Workers take samples from a sewer in Nice, France, in June 2021.

SIGNALS FROM THE SEWER

Measuring virus levels in wastewater can help track the pandemic. But how useful is that?

By **Gretchen Vogel**

In March 2020, the Austrian ski town of Ischgl—known for 239 kilometers of uninterrupted runs and an exuberant après-ski scene—suddenly became infamous as the site of the one of the first COVID-19 superspreading events. Hundreds of infected skiers took the virus home and seeded outbreaks all around Europe.

As the pandemic progressed, however, Ischgl was on the vanguard for a more positive reason: Health officials and scientists in the state of Tyrol were among the first to monitor levels of the pandemic coronavirus in sewage—and base health policy decisions on them. Because the region is so dependent on tourism, officials were eager to know whether the virus was truly on the decline so they could lift key restrictions. They also wanted to catch the earliest possible signals that it might be coming back. Wastewater analysis, which picks up fragments of virus shed in feces, was invaluable, says Stefan Wildt, a wastewater expert at the state's department of water management. Following Tyrol's lead, a national program has recently expanded to cover more than half of Austria's population.

Although wastewater monitoring has been used to track polio and other pathogens for decades, the COVID-19 pandemic has led to an explosion of interest. The technique takes advantage of the fact that SARS-CoV-2 replicates in the digestive system and is shed in high quantities, often before symptoms appear. (The virus is also detected in urine, though not as consistently.) That provides an inexpensive way to monitor infections in thousands or even millions of people without pesky nose or throat swabs, or to predict where cases might be about to surge and hospitals risk getting overburdened. The genetic sequences of the shed virus can also provide hints about how it is evolving.

Scientists in the Netherlands, which has had a nationwide network of wastewater monitoring for decades, were among the first to show fragments of SARS-CoV-2 virus in wastewater samples could accu-

A sewage treatment facility in Austria's Tyrol state, which launched an ambitious wastewater monitoring program early in the COVID-19 pandemic.

PHOTO: STATE OF TYROL/GÜNTER RICHARD WETT

rately reflect its levels in the community (see graphic, p. 1103). Since then, monitoring projects for SARS-CoV-2 have sprung up in at least 58 countries, according to a dashboard set up by Colleen Naughton and colleagues at the University of California (UC), Merced. The European Union recommended all member countries establish monitoring systems for SARS-CoV-2 by October 2021, and 26 of 27 have complied, says Bernd Manfred Gawlik, who is helping coordinate efforts through the European Commission. In the United States, the National Wastewater Surveillance System includes 400 sites in 19 states. Last month, the U.S. Centers for Disease Control and Prevention added a national dashboard of wastewater data, and on 2 March, President Joe Biden's administration said the monitoring system will be part of the effort to detect new variants. In India, a successful project in Bengaluru is expanding to half a dozen new cities.

Still, the jury is out on just how useful the technology is. Reliably determining viral levels in wastewater has posed logistical and technical challenges, and interpreting the data can be difficult. (For one, a good downpour will send virus concentrations in sewers plummeting.) Establishing collection, testing, and reporting systems can be time consuming and expensive as well. And although policymakers have welcomed the results of wastewater monitoring, few have used them to take action; typically, they have waited for cases to rise and intensive care units to fill up.

Shelesh Agrawal of the Technical University of Darmstadt, who has been analyzing water samples from sites across Germany since 2020, says it has been a struggle to convince policymakers the data are useful. "We are the information delivery guys. We deliver to your doorstep, but can't make you eat." Even in the Netherlands, which boasts one of the world's most sophisticated monitoring systems, researchers acknowledge it has had little impact on national policies. Local officials have made use of the Dutch data, however—for example, by ramping up testing in neighbor-

hoods where wastewater suggested cases were being missed.

As the pandemic shifts, however, wastewater could start to play a bigger role in shaping policy. Many countries are not only lifting pandemic restrictions, but abandoning widespread testing of the population, and more people now rely on home tests that aren't reported in official statistics. That makes wastewater a key remaining tool to understand the course of the pandemic, says Heather Bischel, a wastewater expert at UC Davis: "It provides a bigger picture snapshot."

IN THEORY, WASTEWATER testing is straightforward. Like standard clinical tests, it uses a polymerase chain reaction (PCR) assay to search for specific snippets of viral RNA in a sample, which are then copied repeatedly to amplify the signal. The number of cycles, or rounds of copying, needed to detect a signal in a sample is a rough measure of how much virus is there.

But whereas a throat or nose swab contains about the same amount of material from person to person, wastewater samples contain different amounts of feces, depending on the day and time a sample is taken, recent rainfall, and whether the toilets upstream are in homes, offices, or other buildings. All such variables have to be factored in to be able to accurately "read" a sample. How the water is collected, stored, and processed also affects the results. All of these variables make it very difficult to compare data from different sites.

"Some of the stories you read make it sound like you scoop some water out, dip a test stick in, and get your answer," says Hannah Safford, a former student in Bischel's lab at UC Davis and a policy expert at the Federation of American Scientists. "But it's so much harder than that."

Trial and error has helped scientists and technicians refine their techniques during the pandemic. Multiple groups have tested the best way to concentrate samples, comparing, for example, centrifuge times and filtration techniques; they have also identified reference viruses common in wastewater that can help calibrate samples. One unlikely sounding but popular reference is the pepper mild mottle virus (PMMoV). Harmless to humans, it attacks spicy and bell peppers and is ubiquitous in wastewater, passing through the digestive system when we eat infected produce. Because the concentration in human feces stays relatively stable year-round, scientists use it as a proxy for the amount of feces in a sample, reporting results as a ratio of PMMoV levels to SARS-CoV-2 levels.

In Sweden, Zeynep Cetecioglu Gurol and her colleagues at the KTH Royal Institute of Technology discovered that freezing samples, necessary for a while because PCR reagents were in short supply, made virus levels plummet. In Kansas City, Missouri, researchers were puzzled that the viral level in wastewater appeared to increase in a region where clinical cases seemed stable. They found out that repair work had diverted millions of liters of additional wastewater into the sewers from outlying suburbs, skewing their calculations.

Angela Chaudhuri, a public health expert at Swasti Health Catalyst who helped coordinate Bengaluru's monitoring project, says her team had to figure out how levels in open drain systems, common in Bengaluru, compare with closed sewers.

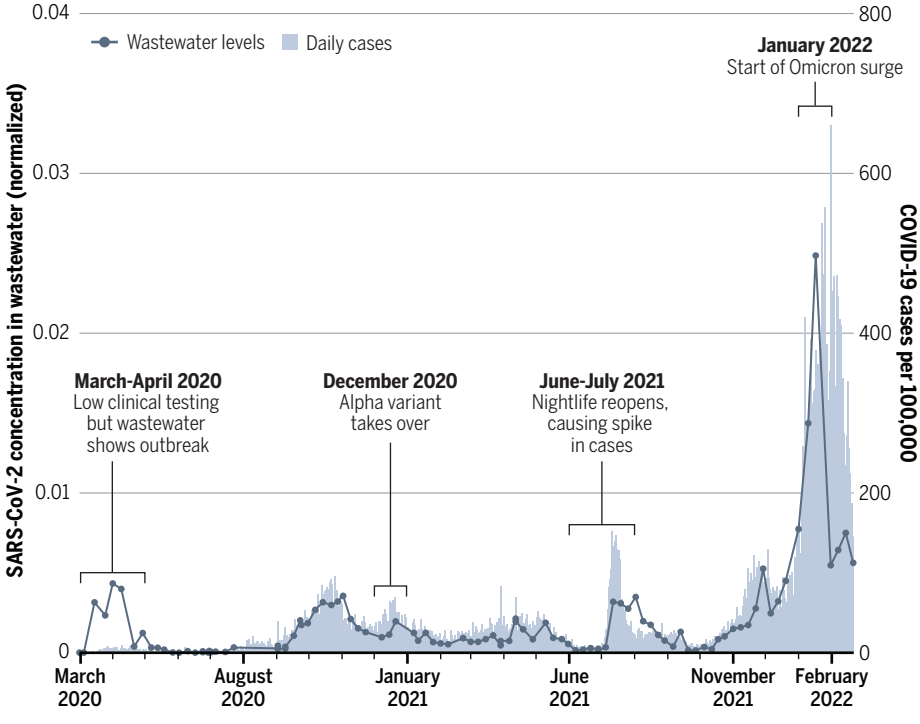
Increasingly, scientists are making their results available directly to the public. The Bengaluru project, for example, has had an online dashboard since May 2021 that shows where virus levels are increasing, decreasing, or remaining stable. "There is mistrust of the government when it comes to COVID," Chaudhuri says. "People think they might be hiding cases. This dashboard gives an independent signal" that can corroborate the official numbers. Marc Johnson, a virologist at the University of Missouri, Columbia, who has helped lead the state's extensive wastewater monitoring, says media and the public have welcomed the data. When the dashboard couldn't be updated on Christmas Eve, he says, the team spoke with journalists eager for information about the appearance of the Omicron variant in sewersheds. "It gives people peace of mind. If you feel like you know what is going on, you have more control," Johnson says.

In addition to tracking the spread of variants, wastewater monitoring has also enabled Johnson and others to spot odd SARS-CoV-2 strains in St. Louis and New York City, never seen in patients, that pose an epidemiological and evolutionary mystery (see sidebar, p. 1102).

SCIENTISTS SAY perhaps the clearest case for wastewater monitoring is in places where there is little virus at all. Australia and New Zealand, for example, used wastewater monitoring as a key part of their zero COVID strategy: As soon as a positive sample appeared, health officials ramped up testing, alerted the public, and, if active cases were confirmed, swiftly imposed restrictions to nip the outbreak in the bud.

Tracking the pandemic's waves

In the Netherlands, which boasts one of the world's most sophisticated monitoring systems, SARS-CoV-2 levels in wastewater have correlated fairly closely with reported COVID-19 cases throughout the pandemic.



CREDITS: (GRAPHIC) K. FRANKLIN/*SCIENCE*; (DATA) GERTJEN MEDEMA/KWR

(Although monitoring continues, both countries have recently given up on zero COVID.) A similar approach has worked on a small scale as well: In fall of 2020, wastewater testing on U.S. college campuses identified infections in residence halls before anyone had tested positive. Testing all the residents enabled health authorities to identify the infected person before the virus spread.

But in regions where cases are high and individual testing common, wastewater may not add significant information. "If you have limited resources, it's important to think about where to best deploy them," Safford says. If wastewater is simply confirming the trends already seen in clinical tests, she says, it may not be the best investment.

Caracas, Venezuela, participated in an international pilot program for SARS-CoV-2 monitoring in wastewater.

And in some cases, politicians simply aren't very interested. In December 2021, wastewater analysis in Florida's Orange county—home to Walt Disney World—showed Omicron, not yet detected in patients, was already the dominant virus. That meant a huge spike in cases was coming—but Florida's hands-off policies meant the find made little difference in public health policies.

ON THE OTHER SIDE of the Omicron wave, however, wastewater has provided reassurance that the decrease seen in tested cases is real, and not just an artifact of maxed-out testing capacity or people's reduced willingness to get tested. And as the pandemic begins to fade into the background in many places, the role of data from wastewater monitoring is likely to grow.

To make the data as reliable as possible, researchers are continuing to improve and standardize their techniques. Especially promising, says Gertjan Medema, an expert on pathogens in wastewater at the KWR Water Research Institute in the Netherlands, are new collecting devices consisting of a container housing magnetic beads or cotton "tampons" to trap the virus. The device is submerged in a sewershed and can collect a sample over hours or days, helping eliminate some of the irregular swings in viral concentration that can result from simply dipping a bottle into the water once a week. Ultimately, Gawlik says, researchers would like to converge on a standard protocol for collection and analysis.

Standardization could also benefit surveillance for other pathogens. Screening for poliovirus has been in place in many countries for decades, and several regions have kept tabs on illicit drugs in wastewater, but the pandemic has increased interest in looking for other diseases. In Bengaluru, Chaudhuri notes, diarrheal diseases are major killers. Wastewater monitoring might allow health authorities to identify outbreaks early and help identify the pathogens at work. Other researchers are adding tests to keep an eye on influenza, rotavirus, norovirus, adenoviruses, respiratory syncytial virus, and antibiotic-resistant bacteria.

Researchers would also like to sequence more of the viruses they catch, because standard PCR assays usually can't distinguish between variants. New York City's program, for example, has discovered Omicron in samples taken on 21 November 2021, several days before scientists in South Africa and Botswana announced they had identified the variant. But the New York City team didn't recognize its find until a week later, after they had sequenced the sample—and knew what to look for.

Sequencing wastewater samples is a challenge because the virus particles are usually degraded and they can come from hundreds or thousands of sources, Johnson says: "You can't figure out which ones fit together" in a whole genome. And because of the way sequencing software works, the most interesting parts of the genome—the regions that change most frequently—can easily be missed.

One way around that is to tailor the sequencing process to capture those fast-changing regions. That's how Johnson's team and another one in New York City identified the mysterious strains: They designed sequencing primers to match the beginning and end of the viral gene that encodes the receptor-binding domain (RBD), the part of the virus that helps it lock onto and infect cells. The technique fishes those RBD sequences out of the genetic soup, allowing the researchers to identify unique patterns of mutations that could provide clues about how the virus is continuing to evolve.

More surprises are likely. The Omicron variant, for example, seems to result in much less virus shedding in feces. In multiple countries, Medema says, wastewater levels lagged slightly behind the explosion in cases. After comparing results, researchers have concluded that, after Omicron took over, wastewater measurements have been underestimating cases—perhaps by a factor of three or four. The reduced shedding might be due to the changes in the virus—or the higher levels of immunity in the population, Johnson says.

Case numbers and wastewater levels in most countries are plummeting in tandem—at least for now. "We all feel it's finally sort of over. Though of course it's not really over, and the virus will continue to be there," Medema says. "We will continue to use wastewater as a sentinel to see what this virus is doing in the population." ■

PHOTO: SEVENTOV/ALAMY STOCK PHOTO

Signals from the sewer

Gretchen Vogel

Science, 375 (6585), • DOI: 10.1126/science.adb1874