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Identifying Local Realities and Anticipating Challenges in Building Capacity of Ontario Municipal Wastewater Systems in Tracking for SARS-CoV-2

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Abstract

One of the biggest challenges that public health experts have ever faced is detecting and mitigating the community spread of COVID-19. Current clinical testing of COVID-19 patients is limited in terms of testing kits available, cost logistics, and detecting individuals that are mildly symptomatic and asymptomatic. False positives and false negatives also cloud the true picture of the pandemic. Ontario municipalities' wastewater systems can provide new testing opportunities for a non-invasive approach in tracking and monitoring the community spread of COVID-19 through sampling raw sludge or untreated wastewater to test for SAR-CoV-2 RNA fragments. Current global and domestic research confirms the effectiveness of wastewater epidemiology surveillance of SAR-CoV-2 and can be detected even before individuals experience symptoms providing a real-time indicator for appropriate public health interventions. In collaboration with the COVID-19 Wastewater Consortium of Ontario (CWCO), an initiative of McMaster University, the objective of this research is to determine the means to optimize the current infrastructure capacity of municipal wastewater systems as an opportunity to monitor and track COVID-19 spread in the community by identifying local realities and risks. To identify local challenges, we distributed a survey amongst Ontario municipalities regarding wastewater treatment plants' characteristics, held focus group discussions, and implemented an eight-week sampling program with CWCO's partners. This report focuses on municipal wastewater treatment plants with in-house laboratory facilities to analyze the current capacity and limitations associated with their sampling and analysis programs. Drawing from survey responses and focus group discussions, we revealed gaps for municipalities to move forward with sample testing and data processing as well as governance challenges.

Keywords: SARS-CoV-2; Covid-19 testing; In-house laboratories; Municipal wastewater systems.

1. Introduction

The outbreak of the coronavirus respiratory disease (COVID-19) was first detected in Wuhan, China in December 2019 and is reportedly caused by the new severe acute respiratory syndrome coronavirus two (SARS-CoV-2) (Mavragani, 2020; Van Caeseele *et al.*, 2020). As the virus spread widely to 114 countries, the World Health Organization (WHO) declared a pandemic on March 11, 2020 (Mavragani, 2020). As of January 31st, 2021, there are 778,972 cases confirmed in Canada, with 35% of reported cases coming from the Province of Ontario (Public Health Ontario, 2020a). Currently, most cases are a result of close contact with infected persons in public spaces, gatherings, along with travel-related cases (Public Health Ontario, 2020b). Table 1 outlines key events in the COVID-19 outbreak in Ontario, starting in March 2020 when the provincial government declared a state of emergency and various measures implemented to manage the virus spread to January 2021.

Table-1. Timeline of events in COVID-19 outbreak in Ontario (March 2020 to February 2021)

Months	Timeline of COVID-19 related events
March	*March 17$^{th:}$* State of Emergency Declared
	March 18th: Canada-US border closed (essential travel only); Announcement of International travel restrictions
	March 20th: Multiple outbreaks in long-term care facilities across GTA
	March 30th: Shutdown of all outdoor amenities and facilities
April	*April 14th:* Extension of state of emergency
May	*May 14th:* Plans underway for the Ontario first stage of economy's recovery - Phase 2: Restart
	May 19th : Elementary, secondary, and post-secondary school to with online

	learning
	May 29th : Open clinical testing announced for public
June	*June 1st:* Face mask or coverage becomes mandated at Toronto's Pearson Airport
	June 12th: Process of Stage 2 reopening for selected municipalities and/or regions
	June 14th : Ontario has conducted over a million of COVID-19 clinical tests
	June 18th : Stage 2 reopening initiated for Durham Region, Haldimand-Norfolk, Halton Region, Hamilton, Lambton, Niagara Region, York Region
	June 23rd: Stage 2 reopening initiated for City of Toronto and Peel Region
July	*July 13th :* Ontario announces various municipalities will proceed into Stage 3 of the recovery plan
	July 24th : Hamilton, the regions of York, Durham, Halton, and Niagara and counties of Haldimand, Horfolk and Lambton enters Stage 3 of the province's reopening plan
	July 31st : Virus hotspot areas, Toronto and Peel Region, moves into Stage 3 of the province's reopening plan
August	*August 20th:* Extension of emergency orders until September 22nd, 2020
September	*Sept 28th:* Ontario officialy declares is entering the second wave of the pandemic with total number of cases passing 50,000
October	*October 2nd:* Implementation of province-wide mask policy
	October 3rd: New restrictions on public spaces and gatherings
	October 9th: Provincial record with 939 cases recorded in a day; Stronger restrictions measures for public spaces in Toronto, Peel Region and Ottawa
	October 31st: Over 1,000 new cases recorded in a day; Total number of cases reaching 75,730
November	*November 3rd:* Introduction of a new five-tiered color-coded system for in managing lockdowns zones , as follows: • Prevent (standard measures – green) • Protect (strengthened measures- yellow) • Restrict (intermediate measures – orange) • Control (stringent measures – red) • Lockdown (maximum measures – grey)
	November 6th: Peel Region placed in red zone; City of Ottawa and York Region placed in the orange zone
	November 10th: Toronto re-enters grey zone (lockdown)
	November 12th: New record of 1,575 COVID -19 cases in a day
	November 20th: Extension of current orders under the Reopening Ontario Act until Dec. 21, 2020
December	*December 6th:* Ontario reports a new record of 1,924 new cases in a day; Total number of cases in the province is 127,309 *December 9th:* Pfizer's coronavirus vaccine approved for use in Canada *December 14th:* 2,275 cases reported, marking a new single-day record *December 17th:* Ontario Hospital Association calls for provincial government to implement a four-week lockdown as a result of infection rate increasing to 0.04% *December 26th:* Start of a province-wide lockdown; • Four weeks in the Southern regions • Two weeks in the Northern regions *December 28th*: A total of 13,200 vaccines have been administered in the province
January 2021	*January 2nd:* A new single-day record of 3,363 COVID-19 cases reported *January 11th:* Province's death toll reaches 5,012 *January 14th:* Province implements stay-at-home order under the *Reopening Ontario Act* with stronger restriction placed *January 15th:* Pfizer to cut vaccines shipments in half for four weeks for plant's maintenance *January 25th:* One-year anniversary on the first case reported in Ontario; Current total of provincial cases reaches 256,960

Source: Nielsen (2020) (Global News Canada)

The current form for testing for SARS-CoV-2 is through clinical viral testing. Viral testing uses nasal or oral swab or saliva as a part of the respiratory system to determine whether SARS-CoV-2 is present (Van Caeseele *et al.*, 2020). Over a year since the emergence of the virus, Ontario has performed more than four million individual testing and continues to lead with the amount of clinical testing performed in Canada (Ontario Office of the Premier, 2020). Clinical testing is effective in isolating symptomatic individuals however struggles to capture mildly symptomatic or asymptomatic individuals that cause community transmission (Wei *et al.*, 2020).

Current global research confirms that the virus can be reliably detected the presence of SARS-CoV-2 the feces of infected individuals including those who have mild or no symptoms. Wastewater surveillance has been successfully used in the past to detect pathogenic enteric viruses, drug use, pharmaceuticals, and others (O'Reilly *et al.*, 2020). The presence of the SARS-CoV-2 in wastewater can be detected before community cases are identified through clinical testing.

In collaboration with the *COVID-19 Wastewater Consortium of Ontario* (CWCO), the objective of this research is to uncover local constraint, risks, and opportunities to adopt COVID-19 wastewater tracking in Ontario's municipal wastewater systems.

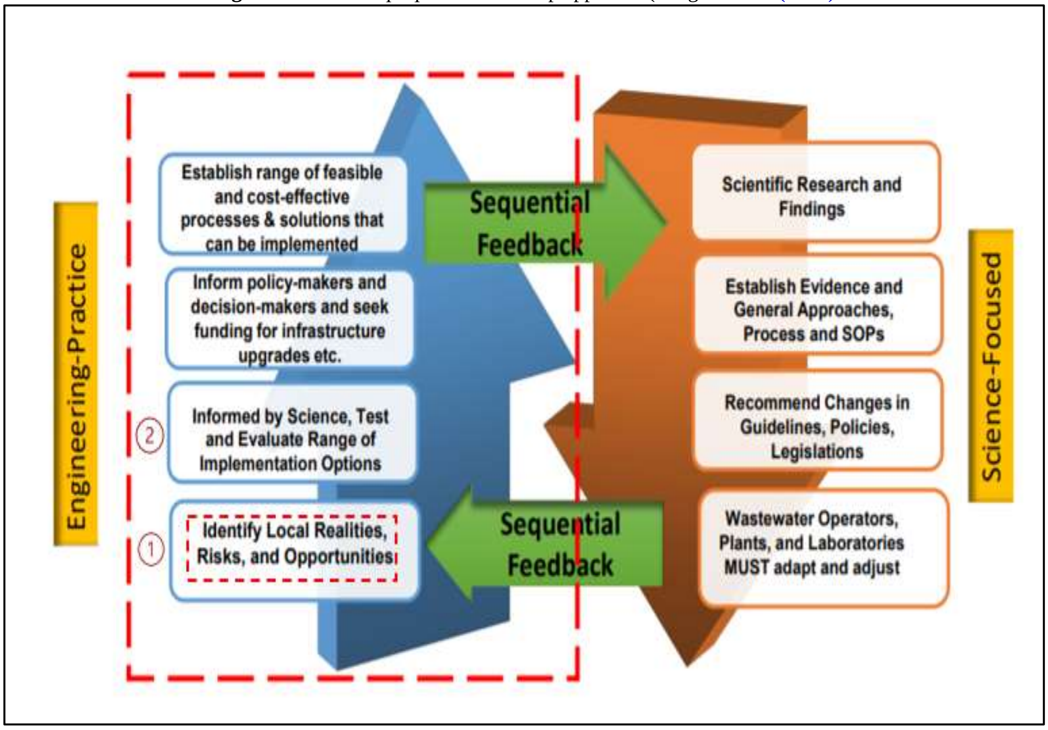
2. CWCO's Approach

The COVID-19 Wastewater Consortium of Ontario (CWCO) is an initiative by McMaster University, currently researching the need to build capacity and provide a roadmap for best practices at a local scale for wastewater tracking of SARS-CoV-2 in Ontario. This project, led by principal investigator, Dr. Gail Krantzberg and Dr. Zobia Jawed of McMaster University began May 2020.

2.1. Bottom-Up Approach

CWCO has employed a bottom-up approach to develop a clear understanding of current municipal partners' wastewater system capacity for sampling and testing for SARS-CoV-2. CWCO's bottom-up approach is shown in Figure 1. There is an urgent need to understand the infrastructure limitations, and then to collectively identify best practices, and effective testing technologies and methods which can be deployed within existing wastewater systems testing frameworks.

Figure-1. CWCO's proposed bottom-up approach (Image: Jawed (2020)



2.2. Governance System of Municipal Wastewater Systems and Treatment Plants

2.2.1. Ontario's Municipality Tier System

According to the Ontario Municipality Act, municipalities are responsible and accountable in providing proper public services such as wastewater management and treatment within their geographic jurisdictions and comply with legislations made by the Government of Ontario (Province of Ontario, 2001) Among Ontario's 444 municipalities, the Municipality Act, 2001 distinguishes among the following three types of municipalities (Province of Ontario, 2020);

- Upper-tier municipalities (regional), found within a two-tier municipalities structure
- E.g., Regional Municipality of Halton (also known as Halton Region)
- Lower-tier municipalities (local), found within a two-tier municipal structure
- E.g., City of Burlington and Towns of Halton Hills, Milton, and Oakville under Halton Region
- Single tier municipalities
- E.g., City of Hamilton

In Northern Ontario, all municipalities (also can be classified as towns, townships, and village) are single tier, however, are often grouped and classified as districts. Districts do not hold any governance structures with the exception of Algonquin District (Province of Ontario, 2020). Indigenous communities or reserves are not classified as municipalities and are their entity. Among the different types of municipalities, legal authority and governance responsibilities vary. In a two-tier government structure, the upper-tier municipalities coordinate area-wide exclusive or non-exclusive service delivery to all lower-tier municipalities.

2.2.2. Ontario Wastewater Legislations

In Ontario, there are 423 sewage treatment plants (Kapitain, 1995). Wastewater systems in Ontario are governed by MOECP and subjected to federal legislation. Components of legislation concerned with sewage treatment are the Fisheries Act (FA), the Canadian Environmental Protection Act (CEPA), and the Canada Water Act (Canadian Institute for Environmental Law and Policy CIELP, 2004). MOECP has mandated inspection on all wastewater systems across Ontario consisting of facility visits, review of information and data for the inspection periods, process operation sampling, and monitoring and corrective actions (Canadian Institute for Environmental Law and Policy CIELP, 2004).

- Environmental Assessment Act
- Environmental Protection Act
- Municipal Water and Sewage Transfer Act
- Nutrient Management Act
- Ontario Water Resources Act
- Planning Act, Sustainable Water, and Sewage Systems Act

Each municipality has its own set of by-laws regarding the operations and services provided by their wastewater treatment plant. Under the Ontario Water Resource (OWR) Act, the overall responsibility for monitoring a wastewater or sewage treatment operations performance through sampling lies under with the plant's operating authority which is either municipal or private.

Under municipal authority, wastewater sampling and testing are either performed by the WWTP in-house laboratories or outsourced to private labs (Ministry of Environment Conservation Parks, 2019). Both in-house and private laboratories conduct environmental monitoring and microbiological assessment to ensure compliance with provincial government regulations. Single-tier and several upper-tier municipalities have in-house labs while lower-tier municipalities outsource samples to private labs. Private labs must be approved and licensed by MOECP before providing their services to a municipality (Ministry of Environment Conservation and Parks, 2020).

3. Wastewater Epidemiology Surveillance for SARS-CoV-2, Current Status

3.1. Sampling Strategy

3.1.1. Sampling Location

The number of people infected and the rate of viral shedding will characterize the prevalence of SARS-CoV-2 concentrations in municipal sewage (Wigginton *et al.*, 2015). An infected body releases viral RNA through his or her feces, even if mildly symptomatic or asymptomatic, thus selecting a sampling site serving a larger population can increase detection of RNA fragments. Studies from Canada (D'Aoust *et al.*, 2020) and Japan (Haramoto *et al.*, 2020) have shown that RNA detection is weak or undetectable from samples with a low incidence of viral load in communities. Ideal sampling sites for a higher incidence of viral load can include manholes or pumping stations serving universities, long-term facilities, hospitals, commercial and industrial buildings, and as well as at the WWTP (Centers for Disease Control and Prevention CDCP, 2020a). Samples that are collected upstream from the WWTP rather than at the treatment facility can pinpoint community hotspots (Centers for Disease Control and Prevention CDCP, 2020b). The outcomes from the sample testing from the described locations should be able to provide quantifiable knowledge of communities at higher risk for the virus spread and optimize timely COVID-19 clinical testing and safety protocols.

3.1.2. Sample Type

Two types of wastewater monitoring samples that are common include untreated wastewater (influent) and primary sludge. Influent includes waste from the use of toilets, showers containing human feces, oils, chemicals and food scraps. In some cases, depending on the municipality's sewage system, stormwater runoff might be combined with untreated wastewater prior to entering the treatment plant. Untreated wastewater can also be sampled from at the plant before primary treatment or upstream in the wastewater pumping stations (Centers for Disease Control and Prevention CDCP, 2020a). In current studies, untreated wastewater is considered as the preferred sample type for wastewater surveillance epidemiology as changes in SARS-CoV-2 RNA concentrations strongly correlated with trends in the number of reported cases and localized the virus hotspots (Medema *et al.*, 2020; Nemudryi *et al.*, 2020).

Primary sludge consists of suspended solids that settle out of wastewater within the primary treatment. Primary sludge samples may be advantageous compared to untreated wastewater since a higher concentration of SARS-CoV-2 RNA fragments concentrate in raw sludge, reducing the sample volume required and possibly eliminate an additional sample concentration step before RNA extraction (Centers for Disease Control and Prevention CDCP, 2020a). Studies have confirmed that primary sludge shows higher concentrations of SAR-CoV-2 RNA fragments than untreated wastewater thereby improving signal detection (D'Aoust *et al.*, 2020; Peccia *et al.*, 2020).

3.1.3. Sample Collection Method

There are two methods for sample collection: grab and composite samples. Grab samples are collected at one point in time while composite samples are multiple grab samples collected over a set time interval at a specified or frequency (Centers for Disease Control and Prevention CDCP, 2020a; CWN., 2020). For untreated wastewater and sludge, grab samples results can lead to inconsistent results due to daily fluctuations in wastewater flow and the rate of viral shedding within the community (Centers for Disease Control and Prevention CDCP, 2020a).

3.1.4. Frequency of Sampling

The Canadian Water Network suggests that the collection of samples on a weekly or biweekly is enough to thoroughly distinguish for the presence of SARS-CoV-2 in wastewater or sludge samples. For early detection, however, increasing sampling frequency is recommended to reveal trends in community outbreaks (CWN., 2020). To date, it still unknown how quickly wastewater concentrations may change under various epidemic scenarios or environmental conditions (Ampuero *et al.*, 2020).

3.1.5. Sample Container and Volume

The samples can be collected in individual sample bottles (Medema *et al.*, 2020) or collected at the site in a larger container (Haramoto *et al.*, 2020) and then split into aliquots in the lab (Nemudryi *et al.*, 2020). Sample container and volume size will depend on the municipality's available resources and storage capacity. Common volumetric sizes of samples range from 50 mL falcon tubes or 250 ml polypropylene bottles. The Canadian Water Network recommends 50mL aliquots as this is more convenient, especially when storage capacity is limited.

3.1.6. Sample Transportation and Storage

If the sample is collected from a sewage treatment plant off-site, it should be transported in a cooler and stored at a minimum temperature of 4°C. Common storage temperatures found in the literature are 4°C (D'Aoust *et al.*, 2020; Medema *et al.*, 2020), -20°C (La Rosa *et al.*, 2020) and –80°C (Peccia *et al.*, 2020). If a sample cannot be processed within 24 hours, it should be frozen at -20°C or -80°C (Hart and Halden, 2020).

3.2. Sample Processing

Sample processing may include viral deactivation, pasteurization, chemical pre-treatment to remove solids via pH adjustment, slow/high-speed centrifugation, or filtration (Centers for Disease Control and Prevention CDCP, 2020b). Concentrating samples before RNA extraction is more applicable to untreated wastewater than primary sludge samples (Centers for Disease Control and Prevention CDCP, 2020b; Michael-Kordatou *et al.*, 2020). Concentration methods that may improve SARS-CoV-2 in RNA recovery in wastewater include ultrafiltration (Medema *et al.*, 2020), filtration via electronegative membrane (Haramoto *et al.*, 2020), polyethylene glycol (PEG) precipitation, skim milk flocculation (Guerrero-Latorre *et al.*, 2020) and ultracentrifugation (Ampuero *et al.*, 2020). The selection of a concentration approach will need to consider the sample type, volume, processing time, and availability of laboratory equipment.

3.3. RNA Recovery Measurement and Quantification

Most studies that we examined use the reverse transcription-quantitative polymerase chain reaction (RT-qPCR) with variations in gene primers or assays to obtain qualitative and quantitative data. Research is underway to determine which detection method is most effective as multiple other factors affect the signaling of SARS-CoV-2 RNA fragments. RNA measurement and quantification are current challenges for setting a standard approach, as each lab adheres to different specific standard operating procedures (SOPs) for sample handling and analysis dictated by their available infrastructure, expertise, and the requirements of laboratories services (Hill *et al.*, 2020).

4. Sampling Strategies in Quantitative Global Research

With the emergence and spread of SARS-CoV-2 in early 2020, many countries have rapidly expanded their viral surveillance systems in wastewater-based epidemiology (O'Reilly *et al.*, 2020). As presented in Table 2, wastewater sampling performed globally has shown that signals of SARS-CoV-2 RNA fragments in municipal wastewater were strongly detected several months before community outbreaks, with a positive correlation with the reported incidence of cases. The collection process and interpretation of the PCR-RT quantitative data of wastewater samples is still an emerging field (O'Reilly *et al.*, 2020).

Table-2. International jurisdictions wastewater sampling strategies and findings in testing of municipal sewage for COVID-19

Country	**Sampling**			**Findings**
	Location	**Type & Collection**	**Frequency & Storage**	
Ottawa, Canada D'Aoust *et al.* (2020)	Two municipal WWTPs serving Ottawa and Gatineau	Primary clarified sludge (PCS) SWastewater influent solids (PGS) Container size: 250 mL	Grab samples PCS collected biweekly Composite PGS samples collected a 24-hour period Stored at 4°C	Sample processing: concentration performed prior to RNA extraction Detection method: RT-qPCR assays & RT-ddPCR Identifies PCS as a preferred solids-rich sample compared to PGS PCS samples showed higher detection of SARS-CoV-2 during low incidence of viral load in communities
Santiago, Chile Ampuero *et al.* (2020)	Two WWTPs in the municipality of Santiago (serves eight million people in total)	Sample Type: composite raw sludge Container size: N/A	Collected from March to June 2020 Frequency: 24 hours composite samples Storage: Sterile propylene bottles	Sample processing: concentrated by ultracentrifugation Detection method: RT-PCR assays Samples collected from March to April tested negative RNA signals Samples collected from May to June had positive results, correlation to the increase number of cases Unknown if virus is viable under environmental conditions
Quito, Ecuador Guerrero-Latorre *et al.* (2020)	-Three sites from the Quito's river	-River samples (Only 3% of sewage is treated) -Container size: collected in 2L manually	Collected in one day (during peak period of outbreak) Stored at 4 °C and processed in less than 3 hours	Sample processing: skimmed milk flocculation using 2L of water Detection method: qRT-PCR SARS-CoV-2 RNA detected in all samples from all three sampling sites Results correlates with number cases in the area -Skimmed milk flocculation highly effective; proposed to be applied in labs lacking specialized equipment for viral concentration -Viability of virus in unknown in polluted water
Yamansashi Prefecture, Japan Haramoto *et al.* (2020)	-WWTP in Yamanashi Prefecture -Fuefuki River	-Grab samples (13 in total) -Influent (5 samples) -Secondary-treated (before chlorination) wastewater (5) -River (3 samples) -Sizes: 1L sterilized plastic bottles	- Collected on 3 different days -Transported to labs on ice and proceed within 6 hours of collection	Sample processing: concentrated electronegative membrane-vortex (EMV) method & membrane adsorption-direct RNA extraction Detection method: RT-qPCR assay -None of the Fuefuki river samples tested positive -SARS-CoV-2 detected in one of the five secondary-treated wastewater samples (only 20%) possibly to low prevalence of cases in area - EMV method outperformed membrane adsorption-direction method where higher RNA concentration was observed in samples
The Netherlands Medema *et al.* (2020)	-WWTPs in two-large and three medium-sized cities and airport from February to March	-Composite sewage samples -Container size: 250mL bottles	-Sampled a 24-hour flow-dependent composite samples -Stored at 4°C	-Sample processing: concentration by ultrafiltration -Detection method: RT-qPCR assay with N1 and N3 primers - High levels of RNA was found at all selected sampling sites and WWTPs -One of the mid-sized municipality (Amersfoort), SARS-CoV-2 virus was detected in wastewater before first set of cases were reported
New Haven,	-Water	-Primary sewage	-Collected from	-Sample processing: not indicated

Connecticut, USA Peccia *et al.* (2020)	pollution abatement facility serving 200,000 residents	sludge (from outlet of a gravity thickener in primary treatment) -Size: N/A	March to May -Stored at -80°C -Frequency: N/A	-Detection method: qRT-PCR - Positive SAR-CoV-2 results in all collected samples - RNA detection peaked three days earlier than hospital admissions and seven days than reported cases (early detection) - Emphasis on primary sludge to be used a sample given its greater solids content than raw wastewater - Notes primary sludge process trains are not uniform and -Notes that clinical testing is prompted by symptoms can lead to underestimated cases.
Bozeman, Montana, USA Nemudryi *et al.* (2020)	-Bozeman Water Reclamation Facility over 52-day time course	- Grab untreated wastewater (downstream from screen and grit washer) -Composite raw influent with automatic flow proportional sampler	-Grab samples collected in triplicate with 15 seconds intervals -Stored 4°C in 500mL aliquots -Processed in under three hours from collection	-Sample processing: filtration and concentration prior to RNA extraction -Detection Method: RT-qPCR using N1 and N2 primers -All samples tested positive for SARS-CoV2 -Concentrations of RNA trended with the reported COVID-19 cases from the hospital in the surrounding area
Northern Italy La Rosa *et al.* (2020)	-Collection from five WWTPS from Milan, Turin, and Bologna from October 2019 to February 2020 (62 samples in total)	-Composite raw sewage samples -Container size: N/A	-Composite samples in 24-hour periods -Stored at -20°C -Extra sanitation on sample bottles as precaution	-Sample processing: prior to concentration, centrifugation viral inactivation treatment was performed -Detection method: RT-qPCR (using two different methods; nested, real-time) - SARS-CoV-2 RNA was detected in 15 out of 62 samples at various levels -Detection was found prior to first reported cases in Milan and Turin - Evidence supports the hypothesis that SARS-CoV-2 had been circulating in Northern Italy as early as the end of 2019
Islamabad, Pakistan Sharif *et al.* (2020)	-Collected from 38 districts across Pakistan -74 samples from polio environment surveillance site -3 from drains in infected area -1 from COVID-19 quarantine drain	-Grab untreated wastewater samples -Container size: N/A	-Collected from April 6th to 28th , 2020 -Stored at 4°C -Processed within 48 hours	-Sample processing: high speed centrifugation -Detection method: RT-qPCR assays -27% of samples from 13 districts tested positive -Notes research is needed on appropriate virus concentration and detection assay to increase signal sensitivity -Development of highly sensitive assay will be an indicator for virus monitoring and to provide early warning signs -Viral load estimation in infected patient still unknown

5. CWCO Research

5.1. Sampling Program

In September 2020, CWCO initiated an eight-week wastewater sampling program across Ontario. Municipalities involved in this sampling program include the Cities of Peterborough, Ottawa, Hamilton, and Niagara Region. The objective of the eight-week sampling program was to collect data before what was then anticipated to be the second wave of COVID-19 in Ontario. The data serves as reference points to analyze community transmission trends and identify community hotspots. The purpose was to provide public health officials relevant data to make informed decisions on deploying clinical testing in community hotspots and implementing appropriate control measures within the province.

5.2. McMaster University COVID-19 Wastewater Tracking

McMaster University is one of the first universities to utilize COVID- 19 wastewater tracking as part of the campus re-opening plans for 2021. Commencing in January 2021, McMaster's Facilities Services collaborated with CWCO by sampling campus wastewater from various locations including McMaster University Children's Hospital, the Student Centre, residences, and several academic buildings. Results from sample testing will determine the need to perform clinical testing and deploy a remedial measure to avoid the spread of COVID-19 on campus. It also establishes a continual campus monitoring program in the event of another viral outbreak in the future.

6. Project Methodology

The scope of this paper is limited to municipal wastewater treatment plants (WWTP) with in-house laboratories. In-house labs are responsible for performing chemical and biological testing on samples and as well as monitoring water quality. As the objective is to develop a better understanding of local infrastructure capacity, we began with municipal WWTPs with in-house labs that already have in place standard operating procedures and laboratory facilities and services to analyze the realities and potential limitations for tracking COVID-19 in wastewater. We partnered with following municipalities: Niagara Region, City of Hamilton, and Peterborough (shown in Figure 2).

Figure-2. Map of Ontario single and upper tier municipalities, with focused municipalities marked in blue (Image: Neptis Foundation (2020)

6.1. General Survey

The general survey served as an initial point of contact with municipalities across Ontario to acquire knowledge of WWTPs characteristics. The general survey was distributed to CWCO's partners and posted on the CWCO's website. The survey link was also distributed by *Ontario Municipal Wastewater Association* (OMWA). The survey included questions regarding general contact information, municipal populations, wastewater treatment plan resources, and sampling/testing resources. It consisted of multiple-choice questions and took approximately 10 minutes to complete. Appendix A.1 shows the survey questions.

6.2. Focus Group Discussions

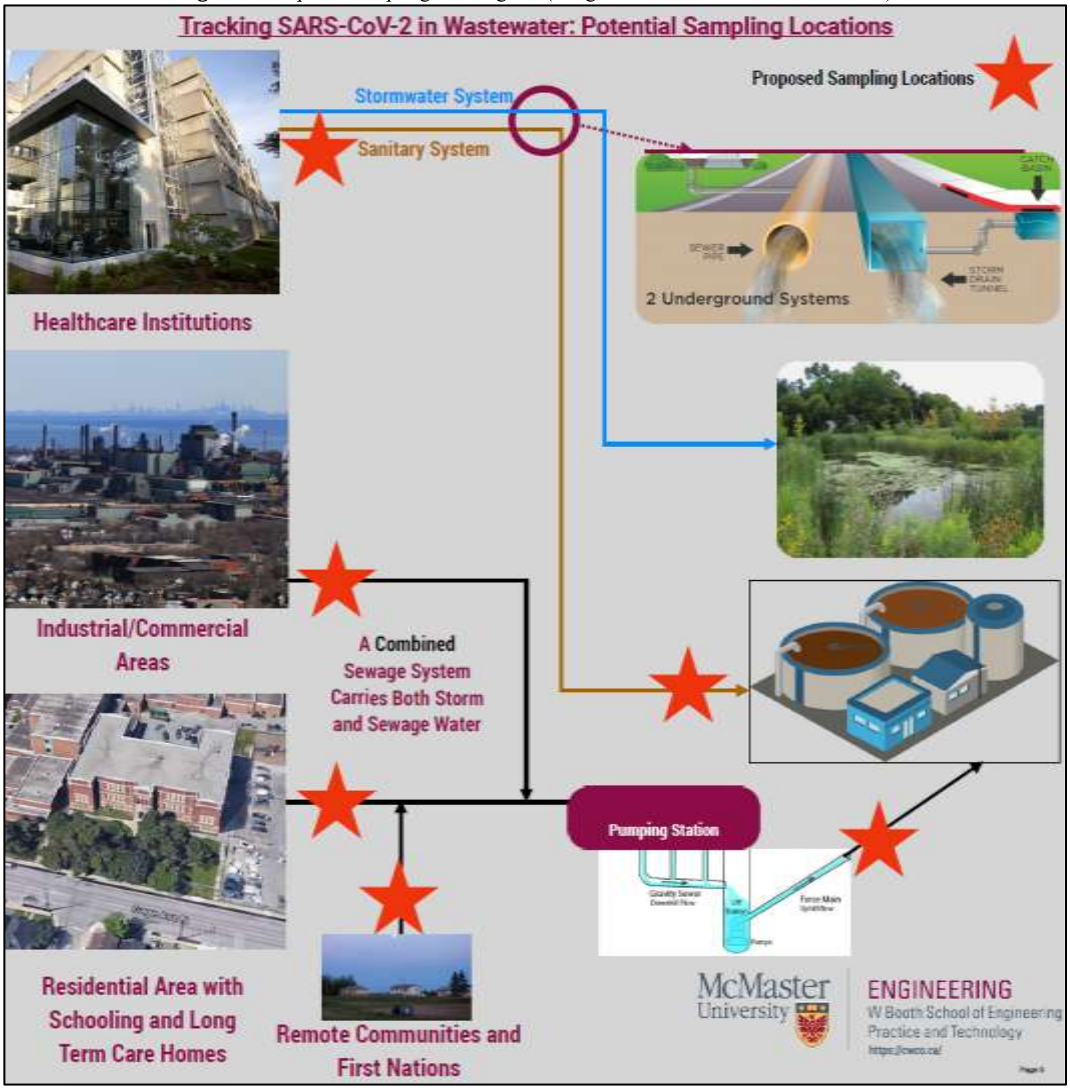
The purpose of our focus groups was to identify local challenges in terms of the capacity associated with municipal wastewater systems' operations. Focus group participants were asked questions in an interactive setting and were encouraged to discuss thoughts freely with other participants to create a collaborative environment. Attendees of focus group discussions involved 13 municipal representatives from various departments including the Cities of Peterborough, Hamilton, Ottawa, and Niagara Region.

7. Focus Group Discussion

A focus group discussion was held on September 3rd, 2020. The objective was to discuss with municipal partners the CWCO's proposed SARS-CoV-2 sampling program. Participants included a diverse representation from operations, quality and control management, laboratory services, and process engineering.

First on the agenda was the discussion of key sampling collection sites. Figure 3 proposed several referral samplings sites such as healthcare institutions, pumping stations, industrial and commercial areas, residential areas with schooling, and long-term care homes. As for sample types, focus group member agreed it optimal to use primary sludge samples given the positive detection results and research conducted by the University of Ottawa. Participants reached consensus on selecting three to five sampling locations that are foreseeable as being a potential viral hotspot and as well as samples collected from the WWTP weekly ranging from 6:30 Am to 10:00 AM

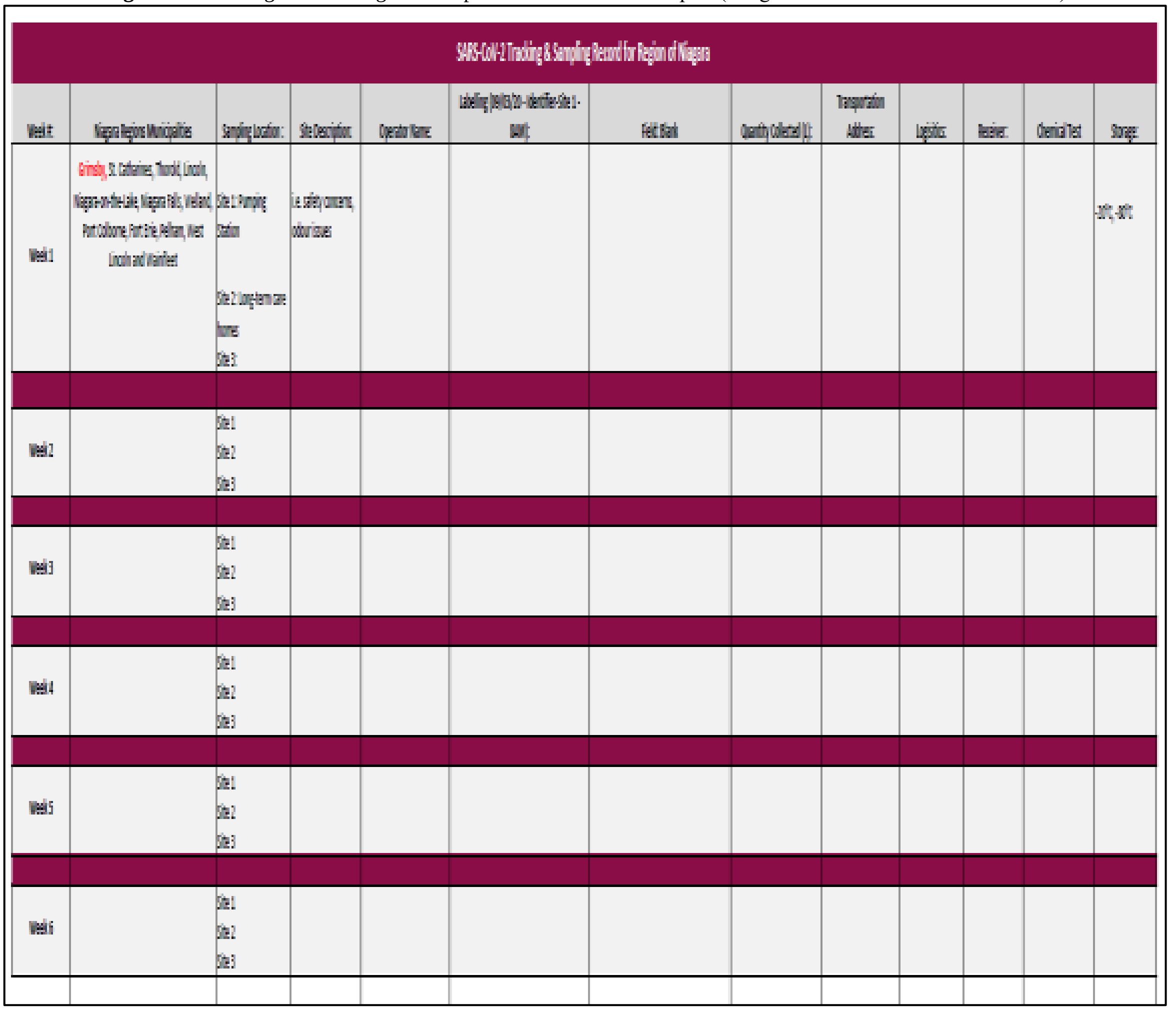
Figure-3. Proposed sampling site diagram (Image Source: CWCO's Student Team)



Storage capacity varied for each municipality. Implications of resource availability were evident. The Focus Group reached consensus on grab sampling in 250mL sterilized containers kept at 4 °C for short-term storage and -20 °C or -80 °C for long-term storage. The utilization of 50 mL falcon containers was agreed upon by the participants given its easiness to store in a limited capacity.

At the time of writing, McMaster University is in the process of standardizing collecting, tracking, and labeling samples and creating a data management portal for municipalities to upload data collection results. Figure 4 presents the master template for sample tracking. Containers were label SARS-CoV-2 wastewater samples with a customized identifier relating to the locations GIS ID.

Figure-4. Labelling and tracking sheet of potential COVID-19 samples (Image Source: CWCO's Student Team)



All municipal Focus Group participants confirmed that no extensive modification was required to existing SOPs, however as a precautionary measure, extra sanitation on surfaces of laboratory equipment was to be integrated. At the end of focus group discussions, the eight – week sampling program begun to test sampling and storage methodologies.

8. Survey Responses

The purpose of the survey was to compile line knowledge of the feasibility, local challenges, and risks for various municipal wastewater COVID-19 testing capacity, marking the first step in CWCO's bottom-up approach. The goal is to understand the resources and infrastructure capacity compared to public health data needs, including modifications to sampling and testing over time with changing scientific knowledge and public health response requirements.

Twenty-eight municipal representatives completed our survey via Microsoft Forms with an average time of 12 minutes to complete. The survey was distributed in October 2020 to over 400 municipalities in Ontario. There was a mixed representation of participants from various departments including wastewater quality and compliance, operations, environmental laboratory services, and engineering. Municipalities who have completed surveys (including non-members of CWCO) were;

Regional Municipality of Durham	City of Cornwall
Town of Midland	Haldimand County
City of Peterborough	Town of Cochrane
City of Greater Sudbury	City of Windsor
Town of Smiths Falls	City of Hamilton
Township of Clearview	The Township of Oro-Medonte
Town of Deseronto	Town of Cobourg
Regional Municipality of Niagara	Municipality of Trent Hills
City of Pembroke	City of Thunder Bay
County of Oxford	Municipality of Kincardine

As focus of this paper is WWTP(s) with in-house testing laboratories, the responses for City of Hamilton, Peterborough and Niagara and Durham Region are presented in Table 3. Survey questions can be found in Appendix A.1.

Table-3. Survey Responses from Niagara Region, City of Hamilton, and City of Peterborough

Regional Municipality of Niagara	
Population	447,888
WWTP(s)	11 in total – all municipally owned St. Catharines, Niagara Falls, Thorold, Port Colborne and Welland. Towns include Niagara-on-the-Lake, Lincoln, Pelham, Grimsby and Fort Erie, and the townships Wainfleet and West Lincoln
Level of WWTP	Secondary
Type of Sewage	Both (combined and separate)
Testing Capacity	In-house labs
Comments	"Equipment not available for COVID-19 testing"
City of Hamilton	
Population	536,917
WWTP(s)	2 in total – all municipally owned
Level of WWTP	Woodward WWTP- Secondary treatment Dundas WWTP- Tertiary treatment
Type of Sewage	Both (combined and separate)
Testing Capacity	In-house labs
Comments	"Do not have the resources/skill to pursue COVID-19 tracking"
City of Peterborough	
Population	85,000
WWTP(s)	City of Peterborough WWTP – municipally owned
Level of WWTP	Secondary
Type of Sewage	Separate
Testing Capacity	In-house labs
Comments	"Do not have on-site testing available for conduct COVID-19 tracking"

Summary of survey responses:

- Only one municipality (Town of Deseronto) serves a First Nation reserve
- Three municipalities (Hamilton, Niagara Region, and Peterborough) have WWTP with in-house laboratories
- Larger sized municipalities or regions have in-house laboratories in their WWTP(s) where all testing is performed
- Smaller-sized municipalities (<100,000) use local private labs to test samples. Private labs are within proximity (less than 50 km)to the WWTP(s) and include: SGS, E3 laboratories, ALS Environmental and Testmark
- Most surveyed municipalities have secondary level WWTPs
- The type of sewage system is nonuniform for each municipality (each system is unique)
- WWTP with in-house labs indicated that COVID-19 tracking is unfeasible for that labs due to a lack of skills, funds, and resources
- WWTPs using private labs are concerned with the potential additional cost-associated or were unaware in sampling and testing wastewater for SARS-CoV-2

Please refer to Appendix A.2 for the detailed survey responses completed by the following municipalities. In Appendix A.3 is the follow-up survey regarding the progression of the sampling program and capacity in testing methodologies.

9. Municipal Challenges

At the time of writing, municipal partners have not performed any RNA measurement. Municipalities with in-house labs have a much greater capacity to perform additional sampling for tracking COVID-19. The most anticipated challenge is the accessibility to funding for testing technology and training to monitor SARS-CoV-2 and build wastewater infrastructure capacity. To overcome limitations in funding, financial support through federal and provincial funding or private investments will likely be required. To date, the federal government has supported the (Canadian Foundation for Innovation, 2020) in funding $28 million for equipment acquisition necessary to expand research related to COVID-19 (CFI, 2020). It was recently announced that Ontario plans to fund $12 million to wastewater testing related projects and research to expand the capacity for the early detection and identification of COVID-19 outbreaks (Ontario Tech University, 2020). There is a need for more such opportunities supported by the federal and provincial governments for municipalities to partake in building municipal wastewater infrastructure capacity.

Currently, operators at municipal WWTPs generally do not have the capacity to perform virological at to test wastewater samples to detect community spread of SARS-CoV-2. This illustrates the benefit of public and academia partnerships to overcome barriers by helping municipalities in the training of virological analysts and assist them in building their wastewater infrastructure testing capacity.

9.1. Data Governance

For wastewater surveillance of SARS-CoV-2 to be effective, it needs to be integrated into other public health initiatives, particularly with Ontario's five-tiered color-coded system. Greater coordination among MOECP and Public Health ministries and commissions in data governance is needed and will directly affect municipal capacity building, if the responsibilities are better defined and there are provisions of financial support for the implementation of SARS-CoV-2 wastewater tracking.

The collaboration or coordination of various ministries to take leadership in providing the necessary resources is an essential component in building municipal wastewater infrastructure capacity. A standardized sampling and testing protocol that is feasible and cost-effective will influence government decision making in allocating the necessary support. Currently, all operation requirements and regulations for Ontario's municipal WWTPs are under the authority of MOECP. Thus, responsible for the potential for wide-scale implementation and allocating financial resources for building municipal wastewater capacity could be managed by the MOECP. Further, the involvement and collaboration with Public Health can increase the availability of funding given that wastewater tracking can be used to monitor the COVID-19 cases and identify outbreak areas. The coordination of MOECP and Public Health (both municipal and provincial) is needed to support municipal acquisition of testing technology, commercial RNA extraction kits, and other necessary tools used for wastewater surveillance.

9.2. Privacy and Confidentiality

The use of wastewater screening to detect SARS-CoV-2 viral RNA to detect, monitor and quantify the community spread of COVID-19, can raise privacy and confidentiality concerns. At the time of writing, no other countries have implemented wastewater epidemiology surveillance as an official form of testing for SARS-CoV-2 thus there is no current reporting of privacy or confidentiality implications. In Canada, sharing data of COVID-19 community hotspots or areas could have compliance issues with the personal information protection and electronic documents act (PIPEDA) and the privacy act. The Office of the Private Commissioner Canada has noted that during this public health crises, privacy laws and rights still apply, but should not be an obstacle to proper information sharing when managing the number of COVID-19 cases (Office of the Private Commissioner Canada OPCC, 2020). To mitigate potential privacy implications, targeted surveillance of high-risk communities should be disclosed and coordinated with the availability of interventions such as clinical testing and measures to reduce viral spread following standards of public legitimacy and ethical guidelines. Table 4 presents a summary of the opportunities to build municipal wastewater systems capacity.

Table-4. Opportunities to build capacity for COVID-19 in wastewater surveillance

Collaboration or Partnerships	To build capacity, municipalities should establish a collaboration or partnership with universities or research groups, private laboratories, and local public health officials to allocate the necessary funds, investments, and resources to enhance sampling processes and transition into testing for SARS-CoV-2 RNA fragments.
Cost-effectiveness	The driving factor to increasing municipal wastewater infrastructure capacity is the cost-effectiveness of the sampling process and implementation of testing technology and methodologies. Considerations involve the acquisition of necessary resources and services that are within the municipalities' budgets or to be funded by financial support other orders of government.
Research	Research is needed to establish standardized sampling and testing strategies that can are feasible for municipal WWTPs with in-house and or private labs without being economically constraining.
Safety	To date, no report has documented aerosol transmission of SARS-CoV2 virus in the collection and sampling of untreated or treated sewage or wastewater. Laboratory processing of wastewater samples should follow existing biosafety standards for handling SARS-CoV-2 samples with extra measures of sanitation of equipment in processing and testing (WHO, 2020).
Privacy Guideline	Considerations of ethical or legal scenarios should be used to address the issues of privacy and confidentiality. With the ability of wastewater tracking to identify community transmitting hotspots, a guideline should be incorporated to ensure that COVID-19 wastewater surveillance is not targeting already-stigmatized communities or neighborhoods (WHO, 2020). Privacy guidelines will be of utmost importance for upper municipalities serving lower-tier municipalities to avoid any forms of stigmatization of an area.
Transparency	The success to adopting a wastewater tracking framework requires public legitimacy and support. Privacy considerations should be incorporated when proposing and building awareness of wastewater surveillance for SARS-CoV-2 integration into public health measures. (World Health Organization WHO, 2020)

10. Conclusion

The detection and quantification of SARS-CoV-2 around the world confirms that wastewater holds great promise for detecting the presence of SARS-CoV-2 infections within a municipality. Further research is needed to standardize wastewater sampling and testing methodologies with improved efficiency and feasibility. To build the capacity of Ontario's municipal wastewater testing systems, it is necessary to address current and foreseeable challenges in accessibility to funding and testing technology, expertise in virological analysis, data governance, political will and public support. Partnerships are proposed to be a starting point for municipalities to build capacity given the current unknown of the feasibility and practicability of wastewater monitoring and tracking of COVID-19 It is important to understand current infrastructural capacities of municipal WWTPs capacity to quantify the magnitude of resources and funding that is required to build local capacity to track the trajectory of the pandemic.

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