

Complete Genome Sequence of a Variant Porcine Epidemic Diarrhea Virus Strain Isolated in China

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Since October 2010, an outbreak of porcine epidemic diarrhea (PED) has been observed in some provinces of China. Here we report the complete genome sequence of porcine epidemic diarrhea virus (PEDV) strain LC, which was recently isolated from sucking piglets that suffered from severe watery diarrhea in Guangdong. It will help in understanding the epidemiological and molecular characteristics of PEDV in China.

Porcine epidemic diarrhea virus (PEDV) is a positive-sense singlestranded RNA virus belonging to group 1a, genus *Coronavirus*, family *Coronaviridae*, which causes porcine epidemic diarrhea (PED), characterized by vomiting, watery diarrhea, and dehydration in swine (6). Since October 2010, outbreaks of PED characterized by high morbidity (nearly 100%) and mortality (80% to 100%) in newborn piglets have emerged in China (7). A virulent PEDV strain, LC, was recently isolated from suckling piglets with severe diarrhea on an immunized-swine breeding farm in Guangdong province. To determine how this isolate evolved in the current epidemics, we sequenced and analyzed the complete genome of LC.

Twelve pairs of oligonucleotide primers for use in amplifying the different regions of LC genomes were designed based on the sequence of PEDV strain CV777. The PCR products were purified and cloned into the pMD18-T vector (TaKaRa, Japan) and sequenced with an automated genome sequencer (3730XL genetic analyzer; Applied Biosystems). The terminal sequences were acquired by using a kit for rapid amplification of cDNA ends (RACE) (TaKaRa, Japan). Sequence alignment was performed using the software program Clustal X 2.1 (9). A phylogenetic tree was constructed using the software program MEGA 4 (8).

The complete genome sequence of LC is 28,035 nucleotides (nt) in length [excluding the poly(A) tail]. Its genome organization is as follows: 5'-replicase (1a/1b)-spike (S)-ORF3-envelope (E)-membrane (M)-nucleoprotein (N)-3', similar to the reported organization of other PEDV genomes (1–5). Untranslated regions (UTR) are found at both ends; the 5' UTR contains 292 nt, and the 3' UTR is 334 nt in length. Seven coding regions, respectively, encode 4 structural proteins (S [nt 20634 to 24791], E [nt 25446 to 25676], M [nt 25684 to 26364], and N [nt 26,376 to 27,701]), 2 nonstructural proteins (replicase 1a [nt 293 to 12601] and replicase 1b [nt 12601 to 20637]]), and the only accessory protein, ORF3 (nt 24791 to 25465).

Compared with CV777, LC has 897 different nucleotides, of which 10 are in the 5' UTR, 522 in ORF1, 262 in the S gene, 23 in ORF3, 5 in the E gene, 12 in the M gene, 55 in the N gene, and 8 in 3' UTR. The complete genome sequence of LC shares 96.9 to 98.9% nucleotide sequence identities with those of other PEDV strains deposited in GenBank (1–5). Three insertion regions (nt 20800, nt 20808 to 20818, and nt 21049 to 21051) and one deletion region (nt 21108 to 21113) were observed in the part of the S gene corresponding to the N-terminal region, and these insertions and the deletion were also observed in three Chinese strains (BJ-2011-1, CH/FJND-3/2011, and GD-B) isolated during 2010 to

2012. Interestingly, a unique deletion (nt 24222 to 24224) was found in the part of the S gene encoding the C-terminal region, and this is not reported for other PEDV strains.

Phylogenetic analysis based on the complete genome shows that LC together with BJ-2011-1, CH/FJND-3/2011, and GD-B forms a new cluster and is distant from vaccine strain CV777. These findings reveals the variant PEDV circulating in swine-producing areas in China and provides molecular epidemiological data for a better understanding of this disease.

Nucleotide sequence accession number. The genome sequence of PEDV strain LC has been deposited in GenBank under the accession number JX489155.

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