

Phylogenetic analysis of 32 porcine circovirus type 2 isolates from Shandong, China

Jianli Shi · Shaojian Xu · Fang Fu · Xiaoyan Cong · Xiaoyuan Yuan ·
Zhe Peng · Jiaqiang Wu · Wenbo Sun · Yijun Du · Jun Li · Baohua Huang ·
Jinbao Wang

Received: 12 September 2014 / Accepted: 28 November 2014 / Published online: 7 December 2014
© Springer-Verlag Wien 2014

Abstract Porcine circovirus type 2 (PCV2), the essential causative agent of postweaning multisystemic wasting syndrome (PMWS), can be divided into distinct genotypes. Thirty-two PCV2 isolates obtained made from pigs in Shandong Province between 2005 and 2013. Complete genome sequences were obtained in three replicates for each virus isolate, and the sequences were submitted to the NCBI database. The ORF1-encoded amino acid sequences had 98.4 %–100 % identity among the 32 isolates, and there were no significant differences among the three potential glycosylation positions: aa 23–25 (NPS), aa 256–258 (NQT)

and aa 286–288 (NAT). The amino acid sequences of ORF2 had 88 %–100 % identity among the 32 isolates and the potential glycosylation position in the cap protein, aa 143–145 (NYS), had no variation. Phylogenetic analysis revealed that the PCV2b/1C genetic lineage was prevalent in swine populations in Shandong Province. It also suggested that selection pressure has made the PCV2 isolates more genetically distant from current vaccine strains.

J. Shi, S. Xu, and F. Fu contributed equally to this work.

J. Shi
College of Life Sciences, Key Laboratory of Animal Resistance
of Shandong Province, Shandong Normal University,
Jinan 250014, China
e-mail: sjl6296@163.com

J. Shi · S. Xu · X. Cong · X. Yuan · Z. Peng · J. Wu · W. Sun ·
Y. Du · J. Li (✉) · B. Huang (✉) · J. Wang (✉)
Division of Swine Diseases, Shandong Key Laboratory of
Animal Disease Control and Breeding, Institute of Animal
Science and Veterinary Medicine, Shandong Academy of
Agricultural Sciences, No. 8 Sangyuan Road, Jinan 250100,
China
e-mail: junli79@163.com

B. Huang
e-mail: jn-hbh@163.com

J. Wang
e-mail: wangjb@saas.ac.cn

F. Fu
Division of Swine Infectious Diseases, State Key Laboratory of
Veterinary Biotechnology, Harbin Veterinary Research Institute,
Chinese Academy of Agricultural Sciences, Harbin 150001,
China

Porcine circovirus (PCV) is the smallest animal virus known and can be divided into two groups: the non-pathogenic PCV1 group and the PCV2 group, whose members are recognized as the causative agents of postweaning multisystemic wasting syndrome (PMWS) [1]. PCV2 belongs to the genus *Circovirus*, family *Circoviridae*. Circoviruses have two major open reading frames (ORF1 and ORF2). ORF1 encodes two replication-associated proteins (Rep and Rep') [6], and ORF2 encodes a major structural capsid protein (Cap) [2].

Studies of genomic sequences show that PCV2 can be further divided into distinct subtypes, also known as genotypes. A PCV2 genotype definition and nomenclature have been proposed, with three genotypes identified to date, including 'a' (PCV2a) and 'b' (PCV2b) with eight clusters (1A to 1C and 2A to 2E), which are the two main phylogenetic groups, and a third genotype, 'c' (PCV2c), which thus far has only been found in Denmark [3–5].

In this study, we analyzed the molecular diversity of and possible recombination events in PCV2 isolates obtained in Shandong Province between 2005 and 2013.

Thirty-two PCV2 isolates were obtained from clinical samples (lymph nodes, spleen, liver, lung and tonsil) collected from different geographical regions in Shandong Province during 2005–2013 (Fig. 2). Primer P1 (5'-GAA

CCGCGGGCTGGCTGA ACTTTTGAAAGT-3') and primer P2 (5'-GCACCGCGGAAATTTCTGACAAACGTT ACA-3') were used to amplify the whole 1.7-kb genome as described by Fenaux et al. [6]. The whole genomes were sequenced three times each and submitted to the NCBI database. Sequences of PCV2 Shandong isolates were compared with other representative PCV2 genotype sequences in GenBank using MEGA V6.0 software.

The amino acid sequences of ORF1 showed 98.4 %–100 % identity among the 32 isolates (Fig. 1). There were no significant differences in the three potential glycosylation positions, aa 23–25 (NPS), aa 256–258 (NQT), aa 286–288 (NAT). The amino acid sequences of ORF2 had 88 %–100 % identity among the 32 isolates, and the potential glycosylation position of the cap protein, aa 143–145 (NYS), had no variation.

The results of phylogenetic analysis showed that the isolates could be divided into two groups, PCV2a and PCV2b. The majority of Shandong strains belonged to PCV2b/1C, but three strains (HM142897, KF027495, and HM142900) belonged to PCV2a. The same conclusion was reached based on the signature motif in ORF2 (amino acids 86–89), which was TNKI in PCV2a, and SNPR or L in PCV2b) [7] (Fig. 2). The PCV2b genetic lineage was found to be prevalent in swine populations in Shandong Province.

Fig. 2 Phylogenetic tree constructed by the neighbor-joining (NJ) method for 32 full-length sequences of PCV2 using MEGA6.0 software. The accession numbers of the 32 Shandong viruses are underlined

The Rep protein, which is associated with virus replication, has a nonvariable amino acid sequence, and the Cap protein, which is associated with pathogenicity, has a variable sequence. In this study, based on amino acid sequence alignment of 32 isolates obtained by our group from Shandong Province, we found that there was only one major variable region in Rep, at amino acid position 6. Some viruses had an Asn residue, and some had a Ser residue. Six major variable regions were identified at positions 8, 51–68, 121–134, 169, 185–191, 206–215, some with the same change, suggesting that these isolates may have had the same origin. The connection between this mutation and virus pathogenicity needs further research.

There are three commercial inactivated PCV2 vaccine strains, SH, LG and DBN, that are used in China. Their position in the phylogenetic tree was PCV2b/1C, PCV2a/2b, PCV2b/1A, respectively. The majority of isolates from 2013 were in PCV2b/1C, which clusters, away from the vaccine strains (Fig. 2). Therefore, a clinical trial is needed

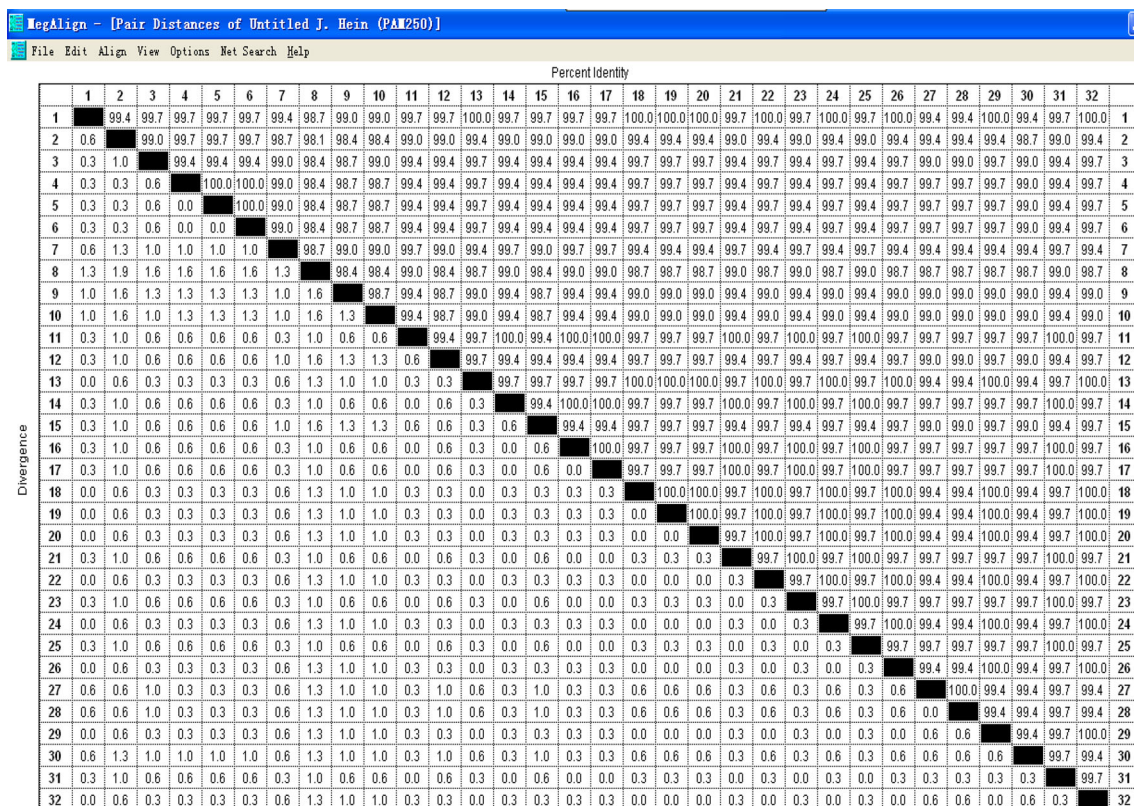
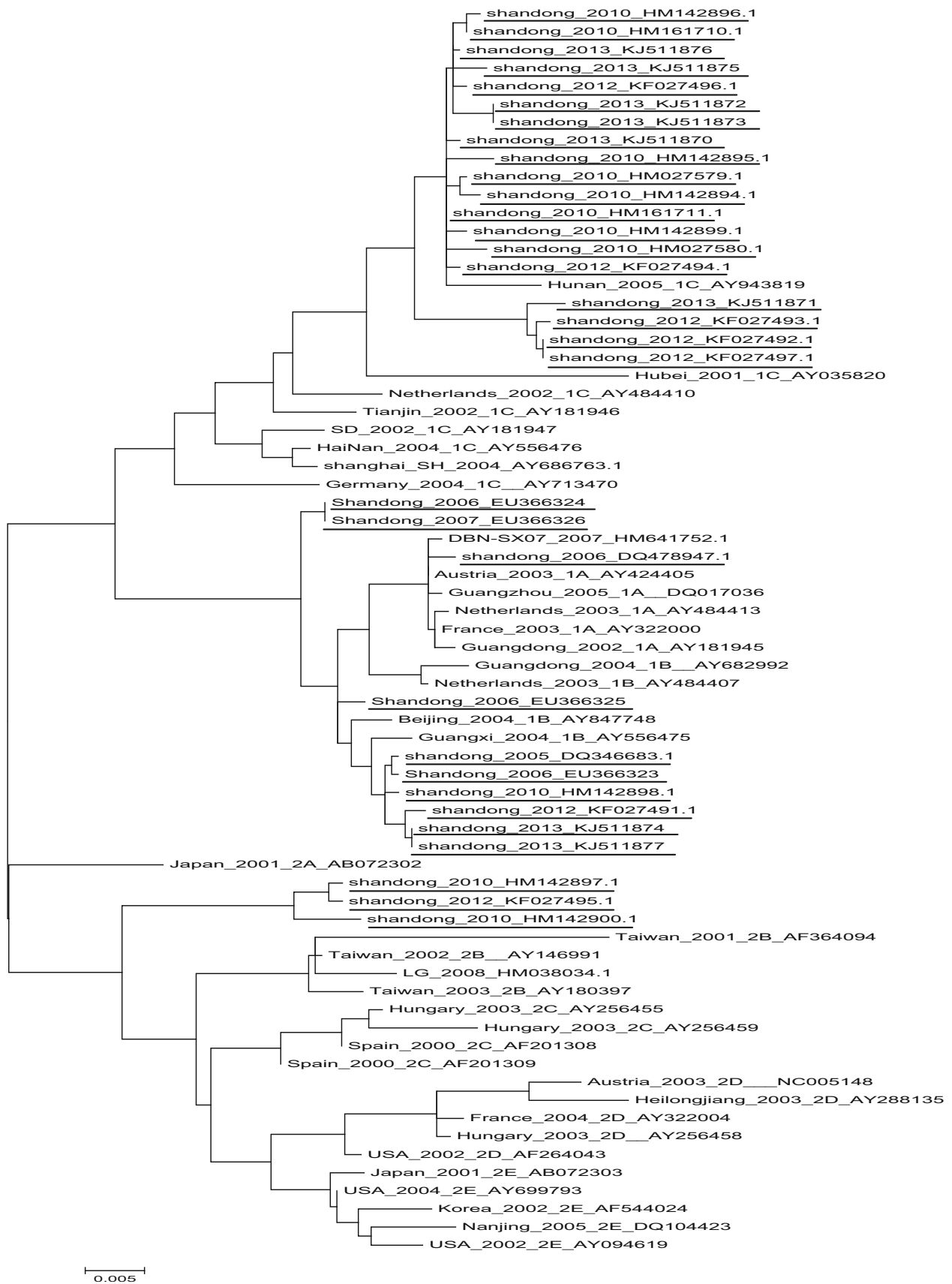


Fig. 1 Comparison of the ORF1 amino acid sequences showing that there was 98.4 %–100 % identity among the 32 isolates



to test the efficacy of this vaccine against the PMWS strain that is now prevalent in Shandong Province.

In conclusion, 32 PCV2 isolates were obtained from pigs in Shandong Province. Phylogenetic analysis revealed that the PCV2b/1C genetic lineage was prevalent in swine populations in Shandong Province. It is suggested that selection pressure has made the PCV2 isolates more genetically distant from current vaccine strains.

Acknowledgments This study was partly supported by the National Natural Science Fund (31201889 and 31170146) and the Youth Scientific Research Foundation of Shandong Academy of Agricultural Sciences (2014QNM40).

References

1. Tischer I, Gelderblom H, Vettermann W, Koch M (1982) A very small porcine virus with circular single-stranded DNA. *Nature* 295:64–66
2. Morozov I, Sirinarumitr T, Soredn SD, Halbur PG, Morgan MK, Yoon KJ, Paul PS (1998) Detection of a novel strain of porcine circovirus in pigs with postweaning multisystemic wasting syndrome. *J Clin Microbiol* 36:2535–2541
3. Olvera A, Cortey M, Segales J (2007) Molecular evolution of porcine circovirus type 2 genomes: phylogeny and clonality. *Virology* 357:175–185
4. Seglaes J, Olvera A, Grau-Roma L, Charreyre C, Nauwynck H, Larsen L, Dupont K, McCullough K, Ellis J, Krakowka S (2008) PCV-2 genotype definition and nomenclature. *Vet Rec* 162:867–868
5. Dupont K, Nielsen E, Baekbo P, Larsen L (2008) Genomic analysis of PCV2 isolates from Danish archives and a current PMWS case-control study supports a shift in genotypes with time. *Vet Microbiol* 128:56–64
6. Fenaux M, Halbur P, Haqshen G, Royer R, Thomas P, Nawagitgul P, Gill M, Toth T, Meng X (2002) Cloned genomic DNA of type 2 porcine circovirus is infectious when injected directly into the liver and lymph nodes of pigs: characterization of clinical disease, virus distribution, and pathologic lesions. *J Virol* 76:541–551
7. Cheun K, Kohutyuk O, Vincent A, Henry S, Baker R, Rowland R, Dunham A (2007) Detection of two porcine circovirus type 2 genotypic groups in United States swine herds. *Arch Virol* 152:1035–1044