Evidence check

11 November 2020

Rapid evidence checks are based on a simplified review method and may not be entirely exhaustive, but aim to provide a balanced assessment of what is already known about a specific problem or issue. This brief has not been peer-reviewed and should not be a substitute for individual clinical judgement, nor is it an endorsed position of NSW Health.

Wastewater surveillance for COVID-19

Evidence check question

What is the evidence for monitoring wastewater as a surveillance strategy for COVID-19?

In brief

- The presence of SARS-CoV-2 in the faeces of infected patients and wastewater has drawn attention to the use of wastewater as an epidemiological tool. Wastewater surveillance of COVID-19 can be an efficient, cost-effective way to survey transmission dynamics of communities as a complementary approach to assessing the prevalence of COVID-19 in a community.(1-3)
- SARS-CoV-2 has been detected in wastewater samples from many regions around the world including; Australia, Spain, Italy, Netherlands, China, the United States of America, Germany, Japan, India, Czech Republic, Brazil and Ecuador.(4-25)
- Often in these reports wastewater samples tested positive before, at the same time, or soon after positive COVID-19 cases were reported in the respective areas.(4, 6, 7, 10-12, 21, 22, 24) In one study, the viral titers observed were significantly higher than expected based on clinically confirmed cases.(17)
- While the majority of studies detect positive SARS-CoV-2 in raw wastewater, there have been some positive tests in treated wastewater.(8, 12) This needs to be balanced against studies that did not detect positive SARS-CoV-2 in treated wastewater.(14, 18)
- The World Health Organization outlines major potential use cases for environmental surveillance for SARS-CoV-2 including: early warning, detection in locations with limited clinical surveillance, monitoring circulation of SARS-CoV-2 and research. Considerations outlined by the World Health Organization for implementing environmental surveillance include: representativeness, coordination, cost-effectiveness, ethical and legal considerations and quality assurance.(26)
- In Australia, the Commonwealth Scientific and Industrial Research Organisation (CSIRO) has a method for monitoring sewage for early detection of COVID-19 outbreaks over 14 days, from people being exposed to SARS-CoV-2, wastewater samples collected and tested, reporting to public health officials, through to clinical nasal swab tests for people who begin to show symptoms.(27)

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Limitations

Many publications looking at empirical data on wastewater surveillance in COVID-19 are published as pre peer review literature.(3) These pre peer review publications have not been included in this evidence check. Studies included in this review use different methods for the collection and analysis of wastewater samples. These methods have not been drawn out or considered as part of this review.

Background

The presence of SARS-CoV-2 in the faeces of infected patients and wastewater has drawn attention to the use of wastewater as an epidemiological tool.(1-3) Wastewater surveillance has the potential to be successful in revealing infection dynamics earlier than diagnostic testing, which could provide public-health officials with near-real-time information on disease prevalence.(28, 29)

Some of the challenges associated with wastewater surveillance include: the interpretation of a single positive wastewater sample is difficult, sample site characteristics affect virus detection, and that laboratory methods for SARS-CoV-2 should be validated.(30) There are also areas which need further consideration, such as the factors influencing persistence of SARS-CoV-2 in wastewater such as temperature, presence of organic matter and suspended solids.(31) An Australian study on decay rates saw that SARS-CoV-2 RNA is likely to persist long enough in untreated wastewater to permit reliable detection.(32)

Different methods used for wastewater concentration and SARS-CoV-2 RNA quantification are described in the literature.(33-35) Currently, there is an absence of an optimised methodological framework concerning the detection and quantification of SARS-CoV-2 in wastewater.(36, 37) Legal and ethical implications of wastewater monitoring such as privacy and autonomy should also be considered.(38)

The purpose of wastewater surveillance needs to be considered, which may vary across countries. In many settings, the use of wastewater surveillance is in adjunct to case finding. While the studies included in this review show that it is possible to detect SARS-CoV-2 in wastewater, there is little literature to date on what difference this has made in the management of COVID-19.

Australian states including New South Wales, Victoria and Queensland are currently undertaking pilot wastewater surveillance programs.(39-41)

Methods (Appendix 1)

PubMed was searched on the 25 September 2020 and Google was searched on the 29 September 2020.

Results

Source	Summary
Peer reviewed sources	
Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics Peccia, et al. 2020 (4)	• Study measured SARS-CoV-2 RNA concentrations in primary sewage sludge in the New Haven, Connecticut, USA, metropolitan area during the coronavirus disease 2019 (COVID-19) outbreak in Spring 2020. • SARS-CoV-2 RNA concentrations in sludge were 0-2 days ahead of SARS-CoV-2 positive test results by date of specimen collection, 1-4 days ahead of local hospital admissions and 6-8 days ahead of SARS-CoV-2 positive test results by reporting date. • In communities facing a delay between specimen collection and the reporting of test results, immediate wastewater results can provide considerable advance notice of infection dynamics.
Quantitative microbial risk assessment of SARS-CoV-2 for workers in wastewater treatment plants Zaneti, et al. 2020 (5)	• This study investigates the potential health risks of SARS-CoV-2 in sewage to wastewater treatment plant workers. A quantitative microbial risk assessment is applied for three COVID-19 scenarios (moderate, aggressive and extreme) to study the effects of different stages of the pandemic in terms of percentage of infected population on the probability of infection to wastewater treatment plant workers. • Estimates of viral RNA in sewage at the entrance of wastewater treatment plants ranged from 4.14 × 101 to 5.23 × 103 genomic copies/ml-1 (viable virus concentration from 0.04 to 5.23 PFU·ml-1, respectively). In addition, estimated risks for the aggressive and extreme scenarios (2.6 × 10-3 and 1.3 × 10-2, respectively) were likely to be above the derived tolerable infection risk for SARS-CoV-2 of 5.5 × 10-4 pppy, thus reinforcing the concern of sewage systems as a possible transmission pathway of SARS-CoV-2.
Metropolitan wastewater analysis for COVID-19 epidemiological surveillance Randazzo, et al. 2020 (6)	• Study used quantitative reverse transcription polymerase chain reaction (RT-qPCR) for SARS-CoV-2 detection in a series of longitudinal metropolitan wastewaters samples collected from February to April 2020, during the earliest stages of the epidemic in the Region of Valencia, Spain. • Researchers were able to consistently detect SARS-CoV-2 RNA in samples taken in late February, when communicated cases in that region were only incipient. • The wastewater viral RNA context increased rapidly and anticipated the subsequent ascent in the number of declared cases. • Results strongly suggest that the virus was undergoing community transmission earlier than previously believed, and

Source	Summary
	suggest that wastewater analysis could be sensitive and cost-effective strategy for COVID-19 epidemiological surveillance.
Temporal detection and phylogenetic assessment of SARS-CoV-2 in municipal wastewater Nemudryi, et al. 2020 (7)	• Untreated wastewater samples were collected on 17 different days over the course of a 74-day period using an autosampler that collects a volume proportional to flow for 24 hr and tested for SARS-CoV-2 RNA. The samples were filtered and concentrated before RNA extraction. • An ultrafiltration system, with spin concentrators that efficiently recover viruses from wastewater, was used to concentrate SARS-Cov-2. Extracted RNA was used as a template for one-step RT-qPCR. • Changes in SARS-CoV-2 RNA concentrations follow symptom onset gathered by retrospective interview of patients but precedes clinical test results. Wastewater surveillance for SARS-CoV-2 foreshadowed new case reports by 2 to 4 days.
Detection of environmental SARS-CoV-2 RNA in a high prevalence setting in Spain Fernández-de-Mera, et al. 2020 (42)	• Overall, 7 (12.28%) of the 57 samples and 6 (26%) of the sites surveyed were positive for SARS-CoV-2 RNA in at least two of the three RT-PCR reactions performed, and all samples were positive for the SARS-CoV-2-specific RdRP-IP4 and RdRP-IP2 PCRs targeting the coronavirus RNA-dependent RNA polymerase. • SARS-CoV-2 RNA was also detected on surfaces in two of the six public service sites, the petrol station and the pharmacy, but virus RNA was not detected in the two wastewater samples.
Detection of SARS-CoV-2 in raw and treated wastewater in Germany - suitability for COVID-19 surveillance and potential transmission risks Westhaus, et al. 2020 (8)	• An extensive sampling campaign, including nine municipal wastewater treatment plants, has been conducted in different cities of the Federal State of North Rhine-Westphalia (Germany) on the same day in April 2020, close to the first peak of the corona crisis. Samples were processed and analysed for a set of SARS-CoV-2-specific genes, as well as pan-genotypic gene sequences also covering other coronavirus types, using RT-qPCR. • Results of the RT-qPCR based gene analysis indicate the presence of SARS-CoV-2 genetic traces in different raw wastewaters. • A comparison of the particle-bound and the dissolved portion of SARS-CoV-2 virus genes shows that quantifications must not neglect the solid-phase reservoir.
COVID-19 surveillance in Southeastern Virginia using wastewater-based epidemiology Gonzalez, et al. 2020 (9)	• In this study, three RT- droplet digital PCR assays (N1, N2, N3) were used to detect SARS-CoV-2 RNA in weekly samples from nine wastewater treatment plants in southeastern Virginia. • In the first several weeks of sampling, SARS-CoV-2 detections were sporadic. Frequency of detections and overall

Source	Summary
	concentrations of RNA within samples increased from mid-March into late July 2020. • During the twenty-one-week study, SARS-CoV-2 concentrations ranged from 101 to 104 copies 100 mL-1 in samples where viral RNA was detected. • Fluctuations in population normalised loading rates in several of the wastewater treatment plant service areas agreed with known outbreaks during the study.
SARS-CoV-2 has been circulating in northern Italy since December 2019: evidence from environmental monitoring La Rosa, et al. 2020 (10)	• The first documented Italian case of COVID-19 was on 21 February 2020. • Authors analysed 40 composite influent wastewater samples collected between October 2019 and February 2020 from five wastewater treatment plants in three cities and regions in northern Italy. • 24 samples collected in the same treatment plants between September 2018 and June 2019 were included as 'blank' samples. • Molecular analysis was undertaken with both nested RT-PCR and real-rime RT-PCR assays. A total of 15 positive samples were confirmed by both methods. • The earliest dates back to 18 December 2019 in Milan and Turin and 29 January 2020 in Bologna, indicating COVID-19 was circulating earlier in Italy that the first reported case.
Post-lockdown detection of SARS-CoV-2 RNA in the wastewater of Montpellier, France Trottier, et al. 2020 (11)	• Authors measured the amount of SARS-CoV-2 RNA at the inflow point of the main wastewater treatment plant of Montpellier, France. • Samples were collected four days before the end of lockdown and up to 70 days post-lockdown. • Increased amounts of SARS-CoV-2 RNA were detected from mid-June 2020. • A surge in the number of new COVID-19 patients started roughly 2 to 3 weeks after the increase of SARS-CoV-2 RNA levels in wastewater.
First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan Haramato, et al. 2020 (12)	• Study used four quantitative and two nested PCR assays. Influent and secondary-treated wastewater samples (collected five times) and river water samples (collected three times) were collected. • Samples were processed using two methods: the electronegative membrane-vortex method (was found superior) and the membrane adsorption-direct RNA extraction method. • SARS-CoV-2 RNA was successfully detected in one of five secondary-treated wastewater samples with a concentration of 2.4 × 103 copies/L by N_Sarbeco qPCR assay following the

Source	Summary
	electronegative membrane-vortex method, with sequence confirmation of the qPCR product, whereas all the influent samples were tested negative for SARS-CoV-2 RNA. • None of the river water samples tested positive for SARS-CoV-2 RNA. • SARS-CoV-2 RNA was detected in the secondary-treated wastewater sample when the COVID-19 cases peaked in the community.
First proof of the capability of wastewater surveillance for COVID-19 in India through detection of genetic material of SARS-CoV-2 Kumar, et al. 2020 (13)	• Wastewater sampling was carried out on 8 and 27 May 2020 at the Old Pirana Waste Water Treatment Plant that receives effluent from Civil Hospital, treating COVID-19 patients. • All three, i.e. ORF1ab, N and S genes of SARS-CoV-2, were found in the influent with no genes detected in effluent collected on 8 and 27 May 2020. • Increase in SARS-CoV-2 genetic loading in the wastewater corresponded with the increase in the number of active COVID-19 patients.
First detection of SARS-CoV-2 RNA in wastewater in North America: a study in Louisiana, USA Sherchan, et al. 2020 (14)	• In this study, untreated and treated wastewater samples were collected on five occasions over four months from January to April 2020. • Two methods were used: concentrated via ultrafiltration, and an adsorption-elution method using electronegative membranes. • SARS-CoV-2 RNA was detected in 2 of 15 wastewater samples using two RT-qPCR assays(CDC N1 and N2). • None of the secondary treated and final effluent samples tested positive for SARS-CoV-2 RNA.
Preliminary study of Sars-Cov-2 occurrence in wastewater in the Czech Republic Mlenjnkova, et al. 2020 (15)	• Samples of untreated wastewater were collected from 33 wastewater treatment plants. • SARS-CoV-2 RNA was concentrated from wastewater and real-time RT-PCR was used to determine viral RNA. • SARS-CoV-2 RNA was detected in 11.6% of samples and more than 27.3% of treatment plants • In some cases, SARS-CoV-2 was detected repeatedly.
Preliminary results of SARS-CoV-2 detection in sewerage system in Niterói municipality, Rio de Janeiro, Brazil Prado, et al. 2020 (16)	• Ultracentrifugation method associated to RT-qPCR was used to detect SARS-CoV-2. • SARS-CoV-2 in 41.6% (5/12) of raw sewage samples obtained from sewage treatment plants and sewers network in the municipality of Niterói, State of Rio de Janeiro, Brazil.

Source	Summary
SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases Wu, et al. 2020 (17)	• Wastewater collected at a major urban treatment facility in Massachusetts, USA was tested. • SARS-CoV-2 RNA from the N gene was detected at significant titers (57 to 303 copies per ml of sewage) in the period from 18 to 25 March 2020 using RT-qPCR. • Viral titers observed were significantly higher than expected based on clinically confirmed cases.
Presence and infectivity of SARS-CoV-2 virus in wastewaters and rivers Rimoldi, et al. 2002 (18)	• Raw and treated samples from three wastewater treatment plants, and three river samples within the Milano Metropolitan Area, Italy, were surveyed for SARS-CoV-2 RNA. • Real time RT-PCR and infectivity test on culture cells was used. • SARS-CoV-2 RNA was detected in raw, but not in treated wastewaters (four and two samples, respectively, sampled on two dates). • The isolated virus genome was sequenced and belonged to the strain most spread in Europe. • RNA presence in raw wastewater samples decreased after eight days. • Virus infectivity was always null, indicating the natural decay of viral pathogenicity in time from emission.
SARS-CoV-2 in river water: implications in low sanitation countries Guerrero-Latorre et al, 2020 (19)	• Three river locations along the urban rivers of Quito were sampled on 5 June 2020 during a peak of COVID-19 cases. • The viral concentrates were quantified for SARS-CoV-2 (N1 and N2 target regions) and human adenovirus as a human viral indicator. • The results showed that SARS-CoV-2 was detected for both target regions in all samples analysed. • Higher values were shown in areas where higher active cases were registered.
Detection of SARS-CoV-2 RNA in commercial passenger aircraft and cruise ship wastewater: a surveillance tool for assessing the presence of COVID-19 infected travellers Ahmed, et al. 2020 (20)	• Aircraft and cruise ship wastewater samples (n= 21) were tested for SARS-CoV-2. • Two virus concentration methods were used: adsorption–extraction by electronegative membrane (n= 13) and ultrafiltration by amicon (n= 8). • Five assays using RT-qPCR and RT-droplet digital PCR were used. • SARS-CoV-2 RNA was detected in samples from both aircraft and cruise ship wastewater, however concentrations were near the assay limit of detection.

Source	Summary
First detection of SARS-CoV-2 in untreated wastewaters in Italy La Rosa, et al. 2020 (21)	• Twelve influent sewage samples, collected between February and April 2020 from wastewater treatment plants in Milan and Rome. • Samples were tested using the standard World Health Organization procedure for poliovirus surveillance. • SARS-CoV-2 RNA detection was accomplished in wastewaters collected in areas of high (Milan) and low (Rome) epidemic circulation, according to clinical data. • Overall, 6 out of 12 samples were positive. One was collected a few days after the first notified case.
SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area Randazzo, et al. 2020 (22)	• Study investigated the occurrence of SARS-CoV-2 RNA in six wastewater treatments plants within the Region of Murcia (Spain), which had the lowest COVID-19 prevalence in the Iberian Peninsula. • Real-time RT-PCR (RT-qPCR) diagnostic panel was used. • The estimated quantification of SARS-CoV-2 RNA titers in untreated wastewater samples of 5.4 ± 0.2 log10 genomic copies/l on average. • Two secondary water samples resulted positive (2 out of 18) and all tertiary water samples tested as negative (0 out 12). • When compared with COVID-19 cases, this data revealed people were shedding SARS-CoV-2 RNA in their stool even before the first cases were reported.
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 Ahmed, et al. 2020 (23)	• Untreated wastewater samples were collected from one suburban pumping station and two wastewater treatment plants representing urban catchments in Southeast Queensland. • Viruses were concentrated using two previously published methods: direct RNA extraction from electronegative membranes and ultrafiltration. • RT-qPCR assays were used. • None of the wastewater RNA samples had RT-qPCR inhibition, as confirmed by the Sketa22 RT-qPCR assay. • Among the nine wastewater samples tested, two (22.2%) samples collected from one wastewater treatment plant on two separate sampling events (27/03/20 and 01/04/20) were positive for SARS-CoV-2 using the N_Sarbeco assay. • Wastewater samples from the pumping station and the second wastewater treatment plant were negative for SARS-CoV-2.

Source	Summary
SARS-CoV-2 in wastewater: potential health risk, but also data source Lodder, et al. 2020 (24)	• From 17 February 2020, onwards, 24-h 10l samples once a week were taken from human wastewater collected at Amsterdam Airport Schiphol (Haarlemmermeer, Netherlands). • Samples tested positive for virus RNA by quantitative RT-PCR four days after the first cases of COVID-19 were identified in the Netherlands on 27 February 2020 (unpublished data).
SARS-CoV-2 RNA detection of hospital isolation wards hygiene monitoring during the coronavirus disease 2019 outbreak in a Chinese hospital Wang, et al. 2020 (25)	• The aim of this study was to monitor the presence of SARS-Cov-2 among hospital environment surfaces, sewage, and personal protective equipment in isolation wards in a hospital in China. • The three sewage samples from the inlet of pre-processing disinfection pool were positive for SARS-CoV-2 RNA and the sample from the outlet of pre-processing disinfection pool was weakly positive, the sewage sample from the outlet of the last disinfection pool was negative. • All of the five sewage samples from various points were negative by viral culture of SARS-Cov-2.
Grey literature	
Status of environmental surveillance for SARS-CoV-2 virus World Health Organization 7 August 2020 (26)	• Major potential use cases for environmental surveillance for SARS-CoV-2 include: early warning, detection in locations with limited clinical surveillance, monitoring circulation of SARS-CoV-2 and research. • Potential considerations for implementing environmental surveillance include: representativeness, coordination, cost-effectiveness, ethical and legal considerations and quality assurance. • Safety considerations and research needs are also outlined.
National wastewater surveillance system (NWSS) - a new public health tool to understand COVID-19 spread in a community Centers for Disease Control and Prevention 17 August 2020 (43)	• Centers for Disease Control and Prevention is currently developing a portal for state, tribal, local, and territorial health departments to submit wastewater testing data into a national database. • Data from wastewater testing is not meant to replace existing COVID-19 surveillance systems, but is meant to complement them by providing: an efficient pooled community sample, data for communities where timely COVID-19 clinical testing is underutilized or unavailable and data at the sub-county level.
Monitoring wastewater Commonwealth Scientific and Industrial Research Organisation (CSIRO) (27)	• CSIRO have a method for monitoring sewage for early detection of COVID-19 outbreaks. • Day 1 - people within a wastewater treatment catchment, or facility, are exposed to SARS-CoV-2. • Day 2-3 - infected people begin shedding the virus in faeces. • Day 3-4 - samples are collected for analysis from wastewater.

Source	Summary
	• Day 4 - the wastewater sample is concentrated. Fragments of the virus's genetic code are then extracted and genetically analysed. • Day 4-6 - detection and quantification of the virus genetic materials are reported to public health officials. • Day 5-14 - people who begin to show symptoms undergo a clinical nasal swab test.

Appendix

PubMed search terms

((2019-nCoV[title/abstract] or nCoV*[title/abstract] or covid-19[title/abstract] or covid19[title/abstract] OR "covid 19"[title/abstract] OR "coronavirus"[MeSH Terms] OR "coronavirus"[title/abstract] OR sars-cov-2[title/abstract] OR "severe acute respiratory syndrome coronavirus 2"[Supplementary Concept])) AND (wastewater)

Google and Twitter search terms

Wastewater surveillance for COVID-19

Inclusion and exclusion criteria

Inclusion	Exclusion
• Studies reporting empirical data in wastewater surveillance for COVID-19 • Studies testing for SARS-CoV-2	• Non-systematic reviews • Editorials and studies not reporting empirical data • Pre peer review studies

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