ENHANCED ENTEROVIRUS SURVEILLANCE IN NEW YORK STATE FOLLOWING A CONFIRMED POLIOMYELITIS CASE IN JULY 2022

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NEW YORK STATE | Department of Health | Wadsworth Center

Introduction

- In July 2022, the Wadsworth Center detected poliovirus type 2 in the stool of an unvaccinated hospitalized patient with paralysis, **see figure 1**.
  - CDC confirmed vaccine-derived poliovirus, Sabin-like virus type 2 (VDPV2).
  - No relevant travel history, implying community acquired VDPV2.
- Wastewater surveillance detected further community VDPV2 transmission within some New York State counties, **see figure 2**.
- To help investigate the extent of virus circulation, the New York State Department of Health initiated an enhanced enterovirus surveillance program.

Enterovirus VP1 Region Gel Electrophoresis

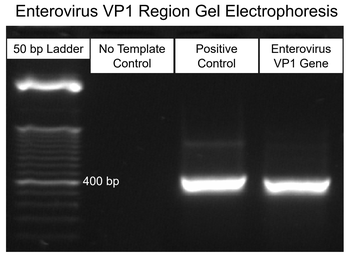

Figure 1. PCR-amplified VP1 region of poliovirus Sabin type 2 in patient sample identified using gel electrophoresis.

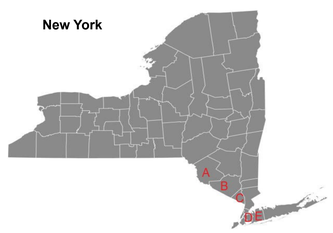

Figure 2. Counties with detections of poliovirus type 2 genetically linked to the virus isolated from the case are indicated in red: Sullivan (A), Orange (B), Rockland (C), Kings and Queens (D), and Nassau (E).

https://www.health.ny.gov/diseases/communicable/polio/docs/waste_water_surveillance_report.pdf

Methods

Testing Strategy

- The preferred sample type was stool, but cerebrospinal fluid (CSF) and respiratory swabs were also received and tested.
- Samples were extracted on the bioMerieux easyMAG® or EMAG®.
- cDNA synthesis was performed using the Quanta qScript™ cDNA Synthesis Kit.
- Real-time RT-PCR for initial enterovirus (EV) detection was performed on Applied Biosystems™ 7500 Dx Real-time PCR System.
- All real-time EV positive samples were reflexed to molecular serotyping by semi-nested conventional PCR, followed by sequencing of a portion of the VP1 gene (modified from Nix *et al.*) from either the 1st or 2nd round of amplification.
- NCBI BLAST analysis of the sequence was used to determine molecular serotype.

Surveillance Strategy

- **Patients Under Investigation (PUIs) included:**
  - Any patient with mild polio-like symptoms, unimmunized or incompletely immunized, who lived or worked in areas of wastewater positivity or low vaccination rates AND tested positive for EV.
  - Any patient with symptoms consistent with acute flaccid myelitis (AFM).
  - EV-positive meningitis cases from counties with polio positive wastewater or low vaccine rates.
- **Diaper Study Patient samples included:**
  - Stool collected from healthy diapered patients at pediatrician well visits.
  - Participants from targeted counties with wastewater positivity.
- **Routine Enterovirus Surveillance included:**
  - Special cases and autopsy samples that tested positive for EV.
  - Enterovirus/rhinovirus positive samples detected on sample-to-answer multiplexed panels that do not distinguish those two viruses.
  - EV testing on hospitalized patients with encephalitis.

Results

Total Enterovirus Real-Time RT-PCR Positive Samples, July 2022-January 2024

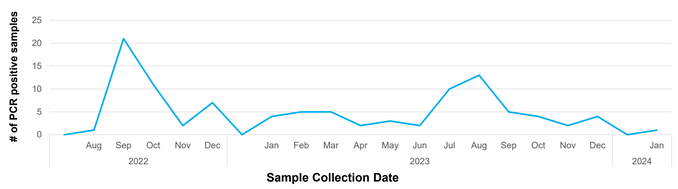

Figure 3. Total number of clinical samples confirmed enterovirus RT-PCR positive at Wadsworth; July 2022-January 2024.

Total Enterovirus Serotypes, by Sample Matrix

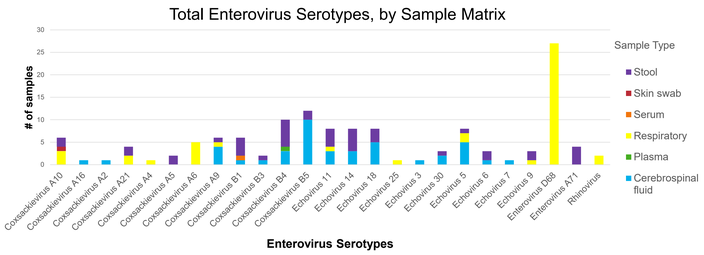

Figure 4. Enterovirus serotypes detected by sample type. EV-D68 was the only serotype exclusively detected in respiratory samples during the surveillance period.

Enterovirus Serotypes from PUIs

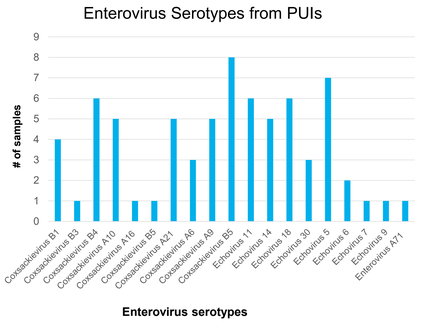

Figure 5. Enterovirus serotypes identified from symptomatic enterovirus surveillance (PUIs). Non-polio enteroviruses accounted for all enterovirus positive cases during the surveillance period.

Vaccination Statuses of PUIs

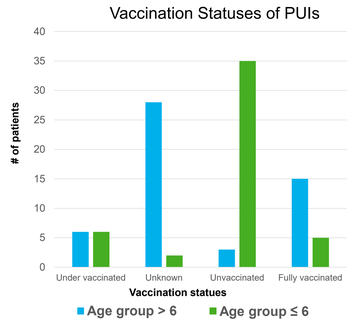

Figure 6. Poliovirus vaccination status across PUIs tested during the surveillance period.

Table 1. Enterovirus serotypes identified from asymptomatic Diaper Study samples.

	NYS Counties		
Enterovirus Serotype	**Orange**	**Rockland**	**Total**
Coxsackievirus A5	2		2
Echovirus 14	1	1	2
Echovirus 6	2		2
Echovirus 9	1		1
Enterovirus A71	1		1
Not Detected	15	9	24
Inconclusive	4		4
TOTAL	26	10	36

Results Continued

VP1 Phylogenetic Comparison of Coxsackievirus B4 Samples

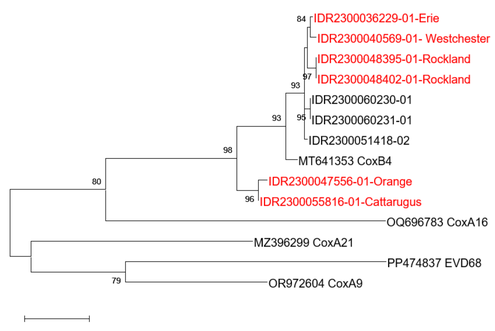

Figure 7. Partial VP1 sequence analysis of CoxB4 samples compared to EV-A (CoxA16), EV-B (CoxB4 & CoxA9), EV-C (CoxA21), and EV-D (EVD68). Evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model (Kimura *et al.*). Percentage of trees in which the associated taxa clustered together is shown next to the branches; values <70 not displayed. There were a total of 220 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.*).

Reduced sensitivity of the VP1 sequencing assay identified variations at the 3' end of the amplicons in some of the CoxB4 samples (Red).

Overall Results

- A total of 445 samples from 368 patients were tested.
- In 60 samples from 91 PUIs a variety of enterovirus serotypes were identified.
- Eight of 36 stool samples from asymptomatic children were enterovirus positive.
- An additional 279 samples were tested under routine surveillance procedures and 63 were enterovirus positive.
- Among EV-positive samples: 20 were species A, 80 species B, 4 species C (non-polio), and 27 species D; but 0 additional polioviruses were identified.
- 15 Coxsackie B4 viruses in 2023 had sequence variations at the 3' end of the VP1 amplicon causing a mismatch and decreased sensitivity for the sequencing primer.

Conclusion

- Even though VDPV2 was detected in a symptomatic patient and wastewater surveillance showed community transmission in 2022, no additional cases of poliovirus were identified during subsequent human testing.
- Surveillance data demonstrated enteroviruses to have multiple tropisms except EV-D68, which was exclusively found in respiratory samples.
- In 2022, EV-D68 circulated statewide but was not detected in 2023, following a known pattern of 2-year epidemic waves.
- In 2023, Coxsackievirus B4 was widely circulating. Sequencing from the larger 1st round PCR product was at times necessary to overcome reduced sensitivity due to changes at the 3' end of the VP1 second round amplicon, identified by sequence analysis.
- Challenge to obtaining Diaper Study samples were low number of participants. However, the 25% EV-positivity rate in these asymptomatic patients was surprising.
- The enhanced EV surveillance improved communication between the Wadsworth Center Virology Laboratory and epidemiology colleagues, resulting in improved AFM surveillance testing in New York State.
- Wastewater surveillance for poliovirus is ongoing in New York. Poliovirus was last detected in a New York State sewershed sample in February of 2023.

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Acknowledgments

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