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Predictive values of time-dense SARS-CoV-2 wastewater analysis in university campus buildings

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HIGHLIGHTS

• Daily building wastewater data matched to twice a week COVID clinical surveillance.
• Concordance analyzed over multiple time windows to optimize predictive values.
• Wastewater data reflects 8 of 11 days with 1–2 clinical cases in the building.
• Two consecutive days of positive wastewater is most predictive of individual case.

ABSTRACT

Wastewater-based SARS-CoV-2 surveillance on college campuses has the ability to detect individual clinical COVID-19 cases at the building-level. High concordance of wastewater results and clinical cases has been observed when calculated over a time window of four days or longer and in settings with high incidence of infection.

At Duke University, twice a week clinical surveillance of all resident undergraduates was carried out in the spring 2021 semester. We conducted simultaneous wastewater surveillance with daily frequency on selected residence halls to assess wastewater as an early warning tool during times of low transmission with the hope of scaling down clinical test frequency. We evaluated the temporal relationship of the two time-dense data sets, wastewater and clinical, and sought a strategy to achieve the highest wastewater predictive values using the shortest time window for agreement.

There were 11 days with clinical cases in the residence halls (80–120 occupants) under wastewater surveillance with 5 instances of a single clinical case and 3 instances of two clinical cases which also corresponded to a positive wastewater SARS-CoV-2 signal. While the majority (71%) of our wastewater samples were negative for SARS-CoV-2, 29% resulted in at least one positive PCR signal, some of which did not correlate with an identified clinical case. Using a criteria of two consecutive days of positive wastewater signals, we obtained a positive predictive value (PPV) of 75% and a negative predictive value of 87% using a short 2 day time window. A conventional concordance over a much longer 4 day time window resulted in PPV of only 60%. Our data indicated that daily wastewater collection and using a criteria of two consecutive days of positive wastewater signals was the most predictive approach to timely early warning of COVID-19 cases at the building level.

Keywords: Residence hall Asymptomatic surveillance Positive predictive value Clinical case
1. Introduction

Infection with SARS-CoV-2 is accompanied by copious and persistent shedding of viral RNA in feces (Lesure et al., 2020; Wölflé et al., 2020) in a large fraction of individuals, estimated to be from 50% (Wu et al., 2020) to 67% (Chen et al., 2020) of subjects. Wastewater monitoring techniques have been used around the world to inform on circulation and risk of viruses such as polio for decades (Pogka et al., 2017; Brouwer et al., 2018) and for SARS-CoV-2 since the early days of the pandemic in 2020 (La Rosa et al., 2020; Medema et al., 2020). Wastewater-based testing has demonstrated the ability to assess COVID-19 prevalence at city and community scales by sampling sewage treatment plants. Importantly, virus shedding occurs not only in symptomatic, but also pre-symptomatic and asymptomatic subjects (Lee et al., 2020) with similar viral load (Kimball et al., 2021). The numbers of asymptomatic and pre-symptomatic are estimated to be the infection source between 43% (Lavezzo et al., 2020) and 79% of observed infections (Li et al., 2020). The significant role of asymptomatic infections in COVID transmission highlights a need for surveillance methods broadly applicable across infections, both symptomatic and asymptomatic. Given the stability of the virus in wastewater (Bivins et al., 2020), surveillance has been extensively implemented for community-scales by sampling wastewater treatment plants influent where results correlate well with clinical testing metrics (Gonzalez et al., 2020; Graham et al., 2020; Greenwald et al., 2021).

Wastewater monitoring implemented at a more granular spatial scale of a building, especially in congregate living settings such as college dorms, can provide actionable public health information where a warning of nascent outbreak guides targeted intervention of isolation and mass testing.

Over 20 universities in the US implemented the use of wastewater monitoring to manage COVID-19 starting as early as May 2020 and continuing throughout the fall 2020 semester (Harris-Lovett et al., 2020). Approaches varied with respect to sampling methods (grab, passive samplers or composite autosamplers), number of sampling sites (from 3 to 15 to over 50 in some cases) and frequency of samples (typically 2 or 3 times a week, with a range from 1 to 7 times/week) (Harris-Lovett et al., 2020). The residence halls housed a number of students ranging from approximately 80 residents (RH2, RH3, and RH4) to 120 residents (RH1) residents. RH5 sampling point collected wastewater from a cluster of three buildings including RH4, and generated from approximately 180 residents with variation over time due to class schedule. In the isolation dorm, the number of residents varied over time from a few units to nearly 30. Sewer connection points were evaluated through as-built engineering drawings and Duke Facilities Management maps and sampling points were selected to ensure they would not be receiving extraneous wastewater from other sources.

Wastewater was collected using 3 portable autosamplers AS950 (Hach, Colorado). Each sampler was powered by solar panel for ease of installation and placed in a lock-box to prevent tampering. A dye test was conducted before installation to ensure collection from each manhole was fed by the intended building. RH2 was sampled from the pipe end in a substation well using a custom trough, while for all other sites, the sewer line was accessed through a manhole. The sampling tube was fed through a pre-existing hole of the manhole cover and placed in the bottom of the sewer line, ensuring placement in the direction of flow. A metal strainer device, custom-designed and provided by the Campus Research Machine Shop of the University of California San Diego was later added to the end of the sampling tubing to prevent clogging from trash and paper products. Time-based composite sample was programmed for 24 h collection of volumes ranging from 20 to 50 mL every 30 min for the isolation dorm, RH2, RH1 (until day 14), and RH3 (until day 26), and collection of 20 mL every 15 min for all other cases.

The sampling frequency of 30 min was used initially per manufacturer recommendation to reduce wear and tear on the autosampler. We changed sampling frequency to 15 min for general population residence halls upon reviewing a study on university campus successfully detecting COVID-19 clinical cases (Colosi et al., 2021).

The autosamplers were loaded with acid-washed 1 gal PET screw-cap containers. The PET containers were swapped daily. The collected wastewater was transported with a dedicated vehicle to the laboratory, poured into a 1 L bottle and pasteurized in a pre-heated water bath programmed at 63 °C for 90 min. The heat inactivation parameters of 60 °C for 90 min were selected based on prior reports on SARS-CoV-2 detection in wastewater (Crist-Christoph et al., 2021; Prado et al., 2021; Bivins et al., 2021). Because of the large volume (1 L) being processed, we ensured 60 °C temperature was achieved inside the container by setting the heating bath to 63 °C.

Pasteurized wastewater underwent ultrafiltration on the same day for 5 days a week and was stored at 4 °C for Saturdays and Sundays and processed on Monday.
Water meters already installed on the water line feeding RH1 and RH3 were read daily for approximately 1 month as a proxy for wastewater volume flowing through the sewer line.

2.2. Wastewater sample concentration

All wastewater processing took place in a biosafety cabinet. Electronegative membrane filtration was carried out according to methods reported in the literature (Ahmed et al., 2020). Bovine Coronavirus Vaccine, BCoV, (Bovilis Coronavirus Calf Vaccine, Merck Animal Health 156,332) was spiked as a process control for the concentration step (50 μL into 100 mL wastewater). Wastewater were amended with MgCl₂ to a final concentration of 25 mM and then incubated at room temperature for 10 min. Highly turbid samples were centrifuged at 6000 RPM for 15 min and the supernatant used for filtration.

Wastewater (100 mL) was added to vacuum filtration assemblies (Eisco Scientific LLC FSAS16) with 0.45 μm pore, 47 mm diameter membrane filters (GN-6 MCE, Pall Laboratory 63,020). After filtration, membranes were folded and placed into 2 mL 0.70 mm garnet PowerBead tubes (Qiagen) and centrifuged at maximum speed for 30 s using a PowerLyzer 24 homogenizer (Qiagen) for 30 s at 4000 rpm and immediately centrifuged at maximum speed for 1 min in a microcentrifuge to pellet the sheared membrane material. 400 μL of the resulting supernatant (the volume that could reliably be recovered from all samples) were transferred to a new collection tube and the remainder of the extraction/purification proceeded according to manufacturer’s instruction, including the optional addition of extra 100% ethanol during nucleic acid precipitation for the recovery of small RNA molecules. RNA was eluted in 100 μL of nuclease-free water and either immediately used for the quantification of selected targets or stored at −80 °C.

2.3. Wastewater RNA extraction

RNA was extracted from the concentrated material on filtration membranes stored in garnet PowerBead tubes using a modified protocol for the RNasey PowerMicrobiome Kit (Qiagen). In order to fully expose samples on the tightly folded membrane to kit lysis reagents, instead of the RNeasy PowerMicrobiome Kit (Qiagen). In order to fully expose samples on the tightly folded membrane to kit lysis reagents, instead of the RNeasy PowerMicrobiome Kit (Qiagen) for 30 s at 4000 rpm and immediately centrifuged at maximum speed for 1 min in a microcentrifuge to pellet the sheared fibrous membrane material. 400 μL of the resulting supernatant (the volume that could reliably be recovered from all samples) were transferred to a new collection tube and the remainder of the extraction/purification proceeded according to manufacturer’s instruction, including the optional addition of extra 100% ethanol during nucleic acid precipitation for the recovery of small RNA molecules. RNA was eluted in 100 μL of nuclease-free water and either immediately used for the quantification of selected targets or stored at −80 °C.

2.4. Wastewater RT-qPCR

The number of targets of the SARS-CoV-2 nucleocapsid gene (N1 target; CDC assay), spiked bovine coronavirus (BCoV), and endogenous pepper mild mottle virus (PMoV) in extracted RNA samples was quantified by RT-qPCR. Primers and Taqman probes for each assay are provided in Table S1. The N2 gene target was initially evaluated but was found to have lower sensitivity than the N1 assay, as reported by others, (Schmitz et al., 2021; Pérez-Cataluña et al., 2021) thus was not considered any further.

Each assay used the Quantitect Probe RT-PCR kit in a final volume of 25 μL including 5 μL of nucleic acid template. Primers and probes for the PMoV and BCoV assays were added to a final concentration of 0.4 μM and 0.2 μM, respectively. SARS-CoV-2 (2019-nCoV) CDC RUO primers and probes targeting the SARS-CoV-2 nucleocapsid gene were purchased pre-mixed from IDT DNA (Coralville, IA, USA) and were added at the recommended final concentrations of 0.5 μM and 0.125 μM, respectively. The one-step RT-qPCR comprised an initial dwell of 50 °C for 30 min, followed by 15 min at 95 °C and 45 cycles of 94 °C for 15 s and 55 °C for 30 s using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad). SARS-CoV-2 N1 assays were singleplex, while PMoV and BCoV were generally run in multiplex. Standard curves for the N1 target were determined from log dilutions of an RNA standard (VR-3276SD; ATCC) with target numbers corresponding to 10 to 10⁷ gene copies per reaction. Standard curves for PMoV and BCoV were calculated from synthetic linear dsDNA gBlocks (IDT DNA) diluted to the same range as the N1 assay control (standard curves of linear dsDNA and ssRNA synthetic templates were not significantly different; data not shown). Standard curves and no-template controls for all assays were included with every qPCR plate run. Also based on these data, qPCRs for N1, PMMoV, and BCoV were considered positive (above the LLOD) at Cq values <40, 42, and 39 cycles, respectively. The lower limit of quantification (LLOQ) for all reactions was 2 × 10³ copies per liter of wastewater. All experimental reactions were performed in triplicate, except for 16 out of 128 that were conducted in duplicate, with average values of the positive reactions reported. All RNA extractions and qPCR reactions were prepared within a biological safety cabinet that had been UV-sterilized and surface treated with an RNase inhibitor.

2.5. COVID-19 clinical monitoring

During the period of this wastewater pilot study (Spring 2021) at Duke University in Durham, North Carolina, all students enrolled in a mandatory SARS-CoV-2 infection surveillance testing program. Duke’s surveillance program started in the fall 2020 and included surveillance of asymptomatic persons using pooled testing and individual testing for symptomatic persons (Denny et al., 2020).

Testing of nasal swabs from symptomatic persons was conducted in a CAP-CLIA-certified laboratory. Pooled testing of asymptomatic persons was conducted after supervised self-collection of a nasal swab using a quantitative laboratory-developed RT-PCR and E_Sarbeco primer-probe set. A five-to-one pooled testing approach was used to achieve high frequency testing of approximately 16,000 students with the following frequencies: residential undergraduates twice weekly (two times in one week), off-campus undergraduates one to two times per week; and graduate and professional students once weekly. Positive pools were deconvoluted on a clinical PCR testing platform to identify positive individual samples in the pool.

All students signed the Duke Compact (https://returnto.duke.edu/the-duke-compact/) agreeing to participation in surveillance testing and compliance with testing on the data requested was approximately 95%. A newly identified positive result triggered contact tracing for quarantine and a focusing of testing either geospatially or within identified cohort (Denny et al., 2020). Students testing positive for COVID-19 and their close contacts entered isolation in residential facilities outside of their assigned residence hall. Students completed isolation per United States Centers for Disease Control and Prevention protocols with at least 10 days from symptom onset for symptomatic cases or 10 days from positive test results for asymptomatic cases. Recovered students returned to their residence hall. Throughout this study we refer to clinical cases as individuals with a COVID-19 infection confirmed by a clinical test, whether or not the individual is symptomatic.

2.6. Ethics

Deidentified data on COVID-19 cases detected by the surveillance program were obtained for the residence halls monitored by wastewater in accordance to an exempt amendment to protocol 0010–7690 approved by Duke University Health System IRB. Clinical data included the date of collection of positive nasal swab test and local address of the individual. To avoid any possible indirect identification, in this report the residence halls are anonymized and the monitoring period is anonymized by the use of elapsed days (0 to 73) rather than dates.

3. Results

This study monitored the isolation dormitory as positive control and, using two portable autosamplers, collected time-weighted 24 h composite samples daily from general population residence halls. The study collected composite wastewater samples over periods of 1 to 3 months for residence halls (RH) 1 through 3 (RH1 - RH3) and for 9 days for RH4 and RH5, for a total of n = 128 samples which were then analyzed for the presence of the SARS-CoV-2 virus by quantitative reverse-transcription PCR (RT-qPCR) of total extracted RNA.
The wastewater analysis results from the general population residence halls were obtained from researchers blinded from the corresponding clinical results data.

This study made consistent use of established methods for wastewater concentration and molecular analysis across all samples. Genes from pepper mild mottle virus (PMMoV), an indicator of human fecal matter in wastewater, were found at a consistently high level in all samples, ranging 5–7.5 log gene copy/L across the residence halls (Fig. S1); these values were comparable to those reported in other building-scale wastewater analyses (Scott et al., 2021). Wastewater samples from the isolation dorm were consistently positive for the SARS-CoV-2 N1 gene target for all technical replicates with typical values between 3 and 4 log10 gc/sample (n = 14; supplementary Fig. S2A). The N1 gene copy number normalized by PMMoV gene copy number was weakly correlated with the number of individuals residing in the isolation building (Fig. S2B), as also observed by (Scott et al., 2021).

Of 114 samples taken from the general population (non-isolation) dorms, 81 (71% of the total) were negative for the N1 gene target for all technical replicates while 32 (29%) had at least one positive qPCR replicate above the limit of detection; of these 13 (11% of the total) were also above the limit of quantification. We note that the threshold of Cq < 40 for N1 positivity was obtained from the average of 12 weeks of standard curves and was robust: all qPCR assays were run for more than 40 cycles and the reported N1 positive results would not be impacted by a higher Cq value threshold. During the period of the study, only 14 clinical cases were recorded in these residence halls while wastewater was being monitored. Of these, on three dates, two subjects tested positive from the same dorm on the same day, resulting in 11 days with positive clinical results, to compare to the detection of SARS-CoV-2 in wastewater.

Fig. 1 summarizes the SARS-CoV-2 detection from wastewater samples and clinical cases by elapsed day for each residence hall (RH) using a color pattern to illustrate strongly positive (all technical replicates positive), weakly positive (at least one technical replicate positive), and negative wastewater results in red, yellow and blue respectively. To account for relationship of signals over time, we also illustrate in gray days when wastewater was not collected. Tables S2–S6 report the copy number values for N1, PMMoV and a bovine coronavirus control with average values of the positive replicates (3 replicates were used, except for 8 samples with only two technical replicates that were either both positive or negative and are reported as red or blue in Fig. 1).

Fig. 1 shows that the clinically-detected COVID-19 cases were mostly in RH1, two were in RH2 and one in RH3. Sampling at RH1 began coincidentally with a cluster of cases on campus that resulted in multiple frequent cases and multiple consecutive days of wastewater testing positive. Of the
11 days with clinical positive cases, 8 days were positive for SARS-CoV-2 in wastewater within 24 h, albeit in half of them the N1 signal was below LLOQ. Specifically, wastewater signal from buildings with 80–120 resident reflected 5 instances with one individual clinical case and 3 instances with two clinical cases.

One of the 3 clinical cases not reflected in N1 wastewater results was on day 39 at RH1. The other two were from RH2 on day 31 and 38. The wastewater access for RH2 was a substation, instead of a manhole, and required a custom-made trough for sampling so, to avoid the additional variable of sample collection geometry that might account for these “misses” cases in wastewater analysis, the autosampler was moved to RH3.

To evaluate effects related to wastewater RNA extraction on the dates of the 3 clinically-positive matches, we examined the percent recovery of the BCoV spiked control and found they were 4%, 6%, and 0.98% on those dates respectively, illustrating successful recovery and quantification of coronavirus RNA in those samples. In fact, our BCoV recoveries for all samples (shown in Fig. S3) has a mean value of 2.4% which is higher than 0.91% reported by an electronegative membrane filtration study (Bivins et al., 2021). Interestingly the recovery of the BCoV control varied between residence halls with the lowest average from RH1, the building (outside of the isolation dormitory) where COVID-19 detection occurred most frequently.

Water use, a surrogate of the amount of wastewater generated, was recorded daily 5 days/week by water meters for RH1 and RH3 and found to be consistent over time (Fig. S4), with water use per person of RH1: 30 ± 5 gal/person/day and RH3: 35 ± 8 gal/person/day. Water use could not be associated to wastewater N1 either positive or negative values.

This study recorded a number of N1 positive wastewater samples not associated with clinical cases. There were three N1 positive wastewater samples in RH1 (day 18, day 21, and day 39) and one in RH2 (day 42) that could have been attributed to recovered cases returning from 10 days of isolation, however it is unclear why these recovered cases would stop shedding exactly one day after their return to residence halls. Thus we believe it is unlikely that our analytical method was able to detect shedding from convalescent cases. We observed in RH1 and RH3 positive N1 signal for 1–2 days after a positive clinical result which could be attributed to residual viral load in sewage, suggesting that in our sewage network one day may be sufficient to clear any residual viral load from a shedding event.

The data from RH4 and RH5, whereby RH5 collected sewer-flow from 3 buildings, one of which was RH4, was collected outside the period of both undergraduate classes and their COVID-19 surveillance. On day 3 one strong positive N1 signal was measured in both dorms and neither days before or after. We interpreted this result as a potential infected visitor to the building/bathroom. Because clinical surveillance was not carried out, we exclude data from RH4 and RH5 from concordance calculations.

3.1. Concordance wastewater-clinical detection over multiple time windows

Concordance metrics of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) and total agreement were calculated according to standard formulas. Because of the high temporal density of our data, different temporal windows including both preceding and following positive clinical results were used to assess concordance metrics for wastewater N1 results.

The rationale for using preceding time window was that shedding in wastewater may occur one or two days prior to an asymptomatic individual being detected as COVID positive by the twice weekly clinical surveillance. The rationale for including one day lagging a positive clinical result was two-fold:

1. the individual received the test result within 24 h from nasal swab collection and, in case of positivity, moved out of the residence hall the day after: thus it is possible that shedding in wastewater occurred the day after a clinical result.

2. it is possible the sewage was not removed by a plug-type flow, thereby traces remained in the sewer system for a period of 24 h (Schmitt et al., 2021)

We also calculated agreement on a two days sliding time window. We noted from Fig. 1 that, apart from the outbreak scenario in days 1–30 in RH1, many weakly positive wastewater signals occurred as a single day event; also in one case in RH3, a weak N1 signal for two consecutive days coincided with a positive clinical COVID-19 case. A two consecutive days time-window was defined by the following criteria i). Each set of two consecutive days with matching wastewater results was counted for either agreement or disagreement with clinical results ii). If two consecutive days of N1 results were neither both positive nor both negative, then no concordance was assigned. iii). In the absence of two consecutive wastewater data, when a positive clinical was recorded a concordance was assigned (either agreement or disagreement). Fig. S5 illustrates two examples for this calculation.

Fig. 2 reports the concordance metrics of PPV, NPV, sensitivity and specificity for these multiple time windows. The temporal window is described with the notation [a, b], where (a) is days preceding and (b) days following a clinical case positive. For example, [−1,0], covers the day preceding and the same day as a positive clinical result while a three-day window both preceding and lagging by one day is [−1,1].

We calculated concordance metrics considering wastewater positive for each of the following cases: 1, 2 or all 3 PCR (R) were positive (Fig. 2).

For same day agreement, time window [0,0], the PPV value was 37% and NPV 94% and total agreement was calculated to be 82%. By extending the time window to preceding days [−1,0] and [−2,0], PPV improved to 47% and 50% respectively. Adding a following day, e.g. in [−1,1], yields an additional gain to PPV = 53% while the NPV is constant at 94%.

The widest time window considered [−2,1] is as wide as 4 days and yields modest concordance agreement relative to the 3 day [−1,1] time window at the price of an additional day in time-to-result.

Notably, the concordance metrics measured under the condition of two consecutive days (2 days total) resulted in the highest PPV of 75% and modestly reduced the NPV to 87% and sensitivity to 43%. Based on the results shown in Fig. 2, the 2 consecutive days’ time-window yields the highest PPV and specificity while using only a 2 day data window and it is therefore the most actionable approach to use wastewater data for surveillance.

4. Discussion and conclusion

Our aim was evaluation of wastewater as a prospective surveillance tool for COVID-19 at the building level. While other research groups have explored different wastewater collection and molecular analysis approaches to increase limit and rapidity of detection, we adopted established methods for wastewater data generation, and sought to optimize the use of time-dense wastewater data to predict the presence of COVID-19 clinical cases in the building as information that would trigger intervention, such as individual screening of targeted building residents.

The outcome of the study was that the isolation dorm wastewater had a consistent quantifiable number of N1 copies, but only 13 out of 32 positive wastewater from general population residence halls were quantifiable. Thus, quantification of N1 did not play a role in the general population surveillance and a signal of presence/absence was used.

This study had 8 instances in which one individual (or at the most two) clinical case(s) corresponded to a positive wastewater N1 signal, indicating that our wastewater processing and PCR analysis was able to detect a single positive individual in a group of 80 to 120 people, as reported by others using similar methods (Gibas et al., 2021). However, at least 3 of the 11
positive COVID-19 cases were not reflected in the wastewater. This was expected as a fraction of COVID-19 infected individuals do not shed virus in detectable quantities in their feces. It is also possible that no bowel movement occurred in the residence hall wastewater stream while the individual was shedding and before they were sent to isolation. Other possible explanations for instances where clinical and wastewater data were not in agreement include the use of facilities by infected non-residents or staff within the monitored buildings, habitation of infected individuals outside of their assigned residence hall, or false positive/negative clinical or wastewater PCR results. However, given these wastewater results for SARS-CoV-2 N1 and associated control genes (for instance, we never observed a positive reaction in any no-template controls), we believe our wastewater collection and analysis methods were of adequate quality. We then sought criteria in the use of a positive wastewater N1 result to minimize the time-to-decision and also minimize the signal noise that may trigger unnecessary interventions. Thus, we were interested in maximizing the PPV parameter that reflects the likelihood of new clinical case in the building after a positive wastewater result. It is well known that predictive values, both positive and negative, depend on prevalence.

The Duke Campus surveillance program resulted in 526 COVID positive cases across a 16,000 student population in spring 2021. The average daily number of new cases campus wide was less than 10 (Fig. S6), except for an outbreak in March, thus this was a low incidence setting.

It is well known in epidemiology that PPV decreases when prevalence decreases and NPV increases (Mausner et al., 1985). This means that, under the same analytical conditions, in a low prevalence setting, a negative wastewater result is more likely to accurately reflect the absence of a COVID-19 case, while a positive N1 wastewater is less predictive of the presence of a COVID-19 case. A low PPV surveillance tool results in false alarms and undue burden of additional screening. Our concordance analysis aimed at finding conditions for maximum PPV. Temporal widening of window of comparison to 3 or 4 days improves agreement metrics but does not provide information for timely intervention. We found that the use of two consecutive day results in a high PPV of 75% in low prevalence setting and using a 2 day time-window, thus intervention can be decided only 24 h after a first positive signal. The high temporal resolution of our data sets, both clinical and wastewater, enabled this finding.

We propose that this temporal criteria can be adopted for wastewater surveillance conducted by a variety of sample collection and molecular analysis methods, and, for practical implementation, it would benefit from integration with methods that scale up and expedite analysis to achieve turn-around of results within 3 to 5 h (Karthikeyan et al., 2021; Bivins et al., 2022).

We expect that our finding will guide strategy for future surveillance of SARS-CoV-2 in wastewater in residential buildings by providing a specific guideline in sampling frequency and exploitation of data temporal relationships. The proposed approach is quite different from surveillance of SARS-CoV-2 at the watershed level, where temporal analysis suggested that twice per week sampling would be adequate for surveillance (Graham et al., 2020).

As an additional finding, we examined systematically the use of 1, 2 or all 3 replicates when considering a positive wastewater result. In building wastewater surveillance, it is not uncommon to have only a partial number of technical replicates being positive. Gibas et al. (2021) used all 3 replicates to define a positive (but had any Cq < 45 as positive) and labelled as “suspicious” results with 1 or 2 positive technical replicates, while other works (Bivins et al., 2022) reported the proportion of the positive triplicate reactions. We found that using 2 positive replicates optimized concordance parameters in the tradeoff between sensitivity and specificity.

As a limitation of this study, we note that analyses of concentrated wastewater samples were performed on a weekly basis, with processed samples (membranes) that had been stored at ~ 80 °C analyzed in batches retrospectively. Samples were generally stored frozen from 1 to 7 days prior to RNA extraction and RT-qPCR, which were typically performed in sequence and on the same day. Storage of samples could have resulted in RNA degradation and reduced the sensitivity of the analyses.

Additionally, a heat inactivation step was used to ensure personnel safety during sample processing. There have been multiple reports that there is no appreciable decrease in N1, PMMoV or BRSV at 60 °C for 90 min (Bivins et al., 2021), or even a small but appreciable increase in recovery for 60 °C at 60 min (Trujillo et al., 2021). However other recent
reports indicate that pasteurization may cause a decrease of N1 counts by a factor 2 (Whitney et al., 2021) or 3 (Islam et al., 2022), depending on conditions. It is likely that a less conservative approach on temperature and duration of heat inactivation may be beneficial to the sensitivity of the analysis.

This study used only one gene target, N1, while other groups have taken advantage of using both N1 and N2 (Colosi et al., 2021; D’Aoust et al., 2021; Scott et al., 2021), although there is no consensus yet on the optimal SARS-CoV-2 assays for wastewater analysis (Ahmed et al., 2022). We believe that our approach is generalizable to use of other assays and additional studies would be needed to determine whether the predictive values are strengthened by the use of multiple gene targets.

In conclusion, we found that two consecutive days of positive signals, even if weak, was the most robust approach to early warning for COVID-19 at building level. A daily sampling strategy accompanied by rapid molecular analysis can make wastewater analysis at building-scale a useful tool for early warning of rising trends when transmission is low and clinical test frequency winds down.

CRediT authorship contribution statement

Claire Welling: Investigation, Resources, Data curation, Formal analysis. David R. Singleton: Investigation, Methodology, Formal analysis, Writing – original draft. Steven B. Haase: Conceptualization, Data curation, Writing – review & editing. Christian H. Browning: Investigation, Formal analysis. Brian R. Stoner: Conceptualization, Funding acquisition. Claudia K. Gunsch: Conceptualization, Methodology, Supervision. Sonia Grego: Methodology, Project administration, Formal analysis, Visualization, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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