Available online at www.sciencedirect.com
ScienceDirect
Veterinary Microbiology 128 (2008) 56–64
veterinary microbiology
www.elsevier.com/locate/vetmic

Genomic analysis of PCV2 isolates from Danish archives and a current PMWS case–control study supports a shift in genotypes with time

K. Dupont [a], E.O. Nielsen [b], P. Bækbo [b], L.E. Larsen [a,*]

[a] *National Veterinary Institute, The Technical University of Denmark, Denmark*
[b] *Danish Meat Association, Kjellerup, Denmark*

Received 23 May 2007; received in revised form 17 September 2007; accepted 26 September 2007

Abstract

Porcine circovirus type 2 (PCV2) is the primary cause of Postweaning Multisystemic Wasting Syndrome (PMWS) in pigs. PCV2, however, is found in both PMWS-affected herds and non-affected herds. The objective of this study was to clarify if PCV2 genome nucleotide sequences isolated from pigs from PMWS-affected herds and non-affected herds cluster phylogenetically in two separate groups. All isolates (45) belonged to PCV2 group 1 and shared a nucleotide sequence identity of 99.4–100% indicating a very homogeneous PCV2 population in Denmark. Phylogenetic analysis of the PCV2 isolates revealed no distinctive clustering of case- and control-herds suggesting that there is no link between PCV2 sequences and herd disease status. The appearance of only PCV2 group 1 isolates in this study (isolates from 2003/2004) led us to determine if PCV2 nucleotide sequences had changed in Denmark over time. Interestingly, all PCV2 isolates from before the first outbreak of PMWS (2001) belonged either to a new PCV2 group identified for the first time in this study and named group 3 (isolates from 1980, 1987 and 1990) or PCV2 group 2 (isolates from 1993 and 1996). The shift from PCV2 group 2 to 1 was confirmed on a more global scale by placing all full genome PCV2 sequences submitted to GenBank from 1997 to 2006 in either of the groups by phylogenetic analysis. The analysis showed that the shift happened in 2003 or even earlier. This may indicate that PCV2 group 1 is a more adapted form of PCV2 and possibly could be more pathogenic.

Keywords: PMWS; PCV2; Case–control study; Phylogeny; Genotypes

1. Introduction

Infection with PCV2 is associated with PMWS in pigs aged 2–4 months. PMWS was first observed in Canada in 1991 (Harding and Clark, 1997) and is now considered as one of the most important swine diseases worldwide. Affected pigs show wasting or unthriftiness, enlarged lymph nodes, and occasionally respiratory distress, jaundice and diarrhea (Segales et al., 2005). The most distinct microscopic lesions in lymphoid organs are lymphoid cell depletion, and formation of multinucleated giant cells as well as basophilic intracytoplasmic inclusion bodies (Segales

* Corresponding author. Tel.: +45 72346274; fax: +45 72346340.
E-mail address: LEL@vet.dtu.dk (L.E. Larsen).

0378-1135/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2007.09.016

and Domingo, 2002). Infection with PCV2 is needed for full expression of PMWS clinically as well as histopathologically. However, far from all pigs infected with the virus develop the full range of symptoms associated with PMWS (Segales et al., 2005).

PCV2 is member of the virus family circoviridae. The virion is icosahedral, non-enveloped and 17 nm in diameter. A single capsid protein of approximately 30 kDa has been identified for PCV2 (Nawagitgul et al., 2000). The genome of PCV2 is circular, covalently closed, single-stranded DNA (ssDNA) of about 1.76 kb (Chae, 2004). Upon infection, the ssDNA genome is converted into a double-stranded intermediate, which serves as template for rolling-circle synthesis of the viral ssDNA strand (Steinfeldt et al., 2006). The genome contains two major reading frames: ORF1 encodes the replicase and ORF2 is encoding the capsid protein. The PCV2 genome is ambisense so that the encapsidated viral DNA strand serves as a template for transcription of the capsid protein gene (ORF2), while the complementary DNA strand of the replicative intermediate functions as a template for transcription of the replicase gene (ORF1) (Mankertz et al., 2004).

The similarity of PCV2 genomes is at least 93% and, phylogenetically, PCV2 can be divided into two major groups (1 and 2) (Olvera et al., 2007). The most distinct nucleotide differences between the two groups are found in the capsid protein gene with 33 differences. In contrast, only 13 nucleotide differences are found in the replicase gene (Olvera et al., 2007). There is apparently no link between PCV2 group and disease status or geographical area (Olvera et al., 2007). However, some countries have observed a shift from PCV2 group 2 to 1. This shift has been reported in Switzerland in 2003 (Wiederkehr et al., 2007), in Canada in 2005 (Ellis et al., 2006) and in USA in 2005 (Cheung et al., 2007).

The aim of this study was to compare the genome nucleotide sequences of 45 PCV2 isolates from Danish pigs originating from PMWS-affected herds (case) and non-affected herds (control), respectively. In Denmark we experienced the first cases of PMWS in 2001 (Vigre et al., 2005). Therefore, PCV2 genome nucleotide sequences from this case–control study (2003/2004) were compared with PCV2 genome nucleotide sequences obtained from Danish pig serum collected in 1980, 1987, 1990, 1993 and 1996. The development in the Danish PCV2 sequences from 1980 to 2004 were placed in a broader context by comparison with the development in PCV2 sequence submissions from other countries to GenBank in the period 1997–2006.

2. Materials and methods

2.1. Sample collection

The case–control study was carried out in 2003/2004 on pigs from 32 PMWS-affected herds and 13 non-affected herds distributed throughout Denmark. PMWS-affected herds were selected according to the definition by Sorden (2000) where the occurrence of PMWS requires that a pig/group of pigs have all the following characteristics: (1) clinical signs characterized by wasting/failure to thrive; (2) histologic lesions characterized by depletion of lymphoid tissues and/or lymphohistiocytic to granulomatous inflammation in any organ, typically lungs and/or lymphoid tissues; and (3) PCV2 within characteristic lesions as determined by immunohistochemistry or in situ hybridization.

Non-affected herds were selected by the absence of clinical signs of PMWS and low mortality. Non-affected herds developing PMWS within 3 months after termination of the study were excluded.

Pig serum samples from Danish archives (1980, 1987, 1990, 1993 and 1996) collected before the first cases of PMWS in Denmark (2001) were included in the study.

2.2. DNA extraction and PCV2 sequencing

DNA was purified from lung tissue and serum with the QIAamp DNA Mini kit (Qiagen, Ballerup, Denmark). The PCV2 genome was PCR amplified as three overlapping PCR products with the primer pairs PCV2-1for (5′-CTTCCGAAGACGAGCGC-3′) and PCV2-1rev (5′-TAGCATTCTTCCAAAATACCAAG-3′) for PCR product 1, PCV2-2for (5′-GTTTACATAGGGGT-CATAGG-3′) and PCV2-2rev (5′-TGCTTCTTCA-CAAAATTAGCG-3′) for PCR product 2, and PCV2-3for (5′-TAGAGACTAAAGGTGGAACTGTA-3′) and PCV2-3rev (5′-TCCTGGGCGGTGGACATG-3′) for PCR product 3. All primers used in this study were delivered by MWG-Biotech AG (Ebersberg, Germany).