



Wastewater Surveillance of SARS-CoV-2 in Three Clemson Area Sewersheds

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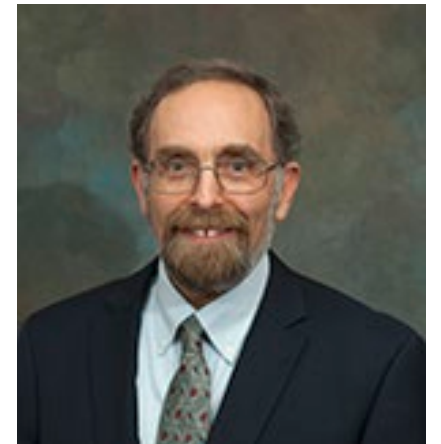
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Department of Environmental Engineering and Earth Sciences

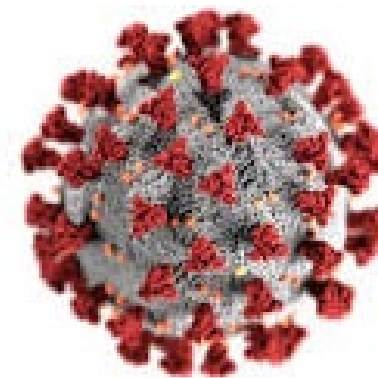
Presented for the Robins AFB Post Monthly Meeting

July 21, 2021



Acknowledgements

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- **Clemson University has funded the wastewater surveillance**



Wastewater-Based Epidemiology

- WBE has been used for decades
 - ❖ **Monitor the effectiveness of polio vaccine:** “Israel experienced an outbreak of wild poliovirus type 1 (WPV1) in 2013–2014, detected through environmental surveillance of the sewage system. . . As we approach global eradication, polio will increasingly be detected only through environmental surveillance.” Brouwer et al., 2018; PNAS
 - ❖ **Monitor the magnitude of opioid use at a community level; most drugs are only partly metabolized and are excreted at detectable levels in sewage**
 - ❖ **Gauge spread of antibiotic-resistant microbes**
- Wastewater is a mirror of important processes that occur within human populations, at the level of individual buildings, neighborhoods, or whole communities

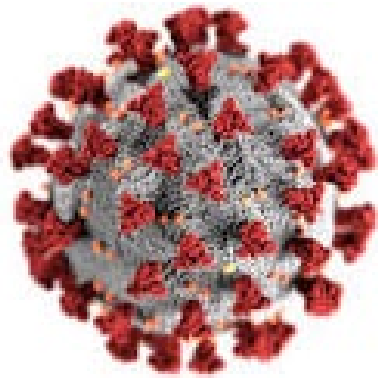


WBE Applied to SARS-CoV-2

- SARS-CoV-2 is the virus that causes the disease COVID-19
- Symptomatic and asymptomatic individuals who are infected with SARS-CoV-2 shed the virus in their feces
- This creates an opportunity to monitor for the presence of the virus in sewage
- Monitoring sewage can indicate at the community level if transmission of the virus is
 - ❖ imminent, since people can shed virus ~1 to 2 weeks before they exhibit clinical symptoms (leading indicator)
 - ❖ underway, especially in the absence of extensive testing of individuals
 - ❖ on the decline, in response to efforts to reduce transmission



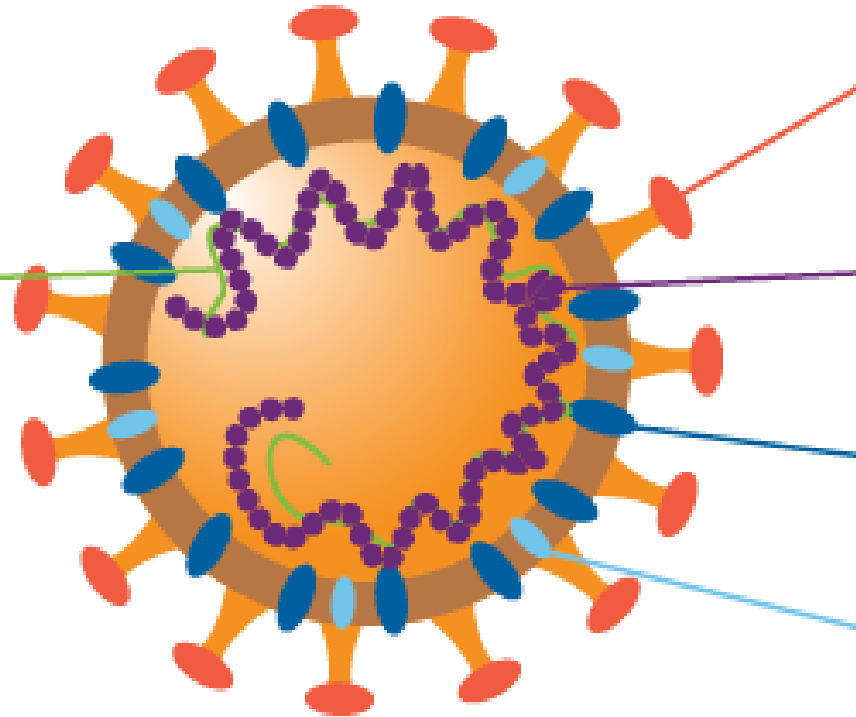
Key Parts of the Virus



✓

ORF-1ab Encoded
Non-Structural
Proteins

RNA



Spike
glycoprotein

✓

Nucleocapsid
protein

✓

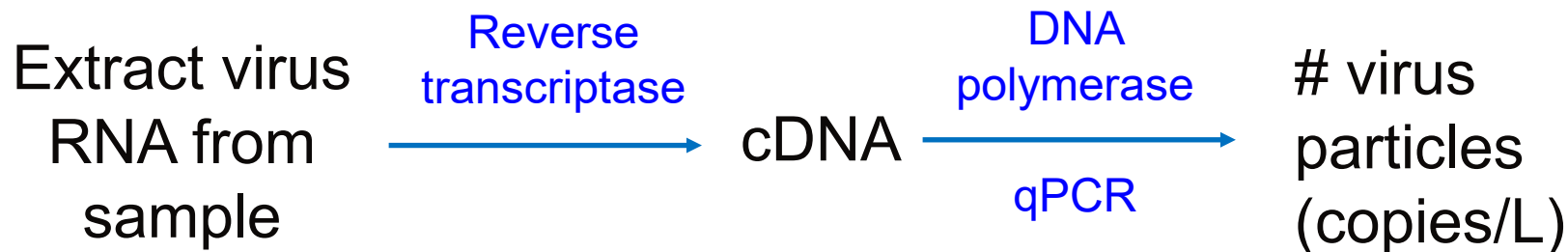
Membrane
glycoprotein

Small envelope
protein

- Strand of RNA encased in an envelope made up of proteins and glycoprotein spikes on the surface
- ✓ = genes used in detecting the virus in wastewater

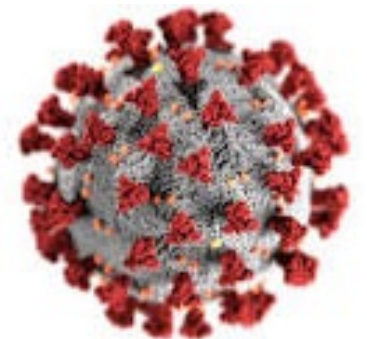
How it Works

- Collect samples of sewage
 - ❖ 24-hour composite; standard practice at “headworks” for WWTPs
 - ❖ Grab sample (mid-morning works best); often the only option for buildings or clusters of buildings; access via manholes
- Samples are analyzed in the laboratory using a procedure similar to what is used to measure SARS-CoV-2 in clinical samples
 - ❖ reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR)



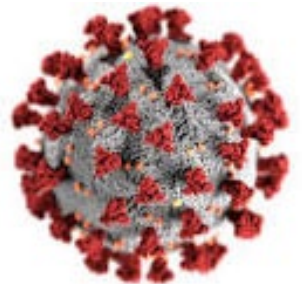
How it Works

- The technique looks for specific, unique sections of the virus' RNA, which is detectable amongst all the other “stuff” present in sewage
 - ❖ Genes that code for the nucleocapsid (N) protein
 - ❖ Genes that code for the spike protein (S) gene primers
 - ❖ The open reading frame 1ab (orf-1ab)
- Positive controls include spikes with MS2 phage RNA and plasmids that contain N gene fragments; no template negative control containing only nuclease free water
- **Outcome:** quantification of how many SARS-CoV-2 particles are present in the sewage, reported in units of “copies per liter”



How it Works

- Once the virus is shed in feces and enters the sewer system, it starts to undergo decay
 - ❖ Rate depends on temperature and residence time
- The envelope is subject to a faster rate of degradation than the RNA, so what arrives at the treatment plant is a combination of RNA strands and intact virus particles
- Important note: The virus is not transmissible in water, so there is no danger of infection by that route; we are only looking for evidence that virus is being shed in the feces by infected individuals



Early Applications

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Spanish researchers develop method to detect coronavirus by analyzing wastewater

The analysis system, which could be used as a method for epidemiologic monitoring, has been tested in Murcia, Spain, and in three treatment plants of the metropolitan area of Valencia.

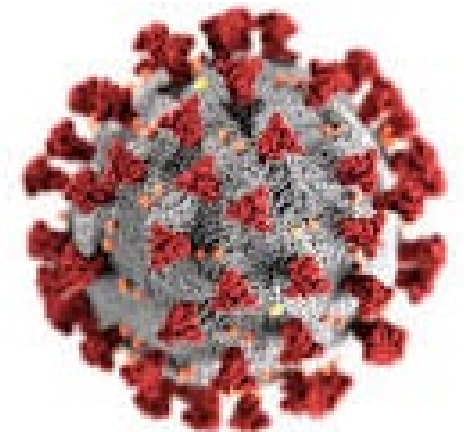
May 12th, 2020

📄 🌐 📧 📷

- WBE was first used to monitor for the virus in Europe, as the pandemic spread there in the Spring of 2020
- Let's look at two examples:

❖ Valencia, Spain

❖ Paris, France



FRANCE 24 LIVE

#CORONAVIRUS #MACRON FRANCE AFRICA US PRESIDENTIAL ELECTIONS CULTURE FIGHT THE FAKE

Coronavirus notice • View the recommendations and information for travellers issued by the French Government →

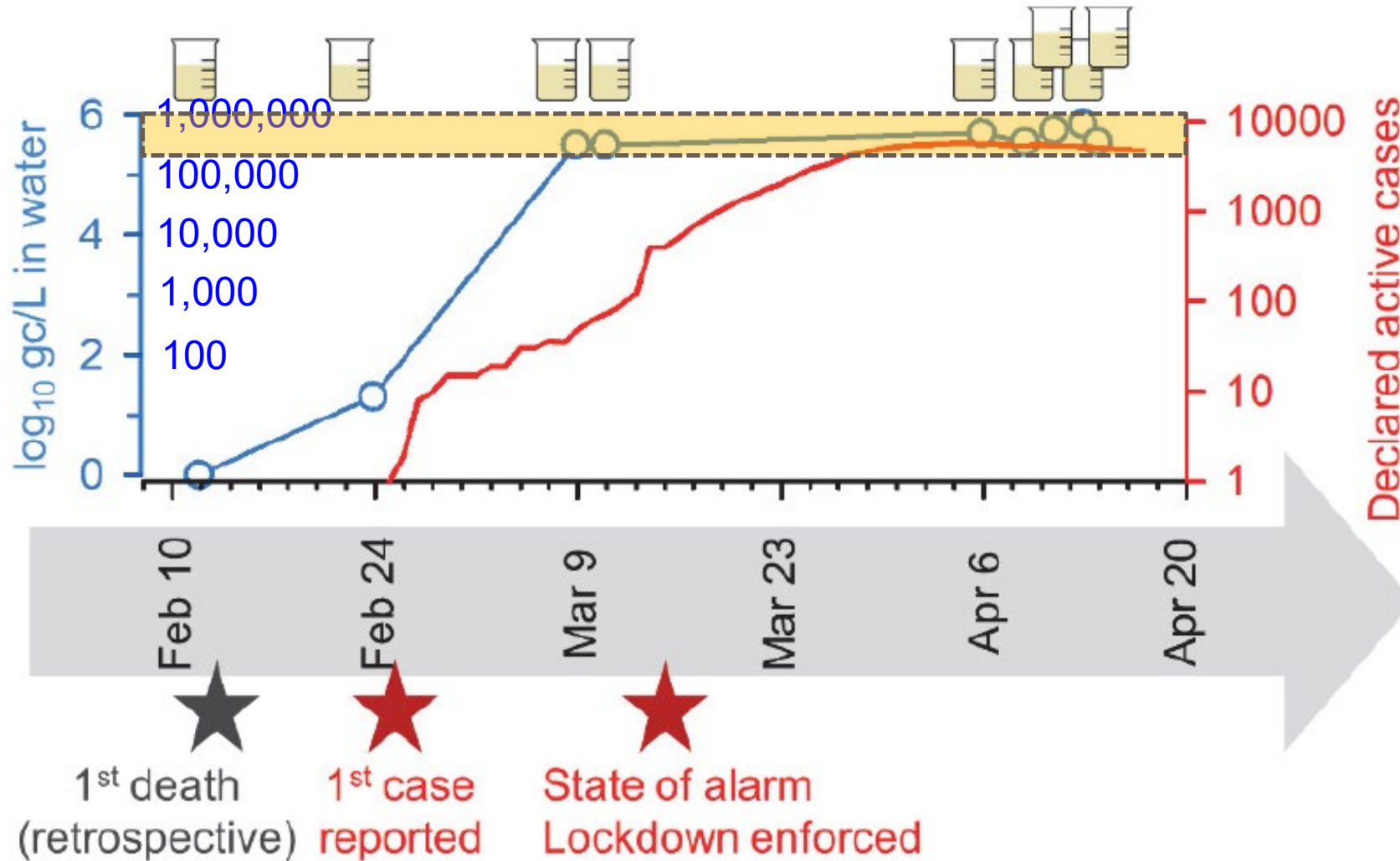
🏠 / Europe

Sewer science: How scientists hope to track Covid-19 through the world's waste

🌐 📧 📷

Issued on: 15/05/2020 - 17:27 Modified: 15/05/2020 - 17:29

Valencia, Spain

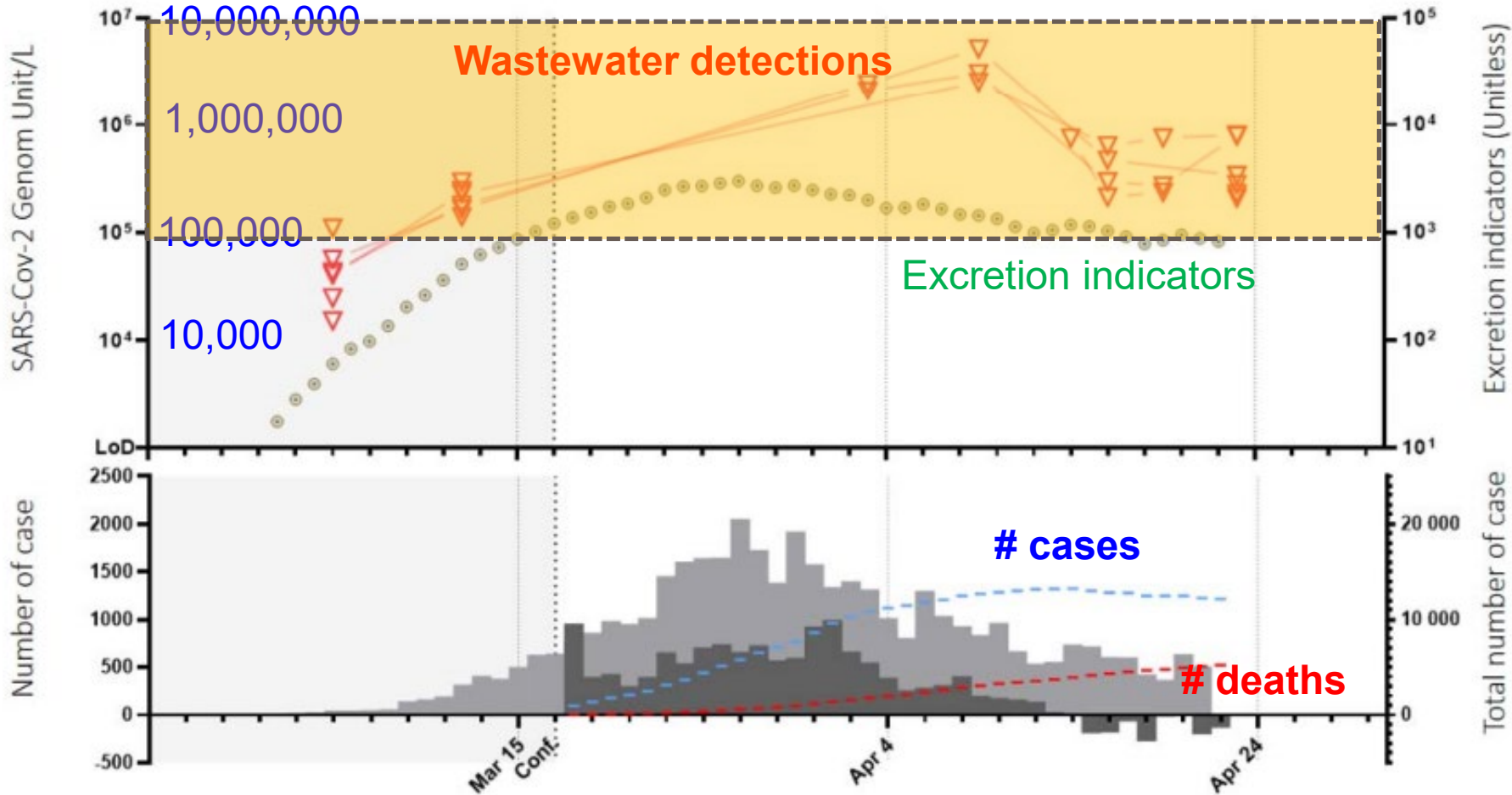


Compare to Local Samples
 $>10^5$ copies/L

- Cochran Rd: 59/97 (61%)
- Pendleton/Clemson: 37/88 (42%)
- Campus: 42/124 (34%)



Paris



Compare to Local Samples
>10⁵ copies/L

- Cochran Rd:
59/97 (61%)
- Pendleton/Clemson:
37/88 (42%)
- Campus:
42/124 (34%)



Initial Clemson Plan (May 2020)

- Objective: Monitor virus levels in the campus wastewater to provide an early indication that active transmission is underway
- The campus wastewater treatment plant offers several advantages
 - ❖ Services only the Clemson campus
 - ❖ Campus wastewater is mostly kept separate from stormwater runoff, so minimal wet weather impacts
 - ❖ Opportunity existed to collect baseline data before the majority of faculty, staff and students returned to campus (initially proposed for June 1)
- ***Early detection in sewage will afford an opportunity to initiate a change in campus operations before the virus becomes widespread***



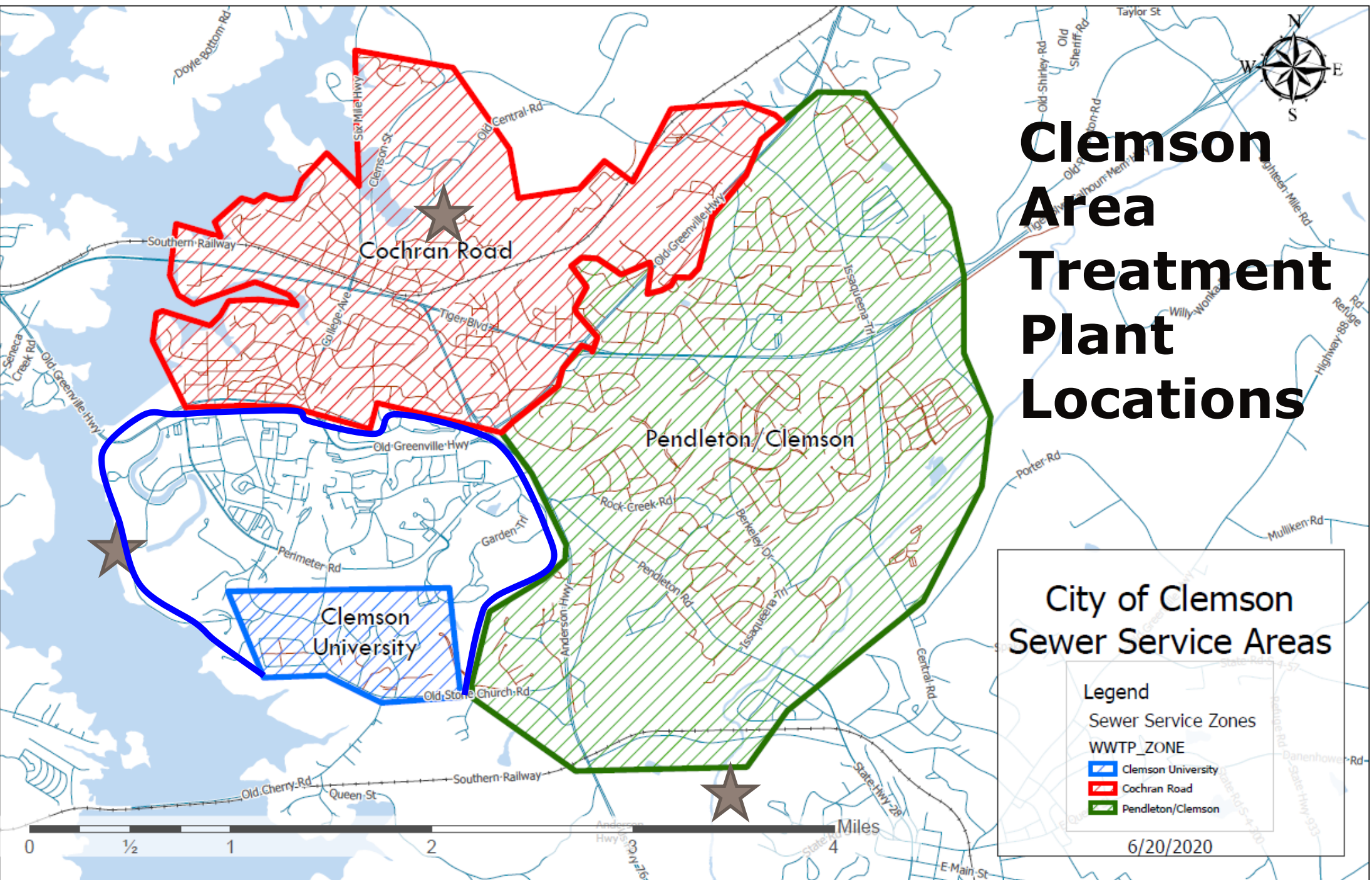
Initial Clemson Plan (May 2020)

- Collect samples from the campus WWTP
 - ❖ Weekly or bi-weekly starting in late May 2020
 - ❖ Additional samples on Saturdays for home football games
- Compare campus results to the City of Clemson
 - ❖ Wastewater at the City's Cochran Road WWTP is dominated by students, faculty and staff affiliated with the university
 - ❖ There are no significant industrial inputs
 - ❖ Trends in the City should be consistent with the campus
 - ❖ **Collect 4 baseline samples in June 2020**
 - ❖ If an increase on virus levels is detected above the baseline on campus, collect samples from the Cochran Road WWTP for two weeks
- Consider extending the sampling to the Pendleton/Clemson WWTP
 - ❖ Includes a mix of residential and student housing





Clemson Area Treatment Plant Locations



City of Clemson
Sewer Service Areas

Legend

Sewer Service Zones

WWTP_ZONE

- Clemson University
- Cochran Road
- Pendleton/Clemson

6/20/2020

Initial Monitoring Plan

- If an increase in virus concentration is detected in campus wastewater, collect additional samples at targeted areas of campus, to help determine the main source of the infected individuals



- Lightsey Bridge Apartment Complex
- Calhoun Courts Apartment Complex
- Thornhill Village Duplex West
- Thornhill Village Duplex East
- Core Campus Complex
- Holmes & McCabe
- Shoe Box Residence Halls
- Stadium Suites
- Lo Rises
- Hi Rises
- Douthit Hill West
- Douthit Hills East



Morning Mix

The University of Arizona says it caught a dorm's covid-19 outbreak before it started. Its secret weapon: Poop.



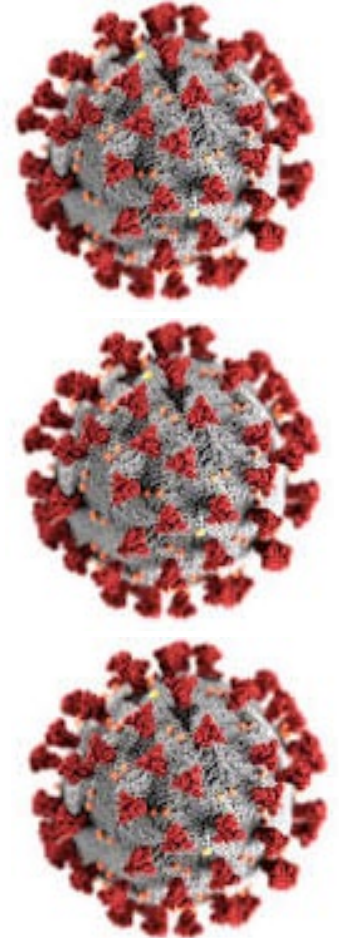
Graduate students and employees process nasal swabs from coronavirus tests in a lab at the University of Arizona in Tucson on Aug. 24. (Cheney Orr/Bloomberg News)

By [Jaclyn Peiser](#)

August 28, 2020 at 5:50 a.m. EDT

As 5,000 students prepared for move-in day at the University of Arizona this week, the school warned they would be tested periodically for the [coronavirus](#). One test, though, doesn't involve a nose swab. The university is regularly screening the sewage from each dorm, searching for traces of the virus.

On Thursday, officials said the technique worked — and possibly prevented a sizable outbreak on campus. When a wastewater sample from one dorm came back positive this week, the school quickly tested all 311 people who live and work there and found two asymptomatic students who tested positive. They were quickly quarantined.



Replicate the surveillance program at the University of Arizona?

- **Trigger point = 10^5 copies/L**
- **Morning grab samples sufficient**

Logistics

Where to Send the Samples

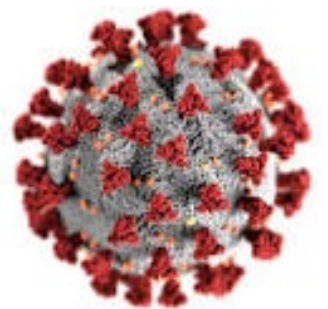
- Many laboratories can do the testing (>40?)
- We considered BioBot, SiREM, and PACE
- Selected SiREM based on cost (\$450/sample) and sensitivity (<1,000 copies/L)
- In-house method still in development

Upstream Sampling

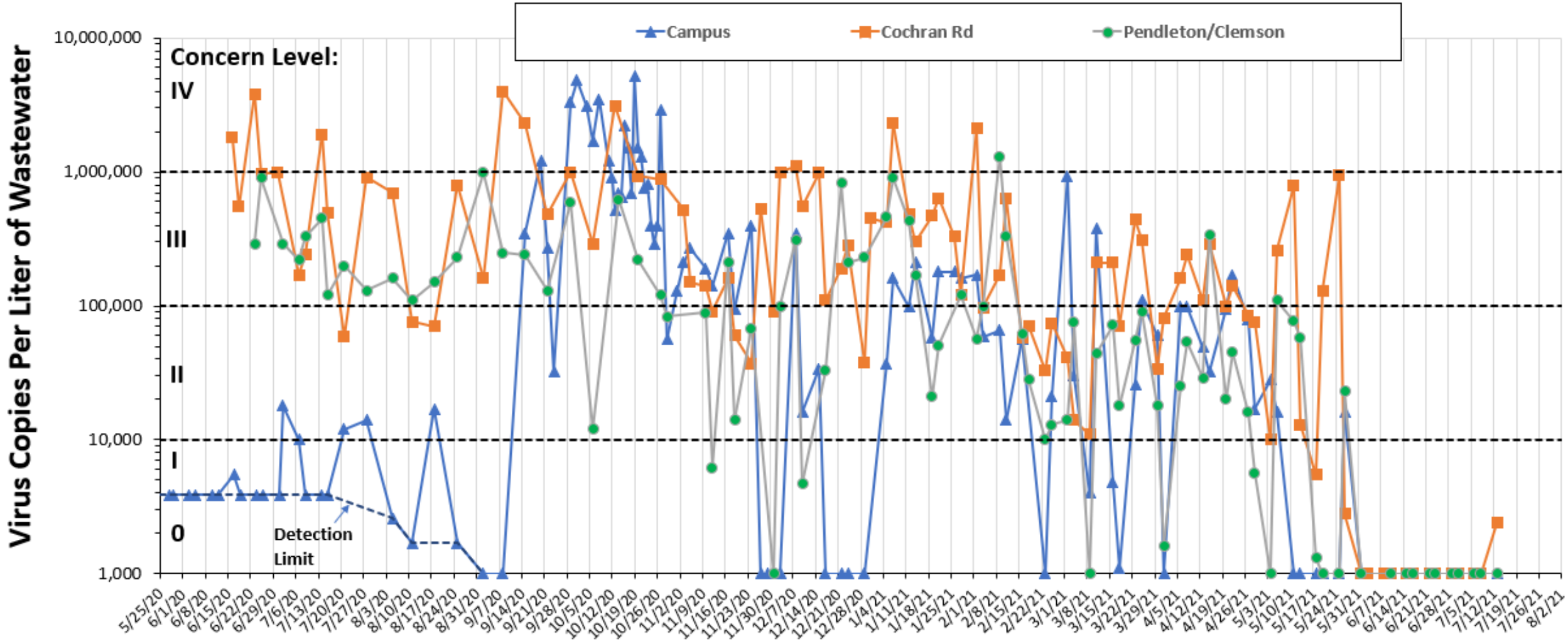
- Validated the use of manhole sampling; 24-hour composite and grab samples

Sample Collection and Handling

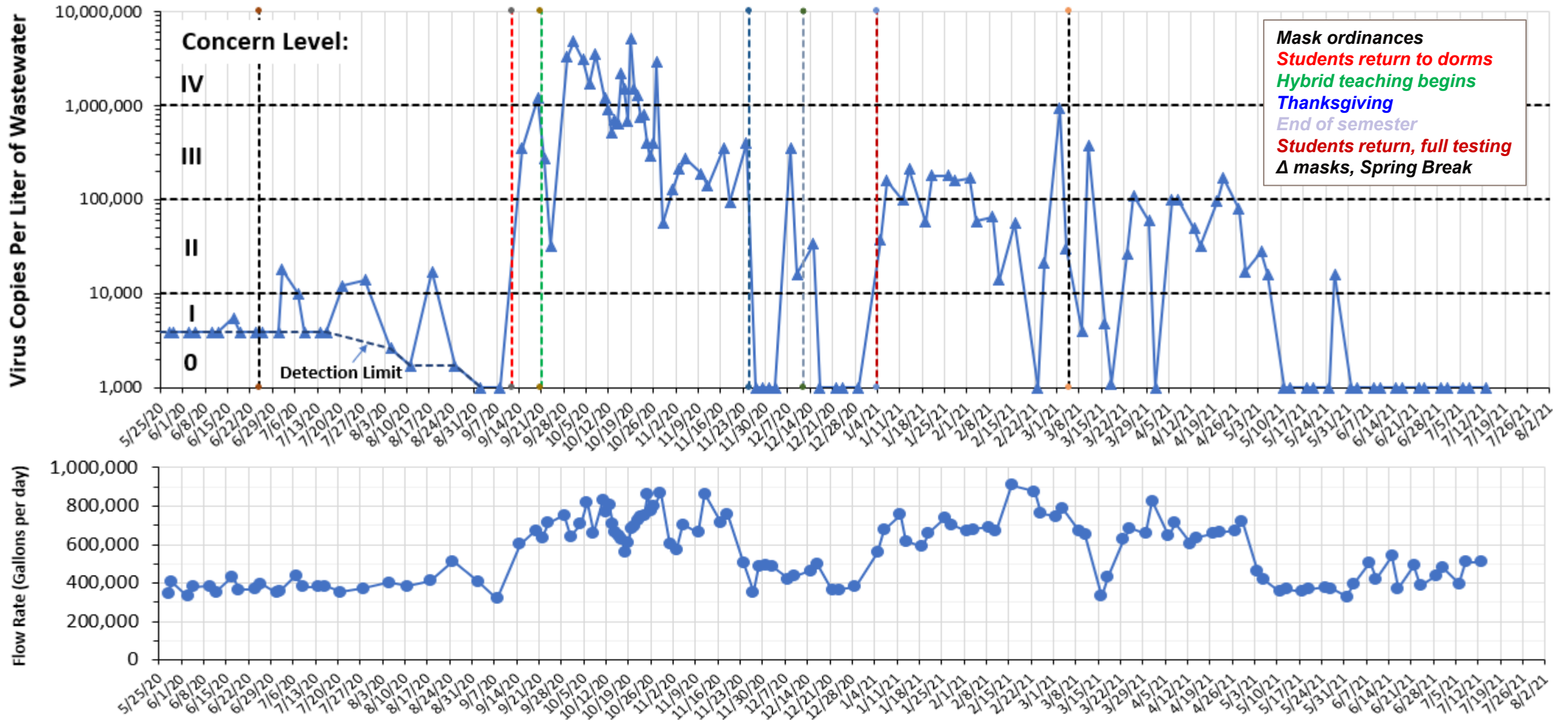
- 500 mL Nalgene bottles
- Ship overnight on ice to Knoxville, TN



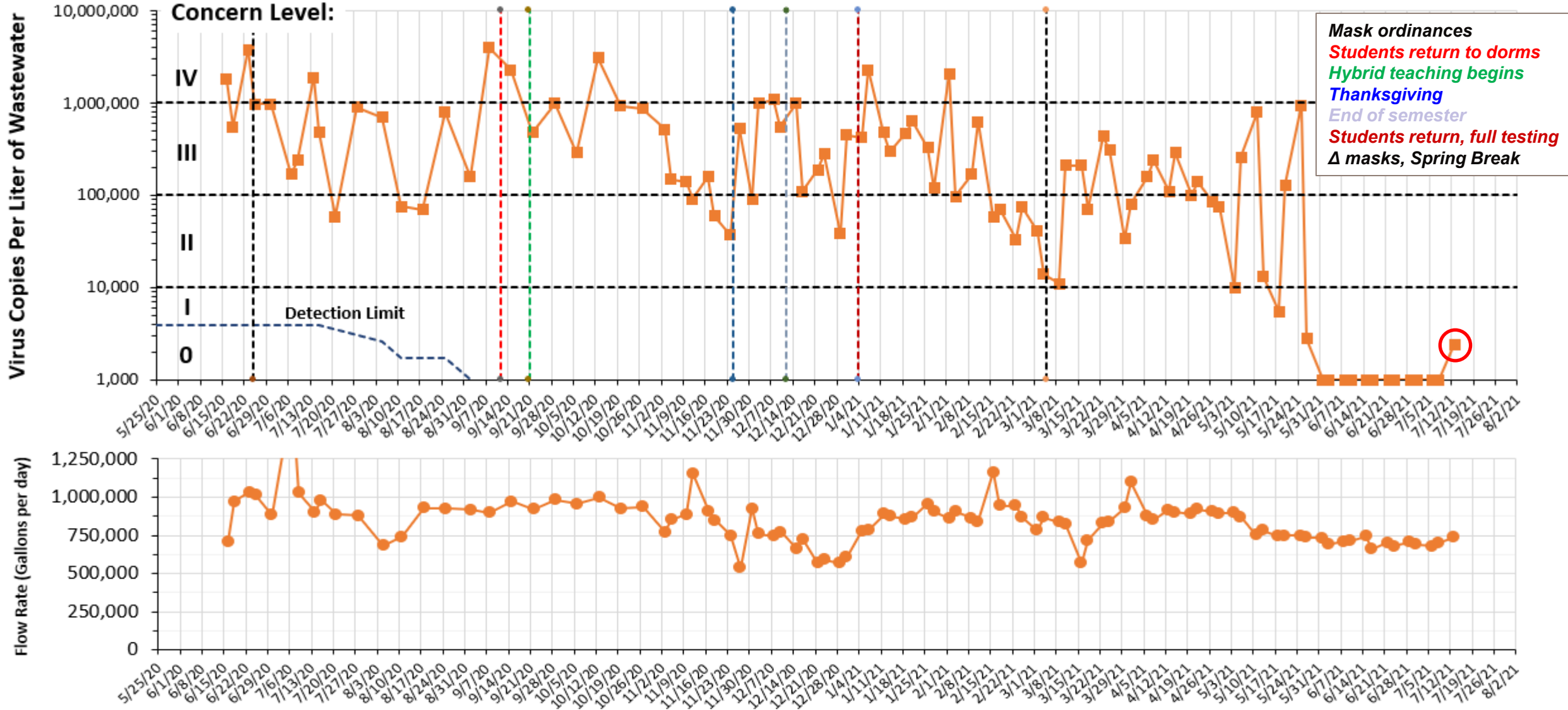
Results to Date



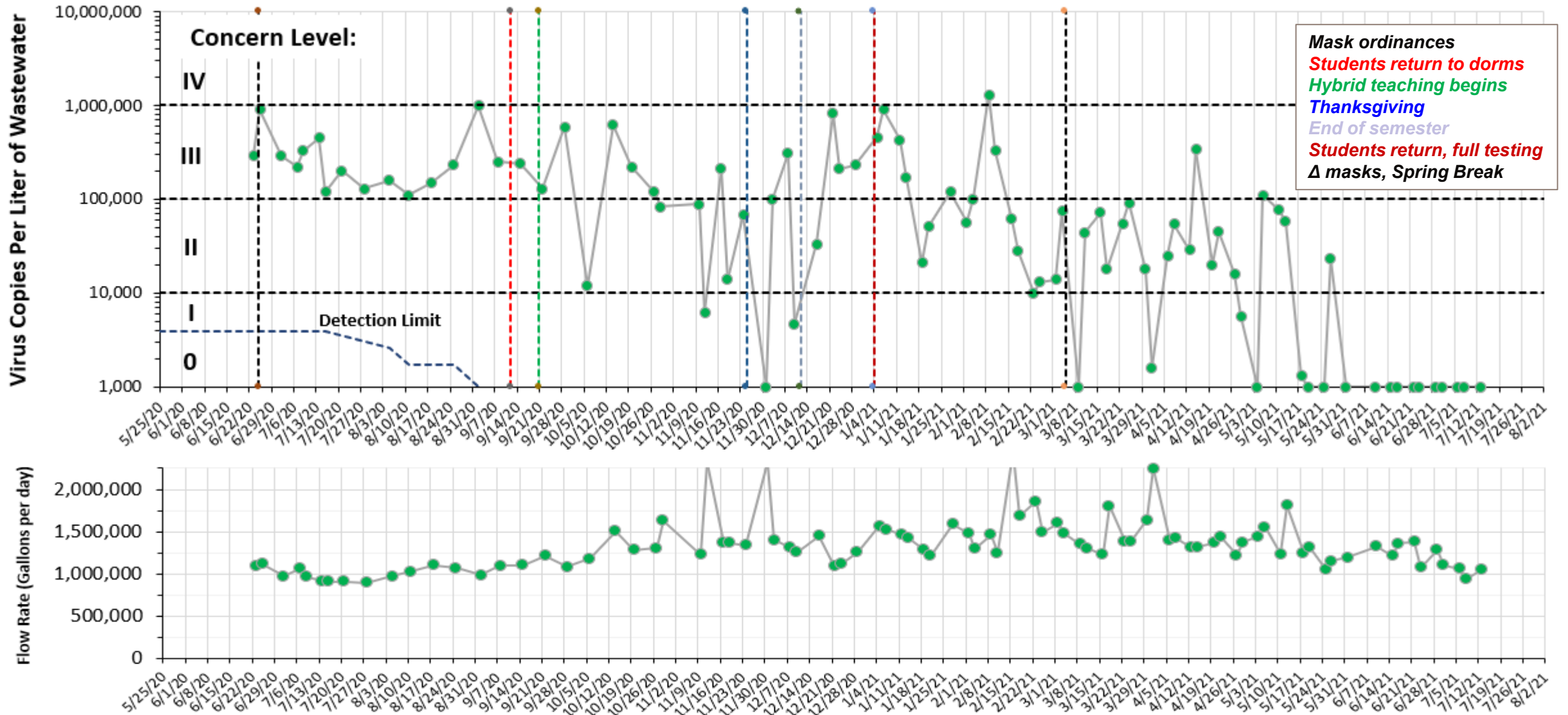
Results to Date: Campus



Results to Date: Cochran Rd



Results to Date: Pendleton/Clemson



Variants

- Detectable based on mutations in the gene for the spike protein
- SiREM recently developed the ability to test for two variants
 - United Kingdom (UK) B.1.1.7
 - South Africa (SA) B1.351
- Both variants increased rapidly after Spring Break, then declined; probably replaced by another variant
- Sequencing of positive samples from infected individuals were consistent with the prevalence of the UK and SA variants in wastewater
- Sequencing of RNA from wastewater is underway; will allow us to have a community level view of the variants that are dominant

Variants

Spring
Break

Date	University WWTP				Cochran Road WWTP				Pendleton/Clemson WWTP			
	Virus Copies Per Liter	Concern Level	% UK B.1.1.7	% SA B1.351	Virus Copies Per Liter	Concern Level	% UK B.1.1.7	% SA B1.351	Virus Copies Per Liter	Concern Level	% UK B.1.1.7	% SA B1.351
3/11/21	380,000	III	BLD	BLD	210,000	III	BLD	BLD	44,000	II	BLD	BLD
3/16/21	4,800	I	BLD	BLD	210,000	III	BLD	BLD	72,000	II	14%	BLD
3/18/21	1,100	I	BLD	BLD	71,000	II	detected	BLD	18,000	II	51%	BLD
3/23/21	26,000	II	28%	BLD	440,000	III	40%	BLD	55,000	II	60%	BLD
3/25/21	110,000	III	71%	BLD	310,000	III	39%	BLD	91,000	II	59%	BLD
3/30/21	60,000	II	50%	BLD	34,000	II	100%	BLD	18,000	II	100%	BLD
4/1/21	<720	0	-	-	81,000	II	97%	BLD	1,600	I	-	-
4/6/21	98,000	II	100%	BLD	160,000	III	92%	8%	25,000	II	100%	BLD
4/8/21	98,000	II	50%	BLD	240,000	III	91%	9%	54,000	II	92%	8%
4/13/21	49,000	II	100%	BLD	110,000	III	34%	20%	29,000	II	100%	BLD
4/15/21	32,000	II	52%	BLD	290,000	III	30%	BLD	340,000	III	7%	BLD
4/20/21	95,000	II	BLD	BLD	100,000	III	17%	BLD	20,000	II	85%	BLD
4/22/21	170,000	III	90%	9%	140,000	III	75%	9%	45,000	II	85%	9%
4/27/21	79,000	II	37%	BLD	85,000	II	100%	BLD	16,000	II	100%	-
4/29/21	17,000	II	59%	41%	76,000	II	24%	17%	5,700	I	60%	40%
5/4/21	28,000	II	13%	13%	10,000	II	61%	39%	960	I	-	-
5/6/21	16,000	II	41%	30%	260,000	III	76%	24%	110,000	III	67%	33%
5/11/21	<790	0	-	-	790,000	III	BLD	BLD	77,000	II	9%	BLD
5/13/21	<720	0	-	-	13,000	II	100%	BLD	58,000	II	20%	BLD
5/18/21	<790	0	-	-	5,500	I	BLD	BLD	1,310	I	-	-
5/20/21	<790	0	-	-	130,000	0	16%	16%	<790	0	-	-
5/25/21	<790	0	-	-	940,000	III	BLD	BLD	<790	0	-	-
5/27/21	16,000	II	BLD	BLD	2,800	I	BLD	BLD	23,000	II	BLD	BLD

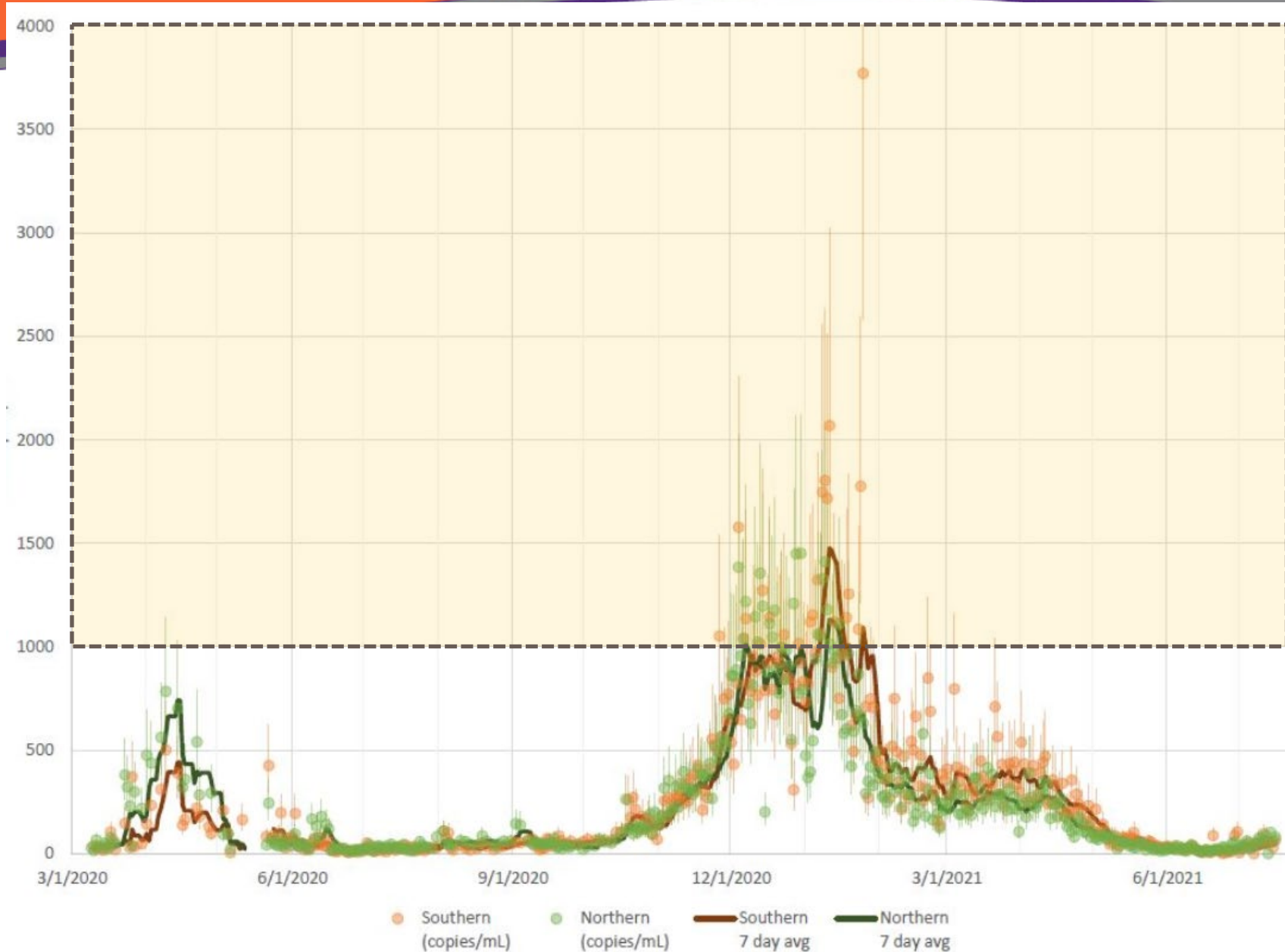
Graduation

Vaccines

Results from Other Cities: Boston, MA

<http://www.mwra.com/>

SARS-CoV-2 (Copies/mL)



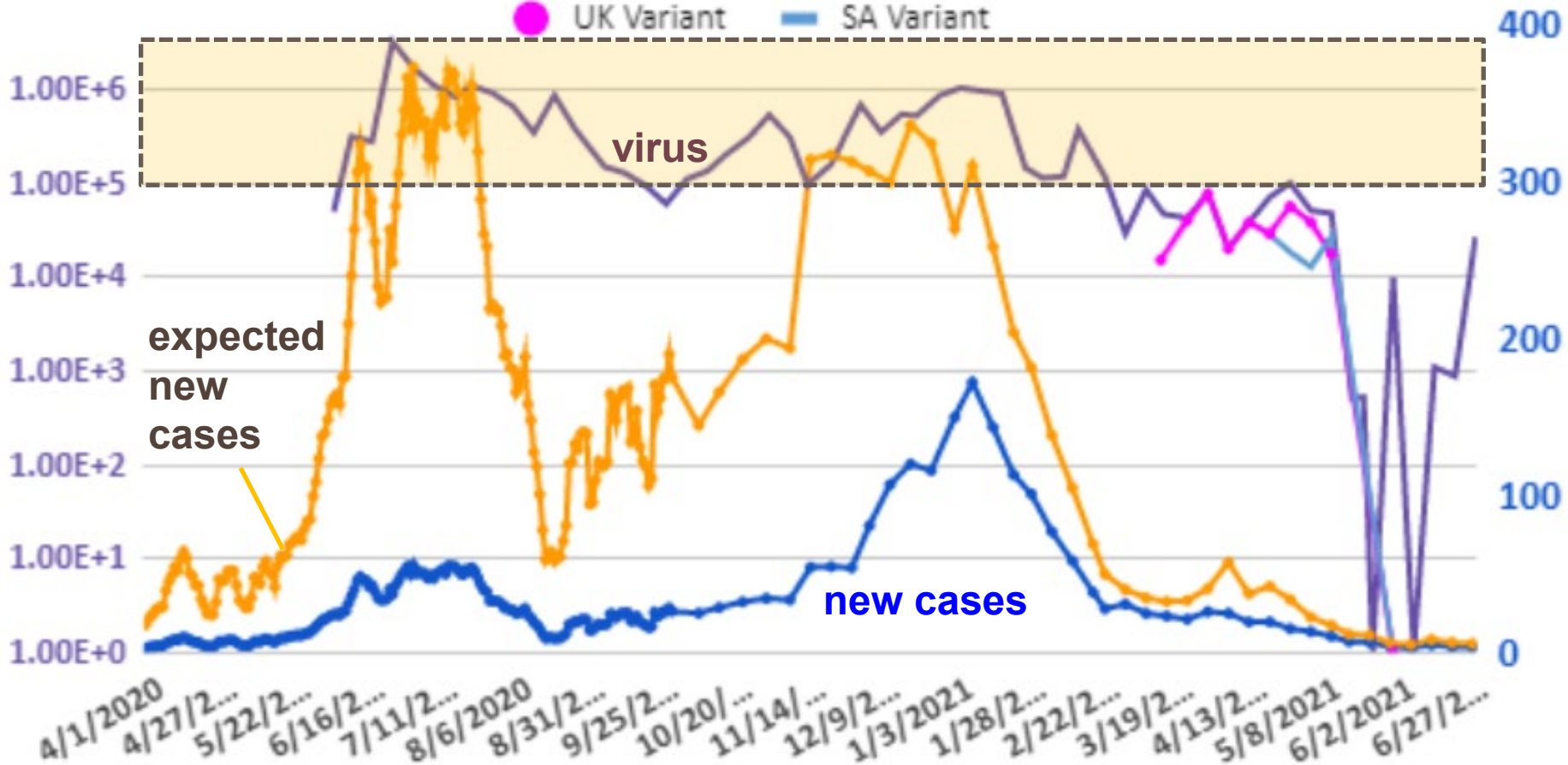
Results from Other Cities:

Greenville, SC

● Average new daily cases
 ● Average expected actual new daily cases
 — Weekly average - Virus concentration in wastewater

● UK Variant
 — SA Variant

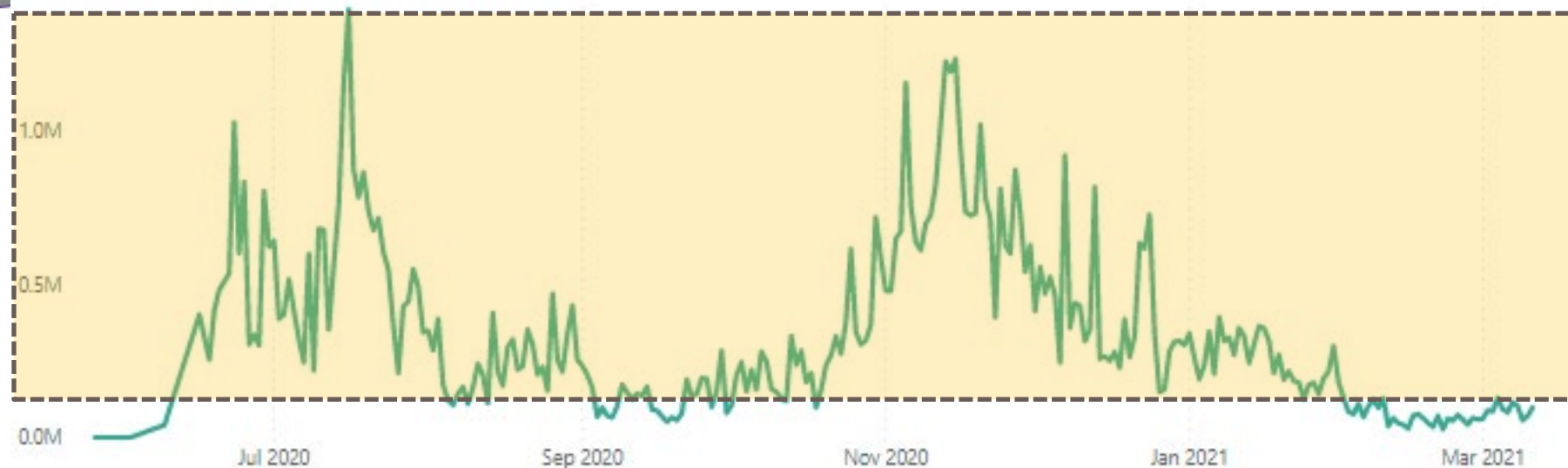
Rolling 7 day virus concentration (copies/L)



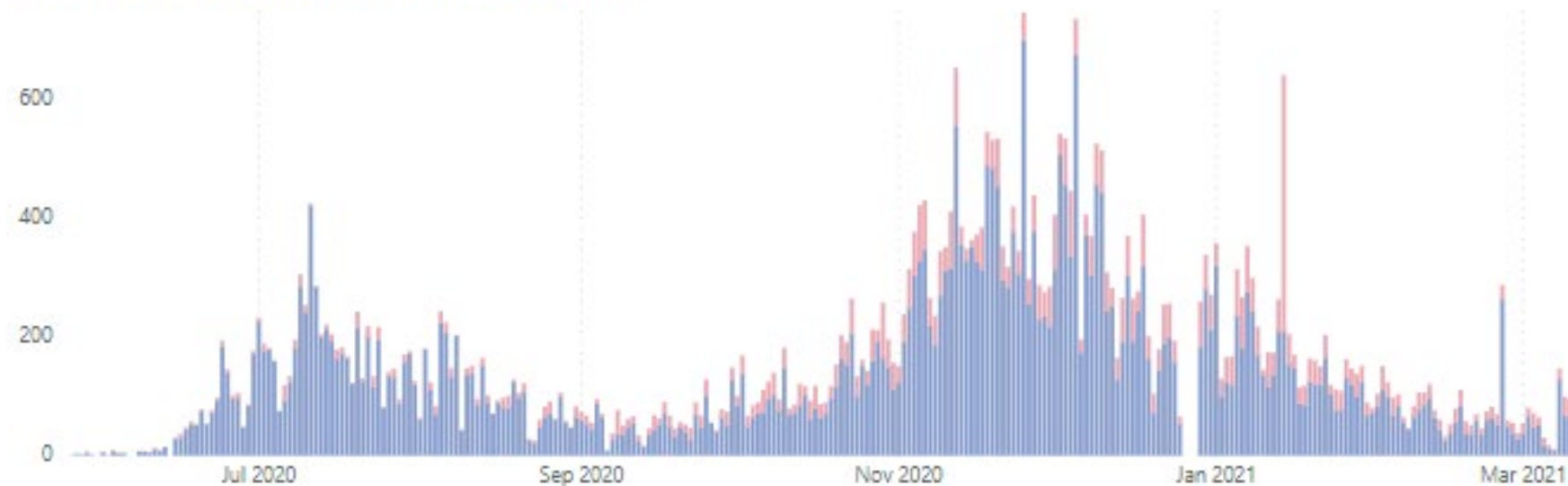
Rolling 7 day average, new cases per day

Results from Other Cities: **Boise, ID**

Wastewater Test Results (virus copies per liter)



Ada County New Cases (confirmed and probable)



Levels of Concern*

- Developed as a communication tool for the public and policy makers
 - ❖ Based on orders of magnitude

0 = virus is below detection; infected individuals may be present, but virus levels are too low to detect ($\sim 10^3$ c/L)

I = virus is above the minimum detection level but still at a low concentration; watch for increasing trends ($>$ detection limit to 10^4 c/L)

II = virus level of concern; pay close attention to a potential for active transmission (10^4 - 10^5 c/L)

III = significant concern; large # of infected individuals likely (10^5 - 10^6 c/L)

IV = major concern; high numbers of infected individuals ($>10^6$ c/L)

*Needs to be adapted to specific sewershed conditions, including dilution

Levels of Concern: Per Person Basis

% Infected	RNA Copies/Person/d	Concern Level
<0.01	<6.0E+05	0
0.01-0.10	6.0E+05 – 6.0E+06	I
0.10-1.0	6.0E+06 – 6.0E+07	II
1.0 – 5.0	6.0E+07 – 3.0E+08	III
>5.0	>3.0E+08	IV

Goal is to reduce the impact of dilution on interpretation of virus loadings in wastewater

Estimating Number of Cases

- Can wastewater data be used to estimate the number of infected individuals in a sewershed?

According to the CDC*: “SARS-CoV-2 RNA concentrations in wastewater cannot currently be used to determine the total number of infected persons in a community or the percent of the population that is infected. More data on SARS-CoV-2 concentrations in the feces of infected individuals are needed to be able to understand the relationship between SARS-CoV-2 RNA concentrations in wastewater and how many people in a sewershed are infected.”

- Really? Let's take a closer look.

* <https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance/developing-a-wastewater-surveillance-sampling-strategy.html>



Estimating Number of Cases

Formula is based on a mass balance:

$$\# \text{ Infected} = \frac{A \times B}{C \times D}$$

A = Flow rate (L/d)

B = Virus copies/L

C = Feces rate (~129 g/person/day)

D = Virus density in feces (copies/g feces); max
~4.7E07 copies/g

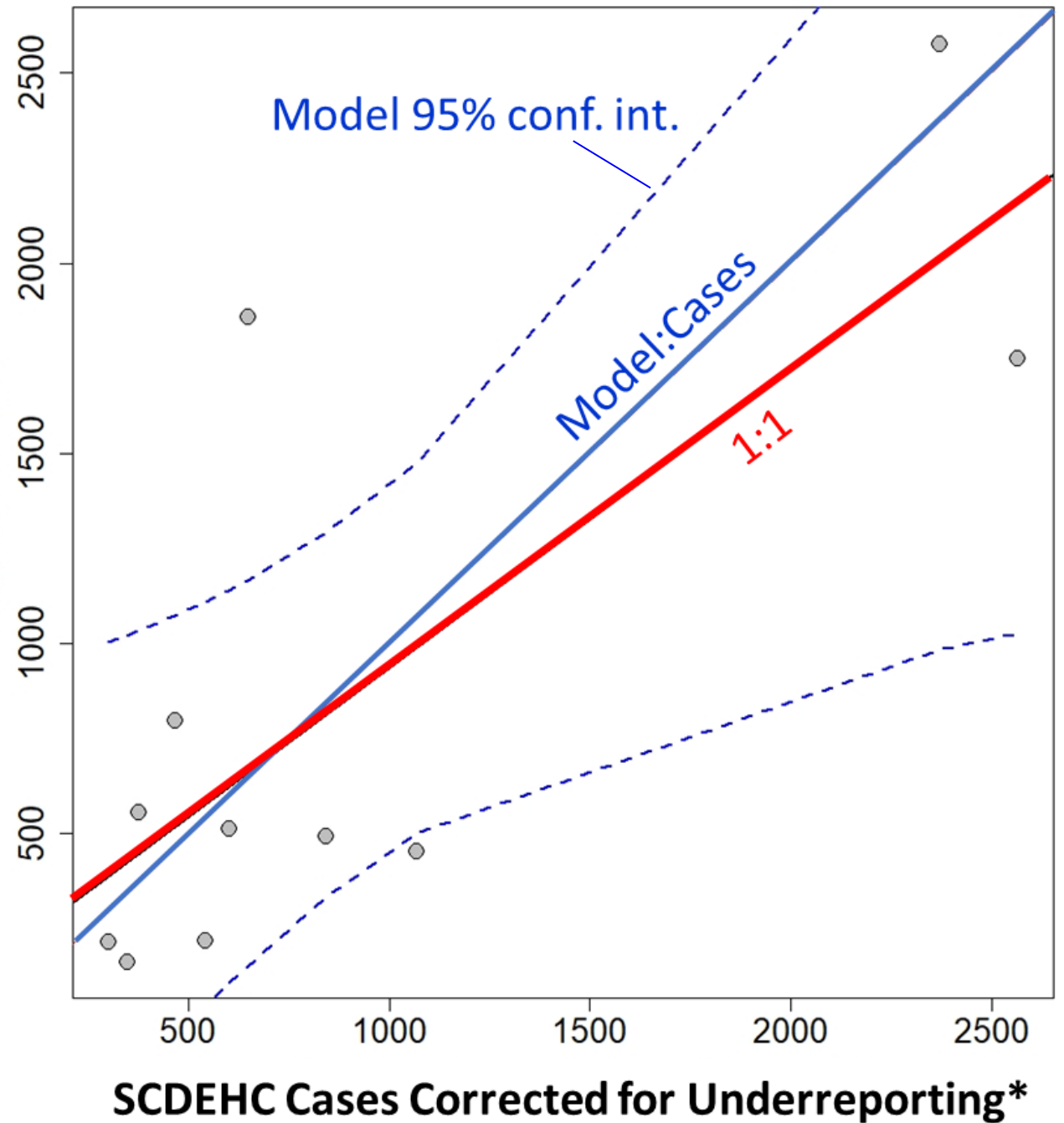
- Use Monte Carlo simulations to factor in uncertainty in C and D
- Does not include decay in sewer system
- Don't forget: DHEC reports cumulative cases; this formula gives infected individuals on any given day



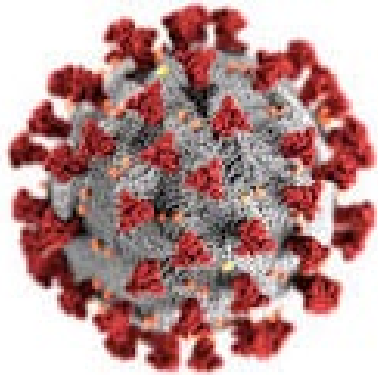
Correlating Reported Cases to Model Predicted Cases

- *SCDHEC reported cases for the 29631 zip code area adjusted by 16:1 based on estimates from Wu et al. for South Carolina
- DHEC uses a multiplier of ~8.

SEIR Model Predicted Cases



Manhole Results



Manhole Results

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M12	M13	M15
Date	Core Campus Complex	Holmes & McCabe	Shoe Box Residence Halls	Stadium Suites	Lo Rises	Hi Rises	Douthit Hill West	Douthit Hills East	Lightsey Bridge Apartment Complex	Calhoun Courts Apartment Complex	Thornhill Village Duplex East	Quad	Martin Inn
9/14/2020	<550, ND	<550, DET	<550, ND	<550, ND	<550, DET	<550, DET	6.00E+06	<550, ND	5.30E+04	8.20E+05	2.30E+04	<550, ND	-
9/17/2020	-	-	-	-	-	-	3.90E+04	-	-	4.60E+05	-	-	-
9/21/2020	4.1E+03	<550, ND	<550, ND	7.0E+03	<550, ND	<550, ND	<550, ND	9.6E+05	<550, ND	2.60E+06	-	1.40E+06	9.60E+05
9/24/2020	4.5E+03	3.8E+03	2.0E+04	<550, ND	<550, ND	3.3E+03	1.2E+04	<550, ND	1.2E+04	3.90E+05	1.60E+05	7.60E+02	2.3E+04
9/28/2020	4.5E+04	1.7E+05	1.2E+05	<840, ND	3.1E+04	3.2E+04	1.3E+04	5.0E+04	1.7E+05	2.9E+04	-	4.6E+04	-
10/5/2020	4.5E+04	2.7E+03	8.3E+04	<840, ND	<840, ND	<840, ND	<840, DET	3.5E+05	1.5E+05	6.5E+05	1.4E+05	<840, ND	-

10⁴ copies/L

≥10⁵ copies/L

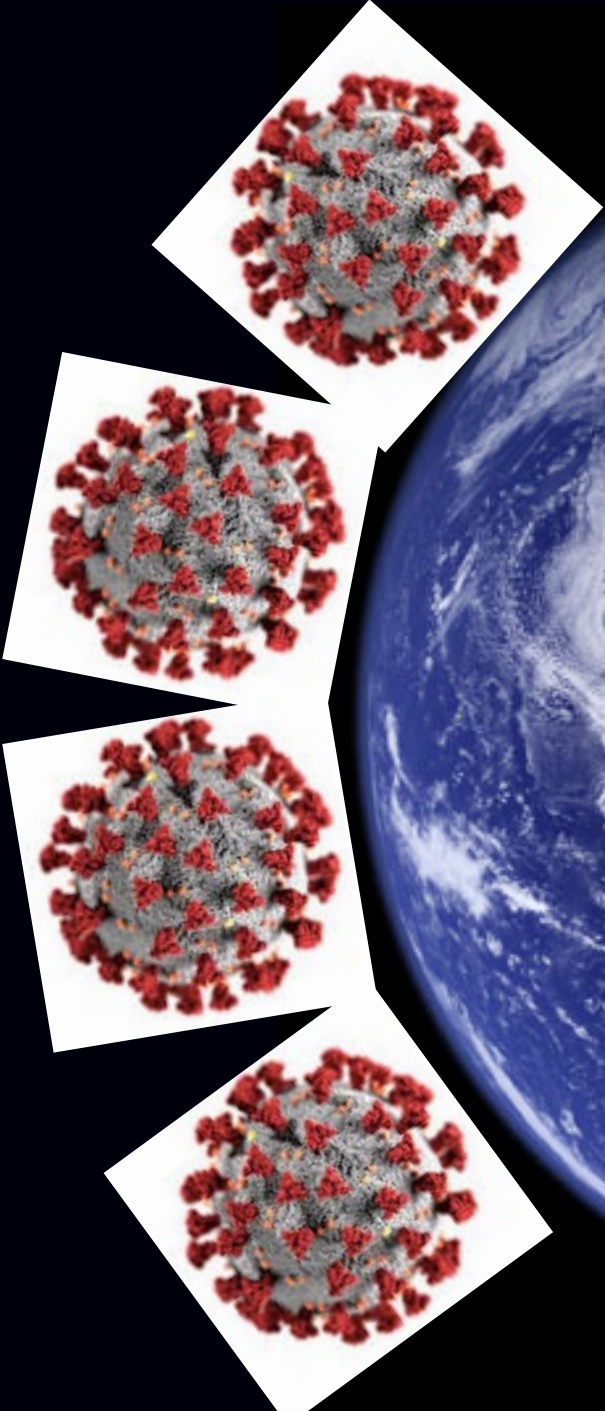
- Likely at least one infected individual in most locations
- Manhole sampling stopped when weekly testing of all on-campus residents started

Summary

- WBE can serve as a leading indicator of COVID-19 infections, especially when individual testing is lacking
- WBE can inform public policy: mask ordinances (Clemson and Central, SC)
- Once transmission is active, virus in wastewater becomes a lagging indicator, but levels do come down when transmission is brought under control
- Virus levels in wastewater can be used to estimate the number of infected individuals, taking uncertainty into account
- Mass rates of RNA likely a better measure of activity versus concentration
- Need to refine levels of concern; helpful for communicating results to public
- Manhole sampling can reveal infected individuals at the building level
- Now possible to track select variants via wastewater
- **WBE is an important tool for assessing the effectiveness of the vaccines**

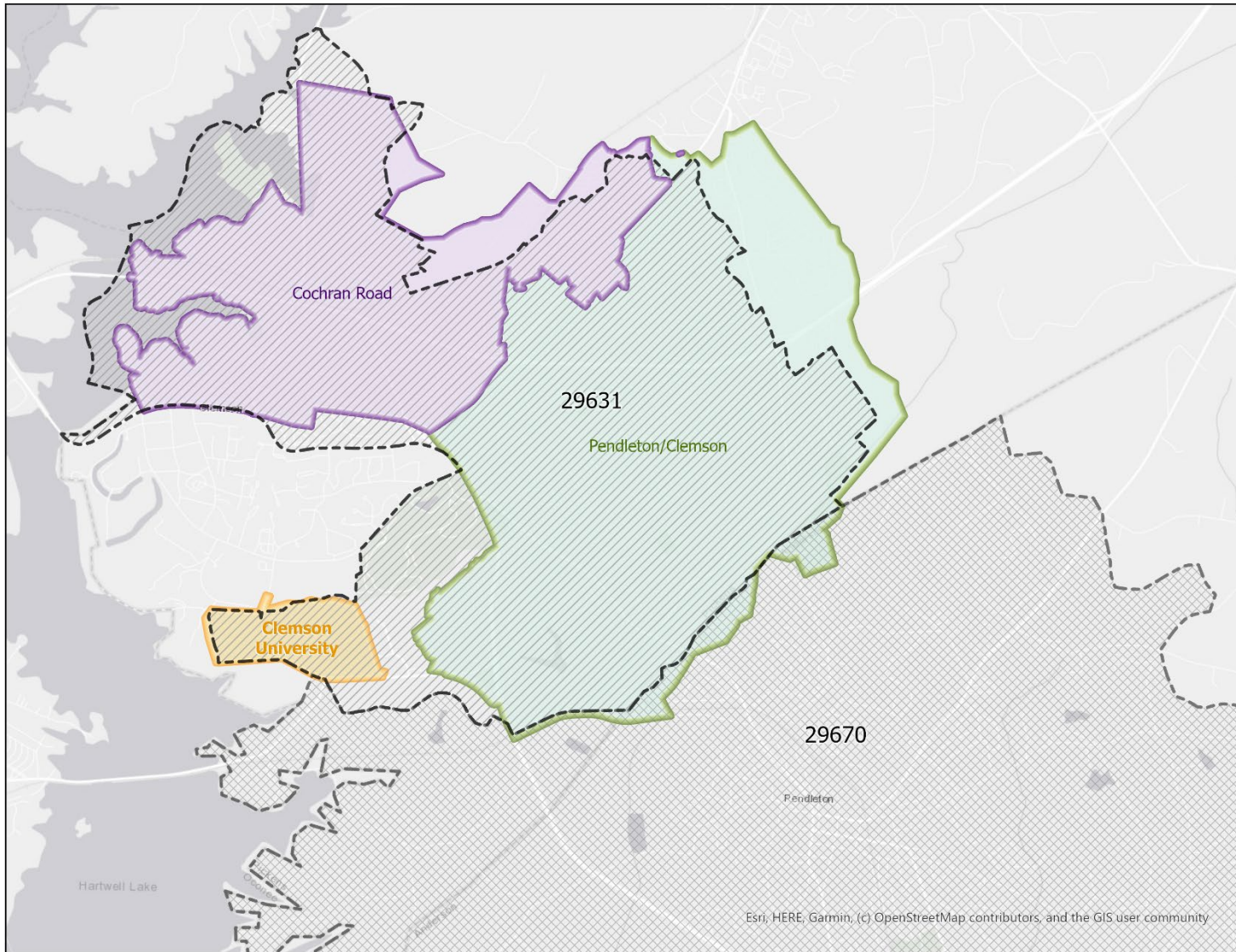
Thank you for listening – Any questions?

Questions?



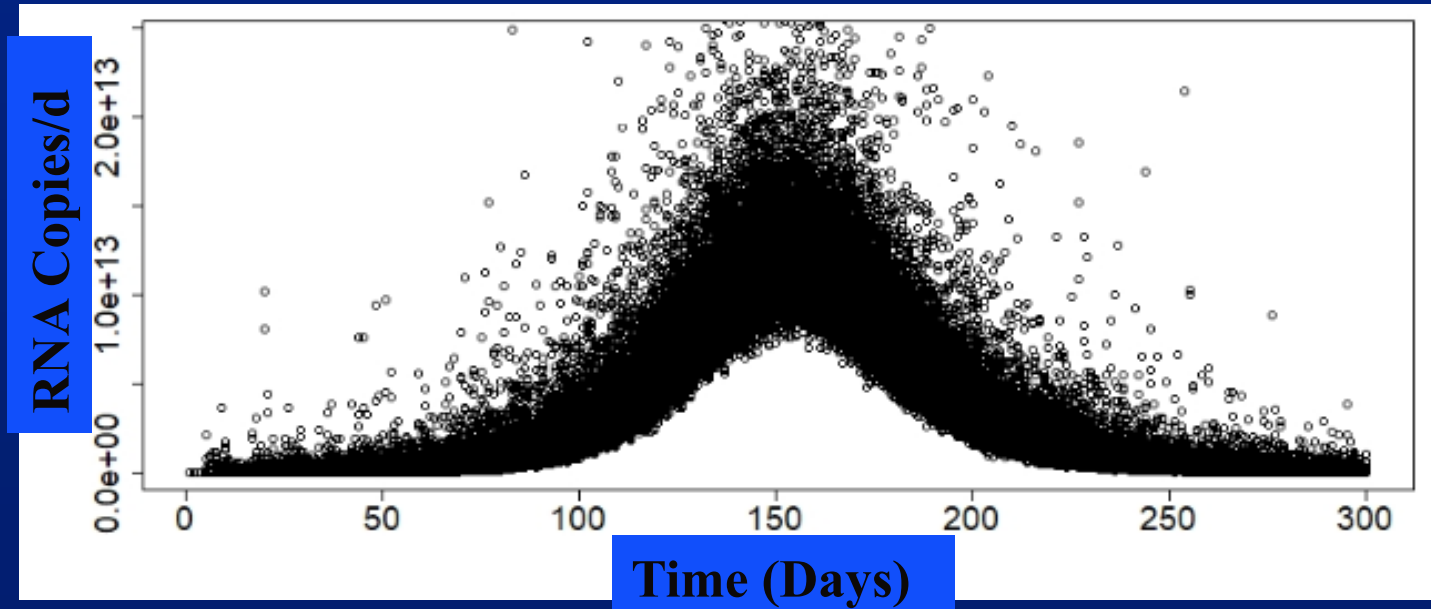
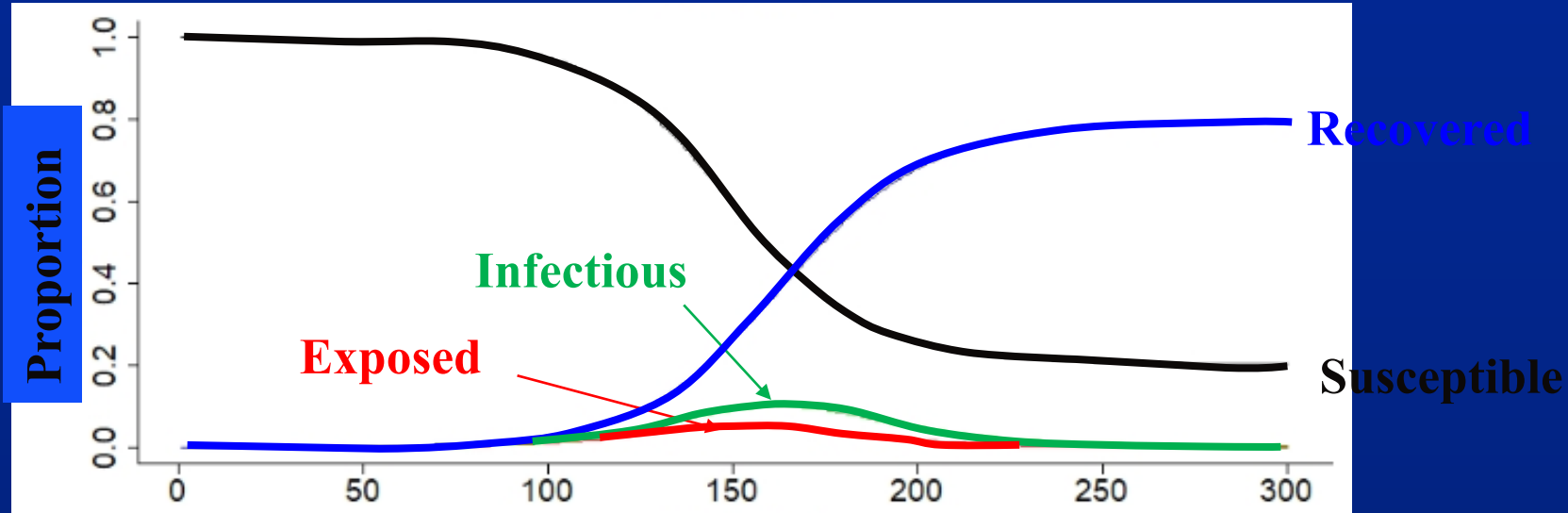
Sewer Sheds and Zip Codes

- 29631 matches up well with Cochran Rd + Pendleton Clemson WWTP sewer sheds
- DHEC provides data on number of COVID-19 cases by zip code



Thanks to Patricia Carbajales of CU and City of Clemson for GIS data

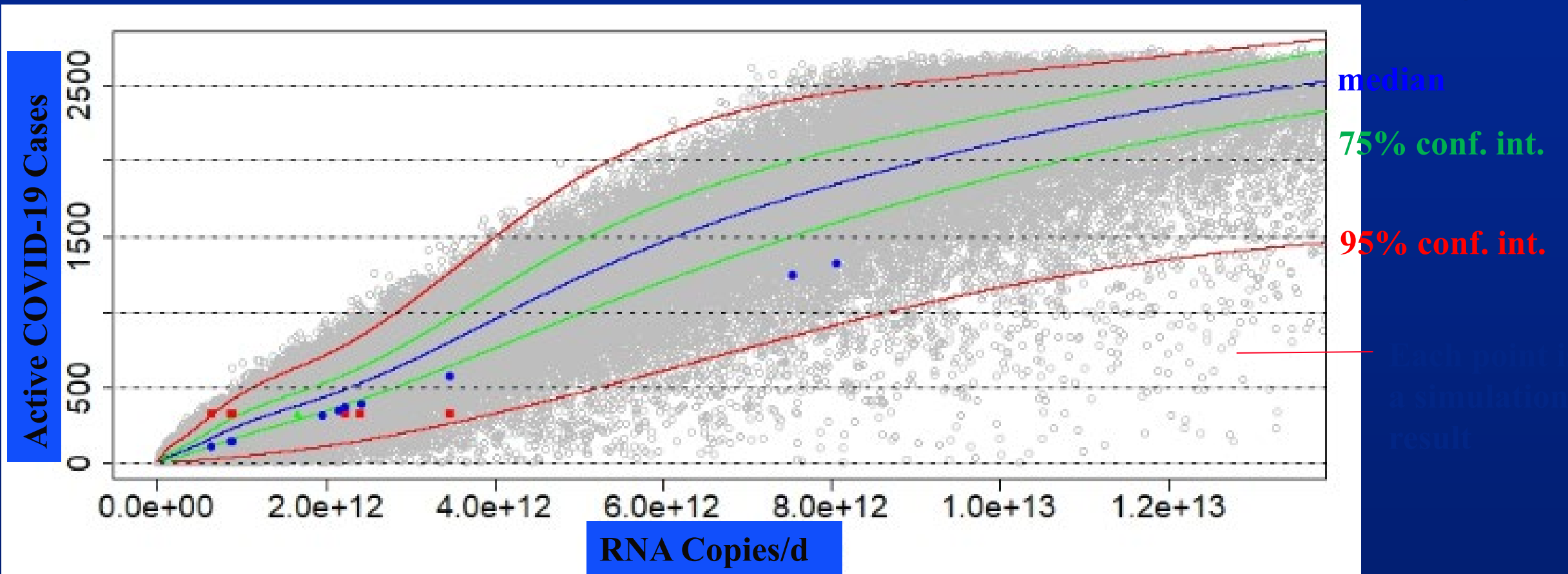




Estimating Number of Cases: SEIR Model Based on RNA Mass Rate

*Predicted cases for 29631 zip code (2
community sewersheds)*

Estimating Number of Active Cases: SEIR Model



■ ~2% infected based on individual testing in July

• Simple estimate

- **Positivity rate gradually declined on-campus, off-campus, and in employees**
- **Infected individuals continue to shed**
- **Virus levels in wastewater lag behind, but will go down as transmission is flattened**

Estimated Prevalence

