Poster # 1
Title: In-pipe flow monitoring in the context of a wastewater-based epidemiology project: methods and applications
Author: William Shuster, James Hartrick, Carrie Turner, Jeffrey Ram
Contact: wshuster@wayne.edu

We have synoptically monitored over the long-term both flow and SARS-CoV-2 viral concentration in urban sewersheds. Flow can be used as a normalization factor to re-scale viral loadings in a given sewershed. We will present on actual monitoring hardware and procedures, calculations, and applications of flow data in this project context.

Poster # 2S

Title: Go With the Flow for Wastewater Data Normalization: Comparing Normalized and Non-Normalized SARs-CoV2 Viral Load and Physiochemical Parameters in Muskegon and Ottawa Counties

Author: Renee Tardani, Alexis Porter, Richard Rediske, Muskegon Resource Recovery Center
Contact: tardanir@mail.gvsu.edu

Wastewater is a complex, ever changing matrix that is reflective of the population utilizing the catchment under evaluation. SARS-CoV-2 RNA residing within wastewater has been extracted and analyzed in droplet digital polymerase chain reaction (ddPCR) to estimate viral incidence trends in a population. Flow rate data has been seen by the CDC as an approved variable to normalize ddPCR wastewater surveillance data which may help assess relationships between clinical cases and viral load in SARs-CoV-2 monitoring. Variables such as flow rate can provide accurate quantification and allow for further statistical analyses of wastewater viral load. Wastewater flow rate data is collected routinely by wastewater treatment plants (WWTP) therefore is a readily available data point for viral RNA concentration. We utilized historic flow rate data to normalize SARs-CoV-2 RNA concentrations from wastewater and explored how data normalization impacts viral load trends for west Michigan sites by doing a comparative trend analysis of the normalized and non-normalized datasets. We then used additional physiochemical parameters routinely gathered by WWTP's to assess potential inhibitors or anomalies that may arise from our unique septic systems and how these results interrelate with molecular evaluation and data normalization. The results indicated that flow data was informative for data normalization compared to non-normalized data. We explored trend relationships between our normalized and non-normalized viral load to a subset of data with physicochemical parameters such as total suspended solids (TSS), conductivity, biochemical oxygen demand (BOD), phosphorus, ammonia, and dissolved metal concentrations for Muskegon Resource Recovery Center, Muskegon County's WWTP. WWTP collaboration utilizing flow and chemical parameters has allowed for the status of our sample matrix to be measured more accurately and assisted troubleshooting sources of inhibition when we compared viral load to flow.

Poster # 3S
Title: Determining Sampling Frequency in the Neighborhood Level Wastewater Surveillance of SARS-CoV-2
Author: Qiuyang Zhang, Anna Kilts, Gina Kittleson, Felipe de Paula Nogueira Cruz, Xavier Jenkins, Kevin Bakker, Michelle Ammerman, Steve Wright, Marisa Eisenberg, and Krista Wigginton
Contact: qiuyang@umich.edu, mammer@umich.edu

Wastewater surveillance is a useful and cost-effective method for monitoring SARS-CoV-2 transmission, without dependence on clinical diagnostic testing. Compared with community level wastewater surveillance, monitoring at the neighborhood level has larger variability due to smaller population size, individual shedding characteristics, and variations in sewage flow dynamics. Determining optimal sampling frequency in the wastewater sample collection process is necessary to ensure all shedding events are captured in the sampling process. To determine the optimum sampling frequency for autosampler grabs, we performed tracer studies using both rhodamine dye and pasteurized bovine coronavirus (BCoV) from 3 different injection sites to a common detection site. BCoV monitoring was assessed to determine if enveloped viruses have a higher adherence to solids, which could alter flow dynamics, compared to dye tracers. Dye flow rates were monitored continuously using a fluorometer and BCoV flow rates were monitored using RNA extraction and digital droplet PCR in pooled grab samples. The mean travel time and time for 90% of dye detection (dispersion) were measured multiple times from all sites. Results suggest buildings with sufficient flow will have similar flow rates primarily determined by dynamics of the primary sewer line. Buildings with low flow will have more variable, building-specific flow rates. Overall, for a given injection site the mean travel time and tracer dispersion were comparable between dye and BCoV. However, there were tests that showed a delay in travel times and increased dispersion for BCoV, likely the result of impeded solids in low flow sewer areas. Our results suggest that dye tracers can be effective for modeling viral flow in sewers, however flow dynamics in building lines can have significant influence on overall flow rates and thus individual buildings should all be tested to ensure events from those locations are factored into determining sampling frequency.

Poster # 4

Title: Which fecal marker should be used for fecal normalization of wastewater pathogens?

Author: Md Alamin, Pelumi Oladipo, James Hartrick, Azadeh Bahmani, Carrie L. Turner, William Shuster, and Jeffrey L. Ram
Contact: alamin081@wayne.edu

Background: Wastewater epidemiology uses the amount of pathogen markers collected at treatment plants or, via manholes, from smaller sewersheds. However, the concentration of pathogen markers will be affected not only by the number of infected people in the sewershed but also by dilution with water for non-toilet personal (e.g., showers, laundry, dishwashing) and business uses and in combined sewer systems, by precipitation. The concentration of fecal matter in the sample can be used to “normalize” the pathogen measurement to the number of people inputting feces to the system. But what is the best measure of the amount of fecal matter? CDC indicates HF183 (human-associated Bacteroides) or PMMoV virus might be used; others have normalized with CrAssphage. E. coli is another indicator of fecal contamination. Methods: Sewershed grab samples were diluted 1:1000 and analyzed by duplex ddPCR for HF183 and CrAssphage; by ddRT-PCR for PMMoV; and E. coli by Quantitray-2000. Results: Values averaged (copies/100 mL, geometric mean of 9 sites) 2.39 x 10^7 for HF183, 1.68 x 10^7 for CrAssphage, and 6.52 x 10^6 for PMMoV; E. coli averaged 1.21 x 10^6 cfu/100 mL. Correlations between methods exhibited R^2 values as high as 0.7 (HF183 v CrAssphage). Ratios of PMMoV to HF183 and CrAssphage were fairly consistent, except that a sewershed in southwest Detroit consistently had ratios averaging 3 times higher than other sewersheds. E. coli correlated best with PMMoV (R^2 near 0.6). Conclusion: HF183 is accepted by CDC and correlates well with CrAssphage; ddPCR is faster and less costly than ddRT-PCR. PMMoV is used by more labs. E. coli Quantitray cost less and can be run simultaneously with SARS-CoV-2 ddRT-PCR. If E. coli is a good measure of total fecal material, it would be preferred. Otherwise, HF183 and CrAssphage by duplex ddPCR measurement seems best.

Poster # 5S
Title: Pepper Mild Mottled Virus as a Monitoring Tool in Wastewater Based Epidemiology
Author: Taylor Segorski, Ashley Thompson, Olatubosun Oyewole, Jack Ruhala, Zane Walters, Puneet Chowdary, Pei-Lan Tsou, Sheila Blackman
Contact: segorskt@mail.gvsu.edu,tsoup@gvsu.edu

Our lab has utilized Wastewater Based Epidemiology (WBE) to monitor SARS-CoV-2 infections in Kent County since the fall of 2020. Any variability in human fecal input in the samples confounds accurate prediction of infection levels and trends using WBE data. Therefore, normalization of wastewater for human fecal input, especially where it varies widely, has emerged as an urgent challenge to improve WBE's utility. Pepper Mild Mottle Virus (PMMoV) is a pathogenic plant virus that is abundant in human fecal matter and harmless to humans. This RNA virus co-purifies with SARS-CoV-2, and thus is ideally suited to normalize counts of this virus for human fecal matter. We developed a reliable and low-cost method to monitor PMMoV in wastewater via qPCR (Taqman) targeting the evolutionarily conserved replicase gene. We found PMMoV concentrations in samples from large Wastewater Treatment Plants to be relatively constant over time whereas concentrations in wastewater samples obtained from manholes were much more variable. Furthermore, PMMoV can be used to identify outliers in fecal content at sample sites and to determine whether sampling variables influence viral RNA recovery from wastewater. Our work demonstrates the need for minimizing variability when sampling for WBE and we conclude that PMMoV is a useful human fecal marker with which to normalize RNA virus concentrations in wastewater and to optimize sampling methodology.

Poster # 6S
Title: Can Fecal Indicator Targets and Flow Normalize Wastewater Insights?
Author: Nicholas Castle, Josie Kuhlman, Michael Vu, John J. Hart, Megan Jamison, David C. Szlag
Contact:ncastle@oakland.edu

During the COVID-19 pandemic, wastewater-based epidemiology (WBE) was used, targeting SARS-CoV-2 viral targets to assess and contain outbreaks at a community wide scale. Current efforts focus on creating models to extrapolate clinical COVID cases from SARS-CoV-2 viral targets signal in wastewater. To facilitate this, most models use a combination of population, daily flow, and the aid of other molecular targets to normalize wastewater fluctuations due to inflow and infiltration (I&I) to normalize viral wastewater signal to correlate with case numbers. Current literature suggests that a variety of DNA and RNA based molecular targets combined with flow can facilitate I&I normalization. Based on the literature, we hypothesized that fecal indicator markers that correlate to flow (PMMoV and HF183) can be used to normalize wastewater data to account for these fluctuations. This study used flow and population data at 6 different sewer sheds in Clinton Township, Michigan where flow ranged from 0.14MGD to 3.09MGD. Data was collected from November 14, 2022, to January 16, 2023. Our data showed little correlation between the fecal indicator markers and flow. This might be due to lower range of flow at these sites compared to other studies. Results suggest that fecal indicator markers combined with flow are unable to act as normalization factors at these sites. This highlights the importance of reviewing literature derived normalization factors prior to implementing them into a normalization model.

Poster # 7
Title: Intelligent Analytics Enables Proactive Management of COVID-19 Hotspots
Author: David Inman
Contact:david@aquasight.io

Aquasight's goal is to provide effective wastewater monitoring solutions for communities and neighborhoods. To achieve this, we developed a state-of-the-art Smart Wastewater Monitoring Platform and tailored our approach to meet the unique needs of each community.

David has extensive experience working with local authorities, businesses, and residents. We engage with them effectively to identify sources of wastewater contamination, track changes in water quality, and provide actionable insights.

Our platform provides real-time data visualization, enabling clients to monitor their wastewater systems in real-time, identify issues quickly, and take proactive measures to prevent contamination. The platform has been approved by leading academic institutions, Michigan State University, Eastern Michigan University, and Oakland University, demonstrating the quality of our technology and expertise.

Our success stories showcase our ability to identify sources of wastewater contamination and provide actionable insights to clients. The tailored approach, combined with our platform, has enabled us to achieve the highest accuracy of wastewater monitoring.

Poster # 8

Title: Surveillance of SARS-CoV-2 in nine neighborhood sewersheds in Detroit Tri-County area, United States: assessing per capita SARS-CoV-2 estimations and COVID-19 incidence

Author: Yabing Li, Brijen Miyani, Liang Zhao, Maddie Spooner, Zach Gentry, Yangyang Zou, Geoff Rhodes, Hui Li, Andrew Kaye, John Norton, Irene Xagoraraki
Contact:liyabing@msu.edu

Wastewater-based epidemiology (WBE) has been suggested as a useful tool to predict the emergence and investigate the extent of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In this study, we screened appropriate population biomarkers for wastewater SARS-CoV-2 normalization and compared the normalized SARS-CoV-2 values across locations with different demographic characteristics in southeastern Michigan. Wastewater samples were collected between December 2020 and October 2021 from nine neighborhood sewersheds in the Detroit Tri-County area. Using reverse transcriptase droplet digital polymerase chain reaction (RT-ddPCR), concentrations of N1 and N2 genes in the studied sites were quantified, with N1 values ranging from 1.92 x 102 genomic copies/L to 6.87 x 103 gc/L and N2 values ranging from 1.91 x 102 gc/L to 6.45 x 103 gc/L. The strongest correlations were observed with between cumulative COVID-19 cases per capita (referred as COVID-19 incidences thereafter), and SARS-CoV-2 concentrations normalized by total Kjeldahl nitrogen (TKN), creatinine, 5-hydroxyindoleacetic acid (5-HIAA) and xanthine when correlating the per capita SARS-CoV-2 and COVID-19 incidences. When SARS-CoV-2 concentrations in wastewater were normalized and compared with COVID-19 incidences, the differences between neighborhoods of varying demographics were reduced as compared to differences observed when comparing non-normalized SARS-CoV-2 with COVID-19 cases. This indicates when studying the disease burden in communities of different demographics, accurate per capita estimation is of great importance. The study suggests that monitoring selected water quality parameters or biomarkers, along with RNA concentrations in wastewater, will allow adequate data normalization for spatial comparisons, especially in areas where detailed sanitary sewage flows and contributing populations in the catchment areas are not available. This opens the possibility of using WBE to assess community infections in rural areas or the developing world where the contributing population of a sample could be unknown.

Poster # 9S
Title: Comparing Normalization Methods for SARS-CoV-2 in Wastewater
Author: Hsinhui Hua, Vanessa Slack, Julie Gilbert, Michelle Ammerman, Krista Wigginton, and Marisa Eisenberg
Contact: hsinhui@umich.edu, mammer@umich.edu

Wastewater surveillance has emerged as an important tool for tracking the prevalence of SARS-CoV-2 in communities since the COVID-19 pandemic began. However, selecting an appropriate normalization method to account for variation in wastewater volume and population size remains a challenging issue. To address this issue, this study compared the performance of different normalization methods for SARS-CoV-2 detection in wastewater, including flow rate, population-flow rate, PMMoV, and volume. The results of this study indicate that flow rate, population-flow rate, and PMMoV methods are highly correlated with volume normalization. Furthermore, the Spearman correlations between the 7-day rolling average of the four different normalization methods and clinical cases were generally high, indicating that all of these methods are useful for tracking the prevalence of SARS-CoV-2 in wastewater. PMMoV-normalized concentration correlations with clinical cases decreased more substantially during times when clinical testing was low compared to other normalization methods. The study also found that the correlations between wastewater data and clinical cases were generally lower in Tecumseh, the smallest sewershed. Overall, these findings suggest that consideration should be given when selecting a normalization method for SARS-CoV-2 detection in wastewater. PMMoV is shown to be a useful method in certain contexts. Flow rate normalization can be used as a reliable alternative to population-flow rate normalization in the absence of real-time population data. Future studies are needed to identify the best normalization method for SARS-CoV-2 surveillance in wastewater.

Poster # 10

Title: Wastewater Monitoring of SARS-CoV-2 at Building Level on a University Campus for COVID-19 Outbreak Assessment

Author: Jianfeng Wu, Xin Li, Thu Le, Olivia Yancey, Christopher Breen, Yili Wang, Peter Song, Joseph Dvonch, Richard Neitzel, Alfred Franzblau, Julie Gilbert, Marisa Eisenberg, Chuanwu Xi*
Contact: wujian@umich.edu, sherli@umich.edu

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to 760 million infections and 6.87 million deaths globally. Early reports that SARS-CoV-2 RNA was detectable in wastewater inspired interest in wastewater-based epidemiology (WBE) as a tool for assisting in the mitigation of COVID-19 outbreaks in a large community. This study investigates the potential of wastewater-based epidemiology (WBE) as a tool for early detection and monitoring of COVID-19 outbreaks on the University of Michigan Ann Arbor campus, a relatively small community with a substantial population of students living in on- and off-campus dormitories. Surveillance was conducted at the building level and covered the period of different policies/stages implemented on campus. Though the correlation at individual building levels varies, the SARS-CoV-2 RNA concentrations in wastewater were highly correlated with a COVID-19 epidemic on the whole campus with a leading period of several days. High residential occupancy and frequent sampling were also found to be critical for more accurate predictions. These findings suggest that WBE can function as an early warning system for COVID-19 outbreaks and can complement existing public health strategies in managing pandemics within universities and other small-community facilities.

Poster # 11
Title: Processing more than 100 samples per week and delivering results the same day: an overview of Hope College's wastewater monitoring process
Author: Daniel Wade1, Adam Slater1, Lauren Cribbs1, Celia Kun-Rodrigues1, Vanessa Kardian1, Dylan Gladysz1, Allaire Schneider1, Grace Goszkowicz1, Louis Kopp1, Benjamin G. Kopek1, Michael J. Pikaart2, Brent P. Krueger2, Aaron A. Best1
Contact: dwade@hope.edu

Wastewater surveillance of SARS-COV-2 has been useful for decision making both at the campus and community levels. In order for the state, our college, and other clients to make best use of our data, we have developed a fast and robust workflow incorporating the 4S method for RNA isolation and qPCR for initial molecular detection that has allowed us to test and report data within 24 hours of receiving the samples, and in some cases within 24 hours of collection. Currently, we monitor approximately 60 unique sampling sites that encompass a range of population sizes. From individual dorms on our campus up to wastewater treatment plants that comprise an entire city, we are able to successfully collect, test, and provide data to inform decision makers about COVID levels in different sized populations. The sites are made up of 17 wastewater treatment plants, 24 sub-sewershed manhole access points (within Holland, Zeeland, and Kalamazoo), 7 assisted living facilities, 5 manholes on Hope's campus, and 5 sewer cleanouts on campus. Our workflow also allows room for some variant testing, sequencing, and the development of passive sampling methods. From 5/20/2021 through 3/21/2023, we have processed and provided data for ~10,000 samples via our online Power BI-based dashboards. We have provided variant data for ~2,800 samples and have sequenced RNA from 47 samples. The passive sampling methods we have been able to test have been compared side by side with autosampler data and have proven to be just as sensitive with less cost per sample and no increase in processing time.

Poster # 12S
Title: A Nested Approach to Identifying Ferris Campus SARS CoV-2 Hotspots
Author: Cameron Priebe, Heather LeFaivre, Ella Connors, Haley Berg, Bailey Copeland, Kassidy Vredeveld, Clifton Franklund, Mary Zimmer, Sky Pike
Contact:priebec2@ferris.edu,skypike@ferris.edu

The sewer system of the Ferris State University campus, west of State Street in Big Rapids, Michigan, is designed in a nested fashion. The sewage of each building flows into the main sewer line with West Campus Apartments being furthest upstream. The Composite 3 sample can be found midway and adds wastewater affluent from a dormitory, classroom building, and public safety. Composite 4, being furthest downstream, includes three additional dormitories. The nested sewage system, accordingly, allows for more precise localization of SARS CoV-2 infections to specific groups of buildings, eliminating the need to test more sample sites individually.

Poster # 13
Title: SARS-CoV-2 Wastewater Monitoring on University Campuses: A Comparative Study on Effective Intervention Strategies for Public Health Guidance
Author: Alexis M. Porter, John J. Hart, Richard R. Rediske, David C. Szlag
Contact: porteal@gvsu.edu

Wastewater surveillance has proven to be a vital tool to provide actionable information to public health officials during the COVID-19 pandemic. This information was especially important for our university institutions due to the suggested heightened presence of pre-symptomatic/asymptomatic cases among the student community sharing living spaces. Through the collaborative efforts of affiliated MiNET universities and participating laboratories, many institutions across the state of Michigan were provided with standardized high quality wastewater insights to complement clinical case efforts. During the onset of COVID-19, universities formed pandemic response committees composed of professors, public health professionals, and other vested parties to facilitate safe learning for students. These committees met on a regular basis to analyze both forms of data, wastewater data and clinical case data, and proposed a variety of mitigation strategies to limit the spread of COVID-19. In this project, we compared wastewater monitoring data for SARS-CoV-2 RNA, clinical case data from university response teams, and mitigation strategies from Grand Valley State University (population 21,648 students) and Oakland University (population 16,108 students) from 11/2020-04/2022. Wastewater positivity rates for both universities ranged from 32.8-46.8%. Peak viral signal for both universities directly corresponded to variant point of entry within the campus populations from 2021-2022. Response and tactics were dependent on the institution and had varying levels of success. Ultimately the majority of response tactics were effective at mitigating the spread of COVID-19 at university institutions. Identifying strategies that were most effective at mitigation is important in preparation for future epidemics. While both institutions had effective strategies at mitigating the spread, our results highlight key differences in clinical case data organization and university public health responses from this information. These highlights offer direction into navigating on-going surveillance and future epidemic response strategies for maximum efficiency in securing student, staff, and faculty health outcomes.

Poster # 14S
Title: Ferris State Campus SARS CoV-2 Trends from November 2020 to April 2023
Author: Allison Patterson, Kendra Hincka, Juliana Carey, Camryn Lowe, Rachel Smith, Kassidy Vredeveld, Clifton Franklund, Mary Zimmer, Sky Pike
Contact:pattea16@ferris.edu,skypike@ferris.edu,

SARS CoV-2 levels for Ferris State University have been monitored in wastewater twice a week from November 2020 to present. Droplet Digital PCR (ddPCR) has been utilized to quantitate SARS CoV-2 in wastewater from select sites within the University demonstrating the SARS CoV-2 campus load. This information provided a preliminary tool to focus campus messaging efforts and clinical SARS CoV-2 testing.

Poster # 15S
Title: SARS-CoV-2 Wastewater Surveillance
Author: Tyler Chlystek
Contact: chlystet@mail.gvsu.edu
Introduction: The COVID-19 pandemic has had a profound influence on public health entities in Michigan over the past 3 years. To gain a better understanding of how the virus affects the population, wastewater surveillance was adopted by public health departments to help quantify the amount of viral RNA present within the population. Wastewater surveillance may offer the ability to track the spread of infection in an effort to keep cases to a minimum, while simultaneously testing for asymptomatic carriers. Since wastewater surveillance can provide data within a couple hours, questions arise on how wastewater data be used to indicate immediate public health action.

Methods: Wastewater data collected by Grand Valley State University's Annis B. Water Resources Institute and COVID case count data from the Department of Public Health Muskegon and Ottawa County will be retroactively cross examined.

Findings: Cross-examination of the data revealed that as viral COVID RNA concentrations increase in the wastewater, so do the number of COVID clinical cases experienced for that given location. When evaluating vaccination data, it was found that vaccines may temporarily increase the amount of viral RNA in wastewater, while simultaneously decreasing the number of confirmed cases.

Discussion: This research can be utilized to further emphasize the importance of vaccines and conducting wastewater surveillance. By conducting surveillance on populations rather than individuals, immediate public health action can be adopted to reduce the level of infection for the larger population. Wastewater data can additionally be used to identify variants, which may affect vaccine evasiveness and efficacy along with symptom onset. Knowing this, health departments can use this information to ensure education and intervention to the community proper action can be taken by the individual based on health department guidance.

Poster # 16

Title: Effectiveness of SARS-CoV-2 Wastewater Monitoring in Rural and Small Metro Communities in Central Michigan

Author: Michael J. Conway, Stephanie Kado, Breanna K. Kooienga, Jacklyn S. Sarette, Michael H. Kirby, Andrew D. Marten, Avery S. Ward, Jackson D. Abel, Steve King, Jacqueline Billette, Elizabeth Braddock, Maggie R. Williams, Rebecca L. Uzarski, and Elizabeth W. Alm
Contact:michael.conway@cmich.edu

The COVID-19 pandemic has threatened the health of millions of people worldwide since 2020. Throughout the pandemic, participating labs across the state of Michigan have been monitoring SARS-CoV-2 levels in wastewater. These samples were found to reflect trends true to the reported cases with some early warning, with a way to include asymptomatic cases that otherwise would not have been reported. However, approximately 35% of Michigan's population uses septic tanks in mostly rural communities and have not been included in the wastewater surveillance. As the pandemic continues there is less testing but there is still a need to keep track of reported cases. The goal of this study was to understand how long the virus signal could last in septage. This observational study examined the degradation of spiked OC43 virus signal in 7 different septage samples over a span of 100+ days. Samples were tested at both room temperature (~22°C) and 4°C. Preliminary results show that over a 2 month period, virus levels in samples kept at room temperature only degraded an average of approximately 2 log gene copies per liter of sample. There is not a significant difference seen in virus concentrations in samples kept refrigerated over the same period of time, suggesting that coronavirus persists for a much longer period in samples kept in a colder environment.

Poster # 17S

Title: Correlation of SARS-CoV-2 Wastewater Concentration and New COVID-19 Cases in Kent County, Michigan

Author: Louis Walter ,Derick Chia ,Dan Adrian ,Sheila Blackman, Pei-Lan Tsou, Puneet Chowdhary
Contact: t_waltelou@gvsu.edu, chiad@gvsu.edu

According to the WHO, over 757 million cases of COVID-19 have been confirmed worldwide. Throughout the pandemic, Wastewater-Based Epidemiology (WBE) has been incorporated with clinical data alongside other surveillance measures to inform decision-making by public health officials as transmission levels changed. Although most non-pharmaceutical preventative measures instituted to slow transmission of the SARS-CoV-2 virus have been relaxed, the virus remains present in the community, presenting a risk to public health. WBE offers a cheap, effective method of tracking the spread of virus in local communities. While both WBE and clinical data were used in decision-making, the relationship between the two is still emerging. Our goal was to utilize 20 months of clinical and wastewater data to elucidate that relationship. SARS-CoV-2 viral concentrations were obtained from wastewater collected from 4 treatment plants across Kent County. RNA from the viral particles were concentrated and extracted, then quantified using droplet digital polymerase chain reaction (ddPCR). Spearman's correlation and linear regression analyses were performed to measure the strength of association and predicted case rates. Significant positive relationships were found between normalized case rates and the average viral gene concentration in wastewater. The relationship between wastewater signal and case counts differed between Omicron and Delta variant dominated periods. Additionally, sample collection method impacted the quality of the relationship in that grab/spot sampling methods had greater variability, hence lower statistical significance than 24-hour composite sampling methods. Our findings provide important insights into the complexities of wastewater data and highlight its importance for public health surveillance.

Poster # 18

Title: Bridging Lab Science and Public Health with WBE: Partnership between KCHD and the Department of CMB at GVSU

Author: Dr. Puneet Chowdhary, Dr. Sheila Blackman, Dr. Pei-Lan Tsou, Sara Simmonds and Andrew Salisbury
Contact:chowdhap@gvsu.edu

Kent County Health Department (KCHD) and Cell and Molecular Biology Department at Grand Valley State University collaborate to monitor infection level of SARS-CoV-2. We partner with 4 different Wastewater treatment plants to surveille community-wide infection and also monitor facilities such as nursing homes, low-income housing, K-12 schools, and university campuses to monitor infection levels in populations that are vulnerable or mobile. Each site is sampled once or twice a week yielding a total throughput of 48 to 60 samples per week. We report the SARS-CoV-2 counts to the county, state, and stakeholders within 72 h of sample pick up. Not only are we successfully analyzing SARS-CoV-2 levels, but we have also developed our method of quality control by including the monitoring of the fecal marker - Pepper Mild Mottled virus. We collaborate with Dr. Marc Johnson (U. Missouri) to sequence the circulating variants of SARS-CoV-2 in our wastewater through targeted sequencing of the Spike protein Receptor Binding Domain. As a result, we were able to quickly identify the onset of the XBB.1.5 wave in December 2022. Our work has been recognized at our university as an exemplar of student engagement in community collaborations and real-life problem solving, as we have trained and employed over 20 masters- and bachelors- level students to carry out our processing. We also employ Masters in Public Health students to facilitate communication between the laboratory, stakeholders and KCHD. This project demonstrates the strength of an academic/health department partnership to - in the near term - surveille for the next infectious pathogen and - in the long term - train a new generation of potential employees skilled and knowledgeable in molecular tools.

Poster # 19
Title: Understanding the Efficacy of Wastewater Surveillance for SARS-CoV-2 in Two Diverse Communities
Author: Matthew Flood, Josh Sharp, Jennifer Bruggink, Molly Cormier, Bailey Gomes, Isabella Oldani, Lauren Zimmy, and Joan B. Rose
Contact: floodmat@msu.edu

During the COVID-19 pandemic, wastewater-based surveillance has been shown to be a useful tool for monitoring the spread of disease in communities and the emergence of new viral variants of concern. As the pandemic enters its third year and clinical testing has declined, wastewater offers a consistent non-intrusive way to monitor community health in the long term. However, understanding the best method for the application of wastewater surveillance in different communities is necessary. This study sought to understand how accurately wastewater monitoring represented the actual burden of disease between communities. Two communities varying in size and demographics in Michigan were monitored for SARS-CoV-2 in wastewater between March of 2020 and February of 2022. Additionally, each community was monitored for SARS-CoV-2 variants of concern from December 2020 to February 2022. Wastewater results were compared with zipcode and county level COVID-19 case data to determine which scope of clinical surveillance was most correlated with wastewater loading. Pearson r correlations were highest in the smaller of the two communities (r = 0.45-0.81) with the highest correlations with zipcode level case data. When comparing the date of cases being reported (referral date) against the date of the onset of symptoms, the smaller community was more highly correlated with the onset date (onset: r¬ = 0.68-0.81 vs. referral: r =0.38-0.48), while the larger community showed little variation (r = 0.62-0.68). A clear difference was seen in cases and in virus signals in wastewater based on vaccine status. This study has demonstrated that wastewater surveillance in different communities is linked to different geographic and temporal scales.

Poster # 20
Title: Monitoring of COVID-19 in wastewater across the Eastern Upper Peninsula of Michigan
Author: Michelle M. Jarvie, Moriah Reed, Benjamin Southwell, Derek Wright, Thu N.T. Nguyen
Contact: mjarvie1@lssu.edu

Wastewater-based epidemiology is being used as a tool to monitor the spread of COVID-19 and provide an early warning for the presence or increase of clinical cases in a community. The majority of wastewater-based epidemiology for COVID-19 tracking has been utilized in sewersheds that service populations in the tens-to-hundreds of thousands. Limited studies have been conducted to assess the usefulness of wastewater in predicting COVID-19 clinical cases specifically in rural areas. This study collected samples from 16 locations across the Eastern Upper Peninsula of Michigan beginning June 2021. Sampling locations included 12 rural municipalities, a Tribal housing community and casino, a public university, three municipalities that also contained a prison, and a small island with heavy tourist traffic. Samples were analyzed for SARS-CoV-2 N1, N2, and variant gene copies using reverse transcriptase droplet digital polymerase chain reaction (RT-ddPCR). Wastewater N1 and N2 gene copies and clinical case counts were correlated to determine if wastewater results were predictive of clinical cases. Significant correlation between N1 and N2 gene copies and clinical cases was found for all sites ($\rho = 0.89$ to 0.48) between June 2021 and February 2022. N1 and N2 wastewater results were predictive of clinical case trends within 0-7 days. However, after February 1, 2022, N1 and N2 gene copies remained steady but reported clinical cases significantly decreased, reducing the predictive correlation ($\rho = 0.55$ to 0.06). The decrease in clinical cases may be a result of at-home testing combined with less severe symptoms produced by new variants. Long-term wastewater monitoring over a large, rural geographic area is useful for informing the public of potential outbreaks in the community regardless of asymptomatic cases, access to clinical testing, or usage of at-home tests.

Poster # 21S
Title: Wastewater SARS CoV-2 Spread in Rural Northern West Michigan
Author: Abigail Proksch, Alex E Guzman-Vargas, My Tran, Hannah Faber, Chandler Hendrickson, Kassidy Vredeveld, Clifton Franklund, Mary Zimmer, Sky Pike
Contact:proksca@ferris.edu,skypike@ferris.edu

SARS CoV-2 levels for North Western cities including Big Rapids, Cadillac, Grayling, Lake City, Ludington, and Reed City have been monitored in wastewater on a weekly basis from June 2021 to present. Droplet Digital PCR (ddPCR) has been utilized to quantitate SARS CoV-2 in wastewater from the treatment plants of the respective cities. Results were reported to local and state stake holders to be utilized for public health decision making.

Poster # 22S
Title: Simple methods for early warnings of COVID-19 surges: Lessons learned from 21 months of wastewater and clinical data collection in Detroit, Michigan, United States

Author: Liang Zhao, Yangyang Zou, Randy E David, Scott Withington, Stacey McFarlane, Russell A Faust, John Norton, Irene Xagoraraki
Contact:zhaolia4@msu.edu

Wastewater-based epidemiology (WBE) has drawn great attention since the Coronavirus disease 2019 (COVID-19) pandemic, not only due to its capability to circumvent the limitations of traditional clinical surveillance, but also due to its potential to forewarn fluctuations of disease incidences in communities. One critical application of WBE is to provide "early warnings" for upcoming fluctuations of disease incidences in communities which traditional clinical testing is incapable to achieve. While intricate models have been developed to determine early warnings based on wastewater surveillance data, there is an exigent need for straightforward, rapid, broadly applicable methods for health departments and partner agencies to implement. Our purpose in this study is to develop and evaluate such early-warning methods and clinical-case peak-detection methods based on WBE data to mount an informed public health response. Throughout an extended wastewater surveillance period across Detroit, MI metropolitan area (the entire study period is from September 2020 to May 2022) we designed eight early-warning methods (three real-time and five post-factum). Additionally, we designed three peak-detection methods based on clinical epidemiological data. We demonstrated the utility of these methods for providing early warnings for COVID-19 incidences, with their counterpart accuracies evaluated by hit rates. "Hit rates" were defined as the number of early warning dates (using wastewater surveillance data) that captured defined peaks (using clinical epidemiological data) divided by the total number of early warning dates. Hit rates demonstrated that the accuracy of both real-time and post-factum methods could reach 100 %. Furthermore, the results indicate that the accuracy was influenced by approaches to defining peaks of disease incidence. The proposed methods herein can assist health departments capitalizing on WBE data to assess trends and implement quick public health responses to future epidemics. Besides, this study elucidated critical factors affecting early warnings based on WBE amid the COVID-19 pandemic.

Poster # 23

Title: Beta and Delta and Omicron, Oh My! : A look into the timeline of the emergence of SARS-CoV-2 variants in West Michigan

Author: Lauren Cribbs, Celia Kun-Rodrigues, Adam Slater, Daniel Wade, Vanessa Kardian, Dylan Gladysz, Allaire Schneider, Grace Goszkowicz, Louis Kopp, Benjamin G. Kopek, Michael J. Pikaart, Brent P. Krueger, Aaron A. Best
Contact: cribbs@hope.edu

The surveillance of SARS-COV-2 in wastewater has played a key role in campus and community level decisions throughout the COVID-19 pandemic. Since the beginning of the COVID-19 pandemic, the virus has mutated time and time again. With the emergence of new variants, it is important to be able to discriminate and detect the various mutations within the spike and nucleocapsid genes of the SARS-CoV-2 virus. We have implemented a workflow in which samples are first screened by our standard qPCR assay. Those found to contain SARS-CoV-2 are then screened again using the GT Molecular ddPCR assay to determine the presence or absence of various genetic mutations. Analysis of variants, from the parental Wuhan strain all the way to multiple sub variants of the Omicron mutation, allow us to assemble an interesting timeline describing the progression of these variants in our local populations.

Poster # 24S
Title: A Timeline of Variant Distribution
Author: Ashlyn Smith, Cassidy Cleary, Natasha Dagrella, Jannifer Tyrell, Tami Sivy
Contact: tsivy@svsu.edu

Like many viruses, SARS-CoV-2 is constantly changing through genetic mutations that can ultimately alter the way it manifests as an illness. As variants of SARS-CoV-2 continue to arise and proliferate, their prevalance and dates of relevance to the Coronavirus cases seen in teh area have become increasingly more complex to analyze. Once variants were detected through genome sequencing and then target primers/probes were developed for each, digital droplet Polymerase Chain Reaction (ddPCR) analysis in sewage samples was implemented in teh lab at Saginaw Valley State University. To maximize useful and informative data in trending variants, it is important to have a general understanding of when these variants began being seen in Coronavirus cases so the proper target primers/probes assays can be run with wastewater sample dates. Based on a rough timeline of the emergence of these variants in the environment, sewage samples were run with the variant kits, Delta/Omicron, BA.1/BA.2, BA.4/BA.5 and XBB in order to visualize a timeline of how the virus mutated in our region.

Poster # 25
Title: Adding wastewater surveillance to the norovirus epidemiological toolbox
Author: Michelle Ammerman, Shreya Mullapudi, Julie Gilbert, Khaitlyn Figueroa, Felipe de Pula Nogueira Cruz, Kevin Bakker, Betsy Foxman, Marisa Eisenberg, and Krista Wigginton
Contact: mammer@umich.edu

Norovirus is the leading global cause of acute gastroenteritis; outbreaks are usually monitored by a combination of case reports and syndromic surveillance, which often lag rather than lead outbreaks, resulting in a significant economic and health burden. Surveillance practices for norovirus vary greatly worldwide, with syndromic surveillance approaches often used by public health officials to monitor outbreaks. Wastewater based surveillance is being used to monitor the prevalence of SARS-CoV-2; our goal was to determine if these methods and samples can be used to monitor other pathogens, including human norovirus GII (HuNoV GII). Norovirus wastewater testing was assessed in influent samples from 5 different wastewater treatment plants (WWTPs) using reverse transcription-digital droplet PCR, and the results obtained were compared to syndromic, outbreak and search term trend data which are currently used for assessing norovirus levels in communities.

Wastewater HuNoV GII RNA levels followed the expected seasonal patterns and were generally comparable across all WWTPs after fecal content normalization, with the smallest WWTP displaying the highest peak levels. HuNoV GII wastewater values typically coincided with or led syndromic, outbreak, and search term trend data. The best correlations were observed when the sewershed population covered in the wastewater data had high overlap with the population included using other monitoring methods. Overall, wastewater-based surveillance of HuNoV GII is feasible using the current wastewater sampling infrastructure and can be a useful complement to existing public health surveillance methods.

Poster # 26
Title: Lessons from a State-wide Wastewater surveillance program (MiNET) for SARS CoV-2 Public Heath use and beyond
Author: N. D'Souza*, A. Porter, J.B. Rose, E. Dreelin, S.E. Peters, P.J. Nowlin, S. Carbonell, K. Cissell, Y. Wang, M.T. Flood, A.T. Rachmadi, C. Xi, P. Song, S. Briggs and the Michigan Network for Environmental Health and Technology (MiNET) consortium
Contact: dsouzan1@msu.edu

Wastewater surveillance is being applied as a valuable tool for pandemic response and management in areas where clinical data and resources are limited. The pandemic produced several large-scale research and surveillance system networks globally. Michigan was one of the first states to initiate the adoption of a larger scale state-wide wastewater monitoring program, inclusive of both community and congregate facility level site sampling. We discuss how Michigan's state-wide laboratory network for monitoring SARS-CoV-2 in wastewater built a growing knowledgebase and summarize results used for public health action. MiNET (Michigan Network for Environmental Health and Technology) consists of 19 laboratories with community partners including 29 local health departments, 6 Native nations, and 60 wastewater treatment plants (WWTPs). MiNET laboratories monitored 214 sites representing 45% of Michigan's population (April 6 and December 29, 2020).

Three datasets were created based on quality criteria. Dataset M was the complete dataset (214 sites, n=2836); Dataset Mp was a subset of Dataset M that passed all quality controls (159 sites, n=2113); and Dataset Mp_wwtp was a subset of Dataset Mp data from WWTPs (53 sites, n=740). Wastewater results that met all QAQC criteria (Dataset Mp) produced stronger correlations with reported clinical cases than analyses using all the results collected (Dataset M), showing a high positive correlation at 17 days lag (rho=0.867, $p < 0.05$) while for Mp_wwtp this was observed at 12 days lag (r=0.867, $p < 0.05$). The strong positive correlations provided confidence in using the wastewater data as an early warning tool for public health use across the state even when strategic monitoring is carried out at sentinel sites.

The project demonstrated the ability to successfully track the virus on a large, state-wide scale and that wastewater surveillance for SARS-CoV-2 provided early warning of increasing COVID-19 cases. MiNET is currently poised to leverage its competency to complement public health surveillance networks through environmental monitoring for new and emerging pathogens of concern and provides a valuable resource to State and Federal agencies to support future responses.

Poster # 27S
Title: Tracking Wastewater PCR Variant Trends Across Michigan from 2021 to 2022
Author: Simran Singh, Nishita D'Souza, Erin Dreelin, Alexis Porter, Kelly Geith, and the Michigan Network for Environmental Health and Technology (MiNET)
Contact: singhsi8@msu.edu

As the COVID-19 pandemic ensued, emerging SARS-CoV-2 Variants of Concern (VOC) swept the nation. Variant waves were initially monitored through clinical sequencing however, this approach has been challenging to implement for widespread community monitoring. Clinical testing is invasive, not available to all individuals, and not pursued by asymptomatic persons and so wastewater monitoring has been used to complement clinical testing for overall community health updates. Analysis of variant data is increasingly important to understand the SARS-CoV-2 virus's case predictions, specialized vaccine development, increased public health communication, and a reserve of knowledge for future pandemics. Viral mutations and shifts in variant dominance can have implications for wastewater surveillance data when not much is known about VOC specific transmission and shedding rates. The objective of this study was to observe trends in variant spread using data collected from monitoring wastewater treatment plants (WWTP) across Michigan from 2021 to 2022 in collaboration with MDHHS. This project involved collating and visualizing the data to help understand trends, transmission patterns, highlight "hotspots" and demonstrate how the data can be used to better inform statewide testing. Labs in the MiNET network collected samples from WWTP across Michigan and preformed concentration, extraction, and PCR. Visualizations of over 1000 data points from 42 WWTP sites were created using R-Studio and Tableau software. The dataset represents 25 counties in Michigan, with population served ranging between 1,000 to 33,000 for each WWTP. These analyses can help guide harmonization of data for future monitoring efforts to determine how to best utilize MiNET resources for variant testing and sequencing.

Poster # 28
Title: Present Challenges and Future Considerations for W-SPHERE: A Global Data Center for SARS-CoV-2 Wastewater Surveillance
Author: Julia Bazner, Joan Rose, Nishita D'Souza, Colleen Naughton, Gertjan Medema, Panagis Katsivelis
Contact: baznerju@msu.edu

Wastewater based surveillance has long been used in public health efforts. Wastewater monitoring is unique in that it can be used as an early warning system that allows for widespread detection of pathogens representing infections within a community. Most recently, this method of population health monitoring has gained public attention with its application to SARS-CoV-2 surveillance in the face of the COVID-19 pandemic. With over 760 million confirmed cases and 6.8 million deaths globally (as of March 2023), numerous cities, states, and countries sought a cost effective and efficient system to measure disease and interventions within their respective communities. However, even with the recent traction of global surveillance, there had yet to be a global data center for wastewater monitoring data until the creation of W-SPHERE (Wastewater SARS Public Health Environmental REsponse). The objectives of this presentation are to share how this data center provides standardized downloadable data, challenges faced, and recommendations for expanding this resource. The W-SPHERE data center currently displays a conglomerate of 27 public datasets spanning 35 countries and totals 1,456 individual sampling sites. COVIDPoops19 is used as the starting point for obtaining datasets from open dashboards and requesting contributions from the scientific community. Received datasets are standardized using data collection form templates that have outlined data dictionaries. This highlights a key challenge as the need for automation is necessary for sustained growth, but datasets are contributed in several formats which creates obstacles in achieving this goal. Presently, W-SPHERE gives a platform to share data on a global scale and provides the necessary information in public health decision making. For future considerations, having a fully automated workflow will only expedite reporting and quality assurance giving users the most up-to-date data for informed decision making. This will be necessary as wastewater surveillance serves future pandemics.

Poster # 29
Title: Changing Tides: West Michigan Based Genomic Analysis of SARS-CoV-2 Variants in Wastewater
Author: Alexis M. Porter, Charlyn G. Partridge
Contact:porteal@gvsu.edu

SARS-CoV-2 wastewater surveillance provides a complimentary approach to public health surveillance and quantification of viral presence within a population. However, robust quantification of variants and de novo detection of emerging variants remains challenging and is ever evolving for existing strategies. Evaluation of SARS-CoV-2 RNA in wastewater within the west Michigan population was conducted from 11/2020-ongoing. Following initial quantification of SARS-CoV-2 utilizing gene targets N1 and N2, samples that exceeded the laboratory threshold-1 of 10,000 gene copies/100 mL underwent variant analysis utilizing discriminatory assays provided by GT-Molecular. Regional genomic targets were determined based on circulating variants of concern as noted by the World Health Organization. Following this criterion, 316 sample extracts were analyzed from 03/2021-03/2023 using droplet digital polymerase chain reaction (ddPCR). Beginning 01/2023, all samples that exceeded the secondary threshold-2 value of 20,000 gene copies/100 mL were evaluated using whole genome sequencing via Illumina's MiSeq System to cross reference with previously analyzed samples that noted ambiguous signal on ddPCR. The bioinformatics pipeline for this analysis was built under guidance provided by the Wisconsin State Laboratory of Hygiene. All samples evaluated came back with detection of targeted gene mutations when using ddPCR. A subset of 8 samples selected from 2023 that met threshold-2 were investigated for sequencing potential. Results revealed detections of new variants of concern entering our population during peaks in wastewater signal. These variants included Alpha, Delta, Epsilon, Omicron, Omicron sub-variants BA.1, BA.2, BA.2.1.2, BA.4, BA.5, BQ.1/BQ.1.1, and XBB/XBB.1.5. Based on clinically available timelines and kit manufacture and distribution, we saw variation of variant point of entry and detection in known clinical cases. Despite challenges in variant quantification for SARS-CoV-2, genomic analysis of SARS-CoV-2 in wastewater can inform epidemiological work and public health officials as a complement to the established infrastructure for viral analysis.

Poster # 30

Title: Whole genome sequencing of SARS-COV-2 from wastewater using two different platforms

Author: Celia Kun-Rodrigues, Adam Slater, Lauren Cribbs, Daniel Wade, Vanessa Kardian, Dylan Gladysz, Allaire Schneider, Grace Goszkowicz, Louis Kopp, Benjamin Kopek, Michael Pikaart, Brent Krueger, Aaron Best
Contact: rodrigues@hope.edu

Wastewater surveillance of SARS-COV-2 has been useful for decision making both at the campus and community levels. Variant detection is routinely achieved with ddPCR kits designed for a specific set of known variants after the variants have been characterized. To be able to detect the emergence of new variants as they appear in populations, we sought to use whole genome sequencing of SARS-COV-2 on our wastewater samples. We employed the widely used ARTIC Network approach on Illumina and Nanopore platforms. A first run with the Illumina COVIDseq kit and the ARTIC primers v4 on the Illumina MiSeq platform provided a 70% of genome coverage for the positive control and clade assignment for 12.8% of the wastewater samples with a genome coverage range between 23.6% and 34.7% and 10 to 47 variants detected that differ from the parental lineage. Results on the Nanopore MinION platform will be discussed and compared to the Illumina data. Nevertheless, following an amplicon approach focused on the S gene for lineage identification and new variant detection might be a better option for wastewater samples; preliminary results for this approach will also be discussed.

Poster # 31S
Title: Variant identification by sequencing of SARS-CoV-2 spike protein receptor binding domain
Author: Elisabeth Hatfield, Elizabeth Cazallis, Louis Walters, Pei-Lan Tsou, Sheila Blackman, Puneet Chowdhary
Contact: hatfiele@mail.gvsu.edu,tsoup@gvsu.edu

Wastewater is a useful tool to monitor SARS-CoV-2 community infection levels, as the virus is shed in feces. RNA extracted from wastewater can be used to track total viral load as well as identify variants. Tracking variants is vital for public health, as mutations (principally in the spike protein Receptor Binding Domain (RBD)) drive antibody escape. There are two common methods to track variants: PCR-based assays (both ddPCR and qPCR) and whole genome next-generation sequencing. Neither is ideally suited to broad deployment: development of effective PCR-based assays cannot keep pace with evolution of the Omicron variant and whole genome next-generation sequencing is costly. To reduce cost, we use a method targeting only the RBD for sequencing. Nested PCR (verified by gel electrophoresis) amplifies the RBD and amplicons are sequenced by Next-Generation Sequencing. Results are analyzed by freely available Python programs to determine proportional variant representations. Using this method we have successfully tracked the disappearance of Omicron BA.4 and BA.5 subvariants, the rise and fall of BQ.1 and its subvariants, and the emergence of the current dominant West Michigan subvariant, XBB.1.5. Our results show that Next-Gen Sequencing targeting only the RBD is a cost-competitive, efficient, and accurate alternative to PCR-based tests and whole genome sequencing for SARS-CoV-2 variant monitoring.

Poster # 32
Title: Method Development and Implementation of Genomic Wastewater Based Biosurveillance
Author: Lindsay Catlin, Angela Minard-Smith, and Rachel R. Spurbeck
Contact: spurbeck@battelle.org

Outbreak tracking is important to identify communities at risk to prevent further spread. However, when the pathogen is novel, the time needed to identify, develop, and validate a diagnostic test to identify infected people can take too long to enable rapid contact tracing and effective quarantine. Wastewater surveillance has emerged as an efficient way to quickly screen large populations for indicators of diseases like COVID-19, monitor their spread over time, and identify whether more virulent strains are becoming more prevalent in the region (Ahmed et al., 2020; Crits-Christoph et al., 2021; Haramoto et al., 2020; Kitajima et al., 2020; Spurbeck et al., 2021). However, most surveillance still utilizes qPCR based diagnostic tests for individual pathogens. Here we have tested several genomic methods, both targeted and untargeted, for wastewater-based biosurveillance to identify the most efficient procedure to identify and track trends in reportable infectious diseases and identify outbreaks of known pathogens as well as potentially novel pathogens or variants on the rise in our communities.

Poster # 33
Title: Methods to test for human virus diversity in wastewater: Towards a practical approach for identifying human viruses with metagenomics
Author: Yabing Li, Brijen Miyani, Kevin Childs, Shin-Han Shiu, Irene Xagoraraki
Contact:liyabing@msu.edu

Considering the overall disease burden caused by diverse pathogenic viruses in wastewater, surveillance tools that are both practical to apply and able to identify diverse viral communities accurately are urgently needed. High throughput metagenomic sequencing opens an opportunity and expands the capabilities of wastewater surveillance. However, there are major bottlenecks in the metagenomic enabled wastewater surveillance, which involve complexities in selecting appropriate sampling and concentration methods, random amplification methods, and bioinformatic analysis of complex samples with low human virus concentrations.
During COVID-19 outbreak, untreated wastewater samples were collected from the Great Lakes Water Authority (GLWA) Wastewater Treatment Facility in Detroit. With a hybrid assessment approach, a practical, accurate and efficient tool, MEGAHIT, was selected to assemble the clean metagenomic reads for the follow-up analysis. To evaluate the abilities of different sampling and concentration methods in virus identification, virus communities concentrated with Virus Adsorption-Elution (VIRADEL) and polyethylene glycol (PEG) were compared. Results indicated that more viral reads were obtained by the VIRADEL concentration method, with an averaging proportion of viral reads in the sample as 2.84 0.57 %, while in samples concentrated with PEG, the averaging proportion of viral reads in the sample was 0.63 0.19%. Taxonomic classification through comparison against the human associated virus protein database indicated the potential prevalence of pathogenic viruses including Orthopoxvirus, Rhadinovirus , Parapoxvirus, Varicellovirus, Hepatovirus, Simplexvirus, Bocaparvovirus, Molluscipoxvirus, Parechovirus, Roseolovirus, Lymphocryptovirus, Alphavirus, Spumavirus, Lentivirus, Deltaretrovirus, Enterovirus, Betacoronavirus, Kobuvirus, Gammaretrovirus, Cardiovirus, Erythroparvovirus, Salivirus, Rubivirus, Orthohepevirus, Cytomegalovirus, Norovirus, Mamastrovirus in the wastewater samples. The study all discusses limitations and other parameters currently under investigation by our team. Integrating innovative environmental microbiology technologies like metagenomic sequencing with viral epidemiology offers a significant opportunity to improve monitoring potential pathogenic viruses in wastewater.

Poster # 34
Title: Interpreting results of wastewater-based SARS-CoV-2 surveillance on a university campus
Author: Ryan Julien; Jade Mitchell
Contact: julienry@msu.edu

Timely public health surveillance data are critical to understand the changing landscape of infection risks and to effectively mitigate the spread of disease. However, gathering case data can be costly, invasive, and provide non-representative data based when participation in such surveillance is voluntary.

Wastewater Based Epidemiology (WBE) has proven to be an effective method to detect the SARS-CoV-2 virus, and has several advantages over monitoring individual people for the virus, including reduced monitoring costs and improved individual participation. However, interpretation of WBE results are less clear. While a wealth of literature attempting to link wastewater results to outbreaks or counts of individual infections has developed since the COVID-19 pandemic began, there is no clear universal framework for interpreting WBE results.

This poster will detail results from a campus-level WBE project for the SARS-CoV-2 virus. Wastewater samples were collected twice weekly from 15 different building-level sites (estimated mean population = 550) and three community-level sites (estimated mean population = 7,000). The number of active infections associated with each sample location were also recorded by university and health department officials through May 2022. Preliminary general linear modeling results indicate weak agreement between wastewater results and case counts. Stochastic modeling was also conducted to evaluate the predictive power of wastewater to case counts. These results show that wide variability in fecal concentrations of the virus introduce a great deal of uncertainty in measured wastewater concentrations, thus complicating case count estimations.

Poster # 35S
Title: Assessing representativeness of wastewater-based epidemiology measurements with dynamic models of fecal shedding
Author: Peter J Arts, Kevin M Bakker, Alexandria B. Boehm, Andrew F. Brouwer, Marisa C. Eisenberg, J Daniel Kelly, Sterling Knight, Krista R Wigginton
Contact:parts@umich.edu

Many wastewater-based epidemiology (WBE) applications assume that longitudinal measurements are representative of all infected individuals in the sewershed equally. However, wastewater samples contain material from numerous individuals at different stages of infection, with likely dramatically different fecal shedding patterns and dynamics. The degree to which individual dynamics of fecal shedding influences the relationship between wastewater concentrations and the number of infected individuals is poorly understood. In this study, we use fecal shedding data recently collected by our team to create a stochastic, longitudinal model of individual fecal shedding of SARS-CoV-2, PMMoV, and crAssphage genomic sequences over the course of 30 days after infection. We then pair dynamic fecal shedding with epidemiological and materials balance mathematical models to mechanistically link individual fecal shedding with community level wastewater concentrations over the course of a simulated outbreak. Using this integrated model, we find that individuals shedding at high concentrations can have a significant influence on resulting wastewater concentrations. Intuitively, this phenomenon is more pronounced when anomalous shedders represent a larger proportion of the population. Additionally, we observe that both crAssphage and PMMoV serve as reasonably reliable correlates to fecal strength only at large population scales. Together, these results highlight biases associated with quantitative WBE data collected at small scales, which are due to dynamics of individual fecal shedding.

Poster # 36S
Title: Selection of Sewage Manholes in Infectious Disease Surveillance
Author: Leyao Zhang, Yahui Zhang, Chuanwu Xi, Peter X.K. Song
Contact: leyaozh@umich.edu

The effectiveness of tracking infected cases with the omicron virus has been greatly compromised due to the availability of at-home COVID test kits in many counties. An alternative solution to monitoring contagions of the COVID disease in the population is to survey viral loads from sewage water systems. The central question of scientific importance is to select those important manholes that are of most relevance to the prediction of confirmed infectious cases in a specific community. To build on a cost-effective monitoring system, we develop a supervised learning paradigm to determine important sampling sites. A novel multi-compartment dynamic model is proposed to simulate viral loads in the wastewater system from the evolution of the pandemic in the population, which is used to evaluate the performance of our proposed model and algorithm. Through extensive numerical experiments, we demonstrated the desirable performances of the proposed MIO approach that outperformed some popular methods in terms of much higher numerical quality in both selection accuracy and estimation accuracy. This proposed methodology is illustrated by a real-world sewage monitoring program run by University of Michigan since 2021, where the proposed MIO approach improved the goodness-of-fit of on-campus COVID-19 confirmed cases.

Poster # 37S
Title: Sewage-Based Surveillance: Using Machine Learning Methods to Address Under-Reporting of Covid Cases.
Author: Yili Wang, Peter Song, Chuanwu Xi
Contact: yiliwang@umich.edu

Attributed to the widespread availability of vaccines and other public health practices, COVID-19 cases have decreased, and many people who contract the virus experience milder symptoms. However, some individuals continue to experience symptoms even after recovering from the acute illness, which has led to the emergence of what is known as "long COVID." Despite the decline in testing rates and reduced concern about the virus, it is still important to monitor the COVID-19 situation to track and prevent further spread in the community. However, the documented confirmed cases alone may not be a reliable indicator due to the decrease in testing rates.

Sewage water has been found to be a valuable tool for monitoring the spread of COVID-19 in the community. Studies have shown a high correlation between the concentration of COVID-19 genetic material in sewage water and the number of confirmed cases during periods of active COVID-19 transmission when testing rates were high and confirmed cases were believed to be an accurate indicator of COVID-19 spread. To leverage this information, Artificial Neural Network and Partial Least Square regression methods have been used to find the relationship between sewage water SARS-CoV-2 concentrations and confirmed cases in the community during active COVID-19 periods. These methods are then used to predict the actual cases when under-reporting has been an issue, providing a more accurate reflection of the actual number of cases in the community. By using sewage water analysis, we can get a better understanding of COVID-19 spread in the community and correct the under-reporting issue. This information is crucial for public health officials to make informed decisions and take appropriate actions to mitigate further transmission of the virus.

Poster # 38S

Title: Wastewater Surveillance Beyond COVID-19: A Ranking System for Communicable Disease Testing in Tri-County Detroit Area, Michigan, USA

Author: Zachary Gentry, Liang Zhao, Irene Xagorarki, Russel A Faust, Randy E David, John Norton
Contact:gentryza@msu.edu

This is a proposed ranking system for prioritizing reportable communicable diseases (CDs) in the Tri-County Detroit Area (TCDA), Michigan, for future wastewater surveillance applications at the Great Lakes Water Authority’s Water Reclamation Plant (GLWA’s WRP). The comprehensive CD wastewater surveillance ranking system (CDWSRank) was developed based on reported incidence data from 2014 to 2021. The CDWSRank system includes 6 binary and 6 quantitative parameters. The final ranking scores of CDs were computed by summing the multiplication products of weighting factors for each parameter, and then were sorted based on decreasing priority. Disease incidence data from 2014 to 2021 were collected for the TCDA, including City of Detroit, as well as Wayne, Macomb, and Oakland Counties, served by the GLWA’s WRP. Disease incidence trends in the TCDA were endowed with higher weights, creating overall ranking scores that prioritize the TCDA over the state of Michigan. The CDWSRank system can be easily adopted to geographical locations beyond the TCDA. The CDWSRank system is the first of its kind to provide an empirical approach to select CDs for wastewater surveillance, specifically in geographies served by centralized wastewater collection in the area of interest. treatment plant.

Poster # 39S
Title: Wastewater-based estimation of the SARS-CoV-2 RNA shedding load distribution
Author: Yahui Zhang; Chuanwu Xi; Peter X.K. Song et al.
Contact: yahuiz@umich.edu

The fact that infected people release RNA from the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) into sewers forms the foundation of the wastewater-based epidemiology approach for COVID-19 surveillance. However, uncertainties remain regarding the intensity and timing with which individuals shed SARS-CoV-2 into wastewater. To address this issue, we estimated the daily number of infections based on the confirmed cases data from a university campus by maximum-likelihood deconvolution. Then we inferred the shedding load distribution based on the wastewater and the estimated daily number of infections using Markov chain Monte Carlo methods. The individual shedding of RNA into wastewater was estimated to peak on average 6 days after infection, and the wastewater measurements might be over-dispersed (negative binomial dispersion parameter, r = 0.20 (95% confidence interval: 0.15 - 0.28)). This result supports the utility of wastewater surveillance and as an early warning of increasing spread in a community.

Poster # 40
Title: Visualizing Transmission Trends: Digital Dashboards in Wastewater-based Epidemiology
Author: Julie Gilbert, Michelle Ammerman, Krista Wigginton, Marisa Eisenberg
Contact: juliegil@umich.edu

Background
The COVID-19 pandemic increased the traffic on and demand for data presented on dashboards by public health departments, governments, and other agencies. This has increased data literacy, while also creating an expectation of transparency and speed in reporting health related information.

Aim or purpose
The goal of this work was to develop a user-friendly dashboard interface for displaying laboratory testing results and increasing the speed and clarity of data exchanged with our stakeholders.

Methods
Automated data pipelines were built in R to minimize human error and increase speed in processing laboratory data. RShiny was used to build the dashboards. Through a data partnership with MDHHS, COVID-19 case data was able to be mapped to catchment areas. Google Trend disease-specific search term data was also integrated. Additionally, new trend and level metrics were developed to aid epidemiological interpretation of wastewater data, based on the Centers for Disease Control and Prevention (CDC) and the Michigan Department of Health and Human Services (MDHHS) methodology.

Findings/results
The dashboard was successfully launched at um.wastewatermonitoring.dataepi.org, and includes wastewater concentration data, clinical case data, and google trend data for SARS-CoV-2 (N1 and N2 targets), norovirus, influenza A, respiratory syncytial virus (RSV), and MPOX. Trend classifications from the CDC and MDHHS were modified and implemented, enabling users to evaluate trends and for SARS-CoV-2, CDC trend indicators and levels were implemented.

Conclusion
Dashboards are a useful way to improve transparency and interpretability of wastewater data, enabling wastewater monitoring to inform public health policy decision-making.

Poster # 41

Title: Triangulating Transmission Trends: Comparing Google Trends and Wastewater Surveillance Data for Multiple Pathogens

Author: Julie Gilbert, Michelle Ammerman, Krista Wigginton, Marisa Eisenberg
Contact: juliegil@umich.edu

Background

Google trends data has been shown to correlate with a wide range of public health and infectious disease patterns, and is part of the growing field of digital epidemiology. Wastewater monitoring can track multiple pathogens without relying on clinical testing or care-seeking behaviors, however there is often no gold standard epidemiological data set with which to validate wastewater data.

Aim or purpose

Here we evaluate correlations between Google Trends data and wastewater monitoring for respiratory and gastrointestinal pathogens across multiple wastewater treatment plants, to explore how different data sets could be used to triangulate disease patterns if there is incomplete clinical surveillance data.

Methods

PMMoV-normalized wastewater concentration data was examined from influent for SARS-CoV-2 and norovirus and from solids for influenza A and RSV from five wastewater treatment plants in southeast Michigan. Google Trends data was evaluated at a range of spatial scales for disease-specific search terms (e.g. “norovirus symptoms”, “stomach flu”). Correlations and cross-correlations were used to determine how the relationship between wastewater and Google Trends data has changed over time since 2021.

Findings/results

We found moderate-to-high correlations between Google Trends and wastewater surveillance data for all pathogens and all sites, suggesting that these data could be used to triangulate disease patterns. However, correlations can be impacted by how distinguishable a given pathogen’s symptoms are from similar pathogens (e.g. RSV vs. other respiratory pathogens). Additionally, correlations between google trends and wastewater can be impacted by digital access levels and public attention—for example, the correlation between SARS-CoV-2 and Google Trends data has decreased substantially as news coverage and public attention has waned.

Conclusion

In diseases with distinct searchable names and symptoms, google trend and wastewater monitoring data has the potential to be used to understand disease patterns, bridging gaps in traditional surveillance data.

Poster # 42

Title: Evaluating the changing relationship between wastewater surveillance, COVID-19 cases, and testing

Author: Julie Gilbert, Michelle Ammerman, Kevin Bakker, Krista Wiggington, Marisa Eisenberg
Contact: juliegil@umich.edu

As clinical testing has waned, wastewater monitoring is increasingly used to discern COVID-19 burden within a community. Because it doesn't rely on clinical testing, symptoms, or effort by individuals in the population, wastewater surveillance can be used to detect COVID-19 transmission and trends without being as affected by changing ascertainment bias and or the lags inherent in hospitalization or mortality data. However, important questions remain about how the relationship between wastewater has changed as testing and variants have shifted, and how that relationship varies across communities and treatment plants with different characteristics. In this study, we explore these questions across five wastewater treatment plants in Southeast Michigan. We examined how correlations between normalized wastewater concentrations and clinical cases have changed over time, and how this relationship depends on testing levels, vaccine coverage, and variant using correlation coefficients, cross correlations, and multivariate regression. We found that while correlations between SARS-CoV-2 wastewater concentrations and clinical cases were generally high, the slope of these correlations depended strongly on the level of testing occurring for a given community and time period. Based on our multivariate regression models, we developed predictive models to quantify the possible impact of lower testing on clinical case surveillance data. Overall, our results help to underscore how wastewater surveillance can provide an important alternative data stream to understand COVID-19 transmission patterns.

Poster # 43S
Title: Five-week warning of COVID-19 peaks prior to the Omicron surge in Detroit, Michigan using wastewater surveillance
Author: Liang Zhao, Yangyang Zou, Yabing Li, Brijen Miyani, Maddie Spooner, Zachary Gentry, Sydney Jacobi, Randy E David, Scott Withington, Stacey McFarlane, Russell Faust, Johnathon Sheets, Andrew Kaye, James Broz, Anil Gosine, Palencia Mobley, Andrea WU Busch, John Norton, Irene Xagoraraki
Contact: Zhaolia4@msu.edu

Wastewater-based epidemiology (WBE) is useful in predicting temporal fluctuations of COVID-19 incidence in communities and providing early warnings of pending outbreaks. To investigate the relationship between SARS-CoV-2 concentrations in wastewater and COVID-19 incidence in communities, a 12-month study between September 1, 2020, and August 31, 2021, prior to the Omicron surge, was conducted. 407 untreated wastewater samples were collected from the Great Lakes Water Authority (GLWA) in southeastern Michigan. N1 and N2 genes of SARS-CoV-2 were quantified using RT-ddPCR. Daily confirmed COVID-19 cases for the City of Detroit, and Wayne, Macomb, Oakland counties between September 1, 2020, and October 4, 2021, were collected from a public data source. The total concentrations of N1 and N2 genes ranged from 714.85 to 7145.98 gc/L and 820.47 to 6219.05 gc/L, respectively, which were strongly correlated with the 7-day moving average of total daily COVID-19 cases in the associated areas, after 5 weeks of the viral measurement. The results indicate a potential 5-week lag time of wastewater surveillance preceding COVID-19 incidence for the Detroit metropolitan area. Four statistical models were established to analyze the relationship between SARS-CoV-2 concentrations in wastewater and COVID-19 incidence in the study areas. Under a 5-week lag time scenario with both N1 and N2 genes, the autoregression model with seasonal patterns and vector autoregression model were more effective in predicting COVID-19 cases during the study period. To investigate the impact of flow parameters on the correlation, the original N1 and N2 gene concentrations were normalized by wastewater flow parameters. The statistical results indicated the optimum models were consistent for both normalized and non-normalized data. In addition, we discussed parameters that explain the observed lag time. Furthermore, we evaluated the impact of the omicron surge that followed, and the impact of different sampling methods on the estimation of lag time.

Poster # 44S

Title: Targeting a free viral fraction enhances the early alert potential of wastewater surveillance for SARS-CoV-2: a methods comparison spanning the transition between Delta and Omicron Variants in a large urban center

Author: Liang Zhao, Qiudi Geng, Ryland Corchis-Scott, R. Michael McKay, John Norton, Irene Xagoraraki
Contact:zhaolia4@msu.edu

Recognizing the benefits of wastewater surveillance as a tool to support public health in tracking SARS-CoV-2 and other respiratory pathogens, numerous wastewater virus sampling and concentration methods were tested for appropriate applications and their significance for actionability by public health practices. Here, we present a 34-week long wastewater surveillance study that covers nearly 4 million residents of the Detroit (MI, USA) metropolitan area. Three primary concentration methods were compared with respect to recovery of SARS-CoV-2 from wastewater: Virus Adsorption-Elution (VIRADEL), polyethylene glycol precipitation (PEG), and polysulfone (PES) filtration. Wastewater viral concentrations were normalized using various parameters (flow rate, population, total suspended solids) to account for variations in flow. Three analytical approaches were implemented to compare wastewater viral concentrations across the three primary concentration methods to COVID-19 clinical data for both normalized and non-normalized data: Pearson and Spearman correlations, Dynamic Time Warping (DTW), and Time Lagged Cross Correlation (TLCC) and peak synchrony. It was found that VIRADEL, which captures free and suspended virus from supernatant wastewater, was a leading indicator of COVID-19 cases within the region, whereas PEG and PES filtration, which target particle-associated virus, each lagged behind the early alert potential of VIRADEL. PEG and PES methods may potentially capture previously shed and accumulated SARS-CoV-2 resuspended from sediments in the interceptors. These results indicate that the VIRADEL method can be used to enhance the early-warning potential of wastewater surveillance applications although drawbacks include the need to process large volumes of wastewater to concentrate sufficiently free and suspended virus for detection. While lagging the VIRADEL method for early-alert potential, both PEG and PES filtration can be used for routine COVID-19 wastewater monitoring since they allow a large number of samples to be processed concurrently while being more cost-effective and with rapid turn-around yielding results same day as collection.

Poster # 45
Title: Point of Care Device as a SARS-CoV2 Wastewater Surveillance Screening Tool
Author: Rebecca Ives, Nishita D'Souza, Joan Rose
Contact:ivesrebe@msu.edu

As other crises emerge, the funding allocated to SARS-CoV2 wastewater surveillance will likely decrease. The qPCR and ddPCR detection methods are time and labor intensive and expensive; an inexpensive screening method would be a useful addition to SARS-CoV2 wastewater surveillance programs. We evaluate the AcculaTM (ThermoFisher Scientific) system, which uses Oscillating Amplification Reaction (Oscar) PCR to produce results in approximately 30 minutes post sample addition, as a screening tool for SARS-CoV2 wastewater surveillance. Sewage samples from two Michigan wastewater treatment facilities and 15 community sewer network locations (n=21) were concentrated using a combination of Polyetheylene glycol (PEG) based precipitation and centrifugation (Borchardt et al., 2017) and underwent paired analysis by ddPCR (Bio-Rad) and the AcculaTM) systems. Molecular quantification of SARS-CoV-2 virus in sewage concentrates was conducted using CDC qPCR detection method adapted to the BIO RAD QX200™ Droplet Digital PCR (ddPCR) analysis platform using Biorad's One-Step RT-ddPCR Advanced Kit for Probes (Corman et al., 2020; Flood et al., 2021). The SARS CoV-2 marker genes are amplified by ddPCR using CDC recommended primer and probe sets listed in Table 1.1.

Paired analysis of the sewage concentrates on the AcculaTM (ThermoFisher Scientific) point of care system found 60% (9/15) of all ddPCR positive samples and 100% (6/6) of all ddPCR negative samples were correctly identified by the AcculaTM system, for an overall agreement of 71.34% with the ddPCR method. The lowest concentrations of N1 and N2 gene copies in the tested samples that produced a positive result on the AcculaTM system were 1.96 x 103 gene copies/100 mL and 1.825 x 103 gene copies/100 mL, respectively. The highest concentrations of N1 and N2 gene copies in the tested samples that produced a negative result on the AcculaTM system were 2.11 x 103 gene copies/100 mL and 3.61 x 103 gene copies/100 mL, respectively, indicating possible matrix interference in certain samples. Further investigation of alternative concentration methods and removal of matrix interference is warranted. The AcculaTM system can be used to quickly check for SARS-CoV2 hotspots in the community sewer network and identify samples that should proceed to more cost intensive analysis. The AcculaTM system shows promise as a screening tool for SARS-CoV2 wastewater surveillance programs.

Poster # 46
Title: Optimizing Polyethylene glycol (PEG) Concentration Methods for SARS-CoV-2 Wastewater Analysis
Author: Rebecca Ives, Nishita D'Souza, Joan Rose, Matthew Flood, Julia Bazner, Spencer Kuehn
Contact: kuehnspe@msu.edu

Optimizing Polyethylene glycol (PEG) Concentration Methods for SARS-CoV-2 Wastewater Analysis

Spencer Kuehn, Nishita D’Souza, Rebecca Ives, Julia Bazner, Joan B. Rose

Traditional polyethylene glycol (PEG) precipitation methods to concentrate SARS CoV-2 from wastewater can be labor-intensive and time consuming. Streamlining the process without compromising on the efficiency of virus recovery would allow for better throughput leading to same day results. Three variations of the method as per (Flood et al., 2021) were compared by varying spin time and centrifugation conditions (n=22). Method A was the original processing method (with a stir time of 2 hours and centrifugation at 4,700 RCF for 45 minutes), Method B altered the stir time to 30 mins and kept centrifugation at 4,700 RCF for 45 minutes, and Method C altered the stir time to 30 mins as well as centrifugation at 10,000 RCF for 25 minutes.

All samples were spiked with a Phi6 bacteriophage (surrogate) suspension at a concentration of 106 copies/mL to determine recovery efficiency. SARS CoV-2 N1 and Phi6 targets were quantified using ddPCR analysis on a BioRad ddPCR system. N1 concentrations were statistically compared using the Spearman Rank Correlation. N1 concentrations between the methods were not significantly different ($p > 0.02$, $\alpha = 0.05$). Phi6 recovery percentages from Methods B and C were higher on average than Method A (methods B and C were 34.1% and 36.5% higher, respectively).

Results indicated that PEG spin time can be reduced by 1.5 hours with no significant impact on recovery. Additionally, the change in centrifugation speed (for Method C) did allow for additional time saving of 10-15 mins which may be considered for laboratories with high-speed centrifuge capacity. This study recommends the use of the optimized PEG method/s to increase throughput in laboratories currently using PEG precipitation for SARS-CoV-2 concentration.

References

Flood et al. (2021) Standard Operating Procedure for Sampling, Concentration and Detection of SARS-CoV-2 in Sewage.

Poster # 47
Title: Comparison of RT-qPCR and RT-Droplet Digital PCR platforms for Quantification of SA
Author: Jianfeng Wu, Xin Li, Thu Le, Olivia Yancey, Christopher Breen, and Chuanwu Xi
Contact: wujian@umich.edu

Wastewater-based epidemiology (WBE) has been used widely for monitoring SARS-CoV-2 at the population level since the outbreak. It is important to develop methods for detection and quantification of nucleic acids with high sensitivity and specificity, better targeting multiple targets in the same run. Here we compared reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and RT droplet digital PCR (RT-dPCR) platforms for the quantification of SARS-CoV-2 RNA in the wastewater collected from the campus in Ann Arbor, MI. 13 wastewater RNA samples were randomly chosen from our lab's collection. Both methods showed a high degree of linearity and quantitative correlation for standards across their detectable ranges. For all the wastewater samples, RT-dPCR generally revealed higher (10-20% more) concentrations than that of RT-qPCR.

Poster # 48S
Title: 3D Passive Sampling for SARS-CoV-2 Detection in Wastewater
Author: Corrine Caponigro, Joan Rose, Rebecca Ives, Nishita D'Souza
Contact: caponig8@msu.edu

New passive sampling methods are being explored for wastewater surveillance. This study compared autosampler composites to passive samplers over 29 days. Forty-three wastewater samples were evaluated across 6 sewer sites (3 community and 3 dormitory) serving populations of 492-10,092. Samples were processed (autosamplers: PEG, passives: bead beating), viral RNA extracted, and N1/N2 genes quantified with ddPCR detecting N1/N2 levels of 5.4x101-1.61x104 gene copies/filter for passives and 3.84x102-3.83x104 gene copies/100 mL for composites. Autosampler composites detected higher concentrations than passive samplers however similar trends were observed using both methods. Passive samplers can be employed as a cost-effective sampling approach to monitor community trends of SARS-CoV-2.

Poster # 49

Title: Passive Swab Versus Grab Sampling for Detection of SARS-CoV-2 Markers in Wastewater

Author: Jeffrey L. Ram a,*, Md Alamina, James Hartrick b, Adrian A. Vasquez a, Azadeh Bahmania, Carrie L. Turner b, William Shuster c, and Nicholas W.
Contact: jeffram@wayne.edu

Background: Early detection of the COVID-19 virus, SARS-CoV-2, is key to mitigating the spread of new outbreaks. As individual testing has decreased, wastewater-based epidemiology has increased in importance as a way to surveil a community while maintaining individual anonymity. However, SARS-CoV-2 markers in wastewater vary diurnally. Grab samples may miss marker presence, and multi-sampling is costly. This study investigates a passive sampling using tampon swabs to accumulate viral material from sewers over time. Methods: Tampons that had been immersed in sewers for 4 hrs were eluted into a 20 – 30 mL Tween-20-containing wash. We analyzed wastewater from six Detroit sewersheds (AS, CS, JV, SG, UC, & WH) with paired swab and grab samples 16-22 times during April – September 2022, and enumerated SARS-CoV-2 markers using ddPCR. We compared the copies/10 mL of swab eluate to the copies/10 mL of paired grab samples. Results: SARS-CoV-2 markers were detected more frequently in swab eluates than in grab samples ($P<0.001$). Swab counts averaged two to three-fold higher than paired grab samples ($p<0.0001$). No significant difference was observed in the recovery of a spiked-in control (Phi6), indicating differences were not due to higher viral recovery or reduction of PCR inhibition. The results vary between sites, with swab samples from smaller sewersheds (<500 people), showing the greatest improvements of swab over grab samples. Other studies show that grab samples from small sewersheds have a greater fluctuation of counts than larger sewersheds. Conclusion: Swab-sampling with tampons provides significant advantages in detection of SARS-CoV-2 wastewater markers. The use of swabs over modest periods of time (4 – 8 hours) improves the detectability of SARS-CoV-2 markers in wastewater and are expected to provide earlier detection of new outbreaks than grab samples, with consequent public health benefits. Follow-up: Recent follow-up studies confirmed the improved sensitivity of swabs at JV and tested another site (PA) for which swabs averaged 5-fold higher than grab samples (n=4 pairs).

Poster # 50

Title: Mapping a Plan to Address Measurement Challenges in the Wastewater Surveillance

Author: Alshae' Logan-Jackson, Nancy J. Lin, Ishi Keenum, Stephanie Servetas, Jason Kralj, Lisa Stabryla, and Scott Jackson
Contact: alshae.logan@nist.gov

Wastewater surveillance is a promising approach to monitor biological and chemical contaminants on a community and national level in support of public health and safety decisions. As one example of the response to the COVID-19 pandemic, The Centers for Disease Control and Prevention (CDC) established the National Wastewater Surveillance System (NWSS) to work with state health departments to determine the SARS-CoV-2 viral load in wastewater in efforts to characterize the spread of the disease. Advances that support detection and quantification of other viral pathogens in wastewater have also been made since the start of the pandemic. Nevertheless, significant measurement challenges remain. Many different protocols and technologies are used throughout the wastewater surveillance workflow, particularly in laboratory steps of sample concentration/extraction and detection, leading to a lack of reproducibility and comparability across laboratories. Novices in the field need guidance, while experts have numerous lessons learned that are yet to be captured in an accessible systematic way. Also, many organizations encounter uncertainty on how best to implement wastewater surveillance for new targets. Our objective is to lead the development of measurement assurance materials and guidance documents to help address some of these issues. Materials under consideration as references include a synthetic wastewater protocol, well-characterized control materials for fecal load normalization and/or recovery testing, and plasmid DNA for antimicrobial resistance gene targets. For guidance documents, the Standards for Wastewater Surveillance (SWWS) Working Group is developing consensus-based best practices for the concentration step of the wastewater surveillance workflow. I will describe our progress toward these measurement assurance materials and guidance document. The anticipated impact of standards such as these is increased confidence and quality assurance for sample concentration/extraction and analyte detection steps and improved comparability and capabilities in wastewater surveillance.

Poster # 51S
Title: Virus genome degradation in untreated wastewater
Author: Katherine R. Harrison, Delaney Snead, Anna Kilts, Michelle L. Ammerman, Krista R. Wigginton
Contact: kateharr@umich.edu

Wastewater-based epidemiology has proven to be a useful tool for tracking viruses in wastewater and is increasingly being used for viruses beyond SARS-CoV-2. However, it is not well understood how the state of the virus (i.e. extraviral or encapsidated) affects the genome signal over time. Additionally, the presence of viral RNA or DNA does not mean an infectious virus is present; better understanding the physical integrity of viruses in wastewater could improve interpretation of molecular measurements in terms of real public health risks.

Here, we used four viruses to assess the impact of the capsid on virus measurements in wastewater. We compared extraviral RNA and DNA decay in wastewater as well as encapsidated RNA and DNA genome persistence. We also examined how amplicon size may bias genome quantification in wastewater. Finally, we compared virus infectivity and genome persistence in wastewater for two viruses.

When the capsid was not present, we found that dsDNA is more persistent than ssRNA. When the nucleic acids were encapsidated, genome decay was slower, with average decay rates on the order of 0.1 days-1. This research demonstrates that the virus capsid provides significant protection to the nucleic acid signals that we rely on for WBE.

We also found that the MS2 amplicon targets degraded at the same rate regardless of size. This finding, coupled with the extraviral nucleic acid results showing that RNA degrades quickly when the capsid is compromised, suggests that the size of the amplicon should not greatly affect WBE findings for ssRNA viruses. These results help us better interpret how virus surveillance is valuable for public health and water safety.

Although wastewater-based surveillance has been successful for SARS-CoV-2 during the global pandemic, this study on both RNA and DNA builds an important knowledge base for all viruses with different genome types and structures.

Poster # 52S
Title: Examining Persistence of Coronavirus in Septage
Author: Emily Zak, Matthew Flood, Rebecca Ives, Nishita D'Souza, Joan Rose
Contact: zakemily@msu.edu
The COVID-19 pandemic has threatened the health of millions of people worldwide since 2020. Throughout the pandemic, participating labs across the state of Michigan have been monitoring SARS-CoV-2 levels in wastewater. These samples were found to reflect trends true to the reported cases with some early warning, with a way to include asymptomatic cases that otherwise would not have been reported. However, approximately 35% of Michigan's population uses septic tanks in mostly rural communities and have not been included in the wastewater surveillance. As the pandemic continues there is less testing but there is still a need to keep track of reported cases. The goal of this study was to understand how long the virus signal could last in septage. This observational study examined the degradation of spiked OC43 virus signal in 7 different septage samples over a span of 100+ days. Samples were tested at both room temperature (~22°C) and 4°C. Preliminary results show that over a 2 month period, virus levels in samples kept at room temperature only degraded an average of approximately 2 log gene copies per liter of sample. There is not a significant difference seen in virus concentrations in samples kept refrigerated over the same period of time, suggesting that coronavirus persists for a much longer period in samples kept in a colder environment.

Poster # 53S
Title: Frequency and Degradation of SARS-CoV-2 Markers N1, N2, and E in Sewage
Author: Michael Vu, John J. Hart, Nicholas Castle, Josie Kuhlman, Megan N. Jamison, Jim N. McNair, David C. Szlag
Contact: michaelvu@oakland.edu

Coronavirus disease 2019 (COVID-19) is an infectious disease which is mainly spread through aerosolized droplets containing severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) and is excreted in feces by infected individuals. Sewage surveillance has been applied widely to obtain data on the prevalence of COVID-19 in whole communities. We used SARS-CoV-2 gene targets N1, N2, and E to determine the prevalence of COVID-19 at both municipal and building levels. Frequency analysis of wastewater testing indicated that single markers detected only 85% or less of samples that were detected as positive for SARS-CoV-2 with the three markers combined, indicating the necessity of pairing markers to lower the false negative rate. The best pair of markers in both municipal and building level monitoring was N1 and N2, which correctly identified 98% of positive samples detected with the three markers combined. The degradation rates of all three targets were assessed at two different temperatures (25 and 35 °C) as a possible explanation for observed differences between markers in frequency. Results indicated that all three RNA targets degrade at nearly the same rate, indicating that differences in degradation rate are not responsible for the observed differences in marker frequency.