Surveillance of wastewater using passive sampling provides an early warning of COVID-19 community transmission

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Highlights

- SARS-CoV-2 detected by low-cost passive samplers in wastewater.
- Passive sampling results reflected clinical diagnosed COVID-19 cases.
- Passive sampler as an early warning tool to prevent severe outbreak.

Introduction

Wastewater-based epidemiology (WEB) provides insights on community living habits and reflects on population health. Virus tracing by wastewater surveillance is a widely accessible, cost effective, ethically practicable way to collect less biased disease prevalence data than clinical testing, hence, it is helpful in understanding disease outbreak when combined with clinical data (Gonzalez et al., 2020).

When there is an epidemic such as COVID-19 prevailing state/national wide, knowing the presence of the virus in areas of interest can provide guidance to relevant authorities on the severity of the epidemic and hence control measures can be taken accordingly (Donner et al., 2021).

Past study by Liu et al. (2020) deployed cotton gauze at building wastewater outflow to detect SARS-CoV-2, sensitive enough to detect 1 or 2 cases in a building by providing a binary result (presence or absence of the virus). Study by Bivins et al. (2021) using tampon swab and RT-LAMP to surveillance 9 residence halls shows a same-day positive predictive value of 33% and negative predictive value of 80%. There is a lack of explicit confirmed case related analysis of passive samplers' performance based on the virus quantity loaded on each passive sampler. In this study, electronegative membrane (Cellulose Nitrate Filter, Sartorius, Germany) is adopted as the virus binding material in passive sampling. This concentration of virus on membranes is linked to active case number to determine the effectiveness of passive sampling in human wastewater as an early warning tool to reflect SARS-CoV-2 infection cases.

Methodology

Sample collection and laboratory analysis

The passive samplers used in this study consists of electronegative membranes held in a 3D printed torpedo shaped housing with open holes that allow water to flow into and out from the sampling device. From January to July, more than 6000 passive samplers were deployed in wastewater through out Victoria. The frequency of the deployment-collection ranges between a day to a week, depending on the prevalence of the epidemic in the state of Victoria. Samples are then transferred on ice into EPHM lab where electronegative membranes are subject to RNA extraction, reverse transcription and qPCR essay, same as described in literature (Schang et al., 2021). Positive detection is defined as when there is at least one replicate of N gene or ORF-1ab gene that has a Cq value < 42.

Data analysis

Positive detections upon passive sampling sites throughout the year have been allocated to a timeline which is also integrated with daily and total confirmed cases, and the lockdown protocol. Once the data of time and location of clinical confirmed cases are available, a multivariable relationship between the passive detection and clinical confirmed cases can be sought. Calibration parameters including but not limited to the infection stages and age of an individual, the distance between the quarantine location and the sampling site, the rainfall during sampling event that might dilute the sewer, the temperature and the chemical used in the sewer that may affect the physical and chemical property of the wastewater, will be taken into consideration in the final presentation of this paper at the conference. The effectiveness of using passive sampling techniques to reflect the COVID-19 cases will be derived from the calibrated statistical model. Further, the concentration of virus (in the unit of copies/sample) on a sample can be estimated based on the Cq value from the qPCR assay. The linkage between the concentration and the infected population in a given catchment will be sought.

Results and discussion

Positive detection by passive sampling is associated with the confirmed cases even in a low prevalence background such as in Victoria, Australia. Figure 1 compares the passive sampling results with contact tracing information of confirmed cases in two different catchments in Victoria. In Case X, passive sampling provides a 2-day in advance early detection when the cases were not yet clinically confirmed. While in the other catchment (Figure 1. left), it shows that there is a general trend that the peak of passive detection concentration occurs approximately a week before the peak of active cases. When the number of active cases is low, copies of virus on the passive may not able to reach the detection limit.

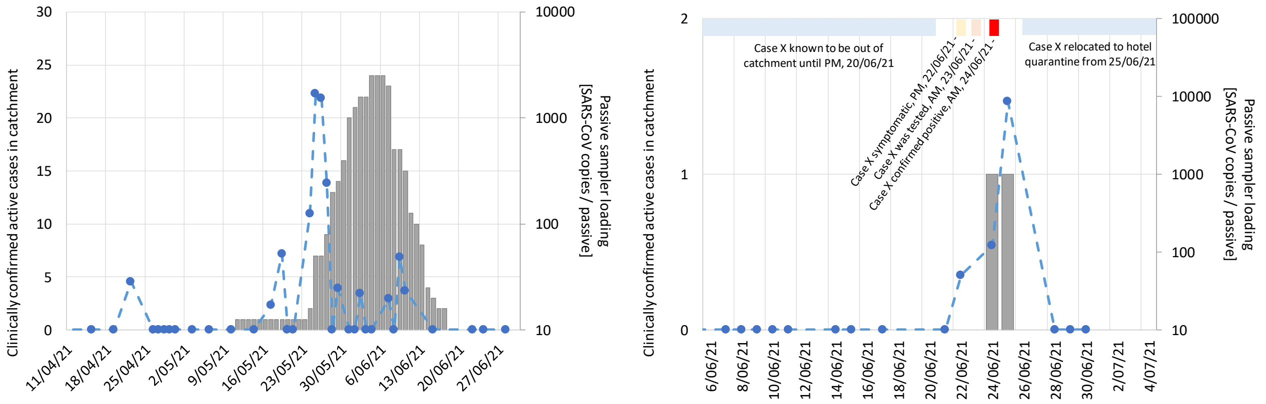

Figure 1. Clinically confirmed active cases and concentrations of SARS-CoV-2 on passive samplers for two catchments in Melbourne: inlet of a small Sewage Treatment Plant capturing wastewater from 100,000 inhabitants (Left) and sewerage network site conveying wastewater from 24,000 inhabitants (right). Active cases are assumed to be shedding for 14 days post-clinical diagnosis or until they leave the catchment (e.g. to enter hotel quarantine). Data on clinical cases and their post-code were obtained from dhhs.vic.gov.au and catchment boundaries for both sewerage systems were approximated using DEM models. Cases were matched to each sewerage system if the catchment boundary included the case's post-code. Information from Case X was obtained from abc.net.au.

Figure 2, compares the passive detection and confirmed cases in the third catchment of interest. Similar as Case X, the early detection using passing sampling method have shown positive results approximately one to two days in advance. Passive detection on 04/06/2021 and 14/06/2021, respectively, corresponding to 4 active cases diagnosed on 06/06/2021 and 4 active cases diagnosed on 15/06/2021. The trend of virus loading on passives goes downwards gradually after the number of active cases peaked in early June, declines along the decrease of active cases, see Figure 2.

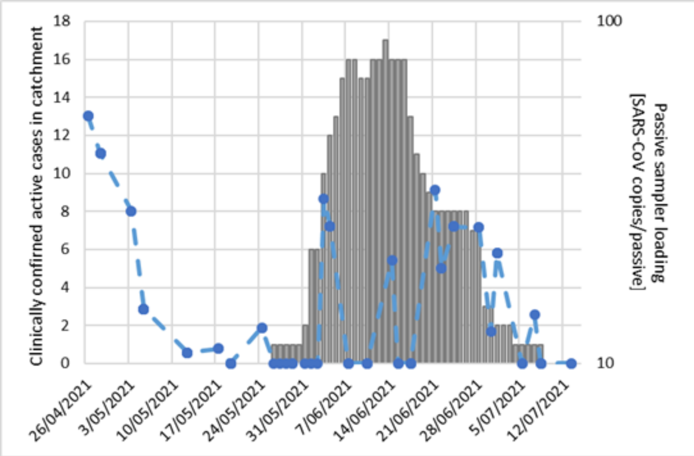

Figure 2. Clinically confirmed cases and SARS-CoV-2 concentration on passive sampler in a densely populated area with more than 166,000 inhabitants.

There are occasions where the passive detection has shown a positive result at a sampling site and a time interval, while there was no diagnosis reported (e.g. Figure 1. left, 22/04/2021, Figure 2. early May). Another occasion includes the detection has not shown a positive result while there is at least one diagnosed case. Sometimes the passive detection shows a positive result a couple of days before or after one or more infection cases are clinically confirmed. This is very likely due to the various faecal shedding characteristics and the virion's routing time in the sewer. Study by Foladori et al. (2020) has reviewed that SARS-CoV-2 virus is not detectable from stool samples of all infected individuals, and positive detection can still be found in faeces weeks after the respiratory tract samples are negative. The exposure rate for passive samplers to viruses from a single shedding event highly depends on the in-sewer distance between quarantine locations of infected individuals and the sampling sites. Current analysis is postcode based with an estimated constant length for all active cases, rather than knowing the exact contact tracing location of infected individuals during the course of infection. More accurate analysis can be achieved with the geographical locations and the length of symptoms and infection of each cases.

Conclusions and future work

Global COVID-19 outbreak gives us a warning that as engineers, we should have handy tools before or in the early stage of the outbreak to alleviate the possible negative impacts to humans and society. Passive sampling in the suburb scale can act as an indicator and provide relevant authorities information on the presence of the virus in a certain area before or in combination with clinical testing results. Future works will focus on the factors that affect the passive sampler performance, binding mechanism between virus and the electronegative membrane, and, determining the best exposure time of the passive samplers in the wastewater.

References

Bivins, A., Lott, M., Shaffer, M., Wu, Z., North, D., Lipp, E., & Bibby, K. (2021). Building-Level Wastewater Monitoring for COVID-19 Using Tampon Swabs and RT-LAMP for Rapid SARS-Cov-2 RNA Detection.

Donner, E., Zamyadi, A., Jex, A., Short, M., Drigo, B., McCarthy, D., ... & Blackall, L. (2021). Wastewater monitoring for SARS-CoV-2. *Microbiology Australia, 42*(1), 18-22.

Gonzalez, R., Curtis, K., Bivins, A., Bibby, K., Weir, M. H., Yetka, K., ... & Gonzalez, D. (2020). COVID-19 surveillance in Southeastern Virginia using wastewater-based epidemiology. *Water research, 186,* 116296.

Foladori, P., Cutrupi, F., Segata, N., Manara, S., Pinto, F., Malpei, F., ... & La Rosa, G. (2020). SARS-CoV-2 from faeces to wastewater treatment: what do we know? A review. *Science of the Total Environment, 743*, 140444.

Liu, P., Ibaraki, M., VanTassell, J., Geith, K., Cavallo, M., Kann, R., & Moe, C. (2020). A Novel COVID-19 Early Warning Tool: Moore Swab Method for Wastewater Surveillance at an Institutional Level. *MedRxiv*.

Schang C., Crosbie N., Nolan M., Poon R., Wang M., Jex A., John N., Baker L., Scales P., Schmidt J., Thorley B., Hill K., Zamyadi A., Tseng K., Henry R., Kolotelo P., Langeveld J., Schilperoort R., Shi B., McCarthy D. (2021). Passive sampling of viruses for wastewater surveillance, DOI: 10.13140/RG.2.2.24138.39367