Spatial and Temporal Distribution of COVID-19 Biomarkers in NH Wastewater Treatment Facilities

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Ongoing Research Projects in the Lab:

1. Geographic variation in SARS-CoV-2 biomarkers in New Hampshire WWTFs (closed)
2. Temporal fluctuations in SARS-CoV-2 biomarkers at WWTF influent (2 locations, ongoing)
3. UNH Durham Campus WW Surveillance Program (began Aug 28, ongoing)
4. SARS-CoV-2 biomarker removal during WW unit treatment processes (ongoing)

University of New Hampshire
Research, Economic Engagement and Outreach

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NEW ENGLAND WATER ENVIRONMENT ASSOCIATION
NEWEA
WORKING FOR WATER QUALITY

NEWEA 21 Annual Conference
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University of New Hampshire
College of Engineering and Physical Sciences
Civil and Environmental Engineering

Project Team

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Special Thanks

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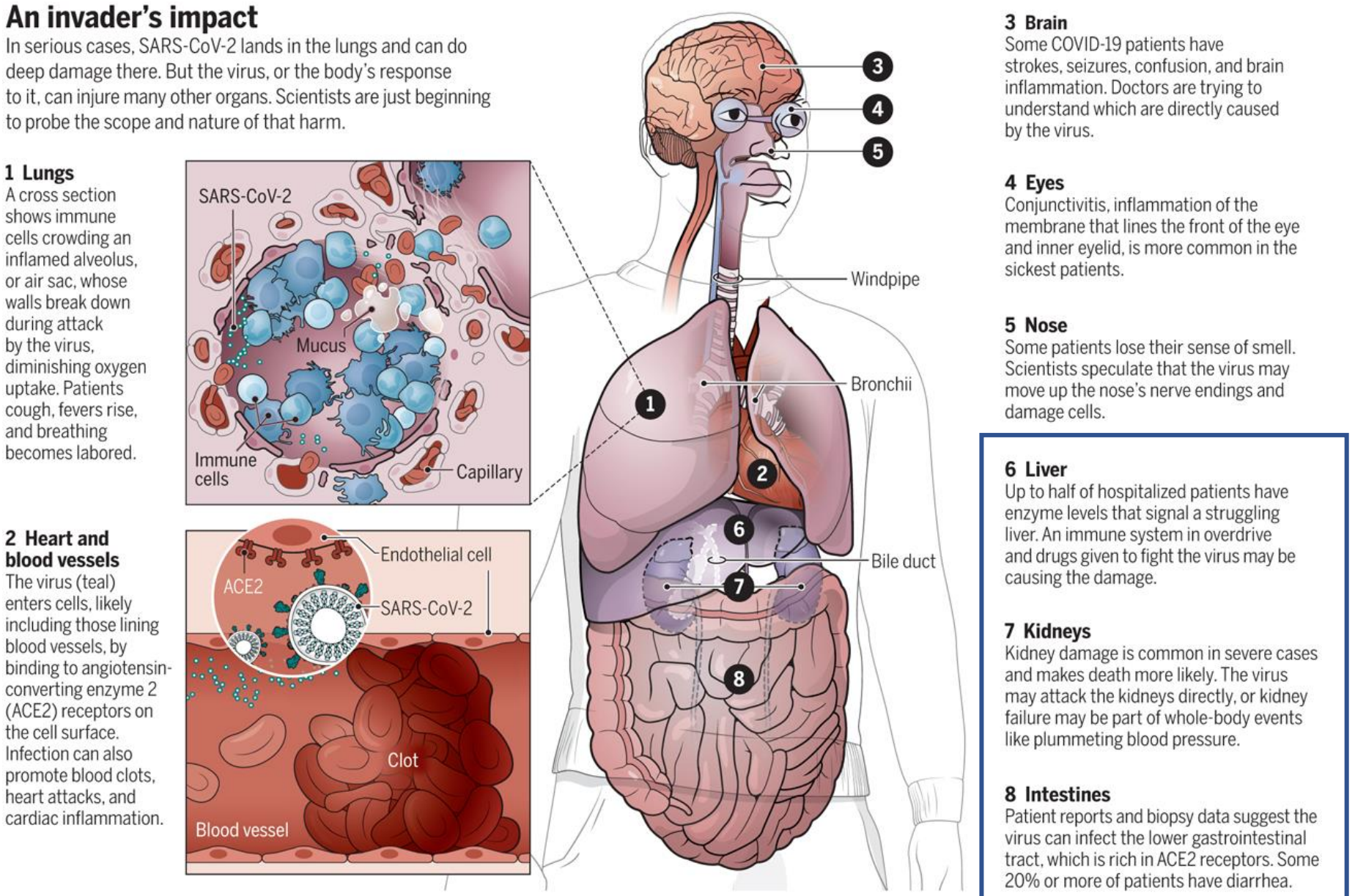
UNH Hubbard Center Genome Studies
NHDES
WWTF Operators

Why is SARS-CoV-2 in Wastewater?



-GI tract plays an important role in purging toxins and viruses from the body

-Also hosts infection

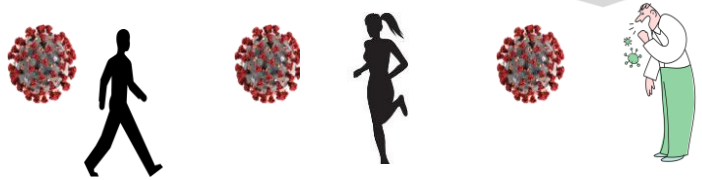
>60% of COVID-19 patients have GI symptoms

>68% of COVID-19 patients test positive for SARS-CoV-2 virus in feces

Lin et al, 2020. Gut. doi: 10.1136/gutjnl-2020-321195

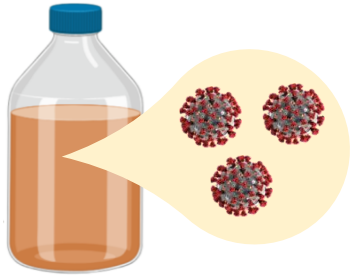
Wadman et al, 2020. Science. DOI: 10.1126/science.368.6489.356

Our Approach

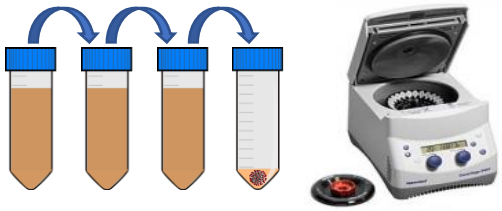


1- COVID-19 prevalence

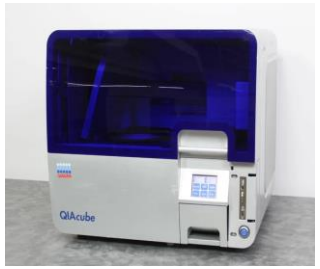
2- Wastewater Treatment Plant

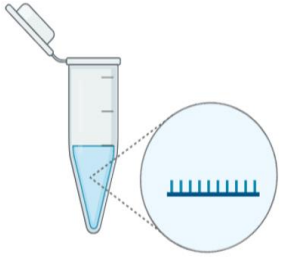


3- SARS-CoV-2 in Wastewater

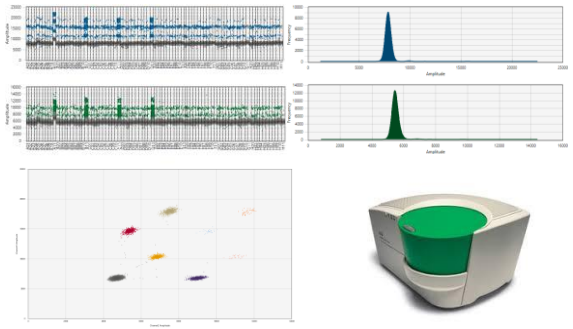


4- Virus concentration and precipitation with PEG/NaCl



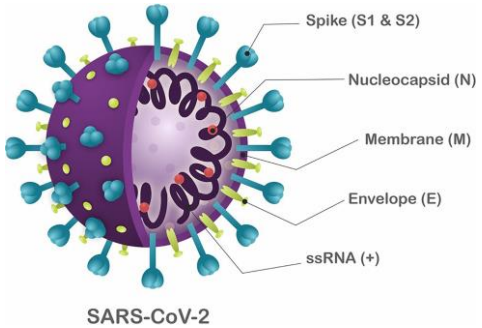


5- Viral RNA extraction



6- Detection and quantification of SARS-CoV-2 RNA with RT-ddPCR

Why ddPCR?



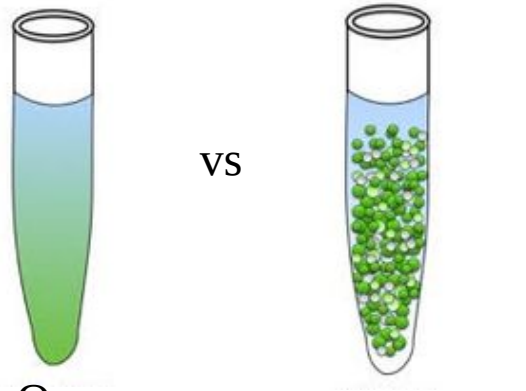
SARS-CoV-2, the virus that causes COVID-19 is a RNA virus (ssRNA)

Nucleocapsid (N) protein is responsible for defense and replication

We use "primers" recommended by the CDC that target N1 and N2 regions of the nucleocapsid (N) gene, and quantify how many copies are present

Taken from Andrade Santos et al., (2020)

RT-qPCR vs **RT-ddPCR**



One fluorescence measurement

Thousands of fluorescence measurement

ddPCR advantages:

- Absolute quantification
- Higher precision
- Higher reproducibility
- Higher sensitivity

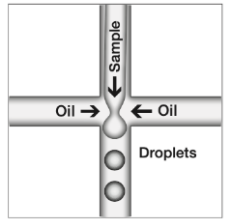
ddPCR disadvantages:

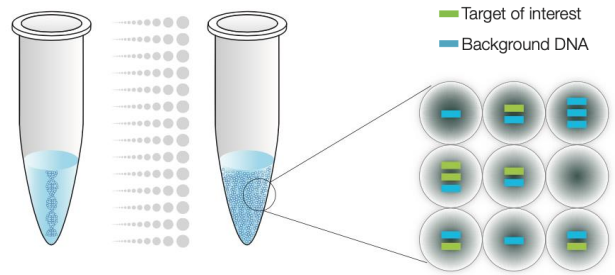
- Cost
- Time
- Specialized expertise to set up the reaction and analyze results

Droplet Generation

Droplet generator partitions each sample into approximately 20,000 nanoliter-sized droplets

RNA is distributed randomly into the droplets during the partitioning process

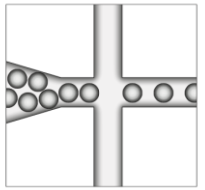




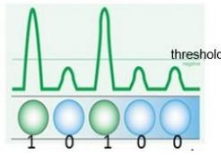
RT and PCR amplification

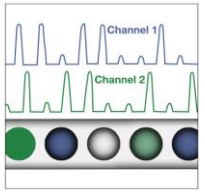
Reverse transcriptase (RT), catalyzes the transcription of RNA into DNA, which is then amplified using polymerase chain reaction (PCR)

Droplet reading



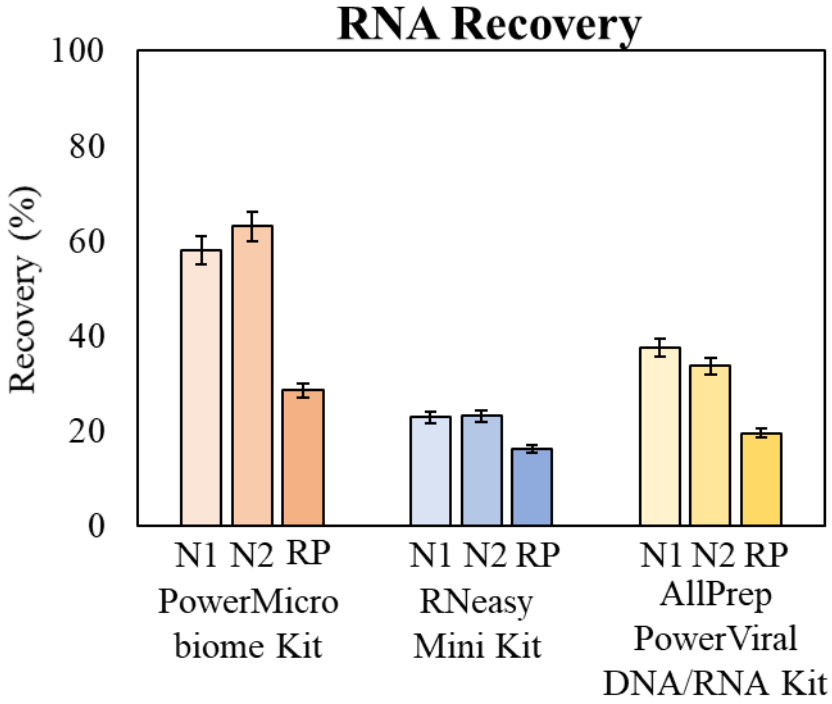
Droplets are spaced out individually for fluorescence reading by the droplet reader

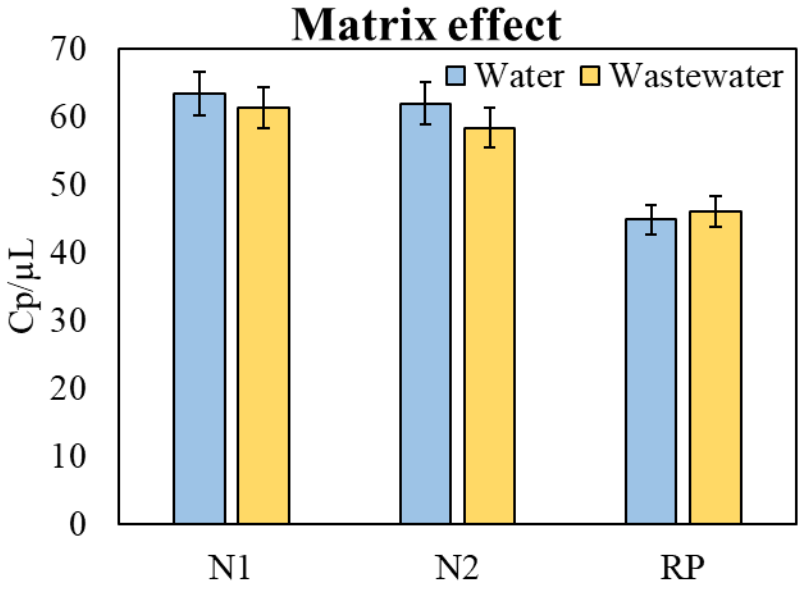


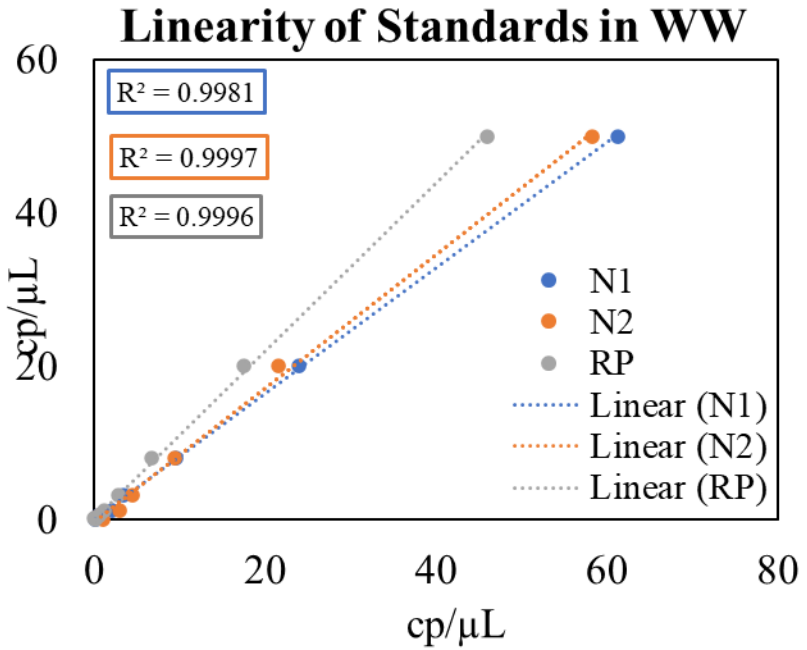


Fluorescence readings are measured for each droplet in two channels

Analytical Performance





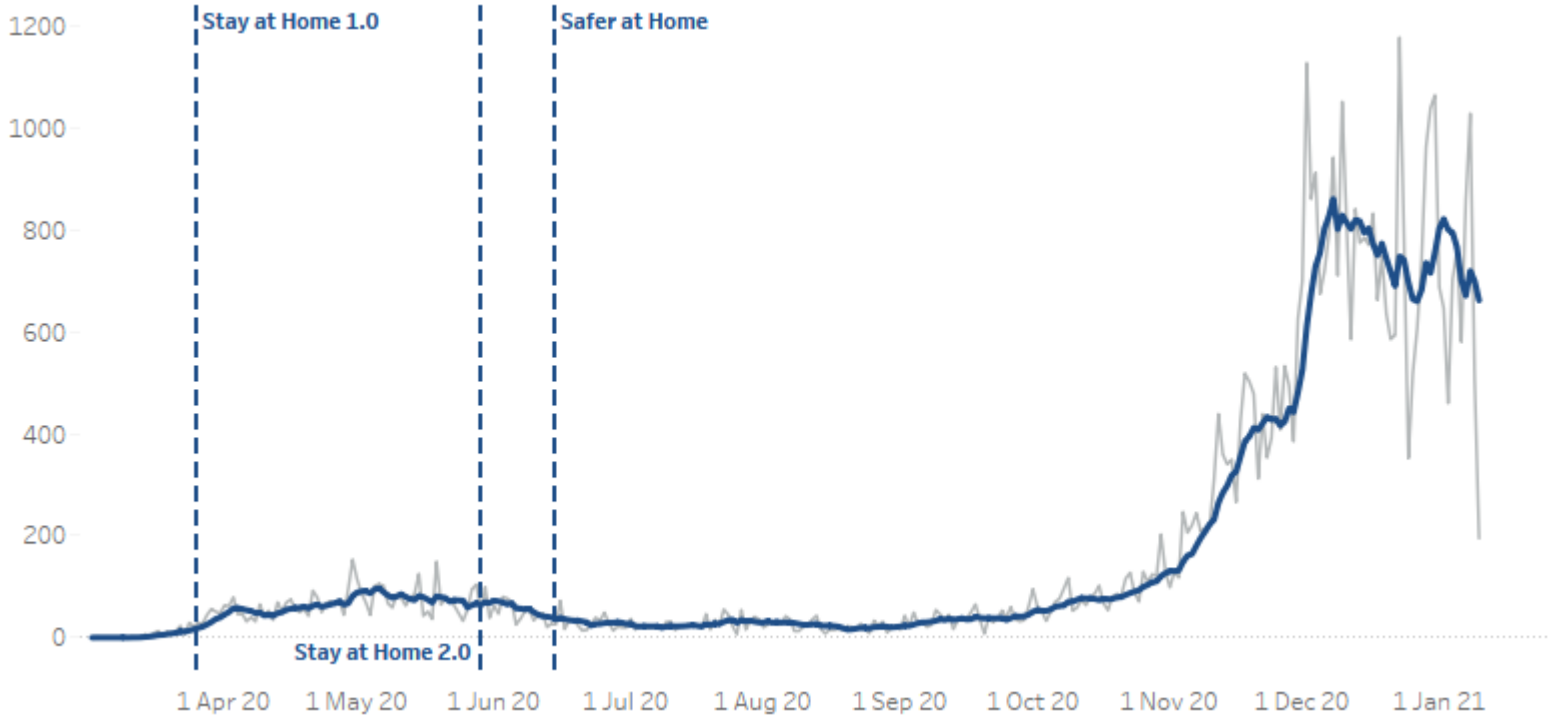


Method standardization included the assessment of

- RNA recovery (~ 48%)
- limit of detection (182 cp/100 mL)
- matrix effect (from 2 to 20% inhibition)

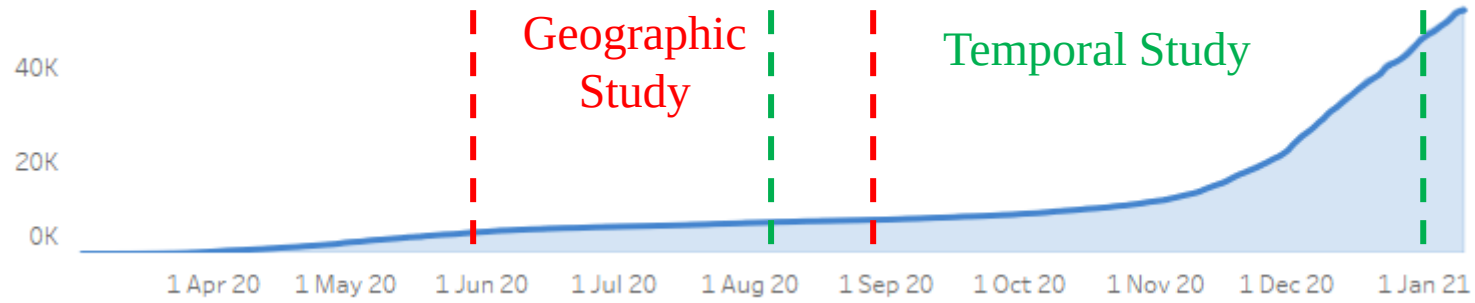
Timeline of COVID-19 cases in New Hampshire, USA

Daily Trends for Cases by Report Date

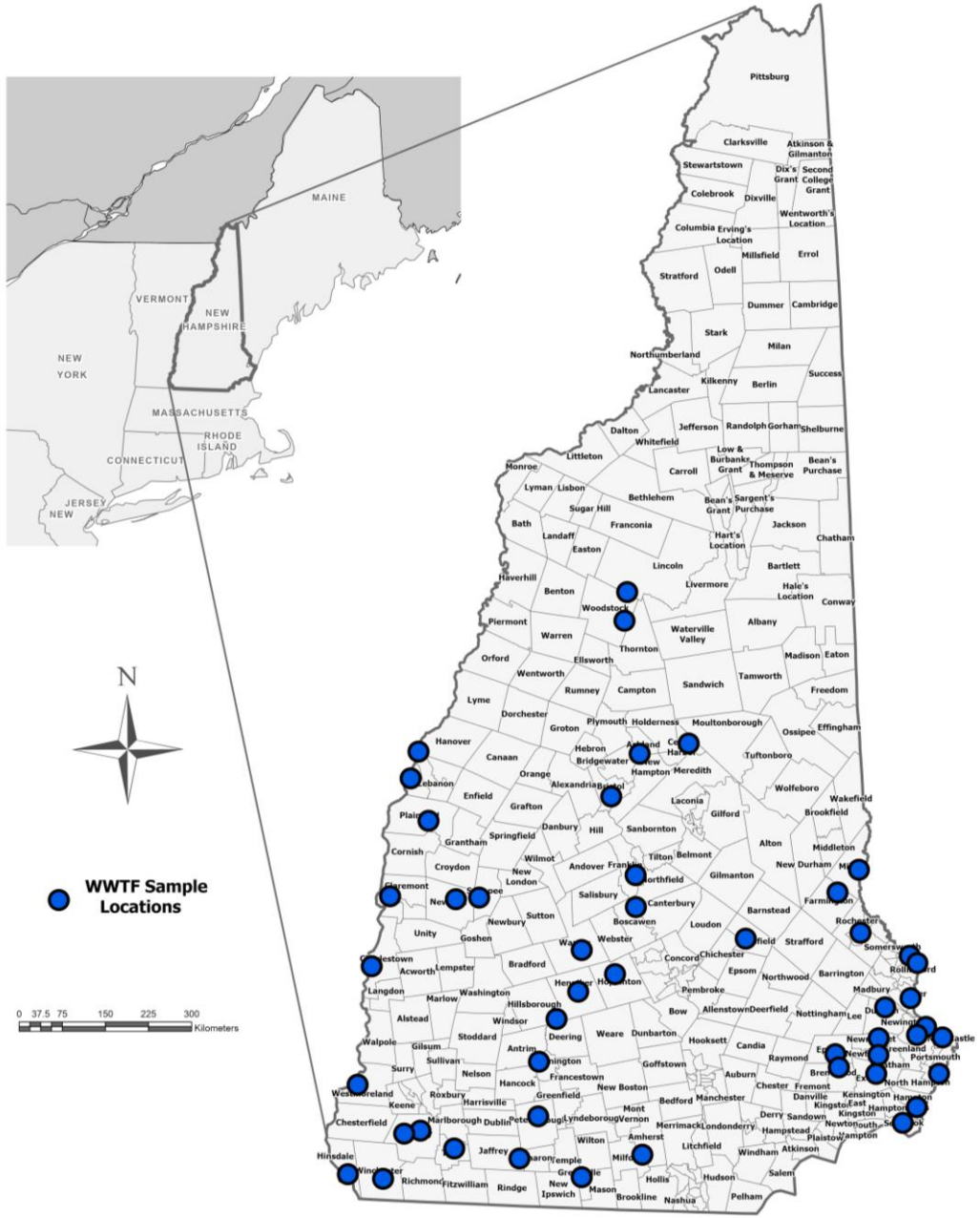


From NHDHHS https://www.nh.gov/covid19/dashboard/trends.htm#dash

Cumulative Total for Cases by Report Date



Geographic Distribution of SARS-CoV-2 in NH WWTFs



Field Sample Approach

52 Municipal WWTF locations in NH

Populations Served: <200 to 29,000 persons

Sample Dates: mid-June through mid-Sept

Facilities "Opted-in" to Sampling

- -Grab samples
- -Collected at influent before solids removal
- -Collected before noon

Results

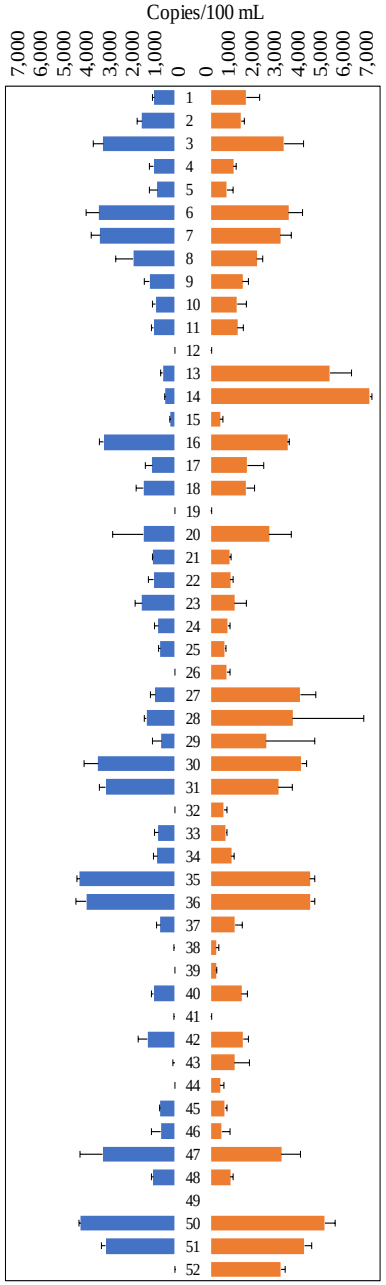
N2 biomarker detected in most facilities

- 92% Positive (48/52)
- 8% Negative (4/52)

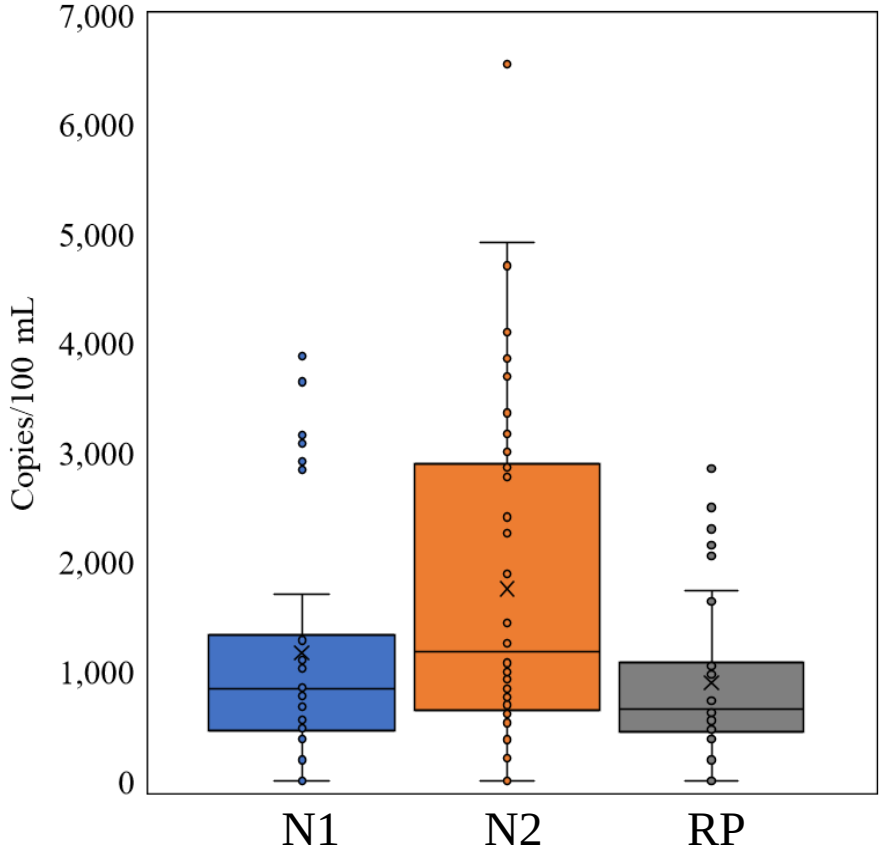
Both N1 and N2 detected in many facilities

- 79% both Positive (41/52)
- 21% one or both Negative (11/52)

Geographic Distribution of SARS-CoV-2 in NH WWTFs

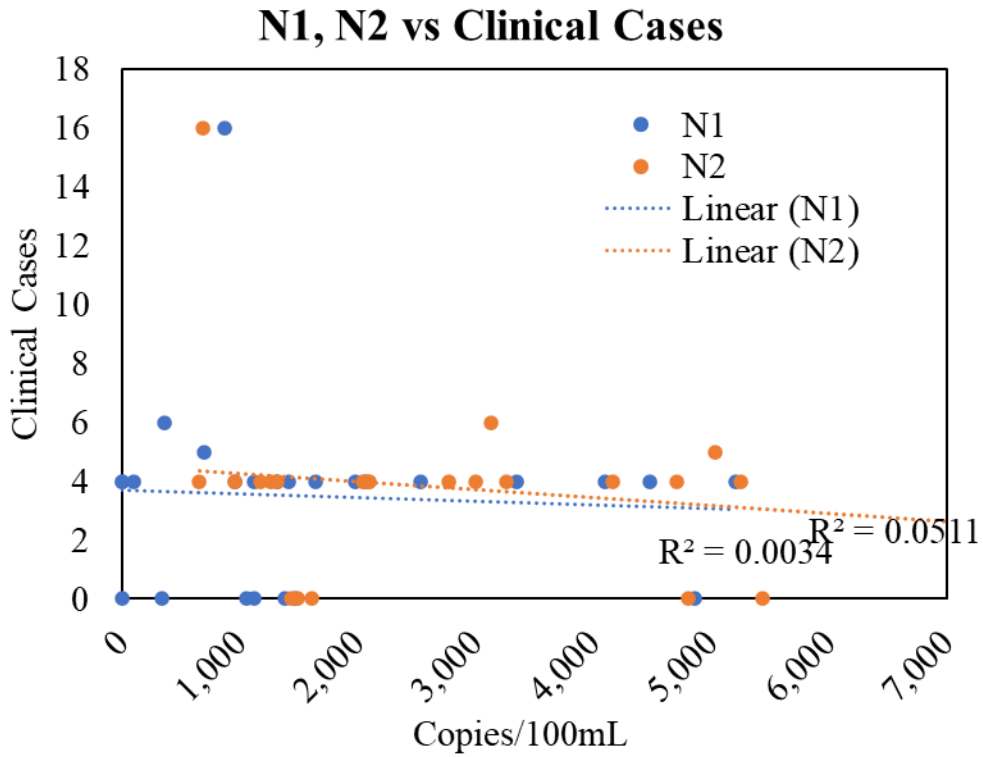


SARS-CoV-2 RNA concentrations in the 52 WWTFs analyzed by RT-ddPCR N1 (blue) and N2 (orange) assays



Box plot showing inclusive mean quartile calculation for N1 (blue), N2 (orange) and RP (grey) genes.

Viral biomarker data lack correlation to current case numbers (very low in NH)



Geographic Distribution of SARS-CoV-2 in NH WWTFs

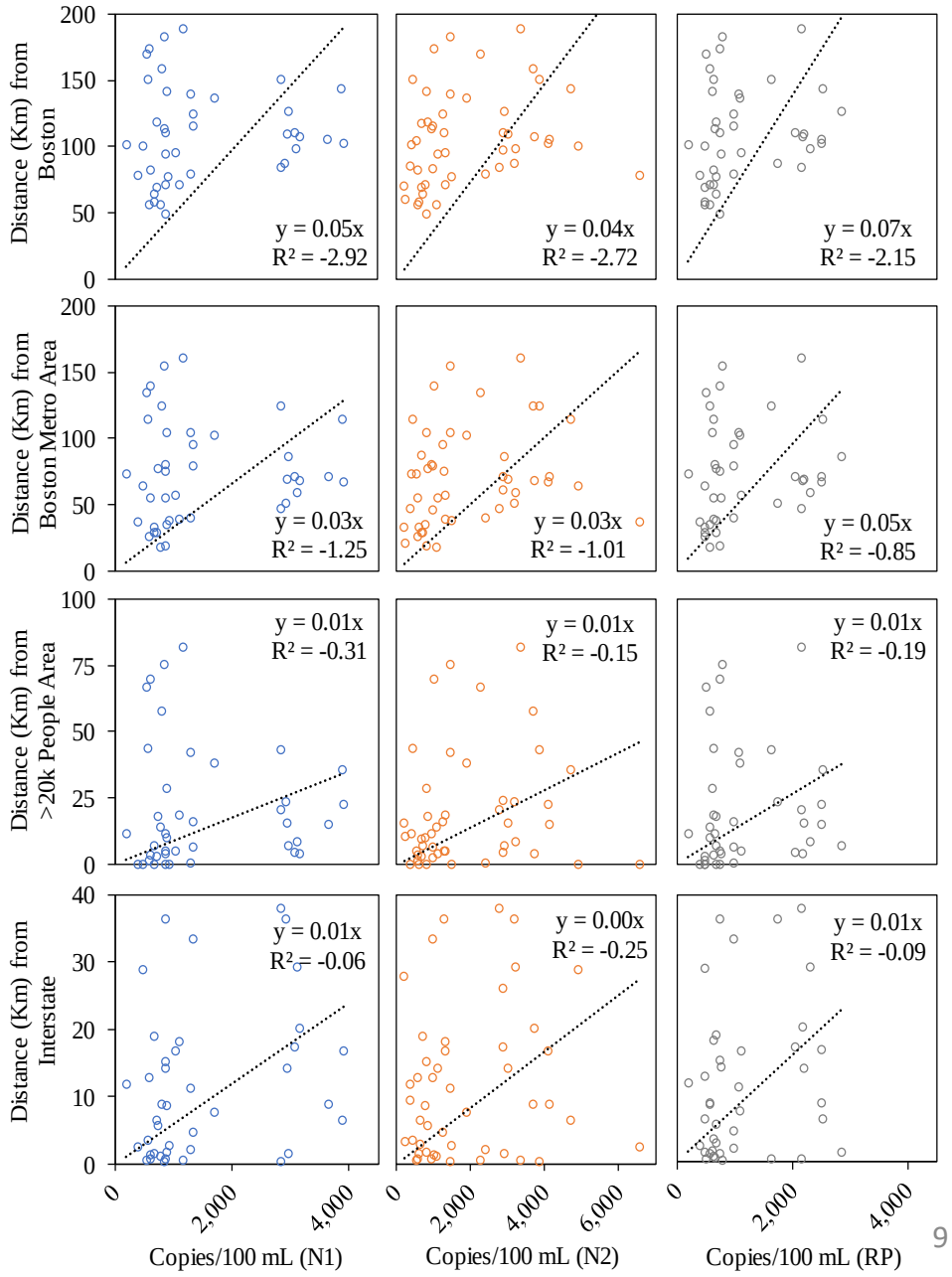
Distance from Boston: NO correlation

Distance from Boston Metropolitan Area: NO correlation

Distance from a city with more than 20k people : NO correlation

Distance from Interstates : NO correlation

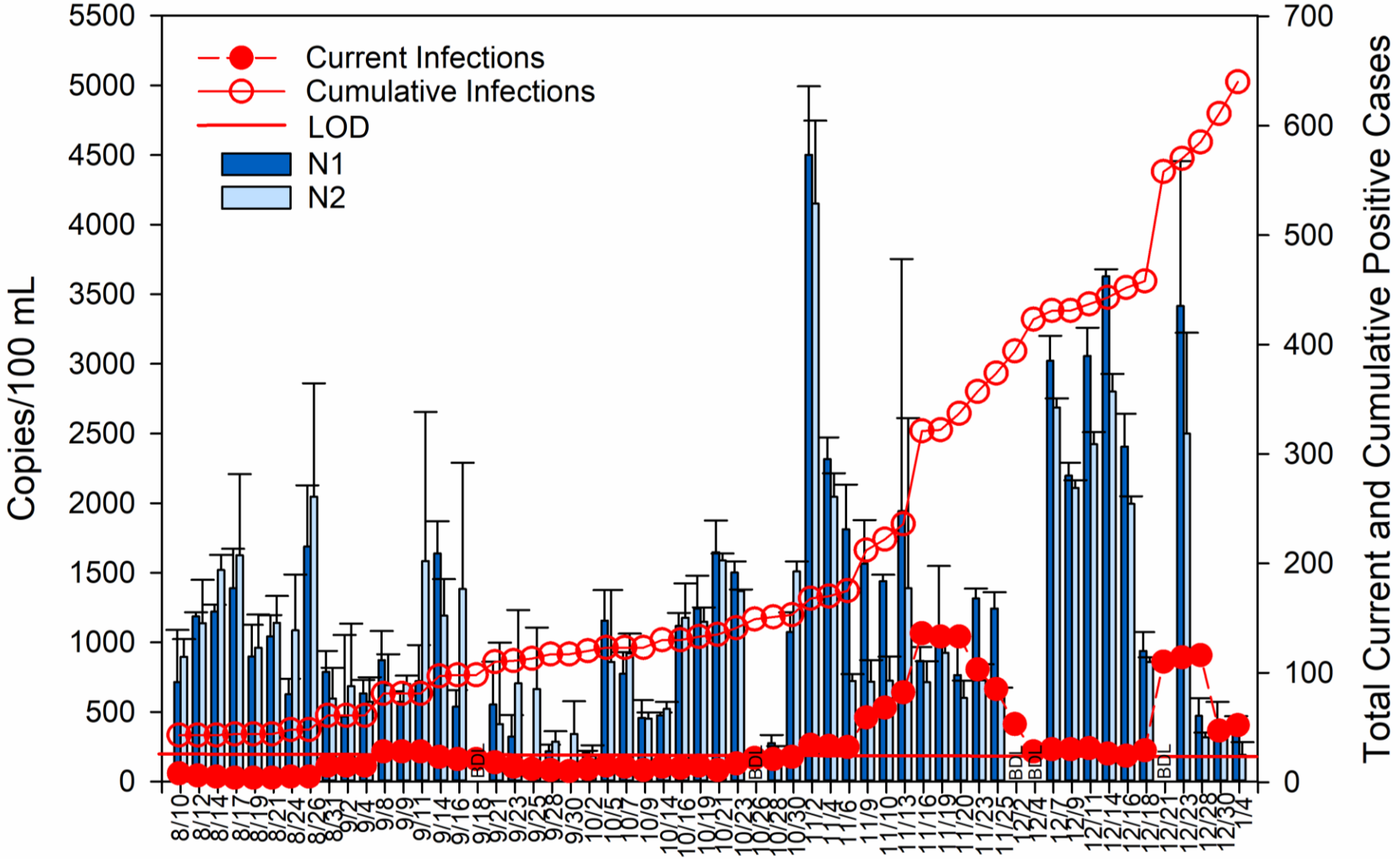
Viral biomarker data lack correlation to current case numbers (very low in NH)



Temporal Fluctuations of SARS-CoV-2 in NH WWTF 1

Field Sample Approach

Population Served: 8,000 to 17,000 persons. Sample Dates: Aug 10 through current. 3x weekly samples (MWF). -24-hour composite sample



Spearman's Rank Correlation Test found a good correlation (p value <0.05) for N1 and RP versus current and cumulative COVID-19 cases

Temporal Fluctuations of SARS-CoV-2 in NH WWTF 1

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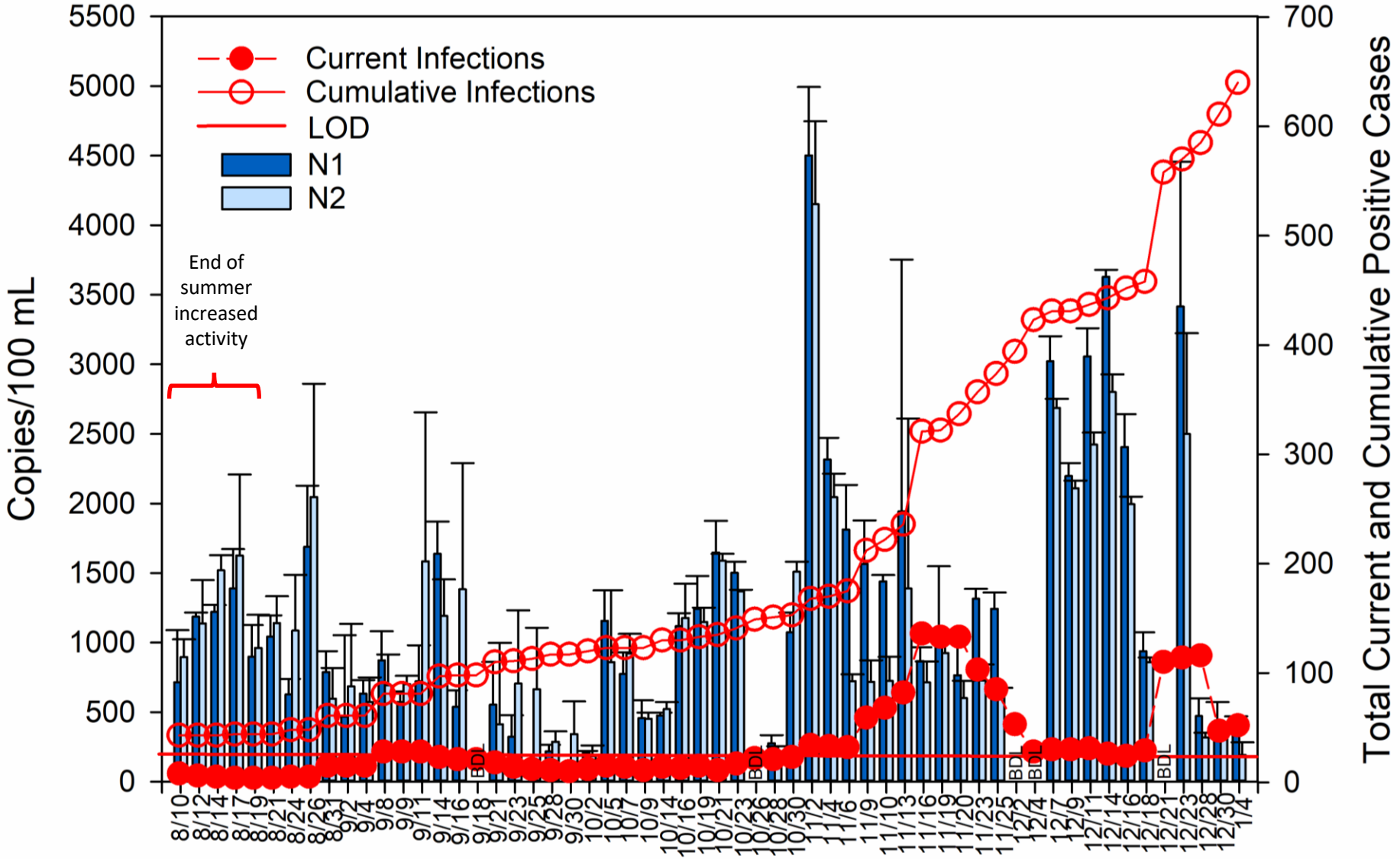

End of summer activities correspond to an increased viral biomarker concentration that is not backed up by clinical data

Temporal Fluctuations of SARS-CoV-2 in NH WWTF 1

Field Sample Approach

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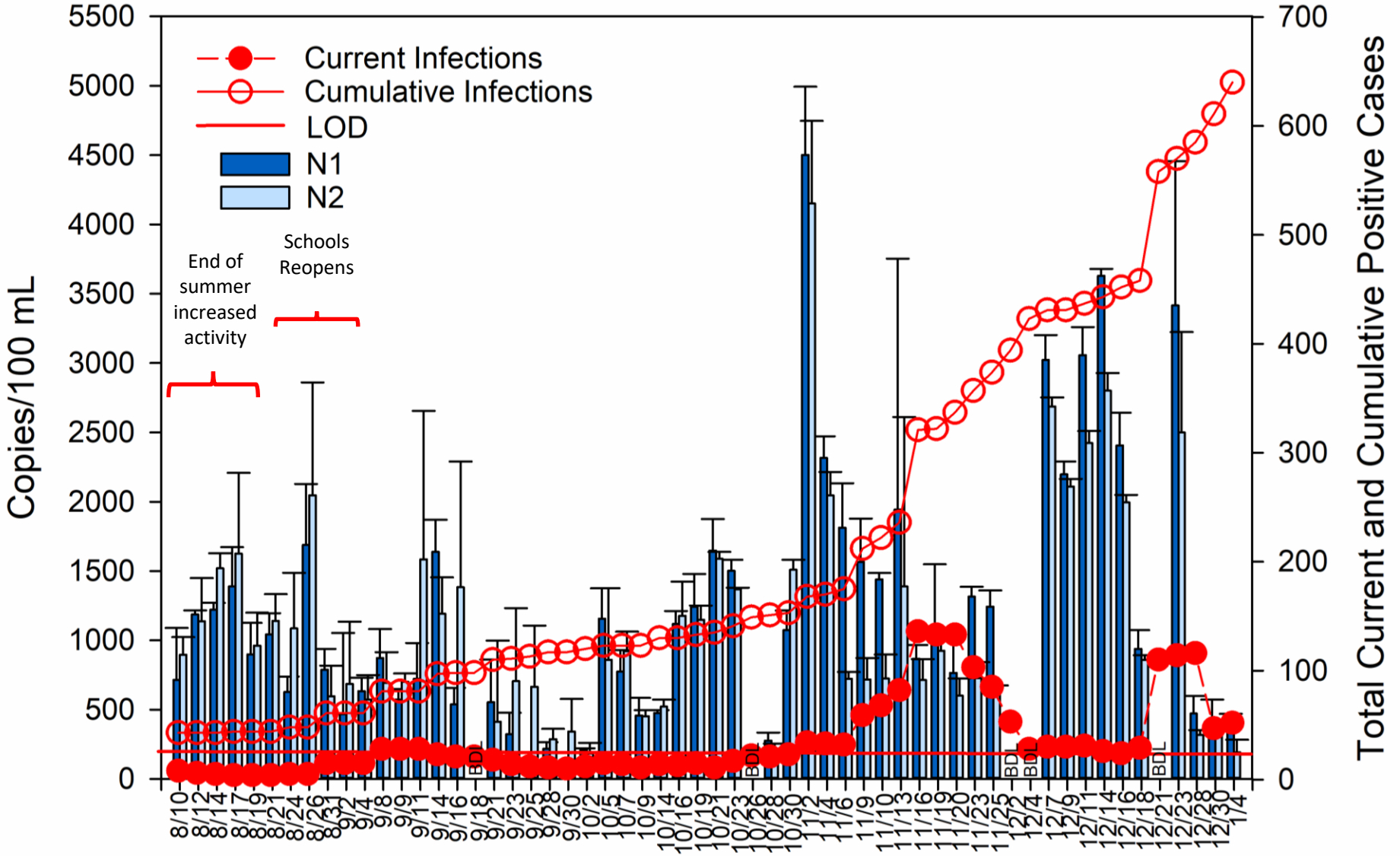


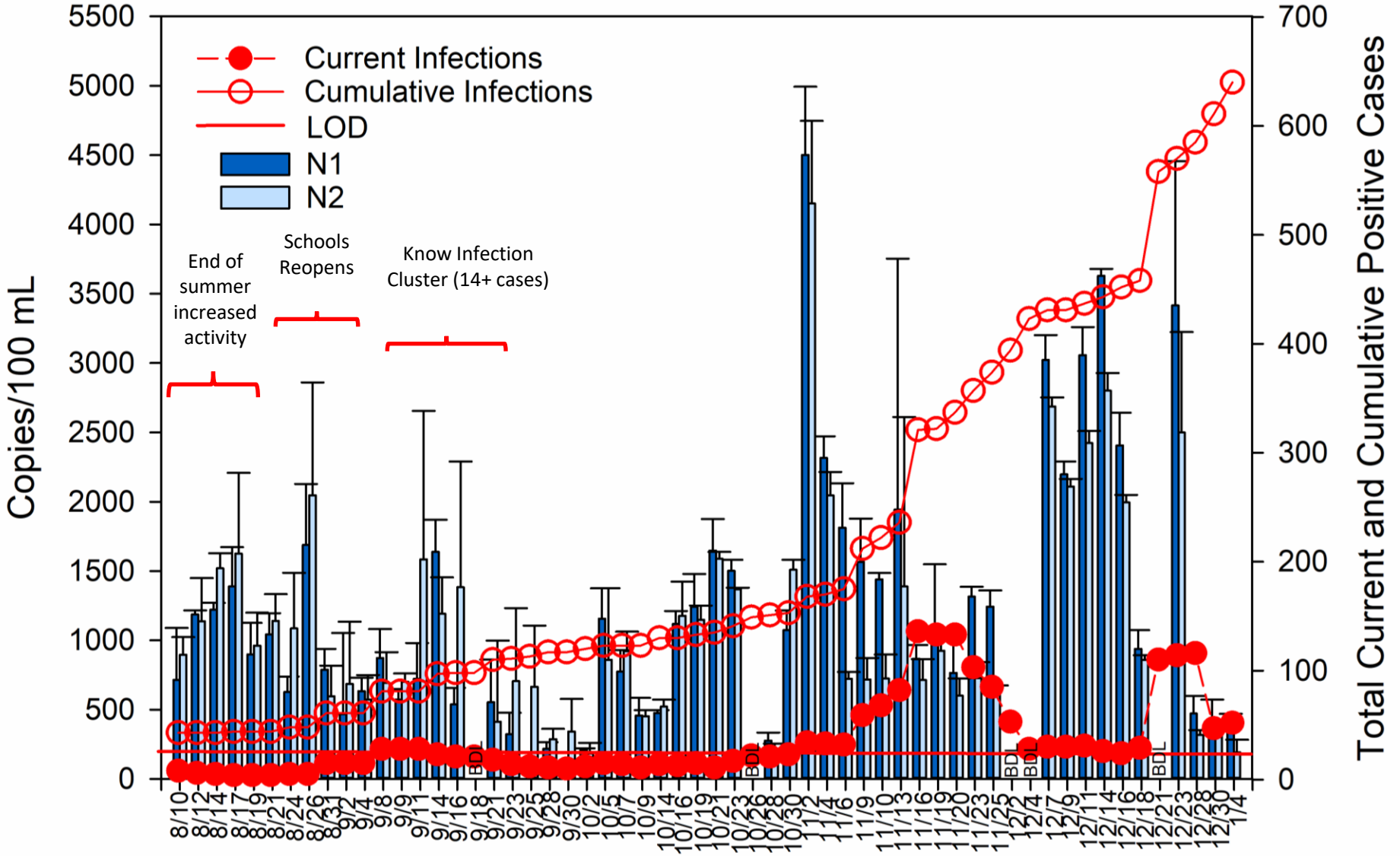
170% increase in viral biomarkers

200% increase in clinical cases after approximately 4 days

Temporal Fluctuations of SARS-CoV-2 in NH WWTF 1

Field Sample Approach

Population Served: 8,000 to 17,000 persons. Sample Dates: Aug 10 through current. 3x weekly samples (MWF). -24-hour composite sample



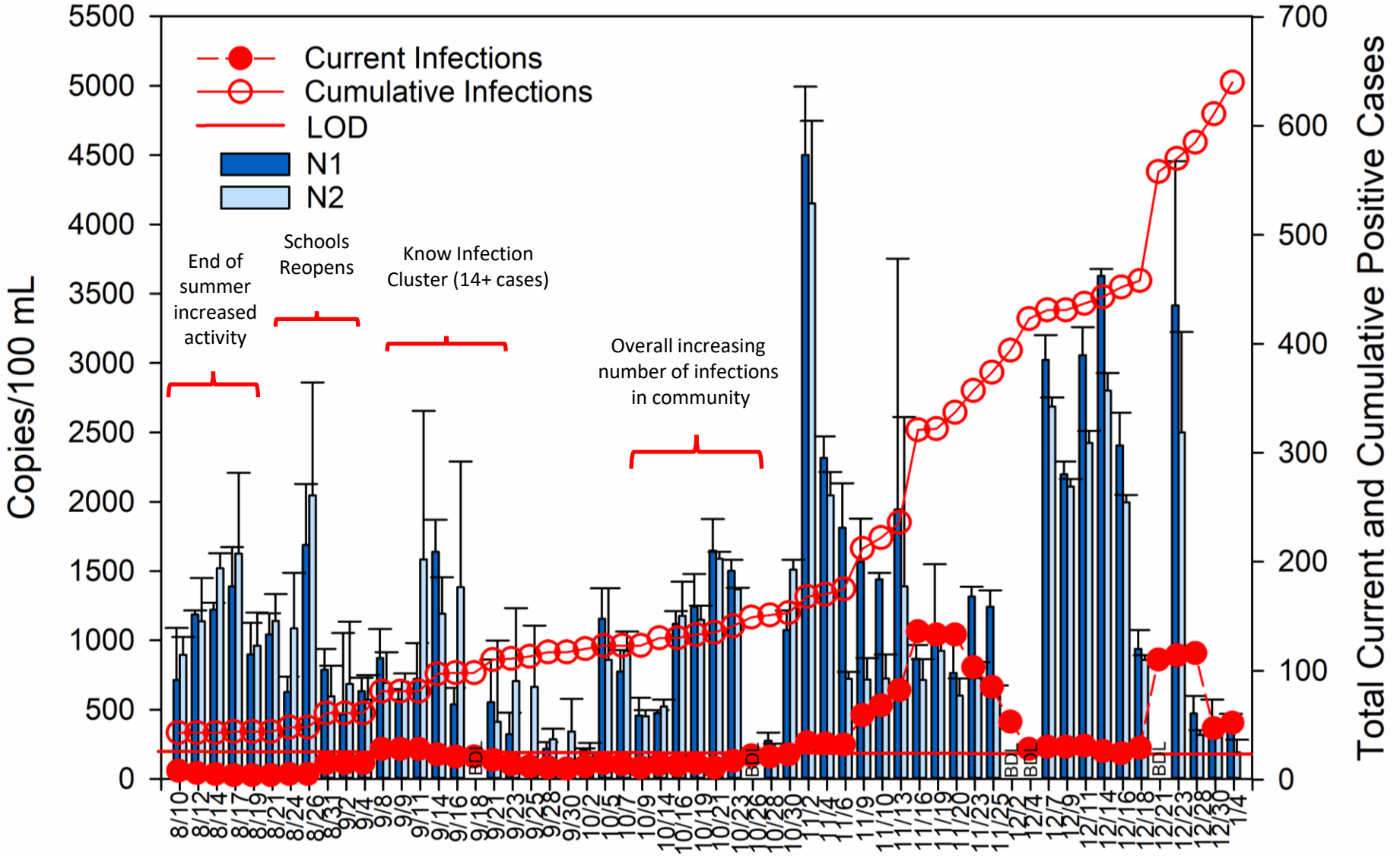
200% increase in viral biomarkers

140% increase in clinical cases

Temporal Fluctuations of SARS-CoV-2 in NH WWTF 1

Field Sample Approach

Population Served: 8,000 to 17,000 persons. Sample Dates: Aug 10 through current. 3x weekly samples (MWF). -24-hour composite sample



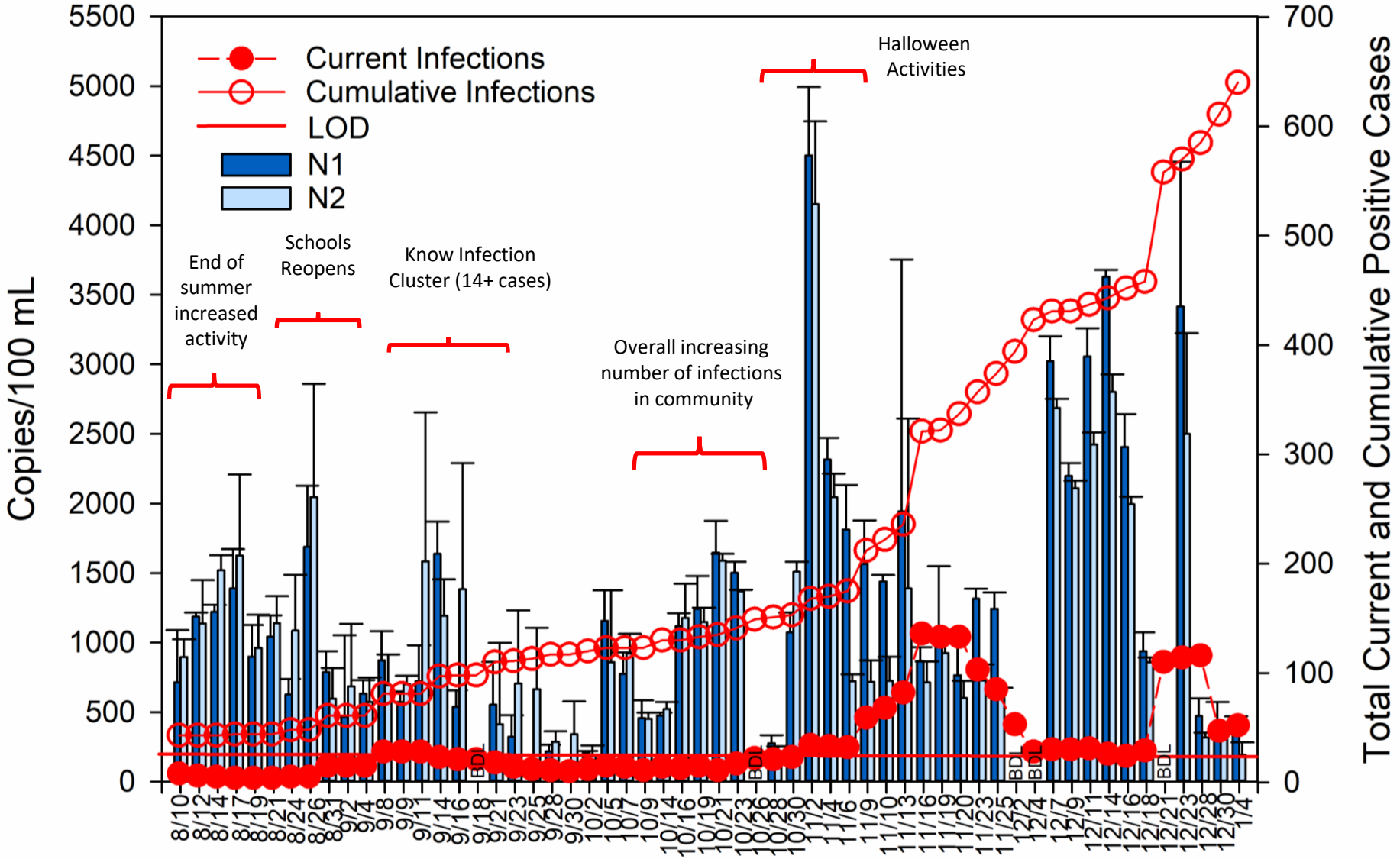
135% increase in viral biomarkers

100% increase in clinical cases

Temporal Fluctuations of SARS-CoV-2 in NH WWTF 1

Field Sample Approach

Population Served: 8,000 to 17,000 persons. Sample Dates: Aug 10 through current. 3x weekly samples (MWF). -24-hour composite sample



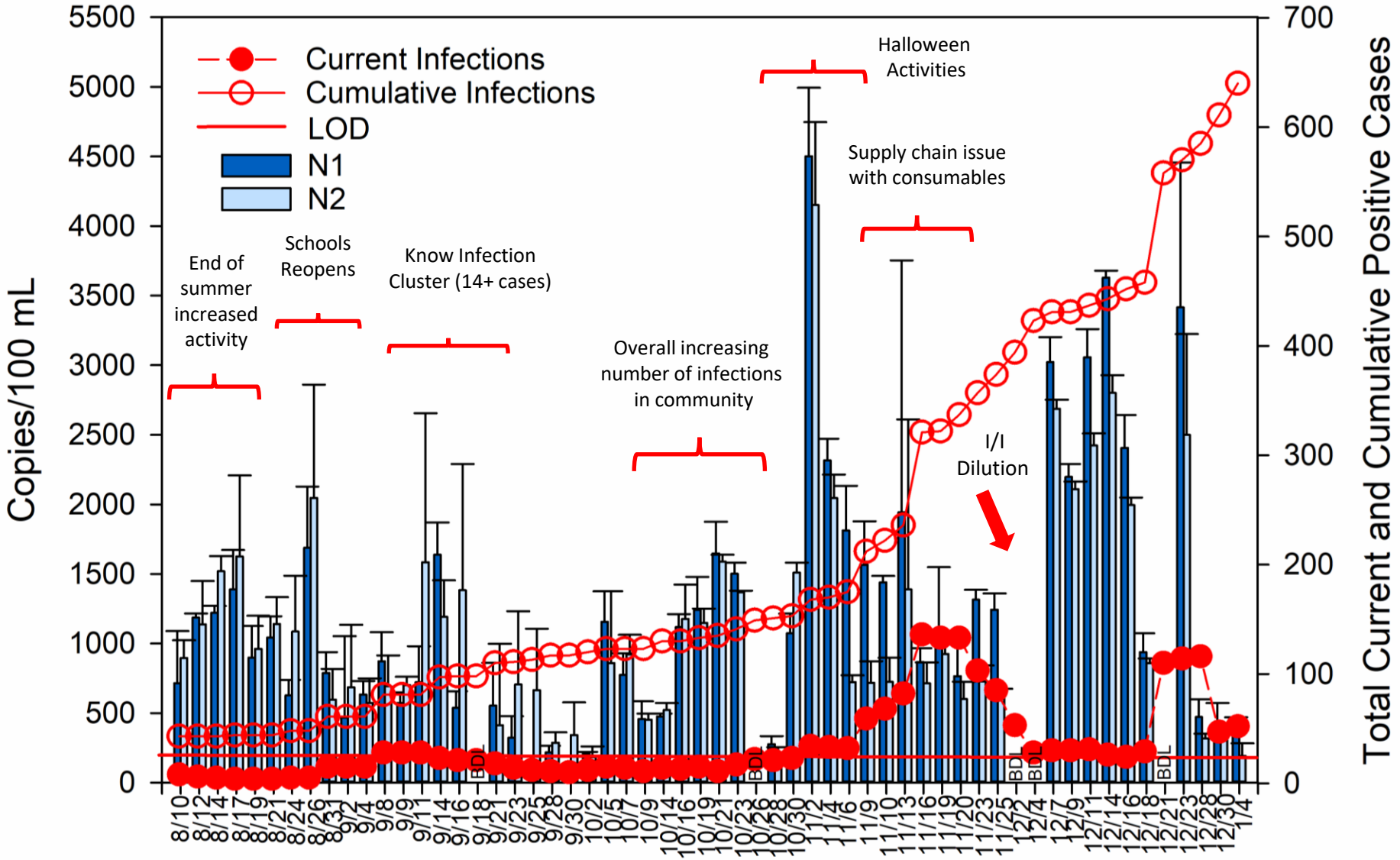
200% increase in viral biomarkers

157% increase in clinical cases

Temporal Fluctuations of SARS-CoV-2 in NH WWTF 1

Field Sample Approach

Population Served: 8,000 to 17,000 persons. Sample Dates: Aug 10 through current. 3x weekly samples (MWF). -24-hour composite sample



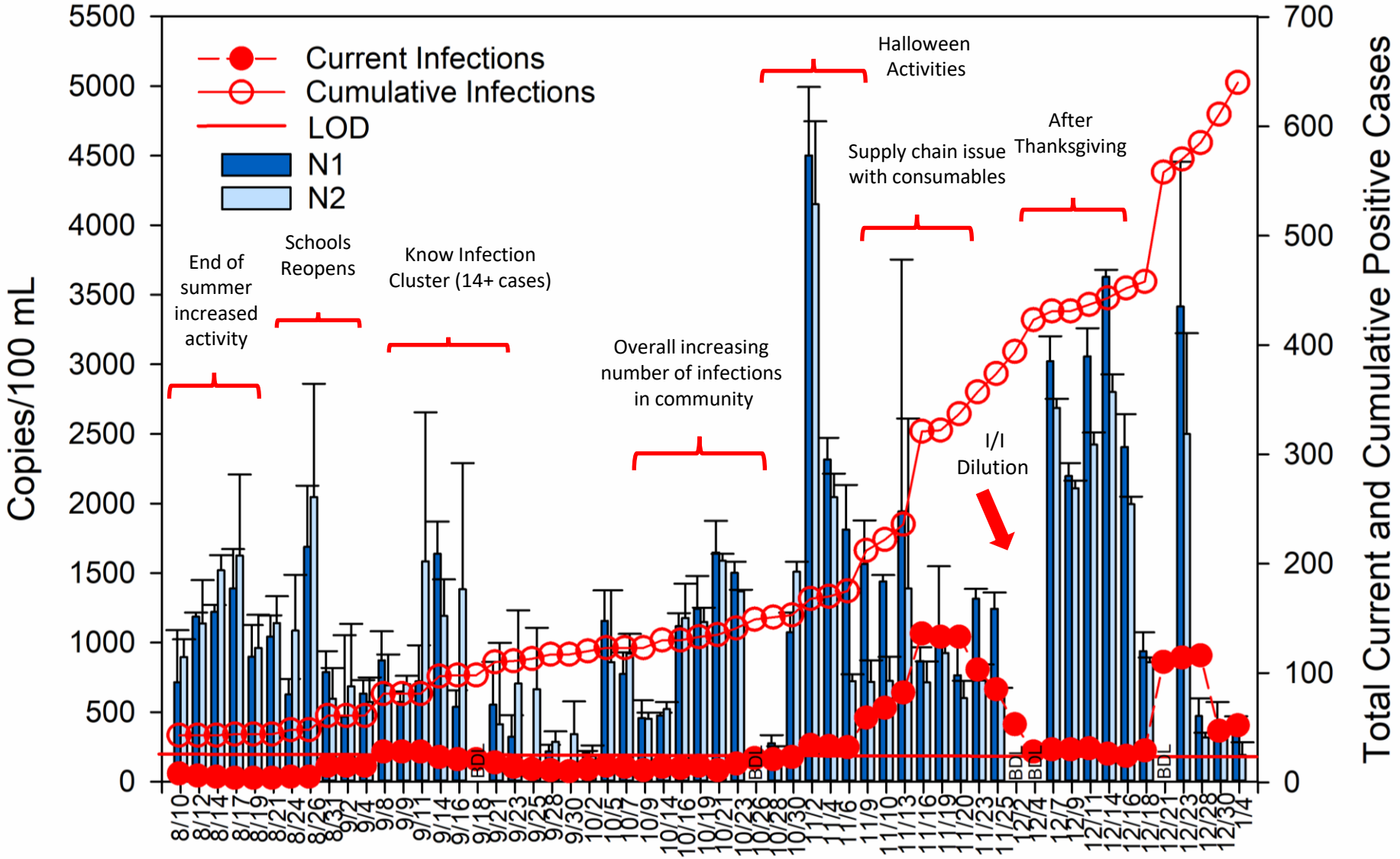
From mid November, while the clinical cases were rising, we had a supply chain issue that resulted in lower RNA recovery.

After this, we also observe a drastic dilution effect caused by high precipitation in the area

Temporal Fluctuations of SARS-CoV-2 in NH WWTF 1

Field Sample Approach

Population Served: 8,000 to 17,000 persons. Sample Dates: Aug 10 through current. 3x weekly samples (MWF). -24-hour composite sample



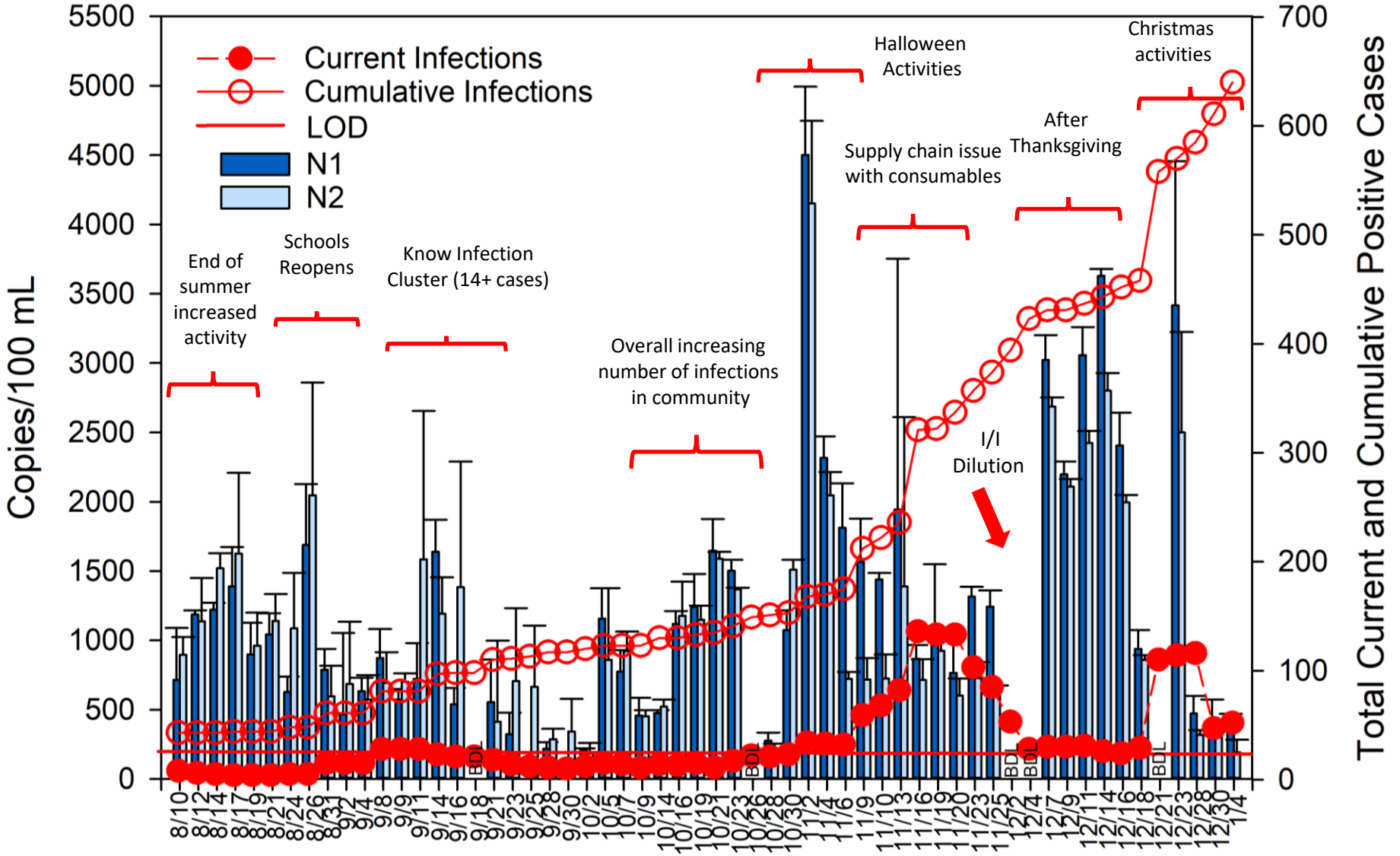
39% increase in viral biomarkers

21% increase in clinical cases

Temporal Fluctuations of SARS-CoV-2 in NH WWTF 1

Field Sample Approach

Population Served: 8,000 to 17,000 persons. Sample Dates: Aug 10 through current. 3x weekly samples (MWF). -24-hour composite sample

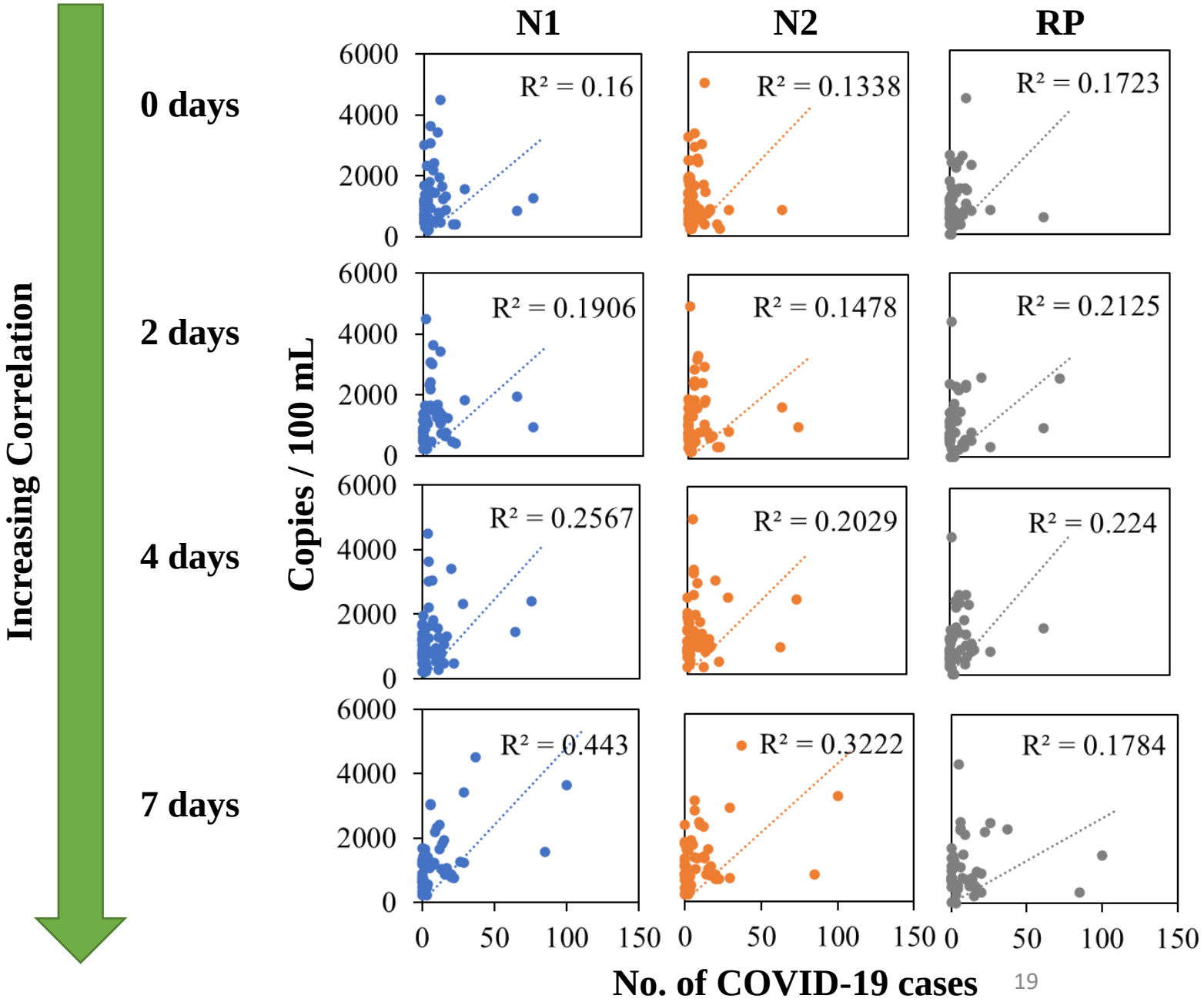


293% increase in viral biomarkers

264% increase in clinical cases

Temporal Fluctuations of SARS-CoV-2 in NH WWTF 1

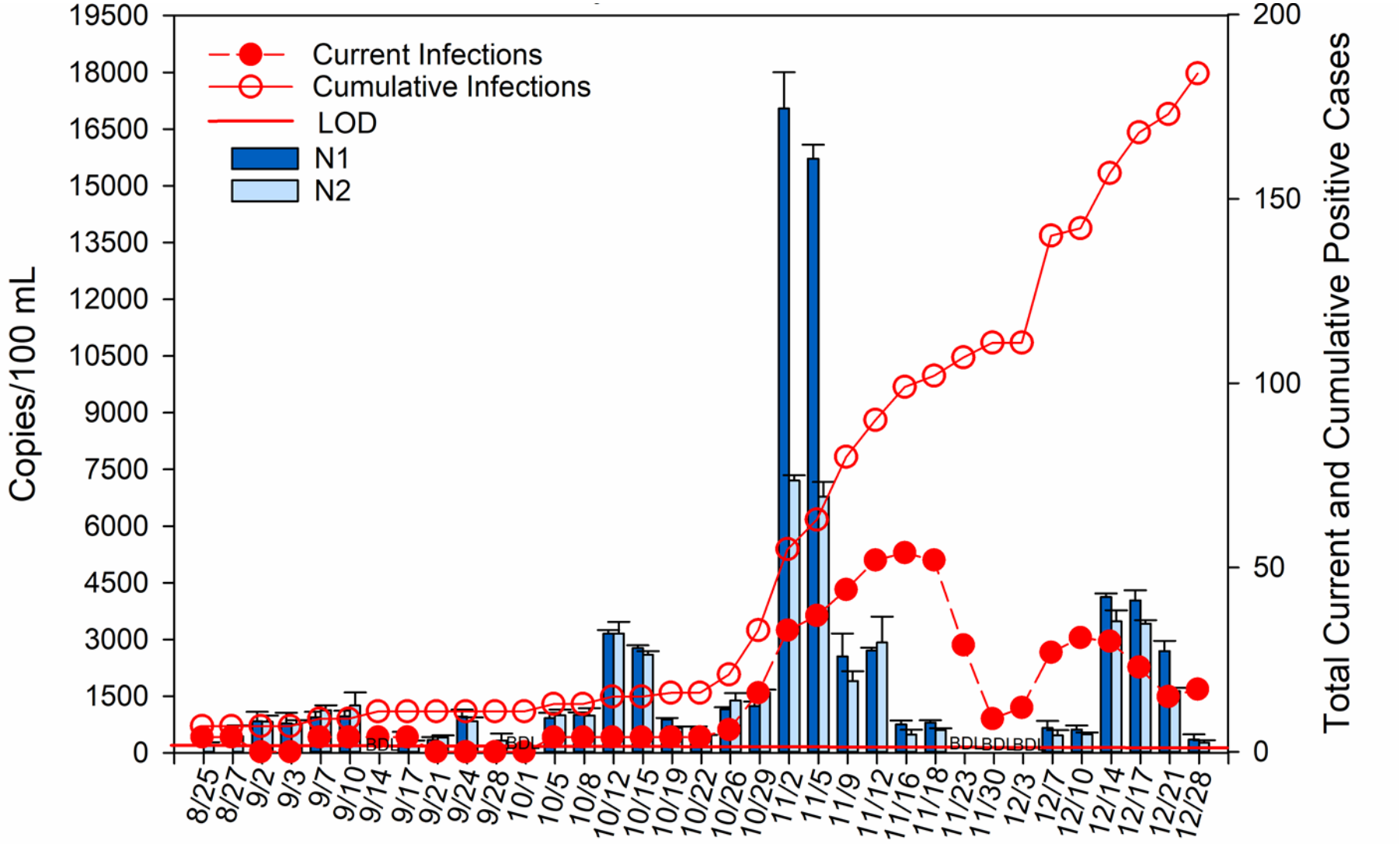
Wastewater surveillance is an early warning tool that can predict COVID-19 outbreak approximately 7 days before clinical testing



Temporal Fluctuations of SARS-CoV-2 in NH WWTF 2

Field Sample Approach

Sample Dates: Aug 25 through current. 2x weekly samples (MTh). -24-hour composite sample. -Collected at influent before solids removal

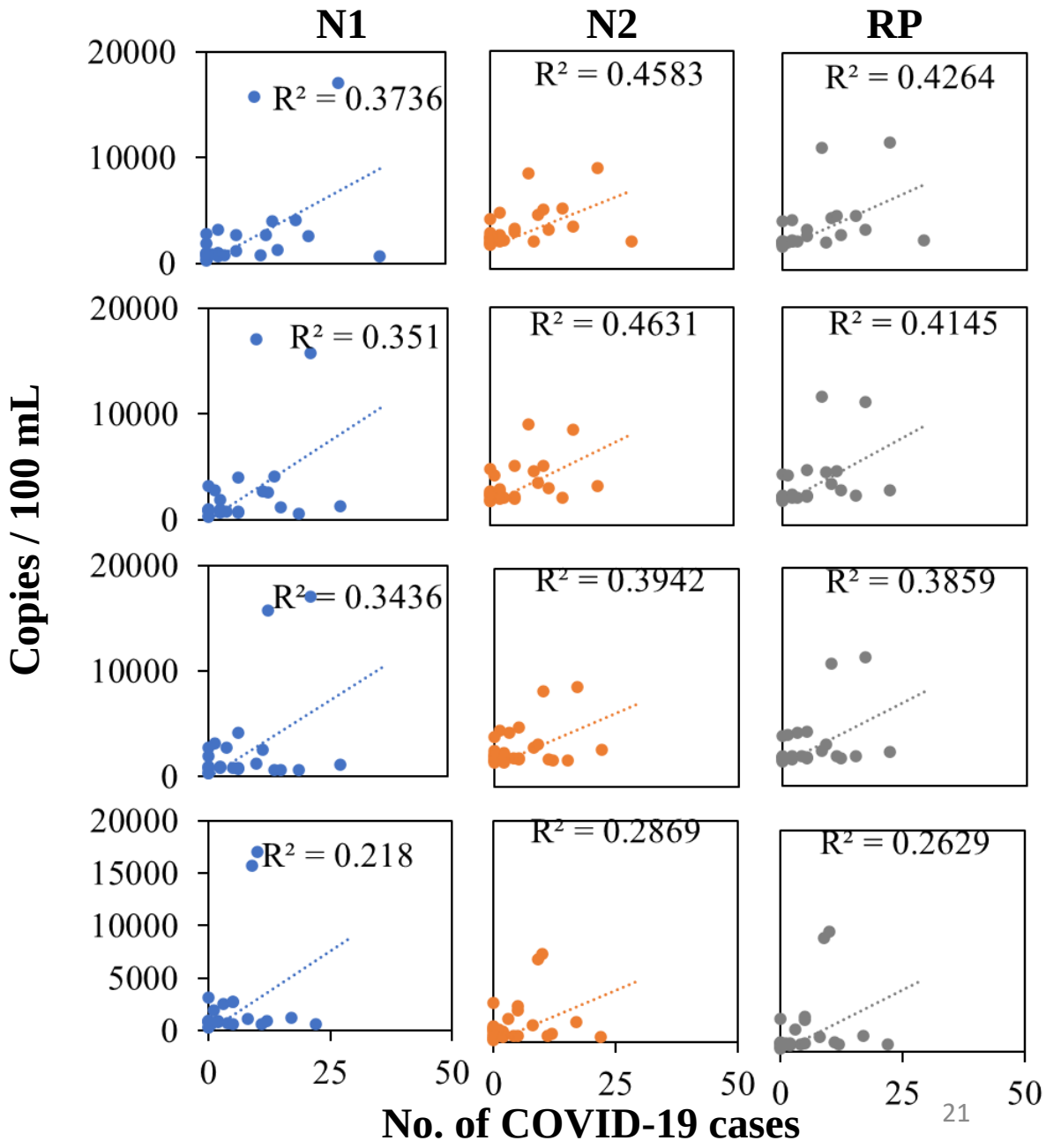


350% increase in viral biomarkers

225% increase in clinical cases

Temporal Fluctuations of SARS-CoV-2 in NH WWTF 2

The lack of clinical data and low number of COVID-19 patients in the area determine a poor correlation between wastewater viral biomarkers and number of COVID-19 cases.



Conclusions

1. The geographic distribution of SARS-CoV-2 in NH WWTFs does not reflect reported cases in a low prevalence area: RNA was detected despite of limited reported case in the vicinity
2. Temporal monitoring of WWTFs unravels the early warning potential of wastewater-based surveillance of COVID-19, and correlates directly to known infections
3. N1 biomarker gene, being more specific, is better correlated to known COVID-19 cases
4. Wastewater biomarkers are detected during initial (or asymptomatic) stage of infection
5. Amount and timing of testing determines “lag” between biomarker data and known infections

Future Steps

- Re-sampling and analysis of the 52 WWTFs selected for the spatial study
- Sequencing of N1 and N2
- Monitoring facilities with high COVID-19 cases in the area
- Removal of SARS-CoV-2: 4 out of 6 selected WWTFs sampled and analyzed

Thank you