WASTEWATER-BASED EPIDEMIOLOGY FOR SARS-COV-2

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Population health surveillance is a fundamental cornerstone of public health intervention in pandemic response and risk management. This surveillance relies on effectively assessing evidence of disease incidence (new cases) and prevalence (total number of cases), as well as geographic distribution, over a given time period in an affected population. The current COVID-19 pandemic has posed an enormous logistical challenge to achieving and maintaining effective population surveillance. In the absence of widespread population immunity, public health measures for limiting the spread of COVID-19 are limited to identifying cases by testing, tracing their contacts and isolating infected individuals from vulnerable, uninfected individuals. The magnitude and rapid growth of COVID-19 infection, particularly during exponential growth of new cases, makes the effective application of these measures a daunting challenge. As of the middle of June, about 2 million Canadians, mostly individuals symptomatic or considered to have been exposed to active cases, have been tested with almost 100,000 Canadians testing positive for COVID-19. The logistical challenges of sampling individuals and performing so many clinical tests have been enormous. The challenge is ongoing; a negative test individual today, could prove to be positive tomorrow. Furthermore, diagnostic tests are not perfect being neither 100% specific nor sensitive (Younes et al., 2020). Resulting false negatives and false positives can misinform public health risk management measures for individuals (Wikramaratna et al. 2020).

Given the daunting challenges to public health risk management posed by these logistics and practical realities, a complementary technique for overall population health surveillance has been attracting rapidly expanding international attention – wastewater-based epidemiology (WBE). The concept is conceptually simple using community sewage as a composite sample of entire communities to obtain quantitative signals of the prevalence of infection. Urban populations in developed nations have comprehensive sewer networks that capture the toilet flushes, laundry and domestic greywater of the entire population, delivering this sewage to a wastewater treatment plant (WWTP) for removal of pollutants before discharge to the environment. Collection of wastewater samples for testing from these WWTPs is ongoing standard practice in response to provincial regulations. A 24-h composite sample of influent sewage provides a representation of the collective faecal discharge of the entire urban community population served by the WWTP over that day - effectively a community-wide swab. As SARS-CoV-2 is discharged in the faeces of infected of individuals (Chen et al. 2020, Cheung et al.,2020, Holshue et al. 2020, Wang W. et al. 2020, Woelfel et al. 2020, Xiao et al. 2020, Xu et al. 2020). sewage samples can potentially provide a signal of COVID-19 infection in the entire population.

Researchers in the Netherlands were early to recognize the potential value of wastewater surveillance. Heijnen and Medema (2011) at the Netherlands Water Recycle Institute (KWR) had already been sampling wastewater to track influenza. Medema et al. (2020a, 2020b) had the presence of mind to start sampling wastewater for genetic signals of SARS-CoV-2 in wastewater of six communities and at Schipol International Airport in early February, 2020 before any clinical cases were detected in the Netherlands. Using a quantitative (q) molecular method known as Reverse Transcriptase-Polymerase Chain Reaction (qRT-PCR), the backbone of clinical testing for the RNA-virus that causes COVID-19, they were able to detect SARS-CoV-2 early. They reported 2.6 to 30 SARS-CoV-2 gene copy fragments per mL in early March at three of the six communities, including one that had no clinical cases as yet reported at the time of sampling. While COVID-19 spread in the Netherlands during March, the wastewater genetic signal increased and correlated with the increasing prevalence of clinical cases. In the 10 weeks since that pioneering Dutch study, there has been a rapidly growing set of preprint and published WBE reports including those from Australia, France, Israel, Italy, Japan, Spain and the USA (Ahmed et al.,2020; Bar-Or et al.,2020, LaRosa et al.,2020; Nemudryi et al.,2020; Peccia et al.,2020; Randazzo et al.,2020; Wu et al.,2020; Wurzer et al.,2020). Enough experience has been obtained to ensure that qRT-PCR analysis of sewage is capable of producing meaningful results, with knowledgeable implementation, for delivering WBE.

WBE has been used for a variety of purposes, such as tracking population drug use (Castiglioni et al. 2013, Choi et al. 2018) and antimicrobial resistance (Hendricksen et al. 2019). In particular, WBE has been used to track viruses, including monitoring effectiveness of polio vaccination programs (Berchenko et al. 2017, Hovi et al., 2012; Lago et al. ,2003; Lodder et al. 2012) and tracking infectious diseases such as hepatitis, (Helmer et al., 2014, LaRosa et al. 2010) norovirus and influenza (Heijnen and Medema, 2011, Kazama et al. 2017, Vincent et al. 2007). Several authors have advocated for widespread adoption of WBE for addressing the COVID-19 pandemic (Daughton 2020, Finkel 2020, Hart and Halden 2020, Mao et al. 2020a,b, Orive et al. 2020, Sims and Kasprzyk-Hordern, 2020.). There are regional and national wastewater surveillance programs being launched or underway by Australia, the European Commission, Finland, Germany, the Netherlands, Singapore, South Africa, Sweden, and the USA. The European NORMAN network of reference labs and research centres is coordinating development of sampling, analysis and data interpretation protocols for SARS-CoV-2 WBE. An international online COVID-19 WBE Collaborative has been launched by 58 researchers representing 13 countries to serve as a hub to coordinate and promote the efforts of research groups undertaking WBE for COVID-19 (Bivins et al. 2020).

Where does Canada stand? The Canadian Water Network (CWN), is an organization that leveraged its experience of 16 years as a federally-funded Networks of Centres of Excellence (NCE), to become a prominent, national not-for-profit curator and broker on knowledge for water decisions. CWN established the Canadian Municipal Water Consortium serving municipal water utilities across Canada, with a Consortium Leadership group comprised of senior water leaders from 23 of the major water utilities across Canada. As the Canadian representative on the Global Water Research Coalition, CWN has direct engagement with the strategic planning of the major water research funding agencies in Australia, France, Germany, the Netherlands, South Africa, Singapore, the United Kingdom and the USA, all of whom are engaged in assessing the potential of WBE to address COVID-19. Since the end of April, international online meetings have been organized to explore the potential applications and challenges of WBE by the Water Research Foundation for investigators and utilities and for U.S. Congressional staff, the Water Science and Technology Board of the U.S. National Academies and the World Health Organization.

In late April CWN launched the COVID-19 Wastewater Coalition to advance the use of WBE for COVID-19 in Canada. A central goal of the Coalition is to facilitate a national proof of concept pilot project to: 1) rapidly assess the ability of WBE to be usefully applied in Canada and 2) identify the key elements of WBE that would need to be built into a national surveillance program. CWN established a National Research Advisory Group to guide progress through the scientific and technical challenges of WBE for SARS-CoV-2 and a National Public Health Advisory Group to advise on the needs of public health decision-makers arising from WBE. The Coalition has produced a set of guiding principles for the pilot study, relevant design concepts for the project and a draft sample collection, processing and analysis protocol. A program of laboratory proficiency testing and inter-laboratory round-robin testing is under development. Given the rapidly evolving international experience, the draft protocol is a living document that will be updated and improved as shared experience dictates. CWN is also coordinating with a recently announced U.S. methods validation study funded by the [Water Research Foundation](https://www.waterrf.org/).

The focus of sewage sampling for WBE is on untreated sewage, allowing WBE to be independent of differing downstream wastewater treatment technologies. Feasibility of collecting meaningful samples is a primary consideration making inlets to centralized wastewater treatment plants the most attractive sampling location. However, if methods and data can be validated and sampling risk minimized, the possibility of sampling from manholes and sewage pump stations may allow for WBE strategies more targeted on suspected high-risk locations within the sewer network. Following decisions to re-open many U.S. university campuses for the fall semester, some researchers are exploring monitoring on-campus sewage to inform decision-making by health officials and campus administrators. Regardless of the sampling strategy there will be a clear obligation to understand the nature of the sewer network being sampled to ensure meaningful interpretation of WBE results.

WBE is not a magic bullet and it certainly cannot replace widespread, ongoing clinical testing, contact-tracing and isolation of infected individuals. Knowledge about which individuals are infected will always be needed. But, the inherent capability of a single WBE composite sample to assess the presence of SARS-CoV-2 in the faecal discharge of thousands to millions of individuals across all socio-economic groups (Murakami et al., 2020) warrants thorough evaluation. The potential of WBE to provide additional, complementary evidence about trends of COVID-19 infection in a community, possibly days to a week in advance of cumulative clinical test data that is normally triggered by the onset of symptoms or exposure to suspect cases, warrants examination. Yet such an approach has not been widely adopted in Canada, perhaps because wastewater management is a different administrative silo than public health surveillance. If there is limited understanding what WBE data may mean, putting WBE to a test is a clear way to determine its value.

The potential to achieve broader and early identification of emerging hotspots, implications of relaxing restrictions on social gathering and border closures and early indications of a need for more intensive focus for clinical surveillance can offer an ability to enhance the effectiveness of conventional public health measures and deployment of resources. A well-organized, carefully executed pilot project can enhance public health decision-makers'confidence in WBE evidence by confirming that Canadian laboratories can achieve meaningful results and timely reporting from WBE. Supporting such a pilot project is a relatively modest investment with a potentially high value return for supporting pandemic public health decision-making. Although the sampling and processing of sewage samples involves additional steps not required with individual clinical tests, the much smaller number of analyses required for WBE is self-evident. Delivering a pilot project in Canada while numbers of new cases are declining is essential before we experience the expected subsequent waves of infection (More et al., 2020).

Interest from investigators across Canada about WBE has been strong and continues to grow. Many municipalities, institutions and even remote industrial camps are inquiring about how to implement WBE. Unfortunately, federal and provincial governments have not yet been able to find the means to support a national proof of concept WBE pilot project, despite expressions of support from various government officials, many municipalities and local public health personnel. A few research groups across Canada have received research project funding that will contribute to advancing WBE in Canada.

The growing interest in WBE is also attracting investigators with widely varying degrees of experience with the necessary techniques, but who may believe they can build the capacity to deliver WBE services to municipalities or institutions. Potential users of WBE need to exercise due diligence in securing wastewater processing and analytical services for SARS-CoV-2 in wastewater. While elements of the necessary techniques are widely used, wastewater is a complex matrix and the combination of knowledge, experience and skills necessary to deliver accurate, reproducible and meaningful results from WBE is not trivial. Rigorous and thorough quality assurance and quality control of sampling, processing and analysis is essential for analytical results to have meaning. Helping Canada to better address and assess what is needed and what the reliable use of the technique could look like is the fundamental rationale for the Canadian COVID-19 Wastewater Coalition and the proposed pilot studies.

The future benefits of WBE can extend beyond the COVID-19 pandemic. Molecular methods for characterizing sensitive markers of pathogens conceptually can allow WBE to be adapted to achieve population surveillance for essentially any pathogen and even for targeted groups of genes (such as antimicrobial resistance). Optimizing procedures for monitoring signals of infective agents in community wastewater could provide effective population surveillance for future disease outbreaks and endemic disease surveillance thereby leaving Canada with an enduring benefit from the COVID-19 pandemic experience.

References cited

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J.W., Choi, P.M., Kitajima, M., Simpson, S.L. and Li, J. 2020. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community. Sci. Total Environ., 728: 138764.

Bar-Or, I., Yaniv, K., Shagan, M., et al. 2020. Regressing SARS-CoV-2 sewage measurements onto COVID-19 burden in the population: a proof-of-concept for quantitative environmental surveillance. medRxiv. doi.org/10.1101/2020.04.26.20073569

Bivins, A., North, D., Ahmad, A. et al. 2020. Wastewater-Based Epidemiology: Global collaborative to maximize contributions in the fight against COVID-19. Environ. Sci. Technol. dx.doi.org/10.1021/acs.est.0c02388

Berchenko, Y., Manor, Y., Freedman, L. S., Kaliner, E., Grotto, I., Mendelson, E. and Huppert, A. 2017. Estimation of polio infection prevalence from environmental surveillance data. Sci. Transl. Med. 9: (383).

Castiglioni, S., Bijlsma, L., Covaci, A., Emke, E., Hernández, F.,Reid, M., Ort, C., Thomas, K. V., Van Nuijs, A. L., De Voogt, P., Zuccato, E. 2013. Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers. Environ. Sci. Technol. 47 (3): 1452–1460.

Chen, Y., Chen, L., Deng, Q., et al. 2020. The presence of SARS-CoV-2 RNA in feces of COVID-19 patients. J. Med. Virol. 92: 833–840 doi:10.1002/jmv.25825

Cheung, K. S.; Hung, I. F.; Chan, P. P., et AL. 2020. Gastrointestinal Manifestations of SARS-CoV-2 Infection and Virus Load in Fecal Samples from the Hong Kong Cohort and Systematic Review and Meta-analysis. Gastroenterology. 2020 doi: 10.1053/j.gastro.2020.03.065

Choi, P.M., Tscharke, B. J., Donner, E., O'Brien, J. W., Grant, S.C., Kaserzon, S. L., Mackie, R., O'Malley, E., Crosbie, N. D.,Thomas, K. V., Mueller, J. F. 2018. Wastewater-based epidemiology biomarkers: past, present and future. Trends Anal. Chem. (TrAC) 105: 453–469.

Daughton, C. 2020. The international imperative to rapidly and inexpensively monitor community-wide Covid-19 infection status and trends. Sci. Total Environ. 726: 138149

Finkel, A. (2020). Advice on the feasibility of monitoring wastewater for early detection and monitoring of COVID-19 in the population. Report from Australia's Chief Scientist to the Australian Minister of Health. 21 April, 2020. Canberra, ACT, Australia.

Hart, O. E. & Holden, R. U. 2020. Computational analysis of SARS-CoV-2/COVID-19 surveillance by wastewater-based epidemiology locally and globally: Feasibility, economy, opportunities and challenges. Sci. Tot. Environ. 730: 138875.

Heijnen, L. and Medema, G. 2011.Surveillance of influenza A and the pandemic influenza A (H1N1) 2009 in sewage and surface water in the Netherlands. J. Water Health 9, 434–442.

Helmer, M., Paxéus, N., Magnius, L. et al., 2014. Detection of pathogenic viruses in sewage provided early warnings of hepatitis A virus and norovirus outbreaks. Appl. Environ. Microbiol. 80(21): 6771–6781.

Hendriksen, R. S., Munk, P., Njage, P., et al. 2019. Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. Nature Commun. 10 (1): 1124.

Holshue, M., DeBolt, C., Lindquist, S., et al. 2020. First case of 2019 novel coronavirus in the United States. N. Engl. J. Med. 382, 929–936.

Hovi, T., Shuman, L. M., Van Der Avoort, H., Deshpande, J., Roivainen, M., De Gourvilles, E. M. 2012. Role of environmental poliovirus surveillance in global polio eradication and beyond. Epidemiol. Infect. 140: 1–13.

Kazama, S., Miura, T., Masago, Y., et al. 2017. Environmental Surveillance of Norovirus Genogroups I and II for Sensitive Detection of Epidemic Variants. Appl. Environ. Microbiol. 83(9): e03406-16

Lago, P., Gary, H.E., Perez, L.S., et al. (2003). Poliovirus detection in wastewater and stools following an immunization campaign in Havana, Cuba. Int. J. Epidemiol. 32: 772–777.

LaRosa, G., Iaconelli, M., Mancinic, P., et al. 2020. First detection of SARS-CoV-2 in untreated wastewaters in Italy. Sci. Tot. Environ. 736: 139652.

LaRosa, G., Pourshaban, M., Iaconelli, M., Vennarucci, V.S., Muscillo, M. 2010. Molecular detection of hepatitis E virus in sewage samples. App. Environ. Microbiol. 76(17): 5870–5873.

Lodder, W.J., Buisman, A.M., Rutjes, S.A., Heijne, J.C., Teunis, P.F., de Roda Husman, A.M. 2012. Feasibility of quantitative environmental surveillance in poliovirus eradication strategies. App. Environ. Microbiol. 78(11): 3800–3805.

Mao, K., Zhang, K., Yang, Z. 2020a. Can a Paper-Based Device Trace COVID-19 Sources with Wastewater-Based Epidemiology? Environ. Sci. Technol. 54: 3733−3735.

Mao, K., Zhang, K., Du, W., Ali, W., Feng, X. & Zhang, H. 2020b. The potential of wastewater-based epidemiology as surveillance and early warning of infectious disease outbreaks. Curr. Opin. Environ. Sci. Hlth. doi.org/10.1016/j.coesh.2020.04.006.

Medema, G., Heijnen, L., Elsinga, G., Italiaander, R., Brouwer, A. 2020a. Presence of SARS-Coronavirus-2 in sewage. medRxiv 2020.03.29.20045880. doi:10.1101/2020.03.29.20045880

Medema, G., Heijnen, L., Elsinga, G., Italiaander, R., Brouwer, A. 2020b. Presence of SARS-Coronavirus-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in the Netherlands. Environ. Sci. Technol. Lett. DOI: 10.1021/acs.estlett.0c00357

Moore, K. A.; Lipsitch, M.; Barry, J. M.; Osterholm, M. T. 2020. COVID-19: The CIDRAP Viewpoint. April 30th, 2020. Part 1: The Future of the COVID-19 Pandemic: Lessons Learned from Pandemic

Influenza; Centre for Infectious Disease Research and Policy: University of MInnesota. https://www.cidrap.umn.edu/sites/default/files/public/downloads/cidrap-covid19-viewpoint-part1_0.pdf

Murakami, M.; Hata, A.; Honda, R.; Watanabe, T., 2020. Letter to the Editor: Wastewater-Based Epidemiology Can Overcome Representativeness and Stigma Issues Related to COVID-19. Environ. Sci. Technol. 54 (9): 5311.

Nemudryi, A., Nemudraia, A., Surya, K., Wiegand, T., Buyukyoruk, M., Wilkinson, R. & Wiedenheft, B. (2020). Temporal detection and phylogenetic assessment of SARS-CoV-2 in municipal wastewater. medRxiv. doi.org/10.1101/2020.04.15.20066746.

Orive, G., Lertxundi, U., Barcelo, D. 2020. Early SARS-CoV-2 outbreak detection by sewage-based epidemiology. Sci. Tot. Environ. 732: 139298.

Peccia, J., Zulli, A., Doug E. Brackney, D.E., et al. 2020. SARS-CoV-2 RNA concentrations in primary municipal sewage sludge as a leading indicator of COVID-19 outbreak dynamics. medRxiv doi.org/10.1101/2020.05.19.20105999

Randazzo, W., Truchado, P., Cuevas-Ferrando, E., Simón, P., Allende, A.,Sánchez, G. 2020. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area, Water Res. doi.org/10.1016/j.watres.2020.115942 .

Sims, N. & Kasprzyk-Hordern, B. 2020. Future perspectives of wastewater-based epidemiology: Monitoring infectious disease spread and resistance to the community level. Environ. Int. 105689. doi:10.1016/j.envint.2020.10568

Vincent, V., Scott, H. M., Harvey, R. B., Alali, W. Q. and Hume, M. E. 2007. Novel surveillance of Salmonella enterica serotype Heidelberg epidemics in a closed community. Foodborne Pathog. Dis. 4(3): 375-85.

Wang, W., Xu, Y., Gao, R., et al. 2020. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA. 323(18): 1843-1844. doi:10.1001/jama.2020.3786

Wikramaratna, P.; Paton, R.S.; Ghafari, M.; Lourenco, J. 2020. Estimating false-negative detection rate of SARSCoV-2 by RT-PCR. MedRxiv. doi:10.1101/2020.04.05.20053355.

Woelfel, R., Corman, V.M., Guggemos, W., et al. 2020. Virological assessment of hospitalized patients with COVID-2019. Nature. 581: 465-469. doi.org/10.1038/s41586-020-2196-x.

Wu, F., Xiao, A., Zhang, J., Gu, X., Lee, W.L., Kauffman, K., Hanage, W., Matus, M., Ghaeli, N. and Endo, N. (2020) SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases. medRxiv.

Wurtzer, S., Marechal, V., Mouchel, J.-M., Moulin, L. 2020. Time course quantitative detection of SARS-CoV-2 in Parisian wastewaters correlates with COVID-19 confirmed cases. medRxiv. doi.org/10.1101/2020.04.12.20062679.

Xiao, F., Sun, J. , Xu, Y. et al. 2020. Infectious SARS-CoV-2 in Feces of Patient with Severe COVID-19. Emerg. Infect. Dis. 26(8).

Xu, Y.; Li, X.; Zhu, B.; Liang, H.; Fang, C.; Gong, Y.; Guo, Q.; Sun, X.; Zhao, D.; Shen, J. 2020. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. Nature Med. 26: 502–505

Younes, N.; Al-Sadeq, D. W.; Al-Jighefee, H.; Younes, S.; Al-Jamal, O.; Daas, H. I.; Yassine, H. M.; Nasrallah, G. K., Challenges in Laboratory Diagnosis of the Novel Coronavirus SARS-CoV-2. Viruses 2020, 12, (6).