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The use of wastewater surveillance to estimate SARS-CoV-2 fecal viral shedding pattern and identify time periods with intensified transmission

Wan Yang[1*], Enoma Omoregie[2], Aaron Olsen[2], Elizabeth A. Watts[2,3], Hilary Parton[2] and Ellen Lee[2]

Abstract

Background Wastewater-based surveillance is an important tool for monitoring the COVID-19 pandemic. However, it remains challenging to translate wastewater SARS-CoV-2 viral load to infection number, due to unclear shedding patterns in wastewater and potential differences between variants.

Objectives We utilized comprehensive wastewater surveillance data and estimates of infection prevalence (i.e., the source of the viral shedding) available for New York City (NYC) to characterize SARS-CoV-2 fecal shedding pattern over multiple COVID-19 waves.

Methods We collected SARS-CoV-2 viral wastewater measurements in NYC during August 31, 2020 – August 29, 2023 ($N = 3794$ samples). Combining with estimates of infection prevalence (number of infectious individuals including those not detected as cases), we estimated the time-lag, duration, and per-infection fecal shedding rate for the ancestral/Iota, Delta, and Omicron variants, separately. We also developed a procedure to identify occasions with intensified transmission.

Results Models suggested fecal viral shedding likely starts around the same time as and lasts slightly longer than respiratory tract shedding. Estimated fecal viral shedding rate was highest during the ancestral/Iota variant wave, at 1.44 (95% CI: 1.35 – 1.53) billion RNA copies in wastewater per day per infection (measured by RT-qPCR), and decreased by around 20% and 50-60% during the Delta wave and Omicron period, respectively. We identified around 200 occasions during which the wastewater SARS-CoV-2 viral load exceeded the expected level in any of the city's 14 sewersheds. These anomalies disproportionally occurred during late January, late April—early May, early August, and from late-November to late-December, with frequencies exceeding the expectation assuming random occurrence ($P < 0.05$; bootstrapping test).

Discussion These estimates may be useful in understanding changes in underlying infection rate and help quantify changes in COVID-19 transmission and severity over time. We have also demonstrated that wastewater surveillance data can support the identification of time periods with potentially intensified transmission.

Keywords Wastewater surveillance, COVID-19, Viral shedding pattern, Variant, Transmission

*Correspondence:
Wan Yang
wy2202@cumc.columbia.edu
Full list of author information is available at the end of the article

Background

Since the early phase of the COVID-19 pandemic, studies have reported that wastewater SARS-CoV-2 viral loads often closely track or lead case and/or hospitalization trajectories and, as such, can serve as a cost-effective surveillance tool for monitoring the COVID-19 pandemic [1–5]. Thus, wastewater-based surveillance systems have been built worldwide on local and national scales. With decreasing clinical testing and genomic sequencing [6, 7], there has been increased interest in wastewater surveillance, given the results are generated independently of clinical testing practice.

Though there are advantages of SARS-CoV-2 wastewater surveillance, a large US national survey of public health agencies completed in 2022 noted the results were often deemed supplementary to surveillance involving clinical laboratory tests [8]. One of the hurdles is that while the trends could indicate changes in SARS-CoV-2 community circulation, it remains challenging to directly translate wastewater SARS-CoV-2 viral loads to a specific number of infections in the population, due to the unclear fecal viral shedding rate (after accounting for the recovery rate of virus genomes) in wastewater samples. In addition, with the fast emergence and turnover of new SARS-CoV-2 variants, it is unclear how fecal shedding of the virus may have altered over time by variant. Further, linking wastewater viral load to clinical health metrics has become more challenging since round 2022 with the decrease in clinical testing and changing reporting requirements. For instance, previous studies [9–11] examining the association between wastewater viral load and clinical health metrics (e.g., cases and/or hospitalizations) have reported decreased strength of correlation, likely to due to changes in test- and healthcare-seeking behavior over time. To address these questions and challenges, we utilize comprehensive wastewater surveillance data and estimates of infection prevalence (versus clinical health metrics in previous work) available for New York City (NYC) to characterize SARS-CoV-2 fecal shedding over multiple COVID-19 pandemic and epidemic waves.

NYC experienced the earliest pandemic wave in the United States (US), and shortly after the initial wave, established a wastewater surveillance program that covers all of its 14 sewersheds which serve over 8 million residents [2, 12]. Since August 31, 2020, the program has continuously measured SARS-CoV-2 viral load weekly. Independently, we have developed and used a comprehensive model-inference system – calibrated to case, Emergency Department (ED) visit, and mortality data – to reconstruct the underlying transmission dynamics and estimate key epidemiological characteristics [13, 14]. In particular, the model-inference system estimates the number of actively infectious individuals including those not detected as cases (i.e., infection prevalence) in each of the city's 42 neighborhoods during each week since March 1, 2020 [13, 14]. Combining the wastewater SARS-CoV-2 viral load data and infection prevalence estimates over a 3-year period (i.e., August 31, 2020 – August 29, 2023), we are able to characterize the viral shedding pattern (i.e., time-lag, duration, and *per-infection* shedding rate) for the ancestral/Iota, Delta, and Omicron variants, separately. Importantly, our estimates are based on *all active* infections regardless of symptoms and test-seeking behavior (i.e., not subject to changes in medical seeking propensity and clinical testing practice) and thus better reflect SARS-CoV-2 variant-specific fecal shedding pattern. Further, linking wastewater viral load to infection prevalence would provide a more complete picture of the underlying transmission dynamics and help to better anticipate future epidemic trajectory, as asymptomatic/mild infections can also transmit SARS-CoV-2 [15]. In addition, we are also able to identify time periods with greater transmission.

Methods

SARS-CoV-2 wastewater surveillance data

The SARS-CoV-2 wastewater surveillance program in NYC started on August 31, 2020 [2, 12]. Wastewater samples were taken at each of the city's 14 wastewater treatment plants, usually twice per week on Sundays and Tuesdays ($N=3794$ samples; see variations and details in Table S1). Specifically, 24-h flow-weighted composite influent wastewater samples were collected from the influent of each wastewater treatment plant; the use of composite samples reduces biases due to sampling time during the day when people may be at work, sleeping, etc. Each composite sample consisted of eight grab samples collected every three hours beginning at 7:00 AM on the sampling date. The volume of each grab sample added to the composite was determined based on the flowrate during the associated 3-h collection period. Samples were transported on ice and stored at 4°C until processing.

SARS-CoV-2 RNA concentration was measured using quantitative reverse transcription polymerase chain reaction (RT-qPCR) assays during August 31, 2020, through April 11, 2023, and reverse transcription digital PCR (RT-dPCR) assays from November 1, 2022, through August 29, 2023. For RT-qPCR, the samples were tested on the ABI 7500 system, with the forward primer (5′- GACCCCAAA ATCAGCGAAAT-3′), reverse primer (5′- TCTGGTTAC TGCCAGTTGAATCTG-3′), and probe (5′-FAM-ACC CCGCATTACGTTTGGTGGACC-BHQ-1–3'). The limit of detection and limit of quantification for the RT-qPCR assay were 180 copies/L of wastewater sample and 590 copies/L of wastewater sample, respectively. For RT-dPCR, samples were tested on the QIAcuity Eight Platform

System, using the GT-Digital SARS-CoV-2 Wastewater Surveillance Assay For QIAcuity™ kit (Catalog #100,179). The limit of detection for RT-dPCR was 0.225 copies/μL, or anything below 3 partitions. All measurements adjusted for sewershed-specific flow rate and service population size. Specifically, per-capita SARS-CoV-2 viral load (RNA copies per day per population) was computed as the viral concentration measure multiplied by the daily sewage flow rate and then divided by the service population size.

For weeks after April 11, 2023, when the samples were measured using RT-dPCR alone, we converted the RT-dPCR measurements to RT-qPCR equivalents, to allow characterization of SARS-CoV-2 viral shedding during the entire Omicron period. Specifically, we first computed the conversion ratio using measurements from November 1, 2022, through April 11, 2023, when both assays were conducted, simply as the mean of all RT-qPCR measurements divided by the mean of all RT-dPCR measurements, during these weeks. That is, the conversion ratio $p = \sum_{i=1}^{n} VL_{\text{i, qPCR}} / \sum_{i=1}^{n} VL_{\text{i,dPCR}}$, where and n is the number of samples measured by both assays and $VL_{\text{i, qPCR}}$ and $VL_{\text{i,dPCR}}$ ($i = 1, 2, \ldots, n$) are the viral load measurements by the two assays for each sample. We then multiplied the RT-dPCR measurements by the conversion ratio to obtain the converted RT-qPCR equivalents. As an alternative, we stratified the data by sewershed and performed the conversion using sewershed-specific conversion ratios (see Sensitivity Analysis). In addition, the RT-qPCR and RT-dPCR measures differed substantially (by a factor of 16.7 based on the aforementioned overlapping measurements), likely due to difference in methodology [12, 16]. To facilitate comparison with studies primarily using RT-dPCR, we also converted all RT-qPCR measurements to RT-dPCR equivalents when reporting the viral shedding rates.

SARS-CoV-2 infection prevalence estimates

Estimated SARS-CoV-2 infection prevalence came from a model-inference system [13, 14, 17], independent of the wastewater surveillance data. Briefly, the model-inference system fit a neighborhood-level Susceptible-Exposed-Infectious-(re)Susceptible-Vaccination (SEIRSV) model to age-grouped, neighborhood-specific COVID-19 case, ED visit, and mortality data, using the ensemble adjustment Kalman filter [18], a Bayesian data assimilation method. The system simultaneously accounted for concurrent nonpharmaceutical interventions, vaccinations, under-detection of infection, and seasonal changes. As reported previously, this model-inference system has been validated using serology data [13] and shown to generate accurate COVID-19 forecasts [19] as well as estimates of key epidemiological variables and parameters (e.g., transmission rate and infection-fatality risk) consistent with other studies [13, 14].

Using the model-inference system, we estimated the number of infectious individuals – i.e., anyone who can actively transmit SARS-CoV-2 and infect others regardless of symptoms and test-seeking behaviors – present in the population, during each week of the study period [17]. That is, similar to the wastewater SARS-CoV-2 viral loads measuring the total population fecal shedding regardless of clinical testing, the infection prevalence estimates included all individuals actively transmitting SARS-CoV-2 (primarily via shedding from the respiratory tracts) regardless of whether they were detected as cases. The infection prevalence estimates are United Hospital Fund neighborhood- [20] and age group specific, and available for each week starting March 1, 2020 (the pandemic onset in NYC) to the week starting August 27, 2023. To match with the sewershed-level wastewater SARS-CoV-2 viral load data, we first mapped each neighborhood (42 in total vs. 14 sewersheds) to the corresponding sewershed based on geolocation; if a neighborhood overlapped multiple sewersheds, we assigned it to the one with the maximal overlap. For each sewershed and week, we then aggregated all estimated infectious individuals from all related neighborhoods.

Estimating the fecal viral shedding time-lag, duration and rate

To analyze the fecal viral shedding pattern by variant, we defined three time periods based on data availability and the predominant circulating variant [21] (i.e., to be more variant-specific): i) the 2nd wave (predominantly the ancestral and Iota variants), from August 31, 2020 (i.e., the first day of wastewater surveillance) through June 26, 2021; ii) the Delta wave (predominantly the Delta variant), from June 27, 2021 (i.e., the first week the share of Delta exceeding 50% among the sequenced specimens) through December 4, 2021; and iii) the Omicron period (predominantly Omicron subvariants and included multiple Omicron-subvariant waves), from December 5, 2021 (i.e., the first week the share of Omicron BA.1 exceeding 25% among the sequenced samples; note that we used a lower threshold here given the milder severity of Omicron BA.1 [22] and thus likely fewer infections detected and sequenced) though August 29, 2023 (i.e., the last wastewater sample during the study period).

SARS-CoV-2 viral load in wastewater represents the pooled fecal shedding of the virus by the population, whereas the infection prevalence represents the proportion of population actively infectious at a given time (i.e., the source of the viral shedding after a potential

time-lag). Thus, to estimate the viral shedding rate for each variant (per the time period defined above), we used a linear regression model, accounting for circulating variants and spatial variations by sewershed, per Eq. 1:

$$VL_{t\in\{t\}+\tau,Sewershed_s,Period_p} = \beta_0 + \beta_{s,1}Sewershed_1 + \cdots + \beta_{s,14}Sewershed_{14} + \beta_{p,1}Period_1 + \cdots + \beta_{p,3}Period_3 + \beta_I I_t + \beta_{IP,1}I_t Period_1 + \cdots + \beta_{IP,3}I_t Period_3 + \varepsilon_t \quad (1)$$

where, $VL_{t\in\{t\}+\tau,Sewershed_s,Period_p}$ is the wastewater SARS-CoV-2 viral load measured during time-window $\{t\}$, adjusted by a time-lag or lead of τ days (see details below); the 2nd and 3rd subscripts denote the corresponding sewershed and time-period, respectively. ε_t is the error term. $Sewershed_s$ is an indicator variable for one of the 14 sewersheds in NYC (see the names of sewersheds in Fig. 1 and Table S2) to account for spatial variation (here, in essence, to allow different intercepts for different sewersheds). $Period_p$ is an indicator variable for one of the three epidemic time periods (i.e., 2nd wave, Delta wave, or Omicron period) as defined above for the corresponding

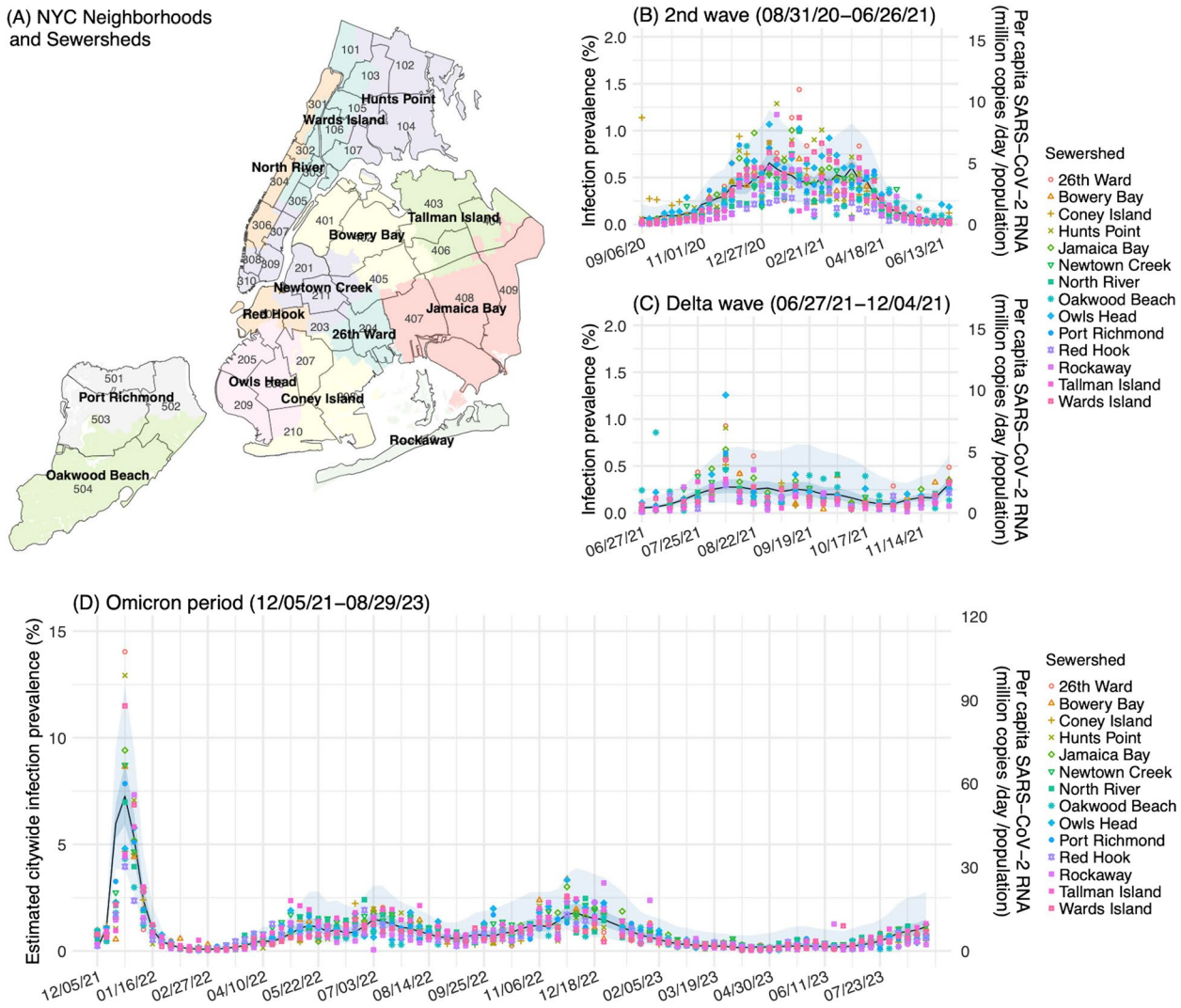

Fig. 1 Trends in wastewater SARS-CoV-2 viral load in the 14 sewersheds in NYC. The map in (**A**) shows 14 sewersheds (delineated by color) and 42 United Hospital Fund neighborhoods (delineated by lines). Dots show the per-capita SARS-CoV-2 viral load in each of the 14 sewersheds (right y-axis, in million copies per day per population by RT-qPCR; color coded per the legend) during the 2nd wave (**B**), Delta wave (**C**), and Omicron period (**D**). For comparison, we overlay the citywide estimates of infection prevalence (left y-axis; blue line = median; darker blue area = 50% CI and lighter blue area = 95% CI)

week, included as a proxy for circulating variants during week-t. I_t is the infection prevalence estimated for week-t. The interaction term $I_t Period_p$ is included to account for potential nonadditive interaction of the two variables (here, in essence, to allow different viral shedding rates by variant). Per Eq. 1, we computed the estimates of fecal viral shedding rate for each variant using the coefficients β_I and β_{IP}'s. For example, using the 2nd wave as the reference period (i.e., $Period_1 = 2^{nd}$ wave, so that $\beta_{IP,1} = 0$), the fecal viral shedding rate is β_I during the 2nd wave, $\beta_I + \beta_{IP,2}$ during the Delta wave (i.e., $Period_2 =$ Delta wave), and $\beta_I + \beta_{IP,3}$ during the Omicron period (i.e., $Period_3 =$ Omicron period).

Given the different surveillance schedules and likely difference between fecal and respiratory viral shedding, we tested three sliding time-windows (i.e., $\{t\}$ in Eq. 1) for matching the wastewater measurements (twice per week, representing fecal shedding) with the infection prevalence estimates (weekly estimates, representing respiratory shedding); specifically, we averaged 2, 3, or 4 consecutive wastewater samples, corresponding to roughly a 1-, 1.5-, or 2-week window, respectively, depending on the wastewater sampling schedule and time-adjustment used. For each time-window $\{t\}$, to identify a proper time-adjustment (τ in Eq. 1), we tested five settings to capture the time difference from becoming infectious via respiratory shedding to fecal shedding per the population-level surveillance data:

i) a 6- to 7-day lead, i.e., the wastewater samples included in time-window $\{t\}$ started from the 1st sample taken *the week before* the infection prevalence estimate; note the 1st sample was taken on Sunday (corresponding to a maximum of 7-day lead) or Monday (corresponding to a maximum of 6-day lead);
ii) a 4- to 5-day lead, i.e., the wastewater samples included in time-window $\{t\}$ started from the 2nd sample taken *the week before* the infection prevalence estimate; note the 2nd sample was taken on Tuesday (corresponding to a maximum of 5-day lead) or Wednesday (corresponding to a maximum of 4-day lead);
iii) concurrent (no time-difference, τ=0), i.e., the wastewater samples included in time-window $\{t\}$ started from the 1st sample taken *the week of* the infection prevalence estimate;
iv) a 2- to 3-day lag, i.e., the wastewater samples included in time-window $\{t\}$ started from the 2nd sample taken *the week of* the infection prevalence estimate (a Tuesday sample corresponded to a 2-day lag and a Wednesday sample corresponded to a 3-day lag); and
v) a 7- to 8-day lag, i.e., the wastewater samples included in time-window $\{t\}$ started from the 1st sample taken *the week after* the infection prevalence estimate (a Sunday sample corresponded to a 7-day lag and a Monday sample corresponded to a 8-day lag).

In addition, we performed variant/period-specific analyses for each of the three time-periods defined above, using a similar model form as Eq. 1 but without the terms related to time-period ($Period_p$). Since the Omicron period included multiple Omicron-subvariant waves, we also performed stratified analyses for the Omicron BA.1 wave (December 5, 2021, through March 4, 2022, i.e., the last week the share of Omicron BA.1 exceeding 50%) and for weeks from March 5, 2022 onwards, separately.

Identifying timings with higher-than-expected transmission

Visual inspection of the wastewater data showed there were occasional spikes in SARS-CoV-2 viral load, potentially due to intensified transmission. Due to the temporal dynamics and sampling noise, it is challenging to distinguish such potential instances (i.e., a true signal) based on the wastewater data alone. Thus, here we used the infection prevalence estimates, which had accounted for the main underlying transmission factors, to construct the expected SARS-CoV-2 viral load for comparison. Specifically, we first computed the daily infection prevalence using the weekly estimates with a spline smoothing function, and then used those as inputs in Eq. 1 to compute the expected daily SARS-CoV-2 viral load (median and 90% confidence intervals [CI]). Given the large variance in both the infection prevalence estimates and SARS-CoV-2 viral load data, we deemed a wastewater measurement higher than expected, if it was higher than the 95th percentile (i.e., the upper bound of the 90% CI) of the expected SARS-CoV-2 viral load.

To examine the timing with higher-than-expected SARS-CoV-2 viral load, we grouped the identified anomaly dates into 10-day bins based on calendar time, i.e., the 1st (early), 2nd (mid), and last (late) 10 days of each month; for example, January 1 of 2021, January 5 of 2022, and January 10 of 2023 would all be grouped as "early-January". This allows recurrent and/or seasonal events to be grouped in the same or nearby bins. To test whether the identified anomalies occurred at random (e.g., due to noise in the data), we further performed a bootstrap test with 5000 random samples. For each bootstrapping set, we randomly sampled $n_{anomaly}$ (i.e., the number of identified anomalies) dates from the wastewater measurements (N=3794), and then grouped the dates into the same

10-day bins as done for the identified anomalies. We then pooled the 5000 sets together to construct the distribution of each timing. For example, for early-January (the first 10-day calendar bin), with n_1, n_2, …, and n_{5000} of the dates falling in that bin for the 5000 sets, the likelihood of having k ($k=0$, …, $n_{anomaly}$, i.e., from none to all) anomalies during early-January would be:

$$P(x=k)=\frac{\text{number of } n_i=\text{k among the 5000 bootsrapping samples}}{5000};$$

and the likelihood of having k or more anomalies during early-January would be:

$$P(x\geq k)=\frac{\text{number of } n_i\geq\text{k among the 5000 bootsrapping samples}}{5000}.$$

Sensitivity analyses

In a first sensitivity analysis, we only included SARS-CoV-2 viral load measured by RT-qPCR (i.e., August 31, 2020– April 11, 2023), to examine if the viral shedding rate estimates were affected by converting RT-dPCR measurements to RT-qPCR equivalents due to changes in testing assays. That is, for this sensitivity analysis, we restricted the study period to August 31, 2020– April 11, 2023, and used the same analysis methods described for the main analysis. In a second sensitivity analysis, we included all SARS-CoV-2 viral load measurements but used the sewershed-specific conversion ratios instead of the citywide conversion ratio for all sewersheds. That is, the conversion ratio (p) was computed for each sewershed using samples taken at the corresponding sewershed alone (vs. pooling all samples from all sewersheds in the main analysis).

Results

General trends in measured wastewater SARS-CoV-2 viral load and estimated infection prevalence

During the 3-year study period (August 31, 2020 – August 29, 2023), trends in wastewater SARS-CoV-2 viral load were generally consistent with the trends in estimated infection prevalence (Fig. 1). Across the 14 NYC sewersheds (Fig. 1A), wastewater SARS-CoV-2 viral load tended to rise and fall around the same time (Fig. 1B-D and Figs. S1-3), indicating epidemic waves were highly synchronized across the city. However, the magnitudes of wastewater SARS-CoV-2 viral load and infection prevalence estimates both varied substantially over time and across sewersheds and may not scale consistently. For example, even though certain sewersheds tended to detect higher SARS-CoV-2 viral loads than others, the rankings changed across different waves (see Fig. S1-3, ranked by average viral load). Similar spatial heterogeneity was apparent in the estimated infection prevalence and the discrepancies between wastewater SARS-CoV-2 viral load and estimated infection prevalence appeared larger during the 2nd wave (Fig. S1). Such spatial heterogeneity is not unexpected, since several factors such as RNA degradation [23] and dilution [23], and the contribution of infected animals [24] could all vary by sewershed, and ultimately affect wastewater measurements. In addition, uncertainty in the infection prevalence estimate could also vary by sewershed (e.g., larger uncertainty for those with smaller population size; see, e.g., the wider uncertainty bounds for Oakwood Beach sewershed in Fig. S1).

Estimated fecal viral shedding patterns

Using the wastewater SARS-CoV-2 viral load data and infection prevalence estimates (i.e., source of fecal viral shedding), we examined fecal viral shedding patterns over the entire study period or stratified by variant/time-period, separately. The estimates from the two analyses are generally consistent; see similar estimated shedding rate, time lag, and number of consecutive samples to aggregate for the best-fit models in Table 1. Among the 15 combinations of fecal viral shedding time-differences and durations tested, the main model (including all waves) identified concurrent infection prevalence estimates (i.e., no time-difference between becoming infectious via respiratory shedding and fecal shedding) and SARS-CoV-2 viral load aggregated over 3 wastewater samples (2 during the same week and 1 in the beginning of the following week, i.e., a 8- to 9- day-time-interval) as the best setting (highest adjusted R-squared; Fig. 2A). Figure 2B shows the model fit and Table S2 shows the estimated coefficients for the best fit model. Using a 4–5-day-lead and aggregation over 4 wastewater samples (i.e., one sample 4–5 days before, two during, and one 1–2 days after the infection prevalence estimate) led to the second-best model fit (Fig. 2A, 2nd dark bar), and was the best setting for the Delta wave and weeks after the BA.1 wave in the stratified analysis (Table 1). Model fit degraded quickly with changing time-differences (both leads and lags), when only 2 (roughly a 1-week duration) or 3 (roughly a 1.5-week duration) wastewater samples were included.

Estimated fecal viral shedding rate was highest for infections during the 2nd wave (mostly due to the ancestral and Iota variants), at 1.44 (95% CI: 1.35 – 1.53) billion RNA copies by RT-qPCR in wastewater per day per infectious person [or 24 (95% CI: 22.49—25.51) billion RNA copies per RT-dPCR conversion; see Methods]. The estimated rate decreased by ~20% during the subsequent Delta wave and by 50–60% during the Omicron period (Table 1). Importantly, we note the lower estimates for Delta and Omicron may in part reflect reduced shedding

Table 1 Estimated patterns of SARS-CoV-2 fecal viral shedding in wastewater. Note in this study, SARS-CoV-2 RNA concentration was measured using quantitative reverse transcription polymerase chain reaction (RT-qPCR) assays during August 31, 2020, through April 11, 2023, and reverse transcription digital PCR (RT-dPCR) assays from November 1, 2022, through August 29, 2023. Based on samples tested using both assays, the RT-qPCR and RT-dPCR measures differed by a factor of 16.7. We used this conversion factor to convert measures from the two methods and provide estimates for RT-qPCR and RT-dPCR assays, separately

Model	Wave	Shedding rate (billion copies per day per infectious person, mean and 95% Confidence interval)	Lag (days)	Number of samples	Adjusted R^2
Include all variant waves	2nd wave (08/31/20-06/26/21)	1.44 (1.35, 1.53) per qPCR; 24.0 (22.49, 25.51) per dPCR[a]	0	3	0.84
	Delta wave (06/27/21-12/04/21)	1.13 (0.86, 1.4) per qPCR; 18.9 (14.45, 23.35) per dPCR[a]	0	3	0.84
	Omicron period (12/05/21-08/29/23)	0.6 (0.59, 0.61) per qPCR; 9.96 (9.76, 10.16) per dPCR[b]	0	3	0.84
Stratified by wave/ period	2nd wave (08/31/20-06/26/21)	1.44 (1.37, 1.52) per qPCR; 24.07 (22.85, 25.28) per dPCR[a]	0	4	0.74
	Delta wave (06/27/21-12/04/21)	1.09 (0.91, 1.27) per qPCR; 18.14 (15.21, 21.08) per dPCR[a]	−5	4	0.37
	Omicron period (12/05/21-08/29/23)	0.6 (0.59, 0.61) per qPCR; 9.98 (9.76, 10.2) per dPCR[b]	0	3	0.86
	Omicron BA.1 (12/05/21-03/05/22)	0.59 (0.56, 0.61) per qPCR 9.78 (9.32, 10.23) per dPCR[a]	0	3	0.91
	After BA.1 (03/06/22-08/29/23)	0.72 (0.7, 0.75) per qPCR; 12.11 (11.72, 12.5) per dPCR[b]	−5	4	0.78

[a] RT-qPCR assays were used to measure SARS-CoV-2 RNA concentration during this period; the dPCR estimates were made by conversion (see Methods)

[b] RT-qPCR assays were used to measure SARS-CoV-2 RNA concentration through April 11, 2023 and RT-dPCR assays were used afterwards; conversion was used to obtain estimates for the entire period (see Methods)

among vaccinees and recoverees, in addition to variant-specific variations.

Timings with higher-than-expected transmission

The infection prevalence estimates have accounted for the general transmission factors (here, population-level mobility, vaccinations, variant-specific properties, and seasonal risk of infection; see Methods), but may have not fully accounted for activities such as increased gatherings during certain time-periods that might increase transmission. In contrast, wastewater SARS-CoV-2 viral load is a composite measure of all transmission events. Thus, comparison of these two quantities could support identification of such events. Following a procedure designed per this mechanism (see Methods), we identified 198 occasions where wastewater SARS-CoV-2 viral loads exceeded the expected levels in any of the 14 sewersheds (see Fig. 3A for identified anomalies for Newtown Creek, the sewershed with the largest service population). These anomalies disproportionally occurred during late January, late April—early May, early August, and mid-November to late-December (Fig. 3B), with frequencies exceeding the expectation assuming random occurrence. Among the 5000 bootstrapping sets, none had as many or more anomalies as observed in early August or late November ($P=0$) and less than 5% had as many or more anomalies as observed in late January, late April, early May, late November, and late December ($P<0.05$ for all these calendar bins; Table S3).

Sensitivity analyses

Results from the two sensitivity analyses are consistent with the main analysis. In the 1st sensitivity analysis (i.e., using SARS-CoV-2 viral load measured by RT-qPCR alone, for a shorter study period from 8/31/20 to 4/11/23), similar fecal viral shedding rates were estimated (Table S4). The 2nd sensitivity analysis (using sewershed-specific conversion ratios to convert the RT-dPCR measurements after 4/11/23, same study period as the main analysis) estimated the same fecal viral shedding rates as the main analysis, and identified three additional anomalies (i.e., 1 in late-January, 1 in mid-August, and 1 in early-July).

Discussion

Wastewater surveillance can be a valuable tool for monitoring SARS-CoV-2 circulation in the population. To further develop understanding of wastewater surveillance data, we have combined independent model-inference estimates of infection prevalence to characterize fecal viral shedding patterns for multiple major SARS-CoV-2 variants. Using NYC as an example, we have also

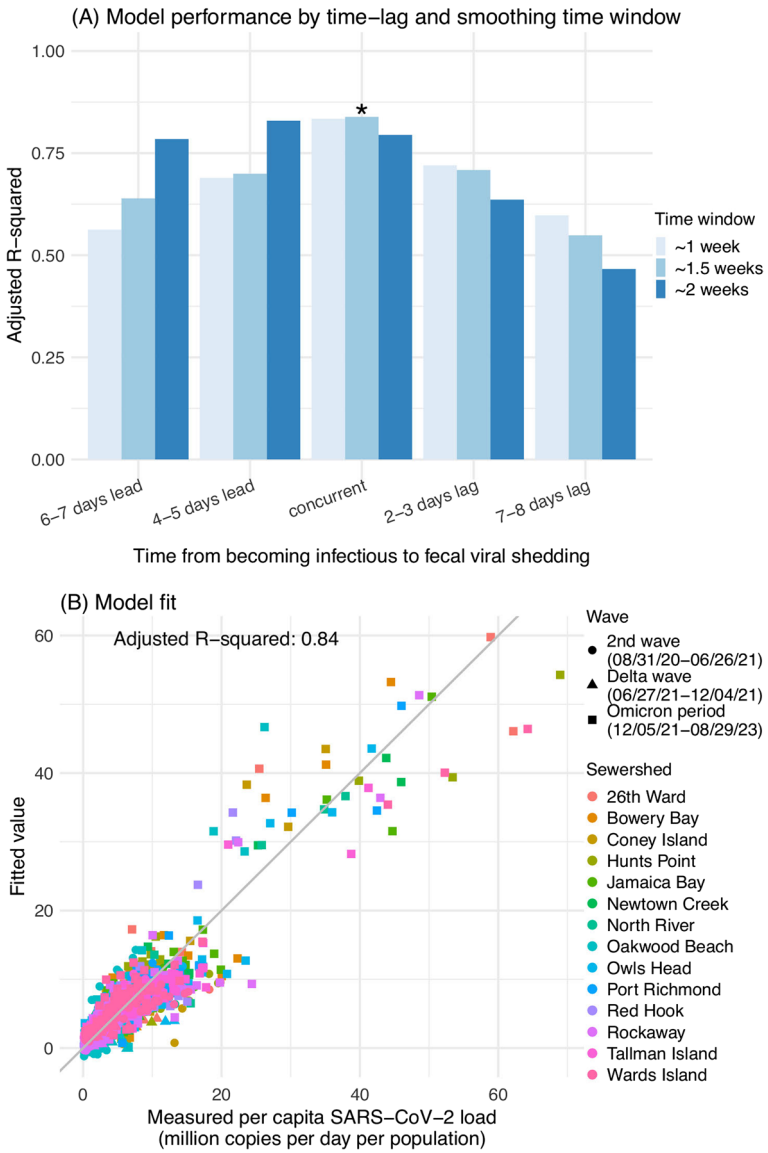

Fig. 2 Model fit. **A** shows model performance based on the adjusted R-squared (higher number represents better performance) for different settings of time from becoming infectious to fecal viral shedding and time window of the wastewater samples are aggregated. The asterisk indicates the setting with the highest adjusted R-squared (i.e., best-fit model). **B** shows the model fit compared to the data

demonstrated that these data and estimates can support the identification of time periods with potentially intensified transmission.

Importantly, here we examined how wastewater SARS-CoV-2 viral shedding is related to estimated infection prevalence, rather than health outcomes as in previous studies. This choice could lead to certain apparent differences but has several advantages. First, previous studies have reported detection of SARS-CoV-2 in wastewater (e.g., an increase in viral load, or the presence of a new variant) several days ahead of the detection of cases, hospitalizations, or deaths, due to the delay in health outcomes [25–27]. Here, infection prevalence is a proxy of respiratory tract shedding, which could precede fecal viral shedding. Indeed, we found wastewater SARS-CoV-2 viral loads measured round 1.5 week of the infection prevalence estimate afforded the best model fit (Table 1). This finding suggests that fecal viral shedding likely starts around the same time an individual becomes infectious and lasts slightly longer than the shedding from respiratory tract. Consistent with our finding, studies have shown that fecal SARS-CoV-2 RNA was detectable in patients within the first week of COVID-19 diagnosis and could last longer than respiratory shedding [23, 28].

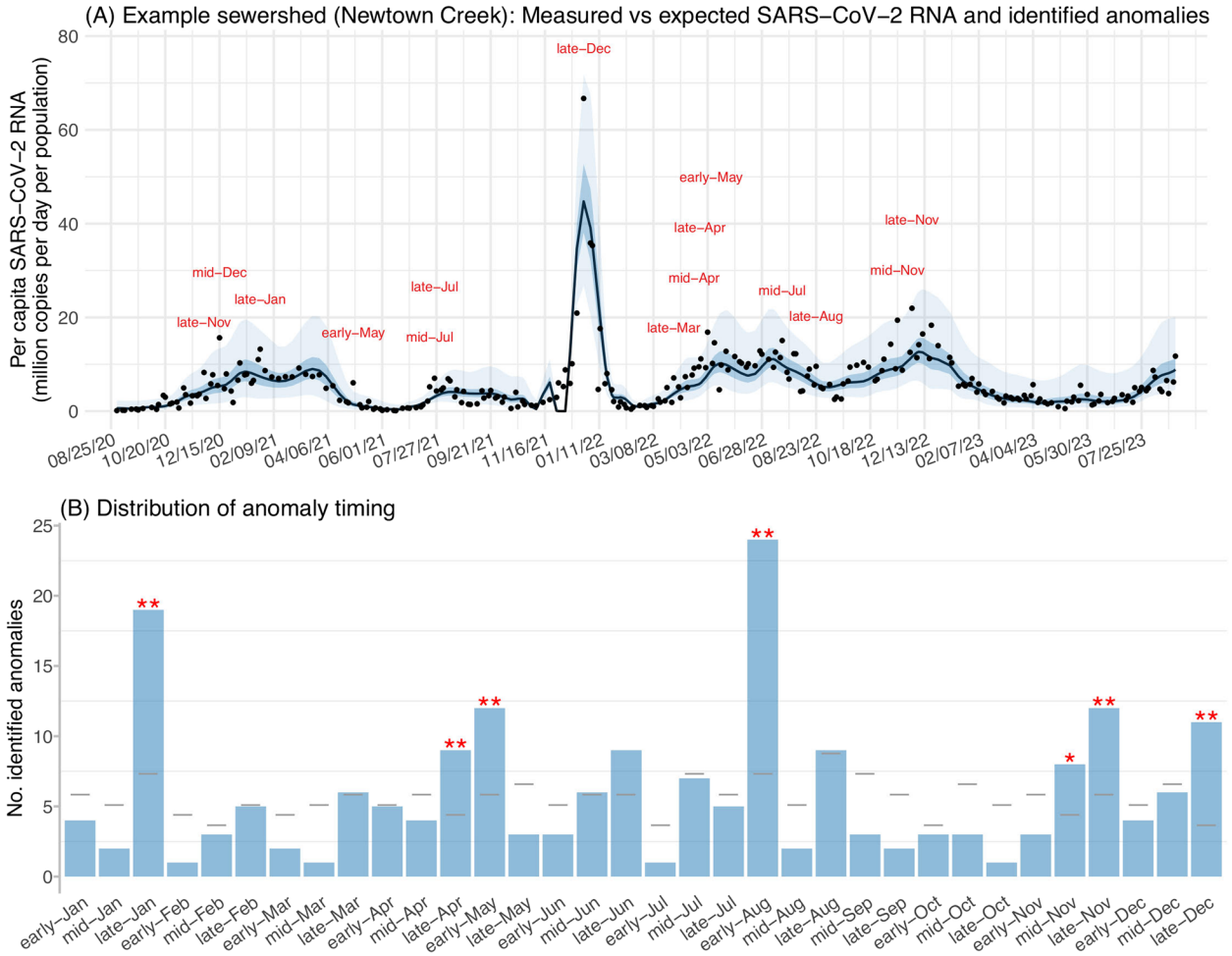

Fig. 3 Identified time-periods with intensified transmission in any of 14 NYC sewersheds. **A** shows an example of the measured (dots) and expected wastewater SARS-CoV-2 viral load (blue line = median; darker blue area = 50% CI and lighter blue area = 95% CI), and identified anomalies with SARS-CoV-2 viral load exceeding the expected (red labels). **B** shows the distribution of all identified anomalies. Asterisks indicate time-periods that exceeded the expected wastewater SARS-CoV-2 viral load with a frequency higher than chance assuming random occurrence per a bootstrapping test (* for $P < 0.1$ and ** for $P < 0.05$). Spatial distribution of the anomalies is shown in Fig. S4

Second, case-, hospitalization-, or death-to-wastewater-viral-load ratio could decrease with increased vaccinations/reinfections and circulation of milder variants (e.g., Omicron) due to reduced severity or testing, and such reductions have been reported [26, 29]. In contrast, as our estimates included all infections regardless of severity or testing, the infection-to-wastewater-viral-load ratio (roughly, the inverse of estimated per-infection fecal viral shedding rate; Table 1) is relatively stable during each variant wave. For example, the wave-stratified analysis estimated similar fecal viral shedding rates for the BA.1 wave and weeks after BA.1 (Table 1). Importantly, using the infection prevalence estimates, we are able to quantify the fecal viral shedding rate for each major SARS-CoV-2 variant/time-period (Table 1). These estimates could be used to help account for changes in underlying infection rate and help examine changes in COVID-19 severity during this study period, when detailed infection estimates are not available; for instance, converting wastewater SARS-CoV-2 viral loads to infection prevalence per Table 1 to help examine changes in hospitalization rate and infection-fatality risk. Such wastewater-viral-load- and infection- based estimates may be more accurate than case-based measures, which are subject to test-seeking biases.

Third, previous studies have measured viral loads in clinical samples from the respiratory tract. Based on the reported cycle threshold (CT) values, the respiratory tract viral load was higher in Delta and Omicron infections than the ancestral variant [30–35], consistent with

the higher infectiousness of these variants of concern. In contrast, fecal viral shedding is not a main mode of transmission [36, 37], and here using variant circulation time-period as a proxy, we estimate that the fecal viral shedding rate was the highest for the ancestral/Iota variants, followed by Delta (~20% lower), and then Omicron (~50–60% lower; Table 1). Early studies of ancestral SARS-CoV-2 infections found that patients with diarrhea shed more viruses than patients without diarrhea (see, e.g., a review in ref [23]), suggesting fecal viral shedding may be associated with diarrhea. In addition, studies found that vaccinations reduced the number of diarrhea episodes [38], and that rates of diarrhea were highest among patients infected with the ancestral SARS-CoV-2, followed by patients infected with Delta and then Omicron [39, 40]. Our estimates are consistent with the fecal viral shedding studies [23, 38–40], and support a difference in viral load between SARS-CoV-2 fecal shedding and respiratory tract shedding, in addition to the timing difference noted above.

In addition to characterizing SARS-CoV-2 fecal viral shedding pattern, we are also able to identify certain time-periods with intensified transmission. In NYC, analysis based on calendar timing showed likely intensified transmission during late-November through December (Fig. 3B). Increased transmission also occurred during early August and late January. It is possible that other factors such as travel, holidays, or specific COVID-19 sub-variants could help explain these periods of intensified transmission, but further investigation is needed to determine their impact.

Lastly, we note several limitations. First, given the biweekly sampling dates for wastewater and weekly estimates for infection prevalence, we were unable to test finer time-differences and durations when examining SARS-CoV-2 fecal shedding pattern. Second, the estimates here were based on population data and thus represent an average of all individuals undergoing different disease stages in the population. As such, the estimated fecal shedding duration may be shorter than that reported in studies based on individual patient data (e.g., days or weeks after respiratory tract samples became negative [23]). Third, our infection prevalence estimates have accounted for the main transmission factors, through the information encapsulated in the COVID-19 case, ED visit, and mortality data used for model estimation. Thus, the expected SARS-CoV-2 viral load constructed using these estimates and in turn the identified anomalies are both conservative estimates and may have missed additional anomalies. In addition, wastewater collected from sewersheds may represent individuals who are residents of NYC as well as outside NYC, while infection prevalence estimates are based on NYC residents only.

Conclusion

In summary, we have characterized the fecal viral shedding pattern of SARS-CoV-2 in wastewater in New York City from 2020–2023. These estimates can be used to account for changes in underlying infection rate and help more accurately quantify changes in COVID-19 transmission and severity over time. We have also demonstrated that wastewater surveillance data combined with model-inference estimates can support the identification of time-periods that potentially intensify transmission. Additional studies are needed to better understand these periods and the potential to mitigate SARS-CoV-2 transmission.

Abbreviations

CI	Confidence interval
COVID-19	Coronavirus disease 2019
ED	Emergency department
NYC	New York City
RNA	Ribonucleic acid
RT-dPCR	Reverse transcription digital PCR
RT-qPCR	Quantitative reverse transcription polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SEIRSV model	Susceptible-exposed-infectious-(re)susceptible-vaccination model

Supplementary Information

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Supplementary Material 1

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Authors' contributions

WY designed the study, performed the analysis, and wrote the first draft; EO, AO, and EAW oversaw provision of the SARS-CoV-2 wastewater surveillance data; HP and EL oversaw provision of the COVID-19 case and emergency department visit data. All authors contributed to the final draft.

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Data availability

The SARS-CoV-2/COVID-19 cases, emergency department visits, mortality, and wastewater surveillance data were used with permission under a Data Use and Nondisclosure Agreement between the NYC Health Department and Columbia University. The NYC Health Department also has a comprehensive, publicly available data website here: https://github.com/nychealth/coronavirus-data.

Declarations

Ethics approval and consent to participate

This activity was classified as public health surveillance and exempt from ethical review and informed consent by the Institutional Review Boards of both Columbia University and NYC Health Department.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY 10032, USA. [2]New York City Department of Health and Mental Hygiene, Queens, NY, USA. [3]Centers for Disease Control and Prevention, Atlanta, GA, USA.

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