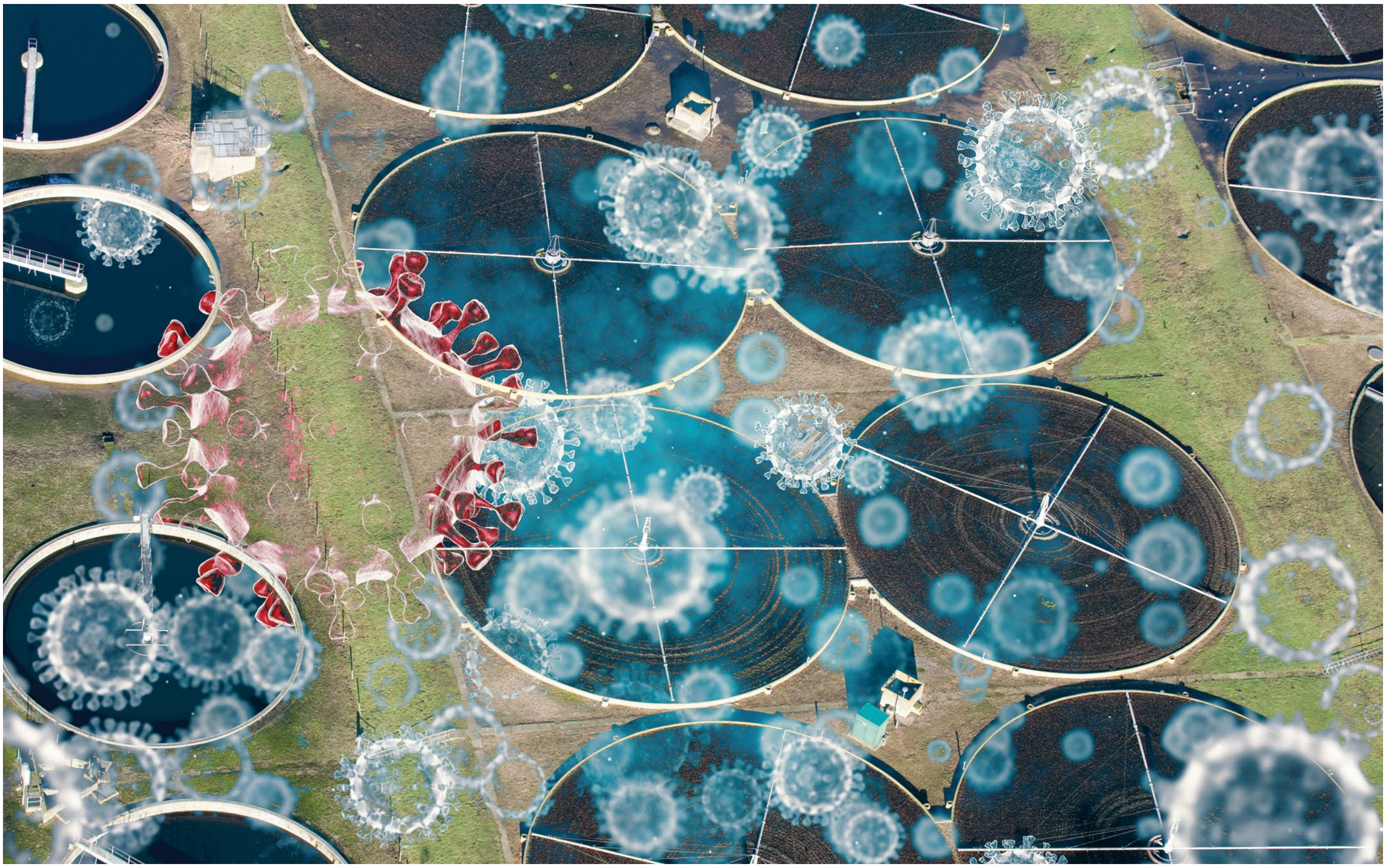

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Watcher in the wastewater

Research groups around the globe are looking to see whether urban wastewater monitoring can be integrated into surveillance systems for SARS-CoV-2 and other pathogens.

Charles Schmidt

After COVID-19 lockdowns eased across the United States in June, some parts of the country began reporting sudden increases in new cases. Texas was among the states leading the surge, and Houston in particular emerged as a national hotspot. Now health officials in Houston are tracking the disease pathogen — SARS-CoV-2 — in the city's vast sewer system. "Any given week we're sampling up to 38 locations," says Anthony Maresso, a microbiologist at Houston-based Baylor College of Medicine who is providing technical support. "And we're finding patterns of activity that flow in waves across the city: one spot may decrease and the spot next to it begins to rise."

Studies from around the world have shown that genetic traces of SARS-CoV-2 correlate with COVID-19 disease trends. The first reported detection of the novel coronavirus in sewage came in February. A study posted on 30 March on the health science preprint server medRxiv reported that a sample collected from Amersfoort, the Netherlands, tested positive for the virus six days before COVID-19 was diagnosed in that city, indicating that "wastewater surveillance can serve as an early warning of SARS-CoV-2 community transmission," says Gertjan Medema, a microbiologist at the KWR Water Research Institute who led the research.

Scientists are increasingly optimistic that wastewater surveillance can fill gaps in what's known about how the virus is moving through populations. As it stands, health officials rely on clinical diagnosis of people who are ill enough to be tested. That approach misses unreported cases and therefore underestimates actual disease rates and future hospital admissions. People will shed SARS-CoV2 in stool regardless of the symptoms (Box 1); therefore, the concentrations in sewage provide an "integrated view of what's happening in the community," says Peter Grevatt, CEO of the Water Research Foundation, a non-profit organization in Denver, Colorado. The evidence so far shows that viral signals in wastewater correlate with trends in clinical cases, he adds, making then leading

Box 1 | The known unknowns

The degree to which SARS-CoV-2 remains infectious in feces and sewage is still uncertain. The virus replicates efficiently in gut epithelial cells that express its binding site — the angiotensin-converting enzyme 2 receptor. During the SARS epidemic in 2002–2003, SARS-CoV was detected in the plumbing system of a Hong Kong high-rise apartment where residents had fallen ill with the disease. It was speculated that some infections may have resulted from their exposure to aerosolized viral particles migrating into the air from bathroom vents and open floor drains, though this was never confirmed. A recent study showed that aerosolized SARS-CoV-2 flung upwards by flushing toilets can hover in an airborne cloud for several minutes. The authors opined that toilets can promote virus transmission. Scientists speculate that fresh stool poses the greatest exposure risk. The risk of contracting COVID-19 from sewage flowing through wastewater systems is likely to be low, according to George Wells, a civil and environmental engineer at Northwestern University in Chicago, who nonetheless cautions that such sources "remain a potential route of transmission that needs to be evaluated further."

Rob Knight, a microbiologist at the University of California, San Diego agrees. But Knight points out that SARS-CoV-2 "may not replicate correctly in gut cells," such that the viral progeny could be unable to infect other people. Moreover, detergents and pH changes can damage the viruses as they travel in sewage, he says, at the same time that dilution reduces their concentrations to levels below infectious doses.

indicators of disease prevalence and sentinels for potential re-emergence after COVID-19 diminishes. The Italian Institute of Health announced that SARS-CoV-2 had been detected in sewage samples collected from Milan and Turin weeks before Chinese officials reported the first cases of COVID-19 on 31 December. In Paris, scientists reported high levels of SARS-CoV-2 RNA in samples collected several days before first cases were diagnosed in that city on 10 March. Might sewage be the way of the future for early detection of emerging pathogens?

An illustrious history

Surveying wastewater for viruses has a long history. In the 1940s, cell-culture methods were used to monitor sewage for polio and other viral pathogens. Scientists later turned to molecular techniques: hybridization with cDNA probes was adopted during the 1980s to monitor sewage for hepatitis A virus, and then in the 1990s scientists began turning to the more sensitive polymerase chain reaction (PCR) methods that are still widely used in the field today.

Sewage surveillance provided early warning of hepatitis A virus and norovirus outbreaks in Sweden in 2013, and it was credited that same year with thwarting a looming outbreak of paralytic polio in Israel. The Israeli Ministry of Health had been monitoring poliovirus in sewage samples collected on a monthly basis from inflows to wastewater treatment plants covering 30–40% of the country's population. After high poliovirus levels were detected in several sites, the Israeli health authorities launched a country-wide vaccination campaign and not a single case of paralytic polio was ever reported.

Eric Alm, a professor of biological engineering at the Massachusetts Institute of Technology, describes the poliovirus intervention as a "shining example of how this technology can be used." Both poliovirus and SARS-CoV-2 have three essential features making them optimal candidates for wastewater surveillance, he says. The viruses are excreted in fecal matter in large amounts; they infect some people asymptomatically (poliovirus produces one case of paralysis for approximately every 100 people), resulting in undiagnosed populations whose effluent allows early warnings; and surveying for them in wastewater generates actionable information.

Sewage surveillance programs

Countries around the globe are now launching wastewater surveillance programs with an aim to forecast new outbreaks, boost preparedness, and inform plans to open schools, restaurants and other public places. Scientists in Spain, for instance, have announced plans to sample 250 wastewater treatment plants twice a week. Israeli researchers at Ben Gurion University and the Ministry of Health started routine sampling wastewater for traces of SARS-CoV-2 in March.

In the United States, a company, Boston-based BioBot Analytics, is leading the charge on wastewater surveillance. At present, it is analyzing samples on behalf of more than 400 sewage treatment plants around the country. Mariana Matus, the company's cofounder and CEO, says sewage sampling provides "an early warning component that's highly valued by our customers," some of which have now begun to pay for the service after a period during which the company provided it pro bono.

"The science of sewage surveillance is still evolving, but there is evidence that it could be a useful indicator of prevalence trends in a given community," says Amy Kirby, an environmental microbiologist with the US Centers for Disease Control and Prevention (CDC). The CDC is now "evaluating the utility of sewage surveillance to provide robust and actionable response information," Kirby adds, "with plans to develop a national surveillance system for housing and interpreting sewage data to inform COVID-19 responses."

These are early days, and Kirby cautions that sewage surveillance alone should not be used to make public health decisions, but rather in "concert with other clinical indicators of the epidemic trends."

Evolving protocols

Developing standard laboratory protocols for detecting SARS-CoV-2 in untreated wastewater has now become a priority for the field. In June the Water Research Foundation began assembling an expert panel tasked with evaluating existing laboratory methods and selecting the ones that generate the most reliable results. "We need a better understanding of how protocols at different laboratories compare in terms of the PCR primer sets they use and the methods for concentrating and preserving the genetic signal," says Grevatt, who is leading the effort.

Sewage samples are generally collected from the untreated influent that flows into wastewater treatment plants or, in some instances, from manholes, pumping stations or plumbing systems in individual buildings, such as hospitals. Organic material content, industrial wastes, humic acids, temperature, rainfall, saltwater and other variables can inhibit, delay or otherwise alter the viral signal, which changes throughout the day. Grevatt says that, for this reason, composite samples collected over multiple hours are more representative than grab samples collected at a single time point.

SARS-CoV-2 virus is so highly diluted in wastewater that concentrating it into smaller liquid volumes is essential to the

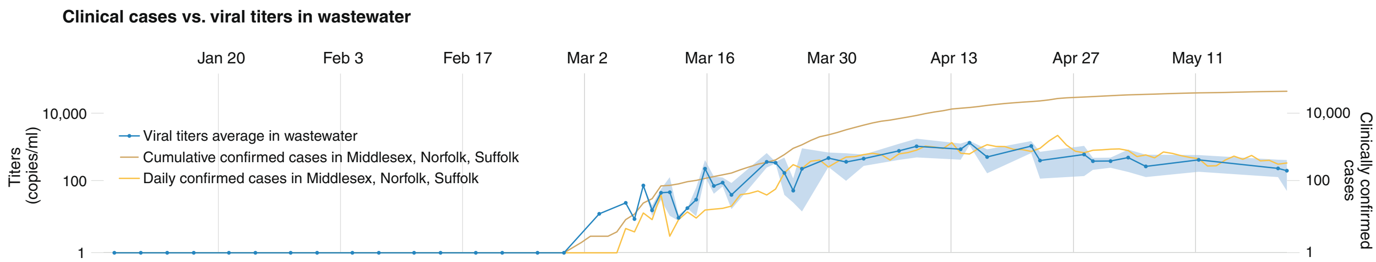

Fig. 1 | Clinical cases versus viral titers in wastewater. Viral titers in wastewater in four counties in Massachusetts (blue line, left vertical axis; shaded area represents the minimum to maximum range of normalized and averaged values), along with daily (orange line) and cumulative (brown line) confirmed cases (right vertical axis). Adapted with permission from F. Wu et al. https://doi.org/10.1101/2020.04.05.20051540 (2020).

analytical process. Some labs concentrate the virus using electrostatic filters to which the negatively charged viruses will adsorb. Ultrafiltration based on size exclusion is another option, as is polyethylene glycol precipitation.

The above methods have a long history in environmental virology, but they were developed for non-enveloped enteric viruses, such as norovirus and hepatitis A virus. In contrast, SARS-CoV-2 is an enveloped virus, meaning that an outer lipid membrane shields its RNA genome. The risk is that current methods are too harsh, such that the virus breaks apart during the concentration step, with some of its RNA being lost before being detected. According to Maresso, however, the existing methods for concentrating SARS-CoV-2 in wastewater appear to be working. “I also think we’ll be able to improve on them,” he adds.

That may also be true of the reverse transcription and PCR (RT-PCR) primer sets selected for viral detection. Most labs today are using primers selected by the CDC for clinical diagnostic purposes — specifically, primers against the SARS-CoV-2 nucleocapsid protein 1 (N1) and 2 (N2) genes, which are conserved regions of the viral genome. According to Kirby, the N1 and N2 primers are highly specific to SARS-CoV-2, although, she says, “the sewage surveillance field is still evaluating whether these clinical targets are the best for environmental samples.”

Parallel trends

So far, the correlations between wastewater sampling results and diagnostic trends look strong. “What’s most encouraging to those of us doing wastewater monitoring of COVID-19 has been the number of independent groups that can demonstrate high-quality correlations between the number of clinical cases identified and the strength of the RNA signal in corresponding sewersheds,” Grevatt says. “We’re seeing that in many countries and also among the utilities collecting data in the United States.”

One such utility, the Hampton Roads Sanitation District (HRSD), operates nine sewage treatment plants in southeast Virginia that collectively serve 1.8 million people. HRSD had been monitoring for enteric pathogens in sewage for years, and “that experience put us in a good position to quickly and efficiently get going on COVID-19,” says Jim Pletl, the company’s director of water quality. HRSD chemists picked up a SARS-CoV-2 signal in early March, when there were just two diagnosed cases of COVID-19 in the region. Analytical trends so far “seem to match up with [diagnostic] information that is provided by the Virginia Department of Health,” he adds. In addition, trends in the strength of the genetic signal vary according to the characteristics of the different service areas, “and there are likely circumstances specific to each service areas that caused these differences,” Pletl says. “Some have more industries and hospitals.” The HRSD team is working to figure out how clinical cases, local demography, and the strength of the viral signal in each area are linked.

A wastewater surveillance program initiated by the city of Tempe, Arizona, also shows how viral loads in sewage vary with the nature of the population: the highest viral loads are in wastewater from the town of Guadalupe, which is located five miles from Tempe and populated almost entirely by Hispanic people and Native Americans from the Yaqui Tribe, many of them essential workers “who rely on daily wages and have to leave their homes to provide for their family members,” says Rolf Halden, director of the Biodesign Center for Environmental Health Engineering at Tempe-based Arizona State University, which analyzes the samples. The COVID-19 rate in Guadalupe is more than four times higher than it is in the surrounding Maricopa county. Furthermore, viral loads in Guadalupe’s wastewater remained consistently elevated, even as levels fell in sewage from wealthier sections of Tempe during lockdowns imposed by city officials in April and May. Sewage surveillance data for the entire region are posted on a public dashboard hosted by the city of Tempe.

BioBot’s Matus describes wastewater surveillance as an effective “hotspotting tool” for monitoring high-risk areas, and adds that by sampling at the street or building level, scientists can gain a finer-grained understanding of local disease trends. The company’s sampling in Massachusetts shows that viral concentrations in wastewater vary by neighborhood according to average household incomes and access to healthcare. “What that tells us is that if we want to deploy resources to fight COVID-19, we need to focus in on underserved communities,” says the Massachusetts Institute of Technology’s Alm, who is also BioBot’s scientific director. “Our interventions are focused on people who can work at home, but the disease is carried mostly by people who lack that luxury — they’re still getting sick before, during and after the stay-at-home orders.”

Getting to the numbers

Alm and Matus say their models are now going beyond trend estimates to infer the number of infected people in a given area. On 23 June, the BioBot team and its collaborators posted a study on medRxiv reporting that viral loads from an urban wastewater treatment plant in Massachusetts serving a combined 2.25 million people shot up in advance of a surge in diagnosed cases (Fig. 1). The virus was first detected in sampling from the plant on 3 March, when just two people in the service area had been diagnosed with COVID-19. The viral loads hovered below 90 copies per milliliter of wastewater until 15 March, after which they began climbing sharply. Four days later, clinical reports of COVID-19 hit a corresponding high, which is consistent with the typical 4- to 5-day incubation period from infection to symptom onset, according to William Hanage, a coauthor and epidemiologist at Harvard University's T. H. Chan School of Public Health.

Alm says that by mathematically fitting viral titers in wastewater to the number of diagnosed cases over time, it's possible to infer shedding rates in stool that correspond with the number of infections. "We found that the shedding function has a huge peak in the very beginning stages of the infection that occurs before the patient presents symptoms and gets tested," he says. "After that, it falls off exponentially." A sudden boost in the genetic signal, he says, "reflects disease incidence — it's actually the number of new cases that will be reported in the future."

Other experts are more skeptical, however, and argue that, given modeling uncertainties, particularly with respect to shedding rates, wastewater surveillance is, for now, better suited to more limited assessments of whether disease rates in particular areas are falling or rising. "There's divergence of opinion in the scientific community," Halden notes. "Some say you can pinpoint the number of infected individuals, but in my view that's overly ambitious." The duration of viral excretion can "last days or months; we don't know," he adds. "And 1 individual may excrete as much virus as 100 others. If we don't fully acknowledge the uncertainty, it may come back to haunt us, and expose us to criticism. We don't want to overstate what the data mean or we'll be at risk of losing credibility."

Lawrence Madoff, medical director of the Bureau of Infectious Disease and Laboratory Sciences at the Massachusetts Department of Public Health, says state officials have access to many different data streams for monitoring COVID-19 and other diseases. Apart from viral testing, syndromic surveillance of social media, web searches and hospital admissions, he says, can also reflect an early increase in disease rates. But Madoff says the resulting data may not be unique to COVID-19, which shares symptoms with other respiratory and gastrointestinal ailments. That's where sewage sampling "hits a sweet spot as a leading indicator," he says. "It's both early and specific to COVID-19."

Madoff says wastewater monitoring can't replace other surveillance methods, all of which he describes as complementary. But he adds that the evidence so far with wastewater surveillance is encouraging and actionable. "It could prompt us to look closer at what's going on in a particular area by looking at other signals, such as testing or hospitalization data," he says. Matthew Meyer, County Executive for New Castle, Delaware, says wastewater surveillance is already informing pandemic responses in his jurisdiction, which has roughly 560,000 residents. County officials are getting weekly sampling updates from 11 sewage treatment substations throughout the region, and according to Meyer, the coverage far exceeds what's feasible with clinical testing. "We've tested about 50,000 people over the last few months, but wastewater surveillance routinely monitors 70% of the population," he says. "We have to make decisions every week about where to locate drive-by testing sites, and the sewage surveillance data are guiding us."

Rob Knight, a microbiologist at the University of California, San Diego, says wastewater surveillance for COVID-19 shows enormous potential. His team plans to monitor sewage sampled from cafeterias, dorms and other campus buildings as part of the university's 'Return to Learn' program, which also includes contact tracing and isolation housing for students who test positive for SARS-CoV-2. But Knight says there's still a need for more data from different populations, especially on shedding rates and what controls them. "We're finding that shedding rates are highly variable by symptom," he says. "What has to happen is not just PCR but also sequencing to see if the loads are high from one individual or low from many." According to Knight, sequencing could potentially pick up viral strains missed by PCR, but it hasn't yet been applied to wastewater sampling of SARS-CoV-2 on a broad scale, and could be useful for interpreting wastewater results. "We're still working on better ways to integrate environmental microbiology with viral epidemiology," Knight says. "This pandemic is compelling us to work together in new ways." ❐

Charles Schmidt
Portland, ME, USA.

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