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Sensitivity of wastewater surveillance: What is the minimum COVID-19 cases required in population for SARS-CoV-2 RNA to be detected in wastewater?

Wastewater surveillance, also referred to as wastewater-based surveillance (WBS) and wastewater-based epidemiology (WBE), has become a powerful tool for the monitoring, control, and prevention of infectious diseases, including COVID-19. Wastewater surveillance is a complementary and unbiased approach to evaluate disease burden at a population level (Hrudey et al., 2022; Kitajima et al., 2020; Kumblathan et al., 2021; Polo et al., 2020). Since the onset of COVID-19 pandemic in 2020, multidisciplinary scientists have developed wastewater surveillance approaches and applied them to gather timely infection data at community levels and to assist public health authorities for decision-making (Hart and Halden, 2020; Hrudey et al., 2022; Kumblathan et al., 2022; Pecson et al., 2021; Prado et al., 2021; Wade et al., 2022). Complementing clinical surveillance, wastewater surveillance has advanced from monitoring the occurrence, dynamics, and trends of infections in communities (Ahmed et al., 2020; Chik et al., 2021; D'Aoust et al., 2021; Gonzalez et al., 2020; Kumar et al., 2021; La Rosa et al., 2021; Medema et al., 2020; Mlejnkova et al., 2020; Peccia et al. 2020; Randazzo et al., 2020; Wu et al., 2020; Zhao et al., 2022) to predicting prevalence and emergence of new variants (Amman et al., 2022; Crits-Christoph et al., 2021; Karthikeyan et al., 2022). Many agencies around the world, such as the World Health Organization (WHO), the Public Health Agency of Canada, the European Commission, and the United States Centers for Disease Control and Prevention (CDC), have recognized wastewater surveillance for SARS-CoV-2 as an important tool for informing COVID-19 response and management (CDC, 2020; Gawlik et al., 2021; Hrudey et al., 2022; WHO, 2022). Despite the importance and large number of published studies on the detection of SARS-CoV-2 RNA in wastewater, the minimum number of COVID-19 cases required in a population to produce a detectable viral RNA signal in wastewater was largely unknown. Li et al. (2023) reported a study in *Journal of Environmental Sciences* to fill this important knowledge gap.

To address the fundamental question of the threshold of COVID-19 cases required in a population for SARS-CoV-2 RNA to be detected in wastewater, Li et al. (2023) obtained and analyzed a unique and large dataset comprised of high numbers of diagnostic testing for COVID-19 and wastewater analyses spanning the first three waves of COVID-19 in Alberta, Canada. Wastewater surveillance was initiated two months after the first case of COVID-19 was identified in Alberta. From May 15, 2020 to June 7, 2021, the authors monitored SARS-CoV-2 RNA in 1842 wastewater samples collected from 12 municipal and regional wastewater treatment plants (WWTPs) that serve 10 cities and towns across the province of Alberta, representing 79% of the total population in the province. During the 14-month period, the data captured the first three COVID-19 waves and had detectable SARS-CoV-2 RNA in almost half of the collected wastewater samples. In the same period, approximately 4.7 million clinical diagnostic tests for COVID-19 were performed using reverse transcription quantitative polymerase chain reaction (RT-qPCR). In a province of 4.4 million people, 4.7 million clinical tests were conducted in the first 14 months, which represented the highest number of COVID-19 PCR tests per capita of any jurisdictions in Canada. The COVID-19 cases in the community were matched by postal codes in the communities served by the WWTPs with population sizes ranging from 13,451 to 1,115,021.

Taking advantages of this large scale of clinical diagnostic testing of COVID-19, matched by large numbers of wastewater samples from WWTPs serving a wide range of populations, the authors were able to assess the sensitivity of SARS-CoV-2 RNA detection in wastewater. They conducted Probit analysis, a specialized regression model for analysis of binomial response variables, to determine the detection threshold from low to high probability. They found that WWTPs serving smaller regions required fewer new cases to detect SARS-CoV-2 RNA compared to larger cities. As few as 2 new cases were required to achieve a positive detection of SARS-CoV-2 RNA in wastewater at 50% probability in the Town of Banff (13,451 residents, the smallest community studied). Approximately 67−77 new cases were needed to achieve positive detections at 50% probability in Edmonton and Calgary (the largest communities in the province, with about 1 million residents in each city). Whereas the absolute number of COVID-19 cases needed for detection varied with the size of the population served by the WWTP, the sensitivity of wastewater detection on the basis of the proportion of COVID-19 cases per 100,000 population was consistent. Overall, a minimum of 4−17 (median

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8), 9–43 (median 18), and 17–97 (median 38) daily reported new COVID-19 cases per 100,000 population were required for SARS-CoV-2 RNA to be detected in the community wastewater at 50%, 80%, and 99% probability, respectively.

The Li et al. (2023) study used larger datasets and covered an earlier and longer period of the COVID-19 pandemic in comparison with many other studies of RT-qPCR detection of SARS-CoV-2 RNA in wastewater. The datasets also captured the first wave when the numbers of infected individuals in the communities served by WWTPs were small. These dataset features are important for estimating the minimum COVID-19 cases required in a population for SARS-CoV-2 RNA to be detected in wastewater. The probability analysis presented the scenarios of SARS-CoV-2 RNA detection in wastewater from high (99%) to low (50%) probability. At low probability, the conservative estimates of the numbers of COVID-19 cases in the community provide early alert for local public health authorities to implement measures for prevention and control.

The authors acknowledged important considerations affecting the detection threshold of SARS-CoV-2 RNA in wastewater. They identified the variability in individual COVID-19 testing protocols between different communities and throughout the timeline of the wastewater sample collection. Hence, the corresponding new and active case numbers used for estimating the detection threshold could be affected by testing policies and case count accuracy. For example, testing of asymptomatic COVID-19 individuals was infrequently performed; therefore, reported COVID-19 cases were lower than the true numbers of infected individuals in the community. In addition, the complex sample matrix of wastewater could present challenges, such as variable levels of degradation of viral RNA and different substances that could inhibit RT-qPCR reactions. Wastewater surveillance is predicated on fecal excretion of viruses; however, fecal shedding of SARS-CoV-2 could vary among individuals. Furthermore, the rate and duration of fecal shedding remains unknown and likely varies with infected host factors. The authors suggest that evaluation of SARS-CoV-2 RNA detection sensitivity in wastewater benefits from large samples sizes over long periods of time.

As infections of the Omicron variant overwhelmed clinical testing capacity in many jurisdictions since late 2021, wastewater surveillance has become a primary approach for assessing the status of community infection. In an effort to manage COVID-19 and improve pandemic preparedness, public health organizations around the world are analyzing challenges and reflecting on lessons learned, including those from wastewater surveillance. In a Royal Society of Canada policy report released on August 8, 2022, the expert panel on wastewater surveillance made the following six recommendations: “Capture useful lessons from wastewater surveillance for SARS-CoV-2; create structures and capacity to sustain capability and develop rapid response to future public health threats; develop frameworks for surveillance program design; develop frameworks for interpretation of surveillance program results; maintain and promote academic partnerships and communication networks that will help identify new opportunities and threats; build upon existing infrastructure and programs” (Hrudey et al., 2022). These recommendations are particularly important for planning and developing relevant public health policies. They also provide useful guidance for future research and implementation of effective and timely wastewater surveillance. The ultimate goal is to protect public health from infectious disease and other environmental factors.

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