic tile	Wood	
1.0	5	–	–	++	#
	24	–	–	++	#
2.0	5	–	–	+	#
	24	–	–	–	#
3.0	5	–	–	–	#
	24	–	–	–	#
4.0	5	–	–	–	#
	24	–	–	–	#

Notes: '–' — the growth of colonies is absent, '+' — the growth to 10 colonies, '++' — the growth from 11 to 20 colonies, '+++' — the growth from 21 to 50 colonies, '#' — the growth more than 50 colonies of mycobacteria

Conclusion. The disinfectant 'Virosan' hills the tuberculosis agent *M. bovis* in a 2.0% concentration of aqueous solution with 24 hours' exposure and 3.0 and 4.0% concentrations with 5–24 hours' exposure can be used for the preventive and forced disinfection of livestock buildings at a rate of 1000 cm^3/m^3.

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SPATIAL AND TEMPORAL PATTERNS OF ENZOOTIC RABIES ON THE TERRITORY OF CHERNIHIV OBLAST OF UKRAINE

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Summary. Rabies is particularly dangerous disease of all warm-blooded animals and humans. Ukraine has been experiencing an uneasy epizootic situation on rabies in recent years. The objective of this study was to determine the spatial and temporal patterns of enzootic rabies in Chernigiv Oblast of Ukraine in 2011–2016. Space-time clusters of rabies cases with a 'sporadic' time trend were identified in central and south-western raions of Chernihiv Oblast. Three clusters of a 'new' time trend in the far south-east of the oblast may be connected with the termination of the 2015 oral immunization of wild carnivores against rabies in Poltava and Sumy oblasts.

Keywords: enzootic rabies, epizootic situation, domestic animals, wild animals, surveillance, space–time cluster

Introduction. At the beginning of XXI century rabies epizootic situation in Ukraine remains insufficiently studied and controlled with permanent and periodic fluctuation of prevalence and significant outbreaks of disease (Pötzsch et al., 2006; Hryshok et al., 2009; Nychyk et al., 2013). The territory in Chernihiv Oblast is not an exception.

By the end of 1960 the main source of rabies were dogs. Later rabies epizooty of the 'natural' type began. Its reservoir presented by the wild carnivores. Due to the adaptation of the virus and the new reservoir of infection there has been a change in the manifestation of epizootic disease by moving its center to the countryside with the active participation of enzootic in domestic and farm animals.

There are more than 10 species of wild carnivores, in Chernihiv Oblast which can be a source of rabies virus in nature. As for the number a red fox takes the first place (*Vulpes vulpes*), then a marten and other members of the marten family (badgers, otters, weasels and minks), then — raccoon dogs, wolves and lynxes. The peculiarity of foxes ecology, their synanthropization, permanent contacts with homeless dogs and cats, uncontrolled breeding, the presence of a source of infection are prerequisites for the emergence of rabies and the formation of stable seats of infection. All these elements together with social and economic factors determine the impossibility of rabies eradication (Nedosiekov et al., 2009).

By rabies enzootic its lethal effect generally reduces the population of the main type of reservoir — a red fox. However, enzootic activity increases when the region is populated by new generations of susceptible hosts, creating cycles of peaks every few years (2006, 2010, 2016) (Golik, Polupan and Nedosekov, 2015).

The analysis of the situation for the rabies demonstrated that in contrast to some European countries where the cases of rabies are registered only among wild animals, in Ukraine dogs and cats are involved actively, so we can observe enzootic 'evolution' towards intensification and consolidation the chains of 'natural' and 'city' rabies.

A separate problem in Ukraine is a large population of homeless animals. According to the Central Sanitary and Epidemiologic Service in Ukraine over the past decade 76,000–78,000 calls from citizens about dog bites (26.3–27.8% stray dogs) and 15,000–18,000 appeals concerning contacts with cats are registered every year.

Radical efforts (oral immunization of wild carnivores — ORV) to curb the spread of rabies epizootic in Chernihiv Oblast were conducted in 2006–2010. Generally there have been held five campaigns (in 2006, 2007, twice in 2008 and 2010) by distributing baits by hand in 2006 and 2007, and using air transport twice in 2008 and 2010. Taken into account the lack of systematic and planned use of ORV significant progress in reduced manifestations of rabies could not be reached.

The cases of rabies which are analyzed in this article were documented in existing surveillance system in Ukraine — identifying sick animals or diagnostic shooting wild animals, pathological material delivery

to the regional veterinary laboratory and the laboratory research method Flourescent Antibody Test (FAT) (in some cases by biotest).

In this study, space-time patterns of the rabies epizooty in Chernihiv Oblast were identified, and described with spatial cluster techniques, to assist in understanding the natural dynamics of rabies.

In order to develop more effective control strategies using parenteral rabies vaccination of pets or ORV, it is necessary to examine the disease patterns in space and time, with the goal of understanding how such patterns might support the development of more efficient rabies control strategies (Recuenco et al., 2007; de Andrade et al., 2016).

The aim of the research was to examine spatio-temporal distribution of rabies outbreaks in 2011–2016 in Chernihiv Oblast.

Materials and methods. The database, containing of 463 animal rabies cases in Chernihiv Oblast of Ukraine for the time period 2011–2016 was constructed. Every case of rabies was georeferenced to the centroid of the nearest village.

Spatio-temporal data analysis was performed with Spatial Statistics, Spatial Analyst, Space Time Pattern Mining toolsets of ESRI ArcMap 10.3.

Standard deviational ellipses (ellipse size = 1 standard deviation, excluding weights) were built to compare central tendency, dispersion, and directional trends of rabies cases for each year.

To estimate where more cases occur during study period we used Kernel density estimation (KDE) method. The optimal search radius for KDE was calculated following Fotheringham, Brunsdon and Charlton (2000):

$$h_{opt} = \left(\frac{2}{3n}\right)^{\frac{1}{4}} \sigma,$$

where σ is the standard distance — a measure of dispersion around the spatial mean of the rabies cases locations. Standard distance was calculated with the Spatial Statistics toolbox in ArcGIS 10.3.

To identify spatio-temporal trends of rabies cases Emerging hotspot analysis was performed. This tool is based on Getis-Ord G* statistic to find out statistically significant clusters of high values (hotspots) and Mann-Kendall statistic to detect time trend in each location. This analysis requires the point data to be aggregated into cells that have specific spatial and temporal size composed into space-time cube in the study area. We chose 14 km spatial cell size (the average rabies-threatened distance) and temporal size — 2 months (average incubation period for rabies). We used 47 km neighborhood distance at which the highest autocorrelation is detected on our data by Spatial Autocorrelation by Distance tool.

Results. The cases of rabies in animals during 2011–2016 were observed in all raions of Chernihiv Oblast except Varvynskyi raion, located in the far south-east of the region. During this period there were registered 463 cases of rabies: 313 cases of rabies among domestic animals and 150 — in wild (Table 1).

Table 1 — Rabies cases, Chernihiv Oblast, 2011–2016

Species	2011	2012	2013	2014	2015	2016	Grand Total
beaver		1		1			2
badger						1	1
wolf		1			3	1	5
cattle	1	10	7	9	8	5	40
boar			1				1
fine cattle		3			1	3	7
raccoon dog	7	3	6	1	14	4	35
horse	1	1	2				4
cat	28	39	30	8	28	47	180
roe					1		1
mole		1					1
marten	1	3					4
fox	16	16	17	8	15	24	96
mouse					1		1
muskrat			1				1
lynx	1					1	2
dog	12	13	19	5	8	25	82
Grand Total	67	91	83	32	79	111	463

During 2011–2016 the laboratory confirmed the diagnosis for rabies in 17 species. However, most of the cases were among domestic animals (dogs and cats) — 262 cases or 56.6%. The percentage of foxes accounted for 20.7% of cases.

In carrying out mapping of all 463 cases of rabies their accumulation was found within Chernihivskyi, Kulikivskyi, Borznianskyi, Nosivskyi, and Bobrovytskyi raions of the oblast (Fig. 1). This can be connected with the higher population density in these areas and therefore greater concentration of domestic animals. Another factor that undoubtedly affects the intensity of epizootic is the volume of specific prevention measures of rabies. However, differences in the implementation of these measures as preventive and necessary in problem areas in different raions of the region were found.

Least number of rabies cases were found in north-eastern and south-eastern regions. One of the theories of uneven rabies epizootic in Chernihiv Oblast, there is an indirect positive impact on the epizootic situation of rabies ORV, constantly carried out on the borders of Sumy and Poltava Oblasts during 2006–2015.

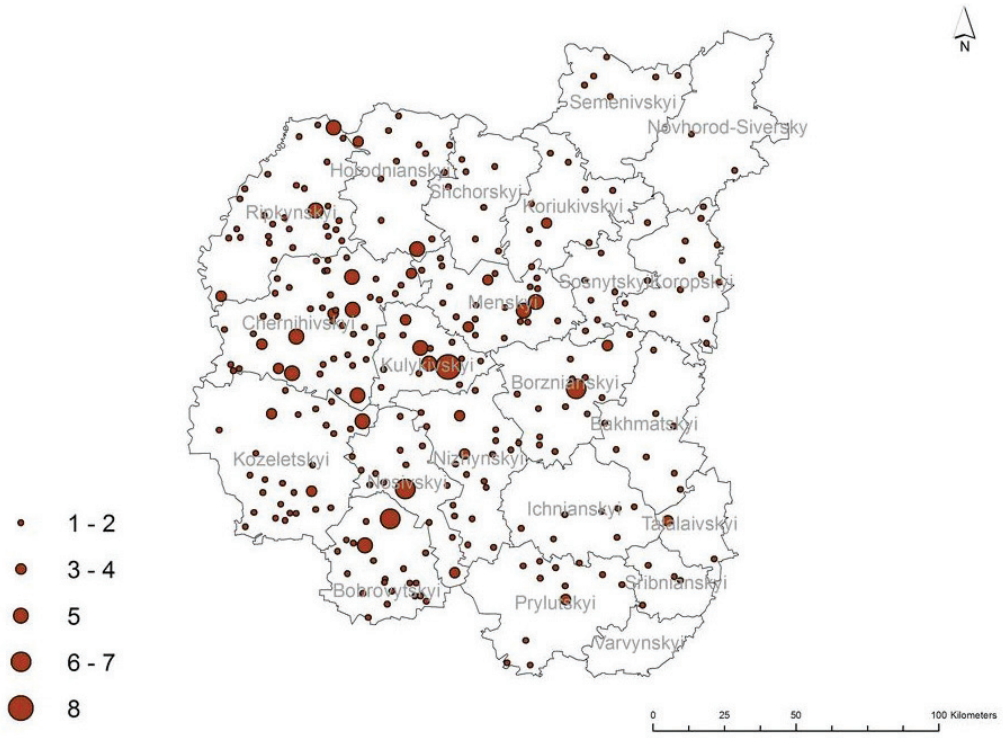

Figure 1. The map of number of rabies cases at sites, Chernihiv Oblast, 2011–2016

Ellipses of standard demonstrated showed that in 2011–2012 spatial trends of rabies cases were lying on the axis of southwest-northeast. The central tendency of rabies cases in 2011 located much farther to the east and north than in later years. Ellipses of 2011–2016 were on the axis of the southeast-north-west, mostly in central and western areas of the oblast. The standard deviational ellipse of rabies cases is located more south than in previous years and the closest to the south-north axis in 2016. Thus, if in 2011, outbreaks of rabies were mainly observed in the northeastern and central regions, in subsequent years, the outbreaks distribution has shifted to west and south (Fig. 2).

The central tendency of the ellipse for 2011–2016 for wild animals was located 23 km further north-west than for domestic ones (Fig. 3).

KDE analysis revealed the spatial clusters of rabies cases located in the central, western and south-western raions. Most rabies density of domestic animals was also observed in the central, western and south-western regions. However, the clusters of cases among wild animals were preferably in the north-western regions which are more forested and have less human population (Fig. 4).

Emerging Hotspot Analysis revealed 18 clusters with spatial temporal trend 'sporadic' (a place in which hotspot appears and disappears; a place where less than 90% of the time intervals were statistically significant hotspots and none of the intervals was statistically significant coldspot) in the central and southwestern areas of the region: Kulikivskyi, Bobrovytskyi, Ichnianskyi, partly Nosivskyi, Nizhynskyi, Kozeletskyi and in the south of Prylutskyi (Fig. 5).

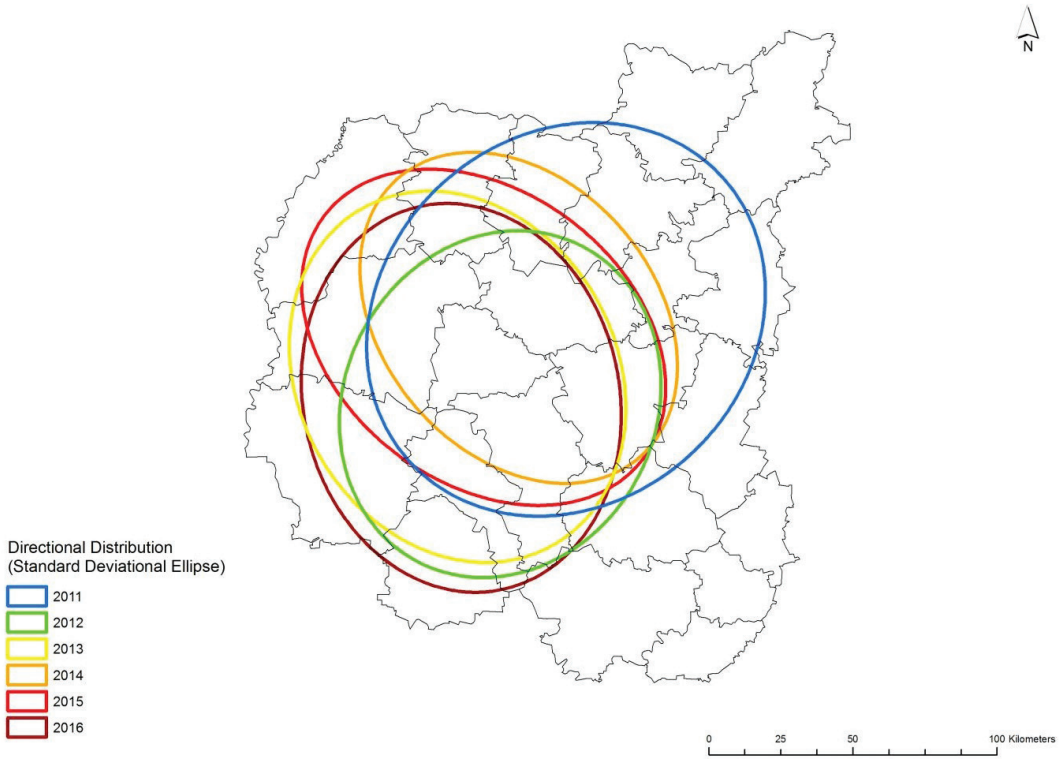

Figure 2. Ellipse of standard deviations (size 1 standard deviation) of rabies cases, Chernihiv Oblast, 2011–2016

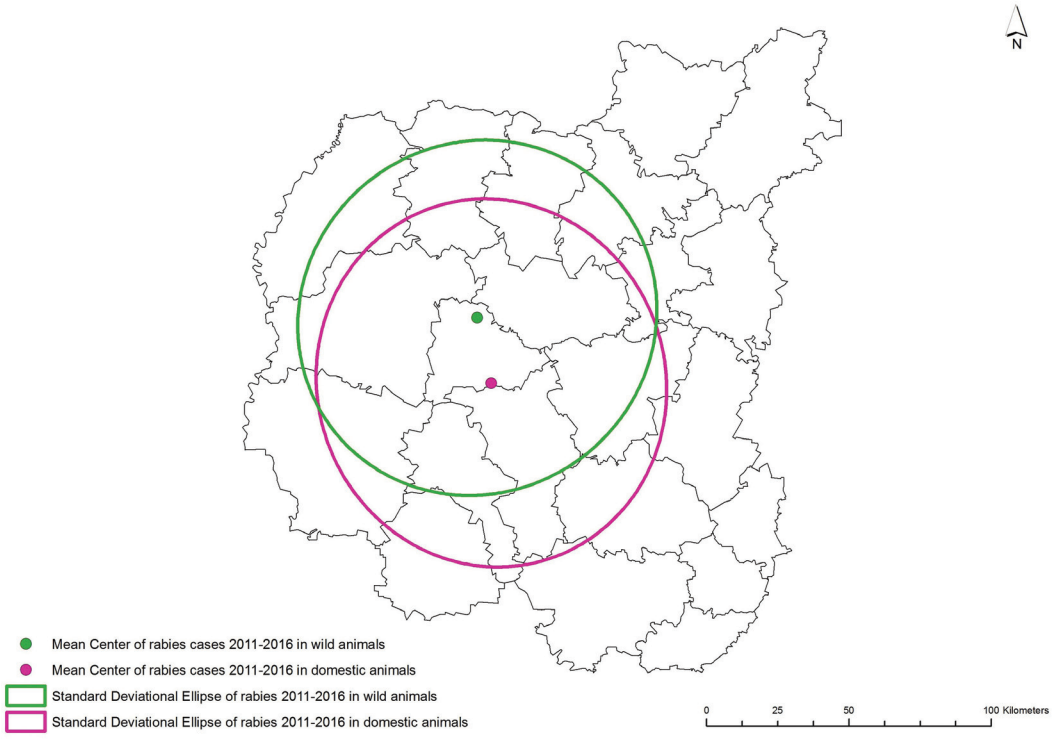

Figure 3. Ellipse of standard deviations (size 1 standard deviation) of rabies cases in wild and domestic animals, Chernihiv Oblast, 2011–2016

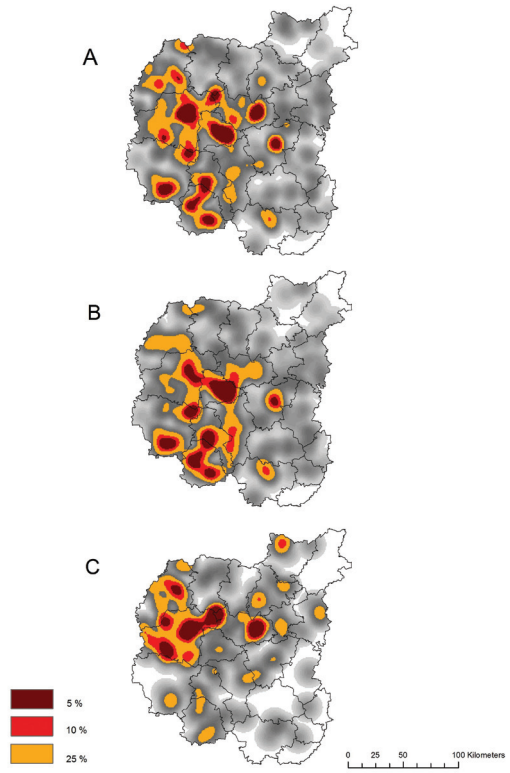

Figure 4. Rabies hotspots derived from kernel density estimation at three different thresholds (upper 5, 10, and 25% of density values), Chernihiv Oblast, 2011–2016: A — total cases, B — cases in domestic animals, C — cases in wild animals

Finding of these 18 clusters may have practical importance in these areas to intensify the work with stray animals, regulation of rabies reservoir species (mainly foxes), increasing amounts of specific prevention of rabies through parenteral immunization of animals and other activities, aimed at the eradication of rabies.

In addition, Emerging Hotspot Analysis revealed three clusters of the 'new' trend (a location is statistically significant hotspot in the last time step and has never been statistically significant hotspot earlier), placed in the far southeastern region on perimeter of Varvynskyi raion. The appearance of these clusters may be linked to termination in recent years ORV on the territory of neighboring Poltava and Sumy oblasts due to the difficult economic situation in Ukraine. So, this may be one more confirmation of theories about the presence of indirect pressure on the epizootic situation of rabies in Chernihiv Oblast of oral immunization of wild animals, which was held in Poltava and Sumy Oblasts.

However, to confirm the existence of ORV pressure on the epizootic situation in related areas additional analysis of earlier periods' data is required — before the event.

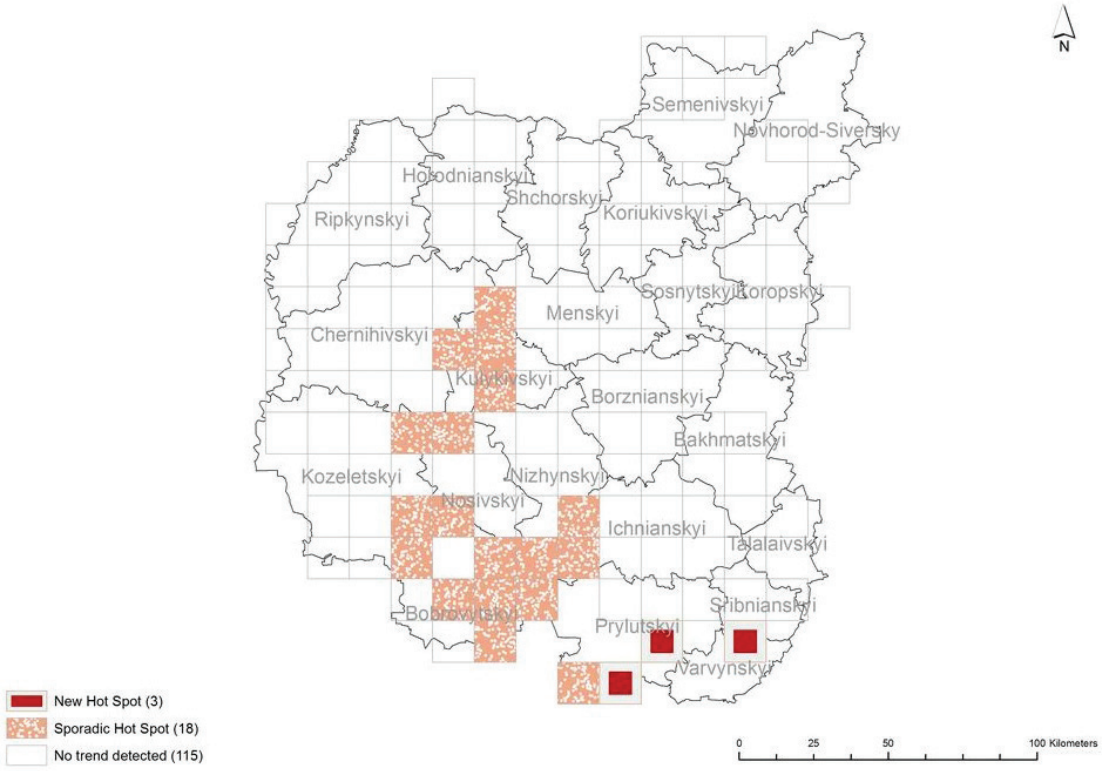

Figure 5. Emerging Hotspot Analysis of rabies cases in wild and domestic animals, Chernihiv Oblast, 2011–2016

Conclusions. Areas with high incidence of animal rabies were identified in Chernihiv Oblast. These areas should activate the work on rabies prevention throughout parenteral vaccination of domestic and agricultural animals, regulate the population of stray animals and wild carnivores.

Three clusters of the 'new' time trend in the far south-east of the oblast may be connected with the termination of oral immunization from rabies of wild carnivores in 2015 in neighboring Poltava and Sumy Oblasts. To validate this hypothesis the data from these oblasts both before and after ORV are needed.

Prospects for further research. Determination of spatial patterns and identification of rabies cases clustering can be useful for making decisions on the efficient allocation of diseases control efforts. We will continue our research with materials from other areas in order to improve the monitoring of rabies in Ukraine and to conduct more effective prevention activities.

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Part 3. Biotechnology and biology

UDC 575.113.1:636.223.1

ANALYSIS OF SNPS *F279Y* AND *S555G* IN GROWTH HORMONE RECEPTOR GENE IN BEEF AND DAIRY CATTLE BREEDS

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Summary. Effect of growth hormone on animal growth and metabolism is mediated by interaction with the specific receptor (*GHR*). Marker-assisted selection programs in cattle include SNPs in *GHR* gene regarding their association with fertility and lactation performance. The aim was to analyze the relation between SNPs *F279Y* and *S555G* in *GHR* gene with growth traits in Aberdeen-Angus and tp draw comparisons with beef and dairy cattle of other countries.

SNP genotyping was performed with PCR-RFLP methods. Statistical methods included Pearson's chi-squared test, Pearson's correlation coefficient *r* and ANOVA.

The allele and genotype frequencies of SNP *F279Y* (*rs385640152*; *g. 914T>A*) are: *T* — 0.69 and *A* — 0.31; *TT* — 62.1%, *TA* — 13.8% and *AA* — 24.1% (n = 58); SNP *S555G* (*rs109300983*; *g. 257A>G*) are: *A* — 0.86 and *G* — 0.14; *AA* — 73.1%, *AG* — 25.0% and *GG* — 1.9% (n = 58). Population is in Hardy-Weinberg equilibrium for *S555G*, contrary to *F279Y*. Animals with *AA*-genotype of SNP *F279Y* are characterized by the higher ADG (+40–100 g/day), as well as body weight at 8 month (+10–30 kg) and 2 years (up to +40 kg). In total dairy breeds group the frequency of T-allele in SNP *F279Y* negatively correlated with milk yield (*r=–0.713*).

Keywords: Aberdeen-Angus breed, growth hormone receptor gene, SNP *F279Y*, SNP *S555G*

Introduction. Growth hormone (*GH*) is a major regulator of postnatal growth and metabolism in mammals, playing essential role in the fertility and lactation in cows (Hadi et al., 2015). Considering that *GH* exerts its effect due to binding with the growth hormone receptor (*GHR*) protein, structure modification of the receptor can affect all *GH*-mediated functions. Known SNPs *F279Y* and *S555G* in *GHR* gene were proved to be associated with milk performance traits and were included in ongoing marker assisted selection (MAS) for commercial purposes in a few countries (Fontanesi et al., 2007; Oleński, Suchocki and Kamiński, 2010).

GHR gene is located on BTA 20. SNP *F279Y* is a *T>A* transversion in exon 8, causing the replacement of a phenylalanine (*F*) to tyrosine (*Y*) in a highly conserved transmembrane domain of the *GHR* protein at position 279 (Fontanesi et al., 2007). The reactive hydroxyl group in aromatic ring of tyrosine decreases hydrophobic properties of the *GHR* protein (Viitala et al., 2006). The A allele of the SNP *F279Y* was shown to be associated with increase of milk yield and lactose content, and decrease of fat, protein, casein content well as somatic cell score (SCS) in dairy cattle (Rahmatalla et al., 2011; Blott et al., 2003) and with an increase in taste panel-assessed odor (Gill et al., 2009). In beef breeds the SNP *F279Y* had shown no significant association with carcass or weight traits, but selection for *TT*-genotype was considered to be advantageous (Tait et al., 2014).

SNP *S555G* is the *A* to *G* substitution in exon 10 of the *GHR* gene, coding tiny glycine (*G*) instead of serine (*S*) in the *GHR* protein cytoplasmic domain at position 555. Exon 10 was considered to be the 'periphery' of conserved regions, suggesting that the variation does not necessarily have functional or structural importance (Viitala et al., 2006). The *A* allele of the SNP *S555G* was shown to have positive effect on milk performance traits — fat yield, protein yield and fat content in dairy breeds. (Oleński, Suchocki and Kamiński, 2010). The relation of SNP *S555G* to growth traits seems to be ambiguous (Waters et al., 2010).

Given the effect of two SNPs on milk performance traits, it is reasonable to assume that this SNP can affect

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average daily gain (ADG) during the preweaning period. The aim of our paper was to analyze the relation between SNPs *F279Y* and *S555G* in *GHR* gene with growth traits in Aberdeen-Angus and compare with beef and dairy cattle of 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 9 time points. Birth weight and ADG data for progeny (n=608; cows: n = 293, bulls: n = 315) of cows tested were included.

DNA was extracted from blood samples using DNA extraction kits 'Diatom DNA Prep 100' ('Isogene', RF). For the SNP genotyping, PCR-RFLP methods were set up, using primer pairs (Viitala et al., 2006) and restriction endonucleases AluI and VspI ('Fermentas', Lithuania).

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

Results. Allele and genotype frequencies of SNPs *F279Y* and *S555G*, growth traits in groups separated by SNPs *F279Y* and *S555G* for Aberdeen-Angus herd studied are given in Table 1.

Table 1 — Allele and genotype frequencies and body weight dynamics in Aberdeen-Angus by SNPs *F279Y* and *S555G*, $\bar{x}\pm s_x$

Parameter	***F279Y***			***S555G***		
Allele	***T***		***A***	***A***		***G***
Frequency	0.667		0.333	0.856		0.144
Genotype	***TT***	***TA***	***AA***	***AA***	***AG***	***GG***
n	32	6	14	38	13	1
%	61.5	11.5	27.0	73.1	25.0	1.9
Body weight						
Birth	30.2±0.7	31.7±0.4	31.3±1.9	30.5±0.7	31.6±1.6	24
ADG, g/day	758±14*	718±33*	811±24*	767±14	782±23	814
8 month	211.2±3.9*	193.8±5.7*	222.5±7.3*	212.1±3.5	213.9±8.6	205
12 month	278.7±5.0	262.4±6.0	280.7±6.0	278.0±4.8	277.6±5.2	290
15 month	325.6±4.7	305.0±5.3	325.6±7.7	323.4±4.3	323.5±7.0	338
18 month	368.2±5.0	346.5±9.1	374.5±10.1	367.1±5.3	368.4±7.2	375
2 year	417.8±5.5*	385.2±3.3*	423.8±11.0*	417.8±5.3	410.3±10.5	427
3 year	443.7±6.9	424.7±10.3	461.9±14.5	449.8±7.2	446.9±14.8	450
4 year	484.0±7.4	462.0±1.7	497.1±16.0	490.2±8.0	476.9±14.9	500
5 year	570.9±16.6	652.5±30.0	522.1±15.3	569.3±14.9	586.0±34.3	565

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

For SNP *F279Y* we found the trend to body weight decrease in all time points *AA*>*TT*>*TA*. In *AA*-group ADG is significantly higher on 40–100 g (*p=0.045*), as well as body weight at 8 month (*p=0.026*) and 2 years (*p=0.032*). Number of animals in group with genotype *GG* for *S555G* is very low, therefore it was excluded from the analysis. Differences between groups *AA* and *AG* were not more than 3% or 1–20 kg, therefore we observed no significant association for *S555G*.

Our findings are consistent with the results published before. *S555G* had no effect on slaughter weight, average daily gain and carcass weight (Sherman et al., 2008), no significant association with IGF-1 concentration, weight gain or off-test hip height (Ge et al., 2003). Therefore, *S555G* does not seem to be a useful marker for traits related to growth.

Preferred alleles for higher fat and protein are *T* and *A* for *F279Y* and *S555G*, respectively, therefore we can suppose that this alleles are likely to be associated with better preweaning growth dynamics based on better nutritive value of the milk (Rahmatalla et al., 2011; Oleński, Suchocki and Kamiński, 2010). In this regard we studied progeny characteristics (Birth weight and ADG) in cows tested (Table 2). As bulls have higher birth weight and ADG characteristics, than cows groups were analyzed separately.

Table 2 — Progeny ADG of Aberdeen-Angus cows tested for SNPs *F279Y* and *S555G*, $\bar{x}\pm s_x$

Parameter	***F279Y***			***S555G***		
Genotype	***TT***	***TA***	***AA***	***AA***	***AG***	***GG***
All						
Birth weight, kg	28.9±0.2	28.3±0.7	29.3±0.2	28.9±0.2	28.8±0.3	30.3
ADG, g	743±5	749±11	753±10	750±5	736±9	727
Cows						
Birth weight, kg	28.1±0.2	27.3±0.4	28.3±0.4	28.0±0.3	28.0±0.4	29.0
ADG, g	718±9	739±1	743±10	727±9	724±11	767
Bulls						
Birth weight, kg	29.8±0.3	29.7±0.8	30.1±0.3	29.9±0.2	29.7±0.4	31.5
ADG, g	765±8	766±11	777±13	776±7	750±11	698

Note: $\bar{x}\pm s_x$ — mean ± standard error

Significant differences between groups within each SNP were absent. But observed trends for ADG in all groups indicate that *A*-alleles in both SNPs show higher values: *AA>TA>TT*, *AA>AG>GG*. *A*-allele of SNP *F279Y* associated with high milk yield and lower somatic cell score (Rahmatalla et al., 2011) and *A*-allele of SNP *S555G* associated with increased fat and protein content probably exert cumulative action in milk quality improvement assessed by ADG values.

Literature data for dairy and beef breeds worldwide are summarized in Table 3 below.

Almost all populations were in Hardy-Weinberg equilibrium, except Aberdeen-Angus group studied for *F279Y* and Holstein group for *S555G* (Hadi et al., 2015). Observed disequilibriun may be a consequence of an unbalanced heterozygous animals number. Essentially all populations within dairy or beef groups considered had similar allelic frequency distribution. Beef breeds (Aberdeen-Angus, Auliekol, Kazakh White-headed, Beef breeds population) were tended to have lower frequency of *T*-allele by SNP *F279Y*, than dairy breeds.

All cattle groups are located in Northern hemisphere from 64°N to 32°N. Therefore, groups can be formed by location (different breeds, long-term selection within relatively small geographic zone) and by breed (as little literature data for each breed available, we considered two groups — beef and dairy breeds). Analysis failed to show the longitudinal pattern for allele frequencies by each SNP or combined. Considering the given frequencies, we assume importance of the geographic distribution, but it is rather determined by isolation and artificial selection in small groups, than by climatic zone. In general, we consider five groups: 'Western-European' (Germany, Italy, Romania), 'Eastern-European' (Poland, Ukraine), 'Northern-European' (Finland, Scotland and Ireland), 'American' (USA), 'Asian' (Iran/Karakhstan for *S555G*/*F279Y*).

Table 3 — Genotype and allele frequencies at SNPs *F279Y* and *S555G* in cattle populations worldwide by climate zones (longitudinal length)

Breed	Breed characteristics	Country	Longitudinal length	n	Genotypes, %			Alleles		HWE	Reference	D
GHR F279Y					***TT***	***TA***	***AA***	***T***	***A***	-		
Aberdeen-Angus	CLM = 500–700 kg BLM = 750–1000 kg BW = 16–25 kg	Ukraine	49°N	58	62.1	13.8	24.1	0.690	0.310	DE	Present study	-
Finnish Ayrshire	CLM = 500–540 kg BLM = 900–1000 kg BW = 32–36 kg Milk = 7400 kg F% = 4.36% P% = 3.36%	Finland	64°N	1528	-	-	-	0.890	0.110	-	Viitala et al., 2006	0.059
Commercial beef breeds, Angus 50%	-	Scotland	55°N	438	76.0	21.0	3.0	0.870	0.130	E	Gill et al., 2009	0.05
Group (5 breeds)[1]	-	Ireland	53°N	22	75.0	23.0	2.0	0.870	0.130	E	Waters et al., 2010	0.05
Jersey	CLM = 400–500 kg BLM = 540–820 kg BW = 25–30 kg Milk = 7700 kg F% = 4.84% P% = 3.95%	Poland	52°N	209	74.0	23.0	3.0	0.850	0.150	E	Komisarek, Michalak and Walendowska, 2011	0.042
Holstein	CLM = 600–800 kg BLM = 1100–1200 kg BW = 32–42 kg Milk = 7340 kg F% = 3.6–3.9% P% = 3.1–3.2%	Germany	51°N	1370	69.1	28.8	2.1	0.840	0.160	E	Rahmatalla et al., 2011	0.038
Auliekol	CLM = 540–560 kg BLM =950–1050 kg	Kazakhstan	48°N	50	-	-	-	0.900	0.100	-	Beyshova, Nametov and Terletskiy, 2016	0.063
Kazakh White-headed	CLM = 540–580 kg BLM =850–950 kg BW = 27–30 kg	Kazakhstan	48°N	50	-	-	-	0.760	0.240	-	Beyshova, Nametov and Terletskiy, 2016	0.012
Romanian Black-and-White	CLM = 650 kg Milk = 8200–8700 kg F% = 3.93–4.12% P% = 3.28–4.65%	Romania	46°N	60	54.5	45.5	0.0	0.770	0.230	E	Carsai et al., 2013	0.015

Romanian Grey Steppe	CLM = 400–500 kg BLM = 650–850 kg Milk = 1400–2200 kg F% = 4.2–5.0% P% = 3.7–4.1%	Romania	46°N	60	100.0	0.0	0.0	1.00	0.000	-	Carsai et al., 2013	0.111
Italian Holstein Friesian	CLM = 600–700 kg BLM = 900–1200 kg BW = 38–48 kg Milk = 8000–9000 kg F% = 3.0–3.1% P% = 3.0%	Italy	43°N	108	50.9	43.5	5.6	0.727	0.273	E	Fontanesi et al., 2007	0.005
Italian Brown	LM = 650–950 kg BW = 45–50 kg	Italy	43°N	104	89.4	10.6	0.0	0.947	0.053	E	Fontanesi et al., 2007	0.085
Italian Simmental	CLM = 550–900 kg BLM =850–1300 kg BW = 34–36 kg	Italy	43°N	104	82.7	16.3	0.1	0.909	0.091	E	Fontanesi et al., 2007	0.067
Jersey	CLM = 400–500 kg BLM = 540–820 kg BW = 25–30 kg Milk = 7700 kg F% = 4.84% P% = 3.95%	Italy	43°N	104	89.4	10.6	0.0	0.947	0.053	E	Fontanesi et al., 2007	0.085
Reggiana	CLM = 650–700 kg BLM = 900–1000 kg Milk = 5240 kg F% = 3.51% P% = 3.38%	Italy	43°N	108	88.0	8.3	3.7	0.921	0.079	E	Fontanesi et al., 2007	0.073
Modenese	CLM = 650 kg BLM = 1050 kg Milk = 4700 kg F% = 3.3% P% = 3.4%	Italy	43°N	66	84.8	15.2	0.0	0.924	0.076	E	Fontanesi et al., 2007	0.074
Rendena	CLM = 490 kg BLM = 500–600 kg Milk = 4733 kg F% = 3.5% P% = 3.36%	Italy	43°N	85	68.2	28.2	3.6	0.824	0.176	E	Fontanesi et al., 2007	0.032
Beef breeds population 1 [2]	-	USA	40°N	556	2.5	18.0	79.5	0.115	0.885	E	White et al., 2007	0.583

Beef breeds population 2 [3]	-	USA	40°N	609	1.5	17.9	80.6	0.104	0.896	E	White et al., 2007	0.604
Breed	Breed characteristics	Country	Longitudinal length	n	Genotypes, %			Alleles		HWE	Reference	D
GHR S555G					***AA***	***AG***	***GG***	***A***	***G***	-		
Aberdeen-Angus	CLM = 500–700 kg BLM =750–1000 kg BW = 16–25 kg	Ukraine	49°N	58	73.1	25.0	1.9	0.855	0.145	E	Present study	-
		USA	40°N	472	-	-	-	0.780	0.220	-	Ge et al., 2000	0.006
Finnish Ayrshire	CLM = 500–540 kg BLM = 900–1000 kg BW = 32–36 kg Milk = 7400 kg F% = 4.36% P% = 3.36%	Finland	64°N	1528	-	-	-	0.870	0.130	-	Viitala et al., 2006	0.000168
Group (5 breeds)[1]	-	Ireland	53°N	22	78.0	21.0	1.0	0.880	0.120	E	Waters et al., 2010	0.000485
Polish Holstein-Friesian	CLM = 600–700 kg BLM = 900–1200 kg BW = 38–48 kg Milk = 8000–9000 kg F% = 3.0–3.1% P% = 3.0%	Poland	52°N	872	74.9	23.2	1.9	0.865	0.135	E	Oleński, Suchocki and Kamiński, 2010	0.001
Holstein	CLM = 600–800 kg BLM = 1100–1200 kg BW = 32–42 kg Milk = 7340 kg F% = 3.6–3.9% P% = 3.1-3.2%	Germany	51°N	315	91.4	7.3	1.3	0.951	0.049	E	Hradecka et al., 2008	0.007
		Iran	32°N	150	30.0	70.0	0.0	0.640	0.360	DE	Hadi et al., 2015	0.061
Piedmontese	CLM = 550–600 kg BLM = 700–850 kg BW = 30–45 kg	Italy	43°N	-	24.0	50.0	26.0	0.490	0.51	E	Di Stasio et al. 2005	0.22

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

[1] Group includes Simmental (n=4), Angus Holstein (n =4), Belgian Blue Holstein (n=6), Holstein (n=4) and Charolais (n=4)

[2] 7 most populous beef breeds in the United States (by annual registration): Hereford, Angus, Red Angus, Simmental, Gelbvieh, Limousin, and Charolais.

[3] Beefmaster, Brangus, Bonsmara, Romosinuano, Hereford, and Angus

Within each group we observe less variation of allele frequency, taking into account the cattle purposes — beef or dairy. The one correlation with the longitude observed for *AA* and *AG* genotypes of SNP *S555G* (*r=0.834* and *r=–0.949, p<0.05*) and for *TT*-genotype of the SNP *F279Y* in dairy breeds group (*r=–0.751, p<0.05*). In dairy breeds *T*-allele of the SNP *F279Y* negatively correlated with milk yield (*r=–0.713, p<0.05*). It is agreed with previously published data (Rahmatalla et al., 2011; Oleński, Suchocki and Kamiński, 2010). Therefore, intra-breed trends can be extended to international cattle population, but extra data are to be included. No trends for growth traits were observed. High percentage of animals with *AA*-genotype by SNP *F279Y* in American beef breeds population (White et al., 2007) are indirectly supported by our findings (pattern for live weight *AA>TT>TA*) or such high frequency of *A*-allele resulted from using in selection *Bos indicus* breeds; however, data on SNPs studied for any *Bos indicus* breeds are not available.

Nei's genetic distances were not powerful enough to indicate inter-breed differences, while the variation for each SNP was low. Within beef breeds group studied is close to Kazakh White-headed breed by SNP *F279Y* (Beyshova et al., 2016) and the highest differences, over 0.6, are observed for American population (White et al., 2007), having extremely low *T*-allele frequency.

Conclusions. The desirable allele A frequencies of SNP *F279Y* and *S555G* are 0.31 and 0.86. Population is in Hardy-Weinberg equilibrium for *S555G*, contrary to *F279Y*. Animals with *AA*-genotype of SNP *F279Y* are characterized by the higher ADG (+40–100 g/day), as well as body weight at 8 month (+10–30 kg) and 2 years (up to +40 kg). In total dairy breeds group the frequency of *T*-allele in SNP *F279Y* negatively correlated with milk yield (*r=–0.713*).

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Part 4. Brief communications

UDC 619:616.98:578.821.2(560)

LUMPY SKIN DISEASE IN TURKEY

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Lumpy skin disease (LSD) is an economically important infection since the presence of the disease affects cattle health and export of cattle products. It is caused by *Capripoxvirus* and shows characteristic skin lesions in infected cattle. The disease was first reported in Zambia in 1929. It then spread to Africa, Middle East and recently to European countries like Bulgaria. The first Turkish outbreak of LSD was reported in 2013 in Kahramanmaras. Until now, many cattle are affected and the disease spread to farms located in different parts of Turkey. After the first outbreak, rapid diagnostic methods have been used in order to identify disease outbreaks. Control and eradication programs have been applied by the Ministry of Food, Agriculture and Livestock of Republic of Turkey including contingency plan, killing and compulsory vaccination.

UDC 619:616.98:578.832.1A:598.2:599

INVESTIGATION OF ADAPTATION OF AVIAN INFLUENZA VIRUSES TO MAMMALIAN SPECIES

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Influenza A viruses are negative-sense, single stranded and segmented RNA viruses. They are zoonotic pathogens that continuously circulate in several animal hosts and undergo genetic drift and shift. These are responsible for causing human epidemics and sometimes pandemics.

Avian influenza H5N1, H7N7, H7N9, and H9N2 viruses have been hypothesized to cause the next pandemic, although there is no clear evidence that they have been successfully maintained in humans. To date, how avian influenza viruses adapt to mammalian species is still not completely understood. We try to answer the following questions:

- How fast do avian influenza viruses adapt to mammalian species?
- How do avian influenza viruses cross the species barrier to adapt to mammalian species?

To answer these questions, we performed three serial passages of a quail H9N2 virus in pigs and sequenced the broncho-alveolar lavage fluid (BALF) from pigs in all three experimental groups using universal and internal primers.

We concluded from virus titration and sequencing results, that the adaptation of an avian H9N2 virus to pigs seems to work. We saw a gradual increase of virus titers in the lung of animals after serial passages. Nucleotide mutations have been found in seven genes analyzed, many of them also leading to amino acid substitutions. The third passage H9N2 isolate induced obvious lesions in pig lungs.

This study was funded by *TÜBİTAK* and Kansas State University.

News

International Scientific-and-Practical Conference
'TRANSBOUNDARY EMERGENT ANIMAL DISEASES (AFRICAN SWINE FEVER, LUMPY SKIN DISEASE, AVIAN INFLUENZA, BLUETONGUE, BRUCELLOSIS ETC.): ACTUAL ASPECTS OF BIOSAFETY AND CONTROL' (June, 5–9, 2017, Odessa)

The International Scientific-and-Practical Conference *'TRANSBOUNDARY EMERGENT ANIMAL DISEASES (AFRICAN SWINE FEVER, LUMPY SKIN DISEASE, AVIAN INFLUENZA, BLUETONGUE, BRUCELLOSIS ETC.): ACTUAL ASPECTS OF BIOSAFETY AND CONTROL'* dedicated to 115th anniversary of birthday of Prof. Isaac Kulesko has been held in the city of Odessa, at the 5th-9th June 2017.

The conference was organized by the National Scinetific Center 'Institute for Experimental and Clinical Veterinary Medicine', Institute for Veterinary Medicine of NAAS, under participation of Ukrainian Biosafety Association, SE 'Gromashevsky Insitute for Epidemiology and Infectious Diceases' of NAMS of Ukraine, SI 'Mechnikov Antiplague Ukrainian Scientific Research Institute' of the MH of Ukraine, and support of State Consumer Protection Service of Ukraine.

The forum was attended by 148 scientists and practicioneries with veterinary and medical background, medical, veterinary and biological scientists, specialists of the Main authorities of the State Consumer Proection Service of Ukraine, diagnostics laboratories, farms' veterinarians, and biological producers for veterinary use. Expanded geography of participants included the representatives of the United States of America, Germany, Poland, Switzerland, Turkey, Georgia, Azerbaijan, Ukraine.

The main areas of the conference were the evaluation of transboundary animal diseases biological risks and their management, including zoonotic diseases; scientific aspects of biosafety and biosecurity; epizootology, epidemiology, monitoring, diagnosis and prevention of the emergence of transboundary infectious diseases; ecology and biology of vector of transboundary diseases; improving national strategies of reduction of biological threats and risks of cross-border spread of diseases with international experience.

The plenary session of the conference contained 22 presentations. Among them were meaningful and informative speeches of academician secretary of the Department of Veterinary Medicine Academy of Agricultural Sciences, Corresponding Member of NAAS Mandyhra M.S., director of the NSC 'IECVM', NAAS academician Stegniy B.T., director, Head of Department of Food Safety and Veterinary SCPS of Ukraine Kobal B.I., director of the State Research Institute of Laboratory Diagnostics and Veterinary Expertise candidate veterinary sciences Novozhytska Yu.M., director of the State Scientific Control Institute for Biotechnology and Microorganism Strains, NAAS academician Golovko A.M. Also aroused great interest the report of foreign members - professors N. Borel and A. Pospishil from the Institute of Veterinary Pathology University of Zurich (Switzerland), Director of the C.E.E.Z.A.D., professor Richt J. (USA), Chairman of the Department of veterinary Medicine of Istanbul University, professor H. Yilmaz (Turkey) and many others. A total of plenary and breakout sessions were made 50 presentations.

The conference was satellited by the exhibition of animal protection means, diagnostic equipment and materials. The materials of the conference were published in interdepartmental scientific collection 'Veterinary Medicine' (№ 103).

It should be noted that the preparation and holding of the conference was provided with substantial support of CBEP DTRA (USA).

Contents