Journal of Water & Health

Journal of Water and Health Vol 22 No 5, 811 doi: 10.2166/wh.2024.293

SARS-CoV-2 wastewater surveillance at two university campuses: lessons learned and insights on intervention strategies for public health guidance

Alexis M. Porter [a,*], John J. Hart[a,b], Richard R. Rediske[a] and David C. Szlag[b]

[a] Robert B. Annis Water Resources Institute, 740 West Shoreline Dr, Muskegon, MI 49441, USA
[b] Department of Chemistry, Oakland University, 146 Library Dr, Rochester, MI 48309, USA
*Corresponding author. E-mail: porteal@gvsu.edu

AMP, 0000-0002-1937-2803

ABSTRACT

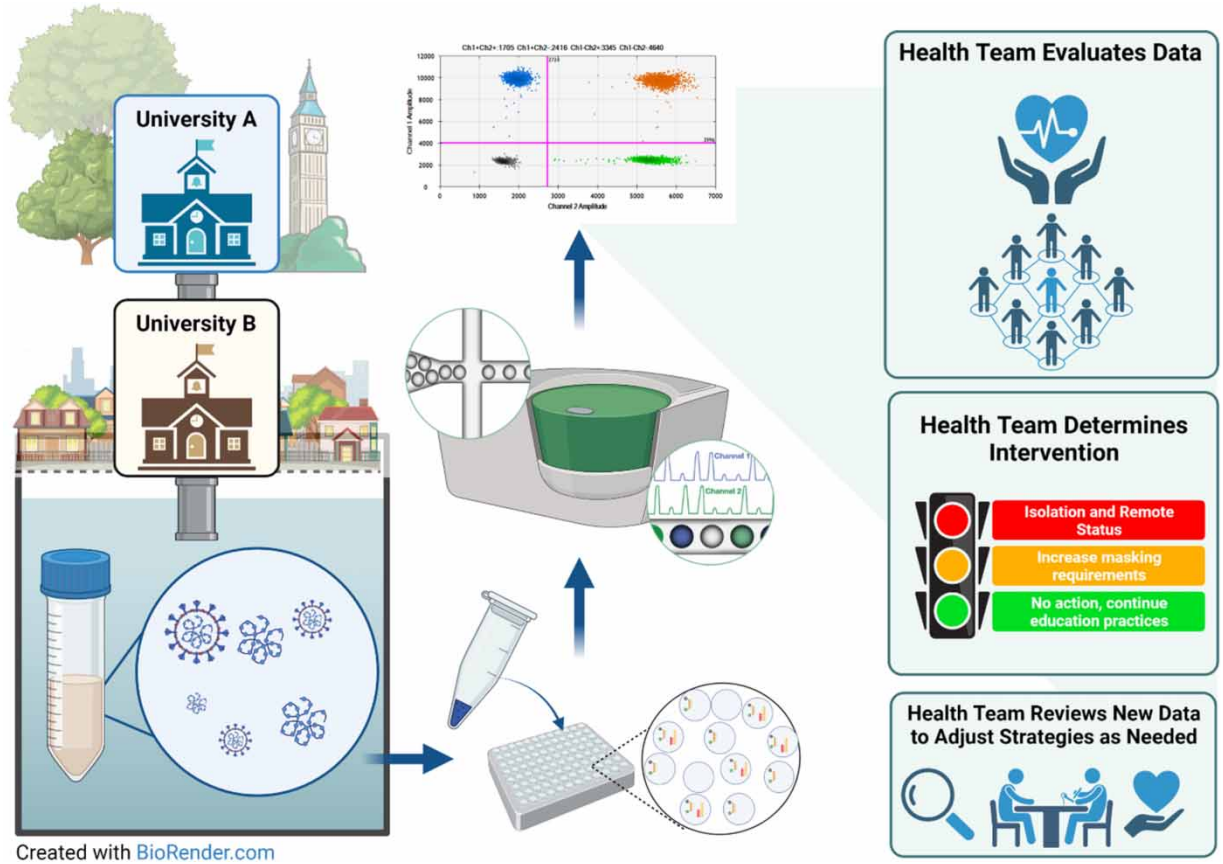
Wastewater surveillance has been a tool for public health officials throughout the COVID-19 pandemic. Universities established pandemic response committees to facilitate safe learning for students, faculty, and staff. These committees met to analyze both wastewater and clinical data to propose mitigation strategies to limit the spread of COVID-19. This paper reviews the initial efforts of utilizing campus data inclusive of wastewater surveillance for SARS-CoV-2 RNA concentrations, clinical case data from university response teams, and mitigation strategies from Grand Valley State University in West Michigan (population 21,648 students) and Oakland University in East Michigan (population 18,552 students) from November 2020 to April 2022. Wastewater positivity rates for both universities ranged from 32.8 to 46.8%. Peak viral signals for both universities directly corresponded to variant points of entry within the campus populations from 2021 to 2022. It was found that the organization of clinical case data and variability of wastewater testing data were large barriers for both universities to effectively understand disease dynamics within the university population. We review the initial efforts of onboarding wastewater surveillance and provide direction for structuring ongoing surveillance workflows and future epidemic response strategies based on those that led to reduced viral signals in campus wastewater.

Key words: COVID-19, ddPCR, epidemiology, intervention, university, wastewater

HIGHLIGHTS

- Viral RNA levels in wastewater tracked the emergence of variants in student populations.
- Intervention strategies suggested reduced numbers in wastewater viral RNA signals prior to variant emergence.
- Public health promotion and educational tools are needed to take complex biological processing to actionable intervention strategies.

GRAPHICAL ABSTRACT



INTRODUCTION

In December 2019, cases of an acute respiratory disease, COVID-19, were determined to be caused by a novel coronavirus named 'Severe Acute Respiratory Syndrome Coronavirus 2' (SARS-CoV-2). SARS-CoV-2 is an enveloped RNA virus that spreads primarily through respiratory droplets (Kim *et al.* 2020; Medema *et al.* 2020). It has been noted that regardless of symptoms displayed, people infected with SARS-CoV-2 shed genetic material in feces and other bodily fluids allowing for wastewater surveillance to be an effective surveillance method for disease patterns and dynamics (Bivins & Bibby 2021; Maryam *et al.* 2023). Persons infected will typically show symptoms within 2–14 days; however, approximately one-third of patients infected with SARS-CoV-2 are estimated to be asymptomatic, complicating the understanding of the spread of the virus (Oran & Topol 2021; Schmitz *et al.* 2021). Asymptomatic patients may not seek medical assistance or participate in clinical testing due to being unaware of illness onset (Espinoza *et al.* 2021; Tay *et al.* 2022). With the onset of at-home testing, positive tests are not regularly reported to health officials or reported at all. This can make gauging public disease spread difficult, making wastewater analysis beneficial for its independence on an individual's health-seeking behavior (Kapoor *et al.* 2022; Hopkins *et al.* 2023). One of the populations that revealed heightened vulnerability and increased presence of asymptomatic disease includes college campus students, staff, and faculty (Lu *et al.* 2022). This population exists within controlled, close quarters and typically sees large gatherings that present a point of entry for disease (Jain *et al.* 2022).

Since SARS-CoV-2 is shed in feces (Jones *et al.* 2020; Wölfel *et al.* 2020), wastewater-based surveillance (WBS) is a tool to guide community decision-making (Wu *et al.* 2021; Wolfe 2022). Due to the onset of the COVID-19 pandemic, it has been used as a tool to support COVID-19 public health decision-making by local, state, and federal institutions (Ahmed *et al.* 2022). Wastewater surveillance results can guide resource allocation and public health decisions at the community level, thus demonstrating effective opportunities for surveillance at building levels throughout college campuses (Harris-Lovett *et al.*

2021; Sharara *et al.* 2021; Lu *et al.* 2022). As this surveillance has been developed, studies suggest wastewater surveillance may be used to address trends in disease that may not be readily available in clinical testing (McMahan *et al.* 2021; Prado *et al.* 2021; Zhu *et al.* 2021).

SARS-CoV-2 RNA has reportedly been noted with increased stability in molecular evaluation compared to infective SARS-CoV-2 (Fukuta *et al.* 2021), with RNA concentrations remaining stable for up to 84 days under laboratory storage conditions and lasting over 14 days *in situ* (Hokajärvi *et al.* 2021; Hart *et al.* 2023). SARS-CoV-2 RNA concentrations were shown to follow infection outbreaks with a rapid rise, plateau, and decline (Ahmed *et al.* 2022). Moreover, dormitory surveillance has been used to test WBS's efficacy and aid prevention strategies in universities, as seen through this study and the State of Michigan's Pilot Project study (Betancourt *et al.* 2021; Corchis-Scott *et al.* 2021; Gibas *et al.* 2021; Sharaby *et al.* 2023). Universities provide an opportunity for WBS studies as sampling occurs in an isolated sewer catchment with controlled populations, so evaluating mitigation strategies and interventions is available (Corchis-Scott *et al.* 2021; Jain *et al.* 2022). From this, WBS was used to guide clinical testing and successfully isolate clinically positive students (Betancourt *et al.* 2021).

To understand how campus public health strategies can utilize WBS, we explored the wastewater surveillance data for SARS-CoV-2 RNA, clinical case data from university response teams, and mitigation strategies from two universities within the State of Michigan, United States. These universities included Grand Valley State University (GVSU) in West Michigan and Oakland University (OU) in East Michigan (Figure S-1). Throughout the academic year of 2020–2022, GVSU saw 21,648 enrolled undergraduate students. The main campus is in a primarily agricultural community within Ottawa County, Michigan. OU is located in a metropolitan community in the Detroit, Michigan area. Throughout the academic year of 2020–2022, OU enrolled 18,552 students. Due to the variations in community dynamics, exposure potential, spatial distribution, and intervention strategies, our goal was to derive lessons learned from the application of wastewater surveillance along with an initial assessment on how this surveillance may supplement clinical case data at two different locations in Michigan. Using the same state standardized methodology for evaluating SARS-CoV-2 RNA in wastewater, results illuminate differences in clinical case data organization and university public health response under this guidance. We highlighted effective mitigation strategies universities can adopt for future epidemics and public health responses and decision-making that ensure student, staff, and faculty safety and well-being.

This study was conducted as part of both the COVID-19 Wastewater Surveillance Pilot Project and, after 2020, the State of Michigan SARS-CoV-2 Epidemiology – Wastewater Evaluation and Reporting (SEWER) Network directed by the Michigan Departments of Health & Human Services (MDHHS) and Environment, Great Lakes, and Energy (EGLE) Partnerships under these projects included other universities, laboratories, health departments, and wastewater utilities. Upon completion of the Pilot Project at the end of 2020, SARS-CoV-2 wastewater surveillance was continued by individual university funding from January 2021 to June 2021, then by the SEWER Project by each university as a goal to seek guidance for outbreaks and disease dynamics within each campus.

METHODS

Sampling

Sites at OU were selected at the building level to gain an accurate representation of the on-campus student population. In 2020–2021, six sample sites were chosen. Five of these sites were the direct output sanitary sewer of individual or combined housing buildings. Due to the locations of the sewers, one site was connected to the main line that received output from dorm buildings, classrooms, and the health center. To account for this, a sixth site was added 'upstream' as a deduct site that collected sewage from the health center and classroom buildings. Sampling for the 2021–2022 school year added two additional sites in common areas to better cover the campus. Here, bi-weekly sampling alludes to sampling conducted twice per week. Bi-weekly sampling occurred at all sample sites during the school year, excluding official university recesses. 24-h composite sampling was collected using ISCO portable samplers. A total of 10 L was collected over the composite sample period. A subsample of 500 mL was collected and transported to the laboratory for further analysis. Sites at GVSU were also selected at the building level to quantify the presence of SARS-CoV-2 on campus. Samples were 500 mL grab samples from eight building effluents. Samples were stored on ice at ~4 °C during sampling events and for transit to the laboratory for further analysis.

Sample preparation

Samples were concentrated and analyzed for the detection and quantification of SARS-CoV-2 using droplet digital polymerase chain reaction (ddPCR) using the standard operating procedure (SOP) provided by Michigan State University (Flood *et al.*

2021) for the MiNET network with a few modifications. To summarize, samples at OU were inverted 10–20 times to ensure homogeneity and 45 mL was added to a 50 mL conical tube. The sample was concentrated via polyethylene glycol (PEG) precipitation (Borchardt *et al.* 2017; Flood *et al.* 2021). Each sample was mixed with 8% (w/vol) molecular biology grade PEG 8000 (Promega Corporation, Madison, WI) and 0.2 M NaCl (w/v). Samples were agitated for 2 h at 4 °C and then centrifuged at 4 °C. Centrifuge parameters were 1 h at 3650 rpm for OU and 4,700 × *g* for 45 min for UA. The supernatant was then removed using a serological pipet, and the final volume of the concentrated pellet was recorded.

Both studies ran a negative processing control (NEG) of deionized water carried through precipitation. In addition to these controls, an RNA extraction blank was prepared (EXT) and analyzed. All samples and controls were then concentrated through QIAamp® Viral RNA mini kit (Qiagen) per manufacturer's instructions and spiked with Phi6 RNA at 10^5 PFU/mL to assess inhibition. Both studies used 200 μL of pellet concentrate for RNA extraction, and a final elution volume of 80 μL was collected. Samples at OU were stored at 4°C and analyzed within 5 h of RNA extraction. GVSU samples were stored at −80°C for ddPCR analysis the following day.

ddPCR analysis

In both surveillance efforts, samples, and quality controls (QC) consisting of a positive, NEG, no-treatment control (NTC), and (EXT) were analyzed in triplicate. RNA viral targets were quantified using Bio-Rad's 1-Step RT-ddPCR Advanced kit with a QX200 ddPCR system (Bio-Rad, CA, USA). The selected SARS-CoV-2 targets were N1, N2, E, and Phi6 for sample inhibition (Gendron *et al.* 2010; CDC 2020; Corman *et al.* 2020). Sequences are provided in Table S-1. Each reaction contained 16.5 μL of an assay mix composed of 5.5 μL of Supermix, 2.2 μL of reverse transcriptase, 1.1 μL of Dithiothreitol (DTT), 900 nm of each primer, 250 nm of each probe, and polymerase chain reaction (PCR) grade water. 5.5 μL of each sample or control was added for a final reaction volume of 22 μL. Droplet generation was performed using a Bio-Rad Automated Droplet Generator System, creating an oil water emulsion of 40 μL. Amplification was performed using a Bio-Rad C1000 Touch™ Thermal Cycler (Bio-Rad Laboratories) under the following conditions: 25 °C for 3 min, 50 °C for 1 h, 95 °C for 10 min, followed by 40 cycles of 95 °C for 30 s and the RNA targets annealing temperature for 1 min with a ramp rate of 2 °C/s followed by a final cycle of 98 °C for 10 min. After thermocycling, the plate is transferred to a QX200 Droplet Reader (Bio-Rad, CA, USA) to quantify RNA concentration.

SARS-CoV-2 variant analysis

During variant emergence in both populations, GVSU and OU utilized the same methodology for testing spike protein mutations on SARS-CoV-2 RNA from wastewater. Both universities utilized GT-Molecular ddPCR SARS-CoV-2 Variant Detection Assay Kits for Bio-Rad QX200 Software. Exact discriminatory kits varied based on circulating variant of concern (VOC) at the time determined based on World Health Organization guidance. During this study period, variant kits from GT-Molecular were utilized for the Alpha, Delta, Epsilon, Omicron, Omicron BA.1, and Omicron BA.2. Table S-2 describes the target spike mutations from each kit for the VOC. Samples that contained the target mutation(s) on the spike protein were identified as containing the VOC. As mutations increased during the Delta and Omicron waves and onward, target mutations became less specific to a particular variant, and suggested presence was noted. Both universities screened samples for variant analysis based on disease severity linked to N1 and N2 gene concentrations when sample concentrations were ≥10,000 gene copies (GC)/100 mL. Samples that met the gene concentration criteria were analyzed on a weekly basis.

SARS-CoV-2 method analysis and quality control

Per state-guided quality control measures, samples with less than 10,000 accepted droplets were excluded from further analysis. Thresholds were set for all wells at approximately 500 florescent units above the negative control cloud. Each sample required a minimum of three positive droplets above the target threshold to be considered positive for an RNA target. Results were calculated as gene copies per 100 mL (GC/100 mL) using Equation S-1. The mean of the triplicate analysis for each sample was reported to stakeholders as wastewater-based insights through either the participating laboratory or associated dashboard provided by a third-party service. All results were sent to the respective university stakeholders within 72 h of sample collection.

SARS-CoV-2 university response teams

Upon the onset of the pandemic, both universities created COVID response teams in the fall of 2020 to guide university-wide intervention strategies on how to ensure the well-being of students and staff and mitigate the impact SARS-CoV-2 had on

respective institutions. OU's response included the creation of a COVID-19 Response Team. This group was composed of the surveillance laboratory's principal investigator, members of university health, members of the administration in campus housing, campus safety, the provost and deans' office, and members of the third-party dashboard service. This group met twice per week as results were available to analyze the stakeholder dashboard's clinical and wastewater insights and discuss the implementation of intervention strategies. GVSU's response was the creation of the virus action team (VAT). This group was composed of administrators and faculty from the provost's office, the school of biomedical sciences, the school of public health, campus health, campus safety, campus housing, and students from the Master of Public Health program. Like OU, these members were staffed positions working on reviewing both the wastewater and clinical insights to mitigate the impact that the SARS-CoV-2 pandemic had at GVSU. In both cases, the respective virus response teams discussed and decided on appropriate mitigation strategies to be implemented on their campuses based on both wastewater surveillance and clinical insights.

Mitigation strategies

Implementation of mitigation strategies at both universities has evolved throughout the progression of the COVID-19 pandemic. Despite this evolution, several mitigation strategies persisted through the total sampling period. At the onset of the pandemic, both institutions started with socially distanced learning to complete the winter 2020 school year, limiting university access to approved researchers. In addition, both universities implemented a social distancing and mask mandate to minimize the risk of spread at their institutions. Before the start of the Fall 2020 school year, both universities mandated that all university housing students be tested and display a negative COVID-19 test before being allowed to move into housing for the year. This mandated testing was also repeated after any long holiday recess from the university. Housing students at both institutions were also required to have an emergency exit plan (EEP) on file with campus health and housing if they tested positive for SARS-CoV-2. OU's primary EEP strategy mandated that students travel home to isolate upon a positive test and not return until a set of criteria is met. Students that could not isolate at home due to distance from the school or other circumstances were given on-campus accommodations. GVSU's primary EEP strategy mandated that students isolate on campus in designated on-campus accommodations until a set of criteria were met supporting student safety.

Both universities required that students, staff, faculty, and guests fill out daily health screeners to intercept suspected positive students before attending in-person classes. University members that did not pass their health screener were instructed to isolate for 24 h and retake the screener the following day. They were also encouraged to go to university health or a third-party provider for testing and further action if they believed their screener was incorrect. In addition, all university members were also encouraged to get tested if they felt unwell from university health or a third-party test provider. In addition to the screener, 1,500 students, staff, and faculty were randomly selected for mandatory testing each day.

Upon the result of a positive test, isolation, and contact tracing occurred. Students living on campus were required to follow their EEP that was on file and could not return to in-person learning or campus housing until a negative COVID-19 test result. Students, staff, and faculty were required to isolate off-campus until similar conditions were met. To maintain transparency OU kept a dashboard displaying the number and breakdown of positive individuals by student, staff, faculty, and guest, the total percentage of students isolating on campus, the percentage of vaccinated campus members, the percentage of vaccine-exempt campus members, and insights on positive cases reported in the surrounding counties. GVSU held all the previously stated information as well as results from their random testing, the breakdown of the nature of vaccine exemptions, positivity rates in local healthcare systems and state regions, and vaccination statistics in the West Michigan region. In addition to the publicly available information, both response teams received a set of data from the campus health department on reported positive cases containing both quantitative variables such as date tested, spike tracking post-holiday events, current isolation status, date of first symptoms, and current vaccination status as well as qualitative variables such as a list of symptoms.

COVID-19 clinical case data

COVID-19 clinical case data were retrieved through open-source data from each university. Both university response teams were responsible for uploading clinical case data to publicly available dashboards (Data Dashboard-Lakers Together-Grand Valley State University 2022). OU had a public dashboard available from the Fall of 2020 through the Winter of 2022, but has since been archived and unavailable for public viewing. Clinical case data were broken down into variations in reporting status by the universities. Cases were reported as confirmed or probable, where probable cases indicate symptom onset but no clinical test confirmation on disease status. Cases considered 'confirmed' were those that indicated a positive

COVID-19 test through either a rapid test and/or a PCR test. Data were organized into two separate temporal indicators. Dates were noted as symptom onset date and reporting date. Reporting dates were utilized in dashboard metrics, as these were the dates of a confirmed case. For all analyses conducted, clinically confirmed COVID-19 cases were used, and dates of clinical results followed the reporting date, as this was the date that would initiate public health response.

Data analysis

University clinical cases were retrieved from each university response team and verified through university COVID-19 dashboards. The daily average of wastewater viral concentrations was calculated from average N1 and N2 gene target concentrations (GC/100 mL) per day using Microsoft Excel V 2102. All statistical calculations were conducted utilizing 'tidyverse' and 'vtable' packages in R (v4.2.2; R Core Team 2022). Data visualizations were created using Tableau 2022.3. All maps and geospatial analyses were conducted using ArcGIS 10.8.2.

RESULTS

Positivity rates

Samples were analyzed from 2 November 2020 to 28 April 2022, covering two academic years. The GVSU campus had a wastewater positivity rate of 32.85% ($n = 554$). Campus population during these years fluctuated between 20,265 and 21,648 students, with a small population increase, not including faculty and staff. Due to GVSU's randomized testing that included testing the total population on campus in a randomly selected lottery, multiple faculty, staff, and students may be tested more than once over the time frame of the study, and members of the campus community may also test multiple times as symptoms persist. With this, total campus positive tests summed 132,392, with a 2.12% positivity rate over the 2020–2022 academic years. The OU campus saw a 46.83% positivity rate in wastewater samples ($n = 679$) with 1,477 positive clinical tests during the 2020–2022 academic years. The OU campus population ranged from 18,552 to 16,108 students, showing a decline in the campus population, not including faculty and staff. Due to the total tests taken not being recorded by the OU COVID-19 Response Team, no positivity rate could be determined.

Descriptive statistics for each university are found in Table 1. OU saw higher standard deviations, showing that the spikes in viral RNA deviated results significantly from the mean, indicating substantial spikes in viral RNA over baseline. OU also saw higher peaks in viral RNA concentrations for both gene targets compared to GVSU, causing a discrepancy in average N1N2 GC/100 mL between the two universities. Though OU had much higher viral concentrations across the surveillance period, OU saw fewer maximum reported clinical cases per day (52), while GVSU saw higher maximum clinical cases reported (92). Daily clinical case data were unavailable from 20 November to 22 April, including 86 entries from GVSU and 296 from OU.

Table 1 | University data comparisons for academic years 2020–2022

Surveillance metrics	GVSU	OU
Campus population (*n*)	21,648	18,552
Wastewater samples (*n*)	554	679
Clinical cases reported (*n*)	2,812	1,477
Total days of clinical cases reported (*n*)	78	101
Average lower limit of detection concentration (GC/100 mL)	1,326 (SD = 493)	999 (SD = 1,308)
Average N1 concentration (GC/100 mL)	6,948 (SD = 29,643)	7,069 (SD = 81,235)
Average N2 concentration (GC/100 mL)	6,590 (SD = 27,495)	6,201 (SD = 71,556)
Maximum N1 concentration (GC/100 mL)	431,947	2,074,074
Maximum N2 concentration (GC/100 mL)	409,067	1,831,111
Maximum clinical cases reported/day	92	52

This shows descriptive statistics for both GVSU and OU wastewater surveillance from academic years 2020 to 2022. Data include both wastewater surveillance data and clinical cases reported by day. Campus populations are as of 2022. Total days of clinical cases reported display the number of daily entries for clinical reporting. Clinical reporting data were not available for all days, as clinical data were subject to testing and case counts per day. Days where no available data varied for each university and were reported as 'NA'. Daily clinical case data where no data were available from November 2020 to April 2022 for GVSU included 86 entries, while OU included 296.

Correlation to clinical cases

University wastewater surveillance results and clinical case counts saw moderate correlative evidence. These results can be seen in Figure 1. GVSU wastewater surveillance saw substantial peaks in viral concentrations on 23 November 2020, 21 March 2021, 29 November 2021, and 17 January 2022. GVSU wastewater signal declined to its lowest during March–April 2022. Maximum case counts for the GVSU clinically confirmed cases fell on 10 January 2022. During the sampling period, the average viral concentration on the GVSU campus was 6,769 GC/100 mL, while the maximum concentration

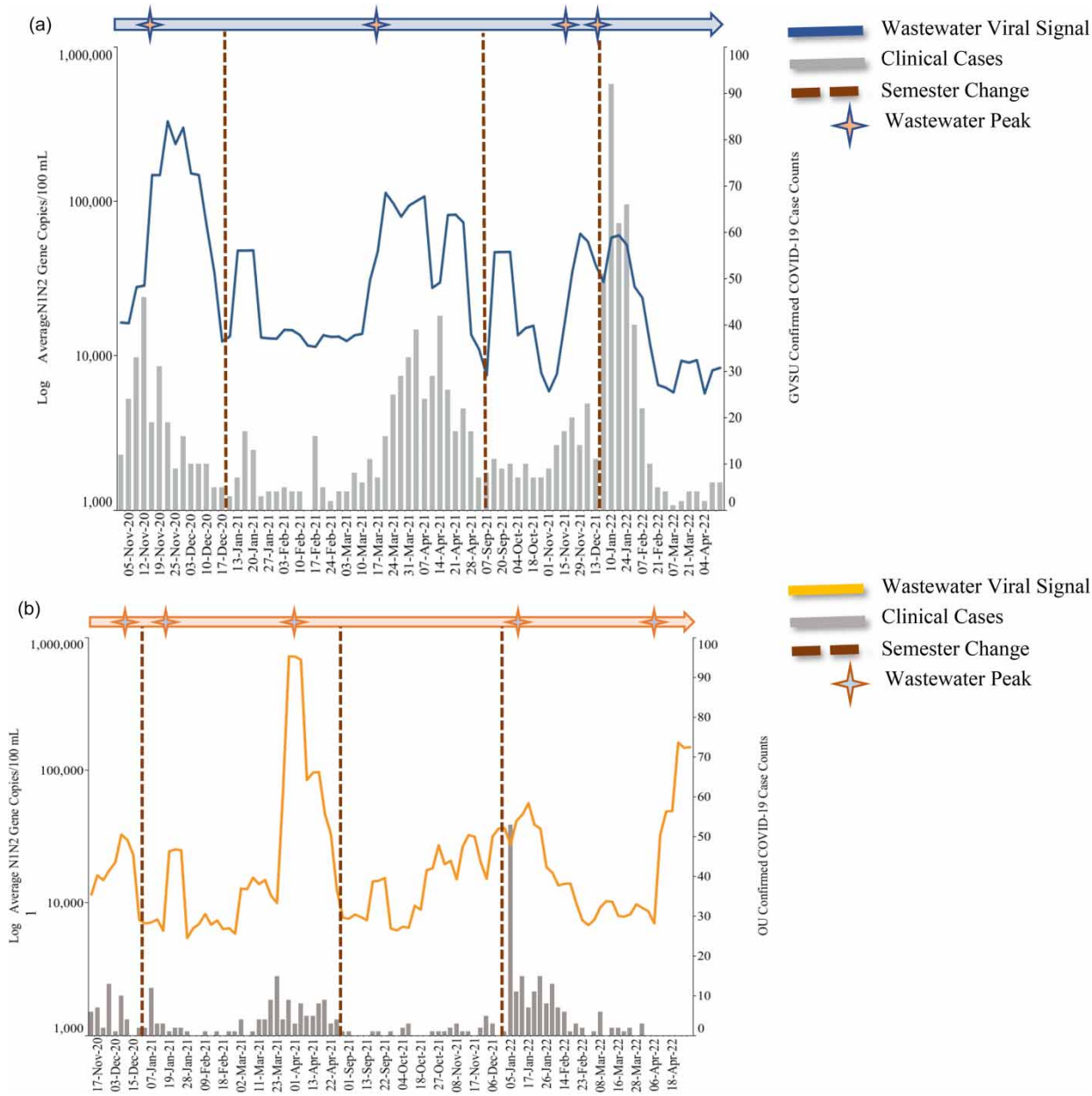


Figure 1 | Fall and winter semester wastewater surveillance and confirmed COVID-19 cases for GVSU (a) and OU (b) for 20 November to 22 April. This displays wastewater surveillance and COVID-19 confirmed case counts. Log_{10} average N1N2 viral concentration (GC/100 mL) is plotted over daily confirmed COVID-19 case counts from each university. (a) GVSU wastewater surveillance data over VAT confirmed COVID-19 clinical case counts from the academic years 2020–2022, beginning 2 November 2020 and ending 18 April 2022. (b) OU wastewater surveillance data over COVID-19 Response Team confirmed COVID-19 clinical case counts from the academic years 2020–2022, beginning 17 November 2020 and ending 22 April 2022. Only fall and winter semesters are shown, as surveillance was not conducted during the spring/summer semesters, nor were university response teams fully active.

was 420,507 GC/100 mL. Moreover, the average case counts during this time were 16, with a daily maximum of 92. OU wastewater surveillance reported higher overall concentrations during the sample period. OU viral peaks occurred on 8 December 2020, 19 January 2021, 30 March 2021, 6 December 2021, 17 January 2022, and 20 April 2022. The average viral signal for OU wastewater surveillance was 6,634 GC/100 mL. This average value displayed similarities to GVSU; however, the maximum concentration during the surveillance period was 1,952,593 GC/100 mL. This suggests similarities in sustained campus disease prevalence; however, OU appears to have increased vulnerability to viral spikes and variant fluctuation in the population. Further, the average OU campus case counts were 4, while the daily maximum was 53 on 5 January 2022, 5 days before the GVSU daily maximum. Unlike GVSU, the OU wastewater signal began to increase around April 2022 as the sampling period began to end. The average of N1 and N2 gene targets was correlated to clinically confirmed daily case counts on campus using Spearman's correlation (p-value < 0.05). GVSU wastewater results were found to weakly correlate to clinical case results for same-day analysis, (rho $= 0.51$, $p < 0.001$) which was also seen with OU results for same-day analysis (rho $= 0.42$, $p < 0.001$). For GVSU, results for a +7-day time shift (clinical data 7 days after wastewater data) were rho $= 0.45$, $p < 0.001$. Results for a −7-day time shift (clinical data 7 days before wastewater data) were rho $= 0.31$, $p = 0.0073$ GVSU.

Variants

Variant analysis was conducted on all samples with N1 and N2 gene concentrations of ≥10,000 GC/100 mL. Wastewater results from Figure 1 showcase wastewater peaks that are seen to align with variant entry into university campuses. Beginning in February 2021, GVSU and OU began testing wastewater sample RNA extracts for the presence of signature mutations on the spike protein tied to the Alpha variant (N501Y, del69-70). Both mutations in the sample indicated a positive detection of the Alpha variant in the campus populations. Both universities noted positive detection in March 2021. Detection of one mutation, but not the other, was then suggestive of a variant, but due to the kit's restrictions, it was unknown which variant was circulating at the time. Concentrations on campus began to decline from April through early fall 2021. Due to limited surveillance from both universities during the spring/summer semesters, data were unavailable for most of the summer months of 2021. As classes began to resume, the emergence of the Delta variant was noted. GVSU observed the initial emergence of the Delta variant on 13 September 2021 on the Allendale main campus that aligned with a spike in N1N2 viral RNA detection. OU saw a delayed identification, noting initial Delta variant detection on 11/05/2021. As testing continued, moderate-high spikes in viral RNA detection were observed to align with the emergence of a new variant in the campus populations. This was seen again during the emergence of the Omicron variant, where GVSU noted detections on 4 January 2022 upon students returning to campus from the winter break. OU noted the initial detection of Omicron on 12 April 2022. GVSU began to analyze sample extracts for the Omicron sub-variants (BA.1 and BA.2) and noted the initial emergence of BA.2 on 11 April 2022. OU did not report the analysis of sub-variants during the time frame of the surveillance period. To understand variant patterns happening on university campuses as a whole, combined variant detections can be found in Figure 2. These detections align with months' peak wastewater signal that was observed across both universities as mentioned earlier.

DISCUSSION

Sampling strategies

Both universities in the present study used bi-weekly sampling to quantify viral signals via wastewater surveillance. OU collected 24-h composite samples, while GVSU used grab samples. In large catchments, composite and grab samples perform similarly to one another (Kmush *et al.* 2022). Grab sample collection methods have been documented to fluctuate more producing results that are less representative of the sampled community in smaller catchments (Polo *et al.* 2020; Ahmed *et al.* 2021). Building-level data are particularly affected due to flow fluctuations which can bias wastewater information (Acer *et al.* 2022). George *et al.* (2022) documented that as catchment size and subsequent population decreased, the percentage of negative grab samples increased even when paired composite sampling indicated the presence of viral signal in wastewater. This has the potential to provide false negative wastewater results. This was suggested at OU, which utilized composite sampling, showcasing increased prevalence throughout the entire sample period while GVSU samples were more prone to fluctuations in viral signal. However, GVSU's sampling timeframe, 8–9 am, has been documented in other studies as a time that shows the best agreement with 24-h composite sampling (Augusto *et al.* 2022).

GVSU's surveillance efforts noted that certain buildings had more consistent reoccurring elevations than off-campus housing, particularly in Freshmen housing. This could be due to the time the wastewater was collected. Mendoza Grijalva *et al.*

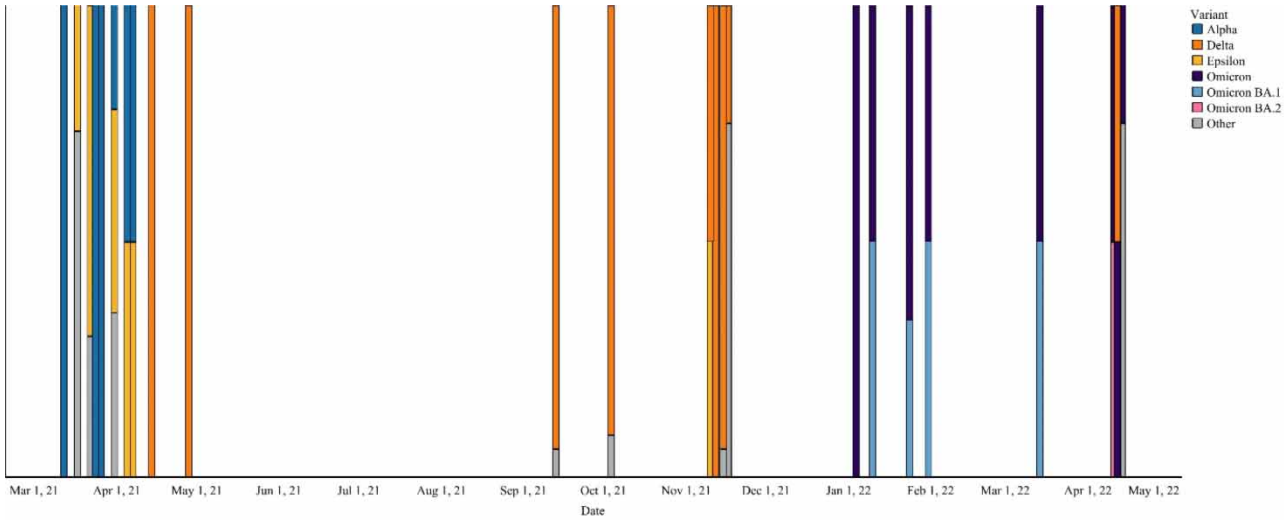


Figure 2 | Variant distributions present in university wastewater testing (GVSU and OU combined), 2021–2022. The distribution of variant detection including both GVSU and OU wastewater surveillance from 2021 to 2022. Variant analysis was conducted by the detection of dominant variant mutations in the wastewater sample. Variant targets were limited based on which mutations the ddPCR variant kits were able to detect at the time of surveillance. Dominant variants found in the wastewater samples were reported as suggestive presence as mutations became more affluent in samples. Others include samples with no mutations detected, following the parental signature.

(2022) found that optimizing grab samples to peak inputs resulted in up to an 82% detection rate in their wastewater system. For those residing in the Freshmen dorms, the time wastewater samples are collected might correspond better with peak flow. While in an extensive sewer system, the best time to collect a grab sample is 8 am–10 am, student demographics and habits may fall outside this typical flow period (Augusto *et al.* 2022). Future testing to indicate peak flow in specific universities could improve the outcomes of grab sample analysis in wastewater surveillance methods.

Each university presented variations in sampling strategies for wastewater collection. Along with this, each university utilized variations in collecting clinical testing data and how these data were stored and shared. Where GVSU utilized a randomized testing lottery that allowed for daily clinical data to be updated and stored through the VAT dashboard, OU relied on self-reporting and testing as a result of symptom onset. This testing was also primarily conducted by nurses and local health departments, making data availability more limited to the university response team. These data also saw delays and gaps in what was available for the OU COVID-19 dashboard. These gaps in data led to limited potential for advanced analysis on the wastewater and clinical case data.

University response

The two universities used differing mitigation and response strategies for managing the risk of transmission at their institutions. On-campus students that yielded a positive test at GVSU were placed into isolated housing. This allowed students to continue to have full access to university resources and provided students with an accessible option in case housing was not readily available off-campus or transportation to safe housing was not readily available. One limitation of this strategy involved the location of isolation housing dormitories. Instead of having dedicated housing for isolation only, GVSU had isolation dormitory rooms inter dispersed with regular dormitory rooms. This resulted in viral spikes in affected wastewater samples. Utilization of separate isolation spaces from health dormitories may have remedied this issue. Various studies report this strategy's effectiveness (Hayden *et al.* 2021; Rennert *et al.* 2021).

At OU, on campus, students living within a 100-mile radius were sent home from campus. Those with extenuating circumstances, living outside the 100-mile radius, or participating in campus athletics were permitted to stay. Several dormitories were selected on campus to be isolation dormitories and were not sampled. Additionally, many students at OU were commuting students, so this mitigation strategy was deemed to have the lowest impact on student populations. The removal or isolation of infected individuals saw immediate drops in viral signals upon subsequent wastewater surveillance data indicating its effectiveness as a mitigation strategy. Here, wastewater surveillance was utilized primarily as a tool to confirm the mitigation strategy for positive case strategies saw a shift in the viral signal detected at the targeted location.

Another aspect of the university's response originated in disseminating information to staff and students. While both institutions had teams dedicated to formatting responses, OU was much more limited in their data dissemination. They also provided less demographic information than GVSU did in their case data. OU also has not provided an archive of past case trends, and their dashboard is no longer available. GVSU provided an archived version of their publicly available data. As of the writing of this study, both universities have suspended updates to their respective dashboards. This has made advanced analysis of data difficult, as a protocol for long-term data storage and clinical data transparency was variable when compared to wastewater surveillance sampling schedules and results. The wastewater surveillance data became the consistent form of data from state dashboards when clinical data were stored or less abundant.

Clinical data uncertainty

The clinical data collected by both universities needed more certainty in accurate clinical insights due to the absence of testing kits at the onset of the pandemic. This issue was compounded due to the suspected high percentage of asymptomatic cases from the student population. Many reviews of asymptomatic patients have reported that younger groups <39 are more likely to be asymptomatic or display mild symptoms upon infection (Kronbichler *et al.* 2020; Chen *et al.* 2021; Ma *et al.* 2021). To conserve resources and gain a better understanding of infected students, GVSU randomly selected a subset of the campus population for mandatory rapid antigen testing and had testing available to any student interested in testing. Testing was also required for students who failed their daily health screening before they were allowed to return to campus activities. This form of random testing was effective in various university studies (Gressman & Peck 2020; Brook *et al.* 2021; Tuells *et al.* 2021). While this random testing and antigen strip testing proved effective at the onset of the pandemic, its accuracy was lessened as mutations persisted and increased the likelihood of inaccurate testing information. Several studies have documented this phenomenon (Adamson *et al.* 2022; Yang *et al.* 2022). Different from GVSU, which was proactive in its testing strategy, OU opted to test students at the beginning of each academic semester and if they did not pass their daily screener. Students that displayed symptoms were instructed to seek testing from the university or a third-party testing organization. This led to many unreported cases to the local university health office and department.

Students at both universities used third-party testing results to be reported to their institutions from public health partners to aid in data collection. This effort was somewhat sporadic and complicated because much of the data from third-party testing locations needed to include important information like the institution attended, leading to many of these cases going underreported. This resulted in significant gaps in available data in both universities making comparative analyses difficult. Due to the complexity of reported data and underrepresented asymptomatic cases, overall clinical data appeared to be underreported on both campuses. Other universities took a much more active role in testing students based on wastewater information. Several studies utilized wastewater data to require mandatory testing in affected buildings (Betancourt *et al.* 2021; Gibas *et al.* 2021). This procedure was documented to help mitigate the risk of outbreaks at their institutions by identifying areas of increased viral presence, testing students in the affected buildings, and isolating infected individuals. Our universities may have benefited from this form of testing.

Overall, as the COVID-19 pandemic evolves and strategies for maintaining community health adapt, it remains pertinent to continue evaluating mitigation strategies, especially for smaller communities like universities. University health officials would benefit from wastewater surveillance as a tool for understanding campus health and disease dynamics for the campus population. Due to the nature of how swift action can be effective for smaller populations such as student bodies, being able to implement public health strategies from lessons learned could improve overall campus health outcomes and lessen the disease burden on college students. With the evolution of wastewater surveillance, other disease-causing organisms could be adopted within the surveillance to check for agents that are commonly affiliated with campus dormitories. Further, campus wastewater surveillance offers the opportunity to evaluate the effectiveness of health forward strategies and allow health officials to improve or continue interventions offering promising initial efforts within the student population, securing student health and well-being.

CONCLUSION

SARS-CoV-2 is an enveloped RNA virus that spreads primarily through respiratory droplets (Kim *et al.* 2020; Medema *et al.* 2020), however bodily fluids with genetic material are shed and collected in domestic wastewater, allowing wastewater tracking of disease patterns and dynamics to be a feasible surveillance method for SARS-CoV-2 (Bivins & Bibby 2021; Maryam *et al.* 2023). With the ability to monitor disease patterns in a population without relying on individual clinical testing,

understanding where effective interventions may be implemented has emerged as a question for navigating the COVID-19 pandemic. One of the populations that revealed heightened vulnerability and increased presence of asymptomatic disease includes college campus students, staff, and faculty (Lu *et al.* 2022). This population exists within controlled, close quarters and typically sees large gatherings that present a point of entry for disease (Jain *et al.* 2022). WBS has been used for surveillance of infectious disease agents, opioid use patterns, chemical contaminants, and drinking water supply quality for years, and due to the onset of the COVID-19 pandemic, it has been utilized as a tool to support COVID-19 public health decision-making by local, state, and federal institutions (Ahmed *et al.* 2022). To understand how campus public health strategies have utilized WBS, we aimed to explore the wastewater surveillance data for SARS-CoV-2 RNA, clinical case data from university response teams, and mitigation strategies from two universities within the State of Michigan, United States. This review has offered insights that suggest the potential success of tracking disease trends in campus populations that can lead to impactful health decisions that secure student, faculty, and staff health outcomes when workflow for wastewater surveillance is well evaluated, tested, and adjusted per the community needs. While opportunities exist to adapt sampling strategies and understand variant evolution, wastewater surveillance for university populations may be a supportive public health tool for campus communities.

ACKNOWLEDGEMENTS

Funding support was provided by the Michigan Department of Health and Human Services, using Federal Financial Assistance from the U.S. Department of Treasury under the Epidemiology and Laboratory Capacity: Enhancing Detection Expansion through Coronavirus Response and Relief (CRR) Supplemental Appropriations Act of 2021 (P.L. 116-260). Grant Awards E20215064-00, E20224041-00, and E20230515-00 to Grand Valley State University and E20224058-00 2022, E20234373-00 2023, E20244361-00 2024 to Oakland State University.

DATA AVAILABILITY STATEMENT

All relevant data are available from an online repository or repositories.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

Acer, P. T., Kelly, L. M., Lover, A. A. & Butler, C. S. 2022 Quantifying the relationship between SARS-CoV-2 wastewater concentrations and building-level COVID-19 prevalence at an isolation residence: A passive sampling approach. *International Journal of Environmental Research and Public Health* **19** (18). https://doi.org/10.3390/ijerph191811245.

Adamson, B., Sikka, R., Wyllie, A. L. & Premsrirut, P. 2022 Discordant SARS-CoV-2 PCR and rapid antigen test results when infectious: A December 2021 occupational case series. *MedRxiv* 2022.01.04.22268770. https://doi.org/10.1101/2022.01.04.22268770.

Ahmed, W., Bivins, A., Bertsch, P. M., Bibby, K., Gyawali, P., Sherchan, S. P., Simpson, S. L., Thomas, K. V., Verhagen, R., Kitajima, M., Mueller, J. F. & Korajkic, A. 2021 Intraday variability of indicator and pathogenic viruses in 1-h and 24-h composite wastewater samples: Implications for wastewater-based epidemiology. *Environmental Research* **193**, 1–27. https://doi.org/10.1016/j.envres.2020.110531.

Ahmed, W., Simpson, S. L., Bertsch, P. M., Bibby, K., Bivins, A., Blackall, L. L., Bofill-Mas, S., Bosch, A., Brandão, J., Choi, P. M., Ciesielski, M., Donner, E., D'Souza, N., Farnleitner, A. H., Gerrity, D., Gonzalez, R., Griffith, J. F., Gyawali, P., Haas, C. N. & Shanks, O. C. 2022 Minimizing errors in RT-PCR detection and quantification of SARS-CoV-2 RNA for wastewater surveillance. *Science of the Total Environment* **805**, 149877. https://doi.org/10.1016/j.scitotenv.2021.149877.

Augusto, M. R., Claro, I. C. M., Siqueira, A. K., Sousa, G. S., Caldereiro, C. R., Duran, A. F. A., de Miranda, T. B., Bomediano Camillo, L., de M., Cabral, A. D. & de Freitas Bueno, R. 2022 Sampling strategies for wastewater surveillance: Evaluating the variability of SARS-COV-2 RNA concentration in composite and grab samples. *Journal of Environmental Chemical Engineering* **10** (3). https://doi.org/10.1016/j.jece.2022.107478.

Betancourt, W. Q., Schmitz, B. W., Innes, G. K., Prasek, S. M., Pogreba Brown, K. M., Stark, E. R., Foster, A. R., Sprissler, R. S., Harris, D. T., Sherchan, S. P., Gerba, C. P. & Pepper, I. L. 2021 COVID-19 containment on a college campus via wastewater-based epidemiology, targeted clinical testing and an intervention. *Science of the Total Environment* **779**, 146408. https://doi.org/10.1016/j.scitotenv.2021.146408.

Bivins, A. & Bibby, K. 2021 Wastewater monitoring during mass COVID-19 vaccination on a college campus. *Environmental Science & Technology Letters* **8** (9), 792–798. https://doi.org/10.1021/acs.estlett.1c00519.

Borchardt, M. A., Spencer, S. K., Hubbard, L. E., Firnstahl, A. D., Stokdyk, J. P. & Kolpin, D. W. 2017 Avian influenza virus RNA in groundwater wells supplying poultry farms affected by the 2015 influenza outbreak. *Environmental Science & Technology Letters* **4** (7), 268–272. https://doi.org/10.1021/acs.estlett.7b00128.

Brook, C. E., Northrup, G. R., Ehrenberg, A. J., Doudna, J. A. & Boots, M. 2021 Optimizing COVID-19 control with asymptomatic monitoring testing in a university environment. *Epidemics* **37**, 100527. https://doi.org/https://doi.org/10.1016/j.epidem.2021.100527.

CDC 2022 *National Wastewater Monitoring System*. Centers for Disease Control and Prevention. Available from: https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/wastewater-surveillance.html.

Chen, C., Zhu, C., Yan, D., Liu, H., Li, D., Zhou, Y., Fu, X., Wu, J., Ding, C., Tian, G., Lan, L., Liu, X., Huang, C., Hecht, R., Li, L. & Yang, S. 2021 The epidemiological and radiographical characteristics of asymptomatic infections with the novel coronavirus (COVID-19): A systematic review and meta-analysis. *International Journal of Infectious Diseases* **104**, 458–464. https://doi.org/https://doi.org/10.1016/j.ijid.2021.01.017.

Corchis-Scott, R., Geng, Q., Seth, R., Ray, R., Beg, M., Biswas, N., Charron, L., Drouillard, K. D., D'Souza, R., Heath, D. D., Houser, C., Lawal, F., McGinlay, J., Menard, S. L., Porter, L. A., Rawlings, D., Scholl, M. L., Siu, K. W. M., Tong, Y., Weisener, C. G., Wilhelm, S. W. & McKay, R. M. L. 2021 Averting an outbreak of SARS-CoV-2 in a university residence hall through wastewater surveillance. *Microbiology Spectrum* **9** (2), e00792-21. https://doi.org/10.1128/Spectrum.00792-21.

Corman, V. M., Landt, O., Kaiser, M., Molenkamp, R., Meijer, A., Chu, D. K., Bleicker, T., Brünink, S., Schneider, J., Schmidt, M. L., Mulders, D. G., Haagmans, B. L., van der Veer, B., van den Brink, S., Wijsman, L., Goderski, G., Romette, J.-L., Ellis, J., Zambon, M., Malik, P., Herman, G., Reusken, C., Koopmans, M. P. G. & Drosten, C. 2020 Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveillance: Bulletin Europeen Sur Les Maladies Transmissibles European Communicable Disease Bulletin* **25** (3). https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045.

Data Dashboard – Lakers Together – Grand Valley State University 2022 Retrieved March 10, 2023. Available from: https://www.gvsu.edu/lakerstogether/data-dashboard-37.htm.

Espinoza, B., Marathe, M., Swarup, S. & Thakur, M. 2021 Asymptomatic individuals can increase the final epidemic size under adaptive human behavior. *Scientific Reports* **11** (1), 19744. https://doi.org/10.1038/s41598-021-98999-2.

Flood, M. T., D'Souza, N., Rose, J. B. & Aw, T. G. 2021 Methods evaluation for rapid concentration and quantification of SARS-CoV-2 in raw wastewater using droplet digital and quantitative RT-PCR. *Food and Environmental Virology* **13** (3), 303–315. https://doi.org/10.1007/s12560-021-09488-8.

Fukuta, M., Mao, Z. Q., Morita, K. & Moi, M. L. 2021 Stability and infectivity of SARS-CoV-2 and viral RNA in water, commercial beverages, and bodily fluids. *Frontiers in Microbiology* **12**. Available from: https://www.frontiersin.org/article/10.3389/fmicb.2021.667956

Gendron, L., Verreault, D., Veillette, M., Moineau, S. & Duchaine, C. 2010 Evaluation of filters for the sampling and quantification of RNA phage aerosols. *Aerosol Science and Technology* **44** (10), 893–901. https://doi.org/10.1080/02786826.2010.501351.

George, A. D., Kaya, D., Layton, B. A., Bailey, K., Mansell, S., Kelly, C., Williamson, K. J. & Radniecki, T. S. 2022 Impact of sampling type, frequency, and scale of the collection system on SARS-CoV-2 quantification fidelity. *Environmental Science and Technology Letters* **9** (2), 160–165. https://doi.org/10.1021/acs.estlett.1c00882.

Gibas, C., Lambirth, K., Mittal, N., Juel, M. A. I., Barua, V. B., Roppolo Brazell, L., Hinton, K., Lontai, J., Stark, N., Young, I., Quach, C., Russ, M., Kauer, J., Nicolosi, B., Chen, D., Akella, S., Tang, W., Schlueter, J. & Munir, M. 2021 Implementing building-level SARS-CoV-2 wastewater monitoring on a university campus. *Science of The Total Environment* **782**, 146749. https://doi.org/https://doi.org/10.1016/j.scitotenv.2021.146749.

Gressman, P. T. & Peck, J. R. 2020 Simulating COVID-19 in a university environment. *Mathematical Biosciences* **328**, 108436. https://doi.org/https://doi.org/10.1016/j.mbs.2020.108436.

Harris-Lovett, S., Nelson, K., Beamer, P., Bischel, H. N., Bivins, A., Bruder, A., Butler, C., Camenisch, T. D., De Long, S. K., Karthikeyan, S., Larsen, D. A., Meierdiercks, K., Mouser, P., Pagsuyoin, S., Prasek, S., Radniecki, T. S., Ram, J. L., Roper, D. K., Safford, H., Sherchan, S. P., Shuster, W., Stalder, T., Wheeler, R. T. & Korfmacher, K. S. Wastewater surveillance for SARS-CoV-2 on college campuses: Initial efforts, lessons learned and research needs. *International Journal of Environmental Research and Public Health* **18** (9), 4455.

Hart, J., Jamison, M. N., McNair, J. N. & Szlag, D. C. 2023 Frequency and degradation of SARS-CoV-2 markers N1, N2, and E in sewage. *J. Water Health* jwh2023314. doi:10.2166/wh.2023.314.

Hayden, M. E., Rozycki, D., Tanabe, K. O., Pattie, M., Casteen, L., Davis, S. & Holstege, C. P. 2021 COVID-19 isolation and quarantine experience for residential students at a large four-year public university. *American Journal of Public Health* **111** (10), 1772–1775. https://doi.org/10.2105/AJPH.2021.3064241.

Hokajärvi, A.-M., Rytkönen, A., Tiwari, A., Kauppinen, A., Oikarinen, S., Lehto, K.-M., Kankaanpää, A., Gunnar, T., Al-Hello, H., Blomqvist, S., Miettinen, I. T., Savolainen-Kopra, C. & Pitkänen, T. 2021 The detection and stability of the SARS-CoV-2 RNA biomarkers in wastewater influent in Helsinki, Finland. *The Science of the Total Environment* **770**, 145274. https://doi.org/10.1016/j.scitotenv.2021.145274.

Hopkins, L., Persse, D., Caton, K., Ensor, K., Schneider, R., McCall, C. & Stadler, L. B. 2023 Citywide wastewater SARS-CoV-2 levels strongly correlated with multiple disease monitoring indicators and outcomes over three COVID-19 waves. *Science of the Total Environment* **855**, 158967. https://doi.org/10.1016/j.scitotenv.2022.158967.

Jain, N., Hamilton, D., Mital, S., Ilias, A., Brinkmann, M. & McPhedran, K. 2022 Long-term passive wastewater monitoring of SARS-CoV-2 for seven university dormitories in comparison to municipal surveillance. *Science of the Total Environment* **852**, 158421.

Jones, D. L., Baluja, M. Q., Graham, D. W., Corbishley, A., McDonald, J. E., Malham, S. K., Hillary, L. S., Connor, T. R., Gaze, W. H., Moura, I. B., Wilcox, M. H. & Farkas, K. 2020 Shedding of SARS-CoV-2 in feces and urine and its potential role in person-to-person

transmission and the environment-based spread of COVID-19. *The Science of the Total Environment* **749**, 141364. https://doi.org/10.1016/j.scitotenv.2020.141364.

Kapoor, V., Al-Duroobi, H., Phan, D. C., Palekar, R. S., Blount, B. & Rambhia, K. J. 2022 Wastewater surveillance for SARS-CoV-2 to support return to campus: Methodological considerations and data interpretation. *Current Opinion in Environmental Science & Health* **27**, 100362. https://doi.org/10.1016/j.coesh.2022.100362.

Kim, D., Lee, J.-Y., Yang, J.-S., Kim, J. W., Kim, V. N. & Chang, H. 2020 The architecture of SARS-CoV-2 transcriptome. *Cell* **181** (4), 914–921. e10. . https://doi.org/10.1016/j.cell.2020.04.011.

Kmush, B. L., Monk, D., Green, H., Sachs, D. A., Zeng, T. & Larsen, D. A. 2022 Comparability of 24-hour composite and grab samples for detection of SARS-2-CoV RNA in wastewater. *FEMS Microbes* **3**, 1–5. https://doi.org/10.1093/femsmc/xtac017.

Kronbichler, A., Kresse, D., Yoon, S., Lee, K. H., Effenberger, M. & Shin, J. 2020 Asymptomatic patients as a source of COVID-19 infections: A systematic review and meta-analysis. *International Journal of Infectious Diseases* **98**, 180–186. https://doi.org/https://doi.org/10.1016/j.ijid.2020.06.052.

Lu, E., Ai, Y., Davis, A., Straathof, J., Halloran, K., Hull, N., Winston, R., Weir, M. H., Soller, J., Bohrerova, Z., Oglesbee, M. & Lee, J. 2022 Wastewater monitoring of SARS-CoV-2 in dormitories as a part of comprehensive university campus COVID-19 monitoring. *Environmental Research* **212**, 113580.

Ma, Q., Liu, J., Liu, Q., Kang, L., Liu, R., Jing, W., Wu, Y. & Liu, M. 2021 Global percentage of asymptomatic SARS-CoV-2 infections among the tested population and individuals with confirmed COVID-19 diagnosis: A systematic review and meta-analysis. *JAMA Network Open* **4** (12), e2137257. https://doi.org/10.1001/jamanetworkopen.2021.37257.

Maryam, S., Ul Haq, I., Yahya, G., Ul Haq, M., Algammal, A. M., Saber, S. & Cavalu, S. 2023 COVID-19 monitoring in wastewater: An epidemiological tool for the monitoring of SARS-CoV-2. *Frontiers in Cellular and Infection Microbiology* **12**. Available from: https://www.frontiersin.org/articles/10.3389/fcimb.2022.978643

McMahan, C. S., Self, S., Rennert, L., Kalbaugh, C., Kriebel, D., Graves, D., Colby, C., Deaver, J. A., Popat, S. C., Karanfil, T. & Freedman, D. L. 2021 COVID-19 wastewater epidemiology: A model to estimate infected populations. *The Lancet Planetary Health* **5** (12), e874–e881. https://doi.org/10.1016/S2542-5196(21)00230-8.

Medema, G., Heijnen, L., Elsinga, G., Italiaander, R. & Brouwer, A. 2020 Presence of SARS-Coronavirus-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in The Netherlands. *Environmental Science & Technology Letters* **7** (7), 511–516. https://doi.org/10.1021/acs.estlett.0c00357.

Mendoza Grijalva, L., Brown, B., Cauble, A. & & Tarpeh, W. A. 2022 Diurnal variability of SARS-CoV-2 RNA concentrations in hourly grab samples of wastewater influent during low COVID-19 incidence. *ACS ES and T Water* **2** (11), 2125–2133. https://doi.org/10.1021/acsestwater.2c00061.

Oran, D. P. & Topol, E. J. 2021 The proportion of SARS-CoV-2 infections that are asymptomatic. *Annals of Internal Medicine* **174** (5), 655–662. https://doi.org/10.7326/M20-6976.

Polo, D., Quintela-Baluja, M., Corbishley, A., Jones, D. L., Singer, A. C., Graham, D. W. & Romalde, J. L. 2020 Making waves: Wastewater-based epidemiology for COVID-19 – approaches and challenges for monitoring and prediction. *Water Research* **186**, 116404. https://doi.org/10.1016/j.watres.2020.116404.

Prado, T., Fumian, T. M., Mannarino, C. F., Resende, P. C., Motta, F. C., Eppinghaus, A. L. F., do Vale, V. H. C., Braz, R. M. S., de Andrade, J. D. S. R., Maranhão, A. G. & Miagostovich, M. P. 2021 Wastewater-based epidemiology as a useful tool to track SARS-CoV-2 and support public health policies at municipal level in Brazil. *Water Research* **191**, 116810. https://doi.org/10.1016/j.watres.2021.116810.

R Core Team 2022 *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available from: https://www.R-project.org/.

Rennert, L., McMahan, C., Kalbaugh, C. A., Yang, Y., Lumsden, B., Dean, D., Pekarek, L. & Colenda, C. C. 2021 Surveillance-based informative testing for detection and containment of SARS-CoV-2 outbreaks on a public university campus: An observational and modelling study. *The Lancet Child & Adolescent Health* **5** (6), 428–436. https://doi.org/https://doi.org/10.1016/S2352-4642(21)00060-2.

Schmitz, B. W., Innes, G. K., Prasek, S. M., Betancourt, W. Q., Stark, E. R., Foster, A. R., Abraham, A. G., Gerba, C. P. & Pepper, I. L. 2021 Enumerating asymptomatic COVID-19 cases and estimating SARS-CoV-2 fecal shedding rates via wastewater-based epidemiology. *The Science of the Total Environment* **801**, 149794. https://doi.org/10.1016/j.scitotenv.2021.149794.

Sharaby, Y., Gilboa, Y., Alfiya, Y., Sabach, S., Cheruti, U. & Friedler, E. 2023 Whole campus wastewater monitoring of SARS-CoV-2 for COVID-19 outbreak management. *Water Science and Technology* **87** (4), 910–923. https://doi.org/10.2166/wst.2023.030.

Sharara, N., Endo, N., Duvallet, C., Ghaeli, N., Matus, M., Heussner, J., Olesen, S. W., Alm, E. J., Chai, P. R. & Erickson, T. B. 2021 Wastewater network infrastructure in public health: Applications and learnings from the COVID-19 pandemic. *PLOS Global Public Health* **1** (12), e0000061. https://doi.org/10.1371/journal.pgph.0000061.

Tay, M. Z., Ang, L. W., Wei, W. E., Lee, V. J. M., Leo, Y.-S. & Toh, M. P. H. S. 2022 Health-seeking behavior of COVID-19 cases during the first eight weeks of the outbreak in Singapore: Differences between local community and imported cases and having visits to single or multiple healthcare providers. *BMC Public Health* **22** (1), 239. https://doi.org/10.1186/s12889-022-12637-8.

Tuells, J., Egoavil, C. M., Pena Pardo, M. A., Montagud, A. C., Montagud, E., Caballero, P., Zapater, P., Puig-Barberá, J. & Hurtado-Sanchez, J. A. 2021 Seroprevalence study and cross-sectional survey on COVID-19 for a plan to reopen the university of Alicante (Spain). *International Journal of Environmental Research and Public Health* **18** (4). https://doi.org/10.3390/ijerph18041908.

Wolfe, M. K. 2022 Invited perspective: The promise of wastewater monitoring for infectious disease surveillance. *Environmental Health Perspectives* **130** (5), 051302.

Wölfel, R., Corman, V. M., Guggemos, W., Seilmaier, M., Zange, S., Müller, M. A., Niemeyer, D., Jones, T. C., Vollmar, P., Rothe, C., Hoelscher, M., Bleicker, T., Brünink, S., Schneider, J., Ehmann, R., Zwirglmaier, K., Drosten, C. & Wendtner, C. 2020 Virological assessment of hospitalized patients with COVID-2019. *Nature* **581** (7809), Article 7809. https://doi.org/10.1038/s41586-020-2196-x.

Wu, J., Wang, Z., Lin, Y., Zhang, L., Chen, J., Li, P., Liu, W., Wang, Y., Yao, C. & Yang, K. 2021 Technical framework for wastewater-based epidemiology of SARS-CoV-2. *Science of the Total Environment* **791**, 148271. https://doi.org/10.1016/j.scitotenv.2021.148271.

Yang, Y.-P., Huang, L.-L., Pan, S.-J., Xu, D., Jiesisibieke, Z. L. & Tung, T.-H. 2022 False-positivity results in rapid antigen tests for SARS-CoV-2: An umbrella review of meta-analyses and systematic reviews. *Expert Review of Anti-Infective Therapy* **20** (7), 1005–1013. https://doi.org/10.1080/14787210.2022.2070152.

Zhu, Y., Oishi, W., Maruo, C., Saito, M., Chen, R., Kitajima, M. & Sano, D. 2021 Early warning of COVID-19 via wastewater-based epidemiology: Potential and bottlenecks. *Science of the Total Environment* **767**, 145124. https://doi.org/10.1016/j.scitotenv.2021.145124.

First received 28 September 2023; accepted in revised form 22 April 2024. Available online 2 May 2024