ALBERTA INNOVATES

CLEAN RESOURCES FINAL PUBLIC REPORT

1. PROJECT INFORMATION:

Project Title:	Detection and quantitation of SARS-CoV-2 in wastewater to conduct surveillance on burden of community infection, identify outbreaks and support public health decision-making on control measures for prevention COVID-19 transmission
Alberta Innovates Project Number:	RES0052543
Submission Date:	August 16, 2021
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Alberta Innovates Funding:	$39,489
AI Project Advisor:	

2. APPLICANT INFORMATION:

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3. PROJECT PARTNERS

This project was funded primarily by CIHR rapid COVID-19 research program and supported by Alberta Health and Alberta Innovates. The partners who have contributed to this large scale and long-term wastewater-based surveillance (WBS) of SARS-CoV-2 in wastewater include the participating wastewater treatment facilities in 10 cities across Alberta and the corporations as EPCOR and Alberta Capital Region Wastewater Commission (ACRWC), Alberta Health, Alberta Chief Medical Office of Health, the National Laboratory of Microbiology, Canadian Water Network, Albert Precision Laboratories, and University of Alberta. The partnership and networks are critical components for delivering this project successfully. Scientific Advisory Committee for this project has provided constant supports for the study along during COVID-19 pandemic. All contributors for this project are acknowledged by the research team.

A. EXECUTIVE SUMMARY

Major project activities and tasks have been completed during this period, which have led to following outcomes as successful milestones of this project.

1) Recruiting 12 wastewater treatment plants (WWTPs) involved with 10 communities across Alberta
2) Establishing a network (provincially and nationally) and setting up monthly Scientific Advisory Committee (SAC) meeting to ensure the study in a right track
3) Developing the standard protocols for the molecular detection of SARS-CoV-2 RNA in sewage using 3 target genes of SARS-CoV-2 to increase detectability
4) Continuing monitoring of SARS-CoV-2 in wastewater samples collected 3 times per week from all WWTPs for 12 months and correlating the data with clinical testing outcomes
5) Data sharing with knowledge users including Alberta Health Ministry Office weekly and some of the cities and towns in Alberta as requested
6) Generating scientific knowledges (2 papers published, 1 submitted and 3 more papers under preparation)
7) Providing evidenced-based support for the latest Alberta Government decision to use WBS for COVID-19 in different communities rather than clinical testing of COVID-19 in this province

B. INTRODUCTION

- **Sector introduction:** Coping with waves of COVID-19 pandemic in Alberta, Alberta Innovates has allocated the funding to support rapid COVID-19 research on right time, including this project of detection and quantitation of SARS-CoV-2 in wastewater to conduct surveillance on burden of community infection, identify outbreaks and support public health decision-making on control measures for prevention COVID-19 transmission. This specific funding was to support this project for one year and was allocated to Dr. Pang and research team as the grant recipient in May 2020 before the 2nd wave of COVID-19 reaches its peak in Alberta. This funding allows the research team to carry on a series of critical research activities of the project before the CIHR funding was allocated to the team via the University of Alberta. This progress report covers these activities and related outcomes during one year from May 2020 to May 2021.
- **Knowledge or Technology Gaps:** This large scale and long-duration study is to address knowledge gaps as follows: 1) what is the true detection of sensitivity of SARS-CoV-2 RNA in wastewater by RT-PCR based methods; and 2) whether WBS of SARS-CoV-2 can be used as a reliable and cost-effective warning system for COVID-19 epidemiology to estimate the prevalence of COVID-19 pandemic in community.

C. PROJECT DESCRIPTION

- **Knowledge or Technology Description:** The original objectives include: 1)Detect and quantify SARS-CoV-2 in wastewater samples from strategically chosen WWTP facilities; 2)Identify temporal fluctuation of SARS-CoV-2 in wastewater from different communities with differing populations and burden of confirmed cases; 3)Investigate the relationship between community-based COVID-19 infections to the levels of SARS-CoV-2 in wastewaters as a potential real-time predictor; and 4)Develop a predictive model for COVID-19 disease burden and early alerts for outbreaks to support informed public health decision-making on pandemic control measures. The goal was to provide evidence-based support for using WBS to monitor COVID-19 activities in the community and assist the decision-making on strengthening or relaxing measures for COVID-19 control via one-year study. RT-PCR based molecular detection method is the core technology on the field of WBS on SARS-CoV-2.
- **Updates to Project Objectives:** The original objectives of the project have been following through without deviations. Another task in the objective of data sharing was added. In collaboration with the National Lab of Microbiology, we have provided our large dataset for modelling analysis together with national data they have obtained in different provinces. This analysis has resulted in a publication entitled "A Wastewater-Based Epidemic Model for SARS-CoV-2 with Application to Three Canadian Cities" which was published in medRxiv online on July 25, 2021. As COVID-19 pandemic goes, the variants of concerns (VOC) have emerged as a new threat to the publics. We have extended the original objective of SARS-CoV-2 detection to VOC strains in wastewater in collaboration with the National Lab of Microbiology. We have used both RT-PCR based method and Next Generation Sequencing (NGS) to detect the variants of SARS-CoV-2 in wastewater.
- **Performance Metrics:** The project specific metrics have not been changed as initially described when the project started. The most critical metric to measure successful delivery of this project is that the knowledge derived from the scientific evidence of this one-year study has been translated to practical application of WBS in this province as announced by Alberta Government recently for monitoring prevalence of COVID-19 in community rather than unaffordable clinical testing of COVID-19 in population. WBS of SARS-CoV-2 is the cost-effective and reliable method for estimate of prevalence of COVID-19 in community.

D. METHODOLOGY

Based on our knowledge and experiences on viral RNA detection in wastewater, we have further developed and validated standard protocols for detection of SARS-CoV-2 RNA in wastewater. The Centricon Plus-70 centrifugal ultrafilter (30-kDa MWCO, Millipore) filtration method was selected as a cost-effective way to concentrate SARS-CoV-2 from 100 ml of wastewater. To increase yielding of SARS-CoV-2 from wastewater and enhance detection sensitivity, we have optimized following procedures: 1) pH adjustment of wastewater samples (raising pH to 9.5 – 10 before the filtration and re-adjusting pH to 7.0 -7.5 after the Centricon device); 2)use of solid portion of wastewater versus liquid portion of wastewater after initial centrifugation; 3) effect of storage conditions of 4 C and -20 C of the samples on SARS-CoV-2 RNA yielding; 4) optimization of viral RNA isolation by selecting different kits including RNeasy Power Microbiome kit (Qiagen, ON, CA), MagMAX-96 viral RNA isolation kit (ThermoFisher, ON, CA), MagMAX Viral/Pathogen nucleic acid isolation kit (ThermoFisher, ON, CA), QIAamp viral RNA mini kit (Qiagen, ON, CA) and ReliaPrep™ RNA Miniprep System (Promega, WI, USA); 5) one-step versus two-step RT-qPCR assays for detecting SARS-CoV-2 RNA; 6) normalization of SARS-CoV-2 RNA signal using quantitation of endogenous PMMoV in the samples; 7) quality control of the process using HCoV-229E lab strain of coronavirus; 8) PCR inhibition monitoring by spiked Salmon DNA; 9) evaluation of sensitivity, specificity and precision of one-step RT-qPCR assay; and 10) selection, validation and incorporation of 3 genomic targets of SARS-CoV-2 N1, N2 and E genes. A standard protocol of detection and quantitation of SARS-CoV-2 was successfully established and implemented for daily performance.

The latest development of methods is direct RT-PCR based detection of VOCs in wastewater. NGS has also been used to confirm the variant strains in wastewater samples which had relatively high copies of SARS-CoV-2 RNA.

E. PROJECT RESULTS

Method validation results: The sample stored at 4 ºC after collection showed enhanced detection of SARS-CoV-2 RNA with an average of 2 PCR-cycle threshold (Ct) value as compared to samples stored at -20 ºC. Pre-adjustment of the raw sample pH to 9.6 followed by readjustment to pH neutral after solid removal increased the recovery of spiked hCoV-229E nearly 13 folds. MagMAX-96 viral RNA isolation kit showed the best recovery of hCoV-229E (50.1 ± 20.1%) among the 5 commercially available RNA isolation kits evaluated. One-step RT-qPCR had better sensitivity than two-step RT-qPCR for quantitation of SARS-CoV-2 RNA in wastewater. The salmon DNA was also included for monitoring PCR inhibition and the pepper mild mottle virus (PMMoV) was measured as a fecal indicator to normalize the SARS-CoV-2 levels in wastewater samples.

SARS-CoV-2 variant of concerns and variant of interest detection: We have further developed the methods allowing us to detect the VOC and VOI in wastewater samples. Q-RT PCR assay and NGS we have developed and applied for detection of VOC and VOI. The results demonstrate that we can identify the VOC and VOI in the wastewater samples with wild-type SARS-CoV-2 RNA positive recently (see attached Table 1).

The results from SARS-CoV-2 RNA in wastewater and COVID-19 active cases in corresponding sewersheds in this study: As more data and analysis obtained, the following trends are clear and meaningful: 1) WBS methods could detect asymptomatic, pre-symptomatic, and symptomatic infections with SARS-CoV-2 shed in wastewater at the community level; 2) the positivity rate of SARS-coV-2 RNA and levels of SARS-CoV-2 RNA in wastewater reflect new and/or active cases in corresponding communities; 3) detection of SARS-CoV-2 RNA in wastewater samples is usually a couple of days ahead of active COVID-19 cases occurrence or peak in that community. Lag-time correlation analysis shows that 5 days prediction is the best fit by the WBS testing before the peak of active COVID-19 cases (Figure 1, 2); 4) fluctuation of COVID-19 active cases in the community is comparable with and follows changes of SARS-CoV-2 RNA in wastewater during and post the 2nd and 3rd waves of COVID-19 in this province; and 5)SARS-CoV-2 RNA levels reflects infected population including mobile workers and /or tourists, which could be mis-interpreted by clinical testing of active COVID-19 rates (infected population per 100, 000) reported based on fixed residents in the related health zone of Alberta.

The detection sensitivity of SARS-CoV-2 RNA in wastewater at 50%, 80% and 99% probability required minimally 85, 195, 385 of new cases, and 105, 215, 410 of active cases of COVID-19 per 100,000 residents in the community, respectively, based on our latest Profit regression analysis of the large dataset.

F. KEY LEARNINGS

Key learnings from this study are as follows:

1) On methodology, we have learned that wastewater samples stored at 4 ºC after collection had enhanced detection of SARS-CoV-2 RNA with an average of 2 PCR-cycle threshold (Ct) value as compared to the samples stored at -20 ºC, and Pre-adjustment of the raw sample pH to 9.6 followed by readjustment to pH neutral after solid removal during sample processing increased the recovery of spiked hCoV-229E, indicating that RT-PCR detection efficacy has been increased. The impact of this gaining of knowledge could significantly improve the detection of viruses in wastewater using WBS approach not only on SARS-CoV-2 but also other pathogenic viruses which could cause frequent outbreaks in community.

2) On the detection sensitivity of WBS RT-PCR based methods for SARS-CoV-2 RNA in wastewater, we by the first time disclosed the true sensitivity of detection of SARS-CoV-2 in wastewater using probit regression analysis based on the large quantity of samples and long-duration of this study. In contrast with large variations of detection sensitivity published in many studies of WBS on SARS-CoV-2 in wastewater, the sensitivity observed in this study is close to the probability of viral presence in nature. A manuscript is under preparation now. This knowledge will answer the critical question regarding the possibility of WBS application and provide the scientific community with evidence as well as confidence on using the WBS surveillance for outbreaks of viral and other infectious diseases.

3) On correlation of SARS-CoV-2 in wastewater and COVID-19 new and active cases in corresponding regions, the study provides large dataset which allows us to do reliable analysis. The results showed that very good correlation was achieved between numbers of COVID-19 cases and fluctuation of SARS-CoV-2 RNA levels in wastewater. The similar analysis was also carried out by the Data analysis group of Alberta Government with the same conclusion. The outcomes from the data analysis have already impacted on the communities which have been involved in the study through the reports to the city and town administrations. They are very interested in hearing from us about the weekly updated results. The latest decision from Alberta Health authority and Alberta Government on replacing clinical COVID-19 testing with WBS of SARS-CoV-2 in wastewater to coping with change of COVID-19 pandemic trend demonstrates real impact of this study on policies and regulations for prevention and control of COVID-19 outbreaks in the community.

G. OUTCOMES AND IMPACTS

1. This project has been a focus of numerous media including national, local, the University of Alberta reports and TV news releases
2. WBS of SARS-CoV-2 in wastewater has been adopted by Alberta Government as a tool to monitoring the prevalence of COVID-19 in selected communities in Alberta post 3rd wave of the pandemic. The funding has been approved and the project will be teamed up by the University of Calgary, University of Alberta, and Alberta Precision Laboratories
3. Publications related to this project as follows (more to come):

    a. A Wastewater-Based Epidemic Model for SARS-CoV-2 with Application to Three Canadian Cities doi: https://doi.org/10.1101/2021.07.19.21260773
    b. Hasing, M.; Yu, J.; Qiu, Y.; Maal-Bared, R.; Bhavanam, S.; Lee, B.; Hrudey, S.; Pang, X. Comparison of Detecting and Quantitating SARS-CoV-2 in Wastewater Using Moderate-Speed Centrifuged Solids versus an Ultrafiltration Method. Water 2021, 13, 2166. https://doi.org/10.3390/w13162166

H. BENEFITS

Economic: The study provides the evidence that WBS of SARS-CoV-2 is a const-effective and affordable way to monitoring the prevalence of COVID-19 in population. It can also be extended to other infectious disease outbreaks at the community level if it can be applied correctly. This project also created several partial-time jobs and jobs for summer students. It is very important during COVID-19 pandemic seasons.

Environmental: Sewage is human waste and impacts environment negatively if it is not treated appropriately. It can also used for obtaining some useful information about human health and disease control and prevention. WBS opens a new avenue on this aspect.

Social: This project has attracted a lot of attentions from various media. News releases have improved the public for understanding of innovations and different approaches against COVID-19 pandemics. It has strengthened stakeholder and health authority involvement and investment on innovations and sciences.

Building innovation capacity: This project has contributed to the training of several HQSPs during periods. Three summer students were also involved and trained to learn new technologies. Another important accomplishment of this project was to establish a network of collaboration on research and innovations. The collaboration network involving different institutions, Universities, City and town administration, corporations, non-profit organizations and researchers across the province and nation is the key for successful delivery of this fruitful project.

I. RECOMMENDATIONS AND NEXT STEPS

- WBS of COVID-19 has improved our understanding of this pandemic and played an increasingly meaningful role in infectious disease control and prevention. Knowledge and key learnings from this study will improve WBS application not only for COVID-19 pandemic but also other outbreaks of viral infectious diseases in long-term. Hopefully it forms a warning system for any potential outbreaks of transmissible diseases in future.

- WBS of SARS-CoV-2 has been adopted by Alberta Government for now (not sure for the terms). Further funding will allow us to advance the WBS for other disease outbreaks, especially norovirus associated gastroenteritis during winter season in this province. This viral outbreak has caused working time lost, some complications and heavy burdens of healthcare resources annually.

- Through this project, we have developed a partnership with the National Lab of Microbiology, which assists us to advance another COVID-19 study funded by PHAC on monitoring SARS-CoV-2 using the site-specific sewage from long-term care facilities in Edmonton to protect the vulnerable population from COVID-19 outbreaks. Another partnership through the study was developed with the University of Calgary, which has led to new WBS program of SARS-CoV-2 that will be launched soon.

J. KNOWLEDGE DISSEMINATION

See the key learning section for details

K. CONCLUSIONS

Having used the public funding from CIHR, Albert Health and Alberta Innovates, our research team has delivered this project of WBS on SARS-CoV-2 in wastewater successfully on planned time frame. This surveillance study covers 10 communities (12 WWTPs) across Alberta for 12 months. Using RT-qPCR base detection on 3 targeting genes of SARS-CoV-2, we demonstrated that the detection sensitivity of SARS-CoV-2 RNA in wastewater at 50%, 80% and 99% probability required minimally 85, 195, 385 of new cases, and 105, 215, 410 of active cases of COVID-19 per 100,000 residents in the community, respectively; and the positivity rates and levels of SARS-CoV-2 RNA in wastewater reflect new and/or active cases in corresponding communities, predicting likely the outbreak for a couple of days ahead of symptomatic COVID-19 cases in that community. This project fills the knowledge gaps on the detection sensitivity of WBS on SARS-CoV-2 in wastewater and demonstrates that WBS of SARS-CoV-2 is a reliable and cost-effective surveillance system for COVID-19 to estimate the prevalence of COVID-19 pandemic in community. The outcomes have provided evidenced-based support for the latest Alberta Government decision to use WBS for COVID-19 in different communities to replace clinical testing of COVID-19 in this province during post-3rd wave of COVID-19 pandemic. This investment has yielded measurable benefits for public health policies and Albertans in addition to gain the national and international-wide reputation of the team in research and innovation on this field.

Attached Table and Figures

Table 1. qPCR-based and NGS-based detection of VOI and VOC in wastewater

Location	Sample Date	PAN-SARS-CoV-2 (E, N1, N2 assays)	qPCR-based assay for VOC	NGS based Assay for VOC
Calgary_site3	21-Feb-2021	Positive	B.1.1.7	N/A
Calgary_site1	28-Feb-2021	Positive	B.1.1.7	N/A
Calgary_site2	28-Feb-2021	Positive	B.1.1.7	N/A
Edmonton	28-Feb-2021	Positive	B.1.1.7	N/A
Red Deer	1-Mar-2021	Positive	B.1.1.7	N/A
Banff	19-Mar-2021	Positive	N/A	B.1.1.7
Edmonton_LTCF#3	30-Mar-2021	Positive	B.1.1.7	B.1.1.7
Calgary site1	4-April-2021	Positive	N/A	B.1.1.7
Calgary site3	4-April-2021	Positive	B.1.1.7	B.1.1.7
Calgary site2	4-April-2021	Positive	B.1.1.7	B.1.1.7
Grande Prairie	9-April-2021	Positive	B.1.1.7	B.1.1.7
Calgary site1	11-April-2021	Positive	N/A	B.1.1.7
Canmore	12-April-2021	Positive	N/A	B.1.1.7
Calgary site2	18-April-2021	Positive	N/A	B.1.1.7
Banff	19-April-2021	Positive	B.1.1.7	B.1.1.7
Red Deer	19-April-2021	Positive	B.1.1.7	B.1.1.7
Edmonton	29-April-2021	Positive	B.1.1.7	B.1.1.7
Fort Saskatchewan	30-April-2021	Positive	N/A	B.1.1.7
Edmonton	2-May-2021	Positive	B.1.1.7	B.1.1.7

Figure 1. Trending of SARS-CoV-2 RNA levels (3 genes) detected in samples collected from the WWTPs of the City of Red Deer and surroundings (population ~190,000) updated to August 6, 2021

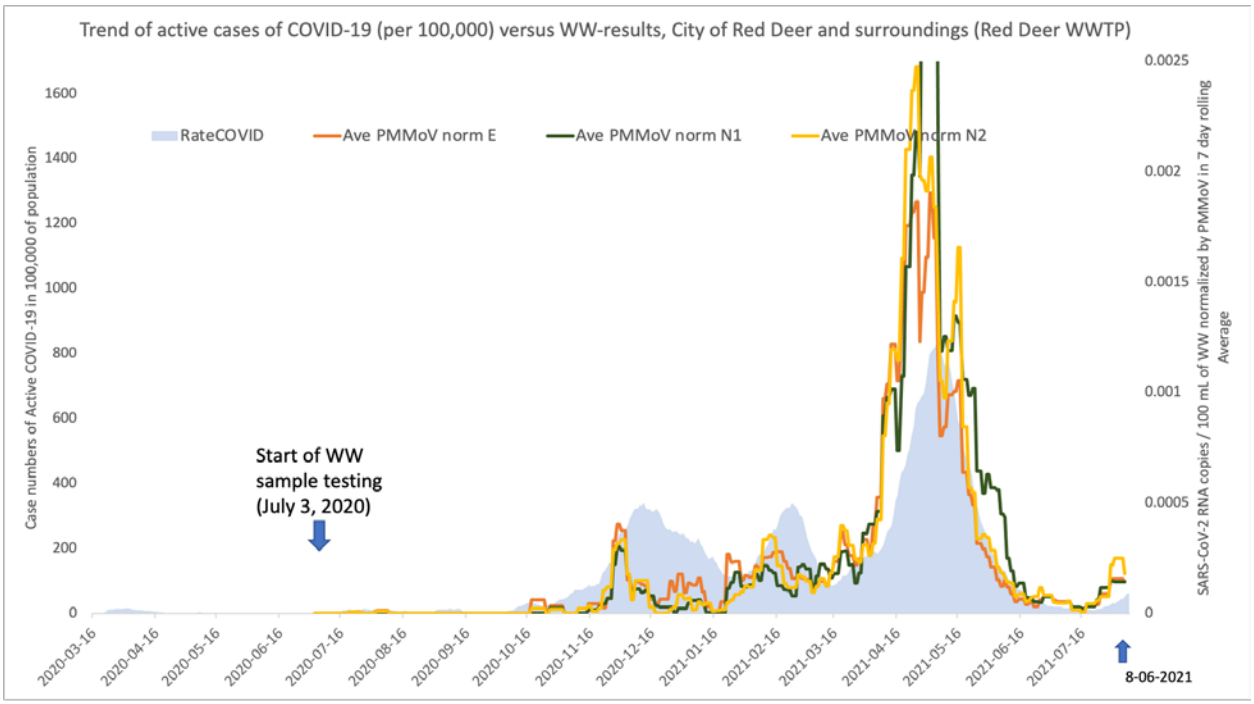

Figure 2. Trending of SARS-CoV-2 RNA levels (3 genes) detected in samples collected from the WWTP of Banff Town (population~13,000) updated to August 3, 2021

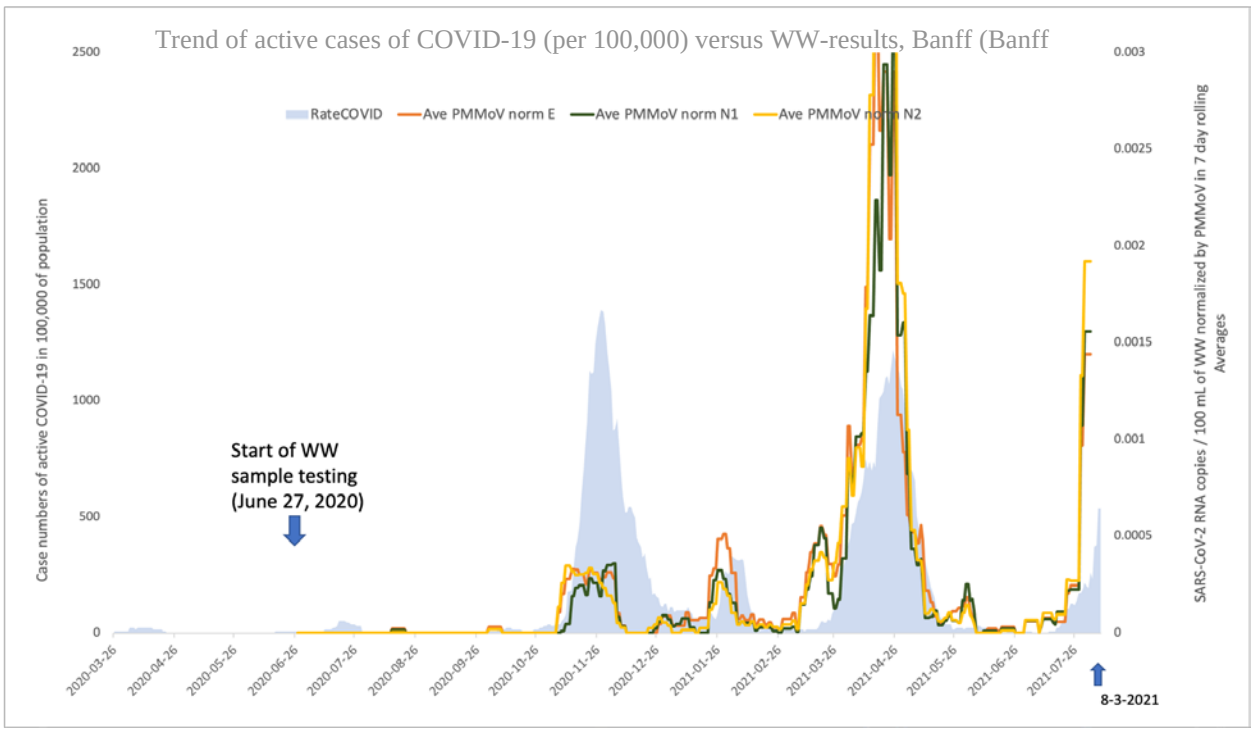

Figure legends: Rate COVID-19: Active cases per 100, 000 in corresponding area; Ave PMMoV Norm E: 7-day rolling average of PMMoV normalized SARS-CoV-2 E gene in wastewater samples; Ave PMMoV Norm N1: 7-day rolling average of PMMoV normalized SARS-CoV-2 N1 gene in wastewater samples; Ave PMMoV Norm N2: 7-day rolling average of PMMoV normalized SARS-CoV-2 N2 gene in wastewater samples.