

Complete Genome Sequence of a Highly Prevalent Isolate of Porcine Epidemic Diarrhea Virus in South China

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A widespread porcine epidemic diarrhea virus (PEDV) occurred in southern China during 2010 to 2012. A virulent field PEDV strain, GD-B, was isolated from a suckling piglet suffering from severe diarrhea in Guangdong, China. We sequenced and analyzed the complete genome of strain GD-B, which will promote a better understanding of the molecular epidemiology and genetic diversity of PEDV field isolates in southern China.

Porcine epidemic diarrhea virus (PEDV), a positive-sense single-stranded RNA virus in the *Alphacoronavirus* genus (family *Coronaviridae*), causes acute diarrhea and dehydration in pigs (5, 6). During 2010 to 2012, a PED outbreak characterized by severe diarrhea, vomiting, and a high mortality rate in suckling piglets emerged in southern China (7). A virulent field PEDV strain, GD-B, was predominantly isolated from suckling piglets with severe diarrhea in Guangdong province in March 2012. In order to identify and characterize the PEDV variants in South China, the sequence of the complete genome of GD-B was determined.

Rapid amplification of cDNA ends (RACE) was performed to capture the 5' and 3' ends of the viral genome, and the complete nucleotide sequence was determined by primer walking strategy. All reverse transcription (RT)-PCR products were gel purified using the QIAquick gel extraction kit (Qiagen) and sequenced on a 3730 DNA Analyzer (Applied Biosystems). The genome was assembled using DNASTar (version 7.0). The complete genomic sequence of GD-B, excluding the poly(A) tail, comprises 28,038 nucleotides (nt), with 5' (292 nt) and 3' (334 nt) ends containing untranslated regions (UTRs). The genomic organization is typical of coronaviruses, having the characteristic gene order (5'-replicase [1a/1b], spike [S], ORF3, envelope [E], membrane [M], and nucleoprotein [N]-3'). The replicase 1a ORF (nt 293 to 12,601) and replicase 1b ORF (nt 12,601 to 20,637) occupy 20.3 kb of the genome. Genes for the major structural proteins S (nt 20,634 to 24,794), E (nt 25,449 to 25,679), M (nt 25,687 to 26,367), and N (nt 26,379 to 27,704) are located downstream of the replicase gene. The ORF3 gene (nt 24,794 to 25,468), which is an accessory gene, is located between the structural genes.

The complete genome sequence of GD-B shares 99%, 97.4%, 97.8%, and 96.8% nucleotide sequence identity with that of CH/FJND-3/2011, CH/S, DR13, and CV777, respectively (1–4). There are four remarkable insertion regions (nt 20,802 to 20,804, nt 20,815 to 20,823, nt 20,844, and nt 21,055 to 21,057) and two deletion regions (nt 20,856 and nt 21,114 to 21,119) in strains GD-B and CH/FJND-3/2011, which were isolated from China during 2011 to 2012. These insertion and deletion sites are all in the N-terminal region of the S gene. Compared with CH/FJND-3/2011, GD-B has 274 single-nucleotide sequence variations, of which 104 variant loci were identified in the S gene and resulted in 40 amino acid substitutions in the S protein.

Phylogenetic analysis of the GD-B genome sequence indicated

that the recent PEDV isolates prevailing in South China were different from foreign and other domestic strains. All new strains were clustered together on the same branch and far from the cluster of vaccine strain CV777. These data showed that the current PEDV isolates were constantly undergoing genetic variation in the continuous transmission, and it may be related to the poor efficacy of vaccine used in China. The present study will promote a better understanding of the molecular epidemiology and genetic diversity of PEDV field isolates in South China and help to prevent and control PEDV infection in future.

Nucleotide sequence accession number. The complete genome sequence of PEDV strain GD-B has been deposited in GenBank under accession no. [JX088695](https://www.ncbi.nlm.nih.gov/nuccore/JX088695).

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