Complete Genome Sequence of a New-Genotype Porcine Norovirus Isolated from Piglets with Diarrhea

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Noroviruses (NoVs) are members of the family *Caliciviridae* and are emerging enteric pathogens of humans and animals. So far, porcine NoVs have been detected exclusively in fecal samples from adult swine without clinical signs. Here we report the genome sequence of a NoV strain isolated from piglets with diarrhea. Experimental infection of miniature pigs with this porcine NoV-positive fecal sample confirmed that this strain can cause diarrhea in piglets. A phylogenetic tree based on the predicted amino acid sequence of the complete capsid region showed that this strain is separate from known porcine GII strains (GII-11, GII-18, and GII-19), constituting the sole member of a new branch.

Noroviruses (NoVs) are members of the family *Caliciviridae* and emerging enteric pathogens of humans and animals (1, 5, 10). Based on the complete capsid sequence, noroviruses were divided into 5 genogroups and at least 29 genotypes (12). Porcine strains are found in GII-11, GII-18, and GII-19 (8). Some porcine NoVs are genetically related to human strains and classified into GII, which contains most strains causing epidemics in humans (2, 8). To date, porcine NoVs have been detected exclusively in fecal samples of finisher pigs without clinical signs (6, 7).

Twelve fecal samples from piglets with diarrhea of unknown etiology were collected from three commercial pig farms in a Shanghai suburb, from May to August 2009. Reverse transcription-PCR (RT-PCR) or PCR assays with different primer sets for the detection of porcine NoVs, and common viruses that can cause pig diarrhea, including porcine circovirus type 2, porcine rotavirus, porcine transmissible gastroenteritis virus, porcine sapovirus, and porcine epidemic diarrhea virus, were performed as previously described (3, 4, 9, 11). Two of the fecal samples were positive for porcine NoV; one was also positive for porcine circovirus, and the other was porcine NoV positive only. The strain obtained from this sample was named pNoVs-Ch6.

In order to elucidate whether pNoVs-Ch fecal samples contained infectious porcine NoV, the fecal specimen was converted to 20% (wt/vol) suspensions in phosphate-buffered saline (PBS, 0.01 M, pH 7.4) and clarified by centrifugation at $10,000 \times g$ for 10 min followed by filtration using microfilters with a pore size of 0.22 μm. A 1.5-ml aliquot of supernatant was used to infect five 15-day-old piglets through oral inoculation. Another three piglets were inoculated with PBS only, as controls. The piglets were checked for outward symptoms every 4 h during the first day, and fecal samples were collected from each piglet every day. All five piglets in the experimental group exhibited mild to moderate diarrhea at 0.5 to 1 day postinoculation. The clinical symptoms persisted for 2 to 6 days. All of the fecal samples from the piglets in the experimental group, but not those from the control pigs, were positive for NoV RNA after 4 to 5 days of infection.

The 3-kb 3′-end fragment of pNoVs-Ch6 was amplified with primers p290 and VN3T20 (8). To amplify the remaining sequence, 6 sets of primers were designed based on the entire porcine NoV genome sequence (AB126320) available in GenBank.

RT-PCR-amplified DNA fragments of the expected sizes were sequenced in a DNA analyzer (Applied Biosystems 3730; Invitrogen) and assembled using CodonCode Aligner.

The entire genome of this porcine NoV strain consisted of 7,531 nucleotides, excluding the poly(A) tail. Similar to previously reported NoV strains, pNoVs-Ch6 has three open reading frames (ORFs), encoding 1,693, 547, and 253 amino acids. It shares the highest (88%) nucleotide homology with AB126320 over the entire genome sequence. Phylogenetic analysis showed that pNoVs-Ch6 was separate from known porcine GII strains (GII-11, GII-18, and GII-19) and forms a new branch, which suggests that this strain may represent a novel genotype in the GII group.

Nucleotide sequence accession number. The virus genome sequence in the present study was deposited in GenBank with the strain name pNoVs-Ch6 and accession no. HQ392821.

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