THE ROLE OF VIRAL INFECTIONS IN THE DEVOLOPMENT OF RESPIRATORY DISEASE IN SWINE

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Abstract
Susceptible categories of pigs for the occurrence of respiratory diseases are categories in postweaning and fattening. The spectrum of clinical symptoms is very wide and includes not only respiratory symptoms, but also many other symptoms that appear as a consequence of systemic disorders, depending on the type of causative agent. Cough can be of different frequency, intensity and productivity, and difficulty breathing can be of different degrees. Respiratory diseases may be accompanied by stunted growth, rough bristles, anorexia, reduced daily gain and lethargy. Disseminated systemic disorders often include symptoms suggestive of central nervous system disorders, swollen joints and lameness, followed by reproductive disorders or sudden death. Pathomorphological changes are also of different character and severity. Non-specific factors predispose to the occurrence of initial damage or contribute to the further spread of already existing lesions. In this article, we wanted to give an overview of our research on respiratory diseases of viral etiology in pigs from commercial farms.

Key words: pigs, respiratory diseases, viral infections

The primary causative agents of swine respiratory diseases are PRRSV, swine influenza virus (SIV), porcine circovirus type 2 (PCV-2), *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *Bordetella bronchiseptica*, and in some cases the virus that causes Aujeski's disease (Pseudorabies Virus - PRV) and the respiratory corona virus (Porcine Respiratory Corona virus - PRCV). Recently, it has been reported that among the infectious agents, the porcine reproductive and respiratory syndrome virus and *M. hyopneumoniae* play a particularly significant role in the etiopathogenesis of respiratory infections (Ivetić V.et.al. 2000, 2005, Došen R.et.al. 2007, Savić B. et.al. 2010, 2012 a,b).

It is characteristic that many of the respiratory pathogens can appear as independent causative agents, or, which is more common, mutually associated in a synergistic action. Their spread and frequency of occurrence varies, both from region to region, and within the region itself, depending on the technological-production group. Many of the mentioned pathogens can be found simultaneously in the same pig farms. In addition to the above, this is also the reason that their classic division into primary and secondary respiratory pathogens is not always completely acceptable (Šamanc, 2009). If it is known that some causative agents, previously designated as secondary pathogens, such as *Pasteurella multocida*, can independently cause the disease and are then considered a primary pathogen (Lončarević, et.al. 1997, Šamanc H. 2009). The interaction of pathogens is very complex, and each of them independently, in synergism or competition, causes a certain manifestation of the disease from the respiratory complex (Radojičić S. et.al. 2011).

Special importance is also attached to non-infectious, i.e. predisposing factors, such as transport, low temeporatures, bad microclimate, deficient nutrition, overcrowding in facilities, frequent exposure to stress factors. The consequence of non-infectious factors is the daily production of a large amount of gases, water vapor, heat and bio-aerosol particles, which directly affect the change in the physical and chemical composition of the air inside the facility (Stanković B. et.al. 2009, Bojkovski J., 2015b). A change in the physical and chemical composition of the air favors respiratory pathogens and their continuous maintenance of a high degree of virulence (Stanković B. et.al. 2008). Dust, which is usually found in large quantities in pig facilities, has a mechanical and chemical effect on the nasopharyngeal and bronchial ciliated epithelium. Due to the increased action of predisposing factors, the epithelium of the mucous membrane of the respiratory system is damaged, its activity decreases, and thus the possibility of continuous elimination of accumulated exudate and inhabited microorganisms (Lončarević et.al. 1997, Kureljušić B. et.al. 2016).As a result of the above-mentioned problems, the occurrence of respiratory infections is more frequent, the control of which is difficult. Technological systems that do not include the "all-in/all-out" procedure , and contain pigs from various locations, introduce gilts into breeding without prior health control and thus form groups of different immune status, which enable infecting the throat with numerous pathogens (Lončarević A. et.al.1997, Šamanc, H. 2009, Weissenbacher-Lang C.et.al.2016). Changes in environmental conditions lead to stress, which, like various infectious agents, can significantly suppress respiratory defense mechanisms in pigs. To that, we should definitely add the fact that the farms where we monitored the health status of the pigs rarely carry out the "facility rest" procedure, which would allow minimal exposure to endemic pathogens and thus lead to the development and uniformity of the immune status of all individuals in the group (Bojkovski J. et.al. 2005, Kureljušić B. et al. 2015).

An important factor in the pathogenesis of the disease is the susceptibility of the blood vessels of the lungs to the action of numerous immune processes. As a result, damage to the walls of blood vessels occurs, their permeability increases, and as a result, edema is created, which provides a suitable basis for the further pathogenic action of many agents (Šamanc,H. 2009). Due to the action of pathogenic microorganisms or their toxic products, the defensive activity in the lungs is impaired, and the circulation is particularly difficult, especially in the parenchyma around the edges of the lung lobes. In this, non-specific factors, such as microclimate and cold, are particularly important. It has been proven that peripheral cooling can cause disruption of blood flow through the lungs, with consequent changes in ciliary activity, mucus production, reduction of local cellular and immune defense activity (ŠamancH. 2009).

The pathomorphological changes that occur in respiratory infections are characteristic and depend on the type of infectious nose, as well as on the routes by which they reach the lungs (Jovanović M. et.al. 2019). The most common route of infection is aerogenous, so the causative agents reach the lungs through the bronchial tree, where they first spread endobronchially, and then secondarily through the lymphatics into the peribronchial spaces. The causative agents can reach the lung tissue and hematogenously, especially after septicemic conditions. First, they settle in the interalveolar, i.e. peribronchial space (Radojičić B. et.al. 2002, Lupulović D. et.al. 2008 ,Radojičić S. et.al. 2011). Regarding the spread of the causative agent and the origin of the infection aerogenously, there are different points of view. It is believed that nasal secretions, as infectious material, can reach the oral cavity and thus allow the causative agents to first settle in the tonsils and pharyngeal mucosa, and from there reach the respiratory tract (Šamanc, 2009, Lipej, 2015, Savić, 2016, Jovanović et al., 2019).

In this article, we wanted to give an overview of our research on respiratory diseases of viral etiology in pigs from commercial farms.

Reproductive-respiratory syndrome of pigs (PRRS)

RESULTS AND DISCUSSIONS

The PRRS virus acts on specific host cells, infecting only specific subsets of porcine macrophages (Šamanc H. 2009). This virus has a special affinity for alveolar macrophages and destroys up to 40% of these cells. The virus leads to the lysis of pulmonary alveolar macrophages and pulmonary intravascular monocytes, which represent the primary sites of virus replication in the lungs, there by further reducing the body's defense ability. The process of PRRS virus entry into macrophages has been described as pH-dependent, receptor-mediated endocytosis. In the first week after infection, there is a dramatic decline in lung alveolar macrophages, and their defensive function is severely impaired. Certain studies also indicate a significant accelerated apoptosis of macrophages in lungs infected with the PRRS virus, which contributes much more to the decline in the number of macrophages if you take into account the fact that only 2% of lung macrophages are directly infected by the virus in the acute phase of inflammation. Damage to pulmonary alveolar macrophages and intravascular monocytes lead to increased susceptibility to bacterial infections from the development of severe pneumonia and septicemia (Šamanc H.2009). Direct contact is the most important way of transmitting the virus. The infection is peroral, aerogenous and coital (including artificial insemination). Pigs infected with the virus that causes PRRS most often exhibit symptoms that include inappetence, elevated body temperature, lethargy and difficulty breathing. Important factors that influence the nature, course and prevalence of the disease are the essence of the population, the quality of the ventilation systems on the farm, the mixing of production categories, the health status of the animals, the way they are kept, the amount and strain of the virus circulating in the group of animals (ŠamancH. 2009, Lipej Z.2009, Radojičić S. et.al., 2011, Bojkovski J.et. a.l 2012, 2015b, Savić B. et. al. 2016).

Sows infected from the 85th to the 90th day of pregnancy can farrow persistently infected piglets in which the RNA virus can be detected up to 210 days after parturition (Petrujikić et.al. 2011). In persistently infected piglets, mortality before farrowing is high and changes in respiratory they are difficult for the organs, and at the same time, such piglets are also a significant source of the dissemination of the virus and its persistence on the farm (Štukelj,M. 2017). More severe clinical symptoms and greater losses are recorded on large farms that do not quarantine newly acquired animals and where zoohygienic conditions are inadequate. However, the PRRS virus causes the most harmful effects in younger categories of piglets and sows. In gilt sows, PRRS infection causes partial placental detachment, which can lead to abortions, premature births, or death and mummification of the fetus. Abortions in late pregnancy were recorded in 30% of infected sows, with the number of stillborn piglets reaching 100%. Liveborn piglets infected prenatally are usually weakly vital and show severe respiratory symptoms, with up to 80% dying on a weekly basis before weaning, so it often happens that only one piglet per litter is weaned (Petrujkić T.et.al. 2011). Diarrhea and severe respiratory disorders caused by lung damage often occur in young piglets infected with the PRRS virus postnatally. In suckling piglets, the infection can be transmitted from infected sows via milk (Petrujkić T. et.al. 2011). At this age, the infection leads to a fatal outcome in as many as 80% of animals after confinement. The mortality rate is decreasing, but the economic losses caused by reduced daily gain and reduced food conversion continue (Petrujkić et al. 2011). A few days after the appearance of a high temperature, a cough begins, and redness and cyanotic changes appear on the skin of the neck, ears, back, peri-anal region and upper parts of the hind limbs. Infection in the middle of the gestation period can be accompanied by abortions, mummification of fetuses, early death of embryos and consequent sterility. A reliable sign of infection in the throat in reproduction is reflected in the reduction of the number of pollinated piglets and the irregular occurrence of estrus, but also as a transition to anorexia and early farrowing (Šamanc,H.2009, Petrujkić T.et.al 2011). In dead animals, pathomorphological changes in the respiratory tract may indicate the disease, but in PRRS there is no pathognomonic finding (ŠamancH. 2009). In newborn piglets and weaned piglets in fattening, moderate to severe, multifocal to diffuse lung changes occur. The most obvious macroscopic change is the enlargement of the lymph nodes, starting on the 10th day after virus inoculation. Microscopic findings on the lungs of young piglets affected by interstitial pneumonia are characterized by infiltration of alveolar septa with mononuclear cells, hypertrophy and hyperplasia of type II pneumocytes and alveolar exudate composed of edema fluid and mononuclear cells. Lymphohistiocytic encephalitis, myocarditis and rhinitis can occur in some cases (ŠamancH. 2009). Changes are equally present in the lung tissue of the apical, medial and caudal lobes. In the case of secondary bacterial infections, the main pathological changes may not be due to infection with the PRRS virus. Changes can be found in the blood vessels in the form of perivasculitis, myocarditis and splenitis with a decrease in the number of lymphocytes (Šamanc H. 2009). Today, PRRS occurs predominantly in Europe as a respiratory syndrome in the category of suckling piglets and in the period of early fattening (Bojkovski J. et.al. 2012 a,b, Štukelj,M. 2017, Obrenović S. et.al. 2019).

In Serbia, the disease was first described clinically in 1998 in pigs in Vojvodina, and during 2000 it was serologically confirmed. It is considered that PRRS is an underestimated and uncontrolled respiratory swine disease in Serbia. According to Petrović T. et al. (2011) the first suspected cases of PRRS in Serbia occurred in 2001, when serious respiratory disorders associated with high mortality affected pigs on two industrial farms located in the Northern region close to the borders with Croatia and Hungary. The suspected cause of the cases was boar semen illegally imported from neighboring countries. Subsequently in 2001-2002, respiratory syndrome with high morbidity and moderate mortality, which was diagnosed as PRRS, occurred on several commercial farms in the Northern Serbian province of Vojvodina and later on in the central part of Serbia (PetrovićT. et.al 2011, Prodanov-Rsadulović, J.et.al. 2020). Severe health problems and high economic losses led the Veterinary Directorate to perform PRRS serology screening in 2002, 2004-2005 and 2006-2007. In the majority of the studied herds, PRRSV antibodies were detected in a very high proportion of fatteners. Monitoring in 2006-2007 revealed PRRSV-positive herds in all Serbian regions at prevalence of 1.56-60.86%. No other monitoring or control program against PRRS was proposed at the national level. However, as a consequence of the first virus introduction and resulting outbreak, an emergency PRRS vaccination campaign was carried out on a small number of commercial farms in Northern Serbia in 2002-2003. The obtained results in the last 15 years suggest that PRRS virus infection is widely distributed in Serbia. Phylogenetic analysis revealed that all genetically typed isolates belong to the EU subtype 1 or Lelystad type viruses that are distributed globally in Europe, as well as in the other parts of the world. This result was expected regarding the results published from surrounding and other EU countries.(Novosel D. et al., 2016). Despite the fact that the disease is widespread in most of the commercial swine farms, there are no legislation procedures regarding PRRSV control in Serbia. To the best of our knowledge, today the vaccination against PRRS virus is used in a number of Serbian commercial pig farms. Also, at the moment PRRS monitoring and surveillance are not undertaken except for the animals imported from another country and in abortion cases that are sent to laboratory testing. However, it needs to be stressed that Serbia as a Western Balkan country annually imports a large number of different categories of live pigs from Western Europe. The preventive measures are only done through serological testing of breeding animals (gilts, sows, boars), farm management and biosecurity protocols.(Prodanov-Radulović J. et al., 2020)

In North Macedonia porcine reproductive and respiratory syndrome virus in was first laboratory detected in 2015 after an acute outbreak of respiratory disease in one pig farm with 650 sows. Twenty animals including weaned and grower pigs were bled and samples were analyzed for PRRSV. After laboratory confirmation on PCR, farm has started vaccinating against PRRSV. Later in 2016, another commercial pig farm with 500 sows in southern region of the country reported clinical outbreak of abortions in sows. After consultation with the Faculty of veterinary medicine in Skopje (FVMS), farm has brought sera samples and vaginal swabs from aborted sows. Samples were tested on PCR and PRRSV was diagnosed. Introducing of the virus in these farms probably has occurred due to importing new gilts for replacement of breeding animals. Additionally, these two farms shared same truck for animal transport from abroad and that was the most likely way of PRRSV introduction into herds. In same 2016, small-scale pig farm complained about poor growth rate, dyspnea, rough hair coat and dehydration in weaning pigs. Farm was visited and blood samples were taken from pigs at 8 to 12 weeks of age. All samples were tested on ELISA and PRRSV antibodies were detected. In 2018, two commercial pig farms with one-site production system (150 to 170 sows) located in the central part of the country had experience with growth retardation and severe respiratory clinical signs. We visited these two farms and necropsy was performed on several carcasses from pigs at 10 to 12 weeks of age. The lesions were suggestive of PRRSV-like induced interstitial pneumonia. Blood samples from weaned pigs were taken in order to be tested on PRRSV. Samples were positive on both ELISA and PCR and mass herd vaccination was recommended.In the same year, small farm with 50 sows located in the west part of the country contacted FVMS and asked for an expert opinion related with poor growth rate, rough hair coat, anorexia and dyspnea in weaned pigs. On the day of farm visit, we take blood samples from several pigs with clinical respiratory sings. Samples were tested on PCR and gave positive results on PRRSV. The PRRSV in this farm was introduced most probably via breeding gilts which were purchased from a farm that vaccinates against PRSSV. Recently in 2021, at request of a farm with 170 sows, 50 blood samples from different category were taken and tested o determine PRRSV circulation. All samples were positive on ELISA and negative on RT-PCR indicating that the farm infection has occurred earlier and the herd has established collective immunity. Very recently in December 2021, large pig farm reported late abortions in sows and cyanosis of ears and skin in finishing pigs. Blood samples from aborted sows and pigs with cyanosis were tested for PRRSV. Samples were analyzed and gave negative results on PCR. However, positive samples on ELISA indicating that the sampled animals most likely had been infected earlier and developed neutralizing antibodies. According to obtained results, PRRSV is probably endemic in most of the commercial pig farms in North Macedonia. Unfortunately, there is no national program for monitoring and surveillance of PRRSV either for commercial pig farms or for imported pigs. National strategy is needed for control and monitoring of PRRSV due to great economic losses that could affect the pig industry. All obtained results regarding PRRSV in North Macedonia are personal data and are not published yet (Angjelovski).

Swine influence

Swine influenza is a highly contagious, acute viral disease of the respiratory system of pigs, present in many countries with developed pig farming (ŠamncH. 2009, Lipej Z. 2015). It is characterized by respiratory disturbances in the form of catarrhal inflammation of the respiratory system, difficult and irregular breathing and strong coughing. The disease appears suddenly with symptoms of elevated body temperature, anorexia, prostration and dyspnea. Morbidity reaches 100%, and sick pigs usually recover quickly. In such cases, mortality is less than 1%. The economic consequences of influenza are significant. Growth is reduced, and deaths are most often the result of secondary bacterial infections (Lončarević A.et al. 1997, ŠamancH. 2009, Maksimović Zorić J et al. 2017).

The three basic subtypes of influenza A virus circulating in pigs are H1N1, H3N2 and H1N2. The most common way of transmitting the virus between a sick and susceptible animal is through aerosols, droplets and feces, and the fastest way of spreading the virus is through nasopharyngeal secretions. (Lončarević et al. 1997, Šamanc, 2009).
After reaching the body of a susceptible individual, the virus is accepted by the cilia of the mucous membrane epithelium of the respiratory system (nose, trachea and bronchi) and after initial replication in individual cells, within 1 to 3 days, it spreads throughout the respiratory system. During the appearance of the most pronounced clinical symptoms, necrosis is emphasized and practically all the cells of the bronchial mucosa are affected. (Lončarević A., et al. 1997, Šamanc H., 2009) At that time, the dominant findings were lobular atelectasis, emphysema, and focal coagulation necrosis of the bronchial epithelium. Within 72 hours, the pathological process reaches its maximum, after which the multiplication of the virus decreases, and the symptoms subside. Bacterial infections, such as *Haemophilus parasuis* and *Bordetella bronchiseptica*, play an important role in the development of the pathological process (LončarevićA. et al. 1997, Šamanc, 2009). In all or most animals in the zoo, clinical symptoms appear suddenly within 1 to 3 days of infection. Prostration, apathy and anorexia are the first symptoms.
Body temperature is elevated. Cough and shortness of breath occur. In addition, rhinitis, nasal discharge, lacrimation and conjunctivitis may occur. Weakness of muscles, loss of body mass is observed, and after being sick for 3 to 6 days, the animal starts to recover. As a rule, all pigs in the barn start eating normally around the seventh day after the appearance of the first symptoms of the disease. Subclinical infections are also possible (Lončarević A. et al. 1997, ŠamancH. 2009). Before and during gestation, infection can cause reproductive problems: infertility, fetal death, abortion, small litters and farrowing of weak piglets. However, in this case it is not a direct effect of the virus, but symptoms that can disrupt the reproductive characteristics of the sow. (Lončarević A. et al. 1997, Šamanc H. 2009).

Macroscopic changes are localized in the lungs, most often in the apical and cardiac lobes. Parts of the lung are consolidated, dark red and purple in color and are clearly demarcated from normal tissue. Hyperemia and the presence of exudate are emphasized. Bronchus and bronchioles are dilated and filled with exudate. Also, in more severe cases, multifocal to diffuse pneumonia may develop with dark red to yellow-brown fields that can affect from 20% to 100% of lung tissue. Hyperemic tracheobronchial and mediastinal lymph nodes are often enlarged. (Lončarević A.et al. 1997, Šamanc, H.2009, Jovanović M. et al. 2019).

Pathohistologically, there are diffuse degenerative changes and necrosis of the epithelium of the bronchi and bronchioles. The lumen of the bronchi, bronchioles and alveoli is filled with exudate containing desquamated cells and neutrophilic granulocytes. Microscopic findings of lung tissue are characterized by broncho-interstitial pneumonia with necrotic bronchitis and bronchiolitis, infiltration of alveolar septa by inflammatory cells, hypertrophy and hyperplasia of type II pneumocytes, and filling of airways and alveolar spaces with liquid containing proteins and various inflammatory cells (Lončarević A.et.al. 1997, Šamanc,H. 2009, Jovanović M. et al. 2019). Although , swine influenza virus is often isolated from individuals suffering from respiratory diseases, and seroconversion has often been demonstrated in growing and fattening pigs, its role in the pathogenesis of diseases from the respiratory complex is still not entirely clear. By damaging the mucociliary apparatus and reducing the function of macrophages, the virus can lead to an increase in the predisposition of pigs to bacterial pneumonia. Also, in combination with other respiratory viruses, swine influenza virus can cause much more severe pathomorphological changes, which will be manifested by more severe clinical symptoms and increased mortality. It has been proven that the influenza virus in co-infection with the PRRS virus or *M. Hyopneumoniae* causes severe morphological changes and prolongs the duration of respiratory diseases in pigs (Lončarević A.et.al. 1997, Šamanc,H.2009, Jovanović M et.al. 2019). Contrary to this, in co-infection with circovirus, influenza virus is isolated much less

Circovirus infection of pigs

Porcine circovirus type 2 (PCV2), from an economic point of view, is one of the most important causative agents of swine diseases. In the group of circovirus diseases, in addition to PMWS, there are also disorders in reproduction, dermatitis - nephropathy syndrome (Porcine dermatitis nephropathy syndrome - PDNS,), as well as respiratory and enteric forms of this disease. Today, these pathological entities are called porcine circovirus associated diseases (PCVAD) by one name (Savić B.et al. 2012a). Based on new knowledge about the pathology of this disease, a new terminology was proposed, the subclinical forms of circovirus infection known today in veterinary practice, then PCV2 systemic disease (Porcine circovirus systemic disease; PCV-2-SD,), respiratory form of PCV2 disease (Porcine circo virus lung disease; PCV- 2-LD), enteric form (Porcine circo virus enteric disease; PCV2-ED), increasingly common reproductive form (Porcine circo virus reproductive diseases; PCV2-RD) and dermatitis nephropathy syndrome (Savić B.et.al. 2012 b).The source of infection is mostly sick animals, and the clinical manifestations depend on the affected organ system. It is not possible to predict which of the systems will be affected, and therefore which manifest form will appear Any manifest form that is involved, damages, both direct and indirect, are always very significant and threaten any rational production of pigs. Of all these, PMWS is the most economically significant disease. This syndrome, as one of the forms of circovirus infections, is present in the pig population in the Republic of Serbia and occurs in pigs between 6 and 16 weeks of age (Ivetić V. et.al., 2004).

Pig circovirus 2 belongs to the genus Circoviruses and causes diseases in pigs, and was isolated in cases of multisystemic stunting syndrome in piglets, followed by the development of porcine dermatitis and nephritis syndrome, i.e. in pigs with reproductive disorders. Three genotypes of the mentioned virus have been described: PCV-2a, PCV-2b and PCV-2c (Ivetić V.et al. 2004., 2005). The oronasal route is considered the most likely and most common route of transmission of PCV2 infections. This claim has been proven by a large number of experimental studies on circovirus infections, which mostly used the intranasal route of inoculation of this virus (Lipej Z., 2015). Although, the oronasal route is considered the most common, the PCV2 virus can also be transmitted by almost all secretions and excreta, tonsillar, bronchial, eye secretions, as well as feces, saliva, urine and sperm (Ivetić, 2005). The presence of PCV2 was also found in colostrum (Petrujkić, 2011), but whether such a finding can lead to infection is still unknown (Petrujkić T.et.al 2011). The pathogenesis of circovirus infections and the cell types that support PCV2 replication are still not fully understood. Lymphocyte depletion and lymphopenia in the peripheral blood are a constant finding in piglets with a developed clinical picture of PCVAD (Šamanc, 2009).

Piglet multisystem wasting syndrome most often occurs in piglets between the ages of 2 and 4 months, although the disease can occur in pigs between the ages of 1 and 6 months. This syndrome is present in almost all types of farms with a capacity of 30 to 10,000 sows. Morbidity and mortality on farms where PMWS occurs is variable, depending on housing, management, co-infections and other factors related to pig production. Morbidity ranges between 3-40% (extremely rarely over 50%) and mortality on affected farms is between 4-20% (IvetićV. et.al. 2004).

The main clinical sign of PMWS is loss of body weight, and often along with this symptom, there are other signs, such as pale skin, difficulty breathing, diarrhea, swelling of the eyelids, and occasionally icterus. Piglets have coarse hair, take a characteristic "buried" or "thoughtful" position with their head down (Ivetić V.et.al.., 2004). Stunted piglets can not recover and have to be removed from the pen due to significant cachexia. A prominent feature in pigs in the early clinical phase of PMWS is an increase in subcutaneous lymph nodes, most often inguinal superficial lymph nodes, although infections are possible without this symptom. Neurological symptoms are observed less often (Ivetić V.et.al. 2004).

On the section of dead piglets, lesions on the lungs and enlargement of lymph nodes (inguinal, sub mandibular, mesenteric and mediastinal) are most often found. The superficial inguinal lymph nodes are most often affected, which on cross-section show a red-brown zone interspersed with bacon fields. In addition to the inguinal, the enteric lymph nodes stand out for their size, so that in some cases they take on the size of a child's forearm. However, these lesions are not always present, so they can not serve as markers for PMWS on pig farm . In a certain number of piglets, necrotic changes in the liver with noticeable discoloration are observed, with jaundice evident. Also, with the piglet, let's take care of the multifocal leaflets in the kidneys. A smaller number of piglets suffering from PMWS may have bronchopneumonia and gastric ulceration in the esophageal serosa that are not directly related to circovirus, but more to secondary infections. These lesions cause internal bleeding that can lead to death in piglets with PMWS and are responsible for pale skin. At the end of this stage, cachexia may develop (Ivetić,V. 2004, Jovanović M. et.al.2019).

Characteristic microscopic lesions can be found in the lymph nodes. These are clearly circumscribed, spherical, basophilic, cytoplasmic inclusions of PCV2 in histiocytes. In the portal zone of the liver, a lymphocytic-histiocytic inflammatory infiltrate, necrosis of individual hepatocytes, etc. can be observed. However, in some cases, generalized perifocal necrosis with massive loss of hepatocytes is observed. Other microscopic changes of a similar nature can be observed in the kidneys, pancreas, intestines and myocardium. Moderate to severe granulomatous enteritis also occurs sporadically (Ivetić V.et al., 2004). In piglets suffering from PDNS, anorexia, depression, loss of consciousness, unsteady gait occur, with individuals having a physiological temperature or mild pyrexia. However, the most obvious sign in the acute phase of the disease is the presence of irregular, red papules and macules on the skin, mainly on the hind limbs and perineal region, which tend to coalesce. Over time, the lesions become covered with dark scabs and gradually fade, some times leaving scars. The cause of death in pigs affected by PDNS is acute renal failure, with usually a very significant increase in serum urea and creatinine (Ivetić V.et.al 2004).

In addition to skin changes, in pigs that die from an acute infection of PDNS, the kidneys are enlarged with numerous cortical hemorrhages and edema of the renal pelvis (Ivetić et.al 2004, Jovanović, 2019). Histologically, non-purulent interstitial nephritis with dilatation of renal tubules can be observed. Kidney and skin lesions are most often present in pigs suffering from PDNS, but there are also rare cases when skin or kidney lesions can occur by themselves (Ivetić et.al. 2004). Renal lymph nodes, as well as other lymph nodes, can be enlarged and red in color due to blood drainage from the affected areas, mainly the skin (Ivetić V.et.al 2004).
In the respiratory form of circovirus disease, the main clinical signs are respiratory disorders and diarrhea (Ivetić 2004). Given that these clinical signs are also present in PMWS, there may potentially be a diagnostic overlap between these forms of circovirus infection (Došen R. et.al. 2005).

Before commercial vaccines became available, successful treatment and control of PCVAD was primarily focused on ensuring good manufacturing practices that minimize stress, eliminate or reduce co-infections, and eliminate potential factors that induce immune stimulation and progression of PCV2 infections. Today, Madek's 20-point plan for controlling PCV2 infections is used, which can be summarized in 4 golden rules, which include: 1) limiting contact with pigs, 2) reducing stress, 3) good hygiene, 4) good nutrition. One of the main points of Madek's plan is to minimize contact between pigs, given that direct contact is one of the most common ways of spreading this infection in the herd. It is recommended to establish solid partitions between boxes, as well as to adopt the "all in, all out" system on farms in order to reduce contact between pigs. The quarantine of newly acquired pigs aims to prevent the introduction of new infections. An important element in the control of PCV2 infections is the suppression of certain diseases such as PPV, PRRS, enzootic pneumonia and swine flu, which increase the severity of PCV2-induced lesions. (Madec F. et al., 1999)

Respiratory form of swine corona virus disease

When we talk about corona virus diseases of pigs, we primarily mean diseases of the digestive system in the form of transmissible gastroenteritis of pigs (Transmissible gastroenteritis - TGE). In general, porcine corona viruses cause vomiting and weight loss in pigs, epizootic diarrhea in piglets, transmissible gastroenteritis and respiratory disease. The porcine respiratory corona virus (PRCV) is only one form of the virus that causes TGE with a changed tropism from the intestinal to the respiratory system. The main difference between the corona virus that causes TGE and the virus that causes respiratory disorders is the deletion in the S gene that exists in the respiratory form of the swine corona virus (Šamanc H, 2009). The change in the antigenic composition, which explains the appearance of the virus that primarily leads to respiratory disorders in pigs, may also be a consequence of the TGE vaccination program (Šamanc H, 2009).

An important mechanism of virus acceptance for the receptive cell is the interaction of the viral S glycoprotein with receptors on the cell surface. These receptors are also glycoprotein in nature and are characteristic of each receptive species. The entire process of viral replication takes place in the cytoplasm. Moreover, this virus can multiply in cells without a nucleus, which is important for the pathogenesis of some diseases caused by corona viruses. The mutant virus that causes changes in the intestines, as most mentioned, can cause I respiratory problems, and it differs only in the E2 viral protein, which is a consequence of the mutation in the S gene (Šamanc H, 2009).

The significance of respiratory corona viruses is manifold. Certain isolates can induce or contribute to the development of viral diseases. Serologically, it is difficult to distinguish respiratory and enteral forms of corona virus. Respiratory corona viruses can induce cross-protection against enteric corona viruses (Šamanc, 2009).

A susceptible animal can become infected by ingesting or inhaling contaminated material. The infectious dose is small and depends on the age of the animal. In the pig population, the disease spreads aerogenically and through contaminated feed. Within a farm and between farms in one region, the disease can also be transmitted by secondary sources of infection, which include means of transport, shoes and clothing of people, such as by air, up to several kilometers. Non-specific factors play a significant role in the development of the disease, especially cold weather, improper diet, frozen or spoiled fodder and other factors that can reduce the animal's resistance. The respiratory form of the disease begins with the primary multiplication of the virus in the mucous membrane of the nose, trachea and lungs. After the primary replication of the virus in the epithelium of the initial parts of the respiratory tract, the virus invades the bronchial and bronchiolar epithelia and further spreads to the peribronchiolar and alveolar spaces. By passage through a greater number of hosts, the virus becomes more pathogenic.

Pigs can be positive for the respiratory corona virus without showing symptoms characteristic of diseases of the respiratory tract. Clinical symptoms are non-specific and manifest as varying degrees of anorexia, lethargy, elevated temperature, difficult and accelerated breathing, weight loss, and in some cases end in death (Šamanc H, 2009). The carcasses of dead piglets are mostly cachectic and dehydrated. Macroscopic lesions, as well as clinical symptoms, vary from inapparent, difficult to detect, to multifocal changes on the lungs, which can progress to complete lung consolidation. A microscopic examination of the changed parts of the lungs reveals a bronchointerstitial pneumonia, characterized by necrosis, metaplasia and proliferation of the bronchiolar epithelium, as well as an increase in the number of neutrophil granulocytes and macrophages in the alveolar spaces (Šamanc H, 2009).

By weakening the defensive function of pulmonary alveolar macrophages, corona viruses lead to a weakening of the overall defensive ability of the respiratory system of pigs. Although, the role of this virus in the complex of swine respiratory diseases is still not entirely clear, it is not unusual for the virus to be isolated together with the PRRS virus and/or the swine influenza virus, all of which can contribute to the faster development of secondary bacterial infections (Šamanc H, 2009).

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REFERENCES

Bojkovski J., Radojičić B.,Petrujkić B. 2005. *Savremeni aspekti u dijagnostici i terapiji uzgojnih bolesti svinja.* Proceedings workshop Clinica Veterinaria Ohrid 3-7 september, 251-257 pp. (on serbian language)

Bojkovski J., Maletić M., Vakanjac S., N. Zdravković N., Vasić A., Maletić J., Apić I.,Vasiljević T., Delić N. 2015a *Breeding and exploitation of boars on commercial farm*, Lucrări ştiinţifice medicină veterinară vol. XLVIII(1), Timişoara pp. 15-25

Bojkovski J., Dobrosavljević I., Delić N., Savić B., Rogožarski D., Petrujkić T., 2012a. *Porcine reproductive respiratory syndrome (PRRS*) Savremena poljoprivreda vol 61.No 1-2, pp.61-67. (on serbian language)

Bojkovski J., Pavlović,I, Savić B.,Rogožarski, D.2012 b. Contribution to knowledge biosecurity on pig farms industrial type Bulltein UASVM,Veterinary Medicine, 69,-

1-2 pp 305-307.

BojkovskiJ.2015b, ,*Biosecurity on pig farm,* Lambert Academic Publishing, monography.

Bojkovski J,Maletić, M.,Zdravković,N.,Nedić S., Pavlović I., 2015c *Pregled bakterijskih i virusnih oboljenja svinja u ekstenzivnom i intenzivnom načinu držanja* Veterinarski žurnal Republike Srpske,(1),144-157.(on serbianlanguage)

Došen R., Prodanov J., Milovanov D.,Stojanov I., Pušić I. 2007. *The bacterial infections of respiratory tract of swine.* Biotechnology in Animal Husbandry, 5-6, 237-243.

Došen R., Ličina J., Petrović T., PolačekV.2005.,*Dijagnostički značaj «Porcine dermatitis and nephropaty sindrome» (PDNS) u regionima gde je klasična kuga svinja(KKS)endemično prisutan*,Simpozijum «Epizotiološki dani»- Jagodina, str: 143.(on Serbian language)

Gagrčin, M.,2009 *Aktuelna znanja o epzootiologiji, kontroli i preventivi cirkovirusnih bolesti svinja,* Sedmi simpozijum„ Zdravstvena zaštita, selekcijai repodukcija svinja", Srebrno jezero,, zbornik radova, str. 6–10. (on serbian language)

Ivetić V., Žutić M., Valter D., Milošević B, 2005 *Kompleks respiratornih bolesti kod svinja, dijagnostika i mere kontrole*, Zbornik radova i kratkih sadržaja 17-og savetovamnja veterinara Srbije sa medjunarodnim učešćem, pp. 190-198. (on setrbian langiuage)

Ivetić V., Savić B., Valter D.2004 Multisistemski *sindrom kržljanja prasadi posle odlučenja (PMWS)* Veterinarski Glasnik, 3-4 2004, str.419-430. (on srerbian language)

Jovanović M., Kovačević –Aleksić S., Knežević M. 2019 *Specijalna veterinarska patologija*, Udruženje veterinarskih patologa Srbije, Beograd

Kureljušić B., Weissenbacher-Lang C., Nedorost N., Stixenberger D., Weissenböck H., 2016 *Association between Pneumocystis spp. And co-infections with Bordetella bronchiseptica, Mycoplasma hyopneumoniaeand Pasteurella multocida in Austrian pigs with pneumonia,* TheVeterinary Journal, 207: 177-179,

Kureljušić B.,Weissenbacher-Lang C., Savić B., Nedorost N., Stixenberger D., Binanti D, Viehman M., Weissenböck H., 2015 *Co-Infection between Pneumocystis spp. and some bacterial lung pathogens in pigs with pneumonia*, Zbornik radovaTrinaestog savetovanja „Zdravstvena zaštita, selekcija i reprodukcija svinja", Srebrno jezero, 28-30. Maj ., 57-61(on serbian language)

Lončarević A., Maričić Z., Toševski J.,Pavlović I. 1997 *Osnove sistematskog zdravstvenog nadzora i programiranje zdravstvene zaštite svinja u intenzivnom odgoju.* U monografiji: A.Lončarević: Zdravstvena zaštita svinjau intenzivnom odgoju, Izd.: Naučni institute za veterinarstvo Srbije, Beograd,517–523.(on Serbian language) **.**

Lipej Z.,:2015 *Bolesti svinja*, Medicinskanaklada, Zagreb.,(on croatian language)

Lupulović D, Prodanov J, Lazić S. 2008 Serološko ispitivanje prisustva virusa reproduktivno respiratornog sindroma svinja (PRRS) u industrijskoj proizvodnji. Zbornik radova i kratkih sadžaja 20-og Savetovanja veterinara Srbije sa međunarodnim učešćem. Vrnjačka Banja, Srbija, 24-27 Septembar, 160. (on serban language)

Maksimović Zorić J., Milićević V., Kureljušić B., Stanojković A., Pepić M., Veljović LJ., Radosavljević V., Savić B., 2017: *Utvrđivanje prisustva influenca A virusa na farmama svinja sa teritorije Republike Srbije*, Zbornik radova Petnaestog savetovanja „Zdravstvena zaštita, selekcija i reprodukcija svinja", Srebrno jezero, 25.-27. Maj ., 45-50 (on serbian language)

Madec F, Eveno E, Morvan P, Hamon L, Morvan H, Albina E, Truong C, Hutet E, Cariolet R, Arnauld C, Jesting A 1999 *La maladie de l'amaigrissement du porcelet (MAP) en France 1 – Aspects descriptifs, impact en élevage*. Journées Rech. Porcine en France.31:347-354.

Novosel D, Petrović T, Acinger-Rogić Ž, Štukelj 2016 *Epidemiology and status of porcine reproductive and respiratory syndrome in the Western Balkan region: challenges and prospects.* Slov Vet Res 2016,53(4):185-93

Obrenović S, Pepić M, Bacić D, Stanković B ,Bojkovski J,Urošević M. VakanjacS., 2017, *Reproduktivni i respiratorni sindrom svinja* " 8 naučni simpozijum repordukcija domaćih životinja" Zbornik predavanja, Divčibare 12-15 oktobar , 263-277(on Serbian language)

Petrujkić T., Bojkovski, J., Petrujkić B. 2011. *Reprodukcija svinja*, Naučni instutut za veterinarstvo Srbije, Beograd. (on serbian language)

Petrović T, Milićević V, Prodanov-Radulović J, Maksimović-Zorić J, Lupulović D, Došen R, Lazić S:2011 *Molecular detection and genetic analysis of Serbian PRRSV isolates*. Proceedings EuroPRRS2011 'Understanding and combating PRRS in Europe' COST Action FA902,50-56.

Prodanov-Radulović J, Vučićević I, Polaček V, Aleksić-Kovačević S:2020 *Current swine respiratory diseases morphology in intensive swine production in Serbia*. Acta veterinaria, 70, 1, 1-36, 2020

Radojičić, S., Valčić, M., i Đuričić, B. 2011. *Infektivne bolesti životinja, specijalni deo*, Beograd, Srbija (on serbian language)

Radojičić B., Đuričić B., Gagrčin M 2002 *Epizootiološki i dijagnistiučki značaj kontrole reproduktivnog i respiratornog sindroma svinja,* Veterinarski glasnik, vol 56,1-2, strr. 21-31 (on serbian language)

Savić B., Milićević V., Jakić-Dimić D., Bojkovski J., Prodanović R., Kureljušić B.,Potkonjak A., Savić B., 2012: *Genetic characterization and phylogenetic analysis of porcine circovirus type 2 (PCV2) in Serbia*, ArchVirol., 157 (1):21-28

Savić B., Radanović O., Žutić M., Kureljušić B., Pavlović I., Bojkovski J.2012.: *Kompleks respiratorne bolesti svinja (PRDC) – prevalenca detektovanih infektivniha genasa*, Zbornik radova Četrnaestog regionalnog savetovanja iz kliničke patologije i terapije životinja, Subotica 14.-16. Jun., 29-34 (on serbian language)

Savić B., Pepić Marijana, Radanović O., Cvetojević Đ., Stevančević O., Kureljušić B., Milićević Vesna, 2016.: *Primo infekcija tovljenika sa virusom reproduktivnog i respiratornog sindroma svinja (PRRSV),* Zbornik radova 14. Savetovanja „Zdravstvena zaštita, selekcija i reprodukcija svinja“, Srebrno jezero, 02. do 04. Jun 2016., 31-35.(on serbian language)

Weissenbacher-Lang C., Kureljušić B., Nedorost N., Matula B., Schießl W., Stixenberger D., Weissenböck H., 2016: *Retrospective analysis of bacterial and viral co-infections in Pneumocystis spp. Positive lung samples of Austrian pigs with pneumonia*, PLoS ONE 11(7): e0158479.

Šamanc,H 2009 *Bolesti Svinja*, ;Naučna, book (on serbian language)

Štukelj M., 2017 Šta smo sve naučili o reproduktivno respiratornom sindromu svinja(PRRS) nakon trideset godina, II međunarodni simpozijum “Zdravstvena zaštita I reprodukcija papkara” ,.11-28(oin serbian language).