Research in Veterinary Science 94 (2013) 789–795

Contents lists available at SciVerse ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

# Prevalence and genetic variation of porcine circovirus type 2 in Taiwan from 2001 to 2011

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ARTICLE INFO

*Article history:*
Received 18 June 2012
Accepted 26 November 2012

*Keywords:*
Porcine circovirus type 2
Genotypes
Phylogenetic analysis
Open reading frame

ABSTRACT

Porcine circovirus type 2 (PCV2) is the major causative agent of postweaning multisystemic wasting syndrome (PMWS) in Taiwanese pig farms. We analyzed the complete genomes of 571 Taiwanese PCV2 isolates in Taiwan from 2001 to 2011 and divided the isolates into 2 distinct genotypes (PCV2a and PCV2b) with 6 clusters (1A, 1B, 1C, 2B, 2D, and 2E). Of the 571 Taiwanese PCV2 isolates, 22.9% (131/571) belonged to PCV2a and 77.1% (440/571) to PCV2b. In this study, PCV2a isolates were the most common in 2001, and then PCV2b isolates became predominate thereafter and widely distributed in pig farms since 2003. Sequence comparisons among the 571 isolates indicated that 89.6–100% had nucleotide identity for complete genome and 87.3–100% for open reading frames 2 (ORF2). The results suggest that a higher genetic variation and shift occurred among PCV2 isolates collected from 2001 to 2011 in Taiwan.



## 1. Introduction

Porcine circovirus type 2 (PCV2) has become a major emerging swine viruses and its associated diseases cause severe economic losses to the global swine industry (Chae, 2005; Segalés et al., 2005; Horlen et al., 2007). This virus is considered as the primary etiological agent of postweaning multisystemic wasting syndrome (PMWS) (Meng, 2012). The main clinical signs of affected pigs include decreased rate of weight gain, dyspnea, paleness, gauntness and icterus (Chae, 2005). Characteristic lesions include macrophage infiltration and depletion of lymphoid follicle in the lymphoid tissues (Chae, 2005). Moreover, PCV2 has been considered as a pathogen causing other clinical syndromes, including porcine dermatitis and nephropathy syndrome (PDNS), porcine respiratory disease complex (PRDC), proliferative and necrotising pneumonia (PNP), congenital tremor (CT) and reproductive failure (Hansen et al., 2010; Krakowka et al., 2008; Saha et al., 2010). All these associated syndromes have been proposed for group as porcine circovirus associated disease (PCVAD) (Segalés et al., 2005; Opriessnig et al., 2007).

Porcine circovirus type 2 is a non-enveloped, circular single-stranded DNA virus belonging to the genus *Circovirus* of the family *Circoviridae* (Meehan et al., 1998; Cheung, 2003). The ambisense genome of PCV2 contains 1767–1768 bases with two major open reading frames (ORFs), namely ORF1 and ORF2 (Cheung, 2003). Open reading frame 1 is believed to encode two replication-associated proteins, the complete Rep and the spliced and hence frame-shifted Rep′ (Cheung, 2003). The ORF2 encodes a major structural capsid protein containing immunologically important epitopes (residues 47–85, 117–131, 165–200 and 230–233) associated with virus neutralization (Truong et al., 2001; Lekcharoensuk et al., 2004; Mahé et al., 2000; Fan et al., 2008). Phylogenetic studies based on the complete genome and the ORF2 region of PCV2 isolates from different countries reveal that the virus isolates have been divided into 3 major genotypes: PCV2a, PCV2b and PCV2c (An et al., 2007; Horlen et al., 2007; Olvera et al., 2007). Previous studies indicated that PCV2a and PCV2b contain five clusters (2A, 2B, 2C, 2D and 2E) and three clusters (1A, 1B and1C), respectively (An et al., 2007; Horlen et al., 2007; Olvera et al., 2007; de Castro et al., 2008; Dupont et al., 2008; Chae and Choi, 2010; Vlasakova et al., 2011). The global genetic shift from PCV2a to PCV2b has been discovered in many countries (Gagnon et al., 2007; Carman et al., 2008; Grau-Roma et al., 2008; Gillespie et al., 2009), but the detailed mechanism of this phenomenon remains unclear (Meng, 2012). PCV2c was isolated only in Denmark in the 1980s and has not been reported in its pathogenicity (Dupont et al., 2008). The

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http://dx.doi.org/10.1016/j.rvsc.2012.11.019

aim of this study is to investigate the genetic diversity of PCV2 among Taiwanese isolates during the past decade by sequence comparison and phylogenetic analysis. Our results not only identified that PCV2a was the most prevalent genotype in 2001 and may became silent isolates in the field beginning in 2003, but also revealed that there has been a shift in genotype from PCV2a to PCV2b since 2003.

## 2. Materials and methods

### 2.1. PCV2 isolates

In this study, 571 PCV2 isolates were isolated at the Division of Hog Cholera, Animal Health Research Institute, Council of Agriculture, Executive Yuan, from pigs at 4–16 weeks of age with PMWS or non-PMWS between 2001 and 2011. The PMWS and non-PMWS were identified using a set of PMWS diagnostic criteria (Segalés et al., 2005). Each isolate was identified as polymerase chain reaction (PCR) (Wang et al., 2004). The numbers of PCV2 isolates in each year were summarized in Table 1.

### 2.2. Viral DNA extraction and PCR

Viral DNA was extracted from each PCV2 isolate by QIAamp Tissue Kit (Qiagen, Valencia, California, USA), according to the manufacturer's instructions. For amplification of the complete genome from the extracted DNA, specific oligonucleotide primer sets were used in the PCR (Wang et al., 2004). A 100 μl PCR mixture consisted of 10 μl of 10 × buffer (100 mM Tris–HCl (pH 8.8), 500 mM KCl, 15 mM $MgCl_2$, and 1% Triton X-100), 1.25 μM of dNTPs, 20 μM of each primer, 1.0 μl of POWER TAQ (2 U/μl; Bertec, Taipei, Taiwan), and 12 μl of the DNA sample. The PCR was initially performed at 95 °C for 5 min, followed by 35 cycles each of denaturation at 95 °C for 1 min, annealing at 58 °C for 1 min, and extension at 72 °C for 1 min, and then a final terminal extension at 72 °C for 10 min. The products were analyzed by electrophoresis using 2% agarose gel containing 0.5 mg/ml SYBR Safe DNA gel stain (Invitrogen, Carlsbad, CA, USA) in Tris–acetate-EDTA buffer.

### 2.3. Bioinformatic analysis of PCV2 complete genome and ORF2 sequence

The PCR-amplified DNA fragments were sequenced by the direct sequencing method using the BigDye™ Terminator Cycle Sequencing kit and the ABI 3730 DNA sequencer (Applied Biosystems, Foster City, California, USA). For sequence comparison, the resultant complete genomic nucleotide sequences of PCV2 were aligned with 48 published genomic sequences available in GenBank (Fig. 1) by the MegAlign program of the DNASTAR package version 8.1 with the Clustal V method (DNASTAR, Madison, Wisconsin, USA). Phylogenetic analysis was performed on the aligned data set, and an unrooted tree was constructed by neighbor-joining method with the software Molecular Evolutionary Genetic Analysis MEGA 4 program package (Kumar et al., 2008). Bootstrap values were calculated based on 1000 repeats of the alignment. The 571 Taiwanese PCV2 isolates were classified and definition as previously studies (An et al., 2007; Olvera et al., 2007). Hydrophobicity profile was generated using DNASTAR package version 8.1 as aforementioned. The differences between nonsynonymous (d*N*) and synonymous (d*S*) substitution rates (d*N*−d*S*) for codon-aligned nucleotide sequences were calculated based on the method of Nei and Gojobori (1986) with the MEGA 4 program package as aforementioned. Amino acid sequence of putative capsid protein of ORF2 entropy calculated using BioEdit (Hall, 1999) was plotted versus the difference between d*N*−d*S* for all the 571 isolates analyzed. These differences between d*N*−d*S* were calculated via the SNAP web utility (http://hcv.lanl.gov/content/hcv-db/SNAP/SNAP.html).

## 3. Results

### 3.1. Phylogenetic analysis

A phylogenetic tree was derived from the complete nucleotide sequences of the 571 Taiwanese PCV2 isolates and the 48 PCV2 published sequences in GenBank (Fig. 1). Among the 571 Taiwanese PCV2 isolates, 22.9% (131/571) were the PCV2a genotype and 77.1% (440/571) were the PCV2b genotype. Of the 131 PCV2a isolates, 84.0% (110/131) of them belonged to cluster 2B, 1.5% (2/131) in cluster 2D, and 14.5% (19/131) in cluster 2E (Fig. 1, Table 1). None of PCV2a isolates in cluster 2A and 2C were obtained (Fig. 1, Table 1). Of the 440 PCV2b isolates, 25.2% (111/440) belonged to cluster 1A, 72.1% (317/440) in cluster 1B, and 2.7% (12/440) in cluster 1C (Fig. 1, Table 1). Of the 571 isolates, 30 representative PCV2 sequences, HQ202944/PT6 to HQ202973/HL2, were randomly selected from each cluster and submitted to GenBank (Table 2). No PCV2c isolate has been found in Taiwan from 2001 to 2011.

### 3.2. Analysis of nucleotide sequences

Pairwise comparison results indicated 89.6–100% nucleotide identity for complete genomes and 87.3–100% for ORF2 among the 571 PCV2 isolates. The analysis showed that all the genomes

**Table 1**
Genotypic analysis of Taiwanese porcine circovirus type 2 (PCV2) isolates collected from 2001 to 2011.

| Year | Number of isolates | PCV2a | | | | | | PCV2b | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2A | 2B | 2C | 2D | 2E | Total | 1A | 1B | 1C | Total |
| 2001 | 66 | | 51 | | 2 | 4 | 57 | 7 | 2 | | 9 |
| 2002 | 60 | | 29 | | | 3 | 32 | 17 | 11 | | 28 |
| 2003 | 69 | | 11 | | | | 11 | 30 | 28[1] | | 58 |
| 2004 | 64 | | 4 | | | | 4 | 7 | 53 | | 60 |
| 2005 | 47 | | 2 | | | 2 | 4 | 6 | 37 | | 43 |
| 2006 | 39 | | | | | | 0 | 7 | 31[1] | 1 | 39 |
| 2007 | 35 | | | | | 1 | 1 | 5 | 26 | 3 | 34 |
| 2008 | 45 | | 5 | | | 1 | 6 | 9 | 27 | 3 | 39 |
| 2009 | 61 | | 2 | | | 4 | 6 | 5 | 48 | 2 | 55 |
| 2010 | 52 | | 4 | | | 3 | 7 | 10 | 33 | 2 | 45 |
| 2011 | 33 | | 2 | | | 1 | 3 | 8 | 21 | 1 | 30 |
| Total | 571 | | 110 | | 2 | 19 | 131 | 111 | 317 | 12 | 440 |

[1] Of the 571 isolates, three PCV2 isolates and one PCV2 isolate were 1778 bases in length and obtained in 2003 and 2006, respectively.