ABSTRACT: Wastewater-based epidemiology (WBE) is a useful complement to clinical testing for managing COVID-19. While community-scale wastewater and clinical data frequently correlate, less is known about subcommunity relationships between the two data types. Moreover, nondetects in qPCR wastewater data are typically handled through methods known to bias results, overlooking perhaps better alternatives. We address these knowledge gaps using data collected from September 2020–June 2021 in Davis, California (USA). We hypothesize that coupling the expectation maximization (EM) algorithm with the Markov Chain Monte Carlo (MCMC) method could improve estimation of “missing” values in wastewater qPCR data. We test this hypothesis by applying EM-MCMC to city wastewater treatment plant data and comparing output to more conventional nondetect handling methods. Dissimilarities in results (i) underscore the importance of specifying nondetect handling method in reporting and (ii) suggest that using EM-MCMC may yield better agreement between community-scale clinical and wastewater data. We also present a novel framework for spatially aligning clinical data with wastewater data collected upstream of a treatment plant (i.e., distributed across a sewershed). Applying the framework to data from Davis reveals reasonable agreement between wastewater and clinical data at highly granular spatial scales—further underscoring the public-health value of WBE.

KEYWORDS: Bayesian analysis, multiple imputation, qPCR nondetects, SARS-CoV-2, wastewater-based epidemiology, wastewater monitoring

INTRODUCTION

Wastewater-based epidemiology (WBE) has become widely recognized as a useful complement to clinical testing for managing COVID-19. Relative to large-scale diagnostic testing, WBE offers a less resource-intensive way to monitor COVID-19 infections and spread among large numbers of people. WBE is also unbiased, capturing data on entire populations rather than just the subset of individuals who come in for clinical testing.1

Most studies to date comparing wastewater and clinical data have focused on the community scale, that is, comparing trends in data collected from the influent to a given wastewater treatment plant (WWTP) to trends in data collected from clinical tests of a subpopulation served by that WWTP.2−4 Such studies have frequently found good agreement between the two data sources. But little is known about relationships between wastewater and clinical data at more granular spatial scales. Part of the challenge in elucidating such relationships is the fact that to protect privacy, clinical-testing results are often geographically aggregated, for example, at the census-block level. A first objective of this study was to develop and test a framework for probabilistically disaggregating clinical-testing data to facilitate comparison with wastewater data collected from sampling sites that strategically isolate different parts of a community sewershed.

Separately, SARS-CoV-2 RNA in wastewater samples is typically quantified using either reverse transcription-quantitative polymerase chain reaction (RT-qPCR) or RT-droplet digital PCR (RT-ddPCR).5 While RT-ddPCR is becoming more popular for wastewater surveillance6 because of its greater specificity and sensitivity,7,8 many laboratories continue to use RT-qPCR due to the higher cost and time requirements.
of RT-ddPCR and the large upfront capital investment of ddPCR instrumentation.

Bivins et al. (2021) recently drew attention to how variability in RT-qPCR methods and reporting affects results and interpretation. An additional and important source of variability not considered by these authors is how nondetects are handled. qPCR nondetects occur routinely for reasons including low or zero target abundance, poor assay design/performance, or human error.

There is no current consensus on how to best manage qPCR nondetects. Researchers, whether through scientific software or manual analysis, typically handle nondetects either using single imputation (setting all nondetects equal to a constant value, such as the mean of detected replicates, half the detection limit, or zero) or by censoring (excluding nondetects from analysis altogether).

Unfortunately, both single imputation and censoring can substantially bias qPCR results. The biasing effect is amplified when, as is often the case for wastewater data, the target is present in low concentrations to begin with. A second objective of this study was to demonstrate how different nondetect handling methods can affect apparent wastewater data trends and to explore whether multiple imputation of nondetects can improve on more commonly used but less sophisticated approaches.

**MATERIALS AND METHODS**

**Study Setting and Design.** We used wastewater data collected through the Healthy Davis Together (HDT) program in Davis—a small city of approximately 69,000 located in northern California—to (1) explore the value of multiple imputation for handling qPCR nondetects and (2) examine relationships between wastewater and clinical data at multiple spatial scales.

HDT was a joint, multipronged initiative between the city of Davis and the University of California, Davis (UC Davis) for local management and mitigation of COVID-19. Beginning in November 2020, HDT made free, saliva-based PCR tests for COVID-19 available to anyone living or working in Davis. Uptake of the clinical-testing program was considerable. The fraction of Davis residents who reported receiving at least one COVID-19 test rose from 30% to 73% from September 2020 to March 2021. As of April 2021, Yolo County had performed the most tests per capita of California’s 58 counties, at a rate quadruple the state median.

HDT also conducted wastewater surveillance at the community, subregional, and building/neighborhood scales (Figure 1). At the community scale, samples were collected from the influent to the City of Davis Wastewater Treatment Plant (COD WWTP). The COD WWTP captures all of Davis’s municipal wastewater, with no contributions from UC Davis or from neighboring jurisdictions. At the subregional scale, samples were collected from sewershed nodes isolating the wastewater contributions of different geographic areas in the city. At the building/neighborhood scale, samples were collected from sewershed nodes isolating high-priority building complexes or neighborhoods identified through discussion with local officials. The HDT WBE program began in September 2020 with weekly samples collected from the COD WWTP. Zones were added and sampling frequency

![Figure 1. Map of subregional (SR; blue) and building/neighborhood (BN; purple) sampling zones for SARS-CoV-2 wastewater-based epidemiology in the city of Davis, CA. Note overlapping zones: in particular, zone SR-M overlaps the entirety of zone BN-F; zone SR-N overlaps a portion of zone SR-O and the entirety of zone SR-M; and zone SR-P overlaps the entirety of zones SR-A through SR-E, as well as zones SR-O, SR-N, and SR-M.](https://doi.org/10.1021/acsestwater.2c00053)
increased over the course of the sampling campaign (Figure S1). At full scale-up, the surveillance program sampled daily from the COD WWTP and 3x/week from each of 16 subregional and seven building/neighborhood zones.

**Sample Collection.** 24-h time-weighted composite samples were collected from each zone using insulated Hach AS950 Portable Compact Samplers (Thermo Fisher Scientific, USA) programmed to collect 30 mL of sample every 15 min. The bulk of samples were processed immediately, with a small number stored at 4 °C for up to 1 week before processing.

**Sample Processing.** Samples were pasteurized for 30 min at 60 °C to reduce biohazard risk while preserving RNA quality. Samples were then spiked with a known concentration of φ6 bacteriophage (strain HB104; generously provided by Samuel Díaz–Muñoz, UC Davis) as an internal recovery control.12,13 The φ6 spike solution was prepared using previously described methods,14 modified slightly by using ATCC Medium 129 in place of LB media. The final steps in the processing pipeline were sample concentration and extraction. From September 2020 through the end of February 2021, concentration was performed via ultrafiltration through 100 kDa Amicon Ultra-15 centrifugal filter devices, and column-based extraction was performed manually using either the NucleoSpin RNA Stool Kit (Macherey-Nagel) or the AllPrep PowerViral DNA/RNA Kit (Qiagen). From February 2021 through June 2021, concentration was performed using Nanotrap Magnetic Virus Particles (Ceres Nanosciences) and the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit (Thermo Fisher) coupled with the KingFisher Flex liquid-handling system (Thermo Fisher). The particle-based method was far more conducive to automation and higher throughput than the ultrafiltration-based method, and the switch was necessary to accommodate greater numbers of samples as the sampling campaign scaled up. Further details on the concentration and extraction protocols are available in SI Materials and methods.

We performed a four-sample comparison of the two methods, utilizing three process replicates and three qPCR technical replicates per method per sample (SI Methods comparison). Two-way ANOVA showed that the ultrafiltration method yielded higher concentrations of the fecal-strength indicator PMMoV while the magnetic-particle method yielded higher concentrations of both the N1 and N2 regions of the SARS-CoV-2 nucleocapsid gene; however, average concentrations of positive replicates for all targets across all samples (Table S1) were generally of the same order of magnitude (with the exception of N1 for Sample 4 and PMMoV for Sample 1, where slightly more than an order of magnitude separated average concentrations of positive replicates for the two methods).

**RT-qPCR.** Sample extracts were analyzed by one-step RT-qPCR for four targets: N1 and N2 targeting regions of the nucleocapsid (N) gene of SARS-CoV-2, φ6 bacteriophage (an RNA virus used as an internal quality control), and pepper mild mottle virus (PMMoV; used for normalization of SARS-CoV-2 results). Additional information on the RT-qPCR assay designs is available in Tables S1–S3. Per Bivins et al. (2021),9 the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) checklist for this study is included as SI MIQE. Inhibition testing (see SI MIQE) was performed on a representative set of six sample extracts, using three different dilutions per sample. Cts increased commensurately with dilution factor, indicating lack of inhibition. Triplicate (technical replicate) wells were run for each target of each sample. Each qPCR run included duplicate of a nontemplate-control (NTC) and a known concentration of positive plasmid to verify consistency of the Ct number between plates.

Because our WBE campaign required preparation and analysis of a large number of qPCR plates, we did not run a standard curve on every plate. Rather, we constructed standard curves for each target (Table S4) on separate plates using seven-point serial dilutions of plasmid containing the targets (ordered from Eurofins Scientific and IDT, who supplied information on starting plasmid concentrations measured through spectrophotometry), with each dilution assayed in triplicate or quadruplicate. Per Kralik and Ricchi (2017),15 the limit of detection (LOD) values were considered “as the minimum concentration of nucleic acid or number of cells, which always gives a positive PCR result in all replicates tested, or in the major part (over 95%) of them”.15 The measured LODs were 2.5 gene copies (gc) per reaction for N1, 10 gc per reaction for N2 and φ6, and 1 × 10² gc for PMMoV (Table S3).

However, Kralik and Ricchi also note that “LOD is not a limiting value and, therefore, that Cq values below the LOD are absolutely valid in terms of microorganism presence; however, the probability of their repeated detection is less than 95%.” Given this, and given that our study assesses how handling of nondetects influences results for very low concentrations of N1 and N2 targets, we also defined a statistical limit of detection (LOD₀) for N1 and N2 using the highest Cts statistically distinguishable from negative controls (Table S3). The LOD₀ values were based on uncertainty in the standard curve as the upper 99th percent confidence interval of the Ct values of either the negative controls (for which signal was detected only for PMMoV) or the Ct of the total number of cycles minus 1 (that is, 44) if no signal was present in the negative controls.16 These values were 42.44 for N1 and 43.07 for N2 (corresponding to LOD₀ values of 0.1 and 0.2 gc/reaction, respectively). All N1 and N2 Cts less than these values were included as valid in our analysis, while Ct values greater than these values were treated as nondetects.

**Nondetect Handling Methods.** Inspired by McCall et al. (2014),11 we developed and applied an expectation maximization-Markov chain Monte Carlo (EM-MCMC) model for multiple imputation of nondetect Ct values in wastewater qPCR data.17 We began by grouping results by sampling zone4 separately for each target (i.e., N1 and N2). Within each zone, we modeled the Ct values (X₀) for each technical replicate (index i) and sampling date (index t) as independent and identically distributed. The values were modeled with a normal distribution characterized by a common variance σ² and common prior probability distribution6 on the mean Ct parameter for a given sampling date across all technical replicates (i.e., the prior on θₜ). The normal distribution is truncated such that it is positive.

We then used an empirical Bayesian approach to learn the prior for the model parameters, enabling discovery of hyperparameters shared by all samples from the same zone via the EM algorithm. The approach reduces variability in estimated mean Ct value for each sample date by specifying a common prior for all samples from a given location. Specifically, we modeled the priors for all θₜ and common σ as two gamma distributions with shape and rate parameters αₙ, βₙ and αₚ, βₚ, respectively. We estimated these hyperparameters’ with the EM algorithm, which alternates between
calculating the posterior distribution for the latent (i.e., model-inferred) parameters given the current hyperparameters (E step) and updating the hyperparameters using maximum likelihood based on the posterior expectation. Because closed forms for the posterior distribution do not exist for this application, we sampled from the posterior using MCMC via Python’s Stan package (pystan).

The EM-MCMC algorithm can be summarized as

(1) Initialize the hyperparameters \( \alpha_i, \beta_i, \alpha_i', \beta_i' \).

(2) Generate \( T \) (a user-defined choice) Monte Carlo samples of the latent parameters \( \theta_i \) and \( \sigma \) within the group using MCMC with the current hyperparameters.

(3) Compute the maximum likelihood estimates of the hyperparameters given the \( T \) sampled latent parameters (solved numerically via the scipy.stats.gamma.fit method).

(4) Repeat steps 2 and 3 until convergence of hyperparameters.

We carried out this process independently for each target and group using the hyperparameter priors \( \alpha_i = 1, \beta_i = 1/35, \alpha_i' = 3, \beta_i' = 1 \). The model was run for 20 iterations, generating \( 10^4 \) MCMC samples per iteration of which the first 500 were dropped. The model was then run again for one iteration (again with \( 10^5 \) MCMC samples and 500 drop samples) using the hyperparameter estimates. The Python script used for implementation, along with a sample data set, is available at https://tinyurl.com/Safford-et-al-EM-MCMC. The model output contained estimated posterior mean \( N_1 \) and \( N_2 \) concentration calculations (and average concentrations of samples with no positive replicates were set to zero).

Data Analysis. \( N_1 \), \( N_2 \), and PMMOV concentrations calculated using each nondetect handling method were converted to gc/L of initial sample based on effective volumes analyzed. MATLAB software (version R2021a; MathWorks) was used for subsequent analysis. \( N_1 \) and \( N_2 \) concentrations were averaged into a single concentration (\( C_{MN} \)) per sample to facilitate data visualization and trend analysis. \( C_{MN} \) values were normalized using PMMoV\textsuperscript{ref} according to the formula

\[
C_{norm} = \frac{C_{MN}}{C_{PMMoV}}.
\]

Normalized data were winsorized (i.e., truncated) at the [1,95] percentile levels to avoid outsized impact of outliers. Finally, relative normalized values were calculated separately for each nondetect handling method using the formula

\[
C_{norm,rel} = \frac{C_{norm}}{C_{norm,max}}
\]

where \( C_{norm,max} \) is the maximum normalized value of all sewershed samples. Relative normalized values were used to visualize and compare trends in wastewater data processed using different nondetect handling methods. Because virus concentrations detected in WWTP influent differed substantially from virus concentrations detected in sewershed samples, these calculations were performed separately on sewershed and WWTP data. Values in between sampling dates were linearly interpolated to

\[
(3) \ [C_{avg}], \text{ censoring nondetects entirely.}
\]

As stated above, serial dilutions of plasmids containing the \( N_1 \) and \( N_2 \) targets indicated that the LOD of our \( N_1 \) and \( N_2 \) qPCR assays were \( \leq 10 \) gc/reaction. For the LOD\textsubscript{0.5} method, then, we set the single imputation value at 0.5 gc/reaction—i.e., half the theoretical LOD of 1 gc/reaction.\textsuperscript{15} This value was substituted as the target concentration for any technical replicate yielding a nondetect. For the \( C_{max} \) method, we similarly substituted 0.010 gc/reaction and 0.047 gc/reaction (values calculated from the master standard curves using the assay’s maximum Ct of 45) as the target concentrations. For the \( C_{avg} \) method, nondetect values were simply dropped from \( N_1 \) and \( N_2 \) concentration calculations (and average concentrations of samples with no positive replicates were set to zero).

Figure 2. (A) Visualization of the connection graph showing all maintenance holes (MHs) in the City of Davis sewershed. Orange dots indicate all MHs upstream of a target MH (indicated as a red square). (B) Illustration of how the connection graph is used to probabilistically assign positive clinical-test results from census blocks to sewershed monitoring zones for the purpose of comparing trends in wastewater data to trends in clinical data. In the illustration, the sewershed monitoring zone covered by the sampler location at bottom and indicated in blue spans two census blocks. The census block on the left has a population of six and one positive test result; the census block on the right has a population of four and no positive test results. Tracking flow through the connection graph results in a predicted 0.33 infections captured by the sampler.
facilitate comparison of wastewater and clinical data, and the MATLAB “smoothdata” function was applied using a centered 7-day moving average.

**Probabilistic Assignment of Clinical Data to Sampling Zones.** All clinical data collected by HDT’s asymptomatic community-testing program since program inception were provided as an anonymized data set indicating the date that each test was administered, the ZIP code and census block corresponding to the testee’s address, and whether the test was positive. Use of these data was deemed exempt from IRB review by the University of California, Davis, IRB Administration. To compare clinical and wastewater data at the city/WWTP scale, we selected a subset of these data comprising all clinical-testing results for Davis ZIP codes (95616, 95617, and 95618). We designed a Python tool (available at https://tinyurl.com/Safford-et-al-Predictive) that combines information on municipal wastewater flows (provided as a File Geodatabase by the City of Davis Public Works Department) with U.S. Census Bureau data to probabilistically assign HDT asymptomatic testing results to sewershed monitoring zones (Figure 2A). Second, we used 2019 American Community Survey (ACS) data from the U.S. Census Bureau (USCB) to estimate the number of people living in each census block included in the HDT clinical-testing data set. We assume that each person in each census block produces the same amount of wastewater (a “unit”) each day, and that each person has an equal probability of discharging the wastewater unit to each MH located within the block. Finally, we used the connection graph to probabilistically assign positive clinical-testing results from census blocks to sewershed monitoring zones, as illustrated and explained in Figure 2B. Results from the probabilistic assignment are contained in SI Clinical model output.

### RESULTS AND DISCUSSION

#### Sample Number, Recovery Efficiency, and Nondetect Prevalence.** We analyzed 964 wastewater samples collected during the sampling campaign, comprising 77 samples from the COD WWTP, 695 from the subregional zones, and 191 from the building/neighborhood zones. φ6 was detected in 889/964 (92.2%) of samples (92.5% of samples processed using ultrafiltration and 92.2% of samples processed using bead-based concentration). Mean φ6 recovery was 1.31 ± 0.15% across all samples where detected, in line with values reported elsewhere.19 Per Kantor et al. (2021), we captured the recovery efficiency for each sample but did not attempt to use this value to correct the concentration data.14

At least one sample from each monitoring site and a total of 377 samples across all sites tested positive for SARS-CoV-2 (i.e., N1 or N2 above LOD in at least one technical replicate). Nondetect replicates were common even among positive samples; only 32 samples were positive for all N1 and N2 technical replicates. N1 and N2 nondetect percentages were similar and inversely proportional to sampling scale (Table S5). This suggests either that reliable detection of SARS-CoV-2 may become more challenging the further upstream in a sewershed that sampling is conducted or that nondetects are simply more frequent for smaller monitoring areas due to lower population sizes. Pepper mild mottle virus (PMMoV) nondetects were never observed. Because PMMoV serves as both an indicator of fecal strength and an internal control, consistent detection of PMMoV suggests that the high percentages of N1 and N2 nondetects are more likely attributable to frequently low abundance of SARS-CoV-2 in the wastewater samples rather than a systematic problem with the viral RNA extraction protocols used. We acknowledge, however, that different qPCR assays can behave differently in response to the same conditions. Confirmation of successful qPCR assays is supported by (1) inclusion of N1 and N2 positive controls for every qPCR run, and (2) the fact that samples yielding higher numbers of positive technical replicates also exhibited lower Cts on average for those replicates (Table S6)—that is, nondetects were more common when the target was present at lower concentrations. We did not observe any systematic correlation between PMMoV concentration and the number of N1 or N2 nondetects.

**EM-MCMC Model Performance.** Trace plots of posterior means generated by the EM-MCMC method over time showed good convergence. Trace plots of the MCMC samples exhibited no obvious patterns, indicating strong mixing of the Markov chains (Figure S2). Table 1 summarizes model output. The table shows that the number of positive replicates for a given sample exhibits a weak negative correlation with average standard deviations of imputed N1 and N2 mean Cts. This indicates that as the number of positive replicates increases, so too does the model’s confidence in its estimate of the “true” Ct. The table also shows that, as we would expect, the more positive replicates of a sample there are, the closer the average of those replicates is likely to be to the imputed mean Ct. The very large values for samples with zero positive replicates indicate that the model, having no information about those samples, simply defaults to the prior specifications placed on it.

**Comparison of Nondetect Handling Methods.** We used COD WWTP data to compare the EM-MCMC method with three other, commonly used methods for handling nondetects in wastewater qPCR data: LOD$_{0.5}$ (single
Handling Method

Normalized WWTP Virus Concentration, by Nondetect

between Community-Level Clinical Cases and Relative

method may not be as robust as alternatives, while also

Table 2. Spearman’s Rank-Order Correlation Coefficients

imputation with half the detection limit), Ct\text{max} (single

Figure 3. Community-level wastewater vs clinical data in Davis, showing effects of different methods of handling nondetects. Symbols represent individual sample results; lines represent trends (as centered 7-day moving averages for all data shown).

quantitative inspection yields a holistic assessment of how well

subcommunity trends in the clinical and wastewater do (or do not) match. Visual inspection enables rapid though subjective

identification of interesting features in the data. The Spearman

correlation analysis, on the other hand, provides a useful

objective framework for interpreting the data but suffers from

limitations. For instance, trends in clinical data collected from

subregional and building/neighborhood scales were frequently

fractional and near zero as a result. For these sampling zones

characterized by sparse positive data, the results of the

Spearman analysis can be significantly affected by only one

or several data points.

Despite these caveats, we find reasonably good agreement

between subcommunity clinical and wastewater data in certain

instances sampling zones at both the subregional and building/

neighborhood scales. Visual inspection shows that zones and
time periods exhibiting greater activity (i.e., more frequent
detections) in clinical data tended to also exhibit greater activity in wastewater data. Moreover, we generally observed much higher Spearman correlation coefficients for the 10 zones where wastewater surveillance began prior to the winter COVID-19 surge. This may be explained by greater activity (in the wastewater and clinical data alike) during the winter surge, as well as by the fact that sampling zones added later in the campaign were generally smaller—and hence, less active—than zones added earlier. The larger data sets available for zones where sampling began early also strengthen the robustness of data comparisons (as indicated by the universally low \( p \)-values of correlation coefficients for these zones). A notable exception to this trend is zone SR-G. We note that this zone largely comprises apartment complexes targeted at low-

considerably lower correlation when using the LOD_{0.5} method and a slightly stronger correlation when using the EM-MCMC method. These correlation coefficients suggest that the LOD_{0.5} method may not be as robust as alternatives, while also indicating the potential value of the EM-MCMC method.

Subcommunity Comparison of Clinical and Wastewater Data. Figure 4 coplots the clinical data and relative normalized virus concentrations (calculated using the EM-MCMC nondetect handling method) for each sampling zone. Table 3 presents the accompanying Spearman’s rank-order correlation coefficients. For these data, coupling visual and

imputation with the maximum qPCR cycle number), and

Ct\text{avg} (censoring nondetects entirely). Figure 3 coplots the community-level clinical data with the relative normalized SARS-CoV-2 concentrations calculated using each method. We see from this plot that while apparent relative normalized virus concentrations are similar when calculated using different nondetect handling methods, they are not the same. Toward the end of the study period, for instance, relative normalized virus concentrations calculated using the LOD_{0.5} method are generally higher than concentrations calculated using the other methods. There are also particular dates when one calculation method yielded much higher values than the other methods (e.g., December 9 for the EM-MCMC method, February 14 for the LOD_{0.5} method, April 20 for the Ct\text{max} method). We applied Spearman’s rank-order correlation to quantitatively assess how well the clinical-data trends match the wastewater-data trends for results obtained using each of the nondetect handling methods tested. The results (Table 2) show a

Table 2. Spearman’s Rank-Order Correlation Coefficients between Community-Level Clinical Cases and Relative Normalized WWTP Virus Concentration, by Nondetect Handling Method\(^\text{a}\)

| non-detect handling method | LOD_{0.5} | Ct_{max} | Ct_{avg} | EM-MCMC |
|---------------------------|-----------|-----------|-----------|----------|
|                           | 0.2175    | 0.5049    | 0.4337    | 0.5457   |

\(^{a}\)All correlations were highly significant (\( p < 0.01 \)).
income renters—a hard-to-count population\(^f\) that may have been underestimated in the USCB data used in this study.\(^{13}\) In multiple zones (e.g., BN-D, BN-E, SR-C, SR-E, and SR-I), even relatively small and isolated spikes in clinical data were
Detailed account of the document content:

- The document discusses the relationship between wastewater testing and clinical case rates, noting increased virus concentrations in wastewater samples during late winter/early spring.
- It highlights the importance of sampling frequency, which increased from November to late January, with weekly sampling during this period.
- The study notes staffing and lab-capacity constraints, limiting sampling frequency.

### Table 3: Spearman’s Rank-Order Correlation Coefficients between Clinical Cases and Relative Normalized WWTP Virus Concentration by Subcommunity Sampling Zone

| Subregional Zone | Correlation Coefficient (p-value) | Zone ID | Correlation Coefficient (p-value) |
|------------------|-----------------------------------|---------|-----------------------------------|
| SR-A             | 0.0199 (0.810)                    | BN-A    | -0.0871 (0.487)                  |
| SR-B             | -0.5986*** (0.000)                | BN-B    | -0.6087*** (0.000)               |
| SR-C             | 0.4793*** (0.000)                 | BN-C    | 0.8216*** (0.000)                |
| SR-D             | -0.0937 (0.509)                   | BN-D    | 0.5270*** (0.000)                |
| SR-E             | -0.6165*** (0.000)                | BN-E    | 0.3883*** (0.000)                |
| SR-F             | 0.4503*** (0.000)                 | BN-F    | 0.3753*** (0.000)                |
| SR-G             | -0.8113*** (0.000)                | BN-G    | -0.7583 (0.000)                  |
| SR-H             | -0.3691*** (0.004)                |         |                                   |
| SR-J             | 0.4067*** (0.000)                 |         |                                   |
| SR-K             | 0.3694*** (0.000)                 |         |                                   |
| SR-L             | 0.3782*** (0.000)                 |         |                                   |
| SR-M             | 0.5927*** (0.000)                 |         |                                   |
| SR-N             | 0.7220*** (0.000)                 |         |                                   |
| SR-O             | -0.3343** (0.025)                 |         |                                   |
| SR-P             | 0.3970*** (0.000)                 |         |                                   |

**Bolded rows indicate zones where wastewater surveillance began prior to the winter COVID-19 surge. p-values are in parentheses: *** p < 0.01, ** p < 0.05, * p < 0.1.**

- The table shows that correlations vary across different zones, with some zones exhibiting high positive or negative correlations, indicating a strong relationship between wastewater testing and clinical case rates.

Further analysis:

- It is noted that there were multiple explanations for mismatches between wastewater and clinical case rates, including changes in disease burden, water use, and movement of people.
- The study emphasizes the importance of sampling frequency, which was increased in late winter/early spring, making it easier to detect positive results.
- The study also highlights the challenges of staffing and lab capacity, which limited the frequency of sampling.

- The document concludes that there are multiple explanations for mismatches between wastewater and clinical case rates, and that further research is needed to better understand these relationships.

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The document discusses the relationship between wastewater testing and clinical case rates, noting increased virus concentrations in wastewater samples during late winter/early spring. The study highlights the importance of sampling frequency, which increased from November to late January, with weekly sampling during this period. The study notes staffing and lab-capacity constraints, limiting sampling frequency. The table shows that correlations vary across different zones, with some zones exhibiting high positive or negative correlations, indicating a strong relationship between wastewater testing and clinical case rates. Further analysis emphasizes the importance of sampling frequency, which was increased in late winter/early spring, making it easier to detect positive results. The document concludes that there are multiple explanations for mismatches between wastewater and clinical case rates, and that further research is needed to better understand these relationships.
Implications for Future WBE Deployment. In this study, we hypothesized that (i) conventional methods of handling qPCR nondetects could substantially bias apparent trends in wastewater data and that (ii) such bias could be minimized by instead using a combined expectation maximization-Markov Chain Monte Carlo (EM-MCMC) strategy to estimate nondetect values. We tested this hypothesis with data collected from November 2020–June 2021 at the City of Davis Wastewater Treatment Plant. Specifically, we compared trends in city/community-level clinical data to trends in WWTP data obtained using four different nondetect handling methods: single imputation with half the detection limit, single imputation with the maximum qPCR cycle number, censoring, and the EM-MCMC method. While results obtained using different nondetect handling methods were more similar than expected, they were not the same. This indicates the importance of specifying nondetect handling method in WBE studies. Though our methods comparison would need to be extended to additional sites to convincingly identify the optimal strategy for handling nondetects, Spearman’s rank-order correlation did show slightly stronger agreement between clinical and wastewater data examined, herein, using the EM-MCMC method. Refinements to the algorithm, tuning parameters, and variable groupings presented herein could further recommend this method for wastewater-data analysis in the future.

We also found that WBE can provide useful information about disease prevalence and trends at granular spatial scales. Visual and quantitative comparison of subcommunity-level data from a large, asymptomatic clinical-testing initiative in Davis, CA, with data from a parallel WBE campaign revealed significant correlations, especially in sampling zones for which greater numbers of data points were available and where COVID-19 burden was relatively high. Our results suggest that strategically geotargeted WBE could support pandemic response by, for instance, informing allocation of resources such as testing, personal protective equipment, and vaccination outreach. In addition, the predictive probability model we developed for spatially aligning clinical and wastewater data by wastewater-sampling zone provides a framework that can be easily extended to support similar analyses in other regions and communities.

We acknowledge two limitations of our work. First, some comparisons presented herein are incomplete because sampling zones were added over time. Only two of the seven sampling zones at the building/neighborhood scale, for instance, were active during the winter pandemic surge. Though this means that our results do not provide deep insight into the value of spatially granular wastewater surveillance during periods of peak disease spread, we note that wastewater surveillance is particularly valuable outside of such periods—for example, as an early warning system when background case levels are low. Second, we did not rigorously test the effect of different data groupings when running the EM-MCMC model. Though grouping data by sampling zone is a logical choice, it is possible that alternate groupings (e.g., grouping by sampling scale, grouping temporally, pooling results from adjacent sites, etc.), coupled with appropriate tuning of model parameters, could further improve model performance.

## CONCLUSIONS

- Because different methods of handling nondetects in wastewater qPCR data yield different results, researchers should specify nondetect handling method used in technical details of future studies.
- Preliminary evidence presented in this Article indicates that coupling the expectation maximization (EM) algorithm with the Markov chain Monte Carlo (MCMC) method to estimate “missing” qPCR values may yield better results than more common and less sophisticated nondetect handling methods. Further work is needed to validate this hypothesis.
- Privacy considerations mean that clinical test results for diseases like COVID-19 may only be available in aggregate, for example, at the census-block level. The predictive probability model presented, herein, is a useful general framework for researchers seeking to spatially align wastewater data from sewershed sampling sites with aggregate clinical data to compare trends.
- Results obtained by applying the predictive probability model to data from the Healthy Davis Together initiative in Davis, CA, show parallels between wastewater and clinical data at the subregional and building/neighborhood scales. These results further underscore the value of wastewater-based epidemiology as a public-health tool, suggesting that sewershed data could help inform allocation of resources (e.g., outreach, mobile testing/vaccination units, distribution of personal protective equipment) on hyperlocal scales.

## ASSOCIATED CONTENT

### Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.2c00053.

- Additional materials and methods, including information on qPCR assay, methods comparison, and sampling zone populations (PDF)
- Raw data and metadata from sample collection and analysis (XLS)
- Probabilistic assignments of clinical-testing results (XLSX)
- Raw data from methods comparison (XLSX)
- MIQE checklist (XLSX)

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https://doi.org/10.1021/acsestwater.2c00053
ACS ES&T Water XXXX, XXX, XXX--XXX
Author Contributions
H.B. and K.S. oversaw the study; M.K. tested and optimized qPCR assays; H.S. and R.Z.-M. performed lab work; X.L. and J.S. designed the predictive probability model; H.S. analyzed data, generated figures, and drafted the manuscript; R.Z.-M., M.K., J.S., K.S., and H.B. contributed text and edits to the final manuscript. All authors have given approval to the final version of the manuscript. CRediT: Hannah Safford conceptualization (lead), data curation (lead), formal analysis (lead), investigation (equal), methodology (equal), project administration (supporting), visualization (lead), writing-original draft (lead), writing-review & editing (supporting); Rogelio E. Zuniga-Montanez conceptualization (lead), data curation (supporting), investigation (equal), methodology (equal), project administration (supporting), validation (lead), writing-review & editing (supporting); Minji Kim methodology (equal), validation (lead), writing-review & editing (supporting); Xiaoliu Wu formal analysis (equal), methodology (equal), software (lead); Lifeng Wei formal analysis (equal), methodology (equal), software (lead); James Sharpnack formal analysis (equal), methodology (equal), software (lead), supervision (lead), writing-original draft (supporting); Karen Shapiro conceptualization (supporting), funding acquisition (supporting), project administration (equal), resources (supporting), supervision (lead), writing-review & editing (supporting); Heather N. Bischel conceptualization (lead), funding acquisition (lead), methodology (equal), project administration (lead), resources (lead), supervision (lead), writing-review & editing (lead).

Funding
Funding to support this project was generously provided by Healthy Davis Together.

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS
We acknowledge the invaluable contributions of the following individuals: L. Rueda, C.W. Bess, R. Pechacek, and M. Clauzel for lab support; N. Krasner for data cleanup and entry; S. Gryzcko, A. Livingston, S. Macomb, and J. Miller for coordinating sample collection; M. Nuño for input on modeling and statistical analysis.

ADDITIONAL NOTES
“The method can accommodate other types of groupings—for example, by sampling scale.

In Bayesian inference, the prior probability distribution (prior) is the probability distribution that would express one’s beliefs about the system before some evidence is taken into account.

A hyperparameter is a parameter used only to influence the learning behavior of a model. Hyperparameter values are not derived from training or experimental data. By contrast, parameters are values determined by the model from analyzing input data.

Though normalization strategies for WBE are still being actively researched, PMMoV normalization is a common current practice to adjust for variability in sample fecal load. D’Aoust et al. (2021) found that PMMoV normalization yielded better results than normalization using alternative indicators, such as Bacteroides 16S rRNA or human 18S rRNA.

UC Davis also conducts a testing program open only to UC Davis students and employees. Data from this program were not included in the data set used for this study for two reasons: (i) the UC Davis testing program is mandatory while the community testing program was not, meaning that inclusion of data from both programs would be likely to result in overrepresentation of UC Davis results, and (ii) a significant percentage of the UC Davis student population lives in on-campus housing. Wastewater from on-campus housing is captured by a sewer system that is entirely separate (including the destination treatment plant) from the community sewer system monitored for this study.

The U.S. Census Bureau has found that the net undercount rate for people living in rental housing is nearly double the net undercount rate for people living in owner-occupied housing (1.1% vs 0.6%). According to the Bureau, furthermore, “households in poverty are traditionally very hard to count.”

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