

Wastewater-Based Surveillance Is an Effective Tool for Trending COVID-19 Prevalence in Communities: A Study of 10 Major Communities for 17 Months in Alberta

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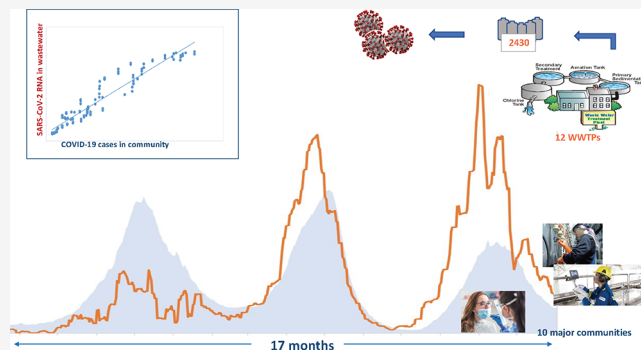
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ABSTRACT: The correlations between SARS-CoV-2 RNA levels in wastewater from 12 wastewater treatment plants and new COVID-19 cases in the corresponding sewersheds of 10 communities were studied over 17 months. The analysis from the longest continuous surveillance reported to date revealed that SARS-CoV-2 RNA levels correlated well with temporal changes of COVID-19 cases in each community. The strongest correlation was found during the third wave ($r = 0.97$) based on the population-weighted SARS-CoV-2 RNA levels in wastewater. Different correlations were observed (r from 0.51 to 0.86) in various sizes of communities. The population in the sewershed had no observed effects on the strength of the correlation. Fluctuation of SARS-CoV-2 RNA levels in wastewater mirrored increases and decreases of COVID-19 cases in the corresponding community. Since the viral shedding to sewers from all infected individuals is included, wastewater-based surveillance provides an unbiased and no-discriminate estimation of the prevalence of COVID-19 compared with clinical testing that was subject to testing-seeking behaviors and policy changes. Wastewater-based surveillance on SARS-CoV-2 represents a temporal trend of COVID-19 disease burden and is an effective and supplementary monitoring when the number of COVID-19 cases reaches detectable thresholds of SARS-CoV-2 RNA in wastewater of treatment facilities serving various sizes of populations.

KEYWORDS: SARS-CoV-2 RNA, RT-qPCR detection, COVID-19 pandemic wave, sewershed populations, correlation, clinical cases



1. INTRODUCTION

Municipal wastewater (sewage) contains a pool of excreted biological markers derived from members of the community served by a centralized wastewater collection system and wastewater treatment plant. This forms the fundamental rationale for wastewater-based epidemiology, where measuring biomarkers in sewage can inform public health decision making at the community level.¹ In 1947, Melnick identified the risk of transmission of poliovirus related to presence of the virus in sewage, providing the first epidemiological insights about viral pathogens in wastewater.² The PCR-based detection of the first re-emergence of polio virus transmission in Israel in 2013–2014 through wastewater monitoring highlighted how wastewater-based surveillance (WBS) could be used to monitor viral pathogens of global importance.³ Over recent decades, WBS has been successfully used to study the circulation of other viruses with gastrointestinal shedding, such as norovirus, adenovirus, hepatitis A and E viruses, and others.^{4,5} Since the start of the COVID-19 pandemic, WBS has

attracted considerable attention and been identified as a valuable complementary tool to monitor trends and estimate the prevalence of COVID-19 at the community level. After the first published study of SARS-CoV-2 RNA in wastewater by Medema et al.,⁶ there were 66 published studies by July 2021 related to WBS SARS-CoV-2 from 26 countries,⁷ covering a wide range of topics, such as methods development, detection sensitivity and performance, correlation with and prediction of COVID-19 disease burden in the community, detection of COVID-19 outbreaks at specific institutions, and study of SARS-CoV-2 variants of interest. This fast-growing and important area of research demonstrates that researchers,

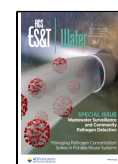
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healthcare professionals, public health officials, and the public are optimistic that WBS could provide valuable information on the prevalence of COVID-19 in communities and assist decision-making for public health measures.

The advantages of WBS SARS-CoV-2 for tracking the trend of endemic waves of COVID-19 include the ability to capture asymptomatic and paucisymptomatic cases that may not be captured by clinical testing.^{8–10} SARS-CoV-2 RNA in wastewater has been shown to correlate well with clinically diagnosed COVID-19 cases during the rise and fall of epidemiological waves of COVID-19.^{11,12} However, long-term studies and large data sets of WBS SARS-CoV-2 using a common and validated laboratory protocol in various sizes of communities with a high rate of clinical testing for COVID-19 cases among sewershed subpopulation remain very limited. Several published studies with SARS-CoV-2 WBS had <6 months of data, including Agrawal et al. (4 months, 2 facilities and 2 samples per week in Frankfurt, Germany),¹³ Gonzalez et al. (21 weeks, 9 facilities and one sample per week in Virginia, USA),¹⁴ Weidhaas et al. (9 weeks, 10 facilities and 2–3 samples per week in Utah, USA),¹⁵ and Graham et al. (89 days with daily samples from 2 facilities in California, USA).¹¹ It would be a reasonable assumption that correlations between measured SARS-CoV-2 levels in wastewater and clinically confirmed COVID-19 cases would be highly reliant on the coverage of the corresponding clinical testing policies. At the start of the pandemic in May 2020, Alberta had among the highest number of COVID-19 tests per capita in Canada coupled with robust contact tracing.¹⁶ By July 29, 2021, asymptomatic testing was no longer recommended, contact tracing of positive cases would occur only when the cases were associated with an outbreak investigation, and quarantines were not legally required for asymptomatic COVID-19 close contacts in the communities.¹⁷ Since the decrease in clinical testing and contact tracing by public health policies, WBS SARS-CoV-2 has become a critical tool to monitor the overall COVID-19 disease burden of communities.^{12,18–20} This study examined the relationship between WBS SARS-CoV-2 and new clinical COVID-19 cases in communities of various sizes by comparing a large number of COVID-19 clinical diagnostic testing results with SARS-CoV-2 RNA levels in wastewater from 12 wastewater treatment plants (WWTP), serving populations across the province of Alberta over a 17-month period. This long-term study aimed to (1) investigate the correlations between the levels of SARS-CoV-2 RNA in wastewater and new COVID-19 cases reported in defined WWTP-served populations for each pandemic wave, (2) identify any differences when SARS-CoV-2 RNA monitoring was applied to small versus large communities with differing geographic scales of sewer systems, and (3) provide further evidence to support WBS SARS-CoV-2 RNA as a tool for estimating the prevalence of COVID-19 infection at the community level effectively.

2. METHODS AND MATERIALS

2.1. Recruitment of Participating WWTPs and Sample Collections. Twelve municipal and regional WWTPs serving 10 major communities (including cities, towns, and counties in their sewersheds) across the province of Alberta with varied populations, including the two major urban centers, were recruited for the WBS SARS-CoV-2 study. Local geographic areas (LGA), created by Alberta Health and Alberta Health Services based on census boundaries, were used to define

boundaries of participating communities served by WWTPs and regional sewershed populations. Postgrit raw influent wastewater samples (500 mL of 24 h composite sample) were collected two or three days per week from May 15, 2020 to Oct 8, 2021. Wastewater samples were initially frozen between May 15, 2020 and Jan 22, 2021 and then changed to storage at 4 °C after collection on the week of Jan 25, 2021, before a once a week shipment of samples to the analytical laboratory during the study. Upon receipt, samples were processed within a median of 24 h (range 4–72 h) for virus concentration, nucleic acid extraction, and real-time RT-qPCR using the two gene targets N1 and N2 of SARS-CoV-2.²¹ Operational parameters such as temperature of the wastewater, average flow rate, pH, and other biological and chemical references on the sampling dates were also collected from the participating WWTPs.

2.2. COVID-19 Pandemic Information in Alberta. Aggregate time series of reported new clinically diagnosed COVID-19 cases and the number of clinical laboratory tests and test positive rate by day stratified by LGA served by corresponding WWTPs were downloaded from the COVID-19 surveillance database of the Government of Alberta.²² The COVID-19 waves were defined as the first (May–Sept 2020), second (Oct 2020–Feb 2021), third (March–June 2021), and fourth (July–Oct 2021) waves in Alberta.

2.3. Wastewater Sample Processing and SARS-CoV-2 Detection. The optimized laboratory protocol to process and test wastewater samples was performed as described by Qiu et al.²¹ Briefly, 100 mL of the sample was centrifuged at 4500g for 10 min. The supernatant was collected, transferred to a Centricon filter cup (30-kDa MWCO, Millipore) and centrifuged at 3000g for 10 min using a refrigerated centrifuge (Allegra X-15R, Beckman Coulter). The concentrated sample was made up to a final volume of 1 mL with phosphate buffered saline. The total nucleic acid (NA) was extracted from 400 μ L of the concentrated sample and eluted at a final volume of 100 μ L. A one-step real time RT-qPCR assay was performed in duplicate to detect N1 and N2 genes of SARS-CoV-2 on an ABI 7500 PCR instrument. An external standard curve was used to quantify the level of SARS-CoV-2 RNA in wastewater samples. The results were expressed as genome equivalent copy numbers per 100 mL of wastewater. The limit of detection was 80 copies (gc) per 100 mL for the gene targets.

For quality control, a known concentration of cultured hCoV-229E (100 μ L, VR-740, ATCC) was added to each sample to measure the recovery efficiency rate of the virus from wastewater and salmon DNA (5 μ L) was spiked into the concentrated sample before NA extraction and quantified by qPCR to assess the degree of PCR inhibition in each sample. Pepper mild mottle virus (PMMoV), an indicator of human feces in wastewater, was used to check the levels of human feces in wastewater samples collected from each WWTP. Negative and positive controls were always included in each step of the analytic process. All quality control criteria must be met before reporting.²³ A positive result (SARS-CoV-2 RNA) was reported when there were two or more positive PCR results out of the four duplicate PCR runs of the two SARS-CoV-2 gene targets.²¹ Ethics and biohazard approvals of this study were obtained from the Health Research Ethics Board of the University of Alberta (Pro001847) and Environment, Health & Safety of the University of Alberta, respectively.

2.4. Data Analysis. Two empirical data sets were generated for each WWTP, including the detection and quantification results of SARS-CoV-2 RNA of N1 and N2

genes in wastewater samples and the daily number of new COVID-19 cases reported for the LGA-matched population served by each WWTP.

The seven-day rolling average was calculated for the average of SARS-CoV-2 RNA levels (copies/100 mL) of N1 and N2 genes in wastewater and for the daily number of new COVID-19 cases reported by the Alberta Government. The calculation was performed by using the data from the 3 days before the date of interest to 3 days after (7 days in total)

$$A_i = \text{SUM}_{j=i-3 \text{ to } i+3}(C_j)/7$$

where A_i is the seven-day rolling average for day i , and C_j is the raw value of day j .

To reflect the provincial trend of COVID-19, the population-weighted mean of a 7-day rolling average of SARS-CoV-2 RNA levels from all participating WWTPs was compiled as

$$C_{\text{Alberta}} = \text{SUM}_{i=1-11}(C_i \times P_i) / \text{SUM}_{i=1-11}(P_i)$$

where C_{Alberta} is the estimated Alberta result for a specific day, C_i is the corresponding SARS-CoV-2 RNA result in wastewater of WWTP _{i} (7-day rolling average), and P_i is the served population of WWTP _{i} .

A Pearson's correlation coefficient analysis was used to measure the linear correlation between the two sets of continuous variables: i.e., the 7-day rolling average of new COVID-19 cases in the population served by each WWTP and the 7-day rolling average levels of SARS-CoV-2 RNA in wastewater of the respective WWTP. For correlation analysis of the compiled data of the whole province and all waves together, the 7-day rolling average of new COVID-19 cases in the sewersheds of Alberta and the population-weighted means of 7-day rolling average SARS-CoV-2 levels from all participating WWTPs were used. Hypothesis tests were done for the significance of Pearson correlation coefficients (r) against the null hypothesis that $r = 0$. Statistical significance testing was adjusted by the degrees of freedom according to Lin et al. for moving average data.²⁴ A significance level was set at a p value <0.001. A conventional approach to describe the strength of correlation, 0.00–0.09 negligible, 0.10–0.39 low, 0.40–0.69 moderate, 0.70–0.89 strong, and 0.9–1.0 very strong, was used to describe the findings of this study.²⁵

3. RESULTS

3.1. Location, Served Population, and Daily Flow Rate of WWTP. The sewersheds of the 12 WWTPs spanned the latitude between 49.69 and 55.17 across Alberta and covered 79% of the provincial population (3458955/4371000). Figure 1 shows the locations of 12 WWTPs in the Alberta Health Zone map and the size of the served population in the corresponding sewersheds. Twelve WWTPs serving the cities/towns/counties and respective daily flow rates of wastewater during the study period are summarized in Table 1, which showed that, as expected, the daily flow rates of wastewater in WWTPs are proportional to the size of the population in communities served by each WWTP.

3.2. Detection and Quantification of SARS-CoV-2 in Wastewater. A total of 2430 wastewater samples were processed and tested for SARS-CoV-2 RNA. The overall positive rate of SARS-CoV-2 RNA detection in wastewater was 56.3% (1367/2430), increasing from 5.6% in the first wave to 61.3% in the second wave, 92.7% in the third wave, and 95.2%

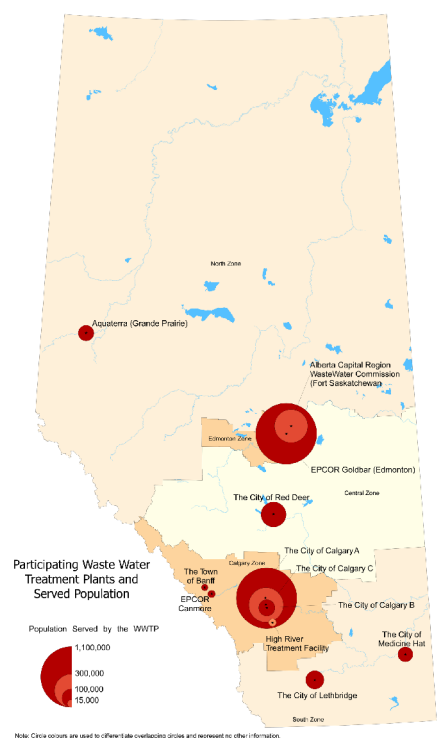


Figure 1. Twelve participating wastewater treatment facilities of the respective cities/towns indicated in the Alberta Health Zone map. The populations in the WWTP sewersheds are presented by different sizes of colored cycles. The legend of colored circles is shown on the left-hand bottom corner of the figure.

in the fourth wave of COVID-19 in Alberta (Table 2). All SARS-CoV-2 RNA positive samples ($n = 1367$) were quantifiable by a real-time RT-qPCR assay. The medians of SARS-CoV-2 RNA genomic copies were 109, 1100, 2784, and 4416 per 100 mL of wastewater for the first, second, third, and fourth waves, respectively (Table 3), showing a remarkable increase of viral loads from the second to fourth waves.

Table S1 shows analytical parameters for quality control of real-time RT-qPCR-based detection for SARS-CoV-2 N1 and N2 genes in wastewater. The recovery ranges of samples spiked in hCoV 229E were relatively large for samples from each WWTP, whereas the proportion of samples with inhibition of the RT-qPCR assay (assessed by spiked salmon DNA) was consistent, indicating a relative similarity of inhibitory effects of wastewater from individual WWTPs on PCR reactions. The standard deviations of PMMoV (by Ct values) were very narrow in the samples from each WWTP and across 12 WWTPs, indicating stable levels of fecal content in wastewater produced by the community. Therefore, PMMoV levels in the samples were not used to normalize the results of SARS-CoV-2 RNA reported in this study.

3.3. Correlation of SARS-CoV-2 in Wastewater to Clinical New COVID-19 Cases. Overall new COVID-19 cases correlated well to SARS-CoV-2 RNA levels in wastewater. A Pearson analysis showed that the correlations between population-weighted SARS-CoV-2 RNA levels in wastewater and the new COVID-19 cases of all the LGA-matched communities served by 12 WWTPs were different in the four pandemic waves, with very strong correlations during the third and fourth waves (0.97 and 0.9, respectively), moderate in the second wave (0.75), and negligible correlation in the

Table 1. Population of the Sewershed and Daily Flow Rate (Average $\pm$ SD) of Wastewater from the Participating Wastewater Treatment Plants (WWTP) across Alberta, Canada, from May 2020 to October 2021

WWTP code	WWTP name	flow rate (m ³ /day)		city/town/county served	population of the sewershed
		ave	SD ($\pm$)		
1	EPCOR Gold Bar, Edmonton	306000	124000	Edmonton, Leduc, Beaumont	1115021
2	The City of Calgary A ^a	346000	44645	Calgary North, Cochrane, Airdrie	1104208
3	The City of Calgary B	89000	13269	Calgary South	398544
4	The City of Calgary C	155000	143075		
5	Alberta Capital Region Wastewater Commission	76000	11190	Fort Saskatchewan, St. Albert, Spruce Grove, Strathcona County (including Sherwood Park), Sturgeon County, Stony Plain, Morinville, Bon Accord, Gibbons	326497
6	The City of Red Deer	55953	8253	Red Deer, Sylvan Lake, Olds, Lacombe, Innisfail	187857
7	The City of Lethbridge	40341	2860	Lethbridge	100655
8	Aquatera	19168	3077	Grande Prairie	74245
9	The City of Medicine Hat	23961	1332	Medicine Hat	68115
10	High River Treatment Facility	3763	332	Town of High River	16922
11	EPCOR Canmore	9078	3733	Town of Canmore	16547
12	The Town of Banff	6346	1578	Town of Banff	13451
total	12			10 majors	3458955

^aThe City of Calgary has three wastewater treatment facilities serving different populations, and the names of the facilities were undisclosed.

Table 2. Detection of SARS-CoV-2 RNA (Number of Positive Samples and Rates of Positive) in Wastewater Samples Collected from 12 Participating WWTPs in Alberta from May 2020 to Oct 2021 Stratified by the Four COVID-19 Pandemic Waves in Alberta

WWTP code	total no. of samples tested	no. tested positive (%)	wave 1 (May–Sep 2020)		wave 2 (Oct 2020–Feb 2021)		wave 3 (Mar – Jun 2021)		wave 4 (Jul – Oct 2021)	
			no. of samples tested	no. tested positive (%)	no. of samples tested	no. tested positive (%)	no. of samples tested	no. tested positive (%)	no. of samples tested	no. tested positive (%)
1	253	156 (62)	105	14 (13)	70	65 (93)	48	47 (98)	30	30 (100)
2	225	142 (63)	83	9 (11)	64	55 (86)	50	50 (100)	28	28 (100)
3	221	128 (58)	81	3 (4)	65	50 (77)	50	50 (100)	25	25 (100)
4	222	124 (56)	83	3 (4)	64	45 (70)	50	49 (98)	25	25 (100)
5	190	140 (74)	46	6 (13)	64	54 (84)	50	50 (100)	30	30 (100)
6	189	122 (65)	45	2 (4)	63	39 (62)	51	51 (100)	30	30 (100)
7	190	104 (55)	47	1 (2)	64	38 (59)	49	40 (81)	30	25 (83)
8	185	106 (57)	45	0	65	32 (49)	47	46 (98)	28	28 (100)
9	185	97 (52)	41	1 (2)	64	23 (36)	50	43 (86)	30	30 (100)
10	185	76 (41)	45	2 (4)	64	20 (31)	48	35 (73)	28	19 (68)
11	191	66 (35)	77	0	64	19 (30)	50	47 (94)	NA	NA
12	194	106 (55)	51	1 (2)	64	35 (55)	49	41 (84)	30	29 (96)
Total (%)	2430	1367 (56.3)	749	42 (5.6)	775	475 (61.3)	592	549 (92.7)	314	299 (95.2)

first wave (Table 4 and Figure 2). Six and three out of the 10 communities had very strong correlations between daily new cases and SARS-CoV-2 RNA level, respectively, the rest showing moderate to strong correlations during the third and fourth waves (Figure 3). In contrast, most of the communities showed negligible and low correlations except for one with moderate correlation ($r = 0.52$) during the first wave. During the first wave, the daily number of new COVID-19 cases per 100000 population in the 10 communities combined was below 5, which was much lower compared with the subsequent second to fourth waves (about 30–50 new COVID-19 cases per 100000) (Figure 4).

Among all the communities during the third and fourth waves, the City of Lethbridge only had moderate correlations in both the third and fourth waves ($r = 0.66$ and 0.54) and the Town of High River only had moderate correlation in the third

wave ($r = 0.65$) (Table 4). The City of Lethbridge was the only one with moderate correlations among five major communities with a sewershed population of over 100000, and the Town of High River was one with a moderate correlation among three small communities with its sewershed population between 10000 and 20000. The rest of the communities with population from 10000 to more than 1 million all had very strong correlations between SARS-CoV-2 RNA levels and new COVID-19 cases during both the third and fourth waves of the COVID-19 pandemic in Alberta. This observation suggested that the size of the population served by a WWTP is not a determining factor on the strength of correlation between the numbers of new COVID-19 cases in the community and SARS-CoV-2 RNA levels in the wastewater collected at the WWTP.

Table 3. SARS-CoV-2 RNA Levels (Medians and Ranges) in Wastewater Samples Collected from 12 Participating WWTPs from May 2020 to Oct 2021 Stratified by the Four COVID-19 Pandemic Waves

WWTP code	no. of positive and quantifiable samples	quantitation of SARS-CoV-2 RNA (copies/100 mL sewage)							
		wave 1 (May–Sep 2020)		wave 2 (Oct 2020–Feb 2021)		wave 3 (Mar–Jun 2021)		wave 4 (Jul–Oct 2021)	
		median	range (n)	median	range (n)	median	range (n)	median	range (n)
1	156	465	147–2605 (14)	1240	124–16850 (65)	2445	153–11105 (47)	3310	124–15100 (30)
2	142	360	206–96150 (9)	773	86–7725 (55)	4295	289–16950 (50)	3793	216–11650 (28)
3	128	385	333–522 (3)	686	149–2695 (50)	1782	127–16350 (50)	3375	80–17150 (25)
4	124	391	221–487 (3)	627	174–1860 (45)	1975	216–17300 (49)	3780	178–14350 (25)
5	140	474	413–879 (6)	594	80–33700 (54)	1039	146–5980 (50)	3838	139–16750 (30)
6	122	321	321–400 (2)	578	216–1800 (39)	1760	149–15060 (51)	7775	143–39100 (30)
7	104	1186	NA (1)	525	80–6350 (38)	838	80–10150 (40)	435	130–4330 (25)
8	106	All Neg	All Neg	795	249–128500 (32)	2648	99–12000 (46)	16600	230–33950 (28)
9	97	54	NA (1)	347	80–1198 (23)	595	80–5085 (43)	7728	201–41200 (30)
10	76	544	544–631 (2)	260	115–1382 (20)	348	80–1640 (35)	474	117–3210 (19)
11	66	All Neg	All Neg	354	83–10720 (19)	1720	80–8785 (47)	NA	NA
12	106	306	NA (1)	591	87–2185 (35)	1389	116–25100 (41)	8975	160–52550 (29)
total	1367	109 ^a	0–7161	1100 ^a	19–3784	2784 ^a	184–10523	4416 ^a	220–14465

^aPopulation-weighted medians of SARS-CoV-2 RNA levels across 12 WWTPs in each wave reflect the trend of COVID-19 burden during specific periods of the COVID-19 pandemic in Alberta.

Table 4. Correlation Analysis between SARS-CoV-2 RNA level in Wastewater (7-Day Rolling Averages) and New COVID-19 Clinical Cases (7-Day Rolling Averages) in Respective Communities during Four Waves of COVID-19 Pandemic in Alberta

WWTP code	city/town/county served	Pearson's correlation coefficient, <i>r</i> value ^a				
		wave 1 (May–Sep 20)	wave 2 (Oct 20–Feb 21)	wave 3 (Mar–Jun 21)	wave 4 (Jul – Oct 21)	during the study period (May 20 Oct 21)
1	Edmonton, Leduc, Beaumont	0.36	0.75 ^c	0.96 ^c	0.87 ^c	0.54 ^c
2	Calgary North, Cochrane, Airdrie	0.02	0.04	0.93 ^c	0.89 ^c	0.51 ^c
3 and 4 ^b	Calgary South	0.13	0.19	0.90 ^c	0.92 ^c	0.77 ^c
5	Fort Saskatchewan, St. Alberta, Spruce Grove, Strathcona County, Sturgeon County, Stoney Plain, Morinville	0.52 ^c	0.81 ^c	0.90 ^c	0.90 ^c	0.86 ^c
6	Red Deer, Sylvan Lake, Olds, Lacombe, Innisfail	0.13	0.70 ^c	0.94 ^c	0.81 ^c	0.80 ^c
7	Lethbridge	0.23	0.29 ^c	0.66 ^c	0.54 ^c	0.67 ^c
8	Grande Prairie	NA	0.32 ^c	0.83 ^c	0.86 ^c	0.60 ^c
9	Medicine Hat	0.03	0.53 ^c	0.83 ^c	0.74 ^c	0.85 ^c
10	Town of High River	0.18	0.31 ^c	0.65 ^c	0.71 ^c	0.68 ^c
11	Town of Canmore	NA	0.09	0.88 ^c	NS	0.60 ^c
12	Town of Banff	0.12	0.80 ^c	0.89 ^c	0.76 ^c	0.52 ^c
	population-weighted <i>r</i> ^d	−0.40	0.75 ^c	0.97 ^c	0.90 ^c	0.64 ^c

^aPearson's coefficient analyses using rolling averages were validated by using adjustment described by Lin et al. (see [Methods and Materials](#)).

^bWWTP ID 3 and 4 were combined for the Pearson's correlation analysis due to crossover of wastewater flows. ^cStatistically significant ($p < 0.001$) against the null hypothesis that $r = 0$. NA: no *r* value was generated from Pearson's correlation analysis. NS: no samples were collected in the wave 4 from this WWTP. ^dPearson's correlation coefficients were calculated by the population-weighted SARS-CoV-2 RNA levels across 12 WWTPs.

The effect of the visiting population on the strength of correlation was also examined for the Town of Banff, a well-known tourist area in Alberta. The estimated visiting population (tourists and seasonal workers) for Banff was 912000 in 2020 based on daily vehicle volumes and visiting occupancy factors (data provided by the Town of Banff), which was 67 times that of registered permanent residents in the town. Since an average stay of days in Banff was unknown for those visitors, their contributions to wastewater of the town were difficult to estimate. However, the correlation between new COVID-19 cases and SARS-CoV-2 RNA levels in wastewater was strong ($r = 0.76–0.89$, $p < 0.001$) from the second to the fourth wave of the COVID-19 pandemic (Table 4 and Figure S1B, Banff).

3.4. Temporal Trends of COVID-19 with SARS-CoV-2 RNA Level Detection.

Temporal trends between new COVID-19 case rates per 100000 population generated from the 10 major communities in Alberta and population-weighted SARS-CoV-2 RNA levels in wastewater are shown in Figure 4 and in Figure S1A–K for each community during the study period. The PCR-based clinical diagnostic testing policies were changed to focusing on symptomatic COVID-19 cases due to increasing case volumes and resource limitations from the second to the fourth wave in Alberta (Figure 4). The daily rolling average of new clinical COVID-19 cases reached 50 per 100000 population during the second wave (13503 ± 4175 daily clinical tests) and third wave (10483 ± 4753 daily clinical tests) and declined in the fourth wave (9630 ± 3789 daily

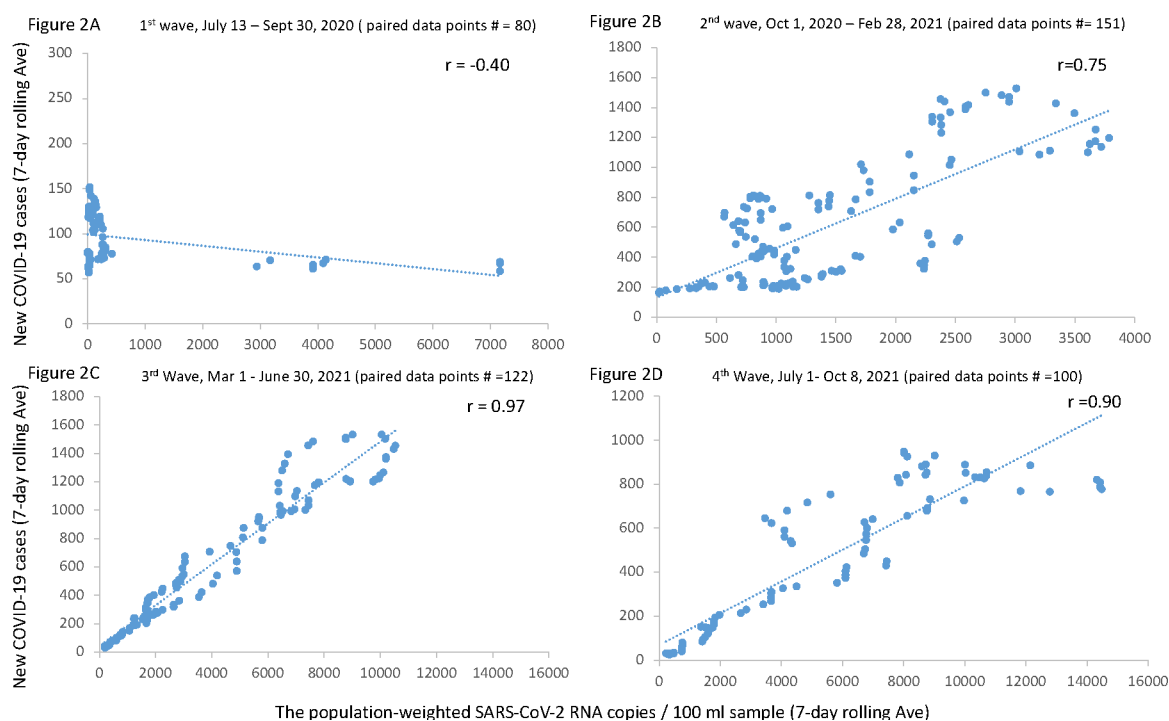


Figure 2. Correlation between SARS-CoV-2 RNA levels in wastewater of WWTP sewersheds and new clinical COVID-19 cases in 10 major participating communities across Alberta during the whole study period of 17 months: (A) correlation in the first wave (July 13–Sept 30, 2020); (B) correlation in the second wave (Oct 1, 2020–Feb 28, 2021); (C) correlation in the third wave (March 1–June 30, 2021); (D) correlation in the fourth wave (July 1–Oct 8, 2021).

clinical tests) because of reduced numbers of clinical tests, while the SARS-CoV-2 RNA levels in wastewater increased remarkably from the second to fourth waves, reaching the highest level in the middle of the fourth wave. Higher levels of SARS-CoV-2 RNA in the third and fourth waves compared to the second wave indicated that COVID-19 prevalence in the communities was higher than clinically reported cases in all of the communities.

4. DISCUSSION

The proof of concept to evaluate WBS SARS-CoV-2 as an effective tool to estimate the prevalence of COVID-19 is to determine whether fluctuations in SARS-CoV-2 RNA concentrations in wastewater can reflect clinical COVID-19 trends in the community.^{6,20,26} As we have learned, the correlation between SARS-CoV-2 RNA in wastewater and clinical COVID-19 case numbers is affected by numerous factors, such as a change in the clinical testing policy, emerging new variants of concern, naturally acquired community immunity, and others including viral loads and target degradation in wastewaters, assay sensitivity, sample frequency, quantity of samples, and duration of surveillance studies. A study with a duration of 21 weeks in 9 regional wastewater treatment plants (one sample per week) showed descriptively an increase prior to lockdown measures, a decrease and a plateau before reopening, and a significant rise in SARS-CoV-2 signal following reopening.¹⁴ Using nearly daily sampling for 89 days from a WWTP, Graham et al. demonstrated parallel trends of rising and falling of the SARS-CoV-2 RNA level in wastewater solids with the rise and fall of clinical COVID-19 case numbers during their study period.¹¹ The statistical power of the data increased exponentially with an increase in study

duration and the number of samples, as shown in two studies among others.^{15,26,27}

Our study has the longest duration of continuous WBS SARS-CoV-2 reported to date covering four waves of the COVID-19 pandemic in Alberta, and we have invested substantial resources to ensure that the reporting area for clinical cases corresponded to the population served by a given sewershed. These data and analyses provided strong evidence that SARS-CoV-2 RNA detection and quantitation were correlated well with temporal changes of clinical COVID-19 cases across the province and in each of the communities engaged in the study (Figure 4 and Figure S1A). The highest correlation was observed in the third wave ($r = 0.97$, $p < 0.001$). The data agreed with the observations of Feng et al. in their WBS SARS-CoV-2 study from 12 WWTPs for 6 months covering only one COVID-19 wave in Wisconsin communities.²⁶ Our findings showed that the fluctuation of the SARS-CoV-2 RNA levels in wastewater mirrored the rise and fall of new COVID-19 cases in directly corresponding communities, implying that WBS SARS-CoV-2 is an effective tool to estimate the prevalence of COVID-19.

We observed large variations in the strength of correlations between new clinical COVID-19 cases and SARS-CoV-2 RNA levels in wastewater among the participating communities during the four pandemic waves in Alberta. There was negligible correlation during the first wave when the number of COVID-19 cases was very low and an overall weak correlation in the second wave as compared to the third and fourth waves of COVID-19 in Alberta. During the first and early part of the second wave, clinical testing of COVID-19 in Alberta was extensive, including symptomatic and asymptomatic testing of close contacts through vigorous contact tracing as well as a pilot project of asymptomatic screening at the

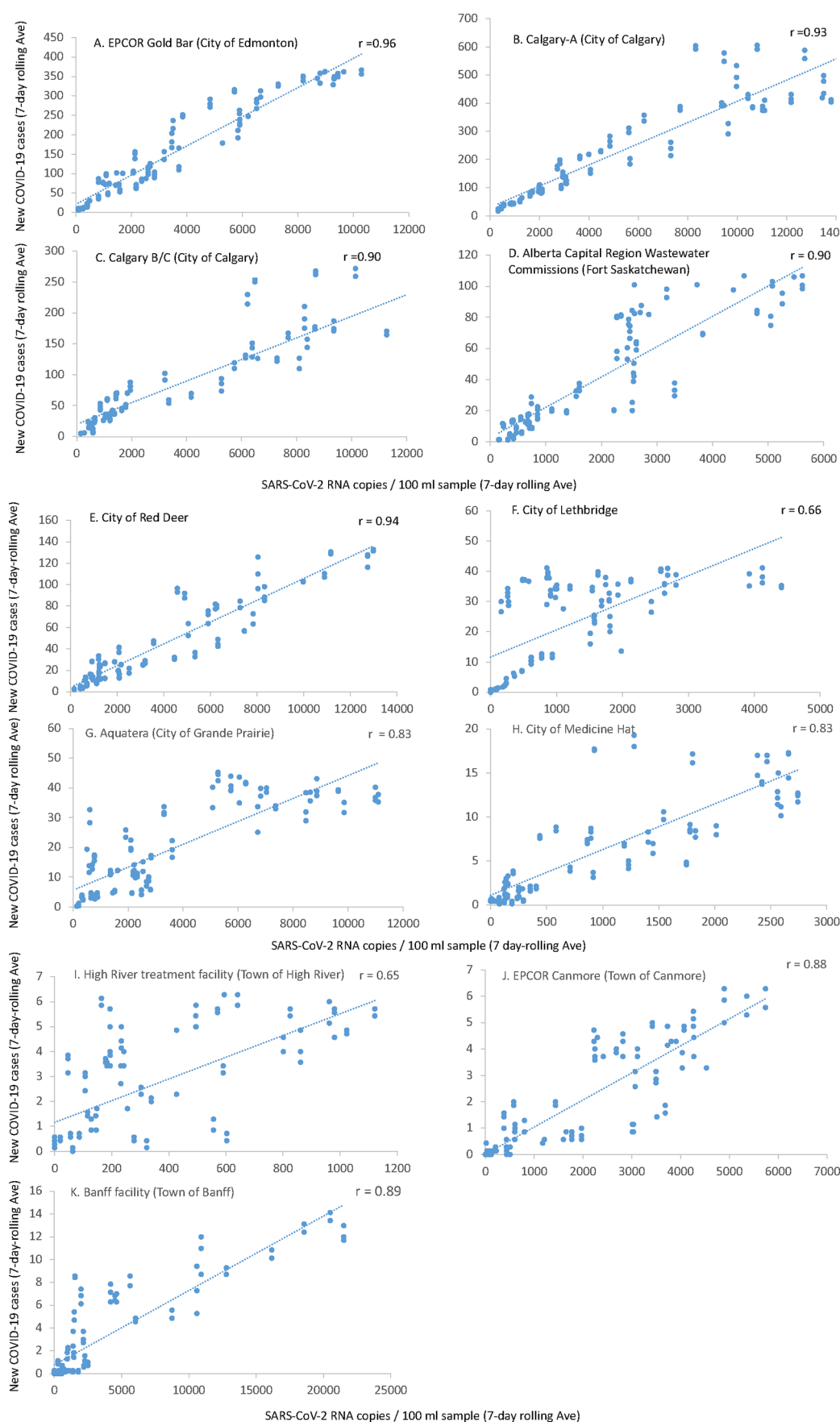


Figure 3. Correlation between SARS-CoV-2 RNA levels in wastewater of 11 WWTPs and new clinical COVID-19 cases in corresponding communities during the third wave of the COVID-19 pandemic in Alberta (March 1–June 30, 2021): (A) EPCOR/the city of Edmonton; (B) Calgary Plant A/the City of Calgary; (C) Calgary Plant B/the City of Calgary; (D) ACRWC/Fort Saskatchewan; (E) the City of Red Deer; (F)

Figure 3. continued

the City of Lethbridge; (G) Aquatera plant/City of Grande Prairie; (H) the City of Medicine Hat; (I) the Town of High River; (J) EPCOR plant/town of Canmore; (K) the Town of Banff.

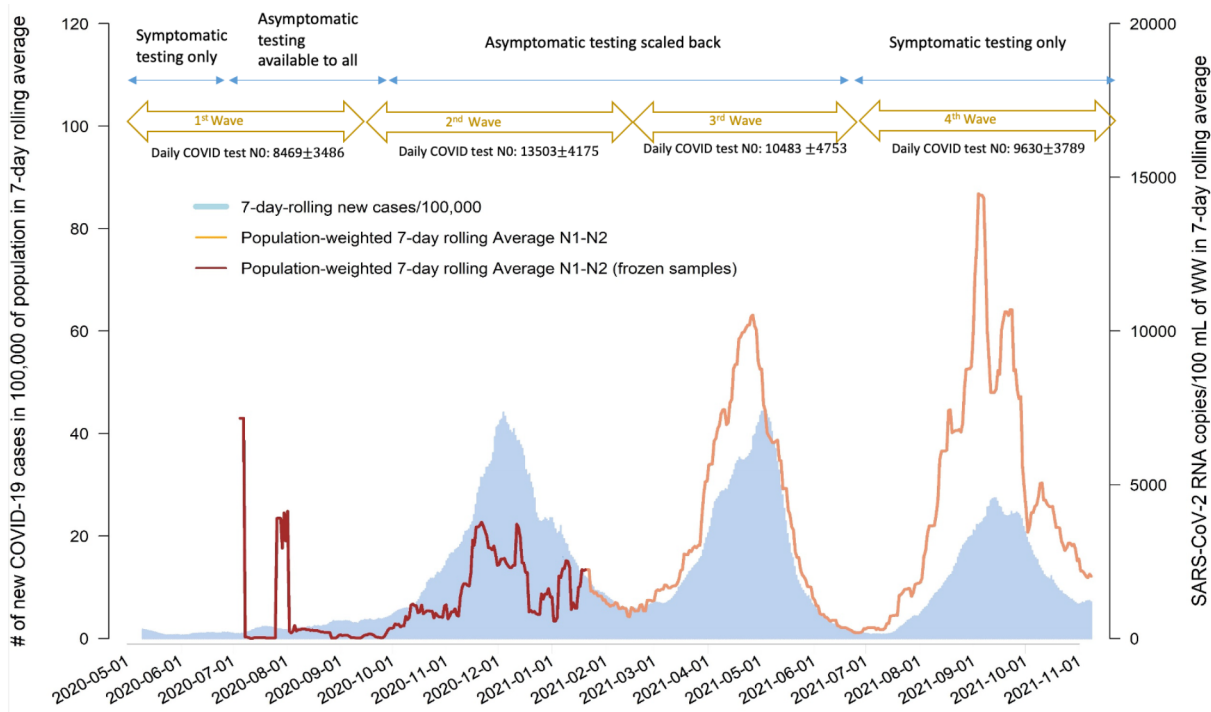


Figure 4. Trends of SARS-CoV-2 levels in wastewater, new COVID-19 cases, and daily clinical testing numbers in different pandemic waves during the study period of 17 months. Public health policy changes during the study period are shown at the top of the figure. The red line in the plot indicated the period when frozen wastewater samples were used for the RT-qPCR assay for SARS-CoV-2 RNA in wastewater.

emergency departments of three acute care facilities in the province. Large numbers of clinical tests were performed daily (13503 ± 4175) in the second wave, revealing a low positivity rate for COVID-19. Our WBS SARS-CoV-2 study started during the initial low incidence period with daily new case rates from 0 to 4 per 100,000 population (the first wave). During the first wave, only 5.6% of wastewater samples tested positive with a quantifiable SARS-CoV-2 RNA, indicating that positive detection of SARS-CoV-2 was truly sporadic from wastewater samples. That would explain the negligible linear correlation in the first wave. Although SARS-CoV-2 positivity in wastewater samples increased to 61.5% during the second wave, there was still either no wastewater sample collected and/or SARS-CoV-2 RNA signal being under the limits of detection at the dates while COVID-19 positive cases were tested and reported when we calculated both trends for new COVID-19 cases and SARS-CoV-2 in wastewater. These asymmetric data undoubtedly affected the estimated r value, resulting in a weak correlation between new COVID-19 cases and SARS-CoV-2 RNA levels in wastewater during the second wave.

There was another possible reason that contributed to the lower correlation observed in both the first and second waves. The processing and RT-PCR testing of the wastewater samples were still being optimized during this phase in the first wave. Wastewater samples collected during 1 week were frozen before a once-a-week shipment to laboratory for testing. A decrease in the SARS-CoV-2 RNA signal was noticed in frozen samples, likely due to RNA degradation caused by freeze–thaw

cycles.²¹ Hence, the storage condition was changed to 4 °C in the middle of the second wave before weekly shipping. The improved sensitivity of our testing process after optimization and altered storage conditions in the later part of the second wave coupled with the increased disease burden during the third and fourth waves of COVID-19 in Alberta (where alpha and delta were the predominant variants) resulted in a higher percentage of SARS-CoV-2 positive detection and levels in wastewater in the third and fourth waves. That resulted in an excellent correlation between new COVID-19 cases and SARS-CoV-2 RNA level in wastewater. Taken together, we postulate that an inherent correlation is present between clinical COVID-19 cases and WBS SARS-CoV-2. Variations can be reduced if the data asymmetry issue is addressed, such as collecting and testing wastewater samples more frequently²⁶ and studying and understanding potential different patterns of viral shedding between the wild-type of SARS-CoV-2 and variants of concern.²⁸

Having an advantage of this long-term and multiparticipant WBS SARS-CoV-2 study, we further analyzed the data to address whether WBS can be used effectively in different communities with varied sizes of population and characters in their sewersheds. The strength of correlation between new clinical COVID-19 cases and SARS-CoV-2 RNA levels in wastewater during the third and fourth waves was used to identify whether the sizes of communities could affect the correlation. Both Lethbridge and High River communities had only moderate correlation ($r = 0.66$ and 0.65 , respectively) among 10 major communities with population in their

sewersheds from 13000 to more than 1 million. We observed that a common feature of these two communities in comparison to others was both having lower clinical COVID-19 cases and lower positive detections/SARS-CoV-2 RNA levels in wastewater among all participating communities.²⁶ We found that the number of new COVID-19 cases per 100000 required to detect SARS-CoV-2 RNA in wastewater at defined probabilities of detection (50%, 80%, and 99%) was higher in smaller communities with <50000 residents than in larger communities with ≥ 100000 residents.²⁹ Lethbridge and High River have populations of 100000 and 17000 in their corresponding sewersheds, respectively. A low occurrence of new COVID-19 cases would increase the asymmetry of data between COVID-19 cases and SARS-CoV-2 RNA levels in wastewater, i.e., fewer data points in pairs, likely prompting the relatively low r values.

The visiting population could result in under-reported new COVID-19 cases in the communities, i.e., some of the COVID-19 cases occurred in the Town of Banff, but the cases were reported in other communities instead based on the permanent residency of the individuals.³⁰ However, the visiting population in Banff seemed not to alter the correlation between new COVID-19 cases and SARS-CoV-2 RNA levels in wastewater. This observation could be explained, as the annual residency equivalent ratio of visitors (estimated 912000 in 2020) was only 1.2 times that of registered permanent residents in the town if an average stay was assumed to be 1 week ($912000/52$ [weeks] = 17538). The correlation between WBS SARS-CoV-2 and clinical COVID-19 cases in Banff was strong from the second to the fourth wave with no visible effects from the visiting population, suggesting that most of the visitors stayed in Banff for a short period of time (1–3 days) and had a limited impact on the local sewage system. Thus, wastewater-based surveillance for SARS-CoV-2 can be applied to communities with varied populations in sewersheds.

While we have shown that WBS SARS-CoV-2 had a strong correlation to the prevalence of COVID-19 in community sewersheds with various population sizes and characteristics when SARS-CoV-2 RNA was detected frequently in the wastewater, a remaining challenge in this unprecedented global COVID-19 pandemic is to accurately determine the true prevalence of COVID-19 at the population level. Clinically reported COVID-19 cases usually represent the demographic of symptomatic COVID-19 patients, while it likely misses most of the asymptomatic or presymptomatic individuals with SARS-CoV-2 infection, which could reach 44%.³¹ In contrast, WBS SARS-CoV-2 can detect the SARS-CoV-2 RNA signal and monitor its levels in wastewater from all infected individuals with or without symptoms of COVID-19 regardless of resource availability or healthcare-seeking behavior.^{8,10,26,32} The outcomes from WBS SARS-CoV-2 provide a less biased data set for the crucial assessment of the population-based prevalence of COVID-19 for epidemiological response.³³

Our study showed that SARS-CoV-2 RNA levels (median genomic copies/100 mL) increased significantly from 1.1×10^3 in the second wave to 2.8×10^3 and 4.4×10^3 in the third and fourth waves, respectively, which were consistent with those reported in different studies.^{32,34–36} On the other hand, the numbers of reported new COVID-19 cases per 100000 were lower in the fourth wave than that in the third wave, while the median levels of SARS-CoV-2 RNA in wastewater were 1.6-fold higher in the fourth wave than in the third wave. There are two possible reasons for the divergent observations: (1)

clinical testing was scaled back to individuals with COVID-19 symptoms only with a decrease in average number of daily tests from 10483 ± 4753 in the third wave to 9630 ± 3789 in the fourth wave and (2) the proportion of vaccinated population had increased, resulting in a higher number of asymptomatic and mildly symptomatic infected individuals who were less likely to seek care and be tested. By October 1, 2021, the vaccination rate of adult Albertans in the participating communities ranged from 46% to 69% (data not shown). The observation and analysis indicated clearly that WBS SARS-CoV-2 has the potential to estimate the prevalence of SARS-CoV-2 infection more accurately than reported clinical COVID-19 cases in the population, especially when the clinical testing number declines because of policy change or limited testing capacity.

In the literature, different approaches have been used to calculate the “true” prevalence of COVID-19 using SARS-CoV-2 RNA levels in wastewater. Prevalence estimates of COVID-19 in the population have ranged from 0.1% to 5%³² and from 2.0% to 6.5%.⁹ These estimates from WBS SARS-CoV-2 usually generated much higher rates in comparison with either confirmed clinical COVID-19 cases or a modeling simulation (0.026% to 0.096%).^{37,38} One published study attempted to use a constant ratio of 0.78:1 between SARS-CoV-2 RNA concentration in wastewater and clinically confirmed COVID-19 case counts to calculate the population-based prevalence of COVID-19 in the community.¹⁵ We calculated the ratios between these two variables using our data set and found that the ratios of SARS-CoV-2 RNA concentration and clinical case counts varied significantly in each of the four waves of COVID-19 in Alberta. Hence, a single ratio should not be used to accurately estimate the true prevalence of COVID-19 in a population for different waves of the pandemic. COVID-19 burdens in the local communities were estimated using the SARS-CoV-2 RNA concentrations in wastewater in a surveillance study of 15 months.³⁹ The estimated incidence of COVID-19 was doubled over reported new cases from clinical testing results, allowing an assessment of potential asymptomatic and mild symptomatic individuals of COVID-19 that were not tested clinically. Nevertheless, the knowledge gaps in SARS-CoV-2 stool shedding kinetics resulted from infections of different variants and the variations of viral RNA degradations under different operating conditions of WWTPs limit the use of WBS SARS-CoV-2 to estimate the true COVID-19 disease burden for a community.^{19,26,40} Sewage travel time before sampling is another factor to consider in terms of the potential degradation of SARS-CoV-2 RNA signal during transportation, which may affect the quantification of SARS-CoV-2 levels in wastewater during surveillance.⁴¹ Further study is needed to address the effect of hydraulic travel time and degradation of SARS-CoV-2 RNA on the correlation with clinically reported COVID-19 cases. Despite the inability to translate outcomes of WBS SARS-CoV-2 into the actual number of COVID-19 cases in a community, the nondiscriminate inclusion of all COVID-19 cases with viral shedding and the strong correlation between WBS SARS-CoV-2 and new COVID-19 cases provides evidence that WBS SARS-CoV-2 can become an effective and supplementary surveillance tool for estimating the prevalence of COVID-19 in a community, especially when used in concert with clinical testing data. The ability to continue monitoring COVID-19 using WBS with the threat of emerging variants of concern

with different transmissibility is clearly valuable in the management of this pandemic.

5. CONCLUSIONS

This wastewater-based surveillance on SARS-CoV-2 addressed 17 months of continuous monitoring during four waves of the COVID-19 pandemic in 10 communities with different sizes of populations in Alberta. SARS-CoV-2 RNA levels in wastewater have a strong correlation with new COVID-19 case numbers in communities with statistical significance once the detection threshold of SARS-CoV-2 RNA in wastewater has been reached. This intrinsic correlation was independent of the size of the population in the sewershed served by the wastewater treatment facility. Continual surveillance on SARS-CoV-2 in wastewater provided temporal trending of the COVID-19 burden in the community, which provides valuable evidence for pandemic preparedness and response. Our work supported that SARS-CoV-2 WBS can be used to monitor COVID-19 in communities of various size served by WWTP, and it provided objective and reliable data with strict quality-controlled analytic procedures for estimating the prevalence of SARS-CoV-2 infection in the community, especially combined with clinical COVID-19 testing and screening.

■ ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its online Supporting Information. We have also deposited the data underlying this study into the public domain with the name of "Online Tracker for COVID-19 in wastewater" <https://covid-tracker.chi-csm.ca>. It is accessible publicly.

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsestwater.2c00143>.

An additional table and figure as described in the text (PDF)

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Notes

The authors declare no competing financial interest.

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