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EDITED BY

Mohammad Hoque,
University of Portsmouth, United Kingdom

REVIEWED BY

Jakariya Md.,
North South University, Bangladesh
Ananda Tiwari,
University of Helsinki, Finland

*CORRESPONDENCE

Clive Lipchin
✉ clivearava@gmail.com
Ariel Kushmaro
✉ arielkus@bgu.ac.il

†These authors have contributed equally to this work

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Wastewater monitoring of SARS-CoV-2 in on-grid, partially and fully off-grid Bedouin communities in Southern Israel

Karin Yaniv^{1†}, Hillary A. Craddock^{2†}, Fared Mahameed³, Marilou Shagan¹, Ikram Salah², Satish Lakkakula¹, Keren Resnick², Corinne Haber², Nadav Davidovitch², Jacob Moran-Gilad², Ariel Kushmaro^{1,4,5*} and Clive Lipchin^{3*}

¹Avram and Stella Goldstein-Goren Department of Biotechnology Engineering, Ben-Gurion University of the Negev, Beer-Sheva, Israel, ²Department of Health Policy and Management, School of Public Health, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel, ³Center for Transboundary Water Management, Arava Institute for Environmental Studies, Ketura, Israel, ⁴The Ilse Katz Center for Nanoscale Science and Technology, Ben-Gurion University of the Negev, Beer-Sheva, Israel, ⁵School of Sustainability and Climate Change, Ben-Gurion University of the Negev, Beer-Sheva, Israel

Background: Wastewater based epidemiology (WBE) has become an important tool in SARS-CoV-2 surveillance and epidemiology. While WBE measurements generally correlate with observed case numbers in large municipal areas on sewer grids, there are few studies on its utility in communities that are off-grid (non-sewered).

Methods and materials: To explore the applicability of wastewater surveillance in our region, five Bedouin communities along the Hebron Stream in Southern Israel (Negev desert) were sampled. One point (El-Sayed) represents a community with partial connection to the sewer grid system and another point (Um Batin) represents a community with no access to the sewer grid system. The towns of Hura, Lakia, and Tel Al-Sabi/Tel Sheva were on-grid. A total of 87 samples were collected between August 2020 to January 2021 using both grab and composite sampling. RNA was extracted from the raw sewage and concentrated sewage. RT-qPCR was carried out with N1, N2, and N3 gene targets, and findings were compared to human case data from the Israeli Ministry of Health.

Results: SARS-CoV-2 was detected consistently over time in on-grid Bedouin towns (Lakia, Tel Sheva/Tel as-Sabi, and Hura) and inconsistently in smaller, off-grid communities (El-Sayed and Um Batin). The trend in maximum copy number/L appears to be driven by population size. When comparing case numbers normalized to population size, the amount of gene copies/L was inconsistently related to reported case numbers. SARS-CoV-2 was also detected from sewage-impacted environmental waters representing communities with no access to the wastewater grid. When grab sampling and composite sampling data were compared, results were generally comparable however composite sampling produced superior results.

Conclusions: The mismatch observed between detected virus and reported cases could indicate asymptomatic or "silent" community transmission, under-testing within these communities (due to factors like mistrust in government, stigma, misinformation) or a combination therein. While the exact reason for the mismatch between environmental SARS-CoV-2 signals and case numbers remains unresolved, these findings suggest that sewage surveillance, including

grab sampling methodologies, can be a critical aspect of outbreak surveillance and control in areas with insufficient human testing and off-grid communities.

KEYWORDS

wastewater monitoring, off-grid, WBE, Bedouin communities, SARS-CoV-2

1. Introduction

While the primary mode of surveillance for SARS-CoV-2 has been individual testing of clinical samples, wastewater sampling to assess community-level COVID-19 activity is gaining in popularity (Ahmed et al., 2020, 2021; Nemudryi et al., 2020; Scott et al., 2021; Yaniv et al., 2021a). Although vaccinations have curtailed disease levels (Fontanet et al., 2021), the emergence of variants of concern as well as surges of cases in unvaccinated populations and reduction in vaccine effectiveness call for continued surveillance. Using a relatively passive, community-level surveillance methodology such as wastewater-based epidemiology (WBE) has the potential to provide beneficial data under such circumstances. WBE is useful for monitoring variants, as surveillance can continue with standard gene targets (which often focus on well-conserved areas of the genome) while variants can be observed via specific PCR screens design (based on specific mutations) or through sequencing efforts.

Although the main SARS-CoV-2 transmission route is through the respiratory system, the virus can be found in other bodily fluids including stool. A study by Zheng et al. (2020), shows that the viral shedding period is longer in stool than in respiratory samples and for the most part can last for up to 30 days after infection. The clinical surveillance for SARS-CoV-2 is limited by personal willingness to be tested, access to testing and overall healthcare system testing capacity. WBE has been previously employed for monitoring other microbial and viral diseases, including the rapid development of assays for emerging diseases such as monkeypox (Tiwari et al., 2022a) and established surveillance protocols for ancient threats like polio, distribution of antibiotic resistance or even chemical water pollution (Asghar et al., 2014; Elkayam et al., 2018; Lorenzo and Picó, 2019; Pruden et al., 2021; Tiwari et al., 2022b). In Israel, virus surveillance in wastewater has been utilized for several decades and played a central role in the response to the silent poliovirus outbreak of 2013/14 (Manor et al., 2014; Shulman et al., 2016; Brouwer et al., 2018). Since the beginning of the COVID-19 pandemic, many studies have been conducted on optimizing and utilizing WBE for estimations of viral burden (Lorenzo and Picó, 2019; Hart and Halden, 2020; Polo et al., 2020; Hemalatha et al., 2021; Wurtzer et al., 2021; Yaniv et al., 2021a,b, 2022), however there is a lack of studies investigating WBE to monitor SARS-CoV-2 in under-resourced and under-served communities (Street et al., 2020). Specifically, there have been few studies investigating WBE in communities not connected or partially-connected to a wastewater grid and research has called for more effort in this regard (Aguilar-Oliveira et al., 2020; Street et al., 2020; Iglesias et al., 2021; Driver et al., 2022; Islam et al., 2023; Naughton et al., 2023).

The Bedouin communities are an indigenous Arab ethnic group residing within Israel that have been documented as facing greater social and health disparities when compared to the majority of the Israeli population (Razon, 2016; Abu-Rabia-Queeder, 2017; Daoud et al., 2018) as well as disparities in terms of infrastructure access (Porob et al., 2020). This represents a situation where there are difficulties in acquiring accurate data via clinical surveillance or wastewater-based surveillance, as individuals may not be willing or able to access healthcare services (Voeten et al., 2009; Hermesh et al., 2020). The COVID-19 response in the region has shown geopolitical discrepancy, potentially fostering similar trends of mistrust (Negev et al., 2021). Even within Israel, a recent study among the various Arab ethnic groups found that there is a discrepancy and mistrust in the healthcare system and increased psychological stress regarding the COVID-19 pandemic was observed in certain groups (Lavie et al., 2020; Satran et al., 2021). Therefore, the Bedouin in Israel represent a population where testing for coronavirus could be impacted negatively by numerous factors, including but not limited to healthcare access disparities, information access, and trust in service providers and the government. Simultaneously, WBE is limited in these communities as they are frequently denied access to the wastewater grid, therefore they will not be adequately represented in national surveillance efforts (Porob et al., 2020; Bar-Or et al., 2021a). In this study WBE was used to assess SARS-CoV-2 in wastewater in on-grid, partially off-grid, and fully off-grid Bedouin communities and compare it to clinical surveillance data in order to assess differences in clinical and sewage data, which has utility both in Israel as well as globally in communities with disparities in healthcare access and infrastructure.

2. Materials and methods

2.1. Sample collection

Samples were collected from five Bedouin communities (Figure 1), all of which are recognized by the Israeli government but represent different levels of connectivity to the wastewater grid. Hura, Tel Sheva/Tel al-Sabi, and Lakia are fully connected to the wastewater grid, El-Sayed village is partially on the wastewater grid and partially off-grid, and Um Batin is a recognized village with no access to the grid. Sample collection methods that this was undertaken with permission and involvement of the Al-Kaysom, Hura, and Lakia regional councils. Off-grid villages are completely without connection to the wastewater grid (i.e., non-sewered); wastewater (including solids and supernatant) is stored in unlined

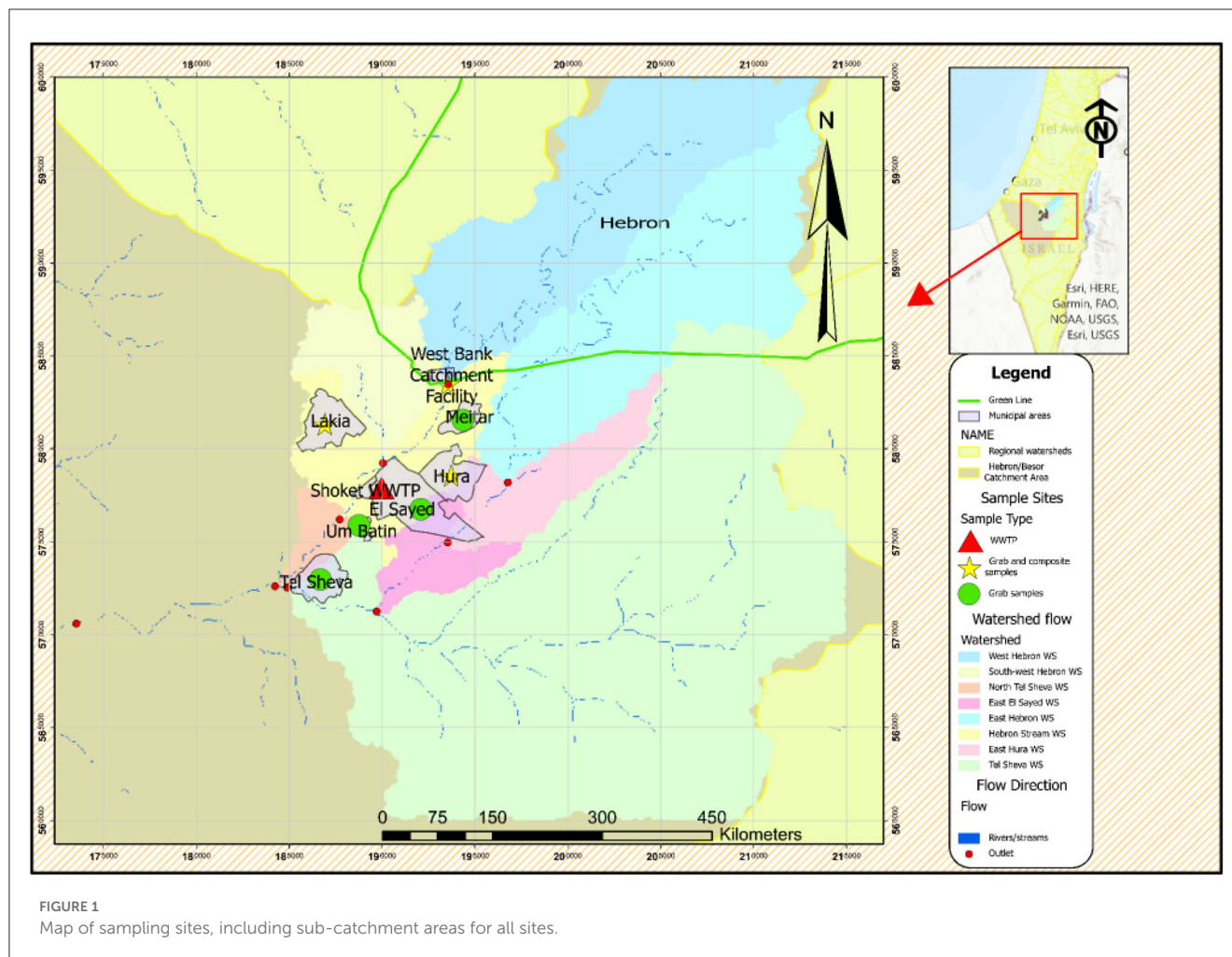


FIGURE 1
Map of sampling sites, including sub-catchment areas for all sites.

cesspits. Wastewater leaches into the ground, and cesspits are sealed when full.

Samples for partially-off grid and on-grid municipalities were collected from the Shoket WWTP. This WWTP treats $\sim 15,000 \text{ m}^3$ per day, and it uses activated sludge for tertiary water treatment. Shoket services municipalities including Meitar, Carmit, Hura, Lakiya, and the wastewater from the southern West Bank that is first received at the West Bank catchment (WBCF) facility located on the Green Line. The WBCF was constructed by Israel to handle the treated sewage, untreated sewage, and industrial effluent discharged from Hebron and surrounding Palestinian areas into the Hebron stream which crosses the Green Line into Israel. The primary purpose of the catchment facility is to lower the turbidity of the sewage which is extremely high due to the large influx of stone cutting slurry that is very high in calcium carbonate. The facility uses a coagulant polymer and mechanical press to lower the turbidity of the sewage. Once this is done the sewage is sent by pipeline to the Shoket WWTP where it is mixed and treated with local municipal sewage.

Estimated populations and spelling of municipality names in Arabic and Hebrew are in [Supplementary Table 1](#) (Israeli Central Bureau of Statistics, 2019). Samples from Um Batin

were collected from an environmental water source (Hebron Stream) via large-volume (100 L) grab sampling methodology. Grab and composite samples were also collected from two upstream sampling points, Meitar, which is upstream of Um Batin, and Hebron Catchment, which is upstream of Meitar) along the Hebron Stream as comparison sites (Figure 1).

Samples from the West Bank Sewage Catchment facility, which represents surface water as well as untreated sewage from off-grid communities gathered at the point where the Hebron Stream crosses the Green Line into Israel, was collected via grab sample and autosampler methodologies and used for protocol development and comparison. Stormwater drains to natural valleys and is only integrated into the wastewater system in the situation of the WBCF. Samples were collected via grab samplers and 18-h composite autosampler methodologies (as described in Yaniv et al. (2021a)). The automated sampler pumped 250 mL every half hour between 5 a.m. to 11 p.m. on a Saturday. Upon arrival at the lab, samples were kept at 4°C for up to 2 days until processing them for virus concentration and RNA extraction. Full details on sampling dates and locations are in [Supplementary Table 2](#).

2.2. Sample processing, concentration, and RNA extraction

For the grab samples, the following protocol was used. Prior to centrifugation of 50 mL to remove solids, 200 μ L of raw sewage was aliquoted for direct extraction and stored at 4°C. The 50 mL aliquot of sewage was spun for 30 min at 4,700 RCF at 4°C over centrifugal devices with a 10 kDa filter size (Pall Corporation, Port Washington, NY, USA and Merck, Darmstadt, Germany) (Ahmed et al., 2020; Gonçalves et al., 2021).

For the composite and surface water samples, the following protocol was used. The samples were concentrated using dialysis filters with 3 to 30 nanometer pore size (recycled dialysis filter, NUFiltration[®], Caesarea, Israel) by “dead end” flow. In the case of “dead-end” flow, the applied pressure drives the entire water flow through a membrane filter (similar to the procedure described in Obayomi et al., 2019). The filter was then back-washed with 0.07–0.1 Liter of distilled water. For composite samples, all initial and final volumes are reported in [Supplementary Table 3](#).

For extraction of all samples, 200 μ L of filtered samples (raw or concentrated) underwent RNA extraction using the Machery-Nagel NucleoSpin RNA isolation kit (catalog number 740955, Düren, Germany). Five microliter of 20 mg per mL Proteinase K (cas number 70663-4, Sigma Aldrich, St. Louis, Missouri, US) and five μ L of MS2 phage at a 10^6 copies per μ L concentration were added to the lysis step. The RNA was eluted with 50 μ L of nuclease-free water.

2.3. RNA quantification

Grab samples were subject to RT-qPCR on the MIC thermocycler (BioMolecular Systems, Upper Coomera, Australia) and composite samples were subject to RT-qPCR on the Applied Biosystems (Thermo Scientific, Waltham, MA, USA) qPCR instrument. N1 and N2 gene targets were quantified on both devices using United States Centers for Disease Control and Prevention primer probe mixtures (catalog number 10006713, IDT, Coralville, IA, USA) and recommended cycling conditions (US Centers for Disease Control Prevention, 2020), and the N3 gene target was quantified with the N3R primer-probe set (Yaniv et al., 2021a). The initial filtration volumes and the final concentrated fraction for each sample were used to calculate the final RNA gene copies/Liter of wastewater. Calibration curves for Thermo Scientific and MIC devices are included in the [Supplementary Figure 1](#). As a part of quality control, we calculated MS2 recovery (Yaniv et al., 2021b) percentage. If the recovery percentage was lower than 5%, a second RNA extraction or sample concentration was performed. Low recovery percentage can imply inhibition presence.

2.4. Epidemiological data and statistical analysis

Israeli cases and test data were extracted from publicly available Israeli Ministry of Health databases. Data extracted

included daily total case numbers by municipality and daily number of tests administered by municipality, and these data were used to calculate cases per day as well as percent positive tests per day by municipality. Data were extracted from relevant date ranges to provide concordant data with sewage sampling dates by municipality. Due to the discrepancy in size among the studied municipalities, case and test data were normalized to population size. All parameters use in this study can be found in the [Appendix](#) (cases and tests per 10,000 people). Statistics including frequency comparison (via chi squared and Fisher's exact tests) and means comparisons (via Mann Whitney and Kruskal Wallis tests) regarding the comparison of composite to grab samples) were carried out in R (Version 4.0.2), specifically the psych package. Maps were generated using ArcGIS Pro (Version: 2.9.5, ESRI, Redlands CA, USA).

3. Results

3.1. Comparison with case numbers and test numbers

[Figure 2](#) presents copy number overlaid with case data for all four sites sampled in the study period (August 2020 to January 2021). Regarding epidemiology parameters as measured by the Ministry of Health (active cases, number of tests, and percentage of tests found to be positive), some fluctuations and patterns can be observed. In Hura, a major virus activity peak was evident from mid-November to mid-December 2020. In Lafia, the main peak was observed from late August throughout September 2020 and a minor peak at the end of 2020 until the beginning of 2021. In Tel Sheva/Tel al-Sabi, more modest case rates were reported, with higher numbers of cases during December 2020 and January 2021. In El-Sayed, no large peaks were observed as the total test number per day remained very low (under 10 tests per day). All peaks correspond to peaks in the total number of tests, suggesting that asymptomatic or subclinical cases may have gone undiagnosed outside of these waves of infection. SARS-CoV-2 was detected in Hura and Lafia during the first sampling efforts in August of 2020, however at this timepoint only Lafia had a wave of testing and subsequent reported cases, indicating the possibility of silent, or unreported, transmission. In Hura, Lafia, and Tel Sheva/Tel al-Sabi, spikes in observed SARS-CoV-2N gene were observed 2–3 weeks before spikes in observed cases. In El-Sayed, spikes in observed SARS-CoV-2N gene were inconsistently associated with observed cases. Given that this community is only partially on-grid, it is possible that at some points positive sewage was not part of the sampled catchment area. Overall, the highest observed copy numbers per sample were observed in the larger communities ([Supplementary Figure 2](#)). When observing trends by, the number of N2 gene copies/L was generally higher in the larger communities. This trend was not as strong in regards to N1 gene copies/L ([Supplementary Figure 2](#)).

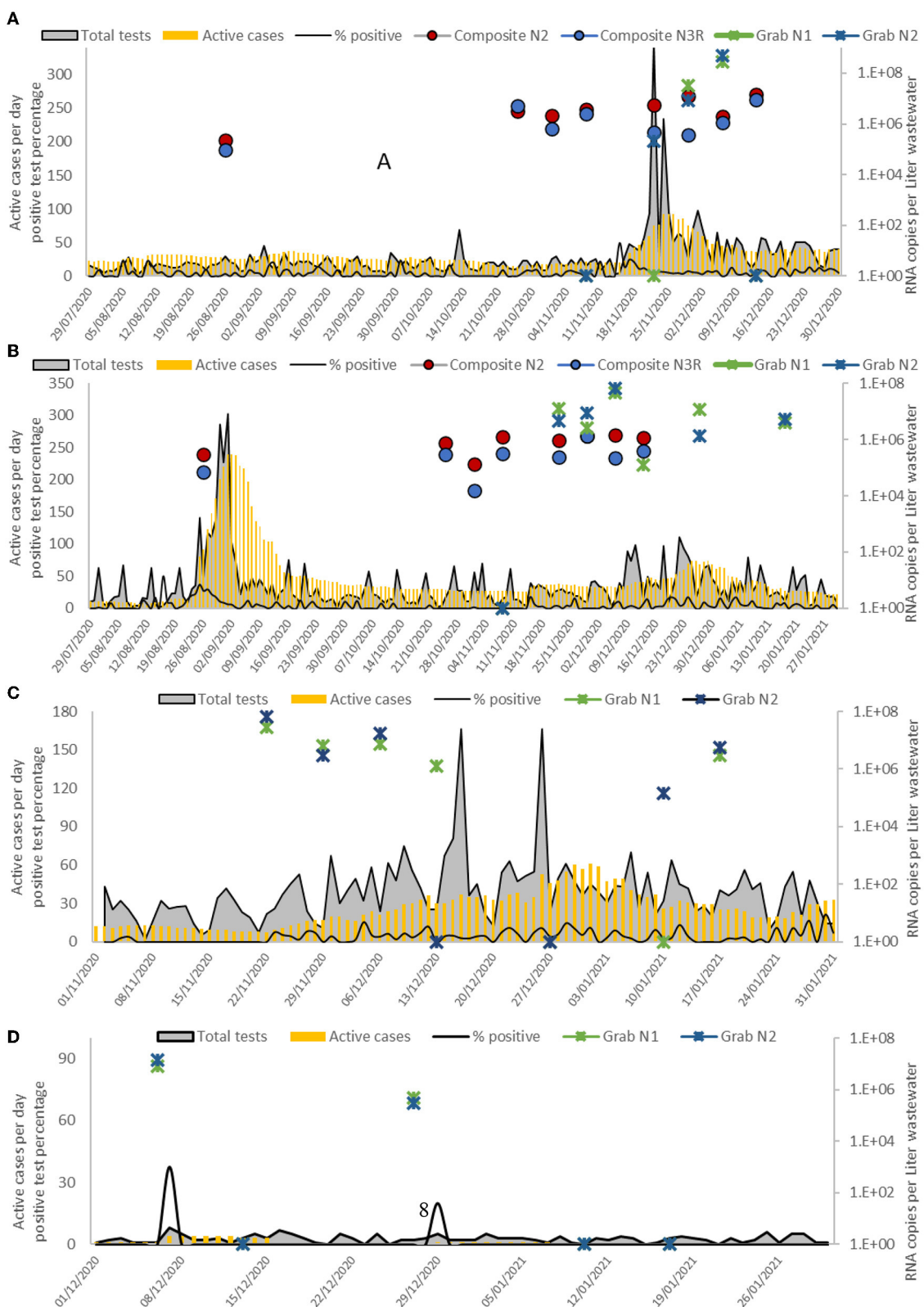
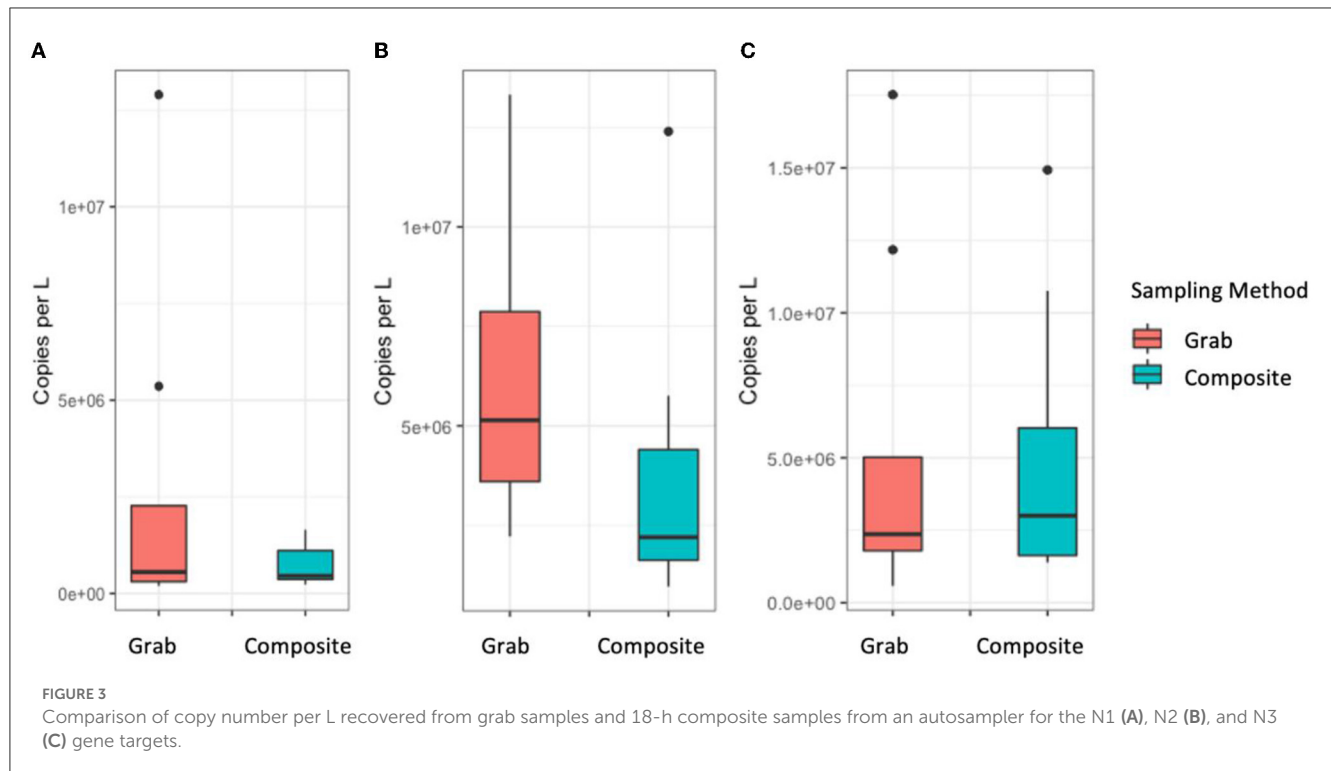


FIGURE 2 SARS-CoV-2 detection in four sampling locations- Hura (A), Lokia (B), Tel-Sheva/Tel al-Sabi (C), and El-Sayed (D) during the study time period (X-axis). Right Y axis presents RNA copy numbers per Liter of wastewater per RT-qPCR results. A Circle icon present composite sampling, blue for N3R detection set and red for N2 detection set. Asterisk icons indicate grab sampling, green for N1 detection set and blue for N2 detection set. In a case of Not Detected result, a value of one appears on the graph. The left Y-axis presents current confirmed cases per 10,000 people (yellow bars), positive test percentage (black line) and total COVID-19 tests performed per 10,000 people (in gray).



3.2. Detection in grab vs. composite samples

A total of 14 pairs of grab and composite samples were available for analysis. Statistically the differences were not significant for N1 ($p = 0.6$) and N3 ($p = 0.3$). N2 ($p = 0.06$) was approaching significance. Copy numbers were comparable for N1 (Composite: Mean: 5.1×10^6 Range: 1.4×10^6 – 9.3×10^6 , Grab: Mean: 5.1×10^6 , Range: 5.8×10^5 – 1.7×10^7) and N3 (Composite: Mean: 6.8×10^5 Range: 2.3×10^5 – 6.5×10^5 , Grab: Mean: 2.7×10^6 Range: 1.9×10^5 – 1.3×10^7), and slightly higher for grab sample data for N2 (Composite: Mean: 3.8×10^6 Range: 4.0×10^6 – 9.6×10^6 , Grab: Mean: 6.5×10^6 , Range: 2.2×10^6 – 1.3×10^7) (Figure 3, Supplementary Table 4). Of the composite samples, 100% were detected with at least one gene target. Of the grab samples, four samples (28.6%) were negative for all gene targets. When frequencies were compared there was not a statistically significant difference in overall detection frequency for the N1 and N2 gene targets (p -value = 0.3 and 0.2, respectively), however the difference was statistically significant for N3 ($p = 0.004$).

3.3. Concentration of environmental waters to test fully off-grid communities

Environmental water samples (Grab samples) were collected from three points along the Hebron River, a transboundary waterway originating near Hebron, West Bank, Palestinian Territories which passes through numerous Israeli towns and villages after crossing the Green Line (Figure 1). Um Batin sewage discharges into the Hebron River, and it was observed that sampling

points near Um Batin were positive for SARS-CoV-2 RNA at one timepoint in October 2020 and one in January 2021, but not at an additional timepoint in January 2021 (Supplementary Table 5). During and in the week preceding the October timepoint there were no recorded active cases in Um Batin, and during the entire January sampling timeframe there were 1–2 recorded active cases in Um Batin (Data not shown). It should be noted that in timepoints where upstream (Meitar) points are positive for SARS-CoV-2, Um Batin is negative, suggesting that dilution effect and/or surface water treatment at the Shoket WWTP (Figure 1) reduced the virus levels below detection limit.

4. Discussion

4.1. Summary of findings

In this study we investigated SARS-CoV-2 in sewage-impacted environmental waters as well as utilizing WBE in on-grid and partially off-grid communities. It is known that the communities sampled experience disparities regarding healthcare access and utilization, ergo it should be noted that peaks in SARS-CoV-2 quantification are not completely aligned with known cases in these communities. However, it should be also noted that testing rates were overall low and case rates were closely associated with testing rates. While large, on-grid municipalities (i.e., Hura) had more robust data, grab samples and composite samples from smaller and partially-off-grid municipalities were able to detect SARS-CoV-2. Copy numbers/L were in line with findings from other municipalities in Israel in other studies (Yaniv et al., 2021a,b). Furthermore, large-volume grab samples of surface water

impacted by sewage from fully off-grid communities were able to detect SARS-CoV-2.

Throughout the COVID-19 outbreak and including the study period described here, Yaniv et al. (2021b) monitored wastewater samples from Beer-Sheva and several other non-Bedouin locations in Israel (August 2020–October 2021) for the presence of SARS-CoV-2 virus using RT-qPCR including CDC's standard detection set. They documented the SARS-CoV-2 RNA levels in wastewater and monitored these values together with the corresponding reported active cases and percent vaccination in the city populations. It was evident that an increase in detected SARS-CoV-2 RNA levels in the city wastewater appeared just prior to every one of the three peaks in reported active cases. Thus, the appearance in the city's wastewater foresaw the increase in reported clinical cases of COVID-19 (Yaniv et al., 2021b). Given the findings of Yaniv et al. (2021a,b) as well as other studies that have noted strong correlations between epidemiological data and sewage data, the lack of alignment of these data in communities with access disparities is noteworthy (Gonzalez et al., 2020; Ahmed et al., 2021; Bar-Or et al., 2021b). The findings of this study support the hypothesis regarding “under the radar” morbidity and emphasize the importance of using WBE to learn about morbidity levels within the community (Hamouda et al., 2021; Karthikeyan et al., 2021; Lee et al., 2022).

4.2. Comparison of composite and grab samples

Composite samples identified SARS-CoV-2 RNA with greater frequency than grab samples, however when compared statistically the detection frequency was not significantly different. While composite sampling is undeniably the preferred standard for wastewater surveillance, it can be logistically and economically difficult to set up in low-income communities, and even if setup is accomplished it may not be sustainable (D'Aoust et al., 2021). Composite sampling raises questions of sustainability in terms of equipment functionality over time, maintenance, access and affordability of replacement parts or units, and costs and access to electricity for running the systems. One question of debate is the relevance of grab vs. composite samples when surveying for SARS-CoV-2 at various catchment scales (George et al., 2022). George et al. (2022) demonstrated that at the high-flow site, grab samples were reasonably comparable to composite samples. However, as the flow rates decreased, the percentage of false-negative grab samples increased and the SARS-CoV-2 concentrations of grab samples varied by up to 1–2 orders of magnitude compared to their respective composite sample concentrations (George et al., 2022). They further conclude that composite sampling is superior to grab sampling, especially as flow decreases. Therefore, for municipalities seeking to augment surveillance for SARS-CoV-2 but without the resources to install and maintain autosamplers, grab sampling is an acceptable method for WBE however flow rates must be considered and areas of higher-flow should be prioritized.

4.3. Implications for off-grid communities and sewage-impacted surface waters

When considering these issues of flow as well as grid access, it is important to consider the implications of these findings for populations not fully connected to the wastewater grid. In this study, we were able to access a WWTP that served a community partially connected to the grid as well as a surface water site known to receive untreated sewage from an off-grid community. This is in line with a recent strategy employed by researchers in Bangladesh, who employed a methodology for off-grid settings that focused on “hotspot” or “nodal” sampling via surface water sites or community gathering places. Using this modality, they were able to effectively track COVID-19 dynamics in connected and unconnected populations (Jakariya et al., 2021). A study from Ecuador investigating rivers impacted by the sewage of cities noted a viral copy load similar to that of raw WWTP influent in other countries and a study of rivers in Brazil also reported observing SARS-CoV-2 loads tracked with population-level case data (Aguiar-Oliveira et al., 2020; Guerrero-Latorre et al., 2020). The level and detection of SARS-CoV-2 genetic material in our surface water sample was low, however considering that our sampling points along the Hebron Stream represented the sewage of a population of under 5,000, the low level of viral material is reasonable. A study in Japan noted that river water receiving fully treated wastewater did not test positive for SARS-CoV-2 RNA (Haramoto et al., 2020), whereas an Italian study investigating river water receiving combined sewage overflow and improperly treated wastewater did identify SARS-CoV-2 genetic material in surface water (Rimoldi et al., 2020). As such, future studies investigating regions with combined on-grid and off-grid populations should consider the presence of improperly treated wastewater in the entire surface water catchment area.

4.4. Limitations and future research needs

This study was limited in terms of numbers of sampling sites as well as in being able to sample in all locations at all timepoints. It represents a proof of concept of what may be accomplished in a pandemic scenario in under-served communities. Such studies may be difficult, in particular during periods of country-wide lockdowns requiring researchers to obtain essential worker permits and due to delays in obtaining reagents due to supply chain disruptions. It is recommended that future WBE research be carried out in non-pandemic scenarios (i.e., with other diseases such as Polio, Brucellosis, or seasonal influenza) with more comprehensive sampling to fully understand how sewer grid access, healthcare access, and health inequality interface in regards to the utility of WBE (Heijnen and Medema, 2011). This study also focused on Israel's southern Bedouin population, and further research even within Israel itself is needed among various rural off-grid populations experiencing infrastructure access and healthcare inequality as well as healthcare system mistrust to establish broad applicability.

5. Conclusions

Numerous studies have emerged detailing the utility of WBE regarding SARS-CoV-2 surveillance as well as surveillance for other pathogenic threats such as antimicrobial resistance (Asghar et al., 2014; Thakali et al., 2022; Tiwari et al., 2022a,b). These studies have primarily been carried out in communities with greater resources primarily representing on-grid communities, however, given the resource limitations and supply chain disruptions observed during the COVID-19 pandemic, it is critical to ensure that all communities can utilize surveillance methods like WBE. The findings of this study suggest that sampling, with grab sample or composite sample methods, can identify SARS-CoV-2 virus in wastewater from communities on the grid, partially on the grid, and off-grid communities. Furthermore, given that the communities of focus in this study experience health inequalities so case data may not represent the full picture of infection in the community. These findings are also useful for SARS-CoV-2 surveillance for communities without infrastructure or equitable access to healthcare resources.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

KY and HC: data curation, formal analysis, investigation, methodology, supervision, visualization, writing—original draft, and writing—review and editing. FM: data curation, investigation, project administration, and writing—review and editing. MS, SL, KR, and CH: data curation, investigation, and writing—review and editing. IS: data curation, formal analysis, methodology, investigation, and writing—review and editing. ND: conceptualization, data curation, and writing—review and editing. JM-G and AK: conceptualization, funding acquisition, methodology, project administration, resources, supervision, writing—original draft, and writing—review and editing. CL: conceptualization, funding acquisition, project administration, supervision, and writing—review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/frwa.2023.1136066/full#supplementary-material>

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