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GENOME ANNOUNCEMENT

Complete Genome Sequences of Novel Canine Noroviruses in Hong Kong

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We report the complete genome sequences of two novel isolates of norovirus isolated from the fecal swab specimens of dogs in Hong Kong. The complete viral genome is approximately 7.6 kb in length and consists of 3 overlapping open reading frames encoding the ORF1 polyprotein, VP1, and VP2, respectively. Analysis of the VP1 sequence suggested that these noroviruses are divergent from known noroviruses and may represent a novel phylogenetic clade within the genus.

Noroviruses (NoVs) are single-stranded positive-sense RNA viruses in the family *Caliciviridae* and are the most important nonbacterial cause of human gastroenteritis worldwide. NoVs are notorious for causing outbreaks, and transmission can occur through a number of routes, including food, the environment, and presumably personal contact (1). They are also an important cause of sporadic gastroenteritis in nonoutbreak settings (2).

There are 5 recognized NoV genogroups (GI to GV), with subdivision into clusters and strains, based on the complete amino acid sequence of VP1 (12). Human NoVs are found in GI, GII, and GIV, while animal NoVs are distributed among different genogroups. Specifically, bovine NoVs are found in GIII, swine NoVs in GII (clusters GII.11, GII.18, and GII.19), and murine NoVs in GV. Canine NoVs were first reported in Italy in 2008 (5) and have since been found in other European countries (4, 6–9). So far, all canine NoVs belong to GIV and the tentative new GVI (7).

As part of a surveillance study of animal viruses, we discovered a novel NoV strain in two canine fecal swab specimens. Complete genome sequences were obtained using previously described strategies for single-stranded RNA viruses (10, 11). Viral RNA was extracted by using the QIAamp RNA blood minikit (QIAgen, Hilden, Germany). Reverse transcription was performed using a SuperScript III kit (Invitrogen, San Diego, CA) according to the manufacturer's instructions for first-strand synthesis. The first-round PCR primers were designed based on the multiple alignment of genomes of other NoVs, while additional primers were designed based on results from previous rounds of genome sequencing. The complete set of primer sequences is available upon request. Sequences from the ends of the viral genomes were obtained by random amplification of cDNA ends (RACE) using a SMARTer RACE cDNA amplification kit (Clontech, Mountain View, CA). PCR products were sequenced twice with an ABI Prism 3730xl DNA analyser (Applied Biosystems, Foster City, CA) using PCR primers. Sequence assembly was performed manually using the software program BioEdit 7.0.9.

The genomes of the present NoVs are 7,637 bases long, with a G+C content of 56.4%. The two genomes are highly similar, with sequence identity of 99.9% and only 7 base differences. The genome organization is similar to that of other NoVs, with 3 overlapping open reading frames encoding the ORF1 polyprotein (1,691 amino acids [aa]), VP1 capsid protein (528 aa), and VP2 minor structural protein (279 aa). The 5′ and 3′ untranslated regions are 4 bases and 151 bases long, respectively. Analysis of the complete VP1 amino acid sequence showed the isolates to be distantly related to other NoVs, including other canine NoVs, with highest sequence identity (51.6%) to an intergenotype GII recombinant human NoV strain (3). The low level of sequence identity suggests that the isolates potentially belong to a novel NoV genogroup, though the classification warrants further sequence and phylogenetic analysis (12).

Nucleotide sequence accession numbers. The complete genomes of the novel canine NoV have been submitted to GenBank under accession no. FJ692500 and FJ692501.

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REFERENCES

1. **Becker KM, Moe CL, Southwick KL, MacCormack JN.** 2000. Transmission of Norwalk virus during football game. N. Engl. J. Med. **343**:1223–1227.
2. **Cheng VC, et al.** 2011. Prevention of nosocomial transmission of norovirus by strategic infection control measures. Infect. Control Hosp. Epidemiol. **32**:229–237.

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3. **Chhabra P, Walimbe AM, Chitambar SD.** 2010. Molecular characterization of three novel intergenotype norovirus GII recombinant strains from western India. Virus Res. **147**:242–246.
4. **Martella V, et al.** 2009. Genetic heterogeneity and recombination in canine noroviruses. J. Virol. **83**:11391–11396.
5. **Martella V, et al.** 2008. Detection and molecular characterization of a canine norovirus. Emerg. Infect. Dis. **14**:1306–1308.
6. **Martella V, Pinto P, Buonavoglia C.** 2011. Canine noroviruses. Vet. Clin. North Am. Small Anim. Pract. **41**:1171–1181.
7. **Mesquita JR, Barclay L, Nascimento MS, Vinje J.** 2010. Novel norovirus in dogs with diarrhea. Emerg. Infect. Dis. **16**:980–982.
8. **Mesquita JR, Nascimento MS.** 11 December 2011. Gastroenteritis outbreak associated with faecal shedding of canine norovirus in a Portuguese kennel following introduction of imported dogs from Russia. Transbound. Emerg. Dis. [Epub ahead of print.] doi:10.1111/j.1865-1682.2011.01284.x.
9. **Ntafis V, Xylouri E, Radogna A, Buonavoglia C, Martella V.** 2010. Outbreak of canine norovirus infection in young dogs. J. Clin. Microbiol. **48**:2605–2608.
10. **Tse H, et al.** 2011. Rediscovery and genomic characterization of bovine astroviruses. J. Gen. Virol. **92**:1888–1898.
11. **Woo PC, et al.** 2012. Natural occurrence and characterization of two internal ribosome entry site elements in a novel virus, canine picodicistrovirus, in the picornavirus-like superfamily. J. Virol. **86**:2797–2808.
12. **Zheng DP, et al.** 2006. Norovirus classification and proposed strain nomenclature. Virology **346**:312–323.