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Building-level wastewater surveillance as an early warning system for COVID-19 outbreaks in congregate living settings†

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The COVID-19 pandemic presented an opportunity to collect wastewater (WW) from a defined population of individuals within a building and monitor the sewage for viral RNA as a leading indicator of COVID-19 infections. The evaluation of the effectiveness of building-level WW surveillance programs as an early warning system has been limited by a lack of frequent asymptomatic surveillance of the defined residential population under WW surveillance. In this study we present the epidemiologic diagnostics of WW surveillance (sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV)) from university residence halls. WW surveillance was layered on top of a rigorous asymptomatic testing program (three times per week) and serves as the gold standard for comparison. This study also spanned across both the Spring 2021 semester when students were unvaccinated and the Fall 2021 semester when >95% of students were vaccinated for COVID-19 to understand how increased immunity may affect viral detection in WW. We analyzed composite WW samples from nine residential buildings that were collected twice weekly. The overall positive WW sample detection rate was 5.5% indicating the low-incidence context of this study population to allow for evaluation of WW surveillance as an early warning system. WW surveillance showed the best performance as a leading indicator of an infected individual when compared in a time inclusive of 1–2 days prior to the date of a clinical positive. The building-level WW surveillance sensitivity and specificity was found to be 60% and 94.9% (PPV: 47.4%; NPV: 96.9%), respectively in the Spring 2021; in the Fall 2021 sensitivity was reduced to 6.3% and specificity remained at a similar level of 97.5% (PPV: 14.3%; NPV: 94.1%). Combined for both semesters, the overall sensitivity and specificity were 32.3% and 96.4% (PPV: 38.5%; NPV: 95.3%). Convalescent shedding may explain up to 31% of false positive WW samples, contributing to decreased surveillance performance. This study demonstrates the greater effectiveness of building-level WW surveillance as an early warning system at the beginning of the COVID-19 pandemic when population-level immunity was naïve and fecal shedding of SARS-CoV-2 was likely more prevalent.

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Water impact

The field of building-level wastewater surveillance is in its nascent stages and much remains to be understood about its effectiveness as a tool for outbreak prevention. This paper advances understanding of the lead-time ability of building-level wastewater surveillance by comparing thrice weekly asymptomatic surveillance of a closed residential population with monitoring wastewater from buildings twice per week.

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1 Introduction

Wastewater (WW) surveillance has proven to be a useful tool to track disease dynamics of COVID-19 infections at the population-level. During the height of the COVID-19 pandemic with reliable clinical surveillance, community-level WW surveillance from treatment plants was extensively compared to reported case data from public health surveillance systems.^{1–5} These analyses established the ability



for a WW sample to serve as a population-level leading indicator of COVID-19 waves. To evaluate WW surveillance as an outbreak prevention tool on a smaller scale, at the building level, it is necessary to have surveillance of both asymptomatic and symptomatic cases for the entire residential population at high frequency (daily to weekly). The use of manholes and building-level WW outlets as collection points has demonstrated its use as a cost-effective and timely tool to comprehensively track disease dynamics in smaller, closed populations.⁶ The experience from the COVID-19 pandemic is informative for future pandemics to determine the utility of building-level WW surveillance as an outbreak prevention tool.

Building-level WW surveillance, also called “near-source tracking”, is the most upstream point of the sewershed to

measure SARS-CoV-2 in the waste stream.⁷ The fecal shedding dynamics of SARS-CoV-2 in infected individuals are critical to understand the utility of WW surveillance in near-source tracking. The virus has been found to be shed in approximately 39–65% of feces, from studies conducted with mostly symptomatic individuals.^{8–10} Sequencing of SARS-CoV-2 from WW has demonstrated unique mutations in the viral genome, suggesting that there are cryptic sites of virus replication in the intestinal track that may lead to the emergence of novel variants resistant to naturally acquired or vaccine-induced immunity.^{11,12} Fecal shedding prior to symptom onset, or for asymptomatic cases, is less well understood. One study found that two of three household contacts that contributed pre-symptomatic fecal samples were positive for SARS-CoV-2 RNA.¹³ Virus shedding in stool

Table 1 Applications of building-level WW surveillance for COVID-19 lead-time analyses in university campus settings.¹⁴ Asymptomatic testing assumes that all building residents are required to be tested at a certain frequency (if all building residents are not tested then “None” is denoted). The independence of clinical surveillance from WW surveillance indicates that the clinical testing occurred on a routine basis and was not contingent on a positive WW detection to initiate clinical testing (i.e. “surge testing”)

Application type	Building-level WW surveillance article in university setting	Frequency of WW testing	Frequency of asymptomatic testing	Clinical surveillance independent of WW
#1 Qualitative detection of disease presence/absence				
	Betancourt <i>et al.</i> , 2021 (ref. 20)	• •	None	No
	Colosi <i>et al.</i> , 2021 (ref. 21)	•	+	Yes
	Kotay <i>et al.</i> , 2022 (ref. 22)			
	Gibas <i>et al.</i> , 2021 (ref. 23)	• • •	None	No
	Godinez <i>et al.</i> , 2022 (ref. 24)	• •	None	Yes
	Solo-Gabriele <i>et al.</i> , 2023 (ref. 25)	• • • •	None	No
	Mangwana <i>et al.</i> , 2022 (ref. 26)	• •	None	No
	Welling <i>et al.</i> , 2022 (ref. 27)	• • • •	+	Yes
	Landstrom <i>et al.</i> , 2022 (ref. 28)	• •	None	Yes
	Rondeau <i>et al.</i> , 2023 (ref. 29)	• • •	+	Yes
#2 Independent, quantitative estimate of community-level disease prevalence and trends				
	Johnson <i>et al.</i> , 2022 (ref. 30)	• • • •	None	Yes
	Reeves <i>et al.</i> , 2021 (ref. 31)			
	Scott <i>et al.</i> , 2021 (ref. 32)	•	+	Yes
	Fahrenfeld <i>et al.</i> , 2022 (ref. 33)	•	+	Yes
	Wang <i>et al.</i> , 2022 (ref. 34)	•	+	Yes
	Karthikeyan <i>et al.</i> , 2021 (ref. 6)	• • • •	+	Yes
	Anderson-Coughlin <i>et al.</i> , 2022 (ref. 35)	^a	^a	^a
	Zambrana <i>et al.</i> , 2022 (ref. 36)	•	+	Yes
	Rainey <i>et al.</i> , 2023 (ref. 37)	•	None	Yes
	Ash <i>et al.</i> , 2023 (ref. 38)	•	None	No
	Sharkey <i>et al.</i> , 2021 (ref. 39)	•	+	Yes
	Amirali <i>et al.</i> , 2024 (ref. 40)			
	Bivins <i>et al.</i> , 2021 (ref. 41)	•	+	Yes
	Lee <i>et al.</i> , 2023 (ref. 42)	• •	None	Yes
	Cohen <i>et al.</i> , 2022 (ref. 43)	• •	None	Yes
	Lu <i>et al.</i> , 2022 (ref. 44)	• •	None	Yes
	Kazenelson <i>et al.</i> , 2023 (ref. 45)	•	None	Yes
	Sellers <i>et al.</i> , 2022 (ref. 46)	• •	None	Yes
	Haskell <i>et al.</i> , 2024 (ref. 47)	• • •	None	Yes
#3 Quantitative estimate of rapid changes in disease incidence				
	Brooks <i>et al.</i> , 2021 (ref. 48)	•	+	No
	Bitter <i>et al.</i> , 2022 (ref. 49)	• •	None	No
	Corchis-Scott <i>et al.</i> , 2023 (ref. 50)	• •	None	No

• • • • 7 days per week. • • • 3 days per week. • • 2 days per week. • 1 or less days per week. + + + + 7 days per week. + + + 3 days per week. + + 2 days per week. + 1 or less days per week. ^a Frequency not provided.



correlates well with viral replication in the upper respiratory tract post-onset of symptoms, making SARS-CoV-2 RNA detection in stool a good proxy for detection in nasal samples.^{9,10,13}

There is vast experience across colleges and universities to demonstrate the utility of WW surveillance as a leading indicator of COVID-19 prevalence. As explained in Olesen *et al.*, the meaning of “leading indicator” depends on the specific application of WW surveillance.¹⁴ The applications can be described in three main categories: i) qualitative detection of disease presence or absence (otherwise known as an “early warning” system); ii) independent, quantitative estimate of community-level disease prevalence and trends; and iii) quantitative estimate of rapid changes in disease incidence. Building-level WW surveillance in the university setting was often implemented on campuses during the COVID-19 pandemic to gain a quantitative estimate of community-level disease prevalence and trends (application #2). Table 1 categorizes the existing studies from the university setting into these three applications to demonstrate the wide use of WW surveillance to understand community-level disease on prevalence and trends. Settings that have applied building-level WW surveillance systems, other than institutions of higher education, include correctional facilities,¹⁵ nursing homes,¹⁶ commercial aircrafts,¹⁷ military settings,¹⁸ and Olympic and Paralympic Villages.¹⁹

To assess whether building-level WW surveillance can be used as a more timely replacement for surveillance based on upper respiratory or saliva samples, the time lag between WW sample collection and analysis must be short. Clinical assessment by nasal or saliva samples during the COVID-19 pandemic was often used to prevent outbreaks in congregate living settings. The frequency of this mass asymptomatic testing of building residents ideally occurred at the shortest interval possible; however, this type of clinical surveillance is resource intensive. To achieve more frequent testing with limited resources, pooling of clinical samples often occurs prior to laboratory analysis.⁵¹ Comparatively, WW surveillance is a naturally pooled sample and can be collected at very short timescales, often hourly. Prior studies have estimated that building-level WW surveillance is able to detect SARS-CoV-2 positivity prior to clinical reporting. However, reported estimates of lead time have been variable, ranging from a 0 to 2 day lead.^{5,52,53} The context of these studies is also highly variable with clinical testing occurring *en masse* in a building only after a positive WW detection occurs.²⁰ This highlights the need to measure clinical symptomatic and asymptomatic surveillance of all building residents in parallel with frequent WW sampling.

This study describes the results of a collaborative, rapid-response effort to implement a comprehensive WW surveillance program for SARS-CoV-2 at the sanitary outflows of residential facilities at the Johns Hopkins University campus complementary to clinical surveillance. The aim of this study was to conduct building-level WW surveillance in

the context of multi-layered COVID-19 mitigation strategies to achieve low-incidence of disease. WW surveillance data is compared with high-frequency asymptomatic clinical testing data to understand its ability to detect a positive case in a low-incidence setting, also known as an “early warning system”.

2 Methods and materials

2.1 Campus response to prevent COVID-19 transmission

At the onset of the pandemic in March 2020, there was a complete shutdown of on-campus activities for the COVID-19 pandemic response. A multi-layered approach was taken to prevent the spread of COVID-19 when students returned in the Spring semester starting January 2021. The goal of the approach was to minimize transmission during on-campus activities and maintain a low-incidence of disease in the student population.

2.1.1 Clinical surveillance and student isolation protocol.

Students housed on-campus were closely surveilled for COVID-19 with both asymptomatic and symptomatic testing requirements. Asymptomatic testing entailed a thrice weekly passive saliva drool, found to be a superior sampling specimen to a nasopharyngeal (NP) swab for early detection.⁵⁴ In brief, 1 mL of saliva was heat inactivated at 95 °C for 30 minutes within 48 hours of collection, after which RNA was extracted from 100 microliters using the MagMAX Viral Pathogen kit II (Thermo Fisher; Waltham, MA) according to the manufacturer's directions. One tenth of the eluate was subsequently analyzed by reverse transcription PCR using the TaqPath COVID-19 Combo kit (Thermo Fisher). Students experiencing COVID-like symptoms were tested at a specialized symptomatic testing site with a rapid PCR nasal swab and if negative it was followed up by an NP swab analyzed by RT-qPCR as described above. Students found to be positive for COVID-19 were removed that day from campus housing and brought to an off-campus hotel for isolation. After an isolation period lasting 10-days, students were allowed to return to their residence halls. If students were still having symptoms after 10 days, a longer isolation period was required. A person determined to be a close contact to the case was also removed from the residence hall and stayed at the hotel for up to 14 days. De-identified clinical surveillance data for students in each residence hall under WW surveillance were tracked to allow for clear timelines to be documented for symptom onset, date of a positive test, and date of departure for isolation.

2.1.2 University policies before and after Fall vaccination mandate. This study investigated two distinct periods before and after COVID-19 vaccines were available. The Spring 2021 semester captured the pre-vaccination period, and the Fall 2021 semester captured the post-vaccination period. COVID-19 vaccination was made available to all adults on campus on April 19th, 2021. Prior to this date students were able to obtain vaccines at large state-run clinics or pharmacies. A university-wide vaccine mandate for students and employees



went into effect on August 8th, 2021, prior to start of the Fall 2021 semester. Vaccination rates in undergraduate students were >95% at the start of the Fall semester. The comparison of these two semesters represents a unique case-study of the utility of WW surveillance in a population with high vaccine uptake to understand how vaccination may affect viral detection in WW. See the ESI† for more details of the policies in place for both semesters.

2.2 WW sampling and collection

WW surveillance was conducted at occupied on-campus housing facilities during the Spring and Fall semesters of 2021. In the initial Spring sampling campaign, deployment of WW samplers occurred in stages, beginning with five initial buildings (A–E) and expanding to a total of eight (A–E, G and H). In the Fall, a previously unoccupied building (F) was re-occupied by students and included in the Fall WW sampling campaign for a total of nine buildings for the Fall 2021 semester. Three residence halls were located on university property (G and H), where underground WW pipes conveying individual building effluent could be accessed through manholes on campus. The remaining six residence halls (A–F) were located off-campus and due to concerns of safety and blocking road and pedestrian traffic WW pipes could not be accessed by manholes. WW samples from these buildings were collected through cleanout ports accessing the main lines inside the basement of the buildings. A summary of each collection site and sample collection method is presented in Table 2.

For each building, the amount of WW conveyed through the outlet sampled was estimated as a percentage of student rooms. Personal communication with facilities personnel and referencing of available building schematics ensured that each building outlet or manhole sampled from only a single residential building of interest. Five buildings were estimated to have 100% coverage. Building D and B were estimated to

cover 50% and 25% of student rooms due to the presence of multiple mains connecting to city sewer lines. Building E covered 50% through the first 3 months of study (site E1) and reached 100% coverage upon installation of a second sampler (E2) in the building's secondary WW effluent pipe (Table 2).

WW samples were collected using ISCO 6712 portable autosamplers (Teledyne ISCO; Lincoln, NE) to pull composite samples through stainless steel strainers (Teledyne ISCO) positioned in the bottom interior of pipes. WW collections were performed twice per week at each tested site by composite sampling over a 15-hour period from 7 AM to 10 PM every Sunday (to capture cases over the weekend) and Tuesday (to deliver timely reports by Friday), with occasional schedule modifications in the event of university holidays. The autosamplers were programmed to draw semi-continuously at discrete 10–20 minute intervals. The following morning after collection, composite sample containers were manually agitated to mix the contents, dispensed into autoclaved 1 L polypropylene screw-cap bottles and delivered to the laboratory for same day processing. Samples were collected on 32 different dates in the Spring and 28 in the Fall, totaling 60 sampling days for this study. Further details describing the WW sampling protocol are presented in the ESI.†

2.3 Processing, extraction, and detection of SARS-CoV-2 RNA

Upon receipt, samples were thermally treated at 75 °C for 2 hours to inactivate WW pathogens before further handling.⁵⁵ Samples were processed in a biosafety cabinet for sub-portioning and concentration by electronegative membrane filtration following a protocol described by Gonzalez *et al.*⁵⁶ Briefly, samples were vigorously shaken, and 125 mL of each sample were aliquoted into an autoclaved glass beaker with a magnetic stirrer. Samples were adjusted to pH 3–3.5 with 2 M HCl and spiked with 25 mM MgCl₂. Samples were spiked

Table 2 Overview of residential hall site testing results during the WW surveillance period

Building code	Sanitary line access	Estimated building sewage coverage	Spring semester (1/25–5/11/2021)				Fall semester (9/5–12/14/2021)			
			# student residents	# clinical positives detected/# unique Pos case dates	# positive WW samples	# WW samples collected	# student residents	# clinical positives detected/# unique Pos case dates	# positive WW samples	# WW samples collected
A	Cleanout	Full	136	1/1	1	32	136	0	0	23
B ^a	Cleanout	Partial	199	2/2	2	31	205	0	0	24
C	Cleanout	Full	253	2/2	4	30	474	7/6	2	23
D	Cleanout	Partial	606 ^d	36/9	6	29	606 ^d	4/3	1	28
E1	Cleanout	Partial → full		4/2	2	30		4/4	0	28
E2	Cleanout	Partial → full			0	6			2	26
F ^b	Cleanout	Full	—	NA	NA	NA	509	4/3	1	27
G ^c	Manhole	Full	93	0	2	18	191	0	0	27
H ^c	Manhole	Full	132	0	2	17	324	3/3	0	27
I ^c	Manhole	Full	49	0	0	18	184	1/1	0	27
Total			1468	45/16	19	211	2629	23/20	7	260

^a Sampler was moved to a different cleanout location within the building between Spring and Fall. ^b Building was unoccupied and not sampled in Spring. Sampling began in Fall 2021. ^c Sampling began later in Spring semester – 03/07/2021. ^d Number of student residents reported as a combined number for buildings D and E, which were part of a connected complex but with separate sewer connections.



with a 1:1000 dilution of internal standard bovine coronavirus (BCoV) (Calf-Guard; Zoetis Animal Health; Parsippany, NJ).

WW aliquots of 50 mL, decreased from 100 mL in the original sample protocol,⁵⁶ were filtered under vacuum through 47 mm 0.2 µm mixed cellulose ester HA membrane filters (Millipore, Billerica, MA). A lower filtrate volume was necessary to minimize filter clogging, which frequently occurred due to high solid content of raw sewage. In 19% of samples ($n = 88$), the entire 50 mL could not be filtered. In these cases, the filter was used with the volume of WW filtered after 2 hours (average 40 mL). In infrequent cases ($n = 10$) when less than 20 mL could be filtered (either due to fouling or low sampled volume), 1 mL aliquots of WW were directly extracted. Filters were folded and placed directly into 2 mL bead-beating tubes after filtration. Synthetic WW (Table S2†) unspiked and spiked with BCoV were processed as extraction blanks and internal standard recovery controls respectively following the same protocols. All the samples were stored at -80°C until RNA extraction.

Sample RNA was extracted from frozen filters and WW aliquots to produce a 100 µL extract using an RNeasy PowerMicrobiome kit (Qiagen; Germantown, MD) with a QIAcube Connect automated extraction system (Qiagen) following the manufacturer's protocol. During extraction, DNase I was applied to remove genomic DNA from the extract. Sample extraction, reaction prep, amplification, and disposal each occurred in separate laboratory spaces to minimize the possibility of cross-contamination.

SARS-CoV-2 detection was based on the CDC 2019-nCoV real-Time RT-PCR diagnostic panel for human specimens (CDC-006-00019, Revision: 06) with modifications. The N1 coding gene coding was exclusively used for SARS-CoV-2 detection. BCoV RNA detection utilized primers and probes described elsewhere.⁵⁶ All primers and probes were obtained from IDT (Coralville, IA) and are listed in Table S1 of the ESI.† RT-qPCR assays were performed as 20 µL (5 µL template) one-step RT-PCR reactions using a QuantStudio 5 thermocycler (Thermo Fisher). PCR reactions were amplified separately (*i.e.* not duplexed) to prevent ablation of the signal due to potential competitive amplification of each target of interest. The thermocycler program was identical to the CDC protocol, except the amplification was run for 40 cycles (instead of 45 cycles).

Extracts from each WW sample were analyzed by RT-qPCR as an undilute template and complementary 10^{-1} diluted template to account for the potential presence of PCR inhibitors. Extracted samples were marked as having potential amplification inhibition if the internal standard BCoV ΔCt values between 10^{-1} and undilute templates deviated at least 2 cycles from a theoretical 10-fold dilution ΔCt of 3.32. In the Fall, the undilute templates were run in duplicate. Stocks of RNA extracted from inactivated SARS-CoV-2 culture and the BCoV vaccine were used as positive controls for their respective assays. SARS-CoV-2/USA-WA1/2020 isolate used as a positive control was obtained from BEI

Resources and grown in Vero cells as previously described,⁵⁷ to an infectious virus titer of 1.58×10^8 50% tissue culture infectious doses per ml ($\text{TCID}_{50} \text{ mL}^{-1}$). Each PCR run contained positive SARS-CoV-2 RNA control templates that were 10-fold serial diluted 3–7 times until extinction into separate reaction wells for quality control. The Ct values for positive control dilution series were fitted to linear calibration curves for the log of each dilution with an average R^2 of 0.991 ± 0.014 (1 S.D.). Average amplification efficiency based on the slopes of these curves was $102\% \pm 15\%$, indicating consistent amplification reactions over the course of the study. The N1 assay limit of detection (LOD) was determined at the highest discrete stock dilution with at least 95% positive detections in the 50 calibration dilution series run during the study.⁵⁸ The LOD was the 10^5 stock dilution (100% detection frequency, average Ct = 33.8), corresponding to an infectious virus titer of $1.58 \times 10^3 \text{ TCID}_{50} \text{ mL}^{-1}$. The next higher dilution (10^6) was detected in only 67% of runs (average Ct = 36.5).

2.4 Data analysis and reporting protocol

The reported WW positivity for SARS-CoV-2 was determined based on threshold criteria for both the N1 and BCoV assays. Samples with a N1 Ct value less than 40 for either undiluted replicate or 10^{-1} diluted template reactions (sample positive) as well as a BCoV Ct value less than 40 (internal standard positive) for the corresponding template dilution were considered positive samples. Due to the high prevalence of sample heterogeneity and PCR inhibition (discussed in section 3.2), quantitative comparisons between sample Ct values were not conducted for positive samples. Instead, WW samples were categorically binned as positive or negative for subsequent lead-time and performance comparisons.

WW collection and analysis was optimized to facilitate reporting to university leadership within 48 hours of initial sample collection. Immediately after data interpretation of a given WW collection, positive results were reported to university leadership and used to make decisions regarding potential outbreaks in individual buildings. Sample results were cross-referenced with individual student testing data reported to the university to help determine early warning of an outbreak. Weekly reports were compiled on Fridays to provide a more formal and comprehensive analysis of the surveillance data. A summary of the weekly WW surveillance workflow and reporting timeline is described in Fig. 1.

2.5 Evaluation of WW surveillance compared to clinical testing

Building-level WW surveillance was evaluated as a leading indicator of infection using aggregated clinical testing results (asymptomatic and symptomatic) as a gold standard benchmark of positive cases. Windows of coincidence were examined 1 to 4 days surrounding the date of a positive clinical detection. The short windows assumed that clinically



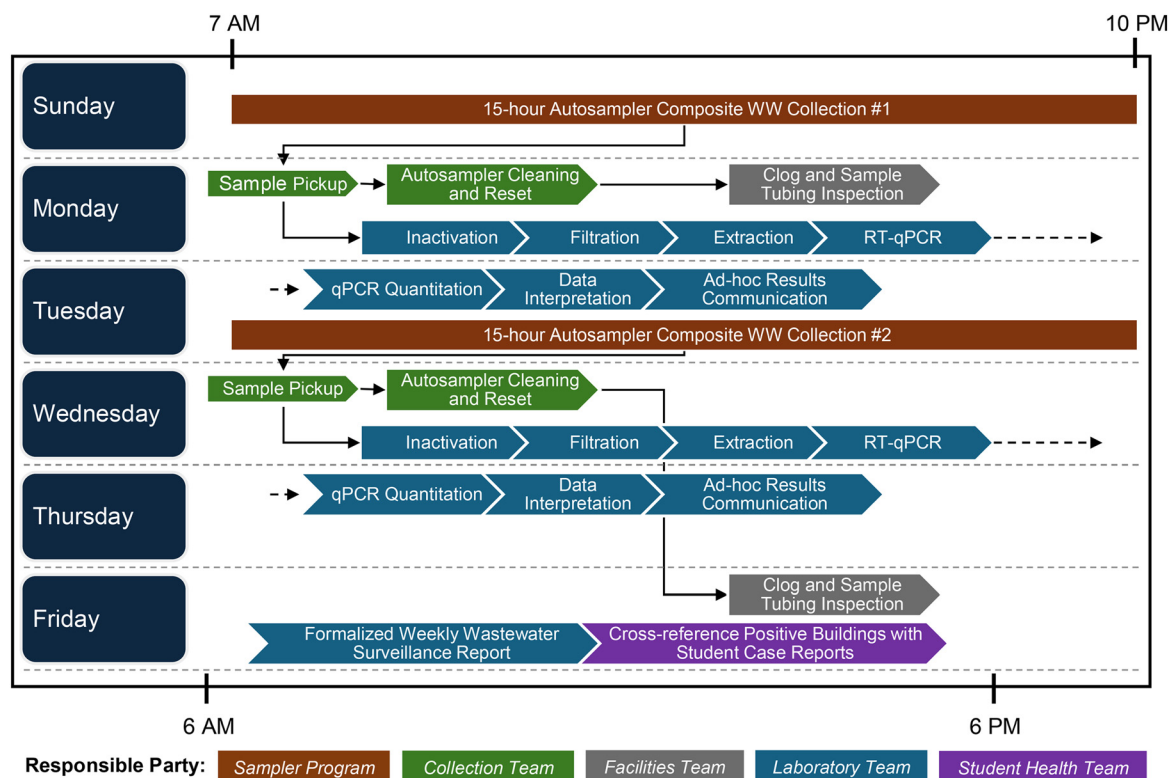


Fig. 1 Weekly protocol for WW surveillance during the study period.

positive students were subsequently placed in separate isolation housing and could no longer generate a SARS-CoV-2 signal in WW. Windows of one and two days prior to the clinical test were used to capture the assumed asymptomatic shedding. A window extending one day after the clinical positive was also investigated in the case of a lagging WW signal resulting from delays in testing and student isolation as well as latent viral material remaining in the sewer system.^{27,59}

Each WW sample was classified as either a true/false positive or true/false negative based on its coincidence with a positive clinical sample (Fig. 2). WW samples that were positive and overlapped with a positive clinical detection were classified as true positives (TPs). If there was a positive clinical detection one to two days prior to a WW sample that was negative it was classified as a false negative (FN). WW samples that were negative and did not overlap with positive clinical detection windows were classified as true negatives (TNs). False positives (FPs) were classified if a WW sample was positive without a positive clinical detection one to two days prior to the WW sample. To understand the accuracy and reliability of WW surveillance as a diagnostic test, sensitivity (*i.e.* the ability for WW to detect a single case in a building) and specificity (*i.e.* the ability for WW to correctly identify that there are no infected individuals in the building) were calculated. Sensitivity was defined as $[TP/(TP + FN)]$.⁶⁰ Specificity was defined as $[TN/(FP + TN)]$.

3 Results

3.1 Clinical surveillance results

Fig. 2 presents chronological results of WW surveillance and positive clinical tests overlaid in each of the nine monitored buildings over the course of Spring and Fall 2021 semesters. Table 2 summarizes case counts that occurred during the WW surveillance period and are reported by building, semester, and across the entire campus.

During the Spring 2021 semester, seven residential buildings were occupied by 1468 students. The eleven students who tested positive prior to the start of building WW surveillance were placed in isolation (Fig. 2). A total of 45 positive clinical cases were reported across 16 unique case-dates (Table 2). At the start of the Spring semester (January 29 to February 15), 40 student residents tested positive, making up the largest cluster of infections in the study (Fig. 2A). Contact tracing attributed this multi-residence hall outbreak event to a large off-campus event on Friday January 29. Multiple students began to test positive (both symptomatic and asymptomatic) on Tuesday February 2 after attending the event. A smaller cluster of 4 positives occurred in a 10-day period April 5–14. This coincided with Easter weekend (April 4), a holiday where many students typically travel and congregate. Between these two clusters, only a single additional positive clinical result was reported for the remainder of the Spring semester, confirming the low-incidence environment within which WW surveillance was conducted.



A. Spring

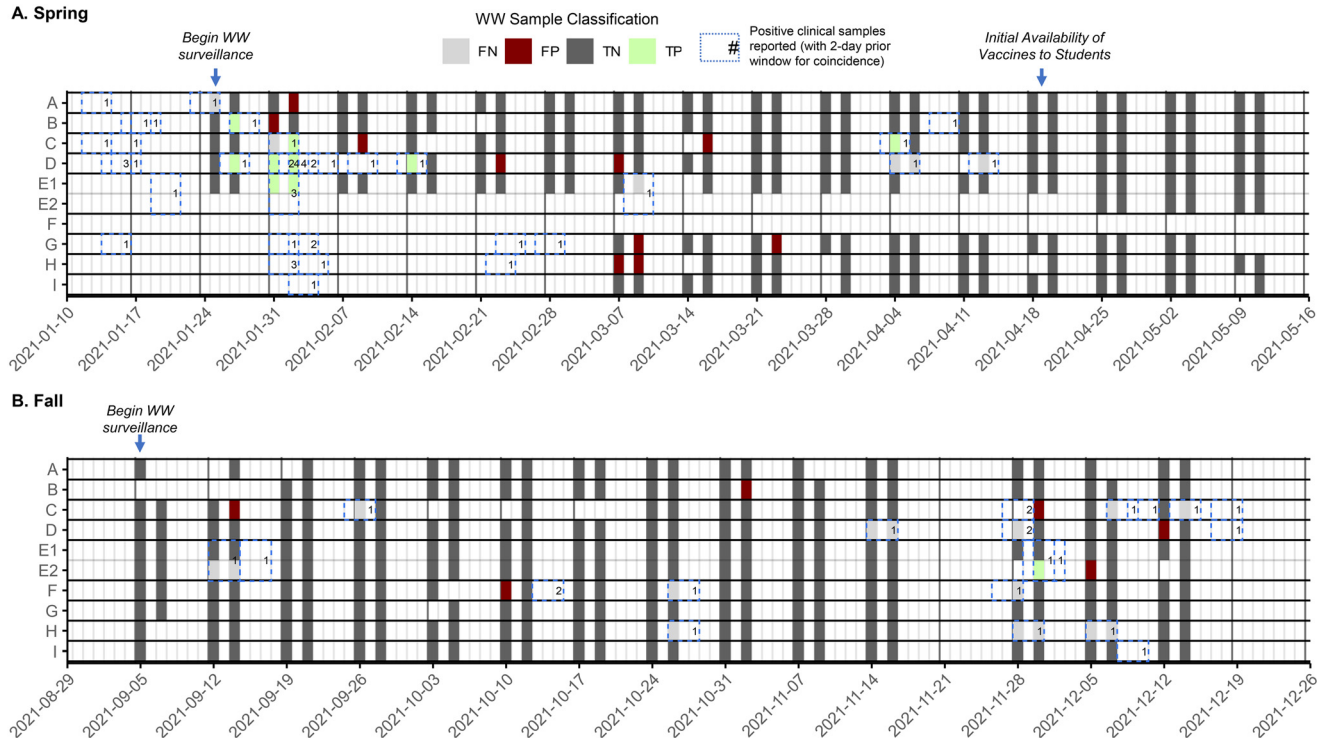


Fig. 2 Chronological results of WW surveillance and clinically positive tests in each monitored building over the course of the A) Spring and B) Fall semesters of 2021. Counts of clinically positive individuals are represented with a number. Collected WW sample tiles were colored based on their coincidence with clinical samples using a comparison window 2 days prior to the clinical sample (dotted boxes). WW sample classifications: FN – false negative, FP – false positive, TN – true negative, TP – true positive.

There was an increase in occupancy in Fall 2021 to 2629 residential students across nine buildings. During this period, 23 positive clinical cases were reported across 20 unique case-dates (Table 2). Eight sporadic cases were reported in various buildings throughout the first 12 weeks of semester prior to the weeklong Thanksgiving break (November 20–27). A cluster of eight positive cases was reported in different buildings in a five-day period immediately following the holiday break. The final two weeks of the semester had 7 positive cases, including a series of 4 individual positives from the same building (building C). There were 3 WW positive samples during this period, with a single occurrence of a positive WW detection of two positive cases (Fig. 2B). No WW collection occurred from November 20–27 over the Thanksgiving holiday.

3.2 WW collection

A total of 471 WW surveillance samples were collected, processed, and analyzed from residential facilities during this study (Table 2). Out of an expected 506 potential samples, the 471 collected WW samples represented a successful collection rate of 93%. Missed samples resulted primarily from clogging of sampler strainers with solid material or movement of strainers to an unwetted area of the sewer pipe. In the event of an uncollected sample, facilities personnel would clean, reposition, and test sample lines to facilitate the

successful collection of subsequent samples. Sample collection rates were identical between semesters (Table 2) despite lower occupancy and qualitatively lower flow observed in building cleanouts during the Spring semester. This suggests that composite WW sampling can be conducted in residential facilities operated at partial occupancy (*i.e.* low-flow environments) without major protocol adjustments. Collected composite sample volumes were variable due to fluctuations in flow conditions throughout the day, which could result in partial or overdraw of the sample. WW samples varied widely in terms of visual quality and consistency, however the measured optical density values of all samples were unassociated with volumes of sample collected or location (ESI† Section S2.1).

Overall, 17% of measured samples ($n = 81$) showed internal standard inhibition during amplification (section 2.3). This rate was consistent across different sample locations (11–23%) and was sporadic across sampling dates. The heterogeneity of samples and high degree of potential PCR inhibition were important considerations in the treatment of data. Prior studies have described inconsistent results from applying internal controls to correct for recovery loss of the SARS CoV-2 signal in WW.^{61,62} Rather than attempt to quantify and normalize gene copy numbers in samples with varying degrees of inhibition, samples were evaluated for the presence/absence of the SARS-CoV-2 signal within the quality control and threshold constraints for the



RT-qPCR assay. This simplified approach provided a rapid, conservative, and uniform method of treating such heterogeneous samples collected at the building level.

A total of 26 collected WW samples were positive for SARS-CoV-2 during this study, with 9 out of 10 sampling locations producing at least one positive sample (Table 2). Measured RT-qPCR results for each positive sample are presented in Table S2.† Among all positive samples, SARS-CoV-2 Ct values ranged from 29.8–38.9 with average and median detected values of 35.7 and 35.9 respectively (Table S2†). A similar fraction of positive samples showed inhibition based on BCoV dilution (23%) compared to the total number of samples (17%), which suggests a lack of a selection bias based on PCR inhibition.

3.3 WW surveillance results

WW surveillance commenced on January 25th one week after student-move in, and the first day of class for the Spring 2021 semester. The positive WW samples detected in buildings B and D on both Sunday, January 31st and Tuesday, February 2nd were associated with positive cases (Fig. 2A). For this outbreak event, WW surveillance was able to detect the SARS-CoV-2 signal two days after the presumed exposure event and two days prior to the clinically positive cases being diagnosed. The total number of positive WW detections during this outbreak event was 12, which spanned across all five buildings under surveillance at the time. Apart from the initial outbreak event, WW surveillance detected 8 additional WW positive samples in the Spring 2021 semester and clinical surveillance detected and isolated 5 clinically confirmed positive cases that resided in buildings with WW surveillance (Fig. 2A). This period of the semester coincided with declining caseloads in the local population and vaccination campaign rollouts across the university and local population.

In the Fall 2021 semester WW surveillance began on September 5th, one week after the beginning of classes on August 30th. Testing and quarantine of students upon move-in was not conducted. Seven single clinically positive cases and three WW positive samples occurred prior to the Thanksgiving holiday (November 20–27), with a single occurrence of a positive WW detection of two positive cases

in building E (Fig. 2B). No WW collection occurred over this holiday period. After this break in the semester, four positive WW detections were found to be associated with three positive clinical tests (Fig. 2B).

The overall positive WW sample detection rate was 5.5%. On a semester basis, the positive detection rate was over three times greater in the Spring (8.9%) than in the following Fall (2.7%). This trend mirrored the overall number of reported positive cases in the residence halls, which dropped from 45 in the Spring to 23 in the Fall. The decreased detection rates occurred despite an increase in student resident density (Table 2) and a relaxation of transmission-reduction protocols within buildings.

3.4 Performance of WW as a leading indicator of a clinical case

WW surveillance was benchmarked against the clinical surveillance data under 5 different scenarios ranging from same-day concurrence [0,0] to a 4-day window spanning 2 days prior to a clinical positive and 1 days after [−2,1] (Table 3). Overall, the sensitivity and specificity of all tested windows were largely similar. Sensitivity was low and ranged from 28.9–36.8%, while specificity was universally high at 95–96.5%. In effect, the WW surveillance rarely produced false positives (FPs) while having a poor ability to capture true positives (TPs). The rates of TP and FN samples increased with increasing window size. Increasing the window prior to the date of a clinical positive allowed for more WW detections to be classified as TP compared to increasing the window 1 day after, indicative of the leading nature of the WW surveillance (Table 3). Based on the temporal data, using a window of concurrence including the date of clinical positive and up to 2 days prior explained the observed WW positives well (Fig. 2). The 2-days prior window yielded the best combination of sensitivity and positive predictive value compared to other comparison windows (Table 3). This 2-days prior window is subsequently used for further discussion in the sections below.

Breaking down the WW surveillance by semester using the 2-days prior window, the Spring 2021 campaign yielded 60% sensitivity and 94.9% specificity (Table 4). Three of the 6 FNs occurred in buildings where full WW coverage was not

Table 3 Evaluation of WW surveillance performance compared to high-frequency clinical testing in residence halls using different windows of coincidence

Comparison window (from date of clinical positive)	Date range	Sample classification				Performance metrics			
		FN	FP	TN	TP	Sensitivity	Specificity	PPV	NPV
Same day	[0,0]	7	23	438	3	30.0%	95.0%	11.5%	98.4%
1-Day prior	[−1,0]	12	19	433	7	36.8%	95.8%	26.9%	97.3%
2-Days prior	[−2,0]	21	16	424	10	32.3%	96.4%	38.5%	95.3%
1-Day prior, 1-day after	[−1,1]	18	18	427	8	30.8%	96.0%	30.8%	96.0%
2-Days prior, 1-day after	[−2,1]	27	15	418	11	28.9%	96.5%	42.3%	93.9%

Abbreviations: FN (false negatives), FP (false positives), TN (true negatives), TP (true positives), PPV (positive predictive value), NPV (negative predictive value). Calculations: sensitivity = $TP/(TP + FN)$; specificity = $TN/(TN + FP)$; PPV = $TP/(TP + FP)$; NPV = $TN/(TN + FN)$.



Table 4 WW surveillance performance using the 2 days prior window broken down by semester and when adjusted after consideration of convalescent shedding

Semester	# student residents	# positive clinical cases	Sample classification				Performance metrics			
			FN	FP	TN	TP	Sensitivity (Se)	Specificity (Sp)	PPV	NPV
Total WW positives										
Spring 2021	1468	45	6	10	186	9	60.0%	94.9%	47.4%	96.9%
Fall 2021	2629	23	15	6	238	1	6.3%	97.5%	14.3%	94.1%
<i>Study total</i>	—	68	21	16	424	10	32.3%	96.4%	38.5%	95.3%
Subtraction of convalescent shedders										
Spring 2021	1468	45	6	5 ⁺	186	9	60.0%	97.4%	64.3%	96.9%
Fall 2021	2629	23	15	5 ⁺	238	1	6.3%	97.9%	16.7%	94.1%
<i>Study total</i>		68	21	10 ⁺	424	10	32.3%	97.7%	50.0%	95.3%

Abbreviations: FN (false negatives), FP (false positives), TN (true negatives), TP (true positives), PPV (positive predictive value), NPV (negative predictive value). Calculations: sensitivity = $TP/(TP + FN)$; specificity = $TN/(TN + FP)$; PPV = $TP/(TP + FP)$; NPV = $TN/(TN + FN)$. + indicates sample classifications that changed due to subtraction of convalescent shedders.

achieved (B, D and E), which suggests that greater sensitivity could have been achieved with complete building coverage. In the highly vaccinated context of the Fall 2021 campaign, there was 6.3% sensitivity for WW surveillance and 97.5% specificity (Table 4). Only a single WW detection qualified as a TP during this period and only three of the 15 FNs occurred in buildings without full WW coverage, indicating that building coverage was less of a factor driving poor sensitivity in a highly vaccinated population. Other factors that could be contributing to lower sensitivity in the Fall semester include changes in asymptomatic testing requirements to 1× per week, overall lower cases, greater dilution effects from increased building water use at full occupancy, and different virus strains that may reduce shedding of the virus.⁶³

Although WW surveillance may be most useful as a leading indicator of infection, it has also been observed to be a lagging indicator of infection when convalescent shedding occurs in a building. One clinical study estimated that approximately 40% of individuals with mild to moderate disease continued to shed RNA in stool up to 14 days after initial diagnosis.⁹ This suggests that students returning to residence halls from 10-day isolation may still shed SARS-CoV-2 virus into WW. WW surveillance performance was subsequently evaluated to determine whether these lag effects could explain FPs observed in WW results. As a follow-up analysis, the effects of convalescent shedding were considered for students who returned to their residence halls from their isolation off-site. A window between the date of return (approximately, but not always 10 days post-clinical positive) and 14 days after a positive clinical test was used to determine potential influence of asymptomatic convalescent shedding on WW positives (Fig. S3†).

Using these criteria, an additional 4 WW samples from the Spring and 1 WW sample from the Fall that were previously classified as FPs could be explained by convalescent shedding (Fig. S3†). This represents a substantial portion of positive WW samples in this study – 31% of all FP samples and 19% of total WW positivity.

Subtracting the effects of convalescent shedding (removing FPs), an “adjusted” WW surveillance performance can be estimated in the absence of post-infection shedding (Table 4). This adjustment led to a modest increase in the specificity (from 96.4% to 97.7%) and positive predictive value (from 38.5% to 50%).

4 Discussion

This study determined that building-level WW surveillance can be an effective early warning tool during outbreaks with high specificity though comparatively low sensitivity. The performance of WW surveillance in an unvaccinated context of Spring 2021 (Se: 60.0%/Sp: 94.9%) was superior when compared to the highly vaccinated context of Fall 2021 (Se: 6.3%/Sp: 97.5%). The three times per week mandatory asymptomatic and symptomatic testing of the student population in Spring 2021 provided an opportunity in this study to compare high frequency clinical diagnostics with WW surveillance at the building level. The multiple layers of mitigation created a low disease incidence setting so that a single clinical case in a residence hall could be analyzed in a one-to-one, time-dependent manner with a positive WW sample in that same building to understand lead time for WW surveillance. The 1–2 day lead time for outbreak prevention can be utilized for future pandemics dependent on the turnaround time from WW sample collection to result, which has been demonstrated to be as low as 5 hours for processing time.⁶ Prior studies that analyzed WW surveillance at the building-level (Table 1) were mostly conducted in student populations with higher disease incidence and lower frequency of clinical testing than this study, only allowing for correlative analyses between clinical and WW surveillance.

The building-level WW surveillance sensitivity and specificity was found to be 60% and 94.9%, respectively in the Spring 2021, when vaccinations were becoming available in the latter half of the semester. In Fall 2021 when the



student population was almost fully vaccinated (greater than 95%), sensitivity was reduced to 6.3% and specificity remained at a similar level of 97.5%. Combined for both semesters, the overall sensitivity and specificity were 32.3% and 96.4%. These findings of low sensitivity and high specificity for building-level WW surveillance are in line with prior studies that used frequent asymptomatic clinical surveillance as the gold standard approach.^{29,64} Of the other COVID-19 university lead-time analyses (Table 1) Rondeau *et al.*²⁹ was most comparable to this study (using thrice weekly WW sampling and twice weekly asymptomatic testing), though they collected a smaller number of WW samples. Other important conditions that made their findings relevant to this study were the rapid isolation of cases, the same timeframe of Spring 2021, the low incidence of disease with one positive case at a time occurring in a building, and similar use of Ct values instead of concentrations to determine a positive detection. Our study findings aligned closely with no correlation between Ct values and case numbers and similar sensitivity, specificity, PPV values. The low sensitivity of WW surveillance as a leading indicator of the presence of an infected individual suggests that its use at hospitals or airports is less effective than clinical surveillance to ensure that a single case is identified.

When convalescent shedding was considered and potential shedders were eliminated from the analysis, WW detection specificity increased marginally to 97.7% overall, while sensitivity remained unchanged (Table 4). Accounting for convalescent shedding greatly reduced FPs in WW samples and increased PPV from 38.5% to 50.0%. This study combined a high frequency of WW testing (2 times per week) with a high frequency of asymptomatic testing (3 times per week) conducted in concurrent but independent programs to produce building-level diagnostic metrics for WW surveillance. The comparatively lower sensitivity of building-level WW surveillance found in this study implies that there were pre-outbreak cases not detected by WW testing. We found that false negatives (WW-/clinical+) were drivers of reduced sensitivity when there was only one individual from a building testing positive. The largest number of true positives occurred when there were multiple cases diagnosed in a building at the same time, such as in early Spring 2021 when there was a known outbreak event. This implies the lower sensitivity of WW testing when disease incidence is low and is aligned with prior work.²⁹ A second possibility for the lower sensitivity in this study is the comparatively high frequency of asymptomatic clinical testing that identified more individual cases compared to approaches that relied on either clinical testing only after a positive WW sample was detected²⁰ or only symptomatic clinical testing.²⁴ This demonstrates the challenge with relying solely on WW surveillance in congregate living settings to identify a single positive case, prior to an outbreak.

The reduced WW testing sensitivity between the Spring and Fall 2021 semesters was significant and can be attributed

to a variety of possible factors. The near-universal vaccination of the student population (>95% coverage) may have substantially reduced the gastrointestinal shedding of virus among positive students in the surveilled population.²⁰ An increase in residential occupancy in the Fall (Table 2) would have also led to an increase in building water usage that could dilute samples and lower RNA concentrations in WW per infected individual, which may fall below limits of detection of the analytical method employed. The lower frequency (once per week for vaccinated and twice per week for unvaccinated) of clinical testing in Fall 2021 may have also reduced the overall sample size of true positives and increased the ratio of false positives to true positives, leading to reduced WW testing sensitivity.

To understand the effectiveness of WW surveillance as an early warning system, convalescent shedding must be accounted for. Clinical studies have shown that post-infection shedding can occur up to months after initial symptoms,⁹ which suggests that students returning to residence halls from isolation could still shed SARS-CoV-2 virus into WW. Continued shedding post-infection from a resident can be mistaken for a new disease case when a previously positive resident returns to the residence hall setting after isolation. After recovery from respiratory illness and the loss of virus in the respiratory tract, convalescent fecal shedding of SARS-CoV-2 RNA has been shown to persist for an average of seven days.⁶⁵ This is critical to understand when evaluating the lead-time capability of building-level WW surveillance given that nasal swabs could be negative but fecal material could still be positive due to cryptic virus replication in the intestinal tract. The potential bias introduced by convalescent shedding can be seen in this study after adjusting for its potential effects and the PPV of a WW sample increased from 38.5% to 50%. This effect was particularly relevant in the Spring semester, when returning students in buildings G and H in early March might have been responsible for a cluster of three false positive WW samples. The ability for WW surveillance to capture convalescent shedders is critical to understand individuals chronically shedding SARS-CoV-2. WW surveillance can also be used to monitor ongoing evolution and mutation of the virus. PCR combined with virus genome sequencing can provide important information on how the virus is changing during acute as well as chronic infection. Since most individuals testing positive for SARS-CoV-2 no longer seek medical care, the sequence of the viruses causing these cases never enters the sequence database, causing our databases to be skewed to variants associated with more severe disease. WW sequencing can capture the sequence of the variants causing mild disease in relatively healthy populations thus improving surveillance efforts. Chronic shedding of SARS-CoV-2 can also result in continued mutation of the genome with the potential to change virus replication and disease potential. In fact, WW surveillance can identify not only population changes in virus sequences, but also help to trace and identify



individuals who are shedding particularly unique variants.^{12,66}

Based on available data, the circulation of SARS-CoV-2 was relatively low in this study setting. Individuals with clinical positives (asymptomatic and symptomatic) were rapidly isolated (within hours) from buildings undergoing WW surveillance (Fig. 2). The overwhelming majority of samples in this study (94.7%) produced no nucleic acid signal for SARS-CoV-2 which contrasts with many other WW surveillance programs on college campuses during the COVID-19 pandemic. Rather than relying on gene copy number or using correlations of WW concentrations with individual numbers of reported cases, we evaluated the temporal overlap between the occurrence of positive WW and clinical samples to assess the use of WW surveillance as an early warning system.

It is important to acknowledge several limitations to the WW surveillance effort that may have influenced the results of this study. First there was an incomplete mapping of residence hall rooms to sewer cleanouts and buildings B and D could only be confirmed to achieve partial coverage of rooms for WW collection sites. This may have led to missed positive samples (decreased sensitivity), though excluding these buildings from analysis did not substantially change the calculated study sensitivity (data not shown). Potential contributors to unexplained false positives in WW surveillance are non-student individuals such as building staff and contractors, who would be excluded from the clinical testing enumeration (leading to false positives). From a sampling perspective, collecting small sample volumes in discrete intervals (10–20 min) may decrease the likelihood of capturing irregular viral loads flowing through the plumbing. Similarly, filtered sub-samples of highly heterogeneous WW samples with high solid content may not necessarily be fully representative of the larger water sample. Additionally, dilution of viral concentrations in WW resulting from higher water use during higher building occupancy (Fall semester) may have contributed to reduced sensitivity of the WW surveillance protocol.

From this work, we have identified several areas of improvement and future research that may better serve future disease surveillance efforts. For sample collection, the commercially available autosamplers used in this study, used commonly in continuously flowing water bodies (such as WWTPs), were poorly suited for the intermittent and confined spaces of premise plumbing. Improved samplers with more customizable sampling programs that approach continuous flow would be highly beneficial to capture more representative samples. Others have already utilized custom-built continuous samplers to this end.³¹ Additionally, samplers capable of using smaller, less obtrusive sample tubing, strainers, and flow sensors would be more readily retrofitted into existing buildings and reduce clogging, which frequently occurs at smaller-diameter pipes and building cleanouts compared to other sample points on campus WW surveillance programs.⁶⁷ In tandem with microbiological

analysis, concurrent measurement and comparison with chemical indicators such as human metabolites or pharmaceutical products could reveal additional insights on building populations and provide new avenues for data normalization to compensate for sample dilution and inhibition.

5 Conclusions

Building-level WW surveillance as a qualitative detection method of disease presence or absence (otherwise known as an “early warning” system) was shown in this study to be a more effective tool at the start of a pandemic when there were more unvaccinated individuals in the population than in the highly vaccinated context. This study presented a unique opportunity to evaluate WW surveillance within a congregate living, university environment that implemented multiple layers of COVID-19 prevention to ensure low levels of disease transmission. The high frequency of asymptomatic testing in the student population allowed for comparison of WW data to clinically confirmed infections (the gold standard) to produce estimates of sensitivity and specificity. Building-level WW surveillance should be evaluated for other potential pandemic pathogens in the context of close clinical surveillance to explore its effectiveness as a tool at the onset of future pandemics.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts of interest to declare.

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References

- 1 X. Fernandez-Cassi, A. Scheidegger, C. Bänziger, F. Cariti, A. Tuñas Corzon and P. Ganesanandamoorthy, *et al.*, Wastewater monitoring outperforms case numbers as a tool



- to track COVID-19 incidence dynamics when test positivity rates are high, *Water Res.*, 2021, **200**, 117252.
- 2 K. E. Graham, S. K. Loeb, M. K. Wolfe, D. Catoe, N. Sinnott-Armstrong and S. Kim, *et al.*, SARS-CoV-2 RNA in Wastewater Settled Solids Is Associated with COVID-19 Cases in a Large Urban Sewershed, *Environ. Sci. Technol.*, 2021, **55**(1), 488–498.
 - 3 C. Hoar, F. Chauvin, A. Clare, H. McGibbon, E. Castro and S. Patinella, *et al.*, Monitoring SARS-CoV-2 in wastewater during New York City's second wave of COVID-19: sewershed-level trends and relationships to publicly available clinical testing data, *Environ. Sci.: Water Res. Technol.*, 2022, **8**(5), 1021–1035.
 - 4 B. A. Layton, D. Kaya, C. Kelly, K. J. Williamson, D. Alegre and S. M. Bachhuber, *et al.*, Evaluation of a Wastewater-Based Epidemiological Approach to Estimate the Prevalence of SARS-CoV-2 Infections and the Detection of Viral Variants in Disparate Oregon Communities at City and Neighborhood Scales, *Environ. Health Perspect.*, 2022, **130**(6), 67010.
 - 5 J. Peccia, A. Zulli, D. E. Brackney, N. D. Grubaugh, E. H. Kaplan and A. Casanovas-Massana, *et al.*, Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics, *Nat. Biotechnol.*, 2020, **38**(10), 1164–1167.
 - 6 S. Karthikeyan, A. Nguyen, D. McDonald, Y. Zong, N. Ronquillo and J. Ren, *et al.*, Rapid, Large-Scale Wastewater Surveillance and Automated Reporting System Enable Early Detection of Nearly 85% of COVID-19 Cases on a University Campus, *mSystems*, 2021, **6**(4), e0079321.
 - 7 F. Hassard, L. Lundy, A. C. Singer, J. Grimsley and M. Di Cesare, Innovation in wastewater near-source tracking for rapid identification of COVID-19 in schools, *Lancet Microbe*, 2021, **2**(1), e4–e5.
 - 8 K. S. Cheung, I. F. N. Hung, P. P. Y. Chan, K. C. Lung, E. Tso and R. Liu, *et al.*, Gastrointestinal Manifestations of SARS-CoV-2 Infection and Virus Load in Fecal Samples From a Hong Kong Cohort: Systematic Review and Meta-analysis, *Gastroenterology*, 2020, **159**(1), 81–95.
 - 9 A. Natarajan, S. Zlitni, E. F. Brooks, S. E. Vance, A. Dahlen and H. Hedlin, *et al.*, Gastrointestinal symptoms and fecal shedding of SARS-CoV-2 RNA suggest prolonged gastrointestinal infection, *Med*, 2022, **3**(6), 371–387.
 - 10 S. Zheng, J. Fan, F. Yu, B. Feng, B. Lou and Q. Zou, *et al.*, Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January–March 2020: retrospective cohort study, *BMJ*, 2020, **369**, m1443.
 - 11 H. M. Machkovech, A. M. Hahn, J. Garonzik Wang, N. D. Grubaugh, P. J. Halfmann and M. C. Johnson, *et al.*, Persistent SARS-CoV-2 infection: significance and implications, *Lancet Infect. Dis.*, 2024, **4**(7), e453–e462.
 - 12 D. S. Smyth, M. Trujillo, D. A. Gregory, K. Cheung, A. Gao and M. Graham, *et al.*, Tracking cryptic SARS-CoV-2 lineages detected in NYC wastewater, *Nat. Commun.*, 2022, **13**(1), 635.
 - 13 P. J. Arts, J. D. Kelly, C. M. Midgley, K. Anglin, S. Lu and G. R. Abedi, *et al.*, Longitudinal and quantitative fecal shedding dynamics of SARS-CoV-2, pepper mild mottle virus, and crAssphage, *mSphere*, 2023, **8**(4), e0013223.
 - 14 S. W. Olesen, M. Imakaev and C. Duvallet, Making waves: Defining the lead time of wastewater-based epidemiology for COVID-19, *Water Res.*, 2021, **202**, 117433.
 - 15 R. M. Klevens, C. C. Young, S. W. Olesen, A. Osinski, D. Church and J. Muten, *et al.*, Evaluation of wastewater surveillance for SARS-CoV-2 in Massachusetts correctional facilities, 2020–2022, *Front. Water*, 2023, **5**, 1083316.
 - 16 L. Davo, R. Segui, P. Botija, M. J. Beltran, E. Albert and I. Torres, *et al.*, Early detection of SARS-CoV-2 infection cases or outbreaks at nursing homes by targeted wastewater tracking, *Clin. Microbiol. Infect.*, 2021, **27**(7), 1061–1063.
 - 17 W. Ahmed, A. Bivins, S. L. Simpson, P. M. Bertsch, J. Ehret and I. Hosegood, *et al.*, Wastewater surveillance demonstrates high predictive value for COVID-19 infection on board repatriation flights to Australia, *Environ. Int.*, 2022, **158**, 106938.
 - 18 G. J. Hall, E. J. Page, M. Rhee, C. Hay, A. Krause and E. Langenbacher, *et al.*, Wastewater Surveillance of US Coast Guard Installations and Seagoing Military Vessels to Mitigate the Risk of COVID-19 Outbreaks, March 2021–August 2022, *Public Health Rep.*, 2024, 333549241236644.
 - 19 M. Kitajima, M. Murakami, R. Iwamoto, H. Katayama and S. Imoto, COVID-19 wastewater surveillance implemented in the Tokyo 2020 Olympic and Paralympic Village, *J. Travel Med.*, 2022, taac004.
 - 20 W. Q. Betancourt, B. W. Schmitz, G. K. Innes, S. M. Prasek, K. M. Pogreba Brown and E. R. Stark, *et al.*, COVID-19 containment on a college campus via wastewater-based epidemiology, targeted clinical testing and an intervention, *Sci. Total Environ.*, 2021, **779**, 146408.
 - 21 L. M. Colosi, K. E. Barry, S. M. Kotay, M. D. Porter, M. D. Poulter and C. Ratliff, *et al.*, Development of Wastewater Pooled Surveillance of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) from Congregate Living Settings, *Appl. Environ. Microbiol.*, 2021, **87**(13), e0043321.
 - 22 S. M. Kotay, K. O. Tanabe, L. M. Colosi, M. D. Poulter, K. E. Barry and C. P. Holstege, *et al.*, Building-Level Wastewater Surveillance for SARS-CoV-2 in Occupied University Dormitories as an Outbreak Forecasting Tool: One Year Case Study, *ACS ES&T Water*, 2022, **2**(11), 2094–2104.
 - 23 C. Gibas, K. Lambirth, N. Mittal, M. A. I. Juel, V. B. Barua and L. Roppolo Brazell, *et al.*, Implementing building-level SARS-CoV-2 wastewater surveillance on a university campus, *Sci. Total Environ.*, 2021, **782**, 146749.
 - 24 A. Godinez, D. Hill, B. Dandaraw, H. Green, P. Kilaru and F. Middleton, *et al.*, High Sensitivity and Specificity of Dormitory-Level Wastewater Surveillance for COVID-19 during Fall Semester 2020 at Syracuse University, New York, *Int. J. Environ. Res. Public Health*, 2022, **19**(8), 4851.
 - 25 H. M. Solo-Gabriele, S. Kumar, S. Abelson, J. Penso, J. Contreras and K. M. Babler, *et al.*, Predicting COVID-19 cases using SARS-CoV-2 RNA in air, surface swab and wastewater samples, *Sci. Total Environ.*, 2023, **857**, 159188.
 - 26 N. Mangwana, E. Archer, C. J. F. Muller, W. Preiser, G. Wolfaardt and B. Kasprzyk-Hordern, *et al.*, Sewage surveillance of SARS-CoV-2 at student campus residences in



- the Western Cape, South Africa, *Sci. Total Environ.*, 2022, **851**, 158028.
- 27 C. M. Welling, D. R. Singleton, S. B. Haase, C. H. Browning, B. R. Stoner and C. K. Gunsch, *et al.*, Predictive values of time-dense SARS-CoV-2 wastewater analysis in university campus buildings, *Sci. Total Environ.*, 2022, **835**, 155401.
 - 28 M. Landstrom, E. Braun, E. Larson, M. Miller and G. H. Holm, Efficacy of SARS-CoV-2 wastewater surveillance for detection of COVID-19 at a residential private college, *FEMS Microbes*, 2022, **3**, xtac008.
 - 29 N. C. Rondeau, O. J. Rose, E. R. Alt, L. A. Ariyan, A. B. Elikan and J. L. Everard, *et al.*, Building-Level Detection Threshold of SARS-CoV-2 in Wastewater, *Microbiol. Spectrum*, 2023, **11**(2), e0292922.
 - 30 W. Johnson, K. Reeves, J. Liebig, A. Feula, C. Butler and M. Alkire, *et al.*, Effectiveness of building-level sewage surveillance during both community-spread and sporadic-infection phases of SARS-CoV-2 in a university campus population, *FEMS Microbes*, 2022, **3**, xtac024.
 - 31 K. Reeves, J. Liebig, A. Feula, T. Saldi, E. Lasda and W. Johnson, *et al.*, High-resolution within-sewer SARS-CoV-2 surveillance facilitates informed intervention, *Water Res.*, 2021, **204**, 117613.
 - 32 L. C. Scott, A. Aubee, L. Babahaji, K. Vigil, S. Tims and T. G. Aw, Targeted wastewater surveillance of SARS-CoV-2 on a university campus for COVID-19 outbreak detection and mitigation, *Environ. Res.*, 2021, **200**, 111374.
 - 33 N. L. Fahrenfeld, W. R. Morales Medina, S. D'Elia, M. Modica, A. Ruiz and M. McLane, Comparison of residential dormitory COVID-19 monitoring via weekly saliva testing and sewage monitoring, *Sci. Total Environ.*, 2022, **814**, 151947.
 - 34 Y. Wang, P. Liu, H. Zhang, M. Ibaraki, J. VanTassell and K. Geith, *et al.*, Early warning of a COVID-19 surge on a university campus based on wastewater surveillance for SARS-CoV-2 at residence halls, *Sci. Total Environ.*, 2022, **821**, 153291.
 - 35 B. L. Anderson-Coughlin, A. E. H. Shearer, A. N. Omar, P. K. Litt, E. Bernberg and M. Murphy, *et al.*, Coordination of SARS-CoV-2 wastewater and clinical testing of university students demonstrates the importance of sampling duration and collection time, *Sci. Total Environ.*, 2022, 154619.
 - 36 W. Zambrana, D. Catoe, M. M. Coffman, M. Kim, A. Anand, D. Solis, M. K. Sahoo, B. A. Pinsky, A. S. Bhatt, A. B. Boehm and M. K. Wolfe, SARS-CoV-2 RNA and N Antigen Quantification via Wastewater at the Campus Level, Building Cluster Level, and Individual-Building Level, *ACS ES&T Water*, 2022, **2**(11), 2025–2033.
 - 37 A. L. Rainey, K. Buschang, A. O'Connor, D. Love, A. M. Wormington and R. L. Messcher, *et al.*, Retrospective Analysis of Wastewater-Based Epidemiology of SARS-CoV-2 in Residences on a Large College Campus: Relationships between Wastewater Outcomes and COVID-19 Cases across Two Semesters with Different COVID-19 Mitigation Policies, *ACS ES&T Water*, 2023, **3**(1), 16–29.
 - 38 K. T. Ash, Y. Li, I. Alamilla, D. C. Joyner, D. E. Williams, P. J. McKay, B. M. Green, C. Iler, S. E. DeBlander, C. M. North, F. Kara-Murdoch, C. M. Swift and T. C. Hazen, SARS-CoV-2 raw wastewater surveillance from student residences on an urban university campus, *Front. Microbiol.*, 2023, **14**, 1101205.
 - 39 M. E. Sharkey, N. Kumar, A. M. A. Mantero, K. M. Babler, M. M. Boone and Y. Cardentey, *et al.*, Lessons learned from SARS-CoV-2 measurements in wastewater, *Sci. Total Environ.*, 2021, **798**, 149177.
 - 40 A. Amirali, K. M. Babler, M. E. Sharkey, C. C. Beaver, M. M. Boone and S. Comerford, *et al.*, Wastewater based surveillance can be used to reduce clinical testing intensity on a university campus, *Sci. Total Environ.*, 2024, **918**, 170452.
 - 41 A. Bivins and K. Bibby, Wastewater Surveillance during Mass COVID-19 Vaccination on a College Campus, *Environ. Sci. Technol. Lett.*, 2021, **8**(9), 792–798.
 - 42 J. Lee, N. Acosta, B. J. Waddell, K. Du, K. Xiang and J. Van Doorn, *et al.*, Campus node-based wastewater surveillance enables COVID-19 case localization and confirms lower SARS-CoV-2 burden relative to the surrounding community, *Water Res.*, 2023, **244**, 120469.
 - 43 A. Cohen, A. Maile-Moskowitz, C. Grubb, R. A. Gonzalez, A. Ceci and A. Darling, *et al.*, Subsewershed SARS-CoV-2 Wastewater Surveillance and COVID-19 Epidemiology Using Building-Specific Occupancy and Case Data, *ACS ES&T Water*, 2022, **2**(11), 2047–2059.
 - 44 E. Lu, Y. Ai, A. Davis, J. Straathof, K. Halloran and N. Hull, *et al.*, Wastewater surveillance of SARS-CoV-2 in dormitories as a part of comprehensive university campus COVID-19 monitoring, *Environ. Res.*, 2022, **212**(Pt E), 113580.
 - 45 J. Kazenelson, T. Jefferson, R. G. Rhodes, L. B. Cahoon and A. R. Frampton, Detection of SARS-CoV-2 RNA in wastewater from an enclosed college campus serves as an early warning surveillance system, *PLoS One*, 2023, **18**(7), e0288808.
 - 46 S. C. Sellers, E. Gosnell, D. Bryant, S. Belmonte, S. Self and M. S. McCarter, *et al.*, Building-level wastewater surveillance of SARS-CoV-2 is associated with transmission and variant trends in a university setting, *Environ. Res.*, 2022, **215**, 114277.
 - 47 B. R. Haskell, H. A. Dhiyebi, N. Srikanthan, L. M. Bragg, W. J. Parker and J. P. Giesy, *et al.*, Implementing an adaptive, two-tiered SARS-CoV-2 wastewater surveillance program on a university campus using passive sampling, *Sci. Total Environ.*, 2024, **912**, 168998.
 - 48 Y. M. Brooks, B. Gryskwicz, S. Sheehan, S. Piers, P. Mahale and S. McNeil, *et al.*, Detection of SARS-CoV-2 in Wastewater at Residential College, Maine, USA, August–November 2020, *Emerging Infect. Dis.*, 2021, **27**(12), 3111–3114.
 - 49 L. C. Bitter, R. Kibbee, G. C. Jiménez and B. Örmeci, Wastewater Surveillance of SARS-CoV-2 at a Canadian University Campus and the Impact of Wastewater Characteristics on Viral RNA Detection, *ACS ES&T Water*, 2022, **2**(11), 2034–2046.
 - 50 R. Corchis-Scott, Q. Geng, A. M. Al Riahi, A. Labak, A. Podadera and K. K. S. Ng, *et al.*, Actionable wastewater surveillance: application to a university residence hall during the transition between Delta and Omicron



- resurgences of COVID-19, *Front. Public Health*, 2023, **11**, 1139423.
- 51 S. Kim and A. B. Boehm, Wastewater monitoring of SARS-CoV-2 RNA at K-12 schools: comparison to pooled clinical testing data, *PeerJ*, 2023, **11**, e15079.
 - 52 K. Bibby, A. Bivins, Z. Wu and D. North, Making waves: Plausible lead time for wastewater based epidemiology as an early warning system for COVID-19, *Water Res.*, 2021, **202**, 117438.
 - 53 P. M. D'Aoust, T. E. Graber, E. Mercier, D. Montpetit, I. Alexandrov and N. Neault, *et al.*, Catching a resurgence: Increase in SARS-CoV-2 viral RNA identified in wastewater 48 h before COVID-19 clinical tests and 96 h before hospitalizations, *Sci. Total Environ.*, 2021, **770**, 145319.
 - 54 R. Ke, P. P. Martinez, R. L. Smith, L. L. Gibson, A. Mirza and M. Conte, *et al.*, Daily longitudinal sampling of SARS-CoV-2 infection reveals substantial heterogeneity in infectiousness, *Nat. Microbiol.*, 2022, **7**(5), 640–652.
 - 55 Y. Ye, R. M. Ellenberg, K. E. Graham and K. R. Wigginton, Survivability, Partitioning, and Recovery of Enveloped Viruses in Untreated Municipal Wastewater, *Environ. Sci. Technol.*, 2016, **50**(10), 5077–5085.
 - 56 R. Gonzalez, K. Curtis, A. Bivins, K. Bibby, M. H. Weir and K. Yetka, *et al.*, COVID-19 surveillance in Southeastern Virginia using wastewater-based epidemiology, *Water Res.*, 2020, **186**, 116296.
 - 57 S. L. Klein, A. Pekosz, H. S. Park, R. L. Ursin, J. R. Shapiro and S. E. Benner, *et al.*, Sex, age, and hospitalization drive antibody responses in a COVID-19 convalescent plasma donor population, *J. Clin. Invest.*, 2020, **130**(11), 6141–6150.
 - 58 K. E. Klymus, C. M. Merkes, M. J. Allison, C. S. Goldberg, C. C. Helbing and M. E. Hunter, *et al.*, Reporting the limits of detection and quantification for environmental DNA assays, *Environ. DNA*, 2020, **2**(3), 271–282.
 - 59 B. W. Schmitz, G. K. Innes, S. M. Prasek, W. Q. Betancourt, E. R. Stark and A. R. Foster, *et al.*, Enumerating asymptomatic COVID-19 cases and estimating SARS-CoV-2 fecal shedding rates via wastewater-based epidemiology, *Sci. Total Environ.*, 2021, **801**, 149794.
 - 60 A. J. McAdam, Discrepant analysis: how can we test a test?, *J. Clin. Microbiol.*, 2000, **38**(6), 2027–2029.
 - 61 S. Feng, A. Roguet, J. S. McClary-Gutierrez, R. J. Newton, N. Kloczko and J. G. Meiman, *et al.*, Evaluation of Sampling, Analysis, and Normalization Methods for SARS-CoV-2 Concentrations in Wastewater to Assess COVID-19 Burdens in Wisconsin Communities, *ACS ES&T Water*, 2021, **1**(8), 1955–1965.
 - 62 R. S. Kantor, K. L. Nelson, H. D. Greenwald and L. C. Kennedy, Challenges in Measuring the Recovery of SARS-CoV-2 from Wastewater, *Environ. Sci. Technol.*, 2021, **55**(6), 3514–3519.
 - 63 M. Garcia-Knight, K. Anglin, M. Tassetto, S. Lu, A. Zhang and S. A. Goldberg, *et al.*, Infectious viral shedding of SARS-CoV-2 Delta following vaccination: A longitudinal cohort study, *PLoS Pathog.*, 2022, **18**(9), e1010802.
 - 64 J. W. Keck, J. Lindner, M. Liversedge, B. Mijatovic, C. Olsson and W. Strike, *et al.*, Wastewater Surveillance for SARS-CoV-2 at Long-Term Care Facilities: Mixed Methods Evaluation, *JMIR Public Health Surveill.*, 2023, **9**, e44657.
 - 65 Y. Zhang, M. Cen, M. Hu, L. Du, W. Hu and J. J. Kim, *et al.*, Prevalence and Persistent Shedding of Fecal SARS-CoV-2 RNA in Patients With COVID-19 Infection: A Systematic Review and Meta-analysis, *Clin. Transl. Gastroenterol.*, 2021, **12**(4), e00343.
 - 66 M. M. Shafer, M. J. Bobholz, W. C. Vuyk, D. A. Gregory, A. Roguet and L. A. Haddock Soto, *et al.*, Tracing the origin of SARS-CoV-2 omicron-like spike sequences detected in an urban sewershed: a targeted, longitudinal surveillance study of a cryptic wastewater lineage, *Lancet Microbe*, 2024, **5**(4), e335–e344.
 - 67 S. Harris-Lovett, K. L. Nelson, P. Beamer, H. N. Bischel, A. Bivins and A. Bruder, *et al.*, Wastewater Surveillance for SARS-CoV-2 on College Campuses: Initial Efforts, Lessons Learned, and Research Needs, *Int. J. Environ. Res. Public Health*, 2021, **18**(9), 4455.

