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J. BOJKOVSKI, V. ILIĆ, A. VASIĆ, J. MALETIĆ, S. VAKANJAC, S. SAVIĆ, M. ŠTUKELJ, N. ZDRAVKOVIĆ, E. D. TZIKA, M. NICLUAE, D. MILANOV

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■ Investigation of Porcine Circovirus type 2 (PCV2) antibodies in clinically healthy boars from Serbian commercial farms

Bojkovski J.^{*1}, Ilić V.², Vasić A.¹, Maletić J.¹, Vakanjac S.¹, Savić S.³, Štukelj M.⁴,
Zdravković N.¹, Tzika E.D.⁵, Nicluae M.⁶ and Milanov D.³

¹University of Belgrade, Faculty of Veterinary Medicine, Bulevar Oslobođenja 18, 11000 Belgrade, Serbia

²University of Belgrade, Institute for Medical Research, Dr Subotića 4, POB 39, 11129 Belgrade 102, Serbia

³Scientific Veterinary Institute Novi Sad, Rumenički put 20, 21000 Novi Sad, Serbia

⁴University of Ljubljana, Veterinary Faculty, Institute for health care of pigs, Gerbičeva 60, 1000 Ljubljana, Slovenia

⁵Aristotle University of Thessaloniki, School of Veterinary Medicine,
Faculty of Health Sciences, 54124 Thessaloniki, Greece

⁶University of Agricultural Science and Veterinary Medicine, Cluj Napoca Romania

ABSTRACT. The aim of the present study was to determine the porcine circovirus type 2 (PCV2) prevalence in boars in 3 farms in Serbia and their possible relation with alterations of reproductive parameters and blood biochemical parameters [total protein, urea, creatinine and aspartate transaminase levels (AST)].

The prevalence of (PCV2) was evaluated by the presence of specific antibodies. An ELISA assay was used for the detection of PCV2 antibodies in 58 boars' blood sera from Serbian commercial farms, among which 48 were from and reared in the Serbian farms, and 10 were imported from different European Union (EU) countries.

Anti PCV2 IgM and/or IgG were detected in sera of 51 (87.93%) boars. Based on the type of antibodies (IgM and IgG PCV2 antibodies), it was concluded that chronic PCV2 infection was the predominant type in tested boars. The imported boars did not have an active infection titer. Biochemical tests in blood didn't show significant differences between PCV2 positive and negative boars. The recorded high prevalence of PCV2 antibodies among boars indirectly proved that PCV2 has been wide spread in the examined farms. Significant difference in the frequency of active, recent and chronic PCV2 infection in boars was found among three farms ($p < 0.05$). Imported boars did not show significant difference in terms of active, recent or chronic PCV2 infection in comparison with boars deriving from Serbian farms

Correspondence:

Jovan Bojkovski,
University of Belgrade, Faculty of Veterinary Medicine,
Bulevar Oslobođenja 18, 11000 Belgrade, Serbia;
Email: bojkovski@vet.bg.ac.rs

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($p > 0.05$). Among boars of three different breeds (Landrace, Duroc and Yorkshire), a significantly higher prevalence of PCV2 antibodies was revealed in Landrace boars ($p = 0.003$). Reproductive parameters of sows that were inseminated with PCV2 positive or negative boars' semen, showed similar farrowing rate, as well as percentage of live and stillborn piglets, thus indicating that the observed chronic PCV2 infection did not affect significantly the breeding capability of boars.

Keywords: boars, farm, porcine circovirus type 2, swine, the Republic of Serbia

INTRODUCTION

Porcine circovirus type 2 (PCV2) is a small (17 nm), non-enveloped, single stranded, circular DNA virus, which belongs to the family *Circoviridae*, genus *Circovirus* (Todd et al., 2005). Among the various disease syndromes and clinical manifestations caused by PCV2, PCV2 - Systemic Disease (PCV2-SD) [formerly known as postweaning multisystemic wasting syndrome (PMWS)] has become a global swine disease with significant economic impact in the swine industry worldwide (Ellis et al., 1998, Hamel et al., 1998, Segales and Domingo, 2002, Segales et al., 2006, Štukelj et al., 2013, Caspari et al., 2014). The occurrence of PCV2-SD associated with PCV2 infection in several intensive porcine farms was recognized in Serbia in 2003 (Toplak et al., 2003).

The PCV2-SD mostly affects piglets aged 2 to 5 months, but pigs from 1 to 6 months can also be affected. Morbidity and mortality are variable, depending on the investigated localities (Vicente et al., 2004). The diagnosis of PCV2-SD is based on certain criteria (Sorden, 2000), which are: weight loss and paleness of skin (respiratory and/or digestive clinical signs may be present as well), moderate to severe lymphocyte depletion with granulomatous inflammation of lymphoid tissues, and moderate to high amount of PCV2 in damaged tissues (Segales and Domingo, 2002). For etiological confirmation of PCV2-SD, the viral antigen must be revealed in more than one lymphoid tissue (lymph node, tonsil, spleen) and at least one other organ (lung, liver, kidney, intestines) (Opriessnig et al., 2008). It is generally accepted that PCV2-SD diagnosis at herd level could be based on the significant increase of mortality associated with clinical signs compatible with PCV2-SD, and on the individual diagnosis in at least one of three to five necropsied pigs (Segales et al., 2005, Grau-Roma et al., 2011).

It has been shown that the oronasal route is the most common route of PCV2 transmission. Infections may come from secretions or contact with diseased animals. PCV2 is also present in semen and artificial insemination can contribute to virus spread (Vicente et al., 2004, Gava et al., 2008). Despite extensive research of PCV2 infection, a precise mechanism of persisting PCV2 in pigs is still unknown (Krakowka et al., 2000). It has been confirmed that the susceptibility to PCV2 infection and development of PCV2-SD is dependent, among other factors, on pig (boar) breed i.e. of their genetic background (Opriessnig et al., 2008). Moreover, the fact that PCV2 needs one or more co-factors for the development of PCV2-SD (Lohse et al., 2008) make etiopathogenesis of PCV2 related diseases even more complex than previously assumed (Magar et al., 2000, Savić et al., 2010, 2012, Vlasakova et al., 2014).

Data from several European countries showed almost 100% herd seroprevalence of PCV2 in both PCV2-SD affected and non-affected farms (Rose et al., 2002). In Serbia, the PCV2 related attention has been focused on the problem of growing pigs, but not on sows in service, even though PCV2 infection can be transmitted by semen (Schmoll et al., 2008). Study of 30 Serbian PCV2 sequences indicated that the predominant genotype in Serbia is PCV2b which is closely related to those previously described in Europe (Toplak et al., 2012; Savić et al., 2012). Vaccination against PCV2 is not obligatory and is being implemented mainly after the occurrence of clear clinical features of the disease. Keeping boars as semen donors for artificial insemination is usual practice in large commercial farms in Serbia and control of their health status is very important (Šamanc et al., 2009).

Table 1. Prevalence of PCV2 IgG and/or IgM antibodies in boars from three Serbian commercial farms.

Group of animals	No	PCV2 Ab negative		PCV2 Ab positive		<i>p</i>
		No	(%)	No	(%)	
Farm A ^a	28	9	32	19	68	0.3087
Farm B ^a	20	3	15	17	85	
Farm C ^a	10	1	10	9	90	
Total number of boars originate from Serbia ^a	58	13	22	45	78	0.7879
Imported boars ^b	10	4	40	6	60	
Total	68	17	25	51	75	

^a The animals born and reared in Serbian farms.

^b Blood samples were taken during quarantine period.

^p *p* value of statistical differences in PCV2 prevalence between A, B and C farms, and PCV2 prevalence between boars of different origin, respectively

The aim of the present study was to determine PCV2 prevalence in boars from three Serbian farms and possible related alterations in reproductive parameters and blood biochemical parameters (as non-specific indicators of health status of boars) indicating PCV2-SD.

MATERIALS AND METHODS

Farm and animals

The study included 58 selected non-vaccinated boars aged 2-3 years of Landrace, Duroc, and Yorkshire breeds and originated from three small one-site commercial farms (A, B and C). In total, 28 boars were from farm "A", 20 from farm "B" and 10 from farm "C". Moreover, 10 out of the total 58 boars in all farms originated from different European Union (EU) pathogen-free herds (8 months old, 5 of Landrace and 5 of Yorkshire breed). Those imported boars at the time of blood sampling were in quarantine (duration of quarantine was 21 days).

Sample collection

Blood samples were taken from the jugular vein. Biochemical analysis of total protein, urea, creatinine and aspartate transaminase (AST) were performed on semi-automatic analyzer (RT-1904C, Rayto, China) with adequate reagents (Clinichem, Hungary) accord-

ing to manufacturer's instructions. After blood coagulation at room temperature, serum was separated by centrifugation (10 minutes on 1000 rpm) and the aliquots were stored at -20°C until serological analysis.

Serology

A commercially available ELISA test ("INGEZIM circovirus IgG/IgM", Inmunologia y genetica aplicada, s.a., Madrid, Spain) was used, according to manufacturer's instructions, in order to assess the prevalence rate of serum PCV2 IgG and IgM antibodies. Stage of infection was determined according to ELISA results as follows: a) PCV2 negative animals: IgM and IgG OD₄₅₀ levels below cut-off values, b) animals with active PCV2 infection (within first 21 days): IgM OD₄₅₀ and IgG OD₄₅₀ levels greater than cut off values, and IgM OD₄₅₀ higher than IgG OD₄₅₀, c) animals with recent (between one and two months) PCV2 infection: IgM and IgG OD₄₅₀ levels higher than cut off values and IgG OD₄₅₀ greater than IgM OD₄₅₀, d) animals with chronic PCV2 infection: IgG OD₄₅₀ above cut-off value, but IgM OD₄₅₀ lower than the respective cut-off values.

Reproductive parameters

The reproductive outcome of sows' insemination with semen from the study boars was evaluated. The measured parameters included: farrowing rate (sows and gilts), live and stillbirth piglets, number of insem-

Table 2. The stage of PCV-2 infections in the boars from three Serbian commercial farms.

Group of animals	No	Active	Recent	Chronic	<i>p</i>			
		infection	infection	infection	(%)	(%)		
Farm A ^a	28	3	16	2	10	14	74	0.0335 ^s
Farm B ^a	20	3	18	3	18	11	65	
Farm C ^a	9	0	0	6	67	3	33	
Total number of boars originate from Serbia ^a	45	6	13	11	24	28	62	0.4259
Imported boars ^b	10	0	0	3	30	3	30	
Total	51	6	12	14	27	31	61	

^aThe animals were born and reared in the Serbian farms.

^bImmediately after arrival in Serbia they were quarantined for 21 days. Blood samples were taken during quarantine period.

^sSignificant

p - *p* value of statistical differences in PCV2 infection stages between A, B and C farms, and boars of different origin, respectively

inated sows and gilts, abortion rate and culling rate of unvaccinated sows.

Statistical analysis

The Fisher's (two-tailed) exact probability test was used for analyzing the parameters between boars' groups. The differences with *p*-values <0.05 were considered significant. Statistical analysis was carried out with an interactive software [Free Statistics Calculators Version 4.0 (Soper, 2006)] where 2x2 contingency tables were used for comparisons between boars of different origin in their respective breeds, 2x3 contingency table was used to assess differences in prevalence of PCV2 between farms, and 3x3 contingency tables were used for estimating the differences among infection stages (active, recent and chronic) in the three tested farms.

RESULTS

PCV2 antibodies were detected in 51 (87.93%) of the sera examined (Table 1). There was no statistically significant difference in PCV2 antibodies prevalence as regard to boars' origin (*p*>0.05).

PCV2 seroprevalence (Table 2) showed that chronic infection mainly occurred in examined boars, characterized by high PCV2 IgG titers and absence of PCV2 IgM antibodies. Significant difference in

the frequency of active, recent or chronic PCV2 infection in boars among the three farms was found (*p*=0.0335). The levels of PCV2 antibodies are presented in Table 3, and both boars deriving from the EU and Serbian farms did not show any significant difference in terms of the type of infection (*p*>0.05). The presence of PCV2 infection did not induce any significant changes in the examined results of biochemical parameters (total protein, urea, creatinine and AST levels) (Table 4). Among the boars of the three different breeds (Landrace, Duroc and Yorkshire) a significantly greater incidence of PCV2 antibodies was revealed in Landrace boars (*p*=0.003). Insignificant differences were noticed in particular reproductive parameters of sows (farrowing rate, live born and still-born piglets) that were inseminated with semen from either PCV2 positive or negative boars (Table 5).

DISCUSSION

According to the results of this study, a large percentage of tested boars was PCV2 positive in the three farms. This finding correlates with the results of Reiner et al. (2010), who reported 100% presence of PCV2 DNA (nested PCR) in boars from 348 examined domestic pigs in Germany. A difference in the frequency of active recent and chronic PCV2 infection was observed in boars of the three different breeds

Table 3. Titers of PCV2 antibodies in sera of PCV2 infected boars from three Serbian commercial farms.

	Relative titer of PCV2 antibodies ^a					
	Active infection		Recent infection		Chronic infection	
	IgG	IgM	IgG	IgM	IgG	IgM
Total number of boars originate from Serbia (n = 48)	1.0 ± 0.3 (0.0 - 0.9)	1.3 ± 0.2 (1.1 - 1.6)	2.8 ± 0.8 (1.6 - 4.4)	1.4 ± 0.3 (1.1 - 1.9)	2.0 ± 0.7 (1.1 - 4.4)	0.7 ± 0.3 (0.1 - 0.9)
Imported boars (n = 10)	n.d	n.d	2.1 ± 1.0 (1.1 - 3.1)	1.2 ± 0.2 (1.1 - 1.9)	2.4 ± 1.0 (1.2 - 4.5)	0.7 ± 0.1 (0.6 - 0.8)

^aCut off values for IgG and IgM set as 1 and relative titer expressed as "fold change" compared to cut-off values. Results are given as mean ± SD and as minimum to maximum range
n.d.: PCV2 antibodies were not detected

Table 4. Results of biochemical blood analysis (total proteins, urea, creatinin, AST) of boars from three Serbian commercial farms (mean ± SD).

	PVC2 negative	PVC2 positive	Reference values
Boars (n=68)	17 (25%)	51 (75%)	
Total proteins (g/l)	71 ± 10	78 ± 13	62 - 82
Urea (mmol/l)	3.3 ± 1.2	3.9 ± 1.2	2.3 - 6.7
Creatinine (mmol/l)	160 ± 77	137 ± 81	80 - 221
AST (U/L)	47 ± 14	43 ± 13	8 - 25

Table 5. Influence of PCV2 infection of boars from three Serbian commercial farms on reproductive parameters of respective inseminated sows (mean ± SD).

	PCV2	
	Positive	Negative
Farrowed sows (%)	87 ± 3	88 ± 3
Live births / litter	13.3 ± 1.1 93 (%)	13.5 ± 1.2 95 (%)
Stillborn / litter	1.0 ± 0.4 7 (%)	0.8 ± 0.3 5 (%)

that were tested (Landrace, Duroc and Yorkshire). A greater prevalence of PCV2 antibodies was revealed in Landrace boars and that was in accordance with previously proved increased susceptibility of Landrace pigs to PCV2-SD (Opriessnig et al., 2006).

The level of PCV2 antibodies can be related to infection stage according to the findings of experimental inoculation that indicate seroconversion between 14 and 21 days post inoculation (Prickett et al., 2011). In the same study, a high level of serum IgG was maintained throughout the study period up to 98 days post inoculation. In naturally infected young pigs' (5-8 week-old), the presence of maternal PCV2 antibodies (IgM) has been detected and seroconversion usually occurs at 8-10 weeks after weaning, which shows that young pigs come in contact with PCV2 during 8-10 weeks of life, a period corresponding to the occurrence of PCV2-SD (Cadar et al., 2009).

The examination of reproductive parameters did not show significant differences between PCV2 positive and negative boars. The PCV2 seroconversion was not related with alterations in biochemical parameters of analyzed boars. It is reported that PCV2 infection has influence on the reproductive characteristics of boars (Madison et al., 2009). Relatively few herds exhibit clinical signs but some affected herds experience massive losses (Opriessnig et al., 2007). Results of this study did not show a sig-

nificant detrimental effect of PCV2 on reproductive parameters and mortality levels during lactation in the tested farms.

The economic significance of PCV2 infection among swine is in reduced weight gain, higher mortality and cost of treatment with early removal of pigs from production. Since 2002, the monthly profit losses of Serbian commercial farms due to PCV2 infection has been increased and estimated up to 9% due to reproductive performance failure and up to 15% due to decrease of fattening population performance (Stankov et al., 2013). As our results show, PCV2-SD in boars remains in focus. Different strategies of preventive approach and reevaluation of cost-benefit matrices in pig meat production should be under consideration.

Conflict of interest

The authors declare that they have no conflict of interest. The experiment was done in compliance with Serbian Law on Animal Welfare (Official Gazette of the Republic of Serbia No 41/09).

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