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Identification of New Parvovirus DNA Sequence in Swine Sera from Myanmar

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Parvoviruses are among the smallest viruses of both vertebrates and invertebrates. During an attempt to identify the hepatitis E virus (HEV) genome, we accidentally PCR-amplified a parvovirus DNA sequence in swine sera from Myanmar. The sequence was only distantly related to the

previously reported porcine parvovirus (1).

Eighty-six swine sera samples were collected in Myanmar. Nucleic acids were extracted from 50 µl of sera with SMITEST ExR&D (Genome Science Laboratories, Fukushima) and reverse-transcribed using random hexamers with SuperscriptIII RNaseH⁻ Reverse Transcriptase (Invitrogen, Carlsbad, Calif., USA). Then cDNA was subjected to a PCR with TaKaRa ExTaq (TaKaRa, Shiga) and primers HE5-1 and HE5-2 (2).

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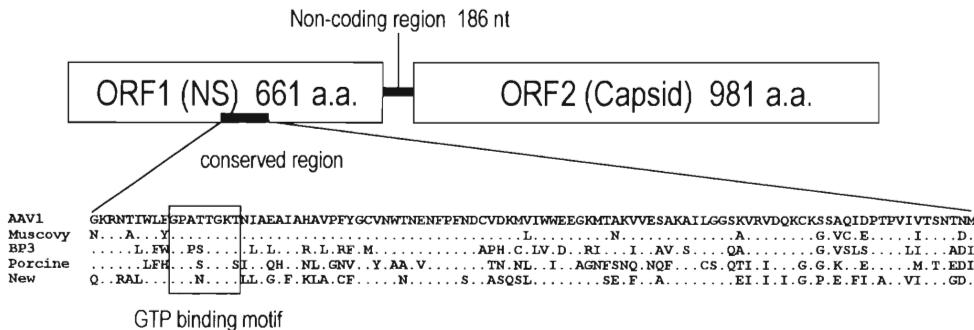


Fig. 1. Overview of the ORFs and sequence alignment of the region conserved in every vertebrate parvovirus nonstructural protein. AAV1, adeno-associated virus 1 (GenBank accession no. AF063497); Muscovy, Muscovy duck parvovirus (X75093); BP3, bovine parvovirus 3 (AF406967); Porcine, porcine parvovirus (M38367); New, the sequence obtained in the present study.

Thermal cycling condition was (95°C 1 min) + (95°C 15 sec, 55°C 30 sec, 72°C 45 sec) × 30 cycles + (72°C 7 min). PCR products were electrophoresed in 2% agarose gels. The expected length of the PCR product for the HEV sequence was 542 bp. However, none of the samples was positive for HEV, and a strong band of approximately 380 bp was observed in three samples. These PCR products were purified with Wizard PCR preps DNA Purification system (Promega, Madison, Wis., USA) and directly sequenced using a BigDye terminator cycle sequencing FS ready reaction kit (Applied Biosystems, Foster City, Calif., USA). The obtained sequence was 339 nucleotides in length and was not identical to any sequences in the database, based a BLAST search (DNA Data Bank of Japan [DDBJ], Shizuoka), and the most closely related sequence was a helicase sequence of adeno-associated virus 4 (GenBank accession no. U89790).

Primers Q1 (5'-GCG CAT TCG CCA AAC TAG CTC-3') and Q2 (5'-GTT TGC CCT TAA TGT CGA TCC-3') were designed based on the obtained sequence, and PCR was carried out without reverse transcription. Nine of the 86 (10.5%) samples were positive. When the extracted nucleic acids were digested with Mung Bean nuclease (TaKaRa) according to the manufacturer's instructions, the sequence could not be amplified. These results suggested that the PCR template was a single-stranded DNA.

A sequence of a total of 5 kb was amplified from a sample by three overlapping PCR reactions with TaKaRa LA Taq (TaKaRa), using primer set Q1 and A2 (5'-ATG TGG AAT CGC AAT GCC AAT-3'), primer set Q2 and A1 (5'-CGA GCT GCA GTG GGC GTG GAC-3'), and primer set Q19 (5'-GGT ACG ACG AGC TCA TCA-3') and Q17 (5'-CCA GAT ATC GTC CTC GTA-3'). Two large open reading frames were found in the 5 kb-sequence (Fig. 1). ORF 1 coded 661 amino acids, and the protein sequence was most closely related to nonstructural protein 1 of Muscovy duck parvovirus (S52209) by a FASTA search (DDBJ). ORF 2 coded 981 amino acids and the protein sequence was most closely related to capsid protein of bovine parvovirus 3 (AF406967) by a FASTA search. In the middle of ORF 1, the helicase sequence, which is most conserved in vertebrate parvoviruses, was found (Fig. 1).

The 5' and 3' non-coding regions including inverted terminal repeats were not amplified in the present study.

Based on the homologies as described above, the sequence was provisionally named porcine parvovirus 2 sequence, though the sequence was only distantly related to the previously reported porcine parvovirus (1). Biochemical properties of virion, its infectivity and pathogenic nature should be determined in the future.

Recently, two novel bovine parvoviruses were discovered from bovine sera that had been used as diluent while attempting to find hepatitis viruses (3). In this study, we found a novel DNA sequence related to parvovirus from swine sera while attempting to amplify an HEV sequence. These unexpected amplifications of parvovirus DNA may indicate that the advent of PCR technology accelerates discoveries of previously unknown viral genomes.

The nucleotide sequence reported in this paper will appear in DDBJ/GenBank/EMBL databases under the accession number AB076669.

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