



Epidemiological Modeling of SARS-CoV-2 in Wastewater

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Wastewater epidemiology can be used to monitor sewersheds for various disease-causing organisms and various chemicals of interest which can aid public health assessment (Weidhaas, et al., 2021). In this study wastewater from Utah sewersheds was monitored for the virus causing COVID-19, namely SARS-CoV-2. Detection of SARS-CoV-2 in feces occurs as a consequence of the virus infecting cells in the gastrointestinal tract (Weidhaas, et al., 2021). After individuals who are ill with COVID-19 shed SARS-CoV-2 virus in their feces to the sewer system, the wastewater travels to a centralized wastewater treatment plant which can be sampled. The collection, processing, and analysis of wastewater samples containing SARS-CoV-2 may provide information that can inform public health managers on how to respond to potential outbreaks and loading of healthcare facilities. These samples are collected from wastewater treatment plants, water reclamation facilities, and sewers to be processed in a laboratory environment. Incubation, filtration, RNA extraction for nucleic acid quantification, and RT-qPCR quantification give an indication of how the sewershed is being impacted by the spread of COVID-19.

Samples of wastewater potentially containing SARS-CoV-2 were collected from influent wastewater streams before entering the treatment facilities. The wastewater samples were transported to the laboratory inside biohazard containers surrounded by ice by Utah Department of Environmental Quality staff. Sample processing was conducted in a laboratory environment at the University of Utah in the Department of Civil and Environmental Engineering. The samples were dropped off, taken into the laboratory environment, and immediately incubated at 65°C for 1 hour. After the wastewater samples were pasteurized, the samples were decanted into sterile centrifuge tubes to be spun for 20 minutes at 3000 rpm for solids separation. The supernatant was decanted into sterile glassware and acidified to a pH of 3.3-3.5. Once acidification was complete, the samples were filtered through 45µm sterile filters to concentrate capture the virus onto the papers. These filter papers were placed inside new, sterile centrifuge tubes and stored in a laboratory freezer for ribonucleic acid (RNA) extraction. The remaining wastewater samples were sterilized and disposed of, and the remaining acidified wastewater filtrate were disposed into hazardous waste storage. RNA extraction was performed on the concentrated filter papers to isolate the SARS-CoV-2 RNA for concentration quantification. Lastly, the concentrated RNA was quantified further using an RT-qPCR machine to relatively determine how the virus spread was going to affect a population and healthcare system within the location.

Quantification using laboratory methods is helpful to understand the effect of the virus on a population and location. Further statistical modeling of the SARS-CoV-2 virus in wastewater is critical to analyze the methodology of wastewater epidemiology. Data on the following parameters were collected: Clinical cases from the Utah Department of Health, average SARS-CoV-2 gene copies/mL and millions of viral gene copies/day/capita from wastewater samples collected by the Utah Department of Water Quality from Salt Lake City Water Reclamation

Facility (SLCWRF, n = 24) and Central Valley Water Reclamation Facility (CVWRF, n = 41/n = 40), and influent parameters from CVWRF. Influent parameters are the measure of quality, quantity, and concentration of wastewater before it flows into a water treatment facility. The parameters that were analyzed include pH, total biological oxygen demand (mg/L), SCBOD (mg/L), carbonaceous biological oxygen demand (mg/L), total suspended solids (mg/L), NH₃ (mg/L), temperature (°C), and chemical oxygen demand (mg/L). The data were analyzed by Pearson's Product-Moment correlation, time series, and lagged Pearson's correlation analyses. The study found no correlation between wastewater data and the concentrations of the virus and wastewater influent parameters (Table 1 and 2). Furthermore, clinical cases decrease with SARS-CoV-2 concentrations in wastewater (Figure 1). Although presence of SARS-CoV-2 in wastewater follow the trend of clinical cases as they were reported during this study and the method of wastewater epidemiology is helpful to track asymptomatic and pre-symptomatic individuals, a long-term study to track this trend could be important for further research. Time series analysis (7-day interval) shows that the cases in the SLCWRF sewershed as a function of time follow the behavior of the wastewater concentrations of the virus, and a lagged correlation analysis does not contribute to the method validation.

The correlations found between the clinical cases of SARS-CoV-2 and wastewater data containing SARS-CoV-2 were found to be insignificant and small, and the lagged correlation analysis does not contribute to the method analysis for wastewater epidemiology. The time series analysis performed on cases in the SLCWRF sewershed increased as concentrations of the virus in the wastewater streams increased. Further statistical analyses may consider the loss of viral abundance in wastewater due to physical constraints like temperature, degradation while moving through pipe systems, and viral decay over time as well as how wastewater epidemiology could be used as an early warning system to predict impact on vulnerable populations and healthcare systems (Hart O. E., et al., 2020).

Appendix

Tables

Table 1: Utah Department of Health clinical cases were correlated with wastewater data collected from Salt Lake City Water Reclamation Facilities using Pearson's Product-Moment correlation.

| SLCWRF sewershed correlations (r-values p value, n = 24) | |
|--|--|
| 3-day average cases vs. gene copies/mL | 3-day average cases vs. MVGC/day/capita |
| -0.09/NS* | -0.08/NS* |
| 7-day average cases vs. gene copies/mL | 7-day average cases vs. MVGC/day/capita |
| -0.17/NS* | -0.16/NS* |
| 14-day average cases vs. gene copies/mL | 14-day average cases vs. MVGC/day/capita |
| -0.23/NS* | -0.23/NS* |
| Lagged cases vs. gene copies/mL | Lagged cases vs. MVGC/day/capita |
| -0.14/NS* | -0.14/NS* |
| Cases vs. gene copies/mL | Cases vs. MVGC/day/capita |
| -0.11/NS* | -0.11/NS* |

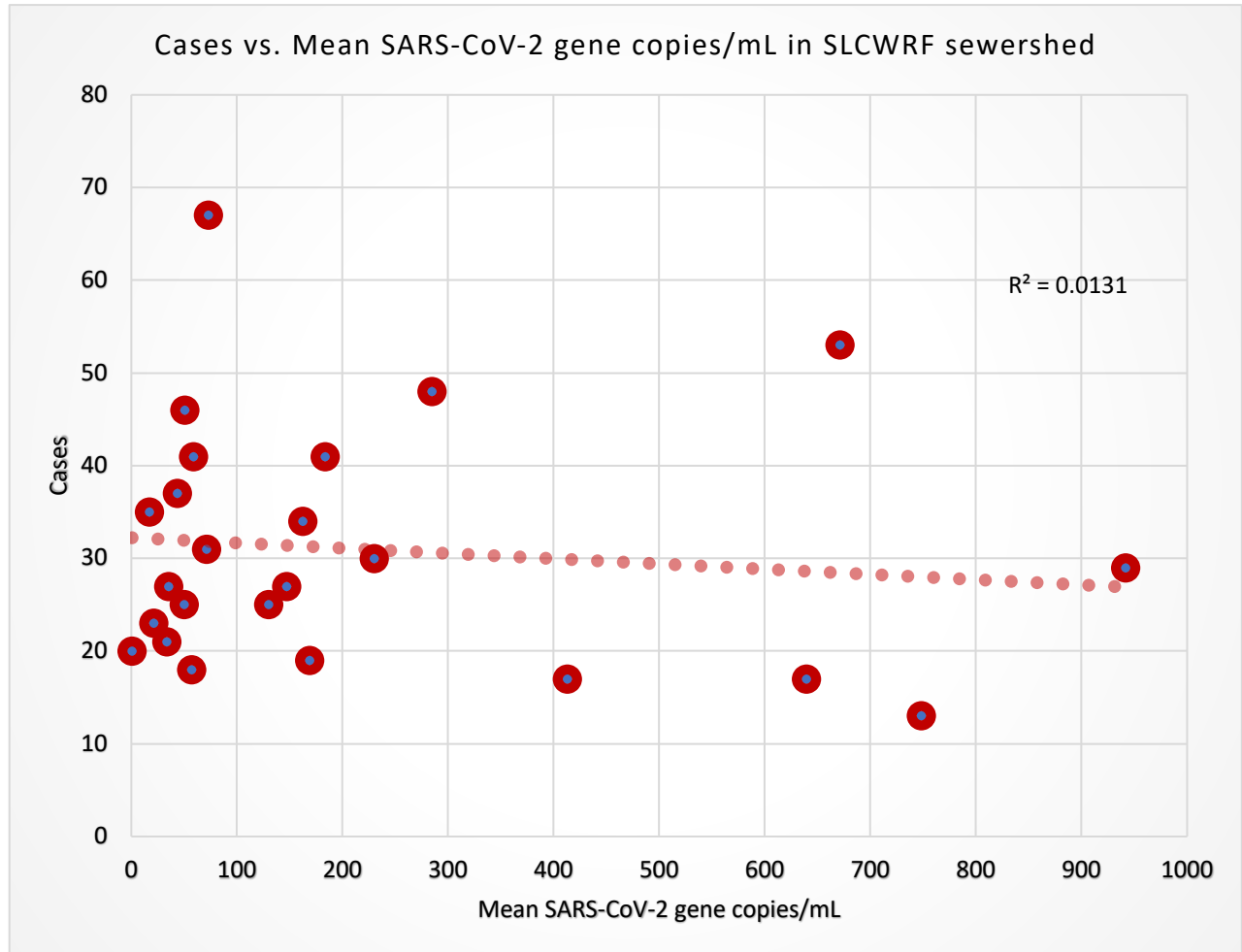
*NS = not significant

Table 2: Wastewater influent parameters were correlated with wastewater data from Central Valley Water Reclamation Facility using Pearson's Product-Moment correlation.

| CVWRF influent parameter correlations (r-values/p value, n = 41 and n = 40) | |
|---|--------------------------------|
| Mean SARS-CoV-2 gene copies/mL vs. pH | MVGC/day/capita vs. pH |
| 0.04/0.84 | 0.02/0.88 |
| Mean SARS-CoV-2 gene copies/mL vs. TBOD mg/l | MVGC/day/capita vs. TBOD mg/l |
| 0.17/0.31 | 0.15/0.36 |
| Mean SARS-CoV-2 gene copies/mL vs. SCBOD mg/l | MVGC/day/capita vs. SCBOD mg/l |
| 0.13/0.37 | 0.14/0.39 |
| Mean SARS-CoV-2 gene copies/mL vs. cBOD mg/l | MVGC/day/capita vs. cBOD mg/l |
| 0.22/0.16 | 0.21/0.19 |
| Mean SARS-CoV-2 gene copies/mL vs. TSS mg/l | MVGC/day/capita vs. TSS mg/l |
| 0.20/0.24 | 0.18/0.27 |
| Mean SARS-CoV-2 gene copies/mL vs. NH3 mg/l | MVGC/day/capita vs. NH3 mg/l |
| 0.20/0.26 | 0.17/0.30 |
| Mean SARS-CoV-2 gene copies/mL vs. Temp °C | MVGC/day/capita vs. Temp °C |
| -0.002/0.95 | 0.02/0.91 |
| Mean SARS-CoV-2 gene copies/mL vs. COD mg/l | MVGC/day/capita vs. COD mg/l |
| 0.06/0.66 | 0.06/0.66 |

Figures

Figure 1: Utah Department of Health clinical cases were plotted with wastewater data collected from Salt Lake City Water Reclamation Facilities.



References:

- Hart, O. E., & Halden, R. U. (2020). Computational analysis of sars-cov-2/covid-19 surveillance by wastewater-based epidemiology locally and globally: Feasibility, economy, opportunities and challenges. *Science of The Total Environment*, 730, 138875. <https://doi.org/10.1016/j.scitotenv.2020.138875>
- Weidhaas, J., Aanderud, Z. T., Roper, D. K., VanDerslice, J., Gaddis, E. B., Ostermiller, J., ... LaCross, N. (2021). Correlation of SARS-CoV-2 RNA in Wastewater with Covid-19 disease burden in sewersheds. *Science of The Total Environment*, 775, 145790. <https://doi.org/10.1016/j.scitotenv.2021.145790>