

Wastewater Epidemiology for SARS-CoV-2 in Southern Nevada: Lessons Learned for Collaboration and Long-Term Implementation

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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 sewerheds 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 (0.344–55%) 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 ~10⁶ 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 sewerheds 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.

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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 (Liu 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, 2020c). 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

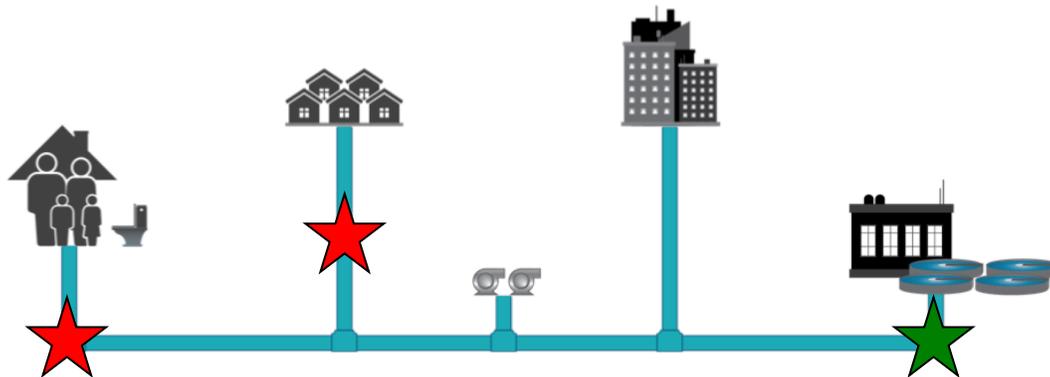
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GLOBAL COLLABORATION

- Virtual International Research Summit in April
- Congressional Briefing in May
- Round Robin Method Comparison
- **WW Surveillance Concepts:**
 - Trend Analysis
 - Community vs. (Sub)sewershed vs. Facility
 - Virus Evolution



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Wastewater Surveillance of the COVID-19 Genetic Signal in Sewersheds

Recommendations from Global Experts

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Congressional Briefing

Environmental Surveillance of the Genetic Footprint of COVID-19 in Sewersheds

Thursday, May 21 | 2:00 PM EDT

Environmental Science
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Reproducibility and sensitivity of 36 methods to quantify the SARS-CoV-2 genetic signal in raw wastewater: findings from an interlaboratory methods evaluation in the U.S.†

Brian M. Pecson,^{†*} Emily Darby,^{†*} Charles N. Haas,^{†b} Yamrot M. Amha,^c Mitchel Bartolo,^d Richard Danielson,^e Yeggie Dearborn,^e George Di Giovanni,^f Christobel Ferguson,^g Stephanie Fevig,^{†g} Erica Gaddis,^{†h} Donald Gray,ⁱ George Lukasik,^j Bonnie Mull,^j Liana Olivas,^c Adam Olivieri,^k Yan Qu^c and SARS-CoV-2 Interlaboratory Consortium§



Potential Applications of SARS-CoV-2 Wastewater Surveillance

SCALE and **TIMING**

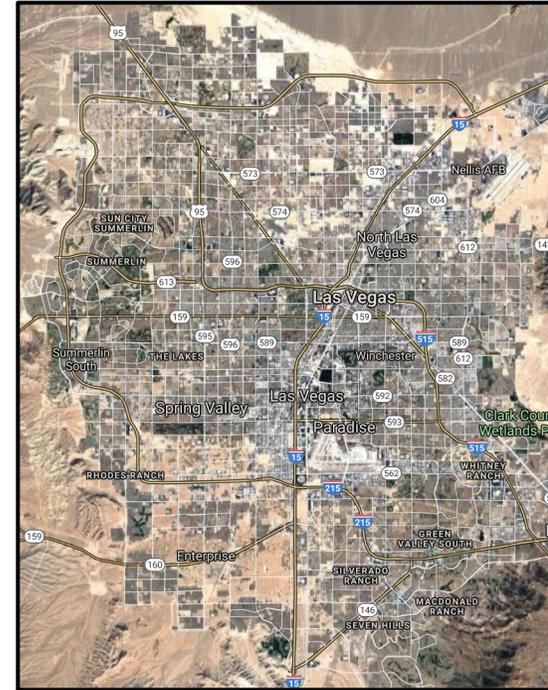
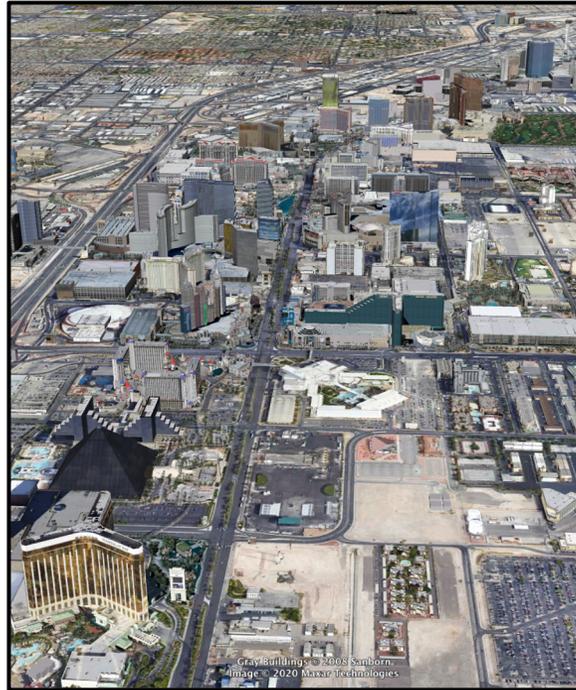


Facility

Sub-Sewershed

Region

SCALE

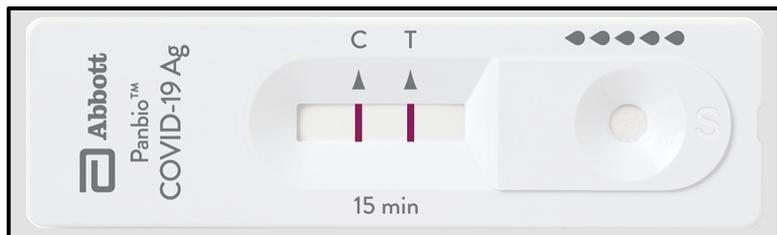


ACTIONABILITY

High

Moderate

Low





A TALE OF TWO COUNTRIES

New cases ▾

 United States ▾

All regions ▾

All time ▾



United States

Population = 330 million

Max Daily Cases = ~300,000

Max Daily Cases = **91 per 100,000**

Note

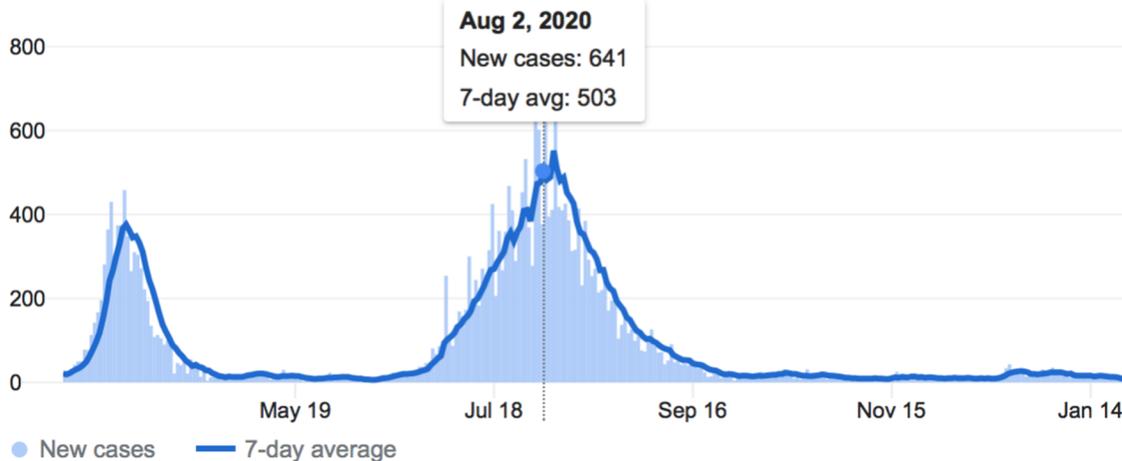
Different

Scales

New cases ▾

 Australia ▾

All time ▾



Australia

Population = 25 million

Max Daily Cases = ~700

Max Daily Cases = **3 per 100,000**



EARLY WARNING FOR RE-EMERGENCE

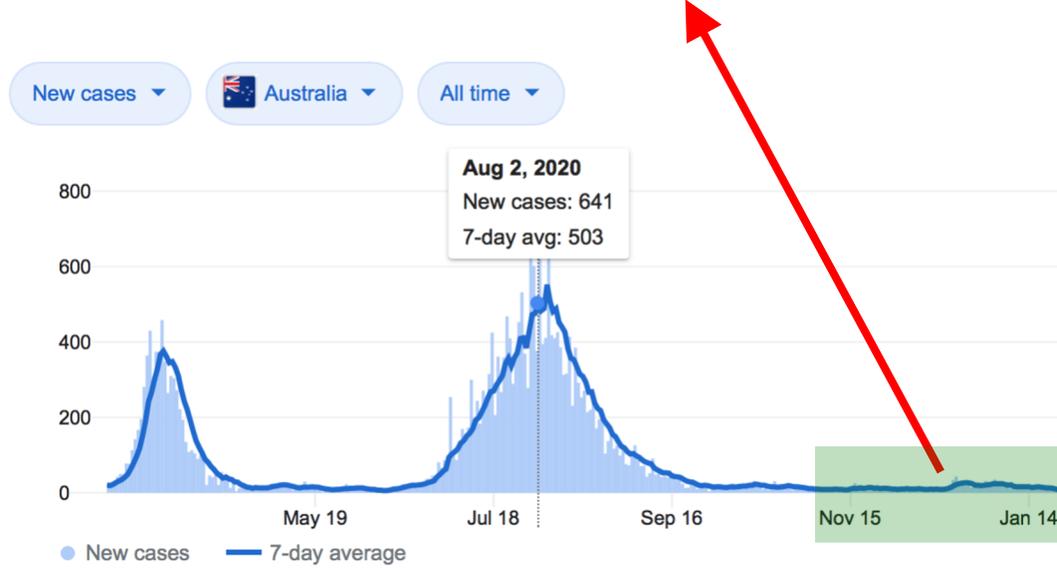
Sewage testing detects COVID-19 fragments

If you live, work or are active in and around the Batemans Bay area

Get tested immediately if COVID-19 symptoms develop.

nsw.gov.au/covid-19





Pop.	Location	Week ending									
		14-Nov	21-Nov	28-Nov	5-Dec	12-Dec	19-Dec	26-Dec	2-Jan	9-Jan	16-Jan
60,514	Blue Mountains (Winmalee)	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
4,681	North Richmond	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
13,052	Richmond	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
110,114	Penrith	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
12,000	Lithgow	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
19,000	South Windsor	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
8,000	McGraths Hill	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
69,245	Warriewood	Green	Green	Green	Green	Green	Red	Red	Red	Red	Red
1,241	Brooklyn	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
31,924	Hornsby Heights	Green	Green	Green	Green	Green	Red	Red	Red	Red	Red
57,933	West Hornsby	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
318,810	Bondi	Green	Red	Red	Red	Green	Red	Red	Red	Red	Red
233,176	Cronulla	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
	Malabar 1	Red	Green	Red	Green	Green	Red	Red	Red	Red	Red
1,857,740	Malabar 2	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
181,005	Liverpool	Red	Red	n	Green	Red	Red	Red	Red	Red	Red
98,743	West Camden	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
6,882	Wallacia	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
14,600	Picton	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
161,200	Glenfield	Green	Green	Green	Green	Green	Green	Green	Red	Red	Red
1,341,986	North Head	Green	Red	Green	Green	Green	Red	Red	Red	Red	Red
	Castle Hill Cattai	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
26,997	Castle Hill Glenhaven	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
163,374	Quakers Hill	Green	Green	Green	Green	Green	Green	Green	Green	Red	Red
119,309	Rouse Hill	Red	Green	Green	Green	Green	Green	Green	Green	Green	Green
37,061	Riverstone	Green	Green	Green	Red	Green	Green	Green	Green	Green	Green
163,147	St Marys	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
73,686	Shellharbour	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
55,000	Wollongong	Green	Green	Green	Green	Green	Green	Green	Red	Red	Red
68,000	Port Kembla	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
93,000	Bellambi	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green

NOT DETECTED (Green background) DETECTED (Red background)

Source: health.nsw.gov.au

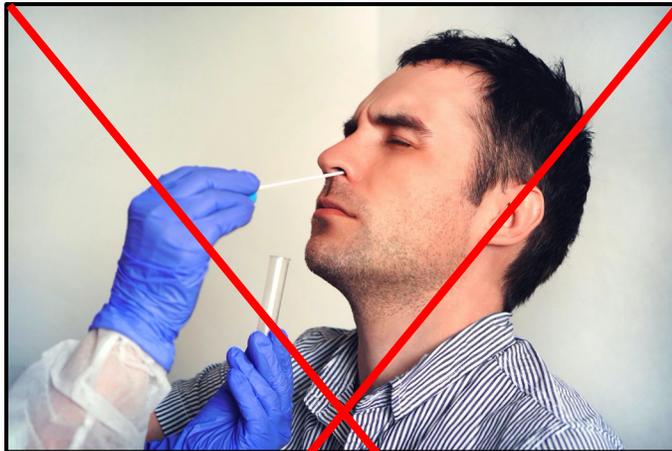


SARS-CoV-2 Wastewater Surveillance Methodology



SARS-COV-2 IN WASTEWATER

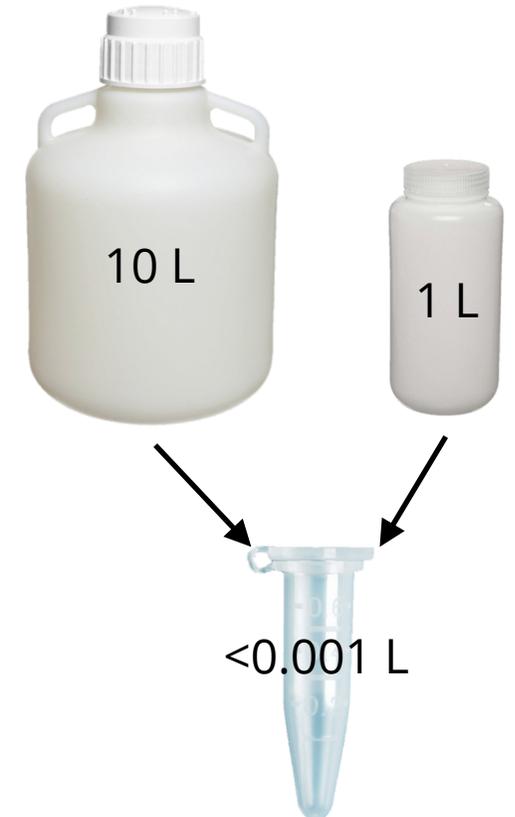
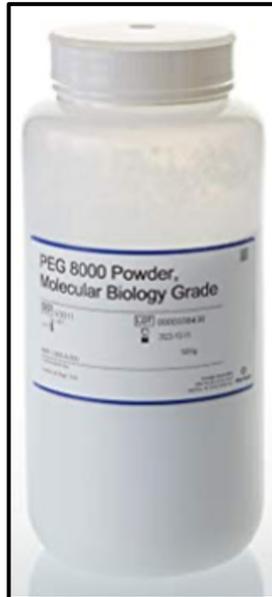
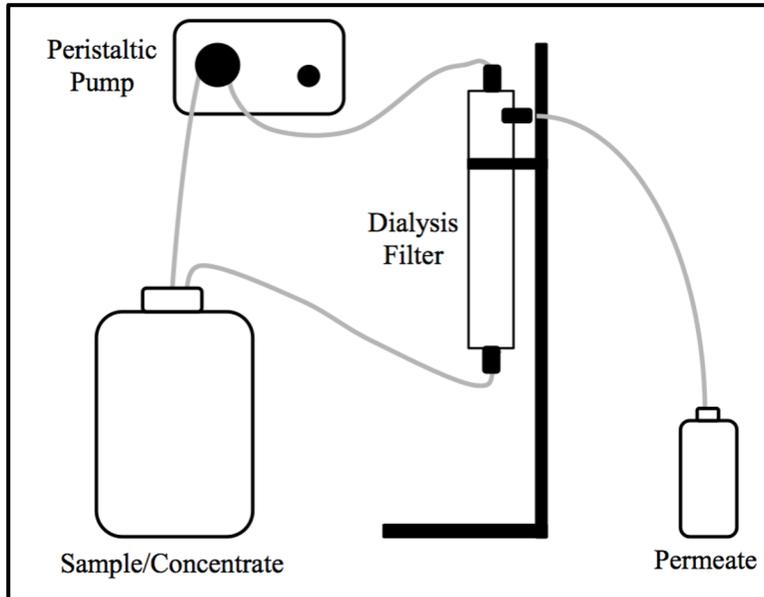
1. Sample Collection (Every Monday Morning)





SARS-COV-2 IN WASTEWATER

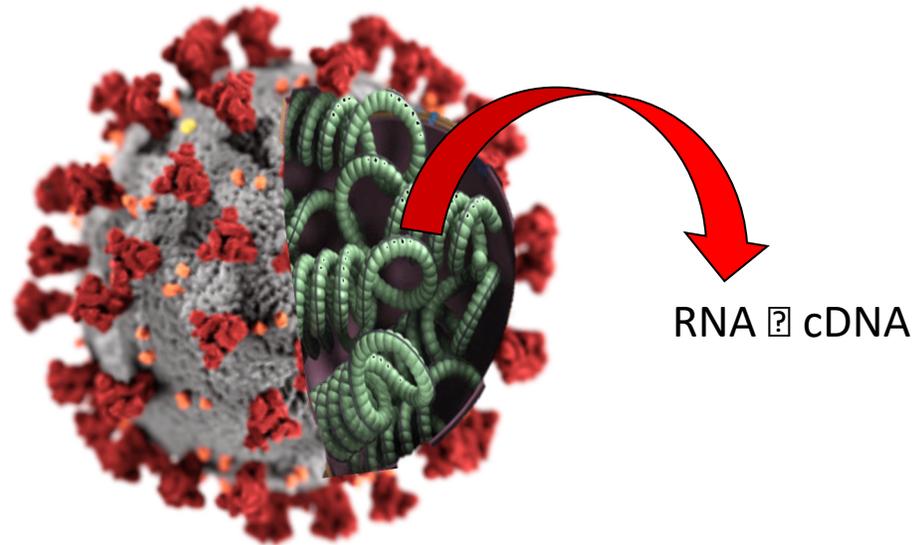
1. Sample Collection (Every Monday Morning)
2. Surrogate Spike for Recovery (Bovine Coronavirus)
3. Sample Concentration





SARS-COV-2 IN WASTEWATER

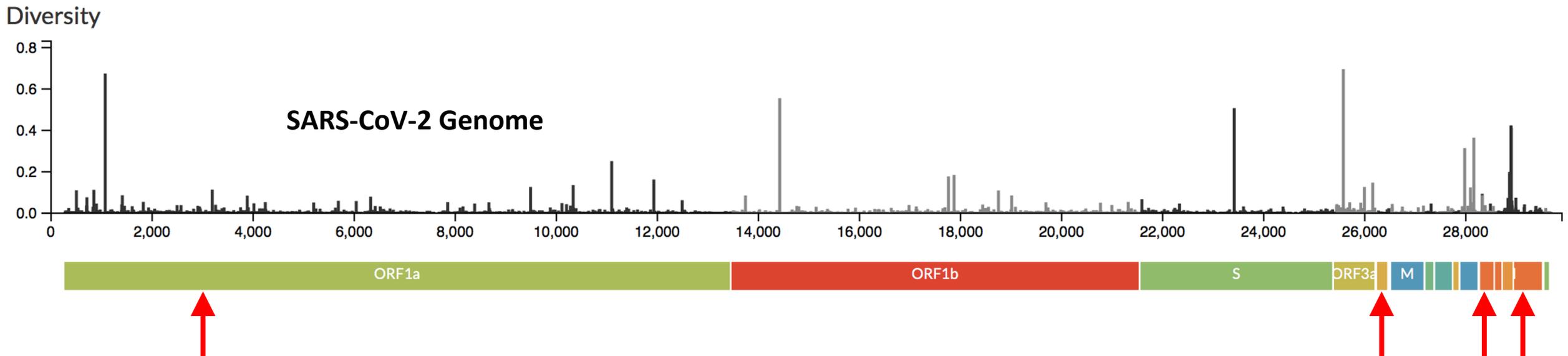
1. Sample Collection (Every Monday Morning)
2. Surrogate Spike for Recovery (Bovine Coronavirus)
3. Sample Concentration
4. Nucleic Acid Extraction and Complementary DNA (cDNA) Synthesis





SARS-COV-2 IN WASTEWATER

1. Sample Collection (Every Monday Morning)
2. Surrogate Spike for Recovery (Bovine Coronavirus)
3. Sample Concentration
4. Nucleic Acid Extraction and Complementary DNA (cDNA) Synthesis
5. Analysis by qPCR with **Gene-Specific** Primers/Probes (4 Different Assays)





SARS-COV-2 IN WASTEWATER

1. Sample Collection (Every Monday Morning)
2. Surrogate Spike for Recovery (Bovine Coronavirus)
3. Sample Concentration
4. Nucleic Acid Extraction and Complementary DNA (cDNA) Synthesis
5. Analysis by qPCR with Gene-Specific Primers/Probes (4 Different Assays)
6. Convert SARS-CoV-2 Concentration to COVID-19 Incidence

$$\text{Infections (persons)} = \frac{\text{Concentration (gene copies/L)} \times \text{Wastewater Flow Rate (L/day)}}{\text{Feces Production Rate (grams/person-day)} \times \text{Fecal Shedding Rate (gene copies/gram)}}$$



SUPPLY CHAIN CONSIDERATIONS

Backordered!



Centricon Plus-70
Centrifugal Filters (100 kDa)



**Raising Cattle to Insure a Future
Water Supply-It's Happening in Nevada**



- Consider required consumables (**cost and availability**) when selecting a method
- Have a method backup plan and understand implications of method changes
- Refrigerated storage may be fine (**avoid freezing**)

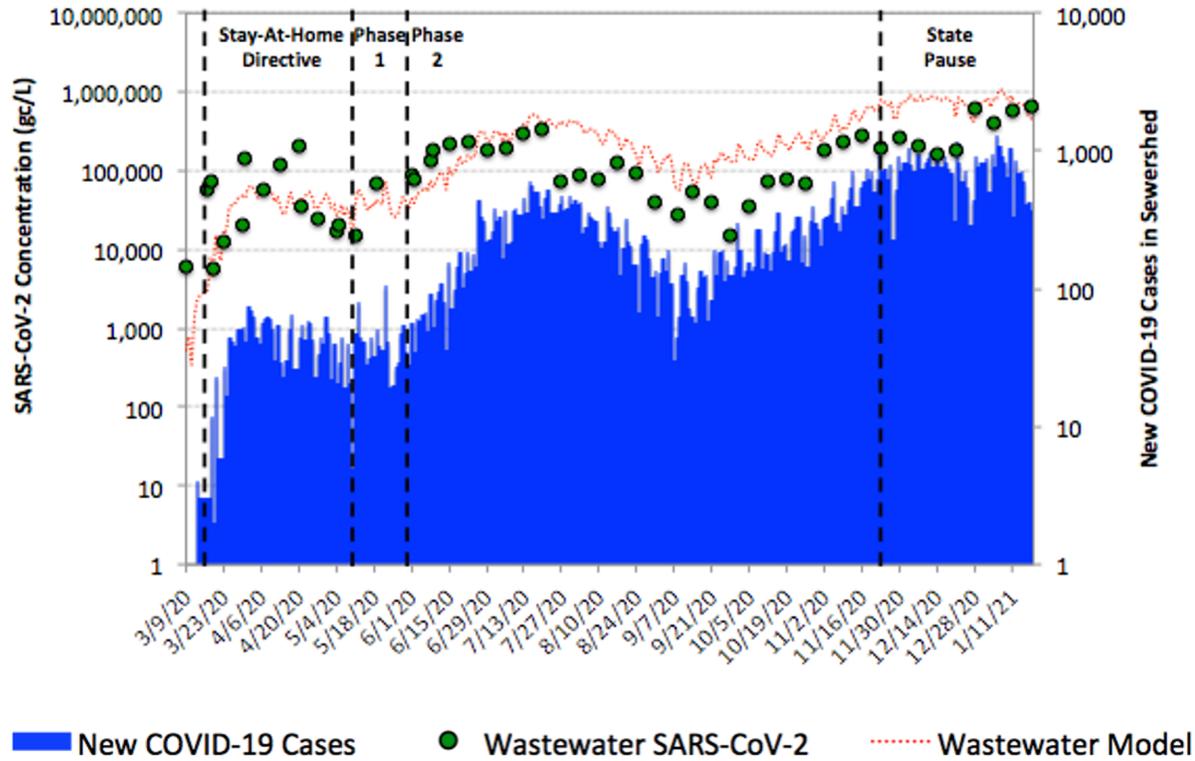


Southern Nevada Wastewater Surveillance Data



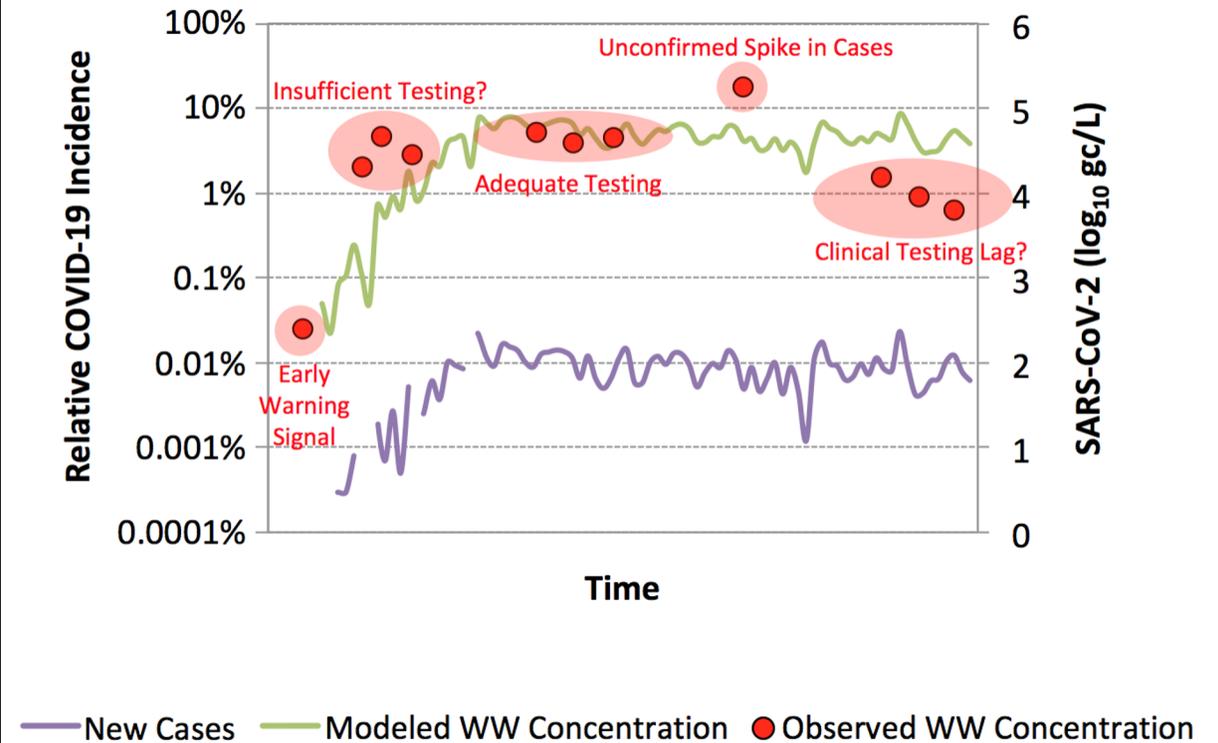
COMMUNITY TREND ANALYSIS

Facility 1: 100 mgd and 1 million people



(mgd = million gallons per day)

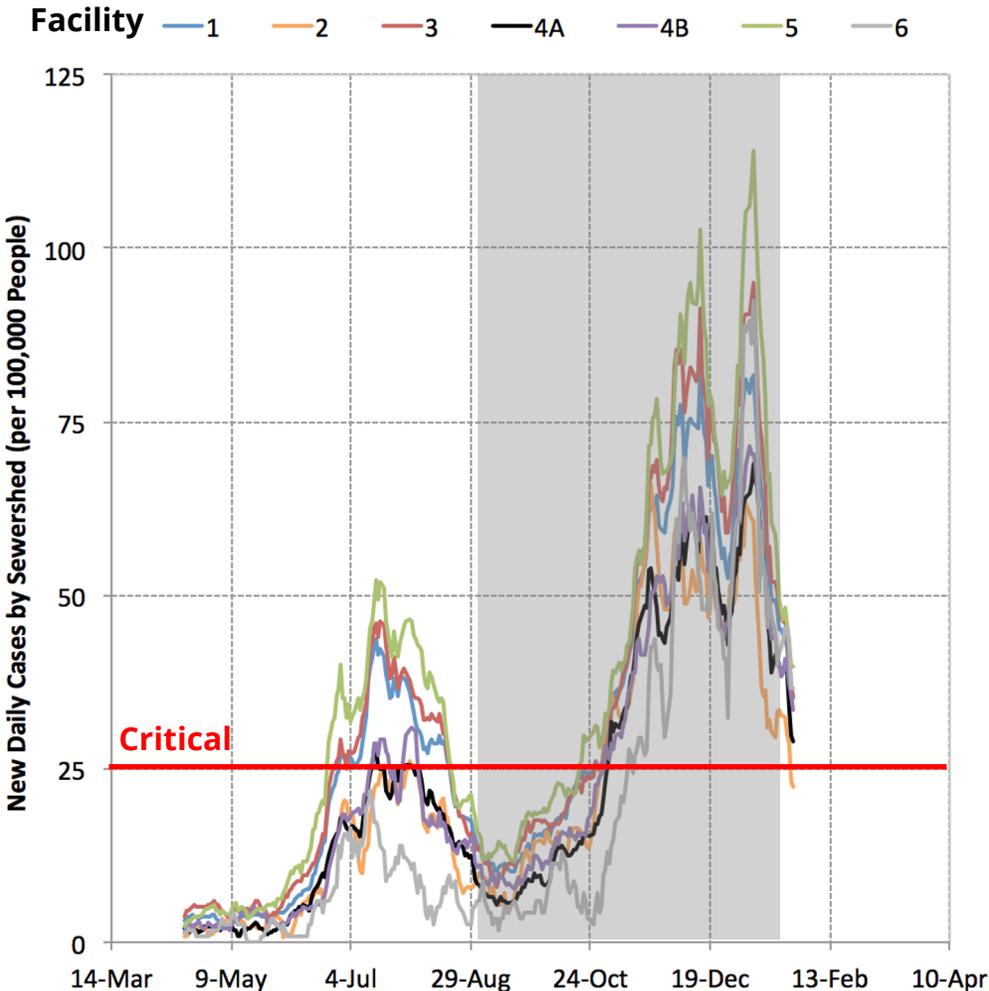
Potential Interpretation of WW Surveillance





(SUB)SEWERSHED TREND ANALYSIS

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



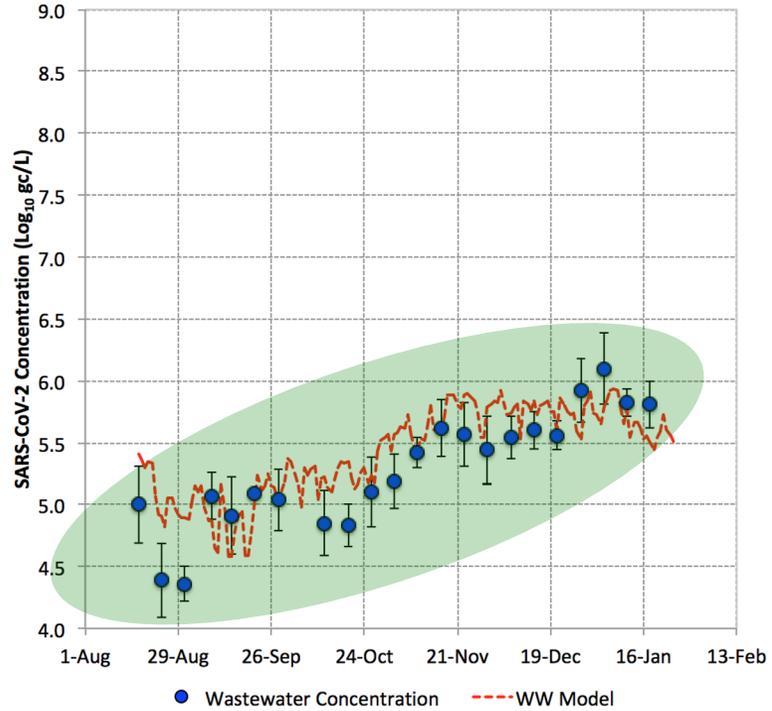
Sewershed SARS-CoV-2 Concentrations (log₁₀ gc/L)

Date	Facility 1	Facility 2	Facility 3	Facility 4A	Facility 4B	Facility 5	Facility 6
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

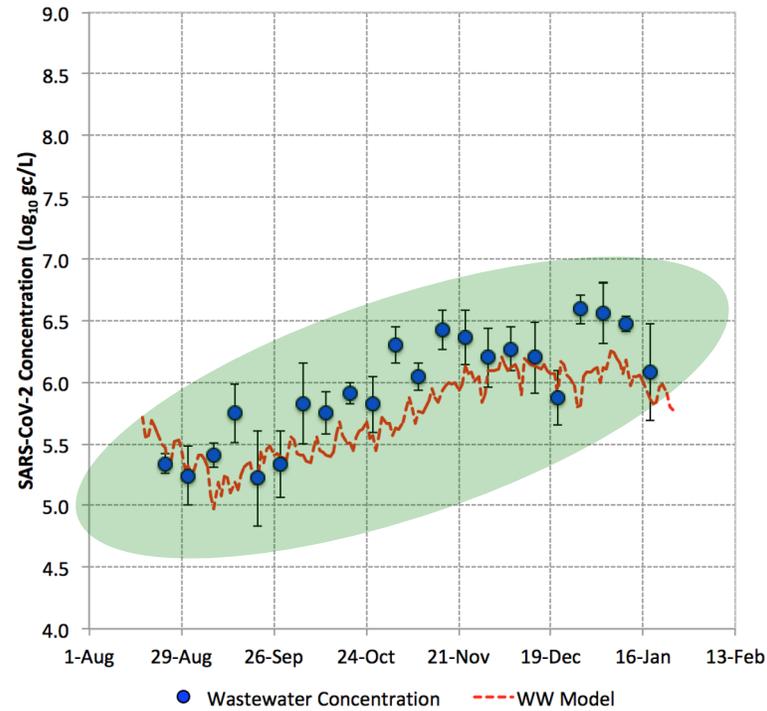


(SUB)SEWERSHED SCALE

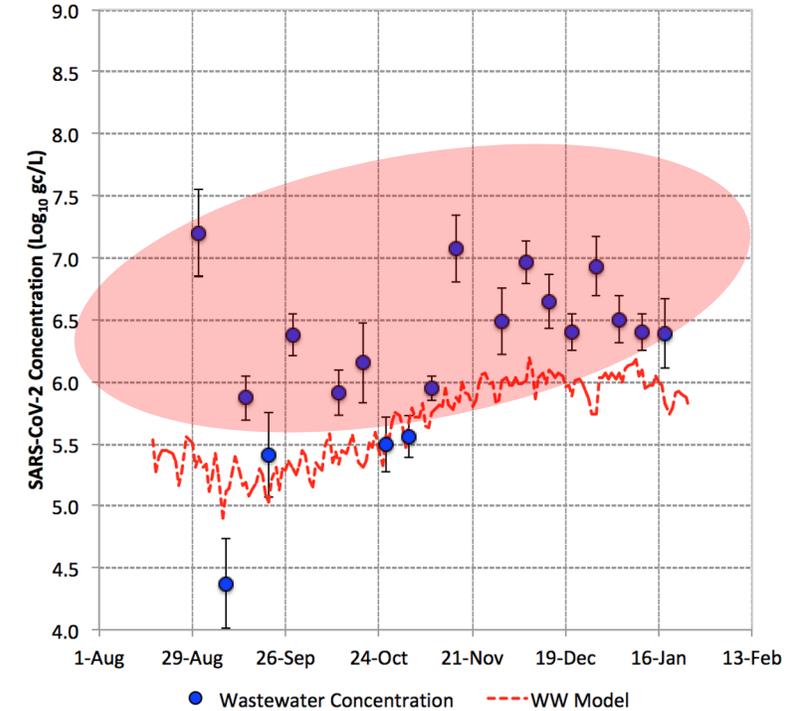
Facility 2



Facility 3



Facility 4B



Facility 4B: Greater discrepancy between wastewater concentrations and clinical case data (i.e., model)





ACKNOWLEDGMENTS

- This work was partially supported by the National Science Foundation under Grant No. 1832713.
- Staff at the collaborating wastewater agencies for their assistance with sample logistics and data access
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- Mark Borchardt and Susan Spencer at the U.S. Department of Agriculture for generously providing the bovine coronavirus vaccine stock.
- Countless research colleagues for their guidance

Questions: daniel.gerrity@snwa.com