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# Measles RNA detection in wastewater solids: longitudinal monitoring at a national scale

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Measles incidence has increased in recent years as vaccination rates have dropped globally, yet clinical surveillance is challenging due to misdiagnoses and a lag between infectivity and symptom onset. In this study, we applied a novel hydrolysis probe digital RT-PCR assay that is specific and sensitive to detect wild-type measles virus RNA in wastewater. Through pilot testing during an outbreak in the United States from December 2024–May 2025, we demonstrated that wild-type measles virus RNA can be detected in wastewater prior to clinical case reporting. We then conducted 6 months of measles RNA monitoring across the United States at 147 wastewater treatment plants including 11 598 samples. We detected measles RNA in 63 samples from 25 wastewater treatment plants across 17 states. Wastewater measles RNA detection was associated with clinical cases within 7 days (odds ratio: 14.7; 95% confidence interval: 8.6–24.6) and in the following 7 days (15.9, 9.6–27.3). Overall, national wastewater monitoring of measles RNA can fill clinical surveillance gaps and provide early warning of cases to supplement measles surveillance.

**Water impact**

This research demonstrates that wastewater testing for measles RNA can be used to identify areas in which measles cases are subsequently reported. Within the context of a recent increase in measles cases across the world, this work provides evidence that monitoring measles RNA in wastewater can help fill surveillance gaps and provide early warning of cases to supplement measles surveillance.

## Introduction

Measles is a highly infectious but vaccine-preventable disease. Despite steady progress on vaccine coverage in the 21st century, vaccine coverage has decreased since the COVID-19 pandemic and global incidence has again increased.[1] The measles virus (recently renamed to *Morbillivirus hominis*) is a single-stranded, negative sense RNA paramyxovirus of the genus *Morbillivirus*.[2] The most common symptoms include fever, rash, cough, rhinitis, and conjunctivitis, with the characteristic rash appearing 3–5 days after symptoms begin.[2] Measles has an average basic reproductive number ($R_0$) between 12 and 18, making it one of the most infectious viruses identified.[3] A vaccine for measles was first licensed in 1963, and measles incidence in the U.S. decreased by more than 95% following its introduction.[2] The vaccine is highly effective; ~95% of those who receive one dose and >99% of those who receive two doses show evidence of measles immunity.[2] However, because measles is highly infectious, community-level vaccine coverage (2 full doses) of ≥95% is needed to prevent outbreaks.[2] Vaccine coverage has fallen below this threshold in many locations, both in the U.S. and globally, since the COVID-19 pandemic, in part due to disruption in routine vaccination of children.[1,4,5]

Falling vaccination rates have resulted in an increase in measles cases worldwide since the COVID-19 pandemic, with large outbreaks reported in 37 countries in 2022.[1] The U.S., where the national vaccination rate is 90.8%,[6] has reported the largest number of cases since 1992, with 2065 cases reported in 2025 across 43 US states and territories, with 49 outbreaks (3+ associated cases) identified.[7] Ninety-three percent of cases in 2025 were among unvaccinated or under-vaccinated individuals; 11% of cases resulted in hospitalizations and three deaths were reported.[7] In the U.S., data on vaccination coverage are not available in many states at a local scale, making it unclear where to target public health resources.

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Clinical surveillance for measles is limited by disease presentation, test availability, and healthcare-seeking behavior. The symptoms of measles are nonspecific prior to the appearance of a rash, and are similar to those of other diseases caused by the dengue, Zika, or Parvovirus B19 viruses. During times of low incidence, measles can be hard to differentiate from other similarly-presenting diseases.[8] In low-resource settings, availability of laboratory reagents and trained staff can make clinical testing difficult to regularly achieve. Regardless of testing resources, only individuals who seek healthcare can be tested and reported, and milder cases or those without access to care may not be included in case counts. In 2022, only half of 144 WHO countries met the measles surveillance target, indicating that enhanced surveillance is needed.[1]

Wastewater monitoring for measles RNA has the potential to fill surveillance gaps and provide critical information on measles virus prevalence globally. Measles RNA is shed in urine, saliva, and sputum.[9] Shedding in feces is currently unknown and requires further investigation, especially given that measles results in systemic infection and diarrhea is a symptom in 1 in 10 measles cases.[2,10] Measles viral RNA partitions preferentially into solids and can persist for days to weeks in wastewater.[11] Measles RNA has been detected in wastewater in Chicago,[11] Texas,[12–14] Belgium,[15] France,[16] the Netherlands,[17] and Switzerland,[18] and the vaccine strain (genotype A) RNA in Ontario wastewater;[19] however, most studies have used an assay that may also detect vaccine strain measles RNA. Most detection of measles RNA has been at concentrations near method detection limits, with measles RNA detectable but in low concentrations in wastewater even during outbreaks. A key challenge in wastewater monitoring for measles has been the differentiation of the vaccine strain and wild-type measles RNA; the vaccine contains a live, attenuated virus that can be shed in recently vaccinated individuals and is genetically similar to the wild-type strains.[19] Because outbreaks often result in increased vaccination efforts, differentiating between vaccine and wild-type measles is critical in understanding outbreak progression. Further, prior studies on measles in wastewater have included few detection investigations and comparisons to clinical cases.

In this study, we (1) evaluated the use of a recently-reported assay specific to wild-type measles for outbreak monitoring by applying it to wastewater testing in an area adjacent to a significant measles outbreak, (2) implemented the assay as part of routine wastewater testing at 147 sites throughout the United States, and (3) compared wastewater results to clinical testing for measles. This study is the first to apply measles wastewater monitoring on a national scale across 147 sites and over 10 000 samples, to include comparison to clinical testing, and to use an assay specific to wild type measles.

# Methods

### Wild type measles assay

We utilized a novel assay specific to wild-type (WT) measles; the assay is a modified version of a previously published measles vaccine-specific assay by Roy *et al.* that targets the 3′ region of the measles N gene.[20] The modified assay is specific to WT measles genotypes B3 and D8, and details of development were reported by Boehm *et al.*[21] We developed the assay in response to requests from our public health partners who expressed concern that results from an assay that cross reacts, even at low levels of efficiency, with the vaccine sequences, such as the Wu *et al.* assay,[11] would reduce the public health utility of the assay in some jurisdictions. The newly designed wild-type measles assay contains modifications to two nucleotides in the probe published by Roy *et al.*[20] The forward primer sequence is AGGATGAGGCGGACCARTACTT, the reverse primer is CRATATCTGAGATTTCCTTGTTCTC, and the probe is CATGAT GATCCAAGTAGTAGTGA. *In silico* and *in vitro* analyses reported in another peer-reviewed publication from our group show that the assay is specific to locally circulating WT measles variants and does not cross react with the vaccine measles sequence and other respiratory viruses and select bacterial pathogens.[21] For reference, the Edmonston-Moraten vaccine sequence is available through NCBI Virus with identification number AF266287.

In the present study, we present additional *in vitro* data illustrating the new wild-type measles assay sensitivity and specificity. Nucleic acid sequences representing genotypes B3 and D8 (WT, gene blocks purchased from IDT, Coralville, IL, Table S1) and genotype A (vaccine strain, purchased from ATCC, VR-24D) were serially diluted in sterile, nucleic acid free water to achieve template concentrations between 0 and 200 copies per reaction and run in triplicate using droplet digital RT-PCR using the wild-type measles assay. VR-24D nucleic acids were extracted from and purified using commercially available kits as described below for the wastewater solids samples. Nucleic acids were used neat as a template in 1-step droplet digital RT-PCR assays. The assay was run in a single well using the cycling conditions and post processing using a QX200 (Biorad) droplet reader in singleplex. No template RT-PCR negative controls were included on each plate.

### Sample collection

For the pilot evaluation, wastewater solids from two neighboring wastewater treatment plants (WWTPs) in Texas (Randall County and Potter County) near an ongoing measles outbreak were tested for measles RNA. Three samples per week collected between 12/29/24 and 1/14/25 (month/day/year format) and two samples per week collected between 1/19/25 and 4/22/25 were retrospectively tested for measles RNA at each site. Prospective testing of 3 samples per week was conducted between 4/27/25 and 5/13/25. The two WWTPs (hereafter referred to as "North" and "South") provided 24 h composited influent from sanitary sewer systems; the North services 60 000 and the South 140 000 residents. Samples were collected using the sterile technique by WWTP staff and


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shipped overnight at 4 °C to the laboratory where processing began within 48 h of sample receipt.

The assay was then added to a national wastewater monitoring program, with samples from 147 sites collected and processed three times per week for most sites (Table S2); this program has been described in detail elsewhere.[21] Site staff provided either "grab" samples from the primary clarifier or 24 hour composite samples of influent. The assay was rolled out to an initial 8 sites on 5/5/2025 and expanded to include all sites on 5/15/2025. See the SI for full details on sites included.

**Testing of wastewater solids for measles RNA.** Sample pre-analytical processing and nucleic acid extraction and purification methods are provided in detail elsewhere.[21] Samples were allowed to sit for 10–15 min and a serological pipette was used to aspirate the settled solids into a falcon tube. Settled solids were centrifuged at 24 000 × *g* for 30 min at 4 °C to dewater the solids. The supernatant was then aspirated using a vacuum and discarded. A 0.5 to 1 g aliquot of the dewatered, wet solids was dried at 110 °C for 19 to 24 h to determine their dry weight.

We extracted and purified nucleic acids from the solids using a Chemagic Viral DNA/RNA 300 Kit H96 (PerkinElmer, Shelton, CT) followed by inhibition removal (Zymo OneStep PCR Inhibitor Removal Kit, Irvine, CA); precise methods are provided on protocols.io[22] and in other publications.[21] The lowest detectable concentration of this method is 500–1000 gene copies per dry gram.

For samples tested retrospectively, nucleic acids were stored at −80 °C for 1–3 months before being tested for measles RNA using the assay. All samples tested retrospectively were also tested for SARS-CoV-2 RNA, and the SARS-CoV-2 RNA concentrations measured in the samples after months of storage were compared to those measured immediately without any storage to assess potential for RNA degradation during storage (see the SI for details).

For samples tested prospectively, the nucleic acids were immediately used neat as a template in RT-droplet digital PCR (RT-ddPCR) format and tested for measles RNA using the modified Roy *et al.* assay. Bovine coronavirus (BCoV) vaccine was spiked into all samples and measured to assess RNA recovery during the pre-analytical and nucleic-acid extraction and purification processes. See the SI for further details on RT-ddPCR reaction chemistry and cycling conditions and processing of machine output.

**Confirmed measles cases.** For the pilot testing, information on the number of confirmed measles cases in 2025 within each of the two counties serviced by the WWTPs in the pilot evaluation (Randall County and Potter County, TX) was gathered from Amarillo Public Health's Public Measles Dashboard.[23] Amarillo Public Health does not report specific case information for cases unless there are more than 6 reported in the county. Information on confirmed measles cases for the entire state of Texas was obtained from the Texas Department of State Health Services (DSHS) Measles page.[4] Confirmed measles cases are reported by the Texas Department of State Health Services (DSHS) by date of rash onset if available; if not available, the symptom onset date, specimen collection date, hospital admission date, or date reported to the region is used in that order of priority.

Daily county-level measles case data for the U.S. were obtained from the publicly available JHU Measles Tracking Team Data Repository at Johns Hopkins University.[24] The repository includes the date a case was reported by health authorities, which may not align with the rash onset date.

### Statistical analysis

For the two sites included in the pilot evaluation, we used the Mann–Kendall trend test as a non-parametric approach to assess whether there was any upward or downward monotonic trend in concentrations measured by ddPCR.

To assess the relationship between reported measles cases and wastewater detection, we linked national measles cases and wastewater data. The counties each WWTP served were identified by merging the shapefiles of each WWTP sewershed with the *tigris* R package US county borders dataset.[25] To correct for potential alignment errors between sewershed and county shapefiles, counties were required to comprise over 5% of a sewershed's area to be included as a county served.

For each observation in the wastewater dataset, we calculated the number of clinical cases within 7 days of the sample collection date (7 days before and after the sample collection date), and in the 7 days following the sample collection date. 7 days were chosen to approximately align with the infectious period of cases (typically beginning 4 days before rash onset) while accounting for delays in case identification and reporting. We also created binary variables indicating the presence of any measles cases over the same time periods. Using the full dataset, we calculated odds ratios (ORs) to investigate whether wastewater measles detection was associated with increased likelihood of clinical case reporting over each time period. We used the *glm* function in R with the family set to binomial and exponentiated the regression coefficients to obtain the ORs; *p* values were adjusted for multiple comparisons using a Bonferroni correction.

## Results

### Assay sensitivity and specificity and QA/QC

Assay specificity and sensitivity have been previously described in the peer reviewed paper by Boehm *et al.*[21] As described in that paper, *in vitro* specificity testing of the wild-type measles assay detected both B3 and D8 but did not produce any positive droplets for the vaccine nucleic-acids. In the present study, we conducted additional validation testing against serial dilutions of B3, D8, and vaccine positive controls. The wild-type measles assay detected the intended targets (B3 and D8) across all tested concentrations. The lowest concentration at which all 3 replicates were positive was 6.25 gene copies per reaction for D8, and 25 gene copies

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per reaction for B3 (Fig. S1). The wild-type measles assay produced zero positive droplets for all concentrations of the vaccine control. The results support the specificity of the assay to genotypes B3 and D8.

Across all experiments, positive and negative controls performed as expected; no amplification was detected in negative controls and all positive controls were positive. The recovery of spiked BCoV RNA across the samples was 0.98 (median, interquartile range (IQR) = 0.76–1.29); recovery higher than 1 is due to spiked BCoV RNA measurement uncertainty. SARS-CoV 2 RNA concentrations measured in the samples used for the retrospective testing (and stored for 1–3 months and subjected to a single freeze–thaw cycle) were compared to SARS-CoV-2 RNA concentrations in the same samples as measured immediately without storage. The ratio of concentrations measured in stored *versus* not stored samples was 1.54 (median, IQR = 1.24–1.91; $n$ = 114, Fig. S2), suggesting limited degradation of RNA during the storage time.

## Pilot testing

**Wastewater results.** Measles RNA was detected in 11/105 samples (10.5%) tested; 5 from the North site and 6 from the South site (Fig. 1). Of the samples that tested positive, concentrations ranged from 3900 gc per dry g to 52 000 gc per dry g (median 6900 gc per dry g). The highest concentration was observed in the first sample that tested positive in Jan 2025 at the North site. There was no significant trend in concentrations of measles RNA in wastewater over time at either site (North $p$ = 0.44, South $p$ = 0.67).

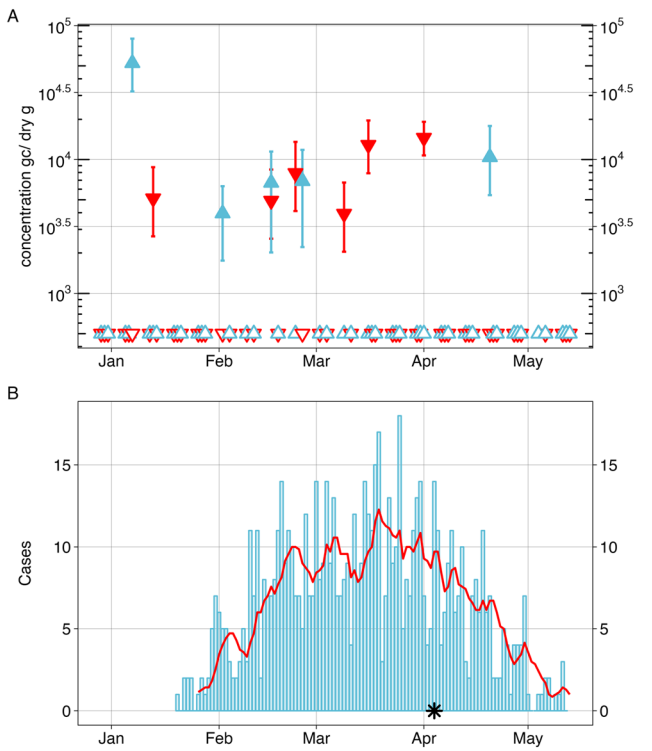


Fig. 1 Wastewater measurements and measles cases in Texas. A) Measles RNA measured in wastewater in cp per g wastewater solids at two WWTPs. Concentrations measured are indicated by filled triangles with the 68% confidence interval represented by bars. Samples with no detection are indicated by open triangles. B) Daily confirmed measles cases by the Texas Department of State Health Services. Daily case counts are represented by blue bars; the red line represents the 7 day rolling average of case counts. The asterisk represents the first confirmed case in Potter or Randall Counties.

**Measles cases.** Texas DSHS reported the first case of measles in late January 2025, and in total 729 cases were reported in Jan–May, 2025. The majority of cases (95%, 692/729) were individuals that were either unvaccinated or had unknown vaccination status. The first measles case in a resident of Potter and Randall Counties was confirmed *via* a public health alert on April 4, 2025.[26] A second case was confirmed in a second public health alert on April 16, 2025.[27] As of May 28, 2025, Amarillo Public Health reported a total of 6 cases in the area, 3 in Randall County and 3 in Potter County.[28]

## National testing

**Wastewater results.** Measles RNA was detected in 63 of 11 598 (0.54%) samples across 17 of 40 states and in 25 of the 147 WWTPs. The median (IQR) concentration in positive samples was 4400 gc per dry g (2800–12 000 gc per dry g; Fig. S3). Sites with multiple detection did not have higher median concentrations of measles RNA measured compared to those with one detection (Wilcoxon rank-sum test, $p$ = 1). Of the 25 WWTPs with measles detection, 18 had one detection, 4 had 2–4 detections, and 3 plants had more than 5 detections (Fig. 2).

**Cases in national wastewater testing areas.** During the study period, 31 measles cases were reported in counties with a WWTP participating in the study that had measles RNA detection and 13 cases were reported in counties with a WWTP participating in the study that did not have measles RNA detection. The 13 cases with no associated WWTP measles RNA detection were across 9 counties with 16 WWTPs enrolled in the study, as multiple WWTPs can serve the same county(ies).

Measles RNA wastewater detection aligned spatially with reported measles cases over the study period. Of the 25 plants with at least one measles RNA detection, 11 (44%) served a county with reported cases and 9 (36%) were adjacent to a county with reported cases; in total, 19 (72%) of plants with measles detection served or were adjacent to counties with cases reported during the study period (Fig. 3).

Wastewater measles RNA detection aligned temporally with clinical cases reported in the same counties. 37% of wastewater detection occurred within 7 days of a reported measles case in the same county (Fig. 3). Wastewater measles RNA detection was associated with increased odds of a measles case in the WWTP county within 7 days (Fig. 3; $n$ = 11 598, $p < 0.001$). Each additional measles case reported in the WWTP county in the 30 days before or after sample collection was associated with a 2-fold increase in measles RNA detection in wastewater (Fig. 3), indicating that


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**Fig. 2** Wastewater detection of measles RNA and confirmed cases in adjacent counties. A) Locations of wastewater treatment plants (WWTPs) participating in the study to detect wild-type measles in wastewater solids. Dark gray shading indicates states with at least 1 site that had positive measles detection in wastewater during the study. The red dots represent the location of the WWTPs where there was positive detection of measles RNA ($n$ = 25), and the light blue dots represent participating WWTPs where no samples were positive for measles RNA ($n$ = 122). B) All participating WWTPs with at least one measles detection during the study period. WWTPs without any measles detection are not depicted. Cases in the county of the WWTP are indicated by red circles, and cases in counties adjacent to the WWTP are indicated by red Xs. The date shown for cases represents when jurisdictions reported the case.

wastewater measles RNA detection was more likely as the number of cases in the WWTP county increased.

Wastewater measles RNA detection also appeared to provide early warning for measles cases. Wastewater detection was associated with increased odds of clinical cases in the WWTP county in the 7 days following sample collection (Fig. 3; $n$ = 11 598, $p < 0.001$). 15 (24%) of WWTPs with measles detection served a county with reported cases in the following 7 days.

## Discussion

This study describes the application of a new wild-type measles specific assay for the detection of measles viral RNA

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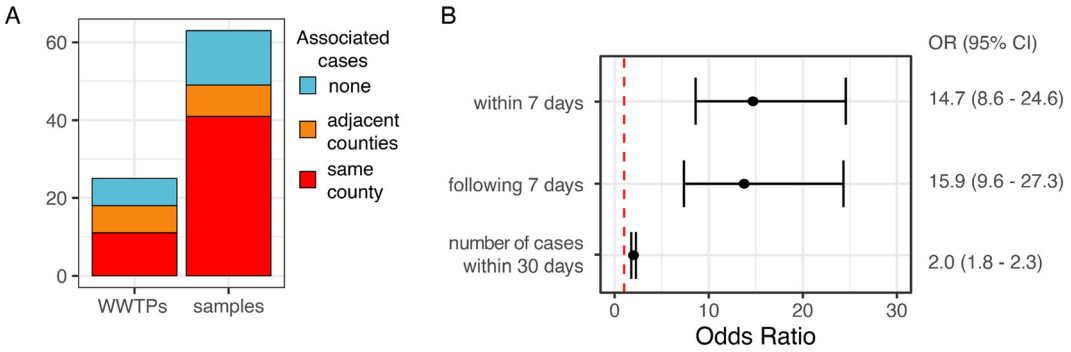


**Fig. 3** Relationship between wastewater detection and clinical cases. A) The frequency of cases associated with wastewater measles RNA detection. The left bar represents the 25 WWTPs with at least one measles RNA detection and the right bar represents the 63 samples positive for measles RNA. Red indicates that a measles case was reported in the same county, orange only in an adjacent county, and blue no associated measles cases in the same or adjacent counties. B) Odds ratios of a reported measles case given wastewater detection. Points represent the odds ratio, and bars represent the 95% confidence interval. The red dashed line indicates an OR of 1. 'Within' indicates a time period including cases the indicated number of days before and after the wastewater sample collection date, while 'following' indicates a time period including cases the indicated number of days following the wastewater sample collection date. For the number of cases, the odds ratio indicates the odds of measles RNA wastewater detection given each additional case.

in wastewater and demonstrates that this approach can be scaled widely and used to detect measles in wastewater during active outbreaks in the region. We found that an assay specific to wild-type measles (and thus not cross-reacting with any RNA from vaccine strains) was able to detect measles in wastewater from treatment plants both with and without reported cases during the study period, and that areas with positive wastewater detection had higher odds of concurrent measles cases.

We developed and applied this assay in response to public health partner feedback expressing a need for an assay specific to wild-type measles that would not cross-react with the vaccine strain. While other assays for measles detection in wastewater have been designed,[11,16] the newly-developed assay presented here is unique in that it is specific to wild-type measles and designed to be integrated into ongoing wastewater monitoring programs, using only one assay for sensitive, specific detection.

During pilot testing, wild-type measles RNA was first detected in wastewater in Texas on 1/7/2025. This was over a week before the first case in the United States in 2025 was confirmed during the week of 1/20/2025 in another region of Texas. Detection throughout the study period was sporadic, without a pattern of increasing or decreasing concentrations over time. While there were cases reported during April and May 2025 of the study period in the study area, there was no sustained outbreak reported in this specific community. Overall, these results suggest that wastewater testing may be able to provide an early indicator of measles circulating in the region and is sensitive to low levels of cases.

Nationally, measles RNA detection in wastewater was associated with subsequent clinical cases identified in the following 7 and 30 days. The odds of measles wastewater detection increased with each additional measles case reported in the WWTP county. These findings demonstrate that wastewater measles RNA detection relates to clinical cases across the country. Still, nearly 25% of our measles detection was in areas with no reported cases. This suggests that wastewater monitoring for measles RNA has the potential to be an early indicator of cases that are missed by clinical surveillance. Although measles presents with a characteristic rash, this rash only develops after 3–5 days of non-specific symptoms. Measles is also typically rare in the United States and outside of known outbreak areas may not be suspected by clinicians. Finally, communities that are at higher risk of outbreaks due to low vaccination rates are also often communities that lack access to diagnosis and treatment for measles, or may be reluctant to access it.

A key limitation of this work is the spatial mismatch in case reporting and sewersheds served by the WWTPs included in this study. The case data were reported at the county level, while WWTP sewersheds served only portions of the relevant counties and often crossed county boundaries. Thus, it was not possible to confirm whether measles cases resided or had spent time in WWTP sewersheds, providing an opportunity for viral shedding into the waste stream. This made it challenging to interpret "false negatives", such as in 9 counties where measles cases were reported with no measles RNA detection at the nearby WWTP in the 7 days before or after the reported case(s). In these situations, cases may include people who do not reside or spend time within the sewershed, or are less likely to shed into wastewater during their illness (*e.g.* young children in diapers) and would not be detected by wastewater monitoring. Because of this, we are unable to determine whether these "false negative" scenarios are because of sensitivity of testing practices or the spatial mismatch. Data that match cases more specifically to sewershed areas would help ascertain when and if cases may be missed by measles wastewater monitoring.

Previous studies have detected measles RNA in wastewater in multiple locations with low case burdens,[11–17] yet evidence

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for the measles wastewater monitoring to provide early warning for cases has been limited.[18] Here, we extend this evidence to demonstrate that, on a national scale across 147 sites and over 10 000 samples, wastewater measles RNA detection is associated with clinical cases and can provide early warning of cases. Since data collection began for this study, results presented here have been utilized by public health departments to issue alerts and initiate response activities, including a June 2025 press release from Yolo County, California[29] and an August 2025 health alert from the Georgia Department of Public Health[30] that both cite wastewater detection of measles. These alerts encourage clinicians to be aware of possible measles cases and advise the general public on the importance of and opportunities for vaccination – a direct impact of information from wastewater on public health practice and outbreak prevention activities.

## Conclusions

Previous studies have reported detection of measles RNA in wastewater during times with known cases.[11–17] This work extends prior measles wastewater work by demonstrating on a national scale the association between clinical cases and wastewater detection. We also present the use of an assay specific to wild-type measles scaled to a national level; by using this new assay, we are able to confirm that detection represents human cases rather than vaccinations, and show that across diverse settings, there is an association between this detection and possible outbreaks. This evidence suggests that wastewater monitoring is an effective tool to supplement measles surveillance and identify areas with possible outbreaks, and early adoption of these results and action by public health departments show that the data have the opportunity to impact public health practice.

## Author contributions

A. P. Paulos: methodology, formal analysis, data curation, writing – original draft, visualization. A. Zulli: methodology, validation. A. Bidwell: data curation, writing – review & editing. S. P. Hilton: data curation, visualization, writing – review & editing. B. Shelden: methodology, validation, investigation, data curation. D. Duong: methodology, validation, investigation, data curation. A. B. Boehm: conceptualization, methodology, validation, resources, writing – review & editing, supervision, project administration, funding acquisition. M. K. Wolfe: conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing – review & editing, visualization, supervision.

## Conflicts of interest

BS and DD are employees of Verily Life Sciences, LLC.

## Data availability

Wastewater data generated for this article as well as matched case data are available at https://purl.stanford.edu/jm571qx0675.

Supplementary information (SI): including SI on molecular analysis and on site characteristics. See DOI: https://doi.org/10.1039/d6ew00020g.

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