




Characterization of the Genome Sequences of Enterovirus C109 from Two Respiratory Disease Cases in Florida, 2016

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ABSTRACT The genomic sequences of two enterovirus C109 isolates (EV-C109 USA/FL/2016-21003 and EV-C109 USA/FL/2016-21002) were obtained during two separate case investigations of respiratory disease in two children. This marks the first description of EV-C109 genomes in the United States.

Enterovirus species C (EV-C) members are in the *Enterovirus* (EV) genus in the *Picornaviridae* family. Currently, there are 23 types of EV-C, which are responsible for a spectrum of human diseases, including respiratory infections and central nervous system diseases. Enterovirus C109 (EV-C109) was first reported in 2010 in a Nicaraguan child with acute respiratory illness during 2008 (1) and was subsequently reported from respiratory samples in Hungary (2), Italy (3), the Netherlands (4), and Denmark (5), as well as from fecal specimens in the Republic of the Congo (6). Currently, only two published sequences contain the complete coding region; these are the prototype strain (Nicaragua/2008, GenBank accession no. [GQ865517](https://doi.org/10.1093/genbank/GQ865517)) and a Hungarian strain (Hungary/2007, GenBank accession no. [JN900470](https://doi.org/10.1093/genbank/JN900470)).

During February and June of 2016, we investigated two separate cases of respiratory disease in two children from Florida, both aged 5 years, with symptoms of fever, cough, and sore throat. Samples from both children were submitted to the Bureau of Public Health Laboratories Tampa Laboratory as outpatient surveillance submissions from the Influenza Incidence Surveillance Program. Nucleic acids extracted from nasopharyngeal (NP) swabs tested positive for enterovirus using pan-enterovirus real-time reverse transcription-PCR (7).

Sanger sequencing targeting the VP1 region (8) identified EV-C109 in both patients, the first two cases detected in the United States. We obtained full genomic sequences of EV-C109 using published protocols (9, 10); in brief, we performed random reverse transcription and PCR amplification, followed by Nextera XT (Illumina) library production and MiSeq reagent V2 (Illumina) sequencing (9). Bioinformatics analysis was conducted with an in-house next-generation sequencing (NGS) pipeline (10). The NGS efficiency (viral reads/total reads) was 7 to 42%; as expected, the efficiency is lower for an NP specimen than for an EV culture (10). The *de novo* assembly efficiency metric UG₅₀% (a metric for comparing assembly results from different samples or studies) (11) was 100%, demonstrating direct full-genome assembly.

EV-C109 USA/FL/2016-21002 and EV-C109 USA/FL/2016-21003 (GenBank accession no. [MH128992](https://doi.org/10.1093/genbank/MH128992) and [MH128993](https://doi.org/10.1093/genbank/MH128993), respectively) are 7,336 and 7,327 nucleotides long, respectively. Compared to the prototype, these two genomes are complete in the 3' nontranslated region (NTR) and missing 17 to 28 nucleotides from the 5' NTR. Our EV-C109 genomes from 2016 share 98% genome-wide nucleotide identity (NI) with

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each other; they share 92% NI with the prototype (Nicaragua/2008, [GQ865517](#)) and the Hungarian strain (Hungary/2007, [JN900470](#)).

Complete VP1 sequences from the Florida/2016 strains share higher NI with each other (98%) than with recent Danish strains (97%; Denmark/2015, GenBank accession no. [KX901640](#)) or to the earlier Hungarian and Nicaraguan strains (91 to 93%). Comparisons of the polyprotein and VP1 regions showed that the two Florida/2016 strains share high amino acid identity (AI) (99.5% polyprotein; 99.7% VP1) with each other and slightly lower AI with the two reference strains (98.2 to 98.5% for polyprotein and 97.0% to 98.0% for VP1). Comparison of the VP1 region of both strains to that of the prototype Nicaragua/2008 showed nonsynonymous substitutions of V25A, S140G, C144S, C145S, and N281K.

The prototype EV-C109 genome was first identified as an EV-C strain harboring a 5' NTR most closely related to an enterovirus A (EV-A) 5' NTR (1), which is attributed to undated recombination events between ancestral strains of both species. The Florida/2016 strains contain no detectable recombinant sites compared to Nicaragua/2008 and Hungary/2007.

Data availability. The sequences of EV-C109 USA/FL/2016-21002 and EV-C109 USA/FL/2016-21003 have been deposited in GenBank under accession no. [MH128992](#) and [MH128993](#), respectively.

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