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Making Waves

Making waves: Plausible lead time for wastewater based epidemiology as an early warning system for COVID-19

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ABSTRACT

Wastewater-based epidemiology (WBE) has emerged as a useful tool in the fight to track and contain COVID-19 spread within communities. One of the motives behind COVID-19 WBE efforts is the potential for 'early warning' of either the onset of disease in a new setting or changes in trends in communities where disease is endemic. Many initial reports of the early warning potential of WBE have relied upon retrospective sample analysis, and delays in WBE analysis and reporting should be considered when evaluating the early warning potential of WBE that enable public health action. Our purpose in this manuscript is to establish a framework to critique the potential of WBE to serve as an early warning system, with special attention to the onset of viral shedding and the differential between results reporting for WBE and clinical testing. While many uncertainties remain regarding both COVID-19 clinical presentation and technical factors influencing WBE results, our analysis suggests at most a modest lead time interval ranging from six days for clinical testing to four days for WBE during community-level wastewater surveillance where clinical testing is accessible on-demand with a rapid time to results. This potential lead time for WBE subsequently increases in settings with limited clinical testing capacity or utilization. Care should be taken when reporting 'early detection' of COVID-19 disease trends via WBE to consider underlying causes (e.g., clinical testing lag or delayed result reporting) to avoid misrepresenting WBE potential.

Wastewater-based epidemiology to monitor COVID-19

As SARS-CoV-2 is excreted in body fluids, such as urine and feces, wastewater-based epidemiology (WBE; also termed wastewater surveillance or wastewater monitoring) has been used globally to monitor public health on a community-wide scale. Compared to individual testing, wastewater provides an aggregated, anonymous, and non-invasive testing opportunity. In WBE applications to monitor COVID-19, the presence and/or concentration of SARS-CoV-2 is monitored in wastewater as an indicator of the presence, potential incidence, and infection trends of COVID-19 within a community.

WBE has gained significant global attention in response to the COVID-19 pandemic (Bivins et al. 2020; Naughton et al. 2021). Among the key challenges in responding to the COVID-19 pandemic have been a lack of clinical testing capacity and disease spread by asymptomatic individuals who may not be ascertained by syndromic surveillance. WBE can be applied to monitor wastewater from the building to the community scale, potentially capturing disease presence and infection trends that may go undetected by clinical testing.

Literature reports of WBE as an early warning system for COVID-19

One of the motivations to develop WBE for monitoring COVID-19 has been the reported ability of WBE to act as an 'early warning system', although many of these reports relied upon retrospective analysis of sewage samples, e.g. (Mao et al. 2020; Randazzo, Cuevas-Ferrando, et al. 2020; Venugopal et al. 2020; Randazzo, Truchado, et al. 2020; Daughton 2020; Zhu et al. 2021). Early warning in this context refers to detecting disease presence or infection trends by WBE prior to clinical reporting. Specific proposed applications include monitoring for outbreak onset (Xagoraraki and O'Brien 2020), disease underreporting (Medema et al. 2020), and disease reemergence (Medema et al. 2020). Current early COVID-19 trend detection reports by WBE have been widely divergent, ranging from a 0-2 day lead time (Peccia et al. 2020; D'Aoust, Graber, et al. 2021) to predicted lead times as high as two (Kumar et al. 2021) and three weeks (Karthikeyan et al. 2021; Ahmed et al. 2021). The context of these studies varies widely, including different transmission patterns while the study was conducted, clinical testing availability and reporting within these communities, sewage

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collection and conveyance systems, and WBE laboratory and analysis techniques. A recent review highlighted some of the knowledge gaps in the potential of WBE as an early warning system, including better quantification of individual SARS-CoV-2 shedding dynamics and the ability to quantify the number of active shedders from WBE data (Zhu et al. 2021). Here, we consider the factors influencing the potential of WBE to serve as an early warning system of disease trends and establish the constraints around when it may serve this role.

Factors influencing WBE COVID-19 'Early Detection'

The goal of the current analysis is to establish a simplified framework to critique the early warning potential of WBE for community-level COVID-19 monitoring applications. There remains significant variability in time to results for both clinical and WBE testing among communities that may impact signal interpretation and reporting time. Potential sources of this variability include the role of asymptomatic transmitters of disease, genetic signal decay during wastewater transport through collection systems, and the efficiency sensitivity of both the clinical and WBE analytical workflows. Our objective here is not to build a comprehensive quantitative model, but rather a framework to interpret previous claims concerning the early warning potential for WBE. Ultimately, accurate presentation and reporting of the technical potential of WBE to identify community disease trends are essential for sustained and continued technical development of WBE applications.

'Early detection' as applied to COVID-19 WBE implies the successful detection and reporting of COVID-19 trends prior to their detection and reporting by clinical surveillance. Early detection via WBE may be driven by two distinct, but potentially related, factors. The first factor is the analysis time differential of WBE and clinical testing results. Factors influencing the response time of clinical testing and reporting have been well documented (Ahmed et al., 2020). Delays inherent to WBE analyses have been less well reported. Importantly, many WBE results are 'back dated' to sampled collection date during trend analysis without further consideration of sample processing time (i.e., when the result was actually reported). This reporting differential is critical for WBE results to provide an actionable lead time.

The second early detection factor is the excretion of SARS-CoV-2 RNA in the stool of infected individuals prior to either nasal viral shedding or symptom onset, and the subsequent clinical detection. In this instance, wastewater monitoring has the potential to detect COVID-19 cases before it would be likely for these cases to be clinically detected.

Delays in WBE data reporting

Similar to delays in clinical diagnostic reporting for COVID-19, there are potential delays in reporting WBE results that influence the relative ability of WBE to precede clinical testing results. Delay in this context refers to the time differential between the shedding of the viral signal in stool and the reporting of the positive SARS-CoV-2 signal in wastewater. While many studies have 'back-dated' WBE results to the date of sample collection to describe the early warning potential, here we describe the factors driving time to initial result reporting prerequisite to any public health decision making.

The first consideration is the time from viral excretion in stool to laboratory receipt of the wastewater sample. Depending on system configuration, wastewater may have significant travel time within the collection system prior to arrival at the wastewater treatment plant or other sampling location, with literature reports of wastewater travel times as high as 35 hours through collection systems (D'Aoust, Mercier, et al. 2021), although this time may be reduced at sub-sewershed sampling locations. In addition, composite wastewater sampling is often employed to capture temporal variability in the virus genetic signal. Composite sampling is most commonly performed in 24-hour increments. In this sampling scheme, subsamples collected early in the 24-hour collection period will be further delayed by this compositing period prior to analysis. Following collection, samples may need to be shipped from the collection site to the laboratory, adding additional delay prior to analysis. Considering all of these factors, the period between initial viral excretion and sample receipt by the analysis laboratory may be as high as two or more days in current sample collection workflows. We note that there is potential viral RNA decay as well during sample collection and transport, so in addition to delaying sample reporting this delay may also alter reporting accuracy (Ahmed et al., 2020, Bivins et al., 2020).

The second consideration is processing time in the laboratory. Typical analytical workflows include sample concentration, nucleic acid extraction, and PCR-based nucleic acid quantification. While there are approaches to make sample concentration more rapid (e.g., the recent analysis by LaTurner et al. (LaTurner et al. 2021)), the overall workflow is limited by the time required for nucleic acid extraction and PCR thermal cycling. In addition, some laboratories do not analyze samples until there is a sufficient batch size to reduce personnel and analytical burden and costs, further delaying results reporting.

Considering all of these factors, current standard sample collection and analytical workflows are reasonably capable of producing WBE results in one to three days following viral excretion in stool, but under some analysis scenarios this timeframe could easily be exceeded. We note that these workflow times are specific to individual WBE applications, and that individual scenarios should be fully described and considered in WBE reports.

Clinical SARS-CoV-2 shedding data

Existing human SARS-CoV-2 viral shedding data is primarily limited to care-seeking COVID-19 cases after the presentation of symptoms and clinical diagnosis, hindering our ability to infer pre-symptomatic viral shedding, as well as to study asymptomatic cases. Animal challenge studies are a useful model for such shedding as they include a well-defined intranasal inoculation time point and longitudinal monitoring of symptoms and viral load following infection. Here, we consider two animal inoculation studies to evaluate viral shedding dynamics following infection to provide insight into the potential for wastewater monitoring to act as an early warning system. In studies by Hartman et al. (Hartman et al. 2020) and Woolsey et al. (Woolsey et al. 2021) investigators examined shedding dynamics in African Green Monkeys (AGMs) following intranasal inoculation with SARS-CoV-2. During both studies, viral load was monitored via nasal and rectal swabs, along with fever onset. Here we use nasal swabs to represent clinical disease positivity, rectal swabs to represent fecal viral shedding, and fever to represent symptom onset. While these data are from an animal disease model, both studies conclude that AGMs are a plausible model to study the clinical course of COVID-19 in humans and subsequent medical therapies.

First, we consider whether fecal viral shedding precedes nasal viral detection. In this case, wastewater monitoring would potentially have the ability to detect cases of COVID-19 prior to the clinical diagnosis via nasal swab. In most cases, nasal viral shedding was detected at the first RNA measurement point two days post inoculation. No fecal sample was positive for RNA prior to detection of the virus in nasal samples. Although, in some cases both the nasal and fecal samples were positive at the initial sampling time point two days post infection. While this data does not allow the definitive exclusion of early viral fecal shedding, it suggests that fecal shedding does not precede nasal shedding and limits the potential fecal shedding lead time to less than two days.

Second, we consider viral detection in stool prior to symptom onset. Symptoms in this case were determined by increased temperature (fever) as defined in the original studies. For animals becoming febrile, virus RNA was detected in stool prior to symptom onset in two of 12 AGMs. In Hartman et al., fecal shedding in animal A4 preceded fever by 1.1 days. In Woolsey et al. (Figure 1B), fecal shedding in AGM3 preceded fever by 0.7 days. In all other cases, (n=10), fecal shedding either trailed

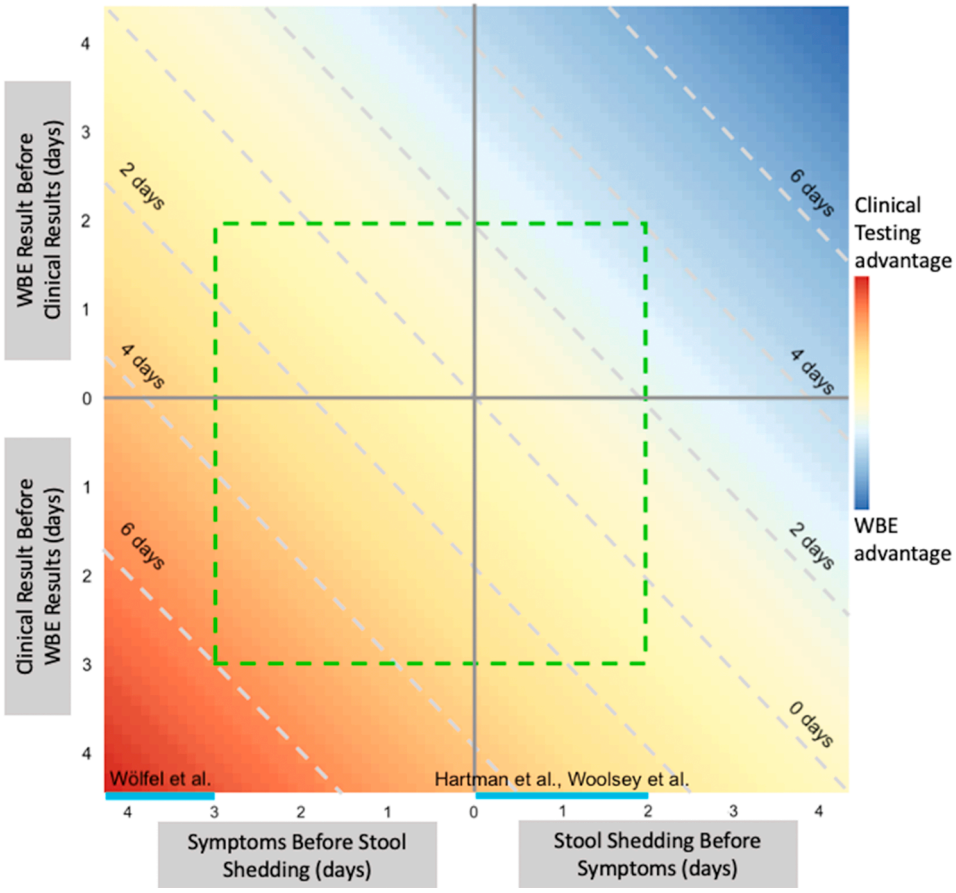

Figure 1. Framework to evaluate the temporal advantage of WBE versus clinical testing. The x-axis represents the onset of symptoms relative to the onset of RNA shedding in stool; the y-axis represents the differential in time to results between WBE and clinical testing. Indicated viral shedding areas are from (Hartman et al. 2020; Woolsey et al. 2021; Wölfel et al. 2020). The dashed green box indicates a representative plausible scenario range as discussed in the text.

symptom onset, or fecal shedding or fever was not observed. Notably, just as in human populations, some animals excreting virus in stool did not exhibit symptoms at any time point.

Alongside these animal studies, it is also useful to consider SARS-CoV-2 shedding during natural human infection to further understand shedding trends in humans. Wölfel et al. (Wölfel et al. 2020) evaluated viral shedding in sputum, stool, and urine of hospitalized COVID-19 patients following symptom onset. The first positive stool samples were observed three days after clinical presentation, and the proportion of patients with a positive stool sample increased through the first week following symptom onset. This suggests that patients were not shedding high viral loads prior to symptom onset. This is consistent with a recent meta-analysis by Miura et al. (Miura, Kitajima, and Omori 2021), which determined that both the incidence and load of viral shedding in stool increased after initial symptom presentation. We note that differing methods may be applied to quantify viral loads in stool and nasal swabs, with the potential for differing conflating factors such as the presence of PCR inhibitors. However, taken together, the evidence suggests that human viral shedding in stool is unlikely to precede symptoms on a timescale of weeks and that viral stool shedding load increases following the onset of symptoms in symptomatic cases.

Finally, we note that there are likely individual exceptions to the trends we outline above, especially within human populations. However, as wastewater monitoring is intended to be a community-level tool, rare individual variations (e.g., early fecal shedding) are not expected to be significant contributors to wastewater as an early warning tool.

Evaluating the early warning potential of WBE for COVID-19 monitoring

As discussed above, the potential for wastewater monitoring to provide early warning depends on both viral shedding dynamics and differentials in clinical and WBE testing and reporting. In Fig. 1, we consider the relative differentials under various shedding and reporting scenarios as discussed above. The x-axis of Fig. 1 is divided into two parts. One denotes the onset of symptoms one to four days before fecal SARS-CoV-2 RNA shedding while the other denotes the onset of fecal shedding one to four days before symptoms. The y-axis is the reporting time differential between WBE and clinical testing results and is also divided into two parts – one with WBE results leading clinical results by one to four days and the other with clinical results leading WBE results by one to four days. Importantly, application of this framework should include relevant ranges rather than specific values for a specific scenario to reflect the uncertainty associated with varying community-level WBE applications.

Restricting this analysis to a representative range of both viral shedding and clinical/WBE testing results differential is a useful exercise to consider the potential of WBE as an early warning tool. Based upon available shedding data, we consider the range of plausible scenarios as initial viral shedding in stool two days preceding symptom onset to three days following symptom onset per observations from COVID-19 patients and animal models. We then consider a scenario where a clinical test is administered at symptom onset, and clinical testing results are reported within zero to three days of collection. As described above, a representative range of WBE reporting times is one to three days from initial signal excretion. In this case, the reporting differential between clinical and WBE testing would range between a two day lead for WBE (i.e., WBE

results reported one day following RNA stool shedding) to a four day lead for clinical testing (i.e., WBE results reported seven days following stool shedding). Considering both the plausible stool shedding trends and reporting differential (highlighted in the green box on Fig. 1), the reasonable range of 'early detection' would be from a four-day lead time for WBE to a six-day lead time for clinical testing, limiting the possibility of longer early warning periods via WBE under this idealized scenario. It should be noted that for asymptomatic infections the onset of RNA stool shedding relative to symptoms would be unbounded toward the right side of the x-axis. In situations where asymptomatic infections are prevalent and unlikely to be detected by clinical surveillance, WBE could be capable of longer lead times for early detection.

Implicit to this analysis are the assumptions that clinical testing is widely available, and that the population is willing to be tested. The reporting advantage of WBE would likely increase as clinical testing is less available and/or unutilized. In addition, this idealized analysis assumes that RNA stool shedding is detected via wastewater without error; false negatives and false positives are an additional concern to be considered when interpreting any disease monitoring approach, including WBE. In addition, this analysis assumes that the major SARS-CoV-2 RNA source in WBE applications is stool, which remains to be confirmed. A final consideration is the performance of asymptomatic surveillance testing, i.e., random individual testing in the absence of symptoms. Under such a surveillance testing scenario, where asymptomatic cases are much more likely to be discovered, advantages in wastewater detection for early warning will likely decrease, especially when surveillance testing is analyzed and reported rapidly.

Conclusions

- The potential for WBE as an early warning system is dependent on both the viral shedding dynamics relative to symptom onset and the differential between WBE and clinical results reporting.
- Delays in WBE analysis and reporting relative to sample collection should be considered when evaluating the early warning potential of WBE.
- Current data suggests that SARS-CoV-2 shedding in stool is typically limited to a maximum of two days prior to symptom onset; however, additional clinical datasets on SARS-CoV-2 RNA excretion in stool and symptom onset will help to refine this estimate.
- Considering an idealized scenario where clinical testing is widely available with relatively rapid result turnaround (0-3 days), the lead time for WBE early detection is constrained to a maximum of four days.
- WBE for COVID-19 has the strongest potential to provide early warning in locations with limited clinical testing capacity or delays in clinical results reporting, or where prevalent asymptomatic infections may evade clinical detection.
- Future claims regarding the potential of wastewater monitoring to provide an early warning system of COVID-19 should provide appropriate contextual information (e.g., due to delayed clinical testing) to allow for a nuanced interpretation of the observations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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