Complete Genome Sequence of a Vero Cell-Adapted Isolate of Porcine Epidemic Diarrhea Virus in Eastern China

Mengjiao Zhao,a Zhen Sun,a Yue Zhang,a,b Guisheng Wang,b Hui Wang,a Fangfang Yang,a Fulin Tian,b and Shijin Jianga

In early 2012, a widespread porcine epidemic diarrhea virus (PEDV) occurred in eastern China. A cell-adapted isolate, SD-M, was at the four-passage level of virulent field strain SD, which was isolated from a 2-day-old dead suckling piglet that had suffered from severe diarrhea in Shandong Province, China. We report here the complete genome sequence of SD-M. This sequence will promote a better understanding of the molecular pathogenesis of PEDV.

Porcine epidemic diarrhea virus (PEDV), a member of the Alphacoronavirus genus in the family Coronaviridae, is an enveloped, single-stranded, positive-sense RNA virus (11). PEDV infection is an acute and highly contagious enteric disease characterized by severe enteritis, vomiting, watery diarrhea, and a high mortality rate in swine (10, 11). Emerging in the 1980s, the disease has become more serious and resulted in severe economic losses in China, and the complete genome sequences of many wild-type PEDV strains have been determined (1–7). To date, only a few studies have focused on the molecular characteristics of cell-adapted PEDV isolates (8, 9, 12). Here we report a new Vero cell-adapted isolate of PEDV.

In February 2012, wild-type PEDV strain SD was obtained from a 2-day-old dead piglet on a commercial swine farm in Shandong Province, eastern China. The mortality of the piglet herd was 100%, the mortality was 90% in suckling piglets, and the sick piglets died in 2 to 3 days with typical watery diarrhea, dehydration, and vomiting. Wild-type PEDV strain SD was successfully passaged in Vero cells. At passage level 4, 36 h after inoculation, the Vero cells produced an obvious cytopathic effect characterized by cell fusion and syncytium formation, and a cell-adapted isolate, SD-M, was obtained. To determine the complete genome sequence of the isolate, 26 pairs of primers were designed on the basis of PEDV isolate CH/FJND-3/2011 (4) to generate overlapping amplicons by reverse transcription-PCR. The 5’ and 3’ ends of the genome of SD-M were confirmed by using a smarter rapid amplification of cDNA ends kit (Clontech, Japan).

The complete genome of SD-M was 27,953 nucleotides (nt) in length without the poly(A) tail. The SD-M genome showed 99.9, 97.8, 97.7, 97.5, 96.9, 97, and 97% nucleotide sequence similarity to those of attenuated DR13, DR13, CV777, CH/S, GD-A, CH/FJND-3/2001, and AJ1102, respectively. The genomic organization of SD-M was similar to that of other reported PEDVs with the characteristic gene order (5’ untranslated region [UTR]-replicase [1α/1β], spike [S], open reading frame 3 [ORF3], envelope [E], membrane [M], nucleoprotein [N]-3’ UTR). The 5’ UTR was 292 nt long, and the 3’ UTR was 334 nt long. The S gene contained 4,149 nt and was 3 nt shorter than that of CV777. The E gene contained 231 nt and was 21 nt longer than that of attenuated DR13. The M gene contained 681 nt, and the N gene contained 1,326 nt. At 276 nt in length, ORF3 of SD-M was as long as that of attenuated DR13 (passage level 100) but 399 nt shorter than those of other wild-type PEDV strains.

SD-M was the Vero cell-adapted PEDV isolate at the four-passage level of a field isolate of PEDV. The genome data of the new cell-adapted PEDV isolate reported in this study will promote a better understanding of the molecular pathogenesis of PEDV and be helpful in preventing and controlling PEDV infections in the future.

Nucleotide sequence accession number. The complete genome sequence of PEDV strain SD-M was submitted to GenBank under accession number JX560761.

ACKNOWLEDGMENT
This work was funded by the Taishan Scholar Project of Shandong Province.

REFERENCES