Complete Genome Sequence of a Novel Picornavirus, Canine Picornavirus, Discovered in Dogs

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We discovered a novel canine picornavirus in fecal, nasopharyngeal, and urine samples from dogs. The coding potential of its genome (5'-VP4-VP2-VP3-VP1-2A-2B-2C-3A-3B-3Cpro-3Dpol-3', where 3Cpro is 3C protease and 3Dpol is 3D polymerase) is similar to those of other picornaviruses, with putative P1, P2, and P3 sharing 54% to 58%, 60%, and 64% to 67% amino acid identities with bat picornavirus groups 1, 2, and 3.

Picornaviruses are positive-sense, single-stranded RNA viruses found in humans and a wide variety of animals (3, 5). Based on genotypic and serological characterization, picornaviruses are currently divided into 12 genera. In the last few years, there has been a surge in the number of novel picornaviruses being discovered and in the number of their genomes being sequenced, including those of three avian picornaviruses, three bat picornaviruses, and one feline picornavirus discovered in our previous studies carried out in Hong Kong (1, 2, 7). We also recently reported the discovery of a novel picornavirus-like virus, canine picodistrovirus, in the picornavirus-like superfamily, with two functional internal ribosomal entry sites (IRES), in dogs (6). During the process of this molecular epidemiology study of picornaviruses in dogs, we discovered a novel picornavirus in fecal, nasopharyngeal, and urine samples from dogs (6). We proposed that this virus be named canine picornavirus (CanPV).

The complete genome of CanPV was amplified and sequenced with an EZ1 virus minikit (Qiagen, Germany), using RNA extracted from the fecal swab of a dog positive for CanPV as a template. RNA was converted to cDNA by a combined random-priming and oligo(dT)-priming strategy. The genome was sequenced by genome walking using degenerate primers and additional primers designed from the results of each round of sequencing (1, 2, 4, 6–9). DNA sequencing was performed using an ABI Prism 3700 DNA analyzer (Applied Biosystems, USA). The 5' end of the viral genome was confirmed by rapid amplification of cDNA ends (RACE) using a SMARTer RACE cDNA amplification kit (Clontech, USA). Sequences were assembled and manually edited to produce the final genome sequence (1, 2, 4, 6–9).

The genome size of CanPV is 7,948 bases, with a G+C content of 41.0% after exclusion of the polyadenylated tract. The genome organization is similar to that of other picornaviruses, with the characteristic gene order 5'-VP4-VP2-VP3-VP1-2A-2B-2C-3A-3B-3Cpro-3Dpol-3' (3Cpro and 3Dpol are 3C protease and 3D polymerase, respectively). Both the 5' (668 bases) and 3' (143 bases) ends of the genome contain untranslated regions (UTRs). The genome contains a single open reading frame of 7,137 bases, which encodes a polyprotein precursor of 2,378 amino acids. In the 5' UTR, the conserved sequence Y15-X83-AUG and a GNRA sequence, a motif conserved among picornavirus IRES, are present. The IRES of CanPV conforms to the structure of type I IRES. A leader protein (L) is present, but it does not possess the characteristic catalytic amino acid residues with proteolytic activity. The 2A of CanPV possesses the characteristic catalytic amino acid residues with trypsin-like proteolytic activity, and its 2C possesses the GXXGXGKS motif for nucleoside triphosphate (NTP) binding and the DDLXQ motif for putative helicase activity. The 3Cpro of CanPV contains the catalytic triad His-Asp-Cys. Like 3Dpol, it contains the conserved KDE(LI)R, GG(LMN)PSG, YGDD, and FLKR motifs. Phylogenetically, CanPV is most closely related to bat picornavirus groups 1, 2, and 3, which we reported previously, with 54% to 58%, 60%, and 64% to 67% amino acid identities between the P1, P2, and P3 of CanPV and those of the three bat picornaviruses, suggesting that they may form a novel genus in Picornaviridae.

Nucleotide sequence accession number. The complete genome sequence of CanPV (strain 325F) has been deposited in GenBank under accession no. JN831356.

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