

Wastewater-Based Surveillance of Norovirus in Wisconsin



#IDLabCon

A Comparison of Wastewater Concentrations with
Clinical PCR Positivity Rates

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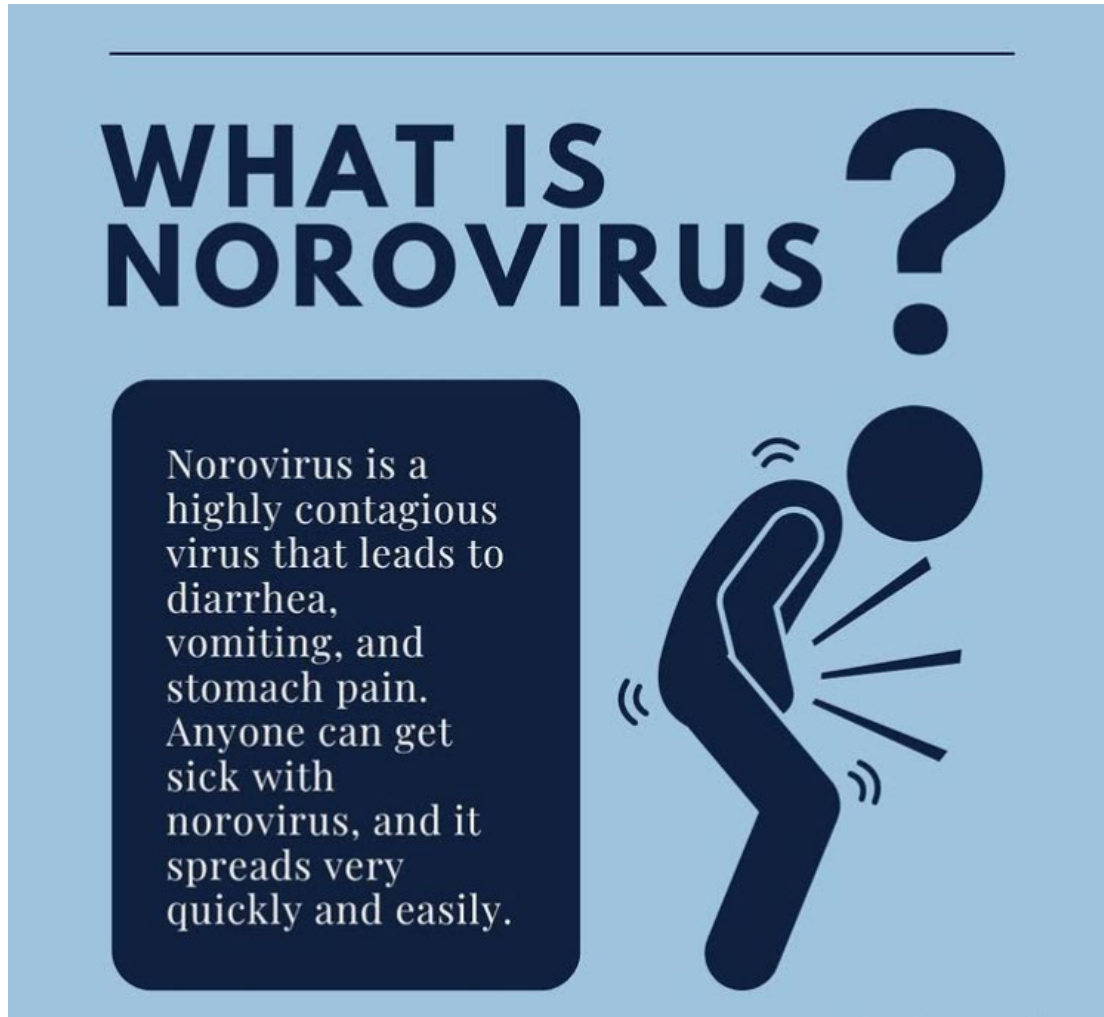
March 25th, 2025

Conflict of Interest Disclosure

The author does not have any conflicts of interest to disclose. While this project is supported by the Centers for Disease Control and Prevention (CDC) of the U.S. Department of Health and Human Services (HHS) as part of a financial assistance award with 100 percent funded by CDC/HHS, the contents are those of the author and do not necessarily represent the official views of, nor an endorsement by, CDC/HHS or the U.S. government.

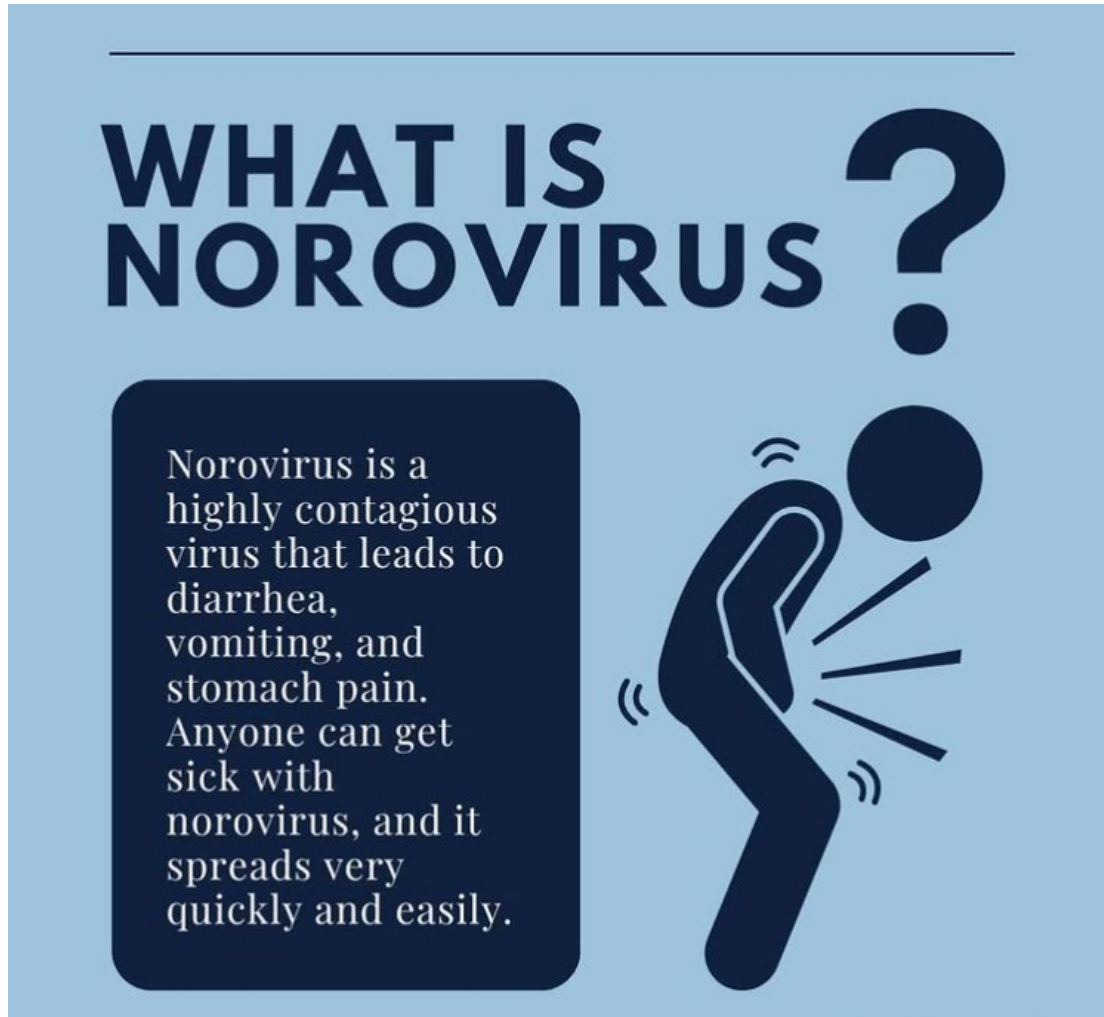


The Basics of Norovirus

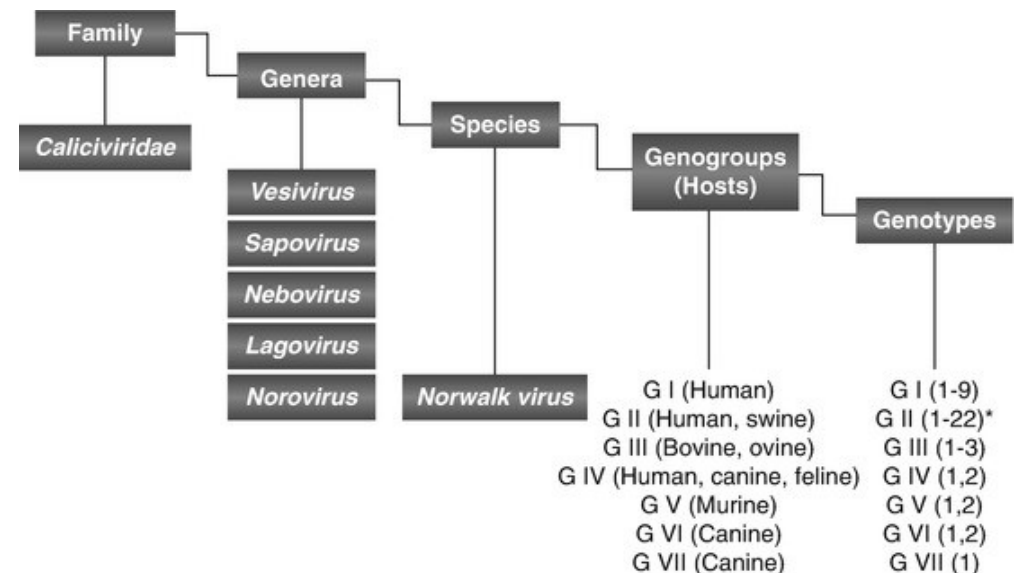


- Leading cause of vomiting and diarrhea in the United States AND leading cause of foodborne illness outbreaks worldwide
- Symptoms develop 12-48 hours after exposure and last for a few days
- Non-enveloped, single-stranded RNA viruses

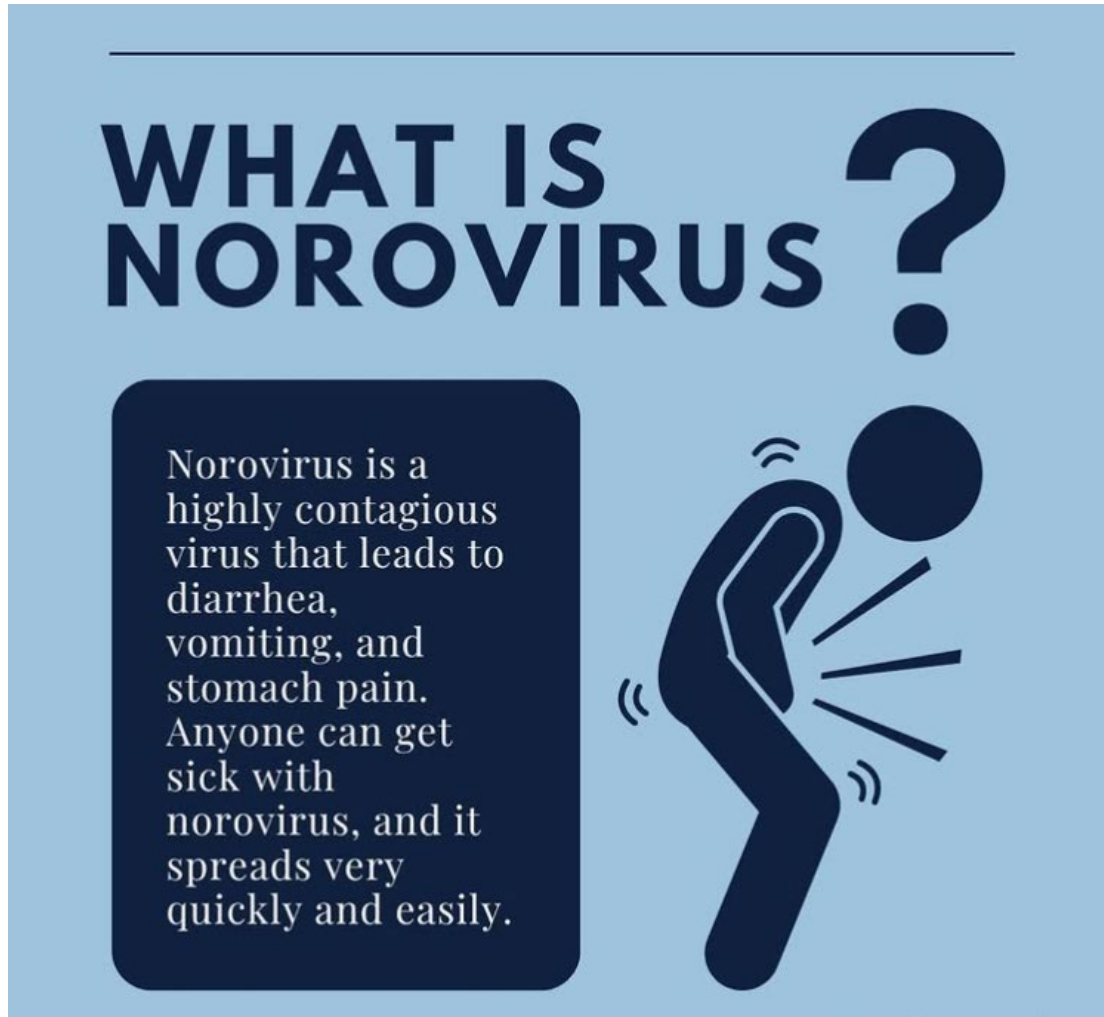
The Basics of Norovirus



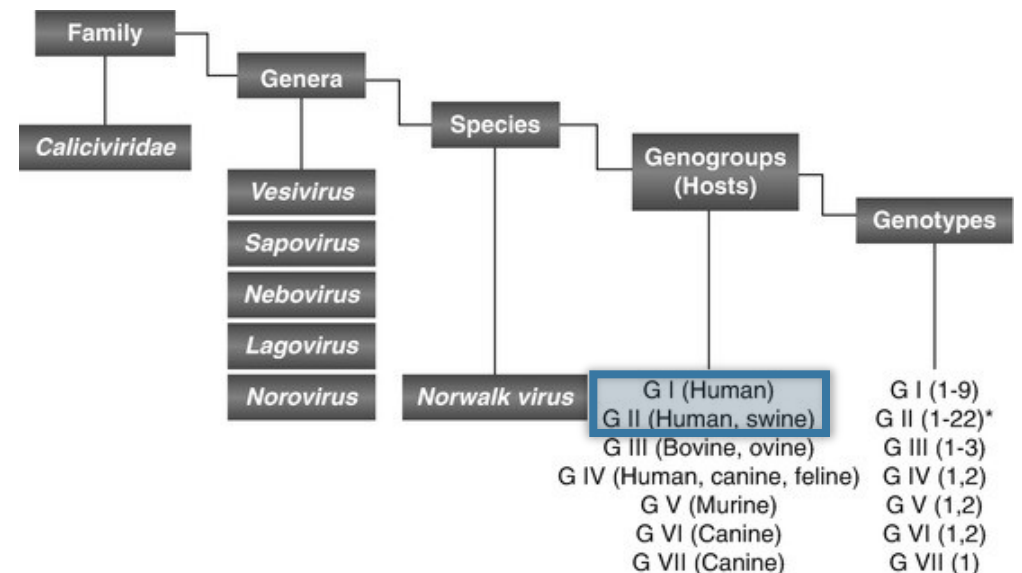
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What Are Noroviruses



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Norovirus Transmission

WHAT IS NOROVIRUS?

Norovirus is a highly contagious virus that leads to diarrhea, vomiting, and stomach pain. Anyone can get sick with norovirus, and it spreads very quickly and easily.

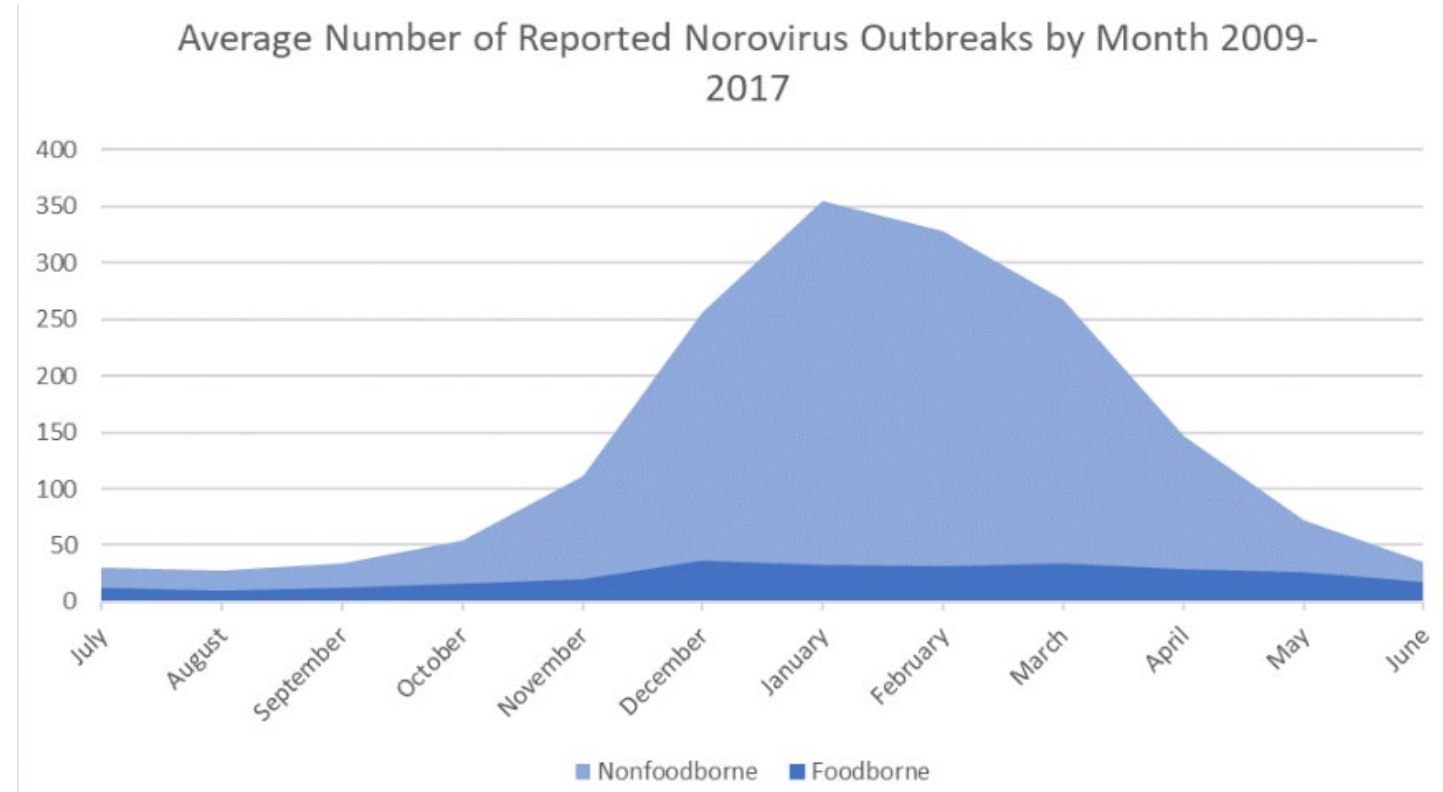


- Extremely contagious
- Fecal-oral transmission
- Spreads through contaminated food, water, and surfaces



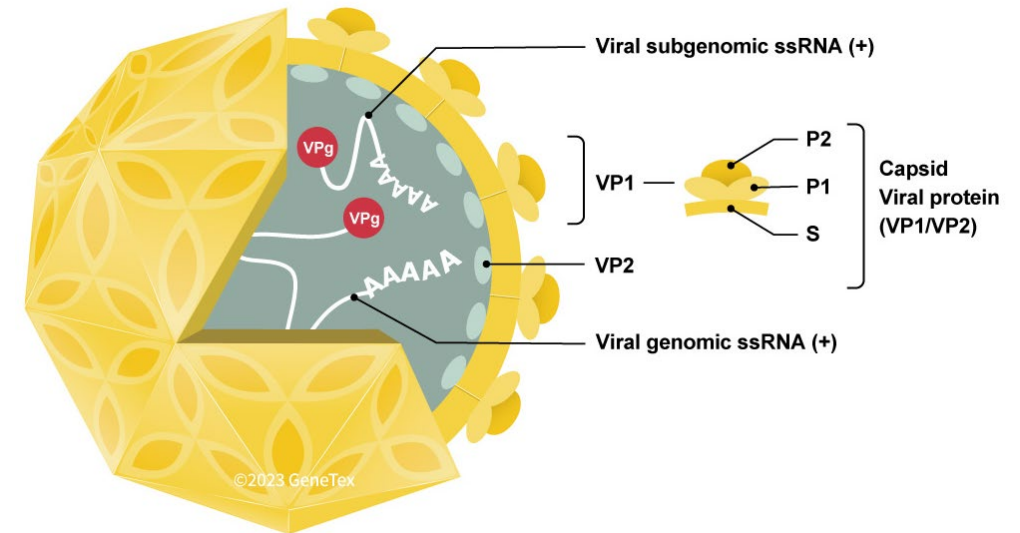
Public Health Impact of Norovirus

- Norovirus genogroup II (NvGII) causes majority of outbreaks
- The CDC's estimated yearly impact of norovirus nationwide:
 - 2,500 reported outbreaks
 - 20 million illnesses
 - 2 million outpatient clinic visits
 - 450,000 ER visits
 - 100,000 hospitalizations
 - 900 deaths
- Currently no vaccine
& no lasting immunity from infection

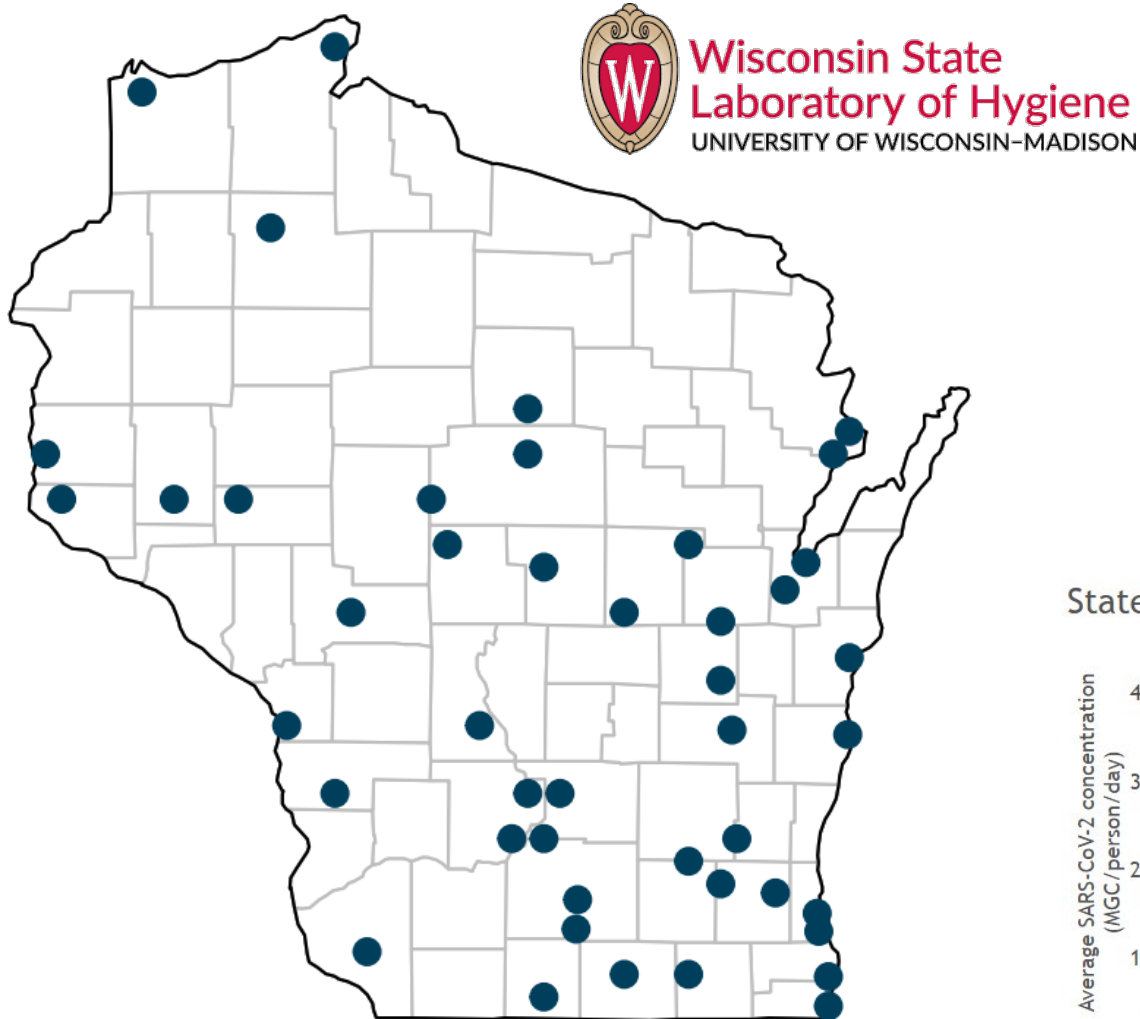


Why Monitor Norovirus in Wastewater?

- Cost-effective and efficient
- Limited clinical data → wastewater has potential to serve as an early warning of transmission and spread to elicit a public health response
- Gastroenteric virus → high concentrations in wastewater → good candidate for sensitive detection in wastewater
- Enclosed by a capsid -> sturdy and survive well in wastewater



WSLH Wastewater Monitoring Program



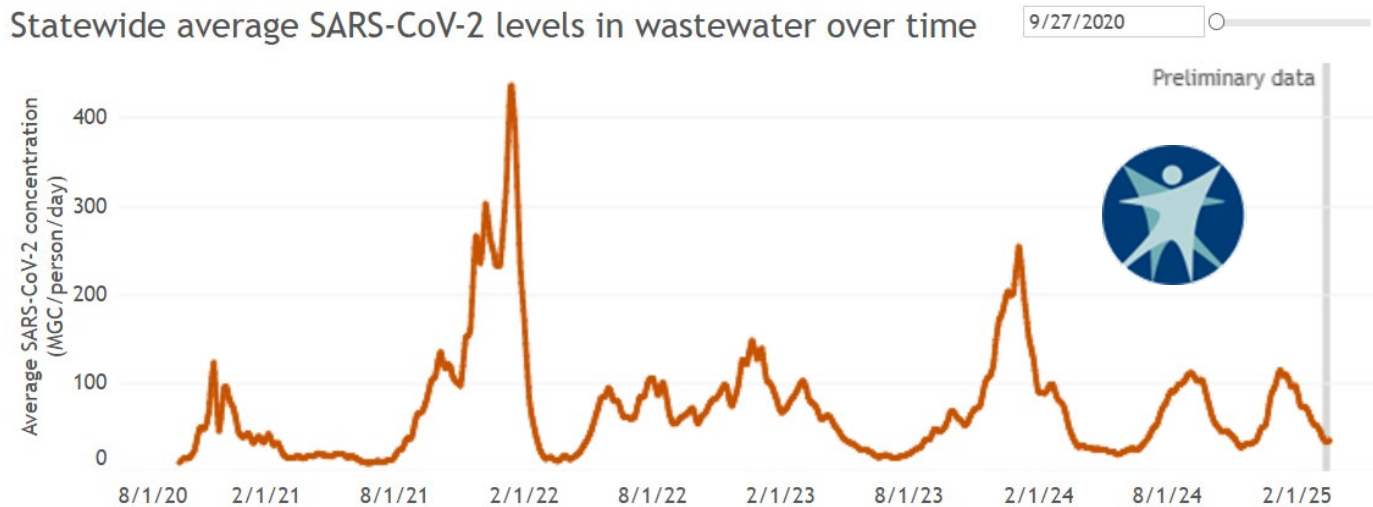
Current network of 44 sites

18,000+ samples collected since 2020

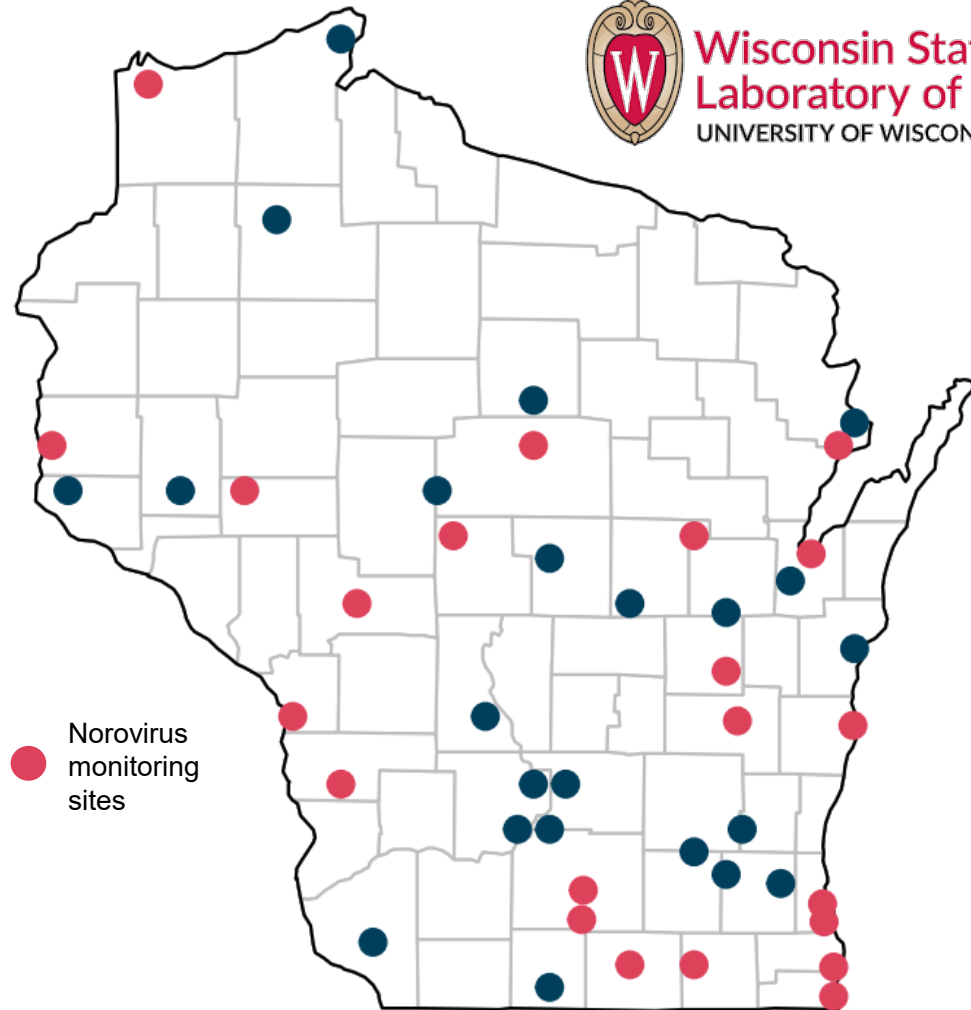
2,500+ sample sequences submitted to NCBI

Serves **~50%** of the state population

Statewide average SARS-CoV-2 levels in wastewater over time



Norovirus Wastewater Monitoring Program

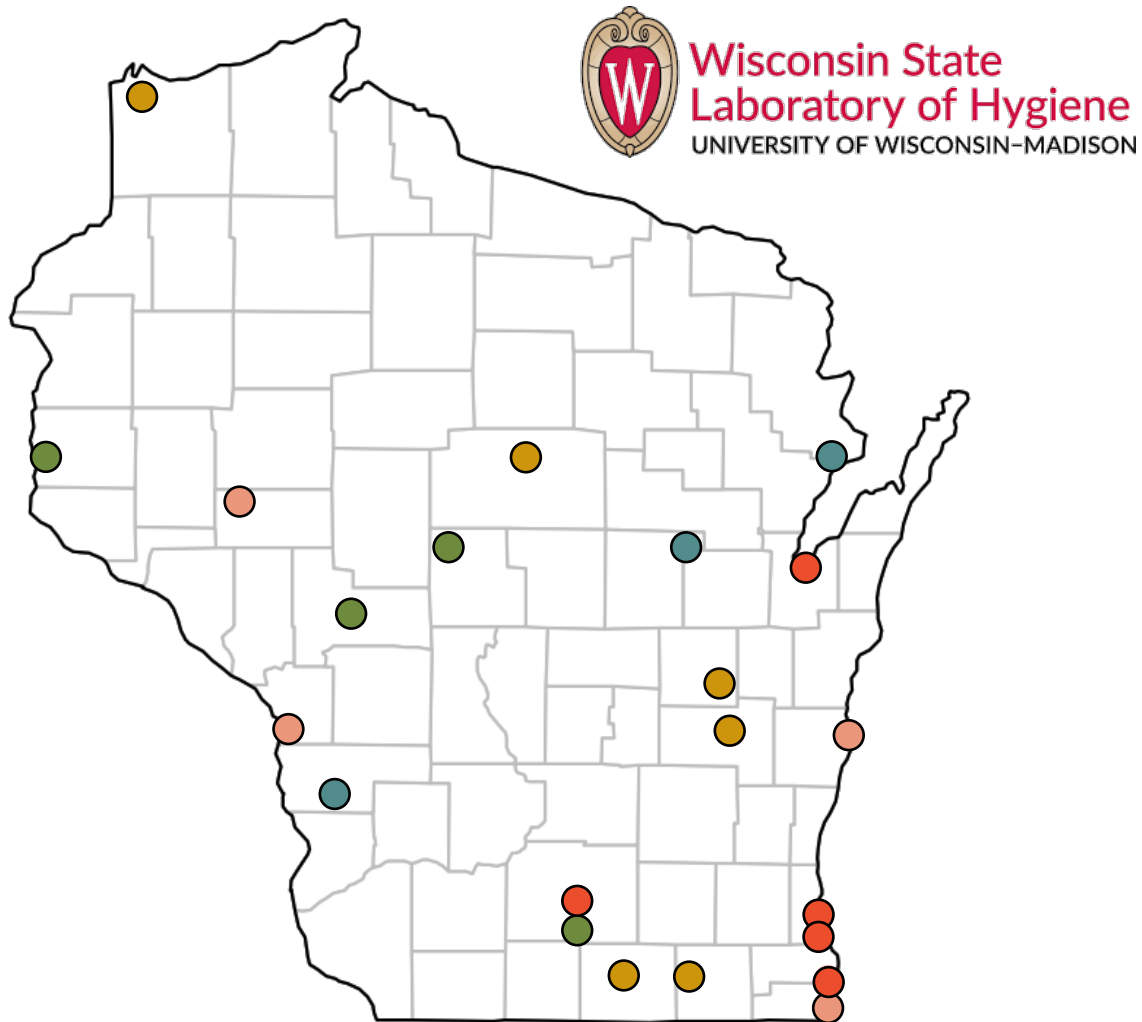


Currently 22 sites monitored

2,500+ samples collected since 2022

Serves **~40%** of the state population

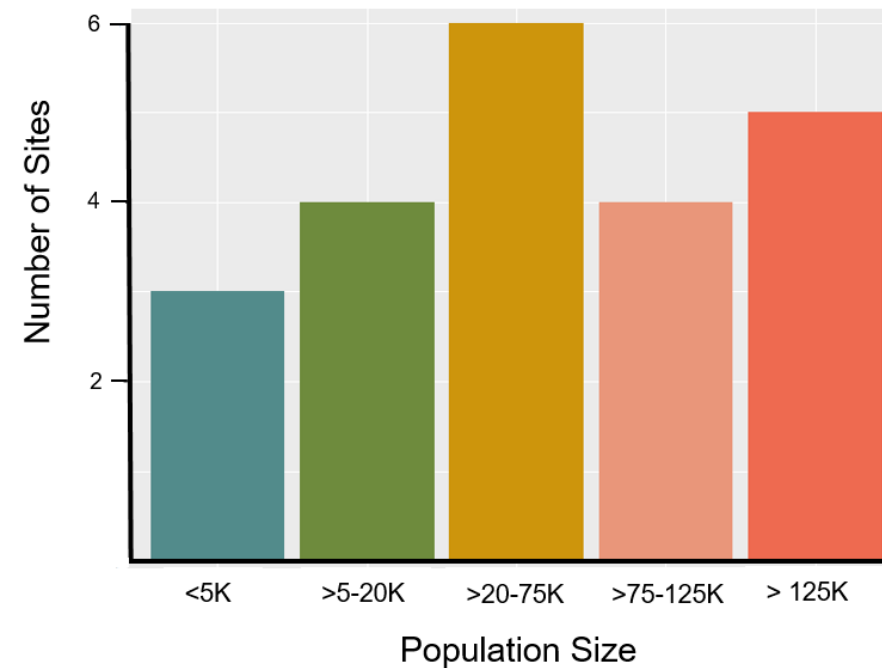
Norovirus Wastewater Monitoring Program



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Typical Sample Processing Workflow



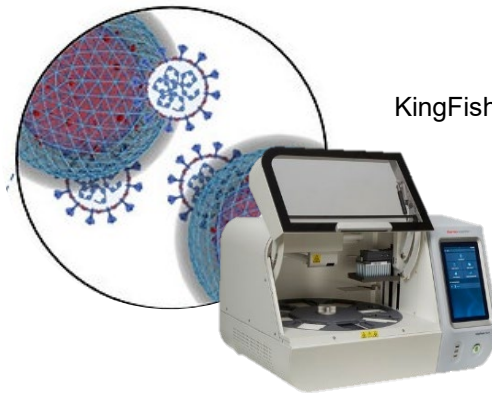
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Laboratory of Hygiene
UNIVERSITY OF WISCONSIN-MADISON



Typical Sample Processing Workflow



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KingFisher Apex

Automated concentration
Nanotrap® ER2 (Ceres)
nanoparticle capture



KingFisher Flex

Automated extraction
Maxwell® HT Viral TNA
kit (Promega)

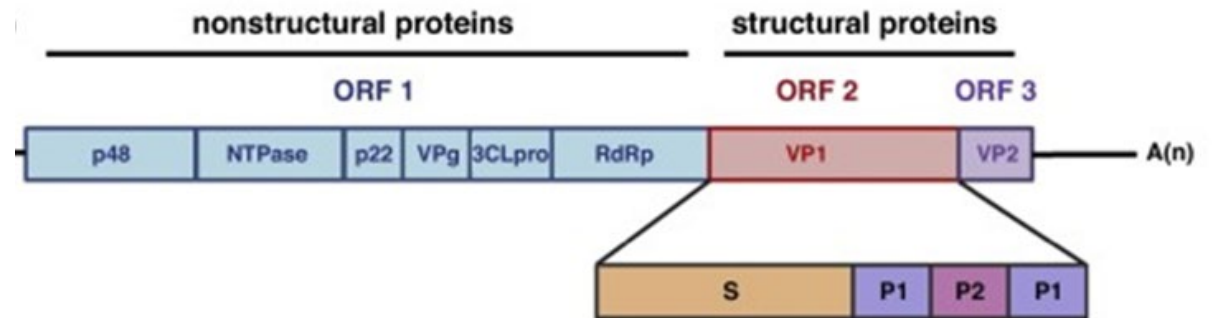
Typical Sample Processing Workflow



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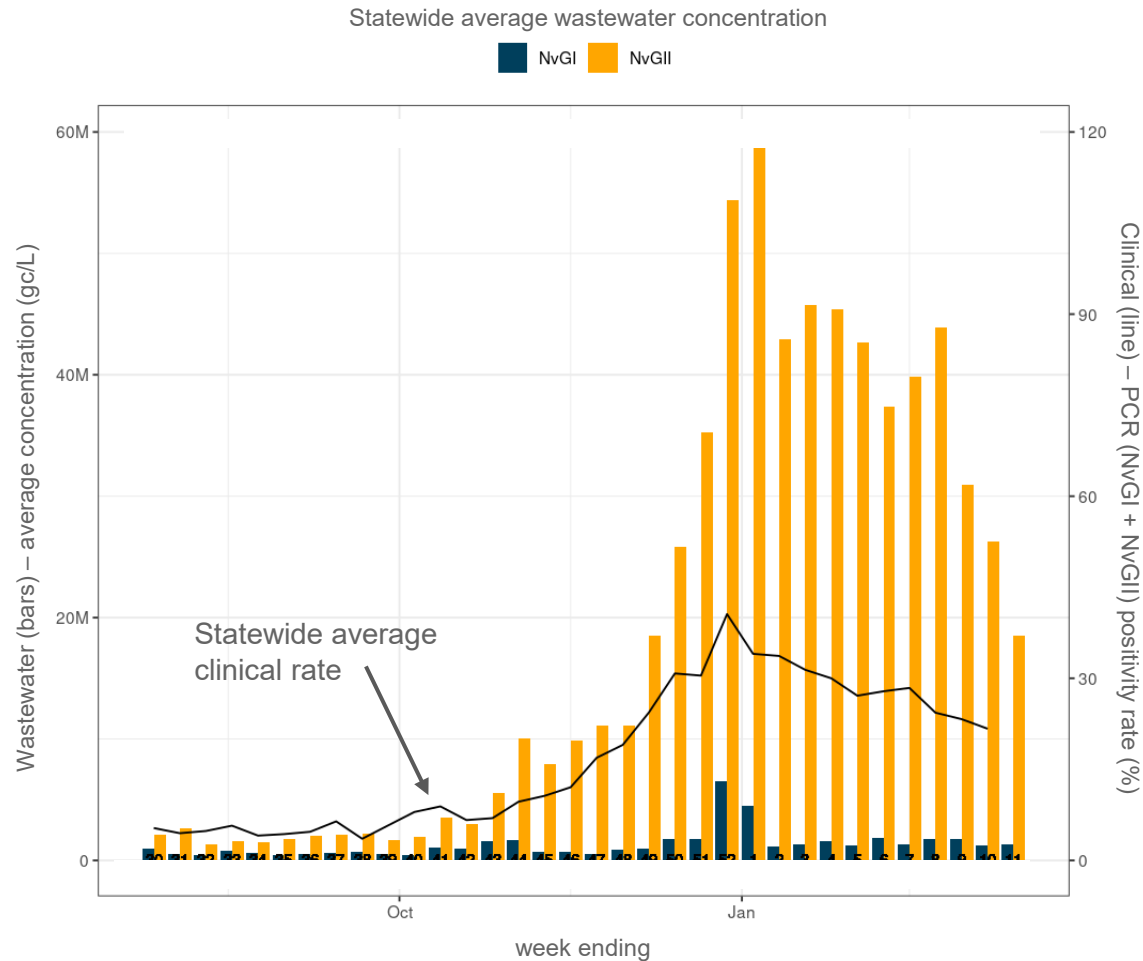


Digital PCR
QIAcuity (Qiagen)
NvGI + *NvGII* assays

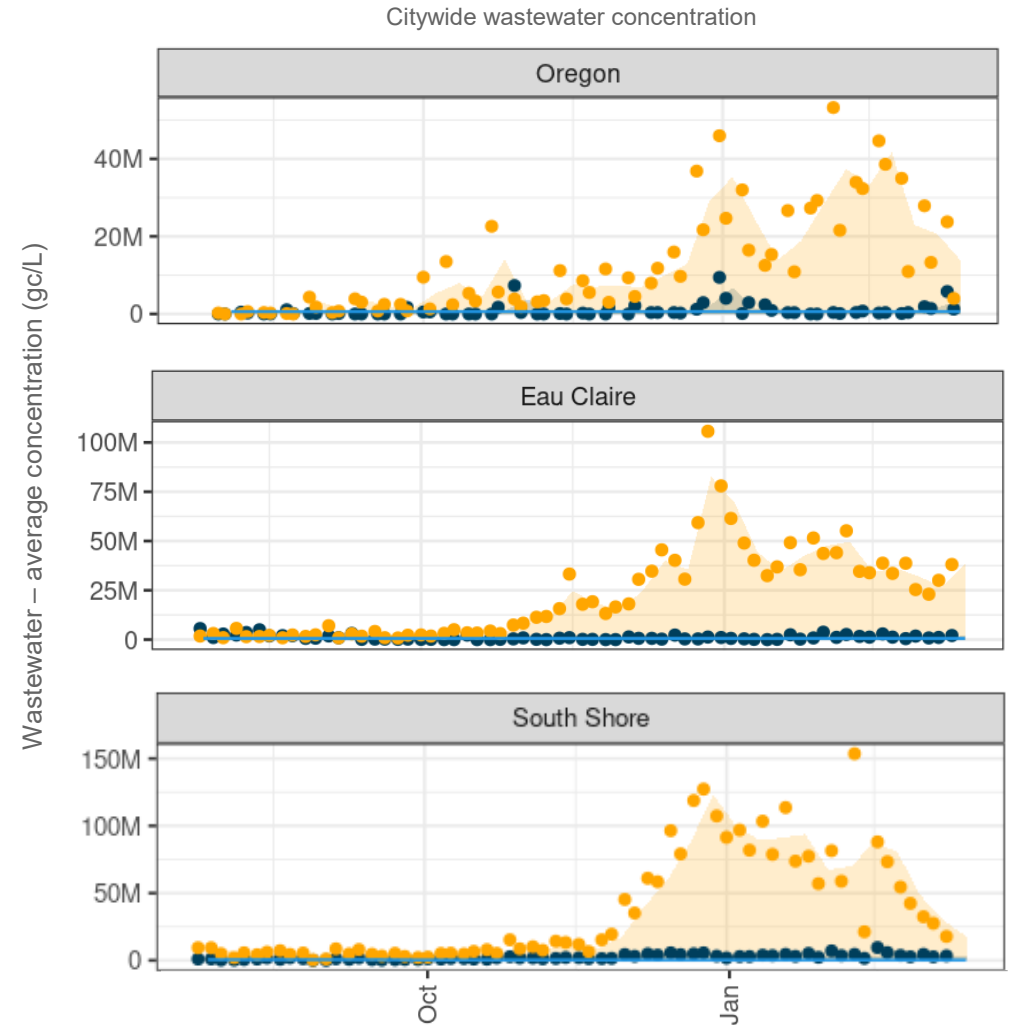


Internal Norovirus Dashboard

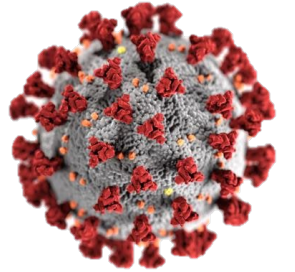
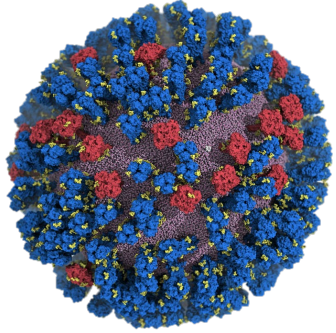
State level



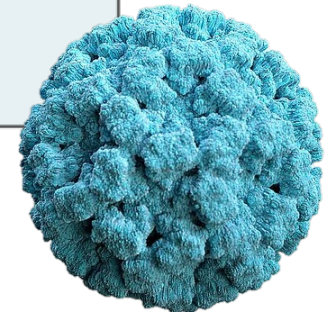
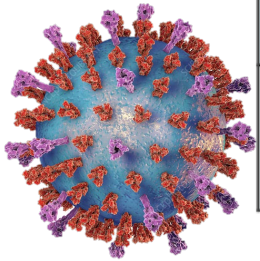
City level



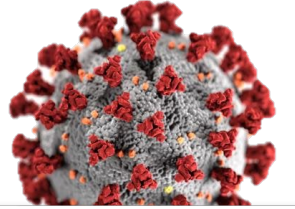
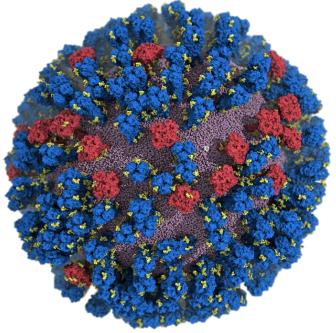
Challenges with Assay Validation



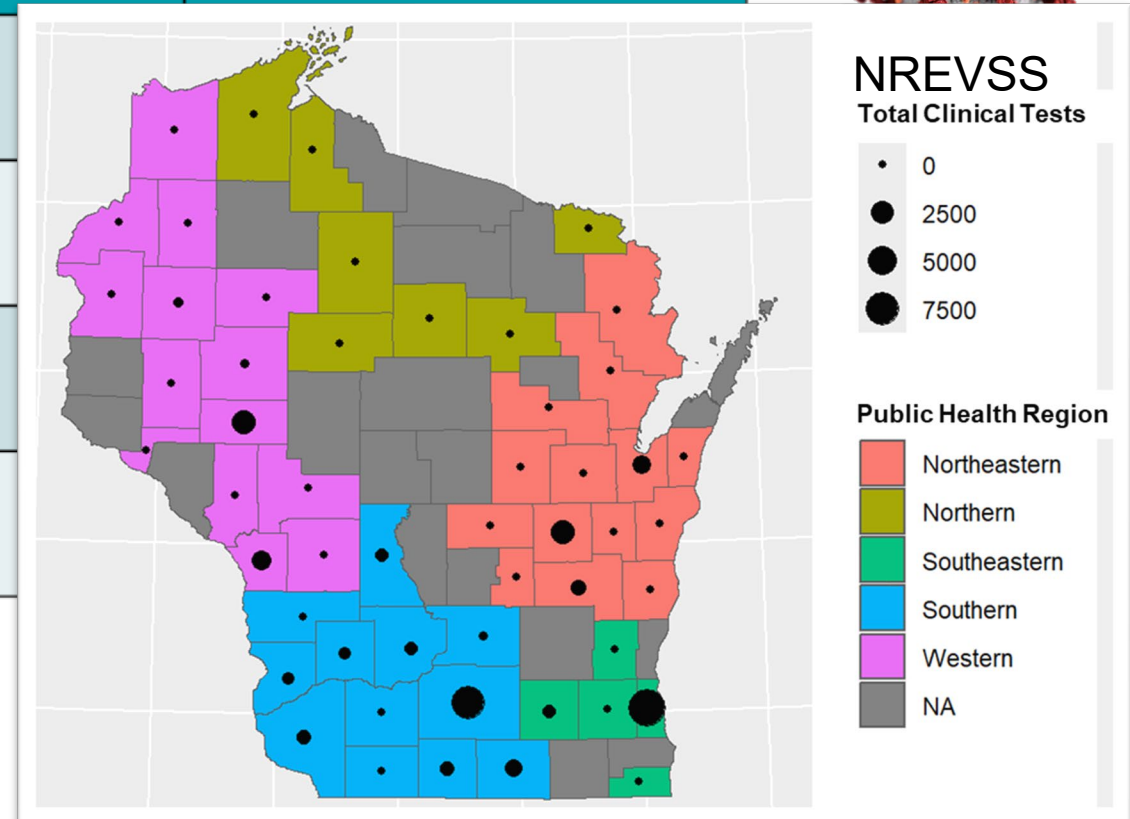
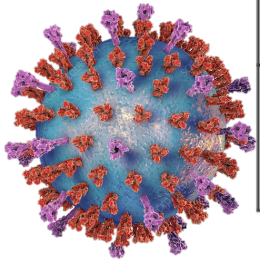
	Clinical data	Wastewater data (sensitivity)
COVID-19	✓	✓
Influenza	✓	✗
RSV	✓	✗
Norovirus	✗	✓



Challenges with Assay Validation

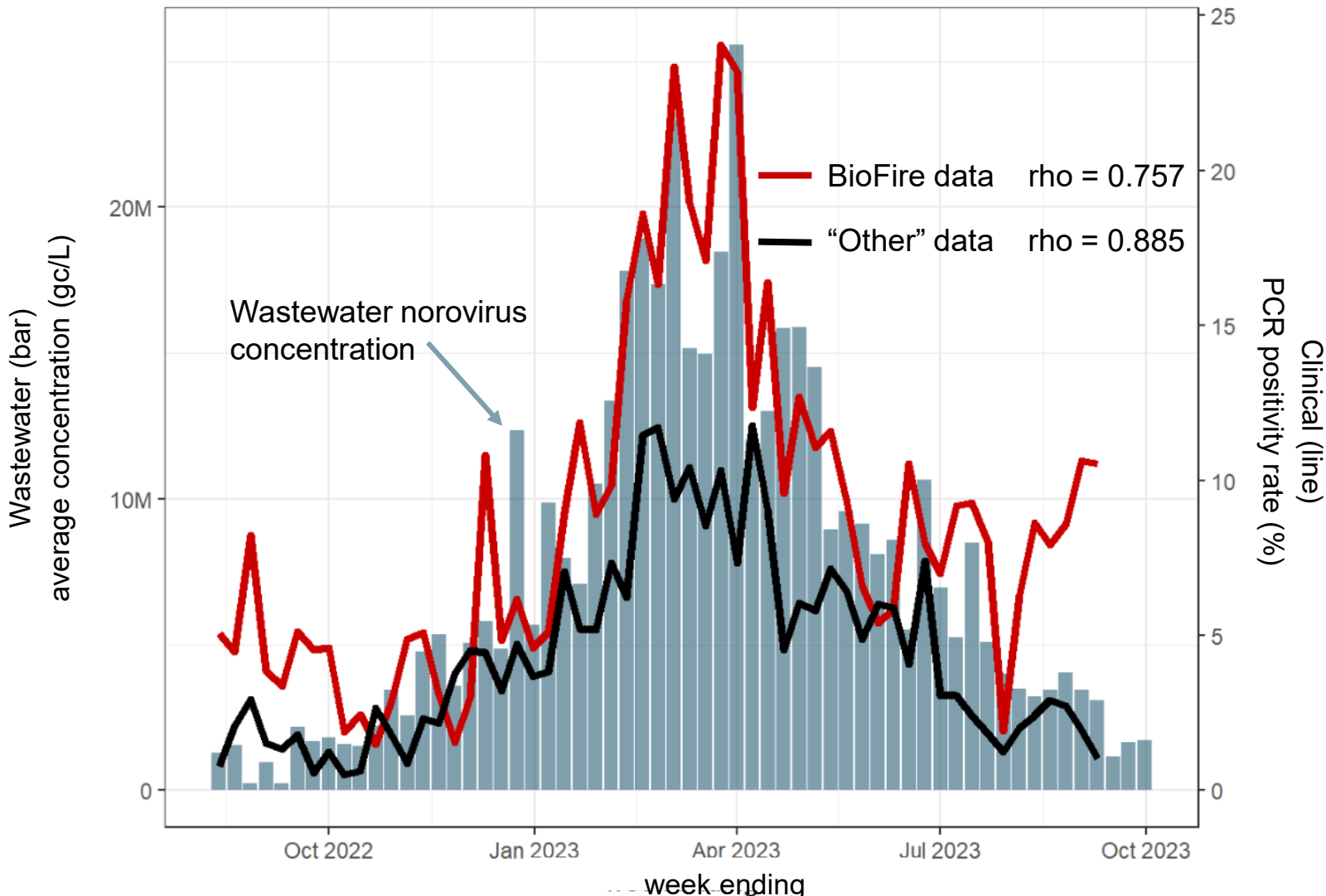


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2022-2023 Retrospective Study in a Large Sewershed

Wastewater Norovirus Levels in Madison, WI vs
Statewide Clinical PCR Positivity Rates

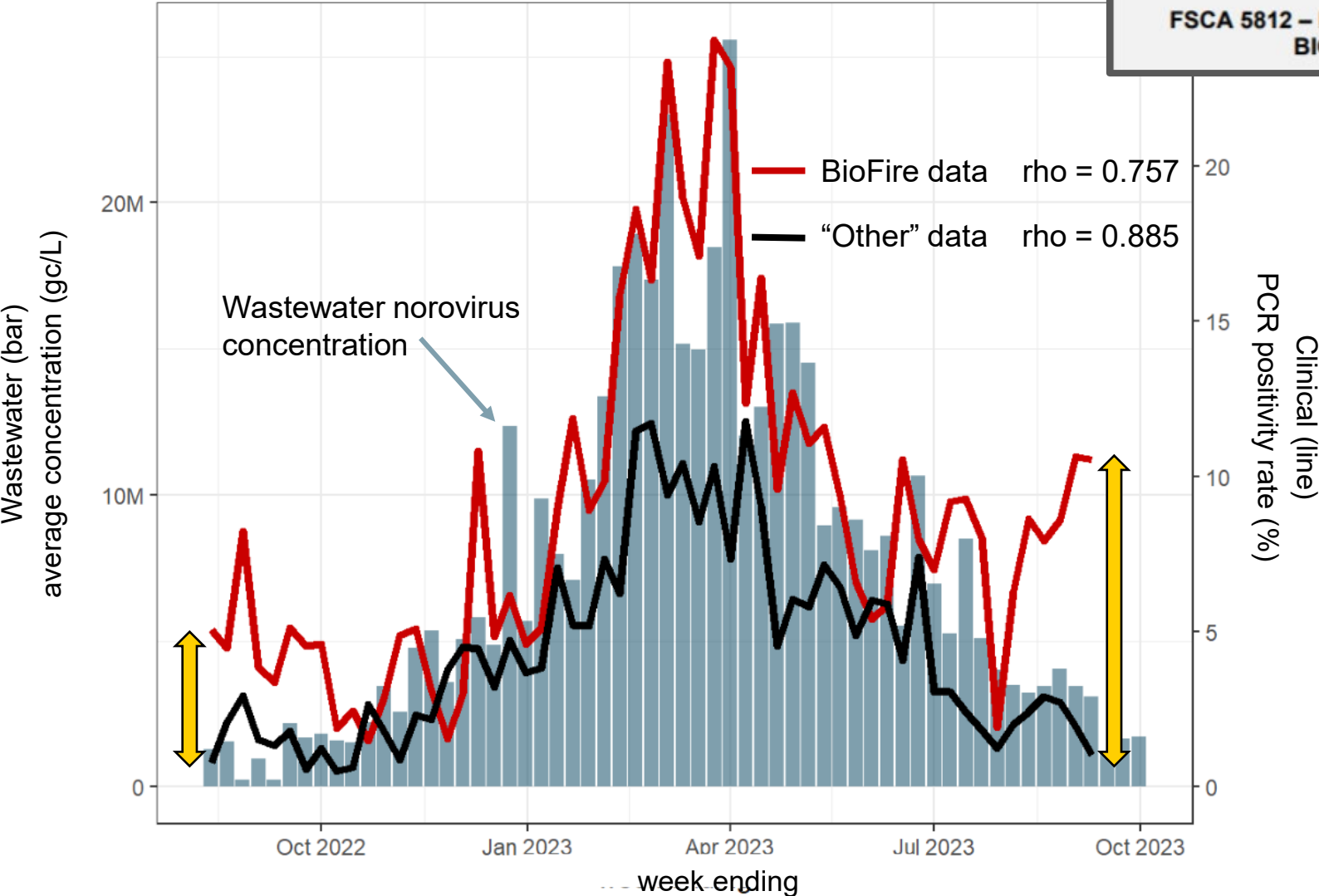


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2022-2023 Retrospective Study

Wastewater Norovirus Levels in Madison, WI vs Statewide Clinical PCR Positivity Rates



IMPORTANT:

URGENT: MEDICAL DEVICE RECALL

BIOFIRE® FILMARRAY® Gastrointestinal (GI) Panel – Ref. Number: RFIT-ASY-0116 & RFIT-ASY-0104

FSCA 5812 – Increased Risk of False Positive Norovirus Results with the BIOFIRE® FILMARRAY® Gastrointestinal (GI) Panel

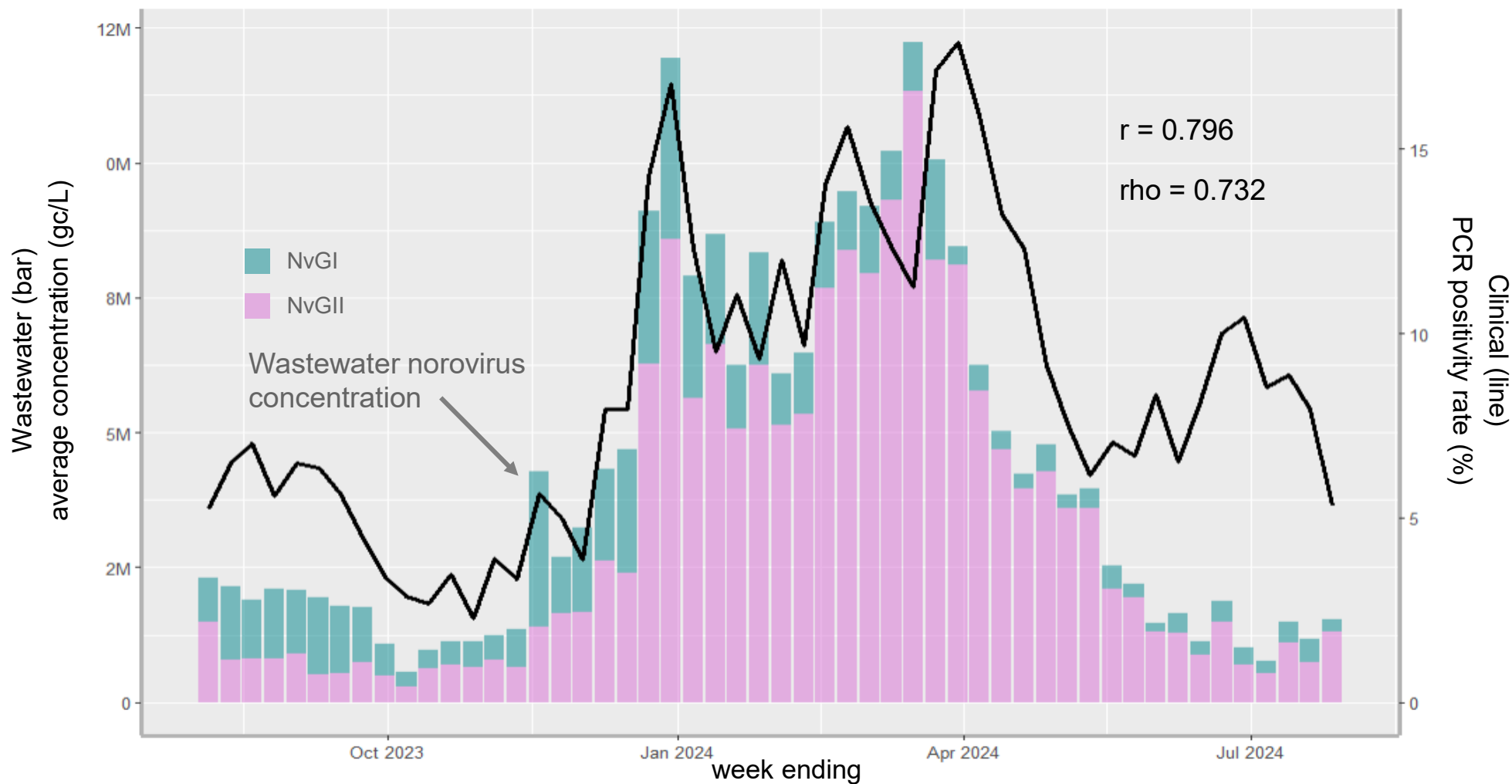


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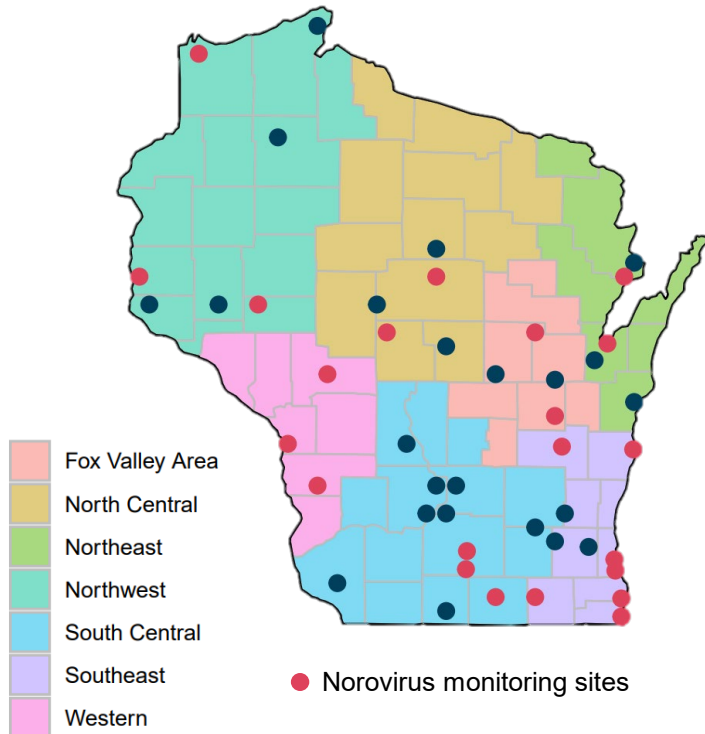


2023-2024 Norovirus Season

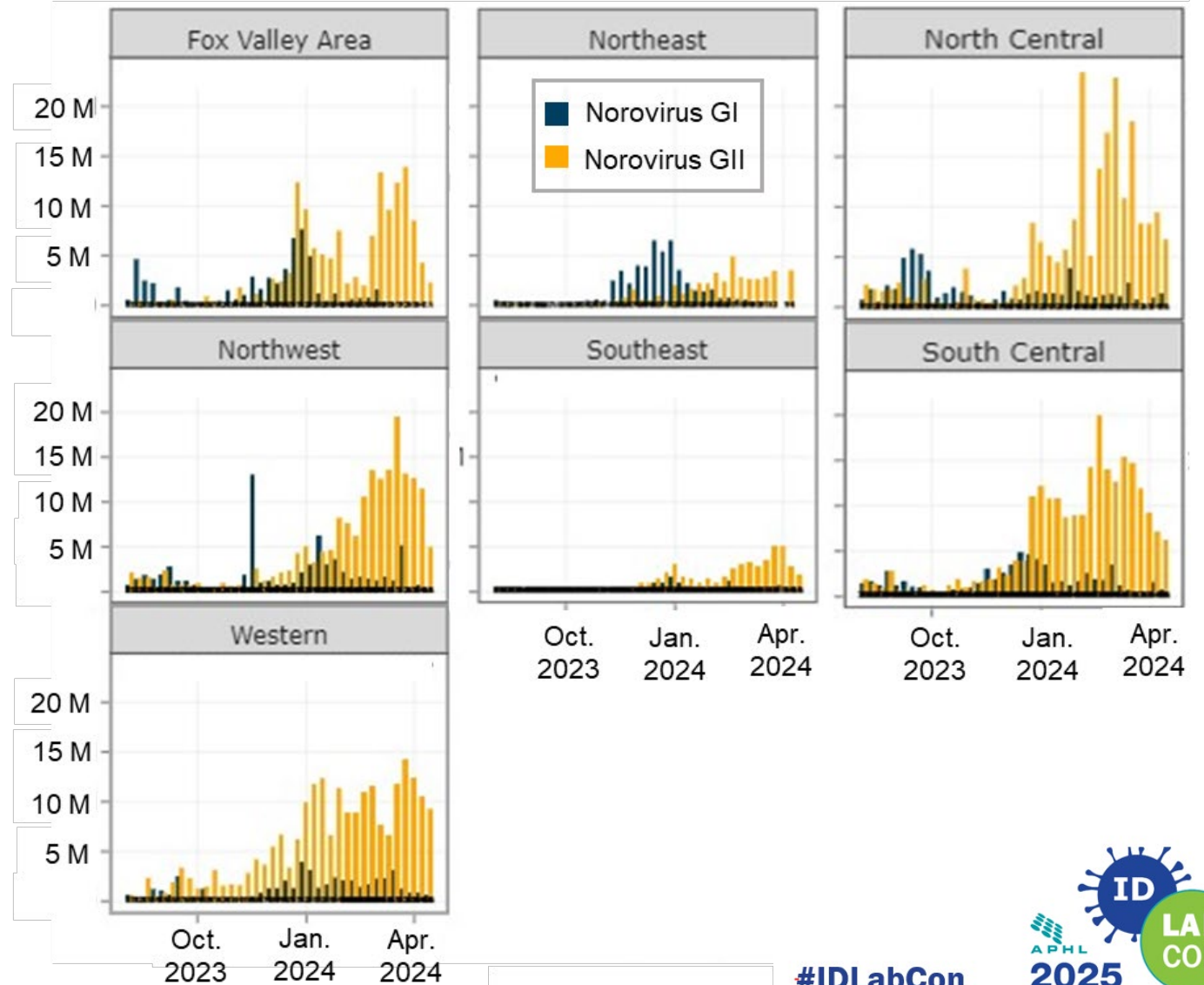
Statewide Wastewater Norovirus Levels vs
Statewide Clinical PCR Positivity Rates



Regional Trends of Norovirus in Wastewater



Weekly Average Wastewater Concentration (gc/L)

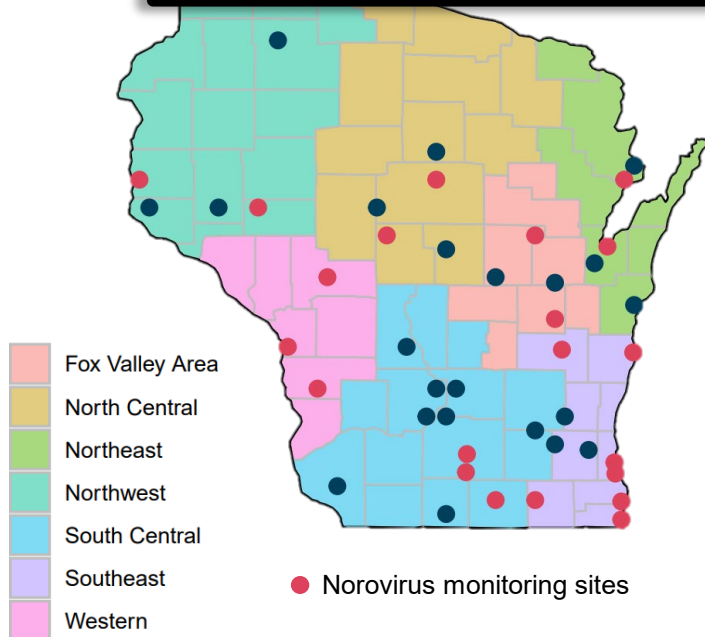


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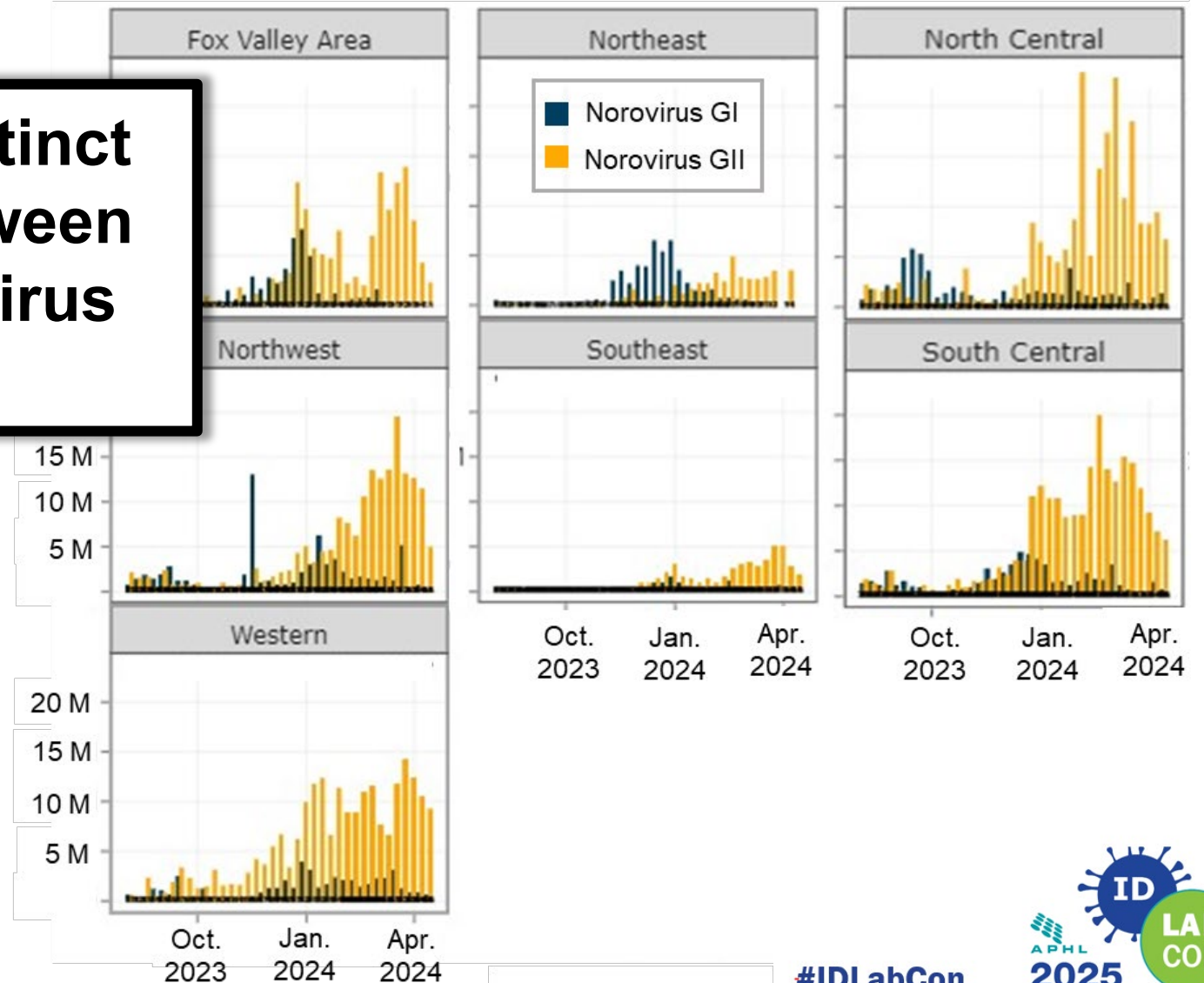


Regional Trends of Norovirus in Wastewater

WW data shows distinct regional trends between the two main norovirus genogroups



Weekly Average Wastewater

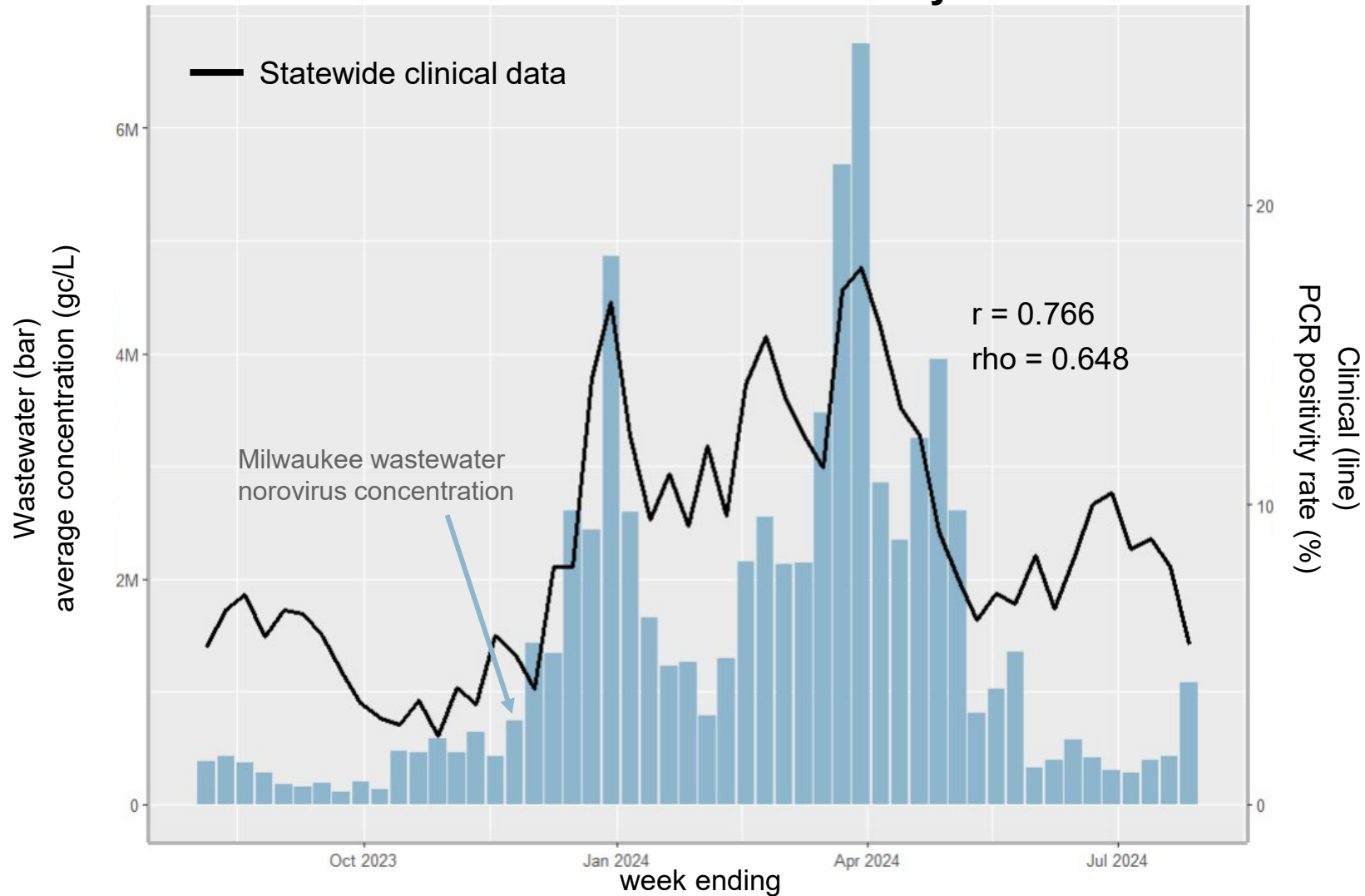


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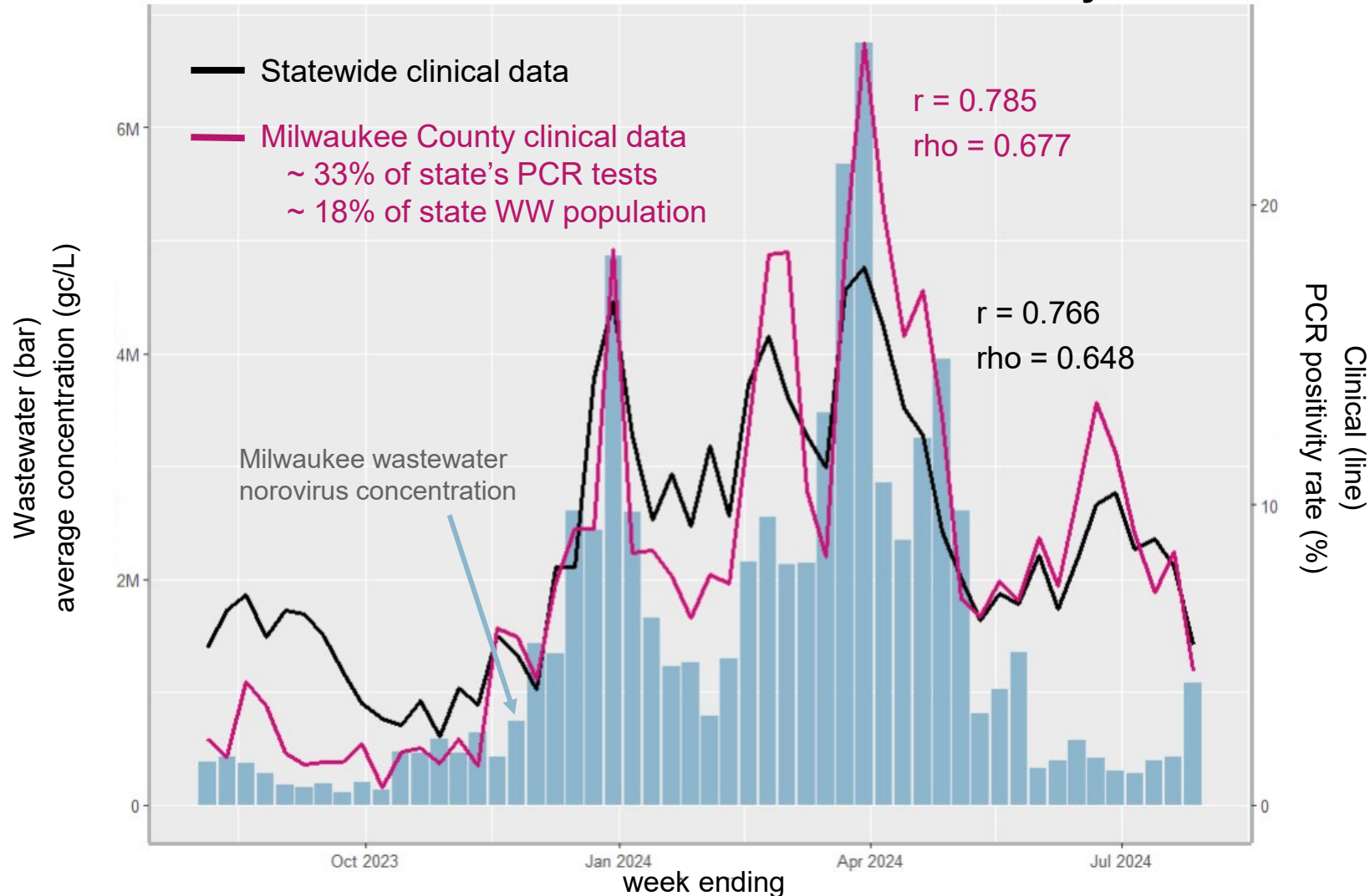
Norovirus Correlations in a Major City

**Milwaukee Wastewater Norovirus Levels vs
Statewide Clinical PCR Positivity Rates**



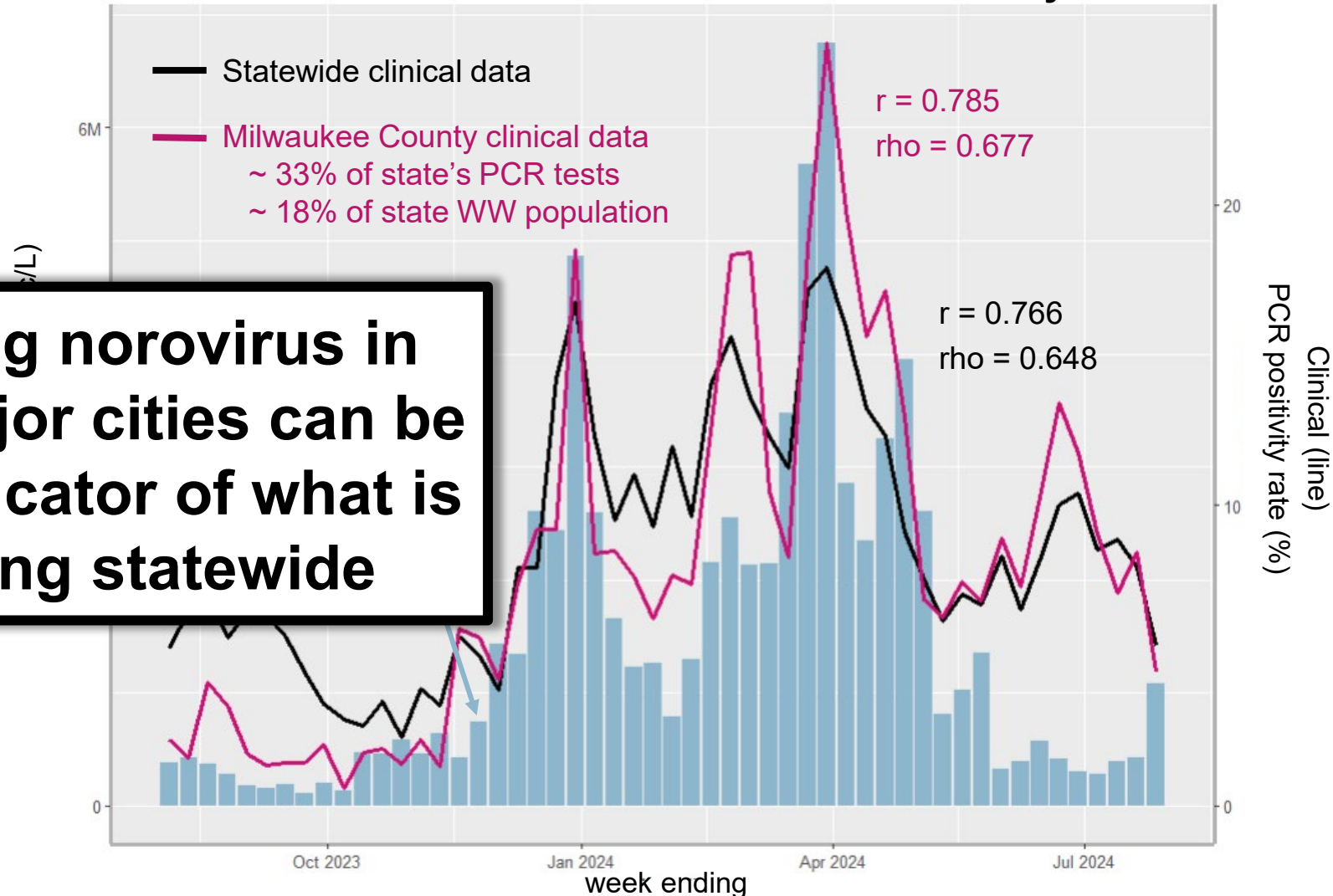
Norovirus Correlations in a Major City

Milwaukee Wastewater Norovirus Levels vs Statewide and Milwaukee Clinical PCR Positivity Rates



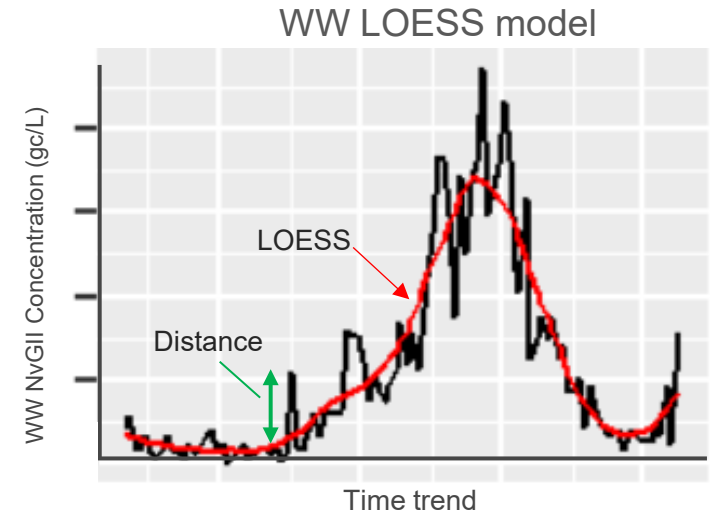
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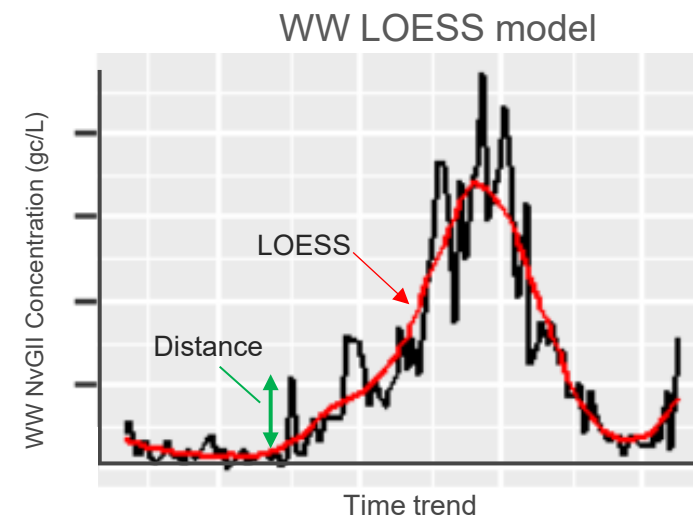
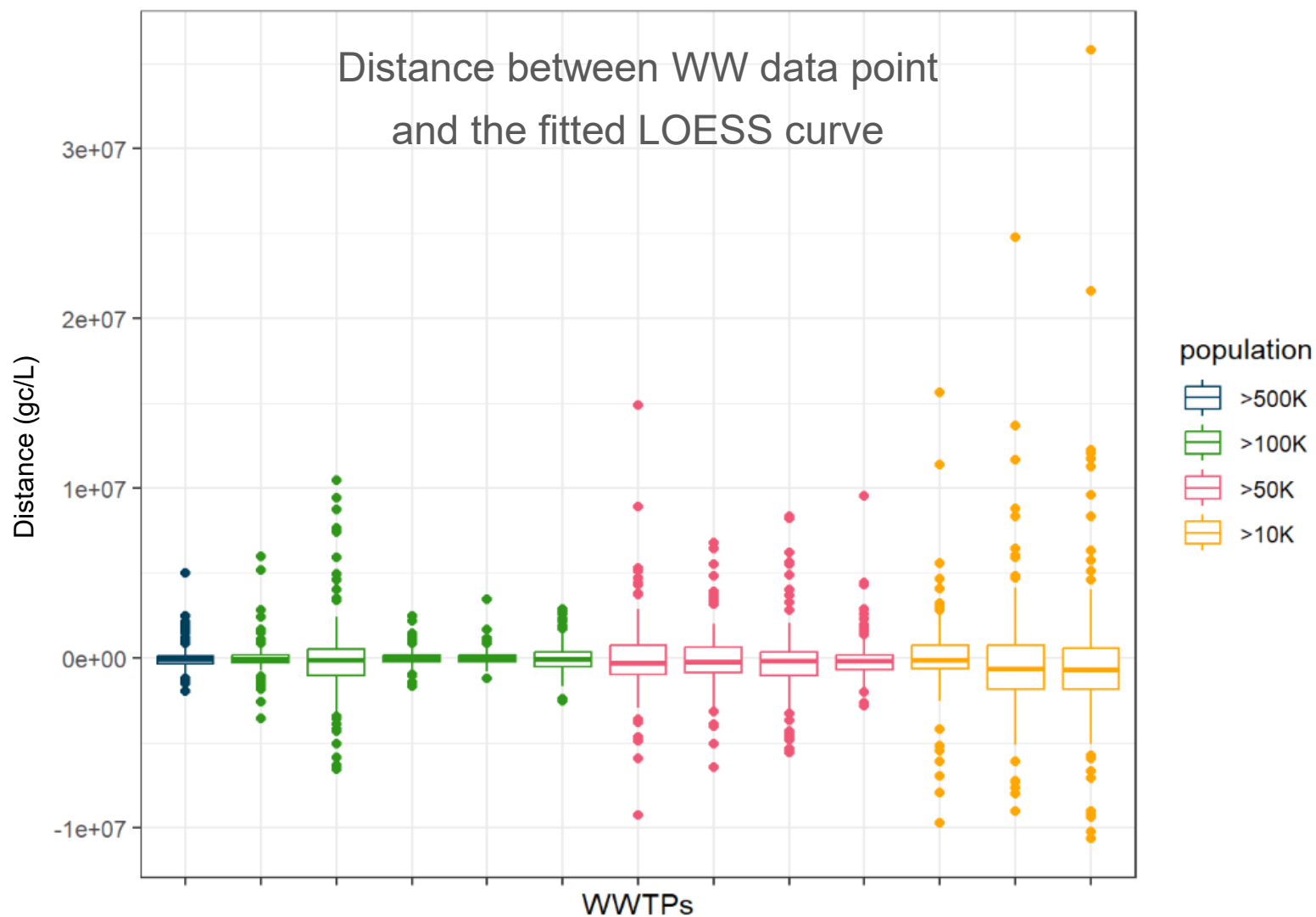


monitoring norovirus in
WW for major cities can be
a good indicator of what is
happening statewide

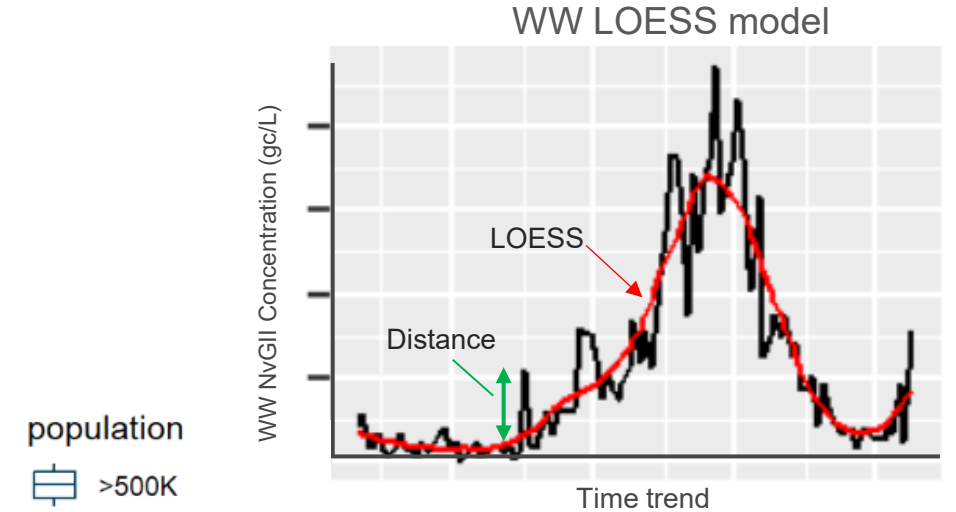
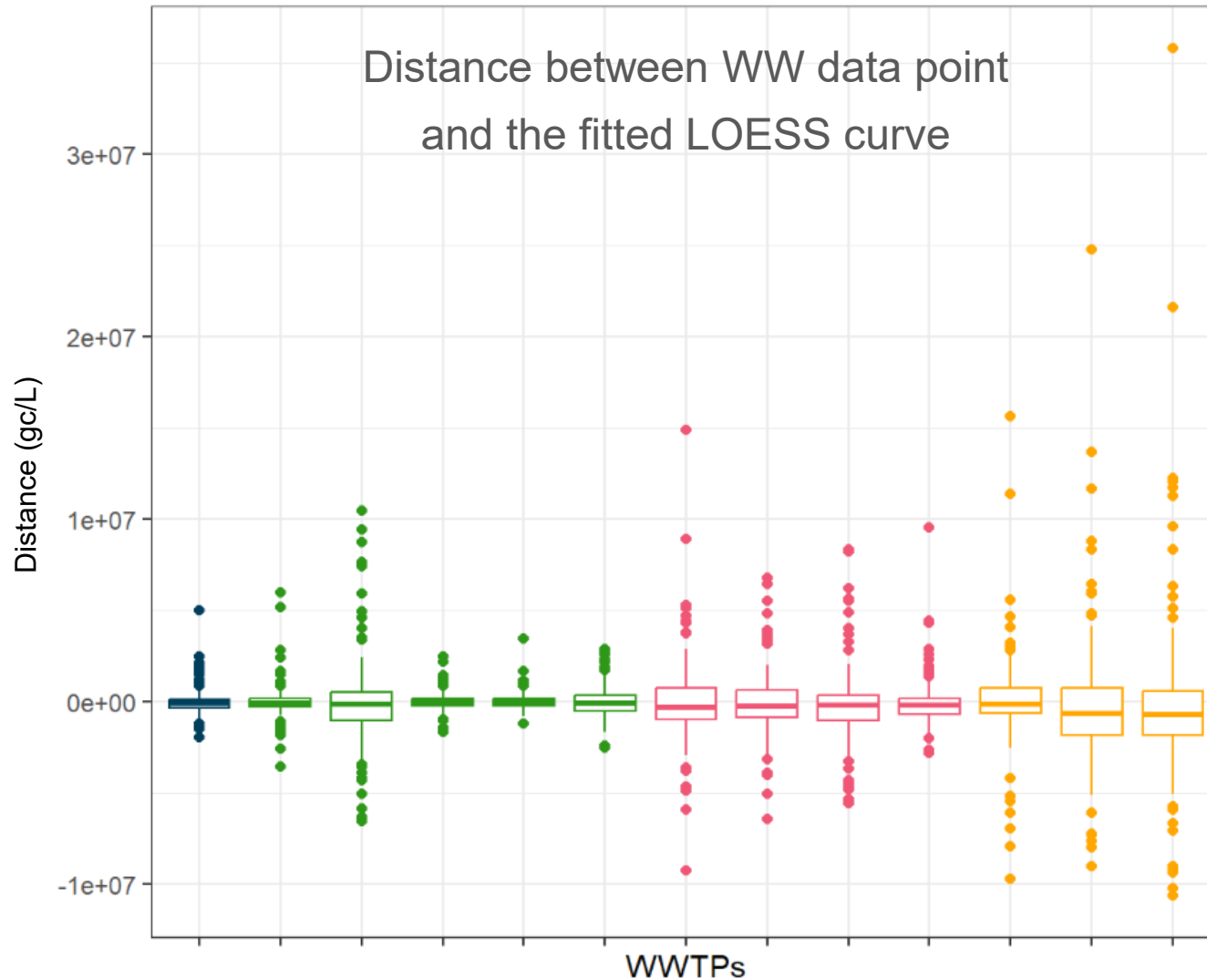
Population Size Effect on Variability



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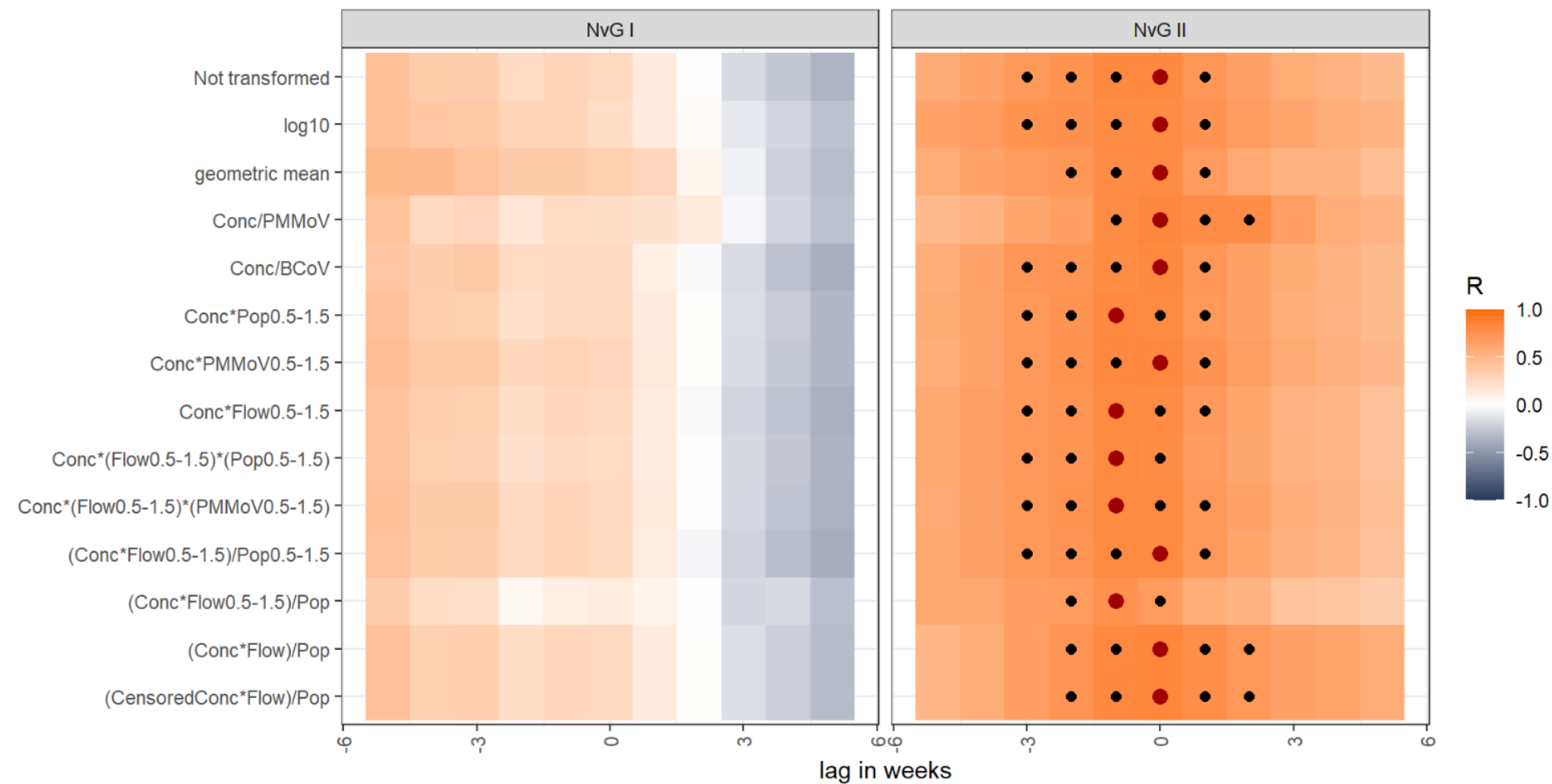


Population Size Effect on Variability

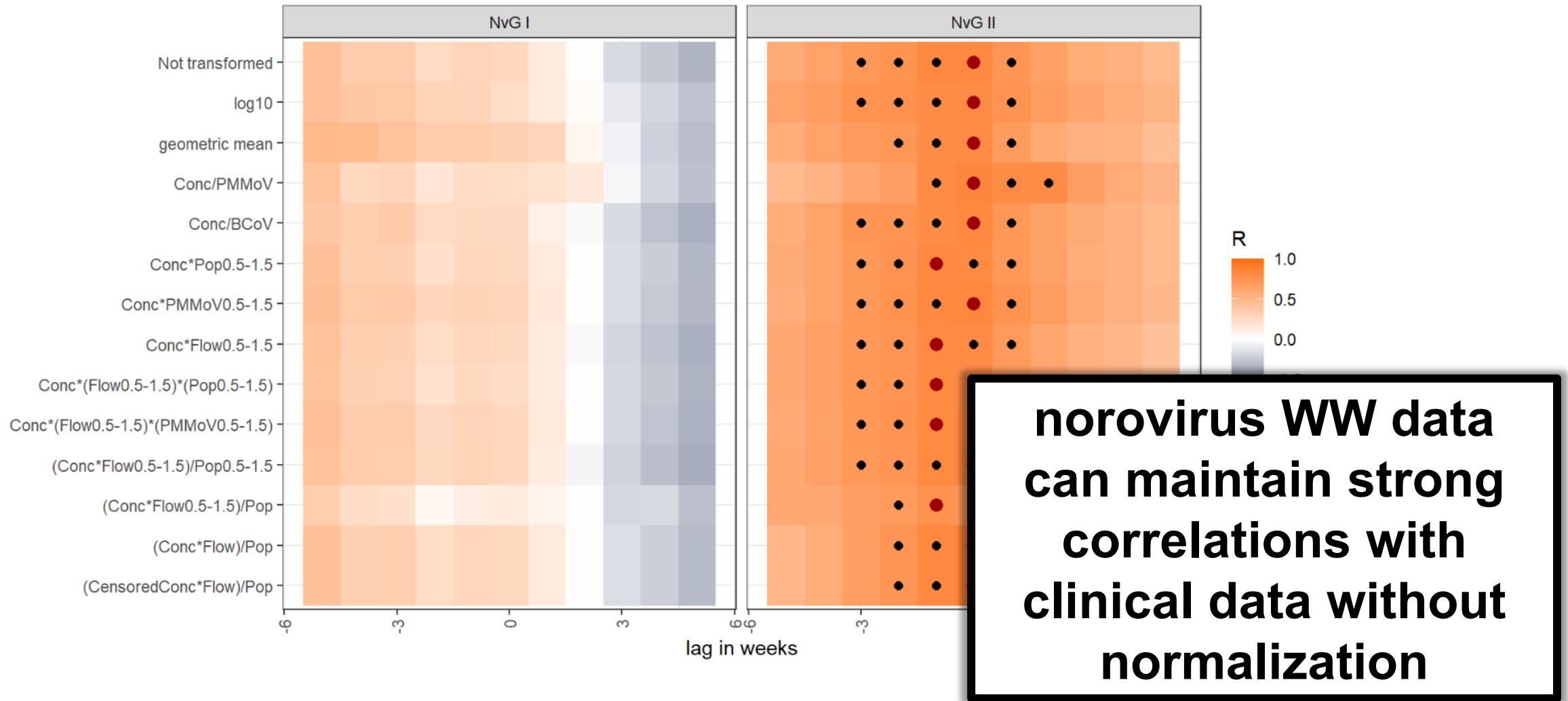


**larger population sizes
served by WWTP = lower
variability in WW trends**

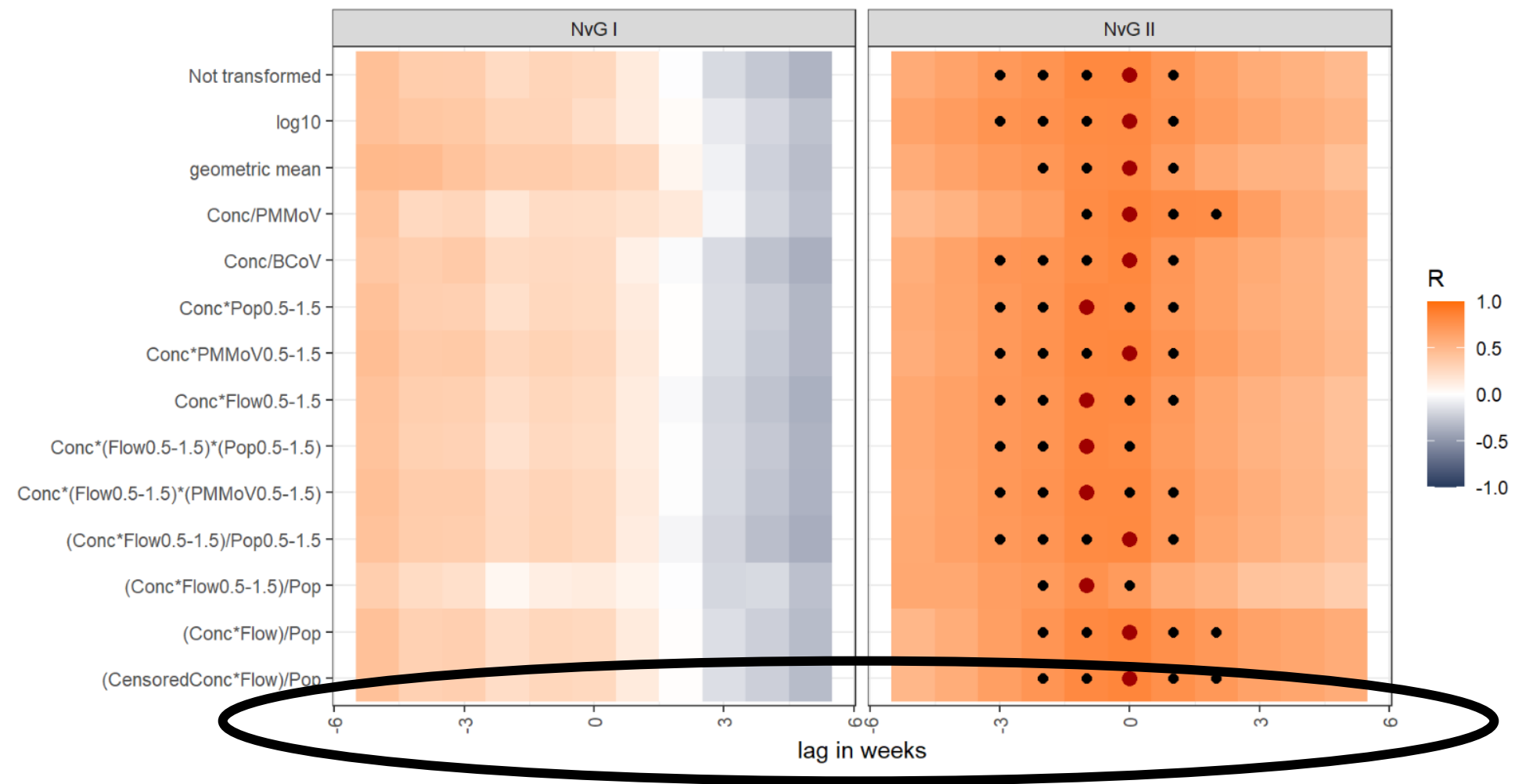
Correlation Strength by Wastewater Normalization Type



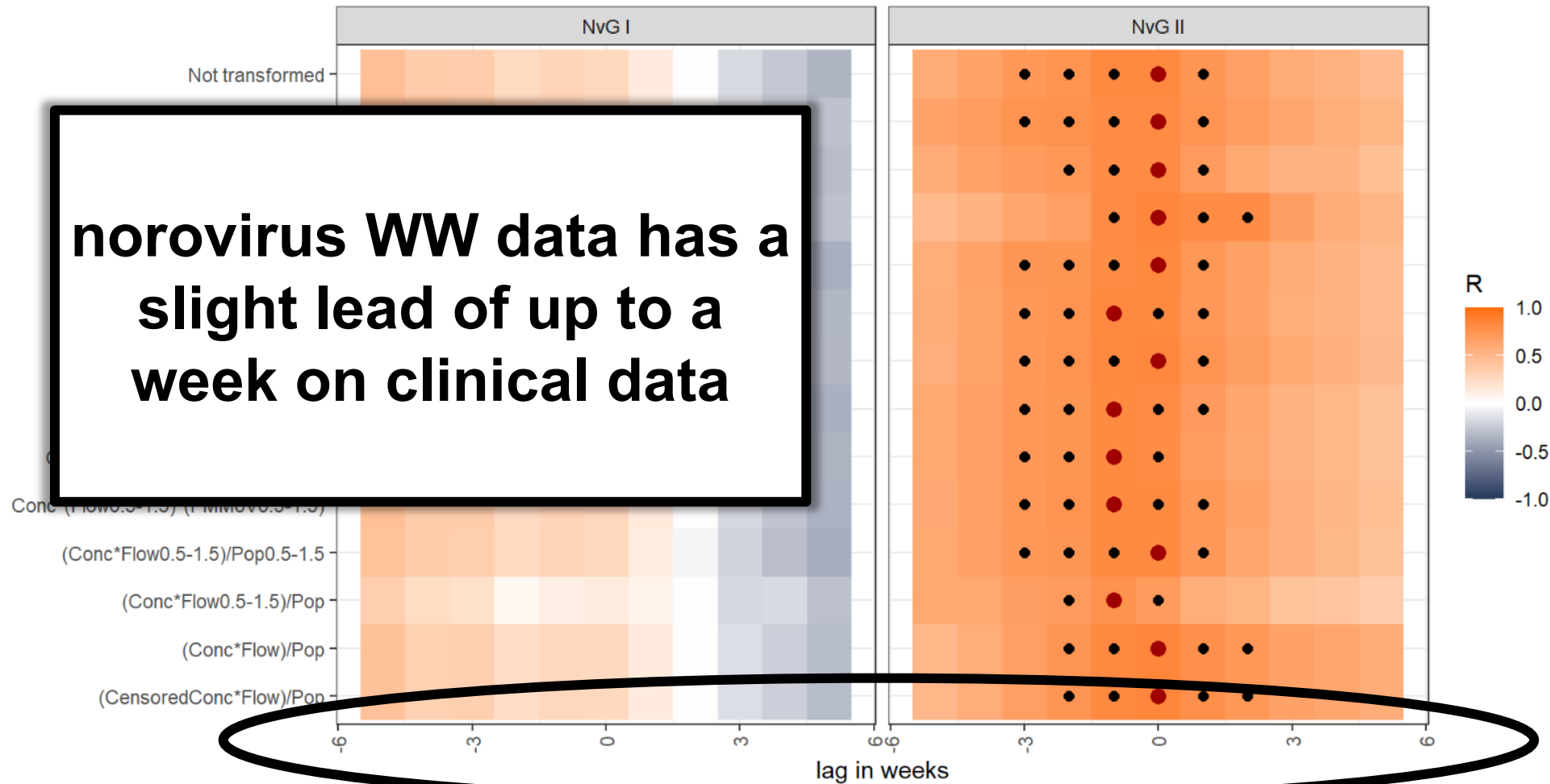
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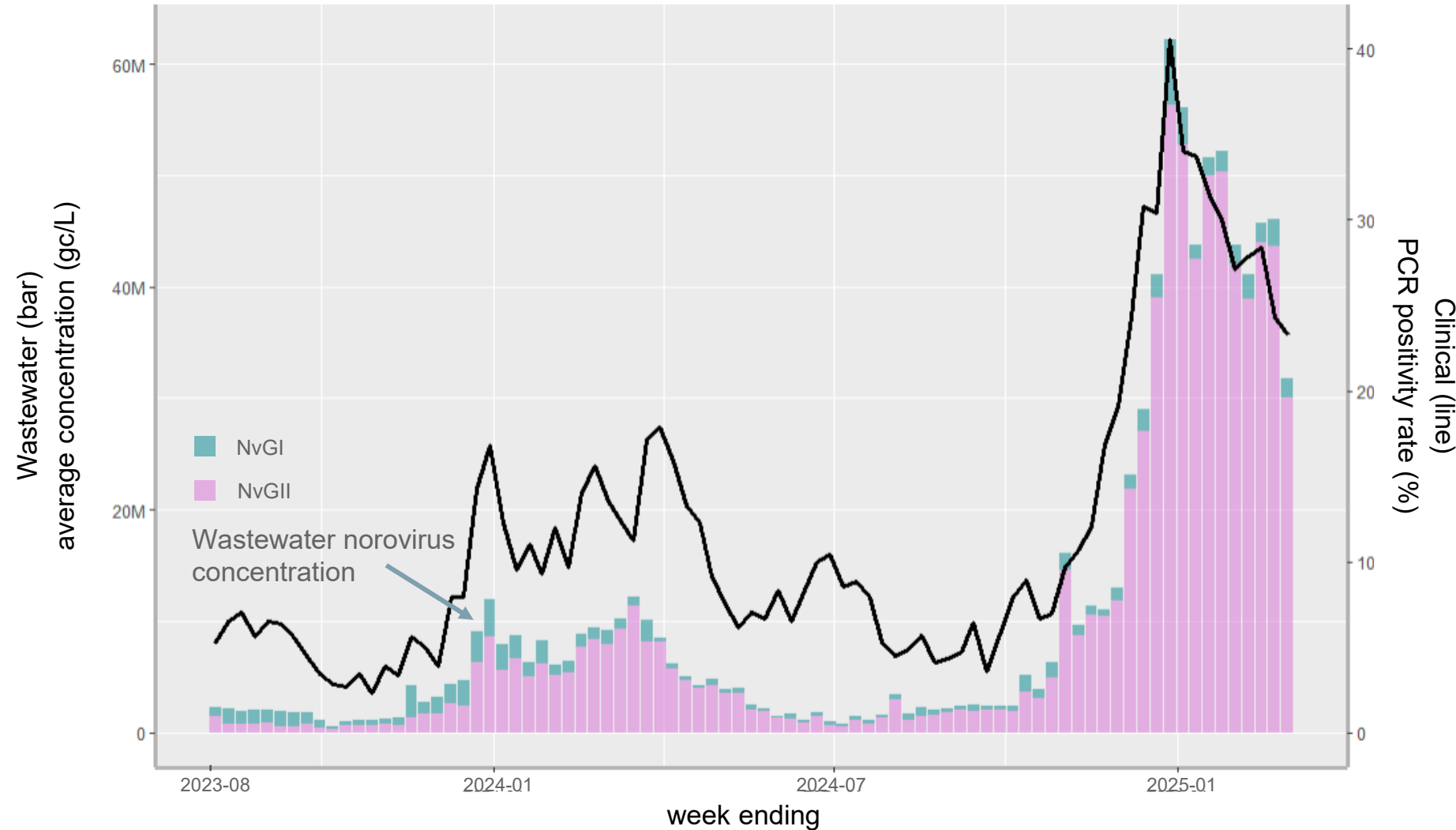


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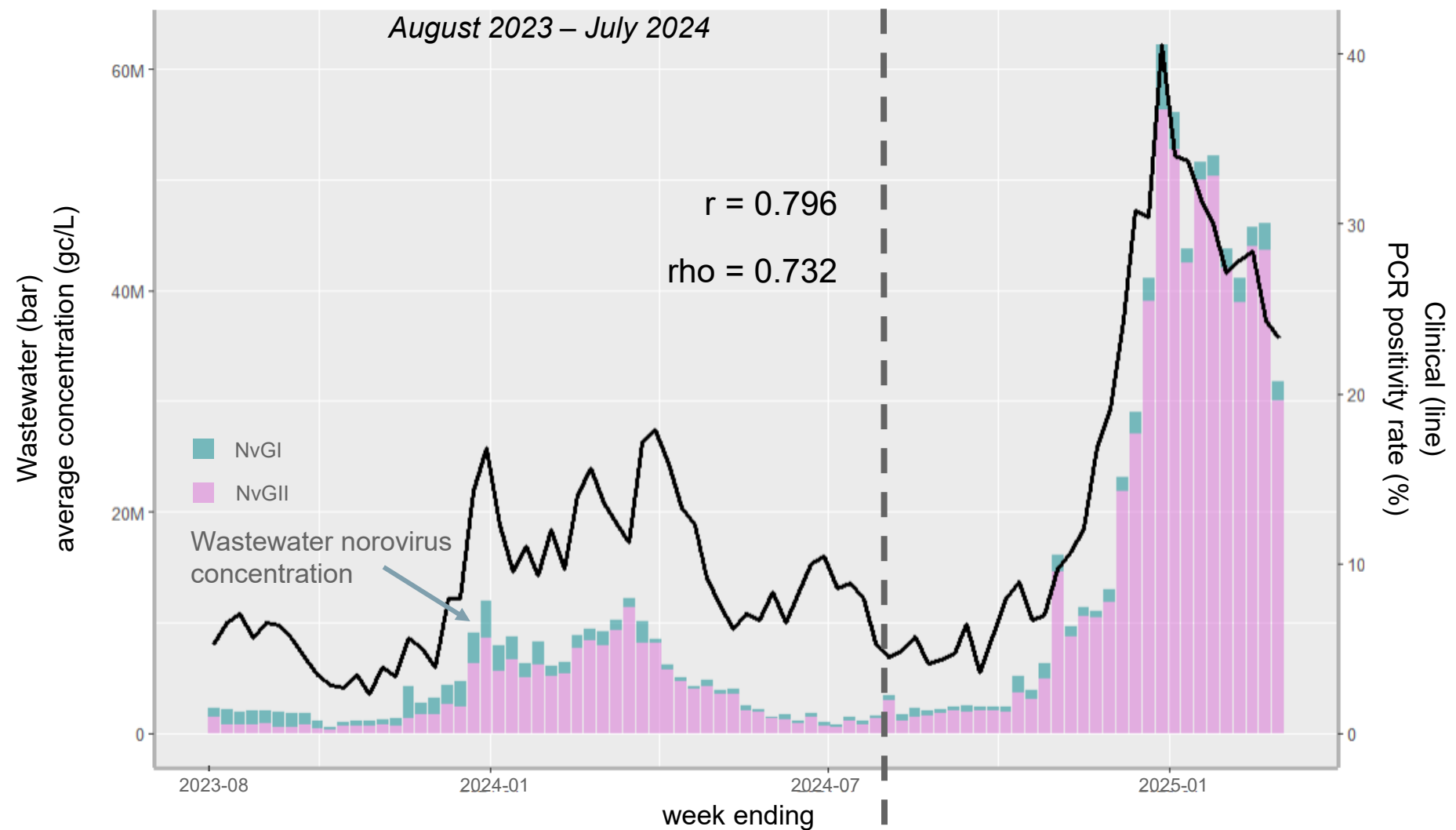
Differences in Norovirus Seasons

Statewide Wastewater Norovirus Levels vs
Statewide Clinical PCR Positivity Rates



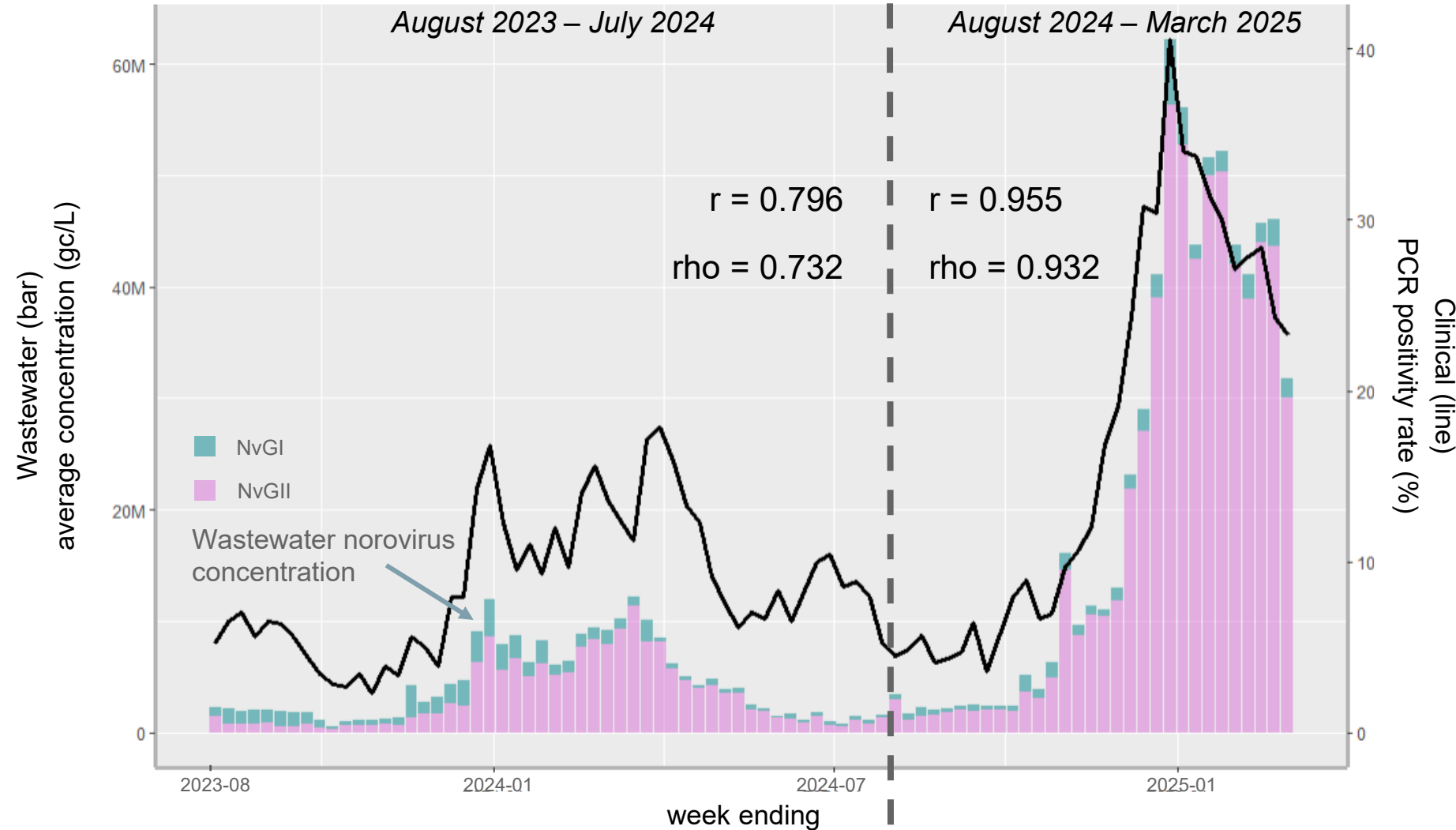
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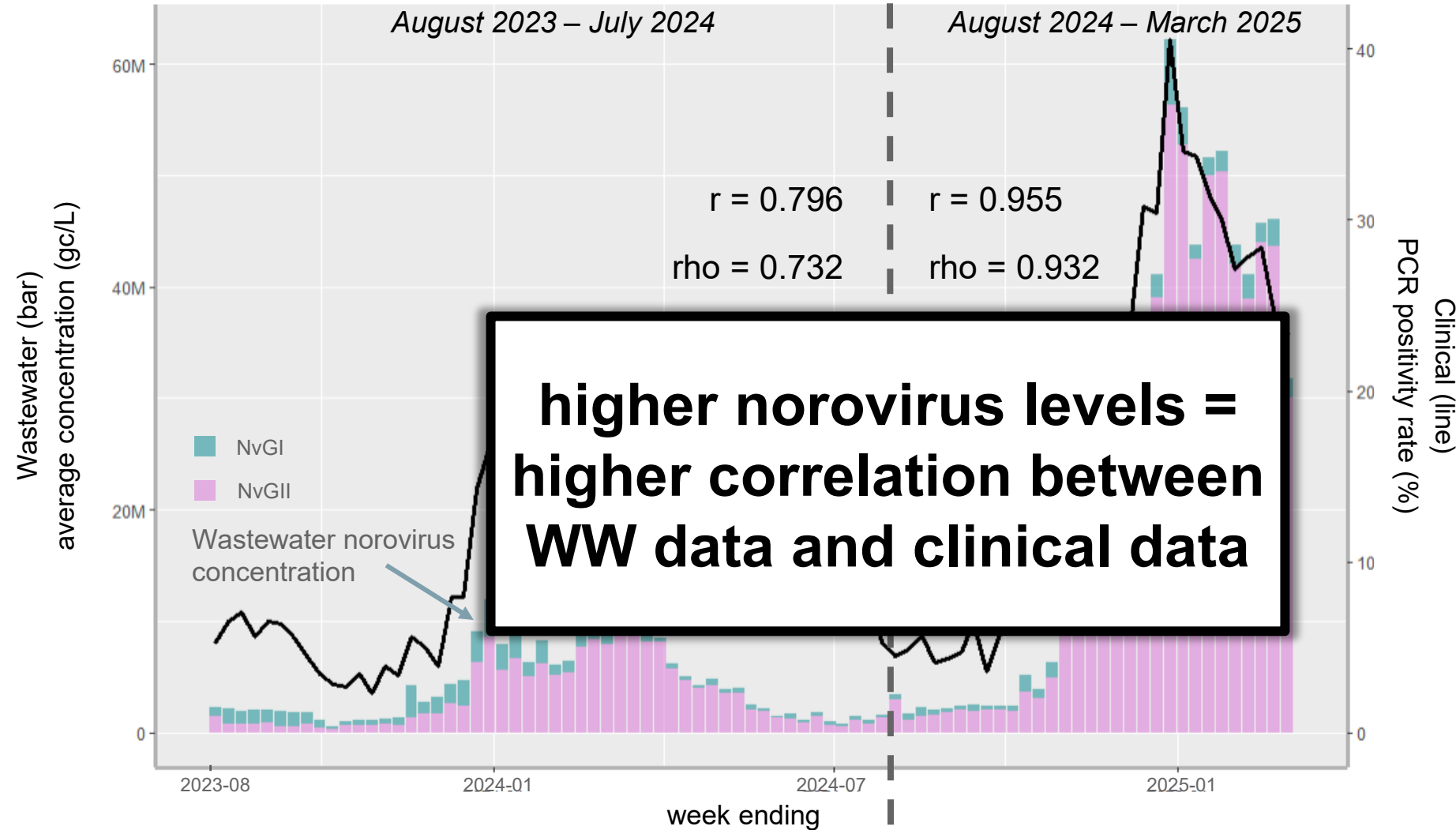
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Conclusions

- Wastewater norovirus data correlates well with clinical PCR positivity rates
- Wastewater data shows distinct regional trends between the two main norovirus genogroups
- Wastewater surveillance is complementary to the clinical data and helps fill in the gaps:
 - e.g., can help investigate discrepancies in public health data
 - wastewater may provide some lead over the clinical data, especially in areas with less clinical testing
- Wastewater based surveillance of norovirus can be difficult to validate due to lack of robust clinical data
- Wastewater monitoring can help support investigations of gastrointestinal illness outbreaks



Wisconsin Wastewater-Based Surveillance Program Team



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CDC Field Officer



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Epidemiologist

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Dagmara Antkiewicz
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Shuchen Feng
Scientist
Angela Schmoltdt

Academic & Other Partners

WWTF Operators



WISCONSIN WASTEWATER
OPERATORS ASSOCIATION

