Canadian COVID-19 Wastewater Coalition
Webinar series – Tuesday, December 1, 2020

WBE in Canada: Use cases, challenges and next steps
2:00 p.m. to 3:30 p.m. EST

CWN Webinars
Connecting water professionals to decision-ready knowledge
Who should read the report?

- Public health leaders seeking to understand the potential (and limitations) of wastewater surveillance
- Decision makers considering the feasibility of wastewater surveillance programs
- Laboratories in the process of developing or adapting SARS-CoV-2 RT-qPCR methods to various wastewater matrices
How can SARS-CoV-2 sewage surveillance best support public health decisions?

• Reflecting asymptomatic and pre-symptomatic in addition to symptomatic individuals?
• Providing an efficient pooled sample?
• Tracking community trends?
• Potential to detect low levels of infection from communities or facilities (sentinel)?
• Potential to better understand spread within a community (support epidemiology)?
Wastewater-based epidemiology in Canada
Use cases, challenges and next steps
Capabilities: Wastewater-based epidemiology

- SARS CoV-2 is shed in faeces — likely whether a person is asymptomatic, symptomatic, infectious or recovering.
- Genetic signals (RNA fragments) of SARS-CoV-2 **CAN** be detected in municipal wastewater.
- There are some indications that SARS-CoV-2 can be detected in community wastewater before clinical cases are reported.
- High hopes for wastewater monitoring to provide an early warning, **BUT**....
- High hopes for wastewater monitoring to inform public health decision-making, **BUT**....
- Potential for using sampling in sewer networks to inform public health decision-making, **BUT**....
Capabilities: Sample processing and analysis

- RT-qPCR can detect SARS CoV-2 in wastewater, but additional processing vs. clinical samples is necessary
- Inhibition of PCR amplification is a problem that is sample matrix-specific and is a pervasive challenge
- Determining recovery of \textit{in-situ} SARS-CoV-2 is challenged by limitations of spiking and choice of spiked standard
- Calibration for the number of gene copies as a function of PCR Ct depends on the choice of agent used for calibration
- Likely to find greater sensitivity possible with an analytical focus on solids
Interpretation of WBE monitoring data

- Measured SARS-CoV-2 signals in municipal wastewater will vary over time, but: How much variation is caused by methods?
- Some compensation for variability in SARS-CoV-2 signals can be provided by correcting for flow, particularly as affected by precipitation / storm flow contribution.
- Further compensation for variability in SARS-CoV-2 signals can be provided by normalizing using indicators of fecal content like the Pepper Mottled Virus (PMMoV) or CrAssphage.
- Smoothing of data by using running averages is helpful for retrospective data presentation, but what choice is best?
- Best case indicator / comparator (e.g. number of cases, per capita cases, % positivity, other?) is not clear on first principles.
Webinar speaker

Robert Delatolla
Associate Professor
Civil Engineering
University of Ottawa
WASTEWATER SURVEILLANCE OF COVID-19 IN OTTAWA

Robert Delatolla, PhD, PEng
Alex MacKenzie, Patrick D’Aoust, Elisabeth Mercier and Tyson Graber
University of Ottawa & CHEO-RI

Research collaborators in this work:
Mark Servos, Nivetha Srikanthan, University of Waterloo
Doug Manual, Warsame Yusuf, Ottawa Hospital
James Brooks, Mike Mulvey, Chand Mangat, PHAC
OTTAWA WASTEWATER INFRASTRUCTURE

Primary Sludge

Legend
- Red: wastewater
- Green: water
- Blue: pipe
- Gray: road
- Yellow: river
- Orange: other
- Purple: building
- Black: text

0 1.25 2.5 5 7.5 10 Kilometers

1:62,500

Ottawa WWTP (ROPEC)
SAMPLE COLLECTION & PROCESSING

Primary clarifier → Centrifugation → Nucleic acid extraction → Quantification

Reduction of noise in data

PMMoV normalization

Copies/L

Copies/PMMoV

[uOttawa] [Canadian Water Network] [CHEO]
TRACKING THE DECREASE IN PREVALENCE (April to June)
EARLY DETECTION OF THE 2ND WAVE (June – August)

WW signal shows early detection

Detection 48 hours before increases in clinical cases

Detection 96+ hours before increases in hospitalizations
TRACKING THE 2ND WAVE (June – November)

https://613covid.ca/wastewater/

New daily cases

N1 and N2 avg. viral signal (7-day midpoint mean)
New reported COVID-19 cases (7-day midpoint mean)

Date

May 1 | June 1 | July 1 | August 1 | September 1 | October 1 | November 1

1×10⁻³ | 6×10⁻⁴ | 5×10⁻⁴ | 4×10⁻⁴ | 3×10⁻⁴ | 2×10⁻⁴ | 1×10⁻⁴

0 | 60 | 120 | 180 | 240 | 300

Lockdown

Testing roll-out at LTC facilities (Mar. 17)
Open testing roll-out for general pop. (May 13)
Phase 1 re-opening (May 19)
Phase 2 re-opening (Jun. 12)
Phase 3 re-opening (Jul. 17)
Transition to appointment-only testing (Oct. 5)
Return to modified phase 2 (Oct. 10)
End of restrictions from modified phase 2 (Nov. 7)

Testing roll-out at LTC facilities
Open testing roll-out for general pop.
NEXT STEPS... KEY SITES IN OTTAWA AND ONTARIO

uOttawa/CHEO study detects COVID-19 signals in wastewater

- Niveau de signal de COVID-19 faible / Low levels of COVID-19 signal
- Niveau de signal de COVID-19 modéré / Moderate levels of COVID-19 signal
- Niveau de signal de COVID-19 élevé / High levels of COVID-19 signal
Webinar speaker

Mark Servos
Professor
Canada Research Chair in Water Quality Protection
University of Waterloo
Catching the Wave?

SARS-CoV-2 Detection in Wastewater

Prof. Mark R. Servos
Nivetha Srikanthan, Hadi Dhiyebi, Patrick Breadner, Sean McKay, Leslie Bragg,
Kirsten Nikel, Erika Burton, Meghan Fuzzen, Paul Craig, Wayne Parker

University of Waterloo

Many research collaborators including:

Robert Delatolla, Patrick M. D’Aoust, Élisabeth Mercier, University of Ottawa.

Municipal Partners:
Region of Peel COVID-19: Currently in Grey (Lockdown)

25,166 total cases

https://www.peelregion.ca/coronavirus/case-status/  Nov. 30, 2020
Clarkson WWTP (228 million L/d)
GE Booth WWTP (469 million L/d)
N-gene (N1, N2) SARS-CoV-2

Method:
- 40 mL whole influent sample
- Cold PEG/NaCl concentration,
- Centrifuge at 12000g
- Power Microbiome Kit/Trizol Extraction
- RT-qPCR

SARS-CoV-2 as of Nov. 25, 2020
PMMoV Normalized N-gene SARS-CoV-2

Endogenous Reference (Fecal Marker)
Mild Mottled Pepper Virus (PMMoV)

11/15 – 11/21

As of Nov. 25, 2020
Webinar speaker

Yuwei Xie
Postdoctoral Fellow
Toxicology Centre
University of Saskatchewan
eRNA based wastewater surveillance of SARS-CoV-2 at Saskatoon

Yuwei Xie, Ph.D., yuwei.xie@usask.ca
John P. Giesy, Ph.D., FRSC, FSETAC, DSAHC, jgiesy@aol.com
Toxicology center, University of Saskatchewan
Nov. 17th 2020
Pipeline

1. Sampling Primary sewage
2. Pasteurization
3. Centrifuge (6000 g for 20 min)
4. Membrane filtering
5. PEG precipitation (25,000 g for 1 hr)
6. Chemistry analyses
7. Acesulfame Creatinine
8. eRNA extraction
9. rRT-qPCR

- SARS-CoV-2 N1 and N2
- Fecal indicator
- Whole processing control
- Internal positive control

Procedure QA/QC Result
eRNA-driven outbreak forecasting of active cases

Forecasting model
For climbing stage of a spike

Virus load in sewer (log10 transformed)
Chemical biomarker for population normalizing
Conclusion

• eRNA based Wastewater-Based Epidemiology revealed the trend of outbreaking in a real-time manner

• Acesulfame is more stable than Creatinine in the primary sewage from Saskatoon WWTP, and can be used for population normalizing
Acknowledgments
Webinar speaker

Robert (Mike) McKay
Executive Director
Great Lakes Institute for Environmental Research
University of Windsor
A Tale of Two Cities: WBE applied to North America’s largest cross-border conurbation

@McKayGLIER

Canadian Water Network
NSERC CRSNG
GLWA
Amherstburg
London, Canada
INNOVATION.CA
Great Lakes Water Authority
GREAT LAKES CENTER FOR FRESH WATERS AND HUMAN HEALTH
bgSU.
GLIER
University of Windsor
Public Safety Alert
MDHHS COVID-19 updated order requiring face masks and limiting gatherings to save lives starts today. New limits on indoor residential gatherings; bars and restaurants open for outdoor dining and carry-out only; colleges and high schools must end in-person classes. For more info, see www.michigan.gov/coronavirus

Michigan COVID-19 data

- **295,177** Total Cases
- **8,377** Total Deaths
- **138,862** Total Recoveries
- **7,205** 7-day case average
- **71** 7-day death average
- **13.41%** 7-day positive test average

All data from MDHHS (Michigan Department of Health and Human Services)
Detroit Metro

Nov 18: Emergency order issued

Confirmed Cases

SARS-CoV-2:PMMoV (mean)

Date

Feb
Apr
Jun
Aug
Oct
Dec

0
500
1000
1500
2000
2500
3000

0.001
0.01

Case data: michigan.gov/coronavirus
Nov 30: Windsor-Essex moves to ‘red’
Yet municipalities of low incidence remain
Webinar speaker

Michael Parkins, MD
Associate Professor
Departments of Medicine and Microbiology, Immunology and Infectious Disease
University of Calgary
Clinic Director
Southern Alberta Adult Cystic Fibrosis Clinic,
University of Calgary Medical Clinics
Monitoring Hospital Wastewater for SARS-COV2 in Calgary

MD Parkins on behalf of a large team from the Faculties of Medicine, Science and Engineering

December 1, 2020
Disclosures

• None related to this talk

• Prior to June – I had no wastewater experience – nor ambition ....
  • What is being presented is on behalf of our entire team

Investigative Team:

HQP:
Why monitor hospital wastewater?

**RATIONALE:**

- Few community cases – when we started....
- Validation - Known Denominator
- Outbreak identification?
- Ease of access/Fixed nodes
- Leverage samples for other studies
Critical Partnerships

- Partnerships are critical for this project.
- Trans-disciplinary approach
  - ACWA
  - Alberta Health Services (AHS)
  - City of Calgary
  - UofC
General workflow

- SARS-CoV-2 wastewater-based epidemiology project:

**01 Sample collection**

Current location sites: Hospital and wastewater treatment plants

**02 Sample Clean up and concentration**

Methodology → Silica column done in ACWA.

40ml of WW samples are collected and spiked with a surrogate organism

**03 Molecular analysis**

RT-qPCR: Detection of viral RNA material, spike (BCoV) and normalization control from wastewater samples.

**04 Data analysis**
Sample Collection

- 24-hour composite samplers
- Placed in or near sewer access ports
Sample Clean Up and Concentration

• Silica column
Molecular analysis –TARGET MONITORING

Target

N1
Nucleocapsid gene of SARS-CoV-2

N2
Nucleocapsid gene of SARS-CoV-2

E
Envelope gene of SARS-CoV-2

BCoV
Transmembrane gene of BCoV strain 339/06

PMMoV
Replicase protein of PMMoV

Primers

CDC, 2020

CDC, 2020

Corman et al. 2019

Decaro et al., 2008

Haramoto et al., 2013

Standard curve

Commercially available

Commercially available

Commercially available

Commercially available

2019-nCoV_N Positive Control plasmid

2019-nCoV_E Positive Control plasmid

This study

This study

Multiplex assays

N1 - N2

E gene

M gene

BCoV, PMMV

Target Primers Standard curve

Commercially available

Commercially available

Commercially available

Commercially available

2019-nCoV_N Positive Control plasmid

2019-nCoV_E Positive Control plasmid

This study

This study

Multiplex assays

N1 - N2

E gene

M gene

BCoV, PMMV
Hospital monitoring of SARS-COV-2 Activity

Aim:

• Pilot Project to assess for SARS-CoV-2 in WW from Calgary hospitals:
  • FMC: Foothills Medical Center ~1200 beds adult hospital NW
  • PLC: Peter Lougheed Center ~700 beds adult hospital NE
  • RGH: Rockyview General Hospital ~700 beds adult hospital SW

• Collection start date:
  • August 5th – one site at each facility
  • October 1st: expansion to two additional locations at FMC (i.e. B and C)

• Sampling time frame:
  • Twice per week (but scalable)
FOOTHILLS MEDICAL CENTRE

- August 5th to Nov 19th

Outbreak

43.8%

93.3%

86.7%
Hospital Based WW SARS-COV2 Monitoring

SARS-Cov2 signal
- August 5th to Nov 19th

PLC 53.1%

RGH 31.3%
Why aren’t we detecting significant community acquired cases in hospital?

Zhou Lancet 2020;395:1054

Natural History of COVID-19

Backer Euro Surv 2020

Zhou Lancet 2020;395:1054
Early Lessons Learned in Hospital

- SARS-COV2 virus can be found in hospital – both % of samples and abundance increasing over time
- Complete capture of hospital system is required
- Monitoring is only effective for hospital “poopers”
  - Hospitalized COVID-19 patients often too sick to self-toilet
  - HCW habits?
- WW SARS-COV2 signal correlates with incident cases of COVID-19 and not prevalent cases
- WW SARS-COV2 signal in hospitals correlates with outbreaks affecting patients and staff (?) – not community acquired cases
- Some hospital samples may not be evaluable .... Why remains to be determined
Acknowledgements
Thank you, webinar speakers!

- Steve Hrudey, COVID-19 Wastewater Coalition
- Robert Delatolla, University of Ottawa
- Mark Servos, University of Waterloo
- Yuwei Xie, University of Saskatchewan
- Mike McKay, Great Lakes Institute for Environmental Research, University of Windsor
- Mike Parkins, University of Calgary
Thank you for attending today’s webinar series.

Slides and recordings will be available next week at: cwn-rce.ca/events/webinars/cwn-webinars
COVID-19 Wastewater Coalition
Phase 2 Inter-Laboratory Study
Estimated start date: February 2021
Insights for the water sector
helping decision-makers move forward

Canadian Water Network frames what is known and unknown in a way that usefully informs the choices being made.

cwn-rce.ca