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.

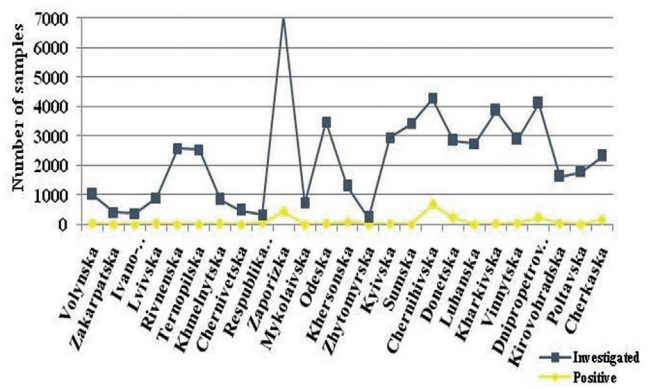

Figure 1. The number of investigated and positive samples of porcine reproductive and respiratory syndrome for the period 2010–2015

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.

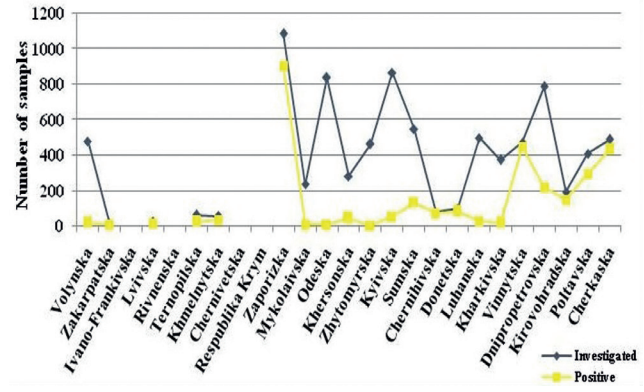

Figure 2. The number of investigated and positive samples of porcine circovirus infection for the period 2010–2015 (the lack of indicators on the chart are due to the absence of these indicators having been investigated and/or lack of positive serum samples of pigs against PCV-2 in certain regions of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

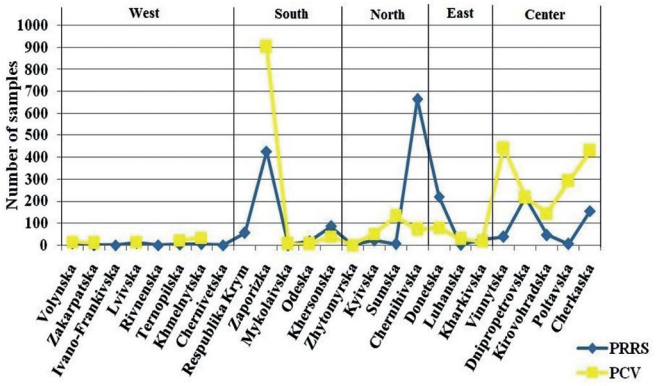

Figure 3. The number of positive blood samples of porcine reproductive and respiratory syndrome and porcine circovirus infection for the period 2010–2015

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).

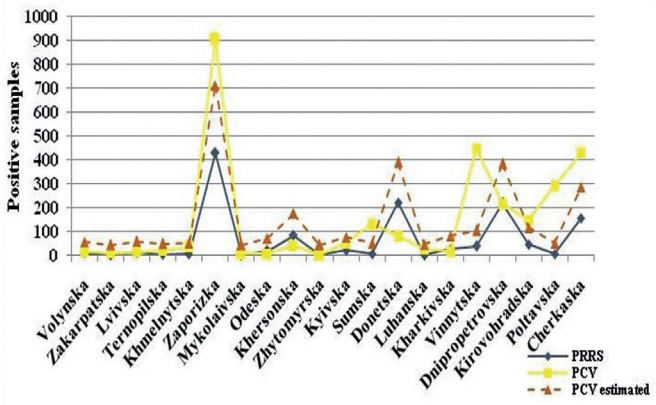

Figure 4. The dependence of porcine circovirus infection and porcine reproductive and respiratory syndrome for the period 2010–2015

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).

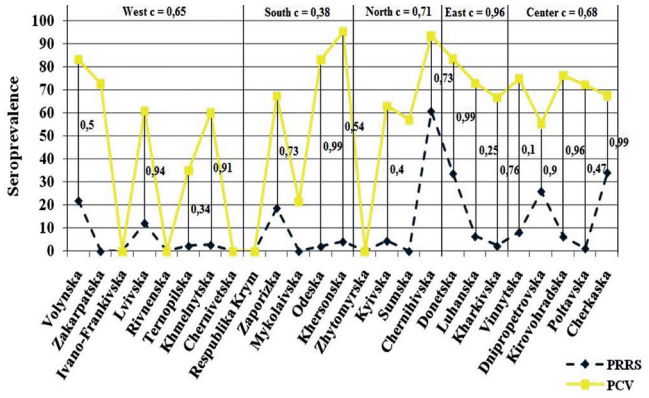

Figure 5. Comparative evaluation of indicators of seroprevalences in the blood serum samples of domestic pigs for PRRS and PCV-2 in regions of Ukraine for the period 2013–2015

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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