

Complete Genome of the Human Norovirus GIV.1 Strain Lake Macquarie Virus

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Norovirus is an important human pathogen that is now recognized as the leading cause of acute gastroenteritis globally. Six viral genogroups have been described, although only genogroups GI, GII, and GIV are known to infect humans, with the GII viruses most commonly identified in both outbreak and sporadic settings. In contrast, infections by GIV viruses are rarely reported, and their overall prevalence in the community is unknown. Here, we report the complete genome sequence of the human GIV.1 strain Lake Macquarie virus, which caused two linked outbreaks of acute gastroenteritis in aged-care facilities in the Hunter region of New South Wales, Australia. The Lake Macquarie virus genome was 7,527 nucleotides (nt) in length and shared highest identity (70%) with the recently completed feline GIV.2 virus genome.

Norovirus (NoV), of the family *Caliciviridae*, is the leading cause of acute gastroenteritis globally (8). A genetically diverse RNA virus, NoV is classified into six genogroups (GI to GVI) (7, 12), which are further divided into at least 36 genotypes (5, 12). The genogroup II, genotype 4 (GII.4) viruses cause 62 to 80% of NoV outbreaks globally (2, 10) and have been associated with five pandemics of acute gastroenteritis (3, 10). In contrast, GIV.1 strains are rarely detected in outbreaks and are generally identified only in sporadic infections or environmental studies (4, 6). Little is known about the origins of the GIV viruses, partly due to a lack of sequence data. Only 50 partial sequences for the human GIV.1 viruses are available in GenBank, and the genogroup is represented by only a single full-length feline GIV.2 genome (9).

During routine surveillance, two NoV-associated outbreaks of acute gastroenteritis were investigated from aged-care facilities in the Hunter region of New South Wales, Australia. One sample from each outbreak was initially genotyped using a reverse transcription-PCR (RT-PCR) targeting the NoV GI and GII capsid gene (3). Both outbreak samples produced weak amplicons using the NoV GII primers. Sequencing revealed that the cause of each outbreak was a virus closely related (99%, 266-bp partial ORF2) to the NoV GIV.1 strain Italy-980/2007/IT (GenBank accession number [FM865412](#)).

Since no full-length NoV GIV.1 genome was available in GenBank, we sequenced the genome from one of our GIV.1 outbreak strains. cDNA was prepared from viral RNA using a tagged oligo(dT) primer that bound to the viral poly(A) tail. In order to amplify ORF1, a forward primer was designed using the sequence at the start of the subgenomic RNA near the ORF1/2 overlap, since in NoV and other caliciviruses, this region is identical to the first 18 nucleotides (nt) of the genome. This primer was then used with a reverse primer located within ORF2. The ORF2/3 region was amplified using a forward primer targeting the end of ORF1 and a reverse primer complementary to the oligo(dT) primer tag sequence. This strategy generated two amplicons, of 5.4 kb (ORF1) and 2.5 kb (ORF2/3), which provided complete genome coverage. Amplicons were sequenced by primer walking using dye-terminator chemistry, and the first 18 nt of the genome was confirmed by

5' rapid amplification of cDNA ends (5'-RACE) PCR. Sequence reads were assembled using MEGA5 (11).

Overall, the Lake Macquarie virus genome was 7,527 nt in length excluding the poly(A) tail and shared highest identity (70%) with the recently completed feline GIV.2 virus genome CU081210E/2010/US (GenBank accession number [JF781268](#)). Similar to other human NoVs, the genome was divided into three open reading frames: nucleotide positions 5 to 5068 (ORF1), 5049 to 6719 (ORF2), and 6719 to 7447 (ORF3). The ORF1-encoded polyprotein contained five predicted cleavage sites at Q³²³/G³²⁴ (NS1-NS2/3), Q⁶⁸⁸/G⁶⁸⁹ (NS3/4), E⁸⁶²/G⁸⁶³ (NS4/5), E⁹⁹⁶/A⁹⁹⁷ (NS5/6), and E¹¹⁷⁷/G¹¹⁷⁸ (NS6/7), which would be expected to yield the same nonstructural proteins as the GII.4 viruses (1).

Nucleotide sequence accession number. The genome sequence of the human NoV GIV.1 strain Lake Macquarie virus was deposited in GenBank with the accession number [JQ613567](#).

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