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Short communication

Porcine circovirus type 2 viremia and seroconversion in pigs from a farm affected by postweaning multisystemic wasting syndrome

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Abstract

The aim of the study was to assess the usefulness of real-time PCR and serological methods as indicators of postweaning multisystemic wasting syndrome (PMWS) occurrence. Significantly higher level of porcine circovirus type 2 (PCV2) viral load in serum and significantly lower titre of specific antibodies in PMWS-affected pigs indicated that combination of quantitative PCR and serological methods may support diagnosis of PMWS.

Key words: PCV2, PMWS, diagnostics, serology, real-time PCR

Introduction

Postweaning multisystemic wasting syndrome is one of the most important diseases associated with PCV2. Diagnosis of PMWS focuses on detection of association between PCV2 and microscopic lesions in lymphoid tissues based on *in situ* techniques (Podgórska and Stadejek 2011). However, those methods strongly depend on the quality of biological material. Recent studies showed that increased level of PCV2 viremia and delayed production of specific antibodies are common features of PMWS (Olvera et al. 2004, Meerts et al. 2006). The aim of this study was to assess the relationship between the PCV2 load in serum and the level of specific antibodies and its possible significance in diagnosis of the syndrome.

Materials and Methods

Seven wasted and 8 clinically normal 11 weeks old pigs from PMWS-affected herd were euthanized and samples of blood and lymph nodes were collected. Sections of lymph nodes were hematoxylin/eosin stained and tested for the presence of PCV2 DNA by *in situ* hybridization (ISH) (Podgórska and Stadejek 2011). Nucleic acid was isolated using MagNA Pure LC Total Nucleic Acid kit (Roche). PCV2 DNA was detected using previously designed primers and TaqMan probe (Opriessnig et al. 2003) and QuantiTect Probe PCR Kit (Qiagen). Reactions were run in Mx3005P® Stratagene (95°C/15 min; 40 cycles: 94°C/15 s, 60°C/1 min). Serum samples were also tested for the presence of porcine reproductive and respiratory syndrome virus

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Table 1. Results of IPMA and real-time PCR analysis of serum samples.

Pig No.	PMWS	IPMA (log[titre])	PCV2 real-time PCR* (DNA copies/ml)	PRRSV real-time PCR (C_t)
1	+	2	8.06×10^{08}	nd**
2	+	2	2.80×10^{09}	31.2
3	+	2	4.96×10^{08}	32.98
4	+	2	6.02×10^{08}	29.87
5	+	4.7	5.77×10^{06}	25.83
6	+	2	5.37×10^{07}	27.36
7	+	3.5	2.90×10^{06}	26.03
8	–	5	8.25×10^{06}	neg
9	–	5	1.31×10^{05}	neg
10	–	5.3	4.83×10^{06}	neg
11	–	3.8	1.20×10^{04}	neg
12	–	3.8	4.16×10^{05}	neg
13	–	2.9	7.50×10^{03}	neg
14	–	4.4	1.06×10^{05}	neg
15	–	3.8	8.40×10^{04}	33.15

* Correlation coefficient (R^2) of real-time PCR method within 10^6-10^1 DNA copies/reaction range reached 0.996; the analytical sensitivity of the test reached 1 DNA copy/reaction
** nd – not determined

(PRRSV) with NextGen PRRSV Viral RNA kit (Tetracore). PCV2 antibodies were titrated with immune peroxidase monolayer assay (IPMA) (Podgórska and Stadejek 2011).

Results and Discussion

In the present study we compared the results of quantitative PCR and IPMA with diagnosis based on ISH. PMWS was confirmed in all wasted pigs based on different degree of lymphocyte depletion, histiocytic infiltrations and moderate to high content of PCV2 DNA within these lesions. Particularly severe PMWS was observed in pigs No. 1, 2, 3, 4 and 6. All clinically healthy pigs were seropositive to PCV2 and viremic, with virus loads of 10^6 DNA copies/ml or lower (Table 1). On the other hand, 5 of 7 diseased pigs having the highest viral load ($\geq 10^7$ DNA copies/ml) had low IPMA titre. Only two PMWS-affected pigs had viral load below 10^7 DNA copies/ml and IPMA titers 4.7 and 3.5 respectively. Mean concentration of PCV2 DNA in PMWS-affected pigs (6.8×10^8 DNA copies/ml) was significantly higher (Cochran and Cox test, $P<0.1$) compared to subclinically infected pigs (1.7×10^6 DNA copies/ml). On the other hand, IPMA titre was significantly lower ($P<0.1$) in diseased pigs (2.6) compared to subclinically infected animals (4.3). These results indicated different dynamics of infection in affected and age-matched subclincally infected pigs. Similar observations were reported before. The mechanisms of this phenomenon are most probably related with immunomodulating activity of PCV2 that may impair humoral response and promote virus replication and development of PMWS (Meerts et al. 2006).

In the present study all the pigs with viral load above 10^6 copies/ml and IPMA titre below 5 were diagnosed as PMWS-affected by ISH. Using these criteria a perfect agreement between combination of PCR/IPMA and ISH was observed (kappa value = 1). These results are consistent with Olvera et al. (2004) who proposed a threshold of 10^7 DNA copies/ml to identify PMWS as well as Wellenberg et al. (2004) who reported that about 90% of PMWS-affected pigs had antibodies titre lower than 5. However, it has to be underlined that under farm conditions pigs of the same age may be at a different stage of the disease and laboratory tests should be based on a sample of a population and not individual pigs.

All PMWS-affected pigs were co-infected with PCV2 and PRRSV while in control group co-infection was identified in only one pig. Obtained results suggest that PRRSV could be involved in the etiology of PMWS in this particular farm. Indeed, eradication of PRRS in that farm coincided with diminishing of

PMWS outbreak what suggests that searching for potential triggering factors of PMWS may contribute to implementation of proper control measures.

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