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ESTIMATION OF INBREEDING DEPRESSION BY CAPN1, CAST, GH, GHR AND CYP3A GENES FOR QUANTITATIVE AND REPRODUCTION TRAITS IN SEVEN ABERDEEN-ANGUS LINES

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Summary. High level of inbreeding has deleterious effects on animal productive and reproductive traits. The effect of inbreeding by *CAPN316*, *CAST282*, *GH L127V*, *GHR F279Y*, *GHR A257G* and *CYP3A28 C994G* markers on reproductive characteristics, weight dynamics (0–5 year age) and exterior traits was studied.

Genealogical analysis showed that population includes seven lines of cattle. For the SNP genotyping, PCR-RFLP methods were set up. Testing deviation from the Hardy-Weinberg equilibrium was performed using Pearson's chi-squared test. The estimation of inbreeding depression by F_{ST} coefficients for SNPs for traits studied was performed via multiple regression models with forward stepwise.

The tested population was analyzed in Herdy-Weinberg equilibrium for all SNPs, except *GHR F279Y*. Lines Bryalhil Sau and Southome Extra considered as the best lines showed the lowest level of heterozygosity. Negative effect of level of inbreeding for different markers were seen for regression coefficients calf birth weight = –1.08±0.30 (*GHR F279Y*), body weight at 8 month = –0.71±0.11 (*CAPN316*), –0.49±0.11 (*GHR F279Y*) and 15 month = –0.54±0.04 (*CAST282*), –0.36±0.06 (*GHR F279Y*). Increased inbreeding had positive influence on regression coefficients for calf average daily gain = 0.86±0.26 (*GH L127V*), weight at 8 and 15 month = 1.43±0.13, 1.05±0.05 (*GHR A257G*) and exterior traits — shoulder, back and low back, rump and hindquarter = 1.09±0.11, 0.77±0.22, and 1.10±0.30 (*CYP3A28 C994G*).

Keywords: Aberdeen-Angus breed, inbreeding, SNPs, *CAPN316*, *CAST282, GH L127V, GHR F279Y, GHR A257G*, *CYP3A28 C994G*

Introduction. Inbreeding has become the classical genetics practice problem since the beginning of the 20th century (Altukhov, 2003). It is defined as the probability that two alleles at any locus are 'identical by descent' and occurs when related individuals are mated (Mc Parland et al., 2008). Inbreeding increases the proportion of homozygous loci in the inbred organisms or population, which can increase the chances of offspring being affected by recessive or deleterious traits. Therefore, the offspring of closely related mates tend to have lower fitness and fitness-related characters because of increased risk of inheriting two copies of recessive deleterious alleles, which would expose the offspring to the full (normally hidden) deleterious effects of those alleles. This effect is called 'inbreeding depression' (Davis and Simmen, 2010).

In many human populations, inbreeding resulting from mating between known relatives is rare due to taboos. Nevertheless, significant positive correlation of close inbreeding has been shown for diseases including CVS diseases — hypertension and heart disease, oncology diseases, mental disorders — schizophrenia and bipolar disorder (lifetime health problems: –0.29) (Verweij et al., 2014), monogenic genodermatosis — epidermolysis bullosa, ichthyosis (r=0.99), sensory system disorders and diseases — isolated neurosensory deafness (r=0.728) (Fedota, 2012) and significant decline in child cognitive abilities and mental retardation (verbal, performance and full scale IQ are –22.00, –26.92, and –24.47) (Farid and Afzal, 2014a). Children of inbred families either showed decline in mean value for height, weight and BMI (–7.318, –6.590, and –2.133, respectively) (Farid and Afzal, 2014b).

In cattle breeding, the moderate level of inbreeding due to using of outstanding sires is considered to be powerful method for concentration of desirable characteristics and creating consistent breeds (Ruban,

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Birukova and Basovskiy, 2013). Line breeding enables the perpetuation of founder's commercially valuable traits in progeny, but potentially leads to inbreeding depression in production traits (at high level of inbreeding), increased homozygosity of recessive genetic conditions and reduction in genetic diversity — loss of favorable alleles that may have existed for some traits. Considering the limited number of bulls' seed it is expected that level of inbreeding will increase in separate groups of animals. Some authors have reported that increased inbreeding of the animal is associated with significant reduced birth weight (Hinrichs et al., 2014; Burrow, 1993), post-weaning growth (Burrow, 1993; Davis and Simmen, 2014), milk yield –9.84 kg, fat yield –0.55 kg, fat% –0.0011% (Miglior, Szkotnicki and Burnside, 1992), protein yield –0.66 kg (Rokouei et al., 2010), reproduction traits — fertility (pregnancy rate –6.37%), increased dystocia +1.67% (Gonzalez-Recio, López de Maturana and Gutiérrez, 2007), decreased calving ease, stillbirth rate (Hinrichs et al., 2014) and exterior traits (Rokouei et al., 2010). Therefore, control of inbreeding in livestock populations is of great importance for prevention a rapid loss of genetic variation and adverse phenotypical effects associated with an inbreeding depression.

The aim of our study was to assess the level of inbreeding in 5 loci by SNPs *CAPN316* in calpain gene, *CAST282* in calpastatin gene, *L127V* in growth hormone (*GH*) gene, *F279Y* and *A257G* in growth hormone receptor (*GHR*) gene and *C994G* in CYP3A28 gene associated with growth, productive, reproduction and exterior traits of commercial value in cattle.

Material and methods. The study object was Aberdeen-Angus breeding herd (n=68) bred at PE 'Agrofirma Svitanok' (Kharkiv region, Ukraine). The Aberdeen-Angus group studied is produced under Canadian selection and predominately includes animals belonging to lines: Bryalhill Sau, Ilinmera Leda, MacHery, Prospectora, Raikina, Raimonda, Southome Extra (Kolisnyk et al., 2014). Reproductive traits assessed included weight at 1st calving, age at 1st calving, pregnancy length (P), calving interval (CI), interval between calving and conception (ICC), calf birth weight (CBW) and calf average daily gain (CADG), calculated for pre-weaning period. Evaluation of growth dynamics was conducted via the control weighing at birth, 8, 12, 15 and 18 months, two, three, four and five years age; ADG was calculated for pre-weaning period. Exterior traits were assessed annually until four year age with 100 point scale according 'Guidance on livestock judging of beef breeds' (Ministry of Agrarian Policy of Ukraine, 2002), including maximum scores for body constitution — 15, muscularity — 10, head and neck — 5, chest — 10, shoulder, back and low back — 15, rump — 10, hind quarter — 10, udder — 15, and extremities — 10.

DNA was extracted from blood samples using DNA extraction kits 'Diatom DNA Prep 100' ('Isogene', Russian Federation). For the SNP genotyping, PCR-RFLP methods were set up, using appropriate primer pairs and amplifiaction modes for *CAPN316* (Miquel et al., 2009), for *CAST282* (Schenkel et al., 2006), for *GH L127V* (Lee et al., 2013), for *GHR F279Y* (Viitala et al., 2006), for *GHR A257G* (Komisarek, Michalak and Walendowska, 2011) and for *CYP3A28 C994G* (Sales et al., 2011). Restriction enzymes used were endonucleases RsaI, BtgI, AluI and VspI ('Fermentas', Lithuania). The digested fragments were electrophoresed on 2.0% agarose gel.

The deviation of allele frequencies from Hardy-Weinberg equilibrium was tested using Pearson's chi-squared test. Population heterozygosity was estimated with F coefficient based on ratio between observed and expected heterozygosity indices for all SNPs tested (Zhivotovsky, 1991). The level of inbreeding was assessed by F_{IS}, F_{IT}, F_{ST} coefficients corresponding to measure of inbreeding for individual/subpopulation, individual/total population and subpopulation/total population (Altukhov, 2003). The estimation of inbreeding depression by F_{ST} coefficients for SNPs for traits studied was performed via multiple regression models with forward stepwise (Khalafyan, 2007). When compared three or more groups ANOVA was used. All values were tested on the significance level of 0.05, 0.01 and 0.001.

Results. Generally, population and each line (subgroup) was in Hardy-Weinberg equilibrium by all SNPs studied, except *GHR F279Y* for total population which showed significant disequilibrium ($\chi^2_{act.}$=14.80, p<0.001). The greatest contribution in disequilibrium by SNP *GHR F279Y* was observed for Ilinmera Leda ($\chi^2_{act.}$=5.95) and Southome Extra ($\chi^2_{act.}$=5.33) lines being considered as the best in preliminary genealogical study (Kolisnyk et al., 2014). The disequilibrium suggests that Aberdeen-Angus breeding herd analyzed is likely to be either under focused selection, stratification or inbred. SNP *GHR F279Y* was shown to be associated with milk quality traits (Komisarek, Michalak and Walendowska, 2011). Our results showed association of SNP *GHR F279Y* with dam reproductive characteristics for CI–$F_{act.}$=3.3, CBW–$F_{act.}$=6.5 and CADG $F_{act.}$=6.1, $F_{st.}$=3.2, p<0.05. Considering that effect of this is SNP is observed at initial stage of calf evaluation, present disequilibrium suggests that homozygotization increases: observed and expected number of heterozygous individuals are 11.5% and 22.9%. Moreover, both homozygous classes are generally superior by reproductive traits and weight dynamics than heterozygous one.

Genetic variation within population studied was measured with heterozygosity (Table 1). The highest observed frequency of heterozygotes were noted in Raikina and MacHery lines, but deviation factor values for these lines we low. High level of average heterozygosity could be expected to correlate with high levels of genetic variation. These lines showed unstable and inferior weight dynamics compared to other lines (Kolisnyk et al., 2014). Conversely, Bryalhill Sau and Southome Extra lines showed the lowest frequency of heterozygotes — this circumstance would be indicative of isolation with the subsequent loss of unexploited genetic potential.

Level of inbreeding was characterized by calculation of the F_{IS}, F_{IT} and F_{ST} (Table 1). The highest inbreeding rate (F_{ST}) is observed in Ilinmera Leda line — the level indicating on inbreeding depression. Two lines showed low inbreeding rate suggesting that traits of commercial value are not affected (Gulisija, Gianola and Weigel, 2007).

Table 1 – Population genetics parameters of Aberdeen-Angus herd in Kharkiv region by line

Parameter	Aberdeen-Angus line							
	Bryalhill Sau (n=10)	**Ilinmera Leda (n=13)**	**MacHery (n=9)**	**Prospec tora (n=21)**	**Raikina (n=3)**	**Raimonda (n=3)**	**Southome Extra (n=9)**	**Total (n=68)**
H_{obs}	0.28	0.37	0.42	0.31	0.44	0.39	0.30	0.34
H_{exp}	0.64	0.66	0.71	0.64	0.70	0.54	0.61	0.68
F, %	55.9	44.1	39.9	51.2	36.8	27.6	51.6	49.5
F_{IS}, %	4.7	7.7	–0.4	–3.4	–2.1	2.4	4.1	1.9
F_{IT}, %	3.8	0.6	–1.7	4.2	–0.6	–1.5	2.4	1.0
F_{ST}, %	13.8	27.5	13.1	19.0	3.8	7.3	15.4	14.3

Notes: Heterozygosity: H_{obs} — observed frequency of heterozygotes in the population, H_{exp} — expected frequency of heterozygotes in the population. F — deviation factor calculated as: $F=(H_{exp}–H_{obs})/H_{exp}$. Inbreeding assessment: F_{IS} — for individual relative to subpopulation, F_{IT} — for individual relative to total population and F_{ST} — for subpopulation relative to total population.

Table 2 – Multiple regression coefficients for reproductive characteristics in Aberdeen-Angus herd, $B±s_B$ ($Beta±s_{Beta}$)

Parameter	Aberdeen-Angus line						
	CAPN316	***CAST282***	***GH L127V***	***GHR F279Y***	***GHR A257G***	***CYP3A28 C994G***	**Intercept**
Weight at 1st calving, kg	–75.88 (–0.43)	195.08 (1.22)	–113.98 (–0.86)	8.67 (0.06)	3.56 (0.02)	119.84 (1.00)	407.08
Age at 1st calving, days	346.83 (0.33)	288.44 (0.30)	–846.66 (–1.07)	248.93 (0.29)	49.13 (0.06)	–116.44 (–0.16)	953.04
P, days	19.68±9.36 (1.12±0.53)	–8.69±8.51 (–0.55±0.53)	—	—	—	—	282.35±0.94 ‡
CI, days	—	—	—	–189.88±84.27 (–0.71±0.31)	—	—	465.07±14.64 ‡
ICC, days	157.37±110.26 (0.48±0.40)	—	—	–233.42±90.45 (–0.88±0.34)	—	—	175.51±17.34 ‡
CBW, kg	—	—	3.86±1.55 (0.70±0.28)	–6.39±1.76 * (–1.08±0.30)	4.30±1.72 (0.71±0.28)	—	28.50±0.24 ‡
CADG, kg/day	87.01±34.96 (0.58±0.23)	—	97.93±29.89 * (0.86±0.26)	–84.42±32.97 –0.68±0.27	—	—	729.10±5.46 ‡

Notes: * — significant at 0.05 level, † — significant at 0.01 level, ‡ — significant at 0.001 level

It is known that increasing of the inbreeding coefficient value is associated with a reduction of a number of economically important traits in cattle (Miglior, Szkotnicki and Burnside, 1992; Gulisija, Gianola and Weigel, 2007). To assess the influence of inbreeding by each SNP on cattle productive and performance characteristics multiple regressions analysis was performed (Tables 2–4).

Reproductive characteristics of the herd studied are considered to be stable for P, CI, ICC, CBW and ADG (Table 2). It was found negative effect of inbreeding for SNP *GHR F279Y* on CBW and positive effect for SNP *GH L127V* on CADG. *CC*-genotype of SNP *GH L127V* is associated with higher BW and milk characteristics (Lee et al., 2013) — last can support and justify increased CADG in *CC* homozygous cows.

Table 3 – Multiple regression coefficients for weight dynamics in Aberdeen-Angus herd, $B \pm s_B$ ($Beta \pm s_{Beta}$)

Parameter	Aberdeen-Angus line						
	CAPN316	***CAST282***	***GH L127V***	***GHR F279Y***	***GHR A257G***	***CYP3A28 C994G***	**Intercept**
AGD, g/day	–192.34 (–0.47)	–82.40 (–0,22)	209.46 (0,68)	–250.89 (–0,75)	447.06 (1,31)	–266.03 (–0,96)	753.99
Birth	—	—	—	9.34±5.25 (0.47±0.26)	—	9.77±4.35 (0.59±0.26)	25.72±0.94 ‡
8 month	–113.02±16.81 † (–0.71±0.11)	—	—	–64.27±13.94 * (–0,49±0.11)	189.80±17.44 * (1,43±0.13)	–12.41±10,76 (–0.11±0.10)	196.01±2.48 ‡
12 month	–113.69 (–0.60)	–16.35 (–0.10)	5.05 (0.04)	–38.08 (–0.25)	229.96 (1.47)	–58.01 (–0.45)	254.70
15 month	—	–91.28±7.41 † (–0.54±0.04)	16.26±7.80 (0.11±0.06)	–54.98±8.58 * (–0.36±0.06)	163.26±8.34 † (1.05±0.05)	—	292.36±1.39 ‡
18 month	9.31 (0.05)	–120.86 (–0.71)	69.22 (0.49)	–91.69 (–0.60)	144.19 (0.92)	–19.00 (–0.15)	339.78
2 year	–183.32 (–0.92)	34.02 (0.19)	79.58 (0.52)	–142.98 (–0.87)	244.96 (1.47)	–57.25 (–0.42)	388.19
3 year	–390.19±154.32 (–1.29±0.51)	222.44±119.38 (0.34±0.81)	—	–181.23±71.36 (–0.73±0.29)	388.27±91,87 (1.55±0.36)	—	398.80±12.22 ‡
4 year	–559.97 (–1.27)	284.41 (0.71)	183.61 (0.55)	–347.16 (–0.96)	552.70 (1.50)	–97.73 (–0.32)	433.67
5 year	450.65 (0.63)	–73.92 (–0.12)	—	469,41 (1.08)	–334.27 (–0.73)	–185.87 (–0.47)	473.93

Notes: * — significant at 0.05 level, † — significant at 0.01 level, ‡ — significant at 0.001 level

High level of inbreeding for SNPs *CAPN316*, *CAST282* and *F279Y* negatively correlated with weight until 15 month age, although increase of inbreeding in SNP *GHR A257G* positively correlated with body weight. First is supported by data on beef cattle that inbreeding of the individual has a consistent adverse effect on growth traits from birth to maturity and on maternal traits. More specifically, for every 1% increase in inbreeding coefficient a decrease of 0.06, 0.44, 0.69 and 1.30 kg in live weight at birth, weaning, yearling and maturity respectively. Additionally, inbreeding in the dam decreased weaning and yearling weights by 0.30 and 0.21 kg respectively for every 1% increase in inbreeding coefficient, probably as a result of decreasing milk yield and reduced maternal value of the inbred dams. (Burrow, 1993). According Davis and Simmen (2010) increased level of inbreeding resulted in lower body weight and average daily gain mediated by IGF-I concentration in blood. The values of inbreeding coefficients in cows and calves (n=3243) were 4.20% and 6.82%, and there was an annual increase for these factors by 0.25% per year and 0.36% per year. The level of inbreeding in the population of the US Angus population was estimated to be in 1985 — 3.3%, in 1995 — 4.7%, in 1999 — 3.5%, and in 2003 — 4,2% (Saatchi et al., 2011).

Table 4 – Multiple regression coefficients for exterior traits in Aberdeen-Angus herd, $B \pm s_B$ ($Beta \pm s_{Beta}$)

Parameter	Aberdeen-Angus line						
	CAPN316	***CAST282***	***GH L127V***	***GHR F279Y***	***GHR A257G***	***CYP3A28 C994G***	**Intercept**
Body constitution	–1.78 (–0.36)	3.07 (0.68)	–1.16 (–0.31)	0.11 (0.03)	–0.22 (–0.05)	3.81 (1.13)	11.39
Muscularity	–1.23 (–0.54)	2.25 (1.08)	–0.85 (–0.49)	–0.49 (–0.26)	–0.55 (–0.29)	1.50 (0.97)	8.04
Head and neck	1.15 (0.58)	0.28 (0.15)	0.96 (0.63)	–1.00 (–0.61)	–1.14 (–0.68)	0.70 (0.51)	3.84
Chest	2.69±1.33 (0.63±0.31)	–	–	–1.24±1.11 (–0.35±0.31)	–1.87±1.39 (–0.52±0.39)	3.00±0.86 (1.03±0.29)	7.59±0.20 ‡
Shoulder, back and low back	–	–	–1.45±0.62 (–0.30±0.13)	–1.58±0.62 (–0.30±0.12)	1.17±0.62 (0.12±0.22)	4.74±0.49 * (1.09±0.11)	11.37±0.08 ‡
Rump	–	–	0.40±0.28 (0.38±0.26)	–0.78±0.25 (–0.69±0.22)	–	0.72±0.21 * (0.77±0.22)	7.99±0.04 ‡
Hind quarter	–	–	–1.61±0.99 (–0.49±0.30)	–	–	3.24±0.89 * (1.10±0.30)	8.17±0.14 ‡
Udder	–1.69 (–0.62)	0.25 (0.10)	–1.24 (–0.61)	0.09 (0.04)	0.29 (0.13)	1.87 (1.01)	12.52
Extremities	–	–	–	0.49±0.37 (0.39±0.29)	–	0.66±0.03 (0.63±0.29)	7.76±0.07 ‡
Total	–7.87 (–0.32)	9.87 (0.44)	–4.32 (–0.23)	–5.13 (–0.25)	1.21 (0.06)	19.09 (1.14)	78.78

Notes: * — significant at 0.05 level, † — significant at 0.01 level, ‡ — significant at 0.001 level

Exterior trait composition is considered to be characteristic for each breed; therefore the level of inbreeding had little influence on their performance. F_{ST} for *CYP3A28 C994G* positively correlated with shoulder, back and low back, rump and hindquarter size and condition.

Conclusion. The population studied is in Herdy-Weinberg equilibrium for all SNPs analyzed, except *GHR F279Y*. Lines Bryalhil Sau and Southome Extra considered as the best lines showed the lowest level of heterozygosity. Negative effect of level of inbreeding for different markers were seen for regression coefficients calf birth weight — –1.08±0.30 (*GHR F279Y*), body weight at 8 month — –0.71±0.11 (*CAPN316*), –0.49±0.11 (*GHR F279Y*) and 15 month — –0.54±0.04 (*CAST282*), –0.36±0.06 (*GHR F279Y*). Increased inbreeding had positive influence on regression coefficients for calf average daily gain — 0.86±0.26 (*GH L127V*), weight at 8 and 15 month — 1.43±0.13, 1.05±0.05 (*GHR A257G*) and exterior traits — shoulder, back and low back, rump and hindquarter — 1.09±0.11, 0.77±0.22, and 1.10±0.30 (*CYP3A28 C994G*).

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IMPROVEMENT AND OPTIMIZATION OF ANTIGENIC COMPOSITION FOR SERODIAGNOSIS OF TUBERCULOSIS

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There is a pressing of needs for improvement existing methods of tuberculosis (TB) diagnosis and screening. New methods should be characterized by high specificity, sensitivity, reliability of results; easiness of implementation; precision interpretation of the results. Regarding the TB, the ELISA test-systems have many advantages over traditional methods of disease diagnostics. However, the most difficult objective of creating such test-systems is the selection of optimal antigenic substance which would characterized by the high antigenicity, and high specificity from the other hand. The aim of the research was to improve previously created antigenic composition using full-size highly immunogenic proteins of *Mycobacterium tuberculosis* MPT63 and MPT83.

The genetic construction pET28a-MPT83(full)-MPT63 was obtain, with further isolation and purification of target protein and compared to its predecessor which based on FLD of MPT83(115–220 aa). Both fusion proteins were tested to the culture medium of hybridomas, which were obtained from mice immunized with a mixture of antigens from mycobacteria, including cell membrane, associated protein MPT83 closest homologue — MPT70. In addition, sera samples from infected *M. bovis* and healthy cattle were tested on several variants of *Mycobacterium fusion* proteins.

These data suggest the need of N-terminal amino acids for protein folding for better antibody recognition. The new antigenic substance gives the best results for serology-based diagnostics of tuberculosis. Using of specific monoclonal antibodies to new obtained antigen as a positive control for the test system can reduce the cost of it and avoid using genuine infected cattle or human serum among ELISA kit reagents.

Keywords: *Mycobacterium tuberculosis, Mycobacterium bovis*, antigenic substance, MPT63, MPT83, chimeric protein, serology-based diagnostics, tuberculosis

Introduction. Mycobacterioses (including TB, leprosy, atypical mycobacterioses, paratuberculosis) are the widespread infectious diseases of human being and animals (Mencarini et al., 2016). TB is a major reason of high levels of morbidity and mortality in the developing countries of the World (Banerjee et al., 2003) and causes a significant damage for livestock (Maia et al., 2014). Current tests most widely used for the detection of tuberculosis both in cattle and humans include measurement of delayed type hypersensitivity (i.e., skin tuberculin testing) to purified protein derivatives (PPD) and *in vitro* assay for gamma interferon (IFN-γ) produced in response to mycobacterial antigen stimulation (Waters et al., 2011). However, these methods are not enough specific, or quite expensive, either requires much time for diagnosis occurrence. Owing to the very high infectious power of pathogenic *Mycobacteria*, early diagnosis is essential to prevent spreading of the disease among the herd or people population.

Serodiagnosis by ELISA has been widely used in the diagnosis of different infectious disease, including TB. The major problem for this method is the optimal choice of highly immunogenic and pure substance for determination of antibodies against the pathogen. The aim of our work is reconstruction unique primary structure of a protein of *M. tuberculosis* MPT83 consisting of the chimeric protein MPT83 (full)-MPT63. Test systems based on specific recombinant antigens of mycobacteria, for example on the basis of two homologous proteins of *M. tuberculosis* MPT70 and MPT83 (Waters et al., 2011), existing in overseas markets. However, the using of proteins with a high degree of homology (Wiker, 2009) is not appropriate to meet the parameters of sensitivity and specificity of serology-based diagnostics. We suggest that the use of fundamentally different antigens (Manca et al., 1997; Chambers et al., 2010) as a parts of fusion protein with spacer link between protein's components can increase exposure of serologically important epitopes, for early diagnosis of the disease in animals and humans.

It was shown dramatically increased yield of MPT83 (full)-MPT63 antigen and it purity in comparison with shorter analogue based on FLD MPT83 (115–220 aa). In addition, used sera from natural infected and relatively healthy cattle have been shown serological value of new obtained antigenic substance. Based on data obtained

previously and these data, ELISA serves as superb method for screening of animals from the herd and potentially people, because antigens of *M. tuberculosis* MPT63 and MPT83 and *M. bovis* MPB63 and MPB83 identical by the amino acids composition in both representatives (Redchuk et al., 2010; Redchuk et al., 2010).

Wild animals such as badgers, deer could be natural foci of TB infection (Miller, Farnsworth, and Malmberg, 2013). Whereas, tuberculosis in humans may result from exposure to any one of the tubercle bacilli included within the *Mycobacterium tuberculosis complex*, including *M. bovis* (Waters et al., 2011). This implies, to abort the One Health TB triad (human-livestock-wildlife) (Wadhwa et al., 2014), there is an urgent need to isolate animals in the early stages to protect human life.

Materials and methods. *Obtaining of genetic construction of the chimeric protein MPT83(full-sized)-MPT63.* The gene encoded full-length soluble protein MPT83 amplified from plasmid DNA p*ET*24*a-mpt83* using pair of oligos which being finished by restriction endonucleases sites *Eco*RI and *Bam*HI — mpt83-SP TGGATCCAGCACCAAACCCGTGTCGCA and mpt83-ASP TAGAATTCTGTGCCGGGGGC. The product of amplification was insert in p*ET*28*a(+)* ('Novagen', Germany) plasmid DNA at the appropriate sites for restriction endonucleases. MPT63 gene was amplified from plasmid DNA p*ET*24*a-mp63* using a pair of primers mpt63-ASP TCAGCTCGAGCGGCTCCCAAATCAGCAGA and mpt63-SPACAAGCTTTTGCTCACCACAATGATC AAGACGGC. The obtained PCR product was nest in plasmid DNA p*UC*-19 ('Novagen', Germany) by blunt-ends cloning. After restriction analysis *mpt63* correct sequences and obtained vector p*ET*28*a-mpt83* were treated with restriction enzyme *Xho*I and *Hind*III. The resulting fragments of p*ET*28*a-mpt83* vector and DNA sequence *mpt63* mixed in a molar ratio of 1:3 and were crosslinked using T4 DNA ligase ('Thermo Scientific', Lithuania). The obtained construct was used to transform *E. coli* Rosetta (DE3) host cells ('Novagen', Germany).

Obtaining and purification of recombinant fusion protein MPT83(full-sized)-MPT63 in procaryotic expression system. Expressed MPT83(full)-MPT63 *E. coli* cells accumulated biomass in LB-Medium ('Carl Roth', Germany) with 50 μg/ml kanamycin and 1% glucose at 37°C and active mixing (250 rpm) to optical density A_{600} 0.3–0.5. The expression of obtained gene construct was induced by 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) ('Thermo Scientific', Lithuania). Target protein was obtained by the method described in (Siromolot et al., 2016) conjunction with use of protease inhibitors cocktail ('Thermo Scientific', Lithuania).

SDS-PAAG electrophoresis. Protein fraction separation was performed in 10% polyacrylamide gels (PAGE), at a voltage of 10 V/cm at the denaturing condition.

Indirect enzyme-linked immunosorbent assay (ELISA). The antigens were sorbed to the 96-well plate at final concentration 5 μg/ml per well in phosphate buffered saline (PBS) (0.8% NaCl, 0.02% KCl, 0.144% Na_2HPO_4, 0.024% KH_2PO_4, pH 7.4) and left for 1 hour at 37°C. As a blocking agent was used non-fat 1% milk in PBS. The incubation with monoclonal antibodies against MPT70 and MPT83 of *M. tuberculosis* in PBS-T (adding Tween-20 to a final concentration of 0.04%) was performed 1 hour. Cattle serum samples were diluted in ratio 1:10. Anti-mouse-HRP conjugate (1:12,000) for mAb and anti-bovine-HRP conjugate for cattle serum Ab detection were used. TMB (3,3',5,5'-tetramethylbenzidine) was used as chromogen substrate. The color reaction was quantified by measuring the absorbency at 490 nm. Sera samples were provided by National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine).

Results and discussion. *Obtaining of genetic construction pET28a-mpt83-mpt63 carrying a full-size gene nucleotide sequence of mpt83.* We have obtained fascyclin-like domain (FLD) of MPT83 (115–220 aa) in our lab previously, which was used for indirect ELISA-based test-system design. The value of a new developed chimeric protein with use recombinant full-length MPT83 consist in playback of a unique immunological properties of the original protein. New improved antigenic substance has been characterized with better antigenic feature that affect the sensitivity and specificity of analysis. The gene *mpt83* was amplified from pDNA construction p*ET*24*a-mpt83* by PCR. Cloning was carried out using *E. coli* Rosetta (DE3) ('Novagen', Germany) and vector p*ET*28*a* (+) (Fig. 1).

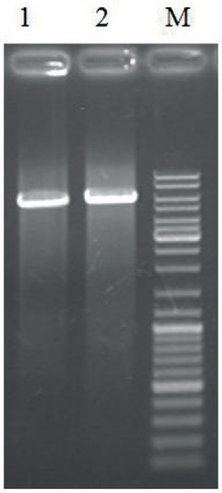

Figure 1. Electrophoregram of selected plasmid vector p*ET*28*a* (1) and created the first stage genetic construction p*ET*28*a*, which carries in itself a full-length gene insertion MPT83 (2)

To further fusion of the *mpt83* and *mpt63* genetic sequences it was decided to create genetic construction p*UC*-19-*mpt63*. Thus, we were extension nucleotide sequences before the restriction enzyme site *Hind*III. The gene *mpt63* cloning was performed by blunt-ends type. The anticipated consequence of cloning by blunt-ends type is a high probability of duplication of gene sequences in plasmid design construct (Fig. 2A). Due to such phenomenon, clone p*UC*-19-(*mpt63*)^2 has been selected in the processing of restriction enzyme *Hind*III on it we get not a single chain rupture with subsequent linearization of DNA but two separate products — *mpt63* and p*UC*-19-*mpt63* (Fig. 2B).

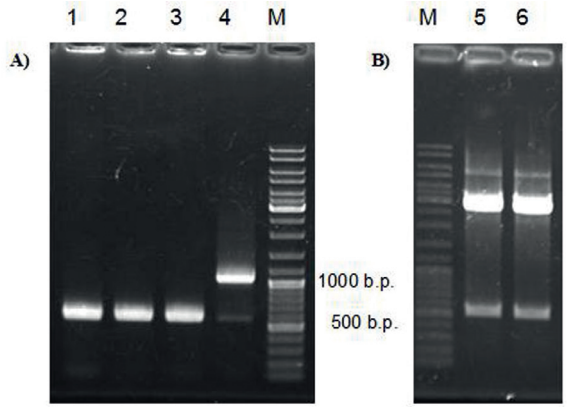

Figure 2. A) Electrophoregram of products of PCR-analysis using primers flanking vector polylinker area: 1–3 — PCR products corresponding to the nucleotide sequence of the gene *mpt63*; 4 — PCR product corresponding to the dimer of two nucleotide sequences of the gene $(mpt63)_2$. B) 5–6 — genetic construction p*UC*-19-$(mpt63)_2$ after treatment with restriction enzyme *Hind*III (circular and linearized form of plasmid DNA and gene *mpt63*)

Thus, resulting nucleotide sequence of the gene *mpt63* treated with endonuclease restriction enzyme *Xho*I to further fusion with previously obtained genetic construct p*ET*28*a*-*mpt83*. To determine the presence of the incorporated nucleotide sequence *mpt63*-*mpt83* consist into genetic construct was performed PCR analysis using primers flanking the gene *mpt63* on one side and the gene *mpt83* on the other. Results of PCR analysis confirmed the presence of the fusion nucleotide sequence *mpt63*-*mpt83* (the size of approximately 1,300 base pairs) in developed p*ET*28*a*-*mpt83*-*mpt63* construction (Fig. 3).

Expression and comparative characteristics of obtained recombinant fusion protein MPT83(full length)-MPT63. The recombinant protein was obtained from 1 ml of *E. coli* culture, which were incubated 4 hours at active mixing at speed of 250 rpm and 30°C and optical density A_{600} 0.3–0.5 in the presence of the inductor 1mM IPTG. The precipitate cells were analyzed for the presence of protein in the soluble and insoluble fraction. Analysis factions of new fusion protein compared to chimeric protein-based FLD MPT83 (115–220 aa) (Fig. 4).

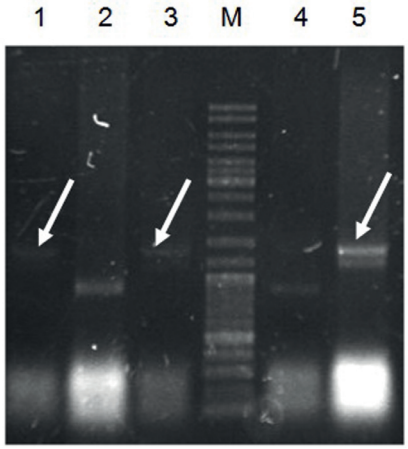

Figure 3. Electrophoregram of PCR-analysis of transformed *E. coli* clones with genetic construct p*ET*28*a*-*mpt83*-*mpt63*. Clones 1, 3 and 5 have the genetic structure of the combined nucleotide sequences of genes *mpt83* and *mp63* (indicated by arrows)

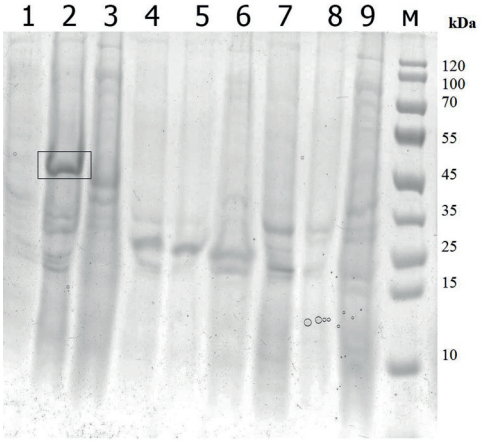

Figure 4. The results of electrophoretic separation of cell lysates of bacteria-producers during the analytic expression of target protein MPT83(full)-MPT63 (1–3), FLD MPT63-MPT83 (115–220 aa) (4–6) (1, 4 — soluble fraction; 2, 5 — insoluble fraction; 3, 6 — total cell lysate). The frame indicate protein product that meets the target protein molecular weight MPT83 (full)-MPT63

Immunological characterization of MPT83 (full)-MPT63. Thus, obtained genetic construction p*ET*24*a*-*mpt83* (full)-*mpt63* made it possible to expression of the functional target protein in the insoluble fraction (inclusion bodies). However, the main purpose of obtaining fusion protein was the creation of antigenic molecules consisting of two highly antigenic

components — full-size MPT83 and MPT63. In addition, the newly created protein characterized by the fact that both antigenic components not only full length, but also insert in plasmid design so that between them inserted spacer (in translational variant polypeptide chain length of 8 amino acid residues), which can affect their folding process biosynthesis. Instead that, our obtained previously fusion protein MPT63-MPT83 (115–220 aa) not contained in the structure spacer link polypeptide chain. Lack of those small sequences areas in the structure of MPT63-MPT83 (115–220 aa) can affect protein folding and, consequently, limit the exposure of diagnostically important epitopes in the structure of the whole molecule.

In order to conduct the comparative characteristics of antigenic properties of two kind fusion protein MPT63-MPT83 (115–220 aa) and MPT83 (full)-MPT63 we conducted indirect ELISA using monoclonal antibodies (mAb) to the N-terminal molecule sites of full-MPT83 of *M. tuberculosis.* The results of ELISA indicated the ability of mAb to the N-terminal molecule sites of full-MPT83 antigen specific recognition of the new recombinant chimeric MPT83 (full)-MPT63. Instead, the recombinant antigen based on the shortened form FLD MPT83 — MPT63-MPT83 (115–220 aa) not specified recognized by mAb (Fig. 5). So, the presence of additional N-terminal site in the structure of MPT83 (full)-MPT63 makes it more attractive and promising antigen composition for serology-based diagnostics.

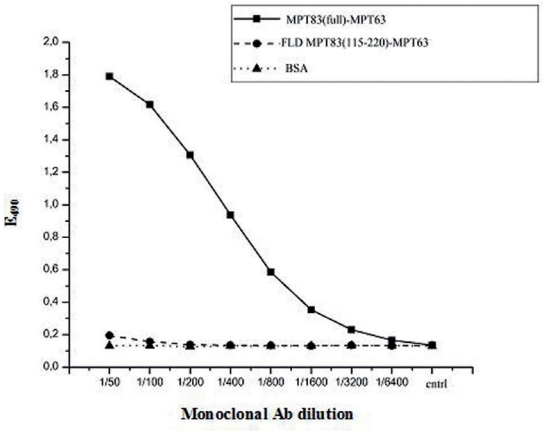

Figure 5. Recognition results of obtained recombinant protein MPT83(full)-MPT63 with monoclonal antibodies to the N-terminal area of MPT83 and MPT70 compared to the shortened form of recombinant antigen FLD MPT83(115-220 aa)-MPT63

Confirming our hypothesis has found in the testing of cattle serum samples of healthy and infected by *M. bovis* animals on fusion proteins consist of not only MPT63, MPT83 and FLD MPT83(115–220 aa) antigens, but also other kind of *Mycobacterium* proteins (Fig. 6).

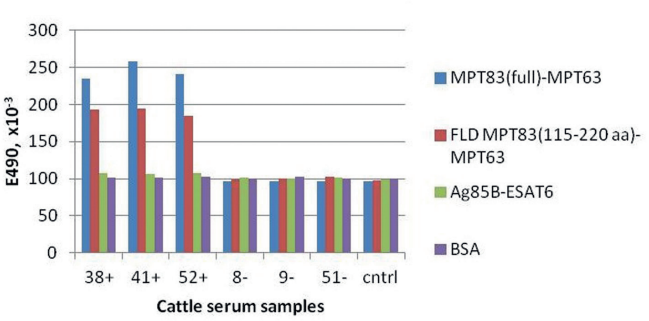

Figure 6. IgG level to the target and control antigens in the cattle serum samples

Screening results show that the improved variant of antigenic substances was able for better identification of sick animals in comparison with 3rd type of chimeric recombinant proteins that have been tested on cattle sera.

Thus, the data indicate that improved antigenic substance consisting of full-size antigens proved itself better for infected animals' detection. Use of mAb which were derived from mice immunized by wide range of *Mycobacterium* proteins, including homologous antigens MPT70 and MPT83 showed the effectiveness of the establishment of the substance based on the whole molecule. Noteworthy, that using a hybridomas culture fluid can replace positive control in the test sets instead a dangerous serum from infected animal or human.

We believe that chosen successful combination of antigens: MPT63 — secretory protein of *M. tuberculosis*, and MPT83 — associated with cell membrane antigen. Therefore, it should be noted that the antigens of *M. bovis* MPB63 and MPB83 and antigens of *M. tuberculosis* MPT63 and MPT83 do not distinguish in functionally product and primary protein structure. This could mean that these fusion proteins can be used to create test systems for the diagnosis of TB in humans.

Conclusions. Conserving of the unique primary protein structure can achieve the best indicators of sensitivity of antigenic substances in the development of test systems and diagnostics to identify markers of infectious diseases. The use of highly immunogenic antigens of *M. tuberculosis* MPT63 and MPT83 which are secretory and miristilated with cell membrane respectively achieves the best parameters of specificity too. It was shown dramatic significance of spatial organization of antigenic composition and its flexibility for exposure of serologically important epitopes.

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INFLUENCE OF PHYSICAL AND EMOTIONAL LOADINGS ON DYNAMICS OF BIOCHEMICAL INDICATORS OF BLOOD SERUM OF RACE HORSES OF GROUP OF UNIVERSAL USE

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Summary. The dates concerning dynamics of level of biochemical indicators of the blood serum of race horses of group of universal use of the Ukrainian riding breed are given in the article. Clinically healthy horses were an object of a research. The samples of blood were taken from the jugular vein to get serum and for further biochemical research. The blood was taken in the condition of relative rest, immediately after ordinary training and after emotional stress during the entertaining performance with participation of a large number of people and loud music. In blood serum the following biochemical indicators were defined: total protein, urea, creatinine, uric acid, total bilirubin and its fractions, glucose, cholesterol, triglycerides, Calcium, Iron, lactate, pyruvate, activity of the ALT, AST, GGT, LDH, ALP. It is established that during training and psycho-emotional loadings of race horses of the Ukrainian riding breed multidirectional changes of level of biochemical indicators in the blood serum were observed that testify the tension of metabolic processes in the animal organism. Emotional loading is the more strong stress factor that causes negative changes in the level of metabolic profile indicators.

Keywords: race horses, biochemical indicators, blood serum, training, stress

Introduction. In recent years the attention to equestrian sport has increased. Experts have complicated challenges: to keep efficiency of animals, to increase their sports longevity, to promote adaptation of a horse to conditions of training process at most, to correct a physiological condition of horses adequately and in time, to avoid an overtraining, to minimize a psychological and physiological stress (Borodkina, 2008; Gorbunova 2009; Mansurova 2009; Polozkov, 1985).

Biochemical methods give a chance to estimate adaptation potential of horses in the conditions of various loadings and to estimate effectively the state of health of horses and their training opportunities (Tkhinvaleli, 2011).

The purpose of the research — to establish the influence of physical and emotional loadings on biochemical indicators of blood serum of horses of the Ukrainian riding breed of group of universal use.

Materials and methods. Twelve race horses of the Ukrainian riding breed were selected for the research. Animals were at the same time used in various performances (show jumping, dressage, horse theater) so horses were brought together in separate group of universal use. All animals were clinically healthy. The samples of blood were taken from the jugular vein to get serum and for further biochemical research. The blood was taken in the condition of relative rest, immediately after physical loading and emotional loading (stress) during entertaining performance with participation of a large number of people and loud music. In blood serum the following biochemical indicators were defined: total protein, urea, creatinine, uric acid, total bilirubin and its fractions, glucose, cholesterol, triglycerides, Calcium, Iron, lactate, pyruvate, activity of the ALT, AST, GGT, LDH, ALP. Defining of biochemical indicators were made according to common used methods.

Calculations of the received results were carried out on the personal computer by means of the statistical program Statistica 7.0 (StatSoft, USA).

Results of research. Dynamic of biochemical indicators of blood serum of race horses of universal group of use with different options of stress loading is given in Tables 1–4.

As it is specified in Table 1, in blood serum of horses changes of level of the total protein with both options of loading are not observed.

It corresponds to absence of changes of urea content after physical loading and to its insignificant reduction after emotional pressure. Obviously, these loadings do not significantly influence the ability of a liver to synthesize proteins of blood serum of race horses of group of universal use adapted to different options of loadings. Concentration of creatinine increases by 21.0% ($p \leq 0.001$) after physical loading of animals while at emotional pressure the indicator increases only by 7.4% ($p \leq 0.001$). Obviously, such distinction is caused by more active participation of muscular system of animals in the conditions of the increased physical activity. However, these fluctuations of concentration of creatinine do not exceed the limit of reference norm for horses and have functional character. The most essential distinctions are established in the analysis of changes of content of uric acid in blood serum of horses in both groups. So at a physical loading concentration of uric acid has increased

by 27.0% (p≤0.05) while at an emotional loading for 74.2% (p≤0.001). Uric acid is a biomarker of an oxidative stress that indicates stronger stressful effect of emotional loading on horses in comparison with physical loading.

The further analysis of results of a research (Table 2) has confirmed this conclusion as the majority of the studied indicators in blood serum of horses of this group after physical loading authentically did not differ from indicators in a condition of relative rest. It concerns such tests as the general bilirubin and its fractions, activity of ALT, AST, GGT. After emotional loading significant increase in the general bilirubin by 105.4% (p≤0.001) at the expense of both of its fractions, and also activity of ALP (p≤0.05) for 28.6% was observed that indicates the stagnation in external bile-excreting channels. Most likely it happens because of spasm after the emotional loading caused by a big congestion of people and loud music. More often it happens with animals which are not adapted to such conditions. At the same time activity of GGT in serum of blood of horses after an emotional loading has gone down for 13.1% (p≤0.01) that does not go beyond reference norm for horses. It is known that increase in serum of blood of this enzyme allows to establish stagnation in the intra hepatic bilious ways and definition of ALP has limited diagnostic value for horses. Therefore it is possible to come to a conclusion that hyperbilirubinemia that was determined in the research is temporary and does not confirm pathological changes of hyperbiliar system after emotional loading.

Table 1 – Indicators of protein metabolism of blood serum of horses in a condition of relative rest, after physical and emotional loadings (n=12)

Indicators		Conditions of relative rest	Conditions after physical loading	Conditions after emotional loading
Total protein, g/liter	M±m	60.2±1.06	67.6±3.04	57.9±0.80
	Lim	54.7–66.4	54.5–81.6	52.8–61.6
Urea, mmol/liter	M±m	7.2±0.17	7.0±0.37	5.8±0.08**
	Lim	6.1–7.9	4.3–8.6	5.3–6.2
Creatinine, μmol/liter	M±m	115.0±1.22	139.2±4.28***	123.6±1.67*
	Lim	106.5–120.6	116.2–156.1	115.3–131.4
Uric acid, μmol/liter	M±m	43.2±3.06	55.0±1.51*	75.3±2.53***
	Lim	25.2–54.1	44.3–61.5	60.6–85.4

Note: * — p≤0.05, ** — p≤0.01, *** — p≤0.001 — in comparison with conditions of relative rest

Table 2 – Indicators of activity of enzymes in blood serum of horses in a condition of relative rest, after physical and emotional loadings (n=12)

Indicators		Conditions of relative rest	Conditions after physical loading	Conditions after emotional loading
Total bilirubin, μmol/liter	M±m	16.7±1.78	14.4±1.23	34.3±0.95***
	Lim	6.5–24.1	8.9–20.5	29.9–39.8
Conjugated bilirubin, μmol/liter	M±m	7.9±1.00	7.2±0.71	16.5±0.63***
	Lim	2.2–11.3	4.2–10.2	11.3–18.8
Unconjugated bilirubin, μmol/liter	M±m	8.8±0.83	7.1±0.63	17.8±0.87***
	Lim	4.3–14.0	4.6–10.3	13.4–23.1
ALT, U/liter	M±m	14.0±0.57	18.9±1.83	13.3±0.20
	Lim	10.4–17.1	10.2–26.0	11.8–14.2

AST, U/liter	M±m	340.4±4.48	320.3±18.96	351.0±7.64
	Lim	315.4–372.6	244.3–399.4	324.5–398.1
ALP, U/liter	M±m	143.8±11.73	167.7±13.92	185.0±7.42*
	Lim	104.6–211.1	83.4–216.9	143.1–215.4
GGT, U/liter	M±m	49.0±0.87	47.1±2.69	42.6±0.74**
	Lim	44.3–53.8	28.5–55.6	38.4–47.1

Note: * — p≤0.05, ** — p≤0.01, *** — p≤0.001 — in comparison with conditions of relative rest

Table 3 – Indicators of carbohydronic and lipidic exchanges in serum of blood of horses in a condition of relative rest, after physical and emotional loadings (n=12)

Indicators		**Conditions of relative rest**	**Conditions after physical loading**	**Conditions after emotional loading**
Glucose, mmol/liter	M±m	4.4±0.16	4.7±0.17	5.4±0.20**
	Lim	3.7–5.1	4.1–5.7	4.7–6.9
LDH, U/liter	M±m	230.8±8.02	322.0±10.61***	298.9±3.97***
	Lim	194.3–270.3	264.3–383.9	275.9–318.0
Cholesterol, mmol/liter	M±m	1.9±0.05	3.2±0.16***	2.3±0.08**
	Lim	1.6–2.2	2.0–3.8	1.9–2.7
Triglycerides, mmol/liter	M±m	1.34±0.042	1.73±0.238	2.06±0.036***
	Lim	1.18–1.55	0.37–2.63	1.85–2.23
Lactate, mmol/liter	M±m	1.40±0.03	3.43±0.29***	2.2±0.05***
	Lim	1.2–1.6	2.0–4.7	1.9–2.4
Pyruvate, mmol/liter	M±m	0.34±0.015	0.17±0.015***	0.26±0.01***
	Lim	0.27–0.42	0.11–0.31	0.21–0.33

Note: * — p≤0.05, ** — p≤0.01, *** — p≤0.001 — in comparison with conditions of relative rest

The analysis of the dates provided in Table 3 has confirmed that emotional loading leads to reaction of an organism caused by an emotional stress in the form of increase in concentration of glucose for 22.6% (p≤0.001) that is not observed after physical loading.

However after both types of loadings reactions of anaerobic glycolysis were observed, and especially after physical loading that is confirmed by increase of activity of LDH for 40.0% (p≤0.001) while after emotional loading — for 29.5% (p≤0.001) that is connected with smaller participation of muscular system at emotional loading of horses. These results coincide with more substantial increase of concentration of a lactate for 145.0% (p≤0.001) after physical loading in comparison with increase of an indicator for 56.0% (p≤0.001) after emotional loading. Concentration of pyruvate also changed. After physical loading it has decreased by 50.0% (p≤0.001) and after emotional loading — for 24.5% (p≤0.001). After physical loading concentration of cholesterol in the absence of change of concentration of triglycerides increases by 69.0% (p≤0.001) while as a result of emotional loading hyperlipidemia occurs due to increase in concentration of cholesterol for 18.9% (p≤0.01) and triglycerides for 53.0% (p≤0.001) due to strengthening of a lipolysis.

According to data given in the Table 4 concentration of general Calcium and Iron was identical at rest and after physical loading. After emotional stress the level of general Calcium increased by 30.8% (p≤0.001) but did not go beyond standard norms.

Thus, physical loading and an emotional stress during tests cause multidirectional changes of level of biochemical indicators of serum of blood in group of race horses of universal use that testifies to tension of metabolic processes in organisms of animals.

Table 4 – Indicators of Calcium and Iron in serum of blood of horses in a condition of relative rest, after physical and emotional loading (n=12)

Indicators		Conditions of relative rest	Conditions after physical loading	Conditions after emotional loading
Calcium, mmol/liter	M±m	1.8±0.08	2.1±0.11	2.4±0.04***
	Lim	1.5–2.4	1.6–2.7	2.2–2.6
Ferum, μmol/liter	M±m	40.0±1.30	37.0±1.30	40.0±0.50
	Lim	33.0–47.0	31.0–45.0	37.0–43.0

Note: * — p≤0.05, ** — p≤0.01, *** — p≤0.001 — in comparison with conditions of relative rest

Conclusions. After physical tension and emotional loading, unlike a condition of relative rest, sports horses of group of universal use have stable or are slightly changed such indicators of protein metabolism as general protein of serum of blood, creatinine and urea.

Concentration of uric acid which is a biomarker of an oxidative stress increases more after emotional loading in comparison with physical loading.

After emotional loading the content of glucose enlarges with the increase of activity of LDH, the content of lactate and decrease of a pyruvate as an indicator of strengthening of anaerobic glycolysis and gluconeogenesis. Lipolysis also amplifies because of cholesterol and triglycerides. After physical loading these changes are less expressed.

After emotional stress the hyperbilirubinemia is developed at the expense of both fractions of bilirubin which is followed by slight increase of activity of ALP and lack of essential changes of activity of GGT that is caused by stagnation in the external bile-excreting ways, most likely because of a short-term spasm.

The short-term physical and emotional loadings for race horses of group of universal use leads to changes of indicators of a metabolic profile of different degree and orientation depending on character of the operating stressor.

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Part 2. Biosafety

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THE EFFECTIVENESS OF APPLICATION ULTRAVIOLET RADIATION FOR THE SANITATION OF PRODUCTION PREMISES OF MEAT PROCESSING ENTERPRISES

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Summary. Bacteriological researches are found ongoing increase of a total number of microorganisms (TNM) in premises during the working day. Air disinfection in premises amounted 98–100% after use of UV radiation. Use of arrangement of closed type (recirculators) allowed reducing TNM in air of working premises on 25.3 %. It is proposed solution of technology of air disinfection in production premises of meat processing enterprises with the help of UVR–recirculators.

Keywords: disinfection, sanitation, ultraviolet, germicidal lamp, UVR recyclers

Introduction. Great attention in the world-wide practice is paid to the problem of obtaining product of high sanitary quality and safety for human at the enterprise of meat and meat products (Bawcom et al., 1995; Castillo et al., 1998; Bolder, 1997).

Large number of microorganisms, including pathogenic — *Escherichia coli* (*E. coli*), *Staphylococcus*, fungi etc., accumulate in the air of meat-shops and meat-processing plants while the slaughter of cattle and meat processing. Microorganisms that are accumulated in the air, on the walls and process equipment, regardless of pathogenicity and their metabolic products (especially microscopic fungi) in case of contact with raw meat may generate a risk to human health due to contamination or food poisoning. Therefore, to obtain safe products of high sanitary quality for human is need to use ecologically safe methods of sanitation of the ambient air in premises of meat enterprise shops (Bohatko and Sakhniuk, 2013; Prokopenko, 2013).

With the aim of disinfection of objects veterinary supervision were developed, tested and proposed for use a wide range of efficient disinfectants and detergent–disinfectants, use of which allows to maintain the veterinary-sanitary status of the processed objects at a high level. However, most of the existing specimens in their physico-chemical and toxicology characteristics do not match any existing today the requirements, especially when they are used in the food industry. Therefore, it is a reasoned necessary search of ecologically safe and highly effective methods of sanitation (Paliy and Paliy, 2016).

Today, ultraviolet germicidal radiation (UV radiation) is one of the most effective preventive sanitary-hygienic means, which suppress the viability of microorganisms in air and water. UVR is widely used abroad as well as in Ukraine at food industry enterprises (shop meat, fish, dairy, bakery, brewery, fruits and vegetables and other products, food bases, warehouses, stores, etc.) for disinfection of air and process equipment surfaces with the aim to compliance the hygienic requirements to the indicator standards of the quality and safety of food raw materials and food products. But the main use of UV in the food industry is disinfection of air in the production area to prevent contamination of the production by airborne organisms (Illarionova, Gymerov and Reshetnik, 2010; Prokopenko, 2013).

UV disinfection has certain advantages in comparing with traditional thermal and chemical disinfectants. So, its bactericidal action more effective at room-temperature, there is no impact on objects, which are processed, satisfy the requirements of environmental safety, has greater producing capacity at a lower laboriousness of the operations for machining, does not require special protective measures, is economically advantageous, and its use eliminates the necessity of usage large quantities of disinfectants (Ivanenko, Khizgiyaev and Mizgaylov, 2006; Tiganov, 2007).

The rational use of UV radiation does not negatively affect the organoleptic (color, smell, taste, texture, appearance) and physicochemical features of foods and raw materials of animal origin (Tiganov, 2007).

The aim of this work was to study the efficiency of disinfection of air in premises of meat enterprise shops of meat processing enterprises with the use of germicidal lamps and UV emitters-recirculators.

Materials and methods. Experimental researches were carried out at Luhansk Meat Packing Plant, PUBJSC according to the current regulatory documents to standard procedures (Antonov and Blinov, 1971).

Sanitary-microbiological parameters of air were studied in the premises of the meat processing enterprises using UV emitters-recyclers (one recirculator per 100 m^3) and the germicidal lamps DB-30-1 that are in block system made of two units. Each lamp power is 30 W, bactericidal flux of 6 W. The average action term of the tested lamps is required current regulatory documents and does not transcend the 5000 hours.

Air samples were taken before process, in 3, 6, and 9 hours and at the end of a shift after carrying out preventive disinfection.

Sanitary-bacteriological studies of the air were studied by sampling the air sedimentation plating technique using meat-peptone agar (MPA) and Sabouraud medium according to the general adopted methodology. Plating was incubated in a thermostat at 37 °C for 2–5 days. Quantity was carried out by the method of counting colony in air per 1 m^3 (Antonov and Blinov, 1971).

Results. Three experiments were carried out to study the sanitary-microbiological background of air at meat enterprise shops of meat processing enterprises.

At the first experiment it was studied sanitary-microbiological air composition in condition of using germicidal lamps DB-30-1 that are in block system made of two units. The results are presented in Table 1.

Table 1 – The efficiency of use of germicidal lamps for air disinfection departments of meat processing enterprises

Research zone	Quantity of microorganisms in the air, ths/m^3		Efficiency of disinfection, %
	before start working germicidal lamps	after using UVR	
Meat-fatty shop			
Cattle and horse processing line	9.1×10^{-3}	1.8×10^{-2}	98
Pig processing line	9.3×10^{-3}	1.9×10^{-2}	98
Preparation of the intestinal sheath line	13.2×10^{-3}	4.0×10^{-2}	97
Shop deboning and trimming of raw meat			
Cattle and horse processing line	5.4×10^{-3}	0	100
Pig processing line	5.5×10^{-3}	0	100
Semi-finished shop			
Central hall	7.2×10^{-3}	4.1×10^{-2}	94.3
Storage of finished products	7.4×10^{-3}	3.4×10^{-2}	95.4
Meat expedition	7.5×10^{3}	3.4×10^{-2}	95.4
Sausage shop			
Vacuum pack department (Cryovac® line)	2.8×10^{-3}	0	100
Vacuum pack department (line Multivac)	2.3×10^{-3}	0	100
Sausage expedition	2.8×10^{-3}	0	100

According to the research results, given in Table 1, it was established that the efficiency of use of UVR is 98% after carrying out preventive disinfecting at the end of the work shift for 1 hour and 30 minutes before the start of process. The disinfection efficiency of 100 % was achieved in the experimental premises of the sausage shop and shop deboning and trimming raw meat.

To our mind the reason for reducing the effectiveness of air disinfection UVR in the meat-fat workshop and the semi-finished products workshop was high humidity 76±2%, that is why the result of transmittance radiation energy is reduced. In addition, the reason might be the lack of control the germicidal lamps.

While setting the second experiment it was studied the changes in sanitary-microbiological composition of the air premises of the meat processing enterprises during working time. Samples were taken in 3, 6 and 9 hours after starting process. Sanitation of air was conducted by UV radiation for 1 hour before starting the experiment. The results of the experiment are shown in Table 2.

Table 2 – The results of sanitary-microbiological control of the indoor air in meat processing plants during the working time

Research zone	Quantity of microorganisms in the air					
	TNM, ths/m³			Fungi and yeast, CFU		
	In/h 3 h	In/h 6 h	In/h 9 h	In/h 3 h	In/h 6 h	In/h 9 h
Meat-fatty shop						
Cattle and horse processing line	5.2×10^{-3}	7.1×10^{-3}	9.3×10^{-3}	5.0×10^{-1}	6.0×10^{-1}	8.0×10^{-1}
Pig processing line	5.3×10^{-3}	8.3×10^{-3}	1.0×10^{-4}	5.0×10^{-1}	7.0×10^{-1}	9.0×10^{-1}
Preparation of the intestinal sheath line	5.4×10^{-3}	1.0×10^{-4}	1.3×10^{-4}	6.0×10^{-1}	10.0×10^{-1}	11.0×10^{-1}
Preparation of the intestinal sheath line						
Poultry processing line	3.1×10^{-3}	4.5×10^{-3}	5.7×10^{-3}	3.0×10^{-1}	5.0×10^{-1}	6.0×10^{-1}
Main process line	3.0×10^{-3}	4.5×10^{-3}	5.5×10^{-3}	3.0×10^{-1}	5.0×10^{-1}	5.0×10^{-1}
Semi-finished shop						
Central hall	5.6×10^{-3}	6.3×10^{-3}	6.9×10^{-3}	3.0×10^{-1}	5.0×10^{-1}	5.0×10^{-1}
Storage of finished products	4.3×10^{-3}	6.2×10^{-3}	7.1×10^{-3}	3.0×10^{-1}	4.0×10^{-1}	5.0×10^{-1}
Meat expedition	5.6×10^{-3}	6.8×10^{-3}	7.4×10^{3}	4.0×10^{-1}	5.0×10^{-1}	7.0×10^{-1}
Sausage shop						
Vacuum pack department (Cryovac® line)	1.6×10^{-3}	2.1×10^{-3}	2.6×10^{-3}	1.0×10^{-1}	2.0×10^{-1}	2.0×10^{-1}
Vacuum pack department (line Multivac)	1.5×10^{-3}	2.0×10^{-3}	2.3×10^{-3}	1.0×10^{-1}	2.0×10^{-1}	2.0×10^{-1}
Sausage expedition	2.1×10^{-3}	2.3×10^{-3}	2.8×10^{-3}	2.0×10^{-1}	3.0×10^{-1}	3.0×10^{-1}

According to the readings given in Table 2 we see that in 3 hours after the start of process the air contamination in industrial premises is in average of $3.9\pm0.15\times10^{-3}$ ths/m³. The number of fungi and yeast is increased almost in 3.3 times. The largest air contamination has a meat-fatty shop; it is $5.2\pm0.1\times10^{-3}$ ths/m³, which is three times more than at the beginning of the experiment. In 6 hours after start process in the workshops of meat-processing enterprises the number of microorganisms in the air grew more in 1.5 times, while the number of fungi and yeast — in 4.9 times. After 9 hours, i.e. at the end of the work shift, the average total number of microorganisms in air is 6.6×10^{-3} ths/m³, and the number of fungi and yeasts reached the level of 5,5±0,5 CFU/m³.

At the third experiment it was studied the sanitary and microbiological indicators of the air in the workshops of meat-processing enterprises in condition of use of UVR-recirculator of closed type when one recirculator is installed at the rate per 100 m³ and additional work of germicidal lamps while 30 minutes before work and then after cleaning the shop at the end of the shift.

Disinfection by UV recirculator was performed continuously during the work shift. Air samples were taken before starting work in the shop and in 3, 6 and 9 hours after turning on recirculator in condition the additional use of germicidal lamps before working in the shops, and after carrying out preventive disinfecting at the end of the work shift. The results are presented in Table 3.

Table 3 – The results of sanitary-microbiological air control of and meat-processing plants in using UVR-recirculators

Research zone	Quantity of microorganisms in the air, ths/m³					
	TNM			Fungi and yeast		
	In/h 3 h	In/h 6 h	In/h 9 h	In/h 3 h	In/h 6 h	In/h 9 h
Meat-fatty shop						
Cattle and horse processing line	5.1×10^{-3}	5.7×10^{-3}	5.9×10^{-3}	5.0×10^{-1}	6.0×10^{-1}	6.0×10^{-1}
Pig processing line	5.2×10^{-3}	5.6×10^{-3}	5.7×10^{-3}	5.0×10^{-1}	7.0×10^{-1}	8.0×10^{-1}
Preparation of the intestinal sheath line	5.4×10^{-3}	6.3×10^{-3}	6.5×10^{-3}	6.0×10^{-1}	7.0×10^{-1}	8.0×10^{-1}
Preparation of the intestinal sheath line						
Cattle and horse processing line	3.1×10^{-3}	3.5×10^{-3}	3.7×10^{-3}	3.0×10^{-1}	5.0×10^{-1}	5.0×10^{-1}
Pig processing line	3.0×10^{-3}	3.5×10^{-3}	3.6×10^{-3}	3.0×10^{-1}	5.0×10^{-1}	5.0×10^{-1}
Semi-finished Shop						
Central hall	5.5×10^{-3}	5.9×10^{-3}	6.0×10^{-3}	4.0×10^{-1}	5.0×10^{-1}	5.0×10^{-1}
Storage of finished products	4.3×10^{-3}	5.2×10^{-3}	5.7×10^{-3}	4.0×10^{-1}	5.0×10^{-1}	5.0×10^{-1}
Meat expedition	5.7×10^{-3}	6.0×10^{-3}	6.2×10^{-3}	5.0×10^{-1}	5.0×10^{-1}	5.0×10^{-1}
Sausage shop						
Vacuum pack department (Cryovac® line)	1.6×10^{-3}	1.7×10^{-3}	1.8×10^{-3}	2.0×10^{-1}	2.0×10^{-1}	2.0×10^{-1}
Vacuum pack department (line Multivac)	1.5×10^{-3}	1.8×10^{-3}	1.8×10^{-3}	2.0×10^{-1}	2.0×10^{-1}	2.0×10^{-1}
Sausage expedition	2.1×10^{-3}	2.3×10^{-3}	2.4×10^{-3}	2.0×10^{-1}	3.0×10^{-1}	3.0×10^{-1}

According to the readings given in Table 3 we see that in 3 hours after the start processing the air contamination of industrial premises is an average $4.25\pm0.15\times10^{-3}$ ths/m³. The number of fungi and yeast was increased almost in 3.3 times. The biggest air contamination was in meat-fatty shop, which was $5.23\pm0.1\times10^{-3}$ ths/m³. In 6 hours after starting work in the shops of meat processing enterprises the number of microorganisms in the air was $4.32\pm0.2\times10^{-3}$ ths/m³, which is on 23% less compared with the second experiment. The number of fungi and yeast increased in 4.7 times, it is on 5% less compared with the second experiment. In 9 hours, i.e. at the end of the work shift, the average total number of microorganisms in the air was $4.9\pm0.15\times10^{-3}$ ths/m³, which is on 25.3% more efficient compared to use only the germicidal lamps. The number of fungi and yeast in 9 hours reached the level 5.1±0.2CFU/m³, which is on 7.3% less compared with the second experiment.

Conclusions. The effectiveness of air disinfection in departments of meat processing enterprises while the use of germicidal lamps DB-30-1 after carrying out preventive disinfection reached 99±1%.

It is investigated that the total number of microorganisms, fungi and yeast in the air while work time at meat processing enterprise increase almost in 3.3 times that does not ensure the stability of microbiological indicators of the air in the production areas and therefore cannot guarantee the quality and safety of sausages and meat semi-finished products in violation of veterinary and sanitary operation mode.

The use of UV-recirculator during the working hours allows maintain the hygienic condition of air while working hours and reduces bacterial air contamination in the industrial premises on 25.3% and on 7.3% in fungi and yeast. The outlook of further studies is using the results to improve the modern system of sanitary-microbiological control at meat processing enterprises in Ukraine.

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Part 3. Veterinary medicine

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AN ANALYTICAL SUBSTANTIATION OF THE CORRELATION BETWEEN PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME AND PORCINE CIRCOVIRUS INFECTION BY USING SEROLOGICAL MONITORING IN UKRAINE

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Summary. The aim of our work was to analytically justify the association between porcine reproductive and respiratory syndrome and porcine circovirus infection accordingly with the results of serological monitoring in Ukraine for the period 2010–2015.

Laboratory studies of blood serum of pigs were conducted at the Scientific-Research Center of Biosafety and Environmental Control Resources DDAEU APC. During the period 2010–2015 25,490 blood serum samples from pigs of PRRS and 8,310 of PCV-2 were examined. In these pig farms preventive vaccination against PRRS and PCV have not been conducted.

The research of presence of specific postinfectious humoral antibodies against the PRRSS virus in the blood serum of the domestic pigs was performed by ELISA using test kits: Ingezim PRRS Universal® 11.PRU.K1 ('Ingenasa', Spain); Porcine Reproductive and Respiratory Syndrome Virus Antibody Test Kit ('IDEXX Laboratories', Switzerland); Swinecheck® PRRSV type 1 and 2 ('Biovet', Canada). PCV diagnosed by ELISA using test-systems 'Porcine Circovirus 2 Antibody Test Kit' ('BioChek'). For the purpose of writing this article data are used from the retrospective serological studies, which were processed by modern statistical methods.

Epizootological and serological monitoring of farms with different form of ownership and maintenance of a livestock of pigs in all regions of Ukraine was carried out for the period 2010–2015. The results have been set associative dependence infectious diseases PRRS and PCV-2. The article describes the identified clinical signs and pathological changes in the studied pig farms with PRRS and PCV-2.

The results of the researches established that seroprevalence to domestic pigs over a period of 6 years (2010–2015) has the following indicators: the percentage of positive samples number tested for PRRS is 3.8%; for PCV-2 — 35.44%. Calculated correlation coefficients of indicators on seroprevalence for PRRS and PCV-2 throughout Ukraine and regions for 2013–2015, namely, in Ukraine r=0.48, and the regions: western r=0.65; southern r=0.38; northern r=0.71; eastern r=0.96; central r=0.68. The highest correlation in the eastern region, and lowest in the south.

Keywords: porcine reproductive and respiratory syndrome, porcine circovirus infection, domestic pigs, association

Introduction. Porcine reproductive and respiratory syndrome (PRRS) is characterized by impaired respiratory, reproductive and digestive functions of pigs (Fan et al., 2013; Li et al., 2007; Baibikov et al., 2001; Kukushkin, 2006; Kukushkin, Baybikov and Fomin, 2008; Lawson et al., 2005). Porcine circovirus infection (PCV), which causes porcine circovirus type 2 (PCV-2) affects the lymphatic and suppresses the body's immune system, opening the gate for other infection (Fan et al., 2013; Malogolovkin, 2008; Sementsov et al., 2009). Complications caused by the PRRS and PCV-2 may increase the severity of the disease. These infections cause serious economic losses in pig industry worldwide. Permanent viruses affect the performance of the herd. Virus PRRS potentially reduces the effectiveness of immunization PCV-2 (Fan et al., 2013; Podgórska et al., 2014). Analyzing literary sources, can be traced to statements regarding the associative flow of PRRS with other viral and bacterial pathogens, in particular PCV (Yastrebov et al., 2005; Lyoo, Park and Park, 2001; Maksimov, 2012). It is also proved that the reproduction of PCV-2 increases by virus PRRS (Sementsov et al., 2009). For co-infections, the causative agent of PRRS has an effect on

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the symptoms of disease (Grebennikova et al., 2005). In addition to PRRS, in the starting mechanisms of complex respiratory disease of pigs leading role of the PCV-2, which is due to the immunosuppressive properties determines the occurrence of respiratory infections, are difficult to diagnose (Sementsov et al., 2009; Blotska, 2008; Opriessnig, Meng and Halbur, 2007; Segalés, Allan and Domingo, 2005).

It is experimentally proved that the reproduction of PCV-2 in piglets is due to the influence of field virus isolates of PRRS, a live vaccine (Sementsov et al., 2009) and adjuvants (Shkaeva et al., 2005).

The relevance of the topic. Associative course of disease of pigs for PRRS and PCV-2 in pork industry of Ukraine. Many researchers have found significant epizootic role of mixed (associated) infections viral pathogens that cause reproductive disorders. Among such associations the most common combination of PRRS and PCV-2 (Fan et al., 2013). Therefore, the study of associative dependence PRRS+PCV-2 is relevant and important.

The aim of our work was to analytically justify the association between porcine reproductive and respiratory syndrome and porcine circovirus infection accordingly with the results of serological monitoring in Ukraine for the period 2010–2015.

Materials and methods. Laboratory studies of blood serum of pigs was conducted at the Scientific-Research Center of Biosafety and Environmental Control Resources DDAEU APC. During the period 2010–2015 25,490 blood serum samples from pigs of PRRS and 8,310 of PCV-2 were examined. In these pig farms vaccination against PRRS and PCV have been conducted.

The research of presence of specific postinfectious humoral antibodies against the PRRSS virus in the blood serum of the domestic pigs was performed by ELISA using test kits: Ingezim PRRS Universal® 11.PRU.K1 ('Ingenasa', Spain); Porcine Reproductive and Respiratory Syndrome Virus Antibody Test Kit ('IDEXX Laboratories', Switzerland); Swinecheck® PRRSV type 1 and 2 ('Biovet', Canada). PCV diagnosed by ELISA using test-systems 'Porcine Circovirus 2 Antibody Test Kit' ('BioChek').

For the purpose of writing this article data was used from the retrospective serological studies, which were processed by modern statistical methods.

Results. The results of the epizootological and serological monitoring of farms with different form of ownership and maintenance of a livestock of pigs in all regions of Ukraine for 2010–2015 established that the structure of viral diseases of pigs are important activators of PRRS and PCV-2 and their association PRRS+PCV-2.

Identified clinical signs and pathological changes in the studied pig farms when:

PRRS. The body temperature rose to 42 °C, the observed decrease in the activity of eating the feed, 2% of piglets were observed cyanosis of the skin of the ears and snout, failure of the respiratory system, cough, pneumonia with a chronic course, significant fatigue, reduction of body weight. Violation of the reproductive function: premature births and abortions was 15% and occurred at the 3rd week after the onset of the disease, the birth of dead and weak piglets. Disorders of the gastrointestinal tract (diarrhea). Discovered pathological changes in pigs with PRRS had the following nature: swelling of the subcutaneous tissue, hemorrhage, degeneration of the liver, hyperemia of the lungs, bronchopneumonia, edema of the subcutaneous tissue and muscles, the presence of transudate in the thoracic and abdominal cavities.

PCV. Pigs were not growing, the skin rash was detected. Observed conjunctivitis, necrosis of the tips of the ears, development of the respiratory syndrome. During the research farms discovered feces black. In some cases, there had been a sudden death of animals. Recorded the defeat of lymphoid tissue, hepatitis, pancreatitis, the death of the fruit. Mortality rate of about 65%. The corpses of pigs with the defeat of virus PCV-2 were depleted with increased 4 times the lymph nodes, especially inguinal that when the incision was sealed with a grayish color. Light muscle consistency. The kidneys are pale, enlarged, with haemorrhages in the cortical layer. Liver with signs of degeneration. The spleen is enlarged.

The retrospective serological monitoring of PRRS in domestic pigs. The statistical data from the period 2010–2015, including our previous investigation (Nevolko and Situk, 2013; Sytiuk et al., 2016), on the number of districts and households in the breakdown by regions of Ukraine, where selected blood serum and their study of antibodies to PRRS virus has been analyzed.

The analysis of indicators of the studied areas confirms the diversity of their magnitude. In 2010, it was investigated 11.22%, 2011 — 28.37%, 2012 — 36.12%, 2013 — 13.14%, 2014 — 9.49%, and in 2015 of 11.42% areas of the total number in Ukraine. On the results of the serological monitoring for 6 years was studied 25,490 blood serum samples of pigs. In 2010 investigated 2,583 serum, 2011 — 9,253, 2012 — 9,185, 2013 — 1,990, 2014 — 1,104, and in 2015 — 1,375 respectively. The highest number of positive blood serum was revealed in 2013 — 354 samples (of 17.79%) and the smallest number in 2010 — 16 (0.62%). The ratio of positive sera of pigs to the total number investigated by PRRS on the results of 6 years of monitoring (2010–2015) is presented in Figure 1.

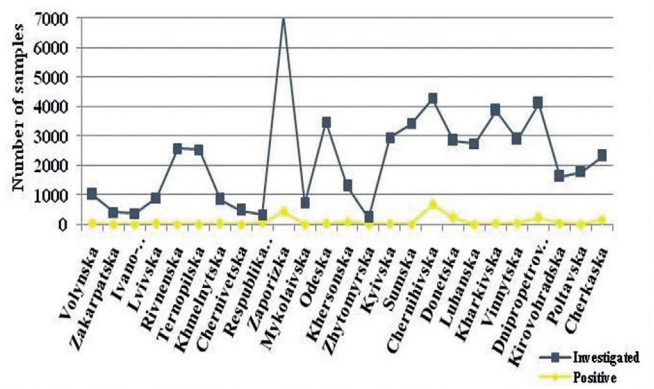

Figure 1. The number of investigated and positive samples of porcine reproductive and respiratory syndrome for the period 2010–2015

The retrospective serological monitoring of PCV-2 in domestic pigs. In 2010, it was investigated 4.9%, 2011 — 2.24%, 2012 — 28.57%, 2013 — 8.57%, 2014 — 5.92%, and in 2015 was 5.31% areas of the total number in Ukraine.

During the period 2010–2015 was studied 8,310 serum samples from pigs. In 2010 881 investigated blood serum, 2011 — 342, 2012 — 5,198, 2013 — 1,024, 2014 — 506, and in 2015 — 359 serum samples.

The highest number of positive blood serum was revealed in 2012 — 1,230 samples (by 23.66%), the least — in 2015 — 299 samples (83, %). In 2011 the result of the research was not positive.

The ratio of positive sera of pigs to the total number investigated by the PCV-2, the results of 6 years of monitoring (2010–2015) is presented in Figure 2.

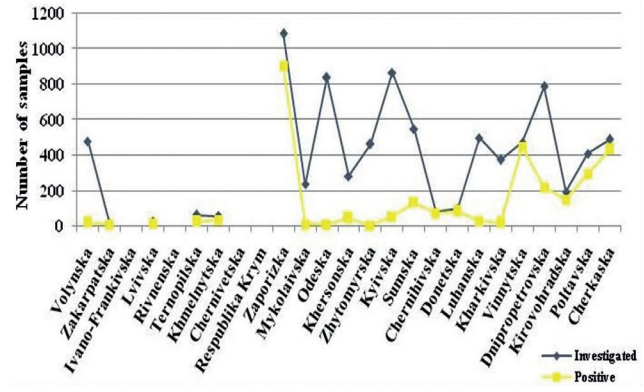

Figure 2. The number of investigated and positive samples of porcine circovirus infection for the period 2010–2015 (the lack of indicators on the chart are due to the absence of these indicators having been investigated and/or lack of positive serum samples of pigs against PCV-2 in certain regions of Ukraine)

The ratio of positive samples of blood serum of pigs for PRRS and PCV-2 for the period of 6 years (2010–2015) is presented in Figure 3.

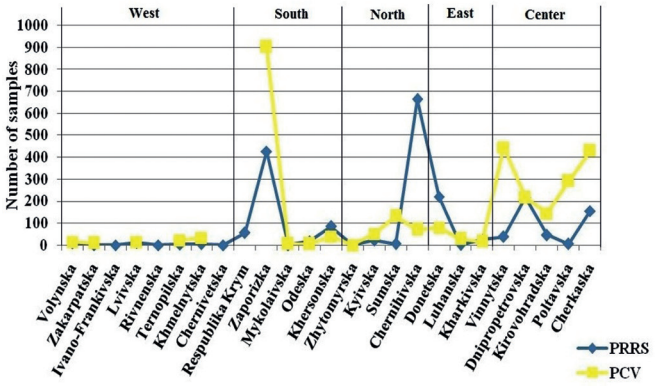

Figure 3. The number of positive blood samples of porcine reproductive and respiratory syndrome and porcine circovirus infection for the period 2010–2015

Further, using correlation and regression we have shown the dependence of one disease to another within each administrative unit (region), based on the number of investigated positive serum samples (Figure 4).

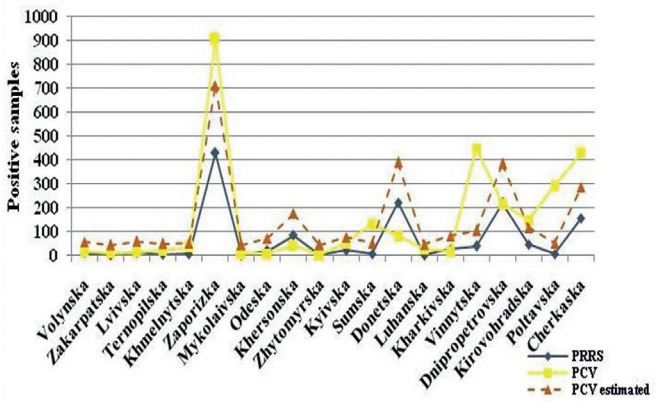

Figure 4. The dependence of porcine circovirus infection and porcine reproductive and respiratory syndrome for the period 2010–2015

Serological indicators of Chernihiv Oblast is unusual for PRRS because they do not meet the conditions of the rules three sigma: x±3σ. They were therefore excluded from the study.

In Ivano-Frankivsk, Rivne, Chernivtsi Oblasts and AR Crimea study over 6 years (2010–2015) was not conducted due to the lack of availability of blood serum samples.

The calculation was performed using the built-in package (Data Analysis) in tabular processor Excel. The regression equation is obtained:

$$y = 42.58 + 1.57 \times x + \varepsilon$$

where: y — PCV-2; x — PRRS; ε — random factors that are not included in the regression equation, explain residual variance.

Coefficient of correlation: r=0.77.

Evaluation of the regression equation using the Fisher test showed that the regression equation is overall significant, well describes the population, because F_e=25.5 and F_t=3.8. That is, F_e significantly exceed F_c (F — Fisher criterion. Fe — estimated. Ft — table. Fc — critical. To — observations. Tc — critical. Tt — table).

Will check its significance using the validation criterion of Student. We calculate T_o using the formula:

$$T_o = R_{xy} \times \sqrt{\frac{n-2}{1-R_{xy}^2}} = 4.91$$

T_c will be taken from the tables of the law Student distribution for a significance level of 0.05 and n–2=19–2=17 degrees of freedom. T_c=2.11.

As you can see, $T_o > T_c$. Therefore, the hypothesis of equality of the correlation coefficient = 0 reject and accept the competing hypothesis that the correlation coefficient is different from 0. The correlation coefficient is significant. Probability is 0.95.

Interval estimation of the correlation coefficient:

$$R \pm T_t \times \frac{1-R^2}{\sqrt{n}}$$,that is 0.57–0.97 in our case.

Samples of blood sera, which were taken during 2013–2015 were investigated with similar methodology and test systems. The results of the research for 2010–2012 were not analyzed and included in the schedule if no results of studies on PCV-2 (Figure 5).

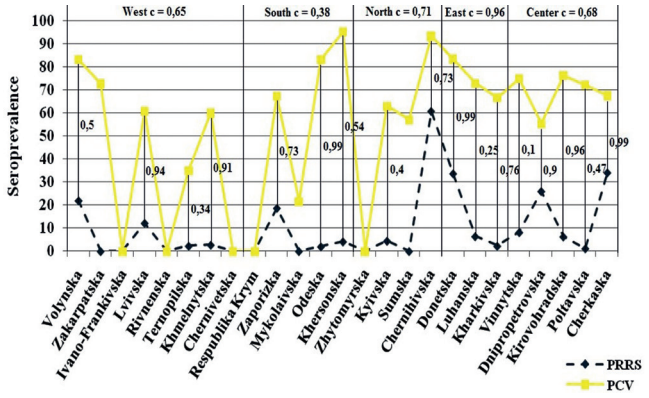

Figure 5. Comparative evaluation of indicators of seroprevalences in the blood serum samples of domestic pigs for PRRS and PCV-2 in regions of Ukraine for the period 2013–2015

Calculated the correlation between PRRS and PCV-2 within the specific Oblast has the following indicators: in the Volyn Oblast r=0.5; Lviv Oblast r=0.94; Ternopil Oblast r=0.34; Khmelnytskyi Oblast r=0.91; Zaporizhia Oblast r=0.73; Odessa Oblast r=0.99; Kherson Oblast r=0.54; Kyiv Oblast r=0.4; Chernihiv Oblast r=0.73; Donetsk Oblast r=0.99; Luhansk Oblast r=0.25; Kharkiv Oblast r=0.76; Vinnytsia Oblast r=0.1; Dnipropetrovsk Oblast r=0.9; Kirovohrad Oblast r=0.96; Poltava Oblast r=0.47, Cherkasy Oblast r=0.99. In other oblasts the correlation coefficient is not statistically significant.

Correlation in the five regions of Ukraine: in the western r=0.65, southern r=0.38, northern r=0.71, eastern r=0.96, central r=0.68.

The overall correlation coefficient for the whole of Ukraine for the period 2013–2015 is r=0.48.

The degree of correlation the retained indicators is divided into 3 levels: low, medium and high. The high level of correlation include the following oblasts: Lviv, Donetsk, Zaporizhia, Odessa, Kharkiv, Dnipropetrovsk, Kirovohrad, and Cherkasy; medium: Volyn, Kherson, Chernihiv, and Poltava; low: Ternopil, Kyiv, Luhansk, and Vinnytsia.

Consequently, the highest correlation is installed in the Lviv Oblast (r=0.94). It is proved that the results of the research between the diseases PRRS and PCV-2 in pig farms of Ukraine is an associative dependence.

Conclusions. By results of researches it is established that seroprevalence to domestic pigs over a period of 6 years (2010–2015) has the following indicators: the percentage of positive samples number tested for PRRS is 3.8%, for PCV — 35.44%.

Calculated correlation indices seroprevalence for PRRS and PCV in Ukraine and regions, namely, in Ukraine r=0.48, and the regions: western r=0.65, southern r=0.38, northern r=0.71, eastern r=0.96, central r=0.68. The highest correlation in the eastern region, and lowest in the southern.

Given the above, in future research, we consider it necessary to confirm the presence of associated infection in the organism of pigs by isolation of different pathogens from biological material from one animal and their subsequent study.

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BACTERIAL BIOFILMS FORMATION OF CATTLE MASTITIS PATHOGENS

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Summary. The results of the microbial biofilms formation by subclinical and clinical forms of cattle mastitis agents were presented. It was determined, those in cattle with subclinical form of mastitis 2.5 times more strains of *Staphylococcus aureus* were allocated. It is occurred, that biofilm density compared to the clinical form of mastitis demonstrates such level of range. In addition, staphylococci, which are the causative agents of cattle mastitis in 1.4–1.7 times more often form the dense biofilm in comparison to streptococci.

The development of the dense staphylococcal biofilms provides their long-term existence on the teats skin and in the breast of carrier's cattle with subclinical form of mastitis. This helps to transform these animals into the pathogen reservoir. Anti-epizootic measures should always be conducted among the animals with clinical form of mastitis for the preventing of infection process chronic transformation. At a subclinical form of mastitis bacteria are located in biofilm matrix and the antimicrobial effect will be less effective.

Keywords: biofilms, cattle, mastitis, *Streptococcus*, *Staphylococcus*

Introduction. The new theory has been formed in the recent years about ecological regularities of microorganisms existence, especially their relationship with the environment, humans and animals body. The main discoveries in this area are associated with learning of microorganism's ability to form biofilms on surfaces of biogenic and abiogenic origin (Flemming and Wingender, 2010). Biofilms is a living set of one or more types or families of the bacteria that are constantly update, is attached to the biogenic or abiogenic surface and surrounded by the polysaccharide matrix (Donlan and Costerton, 2002). Matrix — a mixture of exopolysaccharides, proteins, nucleic acids and other inorganic substances, which protects the bacteria from environmental factors (Costerton, Stewart and Greenberg, 1999; Mah and O'Toole, 2001). Microorganisms in a biofilm 'communicate' to each other about the development, maturation and destruction of the biofilm using secretory mediators, which play an important role in their social behavior (quorum sensing — QS) (Davies et al., 1998). Pores and channels penetrate the biofilms through this structures microorganisms gets the flow of nutrients and exchange metabolic products (Stoodley, deBeer and Lewandowski, 1994).

Accumulated knowledge indicates that bacteria in biofilms are physiologically different from the microbial cells of the same population in free (planktonic) state. Microorganisms generated in biofilms demonstrate increased resistance to antimicrobial agents and cells of the immune system of a living organism (Behlau and Gilmore, 2008; Gilbert, Das and Foley, 1997; Lewis, 2000). The inhibiting of bacterial biofilms formation to this day remains a problem. Microorganisms in the biofilm do not change their individual sensitivity, but better survive under antibiotics action in dose that exceeds the minimum inhibitory concentration (Stewart and Costerton, 2001).

Many bacterial pathogens in animal's body could potentially form biofilms. Caused by them diseases often recur are chronic and difficult to treat (Mah and O'Toole, 2001). The subclinical forms of mastitis are the typical cattle diseases, caused by microorganisms that are able to form biofilm (*Staphylococcus*, *Streptococcus, E. coli*).

The previous our studies have shown (Kukhtyn, 2004) that at dairy farms about 20% of healthy cows carriers *Staphylococcus aureus* on the teats skin and 5% — in the breast. Therefore important now is to examine the factors that are causing the mastitis in cows, as well as environmental peculiarities of mastitis pathogens.

The aim of the study was to determine the ability of cattle mastitis pathogens (isolated from sick and healthy animals) to form the biofilms.

Materials and methods. Work carried out in Institute of Veterinary Medicine of the National Academy of Agrarian Sciences of Ukraine.

Diagnosis of subclinical mastitis in lactating cows was conducted in accordance with guidelines (Deutz and Obritzhauser, 2003). Cattle considered sick on mastitis when pathogens (*Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Escherichia coli* etc.) were detected

in breast secretion. To indicate microorganisms was made bacterial inoculation on meat of Baird Parker agar, *Streptococcus* Selective Agar and on the Endo agar. The generic and specific identification of microorganisms were carried out according to the test systems 'Staphy-test 16', 'Strepto-test 16' and 'Entero-test 24' ('Lachema', Czech Republic).

To determine the ability of microorganism's to form biofilms in sterile plastic Petri dishes full with 5 cm^3 Hottinger's media the 1 cm^3 of daily bacterial cultures were incubated at 37 °C for 24 h. After incubation, washed three times cups from planktonic (unattached) microorganisms by phosphate buffer dried and fixed biofilm formed with 96° ethanol 10 minutes. Then they were stained with methylene blue solution for 10 minutes, washed by the phosphate buffer, dried and stained with fuchsine solution for 2 minutes. After a double rinsing the biofilms were evaluated visually and morphological issues were studied by the microscopy (Stepanović et al., 2000).

To determine the density of the biofilms formation the 96-well plastic plates were used. The 0.1 cm^3 daily culture of microorganisms was plated to the holes and incubated for 3 hours at room temperature. Then 1 cm^3 of meat agar was added and incubated at 37 °C for 24 h. After incubation, the wells were washed three times with phosphate buffer, dried and fixed biofilm formed 96° ethanol for 10 minutes. Then stained with a solution of 0.1% crystal violet for 10 min, again washed with phosphate buffer and dried. In the hole put 96° ethanol and washed them well. Measured optical density of alcohol wash solution spectrophotometric ally at a wavelength of 570 nm (Stepanović et al., 2000).

Electron microscopic study of the formed microorganism biofilms on abiotic surfaces (glass) was performed using a raster electronic microscopy in the mode of secondary electrons at a voltage of 20 thousand and increasing every 20,000 to 30,000 times.

Results. Microorganisms *S. agalactiae, S. dysgalactiae, S. aureus* and *S. epidermidis* were allocated in dairy farms from healthy animals and cattle with clinical and subclinical forms of mastitis. The ability of these bacteria to form biofilms in conditions *in vitro* was studied. The research results presented in the table.

As the table shows, almost all cultures *S. aureus*, which are marked with subclinical form of mastitis, formed dense biofilm. In clinical form of mastitis number of *S. aureus*, which formed biofilm with the density 38.7±3.4%. The same trend have noted with the presence of other mastitis pathogens, which is characterized by an increase in 2.4–3.4 times the number of selected microorganisms which form biofilms in the subclinical form mastitis animals in comparison with animal micro flora from the clinical form. The research results also certify that the *Staphylococcus*, which are the agents of cattle mastitis in 1.4–1.7 times denser biofilm was formed than by *Streptococcus* (Table 1). This indicates that cow's staphylococcus origin bacterial mastitis will be potentially harder in the treatment.

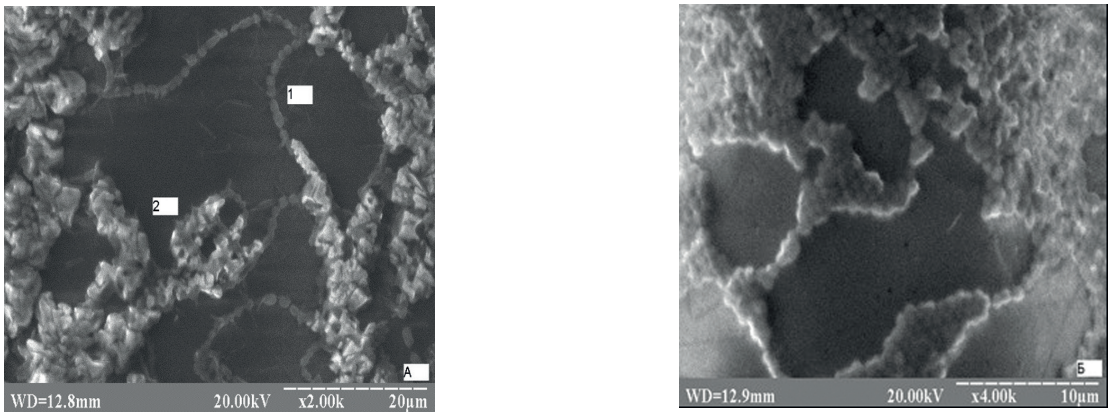
Table 1 – Formation of biofilm by cattle mastitis pathogens, %, M±m, n=80

Type of microorganism	Number of microorganisms which form a dense biofilm in various forms of mastitis and with carriers		
	subclinical	clinical	carriers
S. aureus	97.5±1.6**	38.7±3.4*	94.2±3.2
S. epidermidis	78.3±6.2**	32.4±2.7*	76.3±7.5
S. agalactiae	56.7±2.7	22.3±1.8*	–
S. dysgalactiae	62.5±3.1	18.4±1.5*	–

Note: * — R≤0.01 for subclinical forms of mastitis; ** — R≤0.001 for streptococcal mastitis

The Figure 1 shows the results of electron-microscopic studies *S. aureus* and streptococcus that are in dense biofilm formation.

Discussion and conclusions. The study of *S. aureus* ability for biofilm formation gives us a new look at the cattle mastitis problem. We found that strains of *S. aureus*, which are marked with in cows with subclinical mastitis demonstrated much better colonies forming activity. So at first sight it becomes clear why healthy cows that carriers *S. aureus* (and other staphylococcus) on the teats skin, in the breast, and cows sick on subclinical form of mastitis are less active infection disseminators for some time, compared with sick animals on mastitis clinical form. Obviously, the low ability of cows that are carriers of pathogens, as a source of infection, is because the bacteria carriers are in the biofilm matrix unlike planktonic bacteria that exist in acute clinical mastitis. Staphylococcal biofilm formation provides the long-term existence in animal's carriers. It supports the transform them into a reservoir of the pathogen. Maybe stay of *S. aureus* into biofilm formation in the carrier and in cows suffering from subclinical form of mastitis — is pathogen conservation as a species ensuring on a dairy farm. Cause illness — this is not the main task of microorganisms which are in biofilm formation. The emergence of subclinical forms of mastitis is the manifestation factor of infection. It is well known that the interaction of the microorganism and host depends of his resistance and local and general immunity. *S. aureus* exciting as the biofilm matrix is the reason of practically inaccessible to antibiotics, despite the high sensitivity of planktonic cells to these agents.



a) *S. agalactiae* b) *S. aureus*

Figure 1. Biofilms formation of mastitis pathogens in abiogenically surface (glass): a) 1 — single bacterium *S. agalactiae* outside biofilm; 2 — cells *S. agalactiae*, which are formed in the biofilm that has a three-dimensional surface and solid polysaccharide matrix; b) strains of *S. aureus*, which are in continuous biofilm

Strains of *S. aureus*, which cause clinical form of mastitis, form weak biofilm or its formation required longer period of time (24 hours). However, cows suffering from clinical form of mastitis infection spread much more active for some time, compared to carriers and cows with subclinical form of mastitis. Therefore, we believe all disease control measures should always conduct among patients with clinical form of mastitis preventing 'chronic' process because during subclinical form of mastitis, microorganisms are in biofilm matrix and the antimicrobial effect will be less effective.

Studies of the cow's mastitis pathogens ability to form biofilm are important for effective anti-mastitis measures on dairy farms and the development of new drugs with specific properties that will act on the microorganisms in biofilm.

Author contributions. M. Kukhtyn, A. Bergilevich, and Yu. Horyuk investigated the ability to form biofilm in staphylococci, V. Horyuk and Ya. Stravskyy studied streptococci. Yu. Horyuk and M. Kukhtyn carried out the analysis of research results.

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