

# Complete Genome Sequence of a Novel Porcine Circovirus Type 2b Variant Present in Cases of Vaccine Failures in the United States

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**Two genomes of a new porcine circovirus type 2 (PCV2) strain associated with cases of perceived failure of PCV2 vaccination were sequenced and analyzed. Based on the genome, this is the first report of this mutant of PCV2b in the United States. The genomic knowledge of this mutant PCV2b will improve understanding of the epidemiology of PCV and potentially inform the development of new and more effective vaccines for PCV2.**

Porcine circovirus type 2 (PCV2), a member of *Circoviridae* family genus *Circovirus*, is a small nonenveloped circular virus which was initially discovered in 1998 (6). PCV2 is highly prevalent in the domestic pig population. PCV2 is associated with postweaning multisystemic wasting syndrome (PMWS), enteric disease, respiratory disease, porcine dermatitis and nephropathy syndrome (PDNS), and reproductive failure (6). PCV2 isolates are currently further subdivided into three genotypes: PCV2a, PCV2b, and PCV2c (1, 3).

In this study, two PCV2 strains, US22625-33 and US22664-35, were identified by PCR in cases of suspected vaccine failure in PMWS-affected pigs in a production system located in the United States in June 2012. The complete genome was further sequenced using previously described primers (2). The full genome of the two PCV2s is comprised of 1,767 bp with 10 predicted open reading frames (ORFs). The size of ORF1 is 945 bp, encoding a protein of 314 amino acids (aa), and the size of ORF2 is 705 bp, encoding a protein of 234 aa, which is 1 aa longer than that of the common PCV2 (233 aa). Phylogenetic analysis with the nucleotide sequences of ORF2 of the reference strains PCV2a (AF055392), PCV2b (AF055394), PCV2c (EU148503) (1), and representative U.S. PCV2a (DQ397521) and PCV2b strains (GU799576 and HQ713495) suggested that these newly identified strains are closely related to PCV2b. The predicted amino acid sequence of ORF2 showed higher levels of identity to PCV2b strains (93.6% to 94.9%) than to PCV2a (90.6% to 91%). However, the predicted amino acid sequence of ORF1 of the mutant PCV2 strains showed higher levels of identity (99% to 99.4%) with PCV2a than with PCV2b (98.7%), indicating possible recombination during the origin of the strain US22625-33. Compared with classic PCV2b, a TAA (stop codon) to AAG (Lys) at the 3' end of ORF2 results in one additional amino acid in ORF2.

Further sequence BLAST and comparison showed that both U.S. PCV2 strains had a high level of identity (99.9%) with the PCV2 strain BDH, which is sporadically found in China, reported to belong to genotype PCV2d, and suggested to be of increased virulence (4, 5). One silent mutation (1677A→1677T) in ORF1 was found between BDH and the two U.S. mutant PCV2s. However, according to the new PCV2 genotype definition and nomenclature criteria (1, 7), all of these novel mutant PCV2 strains could be classified into genotype PCV2b, based on the phylogenetic analysis of the nucleotide sequence of ORF2 gene. Notably, in a previous large field investigation conducted in the United States during 2010 to 2011 in which 185 putative amino acid sequences of the PCV2 ORF2 were obtained, aligned, and compared, the novel mutant PCV2b sequence was not present (8).

The present study is the first to identify a new PCV2b mutant in the United States, where it was discovered in cases of suspected PCV2 vaccine failures in pigs with PMWS, indicating the possible antigen drift of this newly identified PCV2b mutant.

**Nucleotide sequence accession numbers.** The complete genome sequences of PCV2 strains US22625-33 and US22664-35 have been deposited in GenBank under the accession numbers [JX535296](#) and [JX535297](#).

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## REFERENCES

1. Cortey M, Olvera A, Grau-Roma L, Segalés J. 2011. Further comments on porcine circovirus type 2 (PCV2) genotype definition and nomenclature. *Vet. Microbiol.* 149:522–523.
2. Fenaux M, Halbur PG, Gill M, Toth TE, Meng XJ. 2000. Genetic characterization of type 2 porcine circovirus (PCV-2) from pigs with postweaning multisystemic wasting syndrome in different geographic regions of North America and development of a differential PCR-restriction fragment length polymorphism assay to detect and differentiate between infections with PCV-1 and PCV-2. *J. Clin. Microbiol.* 38:2494–2503.
3. Grau-Roma L, et al. 2008. A proposal on porcine circovirus type 2 (PCV2) genotype definition and their relation with postweaning multisystemic wasting syndrome (PMWS) occurrence. *Vet. Microbiol.* 128:23–35.
4. Guo L, et al. 2012. A porcine circovirus type 2 (PCV2) mutant with 234 amino acids in capsid protein showed more virulence in vivo, compared with classical PCV2a/b strain. *PLoS One* 7:e41463. doi:10.1371/journal.pone.0041463.
5. Guo LJ, Lu YH, Wei YW, Huang LP, Liu CM. 2010. Porcine circovirus type 2 (PCV2): genetic variation and newly emerging genotypes in China. *Virol. J.* 7:273.
6. Opriessnig T, Langohr I. 14 June 2012. Current state of knowledge on porcine circovirus type 2-associated lesions. *Vet. Pathol.* [Epub ahead of print.] doi:10.1177/0300985812450726.
7. Segalés J, et al. 2008. PCV-2 genotype definition and nomenclature. *Vet. Rec.* 162:867–868.
8. Shen HG, Halbur PG, Opriessnig T. 2012. Prevalence and phylogenetic analysis of the current PCV2 genotypes after implementation of widespread vaccination programs in the United States. *J. Gen. Virol.* 93:1345–1355.

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