BUKTI KORESPONDENSI

ARTIKEL JURNAL INTERNASIONAL BEREPUTASI

Judul artikel	:	Feasibility of SARS-COV-2 surveillance using wastewater and environmental sampling in Indonesia
Jurnal	:	Plos One, Vol/No 17/10, 2022
Penulis korespondensi	:	dr. Indah Kartika Murni, Sp.AK., M.Kes., Ph.D
Penulis lain	:	Indah Kartika Murni, Vicka Oktaria, Amanda Handley, David T McCarthy, Celeste Donato, Titik Nuryastuti, Endah Supriyati, Dwi Astuti Dharma, Hendri Mahindra Sari, Ida Safitri Laksanawati, Jarir At Thobari, Julie E. Bines

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2	Bukti konfirmasi artikel telah sesuai dengan format penulisan jurnal dan pemindahan ke *academic editor* untuk *peer review*	24 Mei 2022
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5	Bukti konfirmasi artikel sedang proses pengecekan kesesuaian format penulisan jurnal	24 Agustus 2022
6	Bukti konfirmasi artikel accepted dan amandemen artikel	6 September 2022
7	Bukti konfirmasi artikel accepted formal	6 Oktober 2022
8	Bukti konfirmasi artikel published online	15 Oktober 2022

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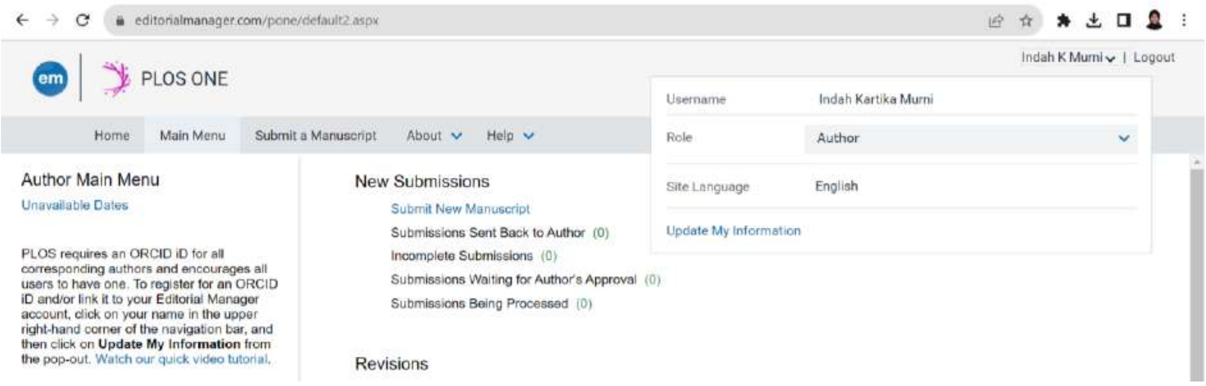

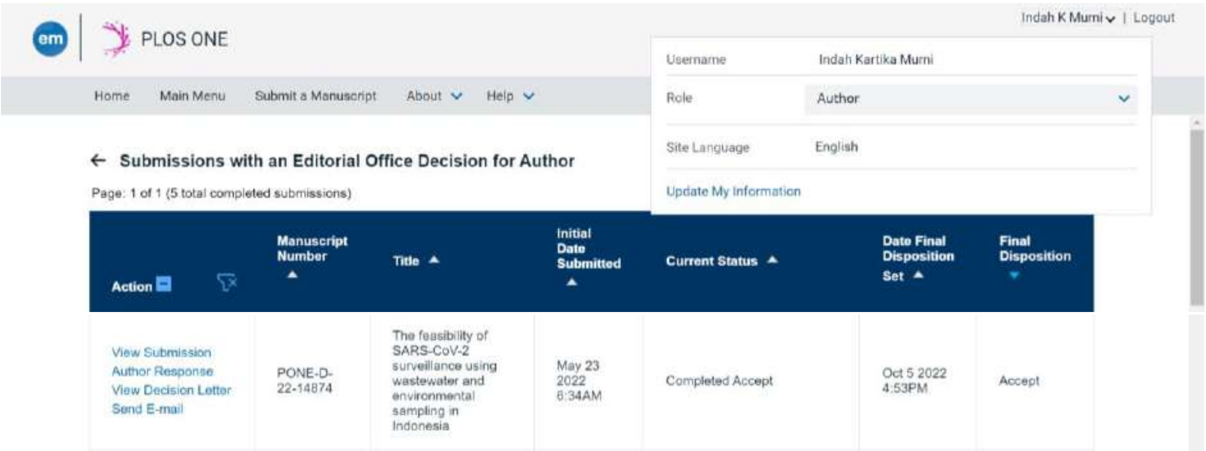

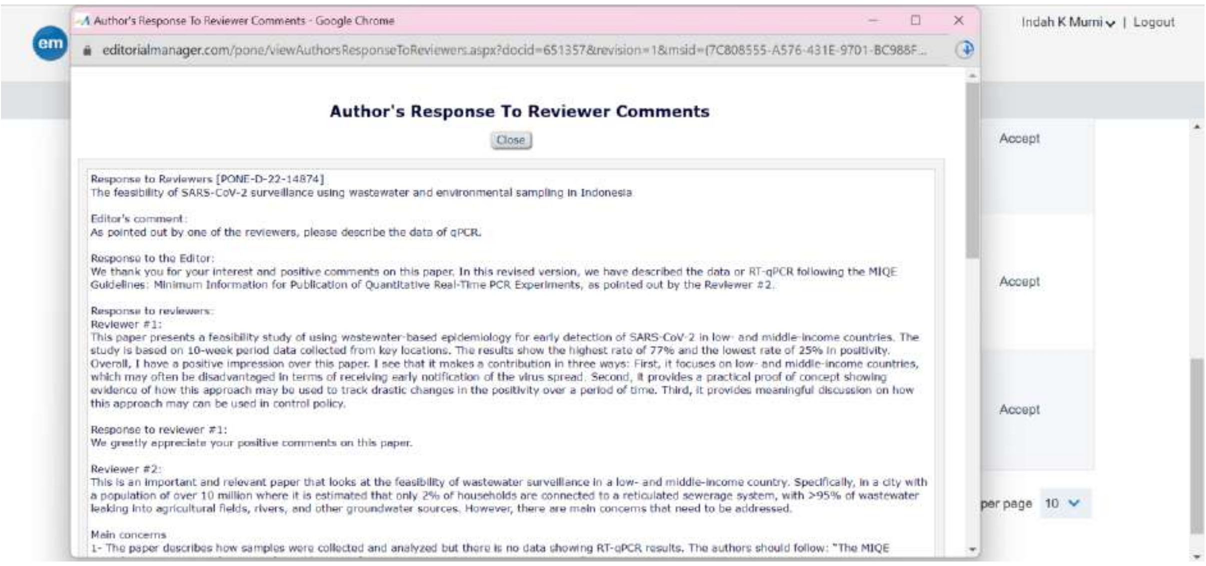

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The feasibility of SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Article
Full Title:	The feasibility of SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia
Short Title:	SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia
Corresponding Author:	Indah K Murni Universitas Gadjah Mada Yogyakarta, INDONESIA
Keywords:	wastewater-based epidemiology surveillance; environmental sampling; SARS-CoV-2; COVID-19; Indonesia
Abstract:	Background Wastewater-based epidemiology (WBE) surveillance as an early warning system (EWS) for monitoring community transmission of SARS-CoV-2 in low- and middle-income country (LMIC) settings, where diagnostic testing capacity is limited, needs further exploration. We explored the feasibility to run a WBE in Indonesia, one of the global epicenters of the COVID-19 pandemic in the middle of 2021, with the fourth largest population in the world where sewer and non-sewered sewage systems are implemented. The feasibility and resource capacity to collect samples on a weekly or fortnightly basis with grab and/or passive sampling methods, as well as to conduct qualitative and quantitative identification of SARS-CoV-2 Ribonucleic acid (RNA) using the real-time RT-PCR (RT-qPCR) in the environmental samples were explored. Methods We initiated a routine surveillance of wastewater and environmental sampling at three predetermined districts in Yogyakarta. Water samples were collected from central and community wastewater treatment plants (WWTPs), including manholes flowing to the central WWTP, and additional soil samples were collected for the near source tracking (NST) locations (i.e., public spaces where people congregate). Results We began collecting samples in the Delta wave of the COVID-19 pandemic in Indonesia in July 2021. From a 10-week period, 54% (296/544) of wastewater and environmental samples were positive for SARS-CoV-2 RNA. The proportion of sample positivity rate decreased in proportion with the reported incidence of COVID-19 clinical cases in the community. The highest positivity rate of 77%, was obtained for samples collected in July 2021 and decreased to 25% by the end of September 2021. Conclusion A WBE surveillance system for SARS-CoV-2 in Indonesia is feasible to conduct for monitoring community burden of infections. Future studies testing the potential of WBE and EWS for signaling early outbreak of SARS-CoV-2 transmissions in the setting are required.
Order of Authors:	Indah K Murni
	Vicka Oktaria
	Amanda Handley
	David T McCarthy
	Celeste M Donato

	Titik Nuryastuti
	Endah Supriyati
	Dwi Astuti Dharma Putri
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Dear Editor,
Wastewater-based epidemiology (WBE) surveillance as an early warning system (EWS) for monitoring community transmission of SARS-CoV-2 in low- and middle-income country (LMIC) settings, where diagnostic testing capacity is limited, needs further exploration. We explored the feasibility to run a WBE in Indonesia, one of the global epicenters of the COVID-19 pandemic in the middle of 2021, with the fourth largest population in the world where sewer and non-sewered sewage systems are implemented. The feasibility and resource capacity to collect samples on a weekly or fortnightly basis with grab and/or passive sampling methods, as well as to conduct qualitative and quantitative identification of SARS-CoV-2 Ribonucleic acid (RNA) using the real-time RT-PCR (RT-qPCR) in the environmental samples were explored. We collected samples in the Delta wave of the COVID-19 pandemic in Indonesia in July 2021. From a 10-week period, 54% (296/544) of wastewater and environmental samples were positive for SARS-CoV-2 RNA. The proportion of sample positivity rate decreased in proportion with the reported incidence of COVID-19 clinical cases in the community. The highest positivity rate of 77%, was obtained for samples collected in July 2021 and decreased to 25% by the end of September 2021.
We found that WBE surveillance system for SARS-CoV-2 in Indonesia is feasible to conduct for monitoring community burden of infections.
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All authors have seen and approved the manuscript, contributed significantly to the work, and the manuscript has not been previously published nor is not being considered for publication elsewhere.

We kindly request two corresponding authors as listed below:
Indah K Murni
Address: Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. Jalan Kesehatan no 1, Sekip, Yogyakarta, 55284, Indonesia
Telephone number: +62274 555455
Email: indah.kartika.m@ugm.ac.id

Vicka Oktaria
Address: Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. Jalan Kesehatan no 1, Sekip, Yogyakarta, 55284, Indonesia
Telephone number: +62274 555455
Email: vicka.oktaria@ugm.ac.id

On behalf of the authors,

Indah K Murni

The feasibility of SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia

Authors

Indah K Murni*[1,2], Vicka Oktaria*[1,3], Amanda Handley[4,5], David T McCarthy[6], Celeste M Donato[4,10], Titik Nuryastuti[7], Endah Supriyati[8], Dwi Astuti Dharma Putri[1], Hendri Marinda Sari[1], Ida Safitri Laksono[1,2], Jarir At Thobari[1], Julie E Bines[4,9,10]

Affiliations

1. Center for Child Health – Pediatric Research Office, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia
2. Child Health Department, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia
3. Department of Biostatistics, Epidemiology and Population Health, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia
4. Enteric Diseases Group, Murdoch Children's Research Institute, Parkville, Victoria, Australia
5. Medicines Development for Global Health, Southbank, Victoria Australia
6. Environmental and Public Health Microbiology Lab (EPHM Lab), Department of Civil Engineering, Monash University, Clayton, Victoria Australia
7. Department of Microbiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia
8. Center for Tropical Medicine, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia
9. Department of Gastroenterology and Clinical Nutrition, Royal Children's Hospital Melbourne, Victoria, Australia
10. Department of Paediatrics, The University of Melbourne, Parkville, Australia

*Corresponding Author

Indah K Murni

Address: Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada.

Jalan Kesehatan no 1, Sekip, Yogyakarta, 55284, Indonesia

Telephone number: +62274 555455

Email: indah.kartika.m@ugm.ac.id

Vicka Oktaria
Address: Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada.
Jalan Kesehatan no 1, Sekip, Yogyakarta, 55284, Indonesia
Telephone number: +62274 555455
Email: vicka.oktaria@ugm.ac.id

The feasibility of SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia

Abstract

Background

Wastewater-based epidemiology (WBE) surveillance as an early warning system (EWS) for monitoring community transmission of SARS-CoV-2 in low- and middle-income country (LMIC) settings, where diagnostic testing capacity is limited, needs further exploration. We explored the feasibility to run a WBE in Indonesia, one of the global epicenters of the COVID-19 pandemic in the middle of 2021, with the fourth largest population in the world where sewer and non-sewered sewage systems are implemented. The feasibility and resource capacity to collect samples on a weekly or fortnightly basis with grab and/or passive sampling methods, as well as to conduct qualitative and quantitative identification of SARS-CoV-2 Ribonucleic acid (RNA) using the real-time RT-PCR (RT-qPCR) in the environmental samples were explored.

Methods

We initiated a routine surveillance of wastewater and environmental sampling at three predetermined districts in Yogyakarta. Water samples were collected from central and community wastewater treatment plants (WWTPs), including manholes flowing to the central WWTP, and additional soil samples were collected for the near source tracking (NST) locations (i.e., public spaces where people congregate).

Results

We began collecting samples in the Delta wave of the COVID-19 pandemic in Indonesia in July 2021. From a 10-week period, 54% (296/544) of wastewater and environmental samples were positive for SARS-CoV-2 RNA. The proportion of sample positivity rate decreased in proportion with the reported incidence of COVID-19 clinical cases in the community. The highest positivity rate of 77%, was obtained for samples collected in July 2021 and decreased to 25% by the end of September 2021.

Conclusion

A WBE surveillance system for SARS-CoV-2 in Indonesia is feasible to conduct for monitoring community burden of infections. Future studies testing the potential of WBE and EWS for signaling early outbreak of SARS-CoV-2 transmissions in the setting are required.

Keywords *wastewater-based epidemiology surveillance, environmental sampling, SARS-CoV-2, COVID-19, Indonesia*

Introduction

Understanding the full extent of COVID-19 pandemic is a major public health challenge. Traditional epidemiological indicators which are based on the number of confirmed clinical cases and deaths due to COVID-19 disease have potential biases and limitations. The capacity for timely diagnosis using laboratory tests may be limited, particularly in low- and middle- income countries (LMICs) during the high seasons. Incidence rates based on hospitalization data lag behind the incidence of infection in the community and lack of representativeness for identification of cases who do not access care, have non-serious illness, or are asymptomatic.

People infected with SARS-CoV-2 shed the virus in stool independently of gastrointestinal symptoms and therefore viral RNA can be detected in wastewater, containing excreta of infected people and sewerage treatment plants.[1–4] Public health surveillance using wastewater is now well established and has been used to monitor communities for the presence of poliovirus, antimicrobial resistant enteric bacteria, and drugs of abuse, e.g. opioids.[5–7] It has been postulated that routine monitoring for the presence of SARS-CoV-2 in wastewater may be useful in predicting an existing or a new potential epidemic.[6,8]

Studies reporting the detection of SARS-CoV-2 RNA in wastewater have been predominantly limited to high-income countries such as Australia, the United States, Japan and a number of European countries,. To date, only a few studies have detected the genetic material of SARS-CoV-

2 in wastewater from low and middle income countries (LMICs), including studiesfrom Argentina, Brazil, Ecuador, India, Pakistan, and South Africa.[9–20] The lack of formal sewerage systems in LMICs, particularly in impoverished areas and informal settlements, has posed a major challenge for SARS-CoV-2 surveillance using wastewater. It is also in these communities where epidemiological surveillance using rates based on disease case capture and death are problematic. The adaptation of environmental surveillance methods suitable for use in LIMCs provides an opportunity to monitor community transmission and inform the public response to SARS-CoV-2 and other future pandemic infections.

This short communication describes the assessment of the feasibility of conducting SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia. The aim was to provide a proof of concept for use of wastewater and environmental surveillance to monitor the community burden of SARS-CoV-2 infection in Indonesia.

Methods

General information on wastewater systems and challenges in Indonesia

In Indonesia, a high proportion of the population is not connected to a sewerage system. In the capital city of Jakarta, a city with a population of over 10 million, it is estimated that only 2% of households are connected to a reticulated sewerage system, with >95% of wastewater leaking into agricultural fields, rivers, and other groundwater sources.[21]

We established the first Indonesian wastewater-based SARS-CoV-2 epidemiology (WBE) surveillance program in Yogyakarta province, one of the locations with the highest number of COVID-19 cases during the COVID-19 Delta wave outbreak. In the Yogyakarta province, only

25,294 households (6% population serviced) are connected to a formal reticulated sewerage system. There are two types of wastewater treatment plants (WWTPs) systems in operation in the province: (a) the central WWTP (*Instalasi Pengolahan Air Limbah Sewon/IPAL* Sewon, Bantul) managed by the provincial government and (b) community WWTPs (IPAL community) that are independently managed by each local community, in addition to individual septic tanks. The service coverage of IPAL Sewon in the Yogyakarta province includes 13 of the 14 sub districts in the Kota Yogyakarta regency, 4 of the 17 sub districts in the Sleman regency and 3 of the 17 sub districts in the Bantul regency. Community WWTPs are used in some suburban areas due to the lack of capacity of the central WWTPs to service their needs and the geography of the region that does not allow passive gravitational flow.

SARS-CoV-2 surveillance on wastewater and environmental sampling in Indonesia (SWESP study)

Routine WBE surveillance (i.e., sewerage and wastewater sites, and waterways) and soil was initiated in three of four districts in the Yogyakarta province (Kota Yogyakarta, Sleman and Bantul districts, **Figure 1)**. One district was not included do to practical challenges due to the geography and relatively sparse population. Identification and mapping of the infrastructure of wastewater system (formal and informal) at provincial and district level was conducted prior to commencing the study**.** Of the total of ten sites, we selected six subdistricts from Yogyakarta city as these areas have the highest coverage of the formal central wastewater system and samples may be considered more representative the broader community. We also selected 12 clustered communities that were served by small community WWTPs. Each community WWTP served between 50-150 households.

Fig 1. Flowchart of sample strategy

We selected ten subdistricts from three out of five districts in Yogyakarta Province (Yogyakarta city, Bantul, Sleman). Samples from three sub districts were taken weekly (as pointed by blue arrows), while others were taken fortnightly. Detailed type and number of samples in each sub district are illustrated in the figure.

We collected samples using either the grab or passive sampling methods. Wastewater from manholes was collected by immersing a ~500ml bottle into the water to a depth of around 20 – 30 cm until the bottle was filled, allowing about 1 cm of air. Recreational water was collected using a-2 L bottle using a similar grab method. Bottles were pre-labelled with sample specific barcodes. A torpedo-style passive sampler with multiple entry points (front, top, sides, and bottom)[22,23] was used to collect samples from septic tanks, rivers, and the central and community WWTPs. Passive samplers were retrieved 24 hours after deployment. Soil samples (20g) and fecal scats (whole pellet) were collected using zip lock bags. Samples were transferred on ice at 2-8°C within four hours to the Microbiology laboratory at the Universitas Gadjah Mada.[24]

Laboratory methods for wastewater and environmental samples

Samples of wastewater (50mL) or recreational water (1000mL) are filtered through a 47mm diameter, 0.45 µm pore size, cellulose nitrate high flow electronegative membrane (Satorius, Germany). The whole filter paper was stored at -80°C until the RNA extraction and RT-qPCR analysis. Soil samples are thoroughly mixed within the collection bag upon arrival in the laboratory. Approximately 0.25 grams of soil were added to DNA/RNA Shield solutions (Zymo Research, USA) and stored at -80°C until the RNA extraction process.

The RNA samples were extracted using the QIAGEN RNeasy PowerMicrobiome kit (QIAGEN, Germany) by replacing the supplied beads with PowerBead Tubes-Garnet beads (QIAGEN, Germany). For every batch of samples processed, a negative control and internal control (MS2 bacteriophage) as supplied in the PerkinElmer SARS-CoV-2 Nucleic Acid Detection Kit (RUO) (PerkinElmer) were included in the RNA extraction process to monitor the RNA extraction performance.

To detect the SARS-Cov2 RNA, a quantitative real-time PCR was conducted using the SARS-CoV-2 Real-time RT-PCR Assay (PerkinElmer, US) and synthetic SARS-CoV-2 RNA Controls MT007544 (Twist Bioscience, California) as the standard curve. The kit is a multiplex assay using primers probes targeting the N gene and ORF1ab region of the SARS-CoV-2 genomes. qRT-PCR assays were performed using two replicates of 5 μL template, with a total reaction volume of 30 μL and a total 45 cycles of amplification. The quantification of the Twist Synthetic SARS-CoV- 2 RNA Control 1 was supplied by the manufacturer. The qRT-PCR assay was performed as described by the manufacturer's instruction using the LightCycler 96 (Roche, Germany).

In order to report the "true" value of SARS-CoV-2 RNA, we calculated the recovery efficiency. In each qPCR run, multiple SARS-CoV-2 RNA controls, a MS2 phage control (to determine the RNA recovery efficiency and as internal control) of different known concentrations and a negative control were included.

The limit of detection (LOD) for the qRT-PCR assay was determined by the analysis of 10 replicates for each dilution of the Twist Synthetic SARS-CoV-2 RNA Control 1 analyzed and was defined as the lowest number of copies of the N gene target and ORF1ab region that could be detected in 80% of the replicates tested. REF The LOD was expressed as the lowest detectable concentration of the N gene target and ORF1ab region in sewage based on the equivalent volume of sewage analyzed in each qRT-PCR assay, not adjusting for any potential loss through the processing of the sewage sample or any potential inhibition of the qRT-PCR assay.[25]

Results

Feasibility of WBE surveillance

The average time from sample collection to availability of the RT-qPCR results was a mean of 64 hours, including the filtration time (3 to 4 hours), RNA extraction and RT-qPCR (2 to 3 hours), and quantification analysis (1 to 2 hours). Both weekly and fortnightly sample collections were practical to conduct. A key challenge was the delay in the importation of critical reagents and consumables exacerbated by the COVID-19 pandemic. As the UGM laboratory is also the central clinical laboratory, priority for the analysis of clinical samples resulted in a delay in wastewater analysis during major clinical peaks in incidence. Initial trials of deployment of the passive samplers were required to limit damage or loss due to difficulties with positioning and securing samplers. We defined criteria for reliable deployment that considered locations with solid ground to safely access, ideally in an inconspicuous position, and using a strong pole or tree to secure the sampler. To avoid samplers being removed we labeled samplers with signs of warning and explanation such as “Sample for Research by Universitas Gadjah Mada and Yogyakarta Government”.

The detection and positivity rates of SARS-CoV-2 RNA

Sample collection commenced on the 27th July 2021 during the Delta wave of the COVID-19 pandemic in Indonesia (**Figure 2**). During the 10-week sampling period, a total of 544 samples were collected with 54% (296/544) of all samples testing positive for SARS-CoV-2 RNA. The highest positivity rate of 77%, was obtained for samples collected in July 2021 and decreased to 25% by the end of September 2021, reflecting the decrease with the incidence of reported COVID-19 clinical cases in the community.

The Nucleocapsid (N) gene was identified in 74% (191/258) of sewage (grab method), 64% (67/104) of NST water samples (passive sampling method), 48% (19/40) of river samples (grab method), and 3% (2/60) of NST soil. This finding was consistent with the open reading frame of 1ab (ORF1ab) gene target but with a higher proportion of soils samples being positive (8%, 5/60) for the ORFlab gene as compared to N gene (what %).

Fig 2. Initial samples were collected at the peak of new confirmed cases during the second wave pandemic of the COVID-19 pandemic in Yogyakarta (available at https://www.worldometers.info/coronavirus/country/indonesia/)

Discussion

WBE surveillance for SARS-CoV-2 RNA was feasible in Indonesia and refected the SARS-CoV-2 clinical burden in the community. The high level of positivity of SARS-CoV-2 RNA in the environment in Indonesia suggests a considerable public health burden and may represent asymptomatic or mild cases that did not access health facilities for testing. Manholes consistently showed higher positivity rates in comparison with river and soil samples. Although river and soil samples showed lower positivity rates, the data are useful to complement the WBE surveillance data particularly in regions where connection to a formal sewerage system is limited. This combination of sampling strategies provides additional insights into the prevalence and distribution of COVID-19 within the community.

In Yogyakarta, many households are not connected to the IPAL Sewon. This may because they were built after the IPAL Sewon infrastructure was established therefore have no connection to the IPAL pipes. Other households were not connected due to technical reasons, such as in lower altitudes and geography that does not support passive gravity flow of wastewater to the central WWTP. However, in this study we managed to collect samples from community WWTPs and septic tanks from NST sites to capture communities that were not served by the central WWTP.

Although we found that both weekly or fortnightly collection frequency with grab and/or passive sampling collection methods as feasible, weekly collections were preferred in order to provide real-time data to inform the public health response. The laboratory capacity to conduct qualitative (positive/negative) and quantitative identification of SARS-CoV-2 RNA in the environmental samples (wastewater and soil) were also feasible although some pre-processing procedures need to be conducted prior to the qRT-PCR procedure (i.e., wastewater filtration and soil homogenization). There were challenges of providing real-time results during peak COVID-19 outbreaks due to overburdened staff and limited access to equipment, and therefore, ideally WBE surveillance should be integrated into the routine surveillance programs with, dedicated staff. Yet the availability of imported reagents have delayed laboratory analysis during periods of high output. Local epidemiological data describing the distribution of COVID-19 cases (symptomatic and asymptomatic) with laboratory confirmed positive tests for SARS-CoV-2 infections by sub district, on a weekly basis, were available to compare with findings from WBE surveillance. However, data analysis to link environmental and community data remain challenging and need further exploration.

Despite efforts there remain practical limitations of WBE surveillance in LIMCs. It is likely that wastewater sampling of the reticulated sewerage system reflects the more modern and affluent sector of the city and may not provide meaningful insights into the presence of SARS-CoV-2 infection within the broader community. Most of the city and rural areas manage human effluent via septic tanks, pit latrines or by open defecation with subsequent contamination of surface water and rivers. Therefore, to understand the distribution of SARS-CoV-2 RNA in environments that reflect the presence of community infections with fragmented wastewater infrastructures, NST sites, and in places where people publicly congregate were selected. These sites include permanent dwellings (apartment and flats), temporary living places (hotels), public spaces (traditional markets, town squares, mosques, and a public swimming pool), rivers, working spaces (both office and factory), and COVID-19 shelters (facilities which are designated as temporary quarantine shelters for people testing positive for COVID-19). This WBE approaches using NST may allow detection of targeted clusters for whom rapid action may reduce or prevent the risk of larger outbreaks within the community.[26]

It has been proposed that WBE surveillance has the potential to act as an early warning system for COVID-19 outbreaks.[27–32] This should be conducted in collaboration with the public health authorities to enable the timely follow up of positive detections by strategies such as contact tracing, strengthening health protocols, or implementing a community lockdown. This could be broadly implemented across the community or in a targeted response depending on the local context and level of concern. For instance, if SARS-CoV-2 RNA is detected (positive result) in the sewerage sample in an area where there had consistently been no detections (negative result), then a lockdown or mass screening could be implemented in the area drained by the sewerage system; or if the result is taken from a closed community (e.g., Boarding school), contact tracing within the community should be conducted immediately (**Figure 3**).

Fig 3. Implementation strategy of the wastewater surveillance program.

Weekly and fortnightly wastewater and environment samples including NST, are collected using grab and passive sampling methods. Further, samples are analysed in the Microbiology Laboratory for detection and quantification of SARS-CoV-2 RNA. Proposed scenario of actions following positive detections of SARS-CoV-2 RNA from wastewater and environmental samples are notification to local public health office and sewerage management facilities for further planning for preventive measures, including contact tracing and breaking transmission chain (i.e., local lockdown).

SWESP: SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia; RNA: Ribonucleic Acid; PCR: Polymerase Chain Reaction

In conclusion, an environmental surveillance system for SARS-CoV-2 in Indonesia is feasible and can be used to monitor the community burden of SARS-CoV-2 infection. However, future research is needed to explore its potential to act as an early warning system for the early identification of SARS-CoV-2 outbreaks within a community, especially in regions with limited access to clinical testing. Although the sewer infrastructure of wastewater systems is quite limited in Indonesia, an expanded sampling approach based on the local context and including NST can support an effective SARS-COV-2 surveillance program.

Acknowledgements

We would like to acknowledge PATH for supporting the study and reviewing the draft manuscript. *Learn more at PATH.org*. We also thank to the wastewater treatment plant team, field assistants and laboratory team for doing sampling collection and lab works.

Role of the funding sources

This Project was funded by the Global Innovation Fund and PATH. The Global Investment Fund had no involvement in study design, data collection or analysis and PATH participated in study design, but had no role in data collection or analysis, writing of the manuscript or the decision to submit it for publication.

Author contributions

Conceptualization - Indah K Murni, Vicka Oktaria, Amanda Handley, David T McCarthy, Celeste M Donato, Julie E Bines

Data curation - Amanda Handley, Endah Supriyati, Dwi Astuti Dharma Putri, Hendri Marinda Sari

Formal analysis - Vicka Oktaria, Dwi Astuti Dharma Putri, Indah K Murni

Funding acquisition - Julie E Bines, Amanda Handley

Investigation - Indah K Murni, Vicka Oktaria, Amanda Handley, David T McCarthy, Celeste M Donato, Julie E Bines

Methodology - Indah K Murni, Vicka Oktaria, Amanda Handley, David T McCarthy, Celeste M Donato, Titik Nuryastuti, Endah Supriyati, Julie E Bines

Project administration - Amanda Handley, Hendri Marinda Sari, Dwi Astuti Dharma Putri, Endah Supriyati

Resources - David T McCarthy, Titik Nuryastuti, Julie E Bines, Indah K Murni, Vicka Oktaria

Supervision - Indah K Murni, Vicka Oktaria, Titik Nuryastuti, Ida Safitri Laksono, Jarir At Thobari, Julie E Bines

Validation - Indah K Murni, Vicka Oktaria, Amanda Handley, David T McCarthy, Titik Nuryastuti, Endah Supriyati, Julie E Bines

Writing - original draft - Indah K Murni

Writing - review and editing - Indah K Murni, Vicka Oktaria, Amanda Handley, David T McCarthy, Celeste M Donato, Titik Nuryastuti, Endah Supriyati, Dwi Astuti Dharma Putri, Hendri Marinda Sari, Ida Safitri Laksono, Jarir At Thobari, Julie E Bines

Conflict of interests

No conflict of interests to declare.

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Figure 1

Click here to access/download;Figure;Figure 1. Flowchart of sample size.tif

	Kasihan	Sewon	Jetis	Danurejan	Wirobrajan	Mantrijeron	Kraton	Gondokusuman	Depok	Mlati
Total Area (km²)	32.38	27.16	1.70	1.10	1.76	2.61	1.4	3.99	55.55	28.52
Total Population (people)	115,050	109,570	23,385	18,670	24,739	33,340	17,943	36,921	131,005	100,524
Total Head of Family (people)	35,548	34,409	9,429	7,299	9,422	12,272	7,568	14,654	41,551	31,399
Population Density	3,197 /km²	3,695 /km²	15,960 /km²	19,395 /km²	15,834 /km²	13,576 /km²	15,594 /km²	10,731 /km²	3,440 /km²	3,229 /km²
COVID on Dec 10, 2021 (people)	1	2	0	0	1	2	2	4	5	0
Incidence Rate*	0.87	1.83	0	0	4.04	6.00	11.15	10.83	3.82	0
Manhole (Grab method)	4	4	5	4	4	4	4	4	3	4
NST Water (Passive & grab method)	2	2	1	2	2	2	1	-	4	2
NST Soil (Grab method)	2	-	-	1	1	1	2	-	1	-
River (Passive & Grab method)	4	2	2	2	-	-	-	-	2	-
Central WWTP Sewon (Passive)	-	4	-	-	-	-	-	-	-	-
Community WWTP (Passive)	3	4	-	-	-	-	-	2	4	1
Total Sampling Location	15	16	8	9	7	7	7	6	14	7

*Estimated number of new cases per 100,000 persons in a population

Blue arrow indicates weekly sampling

Figure 2

Click here to access/download;Figure;Figure 2. First samples collected at the peak of COVID-19.tif

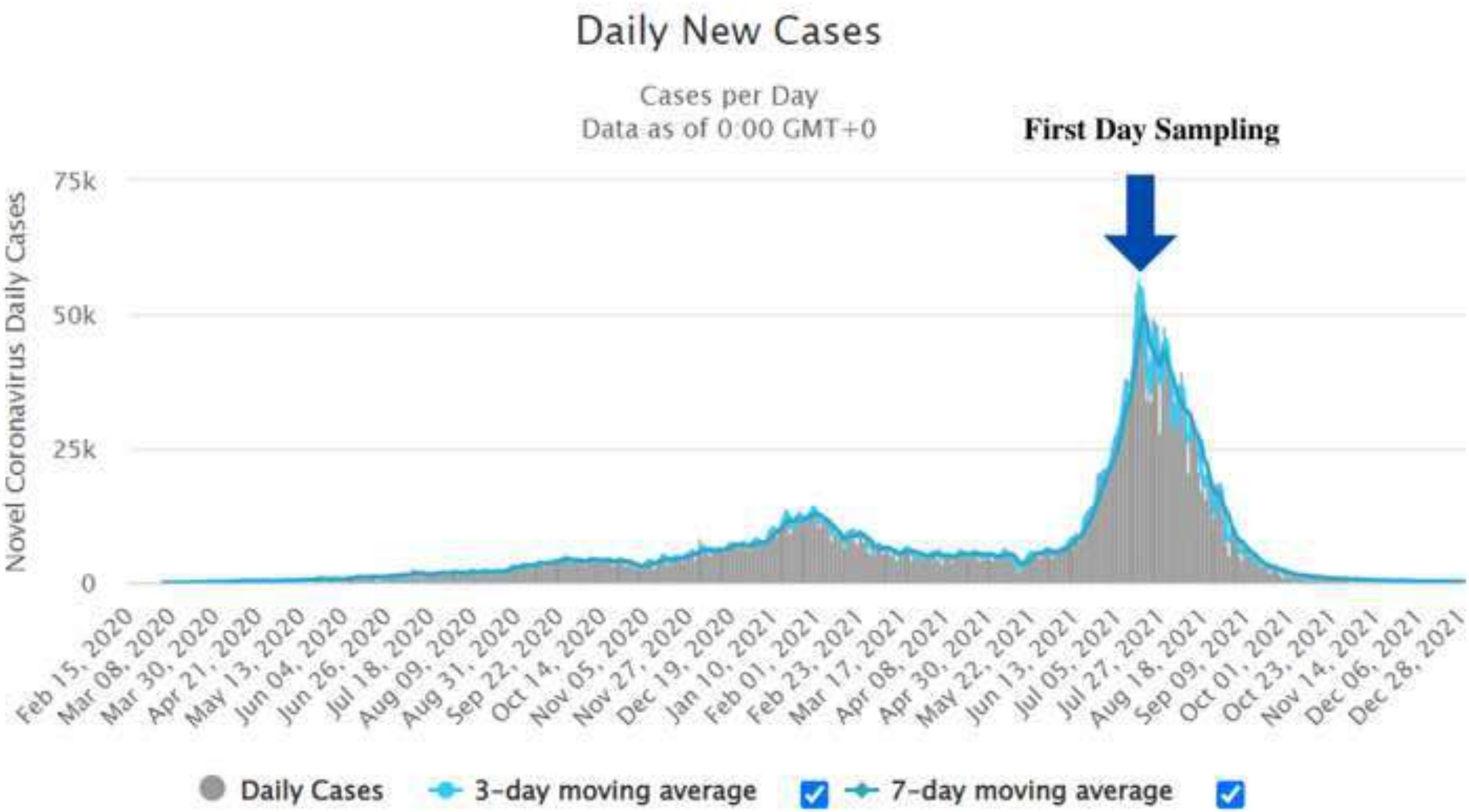

Figure 3

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2. Bukti konfirmasi artikel telah sessuai dengan formal penulisan jurnal dan pemindahan ke academic editor untuk peer review (24 Mei 2022)

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PLOS ONE <em@editorialmanager.com> Tue, May 24, 2022 at 8:15 AM
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To: Indah K Murni <indah.kartika.m@ugm.ac.id>

The feasibility of SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia
PONE-D-22-14874

Dear Dr. Murni,

We're writing to let you know that we've checked your manuscript for our basic formatting requirements and we're now sending it to a staff editor for checks related to our editorial policies. Once this review is complete we will assign your submission to an Academic Editor for peer review. This is part of our standard process for submissions and no action is required from you at this time.

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PLOS ONE Decision: Revision required [PONE-D-22-14874] - [EMID:b43b3a64eca15f64]

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The feasibility of SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia
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Thank you for submitting your manuscript to PLOS ONE. After careful consideration, we feel that it has merit but does not fully meet PLOS ONE's publication criteria as it currently stands. Therefore, we invite you to submit a revised version of the manuscript that addresses the points raised during the review process.

As pointed out by one of the reviewers, please describe the data of qPCR.

Please submit your revised manuscript by Aug 15 2022 11:59PM. If you will need more time than this to complete your revisions, please reply to this message or contact the journal office at plosone@plos.org. When you're ready to submit your revision, log on to https://www.editorialmanager.com/pone/ and select the 'Submissions Needing Revision' folder to locate your manuscript file.

Please include the following items when submitting your revised manuscript:

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Kind regards,

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We note that you have provided funding information that is not currently declared in your Funding Statement. However, funding information should not appear in the Acknowledgments section or other areas of your manuscript. We will only publish funding information present in the Funding Statement section of the online submission form. Please remove any funding-related text from the manuscript and let us know how you would like to update your Funding Statement. Currently, your Funding Statement reads as follows:

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Reviewers' comments:

Reviewer's Responses to Questions

Comments to the Author

1. Is the manuscript technically sound, and do the data support the conclusions?

The manuscript must describe a technically sound piece of scientific research with data that supports the conclusions. Experiments must have been conducted rigorously, with appropriate controls, replication, and sample sizes. The conclusions must be drawn appropriately based on the data presented.

Reviewer #1: Yes

Reviewer #2: Partly

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2. Has the statistical analysis been performed appropriately and rigorously?

Reviewer #1: Yes

Reviewer #2: No

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4. Is the manuscript presented in an intelligible fashion and written in standard English?

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5. Review Comments to the Author

Please use the space provided to explain your answers to the questions above. You may also include additional comments for the author, including concerns about dual publication, research ethics, or publication ethics. (Please upload your review as an attachment if it exceeds 20,000 characters)

Reviewer #1: This paper presents a feasibility study of using wastewater-based epidemiology for early detection of SARS-CoV-2 in low- and middle in come countries. The study is based on 10-week period data collected from key locations. The results show the highest rate of 77% and the lowest rate of 25% in positivity. Overall, I have a positive impression over this paper. I see that it makes a contribution in three ways: First, it focuses on low- and middle-income countries, which may often be disadvantaged in terms of receiving early notification of the virus spread. Second, it provides a practical proof of concept showing evidence of how this approach may be used to track drastic changes in the positivity over a period of time. Third, it provides meaningful discussion on how this approach may can be used in control policy.

Reviewer #2: This is an important and relevant paper that looks at the feasibility of wastewater surveillance in a low- and middle-income country. Specifically, in a city with a population of over 10 million where it is estimated that only 2% of households are connected to a reticulated sewerage system, with >95% of wastewater leaking into agricultural fields, rivers, and other groundwater sources. However, there are main concerns that need to be addressed.

Main concerns
1- The paper describes how samples were collected and analyzed but there is no data showing RT-qPCR results. The authors should follow: “The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments” (https://academic.oup.com/clinchem/article/55/4/611/5631762) when reporting their results.
2- The results provided by the authors appear correct:
- higher positivity rate in wastewater corresponds with clinical data
- higher positivity rate for samples from wastewater treatment plans vs rivers and soil
However, with out the data described above these results can’t be validated.

Minor concerns
1- There are a few typos in the manuscript that suggest that perhaps the authors did not upload the latest version.
2- The resolution of the figures should be improved.
3- A map showing the geographical location of the samples would be helpful to interpret results.

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PONE-D-22-14874R1
The feasibility of SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia

Dear Dr. Murni,

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All the best,
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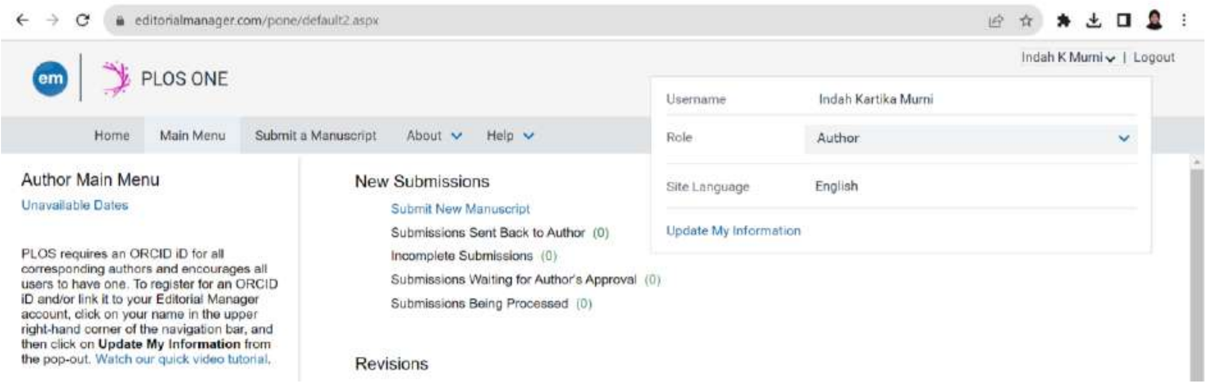
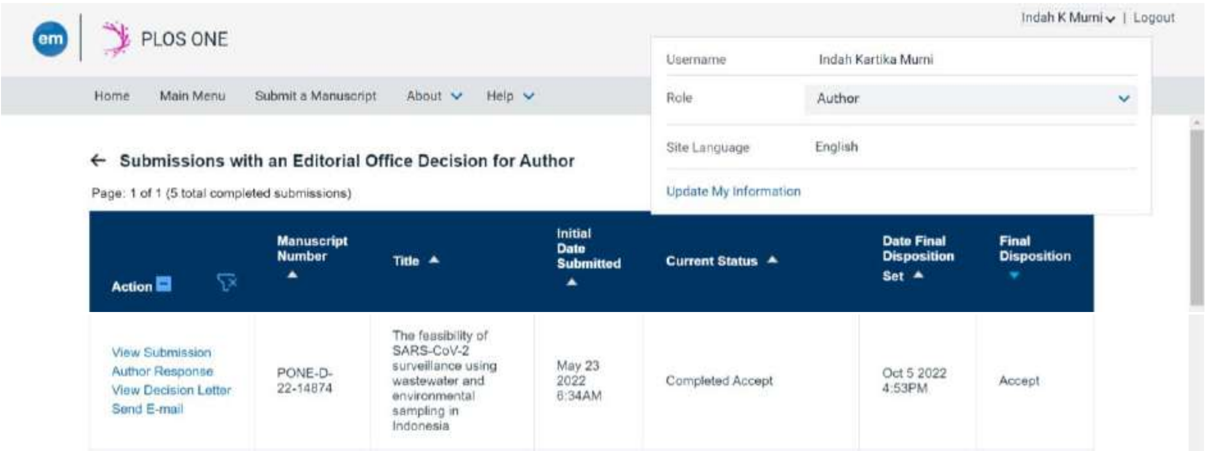
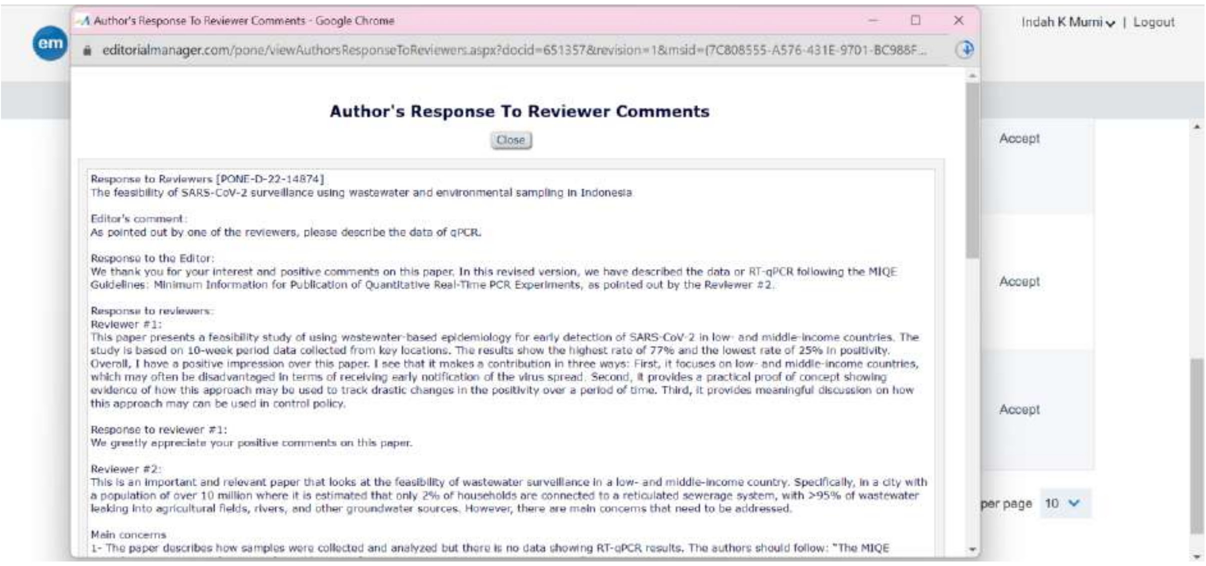
Seluruh dokumen, revisi dan respon kepada reviewer jurnal Plos One dengan judul artikel *"The feasibility of SARS-COV-2 surveillance using wastewater and environmental sampling in Indonesia"* diunggah oleh penulis korespondensi (Indah Kartika Murni) melalui website resmi jurnal Plos One melalui alamat https://www.editorialmanager.com/pone dengan username akun: Indah K Murni







Author's Response To Reviewer Comments

Close

Response to Reviewers [PONE-D-22-14874]
The feasibility of SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia

Editor's comment:
As pointed out by one of the reviewers, please describe the data of qPCR.

Response to the Editor:
We thank you for your interest and positive comments on this paper. In this revised version, we have described the data or RT-qPCR following the MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments, as pointed out by the Reviewer #2.

Response to reviewers:
Reviewer #1:
This paper presents a feasibility study of using wastewater-based epidemiology for early detection of SARS-CoV-2 in low- and middle-income countries. The study is based on 10-week period data collected from key locations. The results show the highest rate of 77% and the lowest rate of 25% in positivity. Overall, I have a positive impression over this paper. I see that it makes a contribution in three ways: First, it focuses on low- and middle-income countries, which may often be disadvantaged in terms of receiving early notification of the virus spread. Second, it provides a practical proof of concept showing evidence of how this approach may be used to track drastic changes in the positivity over a period of time. Third, it provides meaningful discussion on how this approach may can be used in control policy.

Response to reviewer #1:
We greatly appreciate your positive comments on this paper.

Reviewer #2:
This is an important and relevant paper that looks at the feasibility of wastewater surveillance in a low- and middle-income country. Specifically, in a city with a population of over 10 million where it is estimated that only 2% of households are connected to a reticulated sewerage system, with >95% of wastewater leaking into agricultural fields, rivers, and other groundwater sources. However, there are main concerns that need to be addressed.

Main concerns
1- The paper describes how samples were collected and analyzed but there is no data showing RT-qPCR results. The authors should follow: “The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments” (https://academic.oup.com/clinchem/article/55/4/611/5631762) when reporting their results.

Response to reviewer 2:
Thank you for pointing out The MIQE Guidelines for reporting RT-qPCR experiments. We provided the information on RT-qPCR experiments such as details of the samples, nucleic acid extraction, reverse transcription, and qPCR target information under the section ‘Laboratory methods for wastewater and environmental samples’ (page 8-10, line 147-186). We also have amended the paper to describe the data and the RT-qPCR as described in the MIQE Guidelines. Furthermore, we have added the median of RT-qPCR cycle threshold data during the 10-week of sample collection for both the N and ORF1ab genes.
“The median of cycle threshold (Ct) values for positive N and ORF1ab gene results was 35.1 (IQR: 32.1 – 36.9) and 33.9 (IQR: 30.1 – 35.9), respectively.” (results section, page 11, line 212-214)

2- The results provided by the authors appear correct:
- higher positivity rate in wastewater corresponds with clinical data
- higher positivity rate for samples from wastewater treatment plans vs rivers and soil
However, without the data described above these results can’t be validated.

Response to reviewer #2:
We found that the changed trends of the positivity rate detected using N and ORF1ab genes were in alignment with confirmed cases in the community. In order to support this result, we attached the distribution maps of SARS-CoV-2 in Yogyakarta, comparing the detection of SARS-CoV-2 targeting N gene to confirmed community cases (Fig 3).

The distribution maps illustrate three time points, i.e., week 1-2 (Fig 3A), week 5-6 (Fig 3B) and week 9-10 (Fig 3C). The areas were colored with different shades of blue to depict the number of confirmed COVID-19 cases in community, in which the darker color means the higher confirmed COVID-19 cases. The sampling locations were marked using circles (manholes), pentagons (river) and triangles (NST water) which are colored red if detected as positive or colored green if non-detected. In week 1-2, the COVID-19 cases in the community were high (dark blue), almost all sampling locations were positive for COVID-19 (only two green dots/triangles were shown). As the confirmed cases decreased through time, in week 9-10 the areas were shaded with light blue and the number of sampling locations detected as positive was lower. In addition, we revised the relevant results section to summarize this finding, as below.

"The temporal changes in rates of sample positivity correlate with the number of confirmed cases in the community as illustrated in Fig 3. The highest positivity rate of 77%, was obtained for samples collected in July 2021 during week 1 of sample collection and decreased to 25% by the end of September 2021 (corresponding to week 10 of sample collection), reflecting a decreased detection rate correlating with a decrease in the incidence of reported COVID-19 clinical cases in the community." (results section, page 11, line 216-221)

We also added the summary comparing positivity rate between sample types (in results section, page 11, line 214-216) and pie charts of positivity rates of each sample type (Fig 2, see below) to support the finding that river and soil samples showed the lowest positivity rate.
"The highest positivity rate was for manhole samples (74%, 191/258 samples, Fig 2) and the lowest was for soil samples (3%, 2/60 samples, Fig 2)." (results section, page 11, line 214-216)

Minor concerns
1- There are a few typos in the manuscript that suggest that perhaps the authors did not upload the latest version.

Response to reviewer #2:
Thank you for pointing out this matter, we have corrected the typos and ensured that the submitted version is the latest version of our work.

2- The resolution of the figures should be improved.

Response to reviewer #2:
We have re-read the guideline for figures and reviewed our figures following the PLOS ONE requirements using the suggested PACE tool.

3- A map showing the geographical location of the samples would be helpful to interpret results.

Response to reviewer#2:
The newly attached maps of Fig 3 showing the distribution of SARS-CoV-2 in Yogyakarta have addressed this feedback. This figure is a geographical map of Yogyakarta province. As explained previously, we colored the sub-districts included in this study based on the number of confirmed COVID-19 cases. We also marked the sampling location and gave different symbols to each sample type, i.e., circle (manholes), pentagon (river) and triangle (NST water).

Close

PLOS ONE

The feasibility of SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia

--Manuscript Draft--

Manuscript Number:	PONE-D-22-14874R1
Article Type:	Research Article
Full Title:	The feasibility of SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia
Short Title:	SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia
Corresponding Author:	Indah K Murni Universitas Gadjah Mada Yogyakarta, INDONESIA
Keywords:	Wastewater-based epidemiology surveillance; environmental sampling; SARS-CoV-2; COVID-19; Indonesia
Abstract:	Background Wastewater-based epidemiology (WBE) surveillance as an early warning system (EWS) for monitoring community transmission of SARS-CoV-2 in low- and middle-income country (LMIC) settings, where diagnostic testing capacity is limited, needs further exploration. We explored the feasibility to conduct a WBE surveillance in Indonesia, one of the global epicenters of the COVID-19 pandemic in the middle of 2021, with the fourth largest population in the world where sewer and non-sewered sewage systems are implemented. The feasibility and resource capacity to collect samples on a weekly or fortnightly basis with grab and/or passive sampling methods, as well as to conduct qualitative and quantitative identification of SARS-CoV-2 ribonucleic acid (RNA) using real-time RT-PCR (RT-qPCR) testing of environmental samples were explored. Materials and methods We initiated a routine surveillance of wastewater and environmental sampling at three predetermined districts in Special Region of Yogyakarta Province. Water samples were collected from central and community wastewater treatment plants (WWTPs), including manholes flowing to the central WWTP, and additional soil samples were collected for the near source tracking (NST) locations (i.e., public spaces where people congregate). Results We began collecting samples in the Delta wave of the COVID-19 pandemic in Indonesia in July 2021. From a 10-week period, 54% (296/544) of wastewater and environmental samples were positive for SARS-CoV-2 RNA. The sample positivity rate decreased in proportion with the reported incidence of COVID-19 clinical cases in the community. The highest positivity rate of 77% in week 1, was obtained for samples collected in July 2021 and decreased to 25% in week 10 by the end of September 2021. Conclusion A WBE surveillance system for SARS-CoV-2 in Indonesia is feasible to monitor the community burden of infections. Future studies testing the potential of WBE and EWS for signaling early outbreaks of SARS-CoV-2 transmissions in this setting are required.
Order of Authors:	Indah K Murni
	Vicka Oktaria
	Amanda Handley

	David T McCarthy
	Celeste M Donato
	Titik Nuryastuti
	Endah Supriyati
	Dwi Astuti Dharma Putri
	Hendri Marinda Sari
	Ida Safitri Laksono
	Jarir At Thobari
	Julie E Bines
Opposed Reviewers:	
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Additional Information:	
Question	**Response**
Financial Disclosure Enter a financial disclosure statement that describes the sources of funding for the work included in this submission. Review the submission guidelines for detailed requirements. View published research articles from *PLOS ONE* for specific examples. This statement is required for submission and **will appear in the published article** if the submission is accepted. Please make sure it is accurate. **Unfunded studies** Enter: *The author(s) received no specific funding for this work.* **Funded studies** Enter a statement with the following details: • Initials of the authors who received each award • Grant numbers awarded to each author • The full name of each funder • URL of each funder website • Did the sponsors or funders play any role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript? • **NO** - Include this sentence at the end of your statement: *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.* • **YES** - Specify the role(s) played. * typeset	This Project was funded by the Global Innovation Fund and PATH. The Global Investment Fund had no involvement in study design, data collection or analysis and PATH participated in study design, but had no role in data collection or analysis, writing of the manuscript or the decision to submit it for publication.
Competing Interests Use the instructions below to enter a	No competing interest to declare

competing interest statement for this submission. On behalf of all authors, disclose any competing interests that could be perceived to bias this work—acknowledging all financial support and any other relevant financial or non-financial competing interests. This statement is required for submission and **will appear in the published article** if the submission is accepted. Please make sure it is accurate and that any funding sources listed in your Funding Information later in the submission form are also declared in your Financial Disclosure statement. View published research articles from *PLOS ONE* for specific examples. **NO authors have competing interests** Enter: *The authors have declared that no competing interests exist.* **Authors with competing interests** Enter competing interest details beginning with this statement: *I have read the journal's policy and the authors of this manuscript have the following competing interests: [insert competing interests here]* * typeset	
Ethics Statement Enter an ethics statement for this submission. This statement is required if the study involved: • Human participants • Human specimens or tissue • Vertebrate animals or cephalopods • Vertebrate embryos or tissues • Field research	The Medical and Health Research Ethics Committee of Universitas Gadjah Mada, Indonesia approved this study (KE/FK/0514/EC/2022 continuing KE/FK/0426/EC/2021)

Write "N/A" if the submission does not require an ethics statement.

General guidance is provided below. Consult the submission guidelines for detailed instructions. **Make sure that all information entered here is included in the Methods section of the manuscript.**

Format for specific study types

Human Subject Research (involving human participants and/or tissue)

- Give the name of the institutional review board or ethics committee that approved the study
- Include the approval number and/or a statement indicating approval of this research
- Indicate the form of consent obtained (written/oral) or the reason that consent was not obtained (e.g. the data were analyzed anonymously)

Animal Research (involving vertebrate animals, embryos or tissues)

- Provide the name of the Institutional Animal Care and Use Committee (IACUC) or other relevant ethics board that reviewed the study protocol, and indicate whether they approved this research or granted a formal waiver of ethical approval
- Include an approval number if one was obtained
- If the study involved *non-human primates*, add *additional details* about animal welfare and steps taken to ameliorate suffering
- If anesthesia, euthanasia, or any kind of animal sacrifice is part of the study, include briefly which substances and/or methods were applied

Field Research

Include the following details if this study involves the collection of plant, animal, or other materials from a natural setting:

- Field permit number
- Name of the institution or relevant body that granted permission

Data Availability Authors are required to make all data underlying the findings described fully available, without restriction, and from the time of publication. PLOS allows rare exceptions to address legal and ethical concerns. See the PLOS Data Policy and FAQ for detailed information. A Data Availability Statement describing where the data can be found is required at submission. Your answers to this question constitute the Data Availability Statement and **will be published in the article**, if accepted. **Important:** Stating 'data available on request from the author' is not sufficient. If your data are only available upon request, select 'No' for the first question and explain your exceptional situation in the text box. Do the authors confirm that all data underlying the findings described in their manuscript are fully available without restriction?	Yes - all data are fully available without restriction
Describe where the data may be found in full sentences. If you are copying our sample text, replace any instances of XXX with the appropriate details. • If the data are **held or will be held in a public repository**, include URLs, accession numbers or DOIs. If this information will only be available after acceptance, indicate this by ticking the box below. For example: *All XXX files are available from the XXX database (accession number(s) XXX, XXX.*). • If the data are all contained **within the manuscript and/or Supporting Information files**, enter the following: *All relevant data are within the manuscript and its Supporting Information files.* • If neither of these applies but you are able to provide **details of access elsewhere**, with or without limitations, please do so. For example:	The dataset can be found in this link: 10.6084/m9.figshare.19824445

Data cannot be shared publicly because of [XXX]. Data are available from the XXX Institutional Data Access / Ethics Committee (contact via XXX) for researchers who meet the criteria for access to confidential data. *The data underlying the results presented in the study are available from (include the name of the third party and contact information or URL).* • This text is appropriate if the data are owned by a third party and authors do not have permission to share the data. * typeset	
Additional data availability information:	Tick here if the URLs/accession numbers/DOIs will be available only after acceptance of the manuscript for publication so that we can ensure their inclusion before publication.

The Editor,
Plos One
23 May 2022

Dear Editor,
Wastewater-based epidemiology (WBE) surveillance as an early warning system (EWS) for monitoring community transmission of SARS-CoV-2 in low- and middle-income country (LMIC) settings, where diagnostic testing capacity is limited, needs further exploration. We explored the feasibility to run a WBE in Indonesia, one of the global epicenters of the COVID-19 pandemic in the middle of 2021, with the fourth largest population in the world where sewer and non-sewered sewage systems are implemented. The feasibility and resource capacity to collect samples on a weekly or fortnightly basis with grab and/or passive sampling methods, as well as to conduct qualitative and quantitative identification of SARS-CoV-2 Ribonucleic acid (RNA) using the real-time RT-PCR (RT-qPCR) in the environmental samples were explored. We collected samples in the Delta wave of the COVID-19 pandemic in Indonesia in July 2021. From a 10-week period, 54% (296/544) of wastewater and environmental samples were positive for SARS-CoV-2 RNA. The proportion of sample positivity rate decreased in proportion with the reported incidence of COVID-19 clinical cases in the community. The highest positivity rate of 77%, was obtained for samples collected in July 2021 and decreased to 25% by the end of September 2021.
We found that WBE surveillance system for SARS-CoV-2 in Indonesia is feasible to conduct for monitoring community burden of infections.
Given your journal's wide-ranging audience and commitment to publishing work relevant to Global Health we would be pleased if you would consider this paper for publication in the Plos One.
All authors have seen and approved the manuscript, contributed significantly to the work, and the manuscript has not been previously published nor is not being considered for publication elsewhere.

We kindly request two corresponding authors as listed below:

Indah K Murni
Address: Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. Jalan Kesehatan no 1, Sekip, Yogyakarta, 55284, Indonesia
Telephone number: +62274 555455
Email: indah.kartika.m@ugm.ac.id

Vicka Oktaria
Address: Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada. Jalan Kesehatan no 1, Sekip, Yogyakarta, 55284, Indonesia
Telephone number: +62274 555455
Email: vicka.oktaria@ugm.ac.id

On behalf of the authors,

Indah K Murni

14 August 2022

Re: Resubmission to PLOS ONE [PONE-D-22-14874]

Dear Dr. Chenette,

Thank you for the opportunity to revise and resubmit our manuscript "The feasibility of SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia" for your consideration. We have provided responses to the reviewers and believe that the manuscript has been strengthened as a result.

Please find attached:

- Response to reviewers
- Revised manuscript with tracked changes
- A clean version of the manuscript, labeled as 'Manuscript'

As pointed out by the academic editor, Professor Etsuro Ito, we would like to make changes to our financial disclosure into the following:

"This Project was funded by the Global Innovation Fund and PATH (PATH.org). The Global Investment Fund had no involvement in study design, data collection or analysis and PATH participated in study design and reviewing the draft manuscript, but had no role in data collection or analysis, writing of the manuscript or the decision to submit it for publication."

We look forward to your response.

Please address all correspondence concerning this manuscript to me at indah.kartika.m@ugm.ac.id or my colleague dr. Vicka Oktaria, MHP, PhD at vicka.oktaria@ugm.ac.id and do not hesitate to contact us should you have any queries.

Yours sincerely,

dr. Indah Kartika Murni, M.Kes., PhD., Sp.A(K)

Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Indonesia

Email: indah.kartika.m@ugm.ac.id

The feasibility of SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia

Indah K Murni[1,2*], Vicka Oktaria[1,3*], Amanda Handley[4,5], David T McCarthy[6], Celeste M Donato[4,10], Titik Nuryastuti[7], Endah Supriyati[8], Dwi Astuti Dharma Putri[1], Hendri Marinda Sari[1], Ida Safitri Laksono[1,2], Jarir At Thobari[1], Julie E Bines[4,9,10]

[1] Center for Child Health – Pediatric Research Office, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

[2] Child Health Department, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

[3] Department of Biostatistics, Epidemiology and Population Health, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

[4] Enteric Diseases Group, Murdoch Children's Research Institute, Parkville, Victoria, Australia

[5] Medicines Development for Global Health, Southbank, Victoria Australia

[6] Environmental and Public Health Microbiology Lab (EPHM Lab), Department of Civil Engineering, Monash University, Clayton, Victoria Australia

[7] Department of Microbiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

[8] Center for Tropical Medicine, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

[9] Department of Gastroenterology and Clinical Nutrition, Royal Children's Hospital Melbourne, Victoria, Australia

[10] Department of Paediatrics, The University of Melbourne, Parkville, Australia

[*]Corresponding Author

E-mail: indah.kartika.m@ugm.ac.id (IM)

E-mail: vicka.oktaria@ugm.ac.id (VO)

Abstract

Background

Wastewater-based epidemiology (WBE) surveillance as an early warning system (EWS) for monitoring community transmission of SARS-CoV-2 in low- and middle-income country (LMIC) settings, where diagnostic testing capacity is limited, needs further exploration. We explored the feasibility to conduct a WBE surveillance in Indonesia, one of the global epicenters of the COVID-19 pandemic in the middle of 2021, with the fourth largest population in the world where sewer and non-sewered sewage systems are implemented. The feasibility and resource capacity to collect samples on a weekly or fortnightly basis with grab and/or passive sampling methods, as well as to conduct qualitative and quantitative identification of SARS-CoV-2 ribonucleic acid (RNA) using real-time RT-PCR (RT-qPCR) testing of environmental samples were explored.

Materials and methods

We initiated a routine surveillance of wastewater and environmental sampling at three predetermined districts in Special Region of Yogyakarta Province. Water samples were collected from central and community wastewater treatment plants (WWTPs), including manholes flowing to the central WWTP, and additional soil samples were collected for the near source tracking (NST) locations (i.e., public spaces where people congregate).

Results

We began collecting samples in the Delta wave of the COVID-19 pandemic in Indonesia in July 2021. From a 10-week period, 54% (296/544) of wastewater and environmental samples were

positive for SARS-CoV-2 RNA. The sample positivity rate decreased in proportion with the reported incidence of COVID-19 clinical cases in the community. The highest positivity rate of 77% in week 1, was obtained for samples collected in July 2021 and decreased to 25% in week 10 by the end of September 2021.

Conclusion

A WBE surveillance system for SARS-CoV-2 in Indonesia is feasible to monitor the community burden of infections. Future studies testing the potential of WBE and EWS for signaling early outbreaks of SARS-CoV-2 transmissions in this setting are required.

Keywords

Wastewater-based epidemiology surveillance, environmental sampling, SARS-CoV-2, COVID-19, Indonesia.

Introduction

Understanding the full extent of the Coronavirus Disease (COVID-19) pandemic is a major public health challenge. Traditional epidemiological indicators which are based on the number of confirmed clinical cases and deaths due to COVID-19 disease have potential biases and limitations. The capacity for timely diagnosis using laboratory tests may be limited, particularly in low- and middle- income countries (LMICs) during epidemic wave. Incidence rates based on hospitalization data lag behind the incidence of infection in the community and lack of representativeness for identification of cases who do not access care, have non-serious illness, or are asymptomatic.

People infected with SARS-CoV-2 shed the virus in stool independently of gastrointestinal symptoms and therefore viral ribonucleic acid (RNA) can be detected in environmental wastewater, containing excreta from infected people and sewerage treatment plants.[1–4] Public health surveillance using wastewater is now well established and has been used to monitor communities for the presence of poliovirus, antimicrobial resistant enteric bacteria, and drugs of abuse, e.g. opioids.[5–7] It has been postulated that routine monitoring for the presence of SARS-CoV-2 in wastewater may be useful in detecting an existing or predicting a new potential epidemic.[6,8]

Studies reporting the detection of SARS-CoV-2 RNA in wastewater have been predominantly limited to high-income countries such as Australia, the United States, Japan and a number of European countries. To date, only a few studies have detected the genetic material of SARS-CoV-2 in wastewater from LMICs, including studies from Argentina, Brazil, Ecuador, India, Pakistan, and South Africa.[9–20] The lack of formal sewerage systems in LMICs, particularly in impoverished areas and informal settlements, has posed a major challenge for SARS-CoV-2 surveillance using wastewater. It is also in these communities where epidemiological surveillance using rates based on disease case capture and death are problematic. The adaptation of environmental surveillance methods suitable for use in LMICs provides an opportunity to monitor community transmission and inform the public response to SARS-CoV-2 and other future pandemic infections.

This short communication describes the assessment of the feasibility of conducting SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia. The aim was to provide a proof of concept for the use of wastewater and environmental surveillance to monitor the community burden of SARS-CoV-2 infection in Indonesia.

Materials and methods

General information on wastewater systems and challenges in Indonesia

In Indonesia, a high proportion of the population is not connected to a sewerage system. In the capital city of Jakarta, a city with a population of over 10 million, it is estimated that only 2% of households are connected to a reticulated sewerage system, with >95% of wastewater leaking into agricultural fields, rivers, and other groundwater sources.[21]

We established the first Indonesian wastewater-based SARS-CoV-2 epidemiology surveillance program in Special Region of Yogyakarta province, one of the regions with the highest number of COVID-19 cases during the Delta wave. In the Special Region of Yogyakarta province, only 25,294 households (6% population serviced) are connected to a formal reticulated sewerage system. There are two types of wastewater treatment plants (WWTPs) systems in operation in the province: (a) the central WWTP (*Instalasi Pengolahan Air Limbah Sewon/IPAL* Sewon, Bantul) managed by the provincial government and (b) community WWTPs (IPAL community) that are independently managed by each local community, in addition to individual septic tanks. The service coverage of IPAL Sewon in the Special Region of Yogyakarta province includes 13 of the 14 sub-districts in the Yogyakarta city, 4 of the 17 sub-districts in the Sleman district and 3 of the 17 sub-districts in the Bantul district. Community WWTPs are used in some suburban areas due to the lack of capacity of the central WWTPs to service their needs and the terrain of the region that does not allow passive gravitational flow.

SARS-CoV-2 surveillance on wastewater and environmental sampling in Indonesia (SWESP study)

Routine wastewater-based epidemiology (WBE) surveillance (i.e., testing of sewerage and wastewater sites, and waterways) and testing of soil was initiated in three of five districts in the Special Region of Yogyakarta province (Yogyakarta city, Sleman and Bantul districts, **Fig 1**). Two districts were not included due to practical challenges, such as the geography and relatively sparse population. Identification and mapping of the infrastructure of the wastewater system (formal and informal) at provincial and district level was conducted prior to commencing the study. We selected six sub-districts from Yogyakarta city as these areas have the highest coverage of the formal central wastewater system and samples may be considered more representative to the broader community, two from Sleman district, and the remaining two from Bantul district. Within the total of ten sub-districts, we also selected 12 clustered communities that were served by small community WWTPs. Each community WWTP served between 50-150 households.

Fig 1. Flowchart of sample strategy. We selected ten sub-districts from three out of five districts in Special region of Yogyakarta Province (Yogyakarta city, Bantul, and Sleman districts). Samples from three sub-districts were taken weekly (identified by blue arrows), while others were taken fortnightly. Detailed type and number of samples in each sub-district are illustrated in the figure.

We collected samples using either the grab or passive sampling methods. Wastewater from manholes was collected by immersing a ~500 mL bottle into the water to a depth of around 20 – 30 cm until the bottle was filled, allowing about 1 cm of air. Recreational water was collected using a 2 L bottle using a similar grab method. Bottles were pre-labelled with sample specific barcodes. A torpedo-style passive sampler with multiple entry points (front, top, sides, and bottom) [22,23] was used to collect samples from septic tanks, rivers, and the central and community WWTPs. Passive samplers were retrieved 24 hours after deployment. Soil samples (20 g) were collected using zip lock bags. Within four hours of collection, samples were transferred on ice at 2-8°C [24] to the Microbiology laboratory at the Universitas Gadjah Mada Special Region of Yogyakarta, Indonesia.

Laboratory methods for wastewater and environmental samples

The wastewater samples, passive samplers and soil samples were stored in the 4°C fridge upon arrival until the sample processing. Samples of wastewater (50 mL) or recreational water (1000 mL) were filtered through a 47 mm diameter, 0.45 µm pore size, cellulose nitrate high flow electronegative membrane (Sartorius, Germany). This filtration process was performed immediately (<2 hours) once the samples were received at the laboratory. The collection bag containing the soil samples was thoroughly mixed. In a 2 mL tube, 0.25 grams of soil and 2 mL of DNA/RNA Shield solutions (Zymo Research, USA) were added. The passive samplers were opened, and the filter membrane and q-tips were collected.

All of the processed samples from wastewater samples, passive samplers and soil samples were stored at -80°C until the RNA extraction and reverse-transcription quantitative real-time PCR (RT-qPCR) analysis.

The RNA was extracted from samples using the QIAGEN RNeasy PowerMicrobiome Kit (QIAGEN, Germany) following manufacturer's instructions with the exception of replacing the supplied beads with PowerBead Tubes-Garnet beads (QIAGEN, Germany). For every batch of samples processed, a negative extraction controls and internal control (MS2 bacteriophage) as supplied in the PerkinElmer SARS-CoV-2 Nucleic Acid Detection Kit (RUO) (PerkinElmer) were included in the RNA extraction process to monitor the RNA extraction performance.

To detect the SARS-Cov2 RNA, a RT-qPCR was conducted using the SARS-CoV-2 Real-time RT-PCR Assay (PerkinElmer, US) and synthetic SARS-CoV-2 RNA Control 1-MT007544.1 (Twist Bioscience, Australia) as the standard curve. The kit is a multiplex assay using primers and probes targeting the Nucleocapsid (N) gene and open reading frame 1ab (ORF1ab) region of SARS-CoV-2. RT-qPCR assays were performed using two replicates of 5 μL RNA template, with a total reaction volume of 30 μL and a total 45 cycles of amplification. The quantification of the samples was calculated using the synthetic SARS-CoV-2 RNA Control 1-MT007544.1 (Twist Bioscience, Australia) as a standard curve, according to the manufacturer's instruction. The RT-qPCR assay was performed as described by the manufacturer's instruction using the LightCycler 96 instrument (Roche, Germany).

In order to report the actual value of SARS-CoV-2 RNA, we calculated the recovery efficiency. In each qPCR run, multiple SARS-CoV-2 RNA controls, a MS2 phage control (to determine the RNA recovery efficiency and as internal control) of different known concentrations and a negative control were included.

The limit of detection (LOD) for the RT-qPCR assay was determined by the analysis of 10 replicates for each dilution of the synthetic SARS-CoV-2 RNA Control 1-MT007544.1 (Twist Bioscience, Australia) analyzed and was defined as the lowest number of copies of the N gene target and ORF1ab gene that could be detected in 80% of the replicates tested. The LOD was expressed as the lowest detectable concentration of the N gene target and ORF1ab gene in sample based on the equivalent volume of sample analyzed in each RT-qPCR assay, not adjusting for any potential loss through the processing of the sample or any potential inhibition of the RT-qPCR assay.[25] All assays were performed at Microbiology laboratory at the Universitas Gadjah Mada,, Special Region of Yogyakarta, Indonesia.

Ethics

The SWESP study obtained ethics approval from the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada DR. Sardjito General Hospital, Indonesia (KE/FK/0426/EC/2021, KE/FK/0514/EC/2022). Written or verbal consent was not applicable for this study as we did not collect data from individual participants.

Results

Feasibility of WBE surveillance

The average time from sample collection to availability of the RT-qPCR results was a mean of 64 hours, including the filtration time (3 to 4 hours), RNA extraction (2 to 3 hours), and RT-qPCR quantification analysis (3 hours). Both weekly and fortnightly sample collections were practical to conduct. A key challenge was the delay in the importation of critical reagents and consumables exacerbated by the COVID-19 pandemic. As the UGM laboratory is also the central clinical laboratory, priority for the analysis of clinical samples resulted in a delay in wastewater analysis during major clinical peaks in incidence. Initial trials of deployment of the passive samplers were required to limit damage or loss due to difficulties with positioning and securing samplers. We defined criteria for reliable deployment that considered locations with solid ground to safely access, ideally in an inconspicuous position, and using a strong pole or tree to secure the sampler. To avoid samplers being removed we labeled samplers with signs of warning and explanation such as "Sample for Research by Universitas Gadjah Mada and Yogyakarta Government".

The detection and positivity rates of SARS-CoV-2 RNA

Sample collection commenced on the 27th of July 2021 during the Delta wave of the COVID-19 pandemic in Indonesia. During the 10-week sampling period, a total of 544 samples were collected with 54% (296/544) of all samples testing positive for SARS-CoV-2 RNA. The median of cycle threshold (Ct) values for positive N and ORF1ab gene results was 35.1 (IQR: 32.1 – 36.9) and 33.9 (IQR: 30.1 – 35.9), respectively. The highest positivity rate was for manhole samples (74%, 191/258 samples, **Fig 2**) and the lowest was for soil samples (3%, 2/60 samples, **Fig 2**). The temporal changes in rates of sample positivity correlate with the number of confirmed cases in the community as illustrated in **Fig 3**. The highest positivity rate of 77%, was obtained for samples collected in July 2021 during week 1 of sample collection and decreased to 25% by the end of September 2021 (corresponding to week 10 of sample collection), reflecting a decreased detection rate correlating with a decrease in the incidence of reported COVID-19 clinical cases in the community.

Fig 2. Nucleocapsid (N) gene positivity by sample types.

Fig 3. Distribution maps of SARS-CoV-2 in Special Region of Yogyakarta province, comparing detection targeting N gene to community confirmed cases. (A) In week 1-2 of the sample collection. (B) In week 5-6 of the sample collection. (C) In week 9-10 of the sample collection. Community COVID-19 confirmed cases were represented by blue color, the lighter the fewer cases. Detected cases in sampling locations were represented by red colored dots/triangles/pentagons, while non-detected cases were represented by green colored dots/triangles/pentagons. With circles denoting manholes, pentagons denoting river and triangles denoting NST water.

The N gene was identified in 74% (191/258) of sewage samples (grab method), 64% (67/104) of near source tracking (NST) water samples (passive sampling method), 50% (25/50) of river samples (grab method), and 3% (2/60) of NST soil samples. This finding was consistent with the ORF1ab gene target but with a higher proportion of soils samples being positive (8%, 5/60) for the ORFlab gene as compared to the N gene (3%).

Discussion

We successfully demonstrated that WBE surveillance for SARS-CoV-2 RNA was feasible in Indonesia and reflected the SARS-CoV-2 clinical burden in the community. The high level of positivity of SARS-CoV-2 RNA in the environment in Indonesia suggests a considerable public health burden and may represent asymptomatic or mild cases that did not access health facilities for testing. Manholes consistently showed higher positivity rates in comparison with river and soil samples. Although river and soil samples showed lower positivity rates, the data are useful to complement the WBE surveillance data particularly in regions where connection to a formal sewerage system is limited. This combination of sampling strategies provides additional insights into the prevalence and distribution of COVID-19 within the community.

In Special Region of Yogyakarta province, many households are not connected to the IPAL Sewon. This may be because they were built after the IPAL Sewon infrastructure was established and therefore have no connection to the IPAL pipes. Other households were not connected due to technical reasons, such as in lower altitudes and terrain that does not support passive gravitational flow of wastewater to the central WWTP. However, in this study we managed to collect samples from community WWTPs and septic tanks from NST sites to capture communities that were not served by the central WWTP.

Although we found that both weekly or fortnightly collection frequency with grab and/or passive sampling collection methods as feasible, weekly collections were preferred in order to provide real-time data to inform the public health response. The laboratory capacity to conduct qualitative (positive/negative) and quantitative identification of SARS-CoV-2 RNA in the environmental samples (wastewater and soil) were also feasible although some pre-processing procedures need to be conducted prior to the RT-qPCR procedure (i.e., wastewater filtration and soil homogenization). There were challenges in providing real-time results during peak COVID-19 outbreaks due to overburdened staff and limited access to equipment, and therefore, ideally WBE surveillance should be integrated into the routine surveillance programs with dedicated staff. Additionally, the availability of imported reagents has delayed laboratory analysis during periods of high output. Local epidemiological data describing the distribution of COVID-19 cases (symptomatic and asymptomatic) with laboratory confirmed positive tests for SARS-CoV-2 infections by sub district, on a weekly basis, were available to compare with the findings from

WBE surveillance. However, data analysis to link environmental and community data remains challenging and needs further exploration.

Despite efforts, there remain practical limitations of WBE surveillance in LIMCs. It is likely that wastewater sampling of the reticulated sewerage system reflects the more modern and affluent sector of the city and may not provide meaningful insights into the presence of SARS-CoV-2 infection within the broader community. Most of the city and rural areas manage human effluent via septic tanks, pit latrines or by open defecation with subsequent contamination of surface water and rivers. Therefore, to understand the distribution of SARS-CoV-2 RNA in environments that reflect the presence of community infections with fragmented wastewater infrastructures, NST sites, and in places where people publicly congregate were selected. These sites include permanent dwellings (apartment and flats), temporary living places (hotels), public spaces (traditional markets, town squares, mosques, and a public swimming pool), rivers, working spaces (both office and factory), and COVID-19 shelters (facilities which are designated as temporary quarantine shelters for people testing positive for COVID-19). This WBE approaches using NST may allow detection of targeted clusters for whom rapid action may reduce or prevent the risk of larger outbreaks within the community.[26]

It has been proposed that WBE surveillance has the potential to act as an early warning system (EWS) for COVID-19 outbreaks.[27–32] This should be conducted in collaboration with the public health authorities to enable the timely follow up of positive detections by strategies such as contact tracing, strengthening health protocols, or implementing a community lockdown. This could be broadly implemented across the community or in a targeted response depending on the local context and level of concern. For instance, if SARS-CoV-2 RNA is detected (positive result) in the sewerage sample in an area where there had consistently been no detections (negative result), then a lockdown or mass screening could be implemented in the area drained by the sewerage system; or if the result is taken from a closed community (e.g., Boarding school), contact tracing within the community should be conducted immediately.

Conclusions

In conclusion, an environmental surveillance system for SARS-CoV-2 in Indonesia is feasible and can be used to monitor the community burden of SARS-CoV-2 infection. However, future research is needed to explore its potential to act as an EWS for the early identification of SARS-CoV-2 outbreaks within a community, especially in regions with limited access to clinical testing. Although the sewer infrastructure of wastewater systems is quite limited in Indonesia, an expanded sampling approach based on the local context and including NST can support an effective SARS-COV-2 surveillance program.

Acknowledgements

We would like to thank the wastewater treatment plant team, field assistants and laboratory team for doing sampling collection and laboratory works. We thank Rizka Dinari for providing editorial assistance.

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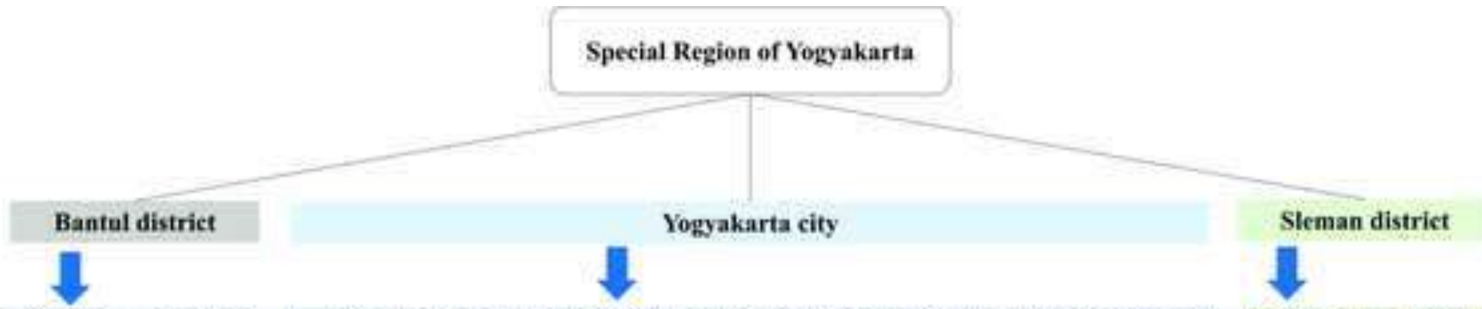
Figure 1



	Sewon	Kasihan	Jetis	Wirobrajan	Danurejan	Mantrijeron	Kraton	Gondokusuman	Mlati	Depok
Total Area (km^2)	27.16	32.38	1.70	1.76	1.10	2.61	1.4	3.99	28.52	35.55
Total Population (people)	109,370	115,050	23,385	24,739	18,670	33,340	17,943	36,921	100,524	131,005
Population Density (per km^2)	3,693	3,197	15,960	15,834	19,395	13,576	15,594	10,731	3,229	3,440
Incidence Rate*	1.83	0.87	0	4.04	0	6.00	11.15	10.83	0	3.82
Manhole (Grab method)	4	4	5	4	4	4	4	4	4	3
NST Water (Passive & grab method)	2	2	1	2	2	2	1	-	2	4
NST Soil (Grab method)	-	2	-	1	1	1	2	-	-	1
River (Passive & Grab method)	2	4	2	-	2	-	-	-	-	2
Central WWTP Sewon (Passive)	4	-	-	-	-	-	-	-	-	-
Community WWTP (Passive)	4	3	-	-	-	-	-	2	1	4
Total Sampling Location	**16**	**15**	**8**	**7**	**9**	**7**	**7**	**6**	**7**	**14**

Figure 2

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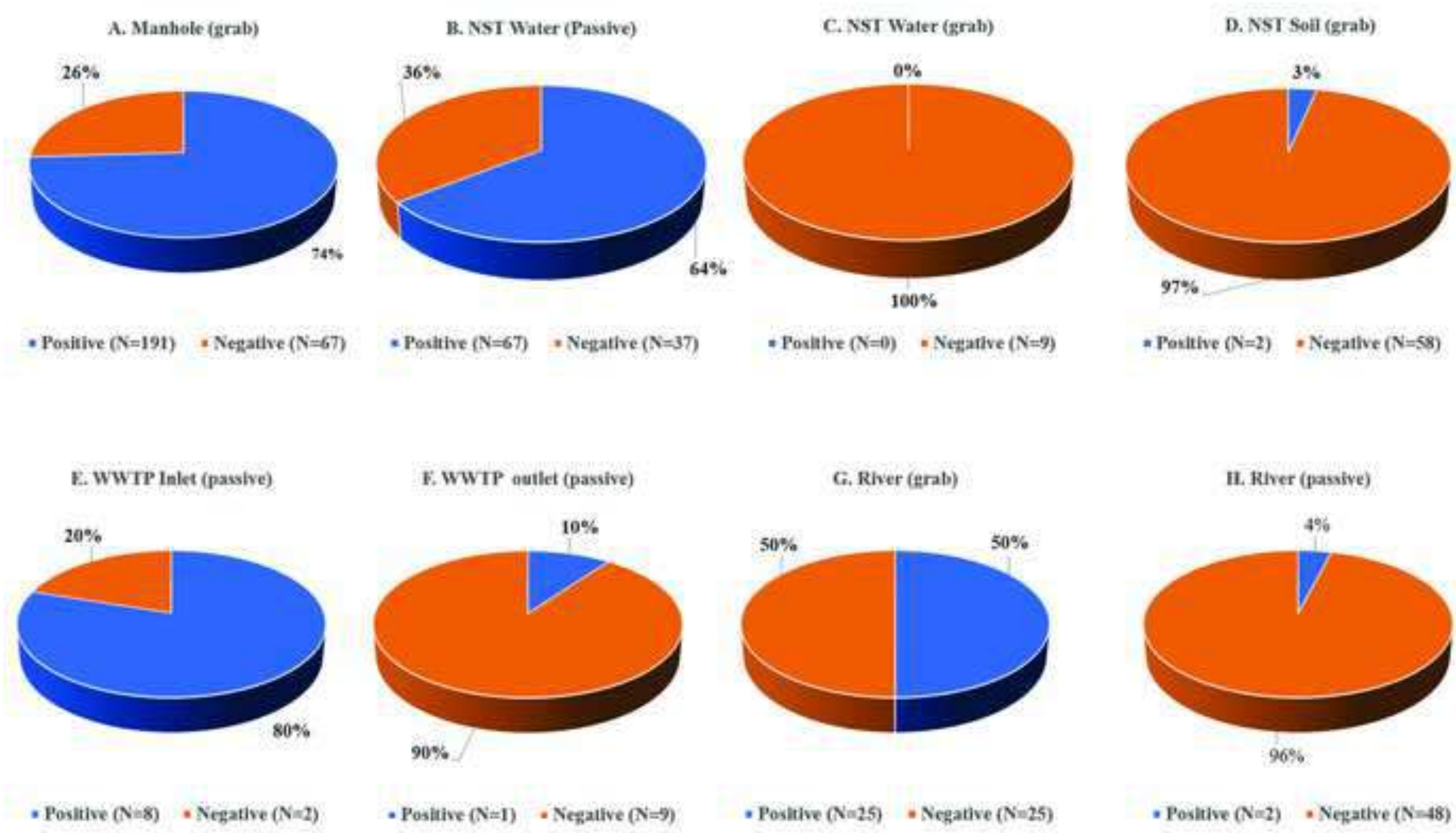
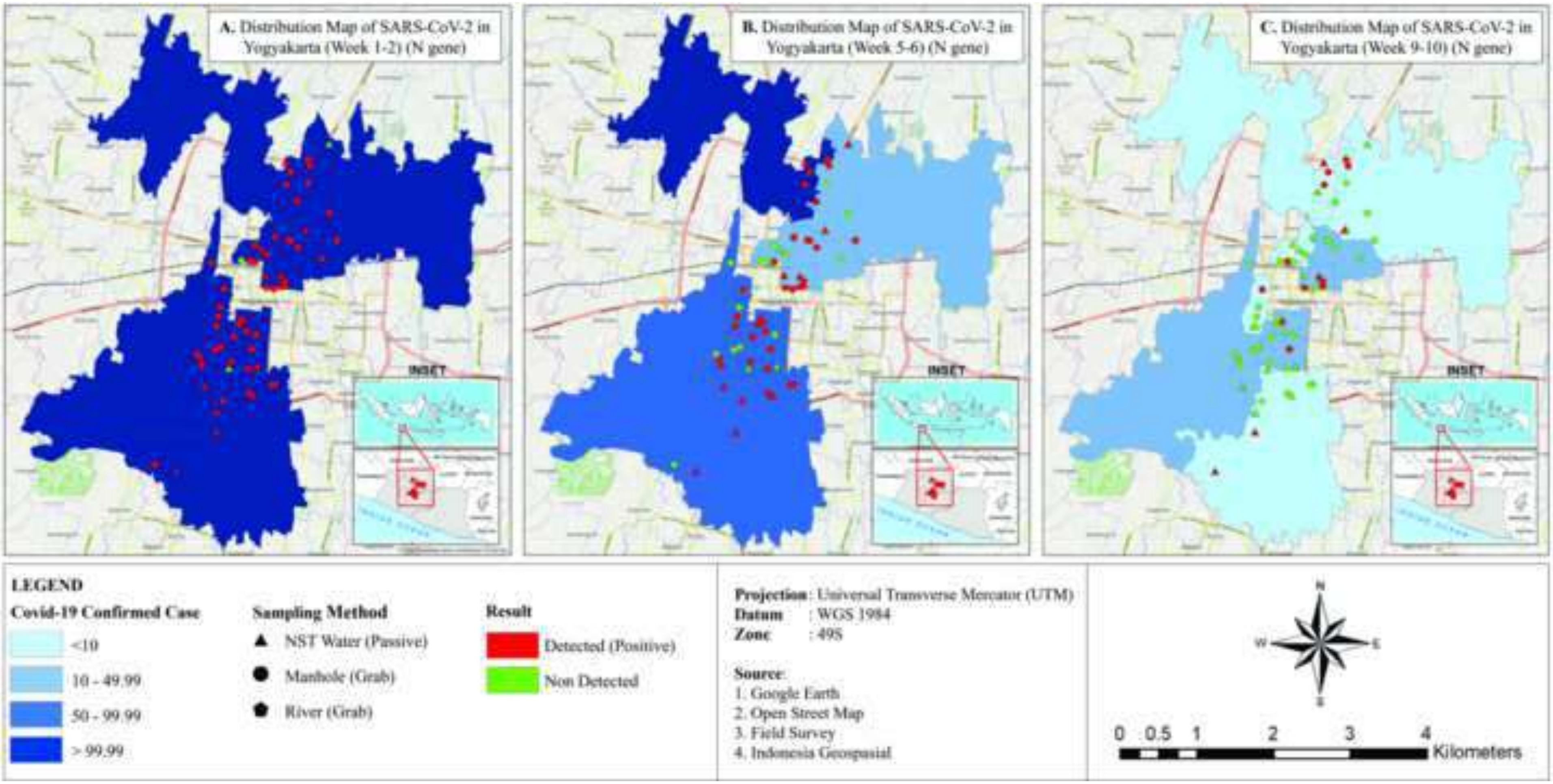


Figure 3



A. Distribution Map of SARS-CoV-2 in Yogyakarta (Week 1-2) (N gene)
B. Distribution Map of SARS-CoV-2 in Yogyakarta (Week 5-6) (N gene)
C. Distribution Map of SARS-CoV-2 in Yogyakarta (Week 9-10) (N gene)
INSET
LEGEND
Covid-19 Confirmed Case
<10
10 - 49.99
50 - 99.99
> 99.99
Sampling Method
NST Water (Passive)
Manhole (Grab)
River (Grab)
Result
Detected (Positive)
Non Detected
Projection : Universal Transverse Mercator (UTM)
Datum : WGS 1984
Zone : 49S
Source:
1. Google Earth
2. Open Street Map
3. Field Survey
4. Indonesia Geospasial
N
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0 0.5 1 2 3 4
Kilometers

The feasibility of SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia

~~Authors~~

Indah K Murni~~*~~[1,2]*, Vicka Oktaria~~*~~[1,3]*, Amanda Handley[4,5], David T McCarthy[6], Celeste M Donato[4,10], Titik Nuryastuti[7], Endah Supriyati[8], Dwi Astuti Dharma Putri[1], Hendri Marinda Sari[1], Ida Safitri Laksono[1,2], Jarir At Thobari[1], Julie E Bines[4,9,10]

[1] ~~Affiliations~~

~~1.~~ Center for Child Health – Pediatric Research Office, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

[2] ~~2.~~ Child Health Department, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

[3] ~~3.~~ Department of Biostatistics, Epidemiology and Population Health, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

[4] ~~4.~~ Enteric Diseases Group, Murdoch Children's Research Institute, Parkville, Victoria, Australia

[5] ~~5.~~ Medicines Development for Global Health, Southbank, Victoria Australia

[6] ~~6.~~ Environmental and Public Health Microbiology Lab (EPHM Lab), Department of Civil Engineering, Monash University, Clayton, Victoria Australia

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[7] 7. Department of Microbiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

[8] 8. Center for Tropical Medicine, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

[9] 9. Department of Gastroenterology and Clinical Nutrition, Royal Children's Hospital Melbourne, Victoria, Australia

[10] 10. Department of Paediatrics, The University of Melbourne, Parkville, Australia

[*]Corresponding Author

Indah K Murni

Address: Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada.

Jalan Kesehatan no 1, Sekip, Yogyakarta, 55284, Indonesia

Telephone number: +62274 555455

E-mail: indah.kartika.m@ugm.ac.id (IM)

Vicka Oktaria

Address: Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada.

Jalan Kesehatan no 1, Sekip, Yogyakarta, 55284, Indonesia

Telephone number: +62274 555455

E-mail: vicka.oktaria@ugm.ac.id (VO)

~~The feasibility of SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia~~

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Abstract

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Background

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Wastewater-based epidemiology (WBE) surveillance as an early warning system (EWS) for monitoring community transmission of SARS-CoV-2 in low- and middle-income country (LMIC) settings, where diagnostic testing capacity is limited, needs further exploration. We explored the feasibility to ~~run~~ conduct a WBE surveillance in Indonesia, one of the global epicenters of the COVID-19 pandemic in the middle of 2021, with the fourth largest population in the world where sewer and non-sewered sewage systems are implemented. The feasibility and resource capacity to collect samples on a weekly or fortnightly basis with grab and/or passive sampling methods, as well as to conduct qualitative and quantitative identification of SARS-CoV-2 r~~R~~ibonucleic acid (RNA) using ~~the~~ real-time RT-PCR (RT-qPCR) testing of ~~in the~~ environmental samples were explored.

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Materials and m~~M~~ethods

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We initiated a routine surveillance of wastewater and environmental sampling at three predetermined districts in Special Region of Yogyakarta Province. Water samples were collected from central and community wastewater treatment plants (WWTPs), including manholes flowing to the central WWTP, and additional soil samples were collected for the near source tracking (NST) locations (i.e., public spaces where people congregate).

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Results

We began collecting samples in the Delta wave of the COVID-19 pandemic in Indonesia in July 2021. From a 10-week period, 54% (296/544) of wastewater and environmental samples were positive for SARS-CoV-2 RNA. The ~~proportion of~~ sample positivity rate decreased in proportion with the reported incidence of COVID-19 clinical cases in the community. The highest positivity rate of 77% in week 1, was obtained for samples collected in July 2021 and decreased to 25% in week 10 by the end of September 2021.

Conclusion

A WBE surveillance system for SARS-CoV-2 in Indonesia is feasible to ~~conduct for~~ monitor the~~ing~~ community burden of infections. Future studies testing the potential of WBE and EWS for signaling early outbreaks of SARS-CoV-2 transmissions in thise setting are required.

Keywords

-W~~w~~astewater-based epidemiology surveillance, environmental sampling, SARS-CoV-2, COVID-19, Indonesia.

Introduction

Understanding the full extent of the Coronavirus Disease (COVID-19) pandemic is a major public health challenge. Traditional epidemiological indicators which are based on the number of confirmed clinical cases and deaths due to COVID-19 disease have potential biases and limitations. The capacity for timely diagnosis using laboratory tests may be limited, particularly

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in low- and middle- income countries (LMICs) during ~~the high seasons~~epidemic wave. Incidence rates based on hospitalization data lag behind the incidence of infection in the community and lack of representativeness for identification of cases who do not access care, have non-serious illness, or are asymptomatic.

People infected with SARS-CoV-2 shed the virus in stool independently of gastrointestinal symptoms and therefore viral ribonucleic acid (RNA) can be detected in environmental wastewater, containing excreta ~~of~~ from infected people and sewerage treatment plants.[~~(~~1–4]~~)~~ ~~1-4~~ Public health surveillance using wastewater is now well established and has been used to monitor communities for the presence of poliovirus, antimicrobial resistant enteric bacteria, and drugs of abuse, e.g. opioids.[~~(~~5–7]~~)~~ ~~5-7~~ It has been postulated that routine monitoring for the presence of SARS-CoV-2 in wastewater may be useful in ~~predicting~~ detecting an existing or predicting a new potential epidemic.[~~(~~6,8~~)~~ ~~6,~~][8]

Studies reporting the detection of SARS-CoV-2 RNA in wastewater have been predominantly limited to high-income countries such as Australia, the United States, Japan and a number of European countries~~,~~. To date, only a few studies have detected the genetic material of SARS-CoV-2 in wastewater from ~~low and middle income countries (~~LMICs~~)~~, including studies from Argentina, Brazil, Ecuador, India, Pakistan, and South Africa.[~~(~~9–20]~~)~~ ~~9-20~~ The lack of formal sewerage systems in LMICs, particularly in impoverished areas and informal settlements, has posed a major challenge for SARS-CoV-2 surveillance using wastewater. It is also in these communities where epidemiological surveillance using rates based on disease case capture and death are problematic. The adaptation of environmental surveillance methods suitable for use in L~~I~~MICs provides an opportunity to monitor community transmission and inform the public response to SARS-CoV-2~~-~~ and other future pandemic infections.

This short communication describes the assessment of the feasibility of conducting SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia. The aim was to provide a proof of concept for the use of wastewater and environmental surveillance- to monitor the community burden of SARS-CoV-2 infection in Indonesia.

Materials and mMethods

General information on wastewater systems and challenges in Indonesia

In Indonesia, a high proportion of the population is not connected to a sewerage system. In the capital city of Jakarta, a city with a population of over 10 million, it is estimated that only 2% of households are connected to a reticulated sewerage system, with >95% of wastewater leaking into agricultural fields, rivers, and other groundwater sources.[(21])[21]

We established the first Indonesian wastewater-based SARS-CoV-2 epidemiology (WBE) surveillance program in Special Region of Yogyakarta province, one of the locations regions with the highest number of COVID-19 cases during the COVID-19 Delta wave outbreak. In the Special Region of Yogyakarta province, only 25,294 households (6% population serviced) are connected to a formal reticulated sewerage system. There are two types of wastewater treatment plants (WWTPs) systems in operation in the province: (a) the central WWTP (*Instalasi Pengolahan Air Limbah Sewon/IPAL* Sewon, Bantul) managed by the provincial government and (b) community WWTPs (IPAL community) that are independently managed by each local community, in addition to individual septic tanks. The service coverage of IPAL Sewon in the Special Region of Yogyakarta province includes 13 of the 14 sub--districts in the Kota

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Yogyakarta ~~regency~~city, 4 of the 17 sub~~-~~districts in the Sleman ~~regency~~ district and 3 of the 17 sub~~-~~districts in the Bantul ~~regency~~district. Community WWTPs are used in some suburban areas due to the lack of capacity of the central WWTPs to service their needs and the ~~geography~~ terrain of the region that does not allow passive gravitational flow.

SARS-CoV-2 surveillance on wastewater and environmental sampling in Indonesia (SWESP study)

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Routine wastewater-based epidemiology (WBE) surveillance (i.e., testing of sewerage and wastewater sites, and waterways) and testing of soil was initiated in three of ~~four~~ five districts in the Special Region of Yogyakarta province (~~Kota~~ Yogyakarta city, Sleman and Bantul districts, **Fig~~ure~~ 1**). ~~One~~ Two districts ~~was~~ were not included due~~o~~ to practical challenges, such as ~~due to~~ the geography and relatively sparse population. ~~-~~Identification and mapping of the infrastructure of the wastewater system (formal and informal) at provincial and district level was conducted prior to commencing the study, W~~Of the total of ten sites, w~~e selected six sub-districts from Yogyakarta city as these areas have the highest coverage of the formal central wastewater system and samples may be considered more representative to the broader community, two from Sleman district, and the remaining two from Bantul district. Within the total of ten sub-districts, w~~W~~e also selected 12 clustered communities that were served by small community WWTPs. Each community WWTP served between 50-150 households.

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Fig 1. Flowchart of sample strategy.

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We selected ten sub-districts from three out of five districts in Special region of Yogyakarta Province (Yogyakarta city, Bantul, and Sleman districts). Samples from three sub--districts were taken weekly (as pointed identified by blue arrows), while others were taken fortnightly. Detailed type and number of samples in each sub--district are illustrated in the figure.

We collected samples using either the grab or passive sampling methods. Wastewater from manholes was collected by immersing a ~500 mLl bottle into the water to a depth of around 20 – 30 cm until the bottle was filled, allowing about 1 cm of air. -Recreational water was collected using a -2 L bottle using a similar grab method. -Bottles were pre-labelled with sample specific barcodes. A torpedo-style passive sampler with multiple entry points (front, top, sides, and bottom) [(22,23])[22,23] was used to collect samples from septic tanks, rivers, and the central and community WWTPs. Passive samplers were retrieved 24 hours after deployment. Soil samples (20 g) and fecal scats (whole pellet) were collected using zip lock bags. Within four hours of collection, -sSamples were transferred on ice at 2-8°C [(24]) within four hours to the Microbiology laboratory at the Universitas Gadjah Mada Special Region of Yogyakarta, Indonesia.(24)[24]

Laboratory methods for wastewater and environmental samples

The wastewater samples, torpedo passive samplers and soil samples were stored in the 4°C fridge upon arrival until the sample processing. -Samples of wastewater (50 mL) or recreational water (1000 mL) weare filtered through a 47 mm diameter, 0.45 µm pore size, cellulose nitrate high flow electronegative membrane (Sartorius, Germany). This filtration process was performed

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immediately (<2~~1~~ hours) once the samples were received at the laboratory.~~-~~ The collection bag containing the soil samples was thoroughly mixed. In a 2 mL tube, 0.25 grams of soil and 2 mL of DNA/RNA Shield solutions (Zymo Research, USA) were added. The ~~torpedo~~ passive samplers were opened, and the filter membrane and ~~cotton buds~~ q-tips were collected.

~~The whole filter paper was stored at -80°C until the RNA extraction and reverse-transcription quantitative real-time PCR (RT-qPCR) analysis. Soil samples~~ we~~are thoroughly mixed within the collection bag upon arrival in the laboratory. Approximately 0.25 grams of soil were~~ was ~~added to DNA/RNA Shield solutions (Zymo Research, USA) and stored at -80°C until the RNA extraction process. This filtration process was performed immediately (<1 hour) once the samples were received at the laboratory.~~ All of the ~~Th~~processed~~e whole filter paper~~samples from wastewater samples, ~~torpedo~~ passive samplers and soil samples were~~as~~ stored at -80°C until the RNA extraction and reverse-transcription quantitative real-time PCR (RT-qPCR) analysis.~~Additionally, the samples were stored for at least 24 hours after sample collection prior to RNA extraction, except for the last 319 samples that were delayed for approximately three months due to discontinued consumable supply.~~

The RNA was extracted from samples ~~were extracted~~ using the QIAGEN RNeasy PowerMicrobiome K~~k~~it (QIAGEN, Germany) following manufacturer's instructions with the exception of ~~by~~ replacing the supplied beads with PowerBead Tubes-Garnet beads (QIAGEN, Germany). For every batch of samples processed, a negative extraction controls and internal control (MS2 bacteriophage) as supplied in the PerkinElmer SARS-CoV-2 Nucleic Acid Detection Kit (RUO) (PerkinElmer) were included in the RNA extraction process to monitor the RNA extraction performance.

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To detect the SARS-Cov2 RNA, a ~~quantitative real-time~~ RT-qPCR was conducted using the SARS-CoV-2 Real-time RT-PCR Assay (PerkinElmer, US) and synthetic SARS-CoV-2 RNA Control~~s~~ 1-MT007544.1 (Twist Bioscience, ~~California~~Australia) as the standard curve. The kit is a multiplex assay using primers and probes targeting the Nucleocapsid (N) gene and open reading frame 1ab (ORF1ab) region of ~~the~~ SARS-CoV-2~~ genomes~~. ~~q~~RT-qPCR assays were performed using two replicates of 5 µL RNA template, with a total reaction volume of 30 µL and a total 45 cycles of amplification. The quantification of the samples was calculated using the ~~Twist~~ s~~S~~ynthetic SARS-CoV-~~-~~2 RNA Control 1-MT007544.1 (Twist Bioscience, Australia) ~~was supplied by the manufacturer~~as a standard curve, according to the manufacturer's instruction. The ~~q~~RT-qPCR assay was performed as described by the manufacturer's instruction using the LightCycler 96 instrument (Roche, Germany).

In order to report the “~~true~~actual” value of SARS-CoV-2 RNA, we calculated the recovery efficiency. In each qPCR run, multiple SARS-CoV-2 RNA controls, a MS2 phage control (to determine the RNA recovery efficiency and as internal control) of different known concentrations and a negative control were included.

The limit of detection (LOD) for the ~~q~~RT-qPCR assay was determined by the analysis of 10 replicates for each dilution of the ~~Twist~~ s~~S~~ynthetic SARS-CoV-2 RNA Control 1-MT007544.1 (Twist Bioscience, Australia) analyzed and was defined as the lowest number of copies of the N gene target and ORF1ab ~~region~~ gene that could be detected in 80% of the replicates tested. ~~REF~~ The LOD was expressed as the lowest detectable concentration of the N gene target and ORF1ab ~~region~~ gene in ~~sewage~~ sample based on the equivalent volume of ~~sewage~~ sample analyzed in each ~~q~~RT-qPCR assay, not adjusting for any potential loss through the processing of the ~~sewage~~ sample or any potential inhibition of the ~~q~~RT-qPCR assay.[~~(~~25] All ~~)25~~assays were performed at

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Microbiology laboratory at the Universitas Gadjah Mada,, Special Region of Yogyakarta, Indonesia.

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-Ethics

The SWESP study obtained ethics approval from the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada DR. Sardjito General Hospital, Indonesia (KE/FK/0426/EC/2021, KE/FK/0514/EC/2022). Written or verbal consent was not applicable for this study as we did not collect data from individual participants.

Results

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Feasibility of WBE surveillance

The average time from sample collection to availability of the RT-qPCR results was a mean of 64 hours, including the filtration time (3 to 4 hours), RNA extraction ~~and RT-qPCR~~ (2 to 3 hours), and RT-qPCR quantification analysis (~~1 to 2~~ 3 hours). Both weekly and fortnightly sample collections were practical to conduct. A key challenge was the delay in the importation of critical reagents and consumables exacerbated by the COVID-19 pandemic. -As the UGM laboratory is also the central clinical laboratory, priority for the analysis of clinical samples resulted in a delay in wastewater analysis during major clinical peaks in incidence. Initial trials of deployment of the passive samplers were required to limit damage or loss due to difficulties with positioning and securing samplers. We defined criteria for reliable deployment that considered locations with solid ground to safely access, ideally in an inconspicuous position, and using a strong pole or tree to secure the sampler. To avoid samplers being removed we labeled samplers with signs of warning and explanation such as “Sample for Research by Universitas Gadjah Mada and Yogyakarta Government”.

The detection and positivity rates of SARS-CoV-2 RNA

Sample collection commenced on the 27th of July 2021 during the Delta wave of the COVID-19 pandemic in Indonesia~~ (**Figure 2**)~~. During the 10-week sampling period, a total of 544 samples were collected with 54% (296/544) of all samples testing positive for SARS-CoV-2 RNA. The ~~mean/~~median of cycle threshold (Ct) values for positive N and -ORF1ab gene results, was 35.1 (IQR: 32.1 – 36.9) and 33.9 (IQR: 30.1 – 35.9), respectively. T~~T~~he highest positivity rate was for manhole samples (74%, 191/258 samples, **Fig 2**) and the lowest was for soil samples (3%, 2/60 samples, **Fig 2**). The temporal changes in rates of sample positivity correlate with the number of confirmed cases in the community as illustrated in **Fig 3**. The highest positivity rate of 77%, was obtained for samples collected in July 2021 during week 1 of sample collection and decreased to 25% by the end of September 2021 (corresponding to week 10 of sample collection), reflecting a ~~the~~ decreased detection rate correlating with a decrease in ~~with~~ the incidence of reported COVID-19 clinical cases in the community.

Fig 2. Nucleocapsid (N) gene positivity by sample types.

Fig 3. Distribution maps of SARS-CoV-2 in Special Region of Yogyakarta province, comparing detection targeting N gene to community confirmed cases. (A) In week 1-2 of the sample collection. (B) In week 5-6 of the sample collection. (C) In week 9-10 of the sample collection. Community COVID-19 confirmed cases were represented by blue color, the lighter

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the fewer cases. Detected cases in sampling locations were represented by red colored dots/triangles/pentagons, while non-detected cases were represented by green colored dots/triangles/pentagons. With circles denoting manholes, pentagons denoting river and triangles denoting NST water.

The ~~The Nucleocapsid (N)~~ N gene was identified in 74% (191/258) of sewage samples (grab method), 64% (67/104) of near source tracking (NST) water samples (passive sampling method), 50~~48~~% (25~~19~~/5~~4~~0) of river samples (grab method), and 3% (2/60) of NST soil samples. This finding was consistent with the ~~open reading frame of 1ab (~~ORF1ab~~)~~ gene target but with a higher proportion of soils samples being positive (8%, 5/60) for the ORFlab gene as compared to the N gene (~~what~~ 3%).

~~**Fig 2**. Initial samples were collected at the peak of new confirmed cases during the second wave pandemic of the COVID-19 pandemic in Yogyakarta (available at https://www.worldometers.info/coronavirus/country/indonesia/)~~

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Discussion

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We successfully demonstrated that WBE surveillance for SARS-CoV-2 RNA was feasible in Indonesia and reflected the SARS-CoV-2 clinical burden in the community. The high level of positivity of SARS-CoV-2 RNA in the environment in Indonesia suggests a considerable public health burden and may represent asymptomatic or mild cases that did not access health facilities for testing. Manholes consistently showed higher positivity rates in comparison with river and

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soil samples. Although river and soil samples showed lower positivity rates, the data are useful to complement the WBE surveillance data particularly in regions where connection to a formal sewerage system is limited.~~ ~~ This combination of sampling strategies provides additional insights into the prevalence and distribution of COVID-19 within the community.

In Special Region of Yogyakarta province, many households are not connected to the IPAL Sewon. This may be because they were built after the IPAL Sewon infrastructure was established and therefore have no connection to the IPAL pipes. Other households were not connected due to technical reasons, such as in lower altitudes and ~~geography~~ terrain that does not support passive gravitational~~y~~ flow of wastewater to the central WWTP. However, in this study we managed to collect samples from community WWTPs and septic tanks from NST sites to capture communities that were not served by the central WWTP.

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Although we found that both weekly or fortnightly collection frequency with grab and/or passive sampling collection methods as feasible, weekly collections were preferred in order to provide real-time data to inform the public health response.~~ ~~ The laboratory capacity to conduct qualitative (positive/negative) and quantitative identification of SARS-CoV-2 RNA in the environmental samples (wastewater and soil) were also feasible although some pre-processing procedures need to be conducted prior to the ~~q~~RT-qPCR procedure (i.e., wastewater filtration and soil homogenization). There were challenges ~~of~~ in providing real-time results during peak COVID-19 outbreaks due to overburdened staff and limited access to equipment, and therefore, ideally WBE surveillance should be integrated into the routine surveillance programs with~~,~~ dedicated staff. ~~Yet~~Additionally, the availability of imported reagents has~~ve~~ delayed laboratory analysis during periods of high output. Local epidemiological data describing the distribution of COVID-19 cases (symptomatic and asymptomatic) with laboratory confirmed positive tests for

SARS-CoV-2 infections by sub district, on a weekly basis, were available to compare with the findings from WBE surveillance. -However, data analysis to link environmental and community data remains challenging and needs further exploration.

Despite efforts, there remain practical limitations of WBE surveillance in LIMCs. It is likely that wastewater sampling of the reticulated sewerage system reflects the more modern and affluent sector of the city and may not provide meaningful insights into the presence of SARS-CoV-2 infection within the broader community. Most of the city and rural areas manage human effluent via septic tanks, pit latrines or by open defecation with subsequent contamination of surface water and rivers. Therefore, to understand the distribution of SARS-CoV-2 RNA in environments that reflect the presence of community infections with fragmented wastewater infrastructures, NST sites, and in places where people publicly congregate were selected. These sites include permanent dwellings (apartment and flats), temporary living places (hotels), public spaces (traditional markets, town squares, mosques, and a public swimming pool), rivers, working spaces (both office and factory), and COVID-19 shelters (facilities which are designated as temporary quarantine shelters for people testing positive for COVID-19). This WBE approaches using NST may allow detection of targeted clusters for whom rapid action may reduce or prevent the risk of larger outbreaks within the community.[(26])[26]

It has been proposed that WBE surveillance has the potential to act as an early warning system (EWS) for COVID-19 outbreaks.[(27–32])[27–32] This should be conducted in collaboration with the public health authorities to enable the timely follow up of positive detections by strategies such as contact tracing, strengthening health protocols, or implementing a community lockdown. This could be broadly implemented across the community or in a targeted response depending on the local context and level of concern. For instance, if SARS-CoV-2 RNA is detected (positive

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result) in the sewerage sample in an area where there had consistently been no detections (negative result), then a lockdown or mass screening could be implemented in the area drained by the sewerage system; or if the result is taken from a closed community (e.g., Boarding school), contact tracing within the community should be conducted immediately~~ (**Figure 3**~~).

~~**Fig 3. Implementation strategy of the wastewater surveillance program.**~~

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~~Weekly and fortnightly wastewater and environment samples including NST, are collected using grab and passive sampling methods. Further, samples are analysed in the Microbiology Laboratory for detection and quantification of SARS-CoV-2 RNA. Proposed scenario of actions following positive detections of SARS-CoV-2 RNA from wastewater and environmental samples are notification to local public health office and sewerage management facilities for further planning for preventive measures, including contact tracing and breaking transmission chain (i.e., local lockdown).~~

~~SWESP: SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia; RNA: Ribonucleic Acid; PCR: Polymerase Chain Reaction~~

Conclusions

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In conclusion, an environmental surveillance system for SARS-CoV-2 in Indonesia is feasible and can be used to monitor the community burden of SARS-CoV-2 infection. However, future research is needed to explore its potential to act as an ~~early warning system~~EWS for the early identification of SARS-CoV-2 outbreaks within a community, especially in regions with limited access to clinical testing. Although the sewer infrastructure of wastewater systems is quite limited in Indonesia, an expanded sampling approach based on the local context and including NST can support an effective SARS-COV-2 surveillance program.

Acknowledgements

We would like to ~~acknowledge PATH for supporting the study and reviewing the draft manuscript. *Learn more at PATH.org*. We also~~ thank ~~to~~ the wastewater treatment plant team, field assistants and laboratory team for doing sampling collection and lab<u>oratory</u> works. <u>We thank Rizka Dinari for providing editorial assistance.</u>

~~Role of the funding sources~~

~~This Project was funded by the Global Innovation Fund and PATH. The Global Investment Fund had no involvement in study design, data collection or analysis and PATH participated in study design, but had no role in data collection or analysis, writing of the manuscript or the decision to submit it for publication.~~

~~Author contributions~~

~~Conceptualization - Indah K Murni, Vicka Oktaria, Amanda Handley, David T McCarthy, Celeste M Donato, Julie E Bines~~

~~Data curation - Amanda Handley, Endah Supriyati, Dwi Astuti Dharma Putri, Hendri Marinda Sari~~

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Formal analysis - Vicka Oktaria, Dwi Astuti Dharma Putri, Indah K Murni

Funding acquisition - Julie E Bines, Amanda Handley

Investigation - Indah K Murni, Vicka Oktaria, Amanda Handley, David T McCarthy, Celeste M Donato, Julie E Bines

Methodology - Indah K Murni, Vicka Oktaria, Amanda Handley, David T McCarthy, Celeste M Donato, Titik Nuryastuti, Endah Supriyati, Julie E Bines

Project administration - Amanda Handley, Hendri Marinda Sari, Dwi Astuti Dharma Putri, Endah Supriyati

Resources - David T McCarthy, Titik Nuryastuti, Julie E Bines, Indah K Murni, Vicka Oktaria

Supervision - Indah K Murni, Vicka Oktaria, Titik Nuryastuti, Ida Safitri Laksono, Jarir At Thobari, Julie E Bines

Validation - Indah K Murni, Vicka Oktaria, Amanda Handley, David T McCarthy, Titik Nuryastuti, Endah Supriyati, Julie E Bines

Writing - original draft - Indah K Murni

Writing - review and editing - Indah K Murni, Vicka Oktaria, Amanda Handley, David T McCarthy, Celeste M Donato, Titik Nuryastuti, Endah Supriyati, Dwi Astuti Dharma Putri, Hendri Marinda Sari, Ida Safitri Laksono, Jarir At Thobari, Julie E Bines

Conflict of interests

No conflict of interests to declare.

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The feasibility of SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia

Indah K Murni[1,2*], Vicka Oktaria[1,3*], Amanda Handley[4,5], David T McCarthy[6], Celeste M Donato[4,10], Titik Nuryastuti[7], Endah Supriyati[8], Dwi Astuti Dharma Putri[1], Hendri Marinda Sari[1], Ida Safitri Laksono[1,2], Jarir At Thobari[1], Julie E Bines[4,9,10]

[1] Center for Child Health – Pediatric Research Office, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

[2] Child Health Department, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

[3] Department of Biostatistics, Epidemiology and Population Health, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

[4] Enteric Diseases Group, Murdoch Children's Research Institute, Parkville, Victoria, Australia

[5] Medicines Development for Global Health, Southbank, Victoria Australia

[6] Environmental and Public Health Microbiology Lab (EPHM Lab), Department of Civil Engineering, Monash University, Clayton, Victoria Australia

[7] Department of Microbiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

[8] Center for Tropical Medicine, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

[9] Department of Gastroenterology and Clinical Nutrition, Royal Children's Hospital Melbourne, Victoria, Australia

[10] Department of Paediatrics, The University of Melbourne, Parkville, Australia

[*]Corresponding Author

E-mail: indah.kartika.m@ugm.ac.id (IM)

E-mail: vicka.oktaria@ugm.ac.id (VO)

Abstract

Background

Wastewater-based epidemiology (WBE) surveillance as an early warning system (EWS) for monitoring community transmission of SARS-CoV-2 in low- and middle-income country (LMIC) settings, where diagnostic testing capacity is limited, needs further exploration. We explored the feasibility to conduct a WBE surveillance in Indonesia, one of the global epicenters of the COVID-19 pandemic in the middle of 2021, with the fourth largest population in the world where sewer and non-sewered sewage systems are implemented. The feasibility and resource capacity to collect samples on a weekly or fortnightly basis with grab and/or passive sampling methods, as well as to conduct qualitative and quantitative identification of SARS-CoV-2 ribonucleic acid (RNA) using real-time RT-PCR (RT-qPCR) testing of environmental samples were explored.

Materials and methods

We initiated a routine surveillance of wastewater and environmental sampling at three predetermined districts in Special Region of Yogyakarta Province. Water samples were collected from central and community wastewater treatment plants (WWTPs), including manholes flowing to the central WWTP, and additional soil samples were collected for the near source tracking (NST) locations (i.e., public spaces where people congregate).

Results

We began collecting samples in the Delta wave of the COVID-19 pandemic in Indonesia in July 2021. From a 10-week period, 54% (296/544) of wastewater and environmental samples were

positive for SARS-CoV-2 RNA. The sample positivity rate decreased in proportion with the reported incidence of COVID-19 clinical cases in the community. The highest positivity rate of 77% in week 1, was obtained for samples collected in July 2021 and decreased to 25% in week 10 by the end of September 2021.

Conclusion

A WBE surveillance system for SARS-CoV-2 in Indonesia is feasible to monitor the community burden of infections. Future studies testing the potential of WBE and EWS for signaling early outbreaks of SARS-CoV-2 transmissions in this setting are required.

Keywords

Wastewater-based epidemiology surveillance, environmental sampling, SARS-CoV-2, COVID-19, Indonesia.

Introduction

Understanding the full extent of the Coronavirus Disease (COVID-19) pandemic is a major public health challenge. Traditional epidemiological indicators which are based on the number of confirmed clinical cases and deaths due to COVID-19 disease have potential biases and limitations. The capacity for timely diagnosis using laboratory tests may be limited, particularly in low- and middle- income countries (LMICs) during epidemic wave. Incidence rates based on hospitalization data lag behind the incidence of infection in the community and lack of representativeness for identification of cases who do not access care, have non-serious illness, or are asymptomatic.

People infected with SARS-CoV-2 shed the virus in stool independently of gastrointestinal symptoms and therefore viral ribonucleic acid (RNA) can be detected in environmental wastewater, containing excreta from infected people and sewerage treatment plants.[1–4] Public health surveillance using wastewater is now well established and has been used to monitor communities for the presence of poliovirus, antimicrobial resistant enteric bacteria, and drugs of abuse, e.g. opioids.[5–7] It has been postulated that routine monitoring for the presence of SARS-CoV-2 in wastewater may be useful in detecting an existing or predicting a new potential epidemic.[6,8]

Studies reporting the detection of SARS-CoV-2 RNA in wastewater have been predominantly limited to high-income countries such as Australia, the United States, Japan and a number of European countries. To date, only a few studies have detected the genetic material of SARS-CoV-2 in wastewater from LMICs, including studies from Argentina, Brazil, Ecuador, India, Pakistan, and South Africa.[9–20] The lack of formal sewerage systems in LMICs, particularly in impoverished areas and informal settlements, has posed a major challenge for SARS-CoV-2 surveillance using wastewater. It is also in these communities where epidemiological surveillance using rates based on disease case capture and death are problematic. The adaptation of environmental surveillance methods suitable for use in LMICs provides an opportunity to monitor community transmission and inform the public response to SARS-CoV-2 and other future pandemic infections.

This short communication describes the assessment of the feasibility of conducting SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia. The aim was to provide a proof of concept for the use of wastewater and environmental surveillance to monitor the community burden of SARS-CoV-2 infection in Indonesia.

Materials and methods

General information on wastewater systems and challenges in Indonesia

In Indonesia, a high proportion of the population is not connected to a sewerage system. In the capital city of Jakarta, a city with a population of over 10 million, it is estimated that only 2% of households are connected to a reticulated sewerage system, with >95% of wastewater leaking into agricultural fields, rivers, and other groundwater sources.[21]

We established the first Indonesian wastewater-based SARS-CoV-2 epidemiology surveillance program in Special Region of Yogyakarta province, one of the regions with the highest number of COVID-19 cases during the Delta wave. In the Special Region of Yogyakarta province, only 25,294 households (6% population serviced) are connected to a formal reticulated sewerage system. There are two types of wastewater treatment plants (WWTPs) systems in operation in the province: (a) the central WWTP (*Instalasi Pengolahan Air Limbah Sewon/IPAL* Sewon, Bantul) managed by the provincial government and (b) community WWTPs (IPAL community) that are independently managed by each local community, in addition to individual septic tanks. The service coverage of IPAL Sewon in the Special Region of Yogyakarta province includes 13 of the 14 sub-districts in the Yogyakarta city, 4 of the 17 sub-districts in the Sleman district and 3 of the 17 sub-districts in the Bantul district. Community WWTPs are used in some suburban areas due to the lack of capacity of the central WWTPs to service their needs and the terrain of the region that does not allow passive gravitational flow.

SARS-CoV-2 surveillance on wastewater and environmental sampling in Indonesia (SWESP study)

Routine wastewater-based epidemiology (WBE) surveillance (i.e., testing of sewerage and wastewater sites, and waterways) and testing of soil was initiated in three of five districts in the Special Region of Yogyakarta province (Yogyakarta city, Sleman and Bantul districts, **Fig 1**). Two districts were not included due to practical challenges, such as the geography and relatively sparse population. Identification and mapping of the infrastructure of the wastewater system (formal and informal) at provincial and district level was conducted prior to commencing the study. We selected six sub-districts from Yogyakarta city as these areas have the highest coverage of the formal central wastewater system and samples may be considered more representative to the broader community, two from Sleman district, and the remaining two from Bantul district. Within the total of ten sub-districts, we also selected 12 clustered communities that were served by small community WWTPs. Each community WWTP served between 50-150 households.

Fig 1. Flowchart of sample strategy. We selected ten sub-districts from three out of five districts in Special region of Yogyakarta Province (Yogyakarta city, Bantul, and Sleman districts). Samples from three sub-districts were taken weekly (identified by blue arrows), while others were taken fortnightly. Detailed type and number of samples in each sub-district are illustrated in the figure.

We collected samples using either the grab or passive sampling methods. Wastewater from manholes was collected by immersing a ~500 mL bottle into the water to a depth of around 20 – 30 cm until the bottle was filled, allowing about 1 cm of air. Recreational water was collected using a 2 L bottle using a similar grab method. Bottles were pre-labelled with sample specific barcodes. A torpedo-style passive sampler with multiple entry points (front, top, sides, and bottom) [22,23] was used to collect samples from septic tanks, rivers, and the central and community WWTPs. Passive samplers were retrieved 24 hours after deployment. Soil samples (20 g) were collected using zip lock bags. Within four hours of collection, samples were transferred on ice at 2-8°C [24] to the Microbiology laboratory at the Universitas Gadjah Mada Special Region of Yogyakarta, Indonesia.

Laboratory methods for wastewater and environmental samples

The wastewater samples, passive samplers and soil samples were stored in the 4°C fridge upon arrival until the sample processing. Samples of wastewater (50 mL) or recreational water (1000 mL) were filtered through a 47 mm diameter, 0.45 µm pore size, cellulose nitrate high flow electronegative membrane (Sartorius, Germany). This filtration process was performed immediately (<2 hours) once the samples were received at the laboratory. The collection bag containing the soil samples was thoroughly mixed. In a 2 mL tube, 0.25 grams of soil and 2 mL of DNA/RNA Shield solutions (Zymo Research, USA) were added. The passive samplers were opened, and the filter membrane and q-tips were collected.

All of the processed samples from wastewater samples, passive samplers and soil samples were stored at -80°C until the RNA extraction and reverse-transcription quantitative real-time PCR (RT-qPCR) analysis.

The RNA was extracted from samples using the QIAGEN RNeasy PowerMicrobiome Kit (QIAGEN, Germany) following manufacturer's instructions with the exception of replacing the supplied beads with PowerBead Tubes-Garnet beads (QIAGEN, Germany). For every batch of samples processed, a negative extraction controls and internal control (MS2 bacteriophage) as supplied in the PerkinElmer SARS-CoV-2 Nucleic Acid Detection Kit (RUO) (PerkinElmer) were included in the RNA extraction process to monitor the RNA extraction performance.

To detect the SARS-Cov2 RNA, a RT-qPCR was conducted using the SARS-CoV-2 Real-time RT-PCR Assay (PerkinElmer, US) and synthetic SARS-CoV-2 RNA Control 1-MT007544.1 (Twist Bioscience, Australia) as the standard curve. The kit is a multiplex assay using primers and probes targeting the Nucleocapsid (N) gene and open reading frame 1ab (ORF1ab) region of SARS-CoV-2. RT-qPCR assays were performed using two replicates of 5 μL RNA template, with a total reaction volume of 30 μL and a total 45 cycles of amplification. The quantification of the samples was calculated using the synthetic SARS-CoV-2 RNA Control 1-MT007544.1 (Twist Bioscience, Australia) as a standard curve, according to the manufacturer's instruction. The RT-qPCR assay was performed as described by the manufacturer's instruction using the LightCycler 96 instrument (Roche, Germany).

In order to report the actual value of SARS-CoV-2 RNA, we calculated the recovery efficiency. In each qPCR run, multiple SARS-CoV-2 RNA controls, a MS2 phage control (to determine the RNA recovery efficiency and as internal control) of different known concentrations and a negative control were included.

The limit of detection (LOD) for the RT-qPCR assay was determined by the analysis of 10 replicates for each dilution of the synthetic SARS-CoV-2 RNA Control 1-MT007544.1 (Twist Bioscience, Australia) analyzed and was defined as the lowest number of copies of the N gene target and ORF1ab gene that could be detected in 80% of the replicates tested. The LOD was expressed as the lowest detectable concentration of the N gene target and ORF1ab gene in sample based on the equivalent volume of sample analyzed in each RT-qPCR assay, not adjusting for any potential loss through the processing of the sample or any potential inhibition of the RT-qPCR assay.[25] All assays were performed at Microbiology laboratory at the Universitas Gadjah Mada,, Special Region of Yogyakarta, Indonesia.

Ethics

The SWESP study obtained ethics approval from the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada DR. Sardjito General Hospital, Indonesia (KE/FK/0426/EC/2021, KE/FK/0514/EC/2022). Written or verbal consent was not applicable for this study as we did not collect data from individual participants.

Results

Feasibility of WBE surveillance

The average time from sample collection to availability of the RT-qPCR results was a mean of 64 hours, including the filtration time (3 to 4 hours), RNA extraction (2 to 3 hours), and RT-qPCR quantification analysis (3 hours). Both weekly and fortnightly sample collections were practical to conduct. A key challenge was the delay in the importation of critical reagents and consumables exacerbated by the COVID-19 pandemic. As the UGM laboratory is also the central clinical laboratory, priority for the analysis of clinical samples resulted in a delay in wastewater analysis during major clinical peaks in incidence. Initial trials of deployment of the passive samplers were required to limit damage or loss due to difficulties with positioning and securing samplers. We defined criteria for reliable deployment that considered locations with solid ground to safely access, ideally in an inconspicuous position, and using a strong pole or tree to secure the sampler. To avoid samplers being removed we labeled samplers with signs of warning and explanation such as "Sample for Research by Universitas Gadjah Mada and Yogyakarta Government".

The detection and positivity rates of SARS-CoV-2 RNA

Sample collection commenced on the 27th of July 2021 during the Delta wave of the COVID-19 pandemic in Indonesia. During the 10-week sampling period, a total of 544 samples were collected with 54% (296/544) of all samples testing positive for SARS-CoV-2 RNA. The median of cycle threshold (Ct) values for positive N and ORF1ab gene results was 35.1 (IQR: 32.1 – 36.9) and 33.9 (IQR: 30.1 – 35.9), respectively. The highest positivity rate was for manhole samples (74%, 191/258 samples, **Fig 2**) and the lowest was for soil samples (3%, 2/60 samples, **Fig 2**). The temporal changes in rates of sample positivity correlate with the number of confirmed cases in the community as illustrated in **Fig 3**. The highest positivity rate of 77%, was obtained for samples collected in July 2021 during week 1 of sample collection and decreased to 25% by the end of September 2021 (corresponding to week 10 of sample collection), reflecting a decreased detection rate correlating with a decrease in the incidence of reported COVID-19 clinical cases in the community.

Fig 2. Nucleocapsid (N) gene positivity by sample types.

Fig 3. Distribution maps of SARS-CoV-2 in Special Region of Yogyakarta province, comparing detection targeting N gene to community confirmed cases. (A) In week 1-2 of the sample collection. (B) In week 5-6 of the sample collection. (C) In week 9-10 of the sample collection. Community COVID-19 confirmed cases were represented by blue color, the lighter the fewer cases. Detected cases in sampling locations were represented by red colored dots/triangles/pentagons, while non-detected cases were represented by green colored dots/triangles/pentagons. With circles denoting manholes, pentagons denoting river and triangles denoting NST water.

The N gene was identified in 74% (191/258) of sewage samples (grab method), 64% (67/104) of near source tracking (NST) water samples (passive sampling method), 50% (25/50) of river samples (grab method), and 3% (2/60) of NST soil samples. This finding was consistent with the ORF1ab gene target but with a higher proportion of soils samples being positive (8%, 5/60) for the ORFlab gene as compared to the N gene (3%).

Discussion

We successfully demonstrated that WBE surveillance for SARS-CoV-2 RNA was feasible in Indonesia and reflected the SARS-CoV-2 clinical burden in the community. The high level of positivity of SARS-CoV-2 RNA in the environment in Indonesia suggests a considerable public health burden and may represent asymptomatic or mild cases that did not access health facilities for testing. Manholes consistently showed higher positivity rates in comparison with river and soil samples. Although river and soil samples showed lower positivity rates, the data are useful to complement the WBE surveillance data particularly in regions where connection to a formal sewerage system is limited. This combination of sampling strategies provides additional insights into the prevalence and distribution of COVID-19 within the community.

In Special Region of Yogyakarta province, many households are not connected to the IPAL Sewon. This may be because they were built after the IPAL Sewon infrastructure was established and therefore have no connection to the IPAL pipes. Other households were not connected due to technical reasons, such as in lower altitudes and terrain that does not support passive gravitational flow of wastewater to the central WWTP. However, in this study we managed to collect samples from community WWTPs and septic tanks from NST sites to capture communities that were not served by the central WWTP.

Although we found that both weekly or fortnightly collection frequency with grab and/or passive sampling collection methods as feasible, weekly collections were preferred in order to provide real-time data to inform the public health response. The laboratory capacity to conduct qualitative (positive/negative) and quantitative identification of SARS-CoV-2 RNA in the environmental samples (wastewater and soil) were also feasible although some pre-processing procedures need to be conducted prior to the RT-qPCR procedure (i.e., wastewater filtration and soil homogenization). There were challenges in providing real-time results during peak COVID-19 outbreaks due to overburdened staff and limited access to equipment, and therefore, ideally WBE surveillance should be integrated into the routine surveillance programs with dedicated staff. Additionally, the availability of imported reagents has delayed laboratory analysis during periods of high output. Local epidemiological data describing the distribution of COVID-19 cases (symptomatic and asymptomatic) with laboratory confirmed positive tests for SARS-CoV-2 infections by sub district, on a weekly basis, were available to compare with the findings from

WBE surveillance. However, data analysis to link environmental and community data remains challenging and needs further exploration.

Despite efforts, there remain practical limitations of WBE surveillance in LIMCs. It is likely that wastewater sampling of the reticulated sewerage system reflects the more modern and affluent sector of the city and may not provide meaningful insights into the presence of SARS-CoV-2 infection within the broader community. Most of the city and rural areas manage human effluent via septic tanks, pit latrines or by open defecation with subsequent contamination of surface water and rivers. Therefore, to understand the distribution of SARS-CoV-2 RNA in environments that reflect the presence of community infections with fragmented wastewater infrastructures, NST sites, and in places where people publicly congregate were selected. These sites include permanent dwellings (apartment and flats), temporary living places (hotels), public spaces (traditional markets, town squares, mosques, and a public swimming pool), rivers, working spaces (both office and factory), and COVID-19 shelters (facilities which are designated as temporary quarantine shelters for people testing positive for COVID-19). This WBE approaches using NST may allow detection of targeted clusters for whom rapid action may reduce or prevent the risk of larger outbreaks within the community.[26]

It has been proposed that WBE surveillance has the potential to act as an early warning system (EWS) for COVID-19 outbreaks.[27–32] This should be conducted in collaboration with the public health authorities to enable the timely follow up of positive detections by strategies such as contact tracing, strengthening health protocols, or implementing a community lockdown. This could be broadly implemented across the community or in a targeted response depending on the local context and level of concern. For instance, if SARS-CoV-2 RNA is detected (positive result) in the sewerage sample in an area where there had consistently been no detections (negative result), then a lockdown or mass screening could be implemented in the area drained by the sewerage system; or if the result is taken from a closed community (e.g., Boarding school), contact tracing within the community should be conducted immediately.

Conclusions

In conclusion, an environmental surveillance system for SARS-CoV-2 in Indonesia is feasible and can be used to monitor the community burden of SARS-CoV-2 infection. However, future research is needed to explore its potential to act as an EWS for the early identification of SARS-CoV-2 outbreaks within a community, especially in regions with limited access to clinical testing. Although the sewer infrastructure of wastewater systems is quite limited in Indonesia, an expanded sampling approach based on the local context and including NST can support an effective SARS-COV-2 surveillance program.

Acknowledgements

We would like to thank the wastewater treatment plant team, field assistants and laboratory team for doing sampling collection and laboratory works. We thank Rizka Dinari for providing editorial assistance.

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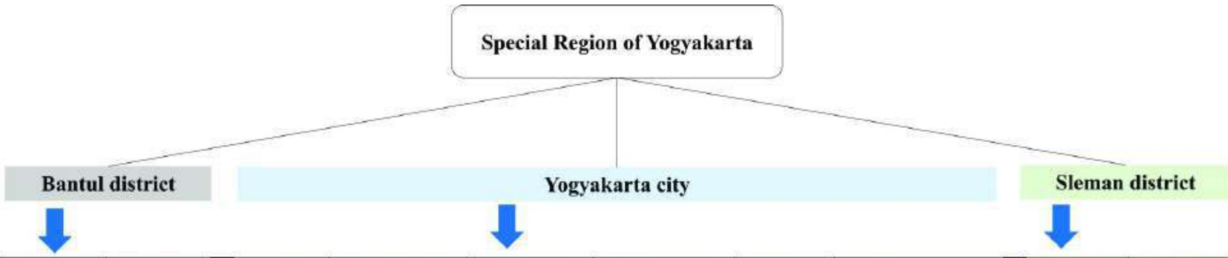
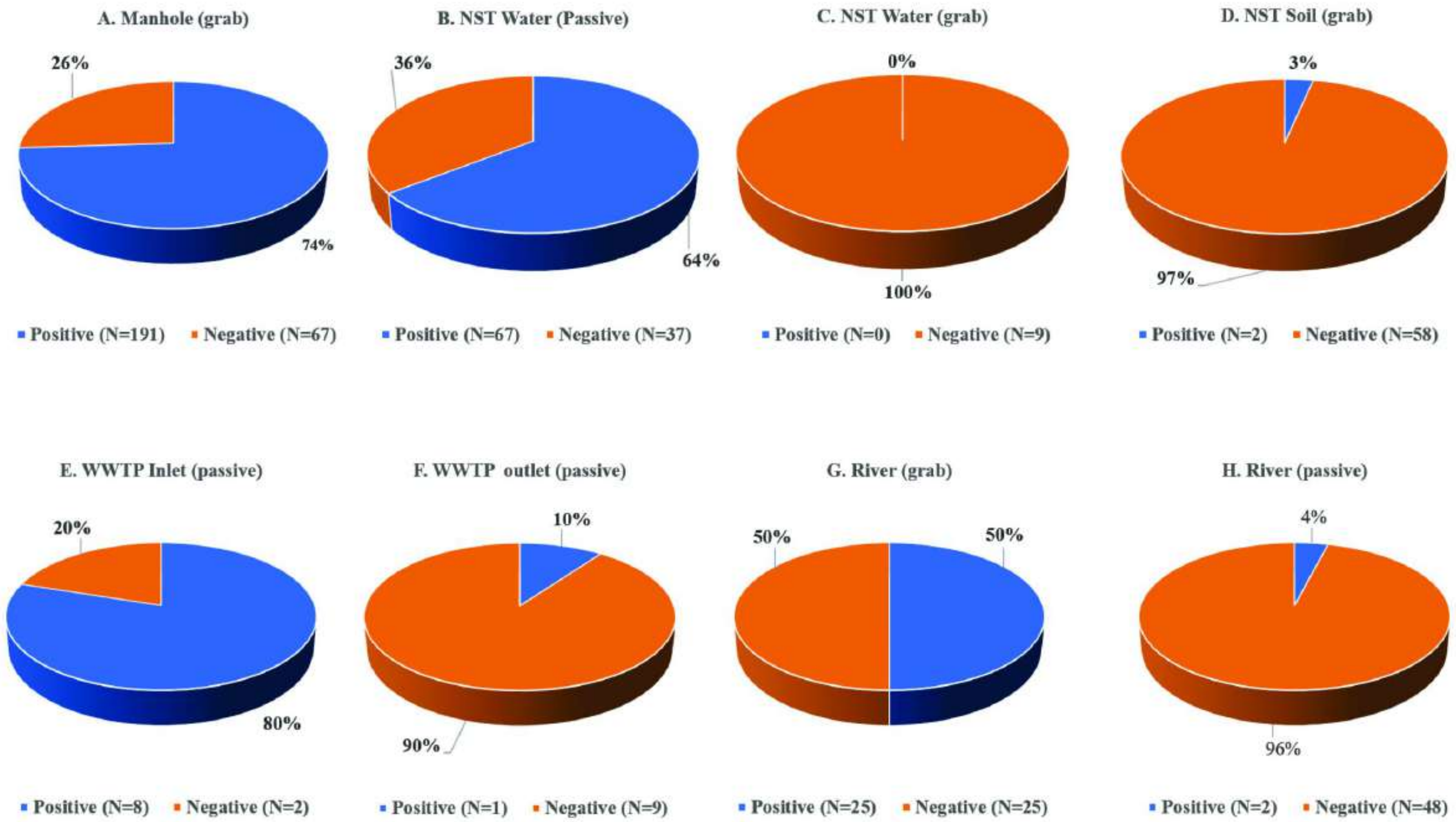
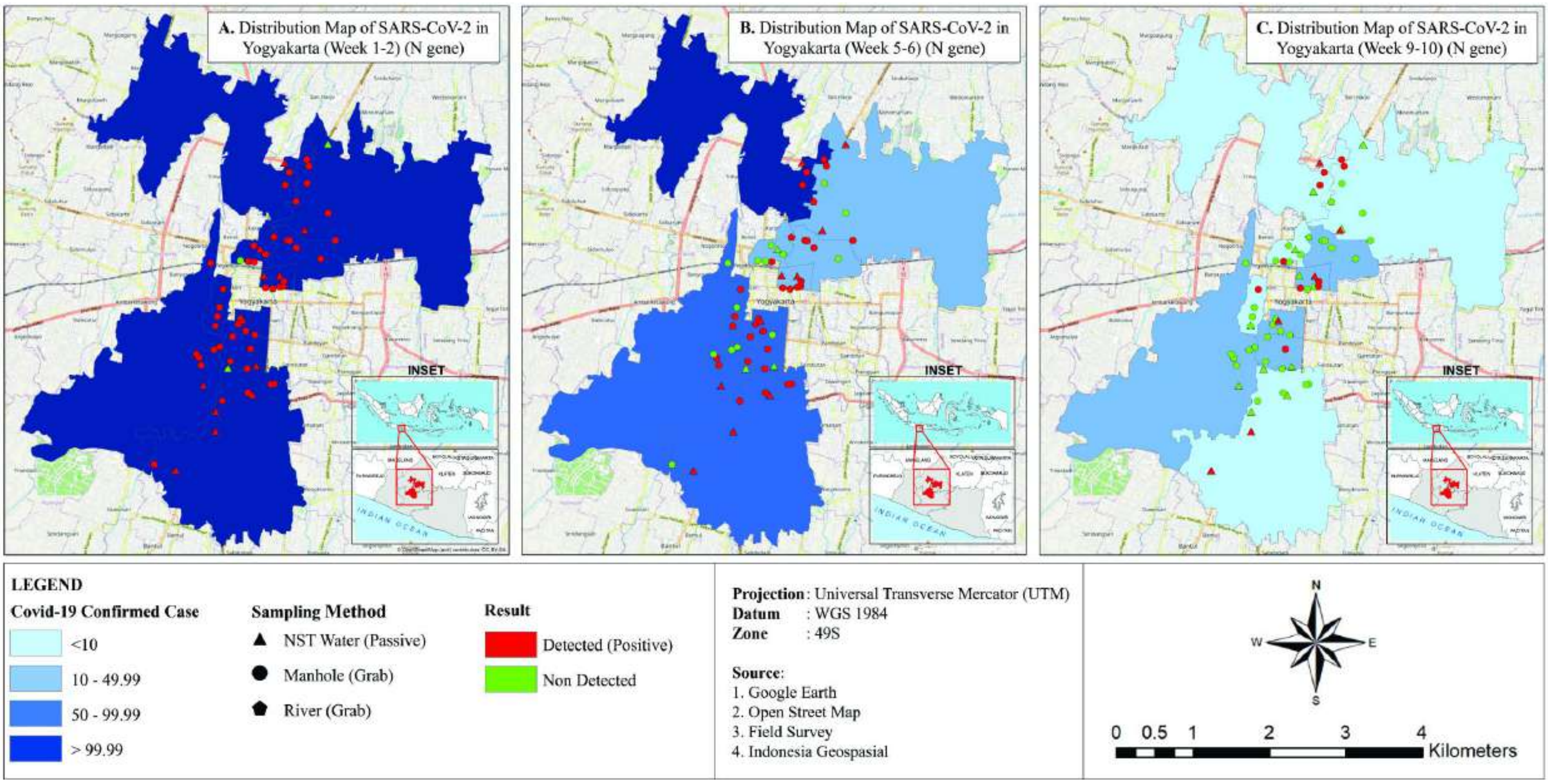


	Sewon	Kasihan	Jetis	Wirobrajan	Danurejan	Mantrijeron	Kraton	Gondokusuman	Mlati	Depok
Total Area (km^2)	27.16	32.38	1.70	1.76	1.10	2.61	1.4	3.99	28.52	35.55
Total Population (people)	109,370	115,050	23,385	24,739	18,670	33,340	17,943	36,921	100,524	131,005
Population Density (per km^2)	3,693	3,197	15,960	15,834	19,395	13,576	15,594	10,731	3,229	3,440
Incidence Rate*	1.83	0.87	0	4.04	0	6.00	11.15	10.83	0	3.82

Manhole (Grab method)	4	4	5	4	4	4	4	4	4	3
NST Water (Passive & grab method)	2	2	1	2	2	2	1	-	2	4
NST Soil (Grab method)	-	2	-	1	1	1	2	-	-	1
River (Passive & Grab method)	2	4	2	-	2	-	-	-	-	2
Central WWTP Sewon (Passive)	4	-	-	-	-	-	-	-	-	-
Community WWTP (Passive)	4	3	-	-	-	-	-	2	1	4
Total Sampling Location	**16**	**15**	**8**	**7**	**9**	**7**	**7**	**6**	**7**	**14**


A. Manhole (grab)
26%
74%
Positive (N=191)
Negative (N=67)
B. NST Water (Passive)
36%
64%
Positive (N=67)
Negative (N=37)
C. NST Water (grab)
0%
100%
Positive (N=0)
Negative (N=9)
D. NST Soil (grab)
3%
97%
Positive (N=2)
Negative (N=58)
E. WWTP Inlet (passive)
20%
80%
Positive (N=8)
Negative (N=2)
F. WWTP outlet (passive)
10%
90%
Positive (N=1)
Negative (N=9)
G. River (grab)
50%
50%
Positive (N=25)
Negative (N=25)
H. River (passive)
4%
96%
Positive (N=2)
Negative (N=48)


A. Distribution Map of SARS-CoV-2 in Yogyakarta (Week 1-2) (N gene)
B. Distribution Map of SARS-CoV-2 in Yogyakarta (Week 5-6) (N gene)
C. Distribution Map of SARS-CoV-2 in Yogyakarta (Week 9-10) (N gene)
INSET
LEGEND
Covid-19 Confirmed Case
<10
10 - 49.99
50 - 99.99
> 99.99
Sampling Method
NST Water (Passive)
Manhole (Grab)
River (Grab)
Result
Detected (Positive)
Non Detected
Projection: Universal Transverse Mercator (UTM)
Datum : WGS 1984
Zone : 49S
Source:
1. Google Earth
2. Open Street Map
3. Field Survey
4. Indonesia Geospasial
N
W
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S
0 0.5 1 2 3 4
Kilometers

Date: Sep 06 2022 07:28AM
To: "Indah K Murni" indah.kartika.m@ugm.ac.id
cc: "Vicka Oktaria" vicka.oktaria@ugm.ac.id, "Amanda Handley" amanda.handley@mcri.edu.au, "David T McCarthy" david.mccarthy@monash.edu, "Celeste M Donato" celeste.donato@mcri.edu.au, "Titik Nuryastuti" t.nuryastuti@ugm.ac.id, "Endah Supriyati" endah.supriyati@worldmosquito.org;endah.supriyati@eliminatedengue.com;endah.supriyati@gmail.com, "Dwi Astuti Dharma Putri" putridhrm@gmail.com, "Hendri Marinda Sari" sarihendrimarinda@gmail.com, "Ida Safitri Laksono" idalaksono@ugm.ac.id, "Jarir At Thobari" j.atthobari@ugm.ac.id, "Julie E Bines" jebines@unimelb.edu.au
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The manuscript must describe a technically sound piece of scientific research with data that supports the conclusions. Experiments must have been conducted rigorously, with appropriate controls, replication, and sample sizes. The conclusions must be drawn appropriately based on the data presented.

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7. Bukti konfirmasi artikel accepted formal

(6 Oktober 2022)

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PONE-D-22-14874R1
The feasibility of SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia

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RESEARCH ARTICLE

The feasibility of SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia

Indah K. Murni[1,2]*, Vicka Oktaria[1,3]*, Amanda Handley[4,5], David T. McCarthy[6], Celeste M. Donato[4,7], Titik Nuryastuti[8], Endah Supriyati[9], Dwi Astuti Dharma Putri[1], Hendri Marinda Sari[1], Ida Safitri Laksono[1,2], Jarir At Thobari[1], Julie E. Bines[4,7,10]

1 Faculty of Medicine, Center for Child Health–Pediatric Research Office, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia, **2** Faculty of Medicine, Child Health Department, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia, **3** Faculty of Medicine, Department of Biostatistics, Epidemiology and Population Health, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia, **4** Enteric Diseases Group, Murdoch Children's Research Institute, Parkville, Victoria, Australia, **5** Medicines Development for Global Health, Southbank, Victoria, Australia, **6** Department of Civil Engineering, Environmental and Public Health Microbiology Lab (EPHM Lab), Monash University, Clayton, Victoria, Australia, **7** Department of Paediatrics, The University of Melbourne, Parkville, Australia, **8** Faculty of Medicine, Department of Microbiology, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia, **9** Faculty of Medicine, Center for Tropical Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia, **10** Department of Gastroenterology and Clinical Nutrition, Royal Children's Hospital Melbourne, Parkville, Victoria, Australia

* indah.kartika.m@ugm.ac.id (IKM); vicka.oktaria@ugm.ac.id (VO)

Citation: Murni IK, Oktaria V, Handley A, McCarthy DT, Donato CM, Nuryastuti T, et al. (2022) The feasibility of SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia. PLoS ONE 17(10): e0274793. https://doi.org/10.1371/journal.pone.0274793

Editor: Etsuro Ito, Waseda University: Waseda Daigaku, JAPAN

Received: May 23, 2022

Accepted: September 5, 2022

Published: October 14, 2022

Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: https://doi.org/10.1371/journal.pone.0274793

Data Availability Statement: The dataset can be found in this link: https://doi.org/10.6084/m9.figshare.19824445.

Abstract

Background

Wastewater-based epidemiology (WBE) surveillance as an early warning system (EWS) for monitoring community transmission of SARS-CoV-2 in low- and middle-income country (LMIC) settings, where diagnostic testing capacity is limited, needs further exploration. We explored the feasibility to conduct a WBE surveillance in Indonesia, one of the global epicenters of the COVID-19 pandemic in the middle of 2021, with the fourth largest population in the world where sewer and non-sewered sewage systems are implemented. The feasibility and resource capacity to collect samples on a weekly or fortnightly basis with grab and/or passive sampling methods, as well as to conduct qualitative and quantitative identification of SARS-CoV-2 ribonucleic acid (RNA) using real-time RT-PCR (RT-qPCR) testing of environmental samples were explored.

Materials and methods

We initiated a routine surveillance of wastewater and environmental sampling at three predetermined districts in Special Region of Yogyakarta Province. Water samples were collected from central and community wastewater treatment plants (WWTPs), including manholes flowing to the central WWTP, and additional soil samples were collected for the near source tracking (NST) locations (i.e., public spaces where people congregate).

Funding: This Project was funded by the Global Innovation Fund and PATH (PATH.org). The Global Investment Fund had no involvement in study design, data collection or analysis and PATH participated in study design and reviewing the draft manuscript, but had no role in data collection or analysis, writing of the manuscript or the decision to submit it for publication.

Competing interests: The authors have declared that no competing interests exist.

Results

We began collecting samples in the Delta wave of the COVID-19 pandemic in Indonesia in July 2021. From a 10-week period, 54% (296/544) of wastewater and environmental samples were positive for SARS-CoV-2 RNA. The sample positivity rate decreased in proportion with the reported incidence of COVID-19 clinical cases in the community. The highest positivity rate of 77% in week 1, was obtained for samples collected in July 2021 and decreased to 25% in week 10 by the end of September 2021.

Conclusion

A WBE surveillance system for SARS-CoV-2 in Indonesia is feasible to monitor the community burden of infections. Future studies testing the potential of WBE and EWS for signaling early outbreaks of SARS-CoV-2 transmissions in this setting are required.

Introduction

Understanding the full extent of the Coronavirus Disease (COVID-19) pandemic is a major public health challenge. Traditional epidemiological indicators which are based on the number of confirmed clinical cases and deaths due to COVID-19 disease have potential biases and limitations. The capacity for timely diagnosis using laboratory tests may be limited, particularly in low- and middle- income countries (LMICs) during epidemic wave. Incidence rates based on hospitalization data lag behind the incidence of infection in the community and lack of representativeness for identification of cases who do not access care, have non-serious illness, or are asymptomatic.

People infected with SARS-CoV-2 shed the virus in stool independently of gastrointestinal symptoms and therefore viral ribonucleic acid (RNA) can be detected in environmental wastewater, containing excreta from infected people and sewerage treatment plants [1–4]. Public health surveillance using wastewater is now well established and has been used to monitor communities for the presence of poliovirus, antimicrobial resistant enteric bacteria, and drugs of abuse, e.g. opioids [5–7]. It has been postulated that routine monitoring for the presence of SARS-CoV-2 in wastewater may be useful in detecting an existing or predicting a new potential epidemic [6, 8].

Studies reporting the detection of SARS-CoV-2 RNA in wastewater have been predominantly limited to high-income countries such as Australia, the United States, Japan and a number of European countries. To date, only a few studies have detected the genetic material of SARS-CoV-2 in wastewater from LMICs, including studies from Argentina, Brazil, Ecuador, India, Pakistan, and South Africa [9–20]. The lack of formal sewerage systems in LMICs, particularly in impoverished areas and informal settlements, has posed a major challenge for SARS-CoV-2 surveillance using wastewater. It is also in these communities where epidemiological surveillance using rates based on disease case capture and death are problematic. The adaptation of environmental surveillance methods suitable for use in LMICs provides an opportunity to monitor community transmission and inform the public response to SARS-CoV-2 and other future pandemic infections.

This short communication describes the assessment of the feasibility of conducting SARS-CoV-2 surveillance using wastewater and environmental sampling in Indonesia. The aim was to provide a proof of concept for the use of wastewater and environmental surveillance to monitor the community burden of SARS-CoV-2 infection in Indonesia.

Materials and methods

General information on wastewater systems and challenges in Indonesia

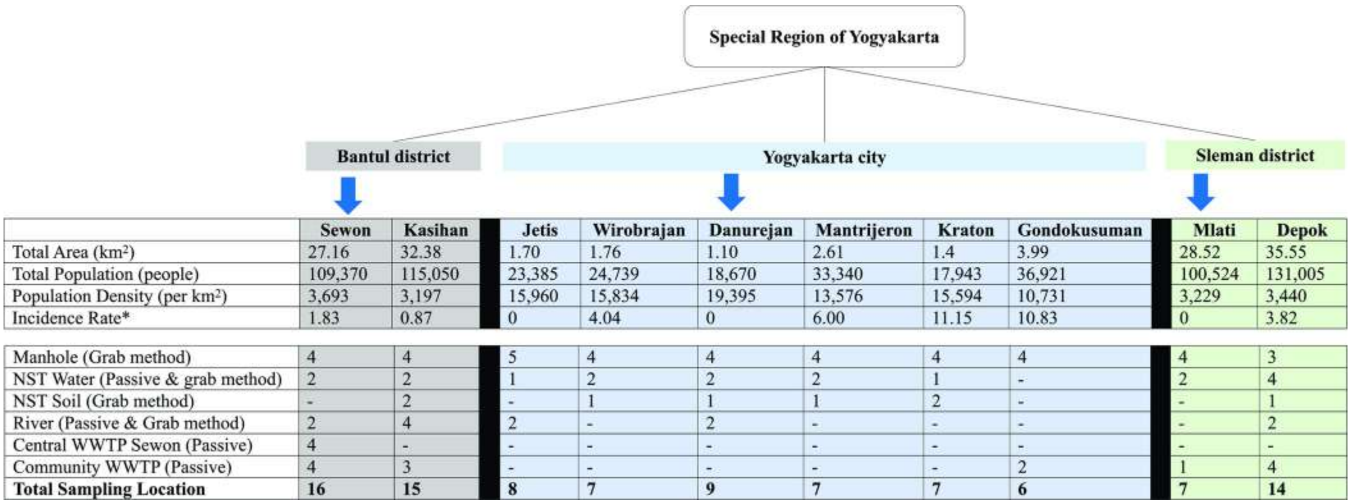
In Indonesia, a high proportion of the population is not connected to a sewerage system. In the capital city of Jakarta, a city with a population of over 10 million, it is estimated that only 2% of households are connected to a reticulated sewerage system, with >95% of wastewater leaking into agricultural fields, rivers, and other groundwater sources [21].

We established the first Indonesian wastewater-based SARS-CoV-2 epidemiology surveillance program in Special Region of Yogyakarta province, one of the regions with the highest number of COVID-19 cases during the Delta wave. In the Special Region of Yogyakarta province, only 25,294 households (6% population serviced) are connected to a formal reticulated sewerage system. There are two types of wastewater treatment plants (WWTPs) systems in operation in the province: (a) the central WWTP (*Instalasi Pengolahan Air Limbah Sewon/ IPAL* Sewon, Bantul) managed by the provincial government and (b) community WWTPs (IPAL community) that are independently managed by each local community, in addition to individual septic tanks. The service coverage of IPAL Sewon in the Special Region of Yogyakarta province includes 13 of the 14 sub-districts in the Yogyakarta city, 4 of the 17 sub-districts in the Sleman district and 3 of the 17 sub-districts in the Bantul district. Community WWTPs are used in some suburban areas due to the lack of capacity of the central WWTPs to service their needs and the terrain of the region that does not allow passive gravitational flow.

SARS-CoV-2 surveillance on wastewater and environmental sampling in Indonesia (SWESP study)

Routine wastewater-based epidemiology (WBE) surveillance (i.e., testing of sewerage and wastewater sites, and waterways) and testing of soil was initiated in three of five districts in the Special Region of Yogyakarta province (Yogyakarta city, Sleman and Bantul districts, **Fig 1**). Two districts were not included due to practical challenges, such as the geography and relatively sparse population. Identification and mapping of the infrastructure of the wastewater system (formal and informal) at provincial and district level was conducted prior to commencing the study. We selected six sub-districts from Yogyakarta city as these areas have the highest coverage of the formal central wastewater system and samples may be considered more representative to the broader community, two from Sleman district, and the remaining two from Bantul district. Within the total of ten sub-districts, we also selected 12 clustered communities that were served by small community WWTPs. Each community WWTP served between 50–150 households.

We collected samples using either the grab or passive sampling methods. Wastewater from manholes was collected by immersing a ~500 mL bottle into the water to a depth of around 20–30 cm until the bottle was filled, allowing about 1 cm of air. Recreational water was collected using a 2 L bottle using a similar grab method. Bottles were pre-labelled with sample specific barcodes. A torpedo-style passive sampler with multiple entry points (front, top, sides, and bottom) [22, 23] was used to collect samples from septic tanks, rivers, and the central and community WWTPs. Passive samplers were retrieved 24 hours after deployment. Soil samples (20 g) were collected using zip lock bags. Within four hours of collection, samples were transferred on ice at 2–8˚C [24] to the Microbiology laboratory at the Universitas Gadjah Mada Special Region of Yogyakarta, Indonesia.



	Sewon	Kasihan	Jetis	Wirobrajan	Danurejan	Mantrijeron	Kraton	Gondokusuman	Mlati	Depok
Total Area (km^2)	27.16	32.38	1.70	1.76	1.10	2.61	1.4	3.99	28.52	35.55
Total Population (people)	109,370	115,050	23,385	24,739	18,670	33,340	17,943	36,921	100,524	131,005
Population Density (per km^2)	3,693	3,197	15,960	15,834	19,395	13,576	15,594	10,731	3,229	3,440
Incidence Rate*	1.83	0.87	0	4.04	0	6.00	11.15	10.83	0	3.82
Manhole (Grab method)	4	4	5	4	4	4	4	4	4	3
NST Water (Passive & grab method)	2	2	1	2	2	2	1	-	2	4
NST Soil (Grab method)	-	2	-	1	1	1	2	-	-	1
River (Passive & Grab method)	2	4	2	-	2	-	-	-	-	2
Central WWTP Sewon (Passive)	4	-	-	-	-	-	-	-	-	-
Community WWTP (Passive)	4	3	-	-	-	-	-	2	1	4
Total Sampling Location	**16**	**15**	**8**	**7**	**9**	**7**	**7**	**6**	**7**	**14**

Fig 1. Flowchart of sample strategy. We selected ten sub-districts from three out of five districts in Special region of Yogyakarta Province (Yogyakarta city, Bantul, and Sleman districts). Samples from three sub-districts were taken weekly (identified by blue arrows), while others were taken fortnightly. Detailed type and number of samples in each sub-district are illustrated in the figure.

https://doi.org/10.1371/journal.pone.0274793.g001

Laboratory methods for wastewater and environmental samples

The wastewater samples, passive samplers and soil samples were stored in the 4˚C fridge upon arrival until the sample processing. Samples of wastewater (50 mL) or recreational water (1000 mL) were filtered through a 47 mm diameter, 0.45 μm pore size, cellulose nitrate high flow electronegative membrane (Sartorius, Germany). This filtration process was performed immediately (<2 hours) once the samples were received at the laboratory. The collection bag containing the soil samples was thoroughly mixed. In a 2 mL tube, 0.25 grams of soil and 2 mL of DNA/RNA Shield solutions (Zymo Research, USA) were added. The passive samplers were opened, and the filter membrane and q-tips were collected.

All of the processed samples from wastewater samples, passive samplers and soil samples were stored at -80˚C until the RNA extraction and reverse-transcription quantitative real-time PCR (RT-qPCR) analysis.

The RNA was extracted from samples using the QIAGEN RNeasy PowerMicrobiome Kit (QIAGEN, Germany) following manufacturer's instructions with the exception of replacing the supplied beads with PowerBead Tubes-Garnet beads (QIAGEN, Germany). For every batch of samples processed, a negative extraction controls and internal control (MS2 bacteriophage) as supplied in the PerkinElmer SARS-CoV-2 Nucleic Acid Detection Kit (RUO) (PerkinElmer) were included in the RNA extraction process to monitor the RNA extraction performance.

To detect the SARS-Cov2 RNA, a RT-qPCR was conducted using the SARS-CoV-2 Real-time RT-PCR Assay (PerkinElmer, US) and synthetic SARS-CoV-2 RNA Control 1-MT007544.1 (Twist Bioscience, Australia) as the standard curve. The kit is a multiplex assay using primers and probes targeting the Nucleocapsid (N) gene and open reading frame 1ab (ORF1ab) region of SARS-CoV-2. RT-qPCR assays were performed using two replicates of 5 μL RNA template, with a total reaction volume of 30 μL and a total 45 cycles of amplification. The quantification of the samples was calculated using the synthetic SARS-CoV-2 RNA Control 1-MT007544.1 (Twist Bioscience, Australia) as a standard curve, according to the manufacturer's instruction. The RT-qPCR assay was performed as described by the manufacturer's instruction using the LightCycler 96 instrument (Roche, Germany).

In order to report the actual value of SARS-CoV-2 RNA, we calculated the recovery efficiency. In each qPCR run, multiple SARS-CoV-2 RNA controls, a MS2 phage control (to determine the RNA recovery efficiency and as internal control) of different known concentrations and a negative control were included.

The limit of detection (LOD) for the RT-qPCR assay was determined by the analysis of 10 replicates for each dilution of the synthetic SARS-CoV-2 RNA Control 1-MT007544.1 (Twist Bioscience, Australia) analyzed and was defined as the lowest number of copies of the N gene target and ORF1ab gene that could be detected in 80% of the replicates tested. The LOD was expressed as the lowest detectable concentration of the N gene target and ORF1ab gene in sample based on the equivalent volume of sample analyzed in each RT-qPCR assay, not adjusting for any potential loss through the processing of the sample or any potential inhibition of the RT-qPCR assay [25]. All assays were performed at Microbiology laboratory at the Universitas Gadjah Mada, Special Region of Yogyakarta, Indonesia.

Ethics

The SWESP study obtained ethics approval from the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada DR. Sardjito General Hospital, Indonesia (KE/FK/0426/EC/2021, KE/FK/0514/EC/2022). Written or verbal consent was not applicable for this study as we did not collect data from individual participants.

Results

Feasibility of WBE surveillance

The average time from sample collection to availability of the RT-qPCR results was a mean of 64 hours, including the filtration time (3 to 4 hours), RNA extraction (2 to 3 hours), and RT-qPCR quantification analysis (3 hours). Both weekly and fortnightly sample collections were practical to conduct. A key challenge was the delay in the importation of critical reagents and consumables exacerbated by the COVID-19 pandemic. As the UGM laboratory is also the central clinical laboratory, priority for the analysis of clinical samples resulted in a delay in wastewater analysis during major clinical peaks in incidence. Initial trials of deployment of the passive samplers were required to limit damage or loss due to difficulties with positioning and securing samplers. We defined criteria for reliable deployment that considered locations with solid ground to safely access, ideally in an inconspicuous position, and using a strong pole or tree to secure the sampler. To avoid samplers being removed we labeled samplers with signs of warning and explanation such as "Sample for Research by Universitas Gadjah Mada and Yogyakarta Government".

The detection and positivity rates of SARS-CoV-2 RNA

Sample collection commenced on the 27th of July 2021 during the Delta wave of the COVID-19 pandemic in Indonesia. During the 10-week sampling period, a total of 544 samples were collected with 54% (296/544) of all samples testing positive for SARS-CoV-2 RNA. The median of cycle threshold (Ct) values for positive N and ORF1ab gene results was 35.1 (IQR: 32.1–36.9) and 33.9 (IQR: 30.1–35.9), respectively. The highest positivity rate was for manhole samples (74%, 191/258 samples, Fig 2) and the lowest was for soil samples (3%, 2/60 samples, Fig 2). The temporal changes in rates of sample positivity correlate with the number of confirmed cases in the community as illustrated in Fig 3. The highest positivity rate of 77%, was obtained for samples collected in July 2021 during week 1 of sample collection and decreased to 25% by

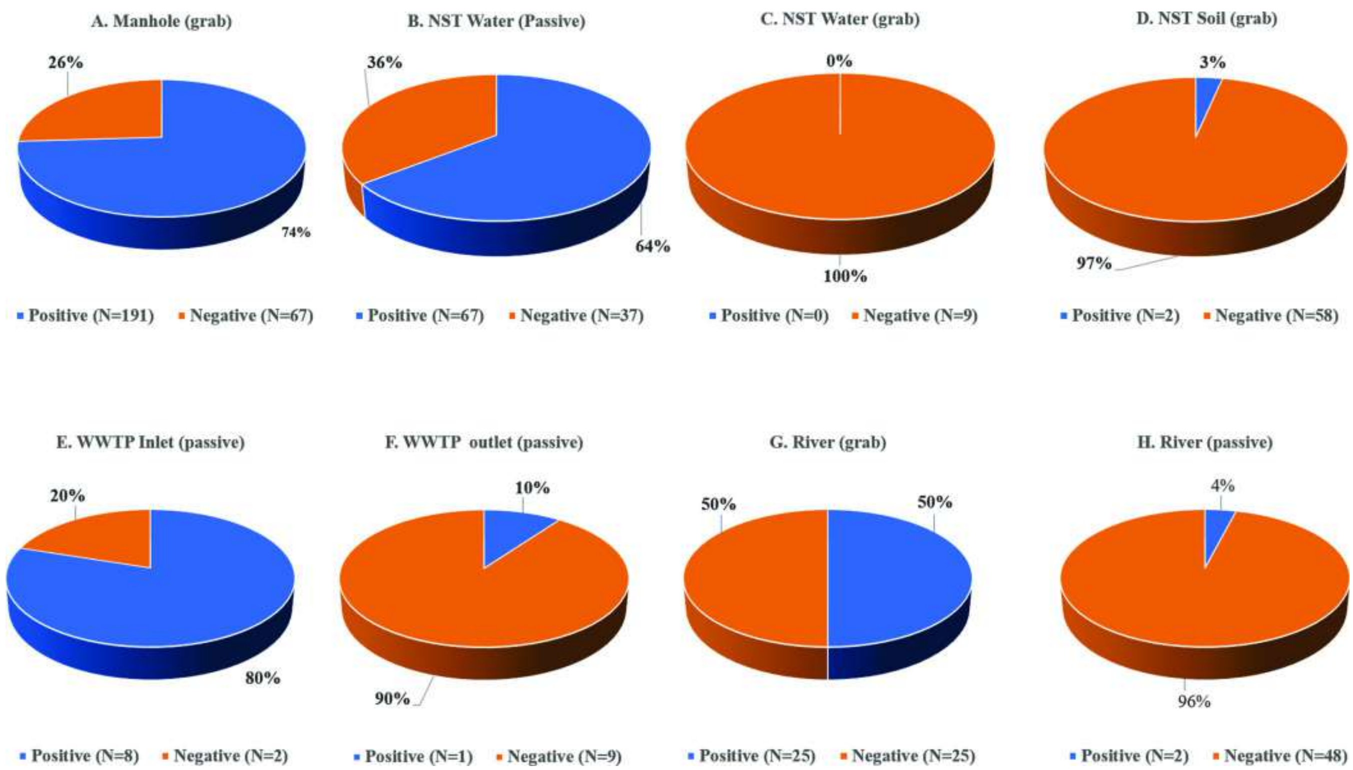


Fig 2. Nucleocapsid (N) gene positivity by sample types.

https://doi.org/10.1371/journal.pone.0274793.g002

the end of September 2021 (corresponding to week 10 of sample collection), reflecting a decreased detection rate correlating with a decrease in the incidence of reported COVID-19 clinical cases in the community.

The N gene was identified in 74% (191/258) of sewage samples (grab method), 64% (67/104) of near source tracking (NST) water samples (passive sampling method), 50% (25/50) of river samples (grab method), and 3% (2/60) of NST soil samples. This finding was consistent with the ORF1ab gene target but with a higher proportion of soils samples being positive (8%, 5/60) for the ORFlab gene as compared to the N gene (3%).

Discussion

We successfully demonstrated that WBE surveillance for SARS-CoV-2 RNA was feasible in Indonesia and reflected the SARS-CoV-2 clinical burden in the community. The high level of positivity of SARS-CoV-2 RNA in the environment in Indonesia suggests a considerable public health burden and may represent asymptomatic or mild cases that did not access health facilities for testing. Manholes consistently showed higher positivity rates in comparison with river and soil samples. Although river and soil samples showed lower positivity rates, the data are useful to complement the WBE surveillance data particularly in regions where connection to a formal sewerage system is limited. This combination of sampling strategies provides additional insights into the prevalence and distribution of COVID-19 within the community.

In Special Region of Yogyakarta province, many households are not connected to the IPAL Sewon. This may be because they were built after the IPAL Sewon infrastructure was established and therefore have no connection to the IPAL pipes. Other households were not connected due to technical reasons, such as in lower altitudes and terrain that does not support

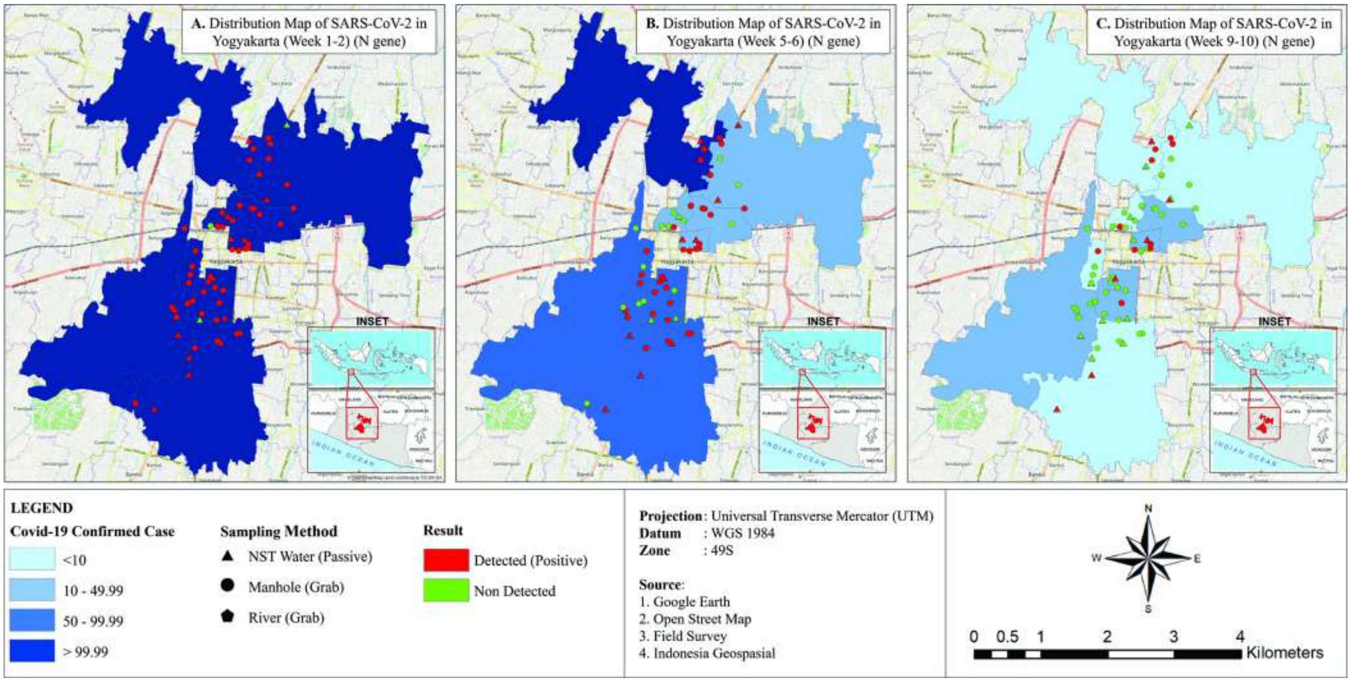


Fig 3. Distribution maps of SARS-CoV-2 in Special Region of Yogyakarta province, comparing detection targeting N gene to community confirmed cases. (A) In week 1–2 of the sample collection. (B) In week 5–6 of the sample collection. (C) In week 9–10 of the sample collection. Community COVID-19 confirmed cases were represented by blue color, the lighter the fewer cases. Detected cases in sampling locations were represented by red colored dots/triangles/pentagons, while non-detected cases were represented by green colored dots/triangles/pentagons. With circles denoting manholes, pentagons denoting river and triangles denoting NST water.

https://doi.org/10.1371/journal.pone.0274793.g003

passive gravitational flow of wastewater to the central WWTP. However, in this study we managed to collect samples from community WWTPs and septic tanks from NST sites to capture communities that were not served by the central WWTP.

Although we found that both weekly or fortnightly collection frequency with grab and/or passive sampling collection methods as feasible, weekly collections were preferred in order to provide real-time data to inform the public health response. The laboratory capacity to conduct qualitative (positive/negative) and quantitative identification of SARS-CoV-2 RNA in the environmental samples (wastewater and soil) were also feasible although some pre-processing procedures need to be conducted prior to the RT-qPCR procedure (i.e., wastewater filtration and soil homogenization). There were challenges in providing real-time results during peak COVID-19 outbreaks due to overburdened staff and limited access to equipment, and therefore, ideally WBE surveillance should be integrated into the routine surveillance programs with dedicated staff. Additionally, the availability of imported reagents has delayed laboratory analysis during periods of high output. Local epidemiological data describing the distribution of COVID-19 cases (symptomatic and asymptomatic) with laboratory confirmed positive tests for SARS-CoV-2 infections by sub district, on a weekly basis, were available to compare with the findings from WBE surveillance. However, data analysis to link environmental and community data remains challenging and needs further exploration.

Despite efforts, there remain practical limitations of WBE surveillance in LIMCs. It is likely that wastewater sampling of the reticulated sewerage system reflects the more modern and affluent sector of the city and may not provide meaningful insights into the presence of SARS-CoV-2 infection within the broader community. Most of the city and rural areas manage human effluent via septic tanks, pit latrines or by open defecation with subsequent contamination of surface water and rivers. Therefore, to understand the distribution of SARS-CoV-2 RNA in environments that reflect the presence of community infections with fragmented wastewater infrastructures, NST sites, and in places where people publicly congregate were selected. These sites include permanent dwellings (apartment and flats), temporary living places (hotels), public spaces (traditional markets, town squares, mosques, and a public swimming pool), rivers, working spaces (both office and factory), and COVID-19 shelters (facilities which are designated as temporary quarantine shelters for people testing positive for COVID-19). This WBE approaches using NST may allow detection of targeted clusters for whom rapid action may reduce or prevent the risk of larger outbreaks within the community [26].

It has been proposed that WBE surveillance has the potential to act as an early warning system (EWS) for COVID-19 outbreaks [27–32]. This should be conducted in collaboration with the public health authorities to enable the timely follow up of positive detections by strategies such as contact tracing, strengthening health protocols, or implementing a community lockdown. This could be broadly implemented across the community or in a targeted response depending on the local context and level of concern. For instance, if SARS-CoV-2 RNA is detected (positive result) in the sewerage sample in an area where there had consistently been no detections (negative result), then a lockdown or mass screening could be implemented in the area drained by the sewerage system; or if the result is taken from a closed community (e.g., Boarding school), contact tracing within the community should be conducted immediately.

Conclusions

In conclusion, an environmental surveillance system for SARS-CoV-2 in Indonesia is feasible and can be used to monitor the community burden of SARS-CoV-2 infection. However, future research is needed to explore its potential to act as an EWS for the early identification of SARS-CoV-2 outbreaks within a community, especially in regions with limited access to clinical testing. Although the sewer infrastructure of wastewater systems is quite limited in Indonesia, an expanded sampling approach based on the local context and including NST can support an effective SARS-COV-2 surveillance program.

Acknowledgments

We would like to thank the wastewater treatment plant team, field assistants and laboratory team for doing sampling collection and laboratory works. We thank Rizka Dinari for providing editorial assistance.

Author Contributions

Conceptualization: Indah K. Murni, Vicka Oktaria, Amanda Handley, David T. McCarthy, Celeste M. Donato, Titik Nuryastuti, Julie E. Bines.

Data curation: Amanda Handley, Endah Supriyati, Dwi Astuti Dharma Putri, Hendri Marinda Sari.

Formal analysis: Indah K. Murni, Vicka Oktaria, Dwi Astuti Dharma Putri.

Funding acquisition: Amanda Handley, Julie E. Bines.

Investigation: Indah K. Murni, Vicka Oktaria, Amanda Handley, David T. McCarthy, Celeste M. Donato, Julie E. Bines.

Methodology: Indah K. Murni, Vicka Oktaria, Amanda Handley, David T. McCarthy, Celeste M. Donato, Titik Nuryastuti, Endah Supriyati, Julie E. Bines.

Project administration: Amanda Handley, Endah Supriyati, Dwi Astuti Dharma Putri, Hendri Marinda Sari.

Resources: Indah K. Murni, Vicka Oktaria, David T. McCarthy, Titik Nuryastuti, Julie E. Bines.

Supervision: Indah K. Murni, Vicka Oktaria, Titik Nuryastuti, Ida Safitri Laksono, Jarir At Thobari, Julie E. Bines.

Validation: Indah K. Murni, Vicka Oktaria, Amanda Handley, David T. McCarthy, Titik Nuryastuti, Endah Supriyati, Julie E. Bines.

Writing – original draft: Indah K. Murni.

Writing – review & editing: Indah K. Murni, Vicka Oktaria, Amanda Handley, David T. McCarthy, Celeste M. Donato, Titik Nuryastuti, Endah Supriyati, Dwi Astuti Dharma Putri, Hendri Marinda Sari, Ida Safitri Laksono, Jarir At Thobari, Julie E. Bines.

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