

PUBLIC HEALTH ALERTS | IN PARTNERSHIP WITH CIDRAP

Detection of a Single Measles Infection Using Untargeted Ultra-Deep Metagenomic Sequencing of Wastewater in Cook County, Illinois

Rachel S. Poretsky, Ph.D.,¹ Vineet K. Dhiman, Ph.D.,² Dylan L. Hendricks, M.S.,² Chi-Yu Lin, M.P.H.,¹ Dolores Sanchez Gonzalez,¹ Stephanie Greenwald,³ Sarah M. Owens, M.S.,³ Charles H. Williams, II, M.P.H.,² Matthew T. Leslie, D.V.M., Ph.D.,² Kelley Bemis, M.P.H.,⁴ Mabel Frias, M.P.H.,⁴ Jeff T. Kaufman,⁵ David H. O'Connor, Ph.D.,⁶ and Marc C. Johnson, Ph.D.⁷

Abstract

Measles is a contagious, vaccine-preventable viral disease that can be shed into wastewater by infected individuals. In September 2025, as part of an ongoing, nontargeted, ultra-deep metagenomic sequencing effort of wastewater in Cook County, Illinois, we detected measles reads from a facility serving more than 1 million people. Out of more than 900 million reads sequenced from wastewater collected on September 14, 2025, 43 matched measles virus genotype B3. Subsequent genomic analysis linked these reads to a confirmed measles infection that was present in the community on that day, demonstrating that untargeted metagenomics appeared to detect a single measles infection in a large municipal wastewater stream.

Introduction

In 2025, measles cases began surging in the United States and 14 people with measles were identified in Illinois. Wastewater monitoring has been shown to be promising for wild-type measles surveillance,¹ with wastewater detections preceding clinical case reporting.^{2,3} Its demonstrated utility has been based on targeted approaches that use quantitative polymerase chain reaction (PCR) or hybrid-capture sequencing to monitor specific pathogens, methods that require specific primers and/or probes. Further, the feasibility of detecting a single measles case in wastewater can be low.⁴ In contrast, metagenomic sequencing (MGS) can broadly capture multiple pathogens simultaneously without a priori knowledge of what might be present and with continually improving costs and turnaround times.^{5,6} Since October 2024, we have been carrying out weekly untargeted ultra-deep MGS of wastewater from 30 sites across 10 states, including four in the Chicago (Cook County), Illinois area as part of CASPER (Coalition for Agnostic Sequencing of Pathogens from Environmental Reservoirs), a wastewater MGS network for pathogen surveillance.⁶ In September 2025, we obtained wild-type measles reads in the MGS dataset from a Cook County, Illinois sample.

The author affiliations are listed at the end of the article.

Rachel S. Poretsky can be contacted at microbe@uic.edu.

Investigation and Outcomes

MGS data from samples collected on September 14, 2025, from a large wastewater treatment plant in Cook County, Illinois were obtained on October 3, 2025. Initial analysis using the NVD2 pipeline (<https://github.com/dhoconno/nvd>) revealed 43 measles genotype B3 MGS reads mapping to the M gene (matrix protein), H gene (receptor binding hemagglutinin), and L gene (polymerase). Upon detection of these reads, local public health departments were notified.

Also in September 2025, 5 months after a previous case was reported, the Cook County Department of Public Health confirmed measles in a second person. The patient was a suburban Cook County child who was not previously vaccinated and contracted the disease during international travel. Follow-up investigation revealed that the patient's home and the hospital at which they sought care lay within the catchment area of the positive wastewater sample. The patient was 2 days after onset, still febrile, and symptomatic on the day the wastewater sample was collected. No other measles cases were identified in the region at that time despite requests for heightened provider surveillance and daily analysis of syndromic surveillance. Although the possibility of unidentified cases occurring within the jurisdiction exists, we believe the likelihood of undetected circulation was low. Therefore, the detection of measles RNA through ultra-deep MGS of municipal wastewater appeared to capture a single case within the population of more than 1 million people and wastewater flow exceeding 300 million gallons per day, represented by approximately 0.000005% of nearly 1 billion sequencing reads.

To validate this finding, we used digital PCR-based quantification of wild-type measles⁷ on samples from September 7, 10, 14, and 21. Only the September 14 sample had detections (two positive partitions; 7425 gene copies/l of wastewater). This sample was then sequenced using a hybrid-capture approach (custom Qiagen xHyb panel), resulting in 6402 measles reads representing approximately 10% genome coverage to all but the N gene.

The patient's clinical specimen was sequenced by the Illinois Department of Public Health laboratory in Chicago. The Bacterial and Viral Bioinformatics Resource Center⁸ Variation Analysis service was used with BWA-mem alignment to identify single-nucleotide polymorphisms (SNPs) between the wastewater reads, the clinical isolate sequence, and a reference measles virus genotype B3 genome from a 2025 California clinical isolate determined by Basic Local Alignment Search Tool (BLAST) to be the closest match to this

Illinois isolate (GenBank accession PV686488). Wastewater reads from both the MGS and the hybrid capture datasets showed no SNPs relative to the clinical sequence with the exception of 3580T>A in all eight MGS reads covering this locus, likely an artifact because it occurred at the end of the read and is not present in any measles genome. In contrast, the clinical isolate had a SNP distance of 187 from closely related measles genomes, eight of which were covered by MGS reads with the same SNPs in genes M and L. Additionally, 19 SNPs spanning five genes (H, F, M, L, and P) had coverage and were present in the hybrid capture panel sequences. These data are consistent with the interpretation that the wastewater sequences originated from the single known clinical case.

Conclusions

This report indicates that ultra-deep MGS successfully detected measles virus with high specificity. Because the local health departments were already performing disease control activities related to this known patient with measles, there was no need to escalate current public health activities based upon the metagenome detection. However, this work suggests the potential utility of untargeted wastewater surveillance for identifying infectious disease.

Disclosures

Author disclosures are available at evidence.nejm.org.

The metagenomics work was supported by Inkfish LLC and Heart of Racing (D.H.O. and M.C.J.) and a gift from Coefficient Giving to SecureBio (J.T.K.). Wastewater surveillance in Chicago and Illinois was supported by the Chicago and Illinois Departments of Public Health (R.S.P., C.-Y.L., D.S.G., S.G., and S.M.O.).

Author Affiliations

¹Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL, USA

²Illinois Department of Public Health, Chicago, IL, USA

³Biosciences Division, Argonne National Laboratory, Lemont, IL, USA

⁴Cook County Department of Public Health, Forest Park, IL, USA

⁵SecureBio, Boston, MA, USA

⁶Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, WI, USA

⁷Department of Molecular Microbiology and Immunology, University of Missouri, Columbia, MO, USA

References

1. Wu J, Wang MX, Kalvapalle P, et al. Multiplexed detection, partitioning, and persistence of wild-type and vaccine strains of measles, mumps, and rubella viruses in wastewater. *Environ Sci Technol* 2024;58:21930-21941. DOI: [10.1021/acs.est.4c05344](https://doi.org/10.1021/acs.est.4c05344).

2. Jensen GM, Gidfar C, Weisbeck K, et al. Notes from the field: wastewater surveillance for measles virus during a measles outbreak — Colorado, August 2025. *MMWR Morb Mortal Wkly Rep* 2026;75:20-22. DOI: [10.15585/mmwr.mm7502a2](https://doi.org/10.15585/mmwr.mm7502a2).
3. Falender R, Sutton M, Cieslak P, et al. Notes from the field: retrospective analysis of wild-type measles virus in wastewater during a measles outbreak — Oregon, March 24–September 22, 2024. *MMWR Morb Mortal Wkly Rep* 2026;75:16-19. DOI: [10.15585/mmwr.mm7502a1](https://doi.org/10.15585/mmwr.mm7502a1).
4. Chen W, Bibby K. Temporal, spatial, and methodological considerations in evaluating the viability of measles wastewater surveillance. *Scie Total Environ* 2025;959:178141. DOI: [10.1016/j.scitotenv.2024.178141](https://doi.org/10.1016/j.scitotenv.2024.178141).
5. Rushford C, Gregory D, Copen E, et al. Untargeted longitudinal ultra deep metagenomic sequencing of wastewater provides a comprehensive readout of expected and unexpected viral pathogens. October 28, 2025 (<https://www.medrxiv.org/content/10.1101/2025.10.27.25338874v1>). Preprint.
6. Justen LJ, Rushford C, Hershey OS, et al. Deep untargeted wastewater metagenomic sequencing from sewersheds across the United States. March 6, 2026 (<https://www.medrxiv.org/content/10.64898/2026.03.05.26345726v1>). Preprint.
7. Paulos AP, Zulli A, Sheldon B, Duong D, Boehm AB, Wolfe MK. Measles RNA detection in wastewater solids. July 21, 2025 (<https://www.medrxiv.org/content/10.1101/2025.07.18.25331801v1>). Preprint.
8. Shukla M, Wattam AR, Aleman A, et al. BV-BRC: a unified bacterial and viral bioinformatics resource with expanded functionality and AI integration. *Nucleic Acids Res* 2026;54:D715-D723. DOI: [10.1093/nar/gkaf1254](https://doi.org/10.1093/nar/gkaf1254).