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

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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.
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 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).

(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
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) according 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 OD450 and IgG OD450 levels greater 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 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 | p  |
|------------------|----|------------------|------------------|----|
| Farm A<sup>a</sup> | 28 | 9 (32)           | 19 (68)          |    |
| Farm B<sup>b</sup> | 20 | 3 (15)           | 17 (85)          | 0.3087 |
| Farm C<sup>c</sup> | 10 | 1 (10)           | 9 (90)           |    |
| Total number of boars originate from Serbia<sup>a</sup> | 58 | 13 (22)          | 45 (78)          | 0.7879 |
| Imported boars<sup>b</sup> | 10 | 4 (40)           | 6 (60)           |    |
| Total | 68 | 17 (25)          | 51 (75)          |    |

<sup>a</sup> The animals born and reared in Serbian farms.

<sup>b</sup> Blood samples were taken during quarantine period.

<sup>p</sup> p value of statistical differences in PCV2 prevalence between A, B and C farms, and PCV2 prevalence between 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 stillborn 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.

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

| Group of animals | Active infection | Recent infection | Chronic infection | p         |
|------------------|------------------|------------------|-------------------|-----------|
|                  | No   | No (% ) | No   | No (% ) | No   | No (% ) |         |
| Farm A           | 28   | 3 (16)  | 2    | 10 (50) | 14   | 74 (52) | 0.0335   |
| Farm B           | 20   | 3 (15)  | 3    | 18 (90) | 11   | 65 (58) |          |
| Farm C           | 9    | 0 (0)   | 0    | 6 (0)   | 67   | 3 (0)   |          |
| Total number of boars originate from Serbia | 45   | 6 (13)  | 11   | 24 (54) | 28   | 62 (50) | 0.4259   |
| Imported boars   | 10   | 0 (0)   | 3    | 30 (100)| 3    | 3 (0)   |          |
| Total            | 51   | 6 (12)  | 14   | 27 (52)| 31   | 61 (50)|          |

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

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

*Cut off values for IgG and IgG 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        |
| Creatinin (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            | 13.5 ± 1.2      |
| 93 (%)                | 95 (%)          |
| Stillborn / litter    |
| 1.0 ± 0.4             | 0.8 ± 0.3       |
| 7 (%)                 | 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 significant 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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REFERENCES

Cadar D, Spinu M, Kiss T, Kobol kuti L, Niculae M, Uricu a A, Sandru D, Brudasca F (2009) Serological profiles of porcine circovirus infection in romanian swine herds. Lucrări Știinifice Medicină Veterinară 1:267-270.

Caspari K, Henning H, Schreiber F, Maass P, Gössl R, Schaller C, Waberski D (2014) Impact of porcine circovirus type 2 (PCV2) vaccination on boar semen quality and quantity using two different vaccines. Theriogenology 21:574-579.

Ellis J, Hassard L, Clark E, Harding J, Allan G, Willson P, Srokappe J, Martin K, McNeill y F, Meehan B, Todd D, Haines D (1998) Isolation of circovirus from lesions of pigs with postweaning multisystemic wasting syndrome. Can Vet J 39:44-51.

Gava D, Zanella EL, Morés N, Janice R, Ciacci-Zanella JR (2008) Transmission of porcine circovirus 2 (PCV2) by semen and viral distribution in different piglet tissues. Pesq Vet Bras 28:70-76.

Grau-Roma L, Fraile L, Segales J (2011) Recent advances in the epidemiology, diagnosis and control of diseases caused by porcine circovirus type 2. Vet J 187:23-32.

Hamel LA, Lin LL, Nayar GS (1998) Nucleotide sequence of porcine circovirus associated with postweaning multisystemic wasting syndrome in pigs. J Virol 72:5262-5267.

Krakowska S, Ellis JA, Meehan B, Kennedy S, McNeill y F, Allan G (2000) Viral wasting syndrome of swine: experimental reproduction of postweaning multisystemic wasting syndrome in gnotobiotic swine by coinfection with porcine circovirus 2 and porcine parvovirus. Vet Pathol 37:254-263.

Lohse LA, Botner AS, Hansen T, Fredriksen K, Dupont CS, Christensen P, Baekbo P, Nielsen J (2008) Examination for a viral co-factor in postweaning multisystemic wasting syndrome (PMWS). Vet Microbiol 129: 97–107.

Madison DM, Patterson AR, Ramamooorthy S, Pal N, Meng XJ, Opriessnig T (2009) Reproductive Failure Experimentally Induced in Sows via Artificial Insemination with Semen Spiked with Porcine Circovirus Type 2. Veterinary Pathology 46:707-716.

Magar R, Müller P, Larochelle R (2000) Retrospective serological survey of antibodies to porcine circovirus type 1 and 2. Can J Vet Res 64:184-186.

Opriessnig T, Fenaux M, Thomas P, Hoogland MJ, Rothschild MF, Meng XJ, Halbur PG (2006) Evidence of breed-dependent differences in susceptibility to porcine circovirus type-2-associated disease and lesions. Vet Pathol 43:281-293.

Opriessnig T, Madison DM, Prickett JR, Kuhar D, Lunney JK, Elsener J, Halbur PG (2008) Effect of porcine circovirus type 2 (PCV2) vaccination on porcine reproductive and respiratory syndrome virus (PRRSV) and PCV2 coinfection. Vet Microbiol 131:103-114.

Opriessnig T, Meng XJ, Halbur PG (2007) Porcine circovirus type 2 associated disease (PCVAD): update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. J Vet Diagn Invest 19:591–615.

Prickett JR, Johnson J, Murtaugh MP, Puvanendiran S, Wang C, Zimmerman JJ, Opriessnig T (2011) Prolonged Detection of PCV2 and Anti-PCV2 Antibody in Oral Fluids Following Experimental Inoculation. Transbound Emerg Dis 58:121–127.

Rose N, Blanchard P, Lourour G, Le Diguer-Her G, Eveno E, Jolly JP, Oger A, Le Dimma M, Jestin A, Madec F (2002) Post-weaning multisystemic wasting syndrome (PMWS) in France: serological profiles of affected versus non-affected herds and preliminary analytical epidemiology. Pig J 50:124–134.

Šamanc H (2009) Bolesli prouzrokovane cirkovirúsim. In: Bolesli svinja, Naučna knjiga, Beograd pp. 385-391.

Savić B, Miličević V, Bojkovski J, Kurelušić B, Ivetić V, Pavlović I (2010) Detection rates of the swine torque teno viruses (TTVs), porcine circovirus type 2 (PCV2) and hepatitis E virus (HEV) in the livers of pigs with hepatitis. Vet Res Commun 34:641-648.

Savić B, Miličević V, Jakić-Dimić D, Bojkovski J, Prodanović R, Kurelušić B, Potkonjak A, Savić B (2012) Genetic characterization and phylogenetic analysis of porcine circovirus type 2 (PCV2) in Serbia Arch Virol 157:21-28.

Schmoll F, Lang C, Steinirgıl AS, Schulze K, Kauffold J (2008) Prevalence of PCV2 in Austrian and German boars and semen used for artificial insemination. Theriogenology 15:814-821.

Segales J, Allan GM, Domingo M (2006) Porcine circovirus disease In: Disease of swine. 9th ed, Blackwell publishing Ltd Oxford: pp 299-308.

Segales J, Domingo M (2002) Post weaning multisystemic wasting syndrome (PMWS) in pigs. Vet Q 24:109-124.

Segales J, Olivierra A, Grau-Roma L, Charryte C, Nauwynck H, Larsen L, Dupont K, Mc Cullough K, Ellis J, Krakowska S, Mankertz A, Fredholm M, Fossum C, Timmusk S, Stockhoce-Zurwieden N, Bettie V, Armstrong D, Grassland B, Baekbo P, Allan G (2008) PCV2 genotype definition and nomenclature. VetRec 162:867-868.

Segalès J, Rodríguez J, Resendes A, Balasch M, Sanz AJ, Plana-Durán J, Venteo A (2005) Humoral immune responses and correlation with viraemia in pigs subclinically infected with porcine circovirus type 2. In: Proceedings of Animal Circoviruses and Associated Diseases conference, Belfast, UK: pp 61.

Soper D, (2006) Free Statistics Calculators Version 4.0 Category: Contingency Table Calculators. Available at: http://www.danielsoper.com/statcalc/category.aspx?id=22. Accessed 12 April, 2016.

Sorden SD (2000) Update on porcine circovirus and postweaning multisystemic wasting syndrome. Swine Health Prod 8:133-136.

Stankov P, Stanimirov D, Mirilović M (2013) The effect of vaccination...
tion of sows against PCV2 viruses on brood size and farrowing rate. In: Proceedings of Health care, selection and reproduction pigs Symposium, Srebrno Jezero, Serbia: pp 49-54.

Štukelj M, Golinar-Oven I (2013) Circovirosis is not a disease: review of PMWS. Savremena poljoprivreda, 62:99-108.

Todd D, Bendinelli M, Biagini P, Hino S, Mankertz A, Mishiro S, Nlel C, Okomoto H, Radial S, Richie BW, Teo GC (2005) Circoviridae. In: Virus taxonomy VIII report of the international committee for the taxonomy of viruses, Elsevier/academic Press, London: pp 327-334.

Toplak I, Lazić S, Lupulović D, Prodanov-Radulović J, Becskei Z, Došen R, Petrović T (2012) Study of the genetic variability of porcine Circovirus type 2 detected in Serbia and Slovenia. Acta Vet Hung 60:409-420.

Toplak I, Lipec Z, Petrović T, Hostnik P, Girom J, Barlić-Maganja D (2003) Detection and phylogenetic analysis of type 2 porcine circovirus (PCV2) from pigs with PMWS in Slovenia, Croatia and Yugoslavia. In: Proceedings of The 4th International Symposium on Emerging and Re-emerging Pig Disease, Rome, Italy: pp 172-173.

Vicente J, Segalés J, Höffle U, Balasch M, Plana-Durán J, Domingo M, Gortázar (2004) Epidemiological study on porcine circovirus type 2 (PCV2) infection in the European wild boar (Sus scrofa). Veterinary Research 35:243–253.

Vlasakova M, Leskova V, Sliz I, Jackova A, Víšek S (2014) The presence of six potentially pathogenic viruses in pigs suffering from post-weaning multisystemic wasting syndrome. BMC Vet Res 10:221-230.