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CONTROL OF *PORCINE CIRCOVIRUS TYPE 2* INFECTIONS IN PIGS

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SUMMARY: Porcine circovirus type 2 (PCV2) is one of the leading viruses that impair the health status of pigs in the last decade and thus call into question the scope and efficiency of pig production. PCV2 infection control means control of all cofactors involved in the development of the disease and the so-called triggers with efforts for their eradication as well as immunoprophylaxis measures which are considered indispensable in controlling this disease. Today, 5 commercial vaccines are available on the European market, and are all based on the genotype of the PCV2 and show good efficacy. Basic issue regarding the successful vaccination is determining the optimal time for administration of the vaccine. Time of vaccination is best determined on the basis of the level of titer of colostrum antibodies in the blood serum of piglets and time of occurrence of PCV2 infections. At this moment eradication of this disease is farfetched, but vaccination is the main form of struggle against PCV2 infection.

Key words: *PCV2, vaccine, control, piglets, immunity.*

INTRODUCTION

Circovirus diseases belong to the group of new-generation diseases of pigs veterinary practice is confronted with in recent years and are considered to be the most

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controversial diseases in the pathology of these types of animals. *Porcine circovirus type 2* (PCV2) is a ubiquitous virus of domestic and wild pigs and is the primary cause of this disease in pigs. Increasing interest in circovirus infections began after the appearance of *Post weaning multisystemic wasting syndrome* (PMWS) in Canada in 1991, and retrospective studies have shown their existence in the late sixties. On the basis of new information about the pathology of the disease a new terminology was suggested, and now subclinical forms of circovirus infection are known in veterinary practice, then *Porcine circovirus systemic disease* (PCV-2-SD) (formerly PMWS), respiratory form of PCV2 disease (*Porcine circovirus lung disease*; PCV-2-LD), enteric form (*Porcine circovirus enteric disease*; PCV2-ED), an increasingly frequent reproductive form (*Porcine circovirus reproductive disease*; PCV2-RD), and *dermatitis nephrosis syndrome* (PDNS) (Segales, 2012). Today, these pathological entities are collectively referred to as Porcine circovirus associated diseases (PCVAD) (Segales et al., 2005; Segales et al., 2009). Which system will be affected, and thus which form of manifestation the disease will take, it is not possible to predict. But whatever form the disease takes, damage both direct and indirect is always very significant and threaten every rational production of pigs (Gagrčin, 2009). Regarding all of that PMWS is considered as economically the most important disease. It was found that direct and indirect costs in the EU amounted to about EUR 600 million per year (Armstrong and Bishop, 2004). In the US, this disease has cost manufacturers an average of 3-4 dollars per pig, with maximum losses ranging up to $ 20 (Gillespie et al., 2009). This syndrome as a form of circovirus infection has expanded among the pig population in the Republic of Serbia and appears in pigs aged 6-16 weeks (Ivetić et al., 2004; Stojanac et al., 2013a). Control of these infections is very complex and difficult. The use of any medications was contraindicated (antibiotics, chemotherapy, etc.), which contributes to even greater complexity of control. However, immunoprophylaxis in terms of administration of specific vaccines has the most frequent application (Llorenc Grau-Roma et al., 2011). Today there are several types of vaccines that are used in the control of these infections in many countries with developed pig breeding (live, inactivated, subunit). The most successful vaccines are those based on the induction of an active immune response to the capsid protein of PCV2. This protein is designated as the immunogen, inducing the formation of protective antibodies (Kixmoller et al., 2008, Tacker et al., 2008). The successful immunization is the one that results in reduction of the clinical forms of the disease, and the prevention of tissue injury.

CONTROL OF CIRCOVIRUS INFECTIONS

Madec's plan. Before vaccines became commercially available, successful treatment and control of PCVAD diseases was primarily focused on providing good production practices which minimize stress, eliminate coinfections or reduce their effect, as well as removing potential factors that induce stimulation of the immune system and the progression of PCV2 infection. Today, Madec's plan with 20 items is used for control of PCV2 infection, which can be summed up in four golden rules which include: 1) limiting of contact between the pigs, 2) stress reduction, 3) good

hygiene, 4) good feed (Madec et al., 1999). At least 16 items from this plan should be applied in order to reduce mortality from 20% to single digits. One of the main items from the Madec's plan is to minimize the contact between pigs, given that direct contact is one of the most common routes of spreading infection in the herd. Establishing firm partitioning walls between the compartments and the adoption of *all in, all out* system throughout the farm is recommended in order to reduce the contact between pigs. Quarantine of newly acquired pigs is aimed at preventing the introduction of new infections (Rose et al., 2003).

Control of coinfections. An important element in the control of PCV2 infection is the suppression of certain diseases such as PPV, PRRS, enzootic pneumonia and swine flu, which intensify the severity of PCV2-induced lesions. Studies on risk factors for PCVAD on 62 farms in Spain indicated that vaccination of gilts against PRRS increases the chances of outbreak of circovirus diseases, while in contrast, vaccination of sows against atrophic rhinitis reduces the chances of developing these diseases (Lo'pez-Soria et al., 2005). Attempts to control PMWS by herd vaccination against PPV in the final fattening stage in the United States, with proven circulation of PPV were successful. However, these positive results have not been confirmed experimentally. The application of immunomodulators is not contested in any segment, but still, abandonment of the application of some vaccines and sanitation programs is too much of a risk. For this reason, and based on the available results, the producers of pigs in PCVAD-positive herds should take into account the determination of the approximate time of appearance of PCV2 infections with the aim to change the time of vaccination as a potential plan for minimizing the disease (Gagrčin, 2009).

Disinfection measures. The use of disinfectants in buildings and vehicles has proved to be effective and is recommended for control of PCV2 infections. *In vitro* reduction of a titer of virus was achieved by using sodium hydroxide, Virkon S, Roccal D Plus, Clorox bleach, 1-Stroke Environ, Fulsan and Tek-Trol under controlled laboratory conditions (Royer et al., 2001). The effectiveness of disinfectants in commercial terms is not known.

Hyperimmune serums therapy. Subcutaneous injection of PCV2 hyperimmune serum of suckling piglets and post-weaning piglets has proved to be effective in reducing mortality on farms with PMWS in France, Spain and the UK (Ferreira et al., 2001; Waddilove and Marco, 2002). However, the success of this procedure is variable, as in some of the farms it did not produce results. Moreover, recent studies have shown that there is no benefit from the application of hyperimmune serum (Hassing et al., 2006; Opriessnig et al., 2006). The mechanism of action of hyperimmune serums therapy is not sufficiently understood.

Nutritional factors. Partial control of PMWS on some farms in the UK was achieved by changes in the diet of diseased pigs (Donadeu et al., 2003). These changes include an increase in the density of feed for young pigs and supplementing with commercial additives, mainly with antioxidant effects. Some studies have shown that conjugated linoleic acid alleviates PCV2 experimental infections (Bassaganya-Riera et al., 2003). On the other hand it was suggested that the addition of vitamin E and/or selenium in feed can

be used on farms affected by PMWS (Baebko et al., 2004). Although these preliminary results, both experimental and field-tested, show that certain nutritional factors can mitigate the effects of PMWS, there is still not enough scientific information to determine the true effects of the diet on this disease.

IMMUNOPROPHYLAXIS OF CIRCOVIRUS INFECTIONS

The introduction of vaccines against PCV2 led to an enormous reduction of PCV2-SD and PDNS, and also one part of the economic justification refers to a significant reduction in PCV2-SI. Today, there are several commercial vaccines used for the prevention of PCVAD in pigs, and all are based on the PCV2 genotype. *Circovac® (Merial)* vaccine consists of an inactivated PCV2 virus and is used for vaccination of piglets older than 3 weeks as well as for vaccination of sows and gilts. The application of this vaccine in piglets is a one-time, while vaccination of sows and gilts requires two applications, one 3-4 weeks before insemination, and another 2-4 weeks before farrowing. *Ingelvac CircoFLEX® (Boehringer Ingelheim), Circumvent® (Intervet/Merck),* and *Porcilis® PCV (Schering-Plough/Merck)* are subunit vaccines based on a PCV2 capsid protein and are used on piglets 2 weeks old, except *Porcilis® PCV (Schering-Plough/Merck)* which is used on piglets older than 3 days. *FosteraTM PCV (Pfizer Animal Health Inc.)* is a vaccine recently introduced to the market, now revised, and previously known as *Suvaxyn® PCV2 One DoseTM (Fort Dodge Animal Health Inc.)* (Nathan et al., 2012). The commercial vaccines are shown in table 1.

In Europe, examples of countries with high rates of vaccination (>80%) are Germany, United Kingdom, Ireland, Austria and Switzerland, while Russia, Denmark and Poland have low rates of vaccination (<30%). US, Canada, Mexico and Chile have a very high rate (80-98%), as well as Korea and Japan (70-90%), while China and Vietnam have a very low rate of vaccination (<5%).

The effectiveness of commercially available PCV2 vaccines has been extensively tested under controlled experimental conditions. Due to the limited cases of clinical diseases caused only by PCV2 virus, most studies on testing efficacy of PCV2 vaccines used model of co-infections with two or three causes. Using the model of coinfection has the advantage, given that it approximately corresponds to the field conditions in which certain pathogens of pigs may contribute to the development of the clinical forms of PCVAD. Co-infection with PRRS increases the severity of PCV2 infection in pigs, leading to increased secretion of circovirus through oronasal route and via faeces (Rovira et al., 2002; Sinha et al., 2011). Vaccination of piglets with Suvaxyn® PCV2 according to the PCV2/PRRS model, induces the generation of neutralizing antibodies with the simultaneous reduction of lung lesions and the quantity of the virus in faeces, serum, and lymph tissue, 28 days after inoculation (Opriessnig et al., 2008b). Several studies compared the efficacy of Suvaxyn® PCV2, Circumvent® and CircoFLEX® vaccines in various models of co-infections. Compared to the unvaccinated pigs, all vaccines induce formation of neutralizing antibodies and reduce quantity of virus in serum and lymph tissue (Opriessnig et al., 2009). Co-infection with *Mycoplasma hyopneumoniae* and

PCV2 also induces the development of clinical forms of PCVAD and was used as a model for testing PCV2 vaccines. The pigs vaccinated according to this model with Suvaxyn® and CircoFLEX® vaccines showed a reduction of PCV2 virus in the serum, reduction of the lymph lesions as well as a gain of body weight as compared to the control group (Kim et al., 2011). Various studies testing vaccine efficacy under controlled conditions clearly demonstrate the usefulness of these commercially available vaccines.

The potential protective effect of piglets after vaccination relies on the protective effect of PCV2 antibodies, either acquired passively (by vaccination of sows) or induced actively (vaccination of piglets). However, the low concentration or absence of antibodies after vaccination does not mean that the animal is not protected from PCV2 infection. Fenaux et al. (2004) found that, after vaccination with chimeric PCV1-2 virus, there was no seroconversion in all pigs, but also no clinical signs of disease were observed in these pigs after exposure to the PCV2 virus, nor PCV2 viremia was observed. These authors suggest the potential role of cell-mediated immunity in protection of pigs after vaccination. Protective immunity generated after vaccination reduces the possibility of an outbreak of PCV2 infection and is expected to protect the pigs in complex terrain conditions (Nathan et al., 2012). The duration of immunity following vaccination has not been well studied. Opriessnig et al. (2009) reported that NA are on a detectable level in piglets 3 months after an one-time vaccination, and that the vaccinated piglets are protected from circovirus infections compared to unvaccinated individuals. Martelli et al. (2011) monitored the serological response in piglets using the ELISA method, from the time of vaccination up to 35th week of life. Vaccinated and control groups had similar titers at the time of vaccination due to residual maternal antibodies, while significant increase in antibody titers in vaccinated piglets was observed two weeks after vaccination. The titer of the antibodies in the vaccinated animals was increased continuously reaching a peak between the 6th and 9th (12 to 13 log2) week after vaccination, after which the level of total antibody level was slightly reduced, never going below 6 log2.

The most successful vaccines are those based on the induction of an active immune response to the capsid protein (Cap) of circovirus type 2. This protein is designated as a major immunogen inducing protective antibodies, in contrast to the Rep protein which is poor immunogen (Kixmoller et al., 2008; Opriessnig et al., 2008b).

Current commercially available vaccines are based on the PCV2 genotype and all show good efficacy, although currently PCV2b genotype is a dominant type of infection worldwide. Subunit and inactivated vaccines have the advantage of stability and safety, but there are other vaccine technologies that are still in experimental stage which have been proven to stimulate anti-PCV2 immune response and prevent PCV2 infections. These are particularly the DNA-based vaccines, modified attenuated live vaccines, marker vaccines and vector vaccines. RNA-based anti-viral therapy and modified live vaccines are able to stimulate cellular and humoral immune responses in contrast to the subunit and inactivated vaccines (Beach et al., 2011; Nathan et al., 2012).

Table 1. Commercially available PCV2 vaccines in Europe

PCV2 Vaccines Company	*Ingelvac CIRCOFLEX®* BOHRINGER INGELHEIM	*Suvaxyn PCV2 One Dose* *FORT DOGE*	*Porcilis PCV® Circumvent®* *INTERVET*	*CIRCOVAC®* *MERIAL*
Antigen	PCV2 Cap protein	Inactivated PCV1-2 chimera	PCV2 Cap protein	Inactivated PCV2
Licensed for:	Piglets (2 weeks old and older)	Piglets (4 weeks old and older)	Porcilis PCV®: Piglets 3 days old and older Circumvent®: (3 weeks old and older)	Sows, gilts
Dosage	One dosage of 1 ml IM	One dosage of 2 ml IM	2 ml IM Porcilis PCV®: Two dosages (3 days and 3 weeks) / One dosage (3 weeks of age) Circumvent®: Two dosage (3 and 6 weeks of age)	2 ml IM Primary vaccination: (3-4 weeks before insemination) Revaccination: 2-4 weeks before farrowing

Immunoprophylaxis in sows and gilts. Vaccination of sows is one of the strategies for prevention of PCVAD in piglets reducing viremia and increasing the amount of specific neutralizing antibodies in the colostrum. Passive immunity in the form of maternal antibodies has been proved to be at least partially protective against PCV2 infections. Under experimental conditions, piglets with high levels of maternal antibodies had lower incidence of PCV2 viremia compared to those with low levels of maternal antibodies, which proves that the protection against PCV2 infection depends on the amount of antibody titers. High titers generally provide solid protection against PCV2 infection, whereas lower titers do not protect against these infections (McKeown et al., 2005; Opriessnig et al., 2008a). Vaccination of pregnant sows on farms with acute PMWS increases the production performance of piglets, reduces the mortality of piglets before weaning and increases the body weight of their offspring (Pejsak et al., 2010). Also, vaccination of sows and gilts increases the number of piglets born alive and number of piglets per sow per year, and reduce the number mummified piglets (Tacker et al., 2008; Stojanac et al., 2013b; Stojanac et al., 2014). In clinical PCV2 infections vaccination boosts the immune response, average daily gain and decreases the fattening period of the

offspring (Kurmann et al., 2011). Vaccination of sows also reduces the quantity of viruses that is transmitted from sows to piglets during gestation and suckling period, but does not completely eliminate the virus excretion via the colostrum (Gerber et al., 2011).

Immunoprophylaxis in piglets. Vaccination of piglets in subclinical cases, leads to improved daily gain and feed conversion and reduces the mortality rate, and in clinical cases further reduces the number of rejects. Today, vaccines are commonly administered to weaned piglets and have been shown to enhance the production parameters in pigs naturally exposed to PCV2 (Stevančević et al., 2013). On farms affected with PMWS, vaccination with a Circovac® vaccine increased average daily gain and reduced the mortality rate of post-weaning piglets (Pejsak et al., 2010). A large number of studies demonstrate a significant reduction of mortality, duration of viremia, and increased daily gain after vaccination with CircoFLEX® vaccine (Fachinger et al., 2008; Kixmoller et al., 2008; Stevančević et al., 2013; Stevančević et al., 2014a). The time of vaccination of piglets is often questionable, considering possible interactions of maternal antibodies which protect the piglets from the development of PMWS (Stevančević et al., 2014b; Stevančević et al., 2014c). High titer of maternal antibodies interferes with the active seroconversion after vaccination, although the vaccine significantly reduces viral load and spread of the virus (Fort et al., 2009). In a study which used Ingelvac Circoflex vaccine, there was no difference in efficacy regardless of whether the pigs were vaccinated in the 3rd or the 6th week of age, indicating that maternal antibodies have no significant impact (Cline et al., 2008). Stevančević et al. (2014d) state that vaccination of piglets on 21st day of life had a certain advantage compared to vaccination on 15th day of life, with the observation that on the 15th day of life there is far greater influence of maternal antibodies on the creation and development of immune response in piglets following vaccination.

CONCLUSION

PCV2 infection control means control of all cofactors involved in the development of the disease and the so-called triggers with efforts for their eradication as well as immunoprophylaxis measures which are considered indispensable in controlling this disease. Basic issue regarding the successful vaccination is to determine the optimal time for administration of the vaccine. It is best to determine the time of vaccination based on the amount of colostrum antibodies titer in the blood serum of piglets and time of occurrence of PCV2 infections. Vaccination presents a measure of long-term protection of animals from weaning to the end of the fattening period and continuous administration of the vaccine in the herd leads to a reduction in infectious pressure in the herd and stabilization of the health status. At this moment eradication of this disease is farfetched, but vaccination is the main form of struggle against PCV2 infection.

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KONTROLA CIRKOVIRUSNIH INFEKCIJA (PCV2) SVINJA

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Izvod

Svinjski cirkovirus tip 2 (PCV2), jedan je od vodećih virusa koji narušavaju zdravstveno stanje svinja u poslednjoj deceniji i time dovode u pitanje obim i ekonomičnost proizvodnje svinja. Kontrola PCV2 infekcija podrazumeva kontrolu svih kofaktora u nastanku bolesti i takozvanih okidača uz napore za njihovo iskorenjivanje kao i mere imunoprofilakse koje se smatraju nezamenjivim u kontroli ove bolesti. Danas je na Evropskom tržištu dostupno 5 komercijalnih vakcina i sve su bazirane na PCV2a genotipu i pokazuju dobru efikasnost. Osnovno pitanje uspešne vakcinacije je odrediti optimalno vreme za aplikaciju vakcine. Vreme vakcinacije najbolje je odrediti se na osnovu visine titra kolostralnih antitela u krvnim serumima prasadi kao i vremenu nastanku PCV2 infekcija. U ovom trenutku iskorenjivanje ove bolesti je neostvarivo, pa vakcinacija predstavlja glavni vid borbe protiv PCV2 infekcija.

Ključne reči: PCV2, vakcina, kontrola, prasad, imunitet.

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