Applying Wastewater-Based Epidemiology to Identify Sites of Active COVID-19 Transmission in Pierce County

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ABSTRACT
Prior to the outbreak of SARS CoV-2, enveloped, RNA viruses had been successfully detected in municipal wastewater. By March 2020, SARS CoV-2 detection via wastewater sampling was demonstrated as predictive, by 3-5 days of subsequent rise in COVID-19 infections in the US and European studies. Thus, measuring municipal wastewater could be a useful epidemiological tool for COVID-19 management. Despite relative validation, these data provided total population level trends, leaving officials little time, or location-specificity, to make use of this warning signal. To address this problem, we partnered with the City of Tacoma Environmental Services Division and Tacoma Pierce County Department of Health on behalf of RAIN, a Tacoma based biotechnology non-profit, and designed a neighborhood-level rapid response plan. By analyzing health disparities data, wastewater infrastructure and active case rates we sought to illuminate sites of active transmission. To conduct this study, we sampled weekly from two wastewater treatment plants in Tacoma, WA, and ten geographically disparate neighborhood sites to monitor SARS CoV-2 levels. We collected population-specific biological samples in each neighborhood for a population-sites to monitor SARS CoV-2 levels. We collected population-specific biological samples in each neighborhood for a population-
specific SARS-CoV-2 analysis. Using quantitative real-time polymerase chain reaction (qRT-PCR), we successfully isolated SARS-CoV-2 at variable levels in all sample populations between April 2020 and March 2021. Taken together, our data reveal infection rates we sought to illuminate sites of active transmission. To conduct this study, we sampled weekly from two wastewater treatment plants in Tacoma, WA, and ten geographically disparate neighborhood sites to monitor SARS CoV-2 levels. We collected population-specific biological samples in each neighborhood for a population-specific SARS-CoV-2 analysis. Using quantitative real-time polymerase chain reaction (qRT-PCR), we successfully isolated SARS-CoV-2 at variable levels in all sample populations between April 2020 and March 2021. Taken together, our data reveal infection trends within a municipal sub-population in absence of individual testing. Moreover, the data provides insight on both symptomatic and asymptomatic infections within the population, informing health officials where specific intervention is necessary to reduce the spread of COVID-19.

METHODS
Sample Procurement and Processing
Sample obtained from wastewater conveyance sidewall buildup and immediately submerged in RNA/DNA shield. Samples were then column extracted using Zymo Research RNA shield. Samples were then column extracted using Zymo Research RNA extraction kit. Purified elution analyzed by RT-qPCR using CDC specified SARS-CoV-2 primers and probes, Rnase P primers and probes, nuclease free water as negative control along with Twist synthetic SARS-CoV-2 RNA as positive control.

RESULTS
• We consistently detected SARS-CoV-2 in neighborhood sized populations (median = 4,854).
• SARS-CoV-2 titers are reduced at Wastewater Treatment Plants during periods of increased rainfall.
• We detect SARS-CoV-2 by our method during periods of increased rainfall at all population levels.
• Coordination and analysis is ongoing with Tacoma Pierce County Department of Health regarding predictive potential of neighborhood sampling.

FUTURE RESEARCH
• Align asymptomatic cases within wastewater surveillance population by nasal swab testing of volunteers.
• Daily sampling for increased accuracy.
• Multiplex assay monitoring SARS-CoV-2 + other viruses

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