EMERGENCE OF PSEUDORABIES VIRUS (MORBUS AUJESZKY) INFECTION AT LARGE SWINE FARMS IN AP VOJVODINA (SERBIA)*

Jasna PRODANOV-RADULOVIĆ♦, Radoslav DOŠEN, Ivan PUŠIĆ, Tamaš PETROVIĆ, Jelena APIĆ, Igor STOJANOV, Vladimir POLAČEK

Summary: The aim of this paper was to evaluate current epidemiological situation concerning occurrence of Aujeszky’s disease (AD) outbreak in large swine farms in Vojvodina Province. The material for this research included the samples from five swine farms, where certain disorders and health problems in suckling piglets i.e. clinical signs suggesting AD were detected. Depending on the specificity of each evaluated case and available material, the applied research methods included: epidemiological and anamnestic evaluation, clinical investigation, gross pathological examination, standard bacteriological examination for detection the presence of aerobic and anaerobic bacteria in the organs and tissue samples (lungs, tonsils, lympho nodes, spleen, cerebral tissue) derived from diseased, died pigs. Viral isolation on the susceptible cell culture and molecular diagnostic method (RT-PCR) were applied. Also, the material for the investigation included 300 blood samples collected from breeding animals, in order to evaluate the presence of specific antibodies against AD virus. The achieved results suggest that swine population in AP Vojvodina province is enzootically infected with AD virus. Besides vaccination, which represents one of the measures in AD eradication, it is necessary to apply strict trade control and checking the health status of herds from where breeding animals are purchased.

Key words: Aujeszky’s disease, Pseudorabies, swine farms, Vojvodina.

INTRODUCTION

Aujeszky’s disease (Pseudorabies, Morbus Aujeszky) is an acute, contagious viral disease, of wide range of domestic and wild-life species (Allepuz et al., 2009). The causative agent is designated as suis alpha herpes virus 1, a member of the Herpes viridae family and Alphaherpes virinae subfamily (Mettenleiter et al., 2012). The Aujeszky’s disease virus (ADV) is an important pathogen of pigs and infects almost all mammalian species except man. However,
pigs are the only animal species that can survive the infection with ADV, which accounts for its ability to be subclinically (latently) infected (Martinez-Lopez et al., 2009) while infected individuals of all other animal species succumb to the disease without shedding the virus (Komáromi and Szabó, 2005).

During the last forty years vaccination against Aujeszky’s disease (AD) on large swine farms in Vojvodina Province was performed with modified-live vaccine (MLV) (Došen et al., 2002; Pušić et al., 2009). However, in Serbia, especially in Vojvodina region, an endemic infection with ADV is a major problem in spite of long lasting vaccination (Pušić et al, 2009; Prodanov-Radulović et al., 2011). Vaccination with MLV alone is unlikely to eradicate the disease due to the problem of latent carriers (Muller et al., 2003). The epidemiological investigation revealed that during the period 2000-2010 the outbreaks of AD were recognized on the territory of 13 municipalities in Vojvodina (Pušić et al., 2007; Pušić et al., 2009). The main reasons for spreading the infection were displacement of latently infected breeding animals from large farms to individual producers and quitting of vaccination on some of the large farms (Pušić et al., 2011). Several years ago the production of MLV type vaccine in Serbia was completely stopped and most of swine farms ceased with vaccination against AD because of lack of vaccine on the market. This resulted in existence of highly susceptible population of pigs on large swine farms in Vojvodina region. As a result, in the beginning of 2011 to 2014, a severe AD outbreaks occurred on several large swine farms.

The aim of this paper was to evaluate current epidemiological situation concerning occurrence of AD outbreak in large swine farms in AP Vojvodina region.

LITERATURE REVIEW

The Aujeszky’s disease has a great economic impact to the swine industry, causing losses due to high mortality in suckling and weaner piglets (Casal et al., 2004; Mettenleiter et al., 2012). Adult pigs do not show obvious clinical signs of infection, except for mild respiratory disorders, fever and weight loss; however, among pregnant females, the disease can cause reproductive disorders (abortion, embryonic death, mummified fetuses) or stillbirths and the birth of avital, weak piglets with neurologic symptoms that lead to death (Stančić et al., 2010; Stančić et al., 2011). The primary means of transmission of the AD among swine herds is thought to be by aerosol suspensions of the virus and direct contact between infected and susceptible pigs. Indirect contacts by vehicles, equipment, personnel or artificial insemination can also spread the virus among herds (Allepuz et al., 2009; Prodanov-Radulović et al., 2011). Inapparent infections rarely occur when ADV is introduced into a herd for the first time where neonatal pigs are present, because pigs of this age are highly susceptible (Casal et al., 2004). Highly virulent strains produce a non suppurative meningoencephalitis that causes fatal disease in piglets, with nervous clinical signs (Martinez-Lopez et al., 2009).

Many European countries have established eradication programs, with the objective of initially controlling and finally eradicating the disease (Martinez-Lopez et al., 2009). The disease has been eradicated from domestic pig populations in most of the European Union (EU) countries, as well as from the United Kingdom, Canada, New Zealand, and the United States (Komáromi and Szabó, 2005; Ketusing et al., 2014). Different Member States of the EU have used different approaches to AD eradication (veterinary administrative measures, serological tests, culling infected animals, using inactivated vaccines, prohibition of use of live vaccines) (Komáromi and Szabó, 2005). Restrictive regulations on the trade of pigs to regions or countries, which are free of ADV have been imposed in the EU. However, the risk of moving infected animals into free or low prevalence areas remains an important problem for the control and eradication of AD (Martinez-Lopez et al., 2009).

The control of the disease in endemic countries is based on compulsory vaccination of the entire swine population (Casal et al., 2004; Müller et al., 2003). In the last 40 years, in Serbia pigs were immunized with MLV to minimise clinical disease and death loss (Došen et al., 2002; Pušić et al., 2011). The virus strain used in this non glycoprotein-E (gE) deleted vaccines was Ercegovac field strain isolated in 1956, and attenuated through 200 passages on chicken fibroblast tissue culture (Pušić et al., 2009). The main goal of this vaccination programs was to reduce economic losses caused by clinical episodes of AD. However, only vaccination for clinical purposes has not been successful in preventing the spread of the virus under field conditions (Ketusing et al., 2014; Müller et al., 2003; Pušić et al., 2011).

By serological examination of blood samples, obtained from unvaccinated pigs in Vojvodina, Pušić et al. (2009) discovered that specific antibodies against ADV were present in 32.7% animals. Relatively high percentage of seropositive breeding pigs owned by individual producers, where no vaccination against AD is performed, suggests that the infection in pig population is present on a significant level in Vojvodina region. The systematic vaccination of breeding pigs, although it reduces the probability of infection and the spread of ADV, generally does not prevent infection and may produce subclinically (latently) infected pigs that will likely not be detected serologically (Martinez-Lopez et al., 2009; Prodanov-Radulović et al., 2011). There is a concern that MLV, after vaccination may...
revert back towards virulence during replication in the host. In such cases, it is possible that the vaccinal virus circulates on farms immunising pigs, although the presence of a field strain of low virulence should not be excluded (Pušić et al., 2009). The vaccinated animals do not suffer from the symptoms of the disease, but they may contract a field infection, excrete live virus and become carriers (Casal et al., 2004; Müller et al., 2003).

Aujeszky's disease is mandatory notifiable disease in Serbia (Pušić et al., 2007). However, there is no special decree issued by the Veterinary Authorities that regulate AD control neither does national or regional eradication program exist. (Pušić et al., 2009). Voluntary vaccination with MLV vaccines precludes the serological diagnosis of infected pigs, thus making eradication efforts impossible (Komáromi and Szabó, 2005). In many EU countries, efforts were made to eradicate MA (Müller et al., 2003). To reach this goal, the development and approval of a gene deleted (gE) vaccine was the decisive breakthrough, as this vaccine made it possible for the first time to distinguish infected from vaccinated pigs (Ketusing et al., 2014). The gE-negative strains are used the most widely, as the absence of the gene fragment encoding the gE protein and the consequent absence of the gE protein itself does not affect either growth of the virus or the protection induced by it. The advantage of such gene-deleted vaccines is that the detection of antibodies to the lost protein by enzyme-linked immunosorbent assay (ELISA test) indicates serological positivity induced by a field virus and not by the vaccine virus (Pasik, 2004). Recently, in Serbia the new type of attenuated vaccine for active immunisation of pigs against ADV was registered. The vaccine contains a modified live viral strain NIA-3, which is gE-antigen and thymidine kinase deleted (a marker vaccine). However, it is not so far, possible to differentiate between vaccinal and infectious titer because the vaccination campaign using marker vaccines in Serbia is not yet operational.

THE RESULTS OF OUR INVESTIGATIONS

Material and methods

The material for this research included the samples from five swine farms, located in Južnobački and Sremskidistrict in Vojvodina, where certain disorders and health problems in suckling piglets i.e. clinical signs suggesting AD were detected. Depending on the specificity of each evaluated case and available material, the applied research methods included: epidemiological and anamnestical evaluation, clinical investigation, gross pathological examination, standard bacteriological examination for detection the presence of aerobic and anaerobic bacteria in the organs and tissue samples (lungs, tonsils, mediastinal and mandibular lymph nodes, spleen, cerebral tissue - cerebellum, medulla oblongata) derived from diseased, died pigs. Viral isolation (VI) on the susceptible cell culture of tissues originating from the dead animals and molecular diagnostic method, reverse transcriptase - polymerase chain reaction (RT-PCR) were applied (Ma et al., 2008). Also, the material for the investigation included blood seras collected from breeding animals, in order to evaluate the presence of specific antibodies against ADV.

History of the examined swine farms: The following details were ascertained by the epidemiological examination and from farm records: number and category of pigs on the unit, production details (nucleus or commercial), disease status, current veterinary health plan (vaccination, medication) and biosecurity protocols used. The control of indoor pig environment was inspected with regards to basic zootechnical conditions for swine (temperature, lighting, ventilation, stocking density, bedding, hygiene). The animals were inspected for clinical signs of disease and abnormal behavior. The clinical inspection was followed by the necropsy of dead pigs.

Bacteriological testing: Isolation of bacteria from clinical samples was performed by aerobic and microaerophilic cultivation. Microscopic examination determined whether the isolated bacteria were Gram positive or not and whether it is a coccoid or rod-like organisms. The determination was carried out by determining the biochemical characteristics of the isolated bacteria (Quinn et al., 2011).

Virology testing - Aujeszky’s disease virus isolation: Isolation of ADV was done by cultivation of tissue samples on cell culture line PK-15 (porcine kidney - ATCC CCL-33). Samples of brain, tonsil, and lung of tested animals were homogenized by mortar and pestle and diluted in PBS 1:10 (1g of tissue and 9 ml of PBS) supplemented with antibiotics (200 IU/ml penicillin; 100 μg/ml gentamicin and 5 μg/ml amphotericin B) to prevent bacterial grow. The tissue homogenate is centrifuged on 2000 g for 10 min and 1 mL of supernatant was used for inoculation of 24 hours old PK-15 cell culture with 75% confluent cell layer in 25 cm² tissue culture flask. Before inoculation, the cell culture growing medium is decanted from the flask and 1 mL of tissue homogenate was added to the cell monolayer, gently shaking to distribute the inoculated material over the whole cell monolayer, and incubated for 1 hour on 37°C. After the incubation 10 mL cell growing medium (Eagle MEM, Sigma) with 10% fetal calf sera (EU grade, PAA, Austria) was added to the cells and cell monolayer was microscopically observed daily for the development of the characteristic herpes virus cytopathic effects (CPE - with rounded birefringent cells, followed by
complete detachment of the cell sheet) in the next 7 days. In the absence of any obvious CPE, after the 7 days incubation period, one blind passage into the new 24 old cell monolayer was performed with 1 ml suspension of the first cell passage after 3 cycles of freezing-towing steps. If the visible CPE is observed, the virus presence was confirmed by neutralization with specific antiserum.

**Serology testing:** The serum neutralizing test (SNT) was applied in order to estimate the specific antibody titer against ADV, following standard procedure as described before (Office International des epizooties, Manual of Epizootic, 2004). A total number of 300 blood samples were examined.

**Results**

The first examined farm represent the modern commercial swine farm. In the time of examination, on the farm the following swine categories were included: 680 sows, 515 pregnant gilts, 50 breeding gilts, 15 boars, 1060 suckling piglets, 5051 weaned piglets and 6050 fatteners. The farm represent the one-site production system (farrowing-to-finish). Applying control of all production stages, the correct stocking densities and housing requirements were detected. The farm have organised own veterinary services and swine health control programme is conducted according to Law (Stojanac et al., 2014). Anamnestically, the health disorders in sows and heigher percent of mortality in their litters were observed. By gross pathological examination of 25 dead suckling piglets the pathology lesions that are considered to be characteristic for ADV infection were detected: diphtheroid and necrotic tonsilitis, swollen and occasionally hemorrhagic lymph nodes, and focal necrosis on the liver and spleen in the form of yellow-white foci size 2-3 mm scattered. By clinical examination in suckling piglets in one chamber, the signs of severe disturbance of the central nervous system (CNS) were observed (hypersalivation, incoordination, trembling, paddling, convulsions, opisthotonus). In some cases the whole litter of piglets died within 48 hours. In lactating sows the signs of inapetence, mild apathy, agalactiae and constipation was noticed. Anamnestically, within the farm perimeter, the carcass of dead cat was found recently. By bacteriological examination of organs and tissues from succumbed suckling piglets *Streptococcus sp.* was isolated. Applying viral isolation (VI), from tissue samples derived from dead piglets, ADV was isolated and by RT-PCR technique the genome of ADV was detected. Serological testing (SNT) of 50 sera samples (sows, gilts, boars) has shown a large variety of specific antibody values against ADV (antibody titer of 1:4 to 1:128). Interestingly, in one boar, beside the serological positive result (titer 1:8), clinically the bilateral testes oedema was noticed. By additional epidemiological examination, it was discovered that all breeding animals on the examined farm are imported from the EU countries 6 -7 years ago and vaccination against AD was never been performed. Also, the serious failures in implementation of biosecurity measures on the swine farm were discovered: the farm-fence is not entirely enclosed, the vehicles for transporting fatteners to the slaughter are entering directly in the farm perimeter, the workers employed to repair some parts of the farm objects are entering without changing the close and boots.

On the other two evaluated commercial swine farms, the capacity 450-500 sows, by pathomorphological examination of 38 carcass of the dead sucking piglets, the existence of gross pathology changes indicative for infection with ADV were detected (*Tonsillitis purulenta et diphtheroides necroticans, Dilatatatio vesicae urinariae porcelli, Necroses submiliares et miliares hepati*). Clinically, the health disturbance was found sporadically in sucking piglets (yellowish diarrhea, paddling, trembling, convulsions). In weaned piglets and fatteners the clinical signs of respiratory disease was noticed (dispnoea, sneezing, coughing). Applying VI, from tissues of dead sucking piglets ADV was isolated. Applying SNT of 100 sera samples, the positive serological finding (antibody titer of 1:4 to 1:128) was detected. By epidemiological evaluation, it was discovered that on the examined swine farms the vaccination against AD was continuously performed in the last 8-10 years. However, because of vaccine shortage on the market, the vaccination was stoped in the last 3 years.

The fourth examined case refers to the modern commercial swine farm, the capacity 2500 sows. The farm represent the one-site production system (farrowing-to-finish). Applying control of all production stages, the correct stocking densities and housing requirements were detected. The farm have organised own veterinary services and swine health control programme is conducted according to Law (Stojanac et al., 2014). The health issue was established at weaning of piglets with clinical signs of respiratory illnesses and CNS diseases (dispnoea, coughing, paddling, trembling, egzophalma). However, gross pathology finding considering the suspicion of AD, was negative. Epidemiological analysis showed that the farm performes vaccination against AD, but the first vaccination of piglets is 10 days before weaning. Serological testing of sera samples derived from weaners (50 samples) and sows (50 samples) has shown a large variety of specific antibody values against ADV (antibody titer of 1:4 to 1:128). From tissue samples of dead weaned piglets in cell culture ADV was isolated and the presence of viral genome was confirmed by RT-PCR.
In the last examined commercial swine farm, the capacity 650 sows, anamnestically, the health disorders in sows and in their litters were observed. By epidemiological investigation it was discovered that on the swine farm in total 50 new sows had been introduced 2 months ago. Serologically, in 25 sows the presence of specific antibodies against ADV was detected (antibody titer from 1:16 to 1:64). However, despite the fact that these animals were serologically positive, the origin of that immunological status from the aspect of AD remained unknown: vaccination or infection. From other hand, the evaluated swine farm 5 years before stoped with the vaccination against AD. By clinical examination in lactating sows the signs of inapetence, mild apathy and agalactiae were observed. In suckling piglets the signs of severe disturbance of the CNS were clinically detected (wide open eyes, paddling, convulsions, ataxia, opistotonus, epileptiform-like seizures). The onset of clinical signs in 2- to 3-day-old piglets was sudden, sometimes spanning 8-10 hours from onset to death. Clinically the fatteners also become anorectic, listless and apathic. The pathomorphological changes that were detected in dead sucklings indicated the lesions characteristic for ADV infection: diphteroid and necrotic tonsilisits, swollen and occasionally hemorrhagic lymph nodes, focal necrosis on the liver and spleen in the form of yellow-white foci size 2-3 mm, oedema and haemorrhages on the meninges and brain tissue. By bacteriological testing on tissue samples from dead piglets the following bacteria were isolated: *Escherichia coli*, *Escherichia coli haemolytica* and *Streptococcus sp.* Applying VI, from tissues from dead piglets ADV was isolated.

**DISCUSSION**

Regarding the occurrence of AD outbreak in large swine farms in Vojvodina Province, the achieved results can be grouped in several categories: the existing problem of farm biosecurity, cessation of vaccination against AD, inappropriate immunoprophylactic program and introducing of new breeding animals on the farm without previous quarantine.

In the first three examined cases of AD, individually non-vaccinated swine herd were surrounded by vaccinated or poorly vaccinated herds and backyard stocks. A primary outbreak of AD in a naïve immunologically unprotected herd can be a devastating event, with spread through the entire herd within 1 week and ending with more than 90% of suckling pigs dead, nursery pigs stunted in their growth, and with respiratory disease in adults (Došen et al., 2007; Mittenleiter et al., 2012). In evaluated farms, the established gross pathology lesions i. e. herpetic and splenic yellow-white foci of necrosis are most frequently seen in young pigs because that lack passive immunity (Mittenleiter et al., 2012). In the examined sera samples, ADV antibodies were detected by SNT as early as 7 days after occurrence clinical disease. The high antibody titre detected in sows gives a rise to suspicion that a mildly virulent strain of wild virus was latently present on the farm, because infection with a mildly virulent strain may pass clinically unnoticed (Müller et al., 2003). However, protection against ADV is not closely related to the level of antibody because the virus is nonviraemic and spreads predominantly by mucosal infection and neuronal innervations (An et al., 2013; Mittenleiter et al., 2012). Quitting of vaccination on some of the large farms in the Vojvodina with the high density pig population without implementation of strict biosecurity measures can be hazardous. The biosecurity and management practices in backyard herds are generally lacking, which makes them a possible threat for large farms (Došen et al., 2012; Prodanov-Raydulović et al., 2011).

In the fourth examined case, an inadequate immunoprophylaxis program against AD was applied and disease occurred primarily in weaning piglets. Generally, vaccines must be used in sows, in order to give protection to the young piglets via the colostrum (Casal et al., 2004; Dosen et al., 2002). The aim of the vaccination programme is to create a good level of immunization in sows and uniform level of protection in the piglets during the first weeks of their life (Casal et al., 2004). One of the main limitations of the immunization of neonates from vaccinated or previously infected mothers is the interference with maternally derived antibodies (MDA). The half-life of MDA that pigs receive in the colostrum of immune sows is approximately 18 days (Mettenleiter et al., 2012). It is considered hat dueto inadequate immunization program, clinical form of the AD occurs primarily in piglets 14 days after weaning. However, in early phase of infection, by gross pathological examination in dead sucklings, often the negative pathological finding can be detected (Došen et al., 2002).

In the last examined case, it was demonstrated that ADV can be latently present in apparently healthy breeding animals without showing any clinical signs. The obtained results indicate that purchasing of breeding animals, with different or unknown health status regarding AD, represents the important route of transmission and spreading of the infection. The outbreak of AD on the swine farm, where immunoprophylaxis was not carried out and purchasing the breeding animals where vaccination against AD was applied, may lead to devastating consequences. The reason for this is the phenomenon of latent infection with reactivation and shedding of the virulent virus (Allepuz et al., 2009; Prodanov-Raydulović et al., 2014). Because of subclinical infection, before purchasing breeding animals, the quarantine measures need to be taken (Došen et al., 2002; Došen et al., 2012). However, during quarantine, latently
infected pigs may not be detected. Even if infected pig shows the signs of the disease during quarantine, these signs may be un especific (respiratory problems, fever, anorexia) and not associated with AD (Prodanov-Radulović et al., 2011). Serological monitoring was shown to be successful in detecting infectious animals (Martínez-López et al., 2009).

Although successful in preventing herds from severe clinical outbreaks, voluntary vaccination in the past did not result in a marked reduction of the spread of ADV in pig-dense parts of Vojvodina region (Pušić et al., 2007; Pušić et al., 2009; Prodanov-Radulović et al., 2011). In addition to the above problems, eradication of ADV from large pig herds in Vojvodina is extremely difficult due to the characteristics of pig production: the dominance of farrow-to-finish type farms, resulting in the simultaneous presence of a large number of susceptible animals; frequent absence of all in-all out principle; the often deficient technological discipline (Prodanov-Radulović et al., 2014). The diversity of production technologies does not make it possible to develop a single procedure that could equally be used on all pig farms (Došen et al., 2007). The herd-specific risk factors such as biosecurity measures, vaccination schemes or the health status of the purchased pigs might be also related to the success of the AD eradication.

**CONCLUSION**

It may be concluded that the swine population in Vojvodina province is enzootically infected with ADV. Individually vaccinated herds are surrounded by non-vaccinated or poorly vaccinated herds and backyard stocks. Such surrounding herds create pockets of susceptibility which can serve as reservoirs of ADV. Also, the obtained results indicate that purchasing of breeding animals, with different or unknown health status regarding MA, represents the important route of transmission and spreading of the infection.

The cessation of vaccination in the regions with the high pig density, if is not supported with the appropriate eradication strategy, represent a great risk from re-introducing of the virus in the herd with all the consequences. Great economic losses, caused by this diseases, as well as restrictions in trade with ADV free countries point on a second stage of eradication programme, the remaining sources of virus need to be traced and eliminated by which would allow regional approach. In the future, the MA eradication programme in Serbia should be based on need of making regulations about prophylaxis and control, as well as to launch a National eradication programme to implement an eradication strategy, represent a great risk from re-introducing of the virus in the herd with all the consequences.

REFERENCES


Aujeskijeva bolest (MA) je infektivno virusno oboljenje, čiji je uzročnik svinjski alfa herpes virus 1, koji inficira široki opseg domaćina izuzev lјude i primata. Svinje predstavljaju jedinu životinjsku vrstu koja može preživeti infekciju sa virusom Aujeskijeve bolesti, nakon čega se uspostavlja latentna infekcija. Cilj rada je bio sagledavanje trenutne epizootiološke situacije, vezano za nedavne slučajeve izbijanja MA na velikim farmama svinja u Vojvodini. Materijal za ispitivanje je obuhvatio uzorke poreklom sa pet farmi svinja, na kojima su registrovani zdravstveni problemi, koji su ukazivali na MA.

Izvođaju se epizootički pregled, standardne laboratorijske metode za utvrđivanje prisustva aerobnih i anaerobnih bacteija, transkripcija-klinički i patomorfološki pregled, standardne laboratorijske metode za utvrđivanje prisustva aerobnih i anaerobnih bacteija, transkripcija-klinički i patomorfološki pregled, standardne laboratorijske metode za utvrđivanje prisustva aerobnih i anaerobnih bacteija, transkripcija-klinički i patomorfološki pregled, standardne laboratorijske metode za utvrđivanje prisustva aerobnih i anaerobnih bacteija, transkripcija-klinički i patomorfološki pregled, standardne laboratorijske metode za utvrđivanje prisustva aerobnih i anaerobnih bacteija, transkripcija-klinički i patomorfološki pregled, standardne laboratorijske metode za utvrđivanje prisustva aerobnih i anaerobnih bacteija, transkripcija-klinički i patomorfološki pregled, standardne laboratorijske metode za utvrđivanje prisustva aerobnih i anaerobnih bacteija, transkripcija-klinički i patomorfološki 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