Pages S1–S120

EMERGING INFECTIOUS DISEASES

A Peer-Reviewed Journal Tracking and Analyzing Disease Trends

Emerging Infectious Diseases is published monthly by the Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop H16-2, Atlanta, GA 30329-4018, United States. Telephone 404-639-1960; email eideditor@cdc.gov

EMERGING INFECTIOUS DISEASES®

Wastewater Surveillance **Summer Supplement, 2026**

On the Cover

Alexander T. Yu, *Tip of the Iceberg*, 2026. Watercolor on paper. 14 in × 17 in (35.6 cm × 43.2 cm). Image courtesy of the artist.

About the Cover p. S119

Building National Wastewater Surveillance for Infectious Diseases in the United States

Jonathan S. Yoder, Nicole Fehrenbach, Alexander Yu, David A. Larsen, Scott Santibanez, Margaret A. Honein

In 2020, the Centers for Disease Control and Prevention (CDC) established the National Wastewater Surveillance System to detect and quantify SARS-CoV-2 RNA through wastewater in the United States. Subsequently, wastewater surveillance (WS) for infectious diseases expanded dramatically from independent, local efforts into a coordinated national system (*1*). This supplemental issue of *Emerging Infectious Diseases* describes the substantial progress during the first 5 years of the National Wastewater Surveillance System, including the dramatic growth of WS epidemiology and the increasing public health applications of WS for infectious diseases. Innovation is occurring in multiple sectors, including frontline health departments, which is reflected in the supplement article authorship. WS has quickly developed into a valuable tool for responding to infectious disease threats.

WS is unique in that it does not depend on whether persons have symptoms, have access to healthcare, visit a provider, or have a clinical diagnostic test performed. As such, it can fill gaps in other surveillance systems, most of which are reliant on clinical diagnostic tests being performed and reported to public health. WS also can act as an early warning system and detect small changes in community transmission of infectious diseases, helping public health agencies take action to prevent further infections.

WS is funded by CDC but relies on a multitude of partners, including academic researchers, commercial laboratories, community stakeholders, wastewater operators and engineers, healthcare professionals, and public health professionals, as seen throughout the articles in this supplement. Key to jurisdictional involvement are the Wastewater Monitoring Centers of Excellence, regional leaders who foster innovation, develop and conduct training, and provide technical assistance to jurisdictions. The 6 Centers of Excellence are led by public health departments through a collaborative partnership with academic and utility partners in California, Colorado, Houston (Texas), New York, North Carolina, and Wisconsin (*1*).

This network of partners (public health, academia, and utilities) is necessary to navigate the complex process of operationalizing WS and making it useful for public health. The WS process involves collecting untreated wastewater from a sewershed, often as it flows into a wastewater treatment plant. Wastewater samples are typically collected by wastewater treatment plant operators and sent to laboratories for testing, which includes sample preparation and concentration and extraction and quantification of viral genetic material, most commonly using reverse transcription digital PCR or reverse transcription droplet digital PCR. Isolates can be further sequenced to compare with clinical sequences and elucidate the spread of variants. Several sequence-based detection approaches (e.g., hybrid capture or shotgun metagenomics) are being explored to increase the usefulness of WS.

Laboratories submit data to the health department, commercial contractor, or academic partner program, who then send data to CDC through the One CDC Data Platform, a cloud-based data integration and management platform. Frontline jurisdictions are able to immediately access and use their data for public health action while sharing it securely with CDC. CDC currently receives data from ≈1,500 wastewater sampling sites across the United States in all 50 states, 7 territories, and some tribal communities.

Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (J.S. Yoder, N. Fehrenbach, S. Santibanez, M.A. Honein); California Department of Public Health, Division of Communicable Disease Control, Richmond, California, USA (A. Yu); Syracuse University Department of Public Health, Syracuse, New York, USA (D.A. Larsen)

DOI: https://doi.org/10.3201/eid3213.260301

As noted in the articles in this supplement, WS data complement, but do not replace, other public health surveillance data. Health departments use wastewater data for a multitude of reasons, including to confirm trends, provide early warning about transmission, inform public health messages, monitor disease activity in communities with limited clinical testing or reporting, direct prevention efforts (e.g., vaccination), inform resource allocation decisions, provide insight on the presence and scope of emerging and rare infectious diseases, and track the emergence of variants. Hospital systems use the information to plan for upcoming increases in visits and hospitalizations, whereas healthcare providers use it to increase their own awareness of locally circulating diseases and refine their differential diagnoses. Individual persons can use WS data to make decisions about their risk for disease and how best to protect themselves and their community. WS is adaptable, can meet changing public health needs, and can rapidly adjust to address emerging and reemerging health threats, such as measles (2). However, that flexibility requires a sustained national WS system; ongoing partnerships; continued investment in innovation for different diseases, approaches (e.g., metagenomics), and settings (e.g., nonsewered communities); ongoing evaluation of population and geographic coverage of the system; improvements in timeliness; and better ways to combine data from multiple disparate laboratory sources.

The authors of the articles in this supplement summarize some of the current perspectives on WS for infectious diseases and implications for public health in the United States. Themes include innovations in WS and epidemiology; comparison of WS data with clinical and syndromic surveillance; evaluation of sampling frequency and cadence; representativeness at county, state, territory, and national levels; case studies of specific programmatic applications in public health surveillance and outbreak response in state and local jurisdictions; and critical gaps and directions for the future, such as the impact and future potential of coordinated WS in shaping public health strategies. As the field of public health evolves to face new threats and challenges, data from WS and other innovative sources will be essential in ensuring we are prepared to address emerging and reemerging infectious diseases in the 21st Century.

References

1. Adams C, Bias M, Welsh RM, Webb J, Reese H, Delgado S, et al. The National Wastewater Surveillance System (NWSS): from inception to widespread coverage, 2020–2022, United States. Sci Total Environ. 2024;924:171566. https://doi.org/10.1016/j.scitotenv.2024.171566
2. Jensen GM, Gidfar C, Weisbeck K, Barnes M, Minnerath E, Matzinger S, et al. Notes from the field: wastewater surveillance for measles virus during a measles outbreak—Colorado, August 2025. MMWR Morb Mortal Wkly Rep. 2026;75:20–2. https://doi.org/10.15585/mmwr.mm7502a2

Address for correspondence: Jonathan Yoder, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop H24-11, Atlanta, GA 30329-4018, USA; email: jey9@cdc.gov

Progress and Expansion of the National Wastewater Surveillance System, United States, 2020–2024

Miguella Mark-Carew,[1] Megan Bias,[1] Stacie Reckling, Kaniz Madhobi, Daniel Cornforth, Igor Volkov, Andrew L. Rainey, Benjamin Lorentz, Kaitlin R. Taibl, Lisa P. Oakley, Rachel West, Rory Welsh, Jeffrey W. Mercante, Niketta Womack, Nicole Fehrenbach, Jonathan Yoder

Wastewater surveillance in the United States has evolved from an emergency response initiative into a valuable component of public health infrastructure. During its first 4 years, the Centers for Disease Control and Prevention's National Wastewater Surveillance System (NWSS) expanded its national testing coverage, suite of pathogen targets, analytics capabilities, data visualizations, and laboratory methods. Collaborations across public, private, and academic sectors continue to drive progress toward standardization of practices, interpretation of wastewater signals, and translation of wastewater surveillance into public health action. This article describes the evolution of NWSS from late 2020 through 2024, highlighting its integration into public health practice and its demonstrated adaptability and scalability as a surveillance system supporting timely, data-driven decision-making to address community-level public health threats.

Wastewater surveillance (WS) has become a valuable component of the United States public health infrastructure since late 2020, when the Centers for Disease Control and Prevention (CDC) launched the National Wastewater Surveillance System (NWSS) to coordinate testing and reporting for SARS-CoV-2 in wastewater (*1*,*2*). NWSS is a partnership between state, tribal, local, and territorial public health departments (hereafter referred to as health departments), commercial entities, and academia to track a range of pathogen targets in sewersheds, or the community area contributing wastewater to a sample site. As a relatively new surveillance system, NWSS has quickly transformed from a rapid-response effort into an adaptive surveillance structure that can support long-term infectious disease monitoring. NWSS complements case-based and syndromic surveillance by providing timely insights into infectious disease signals at the community level (*3*).

Since 2020, NWSS has expanded its wastewater sampling network across the continental United States and its territories, growing from 295 sites representing ≈12% of the US population in 2020 to >1,800 sites representing ≈45% of the US population in 2024 (*4*). During that same period, NWSS broadened its scope from a single-pathogen system tracking only SARS-CoV-2 to one that monitors multiple pathogen targets. In 2022, NWSS added testing for monkeypox virus (MPXV) in response to the 2022 global outbreak and has since expanded to additional targets such as influenza A (and subtypes), influenza B, and respiratory syncytial virus (RSV). That growth has been supported by the One CDC Data Platform (1CDP, formerly DCIPHER), alongside improvements in analytic methods and data visualizations (*3*,*5*). The NWSS program has also fostered collaboration across the public and private sector, including with health departments, commercial laboratories, nonprofit entities, and academic institutions to further WS science. This article describes NWSS from its inception through 2024, updating previously published findings (*4*), with an emphasis on advances in system coverage, pathogen monitoring, analytic capabilities, and public health application during 2023 and 2024.

Materials and Methods

WS data are submitted to NWSS via 1CDP by health departments, CDC commercial contractors, and an

Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (M. Mark-Carew, M. Bias, K. Madhobi, D. Cornforth, A.L. Rainey, K.R. Taibl, L.P. Oakley, R. West, R. Welsh, J.W. Mercante, N. Womack, N. Fehrenbach, J. Yoder); Chenega Corporation, Anchorage, Alaska, USA (S. Reckling, I. Volkov); Goldbelt Professional Services LLC, Chesapeake, Virginia, USA (B. Lorentz)

DOI: https://doi.org/10.3201/eid3213.261118

[1]These first authors contributed equally to this article.

academic partner program (*6*). We limited our analyses to calendar years through 2024, encompassing the first 4 years of NWSS implementation and capturing substantial growth in participating sites and pathogen targets. After downloading concentration data on May 4, 2026, we filtered it to include samples collected from September 1, 2020, through December 31, 2024, and categorized data into 3 time periods: January–December 2023, January–December 2024, and September 2020–December 2024. On May 1, 2026, we downloaded and analyzed sequencing metadata corresponding to paired concentration samples available on June 5, 2025. We included wastewater sites with ≥1 sample collected during each analytic period. Data for other pathogen targets were collected, but our analysis mainly focused on SARS-CoV-2, MPXV, influenza A virus, influenza A(H5) subtype, and RSV, given broad national testing coverage for those pathogens.

We mapped wastewater sampling sites by using the centroid coordinates of the wastewater treatment plant postal (ZIP) codes, stratified by year and number of targets assayed. We assessed county urbanicity by using the US Department of Agriculture 2023 Rural-Urban Continuum Codes (*7*) and calculated counts of NWSS sites by US Census Region (*8*). Assessing data submission timeliness involved parsing dates and excluding records with invalid dates. We used a Python script using Palantir's Foundry API (https://www.palantir.com/docs/foundry/api/v2) and the pandas library (https://pandas.pydata.org) to scan the metadata of archived submissions to determine start dates for automated 1CDP submissions. We conducted descriptive analyses by using R software version 4.4.0 (The R Foundation for Statistical computing, https://www.r-project.org) and Python version 3.12.4 (https://www.python.org) pandas library version 2.2.2 and geospatial analyses using ArcGIS Pro 3.1.7 (https://www.esri.com). We pulled SARS-CoV-2 wastewater sequencing data and metadata submitted to the National Center for Biotechnology Information (NCBI) by health department and commercial contract partners into 1CDP to determine the dominant variant at each participating wastewater site on the basis of the highest relative lineage abundance from mixed SARS-CoV-2 samples. We processed all sequencing samples by using the CFSAN Wastewater Analysis Pipeline (C-WAP; https://github.com/CFSAN-Biostatistics/C-WAP). This activity was reviewed by CDC and conducted consistent with applicable federal law and CDC policy (see, e.g., 45 C.F.R. part 46.102(l)(2), 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq.).

NWSS Site Coverage and Characteristics

NWSS has achieved broad geographic coverage of the United States, representing 178 million persons across 53 states and territories. US population coverage was >45% in both 2023 and 2024 and for the full 2020–2024 period (Table 1). Most wastewater sampling sites were in the South and Midwest US Census Regions across all time periods (Table 1; Figure 1). Wastewater sampling sites were concentrated in highly populated urban counties; in 2023 and 2024, metropolitan counties represented ≈60% of counties served by the NWSS network but 39% of all US counties (Table 1; Figure 2). Approximately 80% of sites were managed by health departments. The number of wastewater sampling sites and counties decreased slightly from 2023 to 2024 (1,967 sites in 2023 to 1,871 sites in 2024 and 1,029 counties in 2023 to 990 counties in 2024).

The number of sites testing for influenza A virus and RSV increased from 2023 to 2024, whereas total sites across all targets and sites testing for SARS-CoV-2 and MPXV decreased slightly from 2023 to 2024 (Table 1; Figure 1). Influenza A(H5) virus testing in wastewater increased substantially from 2023 to 2024 (25 to 551 sites) as multiple sectors collaborated in a One Health approach to respond to the highly pathogenic avian influenza (HPAI) A(H5N1) outbreak in dairy cattle in 2024 (*9*,*10*).

NWSS also worked with health departments and other partners to increase the coverage of sewershed polygons representing the geographic areas contributing to wastewater sampling sites. In 2024, NWSS received 1,233 sewershed polygons representing 82% of CDC-funded sites. That extensive catalog of geospatial data helps NWSS more accurately define communities served by WS for more effective interpretation of pathogen activity.

NWSS Sampling and Pathogen Testing

Data for >456,500 samples from 139 laboratories were submitted to NWSS during 2020–2024. Health departments submitted ≈70% of sample data, including testing results generated by state public health, commercial, and academic laboratories (Tables 1, 2). Sample data submitted by the academic partner program peaked at 18% of total samples reported in 2024 and accounted for 12% of samples submitted for the overall analysis period. The median number of samples reported per site per week was 2 (IQR 1–2) across all analysis periods (Table 1).

Although wastewater sampling and testing methodologies varied across sites and data submitters, ≥70% of samples were from untreated liquid influent wastewater (Table 2). Liquid influent is easier for most

utility operators to sample, whereas other sample types (such as primary sludge) are not available at all treatment plants (*11*,*12*). Of samples collected during 2023 and 2024, >80% were composite samples (e.g., time-weighted, flow-proportional, and manual composite samples). More than two thirds of samples overall were tested using digital or digital droplet PCR, with a notable increase from 2023 (66%) to 2024 (86%).

Endogenous and exogenous controls are important for validating the accuracy, consistency, and reliability of wastewater testing processes, thereby ensuring high-quality and actionable data (*13*,*14*). In 2023 and 2024, ≈65% of samples tested used pepper mild mottle virus as the endogenous control, whereas no control was used in ≈30% of samples (Table 2). Most samples tested in 2023 and 2024 used bovine

Table 1. Characteristics of sites participating in the National Wastewater Surveillance System, United States, 2023, 2024, and 2020–2024*

Characteristics	2023	2024	2020–2024
States and territories, no.	53	52	53
Counties, no.†	1,029	990	1,180
County RUCC, by population, no. (%)‡			
Metropolitan			
>1 million	274 (27)	245 (25)	280 (24)
250,000–1 million	187 (18)	197 (20)	214 (18)
<250,000	156 (15)	155 (16)	175 (15)
Nonmetropolitan			
Adjacent to metro area >20,000	102 (10)	105 (11)	119 (10)
Not adjacent to metro area >20,000	39 (4)	30 (3)	45 (4)
Adjacent to metro area 5,000–20,000	94 (9)	94 (10)	119 (10)
Not adjacent to metro area 5,000-20,000	78 (8)	75 (8)	96 (8)
Adjacent to metro area <5,000	45 (4)	42 (4)	62 (5)
Not adjacent to metro area <5,000	54 (5)	47 (5)	70 (6)
Sites by US Census region, no.§			
Midwest	705	662	829
Northeast	376	340	404
South	480	509	642
West	393	356	443
Site population			
Persons served by all sites, no. (% of US population), in millions¶	163 (49)	150 (45)	178 (54)
Persons served per site, median (IQR), in thousands	24 (8–77)	24 (8–75)	21 (7–67)
Site characteristics			
Sites, no.	1,967	1,871	2,331
Tribal land sites, no.	7	8	14
Site location			
WWTP	1,672	1,605	1,988
Upstream	295	266	343
Targets tested for at site, no.#			
SARS-CoV-2	1,952	1,847	2,308
Monkeypox virus	650	481	731
Influenza A	870	1,301	1,364
Influenza A(H5)	25	551	551
Respiratory syncytial virus	723	1,215	1,256
Site management, no. (%)**			
Local health department	63 (3)	62 (3)	63 (2)
State health department	1,523 (77)	1,551 (83)	1,853 (79)
Commercial national contractor	397 (20)	188 (10)	493 (21)
Academic partner program	198 (10)	192 (10)	199 (9)
Laboratories testing samples from sites, no.	95	102	139
No. samples collected per site per week, median (IQR)††	2 (1–2)	2 (1–2)	2 (1–2)
Automated concentration data submission, sites (health departments), no.	312 (5)	452 (10)	469 (10)
Sewershed boundaries, no. (%)‡‡	1,224 (77)	1,233 (82)	1,407 (73)

*IQR, interquartile range; RUCC, Rural-Urban Continuum Code; WWTP, wastewater treatment plant.
†Counties were included as long as a wastewater site served some part of the county.
‡By 2023 RUCC (https://www.ers.usda.gov/data-products/rural-urban-continuum-codes).
§Some sites are not part of a US Census Region.
¶The US Census Bureau population estimate of 331,449,281 persons (as of April 1, 2020) was used to estimate percent of the US population covered by wastewater sampling sites. That number represents the sum of population served by WWTP sampling locations. Wastewater sewersheds may serve overlapping populations.
#Sites may be testing for ≥1 target; the numbers of sites testing for each target in the table are not mutually exclusive.
**Sites may be managed by ≥1 entity; the numbers of sites managed by each entity in the table are not mutually exclusive. Wastewater data from health departments may reflect testing from public health, commercial, and academic laboratories.
††All samples collected at a site were included in the median of number of samples collected per week regardless of site management.
‡‡Sewershed boundaries are submitted for sites located at a WWTP managed by local or state health departments or the commercial contractor.

coronavirus as the exogenous control organism (57% in 2023 and 74% in 2024) (*14*).

NWSS accelerated pathogen target ingestion in 2023 and 2024, adding 25 (81%) of 31 total targets to 1CDP and broadening coverage beyond MPXV and SARS-CoV-2 to include bacterial, fungal, other viral, and antimicrobial resistance gene targets (Figure 3). Influenza A virus and RSV were 2 of 14 targets added in 2023, and influenza A(H5) subtype was 1 of 11 targets added in 2024. By expanding its pathogen portfolio, NWSS transformed into a flexible, multipathogen surveillance system supporting both routine and emerging infectious disease surveillance. In addition, the existing 1CDP data infrastructure enabled the rapid integration of new targets through standardized data pipelines and provided a common platform for multipathogen WS used by internal and external partners.

NWSS Concentration Data Submission, Quality Control, and Timeliness

NWSS collaborates with data submitters to promote timely and accurate data submissions through automation and integrated quality control measures (*15*). Concentration data were ingested into 1CDP through a kill-and-replace method that overwrites the previous upload, enabling easy error correction and backfilling of historical data. Data submitters were encouraged to upload data on a weekly basis (i.e., samples collected during the previous MMWR reporting week) before 11:59 PM on Wednesdays. An automated pipeline in 1CDP flagged data quality issues and reported them via a quality control dashboard available to jurisdictions soon after data submission. NWSS program staff developed additional tools, including submitter-specific reports and pipeline health checks,

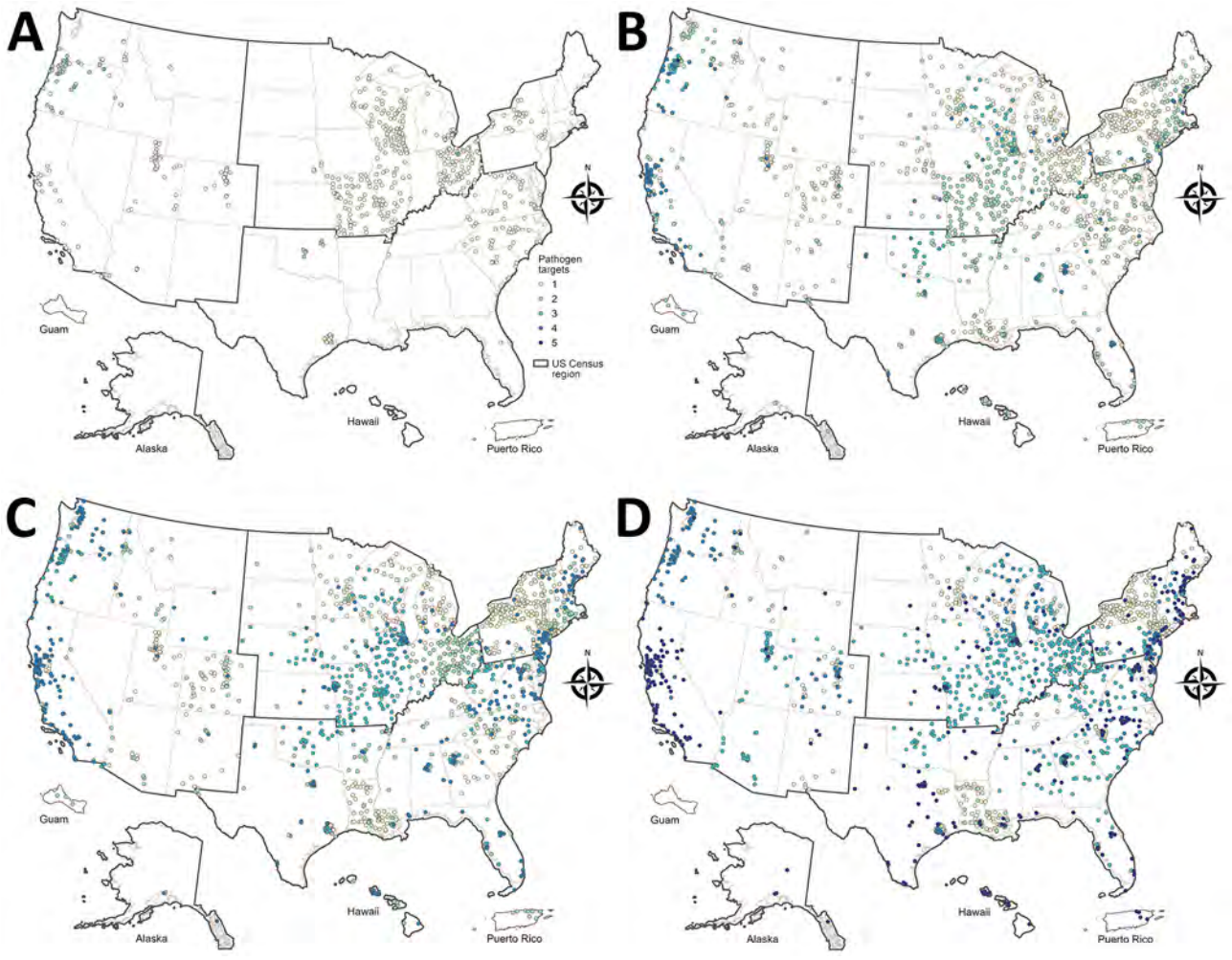


Figure 1. Geographic distribution of National Wastewater Surveillance System testing sites in the United States and territories, September 2020–December 2024. Each site is categorized by the number of viral targets tested during the specified period. Site coverage and viral target testing are shown for September 2020–December 2021 (A), 2022 (B), 2023 (C), and 2024 (D). US Census Regions are available from https://www.census.gov/geographies/mapping-files/time-series/geo/cartographic-boundary.html.

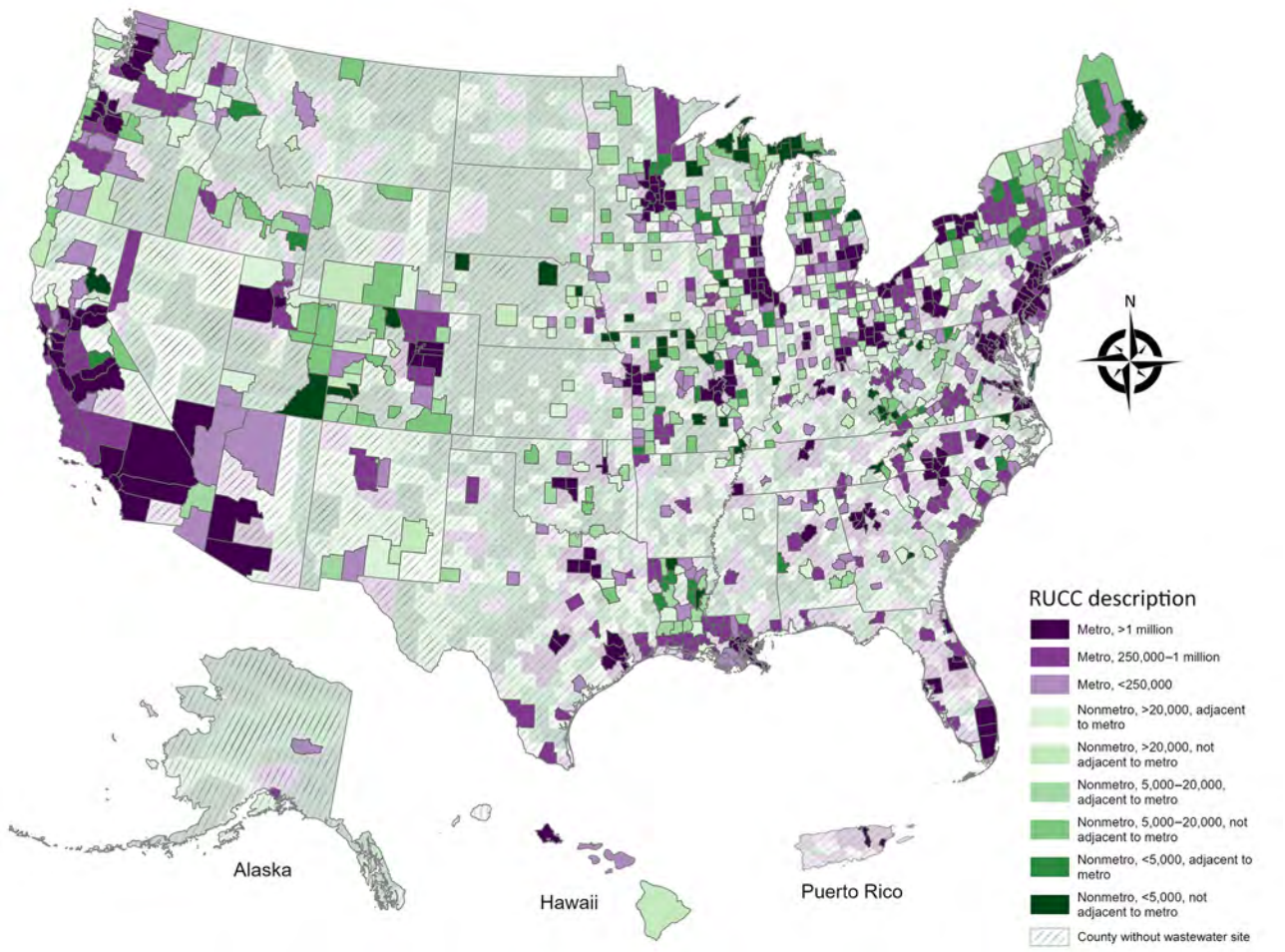


Figure 2. National Wastewater Surveillance System county coverage by US Department of Agriculture RUCC, United States, January 1–December 31, 2024. Counties with ≥1 wastewater site that reported to NWSS are displayed as bolder colors; muted colors with hatching represent no site coverage. RUCC data were from the 2023 RUCC dataset (https://www.ers.usda.gov/data-products/rural-urban-continuum-codes). Metro, metropolitan; RUCC, Rural–Urban Continuum Code.

to triage data quality issues and provide direct technical assistance. We cleaned, parsed, analyzed, and quality-checked wastewater data on Thursdays and made data available for public display and download after successful internal review (*16*–*18*).

Data were submitted to 1CDP via manual upload or automated methods, such as an application programming interface (API) or secure file transfer protocol. All data from academic and national commercial contract partners were submitted via API, whereas most health departments submitted data manually. Automated submissions began with 2 health departments in 2022, expanded to 3 additional health departments in 2023, and added 5 more health departments in 2024, totaling 10 health departments (469 sites) (Table 1).

Although the number of samples and pathogen targets submitted to NWSS increased substantially over time, overall reporting times from sample collection to 1CDP data upload improved markedly; the median decreased from 14 days in 2022 to 7 days in 2023 and 2024 (Table 2; Figure 4). Consistent with recommendations from the Association of Public Health Laboratories, samples from the NWSS laboratory network were processed and tested rapidly, resulting in a median time from sample collection to testing of 2–3 days (Table 2) (*13*). The median time from laboratory testing to 1CDP upload was 4 days in 2023 and 2024 (Table 2). Accordingly, in 2023 and 2024, more than half of sample data submitted were reported within 7 days of sample collection, and >75% of sample data were reported within 14 days of collection (Figure 4).

Wastewater Metric and Data Visualizations Improvements

In 2023, NWSS developed the wastewater viral activity level (WVAL) metric and corresponding

categories to standardize and aggregate SARS-CoV-2 data across state, territorial, regional, and national levels and to compare trends over time, replacing the 15-day rolling detection proportion, percentage change, trend, and percentile metrics (*3*,*4*,*19*). For the 2022 MPXV outbreak, we developed a site-level metric to indicate virus detection in samples collected during the previous 4 weeks (*3*,*20*). That metric categorized detections as persistent detection, detection, no recent detection, or no recent data. In 2024, we developed WVALs for influenza A and RSV and created an avian influenza A(H5)–specific metric to classify sites as having a detection, no detection, or no sample tested by reporting week.

WVAL data visualizations on NWSS CDC webpages display historical national, regional, and state-level trends and include national maps showing state- and territory-level activity. Those visualizations enable comparisons of SARS-CoV-2, influenza A, and RSV activity across geographic scales. MPXV and influenza A(H5) visualizations display site-level detection status to support more localized monitoring of potential disease activity. Corresponding data tables are available for all visualizations, and influenza A(H5) includes an additional table displaying detections over a 6-week lookback period to support interpretation of recent detection patterns.

Wastewater Genomic Sequencing

Wastewater sequencing became a powerful tool for tracking the emergence of genetic SARS-CoV-2 variants over time (*21*). Samples were processed using specialized bioinformatic pipelines that estimate variant proportions in mixed samples, enabling characterization of genetically diverse, mixed populations of multiple co-circulating genomes, rather than determining a single consensus

Table 2. Characteristics of samples submitted to the National Wastewater Surveillance System, United States, 2023, 2024, and 2020–2024*

Samples	2023	2024	2020–2024
Total no. samples tested†	155,165	148,023	456,594
Time from sample collection to testing, d, median (IQR)	3 (1–4)	2 (1–4)	2 (1–4)
Time from sample collection to reporting to 1CDP, d, median (IQR)	7 (5–14)	7 (4–10)	8 (5–22)
Time from sample testing to reporting to 1CDP, d, median (IQR)	4 (2–9)	4 (1–7)	5 (2–17)
Sample collection method, no. (%)‡			
Grab	20,170 (13)	20,073 (14)	58,553 (13)
Manual composite	2,382 (2)	926 (<1)	6,106 (1)
Flow-weighted composite	58,552 (38)	57,404 (39)	189,379 (41)
Time-weighted composite	73,031 (47)	68,635 (46)	200,086 (44)
Passive	1,030 (<1)	984 (<1)	2,462 (<1)
Sample type, no. (%)			
Untreated wastewater	116,347 (75)	104,958 (71)	353,935 (78)
Wastewater post–grit removal	30,286 (20)	34,702 (23)	78,122 (17)
Primary effluent	189 (<1)	186 (<1)	465 (<1)
Primary sludge	8,343 (5)	8,177 (6)	24,072 (5)
Sample management, no. (%)§			
Local health department	3,614 (2)	2,745 (2)	10,429 (2)
State health department	102,602 (66)	105,578 (71)	331,293 (72)
Commercial contractor	23,692 (15)	13,413 (9)	58,332 (13)
Academic partner program	25,553 (16)	26,287 (18)	56,993 (12)
PCR type, no. (%)			
qPCR	51,768 (33)	19,656 (13)	145,494 (32)
dPCR	103,114 (66)	127,891 (86)	310,011 (68)
Multiple PCR types used	283 (<1)	476 (<1)	1,089 (<1)
Endogenous wastewater control, no. (%)			
Pepper mild mottle virus	98,542 (64)	96,592 (65)	277,996 (61)
CrAssphage	9,436 (6)	10,049 (7)	30,962 (7)
Other	268 (<1)	201 (<1)	670 (<1)
None	46,919 (30)	41,181 (28)	146,966 (32)
Exogenous wastewater control, no. (%)			
Bovine coronavirus	88,511 (57)	109,731 (74)	270,720 (59)
Bovine respiratory syncytial virus	29,865 (19)	4,418 (3)	58,258 (13)
Other	23,011 (15)	21,821 (15)	82,648 (18)
None	13,778 (9)	12,053 (8)	44,968 (10)
No. samples sequenced for SARS-CoV-2	45,824	40,748	114,394

*1CDP, One CDC Data Platform (https://www.cdc.gov/data-modernization/php/one-cdc-data-platform/index.html); dPCR, digital PCR (including droplet digital PCR); IQR, interquartile range; qPCR, quantitative PCR.
†Samples were counted based on unique sample identifications; if a sample was tested for multiple targets, the sample was only counted once.
‡Sample collection methods were determined using the most recent collection-method record associated with each sample. Collection method information may be missing for some samples, and a small number of samples were associated with multiple collection methods.
§Sample management counts were not mutually exclusive. Samples could be associated with ≥1 management category.

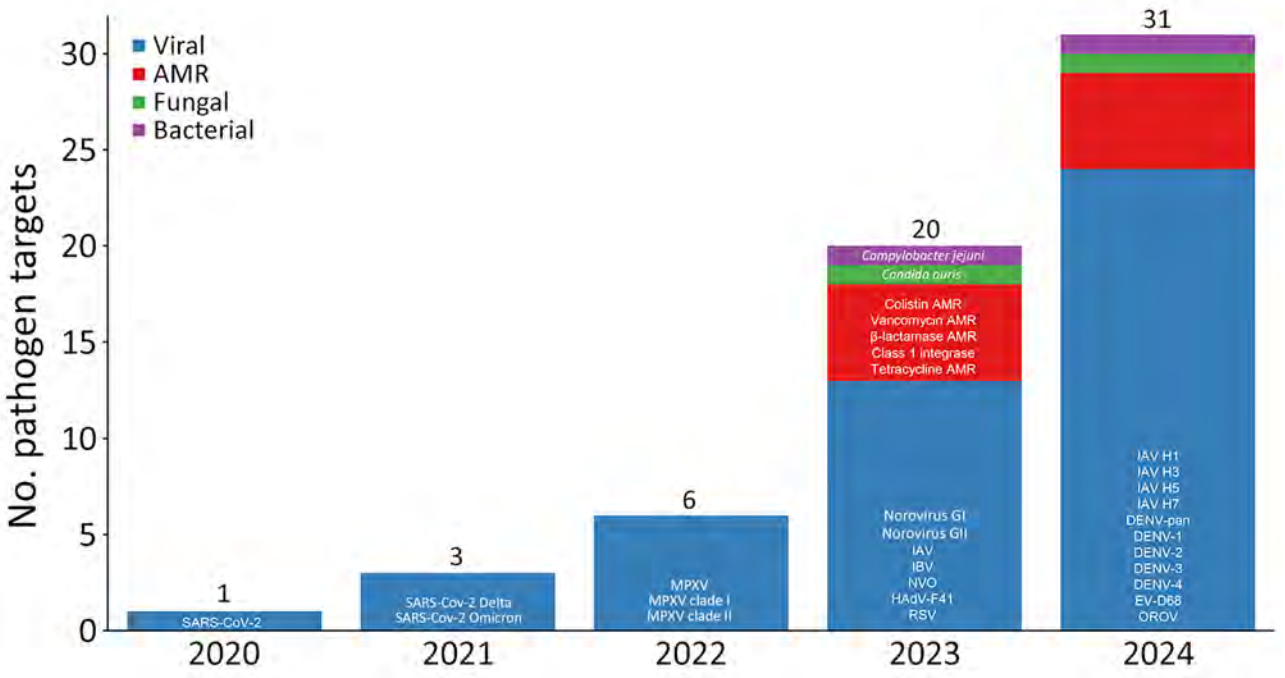


Figure 3. Pathogen targets accepted by the National Wastewater Surveillance System over time, United States, 2020–2024. Stacked bars display pathogen targets for each period, stratified by pathogen target category (viral, fungal, bacterial, and AMR gene targets). Each year's bar is labeled with the pathogen target(s) added that year. Numbers at tops of bars indicate cumulative number of pathogen targets over time. AMR, antimicrobial resistance; DENV, dengue virus; EV-D68, enterovirus D68; HAdV-F41, human adenovirus type 41 species F; IAV, influenza A virus; IVB, influenza B virus; MPXV, monkeypox virus; NVO, nonvariola orthopoxvirus; OROV, Oropouche virus; pan-DENV, all DENV types; RSV, respiratory syncytial virus.

genome, as is common in clinical sequencing analysis (*22*). In 2023 and 2024, approximately half of sites submitted both concentration and sequencing data for SARS-CoV-2 to NWSS, for a total of >86,000 samples (Table 2).

Wastewater whole-genome sequencing at NWSS sites contributed to the early detection and tracking of emerging SARS-CoV-2 variants. NWSS wastewater genomic data demonstrated some of the initial detections of notable lineages, such as initial Omicron variants in 2021, BA.2.86 across multiple US states within weeks of its first identification in August 2023, and JN.1 as it emerged and rapidly attained national predominance in late 2023, paralleling its ascent in clinical genomic surveillance (*23*–*26*).

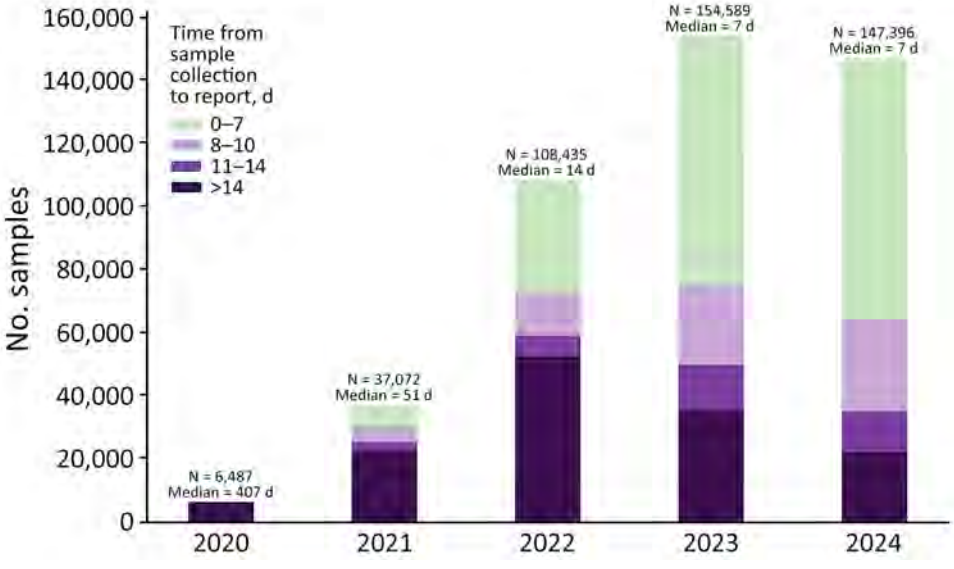


Figure 4. Sample counts and reporting timeliness for the National Wastewater Surveillance System, United States, 2020–2024. Stacked bars display total number of samples submitted during each period stratified by reporting intervals (0–7 days, 8–10 days, 11–14 days, and >14 days from sample collection to reporting). Data include samples tested for SARS-CoV-2, influenza A virus, influenza A(H5) subtype virus, monkeypox virus, and respiratory syncytial virus.

Collaborations and Public Health Capacity Building

The success of NWSS was driven by sustaining collaboration among public and private sector partners and internally with other CDC programs. To strengthen technical capacity across the network, CDC established 4 Wastewater Surveillance Centers of Excellence (CoEs) in California, Colorado, Houston (Texas), and Wisconsin during 2021–2022 and expanded to 6 CoEs in 2024 with the addition of New York and North Carolina (*27*,*28*). Led by state and local public health departments in partnership with academic institutions and wastewater utilities, the CoEs served as regional hubs for technical assistance, workforce development, applied research, and evaluation to advance WS implementation and innovation. Monthly regional calls organized across the 6 CoE regions provided a forum for jurisdictions to exchange surveillance findings, discuss implementation challenges, share best practices, and enhance regional situational awareness.

CDC incorporated WS into its incident management structure during the 2022 MPXV and 2024 avian influenza A(H5) outbreaks, enabling NWSS data to be integrated with epidemiologic, laboratory, and other surveillance information to inform response activities and public health actions. NWSS worked closely with other CDC programs that provide subject matter expertise for each pathogen target supported by the system, ensuring coordinated interpretation of wastewater signals alongside complementary surveillance data (9,*20*). A prime example of cross-program collaboration was NWSS wastewater data for SARS-CoV-2, influenza A, and RSV being displayed with clinical data on CDC's public respiratory illness webpages starting in 2024 to provide a more comprehensive understanding of community-level respiratory disease activity (*29*,*30*). Those efforts have strengthened NWSS's role and utility within CDC's emergency response and routine surveillance frameworks.

Discussion

Since its launch in 2020, NWSS has evolved from a rapid response SARS-CoV-2 surveillance pilot into an adaptable, multipathogen surveillance system to support national public health preparedness and response. During 2020–2024, NWSS received wastewater data from >2,300 sites across 53 states and territories, representing 54% of the US population. Although the system achieved broad geographic coverage, site distribution was concentrated in the South and Midwest and included predominantly urban counties. The robust collection of >1,400 sewershed boundary polygons enhanced the geospatial precision of WS catchment areas, enabling improved characterization of communities and community-level indicators that might influence the public health impact of wastewater detections (*31*).

NWSS expanded nationally coordinated surveillance to include influenza A virus, influenza A(H5) virus, RSV, and MPXV and supported surveillance for a total of 31 pathogen targets through 2024, demonstrating the flexibility and scalability of the system to address local and emerging public health priorities. NWSS also strengthened its laboratory and analytic capabilities through increased adoption of digital and digital droplet PCR methods, expansion of wastewater genomic sequencing to support early detection and monitoring of emerging variants, and implementation of pathogen-specific metrics such as the WVAL and associated visualizations. Together, those advancements improved the standardization and interpretation of wastewater data across jurisdictions and over time.

Enhancements to NWSS's data systems helped improve the timeliness of wastewater concentration data. Greater adoption of automated data submission reduced the time from sample testing to reporting through 1CDP. In addition, code improvements and automated data health checks reduced pipeline run times and improved data quality, further reducing reporting lags. Those improvements have been crucial, given that wastewater signals provide population-level information that can sometimes precede clinical detections and variant identification. That capability supports the utility of NWSS as a public health surveillance system that is independent of health-seeking behavior and testing practices, complements clinical surveillance systems, and can provide early warning of emerging disease activity (*32*,*33*).

The continued growth of NWSS has been supported by sustained collaboration among health departments, wastewater utilities, commercial laboratories, academic institutions, and federal partners. The Wastewater Surveillance CoEs expanded technical capacity, enhanced regional collaboration, and enabled the exchange of best practices across the network. Integration of NWSS into CDC response efforts for mpox and avian influenza A(H5) demonstrated the value of wastewater in monitoring emerging public health threats. NWSS has shown that WS can support coordinated One Health approaches to monitoring zoonotic diseases such as influenza A(H5) while also being sensitive enough to detect emerging infections such as mpox (*9*,*20*,*34*,*35*).

WS has demonstrated substantial progress as a valuable component of public health infrastructure.

Continued development focused on representative coverage, data timeliness, and wastewater sequencing capacities across NWSS will further strengthen its public health utility (*12*,*36*–*39*). Despite NWSS serving ≈178 million persons, coverage gaps persist in some states, rural areas, and unsewered communities. Balancing resource availability with representative coverage and surge capacity needs for emerging threats will remain a critical consideration as jurisdictions continue to optimize WS activities.

Although reporting times have decreased overall, further reductions could strengthen early responses to emerging infectious disease threats. The overall higher reporting times observed in 2020–2022 were partly the result of newly onboarded partners uploading historical sample results. Timeliness of NWSS data could be improved through faster onboarding of data submitters and earlier submission of data after new assays are validated and implemented. Finally, broader application of wastewater genomic sequencing beyond SARS-CoV-2 to include other emerging pathogens may enhance understanding about community transmission patterns and case-based clinical investigation.

In just over 4 years, NWSS matured into a national surveillance platform that complements traditional case-based and syndromic surveillance and can be leveraged to address national public health threats. NWSS expanded national WS coverage to 5 viral pathogen targets during this period and used its scalable data pipeline architecture to support surveillance of additional viral targets, as well as bacterial, fungal, and antimicrobial resistance targets. Strong partnerships with health departments, commercial laboratories, and academic institutions were central to this progress, as were efforts to augment concentration and sequencing data infrastructure to support timely signal interpretation and visualizations for public health decision-making.

Short-term priorities for NWSS include several core system improvements, including optimizing coverage to improve representativeness; increasing standardization of sampling, laboratory, and reporting practices; and scaling up automated data submission to strengthen timeliness and comparability across the network. Future system enhancements will focus on expanding pathogen sequencing capabilities and integrating wastewater data more systematically with clinical and syndromic surveillance data. Sustained funding, workforce capacity building, and continued cross-sector collaboration will be essential to achieving those goals.

Acknowledgments

We acknowledge the valuable contribution of all jurisdictions, territorial partners, laboratories, health systems, and utilities submitting data to the National Wastewater Surveillance System for making this work possible. Specifically for this study, we want to acknowledge: Alabama Department of Public Health; Alaska Division of Public Health; Arizona Department of Health Services; Arkansas Department of Health; California Department of Public Health; Laboratory-Based Surveillance Program, Disease Control & Emergency Preparedness Bureau, Chicago Department of Public Health; Colorado Department of Public Health and Environment and participating wastewater utilities; Connecticut Department of Public Health; Delaware Division of Public Health; Department of Health of the District of Columbia, the District of Columbia Water and Sewer Authority, and the District of Columbia Department of Forensic Sciences, Public Health Laboratory; Florida Department of Health; Georgia Department of Public Health; Guam Department of Public Health and Social Services, Epidemiology and Laboratory Capacity Program and Guam Waterworks Authority; Hawaii State Department of Health; Houston NWSS Center of Excellence, Houston Health Department, Houston Public Works, and Rice University; Idaho Department of Health and Welfare; Illinois Department of Public Health; Indiana Department of Health; Iowa Department of Public Health and the State Hygienic Laboratory at the University of Iowa; Kansas Division of Public Health; Kentucky Department of Public Health, Kentucky Wastewater Surveillance System; Louisiana Department of Health; Maine Department of Health and Human Services; Maricopa County Department of Public Health; Maryland Department of Health; Massachusetts Department of Public Health; Boston Public Health Commission; Michigan Department of Health and Human Services; Minnesota Department of Public Health and the Minnesota Department of Health Wastewater Monitoring Program; Mississippi State Department of Health; Missouri Department of Health and Senior Services; Montana Department of Public Health and Human Services; Nebraska Department of Health and Human Services, Likhitha Duggirala, Derry Stover; Nevada Division of Public and Behavioral Health; New Hampshire Department of Health and Human Services; Department of Health for the State of New Jersey; New Mexico Department of Health, Scientific Laboratory and Environmental Health Epidemiology Bureau; Daniel Lang, Haley Kappus Kron, Shazneen Damani, Paul Dougall, Kristen St. George, Kim Musser, James Chithalen, Sylvia Byun, Samantha Long, Eli Rosenberg; New York City Department of Health and Mental Hygiene and the NYC Department of Environmental Protection; North Carolina

Division of Public Health; North Dakota Department of Health and Human Services, Laboratory Services Section, and Disease Control and Forensic Pathology Section; Ohio Department of Health, Ohio Wastewater Monitoring Network; Oklahoma State Department of Health; Oregon Health Authority, Public Health Division; Pennsylvania Department of Public Health; Philadelphia Department of Public Health; Puerto Rico Public Health Institute; Rhode Island Department of Public Health; South Carolina Department of Public Health (SCDPH) Wastewater Program, SCDPH CDES: Abdoulaye Diedhiou, Laureen Mitchell, Nusaiba Mohamed, Jack Roddey, and Katherine O'Shields-Free, and SCDPH PHL: Jennifer Meredith, Cory J. Weaver, Christy Greenwood, Samuel Evans, and Megan Woronko; South Dakota Department of Health; Tennessee Department of Health; Texas Department of State Health Services; Utah Department of Health and Human Services, Utah Wastewater Surveillance System; Vermont Department of Health, Laboratory Sciences and Infectious Diseases; Virginia Department of Health; Washington State Department of Health, Washington Wastewater-Based Epidemiology Program; West Virginia Bureau for Public Health with Marshall University and West Virginia University as partners in Wastewater Testing for Community Health in WV; Wisconsin Wastewater Monitoring Program, Wisconsin Department of Health Services, Wisconsin State Laboratory of Hygiene, University of Wisconsin–Milwaukee; Wyoming Department of Health. We also acknowledge the contributions of our wastewater partners and collaborators, including CDC-contracted partners, Water Environment Federation, Association of Public Health Laboratories, National Association of County and City Health Officials, Association for State and Territorial Health Officials, and Council for State and Territorial Epidemiologists.

Some of the data used in this study were collected as part of the WastewaterSCAN/SCAN project, a partnership between Stanford University, Emory University, and Verily, funded philanthropically through a gift to Stanford University.

The manuscript was edited using ChatGPT (OpenAI, https://openai.com) for language refinement, formatting assistance, and consistency checks across the manuscript. Authors reviewed and verified the accuracy of the manuscript and approved all edits.

About the Author

Dr. Mark-Carew is an epidemiologist with the Centers for Disease Control and Prevention and senior scientist for the National Wastewater Surveillance System. Her research focuses on infectious disease surveillance and outbreak response. Ms. Bias is an epidemiologist with the Centers for Disease Control and Prevention and senior scientist for the National Wastewater Surveillance System. Her research interests include infectious disease epidemiology and outbreak response.

References

1. Schneider R, Weisbeck K, Sheth K, Sikes P, Domakonda K, Stadler L, et al. Assessment of public health agency and utility training needs for CDC National Wastewater Surveillance System Jurisdictions in the United States. Health Promot Pract. 2024;26:630–6. https://doi.org/10.1177/15248399241275617
2. Kirby AE, Walters MS, Jennings WC, Fugitt R, LaCross N, Mattioli M, et al. Using wastewater surveillance data to support the COVID-19 response—United States, 2020–2021. MMWR Morb Mortal Wkly Rep. 2021;70:1242–4. https://doi.org/10.15585/mmwr.mm7036a2
3. Centers for Disease Control and Prevention. About CDC's National Wastewater Surveillance System (NWSS) [cited 2026 May 25]. https://www.cdc.gov/nwss/about.html
4. Adams C, Bias M, Welsh RM, Webb J, Reese H, Delgado S, et al. The National Wastewater Surveillance System (NWSS): from inception to widespread coverage, 2020-2022, United States. Sci Total Environ. 2024;924:171566. https://doi.org/10.1016/j.scitotenv.2024.171566
5. Centers for Disease Control and Prevention. One CDC data platform [cited 2026 May 25]. https://www.cdc.gov/data-modernization/php/one-cdc-data-platform/index.html
6. Boehm AB, Wolfe MK, Bidwell AL, Zulli A, Chan-Herur V, White BJ, et al. Human pathogen nucleic acids in wastewater solids from 191 wastewater treatment plants in the United States. Sci Data. 2024;11:1141. https://doi.org/10.1038/s41597-024-03969-8
7. US Department of Agriculture. Rural-urban continuum codes—documentation [cited 2026 May 25]. https://www.ers.usda.gov/data-products/rural-urban-continuum-codes/documentation
8. US Census Bureau. Cartographic boundary files [cited 2026 May 25]. https://www.census.gov/geographies/mapping-files/time-series/geo/cartographic-boundary.2020.html
9. Louis S, Mark-Carew M, Biggerstaff M, Yoder J, Boehm AB, Wolfe MK, et al. Wastewater surveillance for influenza A virus and H5 subtype concurrent with the highly pathogenic avian influenza A(H5N1) virus outbreak in cattle and poultry and associated human cases—United States, May 12–July 13, 2024. MMWR Morb Mortal Wkly Rep. 2024;73:804–9. https://doi.org/10.15585/mmwr.mm7337a1
10. Centers for Disease Control and Prevention. H5N1 bird flu surveillance and human monitoring [cited 2026 May 25]. https://www.cdc.gov/bird-flu/h5-monitoring/index.html
11. US Environmental Protection Agency. Wastewater sampling [cited 2026 May 25]. https://www.epa.gov/sites/default/files/2015-06/documents/Wastewater-Sampling.pdf
12. National Academies of Sciences. Increasing the utility of wastewater-based disease surveillance for public health action: a phase 2 report. Washington: The National Academies Press; 2024.
13. Association of Public Health Laboratories. SARS-CoV-2 wastewater surveillance testing guide for public health laboratories [cited 2026 May 25]. https://www.aphl.org/aboutAPHL/publications/Documents/EH-2022-SARSCoV2-Wastewater-Surveillance-Testing-Guide.pdf

14. Borchardt MA, Boehm AB, Salit M, Spencer SK, Wigginton KR, Noble RT. The Environmental Microbiology Minimum Information (EMMI) guidelines: qPCR and dPCR quality and reporting for environmental microbiology. Environ Sci Technol. 2021;55:10210–23. https://doi.org/10.1021/acs.est.1c01767
15. Centers for Disease Control and Prevention. Real-time wastewater data guides public health action [cited 2026 May 25]. https://www.cdc.gov/public-health-data-strategy/php/story/real-time-wastewater-data-guides-public-health-action.html
16. Centers for Disease Control and Prevention. CDC's Wastewater Monitoring Program [cited 2026 May 25]. https://www.cdc.gov/wastewater
17. Centers for Disease Control and Prevention. NWSS public SARS-CoV-2 wastewater metric data [cited 2026 May 25]. https://data.cdc.gov/Public-Health-Surveillance/NWSS-Public-SARS-CoV-2-Wastewater-Metric-Data/2ew6-ywp6/about_data
18. Centers for Disease Control and Prevention. NWSS public SARS-CoV-2 concentration in wastewater data [cited 2026 May 25]. https://data.cdc.gov/Public-Health-Surveillance/NWSS-Public-SARS-CoV-2-Concentration-in-Wastewater/g653-rqe2/about_data
19. Centers for Disease Control and Prevention. CDC's wastewater monitoring data methodology [cited 2026 Jul 29]. https://www.cdc.gov/wastewater/about/data-methods.html
20. Adams C, Kirby AE, Bias M, Riser A, Wong KK, Mercante JW, et al. Detecting mpox cases through wastewater surveillance—United States, August 2022–May 2023. MMWR Morb Mortal Wkly Rep. 2024;73:37–43. https://doi.org/10.15585/mmwr.mm7302a3
21. Centers for Disease Control and Prevention. COVID-19 variants in wastewater [cited 2026 May 25]. https://www.cdc.gov/nwss/rv/COVID19-variants.html
22. Kayikcioglu T, Amirzadegan J, Rand H, Tesfaldet B, Timme RE, Pettengill JB. Performance of methods for SARS-CoV-2 variant detection and abundance estimation within mixed population samples. PeerJ. 2023;11:e14596. https://doi.org/10.7717/peerj.14596
23. Kirby AE, Welsh RM, Marsh ZA, Yu AT, Vugia DJ, Boehm AB, et al.; New York City Department of Environmental Protection. Notes from the field: early evidence of the SARS-CoV-2 B.1.1.529 (Omicron) variant in community wastewater—United States, November–December 2021. MMWR Morb Mortal Wkly Rep. 2022;71:103–5. https://doi.org/10.15585/mmwr.mm7103a5
24. Lambrou AS, South E, Ballou ES, Paden CR, Fuller JA, Bart SM, et al. Early detection and surveillance of the SARS-CoV-2 variant BA.2.86—worldwide, July–October 2023. MMWR Morb Mortal Wkly Rep. 2023;72:1162–7. https://doi.org/10.15585/mmwr.mm7243a2
25. Ma KC, Castro J, Lambrou AS, Rose EB, Cook PW, Batra D, et al. Genomic surveillance for SARS-CoV-2 variants: circulation of Omicron XBB and JN.1 lineages—United States, May 2023–September 2024. MMWR Morb Mortal Wkly Rep. 2024;73:938–45. https://doi.org/10.15585/mmwr.mm7342a1
26. Feistel DJ, Welsh R, Mercante J, Mark-Carew M, Caravas J, Boddapati A, et al. Detection and tracking of SARS-CoV-2 lineages through National Wastewater Surveillance System pathogen genomics. Emerg Infect Dis. 2025;31:57–60. https://doi.org/10.3201/eid3113.241411
27. Centers for Disease Control and Prevention. Wastewater monitoring Centers of Excellence [cited 2026 Jul 30]. https://www.cdc.gov/wastewater/about/centers-of-excellence.html
28. Valencia D, Yu AT, Wheeler A, Hopkins L, Pray I, Horter L, et al. Notes from the field: the National Wastewater Surveillance System's Centers of Excellence contributions to public health action during the respiratory virus season—four U.S. jurisdictions, 2022–23. MMWR Morb Mortal Wkly Rep. 2023;72:1309–12. https://doi.org/10.15585/mmwr.mm7248a4
29. Centers for Disease Control and Prevention. Respiratory virus activity levels [cited 2026 Sep 2]. https://www.cdc.gov/respiratory-viruses/data/activity-levels.html.
30. Centers for Disease Control and Prevention. Respiratory illnesses data channel [cited 2026 Sep 2]. https://www.cdc.gov/respiratory-viruses/data/index.html
31. Reckling S, Reese H, Oakley LP, Mitasova H. A geospatial analysis comparing wastewater-monitored sewershed and statewide populations for 32 states participating in CDC's National Wastewater Surveillance System, 2021-2024. PLOS Glob Public Health. 2026;6:e0006243. https://doi.org/10.1371/journal.pgph.0006243
32. DeJonge PM, Adams C, Pray I, Schussman MK, Fahney RB, Shafer M, et al. Wastewater surveillance data as a complement to emergency department visit data for tracking incidence of influenza A and respiratory syncytial virus—Wisconsin, August 2022–March 2023. MMWR Morb Mortal Wkly Rep. 2023;72:1005–9. https://doi.org/10.15585/mmwr.mm7237a2
33. Gupta P, Liao S, Ezekiel M, Novak N, Rossi A, LaCross N, et al. Wastewater genomic surveillance captures early detection of Omicron in Utah. Microbiol Spectr. 2023;11:e0039123. https://doi.org/10.1128/spectrum.00391-23
34. Xiu L, Yin K. Wastewater surveillance to track influenza viruses. Bull World Health Organ. 2024;102:623–623A. https://doi.org/10.2471/BLT.24.292285
35. Sherchan SP, Solomon T, Idris O, Nwaubani D, Thakali O. Wastewater surveillance of mpox virus in Baltimore. Sci Total Environ. 2023;891:164414. https://doi.org/10.1016/j.scitotenv.2023.164414
36. Parkins MD, Lee BE, Acosta N, Bautista M, Hubert CRJ, Hrudey SE, et al. Wastewater-based surveillance as a tool for public health action: SARS-CoV-2 and beyond. Clin Microbiol Rev. 2024;37:e0010322. https://doi.org/10.1128/cmr.00103-22
37. Levy JI, Andersen KG, Knight R, Karthikeyan S. Wastewater surveillance for public health. Science. 2023;379:26–7. https://doi.org/10.1126/science.ade2503
38. Diamond MB, Keshaviah A, Bento AI, Conroy-Ben O, Driver EM, Ensor KB, et al. Wastewater surveillance of pathogens can inform public health responses. Nat Med. 2022;28:1992–5. https://doi.org/10.1038/s41591-022-01940-x
39. Keck JW, Berry SM. Wastewater surveillance—"messy" science with public health potential. Am J Public Health. 2023;113:6–8. https://doi.org/10.2105/AJPH.2022.307141

Address for correspondence: Miguella Mark-Carew, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop S106-3, Atlanta, GA 30329-4018, USA; email: ocr2@cdc.gov

A Multi-Partner Effort to Launch and Sustain Wastewater Surveillance, United States

Jonathan Yoder, Nicole Fehrenbach, Amanda Raziano, Joseph Kanter, Deise Galan, Rebecca Rainey, Anna Mehrotra, Janet Hamilton, Ewa King, Sarah Wright, Alexandria Boehm, Marlene Wolfe, Rory Welsh, Rachel West, Scott Santibanez, Margaret A. Honein

The National Wastewater Surveillance System represents a groundbreaking initiative aimed at enhancing public health surveillance for infectious diseases through wastewater analyses. We have outlined current and planned collaborative efforts between the Centers for Disease Control and Prevention and key partners, including academic institutions, utilities, jurisdictions, and public health partner networks, to develop, implement, and sustain wastewater surveillance for infectious diseases to inform public health activities. By addressing critical challenges in assay development, community and clinician engagement, governance structures, public health ethics, data system development and integration, and innovation fostering, those partnerships lay the groundwork for a robust public health surveillance framework. Establishing this novel public health surveillance capability required developing new partnerships and skills, incorporating basic principles of public health surveillance, validating assays, and envisioning a system to meet current and future public health challenges. This capability will foster improved public health responses nationwide.

Public health surveillance is essential for monitoring health trends, detecting outbreaks, and informing and evaluating public health responses. A critical public health innovation from the COVID-19 pandemic was the establishment of wastewater surveillance (WS) for infectious diseases in communities across the United States. WS detects pathogens anonymously in community wastewater to increase understanding of community disease spread and transmission. This system complements traditional public health surveillance that relies on clinical testing of symptomatic patients who seek medical care and syndromic surveillance during patient visits to medical facilities. Because WS is a relatively new capability, the Centers for Disease Control and Prevention (CDC) worked with key partners to address multiple challenges to effectively implement and sustain a national public health WS system; validating laboratory assays that could reliably detect pathogens in wastewater and developing reasonable signal thresholds; establishing a network of wastewater utility partners to provide samples; setting up data systems that provide real-time, bi-directional data; increasing WS capacity and training for the public health workforce; developing the science base, innovation, and ethical framework for WS; and providing response tools for state, territorial, local, and tribal (STLT) health departments to respond to infectious disease threats detected through WS, including tools for engagement with local communities and healthcare providers.

The success of adding WS as an innovative public health tool with applicability far beyond COVID-19 is only possible because of the collaborative efforts of multiple stakeholders. Each stakeholder contributes unique expertise and resources to the continued development of this innovation (Figure).

Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (J. Yoder, N. Fehrenbach, A. Raziano, R. Welsh, R. West, S. Santibanez, M.A. Honein); Association of State and Territorial Health Officials, Arlington, Virginia, USA (J. Kanter); National Association of County and City Health Officials, Washington, DC, USA (D. Galan, R. Rainey); Water Environment Federation, Alexandria, Virginia, USA (A. Mehrotra); Council of State and Territorial Epidemiologists, Atlanta, Georgia, USA (J. Hamilton); Association of Public Health Laboratories, Bethesda, Maryland, USA (E. King, S. Wright); Stanford University, Stanford, California, USA (A. Boehm); Emory University, Atlanta, Georgia, USA (M. Wolfe)

DOI: https://doi.org/10.3201/eid3213.260221

Partnerships to Address Key Challenges

Validating WS Assays

Developing specific and sensitive molecular assays for detecting pathogens in wastewater was a substantial challenge. Existing clinical assays needed to be evaluated and adapted for the complex and variable wastewater matrix. In addition, many clinical assays were not in the open knowledge domain, limiting understanding of possible cross-reactions in a complex wastewater matrix. Academic and independent research partners, such as the Research Coordination Network members and WastewaterSCAN, a national program based at Stanford and Emory Universities, played a crucial role in this phase by using their speed and research capabilities to develop, pilot, and validate assays. Concurrently, several state and local public health laboratories began developing analytical capabilities and nurturing relationships with wastewater and public health partners to expand WS nationally. Public health partners field-tested those assays by using their applied technical expertise and resources to ensure the assays were accurate and met the necessary standards for public health surveillance and the public health actions associated with detections. The WS Centers of Excellence, launched in August 2022 and led by public health departments and academic partners

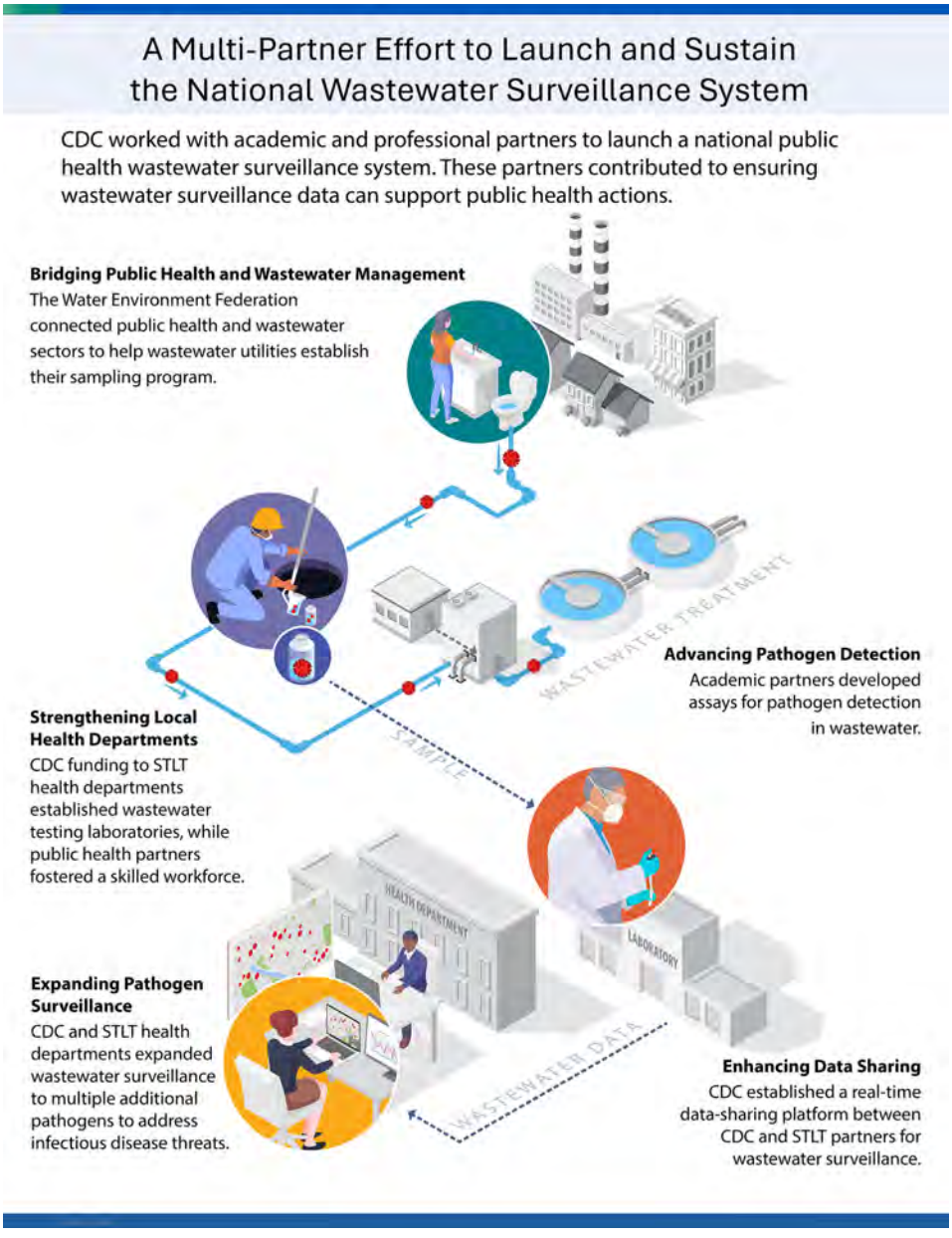


Figure. Visualization of the connections between the multiple partners needed to implement and sustain wastewater surveillance to aid public health actions in the United States. CDC, Centers for Disease Control and Prevention; STLT, state, territorial, local, and tribal.

located in California, Colorado, Texas, New York, North Carolina, and Wisconsin, became learning laboratories for the development and evaluation of assays for new pathogens. Together, those partners established a framework for continuous assay improvement, incorporating feedback from field applications, transparent sharing of available assays, assay validation, and ensuring a nimble response to current infectious disease threats. This approach proved effective during recent outbreaks of monkeypox, influenza A(H5), and measles. Because the Houston Center of Excellence (Houston, TX, USA) developed an assay for measles, they were able to deploy it promptly in Texas and New Mexico. WastewaterSCAN was able to quickly develop, validate, and deploy an assay nationally. CDC used those assays to rapidly implement national surveillance while generating additional validation data and distributing to national testing partners.

Building Relationships with Wastewater Utilities

The involvement of wastewater utilities was essential to the success of WS because WS is not feasible without their routine collection of samples. The Water Environment Federation was a pivotal partner in bridging the gap between the public health and wastewater sectors. Through cross-sector workshops, an annual national wastewater disease surveillance summit, training programs, a community of practice, development of a toolkit to inform health departments collaboration with utilities, podcast episodes and a video series, a utility-focused Web site, and a free autosampler and flow meter initiative, the Water Environment Federation enabled introductions across disciplines and lowered barriers to entry for utilities joining WS efforts. The collaboration enhanced the quality of data collected and promoted a shared understanding of the necessity of WS in public health.

Data Systems for Bidirectional Communication

A robust data infrastructure was necessary to enable bidirectional communication between CDC and STLT health departments, on the basis of the foundational concept that the "basis of effective surveillance is the current and accurate 2-way flow of information among all those who need to know" (*1*). Before the COVID-19 pandemic, public health surveillance platforms were not designed for wastewater data, which includes metadata on the sample, spatial sewershed data, and the ability to collect, analyze, and provision the data in real time to frontline health departments. WS was established on a platform that enabled real-time data sharing between CDC and STLT partners, had the ability to rapidly set up data pipelines for new pathogen targets, and was in alignment with CDC's Data Modernization Initiative (*2*). National public health partners provided guidance on data standardization and interoperability and convened key state and territorial stakeholders to ensure data were available for public health response. The development of plain-language data dashboards enabled the public and public health jurisdictions to visualize trends, make data meaningful for public audiences, and respond promptly to emerging public health threats. This system exemplified the principles of public health surveillance by promoting timely data sharing and actionable insights and communicating findings for public health action (*3*,*4*).

Increasing Capacity, Knowledge, and Training for a WS Workforce

A critical component of WS for infectious diseases is the public health capacity to test and analyze data and interpret results in a way that informs public health action. Funding was provided directly to state, territorial, local, and tribal health departments to establish their wastewater testing laboratories and surveillance programs. Public health partners provided input to guide capacity building by mentoring, developing standards, and addressing public health ethics (*5*). Academic and clinical partners, including those associated with the WS Centers of Excellence, equipped public health professionals with the necessary skills in data interpretation, laboratory techniques, and community and clinician engagement strategies. Communities of practice developed by partner organizations were instrumental in helping the new utility, laboratory and health department workforce connect and share successes and challenges. The Association of Public Health Laboratories (APHL) Wastewater Surveillance Laboratory Community of Practice started in 2020 as a small group of laboratories who met monthly to discuss the challenges of onboarding this testing; it has grown to include 643 members. Monthly presentation- and discussion-based calls cover all aspects of laboratory implementation of WS, including new targets for testing, workflow efficiencies, and sequencing. Other workforce development efforts include APHL and Council of State and Territorial Epidemiologists peer-to-peer network programs that provided bidirectional in-person visits between jurisdictions to learn new techniques and establish collaborative relationships. Those development efforts include APHL fellowship and internship programs that

have sponsored 432 fellows and 298 interns in state and local public health laboratories.

Throughout the United States, local and state health departments are working in collaboration with utilities, academic institutions, and private partners to advance WS. Local health departments (LHDs) play a critical role in those efforts by using wastewater data to inform public health action. Although some LHDs lead WS activities and manage testing directly, others play a more supportive or coordination-based role; however, all contribute critically to developing a comprehensive WS system.

To strengthen local capacity, the National Association of County and City Health Officials (NACCHO), with support from CDC, launched the Wastewater Surveillance Mentorship Program. NACCHO identifies LHDs that have extensive WS experience (mentors) and pairs them with LHDs in the early stages of program development (mentees). Throughout the program, mentors provide tailored technical assistance to help mentees design, implement, and strengthen their WS programs on the basis of their specific goals and jurisdictional needs.

To further expand support and create wider access to WS expertise, NACCHO launched the Fostering Local Wastewater Monitoring Network, a community of practice designed for LHD staff at all experience levels. By investing in workforce capacity in frontline public health, health departments can effectively use wastewater data to inform public health decisions and align with the principles of public health surveillance that continually adapt to evolving health challenges (*6*,*7*).

Developing the Science Base, Innovation, and Ethical Framework

To meet emerging and reemerging infectious disease threats, WS requires continual innovation. Although it began as an innovative system to provide critical data on community levels of COVID-19, WS has expanded to numerous pathogens on the basis of the public health need, most recently including influenza, monkeypox, and measles virus. Innovation requires continued engagement with academic partners such as WastewaterSCAN and the Centers of Excellence to expand the science base.

The collective expertise of academic partners, public health organizations, and wastewater utilities fosters an environment that is conducive to experimentation and adaptation but also promotes ethical public health practice. The Association of State and Territorial Health Officials engaged with CDC and the Centers of Excellence to develop an applied ethical framework (*5*). This framework offers guidance to utility partners, health departments, academic partners, and policymakers involved in protecting privacy, preventing stigma, ensuring proper data stewardship, and helping build trust with contributing communities and clinical professionals to enable the effective use of wastewater data for infectious disease surveillance (*8*,*9*). A strong focus on partnerships enabled the US WS program to embrace new technologies and methodologies and demonstrate leadership in public health innovation, which is a key marker of successful public health innovation (*6*,*7*).

Supporting Public Health Action

The core purpose of public health surveillance is effective and timely public health action. Public health partners provided key support to ensure that wastewater signals informed public health action for each new pathogen target. They developed response checklists, public communication strategies, and data visualizations that enabled health departments to use wastewater data for the early detection of outbreaks, monitoring persistent infectious disease threats, assessing public health interventions, and providing data that the public can use to inform and protect themselves. Partners provided ongoing feedback and input to develop and continually refine those public health action tools.

Future Challenges

The launch of the National Wastewater Surveillance System in the United States underscores the importance of collaborative partnerships in addressing complex public health challenges. By leveraging the respective infrastructure and strengths of diverse partners and working to build community trust, WS enhances our ability to monitor and respond to infectious disease public health threats. Continued innovation in testing methods (e.g., sequence-based or metagenomic approaches), data analysis, and use of wastewater for public health action will be essential to adapt to evolving public health needs and ensure the effectiveness of surveillance efforts. Integration of wastewater data into communication with key partners such as clinical professionals will extend the benefit of these data to protect the health of their patients.

Data sharing among different parties is critical to advance the use of data for public health action and advance science; data sharing protocols are needed to ensure data are used responsibly and to benefit society. Whereas clinical laboratories

generally use US Food and Drug Association–approved methods for diagnostics, WS requires new approaches to ensure data quality. Wastewater matrices are extremely complex and diverse; sometimes specific samples require bespoke methods for analysis. Because methods might be more complicated, it is essential for groups to make methods publicly available to aid data interpretation and advance wastewater-based epidemiology science. Biobanking of wastewater samples is practiced by some groups but not others. Biobanked samples are extremely valuable for retrospective studies, including studies that test new assays, but there are ethical and legal issues associated with the practice that should be addressed. Some sequence-based and metagenomic approaches are being applied to wastewater and might greatly expand the scope and value of WS; those approaches need refinement as public health surveillance tools (*10*).

In conclusion, WS is an emerging field that requires continued investment and robust partnerships to reach its full potential. Wastewater surveillance aligns with the vision of modern public health surveillance that is adaptable, integrated, and capable of addressing emerging health threats (*11*,*12*,*6*). Integration of WS into existing pathogen and genomic public health surveillance systems is an opportunity to fundamentally transform our understanding of infectious disease transmission within communities.

Acknowledgments

We thank Ericka McGowan, Alyssa Boyea, Sidnie Christian, Erin Laird, Kata Chillag, Elizabeth Fenton, Angela Blackwell, Shantrice Jones, Jena Losch, Danielle Kleven, and Luciann Draper for enabling collaboration among partners.

About the Author

Dr. Yoder is the science lead for the National Wastewater Surveillance System, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention. His research interests are focused on infectious disease surveillance and outbreak response.

References

1. Langmuir AD. The surveillance of communicable diseases of national importance. N Engl J Med. 1963;268:182–92. https://doi.org/10.1056/NEJM196301242680405
2. Centers for Disease Control and Prevention. Data modernization [cited 2025 Aug 6]. https://www.cdc.gov/data-modernization/php/one-cdc-data-platform
3. Teutsch SM, Churchill RE, editors. Principles & practice of public health surveillance. New York: Oxford University Press; 2010.
4. German RR, Lee LM, Horan JM, Milstein RL, Pertowski CA, Waller MN; Guidelines Working Group Centers for Disease Control and Prevention. Updated guidelines for evaluating public health surveillance systems: recommendations from the Guidelines Working Group. MMWR Recomm Rep. 2001;50(RR-13):1–35, quiz CE1–7.
5. West R, Moore J, Chillag K, Fenton E, Laird E, Boyea A; Wastewater Ethical Framework Development Workgroup. Development of a new framework to address public health ethical considerations in wastewater surveillance. J Public Health Manag Pract. 2025;31:683–5. https://doi.org/10.1097/PHH.0000000000002167
6. Smith PF, Hadler JL, Stanbury M, Rolfs RT, Hopkins RS, Group CSS; CSTE Surveillance Strategy Group. Blueprint version 2.0: updating public health surveillance for the 21st century. J Public Health Manag Pract. 2013;19:231–9. https://doi.org/10.1097/PHH.0b013e318262906e
7. Foege WH, Hogan RC, Newton LH. Surveillance projects for selected diseases. Int J Epidemiol. 1976;5:29–37. https://doi.org/10.1093/ije/5.1.29
8. Adams C, Horter L, Beekmann SE, Polgreen PM, Ricaldi JN, Louis S, et al. Infectious disease physicians' knowledge and practices regarding wastewater surveillance, United States, 2024. Emerg Infect Dis. 2024;30:2222–3. https://doi.org/10.3201/eid3010.240719
9. Sheth K, Hopkins L, Domakonda K, Stadler L, Ensor KB, Johnson CD, et al. Wastewater target pathogens of public health importance for expanded sampling, Houston, Texas, USA. Emerg Infect Dis. 2024;30:14–7. https://doi.org/10.3201/eid3008.231564
10. Javornik Cregeen S, Tisza MJ, Hanson B, Cook M, Surathu A, Schneider R, et al. Sequencing-based detection of measles in wastewater: Texas, January 2025. Am J Public Health. 2025;115:1115–9. https://doi.org/10.2105/AJPH.2025.308146
11. Buehler JW; Centers for Disease Control and Prevention. CDC's vision for public health surveillance in the 21st century. Introduction. MMWR Suppl. 2012;61:1–2.
12. Adams C, Bias M, Welsh RM, Webb J, Reese H, Delgado S, et al. The national wastewater surveillance system (NWSS): from inception to widespread coverage, 2020–2022, United States. Sci Total Environ. 2024;924:171566. https://doi.org/10.1016/j.scitotenv.2024.171566

Address for correspondence: Jonathan Yoder, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop H24-3, Atlanta, GA 30329-4018, USA; email: jey9@cdc.gov

Implementation and Early Outcomes of Laboratory Proficiency Testing Program for the National Wastewater Surveillance System, United States, 2024

H. Auguste Dutcher, Dagmara S. Antkiewicz, Adélaïde J. Roguet, Martin M. Shafer

Underlying the core goals of the Centers for Disease Control and Prevention National Wastewater Surveillance System (NWSS) in the United States is the need for robust and reliable pathogen data. The rapid build-out of the NWSS network and participation of many laboratories with varying expertise in environmental molecular methods has resulted in a lack of method standardization and poor data comparability. Proficiency testing is a powerful strategy to address this challenge. We describe preliminary outcomes from 2 proficiency testing rounds with 38 total participating laboratories, where reported respiratory virus concentrations from replicate samples spanned roughly 2 orders of magnitude; the program identified key opportunities for improvement. Although still in its infancy, this program proved an effective tool for quantifying variability across the network, offering preliminary insights on method performance, and improving data quality through laboratory support. Our results suggest that wastewater proficiency testing for disease surveillance will improve data quality and strengthen the NWSS.

Wastewater-based surveillance (WBS) for pathogens is increasingly recognized as a valuable complement to traditional clinical monitoring, serving as a leading indicator of increased disease transmission and providing less biased, cost-efficient community monitoring (*1*,*2*). Through the Centers for Disease Control and Prevention (CDC) National Wastewater Surveillance System (NWSS), the United States has established a network of laboratories reporting pathogen concentrations in wastewater, supporting public health decision-making (*3*,*4*). However, the rapid expansion of the network has outpaced the development of standardized protocols, resulting in substantial methodologic heterogeneity across laboratories (*3*,*5*).

With limited tools to assess the existing diverse methods used by laboratories with varied WBS experience, the comparability and utility of the data submitted to the NWSS program are compromised. Many potential sources of data variability in WBS are known, but only a handful of studies have systematically investigated their relative influence on pathogen recoveries (*2*,*6*–*9*). To date, very few interlaboratory comparisons have been conducted (*10*,*11*). Furthermore, many laboratories have insufficient resources for method optimization and operate with limited expertise and support.

A recent report from the National Academies of Sciences, Engineering, and Medicine emphasized the importance of cross-network data standardization for endurance of the network and pointed to proficiency testing (PT) as an avenue to improve data comparability (*12*). PT programs are a well-established mechanism in clinical and environmental testing for benchmarking performance (*13*–*16*), yet to our knowledge no such program exists for pathogens in wastewater, except for the Canada-focused program operated by the Ontario Clean Water Agency (*17*). Moreover, PT programs can provide insights on inter- and intra-laboratory variability, identify low- and high-performing protocols, and guide the establishment of networkwide uncertainty metrics to aid in data interpretation (*5*).

We describe preliminary outcomes of the first 2 rounds of a PT program designed and implemented by the Wisconsin Wastewater Monitoring Program

Author affiliation: Wisconsin State Laboratory of Hygiene, University of Wisconsin–Madison, Madison, Wisconsin, USA

DOI: https://doi.org/10.3201/eid3213.251612

(WWMP), a Center of Excellence in wastewater surveillance (WS) supported by the Centers for Disease Control and Prevention. We report on variability in pathogen quantification, methodologic diversity, and the effect of PT participation on laboratory performance. We identify key factors that contribute to interlaboratory variability, paving the way for future recommendations that could improve cross-network comparability. Finally, we discuss the implications of our findings for future rounds of our PT program as part of a broader effort to strengthen nationwide WS.

Methods

We spiked influent from the same water reclamation facility with inactivated influenza A/B and respiratory syncytial virus and inactivated SARS-CoV-2 B.1.1.7 whole virus (Microbiologics, https://www.microbiologics.com) at levels above the detection limit (Appendix, https://wwwnc.cdc.gov/EID/article/32/13/25-1612-App1.pdf). We added spikes per tube to preserve material (round 1) or to a bulk sample prior to aliquoting (round 2). We evaluated wastewater samples for select physiochemical characteristics, which were consistent with historical norms. We prepared 3 replicate samples per participant by using a polypropylene churn splitter (SP Bel-Art, https://www.belart.com) and shipped all samples chilled for next-day delivery. We verified replicate consistency (sample homogeneity) with the start-to-finish turbidity (Appendix Table 2) and digital PCR analysis. We conducted a 2-week decay study to ensure stability of the viral signal over the assigned sample analysis window. Viral concentrations decreased an average of 30% at 1 week from preparation for influenza and SARS-CoV-2 (Appendix Table 1).

Results and Discussion

The WWMP PT program maintained some elements of traditional PT while incorporating features designed to support laboratories and improve data comparability across the NWSS network. Participating laboratories received wastewater samples, and we instructed them to perform their standard wastewater pathogen protocols (consistent with NWSS, which imposes no prescribed workflow). Alongside quantification results, laboratories submitted detailed metadata of their wastewater processing workflows (https://doi.org/10.6084/m9.figshare.32301843). We used those metadata for quality assurance and to better understand the methodologic factors that can influence performance.

Metadata Overview and Influence of Sample Pretreatment on Data Interpretation

We conducted the first 2 rounds of our WS PT program in 2024 and focused on the respiratory pathogens SARS-CoV-2, influenza A virus, influenza B virus, and respiratory syncytial virus, along with any fecal markers and process recovery controls assessed by participating laboratories. Across both rounds (involving 38 unique laboratories total), 27 participants were state public health laboratories, representing most of the state jurisdictions reporting to NWSS. Enrolled laboratories used a wide variety of concentration and extraction methods (Figure 1; Appendix Tables 4–6).

To assess the effects of viral partitioning between the liquid phase and particulate matter on method performance, we asked laboratories to specify the wastewater phase processed in their workflows. Most laboratories analyzed a bulk sample (18 laboratories in round 1 and 24 in round 2), defined as a sample with no solids removal. Laboratories processing liquid-only samples (11 laboratories in round 1 and 14 in round 2) used a solids removal method such as centrifugation or filtration. Prior work has shown that SARS-CoV-2 and other viruses can associate strongly with solids and that sample prefractionation can substantially influence measured concentrations (*18*,*19*). NWSS uses a purely relative measure to interpret and visualize wastewater data, which does not account for this bulk versus liquid only distinction; however, the magnitude of partitioning between liquid and solid fractions can vary by pathogen and may result in systematically different pathogen recoveries. To investigate this question, we selected results from the most prevalent concentration method (Ceres Nanotrap, https://www.ceresnano.com) and compared data from laboratories that analyzed bulk and liquid fractions. We observed no statistically significant difference in SARS-CoV-2 concentration distributions between the 2 fractions (Appendix Figure 2), although substantial variability across laboratories limits our ability to statistically resolve particle-partitioning effects. In addition, our PT metadata review revealed that some laboratories also pretreat samples with proteases, detergents, or both to help mobilize viral particles into the liquid phase, introducing additional complexity into the interpretation of liquid only versus bulk categories. Because our current definitions of liquid-only versus bulk might not accurately reflect true viral partitioning behavior, we opted to pool results across sample fractions for the purposes of this analysis.

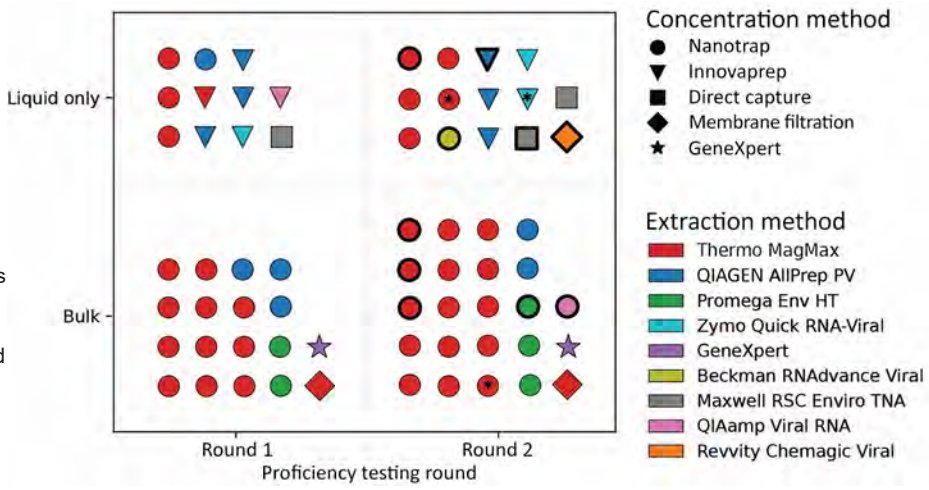


Figure 1. Laboratory workflows represented by the participants of the first 2 rounds of the Wisconsin Wastewater Monitoring Program wastewater proficiency testing program in study of implementation and early outcomes of laboratory proficiency testing program for National Wastewater Surveillance System, United States, 2024. Each symbol represents a single laboratory's wastewater processing workflow (some laboratories tested multiple workflows). Laboratories are separated by the wastewater fraction processed (y-axis). Bold outline indicates new participants in round 2. Asterisks indicate laboratories that implemented a method change from round 1 to round 2. Nanotrap (Ceres Nanosciences, Inc., https://www.ceresnano.com); InnovaPrep (InnovaPrep LLC, https://www.innovaprep.com); Direct Capture (Promega, https://www.promega.com); GeneXpert (Cepheid, https://www.cepheid.com); Thermo MagMAX (Thermo Fisher Scientific, https://www.thermofisher.com); QIAGEN AllPrep Power Viral (QIAGEN, https://www.qiagen.com); Promega HT Env Kit (Promega, https://www.promega.com); Zymo Quick RNA-Viral (Zymo Research, https://www.zymoresearch.com); Beckman RNAdvance Viral (Beckman Coulter, Inc., https://www.beckman.com); Maxwell RSC Enviro TNA Kit (Promega, https://www.promega.com); QIAmp Viral (QIAGEN, https://www.qiagen.com); Revvity Chemagic Viral DNA/RNA 300 Kit (Revvity, Inc., https://www.revvity.com).

Value of Follow-up and Summary Report for Participants

As previously mentioned, our comprehensive data review and follow-up with participating laboratories was a key component of each PT round. Many laboratories benefited from the support provided by the WWMP throughout their participation, including receiving guidance that led to reporting corrections. In round 2, for example, 25 of 36 laboratories required follow-up for calculation, metadata, or other clarifications to ensure accurate reporting (Appendix Table 3). Some important corrections included unit conversion and dilution adjustments and correction of misapplied processing factors, which resulted in substantial changes (sometimes by orders of magnitude) to final pathogen concentrations (Figure 2). In the second round of PT, 3 of 9 new participants required corrections to their pathogen concentration calculations, compared with just 5 of 27 returning participants, highlighting the value of continued engagement.

After data analysis by the WWMP, we sent participating laboratories a report representing compiled data (Appendix Figure 8), including summary statistics for each pathogen and an associated z-score for each laboratory. That report enabled participants to independently assess their performance compared with all laboratories and those laboratories with similar workflows. Several survey respondents highlighted these benefits; one respondent noted, "It was very useful seeing our results plotted out with other labs to see how our results compare," and another wrote, "Having this resource provides us with a clear way to verify that our processing methods are robust." Overall, survey responses indicated that follow-up interactions and PT reports were valuable for participants and useful for cross-laboratory performance comparisons.

Interlaboratory and Intralaboratory Variability

Compiled results showed that measured concentrations for each pathogen, which do not incorporate any recovery corrections (Appendix Figure 6), spanned approximately 2 orders of magnitude in each PT round (Figure 3, panel A), a modest improvement over the wide range reported by an interlaboratory comparison conducted in 2020 (*11*). The range observed in our exercises was comparable to 2 other interlaboratory comparison studies conducted with smaller laboratory cohorts (*17*,*20*). Normalization of pathogen concentrations by the fecal marker pepper mild mottle virus, which is not reported by all laboratories, did not consistently reduce data spread across pathogens (Appendix Figure 1, 3), and variances were not significantly different before versus after normalization, with the exception of SARS-CoV-2 in round 2 ($p = 0.04$ by Brown–Forsythe test). Restricting pathogen concentration comparisons to laboratories by using the most common workflow (bulk wastewater samples processed by using Ceres Nanotrap concentration and MagMAX extraction [Thermo Fisher Scientific, https://www.thermofisher.com]) modestly improved the comparability of reported results (range of 1.4 $\log_{10}$ units for SARS-CoV-2 in round 1,

compared with nearly 2 $\log_{10}$ units for all laboratories [Appendix Figure 4]). However, sample size is limited given the overall diversity of methods used (Figure 1). Recruiting laboratories that all employ the same concentration and extraction method would enable a more rigorous assessment of cross-laboratory variability and could be a valuable design for future PT rounds.

To complement this cross-laboratory analysis, we also examined intralaboratory variation by using the 3 replicate samples included in both rounds of PT (Figure 3, panel B). Across both rounds and all pathogens, the interquartile range of relative SD across replicates was 12%–30%, and roughly 80% of all pathogen results had a relative SD <35%. Although no universally accepted benchmarks for intralaboratory variability exist for WBS, those values are very modest in the context of high interlaboratory variability and suggest that most laboratories can achieve a reasonably high level of reproducibility. Across rounds, SARS-CoV-2 z-scores were moderately correlated (Appendix Figure 7), particularly when laboratories that changed methods between round 1 and round 2 were omitted (adjusted r-squared = 0.669; $p = 6.29 \times 10^{-7}$), supporting NWSS' use of site-relative reporting.

Points of Intervention Identified in First 2 Rounds of PT

A comparative analysis of method performance from the 2 rounds of PT results is inherently limited by the sample size, specifically by the small number of results representing any 1 workflow. Nonetheless, preliminary results suggest some methods might be underperforming; for example, laboratories using the Innovaprep Concentrating Pipette (https://www.innovaprep.com) reported significantly lower pathogen concentrations than those using Ceres Nanotrap particles ($p<0.01$ by 2-sided Mann–Whitney U test) (Appendix Figure 5, panel A). When we compared raw viral concentrations (PCR instrument data reported in gene copies/µL of PCR reaction), that lower performance was statistically significant for SARS-CoV-2 and influenza B virus only (Appendix Figure 5, panel B). Even so, those preliminary results suggest that identifying underperforming methods can be successful within the structure of the program and may be a valuable route to improving cross-network comparability.

We also noted that, for SARS-CoV-2 (the only pathogen quantified by all participating laboratories), the lowest quartile contributed disproportionately to overall variability. In round 1, the lowest 25% of

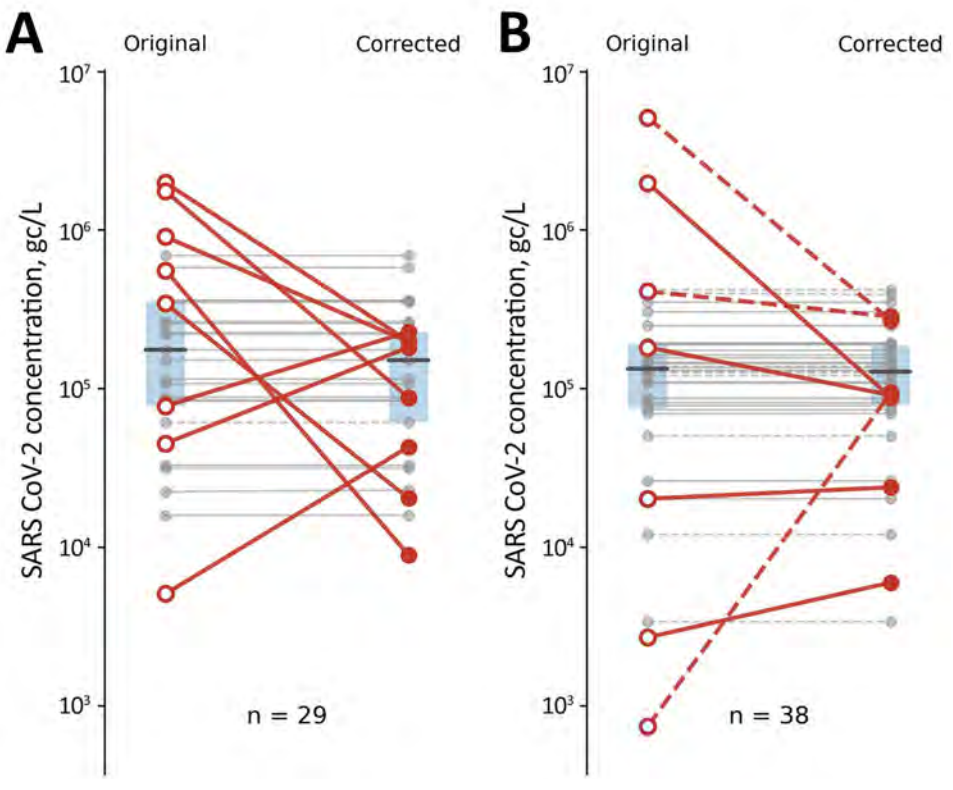


Figure 2. Reported SARS-CoV-2 concentrations in wastewater before and after Wisconsin Wastewater Monitoring Program proficiency testing (PT) intervention and laboratory resubmission of corrected concentrations in study of implementation and early outcomes of laboratory proficiency testing program for National Wastewater Surveillance System, United States, 2024. A) Round A; B) round 20. Each point represents the reported concentration of SARS-CoV-2 from a given laboratory workflow, averaged across gene targets and replicates (n = 3). Total number per PT round is larger than the number of participating laboratories because of multiple workflows tested by some participants. Lines connect results from the same laboratory before and after correction; dotted lines indicate results from new participants in round 2. Red lines highlight results that required substantial correction reflecting issues such as unit conversion errors, misapplied processing factors, or other calculation discrepancies in the initially reported data identified during PT data review; no re-analysis of samples was performed. Blue shaded area denotes the interquartile region across laboratories before and after correction; line denotes median. gc/L, gene copies per liter.

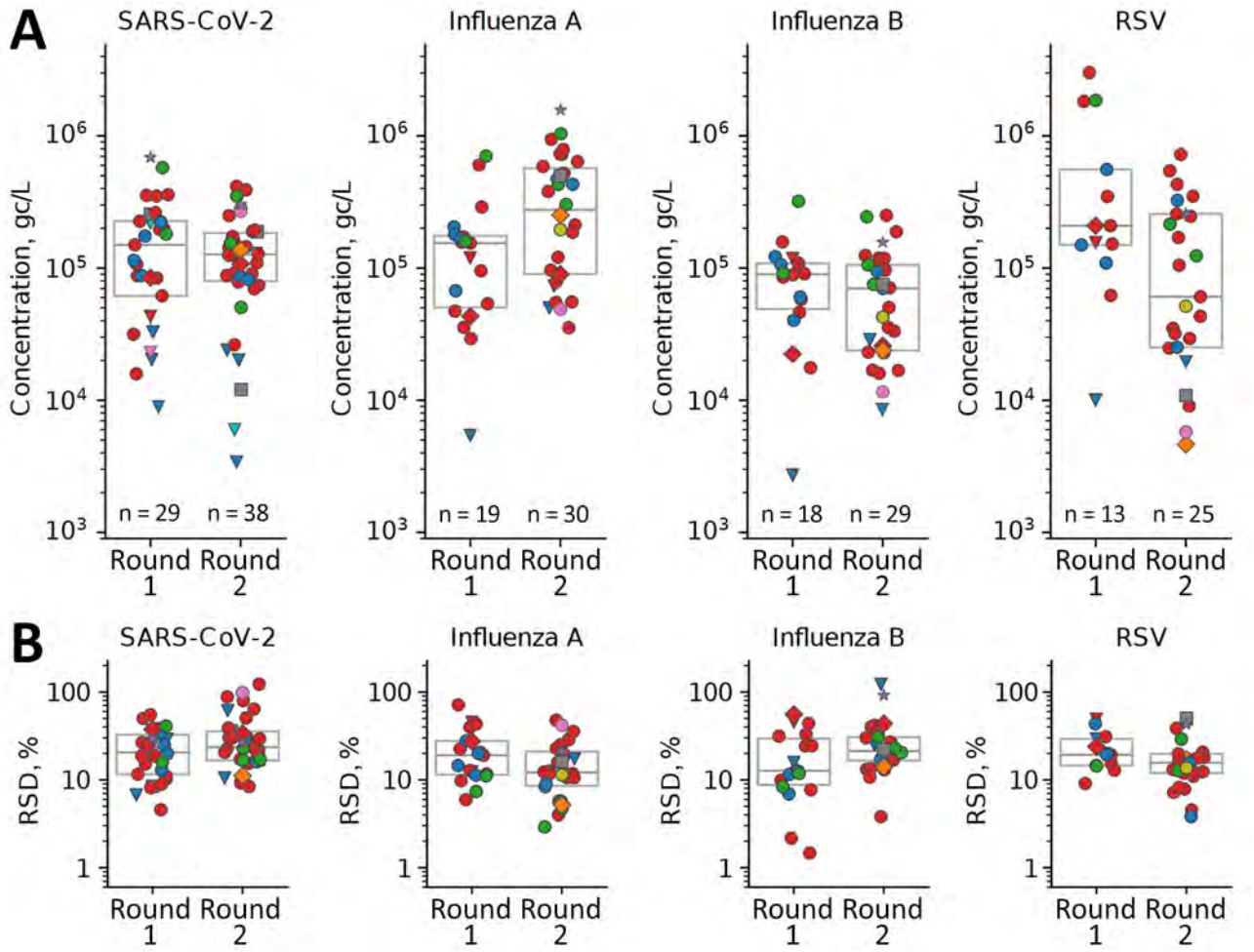


Figure 3. Results from first 2 rounds of Wisconsin Wastewater Monitoring Program proficiency testing, including respiratory pathogens SARS-CoV-2, influenza A, influenza B, and RSV in study of implementation and early outcomes of laboratory proficiency testing program for National Wastewater Surveillance System, United States, 2024. A) Concentrations of pathogens reported by the laboratories participating in proficiency testing rounds 1 and 2. Each symbol represents 1 laboratory's results from a single workflow, averaged over gene targets (if applicable) and replicates (n = 3 for both rounds). B) RSD (%) of pathogen concentrations reported from the replicates in panel A (n = 3). Shapes and colors denote different methods of wastewater concentration and extraction (Figure 1). Horizontal lines within boxes indicate median, top lines of each box indicate 75th percentiles, and bottom lines represents 25th percentiles. gc/L, gene copies per liter; RSD, relative standard deviation; RSV, respiratory syncytial virus.

concentrations spanned approximately 0.8 $\log_{10}$ units, and in round 2, nearly 1.4 $\log_{10}$ units. In contrast, the upper 75% of values ranged just over 1 order of magnitude in round 1 and approximately 0.7 units in round 2 (Figure 3, panel A). That finding suggests that a relatively small subset of results accounts for a substantial fraction of interlaboratory variability. Focusing improvement efforts on laboratories and methods represented in the lowest recovery quartile could prove to be an effective means of improving network data comparability, at least for some pathogens.

Qualitative Outcomes

Results from the follow-up surveys of participants highlight the PT program's ability to identify problem areas and provide critical support to laboratories. Survey responses identified areas for program improvement and mirrored many of our existing plans for program expansion. For example, multiple laboratories suggested that a "PCR quantification only" PT round would be of interest. Narrowing PT exercises to focus on specific steps of the overall method may prove an important strategy to determine sources of variability, a strategy our program has since implemented with a digital PCR–focused round in late 2025. One respondent noted, "It is hard to compare our results to others since our protocol is not aligned with the majority. Maybe including an RNA sample for PCR only so we can determine if our PCR assay is comparable." Another laboratory

expressed interest specifically in high-consequence pathogens: "It would be interesting to investigate how different targets such as mpox and measles compare with the respiratory targets to determine if there are alternate processing methods needed to provide robust data for these targets." Recognizing that need, our recently completed rounds of the PT program have included high-consequence pathogens that are relatively new additions to wastewater testing panels.

Of note, several laboratories reported correcting mistakes or reevaluating internal processes as a result of participation. In the follow-up survey, 4 respondents in round 1 and 5 respondents in round 2 indicated that their laboratories had corrected a mistake in calculations or their reported results (13 survey responses per round). One participant noted in their follow-up survey, "We found many problems with our method which without the PT report we would not have found." Another laboratory reported changing methods to improve pathogen recovery after seeing their laboratory's relative performance. Survey responses confirmed that enrolled laboratories welcome ongoing guidance; one respondent noted, "I'm looking forward to continued guidance on method optimization." Together, those findings confirm that the program not only provides a comparative assessment of method performance but also is a powerful approach to improve data quality and laboratory practices.

Conclusions and Future Directions

Early results from WWMP's wastewater PT program demonstrated the value of structured interlaboratory comparisons in improving overall network data quality. Our findings corroborate previously reported variability in cross-laboratory pathogen quantification from wastewater and highlight the critical role of PT in identifying areas of improvement. From the first 2 rounds alone, we lack sufficient data to confidently reject any specific methods; instead, a key outcome of the initial rounds was identifying reporting errors, often stemming from calculation mistakes or unfamiliarity with standard practices. Incorporating individual consultations and collecting raw data (including metadata) as part of our program design proved essential for improving data quality. For multiple laboratories, the exercise provided an avenue for scientific guidance for their programs, and the act of participating alone improved data quality, suggesting that all laboratories reporting to NWSS would benefit from participating in PT.

Looking ahead, the goals for our wastewater PT program include expanding participation, incorporating additional pathogens, and improving various aspects of program implementation, such as metadata capture and reporting. Progress on those goals has already been made, including implementation of cloud-based data capture in round 3 and a measles-focused PCR quantification–only round 4. Contingent on continued financial support, we aim to continue our program implementation into the foreseeable future, with a long-term goal to make more specific recommendations regarding laboratory methodology and performance. Those efforts will improve the quality and comparability of WS data, strengthening NWSS and ensuring that the underlying data are as robust and reliable as possible for unbiased and defensible public health decision-making.

Acknowledgements

We thank the Wisconsin State Laboratory of Hygiene Proficiency Testing Department for coordinating PT program enrollment and shipping of wastewater samples. We also thank the Wisconsin State Laboratory of Hygiene Wastewater Surveillance Team for support in project implementation. The insightful discussions with Alex Chik on PT program implementations are much appreciated. Many thanks to the Madison Metropolitan Sewerage District for ongoing support of our method development efforts.

This work was supported by the Wisconsin Department of Health Services (DHS) using funds provided by the Centers for Disease Control and Prevention through the Epidemiology and Laboratory Capacity program, including National Wastewater Surveillance System 2 (DHS grant agreement no. 435100-A25-ELCProj-00-M1) and the Epidemiology and Laboratory Capacity program's Enhancing Detection Expansion program (DHS grant agreement nos. 435100-A24-ELCProj-01 and 435100-A25-ELCProj-00).

We acknowledge the use of Microsoft Copilot (GPT-4, OpenAI) for assistance with phrasing and clarity during manuscript preparation.

About the Author

Dr. Dutcher is a scientist at the Wisconsin State Laboratory of Hygiene at the University of Wisconsin, Madison. Together with her colleagues in the Wisconsin Wastewater Monitoring Program, she leads efforts on proficiency testing, multidrug-resistant organism detection, and pathogen sequencing from wastewater.

References

1. Carmo Dos Santos M, Cerqueira Silva AC, Dos Reis Teixeira C, Pinheiro Macedo Prazeres F, Fernandes Dos Santos R, de Araújo Rolo C, et al. Wastewater surveillance for viral pathogens: a tool for public health. Heliyon. 2024;10:e33873. https://doi.org/10.1016/j.heliyon.2024.e33873
2. LaTurner ZW, Zong DM, Kalvapalle P, Gamas KR, Terwilliger A, Crosby T, et al. Evaluating recovery, cost, and throughput of different concentration methods for SARS-CoV-2 wastewater-based epidemiology. Water Res. 2021;197:117043. https://doi.org/10.1016/j.watres.2021.117043
3. Adams C, Bias M, Welsh RM, Webb J, Reese H, Delgado S, et al. The National Wastewater Surveillance System (NWSS): from inception to widespread coverage, 2020–2022, United States. Sci Total Environ. 2024;924:171566. https://doi.org/10.1016/j.scitotenv.2024.171566
4. Valencia D, Yu AT, Wheeler A, Hopkins L, Pray I, Horter L, et al. Notes from the field: the National Wastewater Surveillance System's Centers of Excellence contributions to public health action during the respiratory virus season—four U.S. jurisdictions, 2022–23. MMWR Morb Mortal Wkly Rep. 2023;72:1309–12. https://doi.org/10.15585/mmwr.mm7248a4
5. Keenum I, Lin NJ, Logan-Jackson A, Gushgari AJ, D'Souza N, Steele JA, et al. Optimizing wastewater surveillance: the necessity of standardized reporting and proficiency for public health. Am J Public Health. 2024;114:859–63. https://doi.org/10.2105/AJPH.2024.307760
6. Antkiewicz DS, Janssen KH, Roguet A, Pilch HE, Fahney RB, Mullen PA, et al. Wastewater-based protocols for SARS-CoV-2: insights into virus concentration, extraction, and quantitation methods from two years of public health surveillance. Environ Sci Water Res Technol. 2024;10:1766–84. https://doi.org/10.1039/D3EW00958K
7. Azzellino A, Pellegrinelli L, Pedrini R, Turolla A, Bertasi B, Binda S, et al. Evaluating interlaboratory variability in wastewater-based COVID-19 surveillance. Microorganisms. 2025;13:526. https://doi.org/10.3390/microorganisms13030526
8. Babler KM, Amirali A, Sharkey ME, Williams SL, Boone MM, Cosculluela GA, et al. Comparison of electronegative filtration to magnetic bead-based concentration and V2G-qPCR to RT-qPCR for quantifying viral SARS-CoV-2 RNA from wastewater. ACS ES T Water. 2022;2:2004–13. https://doi.org/10.1021/acsestwater.2c00047
9. Kaya D, Niemeier D, Ahmed W, Kjellerup BV. Evaluation of multiple analytical methods for SARS-CoV-2 surveillance in wastewater samples. Sci Total Environ. 2022;808:152033. https://doi.org/10.1016/j.scitotenv.2021.152033
10. Chik AHS, Ho JJY, Srikanthan N, Dhiyebi H, Servos M. Design and validation of sample splitting protocol for comparison of SARS-CoV-2 quantification in wastewater. J Environ Eng. 2022;148:06022001. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001994
11. Pecson BM, Darby E, Haas CN, Amha YM, Bartolo M, Danielson R, et al.; SARS-CoV-2 Interlaboratory Consortium. Reproducibility and sensitivity of 36 methods to quantify the SARS-CoV-2 genetic signal in raw wastewater: findings from an interlaboratory methods evaluation in the U.S. Environ Sci Water Res Technol. 2021;7:504–20. https://doi.org/10.1039/D0EW00946F
12. Committee on Community Wastewater-based Infectious Disease Surveillance, Water Science and Technology Board, Division on Earth and Life Studies, Board on Population Health and Public Health Practice, Health and Medicine Division, National Academies of Sciences, Engineering, and Medicine. Increasing the utility of wastewater-based disease surveillance for public health action: a phase 2 report. Washington: The National Academies Press; 2024 [cited 2025 Aug 1]. https://doi.org/10.17226/27516
13. Stang HL, Anderson NL. Use of proficiency testing as a tool to improve quality in microbiology laboratories. Clin Microbiol Newsl. 2013;35:145–52. https://doi.org/10.1016/j.clinmicnews.2013.08.007
14. Rosengren A, Heneryd AC. Proficiency testing schemes—their role in quality assurance. Int J Food Microbiol. 1998;45:55. https://doi.org/10.1016/S0168-1605(98)00147-0
15. Babady NE, Bourassa L, Burnham CD, Fisher M, McElvania E, Polage CR, et al. Multicenter evaluation of processing and analysis of College of American Pathologists (CAP) proficiency testing samples by laboratory automation. J Clin Microbiol. 2021;59:e03233-20. https://doi.org/10.1128/JCM.03233-20
16. Van Vliet KE, Muir P, Echevarria JM, Klapper PE, Cleator GM, Van Loon AM. Multicenter proficiency testing of nucleic acid amplification methods for the detection of enteroviruses. J Clin Microbiol. 2001;39:3390–2. https://doi.org/10.1128/JCM.39.9.3390-3392.2001
17. Chik AHS, Glier MB, Servos M, Mangat CS, Pang XL, Qiu Y, et al.; Canadian SARS-CoV-2 Inter-Laboratory Consortium. Comparison of approaches to quantify SARS-CoV-2 in wastewater using RT-qPCR: results and implications from a collaborative inter-laboratory study in Canada. J Environ Sci (China). 2021;107:218–29. https://doi.org/10.1016/j.jes.2021.01.029
18. Roldan-Hernandez L, Boehm AB. Adsorption of respiratory syncytial virus, rhinovirus, SARS-CoV-2, and F+ bacteriophage MS2 RNA onto wastewater solids from raw wastewater. Environ Sci Technol. 2023;57:13346–55. https://doi.org/10.1021/acs.est.3c03376
19. Phaneuf JR, Cha G, Hatt JK, Konstantinidis KT, Graham KE. Decay and solid-liquid partitioning of mpox and vaccinia virus DNA in primary influent and settled solids to guide wastewater-based epidemiology practices. Water Res. 2025;284:123904. https://doi.org/10.1016/j.watres.2025.123904
20. Davis A, Keely SP, Brinkman NE, Bohrer Z, Ai Y, Mou X, et al. Evaluation of intra- and inter-lab variability in quantifying SARS-CoV-2 in a state-wide wastewater monitoring network. Environ Sci Water Res Technol. 2023;9:1053–68. https://doi.org/10.1039/D3EW90014B

Address for correspondence: H. Auguste Dutcher, Wisconsin State Laboratory of Hygiene, 2601 Agriculture Dr, Madison, WI 53718, USA; email: auguste.dutcher@slh.wisc.edu

Targeted Wastewater Surveillance during the World Athletics Championship, Oregon, USA, 2022

Rebecca Falender, Devrim Kaya, Michael Harry, Oumaima Hachimi, Tyler Radniecki, Christine Kelly, Paul R. Cieslak, Andraya Hendrick, David Mickle, Anirudh Bhatia, Brent Kronmiller, Mathew Geniza, Dana Alegre, Justin Elser, Patrick Luedtke, Stacey Jeffries Miles, Nancy Gerloff, Melissa Sutton

Targeted wastewater surveillance during the 18th World Athletics Championships in Eugene, Oregon, USA, in 2022 detected influenza A virus, SARS-CoV-2, and hepatitis A virus. Poliovirus detections were inconclusive. Influenza B, hepatitis E, and measles viruses and Middle East respiratory syndrome coronavirus were not detected. Wastewater surveillance augments traditional surveillance to mitigate risks associated with large multinational gatherings.

During July 15–24, 2022, the University of Oregon (Eugene, Oregon, USA) hosted the 18th World Athletics Championships. More than 1,700 athletes from 179 nations competed, and ≈54,000 persons attended the first 3 days of the event (*1*,*2*). During the event, Oregon Health Authority, in collaboration with Oregon State University, Lane County Health and Human Services, University of Oregon, and the city of Eugene, implemented targeted wastewater testing for common or high-consequence pathogens unlikely to be detected through existing surveillance mechanisms: SARS-CoV-2, influenza A and B viruses, hepatitis A and E viruses, measles virus, Middle East respiratory syndrome coronavirus (MERS-CoV), and poliovirus.

Methods

Study Design

We adapted Oregon Health Authority and Oregon State University's existing wastewater surveillance (WS) system for the event. Wastewater samples were collected from Eugene's wastewater treatment plant (WWTP) influent and 8 microsewershed sites relevant to the event (Figure 1; Appendix Figure, https://wwwnc.cdc.gov/EID/article/32/13/26-0537-App1.pdf). We initiated surveillance ≈2 weeks before the event and continued through 4 weeks after the event (June 28–August 23, 2022). Wastewater samples were collected daily during the event, 2× times/week for 2 weeks before and after the event, and 1× time/week during the final 2 weeks of the surveillance period. We analyzed wastewater data in R Studio version 4.3.1 (https://rstudio.com/products/rstudio). We published results to a public-facing dashboard within 24 hours. We did not make poliovirus results publicly available because confirmatory testing was negative.

Sample Collection and Analyses

We concentrated 24-hour composite wastewater samples, extracted nucleic acids, and quantified viral concentrations, as previously described (*3*), and conducted droplet digital reverse transcription PCR (ddRT-PCR) (Appendix Tables 1–3). We sequenced samples that contained ≥4.0 $\log_{10}$ gene copies/L of SARS-CoV-2, as previously described (*4*). We analyzed all samples and controls in duplicate, using a limit of detection of 3 droplets for positive samples and controls.

Poliovirus Cross-Reactivity Analysis

We evaluated in silico cross-reactivity of the poliovirus ddRT-PCRs against 164 reference genomes obtained from the National Center for Biotechnology Information Virus database (https://www.ncbi.nlm.nih.gov/labs/virus), representing 12 enterovirus C types

Author affiliations: Oregon Health Authority, Portland, Oregon, USA (R. Falender, P.R. Cieslak, A. Hendrick, M. Geniza, M. Sutton); Oregon State University, Corvallis, Oregon, USA (D. Kaya, M. Harry, O. Hachimi, T. Radniecki, C. Kelly, D. Mickle, A. Bhatia, B. Kronmiller, D. Alegre, J. Elser); Lane County Department of Health and Human Services, Eugene, Oregon, USA (P. Luedtke); Centers for Disease Control and Prevention, Atlanta, Georgia, USA (S. Jeffries Miles, N. Gerloff)

DOI: https://doi.org/10.3201/eid3213.260537

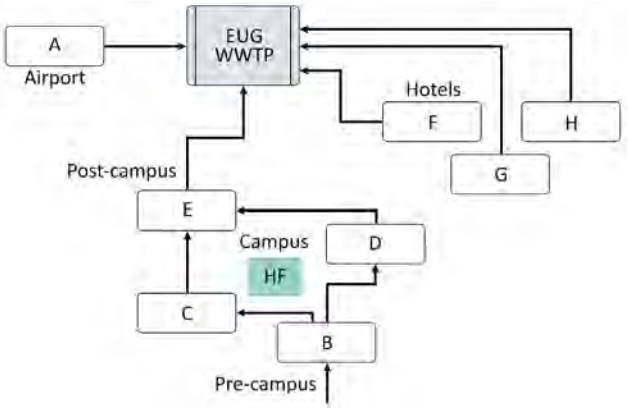


Figure 1. Flow of wastewater and sampling locations during the World Athletics Championship, Eugene, Oregon, USA, 2022. Locations indicate A, Eugene Airport area; B, city of Eugene before water flow enters the University of Oregon campus (precampus); C and D, city of Eugene plus University of Oregon campus (campus); E, city of Eugene, University of Oregon campus, and neighborhood immediately west of the campus (postcampus); F, G, H, hotels and adjacent neighborhood. Location details are available in the Appendix Figure (https://wwwnc.cdc.gov/EID/article/32/13/26-0537-App1.pdf). EUG WWTP, Eugene Wastewater Treatment Plant; HF, Hayward Field.

known to have cross-reactivity with other poliovirus assays. We conducted those analyses using Geneious Prime version 2025.2.2 software (https://www.geneious.com) as described previously (*5*,*6*). Potential cross-reactivity required that the forward primer, reverse primer, and probe were located within the same reference sequence with <4 mismatches each, and none occurring in the last 3 bases of the 3′ end.

Results

We collected a total of 178 samples (Table; Figures 2–5). We detected influenza A in 24 (14%) samples (Table; Figure 2): 1 detection before the event, at multiple sites during the event, and intermittently after the event. Despite microsewershed detections, we detected influenza A only once in WWTP influent. We detected SARS-CoV-2 in 147 (83%) samples (Table; Figure 3); sequencing revealed a decrease in the variants BA.2, BA.4, and BA.5 that were most prevalent in Oregon at that time and an increase of previously abundant variants B.1.1.529 and BA.1 (Figure 6). We detected low concentrations of hepatitis A once, 17 days before the start of the event (Table 1; Figure 5).

We detected panpoliovirus at very low concentrations in 42 (24%) samples; however, none were positive on serotype-specific poliovirus assays (Sabin 1, 2, or 3) (Table; Figure 5; Appendix Table 4). For further characterization, we retested 32 samples with higher concentrations of panpoliovirus; 11 of those were positive (Appendix Table 4). We sent those 11 samples for confirmatory testing to the Centers for Disease Control and Prevention (Atlanta, Georgia, USA), where they tested negative on a panpoliovirus quantitative reverse transcription PCR (qRT-PCR). We observed no cross-reactivity between the poliovirus assays and enterovirus C types (Appendix Table 5). Poliovirus concentrations were insufficient for sequencing. We did not detect influenza B, hepatitis E, measles, or MERS-CoV viruses during the surveillance period. We reviewed and posted results on a public-facing dashboard daily and interpreted them in the context of case and syndromic data.

Discussion

Targeted WS during the WAC documented possible new introductions of influenza A and SARS-CoV-2 variants. Hepatitis A was detected once. Poliovirus detections could not be confirmed. Thirty-three (18%) of 179 participating nations were located entirely in the Southern Hemisphere, where seasonal influenza typically peaks in July and August. After a second cluster of influenza A detections, we found no evidence of further propagation; we detected influenza A once at the WWTP, which suggested that it was not widespread in the community. Trend analysis in influenza percent positivity from 3 clinical laboratories in the region showed no change (*7*). Similarly, we observed no increase in the proportions of emergency department and urgent care visits attributed to influenza-like illness among the 6 facilities within 25 miles of the event (*8*); those results suggested that

Table. Summary of pathogen detections and concentrations in wastewater at the World Athletics Championship, Oregon, USA, 2022*

Pathogen	No. detections	No. sites with detections	Average concentration, log_{10} gene copies/L	Range, log_{10} gene copies/L
Influenza A	24	8	3.70	3.38—4.42
SARS-CoV-2	147	9	4.96	3.34—6.92
Poliovirus†	42	9	3.84	3.43—5.04
Hepatitis A	3	2	3.33	3.26—3.37
Hepatitis E	0	0	NA	NA
Influenza B	0	0	NA	NA
Measles	0	0	NA	NA
MERS-CoV	0	0	NA	NA

*MERS-CoV, Middle East respiratory syndrome coronavirus; NA, not applicable.
†Results not confirmed by Centers for Disease Control and Prevention.

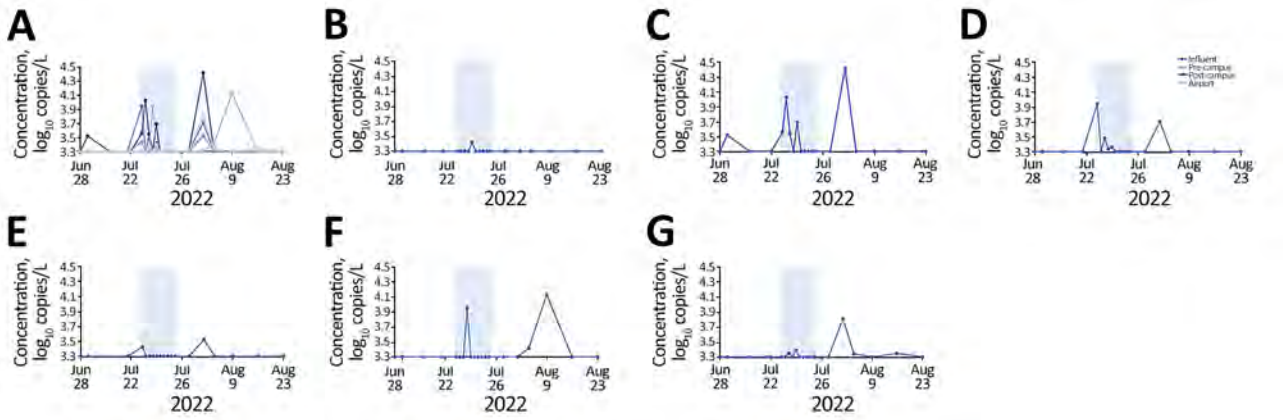


Figure 2. Mean concentrations of influenza A virus detected in wastewater before, during, and after the World Athletics Championship, Eugene, Oregon, USA, 2022. Results are shown by sampling locations: A) all sites; B) Eugene Wastewater Treatment Plant; C) city of Eugene, University of Oregon campus, and neighborhood immediately west of the campus (postcampus); D) city of Eugene plus 2 sites on the University of Oregon campus (on-campus); E) city of Eugene before the point at which water flow enters the University of Oregon campus (precampus); F) Eugene Airport area; G) 3 local hotels and adjacent neighborhood. Location details are available in the Appendix Figure (https://wwwnc.cdc.gov/EID/article/32/13/26-0537-App1.pdf).

targeted WS was more sensitive in identifying influenza A when the pathogen was not widespread in the community.

The WAC reintroduced variants of SARS-CoV-2 that had not been in circulation in Oregon since June 2020 (*9*). Population-level immunity and intervariant competition likely prevented ongoing community transmission (*10*). Most variant proportions returned to preevent baseline proportions after the event.

The detections of panpoliovirus nucleic acids by ddRT-PCR might represent cross-reactivity with nonpolio enteroviruses or true detection of polioviruses at levels too low to be identified by qRT-PCR or serotype-specific assays. WS has documented circulating vaccine-derived poliovirus in several nonendemic countries, including the United States, after importation from countries with ongoing oral poliovirus vaccine use (*11*–*13*). Previous studies have shown ddRT-PCR to have greater analytical sensitivity; limit of detection was significantly lower (in 1 study, more than tenfold lower) than qRT-PCR in detecting wastewater viruses (*14*,*15*). In our study, the poliovirus assays did not cross-react with 12 enterovirus C genomes; it is possible that the assay would cross-react with enteroviruses we did not include in our analysis (Appendix Table 5) (*5*,*6*). No human polio cases were reported in Oregon during the surveillance period.

Because the ddRT-PCR we used was highly sensitive, we inferred that influenza B, hepatitis E, and

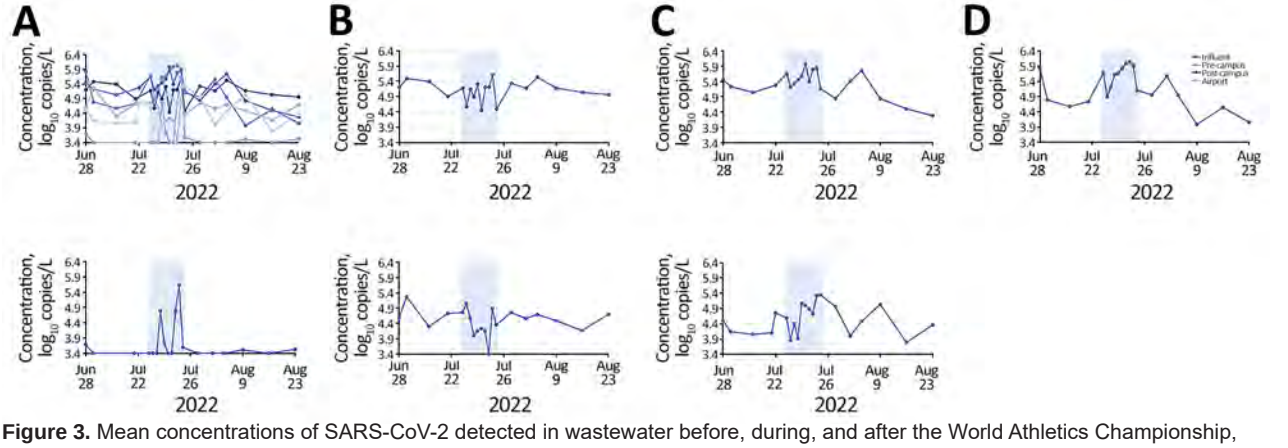


Figure 3. Mean concentrations of SARS-CoV-2 detected in wastewater before, during, and after the World Athletics Championship, Eugene, Oregon, USA, 2022. Results are shown by sampling locations: A) all sites; B) Eugene Wastewater Treatment Plant; C) city of Eugene, University of Oregon campus, and neighborhood immediately west of the campus (postcampus); D) city of Eugene plus 2 sites on the University of Oregon campus (on-campus); E) city of Eugene before the point at which water flow enters the University of Oregon campus (pre-ampus); F) Eugene Airport area; G) 3 local hotels and adjacent neighborhood. Location details are available in the Appendix Figure (https://wwwnc.cdc.gov/EID/article/32/13/26-0537-App1.pdf).

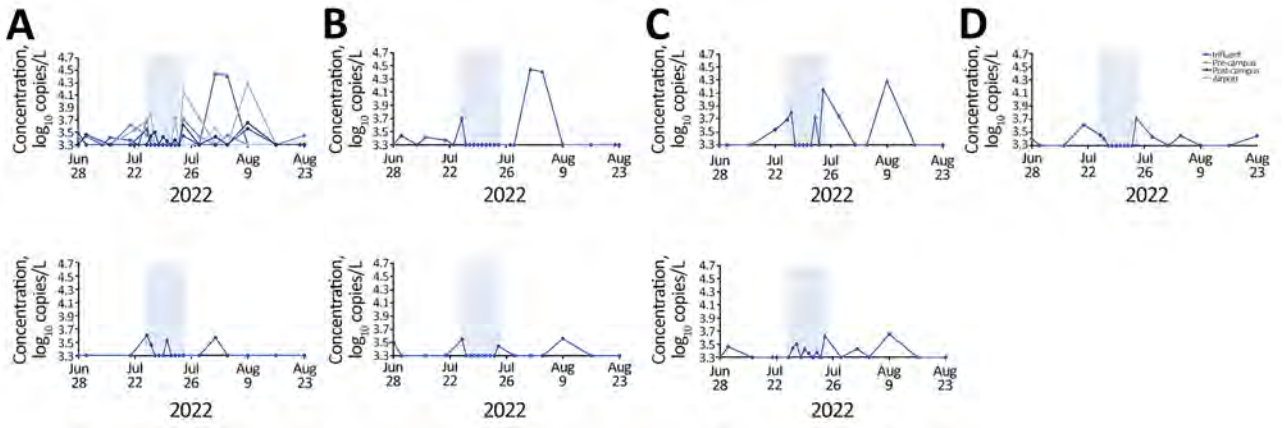


Figure 4. Mean concentrations of poliovirus detected in wastewater before, during, and after the World Athletics Championship, Eugene, Oregon, USA, 2022. Triangles indicate poliovirus samples that tested positive at retest. Poliovirus results were not confirmed by the CDC. Results are shown by sampling locations: A) all sites; B) Eugene Wastewater Treatment Plant; C) city of Eugene, University of Oregon campus, and neighborhood immediately west of the campus (postcampus); D) city of Eugene plus 2 sites on the University of Oregon campus (on-campus); E) city of Eugene before the point at which water flow enters the University of Oregon campus (precampus); F) Eugene Airport area; G) 3 local hotels and adjacent neighborhood. Location details are available in the Appendix Figure (https://wwwnc.cdc.gov/EID/article/32/13/26-0537-App1.pdf).

measles viruses and MERS-CoV were not introduced during the event because they were not detected. No local cases of those diseases were reported to public health during the surveillance period.

This study demonstrated the feasibility and utility of WS for pathogens of public health significance that might not be detected through traditional surveillance systems during a large-scale international event. Through targeted microsewershed surveillance, we detected possible introductions of influenza A and SARS-CoV-2 variants. However, we lacked the metadata to normalize microsewershed viral concentrations by population or flow. Furthermore, because visitors arrived at different times before and during the event, distinctions between event periods are not clear.

Our results strengthen previous studies of waste WS surrounding large events and provide additional considerations regarding panpoliovirus detections in wastewater (*16*). Our findings regarding increased influenza circulation could specifically indicate mitigation efforts to include alerting healthcare providers to potential influenza outbreaks and ensuring supplies of oseltamivir. Overall, targeted WS should be considered during international events as a tool to enable early outbreak detection and response, inform public health efforts, and mitigate the risk of large gatherings.

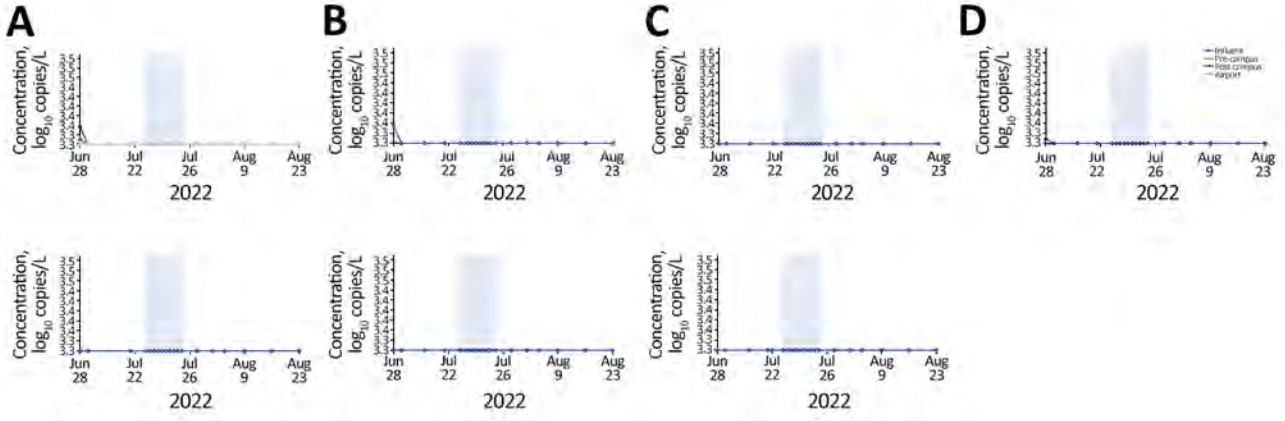


Figure 5. Mean concentrations of hepatitis A detected in wastewater before, during, and after the World Athletics Championship, Eugene, Oregon, USA, 2022. Results are shown by sampling locations: A) all sites; B) Eugene Wastewater Treatment Plant; C) city of Eugene, University of Oregon campus, and neighborhood immediately west of the campus (postcampus); D) city of Eugene plus 2 sites on the University of Oregon campus (on-campus); E) city of Eugene before the point at which water flow enters the University of Oregon campus (precampus); F) Eugene Airport area; G) 3 local hotels and adjacent neighborhood. Location details are available in the Appendix Figure (https://wwwnc.cdc.gov/EID/article/32/13/26-0537-App1.pdf).

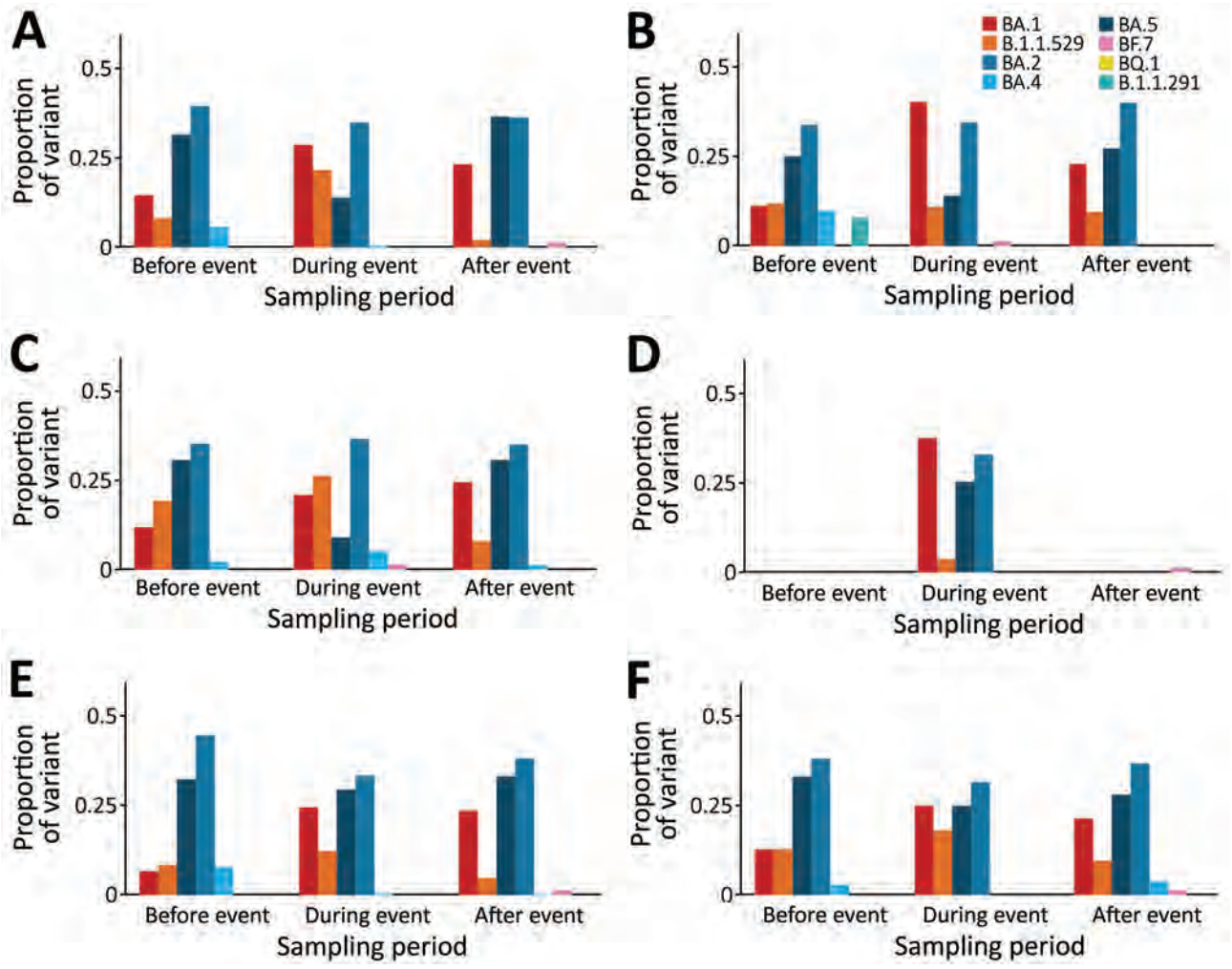


Figure 6. Proportions of SARS-CoV-2 variants detected in wastewater before, during, and after the World Athletics Championship, Eugene, Oregon, USA, 2022. The sampling periods were July 1–14, before the event; July 15–24, during the event; and July 25–August 21, after the event. The variant proportions are aggregated for 3 hotel and 2 on-campus sites. Results are shown by sampling locations: A) Eugene Wastewater Treatment Plant; B) 3 local hotels and adjacent neighborhood; C) Eugene Airport area; D) city of Eugene before the point at which water flow enters the University of Oregon campus (precampus); E) city of Eugene plus 2 sites on the University of Oregon campus (on-campus); F) city of Eugene, University of Oregon campus, and neighborhood immediately west of the campus (postcampus).

Acknowledgments

We thank the staff of the World Athletic Championships and Eugene Wastewater Treatment Plant for participating in this study.

This study was supported in part by an appointment to the Applied Epidemiology Fellowship Program administered by the Council of State and Territorial Epidemiologists and funded by the Centers for Disease Control and Prevention Epidemiology and Laboratory Capacity Cooperative Agreement. T.R. and C.K. also received grant support from the National Science Foundation. T.R. also received grant support from the US Environmental Protection Agency, US Department of Agriculture, Oregon State University Office of Research Advancement, South Dakota State University Sun Grant, and Peaks Renewables Holdco, LLC.

About the Author

Dr. Falender is the senior wastewater epidemiologist with Oregon's wastewater surveillance program. Her research interests include emerging infectious diseases, wastewater surveillance, and One Health.

References

1. World Athletics. Record number of countries win gold at World Athletics Championships Oregon22. 2022 [cited 2026 Mar 19]. https://worldathletics.org/competitions/world-athletics-championships/world-athletics-

championships-oregon-2022-7137279/news/press-releases/athletes-world-shine-wch-oregon22
2. Rogoway M. World Athletics Championships attendance tops 54,000 in first three days. OregonLive. 2022 Jul 19 [cited 2026 Jun 30]. https://www.oregonlive.com/oregon22/2022/07/world-athletics-championships-attendance-tops-54000-in-first-three-days.html
3. Layton BA, Kaya D, Kelly C, Williamson KJ, Alegre D, Bachhuber SM, et al. Evaluation of a wastewater-based epidemiological approach to estimate the prevalence of SARS-CoV-2 infections and the detection of viral variants in disparate Oregon communities at city and neighborhood scales. Environ Health Perspect. 2022;130:67010. https://doi.org/10.1289/EHP10289
4. Sutton M, Radniecki TS, Kaya D, Alegre D, Geniza M, Girard AM, et al. Detection of SARS-CoV-2 B.1.351 (Beta) variant through wastewater surveillance before case detection in a community, Oregon, USA. Emerg Infect Dis. 2022;28:1101–9. https://doi.org/10.3201/eid2806.211821
5. Harrington C, Sun H, Jeffries-Miles S, Gerloff N, Mandelbaum M, Pang H, et al. Culture-independent detection of poliovirus in stool samples by direct RNA extraction. Microbiol Spectr. 2021;9:e0066821. https://doi.org/10.1128/Spectrum.00668-21
6. Holubar M, Sahoo MK, Huang C, Mohamed-Hadley A, Liu Y, Waggoner JJ, et al. Deep sequencing prompts the modification of a real-time RT-PCR for the serotype-specific detection of polioviruses. J Virol Methods. 2019;264:38–43. https://doi.org/10.1016/j.jviromet.2018.11.007
7. Centers for Disease Control and Prevention. The National Respiratory and Enteric Virus Surveillance System (NREVSS). 2024 [cited 2025 May 15]. https://www.cdc.gov/nrevss/php/dashboard/index.html
8. Burkom H, Loschen W, Wojcik R, Holtry R, Punjabi M, Siwek M, et al. Electronic Surveillance System for the Early Notification of Community-Based Epidemics (ESSENCE): overview, components, and public health applications. JMIR Public Health Surveill. 2021;7:e26303. https://doi.org/10.2196/26303
9. Oregon Health Authority. COVID-19 variants in Oregon. 10/25/2023 [cited 2025 May 15]. https://public.tableau.com/app/profile/oregon.health.authority.covid.19/viz/GISAIDVariantDashboardUpdated/OregonVariantDashboard
10. Orf GS, Pérez LJ, Ciuoderis K, Cardona A, Villegas S, Hernández-Ortiz JP, et al. The principles of SARS-CoV-2 intervariant competition are exemplified in the pre-Omicron era of the Colombian epidemic. Microbiol Spectr. 2023;11:e0534622. https://doi.org/10.1128/spectrum.05346-22
11. Klapsa D, Wilton T, Zealand A, Bujaki E, Saxentoff E, Troman C, et al. Sustained detection of type 2 poliovirus in London sewage between February and July, 2022, by enhanced environmental surveillance. Lancet. 2022;400:1531–8. https://doi.org/10.1016/S0140-6736(22)01804-9
12. Huseynov S, Saxentoff E, Diedrich S, Martin J, Wieczorek M, Cabrerizo M, et al. Notes from the field: detection of vaccine-derived poliovirus type 2 in wastewater—five European countries, September–December 2024. MMWR Morb Mortal Wkly Rep. 2025;74:122–4. https://doi.org/10.15585/mmwr.mm7407a4
13. Mercader-Barceló J, Otu A, Townley TA, Adepoju P, Walley J, Okoibhole LO, et al. Rare recurrences of poliomyelitis in non-endemic countries after eradication: a call for global action. Lancet Microbe. 2022;3:e891–2. https://doi.org/10.1016/S2666-5247(22)00253-1
14. Ciesielski M, Blackwood D, Clerkin T, Gonzalez R, Thompson H, Larson A, et al. Assessing sensitivity and reproducibility of RT-ddPCR and RT-qPCR for the quantification of SARS-CoV-2 in wastewater. J Virol Methods. 2021;297:114230. https://doi.org/10.1016/j.jviromet.2021.114230
15. Ding J, Xu X, Deng Y, Zheng X, Zhang T. Comparison of RT-ddPCR and RT-qPCR platforms for SARS-CoV-2 detection: Implications for future outbreaks of infectious diseases. Environ Int. 2024;183:108438. https://doi.org/10.1016/j.envint.2024.108438
16. DeJonge PMV, Pray I, Poretsky R, Shafer M, McLellan SL, Kittner A, et al. Wastewater-based epidemiology to enhance public health preparedness and response during large-scale events: experiences from the 2024 Republican and Democratic National Conventions—Milwaukee, WI and Chicago, IL. Front Public Health. 2026;14:1800385. https://doi.org/10.3389/fpubh.2026.1800385

Address for correspondence: Rebecca Falender, Oregon State University, 116 Johnson Hall, 105 SW 26th St, Corvallis, OR 97331-2409, USA; email: rebecca.falender@oregonstate.edu

Evaluation of Detection Methods for Wastewater Surveillance of Antimicrobial-Resistant Bacteria from Healthcare Facilities

Ean Warren, James VanDerslice, L. Scott Benson, William J. Brazelton, Windy Tanner, Amanda K. Lyons, Florence Whitehill, Angela Coulliette-Salmond, Sydney Fusco, Jennifer Weidhaas

Carbapenem resistance in bacteria is an urgent public health threat. Wastewater surveillance could support antimicrobial resistance monitoring at long-term care facilities. We assessed feasibility of wastewater sampling at such facilities for carbapenemase genes (bla_{KPC}, bla_{VIM}, $bla_{OXA-48-like}$, bla_{NDM}, and bla_{IMP}) detected by quantitative PCR or GeneXpert Carba-R (Cepheid, https://www.cepheid.com) over 16 months and compared those findings with those for clinical infections. The bla_{KPC}, $bla_{OXA-48-like}$, and bla_{VIM} genes were routinely detected in composite wastewater samples, passive samples, and sewer biofilm swab specimens. Wastewater and sewer biofilm resistomes differed, but both contained $bla_{KPC,}$ $bla_{VIM,}$ and bla_{IMP}. Wastewater surveillance for carbapenemases shows promise, but few clinical infections during the study period and contributions from sewer biofilms complicate correlations between wastewater measurements and clinical incidence, underscoring the need for further research.

Antimicrobial-resistant infections caused >35,000 deaths in the United States (*1*) and 1,270,000 deaths worldwide (*2*) in 2017; US costs exceeded $13.5 billion (*1*). By 2050, antimicrobial resistance (AMR)–related deaths are projected to rise by 70%, to >208 million (*3*). Among the Centers for Disease Control and Prevention–classified urgent threats are carbapenem-resistant Enterobacterales (CRE), which are responsible for ≈13,100 hospital-associated infections in the United States annually (*1*). Intermediate and long-term acute care facilities are particularly vulnerable to CRE because of high transmission risk and limited treatment options for antimicrobial-resistant infections (*4*).

Routine patient surveillance for infection and colonization by antimicrobial-resistant pathogens is costly (*5*) and invasive (*6*), whereas monitoring hospital wastewater offers a nonnvasive, cost-effective alternative (*7*,*8*) that captures signals of AMR from large patient populations, including colonized persons. Multiple studies have attempted to correlate wastewater AMR genes with infections or hospitalizations in associated communities (*9*–*11*), although few were longitudinal studies (*12*–*14*). One notable study detected a bla_{NDM}-producing CRE in hospital wastewater that matched a patient isolate (*12*), whereas another found bla_{CTX-M} wastewater concentrations were reflective of the patient population (*11*).

Carbapenem-resistant infections are a growing threat in healthcare settings, highlighting the need for practical surveillance methods to detect outbreaks early. Wastewater surveillance (WS) might be such a solution, although sampling techniques might influence wastewater gene concentrations. To address those concerns, we monitored 5 carbapenemase genes of clinical concern (bla_{KPC}, bla_{VIM}, $bla_{OXA-48-like}$, bla_{NDM}, and bla_{IMP}) (*15*) in wastewater from a rehabilitation hospital over 16 months. We collected samples weekly, twice weekly, or monthly using composite, passive, and biofilm-based methods and analyzed them by quantitative PCR (qPCR), metagenomics, and culturing. We evaluated sampling method, hold time, and storage temperature to determine their effects on data reliability; we focused on inexpensive and simple approaches suitable for facilities without nearby diagnostic laboratories. By examining trends in carbapenemase gene abundance, we aimed to establish a baseline of carbapenemase genes for the facility

Author affiliations: University of Utah, Salt Lake City, Utah, USA (E. Warren, J. VanDerslice, L.S. Benson, W.J. Brazelton, W. Tanner, S. Fusco, J. Weidhaas); Centers for Disease Control and Prevention, Atlanta, Georgia, USA (F. Whitehill, A. Coulliette-Salmond); US Public Health Service, Rockville, Maryland, USA (A. Coulliette-Salmond)

DOI: https://doi.org/10.3201/eid3213.251490

and evaluate whether those trends correlated with clinical infections.

This study used deidentified patient data correlated with wastewater samples. The University of Utah Institutional Review Board determined the protocol to be exempt under Category 4 of 45 CFR 46.101(b) (protocol no. 00177673; exemption date November 26, 2024). All samples were handled in accordance with biosafety precautions.

Materials and Methods

Site Description and Wastewater Parameters

We collected sewer samples from a 5-story rehabilitation hospital with multiple wards in Utah, USA; no other wastewater sources contributed to the flow. With a staff of ≈140, the 75-bed facility provides inpatient and outpatient care (600 outpatient clinical visits per month) and food services. Inpatient care was estimated to contribute 48% of water use, outpatient care 2%, staff 14%, and food services 36% (*16*). Daily visitor count was not known. We confirmed wastewater travel time from various facility floors by flushing dye (Bright Dyes tablet tracer dyes, Kingscote Chemicals, https://www.kingscotechemicals.com) and visually monitoring wastewater in the sewer manhole for arrival of the appropriate dye, as previously reported (*17*).

Limits of Detection of Antimicrobial-Resistant Genes and Cultures in Composite Wastewater

In brief, we serially diluted bacterial cultures with carbapenemase genes in wastewater (n = 5 for each dilution, n = 3 trials per gene). We then processed samples according to standard methods (Appendix, https://wwwnc.cdc.gov/EID/article/32/13/25-1490-App1.pdf) and determined the viable culture and qPCR gene copies (GC) per milliliter for each dilution.

Wastewater Methods

Sample Collection and Processing

We compared 3 sampling methods (Table 1): composite wastewater collected using a peristaltic sampler (Hach, https://www.hach.com) collected over 24 hours (n = 297), passive absorptive samples (tampons) typically deployed for 24 hours except when field conditions did not allow for 24-hour collection (n = 110), and sewer biofilm swabs (World Bioproducts, https://www.worldbioproducts.com) from the periodically wetted area of the sewer pipe above the flowing wastewater (n = 32) (Appendix). We desorbed bacteria from passive samplers and biofilm swabs by vortex at maximum speed for 10 minutes in 1X phosphorus-buffered saline (PBS) (Thermo Fisher Scientific, https://www.thermofisher.com). We added a spike-in recovery control to all samples (Zymo Research, https://www.zymoresearch.com). We precipitated bacteria by adding polyethylene glycol 6000 and 0.9% of NaCl, shaking (80 RPM), centrifuging (typically 10 minutes at 31,400 × *g*), and resuspending in 1X PBS. We subsampled the resuspended pellet for culturing by using MacConkey I (Avantor, https://www.wvr.com) and CHROMagar KPC agar (DRG International, https://store.drg-international.com), analyzed by using GeneXpert Carba-R (Cepheid, https://www.cepheid.com), and extracted DNA (*18*) for qPCR (*19*–*21*) and metagenomics (Appendix).

Comparison of Sample Volume, Handling, and Storage

We investigated the effects of concentrating 40 mL or 500 mL of sample for gene abundance. The treatments compared 40-mL samples centrifuged at 2 different speeds and times with 500-mL samples (Table 2). We tested those speeds and temperatures with the understanding that not all laboratories have access to high-speed, temperature-controlled centrifuges. After centrifugation, we extracted nucleic

Table 1. Sample types, frequency of collection, numbers of samples, and handling methods for trend analysis over 16 months in study of detection methods for wastewater surveillance of antimicrobial-resistant bacteria from healthcare facilities*

Sample type	Frequency of collection	No. days of observations	Replicates	Total no. of samples, including replicates†	Handling method‡
Composite	Weekly or twice weekly	61	Primarily laboratory-split triplicates	297	40-mL concentrated for extraction
Sewer biofilm	Weekly or monthly	16	Field duplicates	32	Sponge shaken in sterile 1X PBS for 10 min, supernatant concentrated for extraction
Passive	Weekly or twice weekly	53	Primarily field duplicates	110	Tampon shaken in sterile 1X PBS for 10 min, supernatant concentrated for extraction

*PBS, phosphate-buffered saline.
†Total number of samples includes days when more replicates were collected (e.g., 6 replicates rather than the typical 3 replicates).
‡All samples were stored at 4°C until processing, which occurred within 24 hours of collection. All samples were processed by concentration with polyethylene glycol/NaCl precipitation for 2 hours at 4°C, centrifugation at 31,400 × *g*, and then nucleic acids were extracted from the pellet (Appendix, https://wwwnc.cdc.gov/EID/article/32/13/25-1490-App1.pdf).

Table 2. Sample handling methods for comparison of sample volumes, preservation method, hold time, hold temperature, and centrifugation speeds in study of detection methods for wastewater surveillance of antimicrobial-resistant bacteria from healthcare facilities*

Sample type	Comparisons	No. for each treatment	Volume, mL	Hold time, d	Hold temp, °C	Centrifuge settings
G	Sample volumes and centrifugation	4	40	NA	NA	10 min at 31,400 × *g*
G	Sample volumes and centrifugation	2	40	NA	NA	1 h at 3,400 × *g*
G	Sample volumes and centrifugation	4	500	NA	NA	1 h at 3,400 × *g*
C	Control, processed immediately	3	40	NA	NA	10 min at 31,400 × *g*
C	Holding times and temperatures of raw samples	3	40	1, 7, or 29	4, −20, or −70	10 min at 31,400 × *g*
C	Effect of preservatives, hold time and temperatures†	3	40	1, 7, or 29	4, −20, or −70	10 min at 31,400 × *g*
C	PEG concentrated and centrifuged sample with hold	3	40	1	4, −20, or −70	10 min at 31,400 × *g*
C	Nucleic acid stability	3	40	7	−20 or −70	10 min at 31,400 × *g*
C	Nucleic acid stability	6	40	29	−20	10 min at 31,400 × *g*

*C, composite; G, grab sample; NA, not applicable; PEG, polyethylene glycol.
†Preservative is 1:1 vol:vol mix of wastewater: sterile 50% glycerol in tris.

acids from the samples (Appendix). We used 1-way analysis of variance (ANOVA) using SigmaStat software version 14.5 (Grafiti, https://grafiti.com) with $p<0.05$ to assess significant differences among mean carbapenemase GC per milliliter when different water volumes were concentrated. If means were significantly different, we conducted multiple comparison tests using the Holm-Sidak or Dunn method.

We evaluated the effect of sample handling conditions, including hold time, temperature, and processing stage, on gene abundance in 5 treatment types, each with ≥2 replicates. We concentrated samples using polyethylene glycol centrifugation and resuspension in 1X PBS as described in the previous section. Samples processed immediately served as controls (Table 2). We compared controls with samples held for different days and temperatures before performing concentration and nucleic acid extraction with and without preservation with 1:1 50% glycerol:10 mM 2-amino-2-(hydroxymethyl)propane-1,3-diol (Tris). We used a general linearized model to determine the effect of sample handling and storage conditions on gene abundance (Appendix).

Wastewater Trends and Alignment with Facility Patient Testing Data

The facility provided monthly average patient census, carbapenem days of therapy, numbers of patients screened for carbapenem-resistant organisms (CRO), numbers of infections with carbapenemase-producing organisms, percentage of patients that were independently performing all steps of toileting and showering, infected patient admission and discharge dates, and building water use. Composite, passive, and sewer biofilm samples were collected weekly during January–December 2023 and then twice monthly during January–April 2024 (Table 1). We determined carbapenemase gene composition in samples by qPCR and Carba-R analysis. We determined total bacteria by quantifying the conserved region of the 16S rRNA gene (Unibac primers) (*25*). We adjusted carbapenemase gene abundance by qPCR for Zymo recovery and $\log_{10}$ transformed or normalization by 16S rRNA GC or wastewater flow (Appendix).

We conducted Spearman rank order correlations of carbapenemase gene detections by method and multivariate linear regressions among antibiotic-resistance gene (ARG) abundance in SAS version 9.4 (SAS Institute Inc., https://www.sas.com). We used a nonparametric regression technique to smooth scatter plots of ARG over time using PROC LOESS in SAS with smoothing parameters from 0.1 to 0.25.

Results

Limits of Detection of Antimicrobial-Resistant Genes and Cultures in Composite Wastewater

The detection limits of bla_{NDM}, bla_{VIM}, and bla_{IMP} in 40 mL of wastewater was 146–5,486 GC/mL with average and SDs of 3 trials (Table 3). We observed variations in cycle threshold (Ct) values for the different dilutions (Figure 1; Appendix).

Table 3. Limits of detection for isolates diluted in wastewater and detected by qPCR and culture-based methods and for qPCR-positive controls diluted in 1X PBS in study of detection methods for wastewater surveillance of antimicrobial-resistant bacteria from healthcare facilities*

Antimicrobial-resistant gene	Observed GC/mL wastewater, mean ±SD	Extrapolated culture detection limit, CFU/mL wastewater		Observed qPCR positive control detection limit, GC/µL qPCR reaction§
		Independent estimate, mean ±SD†	Combined estimate, limit (95% CI)‡	
bla$_{KPC}$	Not estimable	Not estimable		32
bla$_{OXA-48-like}$	Not estimable	Not estimable		34
bla$_{VIM}$	146 ±92¶	38 ±41	26 (2.9–865)	241
bla$_{NDM}$	284 ±154	25 ±24	17 (2.2–1,592)	390
bla$_{IMP}$	5,486 ±3,137	337 ±341	216 (4.2–1,5437)	2200

*Not estimable indicates wastewater contained *bla*$_{KPC}$ and *bla*$_{OXA-48-like}$ genes. GC, gene copies; PBS, phosphate-buffered saline; qPCR, quantitative PCR.
†The culture detection limit for each of 3 trials was estimated independently based on linear regressions and a cycle threshold (Ct) cutoff of 35; then, the mean and SD of those detection limits was estimated.
‡The detection limit for all 3 trials combined was estimated from the linear regression.
§Estimated from qPCR standard curves.
¶Analysis of variance or analysis of variance on ranks was used to determine for each trial the mean Ct value for the lowest dilution that was above a Ct of 35 and at a significantly different concentration than the next lowest dilution. The qPCR standard curve calculation was then used for this Ct to estimate the GC/mL. Mean and SD GC/mL for the 3 trials was then estimated.

Wastewater Methods

Comparison of Composite, Passive and Biofilm Sampling Methods

The *bla*$_{KPC}$, *bla*$_{VIM}$, and *bla*$_{OXA-48-like}$ GC normalized by water usage showed a strong correlation among composite wastewater and passive samples (Spearman ρ<0.05) (Table 4; Appendix Figure 1, whereas *bla*$_{NDM}$ and *bla*$_{IMP}$ were not correlated. In biofilm samples, *bla*$_{OXA}$ was correlated with passive samples and with *bla*$_{KPC}$. We observed inverse correlations between *bla*$_{KPC}$ in biofilms and *bla*$_{VIM}$ in passive samplers but only in 7 samples, thus decreasing the power of the analysis. Regardless of method, fewer gene concentrations were correlated when the data were not water usage–normalized (Appendix Table 5, Figure 2). When normalized to facility water usage, sewer biofilm qPCR concentrations correlated with composite wastewater samples. Deployment of passive samplers for >24 hours (n = 29) was shown to increase only total bacteria (i.e., conserved region of 16S rRNA gene) and *bla*$_{KPC}$ (p<0.05) (i.e., 0.8-day to 7-day deployment with 2.3 ±1.5 day, [average ± SD]), but R^2 values were all <0.15 (Appendix Figure 3).

We correlated CHROMagar KPC and MacConkey I culture counts in passive or composite samples for *E. coli*, *Pseudomonas* spp., and *Acinetobacter* spp. (Appendix Tables 6, 7). Metagenome sequencing revealed a wide range of ARGs (Appendix Figure 4), including detection of *bla* genes in all sample types (Appendix Figure 5). All target *bla* genes were detected in the metagenomes of ≥1 sample (Appendix Figure 4). Passive samples had more *bla*$_{KPC}$ (Appendix

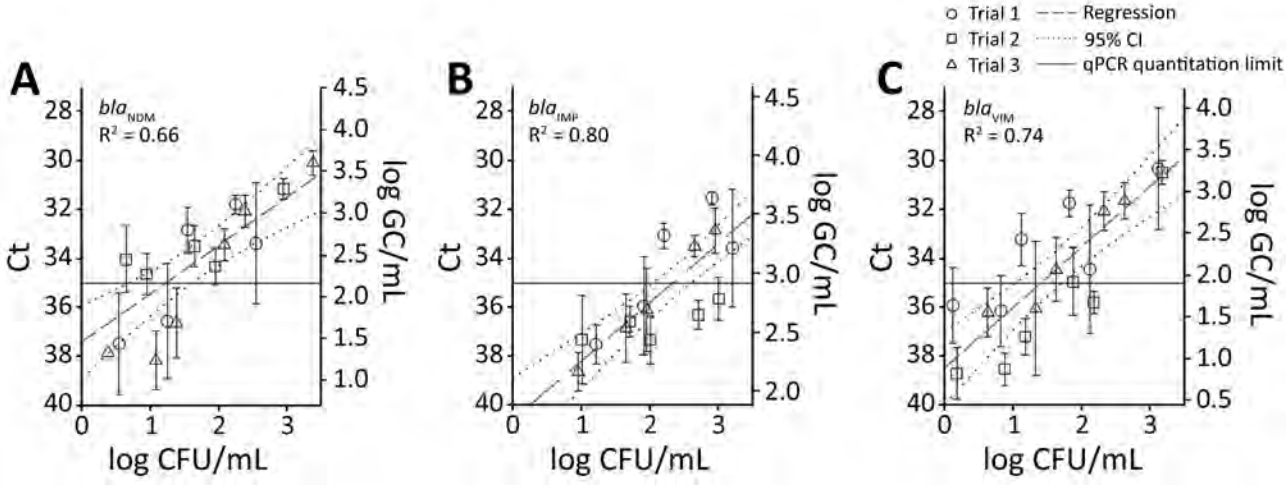


Figure 1. Measured Ct (mean ±SD), calculated log GC/mL, and extrapolated log CFU/mL for detection limit studies in study of detection methods for wastewater surveillance of antimicrobial-resistant bacteria from healthcare facilities. A) *bla*$_{NDM}$; B) *bla*$_{IMP}$; C) *bla*$_{VIM}$. Each point represents 5 replicates. Horizontal line represents a Ct cutoff of 35 cycles. All data points were included in the linear regression for each plot; dashed lines represent 95% CIs of the regression. The log CFU/mL was extrapolated from the initial isolate concentration, which was then serially diluted by 6 log into each of 5 replicates. The log GC/mL was estimated from the Ct response from standard curves using either geneblock DNA or antimicrobial-resistant isolates as positive controls. Ct, cycle threshold; GC, gene copies

Table 4. Spearman rank order correlations among building water usage-normalized *bla*$_{KPC}$, *bla*$_{OXA-48-like}$, and *bla*$_{VIM}$ log gene concentrations for composite, passive, and sewer biofilm sample collection methods in study of detection methods for wastewater surveillance of antimicrobial-resistant bacteria from healthcare facilities*

	Composite			Passive			Biofilm		
Gene	KPC	OXA	VIM	KPC	OXA	VIM	KPC	OXA	VIM
Composite									
KPC	–	ρ = 0.66, p<0.001, n = 58	ρ = 0.56, p = 0.008, n = 21	ρ = 0.40, p = 0.003, n = 51	ρ = 0.41, p = 0.003, n = 50	ρ = 0.68, p = 0.005, n = 15	NS	NS	NS
OXA		–	ρ = 0.64, p = 0.002, n = 21	NS	ρ = 0.4,1 p = 0.003, n = 50	ρ = 0.70, p = 0.004, n = 15	NS	NS	NS
VIM			–	NS	NS	ρ = 0.78, p = 0.005, n = 10	NS	NS	NS
Passive									
KPC				–	ρ = 0.60, p<0.001, n = 51	NS	NS	NS	NS
OXA					–	NS	NS	ρ = 0.67, p = 0.008, n = 14	NS
VIM						–	ρ = −0.821, p = 0.01, n = 7	NS	NS
Biofilm									
KPC							–	ρ = 0.76 p<0.001, n = 15	NS
OXA								–	NS
VIM									–

*Daily values were calculated for composite samples as log(GC/mL)/(monthly average gal × 10^3/d), for passive samplers as log(GC/g)/(monthly average gal × 10^3/d), and for biofilm samples as log(GC/cm^2)/(monthly average gal × 10^3/d); nonnormalized data correlations are shown in Appendix Table 5 (https://wwwnc.cdc.gov/EID/article/32/13/25-1490-App1.pdf). *bla*$_{NDM}$ and *bla*$_{IMP}$ are not included because they were not correlated or only correlated in ≤5 samples. B, sewer biofilm; C, composite; CKPC, *bla*$_{KPC}$; NS, not significant at p<0.05; OXA, *bla*$_{OXA-48-like}$; P, passive; VIM, *bla*$_{VIM}$.

Figure 5) than the biofilm and grab metagenomes, which had very low abundances (1–5 transcripts/million [TPM]). The *bla*$_{VIM}$ gene was most abundant (14 TPM) in the biofilm metagenome. Neither *bla*$_{NDM}$ nor *bla*$_{OXA-48-like}$ genes were detected in any metagenomes, despite detection by qPCR. We examined the relationship between metagenomic sequence coverage and qPCR abundance (Appendix Figure 6) and found a significant correlation for *bla*$_{KPC}$ (R^2 = 0.66). In contrast, *bla*$_{VIM}$ was only detected in 1 of 3 metagenomes, where the qPCR abundance was 4 $\log_{10}$ GC. The *bla*$_{OXA-48-like}$ abundance was 2 to 6 $\log_{10}$ GC and the *bla*$_{NDM}$ abundance was <5 $\log_{10}$ GC, but they were not detected in metagenomes. Therefore, the absence of *bla*$_{OXA-48-like}$ and *bla*$_{NDM}$ genes in the metagenomes is surprising but reasonable because of the limits of detection for metagenomic sequencing.

We sequenced and classified 7 isolates from composite wastewater in January 2023 as *Citrobacter*, *E. coli* (1 isolate; serotype O8 + H19), and *Providencia* (1 isolate). Of 12 isolates sequenced and classified in October 2023, 6 of 12 were also *Citrobacter*, 3 were *Comamonas*, and 3 were *Aeromonas*. The *Aeromonas* and *Citrobacter* isolates had similar ARGs (Appendix Figure 7), including *bla*$_{KPC-2}$, *bla*$_{KPC-3}$, *bla*$_{OXA-2}$, *bla*$_{TEM-1}$, and *bla*$_{SHV}$. The *E. coli* genome includes *bla*$_{CTX-M-15}$, *bla*$_{EC}$, and *bla*$_{TEM-1}$.

Comparison of Carbapenemase Gene Detection Methods

Among all samples and collection methods, there was close agreement between the Carba-R assay and qPCR detections (Appendix Tables 10–12); 551 (85.4%) of 645 replicate carbapenemase gene assay results were either both present or both absent. The *bla*$_{VIM}$ gene was more frequently detected by Carba-R assay than by qPCR; 76 (58.9%) of 129 samples were positive by Carba-R but negative by qPCR. The *bla*$_{IMP}$ gene was not detected by Carba-R, whereas 7% (5/69) of samples were qPCR positive.

Comparison of Composite Wastewater Sample Volume, Handling, and Storage

We conducted studies to evaluate the effect of wastewater sample volume, sample concentration method, and sample handling methods on ARG abundance. In the first study of wastewater sampling volumes, we found no significant difference (p>0.05 by Dunn method) in gene abundance among 2 40-mL samples centrifuged at different speeds (31,400 × *g* or 3,400 × *g*) and times (10 or

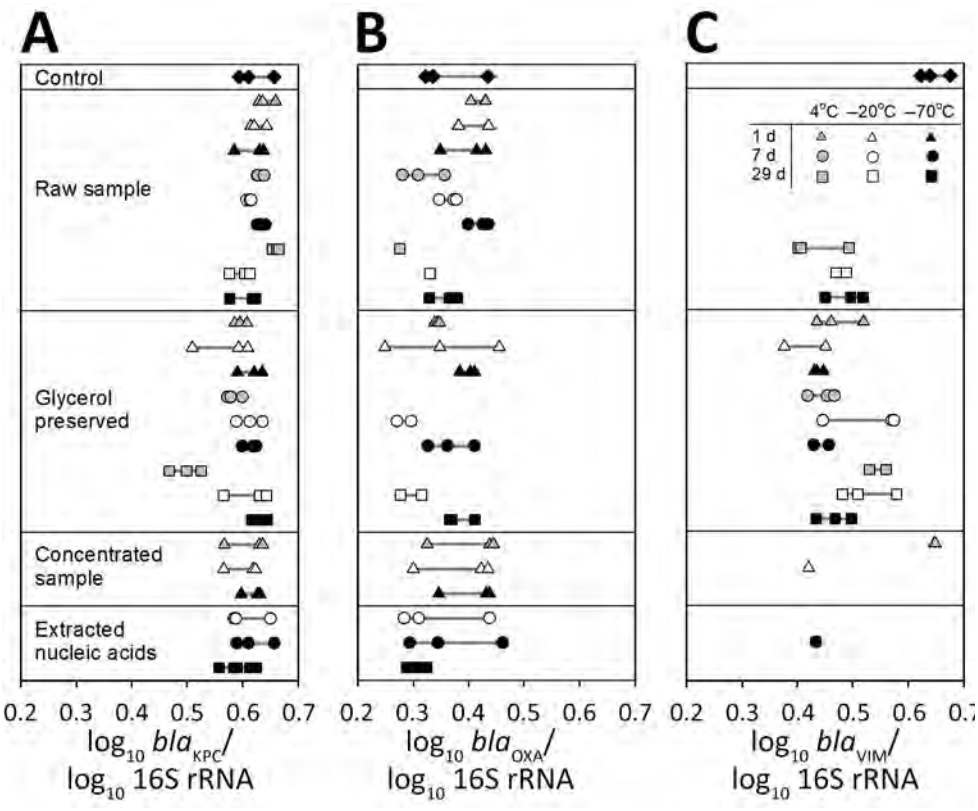


Figure 2. Holding time and preservation temperature comparison for $\log_{10}$ bla_{KPC} (A), $bla_{OXA\text{-}48\text{-}like}$ (B), and bla_{VIM} (C) measured by quantitative PCR in triplicate in study of detection methods for wastewater surveillance of antimicrobial-resistant bacteria from healthcare facilities. Triangles indicate 1 day holding time, circles indicate 7 days, and squares indicate 29 days. Gray symbols indicate 4°C. white symbols indicate –20°C, and black symbols indicate –70°C. All gene copies are normalized to $\log_{10}$ 16S rRNA gene. Horizontal gray lines show the range of values for each treatment. Control sample DNA was extracted immediately upon receipt in laboratory. Raw sample represents processed raw wastewater. Glycerol preserved are unprocessed samples preserved with 50% glycerol with Tris. Concentrated samples were polyethylene glycol precipitated for 2 hours and centrifuged, and the resulting pellet was resuspended and held at the listed temperature.

60 minutes); therefore, we combined those data for further analysis. Overall, 40-mL samples had a significantly higher mean abundance than the 500-mL samples for bla_{VIM} ($p<0.05$ by Holm-Sidak method), 16S rRNA gene ($p = 0.02$ by ANOVA on Ranks), and $bla_{OXA\text{-}48\text{-}like}$ ($p = 0.01$ by ANOVA on Ranks), but not for bla_{KPC} ($p = 0.1$ by ANOVA) (Appendix Figure 8). Therefore, we used 40-mL samples with centrifugation at 31,400 × *g* for 10 minutes.

We compared sample holding times (1, 7, and 29 days), holding methods (concentrated before storage or not), and preservation methods (raw sample or glycerol preserved) with samples extracted and analyzed immediately to determine how sample handling influenced ARG abundance (Table 5, Figure 2). Sample preservation method (Table 5) generated the most variation in gene abundance. Specifically, the sample preservation method accounted for 24%–

Table 5. Effects of sample handling and storage conditions on gene abundance determined by qPCR in study of detection methods for wastewater surveillance of antimicrobial-resistant bacteria from healthcare facilities*

Factor	16S rRNA gene	bla_{KPC}	bla_{VIM}	$bla_{OXA\text{-}48\text{-}like}$	bla_{KPC}/16S rRNA†	bla_{VIM}/16S rRNA†
Overall model F (p value)	1.98 (0.02)	2.23 (0.008)	6.33 (<0.001)	NS	3.99 (<0.01)	9.67 (<0.001)
Source of variation						
Hold time‡	38 (0.002)	25 (0.01)	30 (<0.001)	NA§	NS	31 (<0.001)
Hold temp¶	NS	NS	16 (0.01)	NA	NS	10 (0.008)
Hold method	41 (<0.001)	42 (<0.001)	30.1 (<0.001)	NA	27 (<0.001)	24 (<0.001)
Time × temp	NS	NS	12.5 (0.008)	NA	NS	11 (0.002)
Temp × method	NS	NS	NS	NA	NS	15 (0.007)
Time × method	NS	NS	9 (0.04)	NA	22 (<0.001)	9 (0.006)
Time × temp × method	NS	15 (0.05)	Not estimable**	NA	27 (<0.001)	Not estimable**

*Source of variation is percent of total variation (p value) of type 1 error for the general linearized model. NA, not applicable; NS, not significant; qPCR, quantitative PCR.
†Calculation based on $\log_{10}$ ARG/mL divided by $\log_{10}$ 16S rRNA gene/mL.
‡Hold times were 1, 7, or 29 days.
§The overall model was not significant for this gene.
¶Temperature was 4°C, −20°C, or −70°C.
#Hold methods: extracted immediately versus raw samples held at temperature, glycerol preserved unprocessed samples, polyethylene glycol concentrated and resuspended samples, or nucleic acids extracted and stored.
**These values were not estimable because of limited degrees of freedom.

Table 6. Concentrations of carbapenemase genes in composite wastewater by month and frequency of carbapenemase gene detection over 16 months in study of detection methods for wastewater surveillance of antimicrobial-resistant bacteria from healthcare facilities*

Month	No. sampling dates	log GC/mL, mean ±SD (no. [%] detected)†				
		bla$_{KPC}$	*bla*$_{OXA-48-like}$	*bla*$_{VIM}$	*bla*$_{NDM}$	*bla*$_{IMP}$
2023 Jan	1	7.8 (1 [100])	6.3 (1 [100])	8.7 (1 [100])	ND	ND
2023 Feb	2	7.1 ± 0.1 (2 [100])	6.2 ± 0.4 (2 [100])	7.8 ± 0.3 (2 [100])	ND	ND
2023 Mar	4	7.3 ± 0.3 (4 [100])	5.8 ± 0.2 (4 [100])	7.3 (1 [25])	ND	ND
2023 Apr	3	6.5 ± 0.6 (3 [100])	4.9 ± 0.5 (3 [100])	ND	ND	ND
2023 May	8	6.9 ± 0.5 (8 [100])	5.8 ± 0.8 (8 [100])	6.1 ± 0.6 (2 [25])	ND	ND
2023 Jun	9	6.5 ± 0.4 (9 [100])	5.4 ± 0.5 (9 [100])	5.4 ± 0.5 (2 [22])	ND	ND
2023 Jul	7	6.3 ± 0.6 (7 [100])	5.8 ± 0.6 (6 [86])	4.2 (1 [14])	ND	ND
2023 Aug	6	5.2 ± 0.6 (6 [100])	5.2 ± 0.7 (6 [100])	5.5 ± 0.5 (2 [33])	ND	ND
2023 Sep	4	6.1 ± 0.8 (4 [100])	5.0 ± 0.5 (4 [100])	5.2 (1 [25])	ND	ND
2023 Oct	3	6.3 ± 0.5 (3 [100])	5.4 ± 0.1 (3 [100])	5.3 ± 0.2 (2 [67])	4.6 (1 [33])	ND
2023 Nov	4	6.1 ± 0.4 (4 [100])	4.8 ± 0.3 (4 [100])	4.8 ± 0.2 (3 [75])	5.7 (1 [25])	ND
2023 Dec	2	5.5 ± 0.1 (2 [100])	4.4 ± 0.2 (2 [100])	4.8 (1 [50])	ND	ND
2024 Jan	2	5.6 ± 0.2 (2 [100])	4.8 ± 0.5 (2 [100])	ND	ND	ND
2024 Feb	2	6.1 ± 0.3 (2 [100])	5.7 ± 0.3 (2 [100])	5.6 ± 0.2 (2 [100])	5.1 ± 0.6 (2 [100])	ND
2024 Mar	2	5.8 ± 0.9 (2 [100])	5.1 ± 0.6 (2 [100])	4.8 (1 [50])	4.6 ± 0.6 (2 [100])	6.5 (1 [50])
2024 Apr	2	5.9 ± 0.5 (2 [100])	5.0 ± 0.4 (2 [100])	ND	5.3 ± 0.4 (2 [100])	6.6 (1 [50])
Total (% positive)		61 (100)	60 (98)	21 (34)	8 (13)	2 (3)

*GC, gene copies; ND, not detected.
†Concentrations without SD indicate carbapenemase genes were detected in only 1 sample among multiple sampling dates in a month or only 1 sampling date in a month.

42% of the variation in GC per milliliter. The highest recovery was observed in concentrated samples (57%–121% average recovery of ARG among all temperatures and times) or extracted nucleic acid samples (59%–94% recovery). Sample hold time before handling accounted for 25% to 38% of variability. Hold time significantly influenced ARG abundance (Table 5); shorter hold times are recommended.

Wastewater Carbapenemase Gene Trends and Alignment with Facility Clinical Infections

During the 16-month study, only 5 CRO clinical infections were identified in 4 patients within the facility. Infections 1 and 2 were identified as *Klebsiella (Enterobacter) aerogenes* and *Enterobacter cloacae*, isolated from urine specimens collected 9 days apart in April 2023 from the same patient; both were negative for carbapenemase genes. Infection 3 was a carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) from a wound in April 2023, which was not tested for carbapenemase genes. Infection 4 was a case of CRPA from a urine specimen in December 2023, also not tested for carbapenemase genes. Infection 5 was determined to be *Escherichia coli* and *Citrobacter freundii* and came from a perianal screen in March 2024; it was not tested for carbapenemase genes. Of note, the patient with

Table 7. Concentrations of carbapenemase genes collected with passive samplers by month and frequency of carbapenemase gene detection over 16 months in study of detection methods for wastewater surveillance of antimicrobial-resistant bacteria from healthcare facilities*

Month, no. samples	log GC/mL, mean ±SD (no. [%] detected)†				
	bla$_{KPC}$	*bla*$_{OXA-48-like}$	*bla*$_{VIM}$	*bla*$_{NDM}$	*bla*$_{IMP}$
2023 Jan, n = 1	8.03 (1 [100])	6.8 (1 [100])	7.9 (1 [100])	ND	ND
2023 Feb, n = 2	7.5 ± 0.1 (2 [100])	5.9 ± 0.1 (2 [100])	7.9 ± 0.2 (2 [100])	ND	ND
2023 Mar	Not tested	Not tested	Not tested	Not tested	Not tested
2023 Apr, n = 1	9.0 (1 [100])	6.2 (1 [100])	ND	ND	ND
2023 May, n = 8	8.5 ± 0.6 (8 [100])	7.0 ± 0.7 (8 [100])	ND	ND	ND
2023 Jun, n = 9	8.3 ± 0.5 (9 [100])	6.9 ± 0.4 (9 [100])	6.8 (1 [11])	ND	ND
2023 Jul, n = 7	8.3 ± 0.3 (6 [86])	6.5 ± 0.2 (6 [86])	ND	ND	ND
2023 Aug, n = 7	7.9 ± 0.3 (7 [100])	6.4 ± 0.1 (6 [86])	ND	ND	ND
2023 Sep, n = 3	8.0 ± 0.5 (3 [100])	6.4 ± 0.4 (3 [100])	ND	ND	ND
2023 Oct, n = 3	7.9 ± 0.2 (3 [100])	6.1 ± 0.1 (3 [100])	6.4 (1 [33])	5.4 (1 [33])	ND
2023 Nov, n = 4	8.1 ± 0.4 (4 [100])	6.1 ± 0.2 (4 [100])	6.0 ± 0.2 (3 [75])	6.0 (1 [25])	ND
2023 Dec, n = 2	7.8 ± 0.001 (2 [100])	6.3 ± 0.2 (2 [100])	6.4 ± 0.1 (2 [100])	ND	ND
2024 Jan, n = 2	7.9 ± 0.03 (2 [100])	6.5 ± 0.1 (2 [100])	6.3 ± 0.1 (2 [100])	ND	ND
2024 Feb, n = 2	8.3 ± 0.7 (2 [100])	6.8 ± 0.5 (2 [100])	6.4 ± 0.2 (2 [100])	5.1 (1 [50])	7.9 ± 0.3 (2 [100])
2024 Mar, n = 2	8.2 ± 0.7 (2 [100])	7.0 ± 0.9 (2 [100])	6.2 (1 [50])	5.4 (1 [50])	7.8 (1 [50])
2024 Apr, n = 2	8.0 ± 0.6 (2 [100])	6.7 ± 0.2 (2 [100])	6.2 ± 0.5 (2 [100])	6.1 (1 [50])	7.6 ± 0.2 (2 [100])
Total (% positive), n = 55	54 (98)	53 (96)	17 (31)	5 (9)	5 (9)

*GC, gene copies; ND, not detected.
†Concentrations without SD indicate carbapenemase genes were detected in only 1 sample among multiple sampling dates in a month or only 1 sampling date in a month.

CRE identified in urine specimens in April 2023 used ostomy bags, which might not have been disposed of in the toilet. All other patients were recorded as toileting by nurses. Carbapenem antibiotic use in the facility was documented (Appendix Table 13).

Over 16 months (Tables 6, 7), bla_{KPC} and $bla_{OXA\text{-}48\text{-}like}$ were detected in >98% of composite and passive samples, but bla_{VIM}, bla_{NDM}, and bla_{IMP} were detected less frequently (35% in composite and 31% in passive for bla_{VIM}, 10% in composite and 9% in passive for bla_{NDM}, 3% in composite and 9% in passive for bla_{IMP}). All sewer biofilm samples contained bla_{KPC} and $bla_{OXA\text{-}48\text{-}like}$, but only 24% of samples contained bla_{NDM} and bla_{VIM}, and 18% contained bla_{IMP} (Table 8).

Abundance of bla_{KPC} and $bla_{OXA\text{-}48\text{-}like}$, normalized for facility water usage, increased after April 2023 when CRE- and CRPA-infected patients were present (Figure 3). A general increase in bla_{KPC} and $bla_{OXA\text{-}48\text{-}like}$ abundance was also observed in the last 4 months of the study period (Figure 3, panels A, B, D, and E), although that increase was not attributable to a patient infection. The bla_{VIM} abundance generally decreased over the study period for flow-normalized and non-normalized data (Figure 3, panels G, H, I; Appendix Figure 9). The low incidence of CRO infections (5 over 16 months) and lack of confirmed carbapenemase-producing CRE infections precluded modeling of relationships between wastewater carbapenemase gene abundances and clinical infection occurrence.

Discussion

This study standardized methods for carbapenemase gene detection in hospital wastewater. Sample handling conditions significantly affected gene abundance measurements. Gene recovery was highest when 40 mL of sample was processed using a higher centrifuge speed, compared with 500 mL processed at lower centrifugation speeds. Recovery decreased with prolonged storage or higher temperature unless samples were preserved with glycerol. Those findings emphasize the importance of standardized, practical protocols (*22*). Our results demonstrate that passive sampling is as effective as composite sampling, as suggested by others (*23*), and that storing raw samples at 4°C up to 29 days was comparable to immediate processing. That finding is especially relevant for resource-limited settings, because those facilities can collect and store a passive sample before processing in lieu of composite samples processed the next day.

Over 16 months, 2 carbapenemase genes, bla_{KPC} and $bla_{OXA\text{-}48\text{-}like}$, were consistently detected by qPCR and Cepheid Carba-R assays in each of the 3 collection methods. Among sampling strategies, the composite and passive sampling outperformed biofilm sampling in terms of gene richness and antimicrobial-resistant gene detection frequency. Passive samples also yielded higher concentrations of carbapenemase genes. The sewer biofilm gene concentrations when normalized to building water usage was correlated with wastewater concentrations, suggesting the sewer biofilm might influence the wastewater carbapenemase gene abundance, but additional studies are needed to elucidate that relationship. Differences in gene abundance between sewer biofilms and composite samples might reflect shedding from colonized patients, environmental persistence, or microbial community dynamics.

Table 8. Concentrations of carbapenemase genes observed in sewer biofilms by month and frequency of carbapenemase gene detection over 16 months in study of detection methods for wastewater surveillance of antimicrobial-resistant bacteria from healthcare facilities*

	log GC/cm2, mean ±SD (no. [%] detected)†				
Month, no. samples	bla_{KPC}	$bla_{OXA\text{-}48\text{-}like}$	bla_{VIM}	bla_{NDM}	bla_{IMP}
2023 Jan, n = 1	4.6 (1 [100])	3.9 (1 [100])	6.9 (1 [100])	ND	ND
2023 Feb, n = 1	4.4 (1 [100])	3.9 (1 [100])	6.0 (1 [100])	ND	ND
2023 Mar, n = 1	5.6 (1 [100])	3.5 (1 [100])	ND	ND	ND
2023 Apr	Not tested	Not tested	Not tested	Not tested	Not tested
2023 May, n = 1	6.9 (1 [100])	5.7 (1 [100])	ND	ND	ND
2023 Jun, n = 1	6.0 (1 [100])	5.0 (1 [100])	ND	ND	ND
2023 Jul	Not tested	Not tested	Not tested	Not tested	Not tested
2023 Aug, n = 1	4.5 (1 [100])	4.0 (1 [100])	ND	ND	ND
2023 Sep, n = 1	6.3 (1 [100])	5.6 (1 [100])	ND	ND	ND
2023 Oct, n = 1	5.4 (1 [100])	4.5 (1 [100])	ND	ND	ND
2023 Nov, n = 3	5.6 ± 0.8 (3 [100])	4.2 ± 1.1 (3 [100])	ND	ND	ND
2023 Dec, n = 1	4.8 (1 [100])	4.1 (1 [100])	5.0 (1 [100])	ND	ND
2024 Jan, n = 1	5.0 (1 [100])	4.1 (1 [100])	ND	ND	ND
2024 Feb, n = 1	5.3 (1 [100])	5.5 (1 [100])	ND	6.8 (1 [100])	ND
2024 Mar, n = 1	5.1 (1 [100])	4.8 (1 [100])	ND	4.5 (1 [100])	6.5 (1 [100])
2024 Apr, n = 2	4.3 ± 0.7 (2 [100])	4.5 ± 0.2 (2 [100])	4.2 (1 [50])	5.0 ± 0.1 (2 [100])	6.3 ± 0.2 (2 [100])
Total (% positive), n = 17	17 (100)	17 (100)	4 (24)	4 (24)	3 (18)

*GC, gene copies; ND, not detected.
†Concentrations without SD indicate carbapenemase genes were detected in only 1 sample among multiple sampling dates in a month or only 1 sampling date in a month.

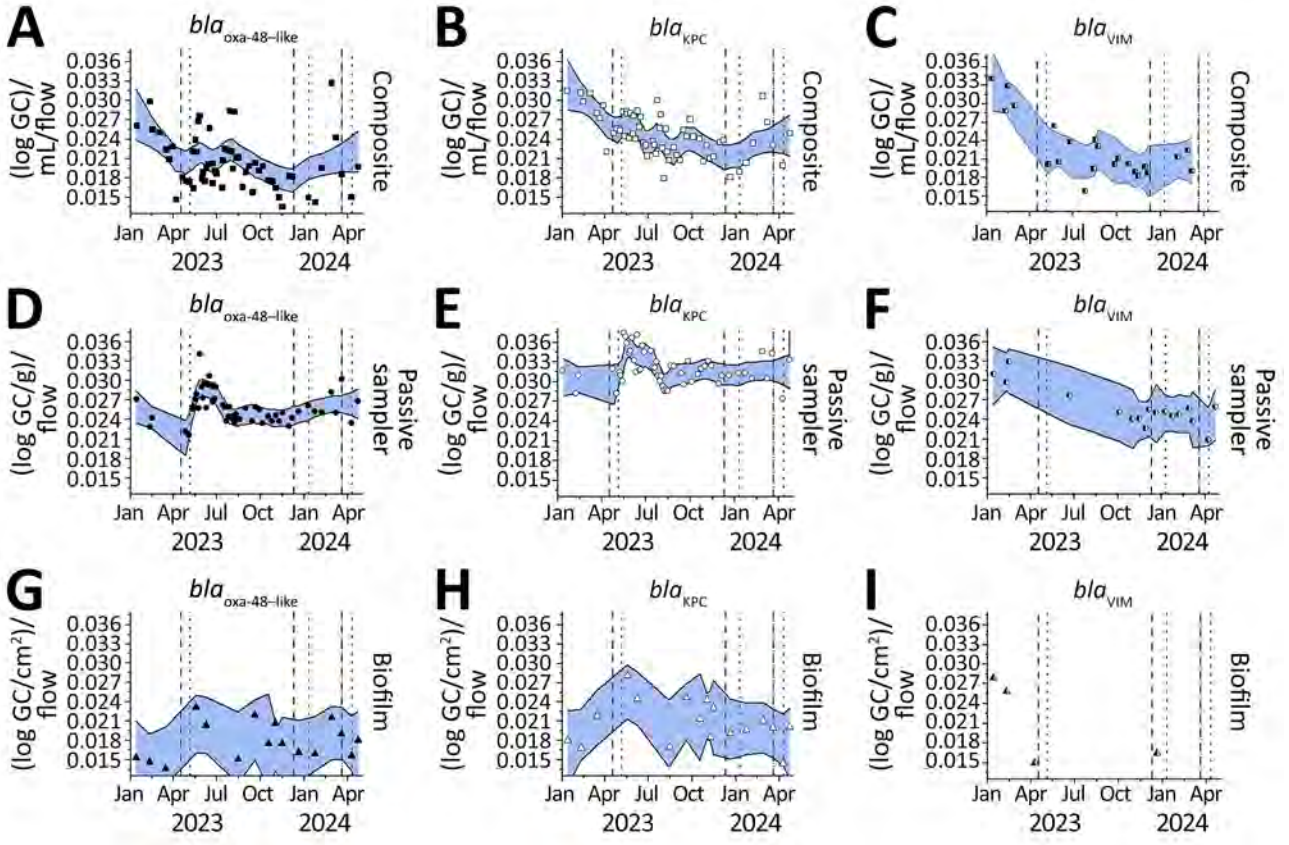


Figure 3. GC of *bla*$_{\text{OXA-48-like}}$, *bla*$_{\text{KPC}}$, and *bla*$_{\text{VIM}}$ in wastewater collected by autosamplers, passive samplers, or swabs of sewer biofilm in study of detection methods for wastewater surveillance of antimicrobial-resistant bacteria from healthcare facilities, January 2023–April 2024. A–C) Composite samples for *bla*$_{\text{OXA-48-like}}$, *bla*$_{\text{KPC}}$, and *bla*$_{\text{VIM}}$. D–F) Passive sampler results for *bla*$_{\text{OXA-48-like}}$, *bla*$_{\text{KPC}}$, and *bla*$_{\text{VIM}}$. G–I) Biofilm samples for *bla*$_{\text{OXA-48-like}}$, *bla*$_{\text{KPC}}$, and *bla*$_{\text{VIM}}$. Values are normalized by recovery control and water usage. Nonparametric regression smoothing factors range from 0.1 to 0.25. Dashed lines (admit date) and dotted lines (discharge date) indicate periods when patients infected with carbapenem-resistant Enterobacterales (CRE) or carbapenem-resistant *Pseudomonas aeruginosa* were in the facility. Infections consisted of *Klebsiella aerogenes*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa* in period 1 (April 2023); *P. aeruginosa* in period 2 (December 2023); and *Escherichia coli* and *Citrobacter freundii* in period 3 (March 2024). The CRE infections in April 2023 were not carbapenemase-producing organisms, based on PCR. The CRE infection in March 2024 was not tested for carbapenemase genes by PCR. None of the carbapenem-resistant *Pseudomonas aeruginosa* were tested for carbapenemase genes.

Temporal trends of gene abundance in wastewater were not correlated with patient infections because of the limited clinical infections during the study. Longer-term sampling is required to evaluate the utility of WS as a complement to hospital AMR surveillance methods. Two critical points require further consideration: first, wastewater results should be interpreted considering reservoirs for antimicrobial-resistant bacteria, such as sinks and sewer biofilms, which were found to harbor carbapenemase genes (Appendix Figure 5) and CRE as reported elsewhere (*24*,*25*); and second, CRE or carbapenemase gene sources in addition to infected patients such as colonized patients, hospital staff, outpatients, and visitors should be considered. Additional research is needed to investigate the contribution of sewer biofilms to wastewater from hospitals, because those biofilm signals might confound temporal gene signals. Identifying the nonpatient contribution of CRE or carbapenemase genes to wastewater will also be critical for successful surveillance efforts.

Because of the limited clinical infections during the study period, it remains an open question whether WS might enhance outbreak detection, support infection control, and aid antibiotic stewardship, and additional study is needed. Further, the contribution of sewer biofilms to wastewater signals needs investigation. In conclusion, wastewater sampling, particularly composite and passive methods combined with sensitive molecular detection, might be a practical, noninvasive approach for monitoring carbapenemase genes in healthcare facilities, as demonstrated by other reports (*26*,*27*). Passive sampling might be suitable in low-resource settings, situations

with space constraints, or situations in which sewer access is limited.

This article was preprinted at https://www.biorxiv.org/content/10.64898/2025.12.14.694051v1.

Acknowledgments

We thank Sophia Reyes, Nathan Hatten, Aspen Dalby, Daniel Jeun, Katherine Reilly, Vivian Marcoux, and the Utah Public Health Laboratory for their valuable assistance.

This work was supported by CDC contract no. 200-2021-12774, Safety and Healthcare Epidemiology Prevention Research Development (SHEPheRD), 2022 Domain 1-A004: Wastewater surveillance approaches for antimicrobial resistant genes and organisms in healthcare settings within the Western US Region, Jennifer Weidhaas, PhD (Civil and Environmental Engineering), Principal Investigator. This work was also supported in part by a first-year fellowship to E.W. from the University of Utah Global Change and Sustainability Center.

J.V., S.B., W.B., W.T., A.L., F.W., A.C.-S., and J.W. conceptualized the study. J.V., S.B., W.T., A.L., F.W., A.C.-S., and J.W. acquired funding. J.V. and J.W. performed project administration. E.W., S.B., W.B., and S.F. curated data. E.W., W.B., and J.W. conducted the formal analysis. E.W. and S.F. were investigators. E.W., W.T., and J.W. constructed the methodology. E.W. and J.W. conducted visualization. E.W. prepared the original draft, and J.V., S.B., W.B., W.T., A.L., F.W., A.C.-S., and J.W. performed review and editing. J.W. supervised the study.

About the Author

Mr. Warren is a PhD student in environmental engineering at the University of Utah and for 25 years was a research hydrologist at the US Geological Survey. His primary research interests are novel community health-related biomarkers for wastewater surveillance.

References

1. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States [cited 2026 Jul 23]. https://www.cdc.gov/antimicrobial-resistance/media/pdfs/2019-ar-threats-report-508.pdf
2. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, et al.; Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399:629–55. https://doi.org/10.1016/S0140-6736(21)02724-0
3. GBD 2021 Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. Lancet. 2024;404:1199–226. https://doi.org/10.1016/S0140-6736(24)01867-1
4. Aung A-H, Kanagasabai K, Koh J, Hon P-Y, Ang B, Lye D, et al. Epidemiology and transmission of carbapenemase-producing *Enterobacteriaceae* in a health care network of an acute-care hospital and its affiliated intermediate- and long-term-care facilities in Singapore. Antimicrob Agents Chemother. 2021;65:e0258420. https://doi.org/10.1128/AAC.02584-20
5. Allel K, Hernández-Leal MJ, Naylor NR, Undurraga EA, Abou Jaoude GJ, Bhandari P, et al. Costs-effectiveness and cost components of pharmaceutical and non-pharmaceutical interventions affecting antibiotic resistance outcomes in hospital patients: a systematic literature review. BMJ Glob Health. 2024;9:e013205. https://doi.org/10.1136/bmjgh-2023-013205
6. Reuben J, Donegan N, Wortmann G, DeBiasi R, Song X, Kumar P, et al. Healthcare Antibiotic Resistance Prevalence–DC (HARP-DC): a regional prevalence assessment of carbapenem-resistant enterobacteriaceae (CRE) in healthcare facilities in Washington, District of Columbia. Infect Control Hosp Epidemiol. 2017;38:921–9.
7. Sanjak JS, McAuley EM, Raybern J, Pinkham R, Tarnowski J, Miko N, et al. Wastewater surveillance pilot at US military installations: cost model analysis. JMIR Public Health Surveill. 2024;10:e54750. https://doi.org/10.2196/54750
8. Yoo BK, Iwamoto R, Chung U, Sasaki T, Kitajima M. Economic evaluation of wastewater surveillance combined with clinical COVID-19 screening tests, Japan. Emerg Infect Dis. 2023;29:1608–17. https://doi.org/10.3201/eid2908.221775
9. Wang J, Chu L, Wojnárovits L, Takács E. Occurrence and fate of antibiotics, antibiotic resistant genes (ARGs) and antibiotic resistant bacteria (ARB) in municipal wastewater treatment plant: an overview. Sci Total Environ. 2020;744:140997. https://doi.org/10.1016/j.scitotenv.2020.140997
10. Wang Y, Han Y, Li L, Liu J, Yan X. Distribution, sources, and potential risks of antibiotic resistance genes in wastewater treatment plant: a review. Environ Pollut. 2022;310:119870. https://doi.org/10.1016/j.envpol.2022.119870
11. Favier C, Toubiana M, Zorgniotti I, Courot O, Durandet F, Licznar-Fajardo P, et al. Could the load of carbapenemase genes in hospital wastewater be a proxy for emerging resistance to carbapenems in humans? Antimicrob Resist Infect Control. 2026;15:26. https://doi.org/10.1186/s13756-026-01697-9
12. Flach CF, Hutinel M, Razavi M, Åhrén C, Larsson DGJ. Monitoring of hospital sewage shows both promise and limitations as an early-warning system for carbapenemase-producing Enterobacterales in a low-prevalence setting. Water Res. 2021;200:117261. https://doi.org/10.1016/j.watres.2021.117261
13. Majlander J, Anttila VJ, Nurmi W, Seppälä A, Tiedje J, Muziasari W. Routine wastewater-based monitoring of antibiotic resistance in two Finnish hospitals: focus on carbapenem resistance genes and genes associated with bacteria causing hospital-acquired infections. J Hosp Infect. 2021;117:157–64. https://doi.org/10.1016/j.jhin.2021.09.008
14. Santiago AJ, Burgos Garay M, Campbell M, Cahela Y, Donlan R, Gable P, et al. Skilled nursing facility wastewater surveillance: a SARS-CoV-2 and antimicrobial resistance detection pilot study. J Water Health. 2025;23:727–42. https://doi.org/10.2166/wh.2025.374
15. Rankin DA, Stahl A, Sabour S, Khan MA, Armstrong T, Huang JY, et al. Changes in carbapenemase-producing carbapenem-resistant Enterobacterales, 2019 to 2023. Ann Intern Med. 2025;178:1818–21. https://doi.org/10.7326/ANNALS-25-02404

16. Roy M-C, Stevens M, Fidsa F. Guide to infection control in the healthcare setting. Boston: International Society for Infectious Diseases; 2018.
17. Coulliette-Salmond A, Whitehill F, Lyons AK, Abera B, Adler C, Walters MS, et al. Healthcare wastewater surveillance: methodological considerations for sampling, feasibility, and implementation. J Water Health. 2026;24:50–64. https://doi.org/10.2166/wh.2025.167
18. Griffiths RI, Whiteley AS, O'Donnell AG, Bailey MJ. Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. Appl Environ Microbiol. 2000;66:5488–91. https://doi.org/10.1128/AEM.66.12.5488-5491.2000
19. Centers for Disease Control and Prevention. Multiplex real-time PCR detection of *Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo-β-lactamase (NDM-1) genes [cited 2026 July 23]. https://www.cdc.gov/gram-negative-bacteria/media/pdfs/KPC-NDM-Protocol-2011-P.pdf
20. Lutgring JD, Zhu W, de Man TJB, Avillan JJ, Anderson KF, Lonsway DR, et al. Phenotypic and genotypic characterization of Enterobacteriaceae producing oxacillinase-48–like carbapenemases, United States. Emerg Infect Dis. 2018;24:700–9. https://doi.org/10.3201/eid2404.171377
21. Prussing C, Canulla T, Singh N, McAuley P, Gosciminski M, King E, et al. Characterization of the first carbapenem-resistant *Pseudomonas aeruginosa* clinical isolate harboring bla_{SIM-1} from the United States. Antimicrob Agents Chemother. 2021;65:e0106621. https://doi.org/10.1128/AAC.01066-21
22. Robins K, Leonard AFC, Farkas K, Graham DW, Jones DL, Kasprzyk-Hordern B, et al. Research needs for optimising wastewater-based epidemiology monitoring for public health protection. J Water Health. 2022;20:1284–313. https://doi.org/10.2166/wh.2022.026
23. Poretsky RS, Gonzalez DS, Horton A, Schoeny M, Lin C-Y, Jarju ML, et al. Establishing a practical approach to sewer monitoring for antimicrobial resistance genes and organisms at healthcare facilities. J Infect Dis. 2026;233:38–48. https://doi.org/10.1093/infdis/jiaf434
24. Kearney A, Boyle MA, Curley GF, Humphreys H. Preventing infections caused by carbapenemase-producing bacteria in the intensive care unit—think about the sink.J Crit Care. 2021;66:52–9. https://doi.org/10.1016/j.jcrc.2021.07.023
25. Ory J, Bricheux G, Robin F, Togola A, Forestier C, Traore O. Biofilms in hospital effluents as a potential crossroads for carbapenemase-encoding strains. Sci Total Environ. 2019;657:7–15.
26. Lenz S, Biswas S, Terry I, Tran-Basha L, Lyons AK, Mazumdar T, et al. Detection of carbapenem-resistant bacteria in skilled nursing facility wastewater. J Infect Dis. 2026;233:59–69. https://doi.org/10.1093/infdis/jiaf534
27. Poretsky RS, Gonzalez DS, Horton A, Schoeny M, Lin C-Y, Jarju ML, et al. Establishing a practical approach to sewer monitoring for antimicrobial resistance genes and organisms at healthcare facilities. J Infect Dis. 2025.

Address for correspondence: Jennifer Weidhaas, University of Utah, Civil and Environmental Engineering, 110 Central Campus Dr, Ste 2000, Salt Lake City, UT 84112, USA; email: jennifer.weidhaas@utah.edu

Temporal Alignment of Wastewater Signals with Clinical Indicators of Respiratory Illness Postpandemic, Texas, USA

Kaiming Bi, Micaela Sandoval, Thuy Nguyen, Isrrael Perez, Lopita Ghosh, Trudy Krause, Cecilia Ganduglia Cazaban, Remy Pasco, Lauren Meyers, Fuqing Wu, Janelle Rios, Michael Tisza, Justin Clark, Anthony Maresso, Eric Boerwinkle, Cici Bauer

In the COVID-19 postpandemic era, declining clinical testing for SARS-CoV-2 has led to the exploration of complementary early detection methods. We evaluated metagenomic wastewater-based epidemiology (WBE) in Texas, USA, by comparing viral signals with the National Syndromic Surveillance Program tracked emergency department visits and Texas All-Payer Claims Database insurance claims across several Texas counties during 2022–2024. By analyzing SARS-CoV-2, influenza, and respiratory syncytial virus, we found moderate-to-strong temporal correlations between wastewater and clinical indicators. Influenza showed the most stable associations ($r \leq 0.98$), whereas SARS-CoV-2 signals generally preceded clinical metrics. Respiratory syncytial virus exhibited higher geographic and temporal heterogeneity, reflecting differences in clinical data capture. Despite sampling frequency and geographic alignment challenges, metagenomic WBE consistently tracked community trends. Our findings suggest WBE can offer valuable situational awareness and a resilient complement to clinical surveillance, supporting public health preparedness in an evolving respiratory disease landscape.

Respiratory viruses, specifically influenza, respiratory syncytial virus (RSV), and SARS-CoV-2, cause a broad spectrum of illness (*1*,*2*), from mild symptoms to severe pneumonia and acute respiratory failure. Those pathogens are primary drivers of global morbidity and mortality, placing immense strain on healthcare systems (*2*). Worldwide, influenza causes ≈400,000 deaths every year (*3*), and the COVID-19 pandemic resulted in over 7.1 million deaths by early 2025 (*4*). Historical outbreaks underscore the critical need for robust, diversified surveillance to track transmission and guide public health interventions (*5*).

Traditional public health surveillance provides the foundation for monitoring those trends. In the United States, the National Syndromic Surveillance Program (NSSP) offers near–real-time indicators of community respiratory activity by tracking emergency department (ED) visits (*6*). However, ED data primarily reflect more severe cases and specific care-seeking behaviors, potentially serving as an incomplete proxy for total community-level infection rates (*7*,*8*). Furthermore, operational differences and reporting inconsistencies can create artificial fluctuations in temporal trends (*9*). To address those gaps, alternative data streams are needed. Administrative health insurance claims data represent a promising but underutilized resource; they capture a broad range of clinical encounters beyond the ED, offering a high-resolution view of healthcare utilization and disease across diverse demographics (*10*).

Wastewater-based epidemiology (WBE) has emerged as a powerful, noninvasive complement to clinical data (*11*). Modern virome sequencing-based WBE enables the simultaneous quantification and variant characterization of thousands of viruses from a single sample (*12*,*13*). Although WBE was

Author affiliations: University of Texas Health Science Center at Houston, Houston, Texas, USA (K. Bi, M. Sandoval, T. Nguyen, I. Perez, L. Ghosh, T. Krause, C. Ganduglia Cazaban, F. Wu, J. Rios, E. Boerwinkle, C. Bauer); Texas Epidemiologic Public Health Institute, Houston (K. Bi, M. Sandoval, T. Nguyen, F. Wu, J. Rios, E. Boerwinkle, C. Bauer); University of Colorado Boulder, Boulder, Colorado, USA (R. Pasco); The University of Texas at Austin, Austin, Texas, USA (L. Meyers); Baylor College of Medicine, USA (M. Tisza, J. Clark, A. Maresso)

DOI: https://doi.org/10.3201/eid3213.260141

Table 1. County population estimates and number of wastewater-based surveillance sites, Texas, USA, 2024

County	Estimated population	No. sampling sites
Cameron	431,874	2
Chambers	31,475	2
El Paso	875,784	4
Fort Bend	958,434	6
Harris	5,009,302	8
Hays	292,029	3
Lubbock	278,902	2
Travis	1,363,767	3
Wichita	137,363	1
Williamson	727,480	3

widely adopted during the COVID-19 pandemic, few studies have rigorously evaluated how metagenomic viral detection in wastewater sequencing data for viruses with pandemic potential, other than SARS-CoV-2, correlates with clinical metrics in the postpandemic context.

We leveraged viral metagenomic sequencing data from a statewide monitoring initiative in Texas, USA, to evaluate the utility of WBE for ongoing respiratory disease monitoring. We compared wastewater signals for SARS-CoV-2, influenza, and RSV across 10 counties to both NSSP syndromic surveillance and health insurance claims data. Our analysis focuses on the strength and consistency of those correlations and the lead and lag timing of wastewater signals relative to clinical indicators across diverse sites and seasons. By integrating insurance claims as a primary comparative metric, we evaluate the potential of this administrative data source to fill gaps in traditional surveillance, ultimately enhancing situational awareness and supporting broader public health monitoring efforts.

Method

Study Region and Population

We conducted a hybrid surveillance validation study across 10 Texas counties (Cameron, Chambers, El Paso, Fort Bend, Harris, Hays, Lubbock, Travis, Wichita, and Williamson) in the Texas Wastewater Environmental Biomonitoring (TexWEB) program (*12*). During June 2022–June 2024, we collected wastewater samples near-weekly and sequenced for SARS-CoV-2, influenza (A and B), and RSV. Coverage for El Paso, Fort Bend, Harris, and Lubbock spans the full 24-month period, whereas data for the remaining counties were available for June 2023–June 2024. Those jurisdictions represent diverse demographic profiles, including major metropolitan areas like Houston (Harris County) (Table 1).

Wastewater Sample Collection

We obtained wastewater samples through the TexWEB network, which monitors 41 publicly owned treatment sites across Texas (*12*). This study included 23 sites within the TexWEB network that maintained near-weekly sampling and consistent sequencing-based reporting for SARS-CoV-2, influenza, and RSV during June 2022–June 2024 (Appendix Table 1, http://wwwnc.cdc.gov/EID/article/32/13/26-0141-App1.pdf). We report viral abundance as reads per million filtered (RPMF), a metagenomic metric that captures a genomewide, variant-inclusive signal. Unlike traditional quantitative PCR–based concentrations (e.g., gene copies per liter), this metagenomic approach enables the simultaneous detection of multiple pathogens and remains resilient to primer-mismatch errors caused by viral evolution, although it measures relative abundance rather than absolute concentration (*14*–*16*). For counties with multiple sampling sites, we calculated a county-level RPMF by using a population-weighted average to ensure a stable comparison with NSSP records and administrative insurance claims.

Validation Clinical Outcome Datasets

We used 2 complementary clinical data sources to validate wastewater signals: the NSSP and the Texas All-Payer Claims Database (TX-APCD). The NSSP Electronic Surveillance System for the Early Notification of Community-based Epidemics provides near–real-time data from participating Texas facilities, capturing ≈90% of healthcare facilities in the Houston region (*17*), enabling the detection of rapid shifts in community respiratory activity (*6*). We derived pathogen-specific counts for COVID-19, influenza, and RSV (June 2022–June 2024) by using

Table 2. Diagnosis and treatment codes used to identify respiratory diseases in the Texas All-Payer Claims Database emergency department claims data, Texas, USA, 2024*

Respiratory disease	Coding system	Codes used
COVID-19	ICD-10	U07.1, U09, U09.9, U04.9, J12.82
	CPT	J0248
	NDC	11 COVID-19–related NDC codes (Appendix Table 2†)
Influenza	ICD-10	J09–J11
	NDC	65 influenza-related NDC codes (Appendix Table 3†)
Respiratory syncytial virus	ICD-10	B97.4, J12.1, J20.5, J21.0

*CPT, current procedural terminology; ICD-10, International Classification of Diseases, 10th Edition; NCD, national drug codes.
†https://wwwnc.cdc.gov/EID/article/32/13/26-0141-App1.pdf.

queries from the Electronic Surveillance System for the Early Notification of Community-based Epidemics that integrate chief complaints with discharge diagnoses, including clinical term codes from the International Classification of Diseases, 10th Revision, Clinical Modification (ICD-10-CM); International Classification of Diseases, 9th Revision, Clinical Modification; and Systematized Nomenclature of Medicine (*18*).

The TX-APCD, managed by UTHealth Houston, offers high-resolution diagnostic confirmation across commercial, Medicaid, and Medicare advantage payers, covering 74% of insured persons from Texas (*19*). We extracted ED claims through June 2024, identifying encounters through ICD-10-CM diagnosis codes supplemented by current procedural terminology and national drug codes for laboratory testing and treatment (Table 2; Appendix Tables

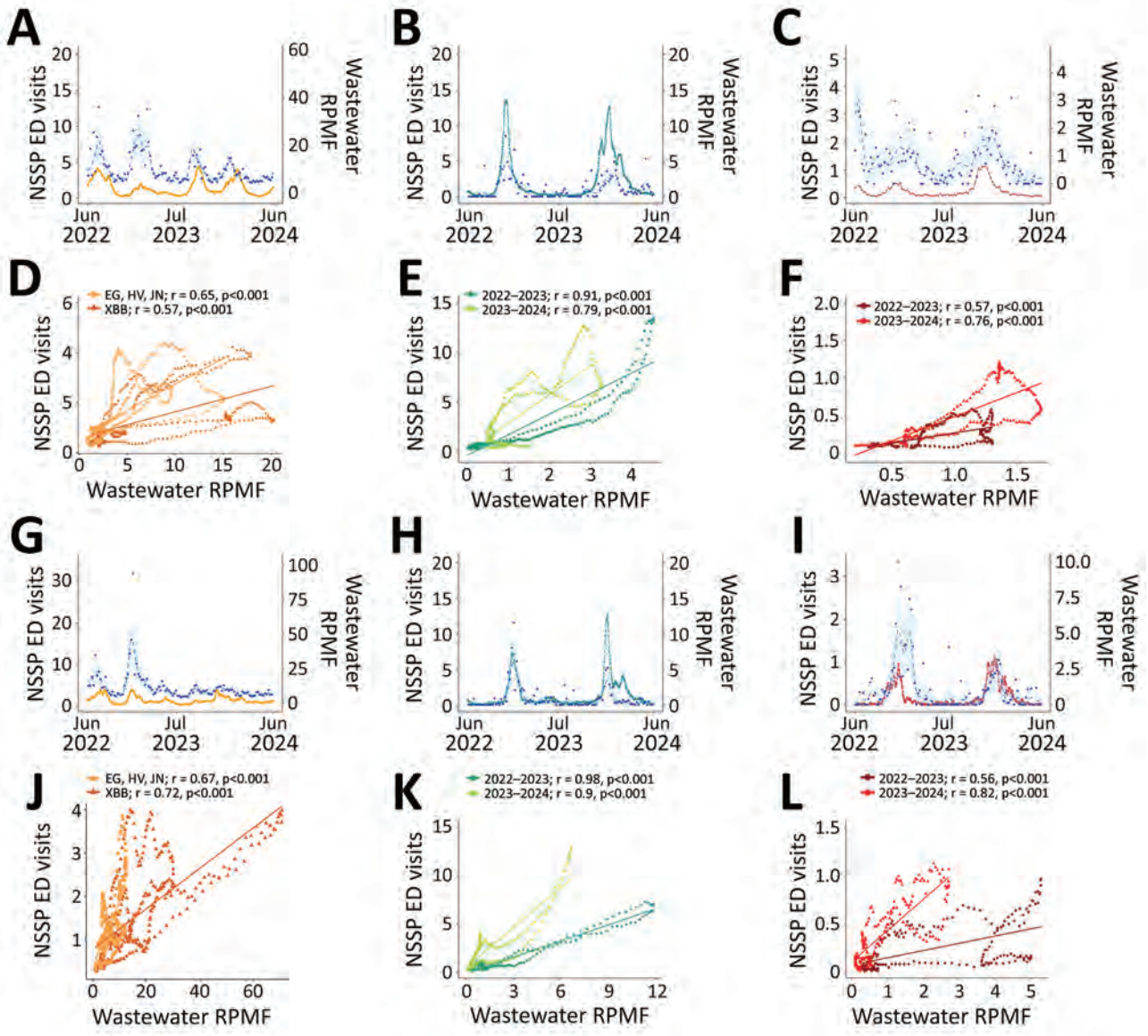


Figure 1. Association between daily wastewater signals (RPMF) and daily NSSP ED visits (per 100,000 population) for SARS-CoV-2, influenza, and respiratory syncytial virus (RSV) in Harris and El Paso Counties, Texas, June 2022–June 2024. A–C) Harris County interpolated daily time series of RPMF values versus NSSP ED visits for SARS-CoV-2 (A), influenza (B), and RSV (C). Solid lines represent ED visits; dashed lines represent RPMF data. D–F) Daily RPMF values versus NSSP ED visits for SARS-CoV-2 (D), influenza (E), and RSV (F). Triangles represent single-day observations. G–I) El Paso County interpolated daily time series of RPMF values versus NSSP ED visits for SARS-CoV-2 (G), influenza (H), and RSV (I). Solid lines represent ED visits; dashed lines represent RPMF data. J–L) Daily RPMF values versus NSSP ED visits for SARS-CoV-2 (J), influenza (K), and RSV (L). Triangles represent single-day observations. Corresponding Pearson correlation coefficients (*r*) and p values are shown. ED, emergency department; NSSP, National Syndromic Surveillance Program; RPMF, reads per million filtered.

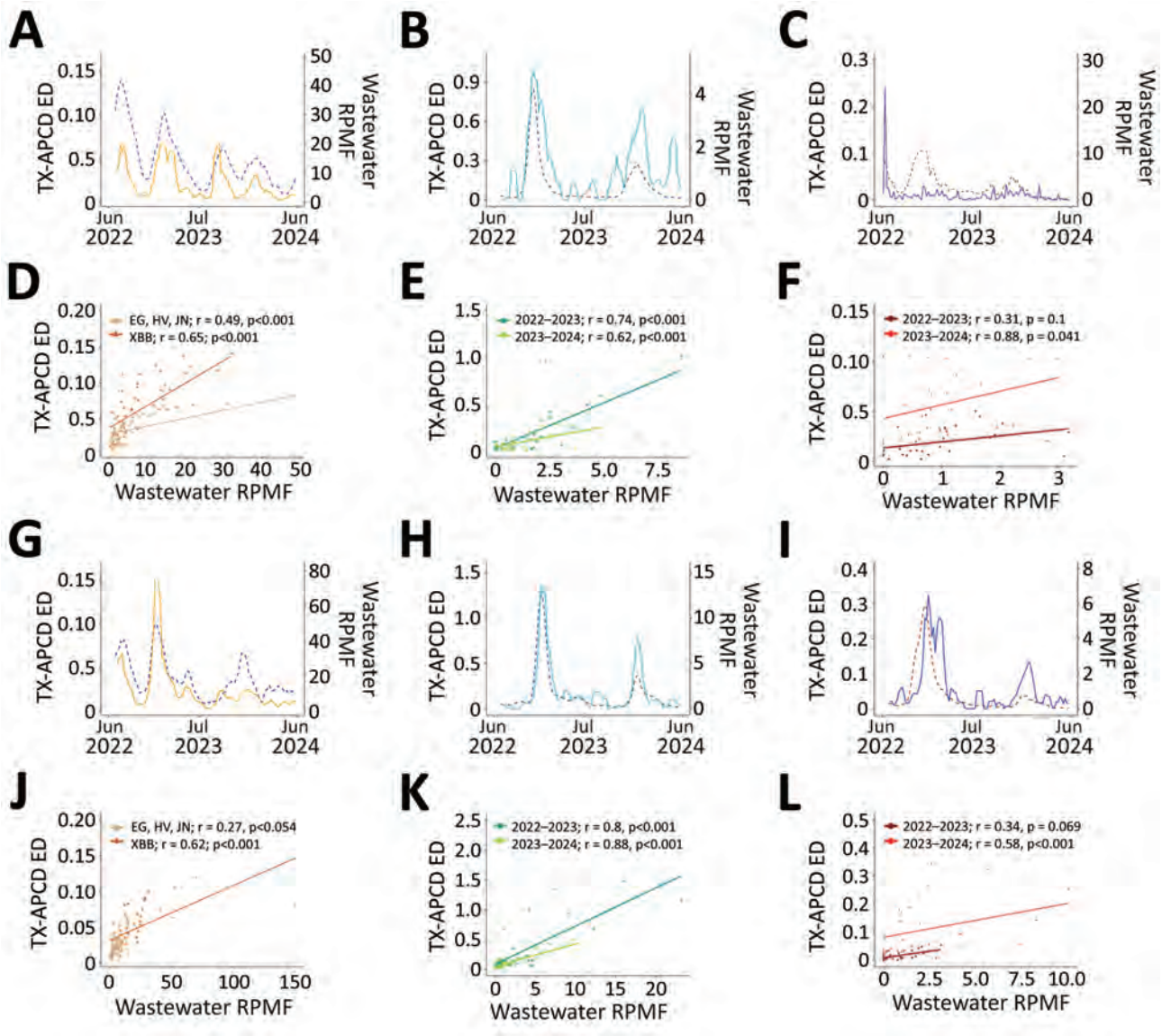


Figure 2. Association between weekly wastewater signals (RPMF) and weekly TX-APCD ED claims for SARS-CoV-2, influenza, and RSV in Harris and El Paso Counties, Texas, June 2022–June 2024. A–C) Harris County weekly trends in wastewater RPMF values versus TX-APCD ED visits for SARS-CoV-2 (A), influenza (B), and RSV (C). Solid lines represent TX-APCD claims; dashed lines represent RPMF data. D–F) Weekly RPMF values versus NSSP ED visits for SARS-CoV-2 (D), influenza (E), and RSV (F). Triangles and diamonds represent single-week observations. G–I) El Paso County interpolated daily time series of RPMF values versus TX-APCD ED visits for SARS-CoV-2 (G), influenza (H), and RSV (I). Solid lines represent TX-APCD claims; dashed lines represent RPMF data. J–L) Weekly RPMF values versus NSSP ED visits for (J) SARS-CoV-2, (K) influenza, and (L) RSV. Triangles and diamonds represent single-week observations. Corresponding Pearson correlation coefficients (*r*) and p values are shown. ED, emergency department; RPMF, reads per million filtered; TX-APCD, Texas All-Payer Claims Database.

2, 3). Although the NSSP excels in timeliness, the TX-APCD provides superior diagnostic specificity across a broad payer mix.

We aggregated claims to weekly counts by residential postal (ZIP) code. To differentiate new infection episodes from follow-up care, we defined a new case by using a 30-day disease-free interval for COVID-19 and influenza and a 45-day interval for RSV. Those thresholds are consistent with established epidemiologic standards for administrative data to minimize duplicate counting of single illness episodes (*20*–*22*). That dual-source approach pairs the broad, population-level coverage of syndromic surveillance with the diagnostic specificity of administrative claims to provide a robust validation of metagenomic wastewater metrics.

Statistical Analysis

We interpolated wastewater viral signals by RPMF to daily resolution by using natural cubic splines to enable comparison with clinical data (*23*). As a global smoothing method, this spline interpolation uses information from both past and future observations; consequently, we can interpret the resulting temporal relationships as descriptive associations rather than strictly real-time predictive leads. For clinical data, we smoothed daily ED visits by using a trailing 7-day moving average (right-aligned) to reduce day-of-week variability while ensuring only past information was used for each data point.

We conducted a time-lagged cross-correlation analysis to evaluate the temporal relationships between wastewater signals and clinical indicators. For the daily NSSP data, we assessed correlations across a ±21-day window. For the TX-APCD administrative claims, which are reported at a lower temporal resolution, we compared weekly aggregated wastewater data to weekly claims data using a ±3-week window. We used Pearson correlation coefficients to quantify those associations, because that metric directly captures the linear relationship and signal intensity (e.g., peak magnitude) between viral concentrations and visit rates. We adjusted CIs for autocorrelation to ensure valid inference.

To account for evolving viral dynamics and spatial heterogeneity, we performed separate analyses for each county and stratified the results by dominant variant or respiratory season. For SARS-CoV-2, we defined 2 major periods: June 2022–June 2023 (XBB variant dominance) and June 2023–June 2024 (emergence of EG.5, HV.1, and JN.1 variants). We stratified influenza and RSV analyses by the 2022–23 and 2023–24 respiratory seasons (*24*). Those stratifications enabled us to evaluate the consistency of wastewater signal associations across shifts in viral lineages and seasonal patterns.

Results

During June 2022–June 2024, wastewater RPMF values demonstrated positive correlations with NSSP ED visits at 0 lag across SARS-CoV-2, influenza, and RSV

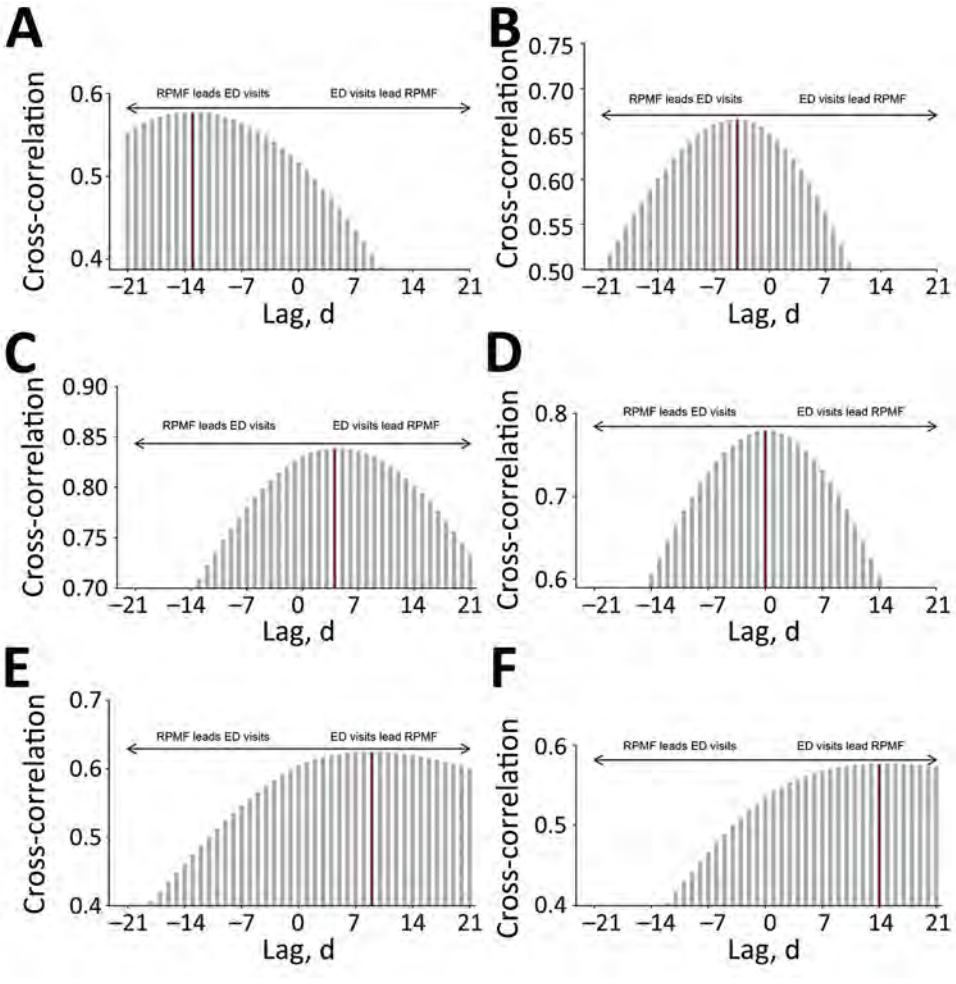


Figure 3. Cross-correlation between daily wastewater signals (RPMF) and National Syndromic Surveillance Program ED visit rates for SARS-CoV-2, influenza, and respiratory syncytial virus (RSV) in Harris and El Paso Counties, Texas, June 2022–June 2024. A) Harris County, SARS-CoV-2. B) El Paso County, SARS-CoV-2. C) Harris County, influenza. D) El Paso County, influenza. E) Harris County, RSV. F) El Paso County, RSV. Wastewater RPMF time series were shifted from −21 to +21 days relative to National Syndromic Surveillance Program ED visits, with Pearson correlation coefficients calculated at each daily lag. Negative lags indicate wastewater signals leading clinical visits. ED, emergency department; RPMF, reads per million filtered.

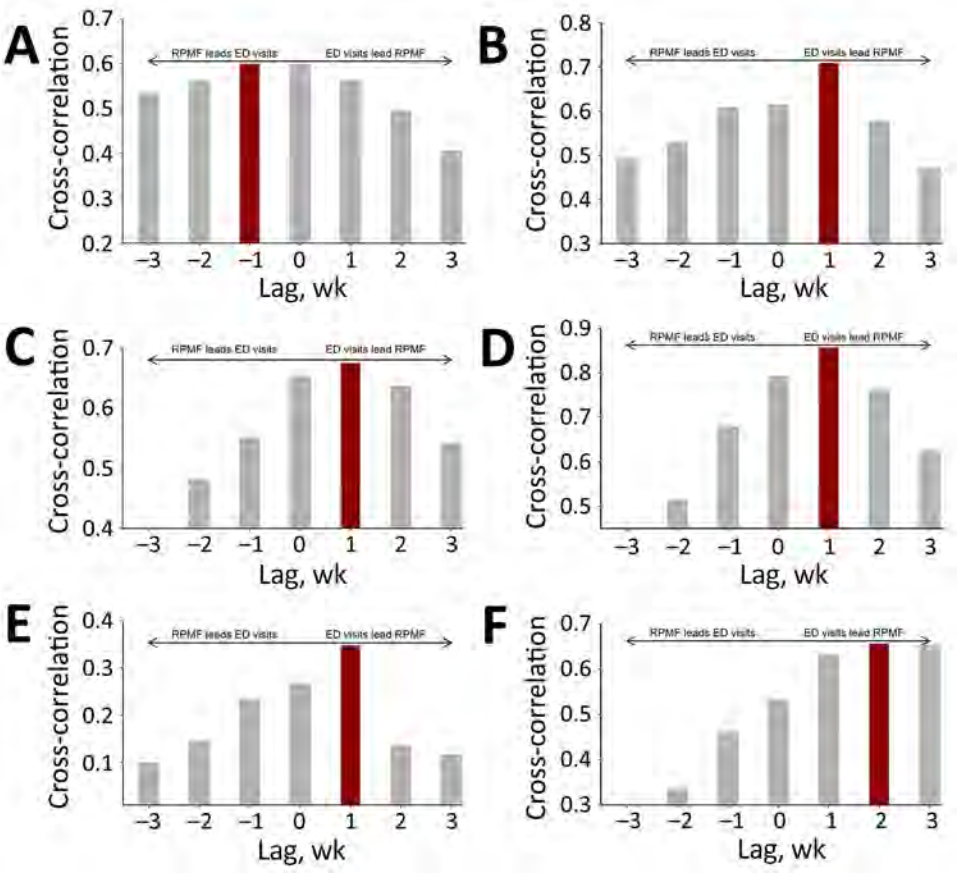


Figure 4. Cross-correlation between weekly wastewater signals (RPMF) and Texas All-Payer Claims Database ED claims for SARS-CoV-2, influenza, and RSV in Harris and El Paso Counties, Texas, from July 2022–June 2024. A) Harris County, SARS-CoV-2. B) El Paso County, SARS-CoV-2. C) Harris County, influenza. D) El Paso County, influenza. E) Harris County, RSV. F) El Paso County, RSV. Wastewater RPMF time series were shifted from –3 to +3 weeks relative to Texas All-Payer Claims Database claims, with Pearson correlation coefficients calculated at each weekly lag. ED, emergency department; RPMF, reads per million filtered.

(Figure 1). In Harris and El Paso counties, Pearson correlation coefficients (r) for influenza were strong over the full study period (r = 0.79–0.98). For those 2 counties, SARS-CoV-2 correlations were moderate to strong (r = 0.57–0.72), whereas RSV associations revealed greater seasonal variability (r = 0.57–0.82). Comparisons between weekly wastewater signals and TX-APCD claims revealed a wider range of associations (Figure 2). Although influenza showed strong concordance in both Harris ($r \geq 0.62$) and El Paso ($r \geq 0.8$) counties, RSV correlations varied widely, including weak associations in Harris County. Those discrepancies were highlighted by divergent peak timings; a June 2022 RSV surge was evident in wastewater and NSSP ED visits but absent in TX-APCD claims.

We identified the lag and lead intervals by using cross-correlation analysis yielding maximum correlation coefficients (r_{max}) for each pathogen (Figures 3, 4). SARS-CoV-2 wastewater signals generally preceded clinical metrics, with leads ranging from 3–15 days for NSSP ED visits and 1 week for TX-APCD claims. Of note, influenza patterns were more synchronous, typically showing r_{max} at 0 or short lags (0–4 days) across counties. In contrast, RSV demonstrated the most variable lead-lag patterns between wastewater and clinical indicators; in most counties the wastewater signal lagged NSSP ED visits, trailing them by up to 2 weeks (Tables 3, 4), and aligned inconsistently with TX-APCD claims.

Table 3. Pearson correlation coefficients between RPMF and 2 ED visits data sources for respiratory syncytial virus during the 2022–23 season across 4 counties, daily NSSP ED visit rates and weekly TX-APCD ED claims rates, Texas, USA*

	RPMF versus NSSP ED visit			RPMF versus TX-APCD ED claims		
County	Original r	Optimal lag, d	r_{max}	Original r	Optimal lag, wk	r_{max}
El Paso	0.56	15	0.61	0.34	2	0.51
Fort Bend	0.61	10	0.64	0.22	3	0.41
Harris	0.57	15	0.75	0.31	6	0.43
Lubbock	0.22	–7	0.24	0.31	6	0.49

*Optimal lag is reported relative to the clinical indicator. A negative value indicates that the wastewater signal leads the clinical indicator; a positive value indicates that it lags. The table shows the correlation at 0 lag (original), the optimal shift required to maximize the association (lag), and the resulting r_{max}. ED, emergency department; NSSP, National Syndromic Surveillance Program; r_{max}, maximum correlation; RPMF, reads per million filtered; TX-APCD, Texas All-Payer Claims Database.

Table 4. Pearson correlation coefficients between RPMF and 2 ED visits data sources for respiratory syncytial virus during the 2023–24 season across 10 counties, daily NSSP ED visit rates and weekly TX-APCD ED claims rates, Texas, USA*

	RPMF versus NSSP ED visit			RPMF versus TX-APCD ED claims		
County	Original *r*	Optimal lag, d	r_{max}	Original *r*	Optimal lag, wk	r_{max}
Cameron	0.56	15	0.83	–0.14	–2	0.44
Chambers	0.64	0	0.64	0.33	4	0.47
El Paso	0.82	6	0.84	0.58	1	0.69
Fort Bend	0.49	15	0.7	0.37	4	0.62
Harris	0.77	13	0.81	0.38	2	0.4
Hays	0.77	12	0.89	0.62	0	0.62
Lubbock	0.95	2	0.96	0.83	0	0.83
Travis	0.72	15	0.87	0.74	1	0.78
Wichita	0.85	3	0.87	0.17	–1	0.49
Williamson	0.65	15	0.81	0.49	4	0.78

*Optimal lag is reported relative to the clinical indicator. A negative value indicates that the wastewater signal leads the clinical indicator; a positive value indicates that it lags. The table shows the correlation at 0 (original), the optimal shift required to maximize the association (lag), and the resulting r_{max}. ED, emergency department; NSSP, National Syndromic Surveillance Program; r_{max}, maximum correlation; RPMF, reads per million filtered; TX-APCD, Texas All-Payer Claims Database.

Across the 10 study counties (Tables 3–8; Appendix Figure), influenza consistently demonstrated the strongest correlations with clinical data; influenza demonstrated 0-day optimal lags relative to NSSP visits in counties such as El Paso and Fort Bend (Tables 7, 8). In contrast, SARS-CoV-2 and RSV displayed greater heterogeneity; SARS-CoV-2 typically led NSSP visits by 3–15 days; r_{max} values reached 0.87 in Cameron County and 0.85 in Harris County during the EG.5, HV.1, and JN.1 SARS-CoV-2 variants period and remaining more moderate in Harris County (r_{max} = 0.58) during the XBB period. RSV demonstrated the longest and most variable lead-lag patterns between wastewater and clinical indicators; in most counties, the wastewater signal lagged NSSP visits, with optimal lags of ≤15 days (Tables 3, 4), and the alignment between wastewater signals and TX-APCD claims was weak and inconsistent. For example, the contemporaneous (0-lag) correlation between wastewater and RSV claims ranged from weakly negative in Cameron County (r = –0.14) to only modestly positive in Wichita County (r = 0.17) and improved only slightly after temporal shifting.

Discussion

WBE can offer a population-level view of respiratory virus circulation that complements comprehensive clinical reporting in a changing surveillance landscape. Although WBE lacks a formal biological denominator to provide absolute prevalence, the use of normalized metrics such as RPMF enables a semiquantitative assessment of community viral load. Our study indicates that metagenomic WBE remains a highly informative tool, although we observed major heterogeneity in both correlation strength and lead-lag timing across diverse pathogens and geographies. Because wastewater data are most actionable for public health when they lead clinical indicators, we emphasize the direction of the wastewater–clinical relationship, and not only its strength.

Influenza exhibited the most consistent contemporaneous (0-lag) alignment with both NSSP ED visits and TX-APCD claims, demonstrating the highest correlation coefficients across the study region. Of note, influenza was often near-synchronous with clinical trends, showing 0-day lags in 5 of 10 counties, which likely reflects its robust seasonal dynamics. Furthermore, the generally shorter wastewater-to-ED lags for influenza compared with SARS-CoV-2 might be attributed to its shorter incubation period (*25*). In contrast, wastewater signals for RSV demonstrated the longest and most variable lag times, generally trailing NSSP ED visits by up to 2 weeks. That extended lag, coupled with inconsistencies between clinical data sources, such as RSV signals lagging NSSP visits while aligning inconsistently with TX-APCD claims,

Table 5. Pearson correlation coefficients between RPMF and 2 ED visit data sources for SARS-CoV-2 during the XBB variant period across 4 counties, daily NSSP ED visit rates and weekly TX-APCD ED claims rates, Texas, USA, 2024*

	RPMF versus NSSP daily ED visit			RPMF versus TX-APCD ED claims		
County	Original *r*	Optimal lag, d	r_{max}	Original *r*	Optimal lag, wk	r_{max}
El Paso	0.72	–5	0.74	0.62	1	0.73
Fort Bend	0.55	–3	0.56	0.7	0	0.7
Harris	0.57	–5	0.58	0.65	0	0.65
Lubbock	0.79	4	0.8	0.61	1	0.66

*Optimal lag is reported relative to the clinical indicator. A negative value indicates that the wastewater signal leads the clinical indicator; a positive value indicates that it lags. The table shows the correlation at 0 (original), the optimal shift required to maximize the association (lag), and the resulting r_{max}. ED, emergency department; NSSP, National Syndromic Surveillance Program; r_{max}, maximum correlation; RPMF, reads per million filtered; TX-APCD, Texas All-Payer Claims Database.

Table 6. Pearson correlation coefficients between RPMF and 2 ED visit data sources for SARS-CoV-2 during the combined EG.5, HV.1, and JN.1 SARS-CoV-2 variant period across 10 counties, daily NSSP ED visit rates and weekly TX-APCD ED claims rates, Texas, USA, 2024

	RPMF versus NSSP ED visit			RPMF versus TX-APCD ED claims		
County	Original *r*	Optimal lag, d	r_{max}	Original *r*	Optimal lag, wk	r_{max}
Cameron	0.86	−4	0.87	0.5	0	0.5
Chambers	0.43	−14	0.56	0.22	2	0.35
El Paso	0.67	0	0.67	0.36	1	0.5
Fort Bend	0.75	−3	0.76	0.61	1	0.62
Harris	0.65	−15	0.85	0.49	−2	0.51
Hays	0.71	−11	0.8	0.62	0	0.62
Lubbock	0.72	−8	0.74	0.35	0	0.35
Travis	0.75	−9	0.81	0.63	0	0.63
Wichita	0.26	7	0.27	0.32	1	0.41
Williamson	0.77	−11	0.84	0.54	−1	0.57

*Optimal lag is reported relative to the clinical indicator. A negative value indicates that the wastewater signal leads the clinical indicator; a positive value indicates that it lags. The table shows the correlation at 0 (original), the optimal shift required to maximize the association (lag), and the resulting r_{max}. ED, emergency department; NSSP, National Syndromic Surveillance Program; r_{max}, maximum correlation; RPMF, reads per million filtered; TX-APCD, Texas All-Payer Claims Database.

might stem from age-specific infection patterns and differences in healthcare-seeking behavior (*26*), particularly among pediatric populations who might contribute less to the wastewater aggregate (*27*); because young children shed comparatively little virus into the wastewater aggregate, such lower shedding might also dampen the wastewater signal and reduce any potential RSV lead time.

Discrepancies between NSSP ED visits and TX-APCD claims in their correlation with wastewater signals highlight the unique limitations of each clinical proxy. Although the NSSP captures encounters regardless of payer status, the TX-APCD is limited to insured populations (*6*,*19*). The lower correlations observed in Cameron County, which has the highest uninsured rate among the studied counties, suggest that socioeconomic factors and insurance coverage majorly influence clinical surveillance sensitivity. Furthermore, whereas the TX-APCD relies on confirmed ICD-10 diagnosis codes, NSSP data represents a hybrid of syndromes and diagnoses, which might introduce nonspecificity.

Our lead–lag findings are broadly consistent with the wider WBE literature (*29*,*30*). For SARS-CoV-2, a systematic review of COVID-19 wastewater studies reported lead times relative to clinical cases that are short and highly variable across settings, from roughly 0 to about 2 weeks, broadly consistent with the 3–15-day NSSP leads we observed (*28*). Direct comparisons are rarer for influenza and RSV, but the available studies likewise report short and inconsistent lead times. Surveillance in Wisconsin found that wastewater influenza A and RSV signals tracked closely with, and did not consistently precede, emergency department visits (*29*), and municipal influenza A wastewater signals have been reported to align with clinical cases only after substantial time-shifting (*30*). Our finding that influenza was near-synchronous and that RSV wastewater signals tended to lag clinical indicators is therefore consistent with that body of work and reinforces the view that metagenomic WBE is best positioned as a complementary, situational-awareness tool rather than a consistently leading early-warning system for these pathogens.

The first limitation of this study is the spatial misalignment between wastewater and clinical catchment areas. Sewershed boundaries often do not follow county lines, meaning the contributing population to a wastewater sample might not perfectly overlap with the persons captured in county-level clinical datasets. That geographic mismatch, combined with variations in sampling frequency and the lack of human-versus-animal source differentiation, might explain some of the observed variability in

Table 7. Pearson correlation coefficients between RPMF and 2 ED visits data sources for influenza during the 2022–23 season across 4 counties, daily NSSP ED visit rates and weekly TX-APCD ED claims rates, Texas, USA*

	RPMF versus NSSP ED visit			RPMF versus TX-APCD ED claims		
County	Original *r*	Optimal lag, d	r_{max}	Original *r*	Optimal lag, wk	r_{max}
El Paso	0.98	0	0.98	0.79	1	0.87
Fort Bend	0.89	0	0.89	0.75	1	0.76
Harris	0.91	0	0.91	0.74	1	0.77
Lubbock	0.68	−13	0.95	0.52	−1	0.68

*Optimal lag is reported relative to the clinical indicator. A negative value indicates that the wastewater signal leads the clinical indicator; a positive value indicates that it lags. The table shows the correlation at 0 (original), the optimal shift required to maximize the association (lag), and the resulting r_{max}. ED, emergency department; NSSP, National Syndromic Surveillance Program; r_{max}, maximum correlation; RPMF, reads per million filtered; TX-APCD, Texas All-Payer Claims Database.

Table 8. Pearson correlation coefficients between RPMF and 2 ED visits data sources for influenza during the 2023–24 season across 10 counties, daily NSSP ED visit rates and weekly TX-APCD ED claims rates, Texas, USA*

	RPMF versus NSSP ED visit			RPMF versus TX-APCD ED claims		
County	Original *r*	Optimal lag, d	r_{max}	Original *r*	Optimal lag, wk	r_{max}
Cameron	0.86	0	0.86	0.25	2	0.46
Chambers	0.84	0	0.84	0.56	1	0.61
El Paso	0.9	0	0.9	0.88	0	0.88
Fort Bend	0.87	0	0.87	0.29	0	0.29
Harris	0.79	10	0.83	0.63	2	0.71
Hays	0.57	−15	0.66	0.45	−3	0.65
Lubbock	0.81	0	0.81	0.4	−2	0.47
Travis	0.53	−15	0.61	0.5	−3	0.54
Wichita	0.68	−14	0.9	0.76	0	0.76
Williamson	0.54	−15	0.63	0.4	−3	0.67

*Optimal lag is reported relative to the clinical indicator. A negative value indicates that the wastewater signal leads the clinical indicator; a positive value indicates that it lags. The table shows the correlation at 0 (original), the optimal shift required to maximize the association (lag), and the resulting r_{max}. ED, emergency department; NSSP, National Syndromic Surveillance Program; r_{max}, maximum correlation; RPMF, reads per million filtered; TX-APCD, Texas All-Payer Claims Database.

lead times. Second, the potential for nonhost genetic material to fluctuate on the basis of environmental factors remains an inherent limitation of metagenomic normalization. Finally, we computed all correlations we report on smoothed signals, wastewater RPMF interpolated to daily resolution with natural cubic splines that draw on both past and future observations, and clinical counts smoothed with a 7-day moving average, rather than on raw data streams. Such smoothing tends to inflate apparent correlation strength relative to unsmoothed data; for SARS-CoV-2, correlations computed on raw wastewater data are typically lower ($r \approx 0.35$). Because the spline interpolation incorporates future and past observations, the lead and lag estimates reported here should be interpreted as descriptive temporal associations rather than strictly real-time predictive leads.

Future systems should prioritize the harmonization of clinical data streams and the refinement of spatial matching at a subcounty level to better account for system-level variability. However, we emphasize that WBE should be used to complement, not replace, traditional case reporting. Our results align with previous city-level studies showing that WBE can be a reliable and timely indicator of respiratory trends (*31*,*32*).

De-identified aggregated data and analytic code will be made available to researchers for the purpose of replicating the results or conducting related public health surveillance research. To request access, interested parties should contact the corresponding author. Access to certain datasets, particularly those derived from the Texas All-Payer Claims Database or sensitive syndromic surveillance, might be subject to a Data Use Agreement and approval from the relevant institutional review boards or data stewards to ensure continued compliance with privacy and security protocols.

This work was supported by the Texas Epidemic Public Health Institute.

About the Author

Dr. Bi is an assistant professor of management, policy, and community health at The University of Texas Health Science Center School of Public Health. His research interests include infectious disease modeling, epidemic forecasting, and supporting public health decision-making.

Reference

1. Bi K, Bandekar SR, Bouchnita A, Fox SJ, Meyers LA. Annual hospitalizations for COVID-19, influenza, and respiratory syncytial virus, United States, 2023–2024. Emerg Infect Dis. 2025;31:636–8. https://doi.org/10.3201/eid3103.240594
2. Hanage WP, Schaffner W. Burden of acute respiratory infections caused by influenza virus, respiratory syncytial virus, and SARS-CoV-2 with consideration of older adults: a narrative review. Infect Dis Ther. 2025;14(Suppl 1):5–37. https://doi.org/10.1007/s40121-024-01080-4
3. Paget J, Spreeuwenberg P, Charu V, Taylor RJ, Iuliano AD, Bresee J, et al.; Global Seasonal Influenza-associated Mortality Collaborator Network and GLaMOR Collaborating Teams. Global mortality associated with seasonal influenza epidemics: new burden estimates and predictors from the GLaMOR Project. J Glob Health. 2019;9:020421. https://doi.org/10.7189/jogh.09.020421
4. Datadot. COVID-19 cases [cited 2026 Jan 9]. https://data.who.int/dashboards/covid19/cases
5. Tempia S, Abou El Naja H, Barakat A, Abubakar A, Khan W. Integrated surveillance for high-impact respiratory viruses: a necessity for better epidemic and pandemic preparedness. BMJ Glob Health. 2022;7(Suppl 4):e009018. https://doi.org/10.1136/bmjgh-2022-009018
6. Centers for Disease Control and Prevention. ESSENCE, 2024 [cited 2026 Jan 9]. https://www.cdc.gov/nssp/php/onboarding-toolkits/essence.html
7. Galanti M, Comito D, Ligon C, Lane B, Matienzo N, Ibrahim S, et al. Active surveillance documents rates of clinical care seeking due to respiratory illness. Influenza Other Respir Viruses. 2020;14:499–506. https://doi.org/10.1111/irv.12753
8. Hiller KM, Stoneking L, Min A, Rhodes SM. Syndromic surveillance for influenza in the emergency department—

a systematic review. PLoS One. 2013;8:e73832. https://doi.org/10.1371/journal.pone.0073832
9. Gu Y, DeDoncker E, VanEnk R, Paul R, Peters S, Stoltman G, et al. Accuracy of state-level surveillance during emerging outbreaks of respiratory viruses: a model-based assessment. Med Decis Making. 2021;41:1004–16. https://doi.org/10.1177/0272989X211022276
10. Preussler JM, Mau LW, Majhail NS, Meyer CL, Denzen EM, Edsall KC, et al. Administrative claims data for economic analyses in hematopoietic cell transplantation: challenges and opportunities. Biol Blood Marrow Transplant. 2016;22:1738–46. https://doi.org/10.1016/j.bbmt.2016.05.005
11. Barcellos DS, Barquilha CER, Oliveira PE, Prokopiuk M, Etchepare RG. How has the COVID-19 pandemic impacted wastewater-based epidemiology? Sci Total Environ. 2023; 892:164561. https://doi.org/10.1016/j.scitotenv.2023.164561
12. Tisza M, Javornik Cregeen S, Avadhanula V, Zhang P, Ayvaz T, Feliz K, et al. Wastewater sequencing reveals community and variant dynamics of the collective human virome. Nat Commun. 2023;14:6878. https://doi.org/10.1038/s41467-023-42064-1
13. Child HT, Airey G, Maloney DM, Parker A, Wild J, McGinley S, et al. Comparison of metagenomic and targeted methods for sequencing human pathogenic viruses from wastewater. MBio. 2023;14:e0146823. https://doi.org/10.1128/mbio.01468-23
14. Simpson JB, Sekela JJ, Graboski AL, Borlandelli VB, Bivins MM, Barker NK, et al. Metagenomics combined with activity-based proteomics point to gut bacterial enzymes that reactivate mycophenolate. Gut Microbes. 2022;14:2107289. https://doi.org/10.1080/19490976.2022.2107289
15. Zhuang X, Vo V, Moshi MA, Dhede K, Ghani N, Akbar S, et al. Early detection of emerging SARS-CoV-2 variants from wastewater through genome sequencing and machine learning. Nat Commun. 2025;16:6272. https://doi.org/10.1038/s41467-025-61280-5
16. Sun Z, Liu J, Zhang M, Wang T, Huang S, Weiss ST, et al. Removal of false positives in metagenomics-based taxonomy profiling via targeting type IIB restriction sites. Nat Commun. 2023;14:5321. https://doi.org/10.1038/s41467-023-41099-8
17. Shenoi RP, Moreland B, Jones JL, Peoples N, Camp EA, Levine N. Using syndromic surveillance for unintentional and undetermined intent drowning surveillance in a large metropolitan area. Inj Epidemiol. 2024;11(Suppl 1):52. https://doi.org/10.1186/s40621-024-00529-x
18. Centers for Disease Control and Prevention. Companion guide: NSSP ED data on respiratory illness, 2026, [cited 2026 Jan 9]. https://www.cdc.gov/nssp/php/onboarding-resources/companion-guide-ed-data-respiratory-illness.html
19. Center for Healthcare Data. Overview TX-APCD, [cited 2026 Jan 9]. https://sph.uth.edu/research/centers/center-for-health-care-data/texas-all-payor-claims-database
20. Chemaitelly H, Ayoub HH, Tang P, Yassine HM, Al Thani AA, Hasan MR, et al. Addressing bias in the definition of SARS-CoV-2 reinfection: implications for underestimation. Front Med (Lausanne). 2024;11:1363045. https://doi.org/10.3389/fmed.2024.1363045
21. Kazaglis L, Edwards G, Butler S, Barkley E. Hospitalizations for flu and its complications higher in the 2024/2025 season than other recent flu seasons, [cited 2026 Jan 9]. https://www.epicresearch.org/articles/hospitalizations-for-flu-and-its-complications-higher-in-the-2024-2025-season-than-other-recent-flu-seasons
22. Li Y, Wang X, Blau DM, Caballero MT, Feikin DR, Gill CJ, et al.; Respiratory Virus Global Epidemiology Network; RESCEU investigators. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in children younger than 5 years in 2019: a systematic analysis. Lancet. 2022;399:2047–64. https://doi.org/10.1016/S0140-6736(22)00478-0
23. Hastie TJ, Tibshirani RJ. Generalized additive models. New York: CRC Press; 1990.
24. Ma KC, Castro J, Lambrou AS, Rose EB, Cook PW, Batra D, et al. Genomic surveillance for SARS-CoV-2 variants: circulation of Omicron XBB and JN.1 lineages—United States, May 2023–September 2024. MMWR Morb Mortal Wkly Rep. 2024;73:938–45. https://doi.org/10.15585/mmwr.mm7342a1
25. Centers for Disease Control and Prevention. Similarities and differences between flu and COVID-19, 2025, [cited 2026 Jan 9]. https://www.cdc.gov/flu/about/flu-vs-covid19.html
26. Abu-Raya B, Viñeta Paramo M, Reicherz F, Lavoie PM. Why has the epidemiology of RSV changed during the COVID-19 pandemic? EClinicalMedicine. 2023;61:102089. https://doi.org/10.1016/j.eclinm.2023.102089
27. Koureas M, Mellou K, Vontas A, Kyritsi M, Panagoulias I, Koutsolioutsou A, et al. Wastewater levels of respiratory syncytial virus associated with influenza-like illness rates in children—a case study in Larissa, Greece (October 2022–January 2023). Int J Environ Res Public Health. 2023;20:5219. https://doi.org/10.3390/ijerph20065219
28. Olesen SW, Imakaev M, Duvallet C. Making waves: defining the lead time of wastewater-based epidemiology for COVID-19. Water Res. 2021;202:117433. https://doi.org/10.1016/j.watres.2021.117433
29. DeJonge PM, Adams C, Pray I, Schussman MK, Fahney RB, Shafer M, et al. Wastewater surveillance data as a complement to emergency department visit data for tracking incidence of influenza A and respiratory syncytial virus—Wisconsin, August 2022–March 2023. MMWR Morb Mortal Wkly Rep. 2023;72:1005–9. https://doi.org/10.15585/mmwr.mm7237a2
30. Mercier E, D'Aoust PM, Thakali O, Hegazy N, Jia JJ, Zhang Z, et al. Municipal and neighbourhood level wastewater surveillance and subtyping of an influenza virus outbreak. Sci Rep. 2022;12:15777. https://doi.org/10.1038/s41598-022-20076-z
31. Anastopoulou Z, Fokas R, Koukouvini K-A, Chatziantoniou A, Vantarakis A. Comparative investigation and trends of respiratory viruses using wastewater-based epidemiological surveillance in Patras, Greece. Food Environ Virol. 2026;18:10. https://doi.org/10.1007/s12560-026-09683-5
32. Ai Y, Davis A, Jones D, Lemeshow S, Tu H, He F, et al. Wastewater SARS-CoV-2 monitoring as a community-level COVID-19 trend tracker and variants in Ohio, United States. Sci Total Environ. 2021;801:149757. https://doi.org/10.1016/j.scitotenv.2021.149757

Address for correspondence: Kaiming Bi, University of Texas Health Science Center at Houston, 7000 Fannin St, Houston, TX 77030, USA; email: kaiming.bi@uth.tmc.edu

Correlations between Wastewater Concentrations of Influenza A and Respiratory Syncytial Viruses and Clinical Laboratory Testing and Hospitalization Data, California, USA

Elisabeth Burnor, Madhura S. Rane, Marisa A.P. Donnelly, Lauren A. White, Cora Hoover, Monica Sun, Caroline Collins, Andrew Abram, Mariah Otstot, Joel Rojas, Cody Berkobien, Seema Jain, Duc J. Vugia, Wayne T.A. Enanoria, Linlin Li, Chad Below, Marlene Wolfe, Alexandria B. Boehm, Tomás M. León, Erin L. Murray, Alexander T. Yu

We evaluated correlations between influenza A virus and respiratory syncytial virus (RSV) wastewater concentrations in California, USA, and 3 clinical laboratory–based disease surveillance datasets: sentinel laboratory surveillance, mandatory electronic laboratory reporting data, and laboratory-confirmed influenza hospitalizations. We evaluated data from 10 counties and 18 wastewater treatment plants in California during July 2022–March 2024. We observed strong, positive, and statistically significant correlations between individual sewershed-level wastewater concentrations and county-aggregated wastewater concentrations of influenza A and RSV and clinical laboratory–based surveillance datasets (median Kendall τ 0.62 [range 0.40–0.78]; $p \leq 0.05$). A lead–lag analysis did not show consistent evidence of wastewater leading other surveillance datasets across all counties and wastewater treatment plants (influenza A –16 to 22 days; RSV –8 to 35 days). Wastewater surveillance can augment other disease surveillance modalities and improve situational awareness of influenza A and RSV transmission in communities.

Influenza virus and respiratory syncytial virus (RSV) cause substantial illness and death in the United States (*1*,*2*). Public health agencies use influenza and RSV surveillance data to track annual seasonal patterns, known as respiratory virus seasons. That surveillance is used for hospital capacity planning, public and healthcare provider awareness, resource allocation, and planning for vaccinations and therapeutics. National influenza and RSV surveillance relies primarily on data from state, tribal, local, and territorial health departments. Those entities, in turn, rely on surveillance drawn from heterogenous data sources, including public health laboratories, clinical laboratories, vital statistics offices, health care providers, emergency departments, and long-term care facilities that variably contribute testing, genomic sequencing, and syndromic, hospitalization, and death data (*3*). Although effective, that surveillance approach has heterogenous coverage gaps and is subject to biases caused by disparate healthcare access and healthcare seeking behavior because persons with mildly symptomatic or asymptomatic infections might not seek clinical testing (*4*).

Wastewater surveillance (WS) could fill some of those gaps in surveillance by providing information

Author affiliations: California Department of Public Health, Richmond, California, USA (E. Burnor, M.S. Rane, M.A.P. Donnelly, L.A. White, C. Hoover, M. Sun, C. Collins, A. Abram, M. Otstot, J. Rojas, C. Berkobien, S. Jain, D.J. Vugia, T.M. León, E.L. Murray, A.T. Yu); County of Santa Clara Public Health Department, San Jose, California, USA (W.T.A. Enanoria, L. Li); San Mateo County Health, San Mateo, California, USA (C. Below); Rollins School of Public Health, Emory University, Atlanta, Georgia, USA (M. Wolfe); Stanford University, Stanford, California, USA (A.B. Boehm)

DOI: https://doi.org/10.3201/eid3213.260306

about disease circulation in areas with limited testing and case reporting, warning of unexpected outbreaks, and helping public health practitioners determine when respiratory virus seasons begin and peak (*5*). WS has been used as a complementary tool alongside COVID-19 clinical surveillance to track trends in SARS-CoV-2 transmission, identify surges or outbreaks, and monitor virus variants (*6*–*13*). When evaluated alongside clinical surveillance data, wastewater data have provided a more complete picture of SARS-CoV-2 transmission in geographic areas with low testing or reporting rates. In addition, evidence suggests that WS might provide an early warning signal for the start of COVID-19 outbreaks (*5*–*7*,*9*,*11*–*13*), which in turn could provide lead time for preparedness measures.

Other viral pathogens, such as influenza A and B viruses, RSV, monkeypox virus, measles virus, human metapneumovirus, and norovirus, have also been successfully detected and quantified in wastewater (*14*–*22*; M.K.Wolfeetal.,unpub.data,https://doi.org/10.1101/2022.09.06.22279312). Previous studies have found that wastewater concentrations of influenza virus and RSV correlated with clinical influenza and RSV metrics (*15*). However, evidence for wastewater as an early indicator for influenza A has been inconsistent; some studies found that wastewater concentrations led clinical indicators for influenza (*20*,*21*,*23*), and other studies found that it lagged (*24*). For RSV, multiple studies have found that wastewater lagged RSV clinical data with variable lag periods (*25*–*27*). Those studies support the feasibility of WS for monitoring influenza and RSV transmission in communities and provide insight into the temporal relationship between wastewater concentrations and clinical indicators. However, additional work with longer study periods and a broad variety of comparative clinical datasets could improve the epidemiologic interpretability of wastewater concentrations and help inform the role that WS can play in overall community-level influenza and RSV surveillance. In this study, we examined correlations between influenza A and RSV wastewater concentrations from 18 California sewersheds and data from California's clinical laboratory–based surveillance systems. We also examined whether wastewater-based surveillance data led or lagged clinical laboratory–based surveillance data.

Materials and Methods

Study Period

We evaluated influenza A and RSV surveillance data collected from sentinel laboratory data, mandatory electronic laboratory reporting (ELR) data, and mandatory hospital reporting data during July 3, 2022–March 16, 2024. Our study's assay for influenza A in wastewater targets the matrix gene, conserved in all influenza A subtypes (e.g., H1, H3, H5) (*28*). We used March 16, 2024, as the cutoff because high H5 virus subtype concentrations have been detected in some California wastewater samples since March 25, 2024. Those high concentrations likely are because of discarded contaminated dairy products or waste from dairy processing facilities, resulting in H5-associated increases in influenza A wastewater concentrations (*29*). To avoid the possibility of nonhuman sources of influenza A in wastewater affecting our correlation analyses, we did not analyze wastewater samples collected after influenza A(H5N1) virus was detected in dairy cattle in California.

Electronic Influenza A Laboratory Results

During the study period, all laboratories in California were required to report all PCR-positive influenza inpatient and outpatient laboratory results to local health jurisdictions via an ELR platform. Reporting of influenza A–negative results and RSV results (positive or negative) was not mandatory during the full study period.

To evaluate correlations between influenza A wastewater and ELR data, we geocoded residential addresses associated with persons who received influenza A virus–positive test results to the spatial boundaries of sewershed catchment areas, as provided by wastewater treatment plant operators (*7*). Each influenza A virus–positive test has an associated specimen date code, which is calculated as the earliest of the following: specimen collection date, specimen received date, and specimen result date. We summed positive tests within each sewershed by using the specimen date code, resulting in daily sewershed-specific ELR counts of influenza A virus–positive specimens. To account for possible day-of-the-week reporting effects while maintaining day-to-day granularity reflecting changes over time, we smoothed daily counts by using a rolling average of positive specimens over the preceding 7 days. Wastewater utilities self-reported population estimates for each sewershed catchment area. Those estimates remained constant over the study period, and we used them to calculate the number of influenza A–positive specimens per 100,000 population.

Sentinel Laboratory Influenza A and RSV Data

The clinical sentinel laboratory network consists of clinical laboratories throughout California that

conduct routine testing as requested by clinicians from inpatient or outpatient settings (*30*). Data reported from those laboratories include the number of positive and negative influenza A and RSV specimens reported to the California Department of Public Health (CDPH) by epidemiologic week (*31*). Sentinel laboratory data are reported by the county where each sentinel laboratory is located, and some test results might represent persons who reside outside the county. During July 3, 2022–March 12, 2023, additional hospital-reported influenza A and RSV test results were available from Santa Clara Valley Medical Center (San Jose, CA, USA) and San Mateo Medical Center (San Mateo, CA, USA), both of which are outside of the sentinel laboratory network. We combined data from those 2 hospitals and from 5 sentinel clinical laboratories in California. We calculated the total number of positive influenza A and RSV samples and percentage test positivity from the hospitals and sentinel laboratories weekly. We calculated percentage positivity for each virus as the sum of positive tests divided by the sum of all tests for that virus. We combined results across all clinical sentinel laboratories and hospitals within each county.

RSV Testing by Age Group

San Mateo Medical Center and Santa Clara Valley Medical Center provided 2,062 RSV-positive test results by age group for patients 0–4 years, 5–17 years, 18–49 years, 50–64 years, and ≥65 years of age. We evaluated weekly age-grouped RSV-positive specimen counts; RSV-negative test results were not available by age group for San Mateo Medical Center, so we did not analyze age-grouped test positivity for this analysis.

Hospital Admission Data

During the COVID-19 public health emergency, all hospitals were mandated to report daily, laboratory-confirmed influenza hospital admissions via the US Department of Human Health and Services Protect/National Hospital Surveillance Network (NHSN) dataset (*32*). Those admission counts did not differentiate between influenza A and B. We aggregated NHSN influenza admission data by the county of the reporting hospital and smoothed by using a rolling sum of the preceding 7 days of daily admissions (i.e., rolling 7-day county-level influenza hospital admissions).

Wastewater Sample Collection and Processing

The WastewaterSCAN program (https://www.wastewaterscan.org), a collaboration between research partners at Stanford University (Stanford, CA, USA) and Emory University (Atlanta, GA, USA) and laboratory implementing partner Verily Life Sciences (https://verily.com), began monitoring for RSV in November 2021, and for influenza A in January 2022 (*14*). WastewaterSCAN processes wastewater samples using previously described methods (*33*). Wastewater settled solids were collected 3–7 times/week from the primary clarifier of each wastewater treatment plant or from liquid wastewater influent samples using an Imhoff cone (*6*,*33*–*35*); samples were transported to the laboratory at 4°C. WastewaterSCAN extracted RNA from settled solids and analyzed viral RNA concentrations by using droplet digital reverse transcription PCR (*33*). We included only WastewaterSCAN data in this study because no other laboratory conducted continuous influenza A and RSV wastewater monitoring in California throughout the full study period. We excluded influenza B from this study because those virus concentrations were only analyzed from a small subset of treatment plants during the full study period.

Wastewater Data Analysis

We analyzed raw, nonnormalized wastewater concentrations of influenza A and RSV RNA. For wastewater samples without detectable virus, we used a single imputation using half of the laboratory-assigned viral limit of detection. For county-level comparisons, we calculated county wastewater aggregates (Appendix, https://wwwnc.cdc.gov/EID/article/32/13/26-0306-App1.pdf). Analyses included influenza A and RSV wastewater concentrations collected during July 3, 2022–March 16, 2024, from 18 sewersheds in California (Appendix).

Correlation Analyses

We used Kendall rank correlation to assess correlations between wastewater concentrations and corresponding clinical data because this method is robust to nonnormal and skewed data distributions (*36*–*38*). We defined strong correlations as Kendall τ values >0.49 (*39*) and considered $p<0.05$ statistically significant. We performed analyses in R version 4.0.4 (The R Project for Statistical Computing, https://www.r-project.org) (Appendix).

Lead–Lag Analyses

To assess whether wastewater served as a leading or lagging indicator of circulating viruses, we calculated the cross correlations of wastewater with

respect to corresponding clinical laboratory-based datasets for influenza A and RSV. Datasets were compared at daily lag intervals, where wastewater data was offset relative to corresponding clinical data. Date offsets ranged from –35 to 35 days. We calculated the maximum Kendall τ correlation value across all lags and determined the range of lags for which the observed τ was within 0.05 of the maximum τ. When analyzing the range of maximum lagged τ, we inferred that ranges including only negative values indicated wastewater data led clinical laboratory–based data. For ranges including only positive τ values, we inferred that the clinical laboratory–based data led wastewater data. For ranges including both negative and positive τ values, we inferred that neither dataset demonstrated a clear leading signal (Appendix).

RSV Sensitivity Analysis

We also conducted an exploratory analysis evaluating county-aggregated weekly average RSV wastewater concentrations and weekly age-grouped RSV-positive specimen counts. For that analysis, we repeated Kendall τ correlation and lead–lag analyses by using the number of RSV-positive patient samples per age group reported from the San Mateo and Santa Clara hospitals.

Results

During the study period, we processed 6,715 wastewater samples from 18 sewersheds for influenza A virus and RSV. A total of 51,847 influenza A virus–positive specimens were reported via ELR and geocoded to included sewersheds. Through clinical sentinel laboratory surveillance, we also included 41,976 influenza A virus–positive specimens from 10 counties and 33,173 RSV-positive specimens from 9 counties. We also included 8,025 influenza hospital admissions from 8 counties reported through NHSN (Table 1).

Influenza A Correlations

We observed strong, positive, and statistically significant correlations between sewershed-level raw wastewater influenza A virus concentrations and the number of ELR influenza A virus–positive specimens per 100,000 persons in the sewershed over the entire study period (median τ 0.64 [range 0.52–0.72]; p<0.001) (Table 2; Figure 1). Of 18 sewersheds assessed for ELR data comparisons, wastewater data led ELR data in 3 sewersheds, ELR data led wastewater data in 3 sewersheds, and neither dataset demonstrated a clear lead in 12 sewersheds (Table 2; Appendix Figure 1).

At the county level, we observed similar results between county-aggregated weekly averages of raw

Table 1. Descriptive characteristics for each county and sewershed included in a study of correlations between wastewater concentrations of influenza A and respiratory syncytial viruses and clinical laboratory testing and hospitalization data, California, USA*

	County-level				Sewershed-level			
			Sentinel laboratory, no. positive specimens					
County	Population	No. influenza hospitalizations	Influenza A	RSV	Sewershed	Estimated population	No. ELR influenza A virus–positive specimens	No. wastewater samples processed
Alameda	1,658,061	991	7,184	5,999	East Bay	740,000	4,057	261
Contra Costa	1,158,249	852	6,686	5,167	Central Contra Costa	484,800	3,460	223
					Richmond	100,000	1,215	207
Marin	254,743	144	703	1,544	Novato	53,000	618	241
Sacramento	1,596,281	1,657	6,692	5,092	Sacramento	1,480,000	16,362	548
San Francisco	845,355	574	2,706	2,847	Oceanside	250,000	1,133	524
					Southeast†	750,000	3,607	541
San Mateo	747,777	293	3,503	2,043	Half Moon Bay	28,000	151	224
					San Mateo	150,000	1,149	232
					Silicon Valley	199,000	1,900	546
Santa Clara	1,921,406	3,066	11,336	8,278	Gilroy/Morgan Hill	110,338	1,631	550
					Palo Alto†	236,000	1,225	549
					San Jose	1,500,000	12,746	550
					Sunnyvale	153,000	998	536
Santa Cruz	263,454	NA‡	113	NA‡	Santa Cruz City	70,000	272	238
					Santa Cruz County	94,000	350	239
Sonoma	482,050	448	2,938	1,957	Petaluma	65,000	641	163
Yolo	224,088	NA‡	115	165	Davis	70,717	332	343
Total	9,151,464	8,025	41,976	33,173	Total	6,689,855	51,847	6,715

*ELR, electronic laboratory reporting; NA, not applicable; RSV, respiratory syncytial virus.
†The San Francisco Southeast sewershed and the Palo Alto sewershed both encompass small portions of neighboring San Mateo County. As a result, the total population coverage for the 2 San Francisco sewersheds is larger than the San Francisco County population, and the total population coverage for the 4 Santa Clara sewersheds is larger than the Santa Clara County population.
‡Numbers insufficient for inclusion in the analysis.

Table 2. Kendall τ correlations between wastewater concentrations of influenza A virus and clinical laboratory testing and hospitalization data, California, USA*

	County-level correlations						Sewershed-level correlations†		
	Sentinel laboratory % positivity		Sentinel laboratory no. positive specimens		Laboratory-confirmed hospitalizations			Population-level	
County	Overall	Maximum lag (range)	Overall	Maximum lag (range)	Overall	Maximum lag (range)	Sewershed	Overall	Maximum lag (range)
Alameda	0.56	0.61 (–2 to 15)	0.62	0.66 (–3 to 12)	0.67	0.67 (–14 to 12)	Alameda	0.6	0.61 (–6 to 6)
Contra Costa	0.53	0.6 (2–8)	0.7	0.75 (–9 to 11)	0.65	0.66 (–15 to 5)	Central Contra Costa	0.72	0.73 (1–5)
							Richmond	0.63	0.66 (–11 to –3)
Marin	0.57	0.57 (–10 to 21)	0.61	0.62 (–8 to 16)	0.5	0.53 (–16 to 12)	Novato	0.64	0.65 (–1 to 5)
Sacramento	0.55	0.57 (–10 to 11)	0.69	0.7 (–12 to 10)	0.65	0.66 (–15 to 9)	Sacramento	0.64	0.64 (–7 to 6)
San Francisco	0.57	0.58 (–8 to 14)	0.69	0.69 (–9 to 12)	0.56	0.57 (–15 to 18)	Oceanside	0.6	0.63 (–7 to –3)
							Southeast	0.64	0.65 (–9 to 7)
San Mateo	0.57	0.58 (–9 to 17)	0.65	0.66 (–9 to 17)	0.67	0.67 (–7 to 8)	San Mateo	0.67	0.67 (–2 to 6)
							Half Moon Bay	0.66	0.67 (–3 to 3)
							Silicon Valley	0.62	0.62 (–2 to 5)
Santa Clara	0.47	0.47 (–16 to 17)	0.67	0.67 (–8 to 7)	0.58	0.59 (–12 to 14)	Gilroy Morgan Hill	0.6	0.6 (–3 to 1)
							San Jose	0.67	0.67 (–5 to 1)
							Sunnyvale	0.7	0.7 (–6 to 2)
							Palo Alto	0.66	0.66 (–1 to 5)
Santa Cruz	0.54	0.61 (1–16)	0.62	0.7 (3–16)	NA	NA	Santa Cruz City	0.7	0.71 (–4 to –1)
							Santa Cruz County	0.75	0.78 (3–9)
Sonoma	0.63	0.69 (2–22)	0.71	0.75 (–3 to 16)	0.64	0.65 (–11 to 6)	Petaluma	0.54	0.57 (3–7)
Yolo	0.57	0.59 (–3 to 19)	0.53	0.55 (–8 to 20)	NA	NA	Davis	0.52	0.53 (–6 to 1)

*Kendall τ correlations between laboratory-confirmed influenza hospital admissions and wastewater influenza A concentrations from ELR, sentinel laboratories, and influenza A–positive specimen counts. Overall correlations represent Kendall τ correlation coefficients for the indicated datasets, calculated over the entire study period with no lags. Maximum lag correlations represent the maximum Kendall τ correlation coefficients for the indicated datasets when comparing lagged clinical datasets to wastewater datasets, including lags of –35 to 35 days. The range of maximum lagged τ (days) represents the range of days around a date of the maximum lagged correlation in which the lagged correlation coefficient is within 0.05 of the maximum τ value. The negative direction indicates that wastewater data is leading clinical data, and the positive direction indicates that wastewater data is lagging clinical data. ELR, electronic laboratory reported.
†Data from mandatory ELR reporting calculated as no. positive specimens/100,000 population.

influenza A virus wastewater concentrations and weekly sentinel laboratory influenza A percentage test positivity (median τ 0.57 [range 0.47–0.63]; p<0.001) (Table 2, Figure 2), influenza A virus–positive weekly specimen counts (median τ 0.66 [range 0.53–0.71]; p<0.001) (Table 2; Appendix Figure 2), and rolling 7-day county-level influenza hospital admissions (median τ 0.65 [range 0.50–0.67]; p<0.001) (Table 2; Figure 3). Of 10 counties assessed for sentinel laboratory influenza A comparisons, percentage test positivity led wastewater in 3 counties and neither dataset demonstrated a clear lead in 7 counties. Influenza A virus–positive specimen counts led wastewater in 1 county and neither dataset demonstrated a clear lead in 9 counties. Of 8 counties assessed for influenza hospital admissions, neither wastewater nor hospital admissions demonstrated a clear lead in any county (Table 2; Appendix Figure 3).

RSV Correlations

We observed moderate to strong, positive, and statistically significant correlations in overall comparisons between county-aggregated weekly averages of raw RSV wastewater concentrations and both weekly sentinel laboratory percent test positivity (median τ 0.45 [range 0.40–0.58]; p<0.001) (Table 3; Figure 4) and RSV-positive specimen counts (median τ 0.70 [range 0.48–0.78]; p<0.001) (Table 3; Appendix Figure 4). RSV percentage test positivity led wastewater concentrations in all 9 counties assessed. RSV-positive specimen counts led wastewater in 3 counties and neither dataset demonstrated a clear lead in 6 counties (Table 3).

Age-Specific RSV Correlations

We observed strong, positive, and statistically significant correlations between county-aggregated weekly

averages of raw RSV wastewater concentrations and weekly RSV-positive specimen counts among all age groups that had enough positive specimens to calculate correlations (Appendix Table 2). In the 0–4 years of age group, RSV-positive specimen counts led wastewater concentrations by 7–21 days across both counties. In the 5–17 years of age group, RSV-positive specimen counts led wastewater concentrations by 12–31 days in Santa Clara, but we noted no clear lead in San Mateo. Only Santa Clara had enough positive specimens from older age groups to calculate correlations. In age groups ≥18 years, we noted no clear lead, but the range of maximum lagged τ shifted towards the negative direction, reducing the lead time of positive

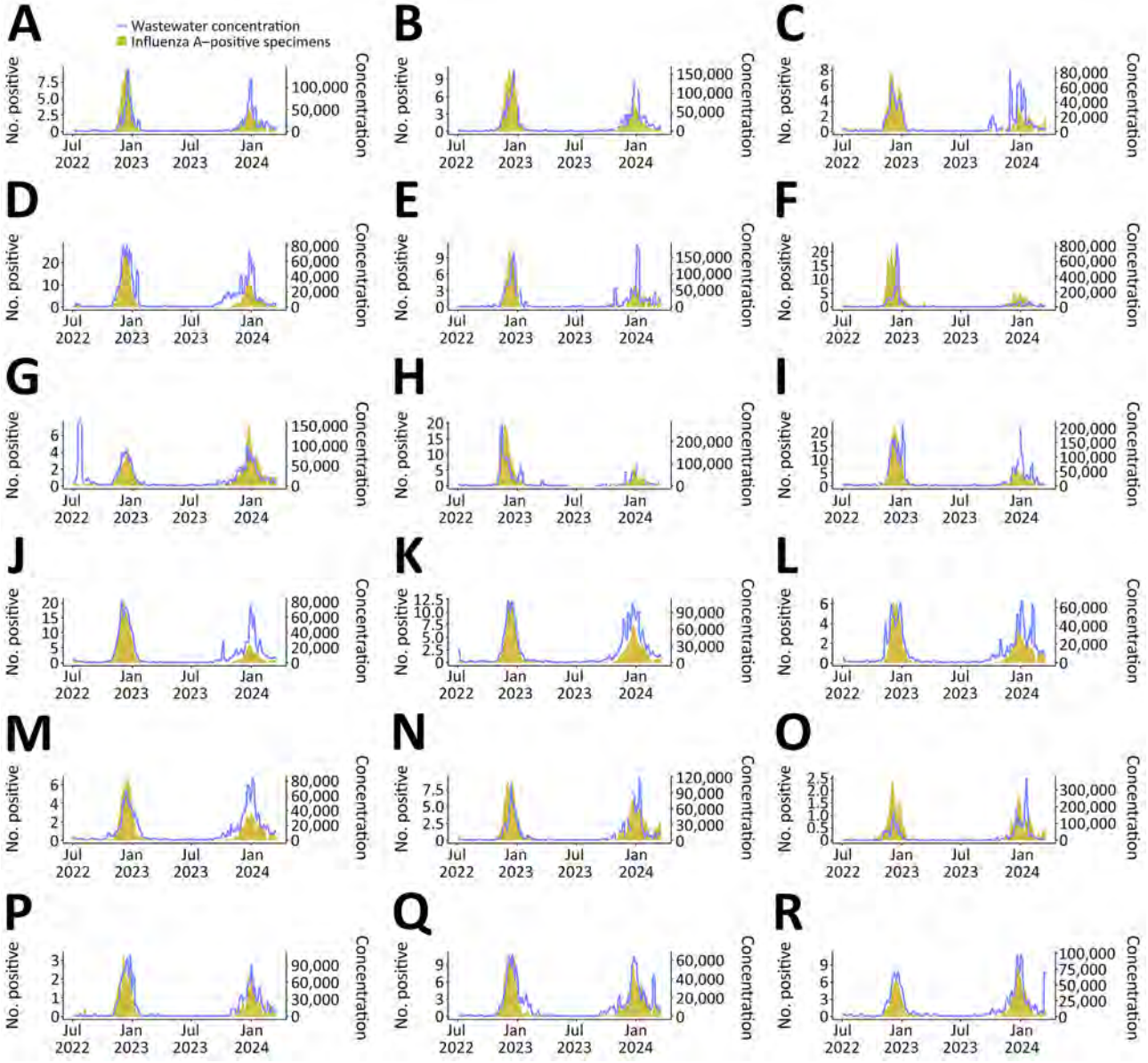


Figure 1. Time series comparison of wastewater and influenza A virus–positive specimens via electronic laboratory reporting from study of correlations between wastewater concentrations of influenza A and respiratory syncytial viruses and clinical laboratory testing and hospitalization data, California, USA, July 2022–March 2024. Reported influenza A virus–positive specimens were geocoded to sewersheds for comparisons. We calculated 7-day rolling average numbers of influenza A virus-positive specimens per 100,000 persons reported via electronic laboratory reporting (yellow bars) and influenza A viral wastewater concentrations in gene copies per gram (light blue line) for 18 sewersheds: A) Alameda (Alameda East Bay); B) Contra Costa (Central Contra Costa); C) Yolo (Davis); D) Santa Clara (Gilroy/Morgan Hill); E) San Mateo (Half Moon Bay); F) Marin (Novato); G) Santa Clara (Palo Alto); H) Sonoma (Petaluma); I) Contra Costa (Richmond); J) Sacramento (Sacramento); K) Santa Clara (San Jose); L) San Francisco (San Francisco Oceanside); M) San Francisco (San Francisco Southeast); N) San Mateo (San Mateo); O) Santa Cruz (Santa Cruz City); P) Santa Cruz (Santa Cruz County); Q) San Mateo (Silicon Valley); R) Santa Clara (Sunnyvale).

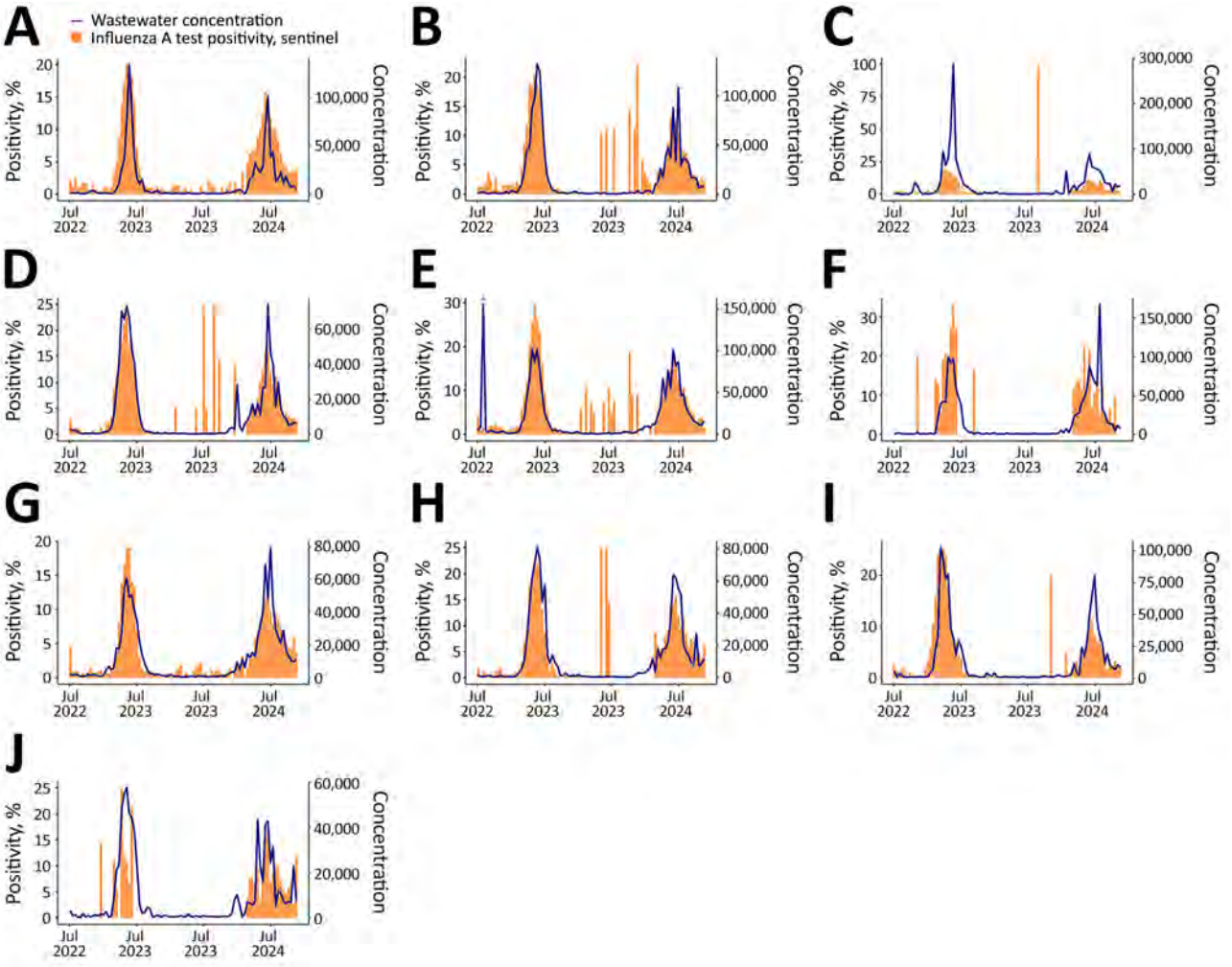


Figure 2. Time series comparison of influenza A virus wastewater concentrations and sentinel laboratory percentage test positivity from study of correlations between wastewater concentrations of influenza A and respiratory syncytial viruses and clinical laboratory testing and hospitalization data, California, USA. Graphs show weekly county influenza A percentage test positivity reported by sentinel laboratories (dark orange bars) and county-aggregated population-weighted averages of nonnormalized influenza A wastewater concentrations in gene copies per gram from all sewersheds sampling within each county (dark blue line) for 10 counties: A) Alameda; B) Contra Costa; C) Marin; D) Sacramento; E) Santa Clara; F) Santa Cruz; G) San Francisco; H) San Mateo; I) Sonoma; J) Yolo.

specimens compared to wastewater (Appendix Table 2, Figure 5).

Discussion

This study demonstrated that influenza A virus and RSV wastewater concentrations correlated with California clinical laboratory–based surveillance datasets for both viruses. Correlation results were consistent across datasets, including laboratory test results received via ELR, sentinel laboratory–reported data, and laboratory-confirmed influenza hospital admissions. We observed strong and statistically significant correlations for both daily and weekly correlation analyses and at the sewershed and county geographic levels. Influenza A wastewater concentrations did not consistently lead or lag clinical laboratory–based data. RSV wastewater concentrations consistently lagged clinical laboratory–based data. Those findings support the consideration of using WS as a complementary public health tool for monitoring these respiratory viruses.

We found that both clinical laboratory–based and wastewater-based surveillance systems were highly correlated in geographic areas and timeframes where both disease surveillance systems are available. That finding supports the combined, multimodal use of laboratory-based and wastewater-based surveillance for a more complete overall picture of disease transmission. Clinical sentinel laboratory surveillance systems are robust for monitoring

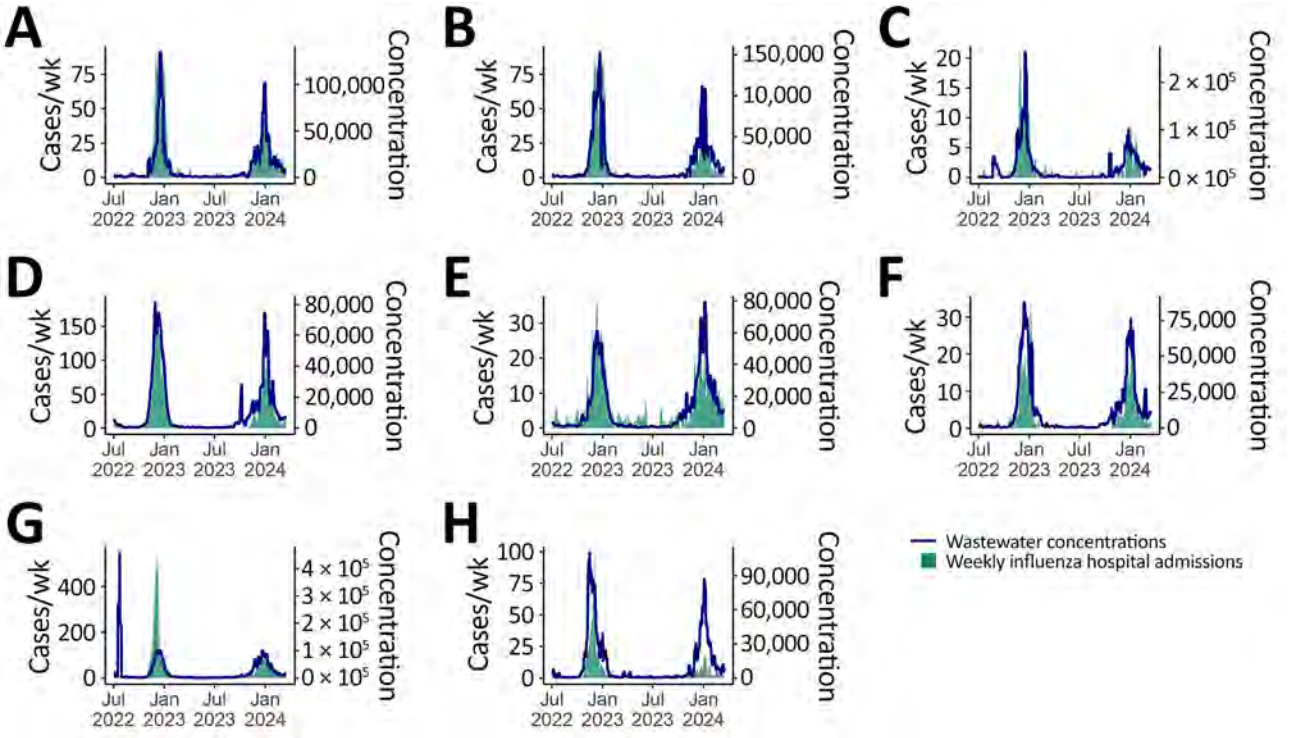


Figure 3. Time series comparison of influenza A virus wastewater concentrations and hospital admissions from study of correlations between wastewater concentrations of influenza A and respiratory syncytial viruses and clinical laboratory testing and hospitalization data, California, USA. Graphs show rolling 7-day county-level influenza hospital admissions (green bars) and county-aggregated population-weighted averages of nonnormalized influenza A wastewater concentrations in gene copies per gram from all sampling sewersheds within each county (dark blue line) for 8 counties: A) Alameda; B) Contra Costa; C) Marin; D) Sacramento; E) San Francisco; F) San Mateo; G) Santa Clara; H) Sonoma.

respiratory viruses but are subject to biases related to testing rates and case ascertainment. Those biases are particularly notable for mildly symptomatic or asymptomatic cases, within populations with lower testing access and utilization, and for illnesses with readily available home testing. Sentinel laboratory surveillance systems for RSV have historically prioritized surveillance in vulnerable pediatric populations and could underestimate RSV burden in older populations (*40*). WS is also subject to limitations because infections are likely to be missed in diapered children who contribute little waste to sewer systems and because wastewater samples collected at wastewater treatment plants will not represent populations that rely on septic systems (*25*–*27*).

Strengths of WS include its ability to track changes in community transmission regardless of symptomatology, healthcare utilization, or clinical

Table 3. Kendall τ correlations between wastewater RSV concentrations sentinel laboratory testing, California*

	% RSV positivity		No. RSV-positive specimens	
County	Overall*	Maximum lag (range)†	Overall*	Maximum lag (range)†
Alameda	0.45	0.65 (16 to >35)	0.64	0.7 (–4 to 18)
Contra Costa	0.46	0.63 (19 to >35)	0.75	0.77 (–2 to 16)
Marin	0.44	0.63 (12 to >35)	0.57	0.65 (2–23)
Sacramento	0.57	0.66 (4 to >35)	0.78	0.82 (–1 to 16)
San Francisco	0.58	0.72 (10 to >35)	0.73	0.75 (–8 to 18)
San Mateo	0.5	0.63 (4–31)	0.7	0.74 (–4 to 10)
Santa Clara	0.42	0.64 (18 to >35)	0.75	0.77 (–5 to 22)
Santa Cruz	0.39	0.48 (1–32)	0.48	0.57 (1–24)
Sonoma	0.4	0.54 (16 to >35)	0.69	0.74 (–2 to 26)
Yolo	0.4	0.64 (28 to >35)	0.48	0.58 (9–34)

*Overall correlations represent Kendall τ correlation coefficients for the indicated datasets, calculated over the entire study period with no lags. Maximum lag correlations represent the maximum Kendall τ correlation coefficients for the indicated datasets when comparing lagged clinical datasets to wastewater datasets, including lags of –35 to 35 days. The range of maximum lagged τ (days) represents the range of days around a date of the maximum lagged correlation in which the lagged correlation coefficient is within 0.05 of the maximum τ value. The negative direction indicates that wastewater data is leading clinical data, and the positive direction indicates that wastewater data is lagging clinical data. RSV, respiratory syncytial virus.

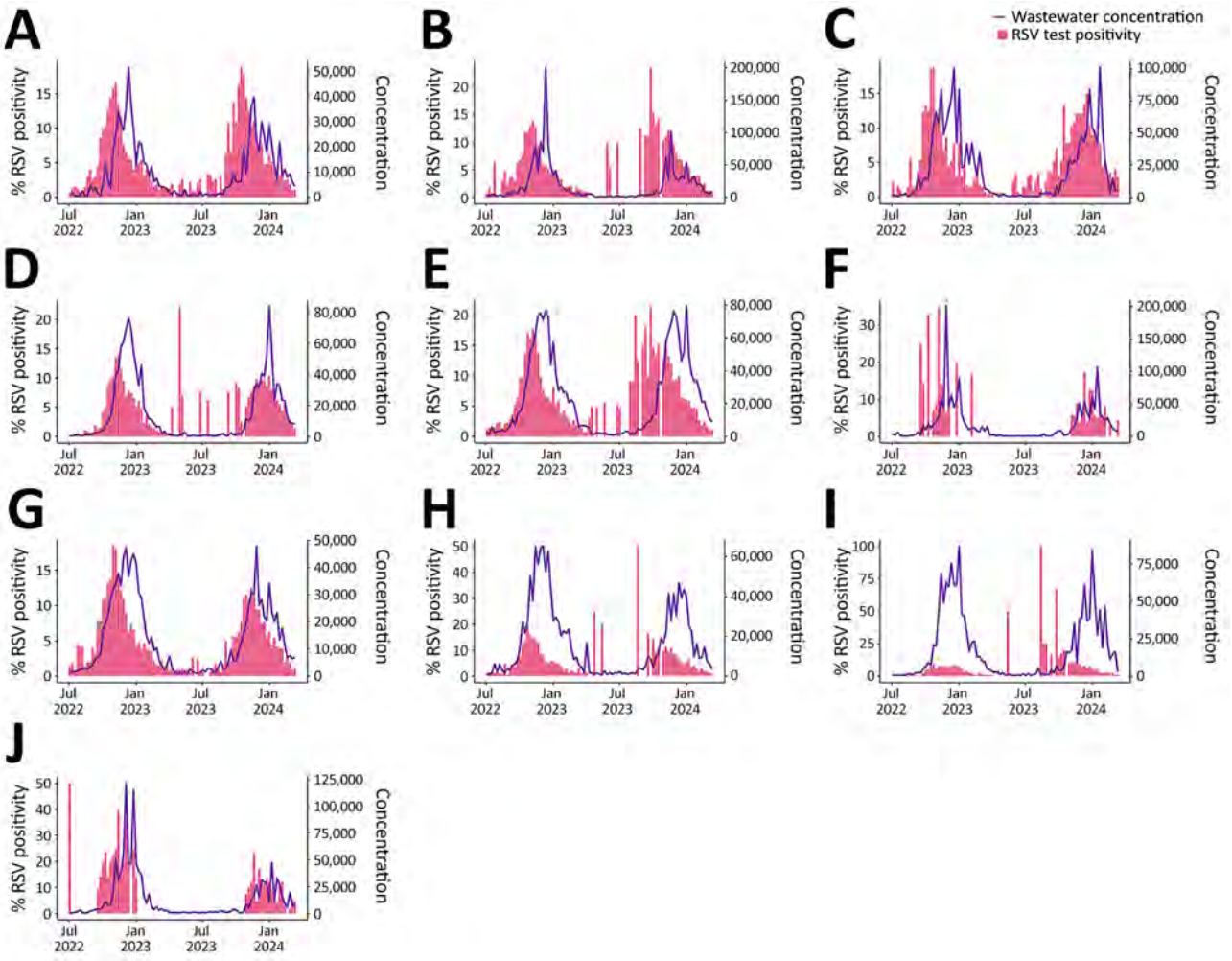


Figure 4. Time series comparison of RSV wastewater concentrations and sentinel percentage test positivity from a study of correlations between wastewater concentrations of influenza A and RSV and clinical laboratory testing and hospitalization data, California, USA. Graphs show weekly county RSV percentage test positivity reported by sentinel laboratories (dark pink bars) and county-aggregated population-weighted averages of nonnormalized RSV wastewater concentrations in gene copies per gram from all sampling sewersheds within each county (purple line) for 10 counties: A) Alameda; B) Contra Costa; C) Marin; D) Sacramento; E) Santa Clara; F) Santa Cruz; G) San Francisco; H) San Mateo; I) Sonoma; J) Yolo. RSV, respiratory syncytial virus.

testing rates. Strategic implementation of WS in locations where clinical laboratory–based surveillance has known gaps, such as rural or medically underserved areas, could serve as a cost-effective way to improve public health surveillance coverage and representation. Of note, small wastewater utilities in rural or underserved areas might not have the resources to easily participate in wastewater monitoring and might require additional financial and logistic support.

This study showed that RSV wastewater data lagged overall clinical laboratory–based data. However, the exploratory age-grouped analyses, although reliant on limited data, suggested that wastewater RSV concentrations were more temporally aligned with RSV-positive specimen counts in age groups ≥18 years (Appendix Table 2, Figure 5). In the Santa Clara age-grouped RSV-positive specimen counts, RSV was detected in children 0–4 years of age before older age groups, a pattern that another study observed in a larger population and across multiple respiratory virus seasons in 2025 (*41*). Although we did not have sufficient age-specific RSV testing data to draw a conclusion from this exploratory age-group analysis, the RSV wastewater lag observed in this study might be partially explained by early RSV infections among young, diapered children that do not contribute to the wastewater signal. In addition, RSV percent test positivity led wastewater by a larger margin than RSV-positive specimen counts, indicating that percent test positivity could be more sensitive to changes in disease dynamics than both wastewater and case

incidence estimates, a dynamic that has also been observed for COVID-19 (*42*).

Although the correlation analyses we used to assess lead–lag patterns did not demonstrate that wastewater provided a leading signal, other wastewater-based data indicators, such as wastewater sample positivity rates, might provide better leading indicators. Another study explored incorporating wastewater sample positivity rates into surge prediction algorithms for COVID-19 (*43*). A similar algorithm could prove useful for providing a leading indicator of the start of influenza and RSV seasons. Also, evaluating correlations and lead–lag patterns during shorter periods within seasons rather than across multiple years might provide more insight into whether wastewater data provides a sensitive or early indicator at timepoints most advantageous to public health, such as the start, peak, and end of each season.

One strength of this study is the inclusion of 20 months of data and multiple clinical laboratory–based surveillance datasets, supporting the robustness of reported results across 2 respiratory virus seasons. This study also implemented a wastewater aggregation method to combine data from multiple sewersheds into county-level wastewater estimates, which enabled more robust comparisons of county wastewater aggregates to county-level clinical surveillance datasets. This analysis focused on data (sewershed and county) at the local level, highlighting that WS can be an effective tool for tracking localized disease transmission.

The first limitation of this study is that we did not normalize raw wastewater data by pepper mild mottle virus (PMMoV), an endogenous human fecal indicator commonly used in WS to normalize viral wastewater concentrations to account for variations in wastewater flow, dilution, and fecal strength (*44*); thus, these study findings might not be directly applicable to surveillance systems that do rely on PMMoV-normalized wastewater concentrations (*44*). A future comparison between raw and PMMoV-normalized data would be valuable, although previous work has shown that PMMoV normalization did not consistently improve correlations between SARS-CoV-2 wastewater concentrations and clinical indicators across all sewersheds evaluated (*44*). Second, the clinical data in this study might have been subject to testing biases, which are challenging to measure and can vary by season and surveillance system. For example, influenza and RSV laboratory testing might have been elevated during the 2022–23 season because of higher demand for testing to identify COVID-19 cases. Thus, correlation estimates reported here might be biased by testing rate variability, potentially reducing the generalizability of these findings to other seasons or geographies. Future analyses should explore how clinical testing rates affect wastewater correlations. In addition, other future analyses could assess correlations and lead–lag patterns when both wastewater concentrations and ELR test results are organized by reporting date, rather than collection date, to clarify wastewater data's utility for real-time public health response. Third, sentinel laboratory surveillance data and hospital admissions potentially were misclassified to the county where a patient received care, rather than the county of residence. Similarly, wastewater concentrations might represent shedding from residents of neighboring counties who routinely cross sewershed and county boundaries because many of the sewersheds included in this study are in areas with high commuting rates. We also relied on county-aggregated wastewater concentrations, even within counties where WS did not cover an entire county. Given those factors, county-level wastewater and clinical datasets compared in this study likely do not represent identical populations, which could affect the strength of correlations observed between datasets and complicate the comparison of correlations between counties. Nonetheless, demonstrating whether those surveillance systems capture similar disease trends, even in somewhat different populations, is still useful. Fourth, this study only included influenza A wastewater concentrations, but hospital admission sums included both influenza A and B admissions, which could skew the observed temporal relationships observed between wastewater concentrations and hospital admissions in the lead–lag analysis. Future work should investigate correlations between influenza B wastewater concentrations and different clinical surveillance systems. Fifth, this study excluded areas with low numbers of positive specimens counts and hospital admissions, which could limit the generalizability of this analysis to communities with small populations, low testing rates, few cases, or a combination thereof. The strong correlations observed in this study provide encouraging evidence that wastewater could serve as a useful proxy for disease activity in populations with lower testing rates (*45*), but more work is needed for confirmation.

In addition to the future analyses already noted, repeating and expanding analyses with wastewater data aggregated to substate regions, states, multistate regions, and national levels could be useful for determining how those aggregates compare to clinical laboratory data combined across broader geographic levels. In addition, cost-benefit analyses could assess the economic effectiveness of WS as a

tool for monitoring influenza and RSV activity and would be useful for jurisdictions considering when and where to implement WS.

In summary, the results from this study support the use of WS for monitoring influenza A virus and RSV activity. WS could bolster existing surveillance and provide improved public health situational awareness of respiratory virus circulation.

Acknowledgements

We thank the participating wastewater treatment plant staff for the indispensable effort and extra work of collecting and shipping samples for this project, including East Bay Municipal Utility District Wastewater Treatment Plant, Central Contra Costa Sanitary District Treatment Plant, West County Wastewater District, Novato Sanitary District Wastewater Treatment Plant, Sacramento Regional Wastewater Treatment Plan, Oceanside Water Pollution Control Plant, Southeast Water Pollution Control Plant, Sewer Authority Mid-Coastside (Half Moon Bay), San Mateo and Estero Municipal Improvement District Water Quality Control Plant, Silicon Valley Clean Water, San Jose Santa Clara Regional Wastewater Facility, City of Sunnyvale Water Pollution Control Plant, Palo Alto Regional Water Quality Control Plant, South County Regional Wastewater Authority, Santa Cruz Wastewater Treatment Facility, City of Petaluma: Ellis Creek Water Recycling Facility, and City of Davis Wastewater Treatment Plant.

The wastewater sampling conducted through WastewaterSCAN was supported by gifts from the CDC Foundation and the Sergey Brin Family Foundation (to A.B.B.). CDPH WS efforts are supported by the Epidemiology and Laboratory Capacity for Infectious Diseases cooperative agreement (grant no. 6NU50CK000539-02) from the Centers for Disease Control and Prevention.

About the Author

Ms. Burnor is an epidemiologist and research scientist currently working in the California Surveillance of Wastewaters Program at the California Department of Public Health. Her research interests include infectious disease surveillance, public health practice, and applied epidemiology.

References

1. Centers for Disease Control and Prevention. Preliminary estimated flu disease burden, 2023–2024 flu season [cited 2024 Nov 7]. https://www.cdc.gov/flu-burden/php/data-vis/2023-2024.html
2. Centers for Disease Control and Prevention. Preliminary estimates of RSV burden [cited 2025 Nov 7]. https://www.cdc.gov/rsv/php/surveillance/burden-estimates.html
3. Centers for Disease Control and Prevention. U.S. influenza surveillance: purpose and methods [cited 2025 Nov 7]. https://www.cdc.gov/fluview/overview/index.html
4. Yang W, Lipsitch M, Shaman J. Inference of seasonal and pandemic influenza transmission dynamics. Proc Natl Acad Sci U S A. 2015;112:2723–8. https://doi.org/10.1073/pnas.1415012112
5. Diamond MB, Keshaviah A, Bento AI, Conroy-Ben O, Driver EM, Ensor KB, et al. Wastewater surveillance of pathogens can inform public health responses. Nat Med. 2022;28:1992–5. https://doi.org/10.1038/s41591-022-01940-x
6. Wolfe MK, Archana A, Catoe D, Coffman MM, Dorevich S, Graham KE, et al. Scaling of SARS-CoV-2 RNA in settled solids from multiple wastewater treatment plants to compare incidence rates of laboratory-confirmed COVID-19 in their sewersheds. Environ Sci Technol Lett. 2021;8:398–404. https://doi.org/10.1021/acs.estlett.1c00184
7. Yu AT, Hughes B, Wolfe MK, Leon T, Duong D, Rabe A, et al. Estimating relative abundance of 2 SARS-CoV-2 variants through wastewater surveillance at 2 large metropolitan sites, United States. Emerg Infect Dis. 2022;28:940–7. https://doi.org/10.3201/eid2805.212488
8. Burnor E, Yu AT, Wolfe MK, Kantor RS, León TM, Ravuri S, et al. Wastewater for public health: timely, sensitive, and reliable SARS-CoV-2 omicron variant monitoring in California. Environ Sci Water Res Technol. 2025;11:876–90. https://doi.org/10.1039/D4EW00845F
9. Duvallet C, Wu F, McElroy KA, Imakaev M, Endo N, Xiao A, et al. Nationwide trends in COVID-19 cases and SARS-CoV-2 RNA wastewater concentrations in the United States. ACS ES T Water. 2022;2:1899–909. https://doi.org/10.1021/acsestwater.1c00434
10. Sharara N, Endo N, Duvallet C, Ghaeli N, Matus M, Heussner J, et al. Wastewater network infrastructure in public health: applications and learnings from the COVID-19 pandemic. PLOS Glob Public Health. 2021;1:e0000061. https://doi.org/10.1371/journal.pgph.0000061
11. Olesen SW, Imakaev M, Duvallet C. Making waves: defining the lead time of wastewater-based epidemiology for COVID-19. Water Res. 2021;202:117433. https://doi.org/10.1016/j.watres.2021.117433
12. Ahmed W, Tscharke B, Bertsch PM, Bibby K, Bivins A, Choi P, et al. SARS-CoV-2 RNA monitoring in wastewater as a potential early warning system for COVID-19 transmission in the community: a temporal case study. Sci Total Environ. 2021;761:144216. https://doi.org/10.1016/j.scitotenv.2020.144216
13. McClary-Gutierrez JS, Mattioli MC, Marcenac P, Silverman AI, Boehm AB, Bibby K, et al. SARS-CoV-2 wastewater surveillance for public health action. Emerg Infect Dis. 2021;27:1–8. https://doi.org/10.3201/eid2709.210753
14. Boehm AB, Hughes B, Duong D, Chan-Herur V, Buchman A, Wolfe MK, et al. Wastewater concentrations of human influenza, metapneumovirus, parainfluenza, respiratory syncytial virus, rhinovirus, and seasonal coronavirus nucleic-acids during the COVID-19 pandemic: a surveillance study. Lancet Microbe. 2023;4:e340–8. https://doi.org/10.1016/S2666-5247(22)00386-X
15. Chassaing M, Walczak C, Sausy A, Le Coroller G, Mossong J, Vergison A, et al. Influenza RNA fluxes monitoring in wastewater as a complementary epidemiological surveillance indicator: a four-year nationwide study in Luxembourg. Sci Total Environ. 2025;982:179621. https://doi.org/10.1016/

j.scitotenv.2025.179621
16. DeJonge PM, Adams C, Pray I, Schussman MK, Fahney RB, Shafer M, et al. Wastewater surveillance data as a complement to emergency department visit data for tracking incidence of influenza A and respiratory syncytial virus—Wisconsin, August 2022–March 2023. MMWR Morb Mortal Wkly Rep. 2023;72:1005–9. https://doi.org/10.15585/mmwr.mm7237a2
17. Hughes B, Duong D, White BJ, Wigginton KR, Chan EMG, Wolfe MK, et al. Respiratory syncytial virus (RSV) RNA in wastewater settled solids reflects RSV clinical positivity rates. Environ Sci Technol Lett. 2022;9:173–8. https://doi.org/10.1021/acs.estlett.1c00963
18. Link-Gelles R, Lutterloh E, Schnabel Ruppert P, Backenson PB, St George K, Rosenberg ES, et al.; 2022 U.S. Poliovirus Response Team. Public health response to a case of paralytic poliomyelitis in an unvaccinated person and detection of poliovirus in wastewater—New York, June–August 2022. MMWR Morb Mortal Wkly Rep. 2022;71:1065–8. https://doi.org/10.15585/mmwr.mm7133e2
19. Markt R, Stillebacher F, Nägele F, Kammerer A, Peer N, Payr M, et al. Expanding the pathogen panel in wastewater epidemiology to influenza and norovirus. Viruses. 2023;15:263. https://doi.org/10.3390/v15020263
20. Mercier E, D'Aoust PM, Thakali O, Hegazy N, Jia JJ, Zhang Z, et al. Municipal and neighbourhood level wastewater surveillance and subtyping of an influenza virus outbreak. Sci Rep. 2022;12:15777. https://doi.org/10.1038/s41598-022-20076-z
21. Schoen ME, Bidwell AL, Wolfe MK, Boehm AB. United States influenza 2022–2023 season characteristics as inferred from wastewater solids, influenza hospitalization, and syndromic data. Environ Sci Technol. 2023;57:20542–50. https://doi.org/10.1021/acs.est.3c07526
22. Wolken M, Sun T, McCall C, Schneider R, Caton K, Hundley C, et al. Wastewater surveillance of SARS-CoV-2 and influenza in preK–12 schools shows school, community, and citywide infections. Water Res. 2023;231:119648. https://doi.org/10.1016/j.watres.2023.119648
23. Ahmed W, Bivins A, Stephens M, Metcalfe S, Smith WJM, Sirikanchana K, et al. Occurrence of multiple respiratory viruses in wastewater in Queensland, Australia: potential for community disease surveillance. Sci Total Environ. 2023;864:161023. https://doi.org/10.1016/j.scitotenv.2022.161023
24. Faherty EAG, Yuce D, Korban C, Bemis K, Kowalski R, Gretsch S, et al. Correlation of wastewater surveillance data with traditional influenza surveillance measures in Cook County, Illinois, October 2022–April 2023. Sci Total Environ. 2024;912:169551. https://doi.org/10.1016/j.scitotenv.2023.169551
25. Koureas M, Mellou K, Vontas A, Kyritsi M, Panagoulias I, Koutsolioutsou A, et al. Wastewater levels of respiratory syncytial virus associated with influenza-like illness rates in children—a case study in Larissa, Greece (October 2022–January 2023). Int J Environ Res Public Health. 2023;20:5219. https://doi.org/10.3390/ijerph20065219
26. Toribio-Avedillo D, Gómez-Gómez C, Sala-Comorera L, Rodríguez-Rubio L, Carcereny A, García-Pedemonte D, et al. Monitoring influenza and respiratory syncytial virus in wastewater. Beyond COVID-19. Sci Total Environ. 2023;892:164495. https://doi.org/10.1016/j.scitotenv. 2023.164495
27. Zulli A, Varkila MRJ, Parsonnet J, Wolfe MK, Boehm AB. Observations of respiratory syncytial virus (RSV) nucleic acids in wastewater solids across the United States in the 2022–2023 season: relationships with RSV infection positivity and hospitalization rates. ACS ES T Water. 2024;4:1657–67. https://doi.org/10.1021/acsestwater.3c00725
28. Boehm AB, Wolfe MK, White BJ, Hughes B, Duong D, Bidwell A. More than a tripledemic: influenza A virus, respiratory syncytial virus, SARS-CoV-2, and human metapneumovirus in wastewater during winter 2022–2023. Environ Sci Technol Lett. 2023;10:622–7. https://doi.org/10.1021/acs.estlett.3c00385
29. Wolfe MK, Duong D, Shelden B, Chan EMG, Chan-Herur V, Hilton S, et al. Detection of hemagglutinin H5 influenza A virus sequence in municipal wastewater solids at wastewater treatment plants with increases in influenza A in spring, 2024. Environ Sci Technol Lett. 2024;11:526–32. https://doi.org/10.1021/acs.estlett.4c00331
30. California Department of Public Health. Influenza surveillance report 2019–2020 season [cited 2025 Jul 11]. https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/Immunization/Annual2019-20_FluReport.pdf
31. Centers for Disease Control and Prevention. National Notifiable Diseases Surveillance System: MMWR weeks definition [cited 2025 Jul 11]. https://ndc.services.cdc.gov/wp-content/uploads/MMWR_week_overview.pdf
32. HealthData.gov. COVID-19 reported patient impact and hospital capacity by facility [cited 2023 Jan 1]. https://healthdata.gov/Hospital/COVID-19-Reported-Patient-Impact-and-Hospital-Capa/anag-cw7u/about_data
33. Boehm AB, Wolfe MK, Wigginton KR, Bidwell A, White BJ, Hughes B, et al. Human viral nucleic acids concentrations in wastewater solids from Central and Coastal California USA. Sci Data. 2023;10:396. https://doi.org/10.1038/s41597-023-02297-7
34. Kadonsky KF, Naughton CC, Susa M, Olson R, Singh GL, Daza-Torres ML, et al. Expansion of wastewater-based disease surveillance to improve health equity in California's Central Valley: sequential shifts in case-to-wastewater and hospitalization-to-wastewater ratios. Front Public Health. 2023;11:1141097. https://doi.org/10.3389/fpubh.2023.1141097
35. Kim S, Kennedy LC, Wolfe MK, Criddle CS, Duong DH, Topol A, et al. SARS-CoV-2 RNA is enriched by orders of magnitude in primary settled solids relative to liquid wastewater at publicly owned treatment works. Environ Sci Water Res Technol. 2022;8:757–70. https://doi.org/10.1039/D1EW00826A
36. Arndt S, Turvey C, Andreasen NC. Correlating and predicting psychiatric symptom ratings: Spearman's r versus Kendall's tau correlation. J Psychiatr Res. 1999;33:97–104. https://doi.org/10.1016/S0022-3956(98)90046-2
37. Field AP. Kendall's coefficient of concordance. In: Encyclopedia of statistics in behavioral science 2005 Oct 15 [cited 2025 Jul 11]. https://onlinelibrary.wiley.com/doi/10.1002/0470013192.bsa327
38. Kendall MG. A new measure of rank correlation. Biometrika. 1938;30:81–93. https://doi.org/10.1093/biomet/30.1-2.81
39. Wicklin R. SAS Blogs, The DO Loop. Weak or strong? How to interpret a Spearman or Kendall correlation. 2023 Apr 5 [cited 2025 Jul 11]. https://blogs.sas.com/content/iml/2023/04/05/interpret-spearman-kendall-corr.html
40. D'Ambrosio F, Lomazzi M, Moore M, Maida A, Ricciardi R, Munno L, et al. Addressing the underestimated burden of RSV in older adults in Europe: epidemiology, surveillance gaps, and public health implications. Vaccines (Basel). 2025;13:510. https://doi.org/10.3390/vaccines13050510

41. Arndt S, Turvey C, Andreasen NC. Exploring RSV transmission patterns in different age groups in the United States. J Infect Dis. 2025;232:700–8. https://doi.org/10.1093/infdis/jiaf230
42. Scobie HM, Panaggio M, Binder AM, Gallagher ME, Duck WM, Graff P, et al. Correlations and timeliness of COVID-19 surveillance data Sources and indicators—United States, October 1, 2020–March 22, 2023. 2023;72:529–35. https://doi.org/10.15585/mmwr.mm7219e2
43. Keshaviah A, Huff I, Hu XC, Guidry V, Christensen A, Berkowitz S, et al. Separating signal from noise in wastewater data: an algorithm to identify community-level COVID-19 surges in real time. Proc Natl Acad Sci U S A. 2023;120:e2216021120. https://doi.org/10.1073/pnas.2216021120
44. Maal-Bared R, Qiu Y, Li Q, Gao T, Hrudey SE, Bhavanam S, et al. Does normalization of SARS-CoV-2 concentrations by pepper mild mottle virus improve correlations and lead time between wastewater surveillance and clinical data in Alberta (Canada): comparing twelve SARS-CoV-2 normalization approaches. Sci Total Environ. 2023; 856:158964. https://doi.org/10.1016/j.scitotenv.2022.158964
45. Hegazy N, Peng KK, D'Aoust PM, Pisharody L, Mercier E, Ramsay NT, et al.; WSI Consortium. Variability of clinical metrics in small population communities drive perceived wastewater and environmental surveillance data quality: Ontario, Canada-wide study. ACS ES T Water. 2025;5:1605–19. https://doi.org/10.1021/acsestwater.4c00958

Address for correspondence: Elisabeth Burnor, California Department of Public Health, 850 Marina Bay Pkwy, Richmond, CA 94804, USA; email: elisabeth.burnor@cpdh.ca.gov

Early-Season Detection of Influenza A(H3N2) Subclade K in Wastewater, Colorado, USA, 2025–2026

Molly C. Hetherington-Rauth, Van Nguyen, Emily Washeleski, Kevin Castro Vital, Gabrielle Lequia, Deborah Aragon, Nisha B. Alden, Kirsten Weisbeck, Laura Bankers, Alexandria Rossheim, Allison Wheeler, Shannon R. Matzinger

We assessed the value of wastewater sequencing for monitoring influenza circulation in Colorado, USA, focusing on the emergence of influenza A(H3N2) subclade K. We detected subclade K in wastewater 31 days before clinical detection. Our results demonstrated that wastewater sequencing can provide real-time situational awareness for public health officials.

Seasonal influenza A and B viruses cause significant illness and death annually (*1*,*2*). During the early 2025–26 influenza season, an antigenetically drifted influenza A(H3N2) strain, known as subclade K, emerged with reduced antibody binding to contemporaneous vaccine strains (*3*,*4*). The emergence of subclade K was correlated with early seasonal influenza activity observed in several countries (*5*). Colorado was among 3 states in the United States to observe early influenza activity based on outpatient respiratory illness reported to ILINet (*6*). By the week ending November 22, 2025, Colorado reported high activity.

The Colorado Department of Public Health and Environment supplemented conventional surveillance, including hospitalization rates, clinical specimen sequencing, and wastewater detection using digital PCR (dPCR), with wastewater sequencing for genetic surveillance. We sequenced the influenza A virus hemagglutinin (HA) gene from wastewater and assessed its added value for monitoring influenza trends during the respiratory illness season.

Author affiliation: Colorado Department of Public Health and Environment, Denver, Colorado, USA

DOI: https://doi.org/10.3201/eid3213.260431

The Study

Hospitalization Cases

Colorado requires the reporting of influenza-associated hospitalizations. We retrieved data for influenza-associated hospitalizations with admission dates of September 1, 2025–February 14, 2026, from the statewide surveillance system. During that period, Colorado recorded 4,426 influenza A–associated hospitalizations and 110 influenza B–associated hospitalizations. Cases began increasing the week ending November 25, 2025, and peaked the week ending December 27, 2025 (Figure 1, panel A).

Clinical Sequencing

The state laboratory received up to 5 influenza A–positive clinical specimens weekly from hospitals of both inpatient and outpatient cases participating in the statewide influenza sentinel surveillance system. We subtyped specimens and sequenced those meeting sequencing criteria (Appendix, https://wwwnc.cdc.gov/EID/article/32/13/26-0431-App1.pdf); we sequenced 812 specimens during September 1, 2025–February 14, 2026. Of those specimens, 720 (89%) were influenza A(H3N2). Of those samples, we characterized 704 (98%) as influenza A(H3N2) subclade K (hereafter, subclade K) (Figure 1, panel B). The first sample characterized as subclade K was collected on October 12, 2025. Colorado's right-size target is 50 samples per week to confidently provide situational awareness; we reached that threshold the week ending December 6, 2025.

Wastewater Detection

Established in 2020, the Colorado Wastewater Surveillance Program monitors wastewater across the

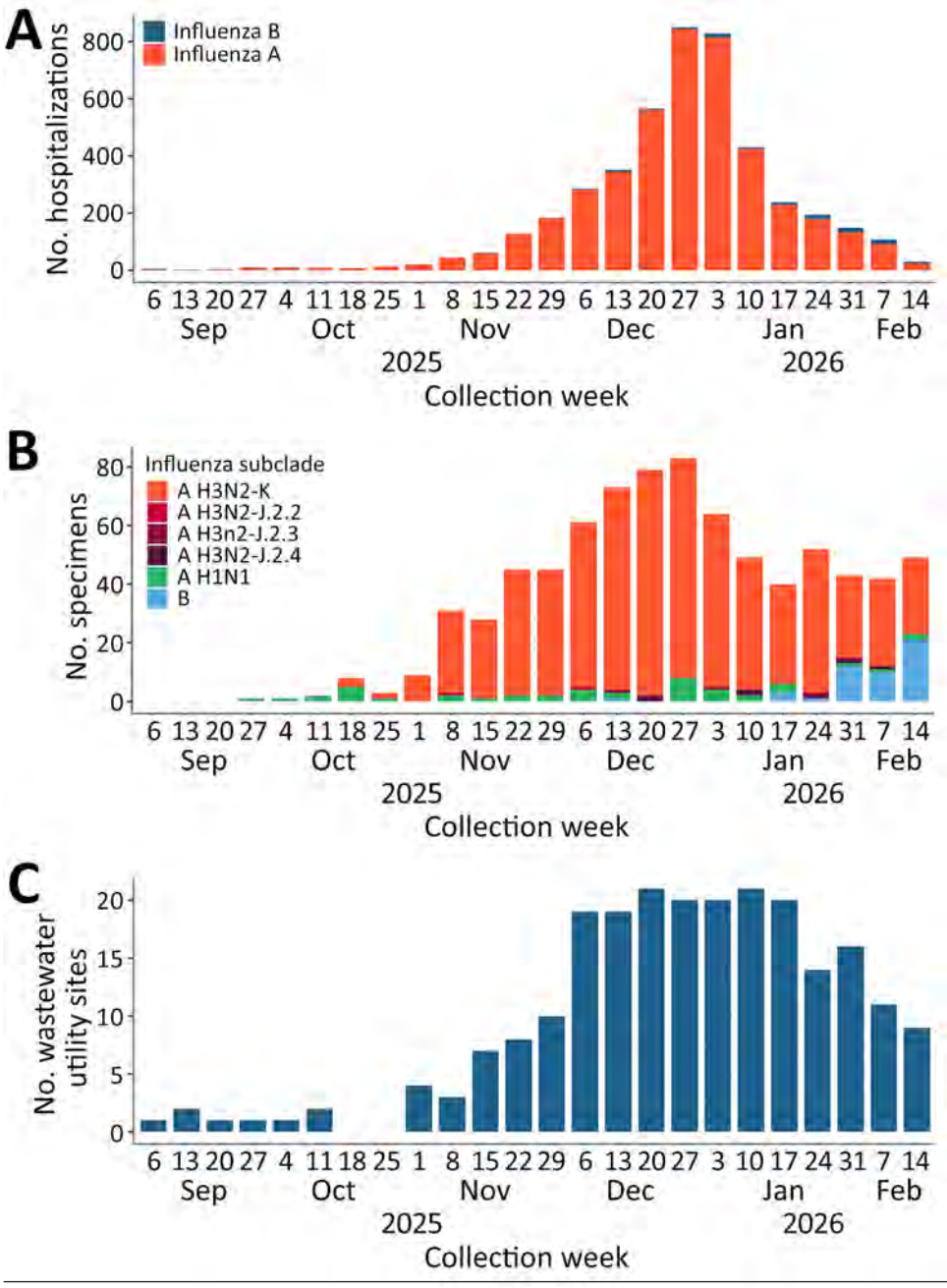


Figure 1. Comparison of hospitalizations, clinical specimens sequenced, and wastewater detection in study of early-season detection of influenza A(H3N2) subclade K in wastewater, Colorado, USA, 2025–2026. A) Influenza hospitalizations of influenza A and influenza B case-patients, by collection week. B) Clinical specimens sequenced and grouped by subclade characterization, by collection week. C) Unique wastewater utility sites at which concentrations of influenza A were above the limit of quantification, by collection week. No sites had concentrations above the limit of quantification for the weeks ending October 18 and 25, 2025. Collection week is shown by the week ending date.

state for respiratory viruses and emerging pathogens using dPCR (*7*). The state laboratory received samples 2×/week from 21 sentinel wastewater treatment facilities. We retrieved wastewater detection data for September 1, 2025–February 14, 2026. We detected and quantified influenza A, influenza A H1, and influenza A H3 by using previously described methods (M.C. Hetherington-Rauth et al., unpub. data, https://doi.org/10.1101/2025.10.15.25338105) (Appendix).

We defined the limit of detection by dPCR as the presence of 1 positive partition. We refer to the limit of quantification by dPCR as 1,200 gene copies (gc)/L. Concentration values calculated that fall below the limit of quantification can be interpreted to mean that target genetic material was present but cannot be accurately quantifiable. Concentration values recorded as 0 /L indicate no positive partitions were observed. The number of sewersheds with influenza A viral concentration above our limit of quantification began increasing the week ending November 8, 2025, and peaked the week ending December 20, 2025, mirroring influenza hospitalization trends (Figure 1, panel C).

The average viral concentration of influenza A in wastewater peaked the week ending December

27, 2025 (Figure 2). Influenza A H3 average viral concentrations mirrored the average influenza A viral concentrations. Influenza A H1 average viral concentrations remained low at or below the level of quantification.

Wastewater Sequencing

The state laboratory sequenced the HA gene from wastewater samples using previously described methods (M.C. Hetherington-Rauth et al., unpub. data, https://doi.org/10.1101/2025.10.15.25338105) (Appendix). We sequenced 649 samples collected during September 1, 2025–February 14, 2026. Of the samples sequenced, 112 (17%) met the 60% coverage threshold of the HA gene required for deconvolution (subclade characterization and abundance estimation) (Appendix). We observed that influenza A viral concentration in wastewater was moderately correlated with percent coverage of the HA gene (0.66 Spearman correlation coefficient) (Figure 3, panel A).

We first identified subclade K in wastewater from a sample collected on September 11, 2025; we detected subclade K by clinical sequencing 31 days later (Figure 3, panel B; Appendix Figure). The sample had 74.4% coverage of the HA gene; subclade K was detected at 100% relative abundance. (Coverage is the percentage of the reference gene sequenced at 10× depth; abundance is the estimated relative frequency of the subclade in the sample.) Viral concentration of influenza A in the sample was 4,300 gc/L. After the initial detection, we observed subclade K at near 100% abundance in nearly all wastewater samples meeting the 60% coverage threshold. We detected subclade J.2.4 in 2 samples at 11% and 10% abundance and subclade J.2.3 in 3 samples at 83%, 64%, and 12% abundance. We observed subclade J.2 and J.2.1 at <1% abundance on average. We detected subclade K in 20 of 21 sentinel sites. We detected subclade K in 13 wastewater samples with influenza A viral concentrations below the limit of quantification.

Conclusions

We assessed the value of wastewater sequencing alongside hospitalization data, clinical sequencing,

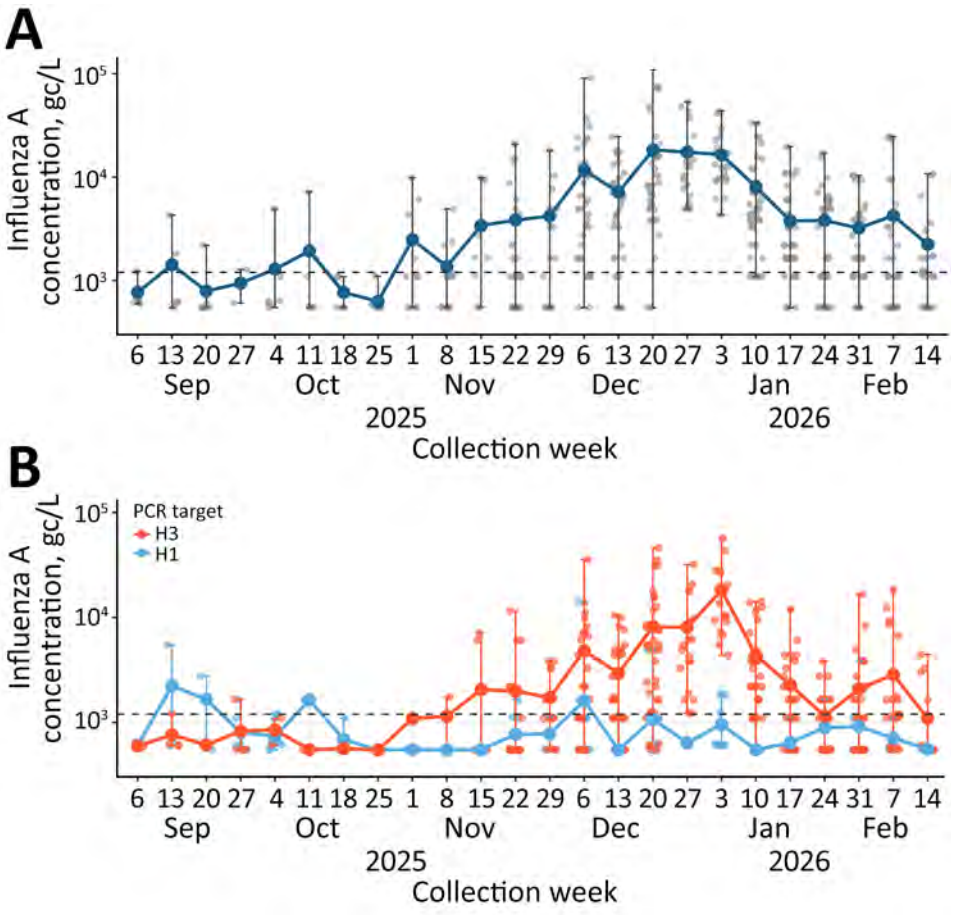


Figure 2. Influenza A viral concentration in wastewater in study of early-season detection of influenza A(H3N2) subclade K, Colorado, USA, 2025–2026. A) Influenza A target concentration. Blue points and connecting lines show the average concentration and trend by week. Gray points show concentrations for each sample collected by week. Error bars show the range of concentration by week. Dashed horizontal line indicates the defined limit of quantification, 1,200 gc/L. No sites had influenza A detection for the weeks ending October 18 and 25, 2025. B) Influenza H1 and H3 concentration in wastewater. Points and connecting lines show the average concentration and trend by week. Error bars show the range of concentration by week. Dashed horizontal line indicates the defined limit of quantification, 1,200 gc/L. No sites had H3 detections for the weeks ending October 4, 11, 18, and 25 and November 1, 2025. There were no H1 detections for the weeks ending October 18 and 25 and November 1, 8, and 15, 2025, or for January 10 and 17 and February 7 and 14, 2026. Concentration is shown in $\log_{10}$ scale. Collection week is shown by the week ending date. gc, gene copies.

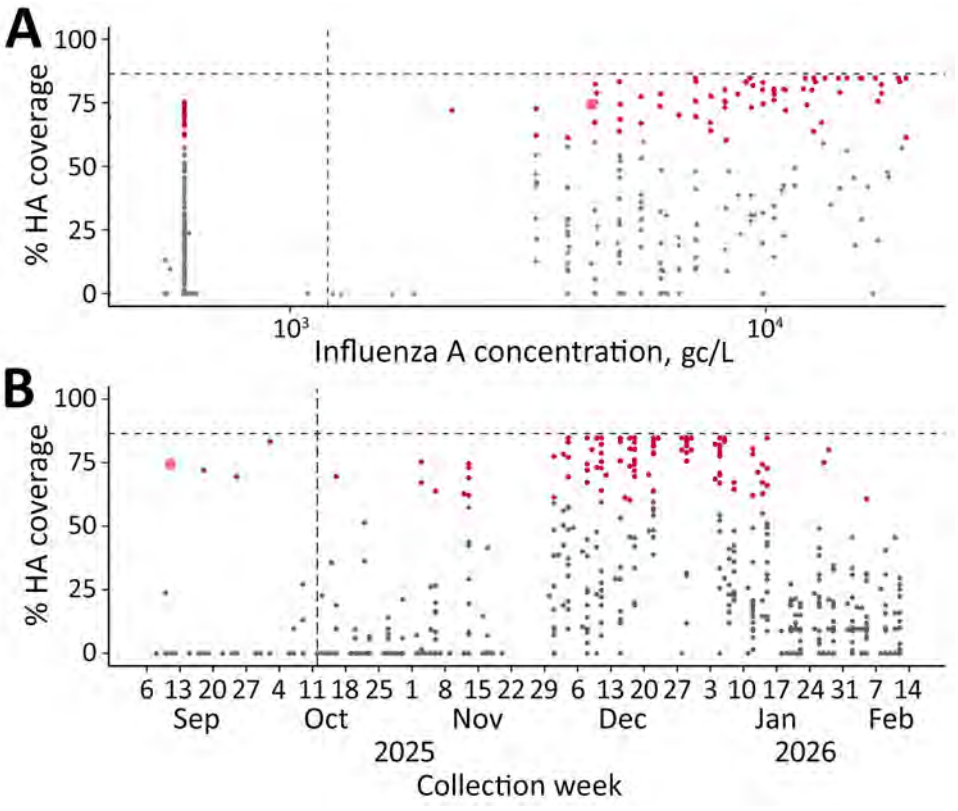


Figure 3. Wastewater sequencing metrics in study of early-season detection of influenza A(H3N2) subclade K in wastewater, Colorado, USA, 2025–2026. A) Percentage coverage of HA gene plotted against influenza A viral concentration. Orange represents sample with the first subclade K detection, collected October 16, 2025. Red represents samples with ≥60% coverage required for performing deconvolution. Gray represents samples with <60% coverage. Horizontal dashed line indicates the tiled amplicon scheme, which covers 86.7% of the HA gene reference sequence. Vertical dashed line indicates the defined limit of quantification, 1,200 gc/L. B) Percentage coverage of HA gene plotted against sample collection date. Horizontal dashed line indicates the tiled amplicon scheme, which covers 86.7% of the HA gene reference sequence. Vertical dashed line indicates the collection date of the first clinical sample characterized as subclade K, October 23, 2025. gc, gene copies; HA, hemagglutinin.

and wastewater viral quantification to track influenza trends during the respiratory illness season. During the early 2025–26 influenza season, wastewater viral concentration trends mirrored hospitalization trends. The characterization of wastewater and clinical samples revealed that the rise in cases correlated with the emergence of subclade K. Using wastewater sequencing, we detected subclade K 31 days earlier than in clinical samples. Furthermore, the detection of subclade K in wastewater samples with influenza concentrations below the limit of quantification by dPCR demonstrates the high sensitivity of wastewater sequencing.

In addition to earlier detection, wastewater sequencing captures asymptomatic and less severe cases, as well as cases from underserved communities and persons who do not seek medical care, compared with conventional genetic surveillance systems that rely on specimens from hospital case-patients (*8*,*9*). Although wastewater samples inherently provide aggregate population-level data, they are subject to factors including the number of infected persons and population mobility, such as tourism and travel between work, home, and school. To clarify how those factors could affect the interpretation of wastewater data, especially for local public health response, future studies could compare clinical and wastewater data at finer geographic scales.

Integrating wastewater sequencing into existing surveillance systems provides an innovative approach to monitor genetic variation, especially early in an influenza season when clinical specimens are typically scarce. Genetic characterization of the virus is integral to monitoring antigenic drift, antiviral resistance markers, vaccine drift, and mutations in primer/probe binding regions of diagnostic assays at national scale. Early characterization of genetic changes of influenza virus through wastewater sequencing can strengthen public health action and reduce response time.

All sequence reads were deposited in the National Center for Biotechnology Information Sequence Read Archive (BioProject PRJNA1458849, accession nos. SRR38394854–5502). All human sequences have been removed from the sequencing reads.

This project was made possible through funding provided under the Epidemiology and Laboratory Capacity for Prevention and Control of Emerging Infectious Diseases (ELC) Cooperative Agreement (no. CK24-0002), Project D:

Advanced Molecular Detection, Project F: National Wastewater Surveillance System to the Colorado Department of Public Health and Environment, and Project J: Viral Respiratory Disease Surveillance.

About the Author

Ms. Hetherington-Rauth is the lead bioinformatics scientist in the genomic surveillance program at the Colorado State Public Health Laboratory. Her primary research interest is the integration of genomic data from clinical and wastewater samples to better understand the evolution and genomic epidemiology of respiratory viruses.

References

1. Centers for Disease Control and Prevention. Influenza. [cited 2026 Apr 30]. https://www.cdc.gov/nchs/fastats/flu.htm
2. World Health Organization. Influenza (seasonal). [cited 2026 Apr 30]. https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal)
3. Centers for Disease Control and Prevention. Weekly US influenza surveillance report: key updates for week 48, ending November 29, 2025. 2025 [cited 2026 Apr 30]. https://www.cdc.gov/fluview/surveillance/2025-week-48.html
4. Scarvaglieri I, De Francesco MA, Alberti M, Cesanelli F, Salvi M, Tiecco G, et al. Influenza A(H3N2) subclade K: epidemiology, molecular evolution and vaccine effectiveness in Europe. Pathogens. 2026;15:474. https://doi.org/10.3390/pathogens15050474
5. World Health Organization. Seasonal influenza global situation. 2025 [cited 2026 Feb 23]. https://www.who.int/emergencies/disease-outbreak-news/item/2025-DON586
6. Centers for Disease Control and Prevention. FluView Interactive. 2025 [cited 2026 Feb 23]. https://www.cdc.gov/fluview/overview/fluview-interactive.html
7. Colorado Department of Public Health. About the wastewater program. 2026 [cited 2026 Feb 23]. https://cdphe.colorado.gov/dcphr/wastewater/about-program
8. Kilaru P, Hill D, Anderson K, Collins MB, Green H, Kmush BL, et al. Wastewater surveillance for infectious disease: a systematic review. Am J Epidemiol. 2023;192:305–22. https://doi.org/10.1093/aje/kwac175
9. Zipfel CM, Colizza V, Bansal S. Health inequities in influenza transmission and surveillance. PLOS Comput Biol. 2021;17:e1008642. https://doi.org/10.1371/journal.pcbi.1008642

Address for correspondence: Shannon Matzinger, Colorado Department of Public Health and Environment, 8100 E Lowry Blvd, Denver CO 80230, USA; email: shannon.matzinger@state.co.us

etymologia revisited

Petri Dish
[pe'tre 'dish]

The Petri dish is named after the German inventor and bacteriologist Julius Richard Petri (1852–1921). In 1887, as an assistant to fellow German physician and pioneering microbiologist Robert Koch (1843–1910), Petri published a paper titled "A minor modification of the plating technique of Koch." This seemingly modest improvement (a slightly larger glass lid), Petri explained, reduced contamination from airborne germs in comparison with Koch's bell jar.

References

1 Central Sheet for Bacteriology and Parasite Science [in German]. Biodiversity Heritage Library. Volume 1, 1887 [cited 2020 Aug 25]. https://www.biodiversitylibrary.org/item/210666#page/313/mode/1up
2. Petri JR. A minor modification of the plating technique of Koch [in German]. Cent für Bacteriol und Parasitenkd. 1887;1:279–80.
3. Shama G. The "Petri" dish: a case of simultaneous invention in bacteriology. Endeavour. 2019;43:11–6. DOIExternal
4. The big story: the Petri dish. The Biomedical Scientist. Institute of Biomedical Science [cited 2020 Aug 25]. https://thebiomedicalscientist.net/science/big-story-petri-dish

Originally published in January 2021

https://wwwnc.cdc.gov/eid/article/27/1/et-2701_article

Wastewater Respiratory Virus Surveillance in Remote Community, Alaska, USA, 2022–2024

Brian Lefferts, Alyssa Leary, Dana Bruden, Ian Blake, Christine Richman, Sarah Sixberry, Michael Vicente, Jennifer Pak, Ellen Hodges, James W. Keck

Wastewater surveillance (WS) is underused in rural communities. We evaluated WS performance in the remote, subarctic, mostly Indigenous, community of Bethel, Alaska, USA, during October 2022–May 2024. Wastewater was collected ≥3 times weekly and underwent on-site PCR testing for SARS-CoV-2, respiratory syncytial virus (RSV), and influenza A and B viruses. We compared WS virus detection with local clinical data by using results from 318 wastewater samples and 7,392 clinical tests. We detected SARS-CoV-2 in 265 (83.3%) wastewater samples, influenza A virus in 128 (40.3%), RSV in 78 (24.5%), and influenza B virus in 29 (9.1%). Wastewater signals correlated with clinical results for influenza B virus (Spearman ρ = 0.85), influenza A virus (ρ = 0.62), RSV (ρ = 0.60), and SARS-CoV-2 (ρ = 0.59), providing corroborating data that informed the timing of seasonal respiratory virus immunization campaigns. Our findings show that WS is feasible and useful for disease surveillance in rural Alaska.

Wastewater surveillance (WS) expanded substantially during the COVID-19 pandemic (*1*). Testing wastewater for the SARS-CoV-2 virus has several advantages over traditional case-based disease surveillance, including its efficiency in monitoring community disease trends, independence from healthcare-seeking behaviors that inform traditional surveillance approaches, and scalability from building-level monitoring to sewersheds serving millions of persons (*2*). WS has yielded actionable COVID-19 public health information across a variety of settings (*3*). Subsequently, WS platforms have expanded to include additional respiratory viruses, such as respiratory syncytial virus (RSV) and influenza virus (*4*), and have supported public health responses during respiratory virus seasons (*5*).

Smaller and remote communities have had lower uptake of WS (*6*). Barriers to adoption in rural and remote areas include limited access to wastewater testing laboratories, costs, insufficient human resources, lack of centralized wastewater collection and treatment systems, and competing priorities (*7*). Local implementation of WS could overcome the high cost and logistical challenges of shipping samples to third-party laboratories, thus improving the timeliness of wastewater data (*8*). Early in the COVID-19 pandemic, a team from Canada demonstrated the feasibility and accuracy of using a rapid and user-friendly clinical PCR platform to measure SARS-CoV-2 virus levels on site in a remote community (*9*). Their WS pilot uncovered previously unknown COVID-19 infections in the community and initiated a public health response. The reported effectiveness and relative simplicity of the WS approach used by Canada team informed the implementation of WS in a remote community in Alaska, USA.

Rural Alaska is home to hundreds of mostly Indigenous communities located off the road system, relying on water and air transportation for travel. Historic inequities and socio-environmental conditions, such as lack of in-home piped water and household crowding, have contributed to high rates of respiratory virus infections, particularly in rural southwest Alaska (*10*). The Yukon-Kuskokwim Health Corporation (YKHC) is a comprehensive regional Tribal Health Organization that provides most of the healthcare in the region. YKHC responded proactively to the COVID-19 pandemic with timely vaccine campaigns, physical distancing measures, and an expansive clinical testing and contact tracing program (*11*,*12*). As pandemic response measures transitioned to routine public health activities, YKHC invested in WS to monitor respiratory virus

Author affiliations: Yukon-Kuskokwim Health Corporation, Bethel, Alaska, USA (B. Lefferts, A. Leary, C. Richman, S. Sixberry, M. Vicente, J. Pak, E. Hodges); Centers for Disease Control and Prevention, Anchorage, Alaska, USA (D. Bruden, I. Blake, J.W. Keck); Alaska Native Tribal Health Consortium, Anchorage (J.W. Keck)

DOI: https://doi.org/10.3201/eid3213.260709

trends in the community. We analyzed 2 years of WS data for SARS-CoV-2, RSV, and influenza A and B viruses and compared wastewater viral signals with clinical testing data from the YKHC-operated YK Delta Regional Hospital.

Methods

Study Setting

YKHC initiated WS on October 19, 2022, in the regional hub community of Bethel, Alaska. Bethel is located 400 miles west of Anchorage in southwestern Alaska and is home to ≈6,300 residents living in 2,450 households; 74% of residents identify as Alaska Native. Many of the additional 22,000 residents in the region regularly pass through Bethel because it serves as the transportation hub and has the only hospital in the region.

Wastewater management in Bethel is complex and is served by 2 different systems: a piped sewer system in the central part of town and a truck-hauled system for households and businesses outside that area. The piped system accounts for 25% of residential and commercial connections and serves 479 customers, including the YK Delta Regional Hospital. Piped services account for ≈65% of the wastewater produced by volume in the community. In contrast, the 1,373 residential and commercial hauled customers account for 75% of connections but produce only 35% of Bethel's wastewater. Hauled customers rely on 3,000-gallon trucks to deliver water to onsite storage tanks; wastewater trucks haul away wastewater to dump into the centralized sewer system or the sewage lagoon. Hauled services are expensive to operate, resulting in higher utility costs, which causes hauled customers to minimize water use.

We obtained wastewater samples from Bethel's last lift station, which pumps raw sewage to a higher elevation so it can flow via gravity into the Bethel sewage lagoon. Previous research identified stronger viral signals from lift station samples than lagoon samples (*13*). Approximately 90% of piped wastewater in Bethel passes through that lift station, including sewage from the regional hospital and long-term care facilities. The only piped wastewater that does not pass through that site is from the Lower Kuskokwim School District campus, which discharges its sewage into the lagoon. Approximately 50% of Bethel's hauled sewage also passes through that lift station.

Wastewater Sampling

During October 2022–May 2024, we passively sampled wastewater by using the Moore swab method (*14*) (Appendix, https://wwwnc.cdc.gov/EID/article/32/13/26-0709-App1.pdf). During October 19, 2022–January 24, 2023, and June 28–September 19, 2023, sampling frequency ranged from 1 to 3 (mean 1.7) times/week. During January 25–June 27, 2023, and September 20, 2023–May 31, 2024, sampling frequency ranged from 3 to 5 (mean 4.7) times/week. Initially, we conducted sampling once per week while we developed collection and processing methods; we also reduced sampling to 1 time/week during periods of low respiratory virus activity. Sampling frequency increased with virus activity but varied depending on staffing and supply constraints. We collected Moore swabs 24 hours after placement except for swabs placed on Fridays, which the team collected the following Monday, resulting in 72-hour samples.

Wastewater Analysis

We analyzed wastewater samples via real-time PCR on a GeneXpert Xpress with Xpert Xpress CoV-2/Flu/RSV plus viral respiratory cartridges (both Cepheid, https://www.cepheid.com), which test for SARS-CoV-2, influenza A and B viruses, and RSV. That system includes sample processing and probe check controls and reports results as cycle threshold (Ct) values. Scientists with the Public Health Agency of Canada validated the use of a clinical testing platform for wastewater analysis (*9*), but YKHC did not validate this laboratory method.

We evaluated a concentration step before PCR analysis in samples collected during October 2022–May 31, 2023 (Appendix). However, we elected to use unconcentrated samples for surveillance because the concentration step required additional resources and time without notable improvement in virus detection (Appendix Tables 1, 2). To analyze the unconcentrated wastewater samples, we added 300 μL of wastewater to a Cepheid GeneXpert cartridge and followed the manufacturer's protocol for testing clinical specimens.

Clinical Testing

Clinical respiratory virus testing followed YKHC standards of care. Patients of all ages with respiratory symptoms in the ambulatory, emergency, or inpatient settings at the YK Delta Regional Hospital (the only hospital in the region) were tested via collection of midturbinate nasal swab sample as part of routine clinical care. Swab samples were analyzed in the hospital's medical laboratory by using the Cepheid GeneXpert respiratory panel kit to detect SARS-CoV-2, influenza A and B viruses, and RSV. We extracted deidentified clinical testing data from YKHC's electronic

health record system. During the study, some community members reported results from SARS-CoV-2 tests to the YKHC Department of Public Health. We conducted a separate correlational analysis by using self-reported SARS-CoV-2 test results. We considered positive virus testing results or self-reported results occurring within 30 days of a prior confirmed positive result for the same virus part of the same illness and excluded those results from the analysis.

Statistical Analyses

We performed analyses for the entire evaluation period, partitioning our data into 2 respiratory virus seasons because of year-to-year variability in respiratory virus circulation. We followed respiratory virus activity levels from the Centers for Disease Control and Prevention to define respiratory virus season as week 27 to week 26 in the following year (*15*) and adjusted for seasonal onset and offset of WS at our site. Hence, the 2022–23 season included wastewater samples collected during October 19, 2022–June 30, 2023, and the 2023–24 season included samples collected during July 1, 2023–May 31, 2024. We calculated 7-day moving averages for virus Ct values from wastewater samples and for clinical test results. For wastewater samples spanning multiple days, we averaged Ct values for each day sampled. Spearman rank-order correlation (ρ) assessed the association between positive clinical test counts and wastewater Ct values. We conducted time-lag correlation analyses for each respiratory virus overall and by season using 7-day moving averages from wastewater and clinical data. We shifted wastewater Ct values from 10 days before (i.e., preceding) to 4 days after (i.e., lagging) the clinical testing data series. Of note, a lower Ct value indicates a higher relative viral concentration in the wastewater sample, which when correlated with the frequency of positive clinical tests yields negative correlation coefficients. We report absolute values of those correlation coefficients in the results for ease of interpretation; a larger coefficient represents a stronger correlation.

We evaluated the sensitivity, specificity, and concordance of the detection of each virus in wastewater with reference to its detection in clinical testing data. We separately aggregated wastewater and clinical data by surveillance week and virus, comparing presence (positive test) and absence (no positive test). We used contingency tables to organize the data with clinical testing serving as the reference standard. Because the GeneXpert platform runs 45 cycles, we defined a Ct value <45 as a positive wastewater test and used unlagged data. Influenza B analysis was limited to the 2023–24 season because influenza B was not detected in clinical or wastewater samples during the 2022–23 surveillance season.

This evaluation was deemed research not involving human subjects (45 CFR 46.102(e)) by the Centers for Disease Control and Prevention. YKHC and the Alaska Native Tribal Health Consortium approved this project.

Results

Wastewater and Clinical Virus Tests

YKHC initiated wastewater sampling 321 times during October 19, 2022–May 31, 2024, and PCR viral test results from 318 samples (99.1%) were available; in 3 instances, the Moore swab was lost in the wastewater stream during the passive sampling process. During the same timeframe, a total of 7,392 clinical respiratory tests detected influenza A virus 543 times (7.3% positivity), SARS-CoV-2 372 times (5.0% positivity), RSV 364 times (4.9% positivity), and influenza B 107 times (1.4% positivity) (Table). YKHC received 779 self-reports of positive SARS-CoV-2 tests.

SARS-CoV-2 Surveillance

In wastewater, SARS-CoV-2 was detected on 91.4% (192/210) of sample days in the 2022–23 season and 73.0% (214/293) of sample days in the 2023–24 season (Table). For clinical samples, SARS-CoV-2 was detected 49.4% (126/255) of days in the 2022–23 season and 32.5% (106/326) of days in the 2023–24 season. In the 2022–23 season, clinical cases were detected throughout the year, and SARS-CoV-2 was detected year-round in wastewater. A notable peak in clinical case detections occurred in late January 2024, and a wastewater concentration peak occurred 8 days later. Correlation of SARS-CoV-2 wastewater and clinical test data was strongest with limited time shift ($\rho = 0.59$; wastewater shifted 1 day before clinical data) (Figure 1). An analysis using self-reported SARS-CoV-2 test results yielded a stronger correlation than observed with healthcare-based testing (Appendix Table 3). WS for SARS-CoV-2 was 95.9% sensitive and 87.1% concordant with weekly clinical testing results (Figure 2; Appendix Table 4).

RSV Surveillance

Overall, RSV was detected in wastewater on 22.1% (111/503) of surveillance days; observed wastewater positivity was lower in the 2022–23 season than in the 2023–24 season (13.8% vs. 28.0%) (Table). Laboratory-confirmed clinical RSV cases occurred on 37.0% (215/581) of days and observed daily clinical

positivity was higher in the 2022–23 season than the 2023–24 season (46.7% vs. 29.4%). RSV circulation seasonality was similar across wastewater and clinical data. In the 2022–23 season, RSV clinical cases occurred during October 29, 2022–June 12, 2023, compared with RSV detection in wastewater during November 7, 2022–April 26, 2023 (Table; Figure 3). In the 2023–24 season, RSV was first detected in wastewater on September 26, 2023, and the first positive clinical test was on October 9, 2023. RSV was detected in wastewater through May 21, 2024, and by clinical testing through May 22, 2024. The correlation between the wastewater RSV signal and clinical testing data increased with increasing time shift of the wastewater data before the clinical data. The peak RSV correlation of 0.60 occurred with 8-day wastewater lead time. RSV correlations were stronger in the 2023–24 season than the 2022–23 season. WS for RSV was 55.0% sensitive and 67.1% concordant with weekly clinical testing results (Figure 2; Appendix Table 4). Sensitivity and concordance of RSV WS was markedly higher in the 2023–24 season: sensitivity increased from 36.7% to 73.3% and concordance increased from 48.6% to 81.3%.

Influenza A Virus Surveillance

Wastewater testing detected influenza A virus on 36.8% (185/503) of surveillance days; detections were more frequent during the 2023–24 season (44.4%; 130/293 days) than the 2022–23 season (26.2%; 55/210

Table. Results of wastewater and clinical respiratory virus testing for surveillance in remote community, Bethel, Alaska, USA, 2022–2024*

Season†	RSV	Influenza A virus	Influenza B virus	SARS-CoV-2
Overall surveillance period				
Samples				
Wastewater, n = 318	78 (24.5)	128 (40.3)	29 (9.1)	265 (83.3)
Clinical, n = 7,392	364 (4.9)	543 (7.3)	107 (1.4)	372 (5.0)
Surveillance days				
Wastewater, n = 503	111 (22.1)	185 (36.8)	41 (8.2)	406 (80.7)
Clinical, n = 581	215 (37.0)	203 (34.9)	49 (8.4)	232 (39.9)
Surveillance weeks, n = 85				
Wastewater	34 (40.0)	52 (61.2)	9 (10.6)	78 (91.8)
Clinical	60 (70.6)	57 (67.1)	12 (14.1)	73 (85.9)
2022–23 season				
Samples				
Wastewater, n = 135	18 (13.3)	33 (24.4)	0	129 (95.6)
Clinical, n = 3,781	228 (6.0)	303 (8.0)	0	193 (5.1)
Surveillance days				
Wastewater, n = 210	29 (13.8)	55 (26.2)	0	192 (91.4)
Clinical, n = 255	119 (46.7)	79 (31.0)	0	126 (49.4)
Surveillance weeks, n = 37				
Wastewater	11 (29.7)	19 (51.4)	0	37 (100)
Clinical	30 (81.1)	21 (56.8)	0	36 (97.3)
First detection date				
Wastewater	2022 Nov 7	2022 Nov 7	NA	2022 Oct 19‡
Clinical	2022 Oct 29	2022 Oct 21	NA	2022 Oct 20
Last detection date				
Wastewater	2023 Apr 26	2023 Jun 14	NA	2023 Jun 26
Clinical	2023 Jun 12	2023 Jun 3	NA	2023 Jun 20
2023–24 season				
Samples				
Wastewater, n = 183	60 (32.8)	95 (51.9)	29 (15.8)	136 (74.3)
Clinical, n = 3,611	136 (3.8)	240 (6.6)	107 (3.0)	179 (5.0)
Surveillance days				
Wastewater, n = 293	82 (28.0)	130 (44.4)	41 (14.0)	214 (73.0)
Clinical, n = 326	96 (29.4)	124 (38.0)	49 (15.0)	106 (32.5)
Surveillance weeks, n = 48				
Wastewater	23 (47.9)	33 (68.8)	9 (18.8)	41 (85.4)
Clinical	30 (62.5)	36 (75.0)	12 (25.0)	37 (77.1)
First detection date				
Wastewater	2023 Sep 26	2023 Jul 3	2024 Mar 21	2023 Jul 3
Clinical	2023 Oct 9	2023 Jul 23	2024 Jan 5	2023 Jul 14
Last detection date				
Wastewater	2024 May 21	2024 May 30	2024 May 20	2024 May 24
Clinical	2024 May 22	2024 May 7	2024 May 31	2024 Apr 23

*Values are no. (%) positive for each virus. NA, not applicable; RSV, respiratory syncytial virus.
†Dates for combine surveillance were October 19, 2022–May 31, 2024; for the 2022–23 season, dates were October 19, 2022–June 30, 2023; and for the 2023–24 season, dates were July 1, 2023–May 31, 2024.
‡Eight SARS-CoV-2–positive clinical test results were detected in the 14 days before wastewater surveillance was initiated.

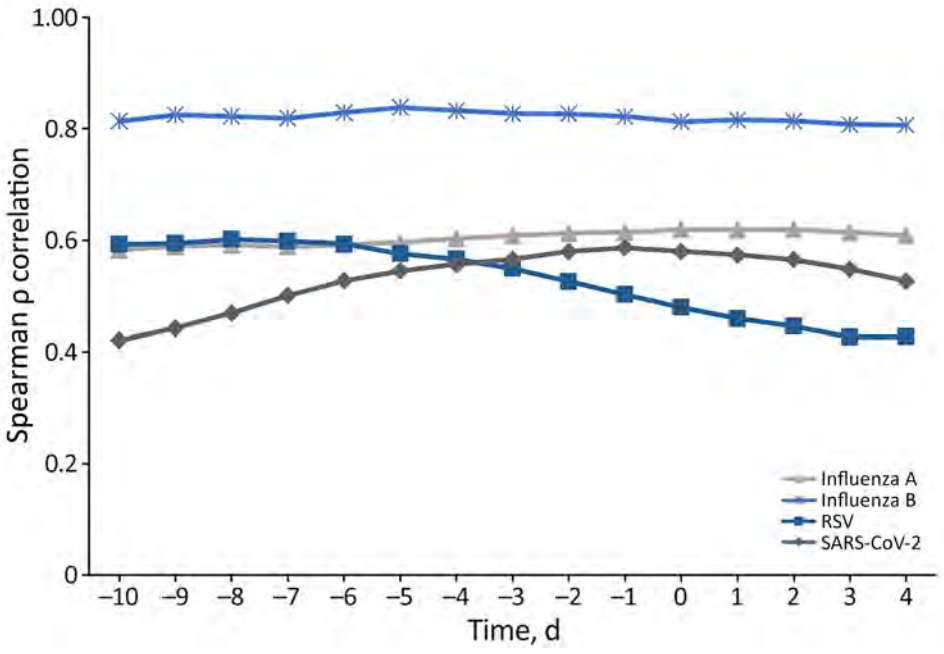


Figure 1. Time-lagged correlations between wastewater respiratory virus concentrations and clinical case counts in remote community, Alaska, USA, 2022–2024. Spearman rank-order correlation (ρ) assessed the association between positive clinical test counts and wastewater cycle threshold values for the number of days wastewater data shifted. For example, the value −7 on the x-axis indicates that wastewater values were shifted 7 days before (earlier) the clinical results from a given date. All correlation coefficients >0.17 are statistically significant at p<0.05. RSV, respiratory syncytial virus.

days). Influenza A virus–positive clinical tests occurred on 34.9% (203/581) of days (Table). Influenza A virus was first detected clinically on October 21, 2022, and in wastewater on November 7, 2022. Wastewater detection patterns mirrored clinical detection patterns (Figure 3). Both surveillance systems identified a wave of influenza A virus during the winter of 2022–23, followed by sporadic cases during the summer of 2023 and several subsequent waves of infection later in the 2023–24 season. We observed consistent, strong influenza A virus wastewater and clinical data correlations, and most lagged correlations were near or above 0.60 (Figure 1). Influenza A virus correlations were stronger during the 2022–23 season and peaked at 0.82 when the wastewater signal shifted 10 days before clinical signal (Appendix Table 3). WS for influenza A virus was 70.2% sensitive and 65.9% concordant with weekly clinical testing results (Figure 2; Appendix Table 4).

Influenza B Virus Surveillance

Influenza B virus was not detected in wastewater or through clinical testing during the 2022–23 season. In the 2023–24 season, influenza B virus was detected in wastewater on 14.0% (41/293) of sample days and clinical tests were positive on 15.0% (49/326) of surveillance days. An initial clinical case of influenza B infection occurred in January 2024 with no concordant detection in wastewater. Additional clinical cases of influenza B infection were identified starting March 17, 2024, and in wastewater on March 21, 2024, and ongoing detection occurred through May 2024 (Figure 3). Influenza B virus wastewater and clinical data had correlations >0.80 across all time shifts and peaked at 0.85 when the wastewater signal shifted 5-days before clinical signal (Figure 1). WS for influenza B virus was 75.0% sensitive and 96.5% concordant with weekly clinical testing results (Figure 2; Appendix Table 4).

Discussion

Implementation of WS by YKHC was unique in several respects. Bethel is a remote community off the road system with limited laboratory capacity and a sanitation system uncommon in the United States. At the time YKHC initiated WS, Alaska as a state lacked a coordinated WS program, necessitating sample shipment to a contracted laboratory, leading to additional costs, delays, and potential sample degradation. YKHC, as the regional Tribal healthcare and public health organization, was well positioned to locally implement WS. The YKHC Office of Environmental Health & Engineering had experience with water quality testing, were trusted by the community, had access to clinical testing data to validate WS, and were able to act on the information generated by WS. The simplicity of the cartridge-based rapid PCR testing system and its performance with unprocessed wastewater contributed to timely results; in most instances, samples were tested the same day they were collected. Local ownership of WS and the associated data likely mitigated community concerns and enhanced program sustainability (*16*).

Given uncertainties in the performance of WS in a subarctic, low water use, partially sewered community, YKHC used the 2022–23 season of WS to internally validate its performance. The strong correlations observed between clinical testing and wastewater results for influenza A virus and RSV during the 2022–23 season encouraged additional investment in WS and led to the creation of a public facing dashboard on the YKHC website (*17*). The dashboard provides background on WS, interpretation of the data, and suggests specific public health actions for community members depending on the wastewater results.

WS data showed moderate to high concordance with laboratory-confirmed clinical instances of respiratory viral infection. The strengths of the correlations were similar to those reported by a shorter study of COVID-19 WS in small, rural communities (*18*). Time-lagged correlation analysis suggests that wastewater detection of RSV could serve as a leading indicator of virus circulation in Bethel. Early identification of RSV circulation enables timely prevention through nirsevimab distribution and clinical and community information sharing. As a current best practice (*19*), YKHC does not use wastewater data in isolation but integrates it with other data sources, such as clinical data, for public health decision making.

Several factors could explain observed discordances between wastewater and clinical data. Wastewater detections in the absence of positive clinical tests suggest circulating virus in the community but no persons ill enough to seek healthcare and receive clinical testing. In addition, some persons shed viral material for prolonged periods following infection, as noted for SARS-CoV-2 (*20*), resulting in wastewater detection in the absence of new clinical cases. A positive clinical test in the absence of a detectable wastewater signal might be because waste from the ill person has not entered the centralized sewer system, viral RNA was diluted in wastewater to below the limit of detection for the assay, or PCR inhibitors were in the wastewater (*21*).

A strength of this study is evaluation of 2 years of comprehensive clinical testing and wastewater data, resulting in a robust dataset. The first limitation of our study is that identification of clinical infections relied upon community members seeking healthcare and receiving viral testing, likely leading to underascertainment of infections. However, YKHC provided care for almost everyone in the region operating the only hospital and emergency department in the Yukon-Kuskokwim Delta. YKHC standard of care was to test all persons with respiratory symptoms, which provided a comprehensive clinical picture of

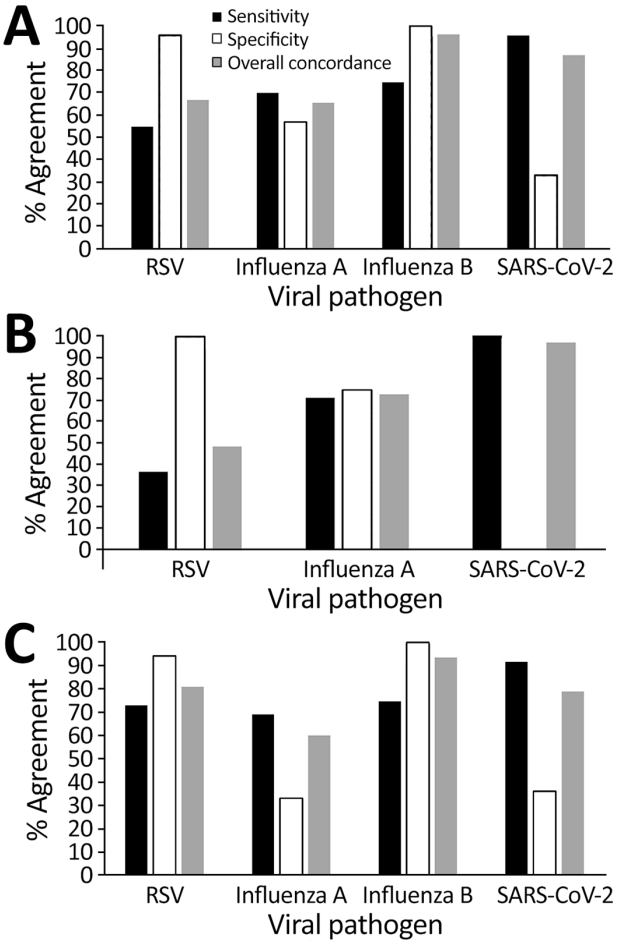


Figure 2. Sensitivity, specificity, and concordance of weekly wastewater testing results compared with weekly clinical testing from wastewater respiratory virus surveillance in remote community, Alaska, USA, 2022–2024. A) Overall study period 2022–2024; B) 2022–23 respiratory virus season; C) 2023–24 respiratory virus season. RSV, respiratory syncytial virus.

SARS-CoV-2, influenza, and RSV circulation in the community. A second potential limitation common to WS evaluations was the possible mismatch between the population accessing clinical testing and the population contributing to the sampled wastewater. Bethel serves as the medical and transportation hub for the region, potentially leading to mismatches in persons receiving clinical testing and contributing to the wastewater system. However, the remote nature of Bethel, which is off the road system and primarily accessed by plane, likely minimizes that population mismatch compared with road-connected communities in the United States with a variety of clinical testing options. Our analysis only used clinical test results from the YK Delta Regional Hospital, meaning all clinical test results came from persons physically in Bethel who could contribute to the city's wastewater.

A third limitation relates to the unique sanitation system in Bethel, where sewage stored at the household level might be introduced into the centralized sewage system days to weeks after it was created. That delay could weaken correlations with clinical testing or decrease the sensitivity of WS because of decay of the viral biomarkers in the wastewater. To explore that issue, we conducted lagged correlational analyses, which found the strongest correlations when the wastewater results were shifted earlier in relation to clinical testing. A fourth limitation is that the passive wastewater sampling method yielded semiquantitative

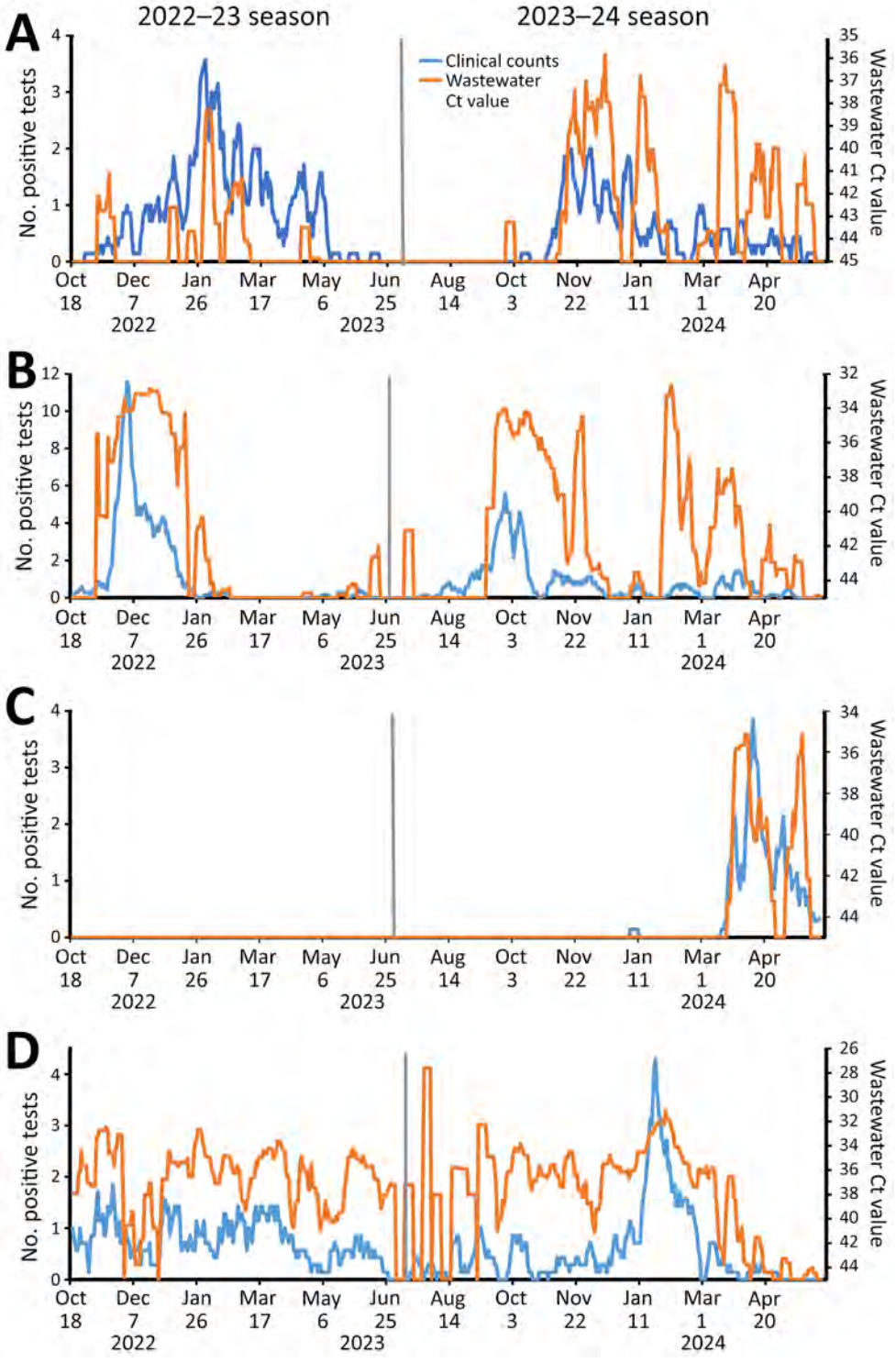


Figure 3. Daily 7-day moving average positive clinical tests and Ct values for respiratory virus surveillance in remote community, Alaska, USA, October 2022–May 2024. A) Respiratory syncytial virus; B) influenza A virus; C) influenza B virus; D) SARS-CoV-2. Ct, cycle threshold.

viral wastewater concentrations. Thus, we report Ct values and not estimated viral concentrations.

In summary, YKHC implemented WS in a rural and remote community midway through the COVID-19 pandemic. That surveillance system frequently identified respiratory viruses in wastewater and demonstrated statistically significant correlations with clinical testing. WS provided YKHC respiratory virus surveillance information that complemented and corroborated traditional clinical surveillance data. WS data helped inform YKHC public health decision-making related to the timing of influenza vaccine campaign and the distribution of nirsevimab, a long-lasting monoclonal antibody used to protect infants from RSV, and supported education and messaging for patients and clinicians. New components of the YKHC WS program include testing for additional respiratory and enteric pathogens, exploratory efforts to enhance active case finding of tuberculosis, and expansion of WS to remote villages in the region. YKHC demonstrated the feasibility of WS in rural Alaska and that correlation between WS and clinical results can support and inform public health activities to protect the community.

Acknowledgments

We appreciate the support of the municipality of Bethel to access their lift station to collect wastewater samples and the technical support provided by scientists with the Public Health Agency of Canada.

This study was supported by grants from the National Science Foundation (grant nos. 2154934 and 2412446) and the Centers for Disease Control and Prevention (grant no. NU38TO00022). The sponsors had no role in the study design, data collection, analysis, and interpretation of data, writing of the report, or decision to submit the article for publication.

About the Author

Mr. Lefferts is the director of public health for the Yukon-Kuskokwim Health Corporation, based in Bethel, Alaska, USA. His research interests include infectious disease surveillance, tuberculosis elimination, and preventive healthcare delivery.

References

1. Adams C, Bias M, Welsh RM, Webb J, Reese H, Delgado S, et al. The National Wastewater Surveillance System (NWSS): from inception to widespread coverage, 2020–2022, United States. Sci Total Environ. 2024;924:171566. https://doi.org/10.1016/j.scitotenv.2024.171566
2. Parkins MD, Lee BE, Acosta N, Bautista M, Hubert CRJ, Hrudey SE, et al. Wastewater-based surveillance as a tool for public health action: SARS-CoV-2 and beyond. Clin Microbiol Rev. 2024;37:e0010322. https://doi.org/10.1128/cmr.00103-22
3. National Academies of Sciences, Engineering, and Medicine; Health and Medicine Division; Division on Earth and Life Studies; Board on Population Health and Public Health Practice; Water Science and Technology Board; Committee on Community Wastewater-based Infectious Disease Surveillance. Wastewater-based disease surveillance for public health action. Washington: National Academies Press; 2023.
4. Centers for Disease Control and Prevention. National wastewater data for respiratory viruses [cited 2026 Jun 14]. https://www.cdc.gov/wastewater/respiratory-viruses/national.html
5. Valencia D, Yu AT, Wheeler A, Hopkins L, Pray I, Horter L, et al. Notes from the field: the National Wastewater Surveillance System's Centers of Excellence contributions to public health action during the respiratory virus season—four U.S. jurisdictions, 2022–23. MMWR Morb Mortal Wkly Rep. 2023;72:1309–12. https://doi.org/10.15585/mmwr.mm7248a4
6. Medina CY, Kadonsky KF, Roman FA Jr, Tariqi AQ, Sinclair RG, D'Aoust PM, et al. The need of an environmental justice approach for wastewater based epidemiology for rural and disadvantaged communities: a review in California. Curr Opin Environ Sci Health. 2022;27:100348. https://doi.org/10.1016/j.coesh.2022.100348
7. Annan J, Henderson R, Gray M, Clark RG, Sarin C, Black K. A review of wastewater-based epidemiology for the SARS-CoV-2 virus in rural, remote, and resource-constrained settings internationally: insights for implementation, research, and policy for First Nations in Canada. Int J Environ Res Public Health. 2024;21:1429. https://doi.org/10.3390/ijerph21111429
8. Kantor RS, Greenwald HD, Kennedy LC, Hinkle A, Harris-Lovett S, Metzger M, et al. Operationalizing a routine wastewater monitoring laboratory for SARS-CoV-2. PLOS Water. 2022;1:e0000007. https://doi.org/10.1371/journal.pwat.0000007
9. Daigle J, Racher K, Hazenberg J, Yeoman A, Hannah H, Duong D, et al. A sensitive and rapid wastewater test for SARS-COV-2 and its use for the early detection of a cluster of cases in a remote community. Appl Environ Microbiol. 2022;88:e0174021. https://doi.org/10.1128/aem.01740-21
10. Bruden DJT, Singleton R, Hawk CS, Bulkow LR, Bentley S, Anderson LJ, et al. Eighteen years of respiratory syncytial virus surveillance: changes in seasonality and hospitalization rates in southwestern Alaska Native children. Pediatr Infect Dis J. 2015;34:945–50. https://doi.org/10.1097/INF.0000000000000772
11. Lefferts B, Bruden D, Plumb ID, Hodges E, Bates E, January G, et al. Effectiveness of the COVID-19 vaccines on preventing symptomatic SARS-CoV-2 infections and hospitalizations in Southwestern Alaska, January–December 2021. Vaccine. 2023;41:3544–9. https://doi.org/10.1016/j.vaccine.2023.04.070
12. Hodges E, Lefferts B, Bates E, Desnoyers C, Bruden D, Bruce M, et al. Use of rapid antigen testing for SARS-CoV-2 in remote communities—Yukon-Kuskokwim Delta region, Alaska, September 15, 2020–March 1, 2021. MMWR Morb Mortal Wkly Rep. 2021;70:1120–3. https://doi.org/10.15585/mmwr.mm7033a3
13. D'Aoust PM, Towhid ST, Mercier É, Hegazy N, Tian X, Bhatnagar K, et al. COVID-19 wastewater surveillance in

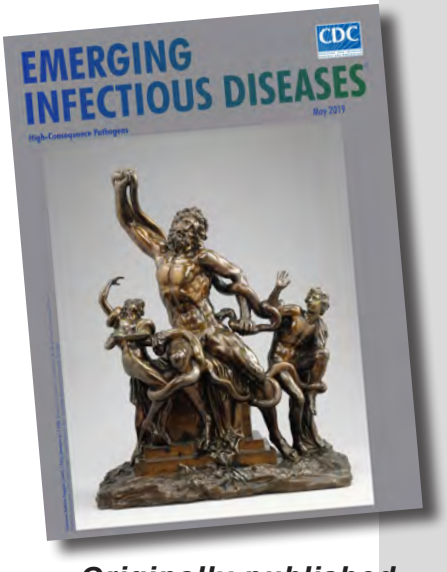
rural communities: comparison of lagoon and pumping station samples. Sci Total Environ. 2021;801:149618. https://doi.org/10.1016/j.scitotenv.2021.149618
14. Liu P, Ibaraki M, VanTassell J, Geith K, Cavallo M, Kann R, et al. A sensitive, simple, and low-cost method for COVID-19 wastewater surveillance at an institutional level. Sci Total Environ. 2022;807:151047. https://doi.org/10.1016/j.scitotenv.2021.151047
15. Centers for Disease Control and Prevention. Respiratory virus activity levels [cited 2026 Jun 14]. https://www.cdc.gov/respiratory-viruses/data/activity-levels.html
16. Holm RH, Pocock G, Severson MA, Huber VC, Smith T, McFadden LM. Using wastewater to overcome health disparities among rural residents. Geoforum. 2023; 144:103816. https://doi.org/10.1016/j.geoforum.2023.103816
17. Yukon-Kuskokwim Health Corporation. Respiratory illness surveillance [cited 2026 Jan 13]. https://www.ykhc.org/respiratory-illness
18. Jarvie MM, Reed-Lukomski M, Southwell B, Wright D, Nguyen TNT. Monitoring of COVID-19 in wastewater across the eastern Upper Peninsula of Michigan. Environ Adv. 2023;11:100326. https://doi.org/10.1016/j.envadv.2022.100326
19. Mathematica. Integrating wastewater and public health data [cited 2026 Jun 15]. https://www.mathematica.org/publications/integrating-wastewater-and-public-health-data
20. Walsh KA, Jordan K, Clyne B, Rohde D, Drummond L, Byrne P, et al. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. J Infect. 2020;81:357–71. https://doi.org/10.1016/j.jinf.2020.06.067
21. Kumblathan T, Piroddi N, Hrudey SE, Li XF. Wastewater based surveillance of SARS-CoV-2: challenges and perspective from a Canadian inter-laboratory study. J Environ Sci (China). 2022;116:229–32. https://doi.org/10.1016/j.jes.2022.01.039

Address for correspondence: Brian Lefferts, Yukon Kuskokwim Health Corporation, PO Box 528, Bethel, AK 99559, USA; email: brian_lefferts@ykhc.org

etymologia revisited

Nipah Virus

[ne´-pə vī´-rəs]



Originally published in May 2019

In 1994, a newly described virus, initially called equine morbillivirus, killed 13 horses and a trainer in Hendra, a suburb of Brisbane, Australia. The reservoir was subsequently identified as flying foxes, bats of the genus *Pteropus* (Greek pteron ["wing"] + *pous* ["foot"]). In 1999, scientists investigated reports of febrile encephalitis and respiratory illness among workers exposed to pigs in Malaysia and Singapore. (The pigs were believed to have consumed partially eaten fruit discarded by bats.)

The causative agent was determined to be closely related to Hendra virus and was later named for the Malaysian village of Kampung Sungai Nipah. The 2 viruses were combined into the genus *Henipavirus*, in the family *Paramyxoviridae*. Three additional species of *Henipavirus*—Cedar virus, Ghanaian bat virus, and Mojiang virus—have since been described, but none is known to cause human disease. Outbreaks of Nipah virus occur almost annually in India and Bangladesh, but *Pteropus* bats can be found throughout the tropics and subtropics, and henipaviruses have been isolated from them in Central and South America, Asia, Oceania, and East Africa.

References:

1. Centers for Disease Control and Prevention. Outbreak of Hendra-like virus—Malaysia and Singapore, 1998–1999. MMWR Morb Mortal Wkly Rep. 1999;48:265–9.
2. Selvey LA, Wells RM, McCormack JG, Ansford AJ, Murray K, Rogers RJ, et al. Infection of humans and horses by a newly described morbillivirus. Med J Aust. 1995;162:642–5.

https://wwwnc.cdc.gov/eid/article/25/5/et-2505_article

Correlation of Norovirus in Wastewater with Gastroenteritis-Related Hospitalizations, New York, USA, 2022–2024

Ozioma F. Nwabor, Patrick Bryant, Dustin Hill, Mohammed Alazawi, Lynsey Schoultz, Aishwarya Jadhav, Ian Bradley, Mila Neyra-Blatz, Yinyin Ye, Kirsten St. George, David A. Larsen

Norovirus is a substantial public health burden, yet surveillance is challenging because infections are underreported. Wastewater surveillance offers a complementary approach. We quantified norovirus genogroups GI and GII from 2,823 wastewater samples collected in New York, USA, during September 2022–July 2024. Across 4 counties in New York, we detected norovirus GI in 100% of samples and GII in 92%–100%. Both genogroups exhibited strong seasonal trends. During the study period, 499 norovirus-specific and 50,004 all-cause acute gastroenteritis hospitalizations occurred. Weekly correlations between GII concentrations and norovirus-specific hospitalizations showed moderate positive relationships (ρ = 0.23–0.28); GI concentrations showed weak correlations (ρ = −0.18 to 0.06). Correlations between norovirus in wastewater and all-cause acute gastroenteritis hospitalizations were weaker. Although wastewater surveillance reliably detects seasonal norovirus circulation in the population, correlations with hospitalizations were moderate. GII showed stronger associations with clinical outcomes than GI, supporting the importance of genogroup-specific monitoring.

Noroviruses are genetically diverse icosahedral viruses belonging to the family Caliciviridae; they consist of a single-stranded, positive-sense RNA genome. They are a leading cause of diarrheal disease. and can infect a wide variety of hosts (*1*). Norovirus consists of 10 genogroups (GI–GX) and 49 genotypes. Genogroups GI, GII, GIV, GVIII and GIX are known to infect humans, whereas genogroup GIII primarily infects cows and GV mice (*1*–*4*). Norovirus infections are highly contagious, causing gastroenteritis that is typically mild and self-limiting in healthy adults but can be severe and even fatal in children, elderly, and immunocompromised persons. In the United States, outbreaks peak during November–April (*5*,*6*) and cause $2.3 billion in direct medical costs and $1.4–$20.7 billion in loss of productivity annually (*7*).

Author affiliations: Syracuse University, Syracuse, New York, USA (O.F. Nwabor, D. Hill, M. Neyra-Blatz, D.A. Larsen); New York State Department of Health, Albany, New York, USA (P. Bryant, M. Alazawi, L. Schoultz, A. Jadhav, K. St. George); University at Buffalo, Buffalo, New York, USA (I. Bradley, Y. Ye); New York State Department of Health, Slingerlands, New York, USA (K. St. George); University at Albany, Albany (K. St. George)

DOI: https://doi.org/10.3201/eid3213.260472

As of August 2026, norovirus surveillance relies primarily on either outbreak detection with case investigations or individual case reporting. Most norovirus infections in healthy adults are either asymptomatic or self-limiting and thus not tested, leading to a large burden of unreported incidence (*1*,*8*,*9*). In most US jurisdictions norovirus is not a notifiable disease. Poor clinical surveillance provides inaccurate estimates of norovirus incidence, which leads to lower prioritization for prevention and control. Understanding the true epidemiologic burden of norovirus requires an approach that addresses those limitations. Wastewater surveillance is one potential data source; it does not depend on symptomatic persons seeking medical care but enables community-level infectious disease surveillance (*10*).

The level of norovirus RNA in wastewater has been associated with the monthly incidence of acute gastroenteritis hospitalizations across diverse geographic regions (*11*). Several studies have found a strong correlation between norovirus genogroup–specific concentrations in wastewater and clinical outcomes (*12*–*15*). GII strains are more strongly associated with large-scale outbreaks and severe gastroenteritis (*16*), but they infect animals as well as humans, which could decrease the utility of wastewater

surveillance (*17*); GI strains are often linked to sporadic cases and specifically infect humans (*18*). Here, we investigate epidemiologic trends of norovirus GI and GII by measuring the concentration of both genogroups in wastewater across 4 counties in the state of New York, USA. We hypothesized that higher wastewater concentrations would correlate with an increase in norovirus and acute gastroenteritis hospitalizations, especially during colder months, when norovirus activity tends to be higher, in light of previous studies linking wastewater viral concentrations to disease incidence. We correlated genogroup-specific norovirus wastewater concentrations with hospitalized cases of acute gastroenteritis to better understand the potential utility of wastewater surveillance to support norovirus surveillance.

Methods

Study Design

We designed an ecologic study to assess the relationship between the concentration of norovirus in wastewater and the incidence of norovirus-attributable and all-cause acute gastroenteritis-related hospitalizations. We conducted the study during September 2022–July 2024, taking samples from 24 wastewater treatment plants in 4 counties in New York: Erie (Buffalo, NY, USA), Onondaga (Syracuse, NY, USA), Jefferson (Watertown, NY, USA), and Westchester (north of New York, NY, USA) (Figure 1).

Setting

The New York State Wastewater Surveillance Network was initiated in March 2020 as a tool to track and address emerging diseases and monitor evolving public health threats (*19*); its purpose is to provide population-level data on community health status and disease burden through sampling and analysis of wastewater from across the state. As part of the study, we sampled 9 treatment plants in Erie County; for each, capacity was 2.20–180 million gallons permitted discharge per day (MGD) and population served was 2,854–431,141, covering ≈83.4% of the county population. In Westchester County, we sampled 7 treatment plants, each with a capacity of 5.0–120 MGD, serving a population of 26,651–523,765, covering ≈83.6% of the county population. In Onondaga County, we sampled 7 treatment plants, each with a capacity of 0.99–84 MGD, serving a population of 3,272–242,377, covering ≈85.7% of the county population. In Jefferson County we sampled 5 treatment plants, each with a capacity 0.06–15.9 MGD, serving a population of 81–26,270, covering ≈46.7% of the county population. The New York State Wastewater Surveillance Network does not routinely collect treatment plant flow data.

Wastewater Sampling and Processing

Plant operators collected 24-hour flow-weighted composite influent (before treatment) wastewater samples 1 or 2 times each week at wastewater plants (WWTPs) participating in the state network (*19*). In Erie County, the samples were processed at an environmental engineering laboratory located at the University at Buffalo. Specifically, we processed 9.75-mL wastewater samples using Nanotrap Microbiome A particles (Ceres Nanosciences, https://www.ceresnano.com), then performed nucleic acid extraction with MagMAX viral/pathogen nucleic acid isolation kits (Thermo Fisher Scientific, https://www.thermofisher.com). We eluted nucleic acids in 50 µL of MagMAX elution buffer. We automated both steps on the KingFisher Apex purification system (Thermo Fisher Scientific), as described previously (*20*). Samples from Jefferson, Onondaga, and Westchester counties were shipped overnight on wet ice to Quadrant Biosciences (Syracuse, NY, USA). In brief, we ultracentrifuged 20-mL samples at 150,000 × *g* at 40°C on a Sorvall WX Ultra series (Thermo Fisher Scientific) with a Sorvall SureSpin 630 (6 × 36 mL) with a sucrose cushion (*21*). We resuspended pellets in 200 µL 1X PBS and transferred them to 1.7-mL microcentrifuge tubes stored at −20°C for <24 h until nucleic acid extraction.

Molecular Testing

We extracted total nucleic acids from pellets using the AllPrep PowerViral DNA/RNA Kit (QIAGEN, https://www.qiagen.com). We eluted nucleic acids in 50 µL elution buffer and synthesized cDNA using the QuantiTect reverse transcription kit (QIAGEN). We quantified total nucleic acid and cDNA by quantitative reverse transcription PCR and quantitative PCR. We used QuantStudio 3 or QuantStudio 5 (Thermo Fisher) real-time PCR systems for all quantitative PCR reactions. We detected and quantified norovirus nucleic acid with 5 µL of the extracted nucleic acid added to 45 µL of norovirus master mix, containing 900 µM of primers and 250 µM of probes with the QIAcuity microfluidic digital PCR instrument (QIAGEN), according to the manufacturer's specifications. The cycling profile was 1 × 50°C for 40 minutes, 1 × 95°C for 2 minutes, and 40 × 95°C for 5 seconds followed by 60°C for 30 seconds. We performed an internal assay validation, including limit of detection, specificity, and reproducibility, before implementation of the pilot study. We empirically determined the limit of detection as 5 gene copies/µL of reaction and quantified mean norovirus GI and GII

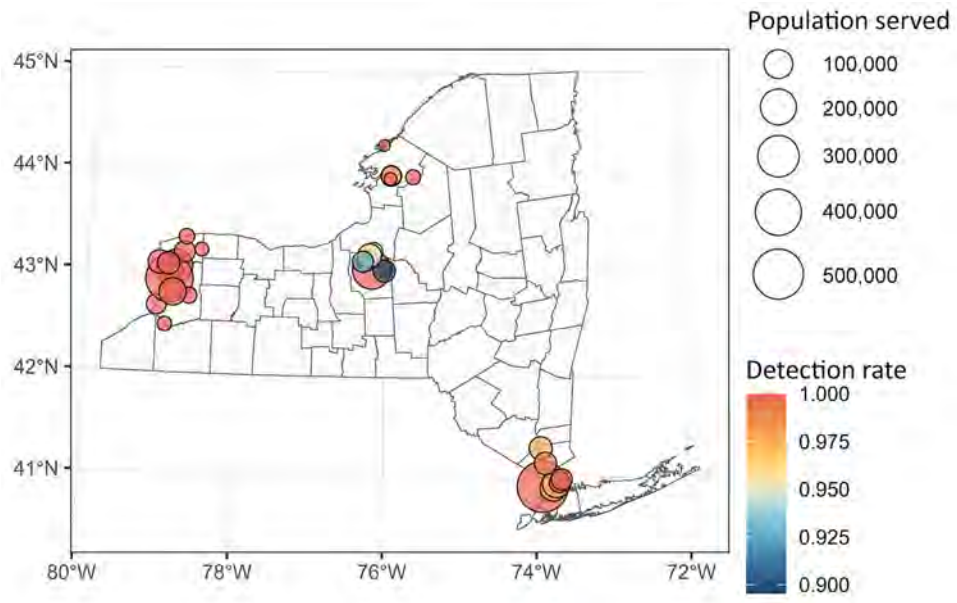


Figure 1. Spatial distribution of sampled wastewater treatment plants in study of correlation between norovirus in wastewater with norovirus-attributable and general acute gastroenteritis–related hospitalizations, New York, USA, 2022–2024. Norovirus genotype I was detected in every sample across the study. Colors indicate detection rate (proportion of samples tested that are positive) of norovirus genotype II across the wastewater treatment plants included in this study.

concentrations per microliter of reaction. The specificity panel for digital PCR detected no cross-reactivity for any of the 23 targets tested (*22*). We included negative and positive controls on all plates. We used norovirus GI- and GII-specific primers and probes implemented for a previously described digital PCR assay for the detection and quantification of hepatitis A and norovirus (*23*) (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/32/13/26-0472-App1.pdf).

Hospitalization Data

We retrieved patient-level acute gastroenteritis hospitalization data from the Statewide Planning and Research Cooperative System that captures hospitalizations from all hospitals in New York. We considered 2 types of hospitalizations: those specifically for norovirus-confirmed acute gastroenteritis and those related to acute gastroenteritis from any cause. We used specific codes for gastroenteritis from the International Classification of Diseases, 10th Revision, Clinical Modification (*24*) (Appendix Table 2), to identify hospital admissions for acute gastroenteritis (AGE). We removed patient records with missing address information, geocoded addresses to latitude and longitude, and aggregated hospitalizations to county level.

Wastewater Measures

We considered 2 different measures of the amount of norovirus in wastewater. First, we used the concentration of norovirus RNA for each genogroup per unit of wastewater. Second, we calculated the norovirus wastewater activity level (WVAL) as a continuous metric with equation 1. WVAL is a metric established by the Centers for Disease Control and Prevention (CDC) to monitor the level of virus in wastewater across diverse geographies and laboratory testing methodologies (*25*). For both approaches, we did not normalize the amount of norovirus in wastewater to the flow of the WWTP. We calculated the wastewater activity level (WVAL) for each wastewater sample *i* in wastewater treatment plant *j* as the concentration of norovirus RNA in a sample (C_{ij}) relative to the mean of norovirus concentrations in the treatment plant (μ_j) and the standard deviation of norovirus concentrations in the treatment plant (σ_j) using this equation:

$$WVAL = \frac{log(C_{ij}) - \mu_j}{\sigma_i}$$

Correlation of Norovirus in Wastewater and Acute Gastroenteritis Hospitalization

We considered 2 different outcomes of AGE hospitalizations: all-cause AGE hospitalizations and norovirus-specific AGE hospitalizations. For each outcome we conducted 2 types of analyses to estimate the correlation of norovirus in wastewater and AGE hospitalizations. First, we compared the mean monthly concentration or WVAL of norovirus in wastewater to the monthly incidence of AGE hospitalizations as previously described (*11*). Second, we compared weekly wastewater norovirus concentrations or WVALs with AGE hospitalizations. We conducted those analyses for both GI and GII norovirus concentrations. We used a Spearman rank correlation coefficient (ρ) to assess relationships between norovirus concentrations in wastewater and AGE hospitalizations. We also conducted cross-correlation analysis to identify potential time lags between wastewater norovirus concentrations and AGE hospitalizations.

To create county-level measures of norovirus concentration and WVAL, we estimated a population-weighted mean concentration of norovirus within a county by weighting each weekly or monthly norovirus concentration by the proportion of the county population served by each WWTP. We then regressed the monthly correlation coefficient between norovirus concentrations in wastewater and AGE hospitalizations to identify county-level factors that may have influenced the relationship. Last, using analysis of variance (ANOVA), we compared seasonal concentrations of GI and GII. We conducted all analyses using R version 4.4.1 (The R Project for Statistical Computing, http://www.r-project.org).

Results

We analyzed 2,823 wastewater samples collected over 2 years. Detection rate for norovirus GI was 100% and for GII 92%–100% (Figure 1). During the study period, we observed 499 norovirus-specific AGE hospitalizations and 50,004 all-cause AGE hospitalizations.

Quantification of Viral Load in Wastewater Samples

Weekly concentrations of norovirus GI and GII in wastewater samples aggregated to the county level revealed strong seasonal trends (Figures 2, 3). GI concentrations were highest in winter (December–February) and lowest in summer (June–August) (Figure 4). GII concentrations were highest in spring (March–May) and also lowest in summer (Figure 4). ANOVA of norovirus GI revealed that the differences in mean virus concentrations across seasons were statistically significant (F[3; 2,816] = 8.054; p<0.001). Tukey post-hoc tests showed significant differences between winter and fall (p<0.01), winter and spring (p<0.01), and winter and summer (p<0.001); we saw higher mean GI concentrations in winter in all comparisons. However, we found no significant differences between spring and fall, summer and fall, or summer and spring (p>0.05 for all). For norovirus GII, ANOVA result showed statistically significant differences in means across seasons (F[3; 2,816] = 34.86; p<0.0001). Tukey post-hoc tests indicated significant differences between spring and fall (p<0.001), summer and fall (p<0.001), summer and spring (p<0.001), winter and spring (p<0.001), and winter and summer (p<0.001). However, we found no significant difference between winter and fall (p = 0.3385). Erie County had an additional surge in norovirus GII wastewater levels during October–December 2023 that we did not observe in other counties (Figure 3); Erie County also had the highest concentrations of both GI and GII norovirus.

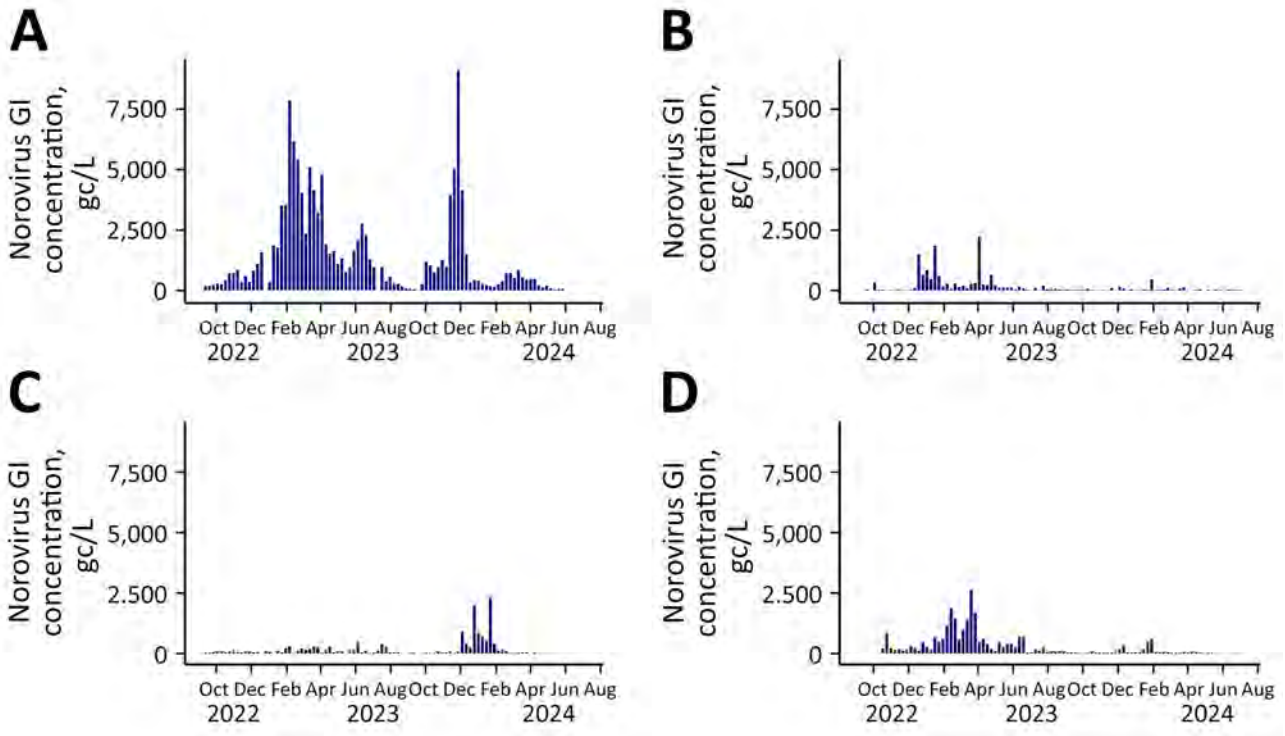


Figure 2. Population-weighted concentration of norovirus genotype I in wastewater over time by area in study of correlation between norovirus in wastewater with norovirus-attributable and general acute gastroenteritis-related hospitalizations, New York, USA, 2022–2024. A) Erie County. B) Jefferson County. C) Onondaga County. D) Westchester County. gc, gene copies.

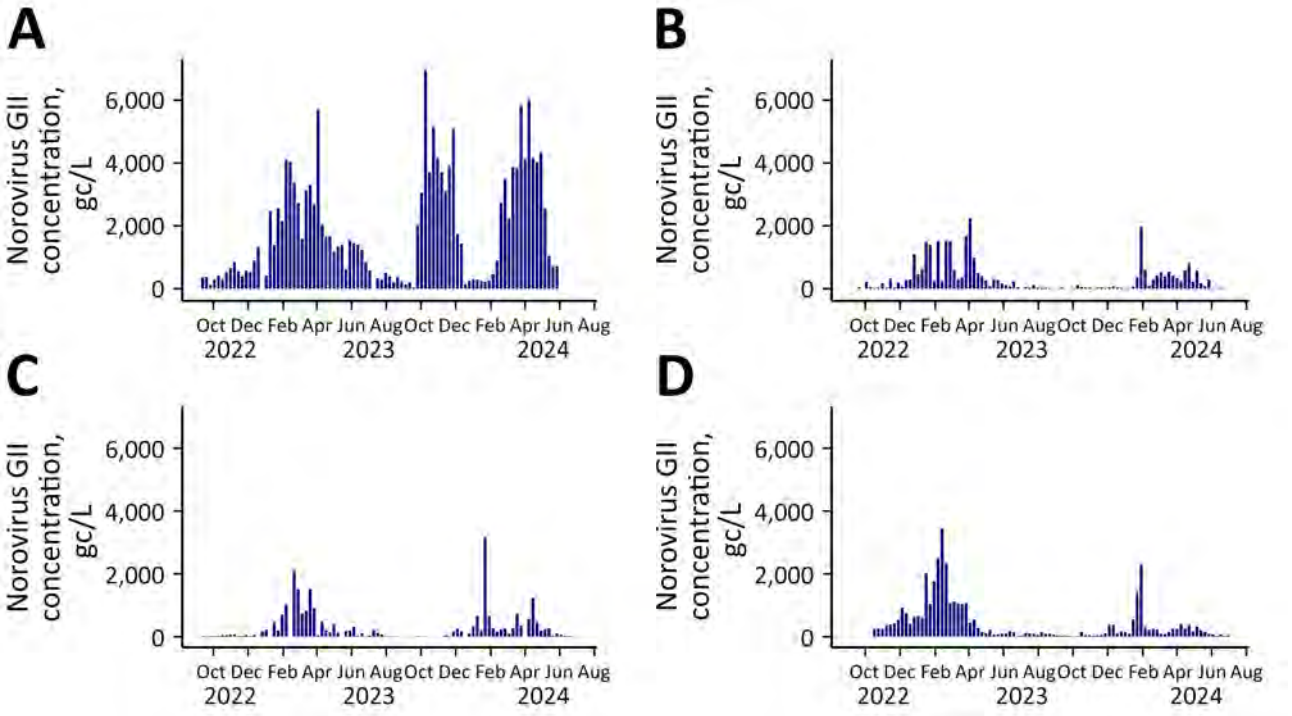


Figure 3. Population-weighted concentration of norovirus genotype II in wastewater over time by area in study of correlation between norovirus in wastewater with norovirus-attributable and general acute gastroenteritis-related hospitalizations, New York, USA, 2022–2024. A) Erie County. B) Jefferson County. C) Onondaga County. D) Westchester County. gc, gene copies.

Wastewater Norovirus Activity Level

In all the counties, both norovirus GI and GII WVAL showed seasonal trends similar to those observed with GI and GII norovirus wastewater trends (Figure 5). Across the counties, GI and GII WVAL were relatively low (mean values <2). A 1-way ANOVA

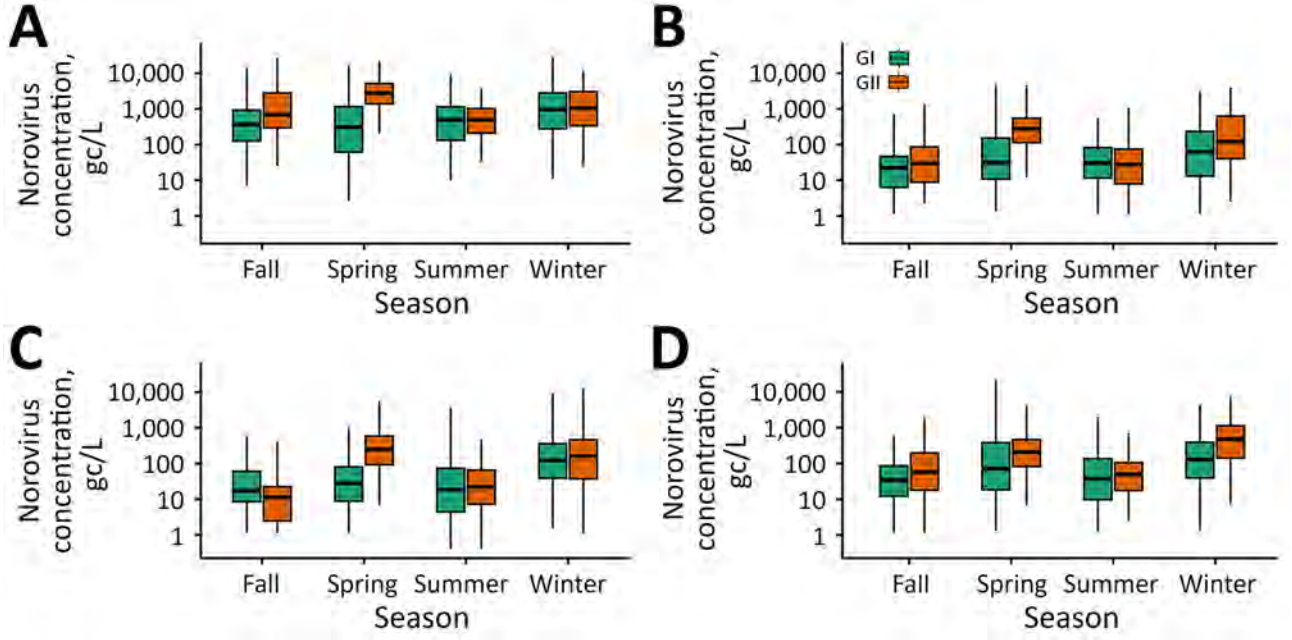


Figure 4. Distribution of norovirus recovered from wastewater by season in study of correlation between norovirus in wastewater with norovirus-attributable and general acute gastroenteritis-related hospitalizations, New York, USA, 2022–2024. A) Erie County. B) Jefferson County. C) Onondaga County. D) Westchester County. Horizontal lines within box plots indicate median concentrations, box tops and bottoms indicate interquartile range, and vertical bars indicate the full range of the data. gc, gene copies.

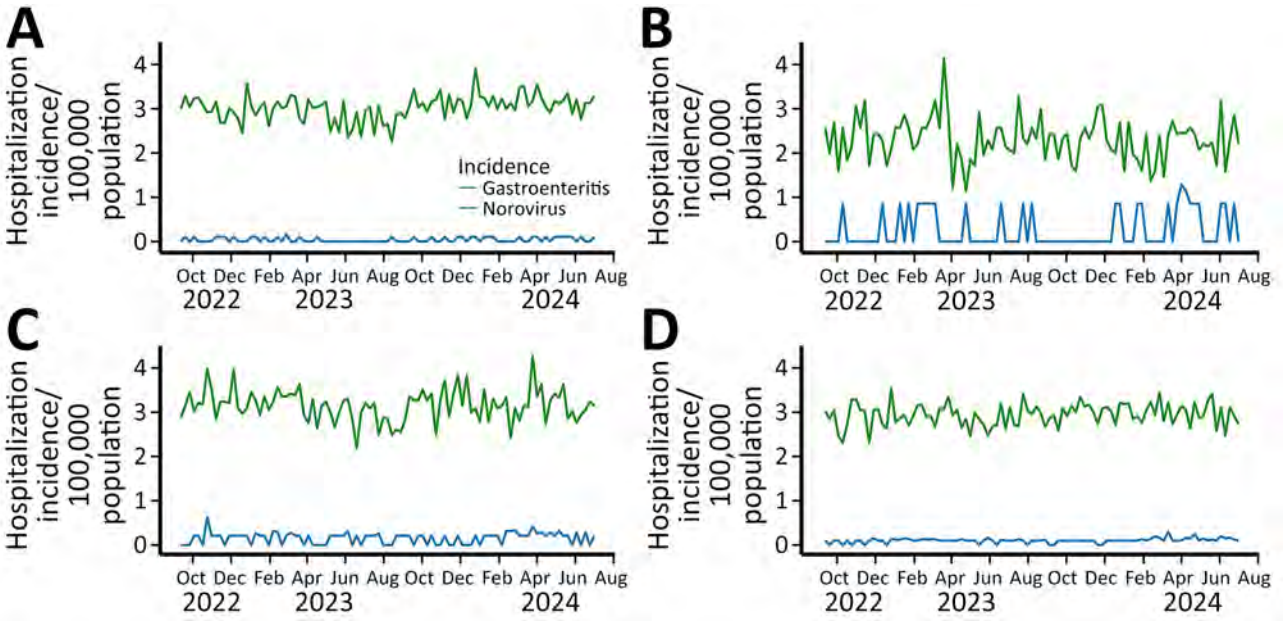


Figure 5. Incidence of all-cause and norovirus-specific hospitalizations for acute gastroenteritis over time in study of correlation between norovirus in wastewater with norovirus-attributable and general acute gastroenteritis-related hospitalizations, New York, USA, 2022–2024. A) Erie County. B) Jefferson County. C) Onondaga County. D) Westchester County.

showed that weighted WVAL GII differed significantly by season (F[3; 796] = 90.33; p<0.001). Similarly, weighted WVAL GI differed significantly by season (F[3; 796] = 23.9; p<0.001). Tukey post-hoc test showed noticeable seasonal structuring for weighted WVAL GII. Winter and spring showed significantly higher values compared with summer and fall. In contrast, weighted WVAL GI revealed a modest seasonal pattern of a visible winter peak and relatively similar values for spring, summer, and fall. Temporal trends of norovirus-specific and all-cause AGE hospitalization incidence with GI and GII WVAL showed no notable similarities (Appendix Figure 1).

Temporal and Spatial Distribution of AGE Hospitalizations

Weekly hospitalizations across the participating counties revealed both the temporal and spatial patterns in norovirus and all-cause AGE hospitalization incidence; incidence of norovirus-specific hospitalizations was slightly higher in the winter and spring (Figure 6). ANOVA indicated statistically significant difference in seasonal norovirus hospitalization incidence (F[3; 376] = 4.666; p<0.01). Tukey post-hoc analysis showed significant differences in norovirus hospitalization incidence between spring and fall (p<0.01) and between winter and fall (p<0.01); we observed no significant differences between other season pairs

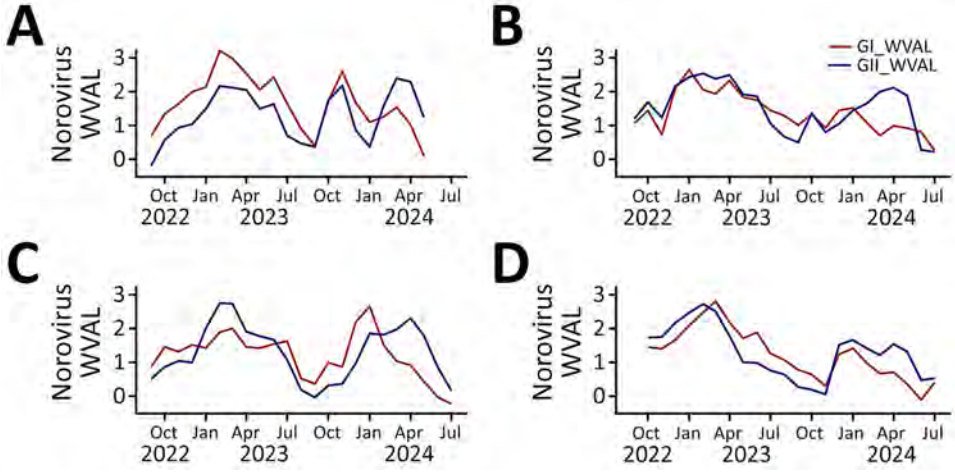


Figure 6. Relative abundance of norovirus in wastewater in study of correlation between norovirus in wastewater with norovirus-attributable and general acute gastroenteritis–related hospitalizations, New York, USA, 2022–2024. Abundance was measured as wastewater activity level, which increased in the winter months and decreased in the summer months. A) Erie County. B) Jefferson County. C) Onondaga County. D) Westchester County. WVAL, norovirus wastewater activity level.

(p>0.05). The incidence of all-cause AGE hospitalizations remained relatively stable over the study period (p>0.05); seasonal mean incidence was 2.87 for spring, 2.87 for winter, 2.91 for fall, and 2.74, for summer. Onondaga County had the highest mean incidence of all-cause AGE hospitalizations (3.15/100,000 population), followed closely by Erie County (3.02/100,000 population), then Westchester County (2.94/100,000 population) and Jefferson County (2.31/100,000 population). Jefferson County had the highest norovirus AGE mean incidence (0.251/100,000 population); Erie County had the lowest norovirus AGE mean incidence (0.039/100,000 population). Mean incidence of norovirus hospitalizations for Onondaga County was 0.159/100,000 population and for Westchester County was 0.103/100,000 population.

GI and GII Concentrations and Norovirus and All-Cause AGE Hospitalizations Incidence

We determined weekly correlations between population-weighted GI and GII concentrations and the incidence of norovirus hospitalizations (Figures 7, 8) or all-cause AGE hospitalizations for the 4 counties (Figures 9, 10). Correlation between population-weighted GI concentrations with incidence of norovirus hospitalizations was −0.18 to 0.06 (Figure 7) and for all-cause AGE hospitalizations was −0.12 to 0.03 (Figure 9). Correlations between population-weighted GII concentrations and incidence of norovirus hospitalizations showed moderate positive relationship in 3 counties; Spearman ρ was 0.23–0.28 (Figure 8). Population-weighted GII concentrations and all-cause AGE were also weakly correlated; Spearman ρ was −0.02 to 0.17 (Figure 10). Monthly correlations between population-weighted GI concentrations and incidence of norovirus hospitalizations or all-cause AGE for the 4 counties showed no significant correlation, whereas correlations between population-weighted GII concentrations and incidence of norovirus hospitalizations and all-cause AGE hospitalizations improved in 3 counties (Appendix Figure 2).

Monthly correlations between norovirus WVAL and the incidence of norovirus-specific and all-cause AGE incidence showed a similar relationship to correlations between norovirus concentrations and incidence (Figures 11–14). County-specific correlations revealed no significant correlations between monthly norovirus incidence (Figure 11) and monthly all-cause AGE hospitalization incidence (Figure 13) with GI WVAL. County-specific association between norovirus incidence and GII WVAL indicated moderate correlation in Jefferson, Onondaga, and Westchester counties (Spearman ρ 0.29–0.38) (Figure 12); correlation with all-cause AGE hospitalization incidence was low (ρ<0.2) for all 4 counties (Figure 14). However, we observed no significant correlations between weekly norovirus GI or GII WVAL and norovirus incidence and gastroenteritis incidence (Appendix Figure 3).

Discussion

We consistently detected GI and GII norovirus in wastewater collected during October 2022–July 2024 across 4 counties in New York: GI in 100% of samples and GII in 92%–100% of samples. We observed

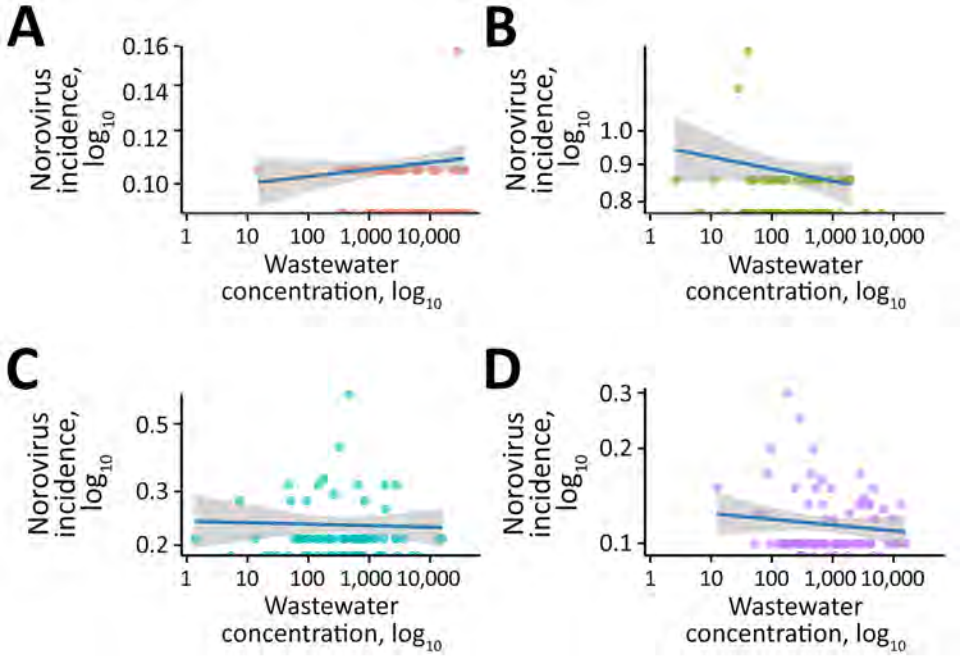


Figure 7. Correlations between population-weighted concentration of norovirus genotype I in wastewater and norovirus-specific incidence of acute gastroenteritis–related hospitalizations in study of correlation between norovirus in wastewater with norovirus-attributable and general acute gastroenteritis–related hospitalizations, New York, USA, 2022–2024. Blue line represents linear regression fit and gray shading indicates 95% CIs. A) Erie County; Spearman rank correlation coefficient (ρ) = –0.18. B) Jefferson County; ρ= 0.06. C) Onondaga County; ρ = 0. D) Westchester County; ρ = –0.1.

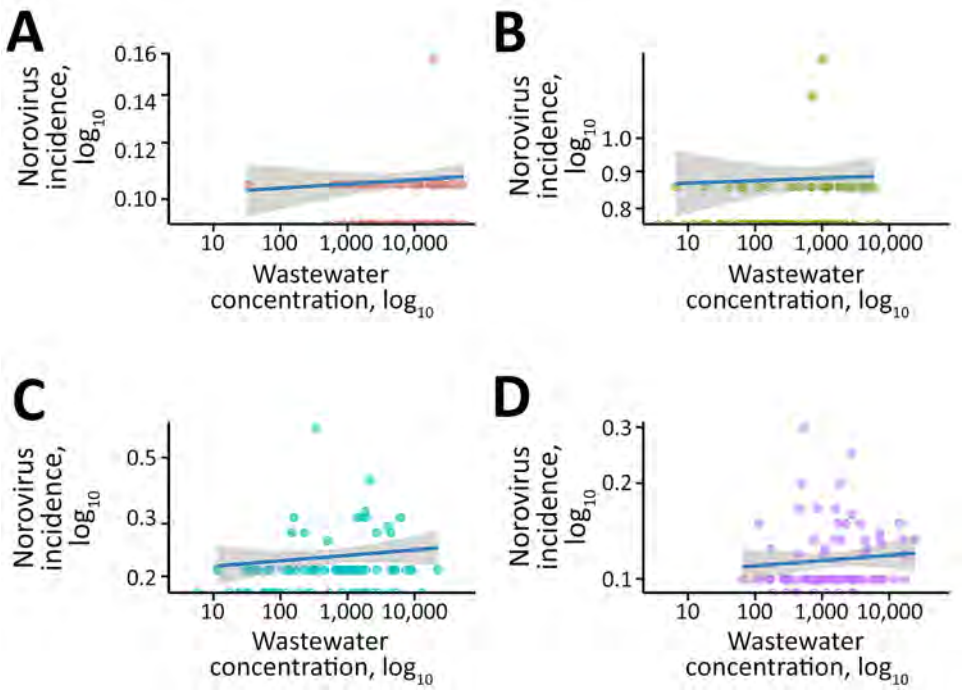


Figure 8. Correlations between population-weighted concentration of norovirus genotype II in wastewater and norovirus-specific incidence of acute gastroenteritis–related hospitalizations in study of correlation between norovirus in wastewater with norovirus-attributable and general acute gastroenteritis–related hospitalizations, New York, USA, 2022–2024. Blue line represents linear regression fit and gray shading indicates 95% CIs. A) Erie County; Spearman rank correlation coefficient (ρ) = –0.03. B) Jefferson County; ρ = 0.26. C) Onondaga County; ρ = 0.28. D) Westchester County; ρ = 0.23.

strong seasonal trends, with higher concentrations of both genogroups in winter and spring and lower concentrations in summer, aligning with the established seasonality of norovirus hospitalizations (*26*,*27*). Hospitalizations showed significant seasonal differences (Figure 5), with higher incidence in winter and spring, which we did not observe for all-cause AGE hospitalizations. Because incidence of norovirus-specific AGE was low, it is difficult to see those differences. Correlations between wastewater concentrations and hospitalization incidence were generally weak to moderate (−0.18 to 0.28). Neither monthly nor weekly correlations were significantly stronger with the WVAL metric.

Our findings are consistent with previous studies that reported weak to moderate positive correlations

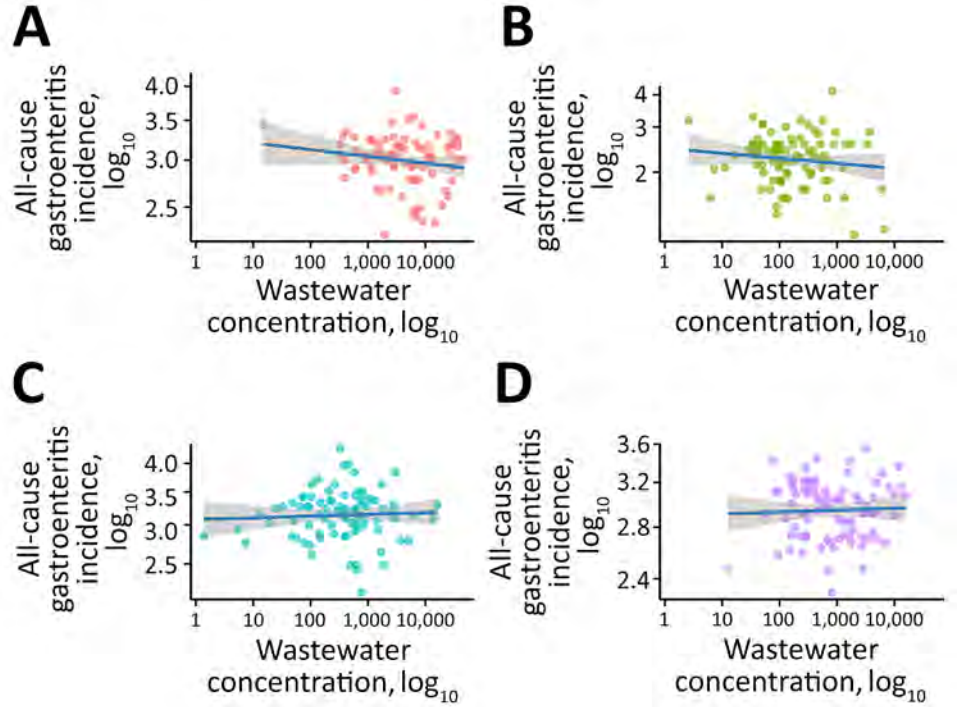


Figure 9. Correlations between population-weighted concentration of norovirus genotype I in wastewater and incidence of all-cause acute gastroenteritis–related hospitalizations in study of correlation between norovirus in wastewater with norovirus-attributable and general acute gastroenteritis–related hospitalizations, New York, USA, 2022–2024. Blue line represents linear regression fit and gray shading indicates 95% CIs. A) Erie County; Spearman rank correlation coefficient (ρ) = –0.12. B) Jefferson County; ρ = –0.07. C) Onondaga County; ρ = 0.03. D) Westchester County; ρ = –0.04.

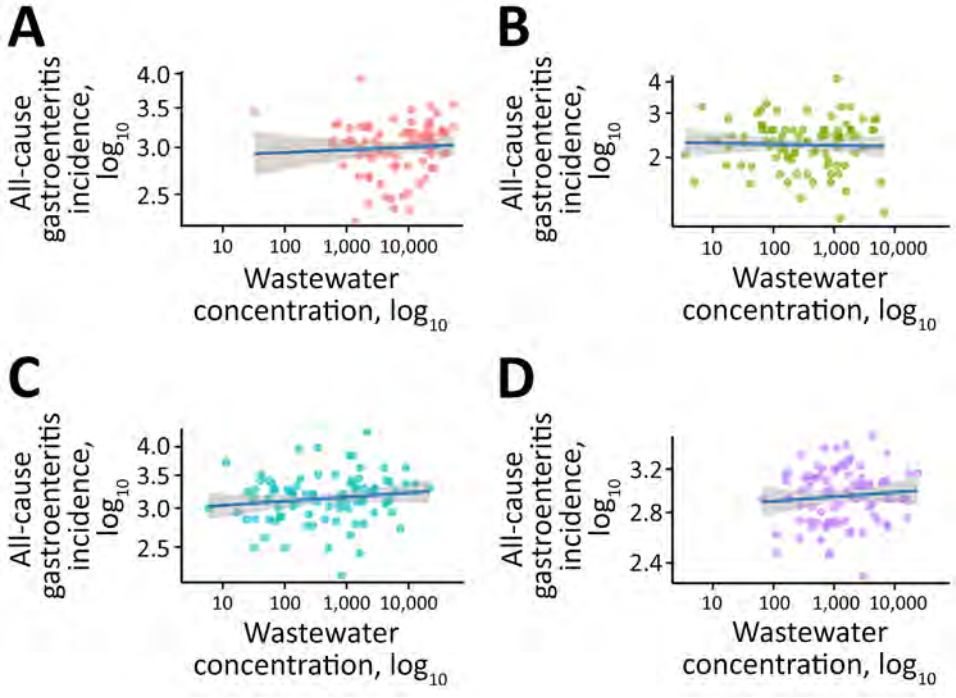


Figure 10. Correlations between population-weighted concentration of norovirus genotype II in wastewater and incidence of all-cause acute gastroenteritis–related hospitalizations in study of correlation between norovirus in wastewater with norovirus-attributable and general acute gastroenteritis–related hospitalizations, New York, USA, 2022–2024. Blue line represents linear regression fit and gray shading indicates 95% CIs. A) Erie County; Spearman rank correlation coefficient (ρ) = 0.12. B) Jefferson County; ρ = 0.02. C) Onondaga County; ρ = 0.17. D) Westchester County; ρ = 0.07.

between norovirus wastewater measures and clinical measures. A study of county data in Michigan, United States (*12*), showed human norovirus GII wastewater levels leading total weekly gastrointestinal illness–related emergency room visits by 2 weeks; correlations were ρ = −0.20 to 0.81. In British Columbia (*14*), norovirus levels in wastewater and weekly norovirus outbreaks were also positively correlated; Kendall τ correlation was 0.21–0.36, depending on normalization method. A linear regression analysis of data in England (*15*) showed a significant linear relationship between norovirus levels in wastewater and weekly cases (R^2 = 0.614). Last, a systematic and meta-analysis study (*11*) of norovirus wastewater correlations with cases and outbreaks reported associations of −0.03 to 0.44. However, difference in scale of spatial aggregation of wastewater data, such as nationwide aggregation (*13*), might suggest that improved correlations are a product of the modifiable area unit problem, whereas ecologic studies are subject to risk masking at regional or higher spatial aggregation (*28*). Large-scale spatial aggregation can

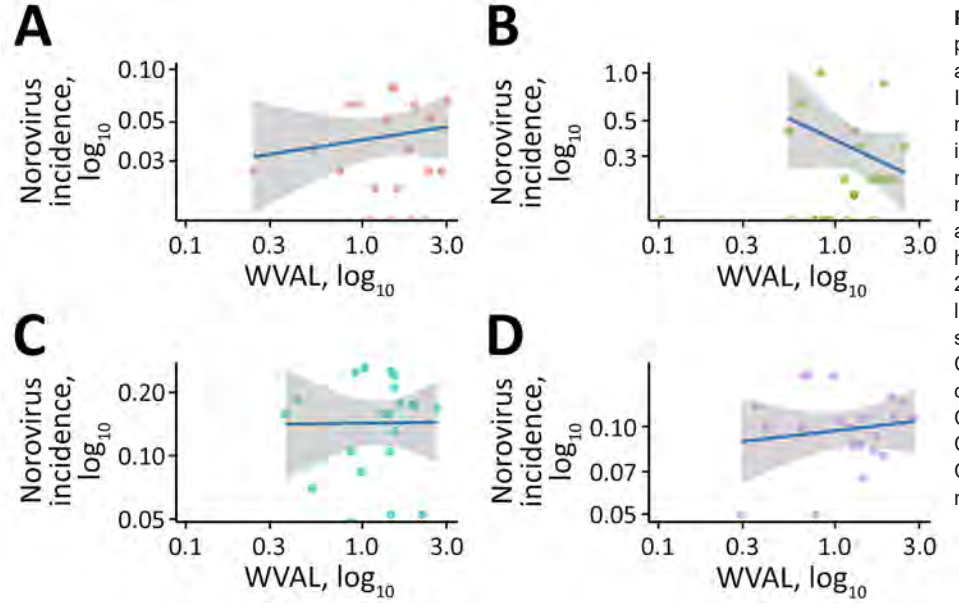


Figure 11. Correlations between population-weighted wastewater activity level of norovirus genotype I in wastewater and incidence of norovirus-specific hospitalizations in study of correlation between norovirus in wastewater with norovirus-attributable and general acute gastroenteritis–related hospitalizations, New York, USA, 2022–2024. Blue line represents linear regression fit and gray shading indicates 95% CIs. A) Erie County; Spearman rank correlation coefficient (ρ) = –0.12. B) Jefferson County; ρ = 0.3. C) Onondaga County; ρ= 0.07. D) Westchester County; ρ = –0.09. WVAL, norovirus wastewater activity level.

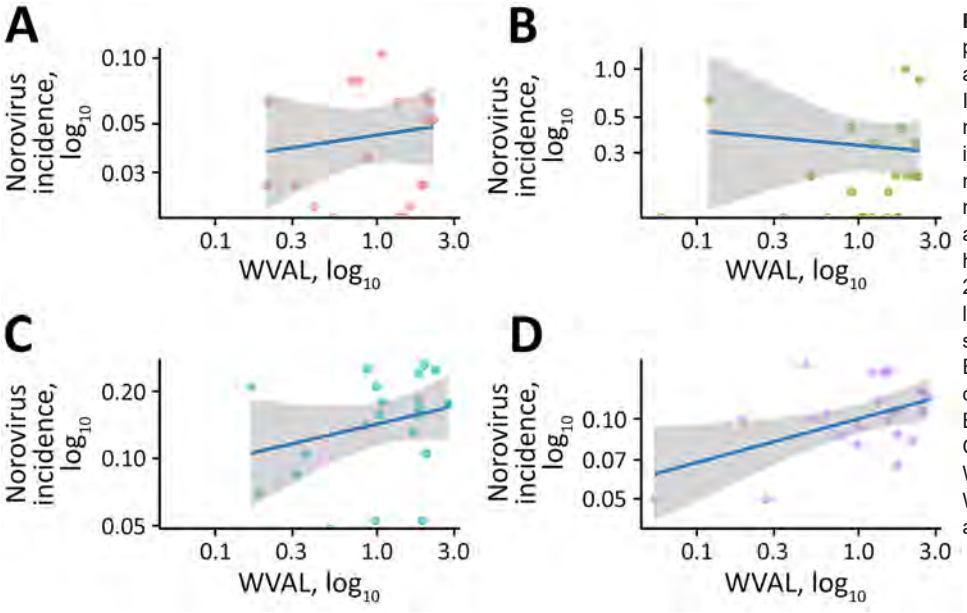


Figure 12. Correlations between population-weighted wastewater activity level of norovirus genotype II in wastewater and incidence of norovirus-specific hospitalizations in study of correlation between norovirus in wastewater with norovirus-attributable and general acute gastroenteritis–related hospitalizations, New York, USA, 2022–2024. Blue line represents linear regression fit and gray shading indicates 95% CIs. A) Erie County; Spearman rank correlation coefficient (ρ) = 0.05. B) Jefferson County; ρ = 0.38. C) Onondaga County; ρ = 0.32. D) Westchester County; ρ = 0.29. WVAL, norovirus wastewater activity level.

affect risk perception and association and is unreliable for local epidemiologic interventions. Similarly, correlations between wastewater and clinical data might vary because of fundamental differences across case, hospitalization, and outbreak data. Also, wastewater data normalization methods using wastewater flow rate in liters/day or population served by each WWTP might affect the relationship between norovirus levels in wastewater and clinical data.

The correlations we observed between norovirus GI and GII in wastewater and hospitalizations were lower than those observed in WBE studies of other pathogens (*29*–*31*). The observed seasonality, with peaks in winter and spring, corroborates established norovirus transmission patterns in temperate regions (*26*,*27*). Higher correlations between hospitalizations and GII concentrations may reflect the dominance of GII strains in large-scale outbreaks (*16*). Weak to moderate correlations between wastewater concentrations (GI or GII) and hospitalization incidence suggest that although WBE can capture population-level norovirus trends, it might not directly predict hospitalizations. That finding is consistent with previous inferences that, although WBE is effective for detecting community-level disease circulation, WBE results might not directly correlate with clinical disease occurrence (*32*,*33*). Possible reasons include temporal misalignment, spatial mismatch, scale of aggregation,

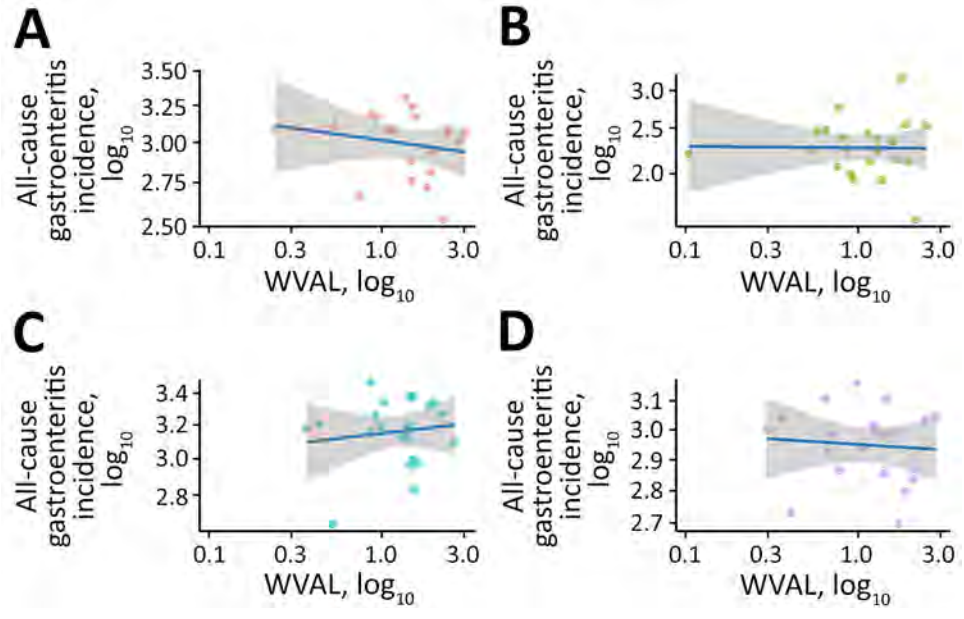


Figure 13. Correlations between population-weighted wastewater activity level of norovirus genotype I in wastewater and incidence of all-cause acute gastroenteritis–related hospitalizations in study of correlation between norovirus in wastewater with norovirus-attributable and general acute gastroenteritis–related hospitalizations, New York, USA, 2022–2024. Blue line represents linear regression fit and gray shading indicates 95% CIs. A) Erie County; Spearman rank correlation coefficient (ρ) = –0.35. B) Jefferson County; ρ = –0.03. C) Onondaga County; ρ = 0.07. D) Westchester County; ρ = –0.1. WVAL, norovirus wastewater activity level.

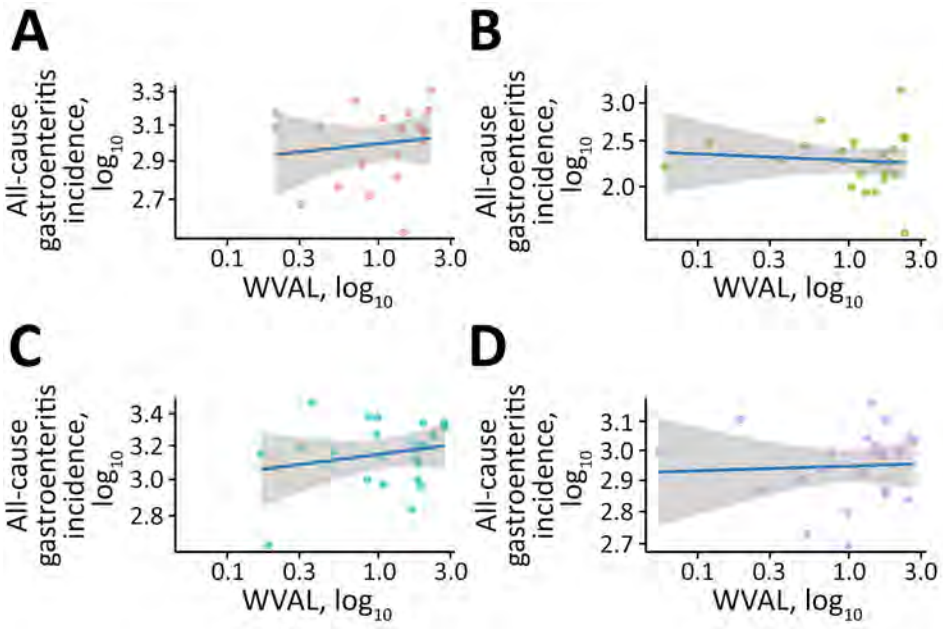


Figure 14. Correlations between population-weighted wastewater activity level of norovirus genotype II in wastewater and incidence of all-cause acute gastroenteritis–related hospitalizations in study of correlation between norovirus in wastewater with norovirus-attributable and general acute gastroenteritis–related hospitalizations, New York, USA, 2022–2024. Blue line represents linear regression fit and gray shading indicates 95% CIs. A) Erie County; Spearman rank correlation coefficient (ρ) = 0.14. B) Jefferson County; ρ = –0.03. C) Onondaga County; ρ = 0.13. D) Westchester County; ρ = 0.14. WVAL, norovirus wastewater activity level.

heterogeneity in pathogen shedding, testing and reporting bias, and environmental and technical noise in wastewater data. Because norovirus infections are often asymptomatic or self-limiting and reporting is not mandatory, cases are underreported and clinical data are underrepresented (*34*). In addition, the lack of strong correlation between wastewater and clinical data likely reflects the self-limiting nature of norovirus and the fact that norovirus cases are underreported in clinical data.

The higher concentrations of norovirus in wastewater from Erie County are likely a result of differences in laboratory processing methods; some samples from Erie County underwent magnetic bead processing. We did not find evidence of elevated numbers of hospitalizations in Erie County, which suggests that the laboratory method is the likely reason for the difference. We observed similar differences in the recovery of SARS-CoV-2 in wastewater with the different concentration methods.

A limitation of our study is that WBE as a surveillance method cannot attribute viral loads to specific persons, which limits its use for providing granular epidemiologic data and follow-up crucial to outbreak investigations (*35*). Second, norovirus-specific hospitalization data are likely an underestimate of norovirus-caused hospitalizations. All-cause AGE is overly broad, and the norovirus-specific AGE International Classification of Diseases codes require laboratory confirmation of norovirus; not all patients will necessarily be tested for all pathogens. Third, wastewater was sampled weekly; however, disease duration of norovirus is often shorter, which might result in a temporal mismatch of data that could obscure finer-scale temporal dynamics and weaken the observed associations between wastewater viral concentrations and hospitalization incidence. We attempted to address that limitation by using the 7-day moving average but found no significant temporal lags or leads. Fourth, our study used 2 different laboratory methods, which made comparison between Erie County and the others more difficult but suggests that the method used to process Erie County samples resulted in greater nucleic acid recovery. Fifth, hospitalizations were relatively rare across the study area and period. It is likely that not all patients who sought care for norovirus infection at a hospital were tested or received diagnosis. Last, the New York state wastewater surveillance network does not routinely collect flow data from participating WWTPs, which precludes any examination of whether normalizing the norovirus concentration in wastewater by flow could improve correlation with hospitalization data.

The results of this study reinforce the ability of WBE to monitor circulating norovirus and suggest that AGE hospitalizations are a small part of the norovirus public health burden. Contrary to its use for other pathogens, such as COVID-19 (*29*), WBE is unlikely to be useful for predicting norovirus hospitalizations. The moderate correlations observed in Erie County and during peak seasons suggest that WBE could be optimized to inform public health interventions, especially during peak seasons, but more evidence is needed. Distinguishing between GI and GII trends highlights the importance of genogroup-specific monitoring; the association of GII with severe

outbreaks (*36*,*37*) could guide resource allocation for outbreak preparedness. In this capacity, WBE is useful for understanding which norovirus genotypes are circulating in a particular population.

Acknowledgments

We thank the WWTP operators who pull samples for the New York state wastewater surveillance network.

This study was supported by the CDC's ELC Program, New York State Unique Federal Award no. NU50CK000516 (New York State Epidemiology and Laboratory Capacity for Prevention and Control of Emerging Infectious Diseases).

About the Author

Dr. Nwabor is a public health scientist with the Onondaga County Health Department. His research interests include infectious disease epidemiology, vectorborne diseases, environmental surveillance, and the application of geospatial and data-analytic methods to public health.

References

1. Carlson KB, Dilley A, O'Grady T, Johnson JA, Lopman B, Viscidi E. A narrative review of norovirus epidemiology, biology, and challenges to vaccine development. NPJ Vaccines. 2024;9:94. https://doi.org/10.1038/s41541-024-00884-2
2. Hall AJ, Vinjé J, Lopman B, Park GW, Yen C, Gregoricus N, et al. Updated norovirus outbreak management and disease prevention guidelines. MMWR Recomm Rep. 2011;60:1–15.
3. Chhabra P, de Graaf M, Parra GI, Chan MCW, Green K, Martella V, et al. Updated classification of norovirus genogroups and genotypes. J Gen Virol. 2019;100:1393–406. https://doi.org/10.1099/jgv.0.001318
4. Doh H, Lee C, Kim NY, Park YY, Kim EJ, Choi C, et al. Genomic diversity and comparative phylogenomic analysis of genus *Norovirus*. Sci Rep. 2025;15:5412. https://doi.org/10.1038/s41598-025-87719-9
5. Donaldson AL, Harris JP, Vivancos R, O'Brien SJ. Can cases and outbreaks of norovirus in children provide an early warning of seasonal norovirus infection: an analysis of nine seasons of surveillance data in England UK. BMC Public Health. 2022;22:1393. https://doi.org/10.1186/s12889-022-13771-z
6. Lopman B, Armstrong B, Atchison C, Gray JJ. Host, weather and virological factors drive norovirus epidemiology: time-series analysis of laboratory surveillance data in England and Wales. PLoS One. 2009;4:e6671. https://doi.org/10.1371/journal.pone.0006671
7. Bartsch SM, O'Shea KJ, Lee BY. The clinical and economic burden of norovirus gastroenteritis in the United States. J Infect Dis. 2020;222:1910–9. https://doi.org/10.1093/infdis/jiaa292
8. Cardemil CV, Parashar UD, Hall AJ. Norovirus infection in older adults: epidemiology, risk factors, and opportunities for prevention and control. Infect Dis Clin North Am. 2017;31:839–70. https://doi.org/10.1016/j.idc.2017.07.012
9. Robilotti E, Deresinski S, Pinsky BA. Norovirus. Clin Microbiol Rev. 2015;28:134–64. https://doi.org/10.1128/CMR.00075-14
10. Larsen DA, Wigginton KR. Tracking COVID-19 with wastewater. Nat Biotechnol. 2020;38:1151–3. https://doi.org/10.1038/s41587-020-0690-1
11. Huang Y, Zhou N, Zhang S, Yi Y, Han Y, Liu M, et al. Norovirus detection in wastewater and its correlation with human gastroenteritis: a systematic review and meta-analysis. Environ Sci Pollut Res Int. 2022;29:22829–42. https://doi.org/10.1007/s11356-021-18202-x
12. Ammerman ML, Mullapudi S, Gilbert J, Figueroa K, de Paula Nogueira Cruz F, Bakker KM, et al. Norovirus GII wastewater monitoring for epidemiological surveillance. PLOS Water. 2024;3:e0000198. https://doi.org/10.1371/journal.pwat.0000198
13. Boehm AB, Wolfe MK, White BJ, Hughes B, Duong D, Banaei N, et al. Human norovirus (HuNoV) GII RNA in wastewater solids at 145 United States wastewater treatment plants: comparison to positivity rates of clinical specimens and modeled estimates of HuNoV GII shedders. J Expo Sci Environ Epidemiol. 2024;34:440–7. https://doi.org/10.1038/s41370-023-00592-4
14. Treagus S, Kopetzky J, Tchao C, Chan T, Bishay F, Jeong HJ, et al. Norovirus trends in British Columbia from 2021 to 2022: the relationship between wastewater surveillance and clinical outbreak data during the COVID-19 pandemic. Sci Rep. 2025;15:22614. https://doi.org/10.1038/s41598-025-06940-8
15. Walker DI, Witt J, Rostant W, Burton R, Davison V, Ditchburn J, et al. Piloting wastewater-based surveillance of norovirus in England. Water Res. 2024;263:122152. https://doi.org/10.1016/j.watres.2024.122152
16. Barclay L, Vinjé J. Increasing predominance of norovirus GII.17 over GII.4, United States, 2022–2025. Emerg Infect Dis. 2025;31:1471–3. https://doi.org/10.3201/eid3107.250524
17. Xu J, Du H, Yu J, Wu R, Zhang Y, Lu Q, et al. Detection of IgG antibody against the porcine norovirus GII.11 in human, domestic and wild animals. Front Microbiol. 2025;16:1567132. https://doi.org/10.3389/fmicb.2025.1567132
18. Hu X, Sun L, Han T, Zhao J, Qi X, Zhang Y, et al. The genetic diversity of genogroup I noroviruses causing acute gastroenteritis outbreaks in Beijing between 2014 and 2023. Heliyon. 2024;10:e39202. https://doi.org/10.1016/j.heliyon.2024.e39202
19. Neyra M, Hill DT, Bennett LJ, Dunham CN, Larsen DA; New York State Wastewater Surveillance Network Team. Establishing a statewide wastewater surveillance system in response to the COVID-19 pandemic: a reliable model for continuous and emerging public health threats. J Public Health Manag Pract. 2023;29:854–62. https://doi.org/10.1097/PHH.0000000000001797
20. Segelhurst E, Bard JE, Gallo S, Huang V, Pohlman A, Yergeau DA, et al. Recovering wastewater RNA for virome sequencing by systematically optimized tangential-flow ultrafiltration and Nanotrap microbiome particles. Appl Environ Microbiol. 2025;91:e00777-25. https://doi.org/10.1128/aem.00777-25
21. Wilder ML, Middleton F, Larsen DA, Du Q, Fenty A, Zeng T, et al. Co-quantification of crAssphage increases confidence in wastewater-based epidemiology for SARS-CoV-2 in low prevalence areas. Water Res X. 2021;11:100100. https://doi.org/10.1016/j.wroa.2021.100100
22. Weidmann MD, Bryant PW, Schoultz L, Jadhav A, Rickerman L, Hill DT, et al. A pilot study to correlate wastewater and clinical surveillance for hepatitis A in

New York state. Epidemics. 2025;53:100867. https://doi.org/10.1016/j.epidem.2025.100867
23. Han Y, Wang J, Zhang S, Yang S, Wang X, Han Y, et al. Simultaneous quantification of hepatitis A virus and norovirus genogroup I and II by triplex droplet digital PCR. Food Microbiol. 2022;103:103933. https://doi.org/10.1016/j.fm.2021.103933
24. Centers for Disease Control and Prevention. International classification of diseases, 10th revision, clinical modification (ICD-10). 2024 [cited 2025 Oct 13]. https://www.cdc.gov/nchs/icd/icd-10-cm
25. Centers for Disease Control and Prevention. Using the wastewater viral activity level. 2026 [cited 2026 Aug 17]. https://www.cdc.gov/wastewater/about/wval.html
26. Kambhampati AK, Calderwood L, Wikswo ME, Barclay L, Mattison CP, Balachandran N, et al. Spatiotemporal trends in norovirus outbreaks in the United States, 2009–2019. Clin Infect Dis. 2023;76:667–73. https://doi.org/10.1093/cid/ciac627
27. Mattison CP, Calderwood LE, Cates JE, Donald J, Hall AJ, Schmidt MA, et al. Seasonality of medically attended norovirus gastroenteritis and its association with climatic factors within an US integrated healthcare system, 2016–2019. PLoS One. 2025;20:e0318077. https://doi.org/10.1371/journal.pone.0318077
28. Zhu Y, Hill DT, Zhou Y, Larsen DA. The effect of the modifiable areal unit problem (MAUP) on spatial aggregation of COVID-19 wastewater surveillance data. Sci Total Environ. 2024;957:177676. https://doi.org/10.1016/j.scitotenv.2024.177676
29. Hill DT, Alazawi MA, Moran EJ, Bennett LJ, Bradley I, Collins MB, et al. Wastewater surveillance provides 10-days forecasting of COVID-19 hospitalizations superior to cases and test positivity: a prediction study. Infect Dis Model. 2023;8:1138–50. https://doi.org/10.1016/j.idm.2023.10.004
30. McCall C, Wu H, Miyani B, Xagoraraki I. Identification of multiple potential viral diseases in a large urban center using wastewater surveillance. Water Res. 2020;184:116160. https://doi.org/10.1016/j.watres.2020.116160
31. Monteiro S, Pimenta R, Nunes F, Cunha MV, Santos R. Detection of dengue virus and chikungunya virus in wastewater in Portugal: an exploratory surveillance study. Lancet Microbe. 2024;5:100911. https://doi.org/10.1016/S2666-5247(24)00150-2
32. Boehm AB, Hughes B, Duong D, Chan-Herur V, Buchman A, Wolfe MK, et al. Wastewater concentrations of human influenza, metapneumovirus, parainfluenza, respiratory syncytial virus, rhinovirus, and seasonal coronavirus nucleic-acids during the COVID-19 pandemic: a surveillance study. Lancet Microbe. 2023;4:e340–8. https://doi.org/10.1016/S2666-5247(22)00386-X
33. Li X, Zhang S, Sherchan S, Orive G, Lertxundi U, Haramoto E, et al. Correlation between SARS-CoV-2 RNA concentration in wastewater and COVID-19 cases in community: a systematic review and meta-analysis. J Hazard Mater. 2023;441:129848. https://doi.org/10.1016/j.jhazmat.2022.129848
34. Centers for Disease Control and Prevention. Norovirus facts and stats. 2024 [cited 2025 Aug 8]. https://www.cdc.gov/norovirus/data-research/index.html
35. O'Reilly KM, Wade MJ, Farkas K, Amman F, Lison A, Munday JD, et al. Analysis insights to support the use of wastewater and environmental surveillance data for infectious diseases and pandemic preparedness. Epidemics. 2025;51:100825. https://doi.org/10.1016/j.epidem.2025.100825
36. Desai R, Hembree CD, Handel A, Matthews JE, Dickey BW, McDonald S, et al. Severe outcomes are associated with genogroup 2 genotype 4 norovirus outbreaks: a systematic literature review. Clin Infect Dis. 2012;55:189–93. https://doi.org/10.1093/cid/cis372
37. Haddadin Z, Batarseh E, Hamdan L, Stewart LS, Piya B, Rahman H, et al. Characteristics of GII.4 norovirus versus other genotypes in sporadic pediatric infections in Davidson County, Tennessee, USA. Clin Infect Dis. 2021;73:e1525–31. https://doi.org/10.1093/cid/ciaa1001

Address for correspondence: Dave Larssen, Department of Public Health, Maxwell School of Citizenship and Public Affairs, Syracuse University, 444 White Hall, Syracuse, NY 13210, USA; email: dalarsen@syr.edu

Monkeypox Virus Surveillance in Wastewater, North Carolina, USA, October 2023–May 2025

Nicole L. Snyder, Thomas Clerkin, Sarah H. Koenigsberg, Mara B. Tate, Ty L. Lautenschlager, Justin Albertson, Emma L. Doran, Erica F. Wilson, Rachel T. Noble, Virginia T. Guidry, Ariel R. Christensen

In September 2023, the North Carolina Department of Health and Human Services identified a new case of mpox clade II 5 months after the last previous report. To assess possible unrecognized transmission, nonvariola orthopoxvirus testing was implemented at select wastewater sites. During October 29, 2023–May 2025, a total of 801 samples were collected across 16 treatment plants serving ≈2 million residents. We analyzed wastewater detections (n = 47) and clinical cases (n = 45) using a ±17-day window. Overall sensitivity of identifying the presence of ≥1 temporally aligned mpox case within a sewershed was 10.6% (95% CI 6.5%–16.7%); specificity was 95.1% (95% CI 93.2%–96.5%), positive predictive value was 31.9% (95% CI 20.4%–46.2%), and negative predictive value was 83.2% (95% CI 80.3%–85.7%). Restricting detections above the limit of detection reduced sensitivity but increased specificity. Overall, wastewater surveillance showed low sensitivity but provided complementary awareness for public health response.

Since cases were first identified outside mpox–endemic regions in 2022 mpox has emerged as an increasing public health concern in the United States. The global outbreak in 2022 affected 75 countries and territories; >2,300 cases had been confirmed in the United States by July 2022 (*1*). Because of evolving transmission dynamics, severity, and uncertainty surrounding social and economic impacts, the World Health Organization declared mpox a public health emergency of international concern on July 23, 2022 (*2*–*3*).

Mpox is an infectious disease endemic to regions of Central and West Africa. It is caused by the enveloped DNA virus monkeypox virus (MPXV) and is characterized by fever, headache, myalgia, lymphadenopathy, and a vesiculopustular rash that can involve the face, extremities, and genital region (*3*). Mpox is primarily transmitted through direct contact with active lesions, contaminated materials, bodily fluids, or respiratory secretions during dose contact (*4*,*5*). Mpox infections are categorized into 2 geneticallly distinct clades, clade I and clade II. Clade II infections, which were responsible for the global outbreak that began in 2022, are generally associated with less severe disease and reduced transmissibility (*5*). Historically, clade I mpox infections have demonstrated greater virulence, mortality, and transmission (*6*).

The Centers for Disease Control and Prevention (CDC) National Wastewater Surveillance System initiated wastewater testing for MPXV in October 2022, five months after the mpox outbreak began in the United States. For underreported and stigmatized infections such as mpox, traditional case-based surveillance can present challenges, including incomplete capture of subclinical or unreported cases, making wastewater surveillance (WS) a valuable complementary approach to clinical mpox surveillance (*5*,*6*). Early work demonstrated that viral shedding through urine, feces, respiratory secretions, and skin lesions can result in detectable MPXV in wastewater, supporting wastewater-based epidemiology as a viable approach for monitoring and quantifying community transmission (*2*,*7*,*8*). In 1 study, MPXV detections in wastewater were shown to precede the identification of the clinical cases, highlighting its potential for early outbreak detection (*9*). Another study reported increases in wastewater viral concentrations ahead of reported clinical cases (*5*). Studies

Author affiliations: Division of Public Health, North Carolina Department of Health and Human Services, Raleigh, North Carolina, USA (N.L. Snyder, S.H. Koenigsberg, M.B. Tate, T.L. Lautenschlager, J. Albertson, E.L. Doran, E.F. Wilson, V.T. Guidry, A.R. Christensen); Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, North Carolina, USA (T. Clerkin, R.T. Noble); Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA (R.T. Noble)

DOI: https://doi.org/10.3201/eid3213.260414

have also documented correlations between wastewater MPXV concentrations and incident mpox cases within corresponding sewersheds, supporting the utility of WS as a proxy for community-level transmission (*10*). Finally, wastewater detections in areas without corresponding clinical reports may help identify communities where mpox transmission might otherwise remain undetected because of social stigma, limited clinical recognition, or constrained diagnostic capacity (*5*,*9*).

Recognizing WS as a valuable strategy for early identification of infectious disease activity, the North Carolina Department of Health and Human Services (NCDHHS) integrated mpox monitoring into its statewide WS program. In September 2023, nonvariola orthopoxvirus testing was implemented at selected WS sites across the state. That assay detects orthopoxviruses including vaccinia, cowpox, and all clades of MPXV. Primary sites were facilities in Asheville, Charlotte, Greensboro, Greenville, and Raleigh; additional periodic testing was conducted at selected sites in Cary, Chapel Hill, North Durham, and South Durham. To evaluate mpox WS program in North Carolina, we conducted this study to estimate sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) relative to confirmed clinical cases.

Methods

Study Area and Surveillance Period

We conducted a retrospective diagnostic performance analysis to evaluate WS for detecting MPXV circulation in North Carolina. For the period October 29, 2023–May 31, 2025, we compared wastewater detections from the North Carolina Wastewater Monitoring Network, operated by the NCDHHS, with confirmed clinical mpox cases using a ±17-day temporal window. We assumed clinical case reporting to be complete for the purposes of this analysis. The study included data from 16 publicly owned wastewater treatment plants (WWTPs) with defined catchment areas serving a combined population of 2,002,864 persons (Table 1). Facilities were in urban and semiurban regions and consisted of 4 plants in Charlotte, 3 plants each in Cary and Raleigh, and 1 plant each in Asheville, Chapel Hill, Greensboro, Greenville, North Durham, and South Durham. During the study period, 45 confirmed mpox cases occurred within the included sewersheds. We excluded sites sampled through CDC-contracted programs (Verily, https://verily.com) and WastewaterSCAN (https://www.wastewaterscan.org) because of differences in laboratory methods, assays, reporting structures, and sampling frequency.

Clinical Case Data

We also obtained clinical case data from NCDHHS. A confirmed mpox case was defined according to the Council of State and Territorial Epidemiologists case definition from 2022 (22-ID-10) as detection of nonvariola orthopoxvirus or MPXV DNA in a clinical specimen by PCR in a patient with compatible symptoms (*11*). We defined case date as date of symptom onset. We geocoded cases using residential address and spatially assigned them to sewersheds by using ArcGIS Pro (Esri, https://www.esri.com).

Wastewater Sample Collection and Laboratory Testing.

Influent wastewater was collected as 24-hour composite samples approximately 1 time/week at each WWTP except for the 3 plants in Cary and those in Chapel Hill, North Durham, and South Durham, which were only sampled selectively over the study period, generally in response to concerns about a confirmed or suspected mpox case in the general area. Samples were transported on ice and processed within 24 hours at the University of North Carolina at Chapel Hill. We quantified MPXV DNA using the CDC Non-variola Orthopoxvirus Generic Real-Time PCR Test (*12*). We originally optimized the assay in a wastewater matrix by conducting a thermal gradient from 62°C–52°C to see which annealing temperature resulted in the greatest separation from positive and negative droplet clusters (Appendix Figure, https://wwwnc.cdc.gov/EID/article/32/13/26-0414-App1.pdf). We reported results as viral gene copies normalized to flow rate and population within the sewershed. We classified samples with concentrations above the assay limit of detection (LOD) as positive (n = 17). We experimentally determined the assay limit of blank (LOB) to be 0 copies/L by using methods adapted from Clinical and Laboratory Standards Institute document EP17 (*13*). Because the LOB was 0, we theoretically estimated the assay LOD to be 500 copies/L, because Poisson distribution requires roughly 3 copies per sample to detect ≥1 copies with 95% probability (*14*,*15*). Using this strategy, we classified samples with concentrations from one half the LOD to the LOD as trace detections (n = 30).

Overall Sensitivity, Specificity, PPV, and NPV

We classified wastewater observations as positive or negative on the basis of laboratory results, as described previously. For each wastewater sample, the presence of ≥1 confirmed clinical case within ±17 days in the

Table 1. Wastewater treatment plant/sewershed characteristics in study of mpox surveillance in wastewater, North Carolina, USA, October 2023–May 2025*

Sewershed	Population served	Days measured	Positive days	Negative days	Clinical cases
Cary 1	84,189	7	2	5	0
Cary 2	74,331	7	0	7	1
Cary 3	75,886	7	1	6	1
Chapel Hill	78,141	2	0	2	0
Charlotte 1	68,685	82	4	78	0
Charlotte 2	182,501	84	6	78	6
Charlotte 3	120,000	78	3	75	11
Charlotte 4	82,158	80	9	71	10
Greensboro	135,821	70	3	67	0
Greenville	89,616	73	2	71	0
MSD Buncombe (Asheville)	173,000	61	2	59	5
North Durham	142,000	7	1	6	0
Raleigh	550,000	83	5	78	11
Raleigh 2	30,655	75	4	71	0
Raleigh 3	7,776	78	3	75	0
South Durham	108,105	7	2	5	0
Total	2,002,864	801	47	754	45

*MSD, Metropolitan Sewerage District.

same sewershed was assessed. We selected a ±17-day alignment window to approximate the biologically plausible period during which MPXV might be detectable in wastewater relative to a reported clinical case. Available data suggest that viral shedding is most likely to occur at 4 to 25 days (*16*,*17*) and might begin before diagnosis. Additional delays related to seeking care, laboratory confirmation, case reporting, and wastewater sampling can create temporal offsets between infection and detection. We selected the ±17-day window to balance those biological and operational considerations while minimizing misclassification. We defined diagnostic categories as follows: true positive (TP), wastewater-positive and ≥1 clinical case within ±17 days; false positive (FP), wastewater-positive and no clinical case within ±17 days; true negative (TN), wastewater-negative and no clinical case within ±17 days; and false negative (FN), wastewater-negative and ≥1 clinical case within ±17 days.

We calculated sensitivity (TP/[TP + FN]), specificity (TN/[TN + FP]), PPV (TP/[TP + FP]), and NPV (TN/[TN + FN]) overall and stratified results by WWTP/sewershed catchment. We calculated 95% CIs using Wilson score intervals because several sites had small denominators and proportions near 0 or 1. The primary analysis classified both positive and trace detections as wastewater-positive (n = 47). A secondary sensitivity analysis restricted wastewater-positive observations to samples exceeding the LOD (n = 17). Because clinical case surveillance is an imperfect reference standard and does not capture all infections, TP/FP/TN/FN classifications should be interpreted as concordance with reported cases rather than true infection status. All analyses were conducted in R version 4.2.3 (The R Project for Statistical Computing, https://www.r-project.org).

Results

During October 29, 2023–May 31, 2025, a total of 801 wastewater samples were collected across 16 WWTPs serving an estimated population of ≈2 million people. Of those, 47 (5.9%) were classified as wastewater-positive (17 above the LOD and 30 trace detections) and 754 (94.1%) were wastewater-negative. During the same period, 45 confirmed mpox cases were reported that fell within sewersheds representing the WWTPs in this study.

Using a ±17-day temporal alignment window, we classified 15 wastewater observations as TPs, 32 as FPs, 627 as TNs, and 127 as FNs (Table 2). The overall sensitivity of wastewater detection identifying the presence of ≥1 temporally aligned reported mpox case within a sewershed was 10.6% (95% CI 6.5%–16.7%). Specificity was 95.1% (95% CI 93.2%–96.5%). The PPV was 31.9% (95% CI 20.4%–46.2%), and the NPV was 83.2% (95% CI 80.3%–85.7%).

Table 2. Diagnostic performance metrics of wastewater monitoring in study of mpox surveillance in wastewater, North Carolina, USA, October 2023–May 2025*

Study	TP	FP	TN	FN	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Detections above LOD and trace detections	15	32	627	127	10.6 (6.5–16.7)	95.1 (93.2–96.5)	31.9 (20.4–46.2)	83.2 (80.3–85.7)
Detections above LOD only	2	15	644	140	1.4 (0.4%–5.0%)	97.7 (96.3–98.6)	11.8 (3.3–34.3)	82.1 (79.3–84.7)

*FN, false negative; FP, false positive; LOD, limit of detection; NPV, negative predictive value; PPV, positive predictive value; TN, true negative; TP, true positive.

At the WWTP sewershed level, sensitivity was highest at Charlotte WWTP 2 (25.0%) and ranged from 0% to 25.0% across sites with clinical activity (Table 3). Several sewersheds had no temporally aligned clinical cases during the surveillance period, resulting in undefined sensitivity estimates. Specificity was consistently high across sites, ranging from 71.4% to 100%. PPV varied widely by facility, ranging from <1% to 100%, reflecting differences in case occurrence and sampling frequency. NPV exceeded 69% at all sites with clinical activity and was 100% at several facilities without aligned cases.

When restricting wastewater-positive observations to samples exceeding the LOD (n = 17), sensitivity decreased from 10.6% (95% CI 6.5%–16.7%) to 1.4% (95% CI 0.4%–5.0%), and specificity increased from 95.1% (95% CI 93.2%–96.5%) to 97.7% (95% CI 96.3%–98.6%) (Table 2). The number of FP observations decreased from 32 to 15, whereas FN observations increased from 127 to 140. PPV decreased from 31.9% (95% CI 20.4%–46.2%) to 11.8% (95% CI 3.3%–34.3%), and NPV was similar (83.2% vs. 82.1%). Site-level performance patterns were unchanged.

Discussion

This evaluation assessed the performance of WS for detecting MPXV across 16 WWTPs in North Carolina during October 2023–May 2025. Using a ±17-day alignment window, wastewater detection demonstrated low sensitivity for identifying temporally aligned clinical cases (10.6% overall when including trace detections and 1.4% when restricted to detections above the LOD) but high specificity (>95%) and moderate NPV of ≈83%. We selected a ±17-day window to account for the expected duration of MPXV shedding and potential delays between symptom onset, clinical diagnosis, and case reporting, while also accommodating once-weekly wastewater sampling. Because wastewater represents a composite, time-integrated signal and samples are collected weekly, this window reduces misclassification that could arise from temporal offsets between individual infections and wastewater detection.

Those findings compare with national estimates reported in another study (*16*) that observed a daily sensitivity of 13.8% for detecting ≥1 person shedding MPXV and a weekly sensitivity of 31.7%. The lower sensitivity observed in North Carolina likely reflects differences in analytic approach, case density, and assumptions about viral shedding. Adams et al. (*16*) assumed complete case ascertainment and uniform 25-day viral shedding after symptom onset of mpox, whereas our analysis relied on confirmed case onset dates and a fixed ±17-day temporal alignment window. This approach likely provides a more conservative estimate of sensitivity because wastewater detections occurring outside the defined window were classified as discordant even if they reflected prolonged or presymptomatic shedding. In addition, only 45 cases were identified across a population of >2 million persons, limiting the probability that wastewater concentrations would exceed detection thresholds.

Despite low sensitivity for detecting single cases, specificity was high (95%–98%), indicating that wastewater-negative observations were generally concordant with the absence of reported cases. The

Table 3. Diagnostic characteristics stratified by wastewater treatment plant/sewershed in study of mpox surveillance in wastewater, North Carolina, USA, October 2023–May 2025

Sewershed	TP	FP	TN	FN	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Cary 1	0	2	5	0	–	71.4 (35.9–91.8)	<1 (0–65.8)	100 (56.6–100)
Cary 2	0	0	7	0	–	100 (64.6–100)	–	100 (64.6–100)
Cary 3	0	1	2	4	<1 (0–49.0)	66.7 (20.8–93.9)	<1 (0–79.3)	33.3 (9.7–70.0)
Chapel Hill	0	0	2	0	–	100 (34.3–100)	–	100 (34.3–100)
Charlotte 1	0	4	78	0	–	95.1 (88.1–98.1)	<1 (0–49.0)	100 (95.3–100)
Charlotte 2	6	0	60	18	25 (12.0–45.0)	100 (93.8–100)	100 (61.0–100)	77.0 (66.4–84.9)
Charlotte 3	3	0	52	23	11.5 (3.5–29.0)	100 (93.1–100)	100 (43.9–100)	69.3 (58.2–78.6)
Charlotte 4	3	6	44	27	10.0 (0.03–25.6)	88.0 (76.2–94.4)	33.3 (12.1–64.6)	62.0 (50.3–72.3)
Greensboro	0	3	67	0	–	95.7 (88.1–98.5)	<1 (0–56.2)	100 (94.6–100)
Greenville	0	2	71	0	–	97.3 (90.6–99.1)	<1 (0–65.8)	100 (94.9–100)
MSD Buncombe (Asheville)	0	2	42	17	<1 (0–18.4)	95.4 (84.9–98.7)	<1 (0–79.3)	71.2 (58.6–81.1)
North Durham	0	1	6	0	–	85.7 (48.7–97.4)	<1 (0–79.3)	100 (61.0–100)
Raleigh	3	2	40	38	7.3 (2.5–19.4)	95.2 (84.2–98.7)	60.0 (23.1–88.2)	51.3 (40.4–62.1)
Raleigh 2	0	4	71	0	–	94.7 (87.1–97.9)	<1 (0–49.0)	100 (94.9–100)
Raleigh 3	0	3	75	0	–	96.2 (89.3–98.7)	<1 (0–56.2)	100 (95.1–100)
South Durham	0	2	5	0	–	71.4 (35.9–91.8)	<1 (0–65.8)	100 (56.6–100)

*Dashes indicate that sensitivity cannot be evaluated in sewersheds with no clinical cases (TP+FN) and PPV cannot be evaluated in sewersheds with no detections (TP+FP). FN, false negative; FP, false positive; MSD, Metropolitan Sewerage District, NPV, negative predictive value; PPV, positive predictive value; TN, true negative; TP, true positive.

NPV exceeded 80% overall and was higher at several individual sites, suggesting that the absence of a wastewater detection was associated with a low likelihood of concurrent reported cases. However, the PPV was modest (31.9% in the primary analysis and 11.8% in the LOD-only analysis), reflecting that some wastewater detections occurred without temporally aligned reported cases. Such detections could represent travel-associated cases, delayed reporting, prolonged shedding, asymptomatic or subclinical infections, or infections among persons residing outside the mapped catchment area. As a consequence, wastewater detections occurring without temporally aligned reported cases should not necessarily be interpreted as analytical FPs and might indicate true but unrecognized infections, suggesting that PPV might be underestimated. Therefore, if even a subset of those observations represented true infections, the PPV of WS would be higher than estimated in this analysis.

Of note, sensitivity increased substantially when trace detections were included, indicating that low-level signals contributed meaningfully to temporal concordance with reported cases. That finding underscores the tradeoff between sensitivity and specificity when defining wastewater-positive thresholds. Restricting classification to concentrations above the assay LOD generally increased specificity but markedly reduced sensitivity, potentially limiting the ability to detect low-level community signals in low-incidence settings. In North Carolina, trace detections are interpreted within a structured public health decision framework rather than as definitive evidence of transmission and might prompt notification of local health departments and wastewater partners, review of recent case activity, enhanced syndromic surveillance monitoring, and coordination regarding case investigation or specimen retesting. Those actions are consistent with our public health action decision tree for confirmed detections (Figure). Confirmed detections might also involve escalation actions such as broader public notification, targeted outreach, and intensified sampling.

Several factors likely contributed to the low sensitivity observed. First, mpox incidence during the study period was low and sporadic, reducing the

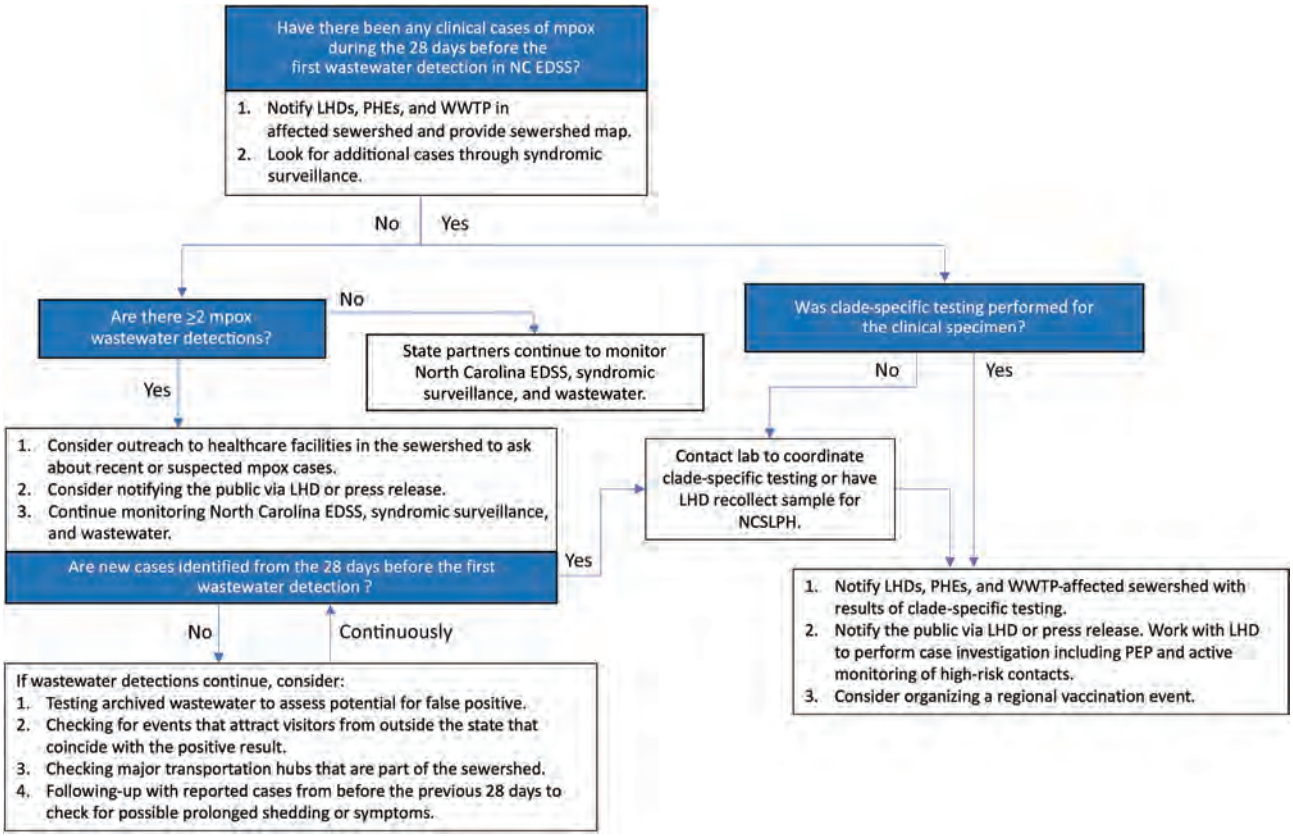


Figure. Public Health Action Decision Tree for monkeypox virus surveillance in wastewater, North Carolina, USA, October 2023–May 2025. Period of 28 days is based on typical lesion healing duration (*16*). EDSS, Electronic Disease Surveillance System; LHD, local health department; PEP, postexposure prophylaxis; PHE, public health epidemiologist; SLPH, State Laboratory of Public Health; WWTP, wastewater treatment plant.

probability that viral shedding might be detectable within large, pooled wastewater catchments. Second, sampling occurred once per week, which might have missed transient shedding signals. Third, wastewater represents an aggregate population-level signal, and the dilution effect within large catchments (some serving >500,000 persons) might limit detection of individual infections. Fourth, alignment relied on residential address assignment; persons infected but not residing within the sewershed would reduce measured concordance.

The first limitation of this study is that reported clinical cases are not a perfect standard for infection. Our intent was to quantify the extent to which wastewater detections were temporally aligned with reported clinical cases within the same sewershed, rather than to provide an estimate of the true diagnostic accuracy of WS for detecting infection. Second, clinical case reporting was assumed to be complete within each sewershed; however, underascertainment of cases could bias estimates of sensitivity and PPV downward while inflating NPV. Several factors might have contributed to incomplete case detection, including mild or atypical symptoms, barriers to healthcare access, stigma, variation in clinical testing practices, and infections occurring among visitors or commuters whose residence falls outside the wastewater catchment. As a result, wastewater detections might occur in the absence of a temporally aligned reported clinical case.

An additional limitation of this study is that viral shedding dynamics were not directly measured, and the ±17-day alignment window might not fully capture the duration or variability of shedding. Wastewater sampling was also conducted only once per week, which might have reduced the likelihood of capturing transient or low-level shedding events and could have contributed to lower observed sensitivity. Finally, although laboratory methods were consistent within this program, sampling frequency and analytic approaches might differ from other surveillance systems and studies, limiting direct comparability with published estimates, including those reported by Adams et al. (*16*).

Although sensitivity for detecting single cases was limited, high specificity and moderate NPV indicate that absence of detection might provide reassurance that widespread community transmission is unlikely. In low-prevalence contexts, isolated wastewater detections should be interpreted cautiously and in conjunction with epidemiologic investigation (*9*). Conversely, sustained nondetection in monitored sewersheds might help inform risk communication and public health response decisions. Continued evaluation across varying transmission intensities and analytic thresholds will help clarify the optimal role of mpox WS in outbreak preparedness and response. Finally, because clinical surveillance does not capture all mpox infections, some wastewater detections classified as FPs might instead represent true but unrecognized infections. Consequently, the sensitivity and positive predictive value reported here should be considered conservative estimates of concordance with reported clinical cases rather than measures of true diagnostic accuracy.

About the Author

Dr. Snyder is lead epidemiologist with the North Carolina Wastewater Monitoring Network through the North Carolina Department of Health and Human Services. Her research interests include wastewater surveillance, infectious disease, and environmental epidemiology.

References

1. World Health Organization. Multi-country outbreak of monkeypox: external situation report #2—25 July 2022 [cited 2026 Feb 28]. https://www.who.int/publications/m/item/multi-country-outbreak-of-monkeypox–external-situation-report–2—25-july-2022
2. Omatola CA, Ogunsakin RE, Olaniran AO, Kumari S. Monkeypox virus occurrence in wastewater environment and its correlation with incidence cases of mpox: a systematic review and meta-analytic study. Viruses. 2025;17:308. https://doi.org/10.3390/v17030308
3. Bowes DA, Henke KB, Driver EM, Newell ME, Block I, Shaffer G, et al. Enhanced detection of mpox virus in wastewater using a pre-amplification approach: a pilot study informing population-level monitoring of low-titer pathogens. Sci Total Environ. 2023;903:166230. https://doi.org/10.1016/j.scitotenv.2023.166230
4. Sherchan SP, Solomon T, Idris O, Nwaubani D, Thakali O. Wastewater surveillance of Mpox virus in Baltimore. Sci Total Environ. 2023;891:164414. https://doi.org/10.1016/j.scitotenv.2023.164414
5. Oghuan J, Chavarria C, Vanderwal SR, Gitter A, Ojaruega AA, Monserrat C, et al. Wastewater analysis of Mpox virus in a city with low prevalence of Mpox disease: an environmental surveillance study. Lancet Reg Health Am. 2023;28:100639. https://doi.org/10.1016/j.lana.2023.100639
6. Mejia EM, Hizon NA, Dueck CE, Lidder R, Daigle J, Wonitowy Q, et al. Detection of mpox virus in wastewater provides forewarning of clinical cases in Canadian cities. Sci Total Environ. 2024;933:173108. https://doi.org/10.1016/j.scitotenv.2024.173108
7. Kim H, Kwon R, Lee H, Lee SW, Rahmati M, Koyanagi A, et al. Viral load dynamics and shedding kinetics of mpox infection: a systematic review and meta-analysis. J Travel Med. 2023;30:taad111. https://doi.org/10.1093/jtm/taad111
8. Piralla A, Mileto D, Rizzo A, Ferrari G, Giardina F, Gaiarsa S, et al.; MPXV Lombardy Network. Dynamics

of viral DNA shedding and culture viral DNA positivity in different clinical samples collected during the 2022 mpox outbreak in Lombardy, Italy. Travel Med Infect Dis. 2024;59:102698. https://doi.org/10.1016/j.tmaid.2024.102698
9. Foulkes D, Kittner A, Korban C, Anderson K, DeJonge PM, Faherty EAG, et al. Using wastewater surveillance for mpox as a complement to traditional case-based reporting—Chicago, March–June 2023. Environ Int. 2024;190:108749. https://doi.org/10.1016/j.envint.2024.108749
10. Wolfe MK, Yu AT, Duong D, Rane MS, Hughes B, Chan-Herur V, et al. Use of wastewater for mpox outbreak surveillance in California. N Engl J Med. 2023;388:570–2. https://doi.org/10.1056/NEJMc2213882
11. Council of State and Territorial Epidemiologists. Mpox case definition from 2022 (22-ID-10) [cited 2026 Aug 13]. https://ndc.services.cdc.gov/case-definitions/mpox-2022
12. Centers for Disease Control and Prevention. Non-variola orthopoxvirus generic real-time PCR test [cited 2026 Feb 28]. https://www.cdc.gov/poxvirus/mpox/lab-personnel/non-variola-orthopoxvirus-generic-real-time-pcr-test.html
13. Clinical and Laboratory Standards Institute. CLSI EP17. Evaluation of detection capability for clinical laboratory measurement procedures. Wayne (PA): The Institute; 2012.
14. Ahmed W, Bivins A, Metcalfe S, Smith WJM, Verbyla ME, Symonds EM, et al. Evaluation of process limit of detection and quantification variation of SARS-CoV-2 RT-qPCR and RT-dPCR assays for wastewater surveillance. Water Res. 2022;213:118132. https://doi.org/10.1016/j.watres.2022.118132
15. Karlin-Neumann G, Bizouarn F, editors. Digital PCR: methods and protocols. Methods in molecular biology, vol. 1768. New York: Humana Press; 2018.
16. Adams C, Kirby AE, Bias M, Riser A, Wong KK, Mercante JW, et al. Detecting mpox cases through wastewater surveillance—United States, August 2022–May 2023. MMWR Morb Mortal Wkly Rep. 2024;73:37–43. https://doi.org/10.15585/mmwr.mm7302a3
17. World Health Organization. Mpox [cited 2026 Feb 27]. https://www.who.int/news-room/fact-sheets/detail/mpox

Address for correspondence: Nicole L. Snyder, North Carolina Division of Public Health, 65 Moore Dr, Durham, NC 27709, USA; email: nicole.snyder@dhhs.nc.gov

Laboratory Assessment of Wastewater as Surveillance Tool for West Nile Virus, United States

Jessica Spring, Audrey Long, Emily Davis, Shannon R. Matzinger, Allison Wheeler, Shelby L. Lyons, Daniel L. Jacobs, Rory Welsh, Jeffrey W. Mercante, Aaron C. Brault, Holly R. Hughes

West Nile virus (WNV) is the most common cause of domestic human arboviral infection in the contiguous United States. WNV surveillance across the United States is not comprehensive and is limited. Nonclinical WNV surveillance is variable, and WNV surveillance in humans is limited by the percentage of symptomatic human infections and time it takes to seek healthcare and diagnosis. Wastewater surveillance has been used to detect viruses shed from asymptomatic and symptomatic persons; therefore, we assessed wastewater as a surveillance tool for WNV tracking. We tested archived RNA and raw wastewater from 6 states with documented human illness during the 2023 WNV transmission season for WNV RNA. WNV RNA was detected in 18% (n = 29/158) of samples from Arizona, California, Colorado, Illinois, Indiana, and Nebraska. Our results highlight the ability to detect WNV in wastewater and the potential of wastewater surveillance to augment current surveillance data.

West Nile virus (WNV) is an enveloped, positive-sense RNA orthoflavivirus transmitted by mosquitoes (*1*). Although several lineages of WNV exist, the most common and virulent for humans are lineage I, found worldwide, and lineage II, found in sub-Saharan Africa and Europe (*2*). Most WNV infections are asymptomatic or mild; however, each year ≈1,300 neuroinvasive disease cases are reported in the United States (*3*–*6*). Symptomatic WNV infection is marked by nonspecific symptoms such as headache, body aches, joint pain, vomiting, diarrhea, or rash (*1*,*2*). A severe neuroinvasive disease develops in ≤1% of those infected with WNV (*1*,*2*). Because no vaccines or therapeutics for WNV have been approved, personal protective behaviors, vector control, and blood and organ donor screening for WNV are essential tools to reduce WNV disease–related illness and death (*7*).

Various modes of surveillance are used to monitor and control WNV transmission, including mosquito, dead bird, and sentinel animal surveillance. However, those surveillance methods are inconsistently used across the United States and rarely performed year-round. Surveillance for WNV in humans includes testing symptomatic persons and monitoring blood donations (*1*,*2*,*8*,*9*). Nevertheless, those methods miss mild infections in persons not seeking medical attention or donating blood, likely underrepresenting the number of WNV cases.

Wastewater surveillance (WS) was successfully implemented to monitor the prevalence and transmission of various viral pathogens including poliovirus, SARS-CoV-2, and monkey pox virus in humans regardless of reported clinical symptoms (*10*–*12*) and sometimes before reported human cases (*13*). WS also proved informative during the COVID-19 pandemic. SARS-CoV-2 virions and viral RNA are shed in patient feces and recoverable in wastewater, which enabled the adaptation of wastewater monitoring to assist in estimating the prevalence of COVID-19 cases and detecting concerning variants (*14*–*16*). Since the COVID-19 pandemic began, WS has rapidly expanded as an effective method to monitor transmission of enteric and respiratory pathogens on a large scale, resulting in multiple countries establishing regional or national level systems (*17*).

Studies have identified measurable levels of WNV RNA in urine from both symptomatic and asymptomatic persons after WNV infection (*9*,*18*–*20*). Because wastewater surveillance was an effective tool for other viruses and WNV has been shown to be

Author affiliations: Centers for Disease Control and Prevention, Fort Collins, Colorado, USA (J. Spring, A. Long, E. Davis, S.L. Lyons, D.L. Jacobs, A.C. Brault, H.R. Hughes); Colorado Department of Public Health and Environment, Denver, Colorado, USA (S.R. Matzinger, A. Wheeler); Centers for Disease Control and Prevention, Atlanta, Georgia, USA (R. Welsh, J.W. Mercante)

DOI: https://doi.org/10.3201/eid3213.260616

shed in urine, we sought to determine the feasibility of WS for WNV. We assessed WNV RNA recovery from spiked wastewater samples and WNV RNA detection from wastewater samples collected in counties with human or nonhuman WNV activity during the 2023 transmission season.

Materials and Methods

Virus Growth

We infected confluent Vero cells (American Type Culture Collection, https://www.atcc.org) in 150-cm^2 flasks with WNV lineage I isolate RO97–50 at a multiplicity of infection of 0.01. We maintained infected cells in yeast extract/lactalbumin media supplemented with 3% (vol/vol) sodium bicarbonate (Invitrogen, https://www.invitrogen.com), 2% (vol/vol) fetal bovine serum (Cytiva, https://www.cytivalifesciences.com), 0.1% (vol/vol) Gentamicin (MP Biomedicals, https://www.mpbio.com), 0.4% (vol/vol) Fungizone (Cytiva), and 1% (vol/vol) Pen/Strep (Invitrogen). We collected the supernatant from infected cells at 80% cytopathic effect and froze the supernatant at −20°C.

WNV Stability in Contrived Wastewater Samples

We spiked wastewater from the Colorado Department of Public Health and Environment (CDPHE) collected outside of typical arbovirus transmission period (collected March 2023) with 10^4–10^7 RNA copies/140 μL of WNV. We included unmanipulated wastewater as a negative control. We pasteurized samples for 30 minutes at 56°C. For time course studies, we left samples at room temperature before pasteurization. We chose a time course to encompass the early time points at which wastewater would be collected and to enable further determination of WNV stability in wastewater. We extracted RNA from 140μL of contrived wastewater samples by using the QIAamp Viral RNA Mini Kit (QIAGEN, https://www.qiagen.com) and eluted in 100 μL of AVE buffer.

RNA Samples Derived from Wastewater

CDPHE provided 48 deidentified, archived RNA samples derived from wastewater on the basis of a request for available samples collected from regions with documented human WNV cases during the summer of 2023. Wastewater was collected from 2 Colorado counties during the summer of 2023. Nanotrap enhancement reagents (CERES Nano, https://www.ceresnano.com) were used to concentrate 40mL of wastewater following manufacturer's instructions. RNA had been previously extracted from concentrated wastewater by using the MagMAX Wastewater Ultra Nucleic Acid Isolation Kit (Thermo Fisher Scientific, https://www.thermofisher.com).

Wastewater Samples and Concentration

The Centers for Disease Control and Prevention's National Wastewater Surveillance System biorepository provided 110 archived raw wastewater samples collected during the 2023 WNV transmission season. The wastewater was provided on the basis of a request for available samples that had been collected in counties or states with documented human WNV cases during the 2023 transmission season.

Volumes of 15 mL per sample were shipped to the Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases, Division of Vector-Borne Diseases, Arboviral Diseases Branch (Fort Collins, CO, USA) and stored at −80°C until processing. We concentrated raw wastewater by using polyethylene glycol precipitation as previously described (*21*). We thawed raw wastewater and waited for debris to settle. From the clarified top section of the sample, we extracted 5 mL and heat treated the sample for 30 minutes at 56°C. We mixed the pasteurized wastewater with polyethylene glycol (0.14 g/mL) and NaCl (0.0117 g/mL). We shook the samples at 200 rpm in a MaxQ 4000 Orbital Shaker (ThermoFisher Scientific) for 4 hours at 4°C and then centrifuged the samples at 6,500 × *g* for 30 minutes at 4°C. We discarded the supernatant and resuspended the pellets in 500 μL sterile phosphate buffered saline. We stored the resuspended pellets at −80°C until RNA extraction. We isolated RNA from resuspended pellets by using the QIAamp Viral RNA Mini Kit (QIAGEN) according to the manufacturer's instructions. We eluted RNA in a total volume of 30 μL AVE buffer (QIAGEN) twice and stored at −80°C.

Digital Reverse Transcription PCR

We conducted digital reverse transcription PCR (dRT-PCR) to quantify WNV RNA copies by using a QIAcuity One Digital PCR System (QIAGEN) and the QIAcuity OneStep Advanced Probe Kit (QIAGEN) according to the manufacturer's instructions. We quantified WNV RNA copies by using envelope-specific primers and probe (forward, 5′-TCAGCGATCTCTCCACCAAAG-3′; reverse, 5′-GGGTCAGCACGTTTGTCATTG-3′; probe, 5′-TGCCCGACCATGGGAGAAGCTC-3′) (*22*). For contrived wastewater samples, we loaded 1 μL RNA in a total mixture volume of 12 μL onto an 8.5K Nanoplate (QIAGEN). For RNA provided by CDPHE and RNA extracted from archived wastewater samples, we loaded 10 μL RNA

in a total volume of 40 μL onto a 26K Nanoplate (QIAGEN). We included primers and probes specific for pepper mild mottle virus (PMMoV) (PMMoV-FP1-rev 5′-GAGTGGTTTGACCTTAACGTTTGA-3′; PMMoV-RP1 5′-TTGTCGGTTGCAATGCAAGT-3′; PMMoV-Probe1 5′-/56-FAM/CCTACCGAA/ZENGCAAATG/3IABkFQ/-3′) when testing RNA extracted from wastewater as positive extraction control. For the PMMV assay, we loaded 1 μL RNA in a total volume of 12 μL onto an 8.5K Nanoplate (QIAGEN). We included a positive WNV control, a negative wastewater control, and a water control on each plate. We considered samples with ≥3 positive partitions positive. Negative and water control wells all had <3 positive partitions.

Real-time Reverse Transcription PCR

We subsequently analyzed samples with ≥3 positive partitions from dRT-PCR testing by using real-time reverse transcription PCR (RT-PCR). We used the Quantitect probe RT-PCR kit (QIAGEN) to detect viral RNA by using the WNV envelope primers and probe (*22*) according to the manufacturer's instructions. We used 10 μL of RNA in a total reaction volume of 25 μL. We included positive and negative controls on each plate. We deemed a cycle threshold value <38 positive (*23*). We only considered samples that were positive by both dRT-PCR and real-time RT-PCR methods positive for WNV RNA.

Surveillance Data

We obtained surveillance data for 2023 from ArboNET, the national surveillance system for arboviral diseases (https://www.cdc.gov/vector-borne-diseases/php/arbonet). State and territorial health departments voluntarily report nonhuman (i.e., positive mosquitoes, sentinel animals, or animal disease cases) and human (disease cases and presumptive viremic donors) data to ArboNET by using standard surveillance case definitions. We compared the timing of positive surveillance events with wastewater testing results by using timelines beginning with the first WNV surveillance event (day 0). Human cases are demarcated by onset date.

Results

WNV Stability in Contrived Wastewater

We determined recovery of WNV RNA from wastewater via dRT-PCR, which has enhanced resistance to inhibitors in complex matrices like wastewater (*24*). Immediate (0 h) isolation of RNA from wastewater spiked with a high concentration (yields 10^6–10^7 RNA copies/140 μL) of WNV resulted in an average of 75% recovery (Figure 1, panel A). Detection dropped to 60% by 4 hours after spiking and 50% by 72 hours after spiking (Figure 1, panel A). As expected, detection of recovered WNV RNA in wastewater spiked with a low concentration (yields 10^4–10^5 RNA copies/140 μL) of WNV was lower, with an average of 50% recovery at 0–4 hours, and down to 35% recovery by 72 hours after spiking (Figure 1, panel B). We detected WNV spiked in wastewater down to a limit of 14 copies/μL of input RNA and observed large deviations in recovery particularly at later timepoints, suggesting variability in the degradation of WNV virions and RNA. Nevertheless, those data indicate WNV can be detected in contrived wastewater samples for up to 72 hours after spiking.

Detection of WNV in Archived RNA

We next investigated whether WNV RNA could be detected in archived RNA derived from wastewater collected during the Colorado arbovirus transmission season of 2023. We tested 48 residual RNA samples from 2 counties for the presence of WNV RNA by dRT-PCR. Five (10.4%) of 48 samples were positive for WNV RNA (Table 1). Of the negative samples, 2 had 2 positive partitions, 9 had 1 positive

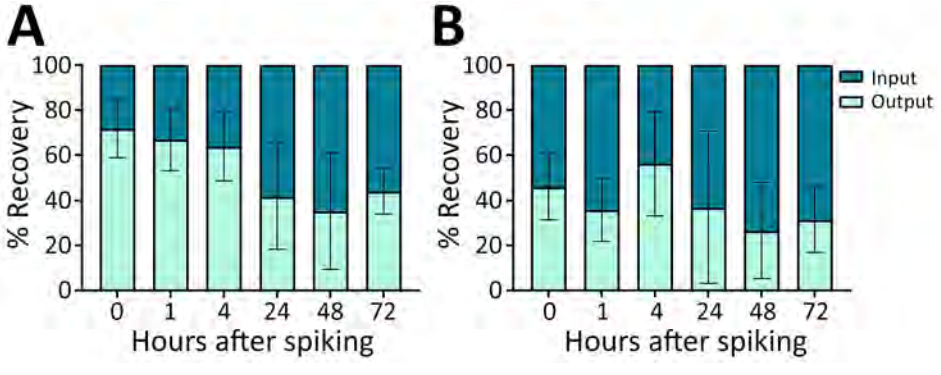


Figure 1. West Nile virus (WNV) RNA detection by digital reverse transcription PCR in spiked wastewater samples up to 72 hours from laboratory assessment of wastewater as surveillance tool for WNV, United States. WNV RNA recovery over time from wastewater spiked with a high concentration of WNV (known yield 10^6–10^7 RNA copies) (A) or a low concentration of WNV (known yield 10^4–10^5 RNA copies) (B). Data are represented as averages from multiple experiments; error bars represent SD.

partition, and 32 had 0 positive partitions. Those data demonstrate WNV RNA can be detected in wastewater by using dRT-PCR.

Detection of WNV in Archived Wastewater Samples

We next examined whether archived wastewater samples collected by the National Wastewater Surveillance System jurisdictions were positive for WNV. We tested wastewater collected during the 2023 WNV transmission season from deidentified counties with or without documented human cases of WNV for the presence of WNV RNA. We then compared results with other reported surveillance data from the same county to determine whether WS could bolster existing surveillance methods (Figure 2).

During the 2023 WNV transmission season, county A in California reported 12 human WNV infections. We tested 46 wastewater samples from the 2 watersheds (data are combined) servicing that county (Table 2). Of those samples, 10 were positive for WNV by dRT-PCR and real-time RT-PCR (Table 3; Figure 2, panel B). Of note, wastewater samples were positive for WNV before positive bird detections by 37 days, sentinel detections by 68 days, and mosquito detections by 29 days (Figure 2, panel A). Furthermore, 5 wastewater samples were positive for WNV up to 27 days before the first documented human WNV case.

Table 1. Archived RNA samples positive by PCR for West Nile virus RNA in a laboratory assessment of wastewater as surveillance tool, United States*

CDPHE sample	No. positive partitions
1	6
2	6
3	12
4	3
5	4

*CDPHE, Colorado Department of Public Health and Environment.

County B in Arizona reported 72 human WNV disease cases and 5 asymptomatic presumptive viremic blood donors during the 2023 transmission season. We excluded 2 human cases that did not have

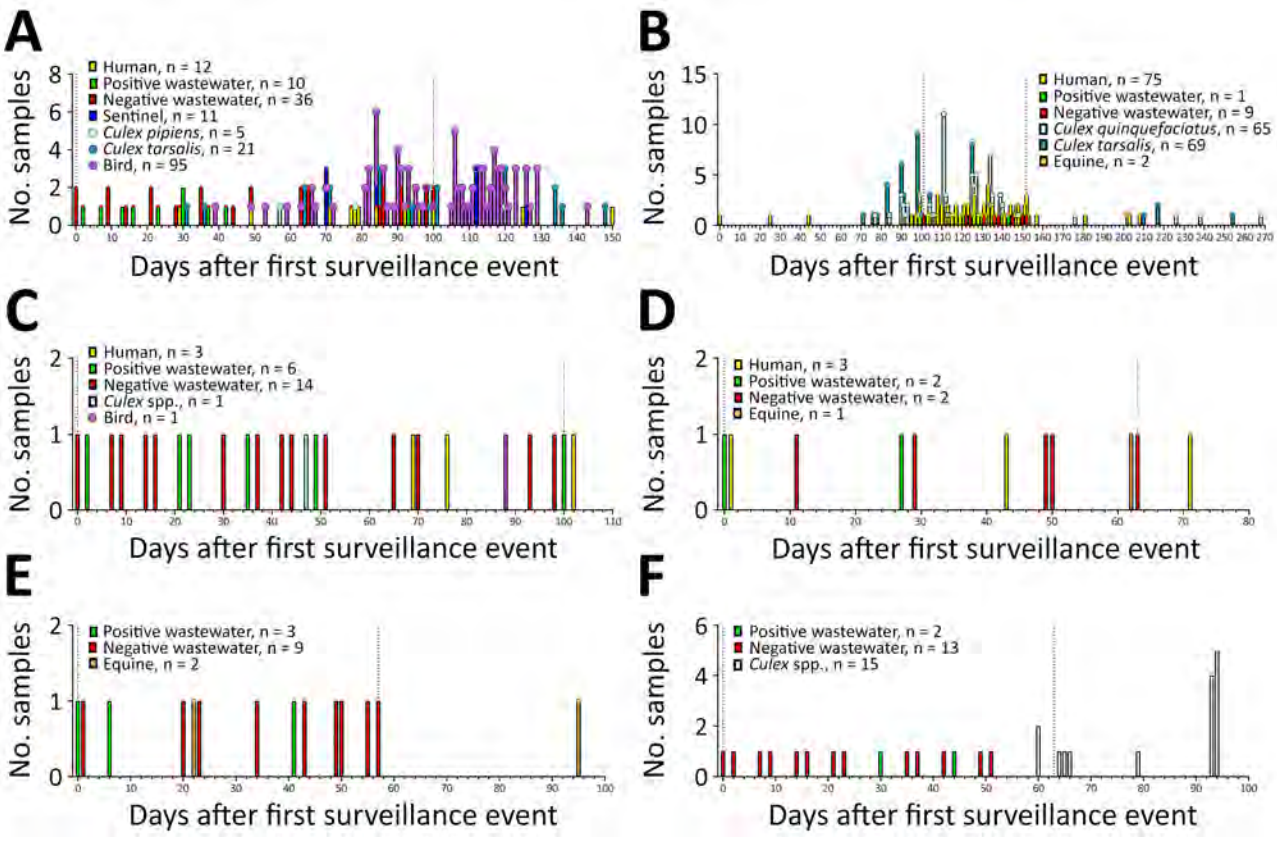


Figure 2. West Nile virus (WNV) RNA detected in wastewater collections from sampled jurisdictions compared with other surveillance methods from laboratory assessment of wastewater as surveillance tool for WNV, United States. A) County A, California, USA. B) County B, Arizona, USA. C) County C, Illinois, USA. D) County D, Nebraska, USA. E) County E, Colorado, USA. F) County F, Indiana, USA. Wastewater samples from each county were tested by digital reverse transcription PCR and real-time reverse transcription PCR for WNV RNA. Wastewater data are compared with the number of other WNV-positive surveillance events reported to ArboNET (y-axis): human, equine, *Culex* mosquito, sentinel, and bird samples. Timeline for samples is dictated as days after first surveillance event (x-axis). Dotted vertical lines mark the time frame for available archived wastewater.

Table 2. Wastewater sample site details including jurisdiction, population size, and total samples collected in a laboratory assessment of wastewater as surveillance tool for West Nile virus, United States

Jurisdiction	Sewershed population	No. samples
County A, CA	709,694	46
County B, AZ	2,400,000	10
County C, IL	240,000	20
County D, NE	4,800	7
County E, CO	7,300	12
County F, IN	28,000	15

reported onset dates from the graph. More than 130 WNV-positive mosquito pools and 2 equine cases were also identified during that time. We assayed 10 wastewater samples for WNV RNA. Although only 1 sample was positive for WNV RNA, it was collected during the time frame when human WNV cases were reported (Table 3; Figure 2, panel B3). The single positive wastewater sample was collected 143 days after the first reported human case, 72 days after the first reported positive mosquito pool, and 59 days before the 2 reported WNV equine cases. The limited number of samples available for testing might have prevented detection of WNV earlier in the season (Figure 2, panel B).

County C in Illinois reported 3 human WNV infections during the 2023 transmission season. Of the 20 wastewater samples available, 6 were positive for WNV (Table 3; Figure 2, panel C). Five wastewater samples were positive for WNV up to 67 days before the first human case and up to 86 days before a WNV positive bird was detected. In addition, 4 wastewater samples were positive for WNV up to 45 days before a single positive mosquito pool.

We tested 7 wastewater samples from county D in Nebraska, which reported 3 human cases of WNV. Of those samples, 2 were positive for WNV RNA (Table 3; Figure 2, panel D), and wastewater was positive for WNV 1 day before the first documented human WNV case and 62 days before a single equine WNV case.

County E in Colorado had no reported human WNV cases during the 2023 transmission season but did report 2 equine cases (Figure 2, panel E). Of 12 wastewater samples available from the county, 3 were positive for WNV RNA, the first of those positive 22 days before the first equine case (Table 3; Figure 2, panel E). County F in Indiana also reported no human cases of WNV during the summer of 2023. However, of the 15 wastewater samples available, 2 samples tested positive for WNV RNA 30 and 16 days before detection of WNV in mosquito pools (Table 3; Figure 2, panel F).

Discussion

WS has proven a useful tool for monitoring circulation of certain infectious agents, prevalence of bacteria with antimicrobial resistance, and traces of narcotics at a population scale (*25*–*27*). For

Table 3. Results for NWSS jurisdiction wastewater samples that were positive by dRT-PCR and real-time RT-PCR collected in a laboratory assessment of wastewater as surveillance tool for West Nile virus, United States*

Sample jurisdiction	First reported nonwastewater surveillance event, d	First reported human disease case, d	RNA copies/5 mL wastewater†	Ct† (*23*)
County A, CA	−27	−27	72.96	36.54
	−22	−22	19.08	32.24
	−16	−16	24.96	35.7
	−13	−13	18.96	37.5
	−6	−6	42.84	35.31
	1	1	184.08	32.44
	1	1	135.72	36.08
	8	8	19.2	35.99
	13	13	30.48	34.97
	64	64	18.96	36.91
County B, AZ	143	143	24.24	36.29
County C, IL	−67	−67	249.72	32.16
	−48	−48	84.84	34.86
	−46	−46	84.84	34.59
	−34	−34	1380	26.91
	−20	−20	38.4	36.53
	31	31	31.68	31.88
County D, NE	−1	−1	44.88	34.14
	26	26	18.24	36.9
County E, CO	−22	NA	28.56	37.09
	−16	NA	57	33.19
	19	NA	18.36	35.26
County F, IN	−30	NA	225.96	32.81
	−16	NA	155.76	34.91

*Ct, cycle threshold; dRT-PCR, digital reverse transcription PCR; NA, not applicable; NWSS, National Wastewater Surveillance System; RT-PCR, reverse transcription PCR.
†RNA copies/5 mL of wastewater from dRT-PCR and Ct values from real-time RT-PCR.

pathogens transmitted by the fecal–oral route, WS has shown to be valuable for detecting outbreaks, often before widespread transmission and clinical manifestations, leading to swift public health responses (*28*–*30*).

WNV RNA was detected in both contrived and archived wastewater samples tested in this study. In total, WNV RNA was detected in 18% (29/158) of samples tested. Five archived RNA samples from Colorado were positive for WNV RNA by dRT-PCR. Furthermore, 24 of 110 raw wastewater samples were found positive for WNV RNA by dRT-PCR and real-time RT-PCR. Of note, many positive wastewater samples were collected before documented human infections (Figure 2, panels A, C, D), indicating WS might provide knowledge of WNV circulation before other surveillance methods or in areas with limited WNV surveillance. Early detection of WNV by using WS might be particularly helpful because lag times often occur between WNV activity detected by existing surveillance efforts (if detected at all) and identification of human cases. Counties without vector surveillance might also lack vector control methods. Regardless, information yielded from WS could be used to inform healthcare providers and the public of the risk for WNV transmission. WNV was also detected in wastewater from counties with robust WNV surveillance before reported detection in the environment (e.g., California and Illinois). It is unknown if environmental surveillance was conducted during those times; nevertheless, WS could bolster existing surveillance strategies. Finally, WNV positive wastewater samples were detected in absence of reported human illness (Figure 2, panels E and F). That finding indicates that WS might detect WNV shed from subclinical human infections. Collectively, our data demonstrate wastewater can provide notice of circulating WNV, perhaps even in advance of other surveillance methods. Nevertheless, additional prospective work is needed to determine site specific applicability of WNV wastewater surveillance to augment WNV prevention and control.

Our results, along with those of other studies, support the feasibility of identifying WNV in wastewater. One study found 16 of 21 wastewater samples positive for WNV from counties in Oklahoma (K.G. Kuhn et al., unpub. data, http://dx.doi.org/10.2139/ssrn.4805820). In that study, the samples were deemed positive if 1 droplet was positive, rather than the 3 positive partitions used with dRT-PCR in this article. Compared with another study, our results indicate the liquid fraction of wastewater is likely more sensitive for detection of WNV because only 2% of 601 settled solid samples were positive for WNV RNA (*31*) compared with 10%–30% positivity found among our samples (Arizona, 10%; California, 22%; Colorado, 25%; Illinois, 30%; Indiana, 13%; and Nebraska, 29%).

Other arboviruses, including Zika virus (ZIKV), Japanese encephalitis virus, and dengue virus (DENV), have been successfully detected in wastewater (*32*–*34*). Those wastewater detections occurred around a small cluster of ZIKV cases (*32*), during an acute outbreak of Japanese encephalitis virus (*33*), and in an area with documented cases and emerging risk for DENV (*34*). In contrast, similar studies were not successful in detecting ZIKV during an outbreak or DENV in an endemic region (*35*,*36*). Discrepancies in detecting arboviral RNA in wastewater were potentially caused by a variety of factors including differences among the targeted viruses, the wastewater systems, sample storage and preparation, and assay specificity and sensitivity.

The first limitation of this study is that wastewater is a complex matrix that greatly hindered our attempts to sequence wastewater samples and further confirm samples. Because of wastewater resistance to inhibitors, we relied primarily on dRT-PCR for detection of WNV RNA in wastewater samples. Further work would be necessary to optimize wastewater sequencing for WNV and reduce potential false positive and to standardize detection of WNV in wastewater, which is influenced by collection volumes, instrument use, and defined limits of detection. Second, because archived wastewater samples were not collected specifically to detect WNV transmission, wastewater samples collected during peak WNV transmission were limited and underwent a freeze–thaw cycle that might have degraded WNV RNA. Third, because of sample volume limitations, we extracted RNA from only 5 mL of wastewater, whereas routine surveillance methods use volumes up to 50 mL. The smaller volume might have been inadequate to detect WNV RNA in some instances. Fourth, the detection of WNV RNA in wastewater was compared with WNV detection by other surveillance methods reported to ArboNET. Because data are voluntarily reported to ArboNET, not all instances of WNV detection by other surveillance methods might be included in our datasets. Finally, we lacked information on the type of wastewater system in each jurisdiction. Wastewater systems are generally separate sanitary sewers (collect wastewater only) or combined sewers (collect both wastewater sewage and stormwater runoff). Certain mosquito

species are known to breed in storm sewer pipes (*37*). Therefore, we cannot rule out nonhuman sources of WNV RNA in wastewater samples. Knowledge of the type of wastewater system at each collection site might have provided more information regarding the source of WNV RNA.

In summary, we were successful in detecting WNV RNA in wastewater across multiple states, often before documented human infection or other ArboNET-reported surveillance methods. Future studies could determine the practicality of WNV WS to support WNV prevention and control in specific jurisdictions. Our results highlight the potential value of WS for WNV, particularly in locations with limited resources for surveillance.

Acknowledgment

We would like to acknowledge the communities in California, Arizona, Illinois, Nebraska, Colorado, and Indiana for their participation in the national wastewater testing contract from which the samples originated. Their work supported wastewater surveillance and this study.

About the Author

Dr. Spring works in microbiology at the Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases, Division of Vector-Borne Diseases, Fort Collins, CO. Her research interests focus on host-pathogen interactions.

References

1. Campbell GL, Marfin AA, Lanciotti RS, Gubler DJ. West Nile virus. Lancet Infect Dis. 2002;2:519–29. https://doi.org/10.1016/S1473-3099(02)00368-7
2. Habarugira G, Suen WW, Hobson-Peters J, Hall RA, Bielefeldt-Ohmann H. West Nile virus: an update on pathobiology, epidemiology, diagnostics, control and "One Health" implications. Pathogens. 2020;9:589. https://doi.org/10.3390/pathogens9070589
3. Centers for Disease Control and Prevention. ArboNET West Nile virus historic data (1999–2022), 2023 [cited 2025 May 1]. https://stacks.cdc.gov/view/cdc/131526
4. Centers for Disease Control and Prevention. ArboNET West Nile virus current year data (2023), 2023 [cited 2025 May 1]. https://stacks.cdc.gov/view/cdc/131926
5. Lim SM, Koraka P, Osterhaus AD, Martina BE. West Nile virus: immunity and pathogenesis. Viruses. 2011;3:811–28. https://doi.org/10.3390/v3060811
6. Gould CV, Staples JE, Guagliardo SAJ, Martin SW, Lyons S, Hills SL, et al. West Nile virus: a review. JAMA. 2025;334:618–28. https://doi.org/10.1001/jama.2025.8737
7. Padda H, Jacobs D, Gould CV, Sutter R, Lehman J, Staples JE, et al. West Nile virus and other nationally notifiable arboviral diseases—United States, 2023. MMWR Morb Mortal Wkly Rep. 2025;74:358–64. https://doi.org/10.15585/mmwr.mm7421a1
8. Hall RA, Blitvich BJ, Johansen CA, Blacksell SD. Advances in arbovirus surveillance, detection and diagnosis. J Biomed Biotechnol. 2012;2012:512969. https://doi.org/10.1155/2012/512969
9. Lee WL, Gu X, Armas F, Leifels M, Wu F, Chandra F, et al. Monitoring human arboviral diseases through wastewater surveillance: challenges, progress and future opportunities. Water Res. 2022;223:118904. https://doi.org/10.1016/j.watres.2022.118904
10. Alygizakis N, Markou AN, Rousis NI, Galani A, Avgeris M, Adamopoulos PG, et al. Analytical methodologies for the detection of SARS-CoV-2 in wastewater: protocols and future perspectives. Trends Analyt Chem. 2021;134:116125. https://doi.org/10.1016/j.trac.2020.116125
11. Ryerson AB, Lang D, Alazawi MA, Neyra M, Hill DT, St George K, et al.; 2022 U.S. Poliovirus Response Team. Wastewater testing and detection of poliovirus type 2 genetically linked to virus isolated from a paralytic polio case—New York, March 9–October 11, 2022. MMWR Morb Mortal Wkly Rep. 2022;71:1418–24. https://doi.org/10.15585/mmwr.mm7144e2
12. Wolfe MK, Yu AT, Duong D, Rane MS, Hughes B, Chan-Herur V, et al. Use of wastewater for mpox outbreak surveillance in California. N Engl J Med. 2023;388:570–2. https://doi.org/10.1056/NEJMc2213882
13. Toribio-Avedillo D, Gómez-Gómez C, Sala-Comorera L, Rodríguez-Rubio L, Carcereny A, García-Pedemonte D, et al. Monitoring influenza and respiratory syncytial virus in wastewater. Beyond COVID-19. Sci Total Environ. 2023;892:164495. https://doi.org/10.1016/j.scitotenv.2023.164495
14. Kitajima M, Ahmed W, Bibby K, Carducci A, Gerba CP, Hamilton KA, et al. SARS-CoV-2 in wastewater: state of the knowledge and research needs. Sci Total Environ. 2020;739:139076. https://doi.org/10.1016/j.scitotenv.2020.139076
15. Chen Y, Chen L, Deng Q, Zhang G, Wu K, Ni L, et al. The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients. J Med Virol. 2020;92:833–40. https://doi.org/10.1002/jmv.25825
16. Fernandez-Cassi X, Scheidegger A, Bänziger C, Cariti F, Tuñas Corzon A, Ganesanandamoorthy P, et al. Wastewater monitoring outperforms case numbers as a tool to track COVID-19 incidence dynamics when test positivity rates are high. Water Res. 2021;200:117252. https://doi.org/10.1016/j.watres.2021.117252
17. Naughton CC, Roman FA Jr, Alvarado AGF, Tariqi AQ, Deeming MA, Kadonsky KF, et al. Show us the data: global COVID-19 wastewater monitoring efforts, equity, and gaps. FEMS Microbes. 2023;4:xtad003. https://doi.org/10.1093/femsmc/xtad003
18. Barzon L, Pacenti M, Franchin E, Pagni S, Martello T, Cattai M, et al. Excretion of West Nile virus in urine during acute infection. J Infect Dis. 2013;208:1086–92. https://doi.org/10.1093/infdis/jit290
19. Lustig Y, Mannasse B, Koren R, Katz-Likvornik S, Hindiyeh M, Mandelboim M, et al. Superiority of West Nile virus RNA detection in whole blood for diagnosis of acute infection. J Clin Microbiol. 2016;54:2294–7. https://doi.org/10.1128/JCM.01283-16
20. Cvjetković IH, Radovanov J, Kovačević G, Turkulov V, Patić A. Diagnostic value of urine qRT-PCR for the diagnosis of West Nile virus neuroinvasive disease. Diagn Microbiol Infect Dis. 2023;107:115920. https://doi.org/10.1016/j.diagmicrobio.2023.115920
21. Philo SE, Keim EK, Swanstrom R, Ong AQW, Burnor EA, Kossik AL, et al. A comparison of SARS-CoV-2 wastewater

concentration methods for environmental surveillance. Sci Total Environ. 2021;760:144215. https://doi.org/10.1016/j.scitotenv.2020.144215
22. Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, et al. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. J Clin Microbiol. 2000;38:4066–71. https://doi.org/10.1128/JCM.38.11.4066-4071.2000
23. Garske T, Van Kerkhove MD, Yactayo S, Ronveaux O, Lewis RF, Staples JE, et al.; Yellow Fever Expert Committee. Yellow fever in Africa: estimating the burden of disease and impact of mass vaccination from outbreak and serological data. PLoS Med. 2014;11:e1001638. https://doi.org/10.1371/journal.pmed.1001638
24. Ahmed W, Smith WJM, Metcalfe S, Jackson G, Choi PM, Morrison M, et al. Comparison of RT-qPCR and RT-dPCR Platforms for the trace detection of SARS-CoV-2 RNA in wastewater. ACS ES T Water. 2022;2:1871–80. https://doi.org/10.1021/acsestwater.1c00387
25. Kilaru P, Hill D, Anderson K, Collins MB, Green H, Kmush BL, et al. Wastewater surveillance for infectious disease: a systematic review. Am J Epidemiol. 2023;192:305–22. https://doi.org/10.1093/aje/kwac175
26. Aarestrup FM, Woolhouse MEJ. Using sewage for surveillance of antimicrobial resistance. Science. 2020;367:630–2. https://doi.org/10.1126/science.aba3432
27. Centazzo N, Frederick BM, Jacox A, Cheng SY, Concheiro-Guisan M. Wastewater analysis for nicotine, cocaine, amphetamines, opioids and cannabis in New York City. Forensic Sci Res. 2019;4:152–67. https://doi.org/10.1080/20961790.2019.1609388
28. Alam MM, Shaukat S, Sharif S, Angez M, Khurshid A, Malik F, et al. Detection of multiple cocirculating wild poliovirus type 1 lineages through environmental surveillance: impact and progress during 2011–2013 in Pakistan. J Infect Dis. 2014;210(Suppl 1):S324–32. https://doi.org/10.1093/infdis/jiu160
29. Asghar H, Diop OM, Weldegebriel G, Malik F, Shetty S, El Bassioni L, et al. Environmental surveillance for polioviruses in the Global Polio Eradication Initiative. J Infect Dis. 2014;210(Suppl 1):S294–303. https://doi.org/10.1093/infdis/jiu384
30. Hellmér M, Paxéus N, Magnius L, Enache L, Arnholm B, Johansson A, et al. Detection of pathogenic viruses in sewage provided early warnings of hepatitis A virus and norovirus outbreaks. Appl Environ Microbiol. 2014;80:6771–81. https://doi.org/10.1128/AEM.01981-14
31. Zulli A, Duong D, Shelden B, Bidwell A, Wolfe MK, White B, et al. West Nile virus (*Orthoflavivirus nilense*) RNA concentrations in wastewater solids at five wastewater treatment plants in the United States. PeerJ. 2025;13:e19748. https://doi.org/10.7717/peerj.19748
32. Wong JCC, Tay M, Hapuarachchi HC, Lee B, Yeo G, Maliki D, et al. Case report: Zika surveillance complemented with wastewater and mosquito testing. EBioMedicine. 2024;101:105020. https://doi.org/10.1016/j.ebiom.2024.105020
33. Fanok S, Monis PT, Keegan AR, King BJ. The detection of Japanese encephalitis virus in municipal wastewater during an acute disease outbreak. J Appl Microbiol. 2023;134:lxad275. https://doi.org/10.1093/jambio/lxad275
34. Wolfe MK, Paulos AH, Zulli A, Duong D, Shelden B, White BJ, et al. Wastewater detection of emerging arbovirus infections: case study of dengue in the United States. Environ Sci Technol Lett. 2023;11:9–15. https://doi.org/10.1021/acs.estlett.3c00769
35. Zhu K, Hill C, Muirhead A, Basu M, Brown J, Brinton MA, et al. Zika virus RNA persistence and recovery in water and wastewater: an approach for zika virus surveillance in resource-constrained settings. Water Res. 2023;241:120116. https://doi.org/10.1016/j.watres.2023.120116
36. Thakali O, Raya S, Malla B, Tandukar S, Tiwari A, Sherchan SP, et al. Pilot study on wastewater surveillance of dengue virus RNA: lessons, challenges, and implications for future research. Environ Chall (Amst). 2022;9:100614. https://doi.org/10.1016/j.envc.2022.100614
37. Durden C, Lopez K, Drexler A. *Culex restuans* (Diptera: Culicidae) in the upper Midwest: a review of ecology, biology, control, and contributions to human WNV in the region. J Med Entomol. 2026;63:tjaf160. https://doi.org/10.1093/jme/tjaf160

Address for correspondence: Holly R. Hughes, Centers for Disease Control and Prevention, 3156 Rampart Rd, Fort Collins, CO 80521, USA; email: ltr8@cdc.gov

Implications of Wastewater Surveillance for Clinical Care among Infectious Disease Physicians, United States, 2024

Shantrice L. Jones, Angela D. Blackwell, Carly Adams, Philip M. Polgreen, Susan E. Beekmann, Chelsea Gridley-Smith, Jessica N. Ricaldi, Scott Santibañez

We analyzed 192 responses from a 2024 survey of Emerging Infections Network members in the United States regarding how wastewater surveillance can affect clinical practice. Four themes emerged: situational awareness, patient counseling, infection control, and diagnostic support. Enhanced collaboration between public health officials and clinicians might optimize utility of such surveillance in clinical practice.

Although wastewater surveillance (WS) (*1*) has a role in outbreak detection (*2*–*4*), integration of WS data into clinical practice remains limited (*5*). Little is known about how infectious disease clinicians perceive WS (*6*). The Centers for Disease Control and Prevention (CDC) and the Infectious Diseases Society of America's Emerging Infections Network (EIN) (https://ein.idsociety.org), a sentinel network of infectious disease physicians and other infectious disease specialists, surveyed EIN member-clinicians in 2024 to generate hypotheses about knowledge of, attitudes toward, and use of WS data (*5*,*7*) Our previously published quantitative results revealed 56% of EIN clinicians did not review WS data regularly, 22% of respondents reviewed it regularly, and 22% were not aware of WS data (*5*). To complement those previous findings, we present an in-depth analysis of qualitative data from the same survey.

Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (S.L. Jones, A.D. Blackwell, C. Adams, J.N. Ricaldi, S. Santibañez); The University of Iowa Roy J. and Lucille A. Carver College of Medicine, Iowa City, Iowa, USA (P.M. Polgreen, S.E. Beekmann); National Association of County and City Health Officials, Washington, DC, USA (C. Gridley-Smith)

DOI: https://doi.org/10.3201/eid3213.260203

Methods

During February–March 2024, we distributed a 9-question, cross-sectional, voluntary internet survey to EIN members. We followed applicable reporting guidelines from The Checklist for Reporting Results of Internet E-Surveys and Standards for Reporting Qualitative Research (*8*). We focused on this open-ended question: "Please provide any specific example(s) of how wastewater surveillance has affected/could affect your clinical practice." Of 1,809 EIN members, 448 (25%) responded to the survey. Of those who responded, 192 (40%) provided free-text comments about clinical impact, with 178 describing how WS does or could affect their clinical practice and 14 indicating WS has no affect on their clinical practice. Using content analysis, analysts iteratively reviewed the 192 responses using an inductive approach, noting multiple response topics. Analysts then coded responses, identified potential themes that aligned with responses, and selected representative quotations from a password-protected file. We reviewed codes and themes and refined them through team discussions to ensure consistency in interpretation. The CDC's National Center for Emerging and Zoonotic Infectious Diseases Human Subjects Advisor reviewed this activity and deemed it nonresearch per applicable federal law and CDC policy.

Results

We compiled key themes based on survey responses (Figure). Of 192 responses, approximately half (47%, n = 91) involved situational awareness and early warning. Respondents stated WS can help to "identify outbreaks earlier," "act like a 'canary in a coal mine' as far as being a harbinger of diseases in a community," and can help to "notify emergency department

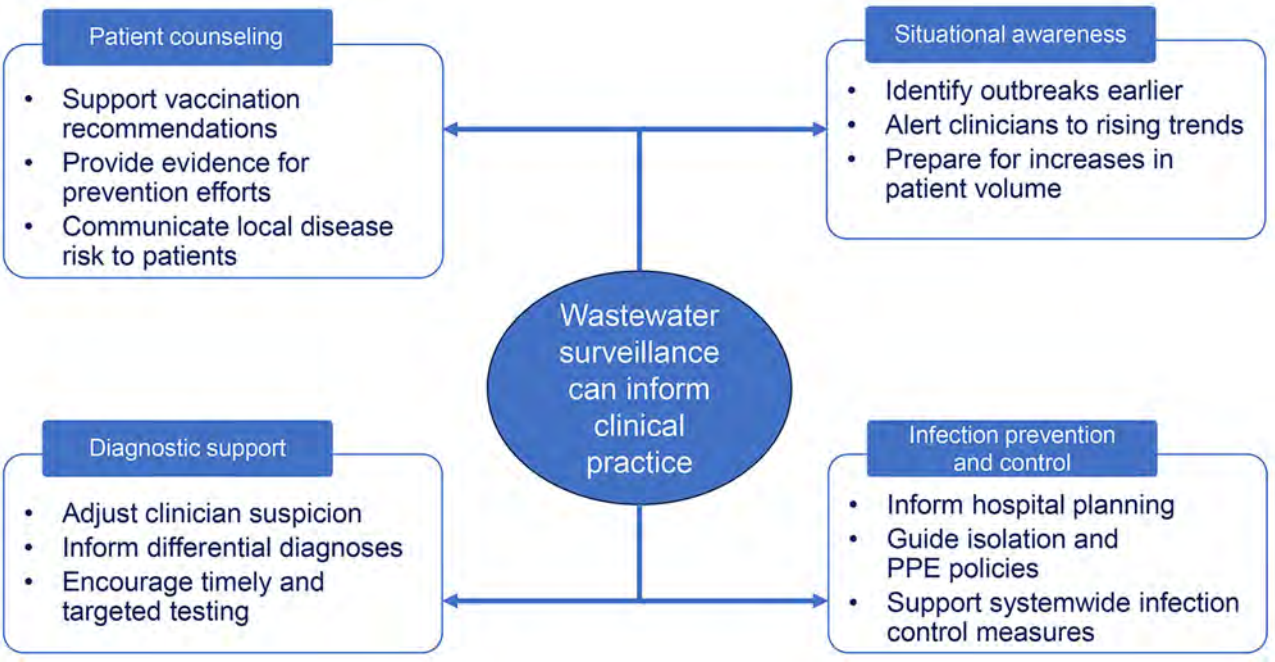


Figure. Wastewater surveillance information utilization inferred from an investigation into the implications of wastewater surveillance for clinical care among infectious disease physicians, United States, 2024. PPE, personal protective equipment.

physicians to be on alert for certain infections." About one quarter (24%, n = 47) of responses pertained to infection prevention and control. One respondent reported using WS "with hospital planning as part of my role as Medical Director for Infection Control in my hospital system." A hospital epidemiologist noted using WS "to help gauge need for sending systemwide updates and reminders about isolation, diagnostic tools, immunizations, etc."

Fifteen percent (n = 29) of responses noted the importance of WS in diagnostic testing and differential diagnoses. Respondents described how awareness "raises or lowers my clinical suspicion" and "can encourage faster relevant testing." Thirteen percent (n = 24) of respondents described the value of WS in educating patients. Specifically, respondents described how WS can provide "evidence for encouraging prevention, including vaccination," can be "helpful [in being] able to cite rising rates when recommending vaccination," and can be "very helpful in encouraging immunizations in those who don't believe a pathogen is a local issue." Last, respondents described how awareness of antibiotic resistance "may lower my threshold to use broader empiric antibiotics" and may aid in "management of vulnerable patient populations, such as transplant recipients and other immunocompromised [patients]."

A minority of respondents expressed uncertainty about WS clinical utility or noted that usefulness depends on specific conditions. For example, some respondents noted that its value depends on timeliness, geographic specificity, and clear clinical guidance. Five respondents indicated that more guidance is needed from health departments. One clinician wrote, "mainly, wastewater surveillance should be [managed by the] health department, with rapid dissemination of info to [infectious disease] docs," and another said WS could provide "data the state health department could use in some manner to provide guidance to physicians and the public."

Discussion

In this analysis, most respondents identified positive elements of how WS can be used. During the analysis and review process, we noted the phrasing of the question did not make a clear distinction between respondents' impressions of how WS findings are actively incorporated into their clinical practice versus their perceptions of how WS can be useful. Although perceived potential usefulness is still valuable, a limitation of our analysis is that findings should not be interpreted as evidence that WS has been broadly adopted. This limitation is supported by the frequent use of conditional language by respondents (e.g., "could," "would," "may"), suggesting that many comments reflected perceived potential rather than current routine use. When considered together

with our previous findings from the same survey (*5*), those comments may help identify hypotheses about potential early uses of WS in select clinical or health-system settings. The brief free-text responses limited the depth of insight. A follow-up study using in-depth interviews or focus groups could help more clearly delineate these aspects. By design, the methods of analysis we employed were intended for hypothesis generation and do not allow the drawing of conclusions. Also, respondents with greater interest in WS may have been more likely to provide comments. Overall, we believe that our findings suggest the potential of WS to inform many aspects of clinical practice.

Acknowledgments

The authors express their gratitude to the survey participants for their openness and the valuable information they provided. They also extend thanks to colleagues at the Infectious Disease Society of America for their insightful review of the manuscript.

About the Author

Ms. Jones is a behavioral scientist and workforce development leader committed to equipping the public health workforce with the skills, tools, and resources needed to realize healthy communities. She currently serves as a public health analyst with CDC's National Center for Emerging and Zoonotic Infectious Diseases.

References

1. National Academies of Sciences, Engineering, and Medicine. Wastewater-based disease surveillance for public health action. Washington: The National Academies Press; 2023. [cited 2026 Sep 25]. https://nap.nationalacademies.org/catalog/26767/wastewater-based-disease-surveillance-for-public-health-action
2. Parkins MD, Lee BE, Acosta N, Bautista M, Hubert CRJ, Hrudey SE, et al. Wastewater-based surveillance as a tool for public health action: SARS-CoV-2 and beyond. Clin Microbiol Rev. 2024;37:e0010322. https://doi.org/10.1128/cmr.00103-22
3. Centers for Disease Control and Prevention. Wastewater surveillance: a new frontier for public health. 2024 [cited 2025 Dec 16]. https://www.cdc.gov/advanced-molecular-detection/php/success-stories/wastewater-surveillance.html
4. Adams C, Bias M, Welsh RM, Webb J, Reese H, Delgado S, et al. The National Wastewater Surveillance System (NWSS): from inception to widespread coverage, 2020–2022, United States. Sci Total Environ. 2024;924:171566. https://doi.org/10.1016/j.scitotenv.2024.171566
5. Adams C, Horter L, Beekmann SE, Polgreen PM, Ricaldi JN, Louis S, et al. Infectious disease physicians' knowledge and practices regarding wastewater surveillance, United States, 2024. Emerg Infect Dis. 2024;30:2222–3. https://doi.org/10.3201/eid3010.240719
6. Gitter A, Ruvalcaba V, Clark K, Tran Carapucci T, Wu F, Hanson BM, et al. Turning analysis into action: opportunities and challenges in implementing wastewater science for public health decision-making. Front Public Health. 2025;13:1562659. https://doi.org/10.3389/fpubh.2025.1562659
7. Pillai SK, Beekmann SE, Santibanez S, Polgreen PM. The Infectious Diseases Society of America Emerging Infections Network: bridging the gap between clinical infectious diseases and public health. Clin Infect Dis. 2014;58:991–6. https://doi.org/10.1093/cid/cit932
8. O'brien BC, Harris IB, Beckman TJ, Reed DA, Cook DA. Standards for reporting qualitative research: a synthesis of recommendations. Acad Med. 2014;89(9):1245-51.

Address for correspondence: Shantrice L. Jones, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop H24-11, Atlanta, GA 30329-4027, USA; email: sjones19@cdc.gov

Public Understanding of Wastewater Surveillance, United States, August 2025

Rieza H. Soelaeman, Diana Valencia, Jena Losch, Danielle Kleven, Jazmyn Moore, Scott Santibañez

We examined public understanding of wastewater surveillance (WS) data and perceptions of its importance by using a nationwide cross-sectional survey. Approximately half of participants were aware of WS, and 79.0% responded that WS information about viruses was important, highlighting the need for community engagement about WS use and value.

The United States National Wastewater Surveillance System, established in 2020, provides public access to timely data about infectious diseases that might be circulating in a community (*1*). Clinicians, public health officials, and the general public can use this information to guide action (*2*,*3*). Despite broad implementation, less is known about how the public interprets wastewater surveillance (WS) data or perceives its value (*4*–*6*). We examined public understanding of WS data and perceptions of its importance.

We collected data by using the PN View survey (https://styles.porternovelli.com/pn-view-panels), a nationwide cross-sectional online survey conducted by Porter Novelli Public Services and Big Village. The voluntary survey was administered from August 22–24, 2025, to adults ≥18 years of age in the United States sampled by using nonprobability quota sampling. Porter Novelli computed survey weights by using a proprietary process to reflect the United States population demographic composition in sex, age, region, race and ethnicity, and education on the basis of Current Population Survey proportions (Appendix Table, http://wwwnc.cdc.gov/EID/article/32/13/26-0559-App1.pdf). We followed the Checklist for Reporting Results of Internet E-Surveys guidelines (*7*).

Participants were asked to select the definition of WS that best matched their understanding, interpret a figure depicting wastewater detections of a hypothetical vaccine-preventable respiratory virus, and identify actions they would take if the virus was detected in their community. We collected participants' opinions on the importance of the information provided by WS by using an open-text field (Appendix). We performed weighted quantitative analyses in Stata version 18.0 (StataCorp, LLC, https://www.stata.com); we analyzed free-text responses in MAXQDA 24 (https://www.maxqda.com/new-maxqda-24).

This activity was reviewed and deemed not research by the National Center for Emerging and Zoonotic Infectious Diseases Human Subjects Advisor at the Centers for Disease Control and Prevention (CDC) and conducted according to CDC Human Research Protection Program Standard Operating Procedures, consistent with applicable federal law and CDC policy. Although Porter Novelli Public Services and Big Village did not require Institutional Review Board review, they adhere to European Society for Opinion and Marketing Research ethical standards.

Of 1,014 participants, 51.0% (95% CI 47.8%–54.2%) were female and 48.5% (95% CI 45.3–51.7) male; 60.8% (95% CI 57.5%–63.9%) were non-Hispanic White, and 45.9% (95% CI 42.7%–49.1%) lived in a suburban community (Table). Nearly half of all participants (50.3% [95% CI 47.1%–53.5%]) defined WS correctly, and 83.3% (95% CI 80.8%–85.5%) of participants interpreted the figure correctly. The most common actions selected by participants were to learn more about the virus and how it spreads (51.1% [95% CI 47.9%–54.3%]), check CDC's website for more information (49.1% [95% CI 45.9%–52.3%]), and consult the local health department (45.7% [95% CI 42.5%–48.9%]) (Figure; Appendix).

Most (79.0%) participants gave free responses that suggested that information from WS about viruses and variants was important. Among the 801 participants who considered WS important, 624 (77.9%) wrote about early detection, outbreak

Author affiliation: Centers for Disease Control and Prevention, Atlanta, Georgia, USA

DOI: https://doi.org/10.3201/eid3213.260559

Table. Public understanding of wastewater surveillance and interpretation of wastewater detection data visualization by demographic characteristic, Porter Novelli View survey, United States, August 2025

Characteristic	No. (%) [95% CI]*	Correctly defined wastewater surveillance, % (95% CI)		p value†	Correctly interpreted the data visualization figure, % (95% CI)		p value†
		Yes, n = 508	No, n = 506		Yes, n = 838	No, n = 176	
Total	1,014 (100.0)	100.0	100.0		100.0	100.0	
Sex				0.53			0.56
M	498 (48.5) [45.3–51.7]	49.9 (45.3–54.4)	47.0 (42.6–51.6)		48.4 (44.9–51.9)	48.8 (41.2–56.5)	
F	506 (51.0) [47.8–54.2]	49.6 (45.0–54.1)	52.5 (48.0–57.0)		51.2 (47.7–54.7)	50.2 (42.5–57.8)	
Missing	10 (0.5) [0.3–0.9]	0.6 (0.3–1.3)	0.4 (0.2–1.1)		0.4 (0.2–0.9)	1.0 (0.3–3.0)	
Age group, y				<0.001			<0.001
18–29	191 (19.1) [16.7–21.8]	16.6 (13.5–20.2)	21.7 (18.1–25.7)		18.0 [15.4–20.9)	24.9 [18.8–32.3)	
30–44	333 (26.7) [24.2–29.4]	22.7 (19.4–26.3)	30.8 (27.0–34.9)		24.6 (22.0–27.6)	37.0 (30.2–44.5)	
45–59	191 (22.8) [20.0–25.8]	23.2 (19.3–27.6)	22.4 (18.6–26.6)		23.6 (20.6–27.0)	18.4 (13.0–25.6)	
≥60	299 (31.4) [28.5–34.5]	37.6 (33.2–42.1)	25.1 (21.4–29.4)		33.8 (30.5–37.2)	19.6 (13.9–26.8)	
Race and ethnicity‡							
Black or African American	116 (12.1) [10.2–14.4]	9.7 (7.3–12.8)	14.6 (11.6–18.2)	0.13	10.7 (8.7–13.1)	19.3 (13.6–26.6)	<0.001
Hispanic or Latino	143 (17.9) [15.4–20.7]	18.3 (14.7–22.5)	17.5 (14.1–21.5)		18.3 (15.5–21.5)	15.9 (10.8–22.8)	
White	641 (60.8) [57.5–63.9]	63.1 (58.5–67.5)	58.4 (53.8–62.8)		63.0 (59.5–66.4)	49.5 (41.8–57.1)	
Other race§	114 (9.2) [7.7–11.1]	8.9 (6.8–11.6)	9.5 (7.4–12.2)		8.0 (6.4–9.9)	15.4(10.8–21.4)	
Marital status				0.31			<0.001
Married or in a union	546 (52.4) [49.1–55.5]	53.7 (49.1–58.2)	51.0 (46.5–55.5)		51.5 (48.0–55.0)	56.6 (48.8–64.1)	
Divorced, widowed, or separated	179 (19.4) [16.9–22.1]	20.2 (16.7–24.3)	18.5 (15.1–22.3)		21.6 (18.7–24.7)	8.2 (4.8–13.4)	
Never married	289 (28.3) [25.5–31.3]	26.1 (22.3–30.2)	30.6 (26.6–34.9)		26.9 (23.9–30.1)	35.2 (28.2–43.0)	
Education				0.005			0.07
High school or less	327 (39.1) [35.9–42.4]	34.7 (30.3–39.4)	43.5 (39.0–48.1)		38.0 (34.5–41.6)	44.7 (37.1–52.5)	
Some college	265 (24.6) [22.0–27.4]	23.9 (20.3–27.8)	25.4 (21.7–29.5)		25.4 (22.6–28.6)	20.6 (15.3–27.2)	
Bachelor's degree	275 (23.8) [21.3–26.5]	27.9 (24.1–31.9)	19.7 (16.5–23.3)		24.8 (22.0–27.8)	18.9 (13.9–25.3)	
Postgraduate degree	147 (12.5) [10.6–14.6]	13.5 (10.9–16.6)	11.5 (9.0–14.4)		11.8 (9.9–14.1)	15.8 (11.2–21.7)	
Children in household				<0.001			0.05
Y	349 (31.2) [28.4–34.1]	25.7 (22.1–29.7)	36.7 (32.5–41.1)		29.9 (26.8–33.2)	37.5 (30.6–45.0)	
N	665 (68.8) [65.9–71.6]	74.3 (70.3–77.9)	63.3 (58.9–67.5)		70.1 (66.8–73.2)	62.5 (55.0–69.4)	
Household income, USD				0.02			0.39
<25,000	182 (20.4) [17.8–23.2]	17.1 (13.7–21.1)	23.7 (19.9–27.8)		20.2 (17.4–23.4)	21.0 (15.3–28.1)	
25,000–49,999	266 (27.6) [24.8–30.6]	25.8 (22.0–30.1)	29.3 (25.3–33.7)		26.6 (23.6–29.9)	32.5 (25.6–40.2)	
50,000–99,999	311 (29.3) [26.5–32.3]	31.6 (27.6–35.9)	26.9 (23.2–31.1)		30.1 (27.1–33.4)	25.1 (19.0–32.4)	
≥100,000	255 (22.8) [20.3–25.5]	25.4 (21.8–29.5)	20.1 (16.8–23.7)		23.0 (20.3–26.0)	21.4 (16.1–28.0)	
Community type				<0.001			0.003
Urban	328 (31.7) [28.8–34.7]	26.7 (22.9–30.9)	36.6 (32.4–41.1)		29.4 (26.3–32.7)	43.1 (35.7–50.9)	
Suburban	463 (45.9) [42.7–49.1]	52.2 (47.7–56.7)	39.5 (35.1–44.0)		47.6 (44.1–51.1)	37.4 (30.3–45.2)	
Rural	223 (22.5) [19.9–25.3]	21.1 (17.6–25.0)	23.9 (20.2–28.0)		23.1 (20.2–26.2)	19.4 (14.1–26.2)	
Region¶				0.36			0.33
Northeast	182 (17.3) [15.0–19.8]	16.2 (13.1–19.8)	18.3 (15.2–22.0)		16.3 (13.9–19.1)	21.9 (16.3–28.8)	
Midwest	199 (20.5) [18.0–23.2]	20.6 (17.2–24.6)	20.3 (16.8–24.3)		21.0 (18.3–24.1)	17.6 (12.4–24.4)	
South	382 (38.6) [35.5–41.8]	37.2 (32.9–41.7)	40.0 (35.7–44.6)		38.6 (35.2–42.1)	38.6 (31.4–46.4)	
West	251 (23.7) [21.1–26.5]	26 (22.2–30.1)	21.3 (17.9–25.2)		24.0 (21.2–27.1)	21.9 (16.3–28.8)	

*Unweighted counts and weighted percentages.
†Statistical significance of differences in responses was determined at $\alpha = 0.05$ by using Pearson χ^2 tests corrected for survey design.
‡Persons of Hispanic or Latino (Hispanic) origin might be of any race but are categorized as Hispanic; all racial group tabulation is limited to those persons reporting being non-Hispanic.
§Includes Middle Eastern or North African persons, persons identifying as another race not listed in the survey, and persons identifying as ≥1 race.
¶Northeast: Connecticut, Maine, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont; Midwest: Iowa, Illinois, Indiana, Kansas, Michigan, Minnesota, Missouri, North Dakota, Nebraska, Ohio, South Dakota, Wisconsin; South: Alabama, Arkansas, District of Columbia, Delaware, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, West Virginia; West: Alaska, Arizona, California, Colorado, Hawaii, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, Wyoming.

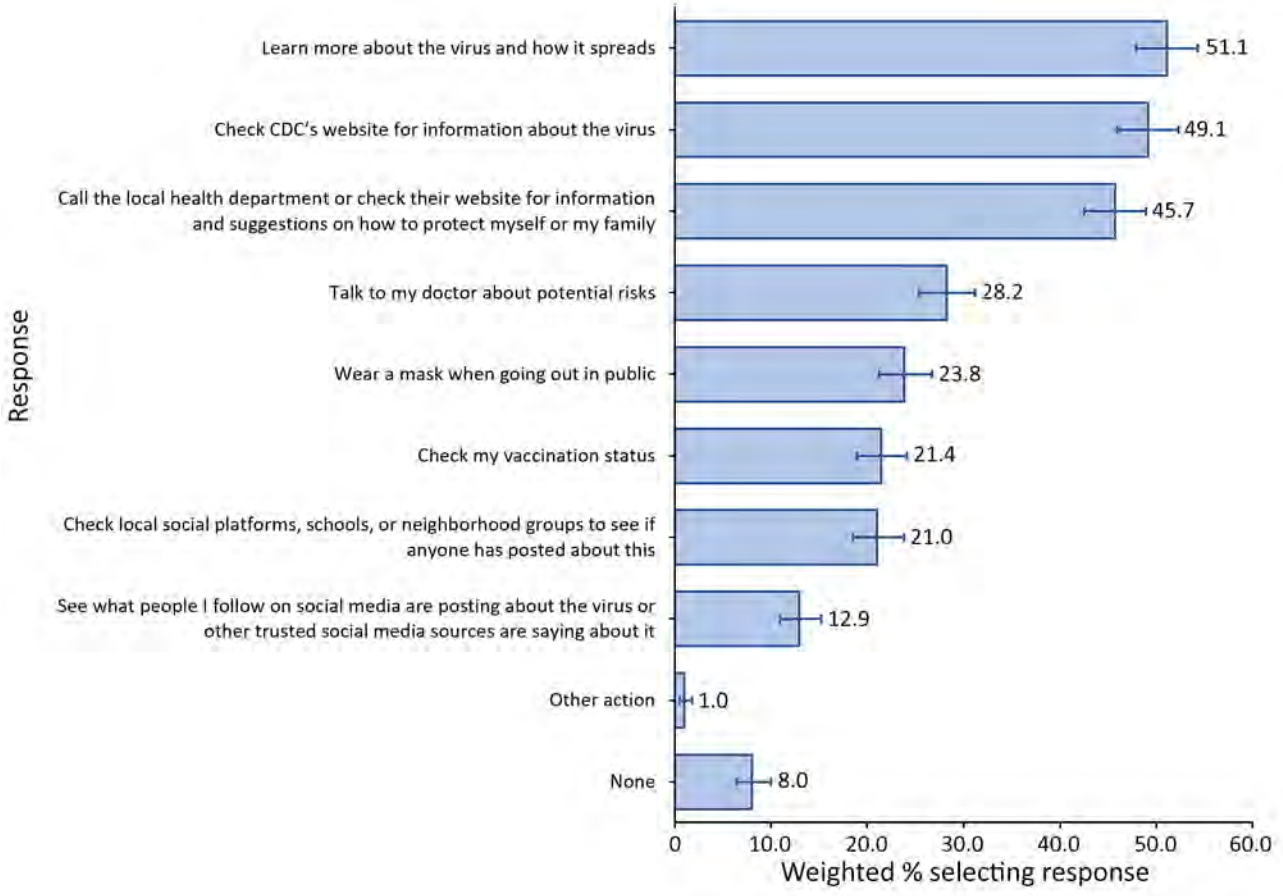


Figure. Actions participants intend to take after wastewater detections of a hypothetical vaccine-preventable virus in their area (n = 1,014) from Porter Novelli View survey, United States, August 2025. Bars indicate the percentage of participants who selected the action in their response to the question, "If wastewater data showed that this virus was present in your community, what steps, if any, would you take?" for which they could select multiple options; error bars indicate 95% CIs. CDC, Centers for Disease Control and Prevention.

prevention, awareness, and family or community safety. Of those, 79 (12.7%) participants specifically mentioned virus strains or vaccinations. One participant stated, "Wastewater monitoring helps detect which viruses are spreading and even identify their variants." Another participant noted, "[WS] can tell you if the strain of the virus is a new one that you may not have been vaccinated against, and you can take the necessary precautions." One participant suggested clear, nonalarming communication for informing the public. Among those who considered WS unimportant, reasons mentioned included uncertainty surrounding actions to take and beliefs that diseases will spread regardless of efforts.

In our survey, participants were moderately aware of WS, with approximately half of participants correctly defining it. That result was consistent with other nationwide surveys reporting awareness levels of 48% in 2022 and 49% in 2023 and a 2024 survey conducted across 5 states that reported 56.0% awareness (*8*,*9*). Furthermore, most participants accurately interpreted a figure depicting wastewater detection data, suggesting that an intuitive graphic can be helpful to convey information. When given background information on the types of infectious disease data WS provides to the public, participant attitudes were generally favorable.

To maximize data usability, health agencies could provide clear, plain-language explanations of WS accompanied by graphics, figures, and guidance for data interpretation for the pathogen. In addition, offering practical protective steps when pathogen detections occur might empower persons to make informed decisions.

Demographic differences in understanding might reflect varying levels of exposure to public health messaging and highlight opportunities for tailored communication (e.g., informing residents of rural communities of the value and use of WS). Study limitations include that persons with interest in public health or WS could have self-selected to participate in the survey and that the framing of the open-ended

question might have led to measurement error in the form of social desirability bias (*10*). During times when WS detects increasing infectious disease activity, health agencies can partner with trusted messengers, including media partners, community organizations, and healthcare providers, who can help amplify and contextualize WS information for their communities and audiences.

Acknowledgments

We thank Nicole Fehrenbach and Jonathan Yoder for the review and feedback provided for this manuscript. We also thank the survey participants for their time.

About the Author

Dr. Soelaeman is a health scientist at the National Center for Emerging and Zoonotic Infectious Diseases, Division of Infectious Disease Readiness and Innovation, Centers for Disease Control and Prevention. Her research interests are in infectious diseases, emergency preparedness and response, and field methods in public health.

References

1. Centers for Disease Control and Prevention. National wastewater surveillance system. 2024 [cited 2024 Sep 30]. https://www.cdc.gov/nwss/index.html
2. Kirby AE, Walters MS, Jennings WC, Fugitt R, LaCross N, Mattioli M, et al. Using wastewater surveillance data to support the COVID-19 response—United States, 2020–2021. MMWR Morb Mortal Wkly Rep. 2021;70:1242–4. https://doi.org/10.15585/mmwr.mm7036a2
3. Valencia D, Yu AT, Wheeler A, Hopkins L, Pray I, Horter L, et al. Notes from the field: the national wastewater surveillance system's centers of excellence contributions to public health action during the respiratory virus season—four U.S. jurisdictions, 2022–23. MMWR Morb Mortal Wkly Rep. 2023;72:1309–12. https://doi.org/10.15585/mmwr.mm7248a4
4. Ni K, Korfmacher KS. A scoping review on the ethics of wastewater surveillance for COVID-19. J Public Health Manag Pract. 2025;31:E387–95. https://doi.org/10.1097/PHH.0000000000002206
5. Soelaeman RH, Kleven D, Losch J, Vega M, Fehrenbach SN, Ricaldi JN, et al. Notes from the field: support for wastewater monitoring and influence on protective behavioral intentions among adults—United States, July 2024. MMWR Morb Mortal Wkly Rep. 2024;73:825–7. https://doi.org/10.15585/mmwr.mm7337a2
6. Soelaeman RH, Valencia D, Horter L, Turner H, Yadav R, Tatti KM, et al. Public awareness and acceptance of wastewater surveillance in the United States, June 2024. Public Health Rep. 2026 Jul 5 [Epub ahead of print]. https://doi.org/10.1177/00333549261457426
7. Eysenbach G. Improving the quality of web surveys: the Checklist for Reporting Results of Internet E-Surveys (CHERRIES). J Med Internet Res. 2004;6:e34. https://doi.org/10.2196/jmir.6.3.e34
8. Holm RH, Anderson LB, Ness HD, LaJoie AS, Smith T. Towards the outbreak tail, what is the public opinion about wastewater surveillance in the United States? J Water Health. 2024;22:1409–18. https://doi.org/10.2166/wh.2024.074
9. Kreuter MW, Garg R, Marsh AK, Olagoke A, Weng O, De La Vega V, et al. Wastewater monitoring: improving public awareness and understanding in the United States, May 2024. Public Health Rep. 2026;141:114–21. https://doi.org/10.1177/00333549251359177
10. Graeff TR. Response bias. In: Kempf-Leonard K, editor. Encyclopedia of social measurement. San Diego, CA: Elsevier Inc; 2005. p. 411–418.

Address for correspondence: Rieza H. Soelaeman, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop H24-11, Atlanta, GA, 30329-4018, USA; email: rsoelaeman@cdc.gov

Exploration of Public Perceptions of Wastewater Surveillance, United States, June 2024

Jazmyn T. Moore, Krysten Rose-McCully, Diana Valencia, Hannah Turner, Kathleen Tatti, Manpreet Kaur, Mariatou Ceesay, Scott Santibanez

Community wastewater surveillance (WS) is a valuable tool for informing public health action, but its effects on individual health behaviors rely on public understanding and awareness. We analyzed 2,024 free text responses from a national survey on perceptions of WS conducted during June 19–23, 2024, to gain insights into public perceptions of WS. Primary themes among 1,181 detailed responses included varying levels of trust in government and authorities, the need for more information about the effectiveness and accuracy of monitoring, desire to understand public health implications, and the need for more education and communication. Those findings could help inform community engagement strategies as WS continues to evolve.

In September 2020, the Centers for Disease Control and Prevention (CDC) launched the National Wastewater Surveillance System (NWSS) to monitor COVID-19 trends in wastewater samples across the United States (*1*). Wastewater surveillance (WS) has since expanded to include monitoring for influenza A, measles, and mpox viruses, as well as other pathogens, across ≈1,500 sites nationwide, including US states, territories, and tribal communities.

Surveillance of infectious diseases in wastewater provides communities with early warning signs of potential outbreaks and information about trends in infections. WS can inform individual protective behaviors and public health actions. A 2024 survey suggested that WS-specific awareness and education can enhance community trust in public health initiatives and motivate persons to take steps to protect themselves (*2*). Without continued efforts to educate and engage communities during the implementation of WS, implementers risk missteps that can harm trust in government and public health. We conducted a qualitative analysis of public perspectives on WS to help inform efforts to engage persons living in communities implementing WS.

Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (J.T. Moore, K. Rose-McCully, D. Valencia, M. Kaur, M. Ceesay, S. Santibanez); Oak Ridge Institute for Science and Education, Oak Ridge, Tennessee, USA (K. Rose-McCully, M. Ceesay); Goldbelt Professional Services, Juneau, Alaska, USA (H. Turner); G²S Corporation, Shavano Park, Texas, USA (K. Tatti); Kennedy Krieger Institute, Dr. James A. Ferguson Emerging Infectious Diseases Graduate Fellowship, Baltimore, Maryland, USA (M. Kaur)

DOI: https://doi.org/10.3201/eid3213.251494

Methods

During June 19–23, 2024, Porter Novelli Public Services conducted a 9-question, nationwide survey among US adults (Appendix, https://wwwnc.cdc.gov/EID/article/32/13/25-1494-App1.pdf). The survey aimed to assess participants' knowledge and attitudes regarding WS, referred to as wastewater monitoring in the survey (Appendix). In this qualitative analysis, we reviewed and categorized 2,024 free text responses to the open-ended question, "Do you have any concerns about wastewater monitoring in your community? Why or why not?"

Three analysts independently coded survey responses and subsequently reached consensus on themes. Each response was classified into the most relevant theme on the basis of its content. We calculated summary statistics to provide an overview of the findings. This activity was reviewed by members of the Information Collections and Human Studies Team in CDC's National Center for Emerging and Zoonotic Infectious Diseases and was determined to be nonresearch in accordance with 45 CFR 46.102(l) on April 11, 2023. The survey was conducted in a manner consistent with applicable federal law and CDC policy.

Results

Of 2,024 write-in responses, a total of 1,342 (66.3%) participants reported no concerns with WS, 285 (14.1%)

expressed concerns, 248 (12.3%) were uncertain or did not understand the question, 110 (5.4%) were unaware of WS initiatives, and 39 (1.9%) relied on private wastewater systems only. From 1,181 responses, we identified the following 4 themes: trust in government and authorities, effectiveness and accuracy of monitoring, public health implications, education and communication (Table 1). Many respondents expressed multiple themes. For example, among 1,342 participants who expressed no concerns, 49% expressed no concerns at all, 43% supported the initiative with conditional trust in government agencies, and 6% expressed no concerns due to their unfamiliarity with the subject.

Trust in Government and Authorities

Respondents expressed a range of views about trust in agencies managing WS. Many voiced confidence in local and state health departments and universities, citing their commitment to evidence-based practice, ethics, and responsiveness to community needs. Others expressed skepticism, raising concerns about transparency, surveillance, potential data misuse, and fears of political influence or excessive government control. Key concerns included uncertainty about who can access data, how data are used, and whether specific communities might be unfairly targeted. For many, distrust was shaped by previous negative experiences with public health programs or messaging. Trust was highest when respondents believed monitoring was transparent, locally led, and focused on public health outcomes.

Effectiveness and Accuracy of Monitoring

Many respondents viewed WS as a valuable tool for tracking community health and providing early warnings about disease trends, especially when conducted by trusted institutions. Concerns included the validity of the testing methods, potential misinterpretation of results, and lack of transparency in data handling or use. Overall, support was strongest when respondents believed monitoring was scientifically rigorous, clearly explained, and overseen with integrity.

Public Health Implications

Several respondents recognized WS as a valuable approach to protecting community health, especially

Table. Key themes from write-in responses describing perceptions and concerns around wastewater monitoring (n = 1,181). Porter Novelli Survey, U.S., June 2024

Theme	Sample quotes	Subthemes
Trust in government and authorities	"I trust the local health department to handle this. They've always been transparent and seem to genuinely care about our community's well-being." "It sounds like something that could be helpful, but only if it's done with complete honesty. Otherwise, I worry it's just another way to monitor communities without telling us the full story."	• Past success during COVID-19 boosted trust in implementation • Concerns about nonhealth use of data • Negative experiences shaped federal government distrust • Fear that monitoring could stigmatize specific communities • Privacy was a key issue, with fears of being monitored
Effectiveness and accuracy of monitoring	"There's a lot of potential in this kind of monitoring, but I'd want to be sure the testing is consistent, and the results aren't being manipulated or misunderstood. Public trust depends on transparency and good science." "My main concern is wondering if the data is accurate or not and is quite possibly skewed by outside sources."	• Allows for real-time or near real-time surveillance • Seen as helpful in tracking patterns over time • Doubts about data accuracy and potential for skewed results • Concerns about misinterpretation or false alarms • Desire for more transparency on data handling and use
Public health implications	"As long as it's used for the right reasons—like tracking disease and helping the community—then I fully support it. It's a smart way to stay ahead of public health threats." "If the goal is public safety and there are clear explanations about what is being done and why, then I think it's a great way to use science to protect the population."	• Seen as useful for early disease detection • Strong support when focused on public health • Belief that data can help prevent outbreaks • Concerned with protecting family and community • More support when purpose is clearly communicated
Education and communication	"Education is key—if you don't tell people what's happening in their own communities and how it affects them, they'll feel left out or even threatened. Communication builds trust." "This is my first time hearing about it. But it sounds like a good idea—if it's explained well and backed up with science. People just need to understand what's going on and why."	• Desire for simple explanations of purpose and process • Trust relied on ongoing, honest communication • Many were unfamiliar with wastewater monitoring • Support linked to clear, transparent messaging • Openness increased with basic information

for early outbreak detection and tracking public health trends. That support was often grounded in the belief that such efforts serve the public good and aim to prevent harm. Participants highlighted the importance of early detection. Confidence in the agencies conducting monitoring also emerged as a key theme, and some participants stated that they trust authorities to do the right thing. Although support was generally strong, a few respondents emphasized that their approval was conditional as long as WS was used for public health reasons. Overall, acceptance was highest when the purpose was clearly linked to community wellbeing and led by trusted institutions.

Education and Communication

Many respondents emphasized the importance of clear communication and public education in building support for WS. Several indicated they were unfamiliar with the practice but expressed openness when given more information. Others stressed the need for accessible explanations and proactive outreach. Trust often hinged on clear and honest communication. Still, concerns about limited awareness remained, with some noting they did not know monitoring was occurring. Overall, respondents viewed education and communication as essential for fostering public understanding, transparency, and trust in WS efforts.

Discussion

In this June 2024 nationwide survey, most respondents did not have concerns about WS for infectious diseases. However, some expressed limited or no awareness of WS and a desire for additional information and improved communication about findings from the initiatives.

Because large-scale use of WS is relatively new, few studies examining public perceptions of these initiatives are available. Results of several national surveys found that about half of respondents reported previous awareness of wastewater monitoring, but most were generally supportive of this public health strategy (*3*–*5*). Another survey found that 65% of participants (n = 516) knew little or nothing about WS and suggested the need for communication about how WS works, how findings are used, and protections against misuse (*6*). Those findings align closely with the results from our analysis and suggest a consistent pattern of support for WS while highlighting the opportunity for continued public education and communication about those efforts.

The findings from this survey highlight the trust that communities have in state and local public health and suggest that communities trust WS when they trust the institutions conducting it. They also suggest that, to further build and maintain trust, WS activities should remain transparent and focused on informing public health action and preventing community harm. The findings also highlight the opportunity for additional efforts to educate the public on why WS is used and how wastewater data are protected.

To engage community members, public health entities can share messages around individual health choices through trusted messengers like healthcare providers, community health workers, and local leaders. Public health entities should also build strong relationships with community and faith-based organizations to better reach local audiences and align communication strategies with the needs and preferences of the populations they serve (*7*–*9*).

The first limitation of this study is that it relied on a convenience sample and an internet-based, English-only survey; persons with limited internet access, less familiarity with online technology, or limited English proficiency might have been excluded. Therefore, the results are not representative of the entire population. In addition, because open-ended questions were optional, responses might not reflect the full range of perspectives on WS.

In conclusion, the survey's findings highlighted opportunities for public health to use WS to build trust. Engaging communities through trusted messengers and organizations can enhance understanding and acceptance, and continued education and transparent communication could increase public awareness and strengthen confidence in public health initiatives. Together, those strategies can support effective individual and community responses to infectious disease threats.

Acknowledgments

We thank Jena Losch, Deanna Amarosa, Rieza Soelaeman, and Emily Nelson for their support of this effort.

This project was supported in part by an appointment to the Research Participation Program at the Centers for Disease Control and Prevention administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the US Department of Energy and Centers for Disease Control and Prevention (E.F.S.). This manuscript was supported by funding from the Centers for Disease Control and Prevention (cooperative agreement no. NU50CD300866).

About the Author

Ms. Moore is a health scientist at the Centers for Disease Control and Prevention in Atlanta, Georgia, USA. Her primary research focuses on parasites and infectious diseases, with a particular emphasis on their social and environmental determinants.

References

1. Adams C, Bias M, Welsh RM, Webb J, Reese H, Delgado S, et al. The National Wastewater Surveillance System (NWSS): from inception to widespread coverage, 2020–2022, United States. Sci Total Environ. 2024;924:171566. https://doi.org/10.1016/j.scitotenv.2024.171566
2. Soelaeman RH, Kleven D, Losch J, Vega M, Fehrenbach SN, Ricaldi JN, et al. Notes from the field: support for wastewater monitoring and influence on protective behavioral intentions among adults—United States, July 2024. MMWR Morb Mortal Wkly Rep. 2024;73:825–7. https://doi.org/10.15585/mmwr.mm7337a2
3. LaJoie AS, Holm RH, Anderson LB, Ness HD, Smith T. Nationwide public perceptions regarding the acceptance of using wastewater for community health monitoring in the United States. PLoS One. 2022;17:e0275075. https://doi.org/10.1371/journal.pone.0275075
4. Holm RH, Brick JM, Amraotkar AR, Hart JT, Mukherjee A, Zeigler J, et al. Public awareness of and support for the use of wastewater for SARS-CoV-2 monitoring: a community survey in Louisville, Kentucky. ACS ES T Water. 2022;2:1891–8. https://doi.org/10.1021/acsestwater.1c00405
5. Holm RH, Anderson LB, Ness HD, LaJoie AS, Smith T. Towards the outbreak tail, what is the public opinion about wastewater surveillance in the United States? J Water Health. 2024;22:1409–18. https://doi.org/10.2166/wh.2024.074
6. Kreuter MW, Garg R, Marsh AK, Olagoke A, Weng O, De La Vega V, et al. Wastewater monitoring: improving public awareness and understanding in the United States, May 2024. Public Health Rep. 2026;141:114–21. https://doi.org/10.1177/00333549251359177
7. Santibañez S, Lynch J, Paye YP, McCalla H, Gaines J, Konkel K, et al. Engaging community and faith-based organizations in the Zika response, United States, 2016. Public Health Rep. 2017;132:436–42. https://doi.org/10.1177/0033354917710212
8. West R, Moore J, Chillag K, Fenton E, Laird E, Boyea A. Development of a new framework to address public health ethical considerations in wastewater surveillance. J Public Health Manag Pract. 2025;31:683–5. https://doi.org/10.1097/PHH.0000000000002167
9. Chillag K, Fenton E. Framework for addressing ethical considerations in infectious diseases public health wastewater surveillance. Arlington (VA): Association of State and Territorial Health Officials; 2025.

Address for correspondence: Jazmyn Moore, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop H24-3, Atlanta, GA 30329-4018, USA; email: vin2@cdc.gov

Alexander T. Yu, *Tip of the Iceberg*, 2026. Watercolor on paper. 14 in × 17 in. Image courtesy of the artist.

Under the Iceberg

Alexander T. Yu

The cover illustration for this supplement to *Emerging Infectious Diseases* depicts a city resting atop an iceberg floating in the ocean. The iceberg is a familiar public health metaphor in infectious disease epidemiology where cases known to traditional public health surveillance systems are often described as the tip of the iceberg—the small visible portion of a much larger burden of disease that remains unseen. Beneath the surface are undiagnosed infections, asymptomatic cases, spontaneous recoveries, unrecorded deaths, and other uncounted incidences due to barriers to healthcare access, limitations of testing, and a multitude of other reasons.

As a public health medical epidemiologist and infectious diseases physician, I have spent the past decade trying to illuminate what lies underneath the tip of the iceberg. Wastewater surveillance (WS), the focus of this supplement issue, has been an incredible tool, allowing public health to do just that. WS works by collecting samples from

Author affiliation: California Department of Public Health, Richmond, California, USA

DOI: https://doi.org/10.3201/eid3213.AC3213

sewage, which everyone living in sewered communities contributes to, and testing it for the presence and quantity of different pathogens. As such, WS can capture information from entire communities, regardless of symptoms or healthcare-seeking behavior. That process contrasts with traditional surveillance systems, which rely on test results from individual persons. WS can provide early warning of outbreaks, identify emerging pathogens, monitor disease trends, and complement traditional clinical surveillance. This form of surveillance can also generate meaningless, difficult to interpret, or inactionable data. As the illustration depicts, the sewershed is large and diverse. Interpreters of the data the system generates may not be able to readily discern the source(s) of salient findings, be they from homes, hospitals, or farms, or even whether they originate from humans or animals.

WS is exciting but still relatively novel, unheard of by many, including those in healthcare and public health. There remains the basic need to understand how to translate noisy wastewater data that is meaningless by itself into a clear message interpretable to all (e.g., public health, health care providers, the public). Before I began a career in medicine and public health, I worked as a freelance cartoonist and illustrator for a variety of newspapers. Now, as a public health practitioner often trying to communicate about public health and WS findings, I have found myself leaning on lessons learned from that time as a cartoonist and illustrator. An essential element of cartooning and illustration is to convey simple and clear messages or stories that can be interpreted similarly by many different viewers and still carry nuance and complexity.

To capture this moment in the WS field and the essence of what this supplement hopes to improve, I wanted to create an illustration that could communicate simply what WS is and the role it can play in public health by leaning on the familiar iceberg metaphor. In the illustration, a magnifying glass focused under the iceberg reveals a network of wastewater pipes and the microorganisms flowing through them. This hidden infrastructure carries traces of entire communities, creating an opportunity to observe population health from a different perspective. By revealing part of the iceberg that has long remained unseen beneath the surface, WS offers a new perspective on an old challenge: understanding the health of populations through signals that are often hidden from view.

ChatGPT (OpenAI, https://www.openai.com) was used in a limited way to assist in the writing of this essay. No artificial intelligence tools were used in the creation of the illustration.

Address for correspondence: Alexander T. Yu, California Department of Public Health, Center for Infectious Diseases, 850 Marina Bay Pkwy, Bldg P, 2nd Fl, Office P-2330, Richmond, CA 94804, USA; email: alexander.yu@cdph.ca.gov