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Circulation of Enterovirus D68 during Period of Increased Influenza-Like Illness, Maryland, USA, 2021

Appendix

Patients' Clinical Data

Demographic and clinical data were collected through bulk query from the Johns Hopkins Hospital electronic medical record system. Influenza-like illness encounters were defined based on the following criteria: all emergency department (ED) visits (regardless of age across the entire system) where: (1) patients presented with a chief report of upper respiratory infection (URI), pneumonia, influenza, flu-like symptoms, cough, or fever and sore throat (2) ED diagnosis of B97.89 (other viral agents as the cause of diseases classified elsewhere). Diagnosis codes are detailed in Appendix Table 1. Clinical data presented in Appendix Table 2 were collected by detailed manual chart reviews from the electronic patients' records.

Specimens and Diagnostic Testing

The Johns Hopkins Microbiology laboratory serves a wide geographic area that includes Baltimore, Virginia, and Washington, DC. Testing for enterovirus/rhinovirus is performed as a part of multiplex respiratory viral panels using the GenMark ePlex RP1 and RP2. These panels do not differentiate between enteroviruses and rhinoviruses (Figure, https://wwwnc.cdc.gov/EID/article/28/7/21-2603-F1.htm) and detect Adenovirus, HCoV-229E, HCoV-HKU1, HCoV-NL63, HCoV-OC43, human Metapneumovirus (HMPV), enterovirus/rhinovirus, influenza A/A H1/A H1–2009/A H3, influenza B, parainfluenza (HPIV)1–4, RSV A, RSV B, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae* in addition to SARS-COV-2 (in RP2 only) (*1*,*2*).

Genomic Sequencing for Genotyping

RNA was extracted from 300µL of clinical specimens using the Chemagic 360 system (Perkin Elmer) according to the manufacturer's specifications. RNA was eluted with 60µl nuclease-free water and stored at −80°C until use. cDNA synthesis has been performed using RevertAid First Strand cDNA Synthesis Kit (ThermoFisher Scientific) following the manufacturer's protocol. The amplification of the 5′ half of the genome (~4500 nt) was performed as previously described (*3*). PCR products were barcoded using the Native barcoding genomic DNA kit (EXP-NBD196) according to the manufacturer's instructions and sequenced using R9.4.1 flowcells on a GridION (Oxford Nanopore Technologies). For the FASTQ files analysis, low-quality reads were filtered, and adapters trimmed with Trimmomatic. Denovo assembly was performed using metaSPAdes. Identification of the enterovirus types was carried out with the RIVM genotyping tool (http://www.rivm.nl/mpf/enterovirus/typingtool/). Sequence alignment and phylogeny were performed using MEGA 7.0. The robustness of the ML tree was assessed by bootstrap analyses of 1,000 replicates and the Fermon strain, collected in 1962 was used to root the tree. The evolutionary distances were derived using the Tamura 3 parameter method. BLAST analysis showed >99% homology with strains detected in Europe in 2019 and with a bootstrap of 98%.

References

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Appendix Table 1. Diagnosis codes used to categorize influenza-like illness encounters (*4*)

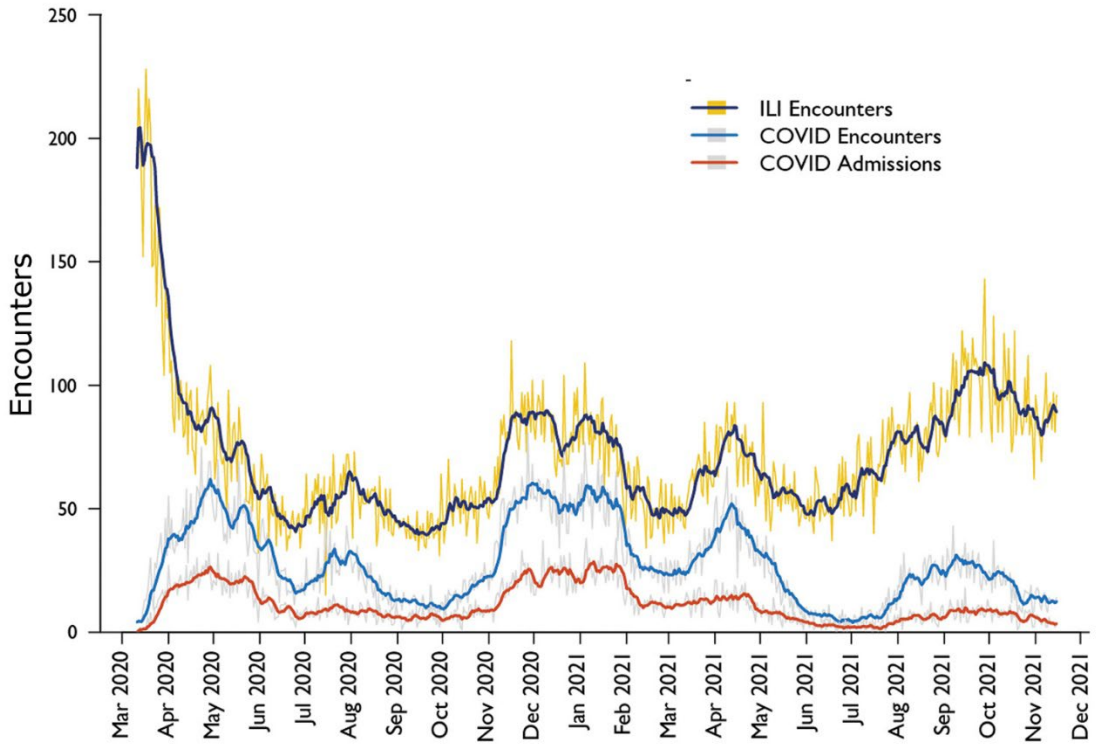
Code	Diagnosis
B97.89	Other viral agents as the cause of diseases classified elsewhere
H66.9	Otitis media, unspecified
J06.9	Acute upper respiratory infection, unspecified
J00	Acute nasopharyngitis; common cold
J01.9	Acute sinusitis, unspecified
J09.X	Influenza due to identified novel influenza A viruses
J10.0	Influenza due to identified novel influenza A viruses
J10.1	Influenza due to other identified influenza virus with other respiratory manifestations
J10.2	Influenza due to other identified influenza virus with gastrointestinal manifestations
J10.8	Influenza due to other identified influenza virus with other manifestations
J11	Influenza due to unidentified influenza virus
J12.89	Other viral pneumonia
J12.9	Viral pneumonia, unspecified
J18	Pneumonia, unspecified organism
J20.9	Acute bronchitis, unspecified
J40	Bronchitis, not specified as acute or chronic
R05	Cough
R50.9	Fever, unspecified
J22	Unspecified acute lower respiratory infection
B34[0–9]	Viral infection of unspecified site [adenovirus, enterovirus, coronavirus, parvovirus, papovavirus, other viral infections
U07.1	COVID-19
R68.89	Diagnosis reported 'Flu-like symptoms' or 'Influenza-like symptom

Appendix Table 2. Clinical and demographic characters of enterovirus infected patients in September and October 2021

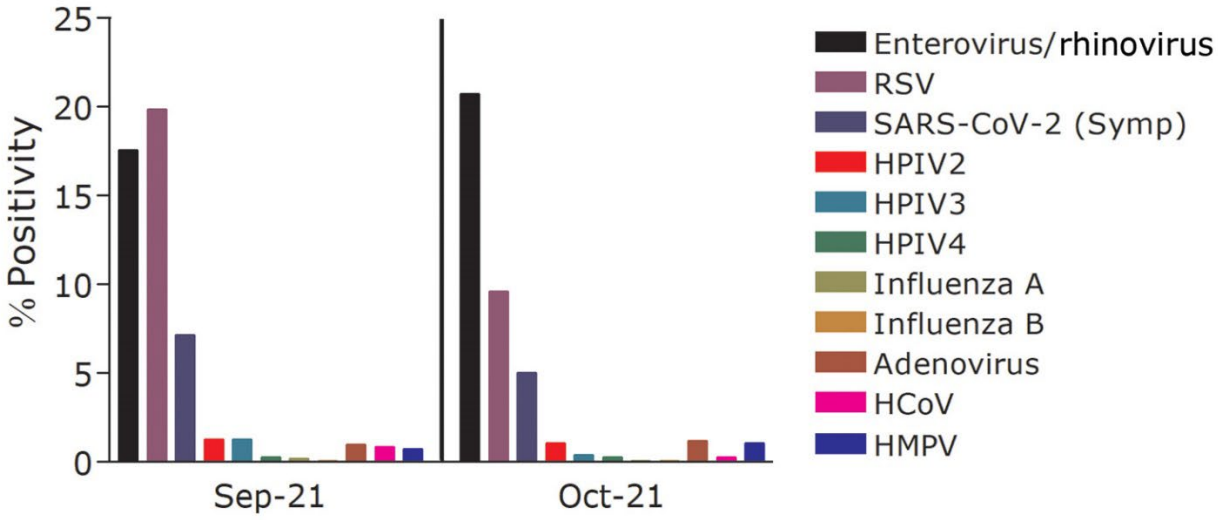
Genome ID	Enterovirus	Age range, y	Gender	Respiratory/viral Complaint	Admitted	ICU	Supplemental Oxygen
JH-EV-0001/2021	EV-D68	<1	Female	Breathing problem	Yes	Yes	Yes
JH-EV-0002/2021	EV-D68	45–48	Male				
JH-EV-0003/2021	EV-D68	<1	Female		Yes	Yes	Yes
JH-EV-0004/2021	EV-D68	8–10	Male	Viral illness	Yes	Yes	Yes
JH-EV-0005/2021	EV-D68	23–25	Female				

Genome ID	Enterovirus	Age range, y	Gender	Respiratory/viral Complaint	Admitted	ICU	Supplemental Oxygen
JH-EV-0006/2021	EV-D68	1–3	Male	Rhinorrhea			
JH-EV-0007/2021	EV-D68	<1	Male	Cough, Bronchiolitis, Acute respiratory distress	Yes		Yes
JH-EV-0008/2021	EV-D68	4–6	Female	Fever, loss of appetite, cough, runny nose			
JH-EV-0009/2021	EV-D68	<1	Female	Fever, irritability, congestion, rhinorrhea, sneezing, cough			
JH-EV-0010/2021	EV-D68	1–3	Male	Cough, rash			
JH-EV-0011/2021	EV-D68	1–3	Female	Respiratory distress, viral pneumonia, enterovirus bronchiolitis	Yes	Yes	Yes
JH-EV-0012/2021	EV-D68	1–3	Male	Cough and nasal congestion			
JH-EV-0013/2021	EV-D68	1–3	Male	Fever, productive cough, runny nose			
JH-EV-0014/2021	EV-D68	<1	Female	Nasal congestion, cough			
JH-EV-0015/2021	EV-D68	1–3	Male	Congestion and cough			
JH-EV-0016/2021	EV-D68	1–3	Male	Cough, rhinorrhea, fever, respiratory distress, pneumonia	Yes	Yes	Yes
JH-EV-0017/2021	EV-D68	1–3	Female	Nasal congestion and fever			
JH-EV-0018/2021	CV-A6	32–35	Male	Rash			

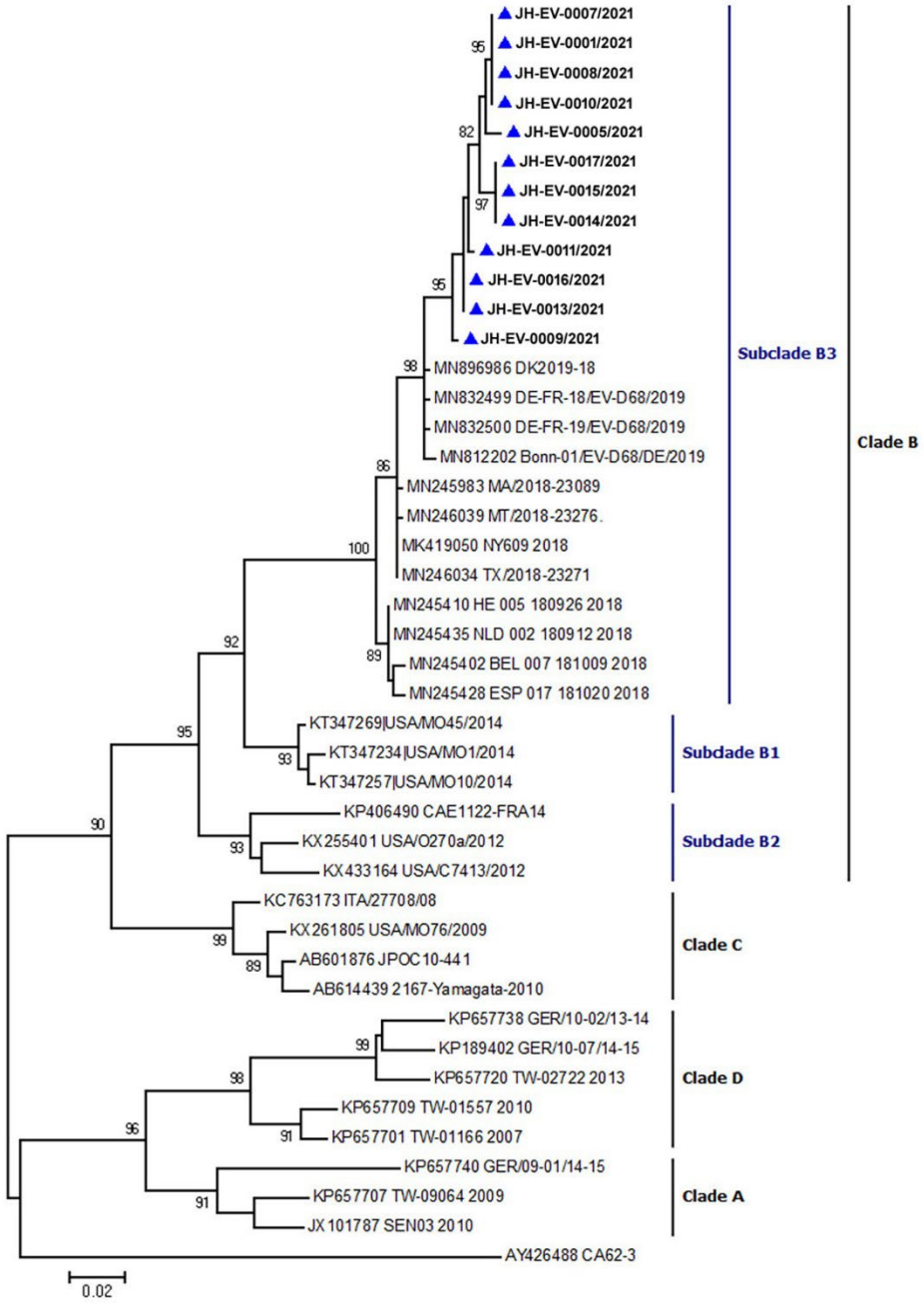
*ICU, intensive care unit.



Appendix Figure 1. Encounters of influenza-like illness (ILI), COVID-19 encounters, and admissions since the beginning of the COVID-19 pandemic at Johns Hopkins Hospital system.



Appendix Figure 2. Positivity of respiratory viral targets at Johns Hopkins Medical Microbiology laboratory in September and October 2021. HPIV (human parainfluenza virus), RSV (respiratory syncytial virus), HMPV (human metapneumovirus), SARS-CoV-2 (symp) indicates symptomatic testing positivity.



Appendix Figure 3. Phylogenetic relationships of EV-D68 strains identified from the Johns Hopkins Medical Microbiology laboratory between September and October 2021 (marked with blue triangles). The phylogenetic tree constructed on complete 5′ half of the genome of EV-D68 strains was generated using the Maximum Likelihood method based on the using the Tamura 3 parameter method in MEGA7. The phylogenetic tree is rooted by the oldest EV-D68 sequence in GenBank, the Fermon strain. We performed 1,000 bootstrap replicates to determine the consensus tree; support for nodes present in >70% of the trees are annotated.