Differentiating between the possibility and probability of SARS-CoV-2 transmission associated with wastewater: empirical evidence is needed to substantiate risk

Warish Ahmed[1], Kyle Bibby[2], Patrick M. D'Aoust[3], Robert Delatolla[3], Charles P. Gerba[4], Charles N. Haas[5] Kerry A. Hamilton[6], Joanne Hewitt[7], Timothy R. Julian[8], Devrim Kaya[9], Paul Monis[10], Laurent Moulin[11], Colleen Naughton[12], Rachel T. Noble[13], Abhilasha Shrestha[14], Ananda Tiwari[15], Stuart L. Simpson[16], Sebastien Wurtzer[11], Aaron Bivins[2]

[1] CSIRO Land and Water, Ecosciences Precinct, 41 Boggo Road, QLD 4102, Australia[2] Department of Civil & Environmental Engineering & Earth Sciences, University of Notre Dame, 156 Fitzpatrick Hall, Notre Dame, IN 46556, USA
[3] Department of Civil Engineering, University of Ottawa, Ottawa, ON, Canada
[4] Department of Environmental Science, Water and Energy Sustainable Technology Center, University of Arizona, 2959 W. Calle Agua Nueva, Tucson, AZ 85745.
[5] Drexel University, Philadelphia, Pennsylvania, USA.
[6] School of Sustainable Engineering and the Built Environment and the Biodesign Institute Center for Environmental Health Engineering, Arizona State University, Tempe, AZ 85287, USA.
[7] Institute of Environmental Science and Research Ltd (ESR), Porirua, 5240, New Zealand
[8] Eawag, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf CH-8600, Switzerland
[9] School of Chemical, Biological, and Environmental Engineering, Oregon State University, 105 SW 26th St #116, Corvallis, OR 97331, USA
[10] South Australian Water Corporation, Adelaide, Australia
[11] Eau de Paris R&D Laboratory. 33 Av. Jean Jaures 94200 Ivry/seine France.
[12] University of California Merced Department of Civil and Environmental Engineering 5200 N. Lake Rd. Merced, CA 95343.
[13] University of North Carolina Institute of Marine Sciences, Morehead City, NC, United States of America.
[14] Division of Environmental and Occupational Health Sciences, School of Public Health, University of Illinois Chicago, Chicago, IL, USA
[15] Finnish Institute for Health and Welfare, Expert Microbiology Unit, Kuopio, Finland.
[16] CSIRO Land and Water, Lucas Heights, NSW 2234, Australia.

*Corresponding author. Warish Ahmed. Mailing address: Ecosciences Precinct, 41 Boggo Road, Dutton Park 4102, Queensland, Australia Tel.: +617 3833 5582; E-mail address: Warish.Ahmed@csiro.au

All authors equally contributed to this manuscript.

People infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) shed the virus and its genetic material via their sputum, nasopharyngeal secretions, saliva, urine, and feces (Cevik et al. 2020). Hence, public health and water quality scientists throughout the world have been monitoring untreated and/or primary treated wastewater and sludge for the surveillance of SARS-CoV-2 in communities (https://arcg.is/1aummW). Numerous reviews have discussed the possibility of SARS-CoV-2 transmission to humans from exposure to wastewater or waters receiving untreated or inadequately treated wastewater based on limited empirical evidence (Adelodun et al. 2020; Bilal et al. 2020; Elsamadony et al. 2021; Khorram-Manesh; Olusola-Makinde; Shutler et al. 2021). Multiple transmission routes have been suggested, including waterborne transmission, airborne transmission, contact with contaminated surfaces (fomites), and subsequent touching of mucous membranes such as the mouth, nose, or eyes. Herein, we briefly summarize the empirical evidence pertaining to the transmission of SARS-CoV-2 associated with wastewater exposure.

SARS-CoV-2 RNA has been detected at high concentrations in stool of COVID-19 patients (10^8 genome copies (GC)/g) and COVID-19 patients are reported to shed RNA in their stool >30 days (Li et al. 2020). In contrast, infectious SARS-CoV-2 is typically only isolated from nasopharyngeal specimens during the first nine days of infection (Cevik et al. 2020). Studies have reported the isolation of infectious SARS-CoV-2 from the feces and urine of COVID-19 patients (Sun et al. 2020; Xiao et al. 2020); however, this is rare. For example, Wang et al. (2020) screened 153 stool samples and isolated infectious SARS-CoV-2 from only four specimens with “high copy numbers”. Wölfel et al. (2020) failed to detect infectious SARS-CoV-2 in 13 samples collected from four patients over six days. Another study found that infectious SARS-CoV-2 was rapidly (i.e., 5-fold within 1 h and loss of 80% viral infectivity after 24 h) inactivated by simulated colonic fluid (Zang et al. 2020). Similarly, during longitudinal studies of monkeys inoculated with SARS-CoV-2, infectious SARS-CoV-2 was isolated from stool two to seven days post-infection from one of six monkeys at concentrations four orders of magnitude lower than RNA (Woolsey et al. 2020). Collectively, the available data indicate that for each shedding route, infectious SARS-CoV-2 is shed for shorter durations and at lower prevalence and concentration than SARS-CoV-2 RNA.

SARS-CoV-2 RNA has been commonly detected at concentrations ranging from 20 to more than 10^6 GC/L in untreated wastewater and $>10^8$ GC/L in primary sludge (Ahmed et al. 2020a; Mlejnkova et al. 2020; Peccia et al. 2020). SARS-CoV-2 RNA has been detected in 25% of final treated effluent samples at concentrations ranging from 1.3 to approximately 10^5 GC/L (Ampuero et al. 2020; Balboa et al. 2021; Carrillo-Reyes et al. 2020; Rimoldi et al. 2020; Saguti et al. 2020; Sherchan et al. 2020; Kumar et al. 2021a; Westhaus et al. 2021).

SARS-CoV-2 RNA has also been detected in environmental waters. For surface waters receiving untreated wastewater in areas with poor sanitation, RNA has been detected in 100% samples ($n = 18$) at concentrations over 10^6 GC/L (Guerrero-Latorre et al. 2020; Iglesias et al. 2020). Whereas for surface waters receiving treated wastewater, SARS-CoV-2 RNA has been collectively detected in 3/7 (43%) samples, but Cq values or concentrations were not reported (Haramoto et al. 2020; Rimoldi et al. 2020). However, attempts to detect infectious SARS-CoV-2 from six untreated wastewater samples, four treated wastewater, and six river water samples in Italy were not successful (Rimoldi et al. 2020). Westhaus et al. (2021) could not detect infectious SARS-CoV-2 in untreated and treated wastewater in Germany. Additionally, Desdouits et al. (2021) did not detect SARS-CoV-2 RNA in shellfish in 187 samples across 37 sites along the French coast between April and August 2020.

Inactivation and decay studies demonstrated that SARS-CoV-2 RNA persisted longer (T_{90} =18-25 days) (Ahmed et al. 2020b) than infectious viruses (T_{90} = 1.2-1.9 days) (Bivins et al. 2020; de Oliveira et al. 2021; Sala-Comorera et al. 2021) when seeded in wastewater and surface water and seawater. This persistence differential leads to a decreasing ratio of infectious virus/RNA GC over time. For instance, during a 7-day period in seeded wastewater, the median 50% tissue culture infectious dose ($TCID_{50}$)/GC ratio decreased from 1 to 100 to less than 1 to 10,000 (Bivins et al. 2020). A recent preprint suggested that evaluating total RNA overestimated the number of intact viruses within wastewaters (Wurtzer et al. 2021). Enveloped viruses are considered less stable in the environment than non-enveloped viruses, such as human enteric viruses (e.g., norovirus), typically transmitted via the faecal-oral route and associated with waterborne transmission (Casanova and Weaver 2015). These observations align with recent opinions from water microbiologists and wastewater professionals (Maal-Bared et al. 2020) that wastewater does not appear to be a significant transmission route for SARS-CoV-2. Furthermore, presence of RNA in a sample is insufficient to infer the magnitude of the risk of waterborne transmission via wastewater or environmental waters.

Despite caveats associated with using RNA concentration for risk assessment, several studies have conducted quantitative microbial risk assessment (QMRA) for SARS-CoV-2 transmission from exposure to wastewater via oral or inhalation exposure routes (Dada and Gyawali 2021; Gholipour et al. 2021; Kumar et al. 2021b; Shutler et al. 2021; Yang et al. 2020; Zaneti et al. 2021).

QMRA models are inherently limited by assumptions and uncertainties, including SARS-CoV-2 shedding rates and durations, the persistence of infectious SARS-CoV-2 in wastewater and wastewater aerosols, dilution rates in wastewater collection systems, and ratios of RNA to infectious viruses (the models assumed ranges from 1000 to 1 and 29 to 1). Furthermore, each model uses a SARS-CoV-1 dose-response model (Watanabe et al. 2010) and some apply this inhalation dose-response model to other routes of exposure such as oral ingestion (Zaneti et al. 2021). There is also uncertainty surrounding morbidity ratios among those infected. While, multi-pathway risk assessments have not been conducted for wastewater exposures, QMRAs of multiple exposure routes among health care workers indicated the dominance of aerosol exposures as risk drivers (Mizukoshi et al. 2021; Jones 2020).

Several risk analyses have also raised concerns regarding wastewater-impacted surface waters (Kumar et al. 2021b, Shutler et al. 2021, Yang et al. 2020). Shutler et al. (2021) conducted a relative risk analysis of countrywide surface waters, calculating concentrations in receiving rivers after a sewage spill, highlighting situations where high infection rates among the wastewater-producing population and low dilution rates in the environment that could result in river water “infectious doses” of SARS-CoV-2 RNA >40 GC/100 mL, which is reflective of sewage impacted waterways, but does not account for RNA to infectious virus ratios. Kumar et al. (2021b) estimated that per event infection risks from incidental ingestion of recreational water can range from $10^{-5.84}$ to $10^{-2.61}$ for swimming and fishing, respectively. Yang et al. (2020) combined these approaches and estimated per-exposure infection risks ranging from 10^{-12} to 10^{-10} across various inhalation scenarios. Across the three analyses, improved understanding of viral fate, transport, and viability, as well as a better understanding of relevant exposure routes, were highlighted as limitations.

The frequent detection of SARS-CoV-2 RNA in wastewater combined with the rare observations of infectious SARS-CoV-2 in feces/urine, the transmission of COVID-19 via wastewater is possible. However, given the limited persistence of infectious SARS-CoV-2 in wastewater, and the well-established inhalation exposure route, the most probable transmission scenario is fecal aerosols generated from fresh wastewater escaping to air via defective building plumbing as implicated previously for SARS-CoV-1 (McKinney et al. 2006). However, based on the current evidence, we assert that fecal-oral transmission of SARS-CoV-2 associated with wastewater is likely to be low compared to well-documented person-to-person transmission via respiratory droplets/aerosols. This assertion is largely premised on the failure to isolate infectious SARS-CoV-2 from wastewater or environmental waters in two peer-reviewed studies totalling 19 samples (Rimoldi et al. 2020; Westhaus et al. 2021). Furthermore, the low ratios of infectious SARS-CoV-2 to RNA in clinical samples, the limited persistence of infectious SARS-CoV-2 in environmental waters as observed during three studies, and the efficacy of most WWTPs in virus reduction act as barriers to substantially reduce risks. SARS-CoV-2 infection risks from untreated and treated wastewaters and wastewater-impacted environmental waters are likely lower than other fecally-excreted pathogens such as norovirus and hepatitis A virus. Existing water quality regulations, biosafety protocols, and procedures, which have been designed for waterborne pathogens, along with masks as recommended by the CDC (2021), are sufficient to ensure the safety of the public and wastewater professionals (Brisolara et al. 2021).

Definitive conclusions about risk from wastewater exposures are constrained by the limited sample size of the research performed to date. For example, to the best of our knowledge, only two studies, with a total sample size of 19, have tested for SARS-CoV-2 infectivity in wastewater and environmental waters (Rimoldi et al. 2020; Westhaus et al. 2021). Negative results, which are critical to establish upper bounds of risk (e.g., 0 in 20 is not equivalent to 0 in 1,000), are less likely to be published due to bias against such results (Anonymous 2020). Additional empirical observations of infectious SARS-CoV-2 in wastewater or environmental waters, including negative results if available, are needed.

Given the finite resources available for responding to the COVID-19 pandemic, possible transmission routes must be examined, considering their probability of contributing to disease. Researchers should continue to exercise caution and communicate the uncertainty and assumptions of their studies when leveraging models based on limited empirical evidence particularly during a global pandemic when these results can be misinterpreted.

References:

1. Adelodun B, Ajibade FO, Ibrahim RG *et al.* Snowballing transmission of COVID-10 (SARS-CoV-2) through wastewater: Any sustainable preventive measures to curtail the scourge in low-income countries? *Sci Total Environ* 2020:742:140680.
2. Ahmed W, Angel N, Edson J *et al.* 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* 2020a;728:138764.
3. Ahmed W, Bertsch PM, Bibby K *et al.* Decay of SARS-CoV-2 and surrogate murine hepatitis virus RNA in untreated wastewater to inform application in wastewater-based epidemiology. *Environ Res* 2020b;191:110092.
4. Ampuero M, Valenzuela S, Valiente-Echeverria F *et al.* SARS-CoV-2 detection in sewage in Santiago, Chile - preliminary results. *MedRxiv* 2020; https://doi.org/10.1101/2020.07.02.20145177.
5. Anonymous. In praise of replication studies and null results. 2020;578:4890490.
6. Balboa S, Mauricio-Iglesias M, Rodriguez S *et al.* The fate of SARS-COV-2 in WWTPS points out the sludge line as a suitable spot for detection of COVID-19. *Sci Total Environ* 2021;772:145268.
7. Bilal M, Nazir MS, Rasheed T *et al.* Water matrices as potential source of SARS-CoV-2 transmission - An overview from an environmental perspective. *Case Stud Chem Environ Eng* 2020;2:100023.
8. Bivins A, Greaves J, Fischer R *et al.* Persistence of SARS-CoV-2 in water and wastewater. *Environ Sci Technol* 2020;7:937-942.
9. Brisolara KF, Maal-Bared R, Sobsey MD *et al.* Assessing and managing SARS-CoV-2 occupational health risk to workers handling residuals and biosolids. *Sci Total Environ* 2021;774:145732.
10. Carrillo-Reyes J, Barragán-Trinidad M, Buitrón G. Surveillance of SARS-CoV-2 in sewage and wastewater treatment plants in Mexico. *J Water Process Eng* 2021;40:101815.
11. Casanova LM, Weaver SR.Inactivation of an enveloped surrogate virus in human sewage. Environ Sci Technol Lett 2015;2:76-78,
12. Cevik M, Tate M, Lloyd O *et al.* SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamis, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. *Lancet Microb* 2021;2:E13-E22.
13. Dada AC, Gyawali P. Quantitative microbial risk assessment (QMRA) of occupational exposure to SARS-CoV-2 in wastewater treatment plants. *Sci Total Environ* 2021;763:142989.
14. de Oliveira LC, Torres-Franco AF, Lopes BC *et al.* Viability of SARS-CoV-2 in river water and wastewater at different temperatures and solids content. *Water Res* 2021;195:117002.
15. Desdouits M, Piquet J-C, Wacrenier C *et al.* Can shellfish be used to monitor SARS-CoV-2 in the coastal environment? *Sci Total Environ* 2021;775:146270.
16. Elsamadony M, Fujii M, Miura T *et al.* Possible transmission of viruses from contaminated human feces and sewage: Implications for SARS-CoV-2. *Sci Total Environ* 2021;755:142575.
17. Gholipour S Mohammadi F, Nikaeen M *et al.* COVID-19 infection risk from exposure to aerosols of wastewater treatment plants. *Chemosphere* 2021;273:129701.
18. Guerrero-Latorre L, Ballesteros I, Villacres-Granda I *et al.* SARS-CoV-2 in river water: Implications in low sanitation countries. *Sci Total Environ* 2020;743:140832.

19. Haramoto E, Malla B, Thakali O *et al.* First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan. *Sci Total Environ* 2020;737:140405.
20. Iglesias NG, Gebhard LG, Carballeda JM *et al.* SARS-CoV-2 surveillance in untreated wastewater: first detection in a low-resource community in Buenos Aires, Argentina. *medRXiv* 2020;https://doi.org/10.1101/2020.10.21.20215434.
21. Kolorevi S, Micsinai A, Szanto-Egesz R *et al.* 2021. Detection of SARS-CoV-2 RNA in the Danube River in Serbia associated with the discharge of untreated wastewater. Sci. Total Environ. 783:146967.
22. Khorram-Manesh A, Goniewicz K, Burkle F. Unrecognized risks and challenges of water as a major focus of COVID-19 spread. *J Glob Health* 2021:11:03016.
23. Kumar M, Kuroda K, Patel Ak *et al.* Decay of SARS-CoV-2 RNA along the wastewater treatment outfitted with Upflow Anaerobic Sludge Blanket (UASB) system evaluated through two sample concentration techniques. *Sci Total Environ* 2021a;754:142329.
24. Kumar M, Alamin M, Kuroda K *et al.* 2021. Potential discharge, attenuation and exposure risk of SARS-CoV-2 in natural water bodies receiving treated wastewater. npj Clean Water 2021b;4:1-11.
25. Li N, Wang X, Lv T. Prolonged SARS-CoV-2 RNA shedding: Not a rare phenomenon. *J Med Virol* 2020;92:2286-2287.
26. Maal-Bared R, Sobsey M, Bibby K *et al.* Letter to the editor regarding Marhavarajah et al. (2020) Pandemic danger to the deep: The risk of marine mammals contracting SARS-CoV-2 from wastewater. *Sci Total Environ* 2020;144855.
27. McKinney KR, Gong YY, Lewis TG. Environmental transmission of SARS at Amoy Gardens. *J Environ Health* 2006;68:26-30.
28. Mizukoshi A, Nakama C, Okumura J *et al.* Assessing the risk of COVID-19 from multiple pathways of exposure to SARS-CoV-2: Modeling in health-care settings and effectiveness of nonpharmaceuticals interventions. *Environ Int* 2021:106338.
29. Mlejnkova H, Sovova K, Vasickova P *et al.* Preliminary study of SARS-CoV-2 occurrence in wastewater in the Czech Republic. *Int J Environ Res Public Health* 2020;17:5508.
30. Olusola-Makinde OO, Reuben RC. Ticking bomb: Prolonged faecal shedding of novel coronavirus (2019-nCoV) and environmental implications. *Environ Poll* 2020;267:115485.
31. Peccia J, Zulli A, Brackney DE *et al.* Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics. *Nat Biotechnol* 2000;38:1164-1167.
32. Rimoldi SG, Stefani F, Gigantiello A *et al.* Presence and infectivity of SARS-CoV-2 virus in wastewaters and rivers. *Sci Total Environ* 2020;744:140911.
33. Saguti F, Magnil E, Enache L et al. Surveillance of wastewater revealed peaks of SARS-CoV-2 preceding those of hospitalized patients with COVID-19. *Water Res* 2021;189:116620.
34. Sala-Comorera L, Reynolds LJ, Martin NA et al. 2021. Decay of infectious SARS-CoV-2 and surrogates in aquatic environments. Water Res. 2021;29:117090.
35. Sherchan S, Shahin S, Ward LM *et al.* First detection of SARS-CoV-2 RNA in wastewater in North America: A case study in Louisiana, USA. *Sci Total Environ* 2020:743:140621.
36. Shutler JD, Zaraska K, Holding T *et al.* Rapid Assessment of SARS-CoV-2 transmission risk for fecally contaminated river water. *ACS ES&T Water* 2021; https://doi.org/10.1021/acsestwater.0c00246.
37. Sun J, Zhu A, Li H *et al.* Isolation of infectious SARS-CoV-2 from urine of a COVID-19 patient. *Emerg Microbes Infect* 2020;9:991-993.
38. Wang D, Xu Y, Gao R *et al.* Detection of SARS-CoV-2 in different types of clinical specimens. JAMA 2020;323:1843-1844.
39. Watanabe T, Bartrand TA, Weir, MH *et al.* Development of a Dose-Response Model for SARS Coronavirus. *Risk Analysis* 2010; 30:1129-1138.
40. Westhaus S, Weber F-A, Schiwy S *et al.* Detection of SARS-CoV-2 in raw and treated wastewater in Germany - suitability for COVID-19 surveillance and potential transmission risks. *Sci Total Environ* 2021:751:141750.

41. Wölfel R, Corman VM, Guggemos W *et al.* Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020;581:465-469.
42. Woolsey C, Borisevich V. Prasad AN *et al.* Establishment of an African green monkey model for COVID-19 and protection against re-infection. *Nat Immunol* 2021;22:86–98.
43. Wurtzer S, Waldman P, Ferrier-Rembert A *et al.* Several forms of SARS-CoV-2 RNA can be detected in wastewaters: implication for wastewater-based epidemiology and risk assessment. *MedRxiv* 2021.
44. Xiao F, Sun J, Xu Y *et al.* Infectious SARS-CoV-2 in feces of patients with Severe COVID-19. *Emerg Infect Dis* 2020;26:1920-1922.
45. Yang B, Li W, Wang J *et al.* Estimation of the potential spread risk of COVID-19: Occurrence assessment along the Yangtze, Han, and Fu River basins in Hubei, China. *Sci Total Environ* 2020;746:141353.
46. Zaneti RN, Girardi V, Spilki FR *et al.* Quantitative microbial risk assessment of SARS-CoV-2 for workers in wastewater treatment plants. *Sci Total Environ.* 2021;754: 142163.
47. Zang R, Castro MFG, McCune T *et al.* TMPRSS2 and TMPRSS4 promote SARS-CoV02 infection of human small intestinal enterocytes. Sci. Immunol. 2020;5:eabc3582.