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Number of COVID-19 cases required in a population to detect SARS-CoV-2 RNA in wastewater in the province of Alberta, Canada: Sensitivity assessment

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ABSTRACT

With a unique and large size of testing results of 1,842 samples collected from 12 wastewater treatment plants (WWTP) for 14 months through from low to high prevalence of COVID-19, the sensitivity of RT-qPCR detection of SARS-CoV-2 RNA in wastewater that correspond to the communities was computed by using Probit analysis. This study determined the number of new COVID-19 cases per 100,000 population required to detect SARS-CoV-2 RNA in wastewater at defined probabilities and provided an evidence-based framework of wastewater-based epidemiology surveillance (WBE). Input data were positive and negative test results of SARS-CoV-2 RNA in wastewater samples and the corresponding new COVID-19 case rates per 100,000 population served by each WWTP. The analyses determined that RT-qPCR-based SARS-CoV-2 RNA detection threshold at 50%, 80% and 99% probability required a median of 8 (range: 4-19), 18 (9-43), and 38 (17-97) of new COVID-19 cases /100,000, respectively. Namely, the positive detection rate at 50%, 80% and 99% probability were 0.01%, 0.02%, and 0.04% averagely for new cases in the population. This study improves understanding of the performance of WBE SARS-CoV-2 RNA detection using the large datasets and prolonged study period. Estimated COVID-19 burden at a community level that would result in a positive detection of SARS-CoV-2 in wastewater is critical to support WBE application as a supplementary warning/monitoring system for COVID-19 prevention and control.

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Introduction

Studies have demonstrated that shedding of SARS-CoV-2 in stools occurs in both symptomatic and asymptomatic COVID-19 cases ranging from 7-21 days depending on timing of collection and testing of stool samples (Weiss et al., 2020; Foladori et al. 2020; Amirian, 2020; Cevik et al., 2021; Walsh et al., 2020). The transmission efficiency of pre-symptomatic and asymptomatic COVID-19 cases has yet to be delineated but these individuals can be an important source of community close contact transmission in various settings (Gandhi et al., 2020; He et al., 2020). At the start of the pandemic in 2020, Alberta has the highest number of COVID-19 tests per capita than any other jurisdictions in Canada (Alberta Government, 2021). The total number of COVID-19 tests performed in Alberta with a total population of 4.4 million was 4,703,047 up to July 1, 2021. This study utilized the dataset of high numbers of diagnostic testing for COVID-19 in Alberta during the pandemic and the early implementation of wastewater-based epidemiology surveillance (WBE) SARS-CoV-2 in 12 WWTP across the province to perform unique analyses to determine the population-based COVID-19 burden required for the detection of SARS-CoV-2 RNA in wastewater at different estimated detectability.

It is unfeasible to identify and trace asymptomatic and pre-symptomatic individuals early enough to prevent spread of SARS-CoV-2, which explains one of the reasons for the high prevalence of COVID-19 in general population. A key advantage of WBE on the level of SARS-CoV-2 RNA over clinical diagnostic testing for COVID-19 is the comprehensive capture of all clinical stages of COVID-19 including asymptomatic, pre-symptomatic and symptomatic infections in the community. There is no selection bias with WBE as compared to clinical testing that is affected by testing policies, symptom recognition, symptom severity, and access to testing, etc. (Li et al., 2022). Quantitative molecular detection assays, e.g., reverse transcription-quantitative polymerase chain reaction (RT-qPCR) has been used to detect and quantify the level of SARS-CoV-2 RNA in wastewater as a proxy for community-based transmission dynamics and to identify new waves of COVID-19 (Ahmed et al., 2020; Gonzalez et al, 2020; Weidhaas et al., 2021). However, accurate detection and quantification of SARS-CoV-2 RNA in wastewater is challenged by the complex matrix of wastewater including a high content of organic matter and a wide range of PCR inhibitory substances, which can vary significantly within hours (Schrader et al., 2012; Ahmed et al., 2021). Moreover, the level of SARS-CoV-2 RNA detected in the feces was much lower than respiratory specimens and the positive rates of feces ranged from 29% to 53% of COVID-19 cases (Parasa et al., 2020). Understanding the performance characteristics of WBE on SARS-CoV-2 RNA is critical for adapting it as a reliable tool for COVID-19 surveillance at community level. The number of COVID-19 cases required in a population to produce a detectable viral RNA signal in wastewater is largely unknown despite the large number of published studies on the detection of SARS-CoV-2 RNA in wastewater (Ahmed et al., 2020; Schmitz et al, 2021; Haramoto et al., 2020; Kumar et al., 2020; Medema et al., 2020; Randazzo et al., 2020; D'Aoust et al., 2021). Large variations of the positive detection rates (from 0.00005% to 0.88%) have been reported in these studies when different gene targets were used to detect SARS-CoV-2 RNA in wastewater (Hart and Halden, 2020). While correlation between COVID-19 burden and WBE SARS-CoV-2 RNA was observed in these studies, it is not possible to determine the threshold for detecting SARS-CoV-2 RNA in wastewater which allows us to project the population-based COVID-19 rates.

In this study, we investigated the sensitivity of RT-qPCR detection of SARS-CoV-2 RNA in wastewater associated with the numbers of new COVID-19 cases by using high numbers of clinical COVID-19 tests in each community and a large dataset of SARS-CoV-2 RNA results from corresponding sewersheds. Understanding of the COVID-19 disease burden at a community level that would result in a positive detection of SARS-CoV-2 RNA in wastewater is essential to support WBE application.

1. Materials and methods

1.1. *Recruitment of participating WWTPs and sample collections*

Twelve municipal and regional WWTPs serving 10 cities/towns across the province with geographic representatives, including the two major urban centers, were enrolled in this study. The communities and associated population served by respective WWTP were identified based on the sewersheds' information provided by each WWTP so that reported new and active COVID-19 case numbers of the corresponding areas were determined from COVID-19 surveillance database of the Government of Alberta. The COVID-19 endemic waves were defined as the first (May – Sept 2020), second (Oct 2020 – Feb 2021) and third (March – June 2021) in Alberta. The mean daily flow rate was also collected from each WWTP.

Post-grit raw influent wastewater samples (500 mL of 24-hr composite sample in frequency of every 2 hours-collection setting of the autosampler) were collected two or three days per week from May 15, 2020 to June 7, 2021. Wastewater samples were stored at 4°C on collection sites until transport once per week to the analytical laboratory and processed upon receiving for virus concentration, nucleic acid extraction and RT-qPCR using three gene targets of SARS-CoV-2.

1.2. *Wastewater sample process and SARS-CoV-2 RNA detection*

An optimized laboratory protocol for processing of wastewater samples was described by Qiu et al. (2021). Briefly, 100 mL of sample was centrifugated at 4500 x*g* for 10 min. The supernatant was collected, transferred to the Centricon filter cup (30-kDa MWCO, Millipore) and centrifuged at 3000 x*g* 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 and either processed for nucleic acid extraction or stored at −70 °C for later use. The total nucleic acid was extracted from 400 µL of the concentrated sample using MagMAX-96 viral RNA isola-

tion kit on the automated KingFisher™ Flex instrument and eluted at a final volume of 100 µL. One-step RT-qPCR assay was performed in duplicates to detect E, N1 and N2 genes of SARS-CoV-2 on an ABI 7500 PCR instrument (Qiu et al., 2021). To quantify the level of SARS-CoV-2 RNA in wastewater samples, an external standard curve was prepared by 10-fold series of dilution from 1.66×10^2 to 1.66×10^6 copies of the SARS-CoV-2 RNA fragment containing the RT-qPCR targets. The results are expressed as genome equivalent copy numbers per 100 mL of wastewater. The limit of detection was 80 copies per 100 mL for all three targets.

1.3. *Quality controls*

Known concentration of cultured hCoV-229E (100 µL, VR-740, ATCC) was spiked into each unprocessed sample and the recovery efficiency rate of the virus from wastewater was calculated as described. The recovery rate (%) = amount of hCoV 229E detected in the spiked sample / amount of virus detected in the baseline sample x 100. Salmon DNA (5 µL) was spiked into concentrated sample before the nucleic acid extraction and quantified by qPCR to assess the degree of PCR inhibition. Inhibition was defined as a delay of Ct by 3 cycles as compared to a distilled water control spiked with the same amount of salmon DNA. Pepper mild mottle virus (PMMoV), an indicator of human feces in wastewater (Bivins et al., 2020), was quantified using RT-qPCR alongside the 3 gene targets of SARS-CoV-2. Negative and positive controls were always included during sample concentration, nucleic acid extraction and RT-qPCR. All quality control criteria must be met before reporting. Positive result (SARS-CoV-2 RNA) was reported when there were two or more positive PCR tests out of the six duplicate PCR runs at two for each of the three SARS-CoV-2 gene targets (Qiu et al., 2021).

1.4. *Probit regression analysis and the sensitivity of detection*

Two empirical datasets were generated for each WWTP over the sample collection dates including the detection results of SARS-CoV-2 RNA in wastewater (positive or negative) and the rates of diagnosed cases of COVID-19 reported from its corresponding served population. Descriptive data analyses were performed using new cases in a 7-day average positive rate to observe whether a sigmoid trend of positive rate presents. A statistical method of Probit Analysis was then used to analyze the probability of detection of SARS-CoV-2 RNA in wastewater in relation to the number of new and active COVID-19 cases per 100,000 population (Finney, 1952). R package "brglm" was used for the Probit Analysis, which has the bias-reduction method to solve the quasi-complete separation issue in regression. Due to the heterogeneity of served populations and wastewater treatment capacities, data for each WWTP was also analyzed separately.

Required number of new COVID-19 cases per 100,000 population to detect SARS-CoV-2 RNA in wastewater at a probability of 50%, 80% and 99% were estimated using the fitted Probit Regression relationships for new COVID-19 cases rates. Furthermore, Probit analysis was also performed by clustering the data based on sizes of the population served by WWTPs as small (<50,000), moderate (50,000 – 150,000), and large (>150,000).

Since abundant viral shedding of SARS-CoV-2 in faeces was reported occurring in the early infection course (Hoffmann and Alsing, 2021; Wu et al, 2022), new cases of COVID-19 are used as independent variable in the Profit analysis. However, actual level of SARS-CoV-2 RNA in wastewater of each community is proportional to viral shedding from both new and active cases of COVID-19. Active cases of COVID-19 (defined as 14 days from the onset) represent a wide range of infected individuals and have autocorrelation for the continuous data of viral shedding although the dependency of active case is not met with the Probit regression analysis. Therefore, it is interesting to use active cases as a deductive independent variable to run the Probit analysis and to compare the outcomes of the probability of SARS-CoV-2 RNA detection in wastewater by new versus active COVID-19 cases. The difference of the probabilities between new and active COVID-19 cases for SARS-CoV-2 RNA detection in wastewater were compared using paired t-tests. The statistical significant level was set at $p < 0.05$.

2. Results

2.1. *Location and characteristics of WWTPs*

Twelve WWTP serving the cities/towns and its surroundings were summarized in Table 1. The areas served by 12 WWTPs covered 79% the provincial population of Alberta (3,458,955/4,371,000). These WWTP had mean daily flow rate from 3,763 ± 332 (smallest plant) to 346,000 ± 44645 (largest plant) m^3/day during the study period.

2.2. *Detection of SARS-CoV-2 RNA in wastewater*

A total of 1,842 wastewater samples were processed and tested for SARS-CoV-2 RNA. The overall positive rate of SARS-CoV-2 RNA detection in wastewater was 49.84% (918/1842), varying from 6.47 % to 95% during different COVID-19 waves in Alberta. The third wave showed the highest positive rate of SARS-CoV-2 RNA in wastewater samples (Table 1). Overall recovery of hCoV 229E had relatively large variations. The levels of PMMoV (by Ct values) and the proportion of samples with inhibition (by salmon DNA) were consistent (Appendix A Table S1), indicating relatively stable levels of fecal content in wastewater and equivalent intrinsic inhibitory effect of wastewater from individual WWTP.

2.3. *Sensitivity of SARS-CoV-2 RNA detection in wastewater versus COVID-19 cases*

The results of the probit analysis showed that the threshold of RT-qPCR detection of SARS-CoV-2 RNA in wastewater at 50%, 80% and 99% of probability required median (range) 8 (4-19), 18 (9-43), and 38 (17-97) daily reported new cases (Table 2 and Fig. 1); and 108 (42-164), 166 (94-414), and 318 (162-991) daily reported active cases (Appendix A Table S2/Fig. S1) of COVID-19 per 100,000 residents served by WWTP, respectively. Namely, the detection of SARS-CoV-2 RNA in wastewater at 50%, 80%

Table 1 – Overview of detecting SARS-CoV-2 RNA in wastewater collected from participating WWTPs in Alberta.

WWTP ID	[a] City/Town/County served	Start date (2020)	Tested no.	Positive no. (%)	Stratified by the three COVID-19 pandemic waves in Alberta					
					Wave 1 (May - Sep 2020)		Wave 2 (Oct 2020 - Feb 2021)		Wave 3 (Mar - Jun 2021)	
					Tested no.	Positive no. (%)	Tested no.	Positiveno. (%)	Tested no.	Positive no. (%)
1	Edmonton, Leduc, Beaumont	May 18	215	119 (55.3)	105	14 (13.3)	70	65 (92.9)	40	40 (100)
2	[b]Calgary North	May 10	189	106 (56.1)	83	9 (10.8)	64	55 (85.9)	42	42 (100)
3&4	[b]Calgary South	May 10	192	106 (55.2)	85	7 (8.2)	65	57 (87.7)	42	42 (100)
5	St. Alberta, Spruce Grove, Strathcona County, Fort Saskatchewan, Sturgeon County, Stoney plain, Morinville, Bone Accord, Gibbons	July 6	153	103 (67.3)	46	6 (13)	64	54 (84.4)	43	43 (100)
6	Red Deer, Sylvan Lake, Olds, Lacombe, Innisfail	July 6	151	84 (55.6)	45	2 (4.4)	63	39 (61.9)	43	43 (100)
7	Lethbridge	July 8	152	77 (50.7)	47	1 (2.1)	64	38 (59.4)	41	38 (92.7)
8	Grande Prairie	July 10	151	73 (48.3)	45	0 (0)	65	32 (49.2)	41	41 (100)
9	Medicine Hat	July 13	148	61 (41.2)	41	1 (2.4)	64	23 (35.9)	43	37 (86)
10	High River	July 12	150	54 (36)	45	2 (4.4)	64	20(31.3)	41	32 (78.1)
11	Canmore	May 25	184	61 (33.2)	77	0 (0)	64	19 (29.7)	43	42 (97.7)
12	Banff	June 30	157	74 (47.1)	51	1 (2)	64	35 (54.7)	42	38 (90.5)
Total			1842	918 (49.8)	670	43(6.4)	711	437(61.5)	461	438(95)

WWTP: Wastewater Treatment Plant
[a] Numerical labels were assigned to each WWTP for confidentiality
[b] City of Calgary has three wastewater treatment facilities participated in the study, WWTP 2 serves more population than WWTP 3&4

Table 2 – Number of COVID-19 new cases per 100,000 population required to detect SARS-CoV-2 RNA in wastewater at a probability of 50%, 80% and 99%.

WWTP ID	Number of COVID-19 new cases (per 100,000 population) required		
	50%	80%	99%
1	6	10	17
2	7	11	21
3&4	6	9	17
5	4	9	22
6	8	14	27
7	13	23	46
8	12	20	38
9	8	18	40
10	14	27	59
11	19	43	97
12	12	34	85
Median (range)	8 (4-19)	18 (9-43)	38 (17-97)

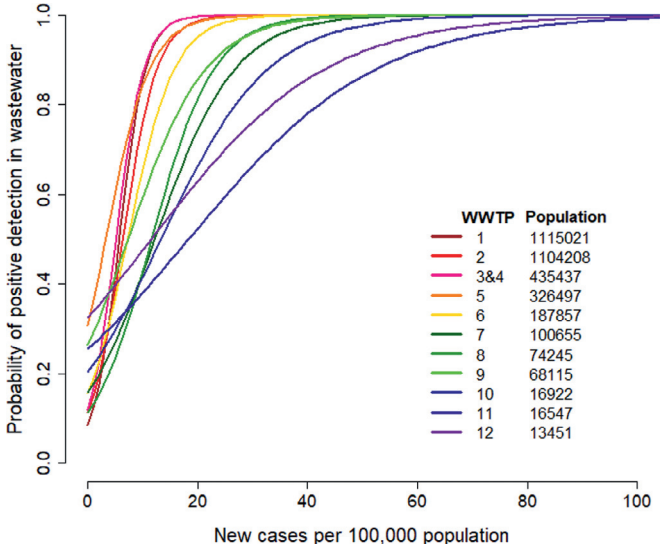

Fig. 1 – Probability of positive detection of SARS-CoV-2 RNA in wastewater versus the number of clinical diagnosed COVID-19 new cases per 100,000 population in each community served by corresponding WWTP.

and 99% probability required one new case of COVID-19 out of 9091 (0.01%), 4762 (0.02%), and 2564 (0.04%) of individuals (infected and non-infected) in the community averagely. The number of diagnosed COVID-19 cases required to detect SARS-CoV-2 RNA in wastewater at defined probability was different based on actual residents in the cities/towns served by its WWTP, i.e., the large facilities required higher numbers of new COVID-19 cases to detect SARS-CoV-2 RNA than the WWTPs serving a smaller number of residents (Table 3). As few as 2 new cases were required to achieve a positive detection of SARS-CoV-2 RNA at 50% probability in the smallest community (Town of Banff) while 67 to 77 new cases to achieve the same level of positive detection in the largest

Table 3 – Number of COVID-19 new cases required by the size of the population in each community served by the WWTP to detect SARS-CoV-2 RNA in wastewater at a probability of 50%, 80% and 99%.

WWTP ID	Population served by each WWTP	Number of COVID-19 new cases required by the size of population served by the WWTP		
		50%	80%	99%
1	1,115,021	67	112	190
2	1,104,208	77	121	232
3&4	435,437	26	39	74
5	326,497	13	29	72
6	187,857	15	26	51
7	100,655	13	23	46
8	74,245	9	15	28
9	68,115	5	12	27
10	16,922	2	5	10
11	16,547	3	7	16
12	13,451	2	5	11
Total	3,458,955			

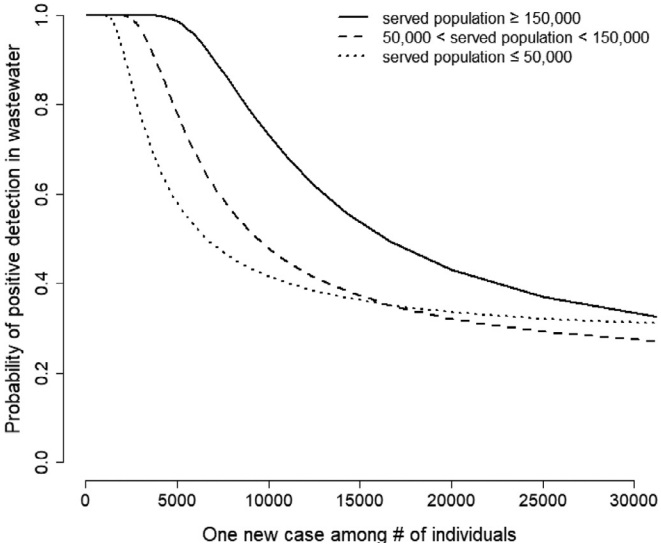

Fig. 2 – Probability of positive detection of SARS-CoV-2 RNA in wastewater when there is one new case in given number of individuals (infected and non-infected) in the community.

communities (Edmonton and Calgary). When the WWTPs are grouped into large, moderate, and small facilities based on its served population, the difference of detection sensitivity of SARS-CoV-2 RNA in wastewater became clearer, i.e., higher sensitivity in the large facilities compared to small ones using 100,000 population as denominator (Fig. 2 and Table 4). The trends of SARS-CoV-2 RNA positive detection rates in wastewater had changed over-time, corresponding to the fluctuation of COVID-19 burden in each community in this study (Appendix A Figs. S3-1 to S3-11). Using the rate of reported active COVID-19 cases per 100,000 population for the probit regression analysis, the results showed the same trend as the rate of new COVID-19 cases per 100,000 population in corresponding community (Appendix A Tables S2 to S4 /Figs. S1 to S2). However, the sensitivity of detection was increased significantly using the rates of new cases compared to the rates of active cases per 100,000 population ($p<0.05$).

3. Discussion

WBE on SARS-CoV-2 RNA has become a useful supplementary tool to monitor the changes of COVID-19 burden in the population (Ahmed et al., 2020; Gonzalez et al, 2020; Weidhaas et al., 2021; Kumar et al., 2020; Medema et al., 2020; Wu et al., 2021). This study started two months after the first case of COVID-19 was identified in Alberta. The overall positive detection rate of SARS-CoV-2 RNA in wastewater was 49.8 % of all samples collected from 12 WWTPs. The positive detection rates of SARS-CoV-2 RNA in wastewater were aligned well with the three waves of COVID-19 pandemic; and the levels of SARS-CoV-2 RNA correlated with the COVID-19 burden in Alberta (data will be shown in another publication). The large number of wastewater samples were collected from the participating WWTPs serving the communities with population sizes ranging from 13,451 to 1,115,021 while the matched new and active COVID-19 cases (by postal codes) reported in corresponding sewersheds were extracted and analyzed together with SARS-CoV-2 RNA results using Local Geographic Area mapping. The enriched datasets have allowed us to assess the sensitivity RT-qPCR detection on SARS-CoV-2 RNA in wastewater with much less bias.

The fundamental question is: What is the threshold of COVID-19 cases required in population for SARS-CoV-2 RNA to be detected in the wastewater? Large variations of detection sensitivity of SARS-CoV-2 RNA in wastewater ranging from 0.00005% to 0.88% have been reported in different studies, which was equivalent to one COVID-19 case in a population of 114 to 2,000,000 as a threshold to detect SARS-CoV-2 RNA in wastewater (Hart and Halden, 2020; Chik et al., 2021; Kumblathan et al., 2022). There are three key factors which can affect the threshold for SARS-CoV-2 RNA detection in wastewater. The first factor was the wide extent of clinical testing approaches for COVID-19 from one jurisdiction to another over times. In Alberta, testing policy has changed several times during the period of COVID-19 pandemic. The reliable number of diagnosed COVID-19 cases and identification of probable COVID-19 cases influence inevitably the accuracy of COVID-19 cases required to detect SARS-CoV-2 RNA in wastewater. The second factor entangles with the effect of intrinsic characteristics of wastewater matrix on RT-qPCR detection of SARS-CoV-2 RNA, including uncertainty of degradation and dilution of the virus in wastewater under various weather conditions, and presence of inhibitory substances to RT-qPCR performance (Schrader et al., 2012). One report, summarizing 13 studies, found that only 29%-55% of COVID-19 cases shed their viruses in stool specimens with much lower viral load in feces compared to respiratory specimens (Foladori et al., 2020). Some studies observed that SARS-CoV-2 can persist in feces up to 33 days (La Rosa et al., 2020). Degradation of viral RNA at different temperatures in wastewater were also

Table 4 – Number of COVID-19 new cases required, and the number of individuals required with one new COVID-19 case among them to detect SARS-CoV-2 RNA in wastewater at defined probabilities by different population size served by the WWTP

WWTP ID	The population scale	Number of COVID-19 new cases per 100,000 population required			One new case out of number of individuals in the community		
		50%	80%	99%	50%	80%	99%
1, 2, 3&4, 5, 6	> 150,000	7	12	21	14,286	8,333	4,762
7, 8, 9	50,000 -150,000	11	21	39	9,091	4,762	2,564
10, 11, 12	< 50,000	16	36	71	6,250	2,778	1,408

reported (Aboubakr et al., 2021). These intrinsic characteristics create uncertainties for the detectability of SARS-CoV-2 RNA. The third factor was the study design. Most WBE studies reported RT-qPCR testing sensitivity of SARS-CoV-2 RNA in wastewater either using a limited number of wastewater samples or only short periods of observation time (Ahmed et al., 2020; Medema et al., 2020; Randazzo et al., 2020; Hart and Halden, 2020; Wu et al., 2021; Hong et al., 2021; Chavarria-Miró et al., 2021). These deficiencies in study designs and data analysis had limited the representativeness of the datasets for assessing the detection sensitivity of SARS-CoV-2 RNA in wastewater.

Detection of SARS-CoV-2 RNA in wastewater reflects overall burden of all COVID-19, including asymptomatic, pre/post-symptomatic and symptomatic individuals (Wölfel et al., 2020). Having taken advantages of large scale of diagnostic testing of COVID-19 in Alberta, large numbers of wastewater samples collected in this study encompassed a wide range of populations served by participating WWTPs, presenting continuously monitoring changes of SARS-CoV-2 in wastewater. Rationale of the study design was that effects of intrinsic characteristics of wastewater on the detection of SARS-CoV-2 RNA are relatively unchanged during study period. This allows us to relate the positive rate of SARS-CoV-2 RNA per 100,000 population in wastewater of WWTP sewershed to new COVID-19 cases in corresponding community to assess the detection sensitivity via the probit regression analysis. Probit analysis is a specialized regression model to analyze binomial response variables and has been used widely, especially in toxicology, biology, and health sciences. This RT-qPCR detection sensitivity was similar to the sensitivity reported by Ahmed et al. using new cases in analysis (0.026% – 0.028%) (Ahmed et al., 2020; Wu et al., 2021), higher than reported by Chavarria-Miró et al. (2021) (0.09%) and Xagoraraki and O'Brien (2020) (0.64%), and lower than that estimated by Hart and Halden (2020) (0.00005%) by using modeling. The Probit analysis provided us with the scenarios of SARS-CoV-2 RNA detection threshold in wastewater from low (50%) to high (99%) probability. At low probability, the threshold estimates conservatively the numbers of COVID-19 cases in the community under WBE surveillance, allowing early alert for local public health to implement measures for prevention and control. To our knowledge, this study uses the larger datasets and covers the longer period of the COVID-19 pandemic in comparison with other studies of RT-qPCR detection on SARS-CoV-2 RNA in wastewater. Thus, the detection sensitivity obtained from the study provided a reliable reference for understanding of WBE on SARS-CoV-2.

The rates of active COVID-19 cases were imputed using Probit analysis and the results were compared with the rates of new cases for the detection sensitivity of SARS-CoV-2 RNA in wastewater. Not unexpectedly, a significantly higher threshold of active COVID-19 cases was required for detection of SARS-CoV-2 RNA as compared to new COVID-19 cases ($p<0.05$). Arguments on this finding were that rapid degradation of RNA viruses occurred in wastewater (Hoffmann and Alsing, 2021) and/or short duration of SARS-CoV-2 viral shedding from the feces (median 7 days) after negative conversion of COVID-19 in pharyngeal swabs regardless of COVID-19 severity (Chen et al., 2020), resulting in decrease in the level of detectable SARS-CoV-2 RNA even though there were more active than new case presence in the community. This also implies that new COVID-19 cases are likely shedding more virus in feces (Wu et al., 2022). However, active cases provide more relevant estimates on demands for healthcare, which is an important outcome measure and target for prevention in COVID-19 pandemic management. Several studies using new case rates to define threshold of SARS-CoV-2 RNA detection demonstrated large variations regarding to detection sensitivity (Hart and Halden, 2020). Further study is needed to assess the relevance of active versus new cases to measures and management of COVID-19 pandemic.

There are some limitations of this study, which may influence the thresholds of SARS-CoV-2 RNA detection in wastewater. Even though there were pilots of testing asymptomatic individuals for COVID-19 in Alberta, these pilots were only performed for a very short period, so the reported rates of clinical COVID-19 cases underestimated the true rates of COVID-19 infection in population. Virus shedding from all individuals with COVID-19 infection could be detected in wastewater, but individuals might not be diagnosed by clinical testing. Lack of a precise SARS-CoV-2 fecal shedding rate and duration is the current limit in the use of SARS-CoV-2 RNA results in wastewater to calculate accurate numbers of COVID-19 cases in a community (Hong et al., 2021). Travelers are another factor contributing to the variations of COVID-19 cases related to the detection threshold of SARS-CoV-2 RNA as observed in certain communities (e.g. Town of Banff). In consideration of this large-scale study across the province and restrictions for travels during pandemic, the impact of travelers on the analyses of detection sensitivity of SARS-CoV-2 RNA in wastewater is minimal.

4. Conclusions

Using the Probit analysis, we determined that RT-qPCR detecting SARS-CoV-2 RNA in wastewater at 50%, 80% and 99% probability required a median of 8, 18, and 38 of new COVID-19 cases per 100,000 population in a community through this study with more than 1,800 composite wastewater samples over 14 months covering the duration of the three waves of COVID-19 pandemic. The probability enables us by the first time to estimate the COVID-19 burden in communities from results of SARS-CoV-2 RNA in wastewater numerically. Imputed sensitivity of SARS-CoV-2 RNA detection provides reliable estimate of COVID-19 case numbers and will promote WBE application for SARS-CoV-2 as a complementary approach to diagnostic testing during the pandemic.

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Appendix A Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2022.04.047.

REFERENCES

Aboubakr, H.A., Sharafeldin, T.A., Goyal, S.M., 2021. Stability of SARS-CoV-2 and other coronaviruses in the environment and on common touch surfaces and the influence of climatic conditions: a review. Transbound. Emerg. Dis. 68, 296–312. doi:10.1111/tbed.13707-35.

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J.W., et al., 2020. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. Sci. Total Environ. 728, 138764. doi:10.1016/j.scitotenv.2020.138764.

Ahmed, W., Bivins, A., Bertsch, P.M., Bibby, K., Gyawali, P., Sherchan, S.P., et al., 2021. Intraday variability of indicator and pathogenic viruses in 1-h and 24-h composite wastewater samples: implications for wastewater-based epidemiology. Environ. Res. 193, 110531. doi:10.1016/j.envres.2020.110531.

Amirian, E.S., 2020. Potential fecal transmission of SARS-CoV-2: current evidence and implications for public health. Int. J. Infect. Dis. 95, 363–370.

Bivins, A., Crank, K., Greaves, J., North, D., Wu, Z.Y., Bibby, K., 2020. Cross-assembly phage and pepper mild mottle virus as viral water quality monitoring tools - potential, research gaps, and way forward. Cur. Opinion Environ. Sci. Health 16, 54–61. doi:10.1016/j.coesh. 2020.02.001.

Cevik, M., Tate, M., Lloyd, O., Maraolo, A.E., Schafers, J., Ho, A., 2021. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. Lancet Microbe 2, e13–e22.. doi:10.1016/S2666-5247(20)30172-5, doi.org/.

Chavarria-Miró, G., Anfruns-Estrada, E., Martínez-Velázquez, A., Vázquez-Portero, M., Guix, S., Paraira, M., et al., 2021. Time evolution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in wastewater during the first pandemic wave of COVID-19 in the metropolitan area of Barcelona, Spain. Appl. Environ. Microbiol. 87, e02750. doi:10.1128/AEM.02750-20, -20..

Chen, Y, Chen, L, Deng, Q, Zhang, G, Wu, K, Ni, L, et al., 2020. The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients. J. Med. Virol. 92, 833–840. doi:10.1002/jmv.25825.

Chik, A.H.S., Glier, M.B., Servos, M., Mangat, C.S., Pang, X.-L., D'Aoust, P.M., et al., 2021. Canadian SARS-CoV-2 inter-laboratory consortium. Comparison of approaches to quantify SARS-CoV-2 in wastewater using RT-qPCR: results and implications from a collaborative inter-laboratory study in Canada. J. Environ. Sci. 107, 218–229.. doi:10.1016/j.jes.2021.01.029, doi.org/.

Finney, D. J., 1952. Probit Analysis. Finney DJ ed., 2nd, Cambridge University Press, New York. doi: 10.1002/jps.3030411125

D'Aoust, P.M., Mercier, E., Montpetit, D., Jia, J.J., Alexandrov, I., Neault, N., et al., 2021. Quantitative analysis of SARS-CoV-2 RNA from wastewater solids in communities with low COVID-19 incidence and prevalence. Water Res. 188, 116560. doi:10.1016/j.watres.2020.116560.

Foladori, P., Cutrupi, F., Segata, N., Manara, S., Pinto, F., Malpei, F., et al., 2020. SARS-CoV-2 from faeces to wastewater treatment: what do we know? Sci. Total Environ. 743, 140444. doi:10.1016/j.scitotenv.2020.140444.

Gandhi, M., Yokoe, D.S., Havlir, D.V., 2020. Asymptomatic transmission, the Achilles' heel of current strategies to control COVID-19. N. Engl. J. Med. 382, 2158–2160. doi:10.1056/NEJMe2009758.

Gonzalez, R., Curtis, K., Bivins, A., Bibby, K., Weir, M.H., Yetka, K., et al., 2020. COVID-19 surveillance in Southeastern Virginia using wastewater-based epidemiology. Water Res. 186, 116296. doi:10.1016/j.watres.2020.116296.

Haramoto, E., Malla, B., Thakali, O., Kitajima, M., 2020. First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan. Sci. Total Environ. 737.

Hart, O.E., Halden, R.U., 2020. Computational analysis of SARS-CoV-2/COVID-19 surveillance by wastewater-based epidemiology locally and globally: Feasibility, economy, opportunities and challenges. Sci. Total Environ. 730, 138875. doi:10.1016/j.scitotenv.2020.138875.

He, X., Lau, E.H.Y., Wu, P., Deng, X., Wang, J., Ha, o X., et al., 2020. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat. Med. 26, 672–675.

Hoffmann, T., Alsing, J. 2021. Faecal shedding models for SARS-CoV-2 RNA amongst hospitalised patients and implications for wastewater-based epidemiology. Preprint from medRxiv. doi: https://doi.org/10.1101/2021.03.16.21253603

Hong, P.Y., Rachmadi, A.T., Mantilla-Calderon, D., Alkahtani, M., Bashawri, Y.M., Al Qarni, H., et al., 2021. Estimating the minimum number of SARS-CoV-2 infected cases needed to detect viral RNA in wastewater: to what extent of the outbreak can surveillance of wastewater tell us? Environ. Res. 195, 110748. doi:10.1016/j.envres.2021.110748.

Kumar, M., Patel, A.K., Shah, A.V., Raval, J., Rajpara, N., Joshi, M., et al., 2020. First proof of the capability of wastewater surveillance for COVID-19 in India through detection of genetic material of SARS-CoV-2. Sci. Total Environ. 746, 141326. doi:10.1016/j.scitotenv. 2020.141326.

Kumblathan, T., Piroddi, N., Hrudey, S.E., Li, X.-F., 2022. Wastewater based surveillance of SARS-CoV-2: challenges and perspective from a Canadian inter-laboratory study. J. Environ. Sci. 116, 229–232. doi:10.1016/j.jes.2022.01.039.

La Rosa, G., Bonadonna, L., Lucentini, L., Kenmoe, S., Suffredini, E., 2020. Coronavirus in water environments: occurrence, persistence and concentration methods - a scoping review. Water Res. 179, 115899. doi:10.1016/j.watres.2020.115899.

Li, L., Mazurowski, L., Dewan, A., Carine, M., Haak, L., Guarin, T.C., et al., 2022. Longitudinal monitoring of SARS-CoV-2 in wastewater using viral genetic markers and the estimation of unconfirmed COVID-19 cases. Sci. Tot. Env. 817, 152958. doi:10.1016/j.scitotenv.2022.152958.

Medema, G., Been, F., Heijnen, L., Petterson, S., 2020. Implementation of environmental surveillance for SARS-CoV-2 virus to support public health decisions: opportunities and challenges. Curr. Opin. Environ. Sci. Heal. 17, 49–71.

Parasa, S., Desai, M., Chandrasekar, V.T., Patel, H.K., Kennedy, K.F., Roesch, T., et al., 2020. Prevalence of gastrointestinal symptoms and fecal viral shedding in patients with coronavirus disease 2019: a systematic review and meta-analysis. JAMA Netw. Open 3, e2011335. doi:10.1001/jamanetworkopen.2020.11335.

Qiu, Y., Yu, J., Pabbaraju, K., Lee, E.B., Gao, T.J., Ashbolt, J.N., et al., 2021. Validating and optimizing the method for molecular detection and quantification of SARS-CoV-2 in wastewater. Sci. Total Environ., 151434 doi:10.1016/j.scitotenv.2021.151434.

Randazzo, W., Truchado, P., Cuevas-Ferrando, E., Simón, P., Allende, A., Sánchez, G., 2020. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. Water Res. 181, 115942. doi:10.1016/j.watres.2020.115942.

Schrader, C., Schielke, A., Ellerbroek, L., Johne, R., 2012. PCR inhibitors - occurrence, properties and removal. J. Appl. Microbiol. 113, 1014–1026.

Schmitz, B.W., Innes, G.K., Prasek, S.M., Betancourt, W.Q., Stark, E.R., Foster, A.R., et al., 2021. Enumerating asymptomatic COVID-19 cases and estimating SARS-CoV-2 fecal shedding rates via wastewater-based epidemiology. Sci. Total Environ. 801, 149794. doi:10.1016/j.scitotenv.2021.149794.

Alberta Government, 2021. Symptoms and testing Available from.

Walsh, K.A., Jordan, K., Clyne, B., Rohde, D., Drummond, L., Byrne, P., et al., 2020. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. J. Infect. 81, 357–371.

Weidhaas, J., Aanderud, Z.T., Roper, D.K., VanDerslice, J., Gaddis, E.B., Ostermiller, J., et al., 2021. Correlation of SARS-CoV-2 RNA in wastewater with COVID-19 disease burden in sewersheds. Sci. Total Environ. 775, 145790. doi:10.1016/j.scitotenv.2021.145790.

Weiss, A., Jellings, M., Sommer, M.O.A., 2020. Spatial and temporal dynamics of SARS-CoV-2 in COVID-19 patients: a systematic review and meta-analysis. EbioMedicine 58, 102916. doi:10.1016/j.ebiom.2020.102916.

Wölfel, R., Corman, V.M., Guggemos, W., Seilmaier, M., Zange, S., Müller, M.A., et al., 2020. Virological assessment of hospitalized patients with COVID-2019. Nature 581 (7809), 465–469. doi:10.1038/s41586-020-2196-x.

Wu, F., Xiao, A., Zhang, J., Moniz, K., Endo, N., Armas, F., et al., 2021. Wastewater surveillance of SARS-CoV-2 across 40 U.S. states from February to June 2020. Water Res. 202, 117400. doi:10.1016/j.watres.2021.117400.

Wu, F, Xiao, A, Zhang, J, Moniz, K, Endo, N, Armas, F, et al., 2022. SARS-CoV-2 RNA concentrations in wastewater foreshadow dynamics and clinical presentation of new COVID-19 cases. Sci. Total Environ. 805, 150121. doi:10.1016/j.scitotenv.2021.150121.

Xagoraraki, I., O'Brien, E., 2020. Wastewater-based epidemiology for early detection of viral outbreak. In: Bannon, D.E. (Ed.), Women in Water Quality. Springer, pp. 75–97. doi:10.1007/978-3-030-17819-2_5.