

CURRENT STUDY OF PORCINE CIRCOVIRUS TYPE 2 IN CROATIAN PIG POPULATIONS

BESI ROIĆ, LORENA JEMERŠIĆ, SVJETLANA TERZIĆ,
ANDREJA JUNGIĆ, DRAGAN BRNIĆ¹

SUMMARY: Porcine circovirus type 2 has been circulating in pigs for many years before being linked to disease. In the last ten years porcine circovirus 2 (PCV2) has moved from the arcane to near center stage in veterinary virology. In Croatia the main epidemic outbreak of PCV2 infection happened in 2004 and caused great economic losses in pig industry. The purpose of this paper was to investigate the current situation regarding PCV2 infection in Croatia. During 2007-2013 a total of 2518 samples of pig blood serum were tested by ELISA. Antibodies to PCV2 were detected in 398 (15.80%) of the tested sera and established in 5 of 9 investigated regions. Virus circulation was confirmed by PCR. The obtained results showed that 100 (53.19%) of 188 examined tissue samples were PCV2 PCR positive. Our results provide information on the current disease exposure to PCV2 infection in Croatian pig populations. PCV2 is not a notifiable disease and obligatory national program is carried out in Croatia but effective monitoring of the health control should be applied.

Key words: PCV2 (Porcine circovirus type 2), pig, Croatia, vaccine.

INTRODUCTION

Porcine circovirus type 2 (PCV2) is an economically important swine pathogen which represent a threat to the competitiveness of swine farming worldwide through reduced growth and mortality (McKillen et al., 2007). PCV (Porcine circoviruses) are members of the genus *Circovirus*, family *Circoviridae*. These are very small, non-enveloped icosahedral viruses with a single stranded circular DNA genome of about 1.7 kb organized in an ambience direction (Mankertz et al., 1998). There are two types of

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¹Dr.sc. Besi Roić, scientific advisor, Doc.dr.sc. Lorena Jemeršić, scientific advisor, Virology department, Croatian Veterinary Institute, Zagreb. Dr.sc. Svjetlana Terzić, scientific advisor, Department of Veterinary Public Health, Zagreb, Croatia. Andreja Jungić, scientific assistant, Virology department, Croatian Veterinary Institute. Dragan Brnić, scientific assistant, Virology department, Croatian Veterinary Institute, Zagreb.

Corresponding author: Besi Roić, Croatian Veterinary Institute, Savska cesta 143, 10000 Zagreb, Croatia, email: roic@veinst.hr, Phone: +385 1 6123 674.

porcine circovirus: Type 1 (PCV1) is widespread and apparently do not cause clinical problems in pigs and was first reported as a persistent contaminant in laboratory PK15 cell lines (Crowther et al., 2003), and Type 2 (PCV2) considered as an important emerging pathogen associated with a number of different syndromes and diseases in pigs; for instance PMWS (post-weaning multisystemic wasting syndrome), PDNS (dermatitis and nephropathy syndrome), respiratory disease complex, granulomatous enteritis, exudative epidermis, necrotizing lymphadenitis, congenital tremor (Chae, 2005), reproductive failure (Hansen et al., 2010), and infection in immune response (Opriessning et al., 2006). In the last ten years porcine circovirus type 2 (PCV2) has moved from the arcane to near center stage in veterinary virology. This has been associated with dramatic increase in the incidence, to sometimes epidemic proportions, of porcine circovirus diseases (PCDV), notably post-weaning multisystemic wasting syndrome (PMWS). Although tremendous progress has been made since the discovery of PCV2 in 1998, there are many important questions to remain (Meng, 2013).

PCV2 infection in pigs related with PMWS and PDNS in Croatia have been reported by Jemeršić et al. (2004) and Lipej et al. (2005). An epizootic disease characterised by depression, weight loss, respiratory and/or enteritic disorders and erythematous skin lesions of which some had progressed to dermal necrosis, among 4- to 16- week-old pigs (nursery and fattening units) was recognised during 2002 in several farrow-to-finish farms located in eastern and western part of Croatia. The morbidity in all studied farms was from 15-30%, while the mortality ranged from 9.3% to 23.8%. In general, affected animals do not respond to antibiotic treatment. The high prevalence of viral circulation has been confirmed by the use of PCR method. It was assumed that disease was introduced to Croatia by importation breeding stock (gilts and boars) from different European countries, such as Spain, Austria, France and Hungary where the disease was previously diagnosed (Segales and Domingo, 2002). After the first outbreak disease spread within the whole country causing enormous economic losses on commercial swine farms. We don't have information of the total loss during an outbreak of PCV2, but the obtained results in affected farms showed that morbidity varied from 10 to 60% (Lipej et al., 2004). These results lead us to conclude that PCV2 infection had devastating economic impact on the Croatian pig husbandry at that time.

Concerning control of PCV2 vaccination has become very popular during the last 10 years. Benefits of vaccination do not only come from preventing PCV2, but mostly from preventing the effects of PCV2 subclinical infection. Commercial PCV2 vaccines for use in growing pigs and breeding animals were introduced in Croatia in 2009 and to date 3 products are available (authorised: <http://www.veterinarstvo.hr/default.aspx?id=140>).

The objective of this study was to investigate the current disease widespread of PCV2 infection in pig populations in Croatia.

MATERIAL AND METHODS

From January 2007 to April 2013 a total of 2518 blood samples from pigs of different age were examined. Samples were delivered to the Virology department, Serology laboratory for viral diseases, of the Croatian Veterinary Institute in Zagreb during regular health program of controlling the disease based on serological testing. Samples were submitted from 22 farms in 9 counties of Republic of Croatia (Table 2). Sera were

obtained by centrifugation for 10 min at 2000 rpm and stored at -20°C until analysis. The test used was commercial enzyme-linked immunosorbent assays (ELISA) Ingezim Circovirus IgG/IgM 11.PCV.K2. (Ingenasa, Madrid, Spain), which detects antibodies against IgG and IgM, allowing the determination of the timing of PCV2 infection: IgM values $>$ IgG values: active infection, IgG values $>$ IgM values: recent infection (between 1-2 month), High IgG values and negative IgM values: old infection.

The test was carried out, and the results were interpreted according to the manufacturer's recommendations. Optical density was read on a Tecan Sunrise Basic spectrophotometer (Austria) at 450 nm.

In addition, 188 samples of lymph nodes, lung and spleen tissue originated from domestic pigs were tested by PCR (Gene Amp[®] PCR System 9700, Applied Biosystems, USA). Total DNA was extracted from tissue samples using QIAmp[®] DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The oligonucleotide primers (PCV2-2A, PCV2-2B) used for detection of PCV2 DNAs was previously described by Sandvik et al. (2001). The tissue samples were collected during the period 2008 to 2013 in 6 Croatian counties and delivered to the Laboratory for CSF, molecular virology and genetics of Croatian Veterinary Institute.

RESULTS

From the total number of 2518 blood sera tested, PCV2 specific IgG and IgM antibodies were detected in 398 (15.80%). All positive animals had high levels of IgM (IgM $>$ IgG OD value) values suggesting active infection. Antibody prevalence showed difference among counties and investigated period. The highest antibody prevalence of 25% was detected in 2011, followed by 15.64% positive animals in 2012. In contrast, the absence of antibodies to PCV2 was recorded in 2009. According to the county distribution, as expected the antibody prevalence was higher in counties in eastern part of continental Croatia, a region characterized by high density of commercial pig production. The highest antibody prevalence was detected in Osijek and Baranja County where of 1173 sera tested 239 (20.37%) were positive to PCV2. In additionally, in Vukovar and Srijem County we found 108 positive samples (15.14%) out of 713 tested. In other counties was recorded a low prevalence or negative findings to PCV2 antibody. The analysis showed that 5 of 9 (55.55%) regions were positive. These results are summarized in Table 1.

Of 188 examined tissue samples by PCR, 100 (53.19%) were PCV2 PCR positive. The majority of PCV2 positive results were recorded in eastern part of the country. The obtained results showed high level of positive samples in Osijek and Baranja County, especially in 2010 (54.55%) and 2011 (66.66%). The results of PCR PCV2 investigation are shown in Table 3.

Table 1. Results of pig sera tested for specific IgG and IgM antibody to PCV2 in different Croatian counties during the period 2007- 2013

County	Year						
	2007	2008	2009	2010	2011	2012	2013
	tested/ pos	tested/ pos	tested/ pos	tested/ pos	tested/ pos	tested/ pos	tested/ pos
Zagrebačka		92/28	20/0	115/1	2/0	26/2	
Varaždinska					4/0	4/0	
Koprivničko-križevačka	54/0	70/0	34/0	30/0	39/0		
Bjelovarsko-bilogorska	3/2			5/0			
Virovitičko-podravska			6/0				
Brodsko-posavska	108/18			10/0			
Osječko-baranjska	117/29	47/0	64/0	210/39	254/92	389/62	92/17
Vukovarsko-srijemska				120/0	328/65	265/43	
Međimurska	10/0						
<i>Total</i>	292/49	209/28	124/0	490/40	627/157	684/107	92/17

Table 2. Number of pig farms examined to PCV2 antibody during the period 2007- 2013 in different Croatian counties

County	Year Godina						
	2007	2008	2009	2010	2011	2012.	2013
The city of Zagreb/		2	1	1	1	2	
Varaždinska					1	1	
Koprivnica and Krizevci/	1	1	1	2	1		
Bjelovar and Bilogora/	1			1			
Virovitica and Podravina/			1				
Slavonski Brod and Posavina	1			1			
Osijek and Baranja	4	3	2	7	5	8	4
Vukovar and Srijem				3	5	2	
Međimurje	1						
Total	8	6	5	15	13	13	4

Table 3. Results of PCV2 analysis obtained by PCR in different Croatian counties during 2008-2012

County	Year				
	2008	2009	2010	2011	2012
Županija	tested/pos	tested/pos	tested/pos	tested/pos	tested/pos
<i>The city of Zagreb</i>					1/1
Osijek and Baranja		5/2	44/24	33/22	69/27
Vukovar and Srijem	15/5	3/3			
Medjmurje		2/0			
<i>Krapina and Zagorje</i>		2/2			
Sisak and Moslavina		14/14			
Total	15/5	26/21	44/24	33/22	70/28

DISCUSSION

Outbreaks of disease in the world's growing livestock sector present significant potential costs – at the farm level through losses in production and productivity, and at the national level by disruption to markets and international trade. Porcine circovirus type 2 (PCV2) is responsible for various symptoms that impair pig growth and are described as PCV2 diseases (PCVD). It has been estimated that PCVD costs (direct and indirect losses) around 600 million Euros per year to the European Union (Segales et al., 2007). Concerning control of PCV2 infection the losses can be limited by strict application of general prophylactic measures. Before the vaccines became available, much focus was on good production practice and on the control of other diseases. Introduction of the PCV2 vaccines indeed changed the global situation obtaining significant reductions in mortality in the postweaning area (Baekbo et al., 2011). Since the beginning of observation PCV2 suspected animals in Croatia high percentage of animals with clinical symptoms was recorded (Lipej et al., 2004). PCV2 took a wide spread and reduced pig production. In 2009 Croatia implemented vaccination policy as an important measure for limiting the losses. Presently three commercial PCV2 vaccines are available: one sow vaccine (Circovac, Merial), and 2 piglets vaccines (Ingelvac Circoflex, Boehringer and Porcilis PCV, Intervet). Vaccination and good farm management practice significantly reduced the total losses after an outbreak.

In our study antibody prevalence to PCV2 varied among areas and testing period. During the period 2007-2013 antibodies to PCV2 have been detected in 398 (15.80%) of tested blood samples. The absence of antibodies to PCV2 was recorded only in 2009. The antibody prevalence was higher in Osijek and Baranja County a region important for the Croatian pig industry, because the majority of pig farms are located in this region. We found 239 positive samples (20.37%) to PCV2 of 1173 tested. In other counties was recorded a low prevalence or negative findings for PCV2 antibody. The PCV2 IgG/IgM ELISA as used in this study provides a method to characterize the humoral immune response of PCV2 infected pigs. Animals found positive have experienced ac-

tive infection, as indicated by the presence of specific IgM. IgM are first detectable 7-10 days following infection and remain detectable until 50-60 post infection. On the contrary, IgG are first detectable 12-15 days post infection and remain detectable for years. During regular health program of controlling the disease in period from 2008 to 2012 188 tissue samples were delivered at Croatian Veterinary Institute and tested by PCR in order to determine whether PCV2 virus is circulating in pig herds. Analysis showed that 100 samples (53.19%) were PCV2 PCR positive. The majority of PCV2 positive results were recorded again in eastern part of the country. The obtained results showed high level of positive samples in Osijek and Baranja County, especially in period 2010 (54.55%) and 2011 (66.66%). In contrast to serological findings, in 2009 with PCR 21 samples out of 26 (80.76%) were PCV2 PCR positive. In Croatia PCV2 is not a notifiable disease and obligatory national program is carried out, but about each positive result CVI is obligated to inform Ministry of Agriculture, Veterinary Directorate must be informed.

According to our results based on serology and PCR we can conclude that PCV2 is present in Croatian pig farms. Although the results show that it is not so widespread and does not cause great economic losses still remains an important disease having an impact on the swine industry in Croatia. Since 1990 in Croatia the number of farms and pigs has been considerably reduced, and the tendency of declining in pig industry is still continuing. In a region with high pig density and commercial farms a high health status of all pigs must be based on good farm management practice, on the control of the concurrent diseases and introduction of PCV2 vaccine. Although tremendous progress has been made toward understanding PCV2 pathogenesis and immune interactions, many important questions remain. PCV2 is an important pathogen that is causally associated with important emergent disease syndromes in swine.

Due to the information obtained from this study it can be concluded that the implementation of the monitoring of the health condition of pigs in large agglomerations and the systematic recording of health indicators is fundamental for control the incidence of infection and prevent it spreading to other animals.

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PROŠIRENOST CIRKOVIRUSA TIP 2 (PCV2) U SVINJOGOJSKIM UZGOJIMA U HRVATSKOJ

BESI ROIĆ, LORENA JEMERŠIĆ, SVJETLANA TERZIĆ, ANDREJA JUNGIĆ,
DRAGAN BRNIĆ

Izvod

Svinjski cirkovirus tip 2 prisutan je u uzgoju svinja diljem svijeta dugi niz godina prije nego što je povezan sa izbijanjem ove bolesti. U zadnjih deset godina PCV2 se polako iz skupine najznačajnijih bolesti u veterinarskoj virologiji svrstava u manje značajne

bolesti. Bolest je prvi puta u Hrvatskoj dokazana 2004. godine kada je nanijela velike gospodarske štete. U razdoblju od 2007. godine do 2013. godine pretraženo je 2518 uzoraka krvi svinja na prisustvo protutijela za PCV2 i temeljem seroloških pretaga u njih 398 (15.80%) dokazana su protutijela. Prisustvo virusa potvrđeno je i metodom PCR. Od 188 pretraženih uzoraka tkiva njih 100 (53.19%) bilo je pozitivno na PCV2 virus. Ostvareni rezultati upućuju na činjenicu da infekcija svinjskim cirkovirusom tip 2 i dalje prisutna u svinjogojskim uzgojima u Hrvatskoj. Iako ova bolest nije u „Naredbi o mjerama zaštite životinja od zaraznih i nametničkih bolesti i njihovom financiranju“ i ne postoji nacionalni plan za suzbijanje ove bolesti, dobiveni rezultati ukazuju da postoji potreba programa praćenja ove bolesti u obliku sustavnog pretraživanja stada svinja što bi osiguralo bolji epidemiološki uvid u bolest u populaciji svinja.

Ključne riječi: PCV2 (svinjski cirkovirus tip 2), svinja, Hrvatska, vakcina.

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