Evidence-based wastewater monitoring and policy for addressing the COVID-19 pandemic in Israel

This study demonstrates our ability to detect SARS-CoV-2 virus RNA in sewage using qPCR in (recognized Bedouin villages connected to the grid) and also in off grid areas (Figure 1 & Figure 2). While most of the world targets wastewater treatment plants (WWTP) for monitoring surveillance of SARS-CoV-2 virus, here we tracked the virus throughout the wastewater system and natural resources. In our attempts to improve the limits of SARS-CoV-2 detection in sewage samples, we employed dialysis concentration method using NUFiltrartion© filters. To establish this monitoring method, we tested samples from the different areas starting October 2020 through January 2021.

Figure 1: Sampling sites of the Bedouin villages. three composite samples were sampled using automatic sampler (KanDo) and they are: Hura (23062 inhabitants) , Lakia (which was sampled at Shoket WWTF , 14617 inhabitants) and the sewage catchment facility (mixed sewage) located on the Green Line. 100 litres per sample per site were taken from two off grid sites (Um Batin 1 & Um Batin 2) and a third was taken from Meitar/Hura off grid site.

Figure 2: Sampling sites of the Bedouin villages. two composite samples were sampled using automatic sampler (KanDo) and they are: Hura (23062 inhabitants), Lakia (which was sampled in Shoket WWTF , 14617 inhabitants). Grab samples were taken from all the Bedouin villages on the map: Hura, Lakia, Elsayed (6059 inhabitants), Tel-Elsabee (Tel-Sheva-21531 inhabitants) and Um-Batin (4481 inhabitants).

To achieve our goal, we have monitored the villages of Hura, Lakia and the West Bank catchment facility on the Green Line (Figure 3) and along the Hebron River (Table 1). Samples were taken each week or more from October 2020 to January 2021 as indicated next to each location. All samples were tested for SARS-CoV-2 RNA in both raw sewage and concentrated (dialysis). Copy number per Liter was calculated according to calibration curves and volume taken from each sampling point. For raw sewage, limit of detection is $6.37*10^4$ copies/L and $1.3 \cdot 10^3$ copies/L for concentrated sewage. Figure 2 presents the N gene copy number in different dates for the sampled areas this research tested. We can see that only one sample point was not detected (ND) at the raw sewage level in the West Bank catchment facility location, while all sampling points throughout October 2020 and November 2020 showed positive detection at the raw and concentrated sewage level. Although concentration efficiency differed from sample to sample, results showed 10-fold reductions in limit of detection, which means better detection sensitivity that will result in better monitoring and early warnings of disease outbreaks. This analysis provides a proof of concept for monitoring viral load trends during the sampling period and comparison between sampling sites.

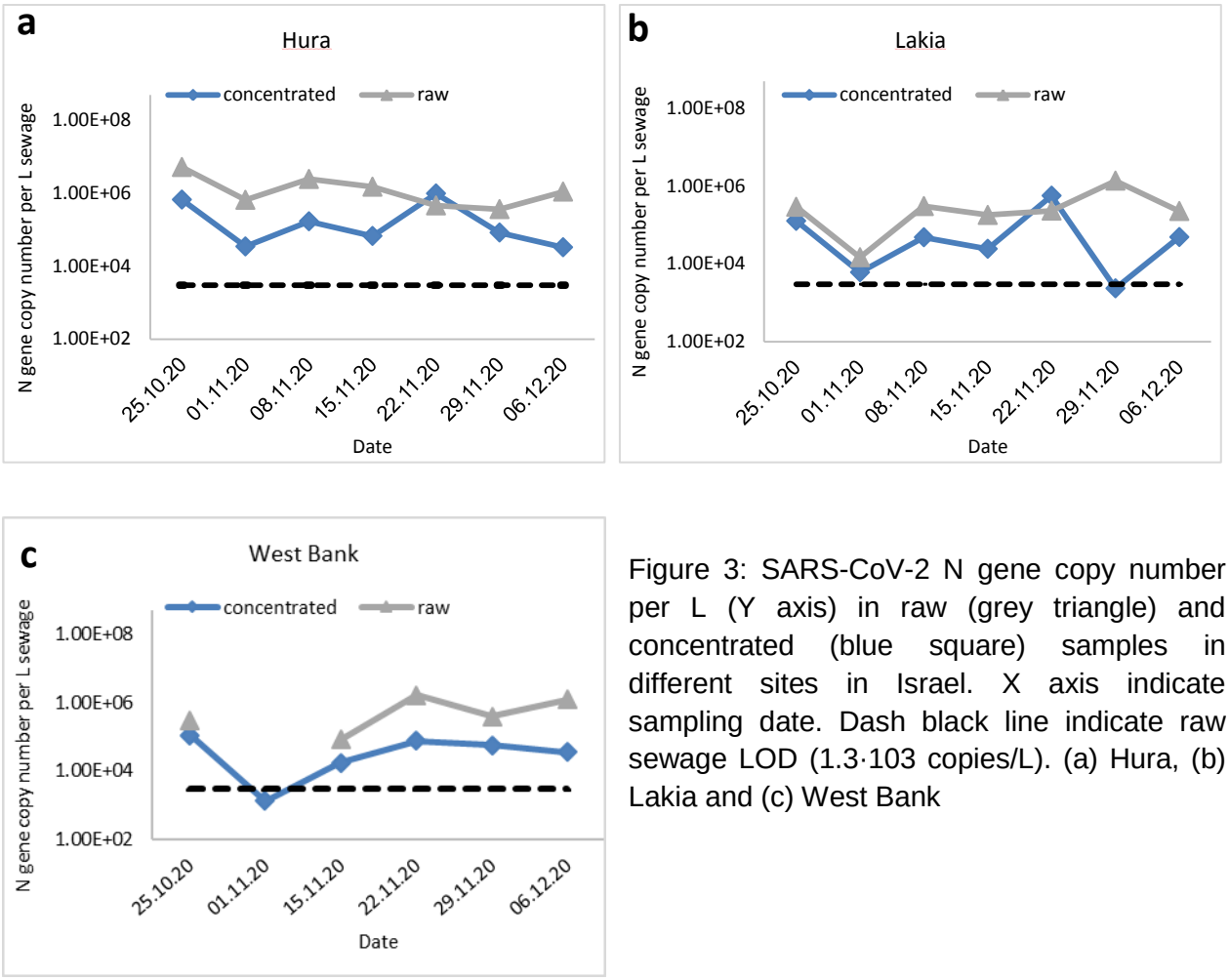


Figure 3: SARS-CoV-2 N gene copy number per L (Y axis) in raw (grey triangle) and concentrated (blue square) samples in different sites in Israel. X axis indicate sampling date. Dash black line indicate raw sewage LOD (1.3·103 copies/L). (a) Hura, (b) Lakia and (c) West Bank

Understanding the dynamics of SARS-CoV-2 in human sewage could lead to efficient monitoring and surveillance of this virus as well as to underpin environmental surveillance. Our results demonstrate the need for recognized Bedouin villages connected to the grid and off grid sewer sampling efforts to overcome dilution effects that can eventually affect the sensitivity and validity of this surveillance method. Such sewer study sampling may assist in geographical localization of disease hotspots. This approach should be further studied and validated as an early warning for SARS-CoV-2 resurgence in non-urban settings and as a national decision support tool.

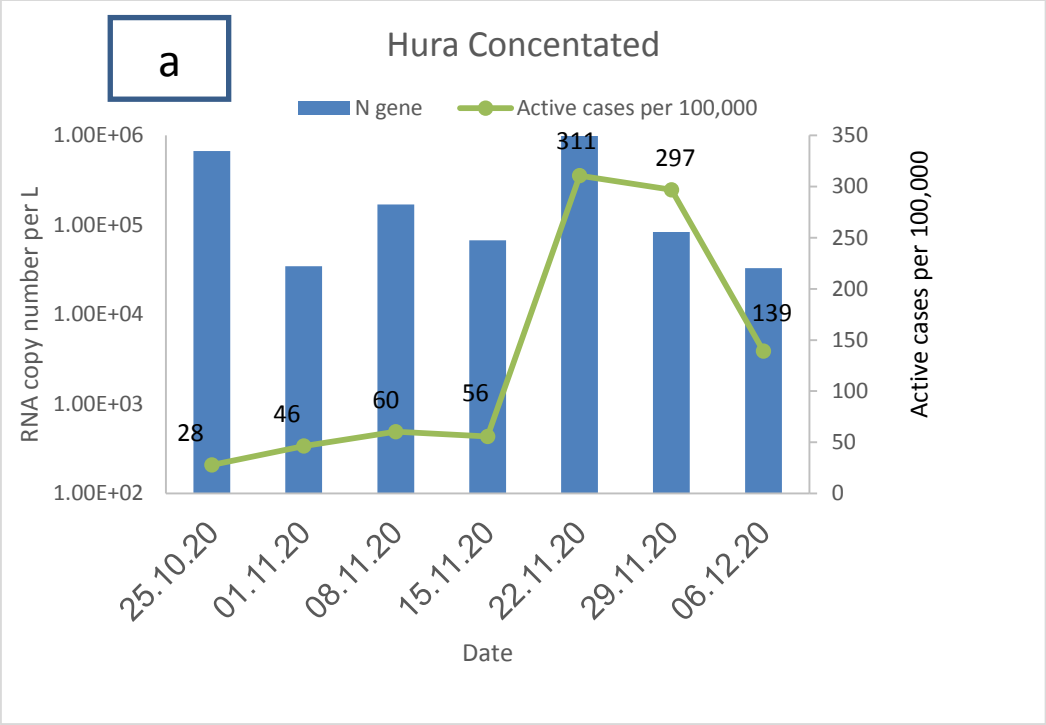
Table 1: SARS-CoV-2 detection and chemical analysis results from the Hebron River sampling sites.

Date of Sampling	Sampling Point	Sewage treatment description	Concentration factor	N2 copy number per L (Ct N2)	N3R copy number perL (Ct N3R)	TSS mg/L	COD mg O2/L ±12.3	TOC mg\L	BOD mg\L	Conductivity (ms)

14.10.20	Um Batin #1	Raw	1	ND	ND	NA				
		Concentrated	100	ND	1.12E03 (Ct=39.24)					
	Um Batin #2	Raw	1	ND	ND	NA				
		Concentrated	100	1.02E03 (Ct=39.24)	1.17E04 (Ct=36.12)					
03.01.21	Um Batin #1	Raw	1	3.76E05 (Ct=38.5)	1.95E05 (Ct=37.41)	0.128	102.5	21.65	7.35	1.66
		Concentrated	1000	1.34E02 (Ct=37.42)	4.41E02 (Ct=38.86)					
	Um Batin #2	Raw	1	ND	ND	0.016	77.90	22.49	9	2.02
		Concentrated	1000	6.03E01 (Ct=38.91)	1.44E02 (Ct=39.98)					
10.01.21	Metar	Raw	1	3.76E05 (Ct=38.5)	1.95E05 (Ct=37.41)	0.136	94.30	43.37	76	1.90
		Concentrated	900	1.34E02 (Ct=37.42)	4.41E02 (Ct=38.86)					
	Um Batin #2	Raw	1	ND	ND	0.016	82.00	17.20	8.6	0.64
		Concentrated	900	ND	ND					
17.01.21	Metar	Raw	1	ND	ND	0.9222	147.6	59.5	120.3	3.27
		Concentrated	1000	4.52E03 (Ct=39.24)	4.52E03 (Ct=36.64)					
	Um Batin #2	Raw	1	ND	ND	0.052	5>	21.77	NA	1.42
		Concentrated	900	ND	ND					

*ND=Not Detected; NA= Not Available

Although there are still no correlation methods between Sars-Cov-2 viral RNA concentration in the sewage and morbidity, active cases (figure 4) and mortality, it is still worth trying to compare with the official data obtained from the Ministry of Health and try to look for temporal patterns in the sampled communities. Until a validated correlation method will be developed, it will be of our utmost interest to utilize the findings of our studies as a potential for the surveillance of the virus in the sampled communities.



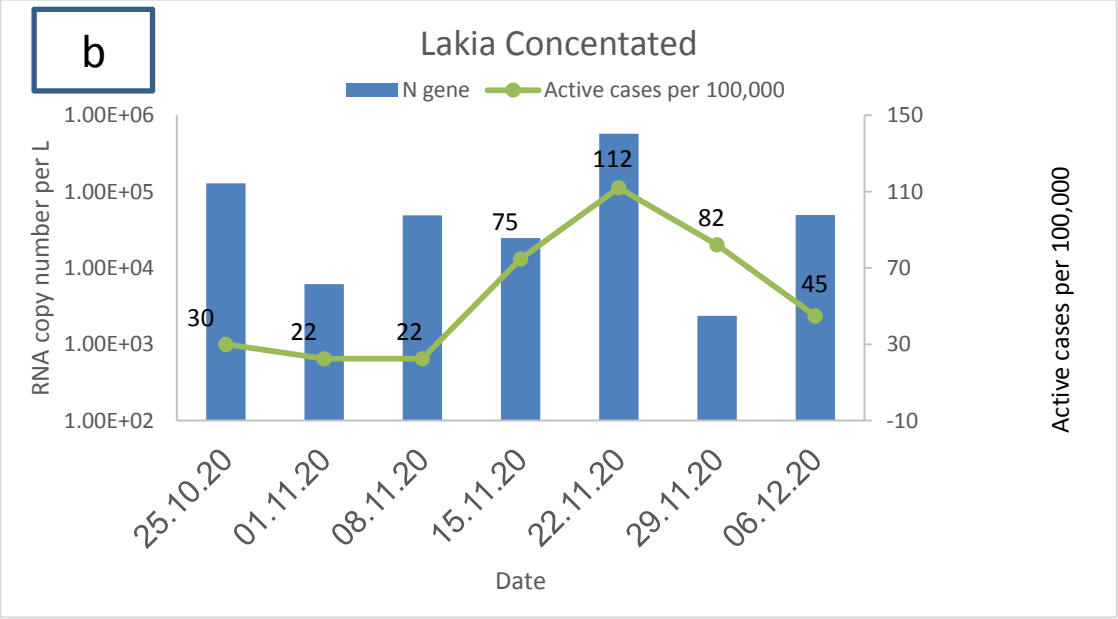


Figure 4: Y axis indicates SARS-CoV-2 N gene copy number per L green line and dots compared to the Cov-19 Active cases per 100,000 according to the Israeli Ministry of Health data (blue columns). X axis indicates sampling date. (a) for Hura & (b) for Lakia. Graph "a" in Hura the virus RNA concentration was high in October & mid-November and the active number cases per 100,000 was low, after the 15th of November the active cases per 100,000 increased and there was some sort of alignment between the two figures.

Graph“b” in Lakia the virus RNA concentration was high in October & mid-November and the active number cases per 100,000 was low, after the 15th of November the active cases per 100,000 increased and there was some sort of alignment between the two figures.