Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

The challenges of informative wastewater sampling for SARS-CoV-2 must be met: lessons from polio eradication

Lancet Microbe 2020
July 28, 2020
https://doi.org/10.1016/S2666-5247(20)30100-2

Since the emergence and spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has caused the COVID-19 pandemic, many countries have rapidly expanded their viral surveillance systems. Wastewater sampling has been increasingly implemented, as substantial quantities of SARS-CoV-2 are shed in the stool of infected individuals.[1] So far, wastewater sampling has retrospectively shown that virus is present in cities several months before large COVID-19 outbreaks,[2] that there is a correlation between quantitative RT-PCR data and the reported incidence of cases,[3] and that the presence of SARS-CoV-2 in wastewater is ubiquitous.[4] There are numerous benefits of wastewater sampling, but the collection and interpretation of data is an emerging field. Within the Global Polio Eradication Initiative, wastewater sampling has successfully been used to detect polioviruses and inform eradication for several decades.[5] This virological analysis and investigation of wastewater has been done by the Global Polio Laboratory Network and independent laboratories. Here, we highlight several challenges of wastewater sampling for SARS-CoV-2 and outline lessons that can be learnt from polio eradication.

Interpretation of a single positive wastewater sample is difficult; longitudinal sampling alongside clinical surveillance is more informative. One positive sample merely suggests that at least one individual has shed virus upstream from the sampling site, and does little to inform epidemiology. Quantitative data (eg, quantitative PCR, metagenomics), and longitudinal sampling from the same site can provide more context. In the COVID-19 pandemic, the interpretation of positive samples will vary according to local assessment of the epidemiology: from an importation event to continued infection in the community. Development of protocols for interpreting and responding to positive SARS-CoV-2 samples are essential even in the early stages of use. RNA from the virus has been detected in stool (via RT-PCR amplification), but there is little evidence for infectious virus in stool,[1,6] meaning that whether stool is a source of new infections is unclear.

Sample site characteristics affect virus detection and require further study. Locations include the entrances of sewage treatment works, upstream pumping stations, or direct collection at rivers or latrines. Industry effluence, runoff from excess rain, and the pH of the sample[7] can all affect sample quality, which might influence the ability to detect and isolate virus. The method of sampling (eg, 24-h composite samples *vs* periodic grab samples), population demographics of the catchment area, and local epidemiological factors are important for planning environmental surveillance. The method and volume of sample is also important: several approaches (eg, bag filtration and composite sampling) are used to increase the volume of a sample.[8] Although large sample volumes might increase identification of virus in wastewater, this can make samples increasingly intractable to handle and process in laboratories. Wastewater sampling in cities requires good maps of sewer networks to understand what population is being represented. In very mobile populations (exemplified in Pakistan) sampling might indicate the presence of virus but not the affected population. Outside of dense populations there are fewer converging sewer networks that enable informative wastewater sampling; alternative sampling strategies for remote settings are a recognised need.

Laboratory methods should be validated, and for the assays that are used, analytical specificity and limits of detection should be reported.[9] Suitable process controls should be defined to validate results, identify false negatives, and minimise cross-contamination. In polio surveillance, isolation of the related non-polio enteroviruses (a group of ubiquitous enteric viruses) has been a useful quality indicator for field samples and testing performance. WHO protocols suggest that at least 10–30% of samples should reveal non-polio enteroviruses, and sites can be rejected if isolation is not possible. A clear separation in sample handling and processing is needed to reduce the risk of cross-contamination. Generally, samples are processed within biosafety level two laboratory conditions, separate from clinical samples, where the standard process involves a two-phase separation procedure (for virus concentration), followed by virus culture and isolation, as well as molecular methods that offer genome detection.

Increasingly, specialist laboratories are also adopting modern sequence-based methods for wastewater surveillance, such as metagenomics or nanopore sequencing,[10] which generates high-resolution genomic information that offers detailed insights into possible virus origins.

Despite the challenges, wastewater sampling has long been an important supplement to clinical surveillance in polio eradication and has the potential to inform the epidemiology of COVID-19. Wastewater sampling can act as an early warning system for local infection, and support clinical surveillance to confirm local elimination through negative samples. To be an informative mode of surveillance, it will be essential to set minimum criteria for surveillance sites, develop a consistent sampling strategy, establish laboratory testing protocols to enhance sensitivity and minimise the risks of cross-contamination, and to collaborate internationally.

We declare no competing interests.

Kathleen M O'Reilly, David J Allen, Paul Fine, Humayun Asghar
kathleen.oreilly@lshtm.ac.uk

Centre for Mathematical Modelling of Infectious Diseases, Department of Infectious Disease Epidemiology, Faculty of Epidemiology and Population Health (KMO, PF) and The Vaccine Centre, Department of Infection Biology, Faculty of Infectious and Tropical Diseases (DJA), London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK; and Polio Eradication Program, WHO Regional Office for East and Mediterranean, Amman, Jordan (HA)

1 Xu Y, Li X, Zhu B, et al. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. *Nat Med* 2020; **26:** 502–05.
2 Kelland K. Italy sewage study suggests COVID-19 was there in December 2019. Reuters. June 19, 2020. https://uk.reuters.com/article/us-health-coronavirus-italy-sewage-idUKKBN23Q1J9 (accessed June 22, 2020).
3 Wu F, Xiao A, Zhang J, et al. SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases. *medRxiv* 2020; published online April 7. https://doi.org/10.1101/2020.04.05.20051540 (preprint).
4 Medema G, Heijnen L, Elsinga G, Italiaander R, Brouwer A. Presence of SARS-Coronavirus-2 in sewage. *medRxiv* 2020; published online March 30. https://doi.org/10.1101/2020.03.29.20045880 (preprint).
5 Asghar H, Diop OM, Weldegebriel G, et al. Environmental surveillance for polioviruses in the global polio eradication initiative. *J Infect Dis* 2014; **210** (suppl 1)**:** S294–303.
6 Xiao F, Sun J, Xu Y, et al. Infectious SARS-CoV-2 in feces of patient with severe COVID-19. *Emerg Infect Dis* 2020; published online May 18. https://doi.org/10.3201/eid2608.200681.
7 Hamisu AW, Blake IM, Sume G, et al. Characterizing environmental surveillance sites in Nigeria and their sensitivity to detect poliovirus and other enteroviruses. *J Infect Dis* 2020; published online April 9. https://doi.org/10.1093/infdis/jiaa175.
8 Manor Y, Handsher R, Halmut T, et al. Detection of poliovirus circulation by environmental surveillance in the absence of clinical cases in Israel and the Palestinian authority. *J Clin Microbiol* 1999; **37:** 1670–75.
9 Lodder WJ, Buisman AM, Rutjes SA, Heijne JC, Teunis PF, de Roda Husman AM. Feasibility of quantitative environmental surveillance in poliovirus eradication strategies. *Appl Environ Microbiol* 2012; **78:** 3800–05.
10 Shaw AG, Majumdar M, Troman C, et al. Rapid and sensitive direct detection and identification of poliovirus from stool and environmental surveillance samples using nanopore sequencing. *bioRxiv* 2020; published online April 28. https://doi.org/10.1101/2020.04.27.053421 (preprint).