Complete Genome of a Novel Porcine Astrovirus

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Astroviruses have been widely described in mammalian and avian species. Here, we report a complete genome sequence of a novel porcine astrovirus (PoAstV) isolated from a porcine fecal sample in China. The genome consists of 6,611 nucleotides, excluding the 3’ poly(A) tail, and has two open reading frames (ORFs). ORF1 maps between nucleotide positions 19 and 4211 and encodes a 1,396-amino-acid (aa) polyprotein precursor consisting of nonstructural protein and putative RNA-dependent RNA polymerase, and ORF2 maps between nucleotide positions 4202 and 6531 and encodes a 775-aa polyprotein which is a capsid precursor protein. The genome sequence of the virus was distinct enough from those of the known PoAstVs to be considered a novel sequence. Phylogenetic analysis based on the predicted amino acid sequence of the complete capsid region showed that this strain may be a novel porcine astrovirus.

Astroviruses, belonging to the family Astroviridae, are nonenveloped viruses with a single-stranded positive-sense RNA genome that ranges in size from 6.4 to 7.3 kb (13). The genome contains two large open reading frames (ORFs) that encode the nonstructural polyprotein and the capsid structural polyprotein. The family Astroviridae consists of two genera, Mamastrovirus and Avastrovirus, whose members infect humans, swine, sheep, deer, mink, dogs, sea lions, bats, ducks, pigeons, chickens, and turkeys (1, 2, 5–7, 9, 10, 12, 15, 16, 19–24). Recently, a group of novel astroviruses which were highly diverse was identified in humans and swine (3, 4, 8, 11, 14, 18). To enlarge the numbers of sequenced porcine astroviruses (PoAstVs), this study describes a complete genome of a novel porcine astrovirus in China.

Seventeen porcine stool samples from healthy pigs were collected from 17 commercial porcine farms in five provinces (Shanghai, Jiangsu, Anhui, Shandong, and Guizhou) of China from August 2008 to October 2009. The samples were suspended in phosphate-buffered saline (PBS) (0.01 M, pH 7.3), vortexted, centrifuged at 15,000 × g for 10 min, and filtered. The filtrates were then treated with Benzonase, DNase, and RNase. Viral nucleic acids were then extracted using the TIANamp virus DNA/RNA kit (Tiangen Biotech, Beijing, Co., Ltd.). Viral cDNA synthesis and PCR were performed, and PCR products were analyzed as described previously (12, 17, 18). The sequences were then screened for sequence similarities using BLASTn and BLASTx in GenBank databases. Three sequences of one sample collected from Shanghai matched porcine astrovirus (AB037272 and Y15938) and human astrovirus 1 to 8, with a low degree of identity with them. Based on the three sequences, specific primer pairs were designed to determine the complete nucleotide sequence of the astrovirus by reverse transcriptase PCR (RT-PCR) to clone gaps and the genome-walking method to clone unknown regions. The 5’-3’ rapid amplification of cDNA ends (RACE) PCR method was used to clone the 5’ and the 3’ ends of the genome.

The complete genome of this PoAstV strain consisted of 6,611 nucleotides, excluding the poly(A) tail, which contains a 19-nucleotide (nt) region of the 5’untranslated region (UTR) ORF1 and ORF2 and the 3’ UTR. ORF1 begins at nt 20 and ends at nt 4210 (4,191 nt in length) and encodes 1,396 amino acids (aa). ORF2 (nt 4203 to 6530) comprises 2,328 nt and encodes 775 aa. There is an overlap (nt 4203 to 4210) between ORF1 and ORF2. ORF2 of the PoAstV strain showed a lower degree of nucleotide identity (76.8%) with ORF2 of porcine astroviruses (AB037272 and Y15938) available on GenBank, which made it distinct sufficiently from known PoAstV types to be a candidate novel genotype. Phylogenetic analysis obtained for the complete capsid precursor protein of the PoAstV strain and related astrovirus strains available in GenBank showed that the PoAstV strain was separate from known astroviruses and formed a novel branch, which suggests that this strain may represent a novel genotype of PoAstV.

Nucleotide sequence accession number. The virus genome sequence described here has been deposited in the GenBank database and assigned accession no. GQ914773.

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REFERENCES


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