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Prevalence of porcine circovirus 2 infection in pig population in Slovakia

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Summary. – The prevalence of porcine circovirus 2 (PCV-2) infection in the pig population in Slovakia was investigated. Sera from pigs suspected for post-weaning multisystemic wasting syndrome (PMWS) as well as clinically healthy pigs were tested for viral DNA and specific IgM and IgG antibodies. Pigs (n = 198) were categorized to weaning, grower and fattening ones and sows. The results showed that PCV-2 antibodies were present in 53.4% of PMWS-suspects, in 50.0% of healthy pigs and in 69.0% of sows. In PMWS-suspect grower pigs, 40.7% were positive for IgM+IgG antibodies and 22.2% for viral DNA. In PMWS-suspect fattening pigs, 50.0% were positive for IgM+IgG antibodies and 25.0% for viral DNA. In healthy fattening pigs, almost 90.0% were positive for IgG antibodies and 38.5% for viral DNA. The highest proportion of PMWS-suspects was in grower pigs and specific antibodies were increasing with the age of pigs. A combination of positivities for IgG+IgM antibodies and viral DNA was a highly significant marker of PMWS. Viral DNA was detected in seropositive as well as seronegative PMWS-suspects. Overall, in all categories of pigs tested, specific antibodies and viral DNA were detected in 54.0% and 35.5%, respectively.

Keywords: porcine circovirus 2; pigs; prevalence; Slovakia

Introduction

PCV-2 is a non-enveloped, single-stranded circular DNA virus and belongs to the family *Circoviridae* and the genus *Circovirus* (Fenaux *et al.*, 2000; Mankertz *et al.*, 2000). There are two phenotypically different and genetically related species namely PCV-1 and PCV-2. PCV-1 is generally considered as non-pathogenic, whereas PCV-2 is associated with PMWS (Harding and Clark, 1997; Allan *et al.*, 1998; Allan and Ellis, 2000). PMWS is clinically characterized by progressive weight loss, dyspnea, pallor, diarrhea and jaundice from suckling to the growing stages of pigs, typically affecting animals between 7 and 15 weeks of age (Harding, 2004). However, PCV-2 is ubiquitous and can be isolated from both diseased and healthy pigs (Allan and Ellis, 2000). It is the cause of major economical losses in pig industry.

Segalés (2007) in his review classified Slovakia as a country with unknown PMWS status. Two years later the first official data on detection of PCV-2 in PMWS-affected pigs at antigenic and genetic level was described in Slovakia (Pistl *et al.*, 2009). In the last decade, several studies were published, which document PCV-2 infection and the occurrence of PMWS in domestic or feral pigs in Poland (Stadejek *et al.*, 2006), Hungary (Kiss *et al.*, 2000; Tibor, 2004), Romania (Cadar *et al.*, 2007), Slovenia (Toplak *et al.*, 2004), Croatia (Lipej *et al.*, 2005), Greece (Sofia *et al.*, 2008), Czech Republic (Celer and Carasova, 2002; Sedlak *et al.*, 2008) and Austria (Schmoll *et al.*, 2002, 2003).

Till to date, no data is available that presents the prevalence of PCV-2 infection in the pig population in Slovakia. Therefore, the aim of this study was to determine the prevalence of PCV-2 infection in PMWS-suspect as well as clinically healthy pigs of different age categories. For this purpose, serum samples from total of 198 pigs were collected and subjected to ELISA for specific IgM and IgG antibodies and PCR for viral DNA.

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Abbreviations: MAb(s) = monoclonal antibody(ies); OR = odds ratio; PCV-2 = porcine circovirus 2; PMWS = post-weaning multisystemic wasting syndrome

Table 1. Prevalence of PCV-2 antibodies in different age categories of PMWS-suspect and healthy pigs in Slovakia

Pig category	No. of sera		No. (%) of antibody-positive sera						No. (%) of antibody negative sera	
			IgM+IgG		IgM		IgG			
	PMWS	H	PMWS	H	PMWS	H	PMWS	H	PMWS	H
Weaning	22	32	0	0	2 (9.1)	0	4 (18.2)	2 (6.3)[a]	16 (72.7)	30 (93.8)
Growers	27	25	11 (40.7)	6 (24.0)	1 (3.7)	0	0	3 (12.0)[b]	15 (55.6)	16 (64.0)
Fattening	24	39	12 (50.0)[*]	2 (5.1)	1 (4.2)	0	8 (33.3)	35 (89.7)[a,b]	3 (12.5)	2 (5.1)

PMWS = PMWS-suspects; H = healthy pigs; [a]P <0.001; [b]P <0.001; [*]P <0.05 (OR = 12.67).

Materials and Methods

Serum samples. Total 198 serum samples from 16 farms located in different regions of Slovakia were collected during years 2009–2010. Sera were obtained from PMWS-suspect (n = 73) as well as healthy (n = 96) pigs. As PMWS clinical signs like wasting, enlarged inguinal lymph nodes and dyspnea are restricted to the nursery and early grower stages (Harding, 2004), sera from sows (n = 29) were excluded from categorization based on clinical picture. Animals were further categorized into weaning (3–6-week-old; n = 54), grower (7–12-week-old; n = 52); fattening (>13-week -old; n = 63) pigs and sows. None of the tested animals was vaccinated against PCV-2.

ELISA. Specific PCV-2 IgM and IgG antibodies were determined by commercially available capture ELISA (INGENZIM Circovirus IgG/IgM, Ingenasa, Spain) according to manufacturer´s instructions. The ELISA kit contained two different plates with fixed pig-specific IgM and IgG monoclonal antibodies (MAbs). When a pig serum is added on each plate, the IgM and IgG antibodies present in the sample are captured by MAb adsorbed on the plate. After washing, the added PCV-2 antigen (recombinant empty capsid) is captured by the IgM and/or IgG in the serum sample. In the next step, HRP-conjugated PCV-2 MAb was used. The colorimetric reaction was measured after addition of TMB substrate by LEDETECT 96 Microplate Reader. Samples with A_{450} lower than cut off levels of positive controls were considered as negative for PCV-2 IgM and/or IgG antibodies.

PCR. Total DNA was isolated from 200 µl of serum. Briefly, after overnight digestion at 50°C in SDS/Proteinase K solution (SDS 0.1 g/ml, Proteinase K 0.5 mg/ml) the DNA was extracted with phenol-chloroform and ethanol-precipitated. PCR mixture (25 µl) contained 0.5 µl of extracted DNA, 1 × PCR reaction buffer, 3 mmol/l $MgCl_2$, 0.1 mmol/l of each dNTP, 0.3 µmol/l of each primer (ORF2.PCV2.S4 and ORF2.PCV2.AS4; (Ouardani *et al.*, 1999) and 0.5 U of Taq DNA Polymerase (Invitrogen, Brazil). Amplification was performed with an initial denaturation at 94°C for 5 mins, followed by 35 cycles of amplification (94°C for 1 min, 60°C for 1 min, 72°C for 1 min), with a final extension at 72°C for 7 mins. PCR products of 494 bp size were separated on 1.5% agarose gel at 80 V, stained with ethidium bromide and visualised using an ultraviolet transluminator.

Statistical analyses. Association between the presence of both PCV-2 antibodies and viral DNA in PMWS-suspect pigs was calculated by odds ratio (OR) supported by chi-square test with Fisher´s exact test. The association was significant if OR >1 and P <0.05 and not significant when OR ≤1 and P >0.05. To assess the differences in positivity for PCV-2 antibodies and viral DNA, and distribution of PMWS-suspects in different age groups, chi-square test and Fisher´s exact test was used.

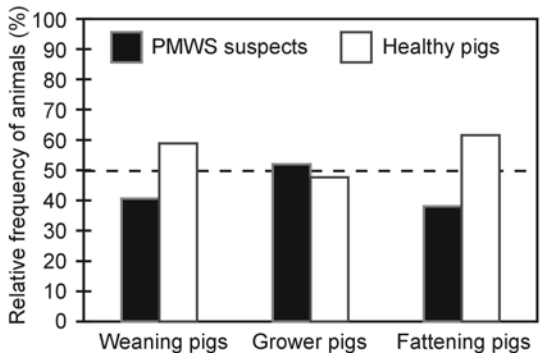

Fig. 1

Age distribution of PMWS-suspect and healthy pigs

PMWS-suspect pigs (full columns); healthy pigs (empty columns).

Results

Age distribution of PMWS-suspect and healthy pigs

Based on clinical picture, the distribution of PMWS-suspects in different age categories was determined (Fig. 1). There was no significant difference between the prevalence of PMWS-suspect pigs in age groups.

Prevalence of specific IgM and IgG antibodies and viral DNA in PMWS-suspect and healthy pigs

Altogether 53.4% serum samples of PMWS-suspect pigs and 50.0% of healthy pigs were seropositive for PCV-2 antibodies. Most of the PMWS-suspect and healthy pigs

Table 2. Prevalence of PCV-2 antibodies and PCV-2 DNA in different age categories of PMWS-suspects and healthy pigs in Slovakia

Pig category	No. of sera		No. (%) of antibody-positive and viral DNA-positive sera						No. (%) of antibody-negative and viral DNA-positive sera	
			IgM+IgG		IgM		IgG			
	PMWS	H	PMWS	H	PMWS	H	PMWS	H	PMWS	H
Weaning	22	32	0	0	2 (9.1)	0	0	0[a]	0	0
Growers	27	25	6 (22.2)	2 (8.0)	1 (3.7)	0	0	2 (8.0)[b]	5 (18.5)	0
Fattening	24	39	6 (25.0)[*]	1 (2.6)	0	0	1 (4.2)	15 (38.5)[a,b,c]	1 (4.1)	0

PMWS = PMWS-suspects; H = healthy pigs; [a]P <0.001; [b]P <0.01; [c]P <0.01 between sows (Table 3); [*]P <0.05 (OR = 12.67).

Table 3. Prevalence of PCV-2 antibodies and PCV-2 DNA in different age categories of pigs in Slovakia

Pig category (No. of sera)	PMWS and healthy pigs		PMWS pigs
	No. (%) of positive sera		
	Specific antibodies	Specific antibodies + viral DNA	
Weaning (54)	8	2	2
Growers (52)	21	11	7
Fattening (63)	58	23	7
Sows (29)	20	2[c]	–
Total (198)	107 (54.0)	38 (35.5)	16 (42.1)

PMWS = PMWS-suspects; [c]P <0.01 between fattening pigs (Table 2).

in weaning groups were seronegative; however, in growers showing PMWS symptoms we observed increase of PCV-2 IgM+IgG- and PCV-2 DNA-positive serum samples (Table 1). In the fattening pigs, significant association (P <0.05, OR = 12.67) was observed between PMWS clinical signs and the presence of PCV-2 IgM+IgG antibodies as well as viral DNA (Tables 1 and 2). The highest number of PCV-2 antibody- and viral DNA-positive serum samples was detected in the fattening pigs (Table 3). Almost 90.0% of serum samples of healthy fattening pigs were PCV-2 IgG positive, which differs significantly from healthy weaner and grower pigs (P <0.0001; Table 1). In addition, the presence of PCV-2 DNA in the same age group was also significantly higher in comparison to all age categories of pigs (Tables 2 and 3).

Prevalence of PCV-2 antibodies was 54.0% of total number of pigs from different regions of Slovakia (Table 3). PCV-2 DNA was detected in 35.5% of the seropositive samples but 6 seronegative PMWS-suspect sera showed presence of viral DNA (Table 2). None of seronegative sample from the clinically healthy group was positive for PCV-2 DNA (Table 2).

Discussion

The highest number of PMWS-suspects was observed in weaning and growing pigs. These results are in parallel with the findings reported by Segalés and Cortey (2010), where more than 80.0% of PMWS-affected pigs fall between two and four months of age. Low number of PCV-2 IgG-positive pigs in the weaning group implies that these animals had low, if at all, colostral antibodies against PCV-2. This fact is also supported by 31.0% seronegativity in the sows. Pigs have epitheliochorial placenta impermeable to immunoglobulins and are therefore hypo- or agammaglobulinemic at birth (Kim, 1975). Thus, the survival of neonatal piglets depends upon their ingestion of colostrum during the first hours of life (Salmon *et al.*, 2009). The amount and persistence of colostral PCV-2 IgG in the serum of piglets depends on their level in the blood of sows. The mean PCV-2 antibody half-life was estimated to be 19 days, and the level of passively acquired antibodies decay below ELISA cut off levels approximately at five weeks of age in weanlings derived from sows with low PCV-2 IgG (Opriessnig *et al.*, 2004).

Most of the PCV-2 IgM- and IgG-positive pigs were detected among grower and fattener pigs regardless of the clinical status. High prevalence of IgM+IgG-positive animals in our study confirms that pigs get infected after the weaning period (Harding, 2004). Detection of both PCV-2 antibodies and viral DNA in PMWS-suspects suggest acute infection at the time of sampling. A combination of the presence of both IgM and IgG antibodies, and viral DNA can be a significant marker for detection of PMWS-suspect fattening pigs.

Viral DNA in combination with PCV-2 antibodies had the highest prevalence within the group of fattening pigs. Surprisingly, despite of a repeated PCR, we noted low number of PCV-2 DNA-positive samples in all age categories. This event can be explained with low amount of virus in the blood at time of sampling, which cannot be detected by conventional PCR. The same pattern was observed in a study by McIntosh *et al.* (2006),

where higher detection rate was reached by nested-PCR. PCV-2 is present in PMWS-free as well as affected farms (Larochelle *et al.*, 2003; Sibila *et al.*, 2004; Meerts *et al.*, 2006), what supports our results, since approximately 50.0% of healthy and PMWS-suspect pigs were seropositive and a high number of healthy ones was PCV-2 DNA-positive too.

Overall, among all four age categories, specific PCV-2 antibodies were detected in 54.0% of samples, which is lower in comparison with data from other European countries with intensive pig industry, like Spain with 72.7% seroprevalence (Rodriguez-Arrioja *et al.*, 2003), France with 80.0% (Blanchard *et al.*, 2003), Belgium with 100.0% (Lefebvre, 2008), Austria with 60.0% (Schmoll *et al.*, 2008), but also Canada with 82.4% (Liu *et al.*, 2002) and USA with 80.0% seroprevalence (Nawagitgul *et al.*, 2002).

From the results we conclude: i) the development of PCV-2 antibodies was age dependent, ii) the prevalence of PCV-2 antibodies was almost the same in PMWS-suspect and healthy pigs, iii) PMWS-suspect pigs were mostly PCV-2 IgM+IgG-positive, while in healthy pigs, IgG were prevalent, which shows a different trend in the distribution of PCV-2 antibodies between PMWS-suspects and healthy animals, iv) simultaneous detection of IgM+IgG antibodies and viral DNA can be a significant marker for PMWS-suspect pigs, and v) the prevalence of antibodies against PCV-2 in the Slovak pig population was 54.0%, which is lower in comparison with countries with enzootic PCV-2 infection.

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