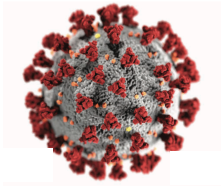
SOUTHERN NEVADA WATER AUTHORITY



Wastewater Surveillance of SARS-CoV-2 in Southern Nevada

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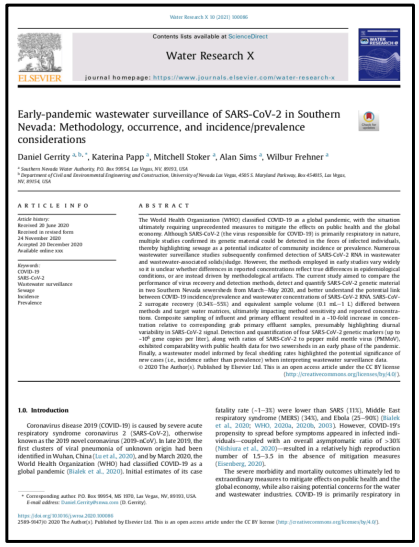

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Early-pandemic wastewater surveillance of SARS-CoV-2 in Southern Nevada: Methodology, occurrence, and incidence/prevalence considerations

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ABSTRACT

The World Health Organization (WHO) classified COVID-19 as a global pandemic, with the situation ultimately requiring unprecedented measures to mitigate the effects on public health and the global economy. Although SARS-CoV-2 (the virus responsible for COVID-19) is primarily respiratory in nature, multiple studies confirmed its genetic material could be detected in the feces of infected individuals, thereby highlighting sewage as a potential indicator of community incidence or prevalence. Numerous wastewater surveillance studies subsequently confirmed detection of SARS-CoV-2 RNA in wastewater and wastewater-associated solids/sludge. However, the methods employed in early studies vary widely so it is unclear whether differences in reported concentrations reflect true differences in epidemiological conditions, or are instead driven by methodological artifacts. The current study aimed to compare the performance of virus recovery and detection methods, detect and quantify SARS-CoV-2 genetic material in two Southern Nevada sewersheds from March–May 2020, and better understand the potential link between COVID-19 incidence/prevalence and wastewater concentrations of SARS-CoV-2 RNA. SARS-CoV-2 surrogate recovery (1.34%–55%) and equivalent sample volume (0.1 mL–1 L) differed between methods and target water matrices, ultimately impacting method sensitivity and reported concentrations. Composite sampling of influent and primary effluent resulted in a >10-fold increase in concentration relative to corresponding grab primary effluent samples, presumably highlighting diurnal variability in SARS-CoV-2 signal. Detection and quantification of four SARS-CoV-2 genetic markers (up to ~10^6 gene copies per liter), along with ratios of SARS-CoV-2 to pepper mild mottle virus (PMMoV), exhibited comparability with public health data for two sewersheds in an early phase of the pandemic. Finally, a wastewater model informed by fecal shedding rates highlighted the potential significance of new cases (i.e., incidence rather than prevalence) when interpreting wastewater surveillance data.

1.0. Introduction

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), otherwise known as the 2019 novel coronavirus (2019-nCoV). In late 2019, the first clusters of viral pneumonia of unknown origin had been identified in Wuhan, China (Lu et al., 2020), and by March 2020, the World Health Organization (WHO) had classified COVID-19 as a global pandemic (Bialek et al., 2020). Initial estimates of its case fatality rate (~1–3%) were lower than SARS (11%), Middle East respiratory syndrome (MERS) (34%), and Ebola (25–90%) (Bialek et al., 2020; WHO, 2020a, 2020b, 2003). However, COVID-19's propensity to spread before symptoms appeared in infected individuals—coupled with an overall asymptomatic ratio of >30% (Nishiura et al., 2020)—resulted in a relatively high reproduction number of 1.5–3.5 in the absence of mitigation measures (Eisenberg, 2020).

The severe morbidity and mortality outcomes ultimately led to extraordinary measures to mitigate effects on public health and the global economy, while also raising potential concerns for the water and wastewater industries. COVID-19 is primarily respiratory in

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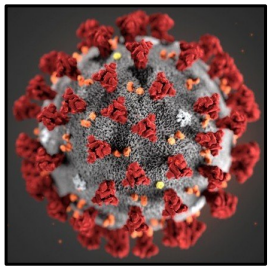
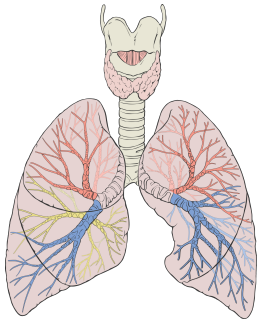
2589-9147/

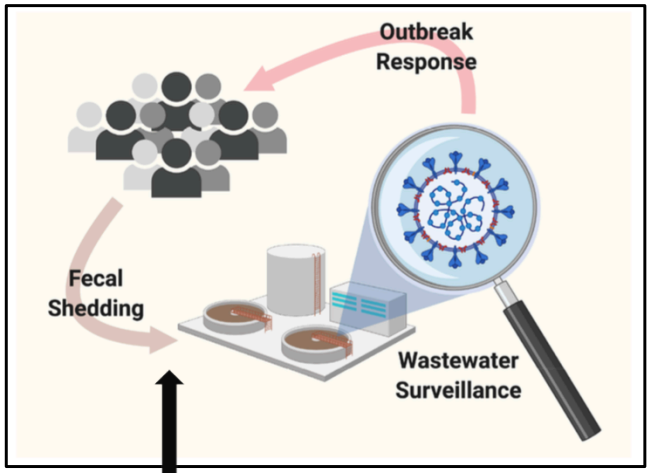
Wastewater-Based Epidemiology: Overview

Wastewater-Based Epidemiology for COVID-19

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)







≈ 200 billion SARS-CoV-2 viral particles per infection

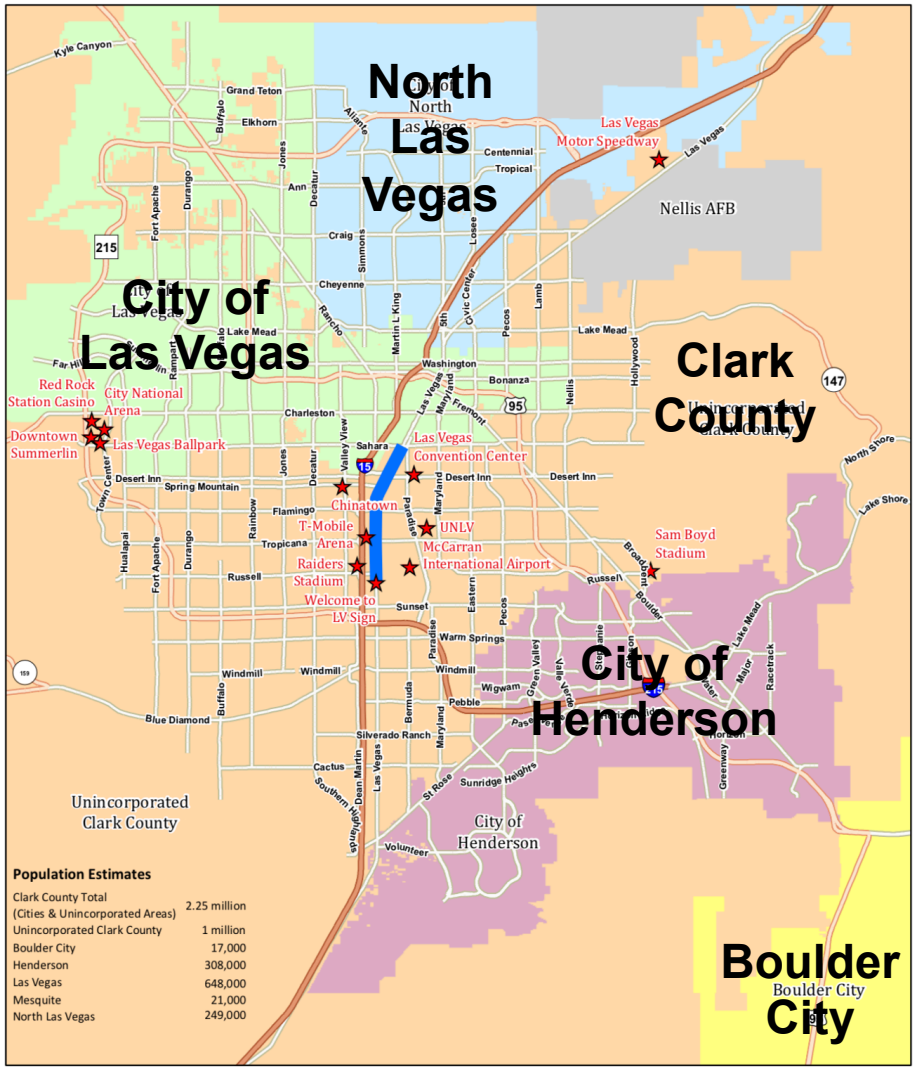
Wastewater Surveillance in Southern Nevada

SNWA/UNLV Study:

- Samples collected every Monday morning
- 2 sites since onset of pandemic, 4 since August, 1 since December
- **No hits in treated wastewater (WW), Las Vegas Wash, Lake Mead**
- UK variant of concern detected in WW before clinical samples
- Participated in Water Research Foundation methods comparison

Southern Nevada Study

Facility	Flow (mgd)	Population	Sample Type
1	100	872k	**Grab** Primary Eff.
2	42	86k	Composite Influent
3	20	757k	Composite Influent
4	5	134k	**Grab** Influent
5	15	115k	**Grab** Influent
6	6	255k	Composite Influent
7	0.8	16k	**Grab** Influent



Southern Nevada Study

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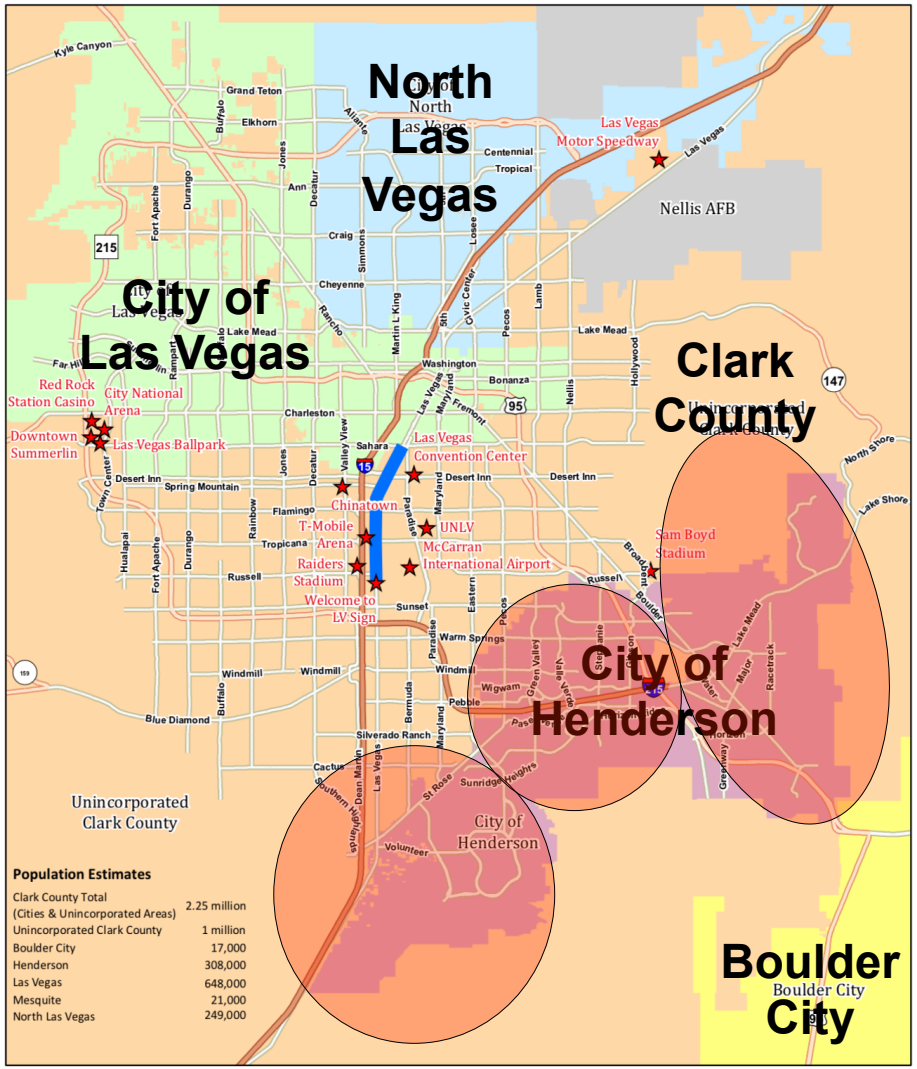
5	15	115k	**Grab** Influent
6	6	255k	Composite Influent
7	0.8	16k	**Grab** Influent

Additional Manhole Sites:

- Homeless Shelter
  - Sampling aligned with ongoing outbreak
- Public Schools (led by UNLV)
  - Evaluation of health disparities by location
  - Schools categorized based on reduced lunch rate

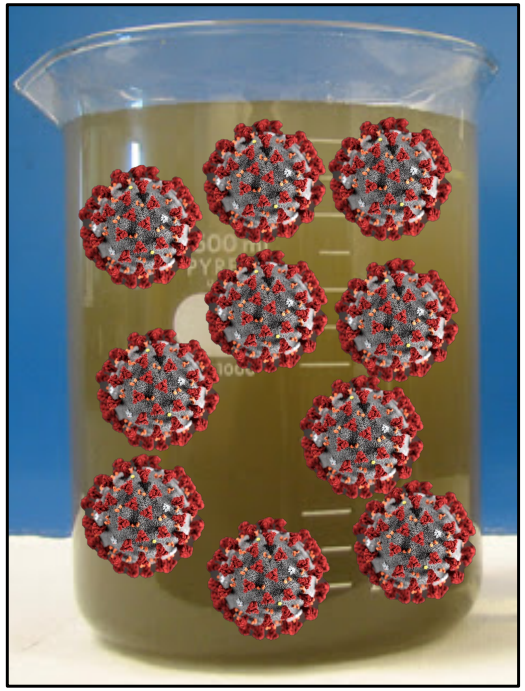


SARS-CoV-2 Concentrations vs. Sequences

Dr. Katerina Papp

Postdoc

SNWA

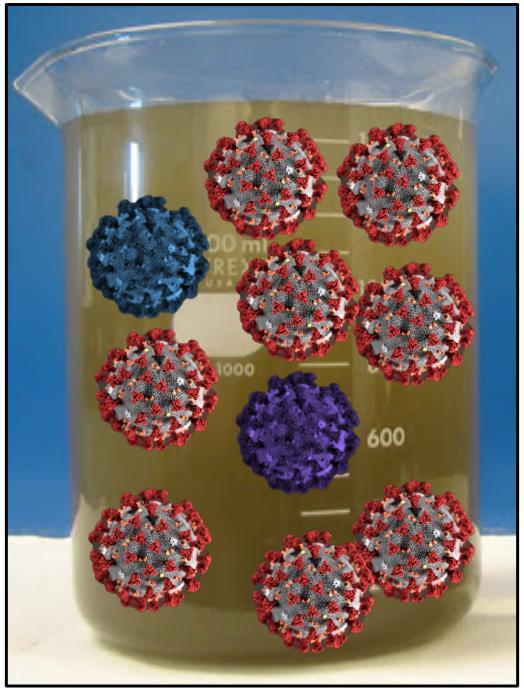


Example: 10 genome copies
(viruses) per liter
of sewage

Dr. Edwin Oh

Associate Professor

UNLV

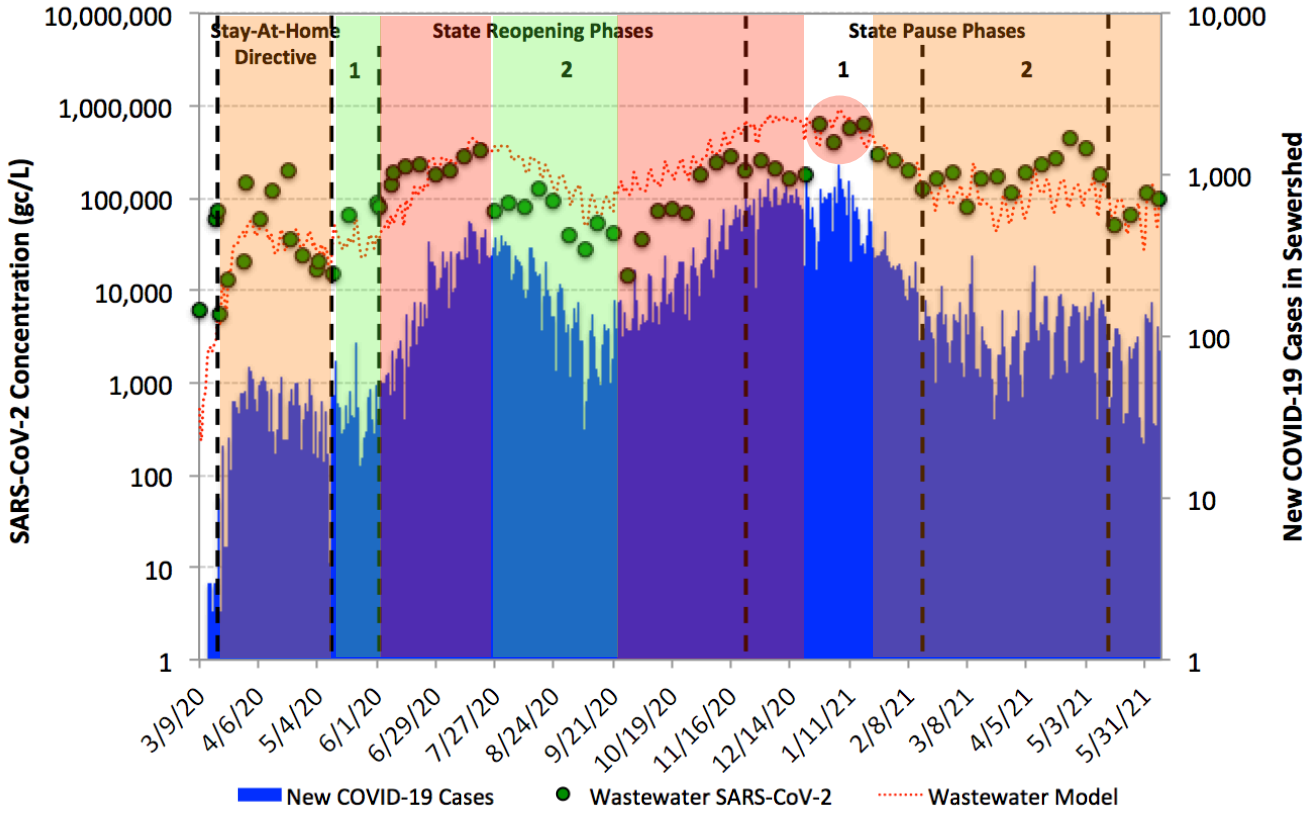


Example: 80% Wild Type
10% UK Variant
10% California Variant

Wastewater Surveillance in Southern Nevada: Data Summary

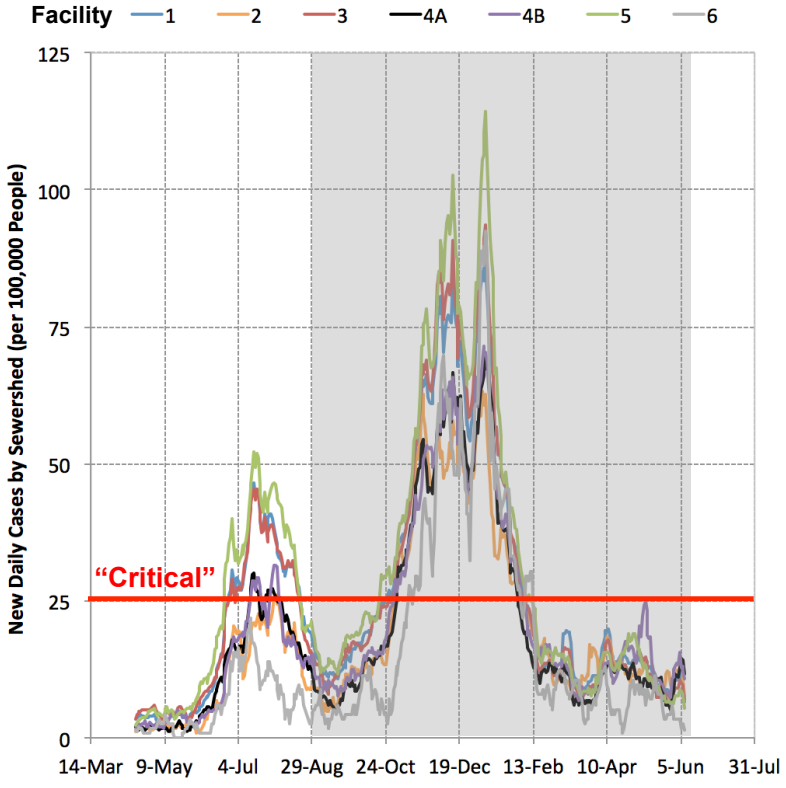
Community Trend Analysis

Facility 1: 100 mgd and ~1 million people



Sewershed Trend Analysis

Sewershed COVID-19 Cases (per 100,000 people)



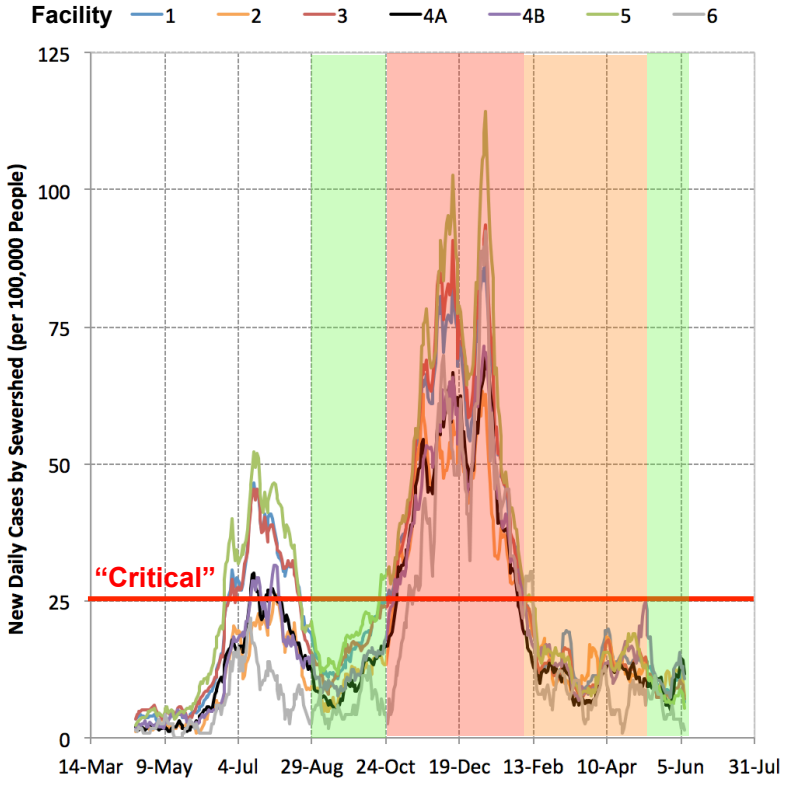
Sewershed SARS-CoV-2 Concentrations (log_{10} gc/L)

Date	Facility 1	Facility 2	Facility 3	Facility 4	Facility 5	Facility 6	Facility 7
Sample	Grab Prim.	Comp. Inf.	Comp. Inf.	Grab Inf.	Grab Inf.	Comp. Inf.	Grab Inf.
Mon. 8/24	5.0	4.4	5.3	No Sample	No Sample	No Sample	No Sample
Mon. 8/31	4.6	4.4	5.2	5.3	7.2	5.8	No Sample
Tue. 9/8	4.5	5.1	5.4	4.8	4.4	5.3	No Sample
Mon. 9/14	4.7	4.9	5.8	5.1	5.9	5.8	No Sample
Mon. 9/21	4.6	5.1	5.2	5.3	5.4	5.4	No Sample
Mon. 9/28	4.2	5.0	5.3	5.2	6.4	5.4	No Sample
Mon. 10/5	4.6	No Sample	5.8	No Sample	No Sample	No Sample	No Sample
Mon. 10/12	4.9	4.9	5.8	5.9	5.9	5.8	No Sample
Mon. 10/19	4.9	4.8	5.9	5.6	6.2	5.6	No Sample
Mon. 10/26	4.8	5.1	5.8	5.5	5.5	5.6	No Sample
Mon. 11/2	5.3	5.2	6.3	6.5	5.6	6.3	No Sample
Mon. 11/9	5.4	5.4	6.0	6.7	6.0	6.0	No Sample
Mon. 11/16	5.5	5.6	6.4	5.8	7.1	6.0	No Sample
Mon. 11/23	5.3	5.6	6.4	6.5	No Sample	6.2	No Sample
Mon. 11/30	5.4	5.4	6.2	5.9	6.5	6.0	No Sample
Mon. 12/7	5.3	5.5	6.3	6.5	7.0	6.3	No Sample
Mon. 12/14	5.2	5.6	6.2	6.5	6.7	6.4	5.9
Mon. 12/21	5.2	5.6	5.9	5.9	6.4	6.1	5.9
Mon. 12/28	5.8	5.9	6.6	6.4	6.9	6.4	8.7
Mon. 1/4	5.6	6.1	6.6	6.6	6.5	6.3	6.4
Mon. 1/11	5.8	5.8	6.5	6.2	6.4	6.5	6.0
Mon. 1/18	5.8	5.8	6.1	5.9	6.4	6.0	6.1
Mon. 1/25	5.5	5.9	6.1	6.2	6.2	5.9	6.5
Mon. 2/1	5.4	5.4	6.1	5.7	6.1	5.9	5.5
Mon. 2/8	5.3	5.4	6.0	6.1	6.0	5.3	6.2
Mon. 2/15	5.1	5.1	6.5	5.6	5.9	6.1	6.3
Mon. 2/22	5.2	5.4	5.6	6.0	6.0	5.7	5.4
Mon. 3/1	5.3	5.0	5.8	5.8	5.4	6.1	5.3
Mon. 3/8	4.9	4.9	5.5	5.5	5.4	5.6	5.5
Mon. 3/15	5.2	5.5	5.9	5.2	6.4	Non-Detect	6.4
Mon. 3/22	5.2	5.2	5.9	5.5	5.8	Inconclusive*	6.8
Mon. 3/29	5.1	5.0	5.8	6.0	5.1	5.6	5.0
Mon. 4/5	5.3	5.0	5.9	5.2	6.2	6.3	5.5
Mon. 4/12	5.4	5.1	5.7	6.2	5.4	5.9	5.4
Mon. 4/19	5.4	5.1	6.0	5.9	6.3	6.0	5.6
Mon. 4/26	5.6	5.2	6.1	6.1	5.5	5.5	5.4
Mon. 5/3	5.5	5.4	5.9	6.5	6.1	5.8	5.5
Mon. 5/10	5.2	4.9	5.8	6.5	5.8	5.5	Non-Detect
Mon. 5/17	4.7	4.8	5.5	5.5	5.6	5.5	Non-Detect
Mon. 5/24	4.8	5.1	5.3	4.6	5.5	5.6	4.8
Tue. 6/1	5.1	4.7	5.1	5.3	5.2	4.8	4.8
Mon. 6/7	5.0	5.5	5.2	5.9	5.9	5.2	4.5

*Only 1 of 8 analytical replicates positive

Sewershed Trend Analysis

Sewershed COVID-19 Cases (per 100,000 people)



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Mon. 11/23	5.3	5.6	6.4	6.5	No Sample	6.2	No Sample
Mon. 11/30	5.4	5.4	6.2	5.9	6.5	6.0	No Sample
Mon. 12/7	5.3	5.5	6.3	6.5	7.0	6.3	No Sample
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Mon. 1/25	5.5	5.9	6.1	6.2	6.2	5.9	6.5
Mon. 2/1	5.4	5.4	6.1	5.7	6.1	5.9	5.5
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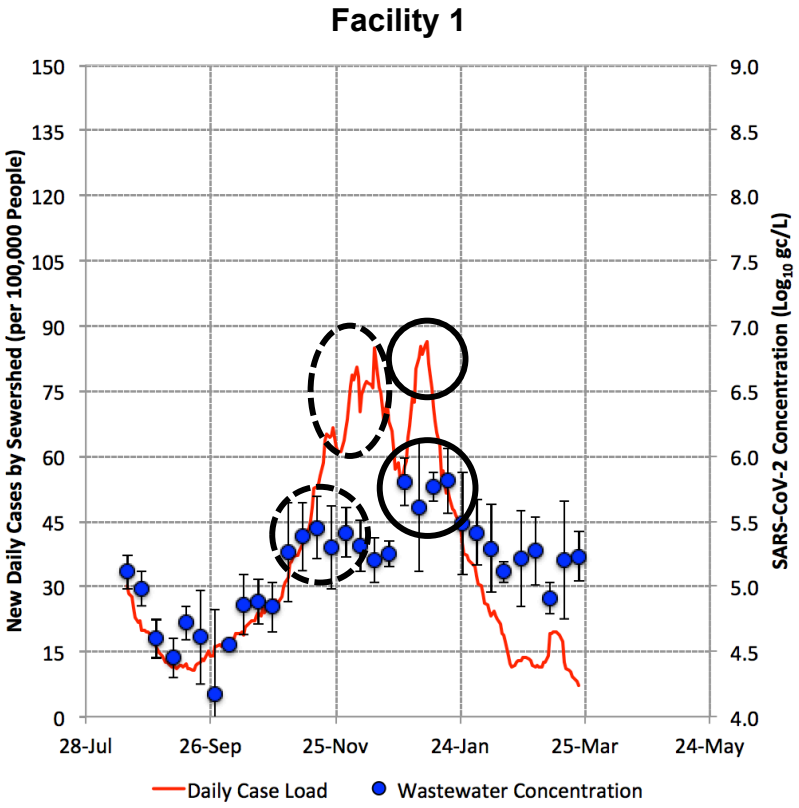
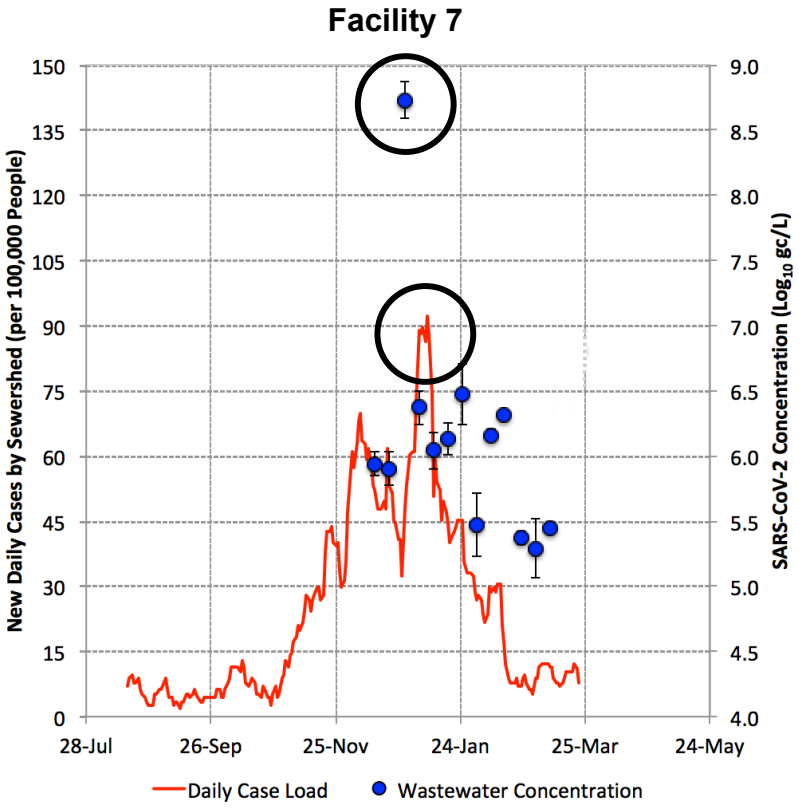
Mon. 6/7	5.0	5.5	5.2	5.9	5.9	5.2	4.5

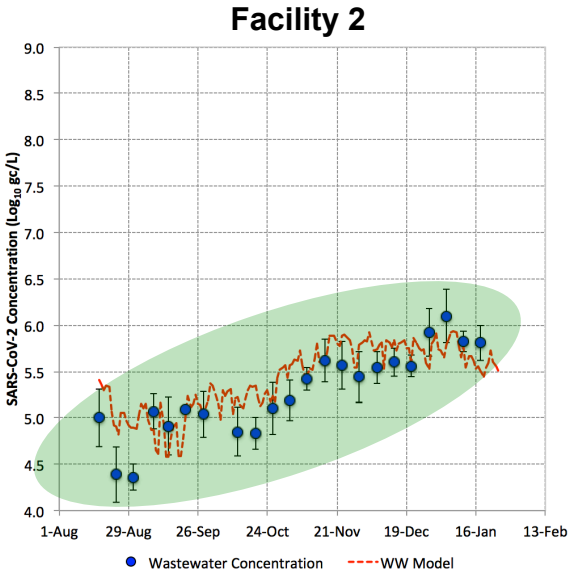
*Only 1 of 8 analytical replicates positive

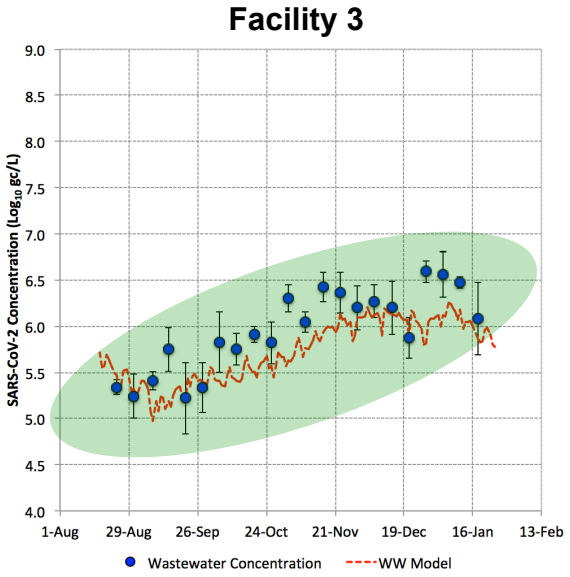
Predicting the Holiday Surge

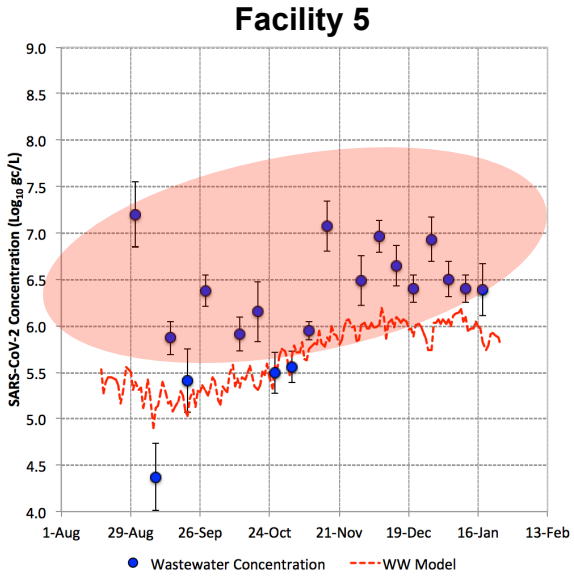




Are the Data Actionable?

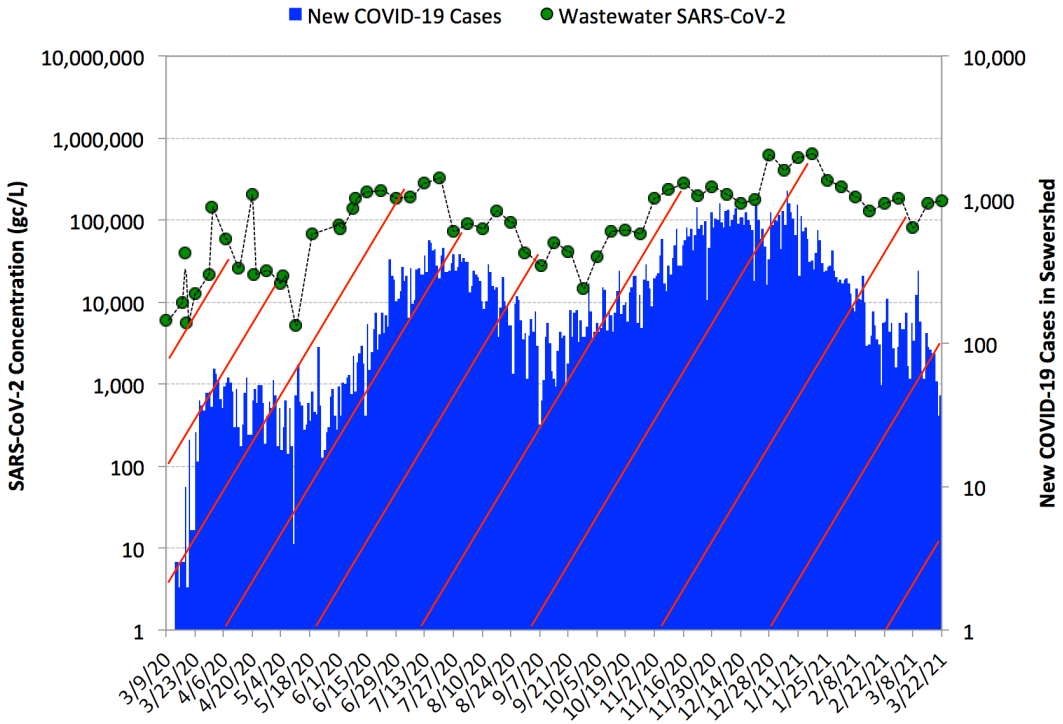






Facility 5: Greater discrepancy between wastewater concentrations and clinical case data (i.e., model)

Retrospective Infection Estimate



Area under wastewater curve = total SARS-CoV-2 in wastewater since March 2020

Flow rate = 100 million gallons per day | Concentrations adjusted for diurnal variability

= 9.4×10^{16} gene copies into Facility 1 from March 2020 – May 2021

Retrospective Infection Estimate

Based on Facility 1 (as of May 2021):

- A = Total SARS-CoV-2 into WWTP = 9.42×10^{16} gc
- B = Total SARS-CoV-2 per Infection = 2.42×10^{11} gc/infection → **still somewhat uncertain**
- A/B = Estimated Total Infections since March 2020 = 389,445 infections

All Sewersheds (as of May 2021):

- WW Estimated Infections in Southern Nevada = **915,493 = ~40% of Southern NV**
- Antibody Estimated Infections in Southern Nevada = **575,000 = ~25% of Southern NV**
- Confirmed Infections in Southern Nevada **= 239,702 = ~10% of Southern NV**
- Assume 90% for herd immunity → **2.1 million infections + vaccinations**
- 'Non-Infected' Vaccinations Needed = **1.2 million | 1.5 million | 1.9 million**
- Vaccinations Completed/Initiated = **0.8 million | 1.1 million (some vax previously infected)**

Wastewater-Based Epidemiology: Path Forward

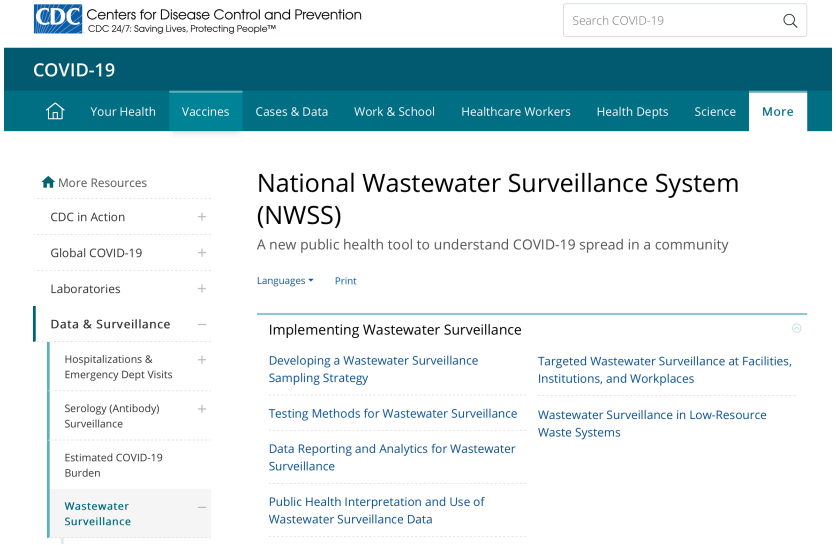
Increasing Accessibility/Leveraging Momentum

SNWA Collaboration with Hach/Luminultra

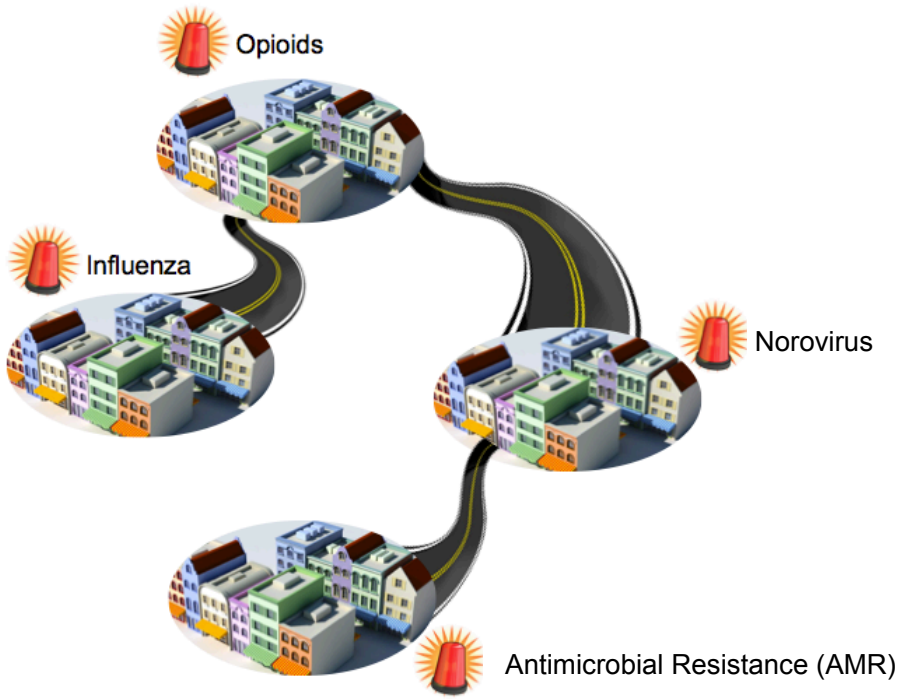
- Portable qPCR Instrument ≈ $16,000
- SARS-CoV-2 Kit ≈ $40/sample

CDC National Wastewater Surveillance System (NWSS)

- Developing national database for SARS-CoV-2
- Expand to other targets in the future?



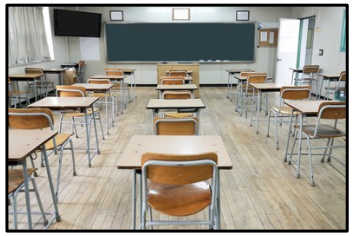
Path Forward for Wastewater Epidemiology



Alternative Data Sources

CVS pharmacy®

SNHD Southern Nevada Health District



Acknowledgments

- Staff at the collaborating wastewater agencies: Clark County Water Reclamation District, City of Henderson, City of Las Vegas, City of North Las Vegas, Boulder City
- Staff at the Southern Nevada Water Authority for ensuring a safe work environment during the pandemic
- Countless research colleagues for their guidance
- This work was partially supported by the National Science Foundation under Grant No. 1832713

- **For additional information:**
  - SNWA Podcast: Poops Don't Lie – Tracking a Pandemic Using Wastewater[a]
  - Email: Daniel.Gerrity@snwa.com

[a]https://watersmarts.buzzsprout.com/1568941/8125328-poops-don-t-lie-tracking-a-pandemic-using-wastewater