



SARS-CoV-2 Variant Tracking in Ohio Wastewater

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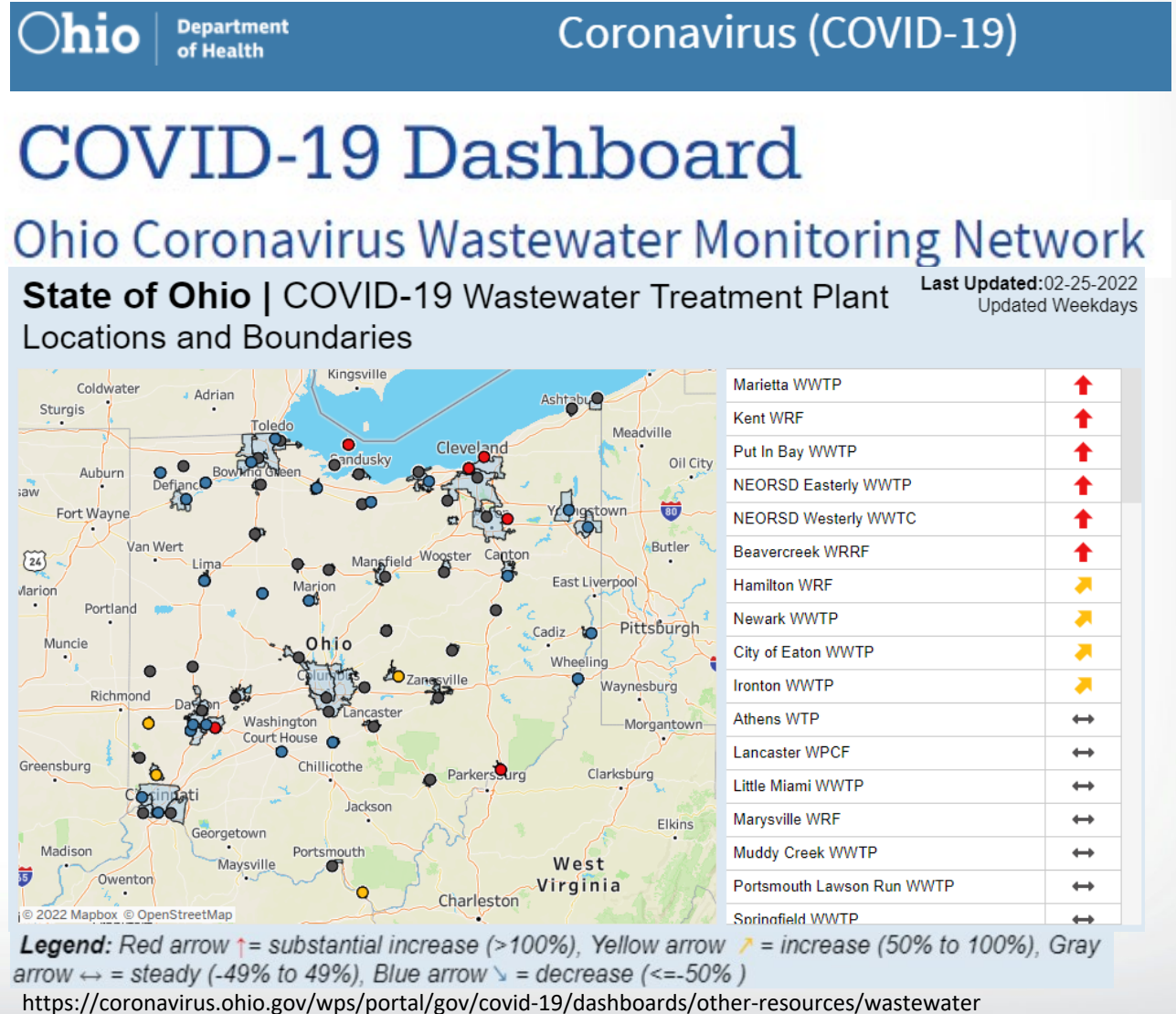
Ohio Wastewater Monitoring Network (OWMN)

- **Statewide Network**

- Started July 2020
- Coordinated by Ohio Water Resources Center at OSU
- Leveraged expertise and resources
- 67 utilities, twice a week
- 9 labs (university, commercial, private, government)
- Dashboard with results updated daily

- **Goal**

- Monitor trend of SARS-CoV-2 RNA in sewersheds
- 2021: Screen for presence of SARS-CoV-2 variants of concern/interest (VOC/VOI) by sequencing and RT-ddPCR





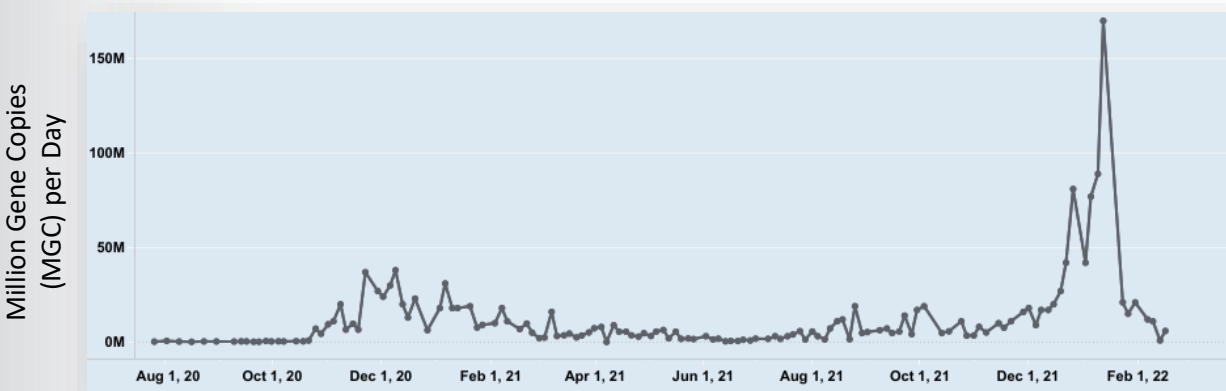
OWMN Dashboard

Mill Creek WWTP

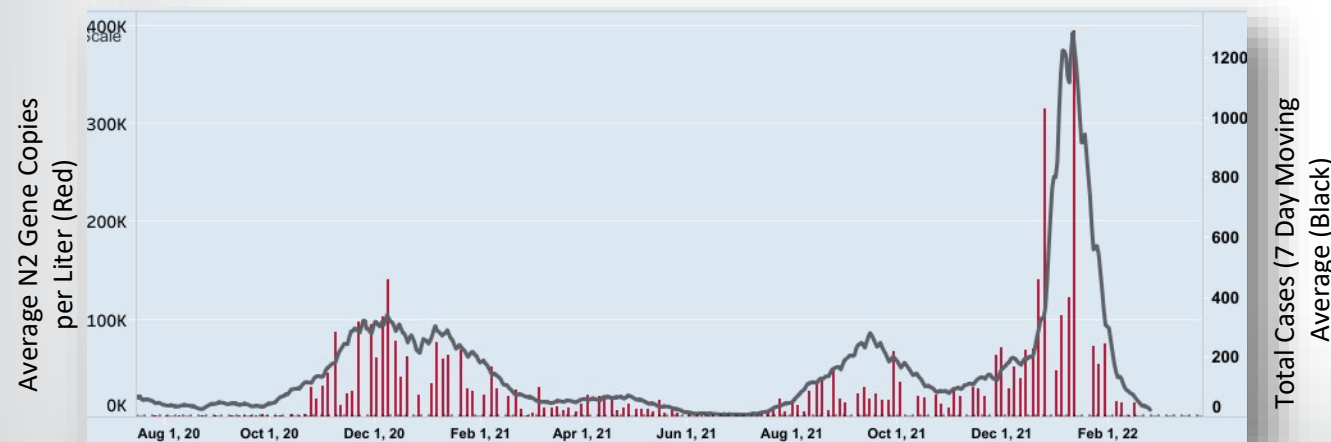
Levels Decreasing

Calculated based on: 2/6/2022 - 2/16/2022

Wastewater Virus Load



Wastewater and Case Data



Application

- The focus is on trends or significant changes in the number of viral gene copies detected at each site
- Action is taken when at least 3 samples show a sustained increase of at least 10-fold (1 log)

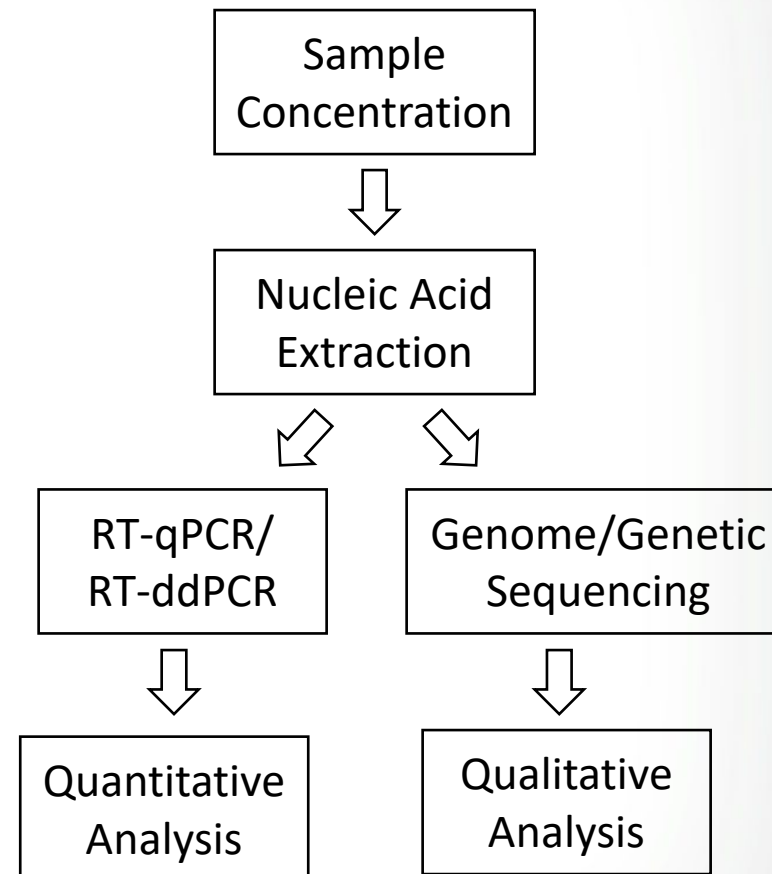
Accomplishments

- Statewide network represents wastewater flow from nearly 5 million residents
- ~2 years of twice weekly data collected
- Provided nearly 1000 warnings to local health communities



Lab Method Logistics

- Report quantitative data within 2 days of sample receipt
- No prescribed method; labs decide
- Sample hold time: 4°C - 72 hours
- No sample pasteurization
- QA/QC (informed by NWSS)
 - Matrix spike to assess method recovery efficiency
 - Coronavirus recommended: human (OC43), murine (MHV), bovine (BCoV)
 - PCR Inhibition control
 - RT-qPCR standards/RT-ddPCR positive control
 - Human fraction measurements
 - crAssphage
 - pepper mild mottle virus (PMMoV)





OH Network Lab Methods

LAB	LOD (copies/L)	Processing Method	Nucleic Acid Extraction	Quantitative Analysis Method	RT-PCR Standard Curve/ Control	Inhibition Control	Matrix Spike	Fecal Indicator	Library Preparation	Sequencing
A	1200	Centrifugation, filtration	Qiagen RNeasy PowerWater Kit	RT-qPCR	DNA plasmid	Dilution	MHV	crAssphage	ARTIC v3/v4 Illumina Tagmentation	NextSeq2000
B	170	Centrifugation, filtration	Qiagen Allprep DNA/RNA Kit	RT-qPCR	DNA plasmid	Dilution	BCoV	crAssphage	ARTIC v3/v4 Illumina Tagmentation	NextSeq2000
C	133	Tween, solids removal, hollow fiber ultrafiltration (InnovaPrep)		RT-ddPCR	DNA plasmid	Luciferase Control RNA	OC43	crAssphage	ARTIC v3/v4 Illumina Tagmentation	NextSeq2000
D	4050	Filtration	Trizol, garnet bead beating, alcohol precipitation	RT-qPCR	DNA plasmid	Dilution	BCoV	PMMoV	ARTIC v3/v4 Illumina Tagmentation	NextSeq2000
E	500	Centrifugation, filtration	TRIZol and RNA purification kit	RT-qPCR	Synthetic RNA	Luciferase Control RNA	BCoV	crAssphage	SWIFT/IDT SARS-CoV-2 Panel	NextSeq550
F	3,000	Promega, add protease, supernatant through GFA/silica column	Promega Wastewater Large Volume TNA Capture Kit	RT-qPCR	DNA plasmid	Promega probe	OC43	PMMoV	ARTIC v3/v4 Illumina Tagmentation	NextSeq2000
G	231	Centrifugation, filtration	TRIZol-chloroform	RT-ddPCR	SARS-CoV-2 genomic RNA	Luciferase Control RNA	OC43	crAssphage	ARTIC v3/v4 Illumina Tagmentation	NextSeq2000
H	891	Acidification, Filtration, extract filter	Qiagen Allprep PowerViral DNA/RNA Kit	RT-qPCR	Synthetic RNA	Mouse lung RNA	OC43	PMMoV	ARTIC v3/v4 Illumina Tagmentation	NextSeq2000



Sequence Analysis

- Lab specific analytical pipelines
- Shared list of substitutions*; focus on spike region for Variants of Concern
- Depth of sequence read coverage
- Presence of variant-specific substitution = Alternate allele frequency > 1%
- Lineage presence = 50% of variant-specific substitutions

*<https://outbreak.info/situation-reports>



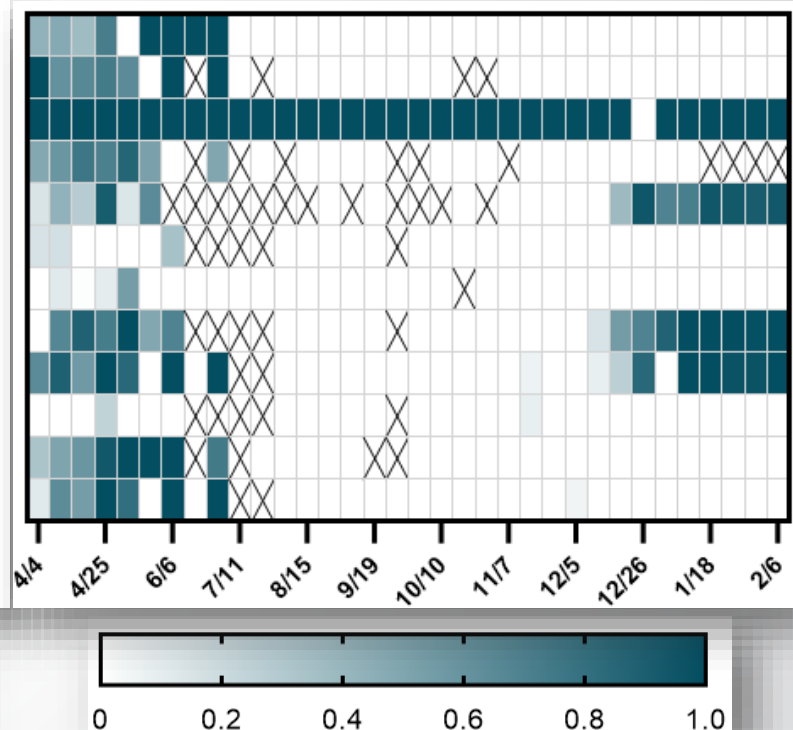
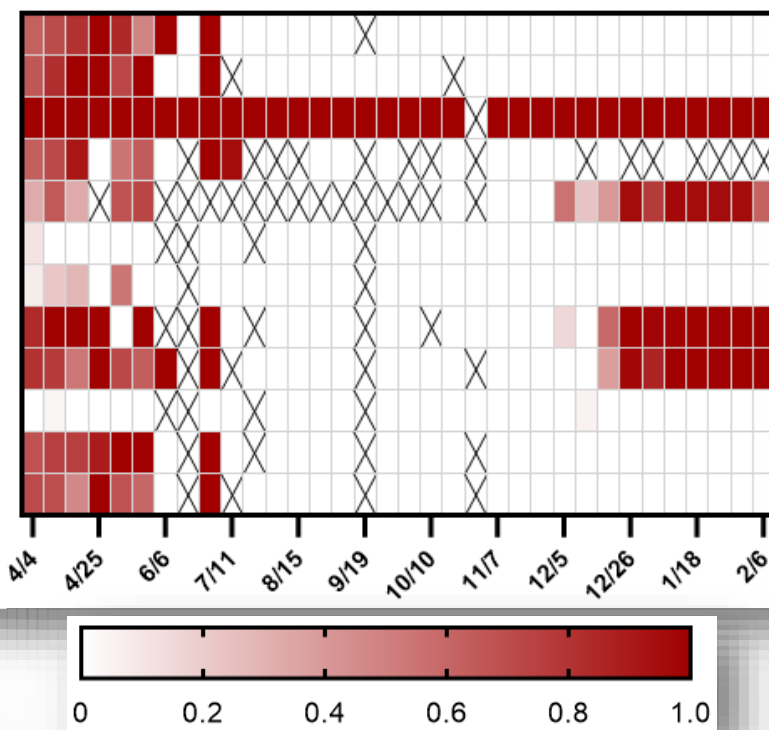
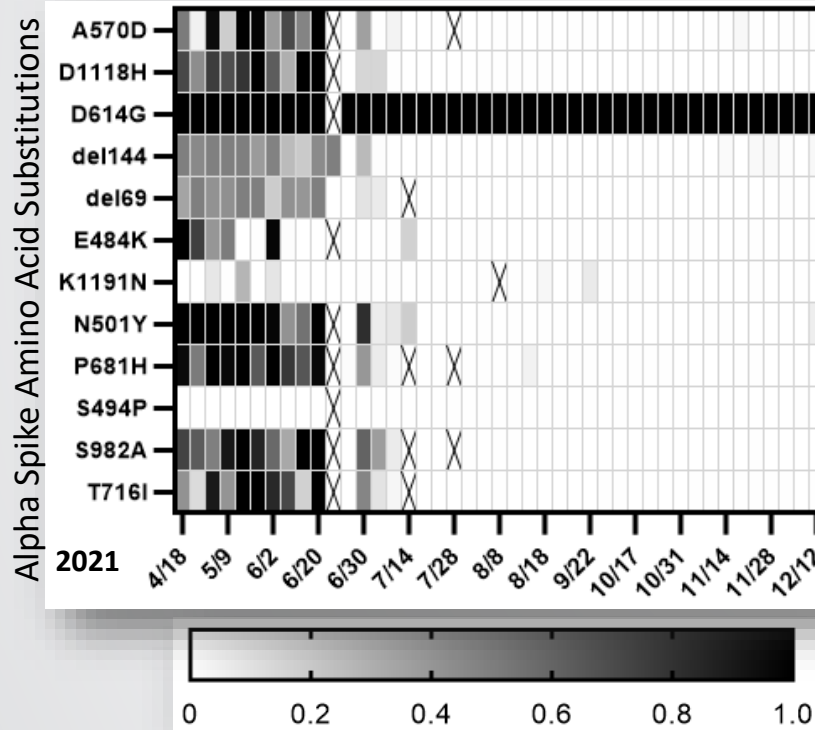
Alpha in OH Wastewater

These WWTPs combined represent 1,658,094 (14%) OH residents

WWTP A

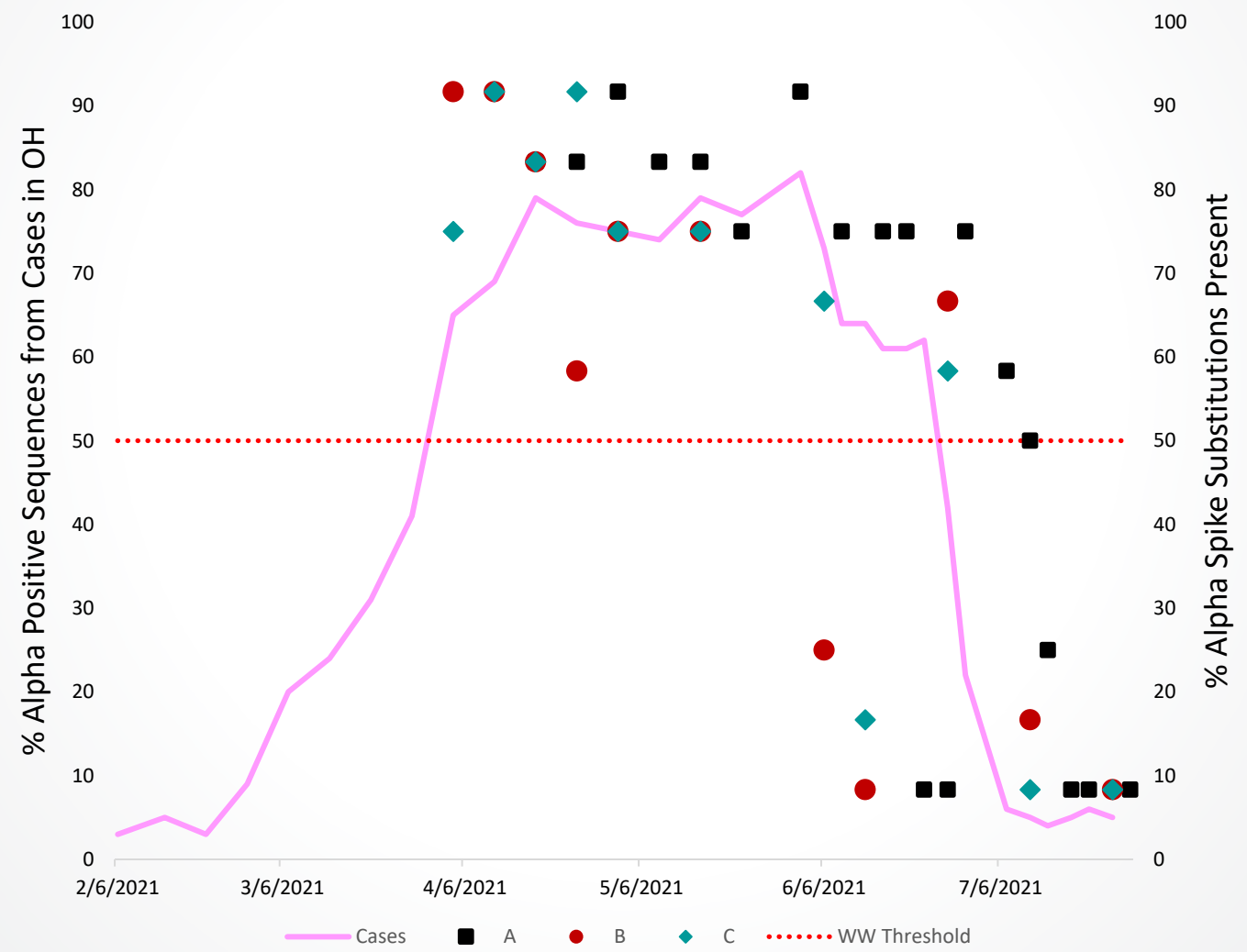
WWTP B

WWTP C





Alpha: OH Wastewater and Case Data





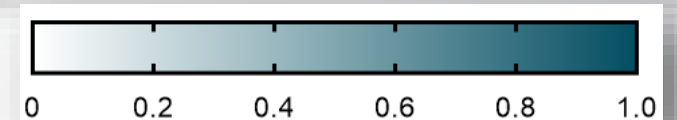
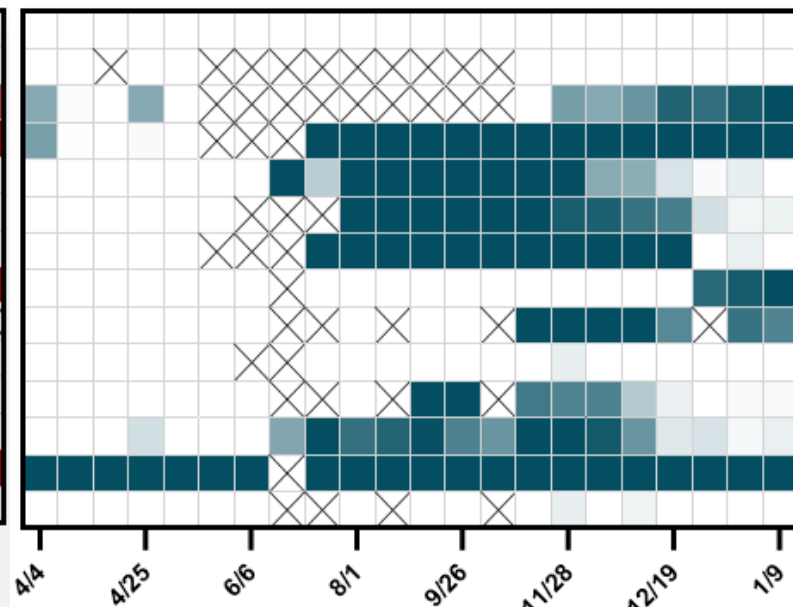
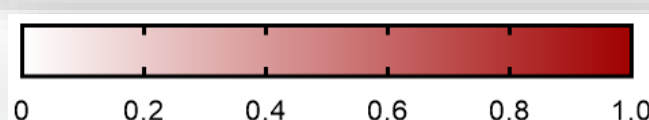
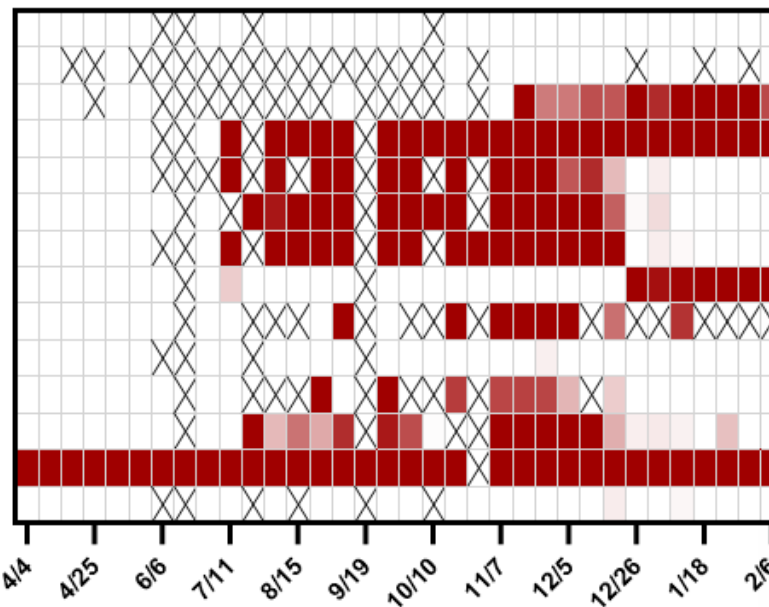
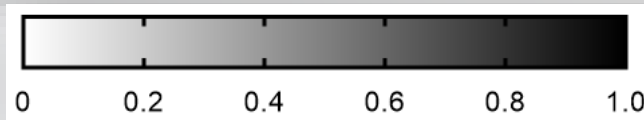
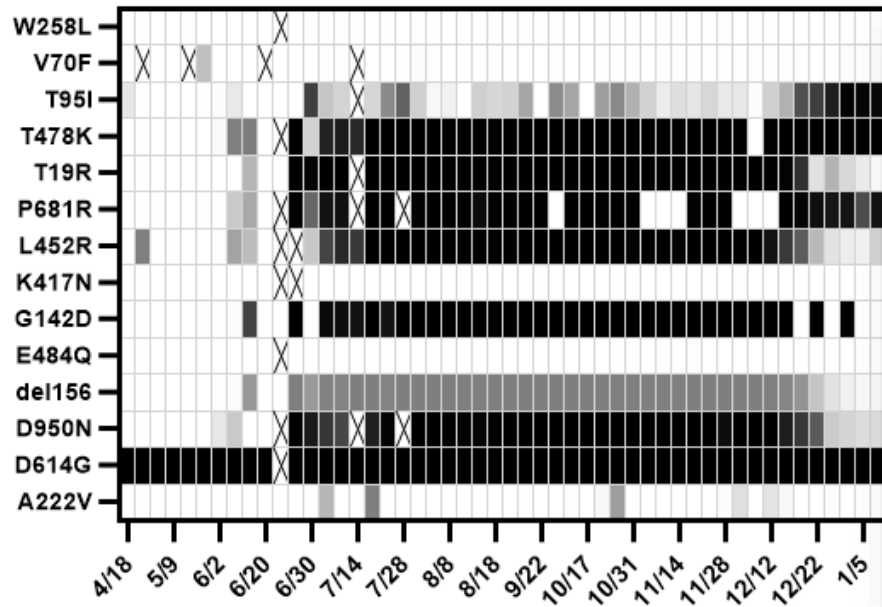
Delta in OH Wastewater

WWTP A

WWTP B

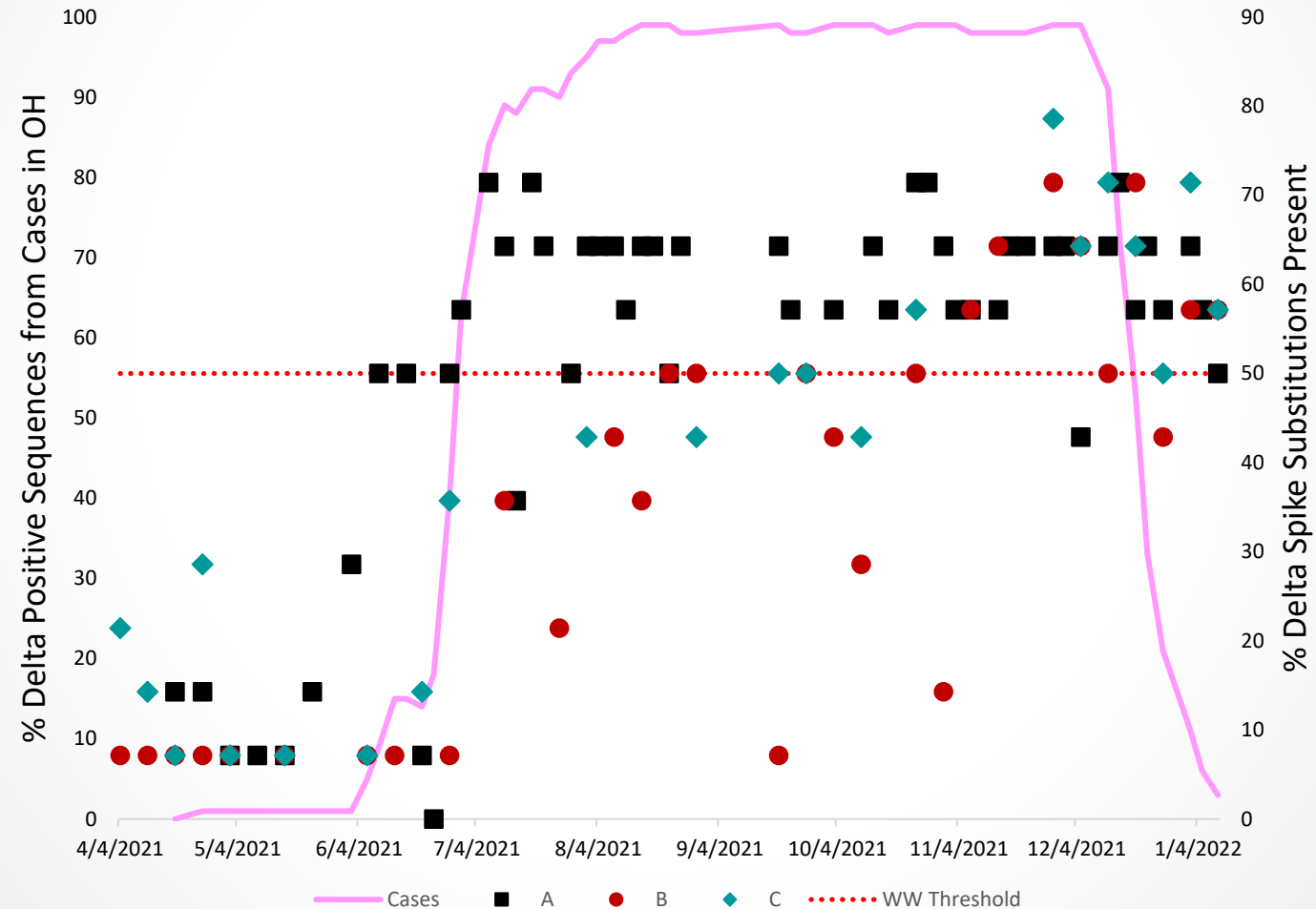
WWTP C

Delta Spike Amino Acid Substitutions





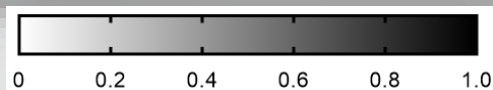
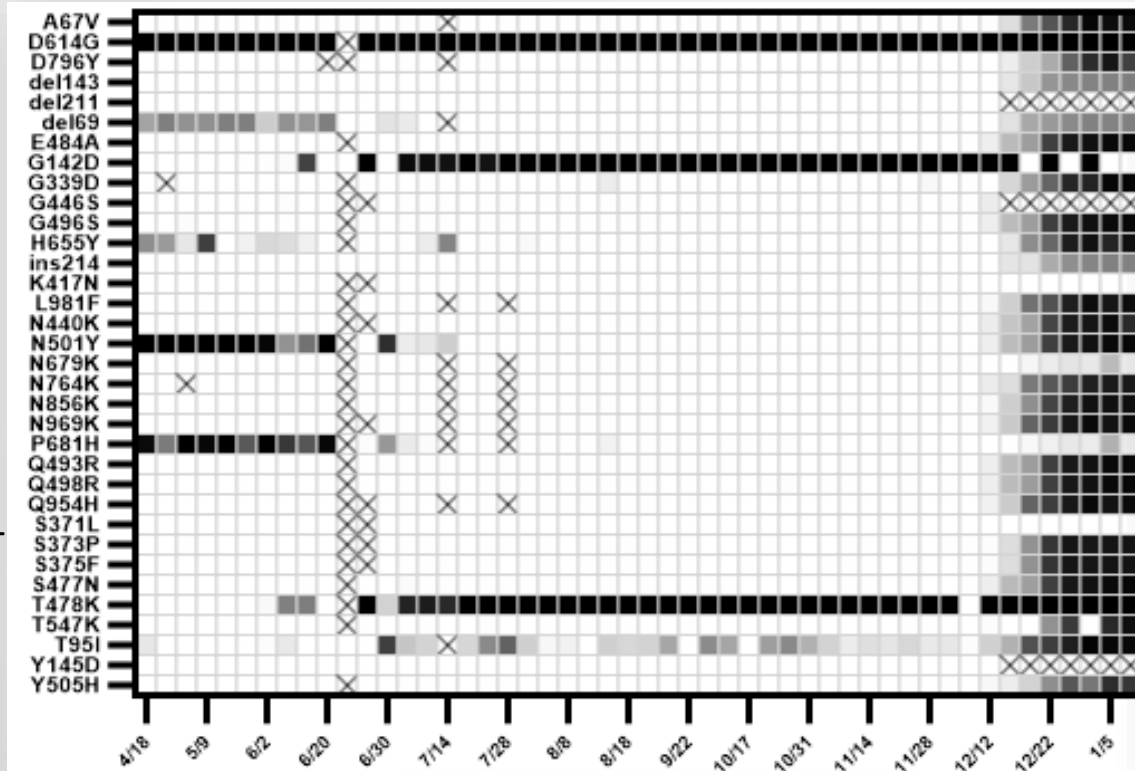
OH Wastewater and Case Data



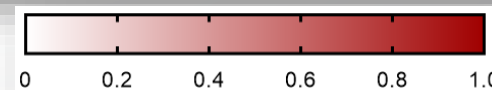
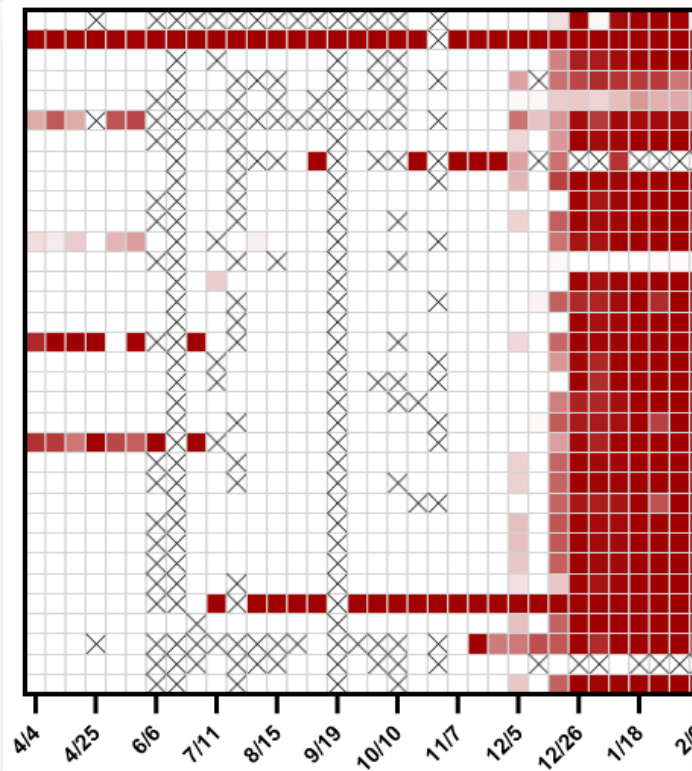


Omicron in OH Wastewater

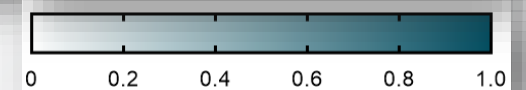
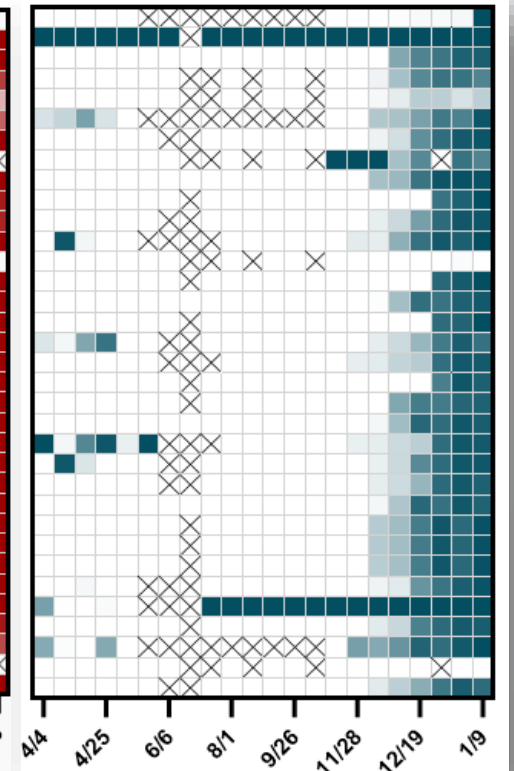
WWTP A



WWTP B



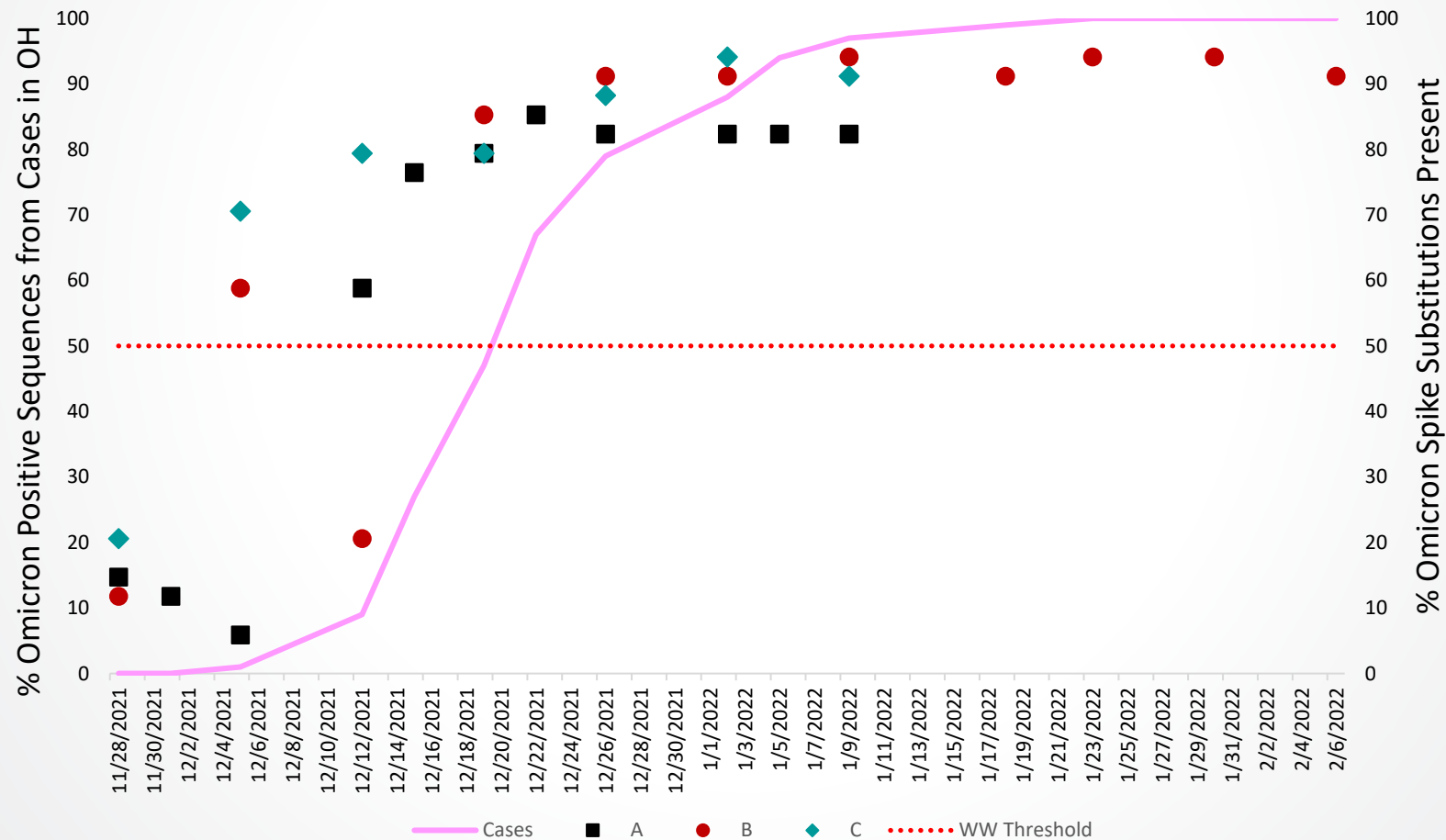
WWTP C



Omicron Spike Amino Acid Substitutions



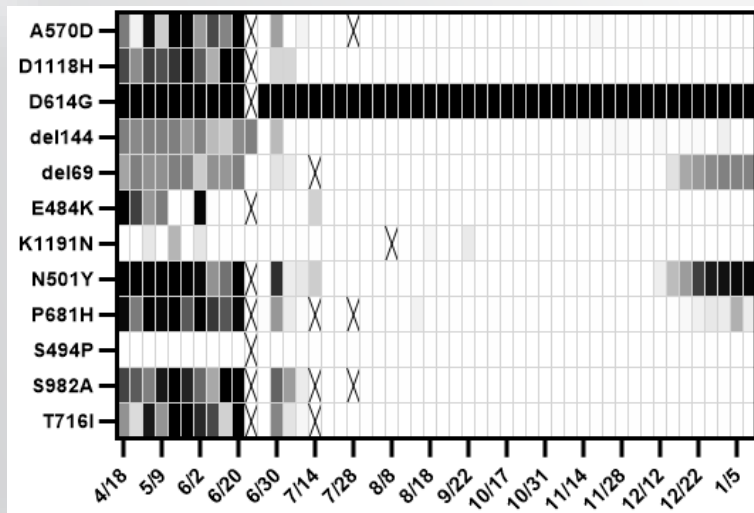
Omicron: OH Wastewater and Case Data



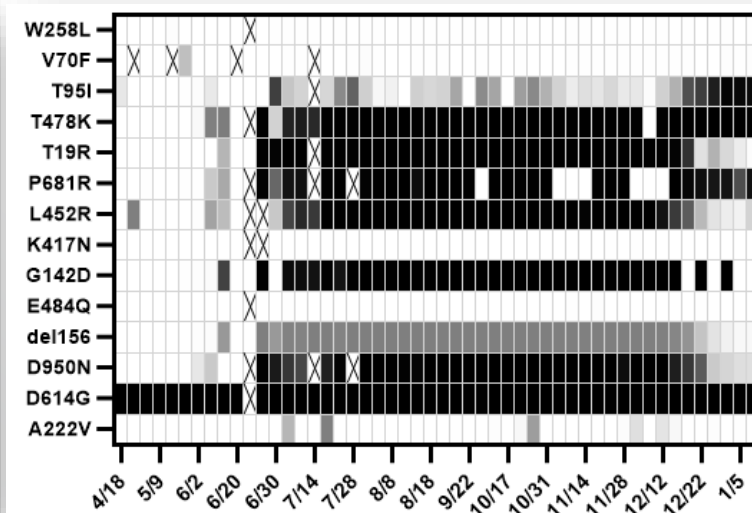


WWTP A

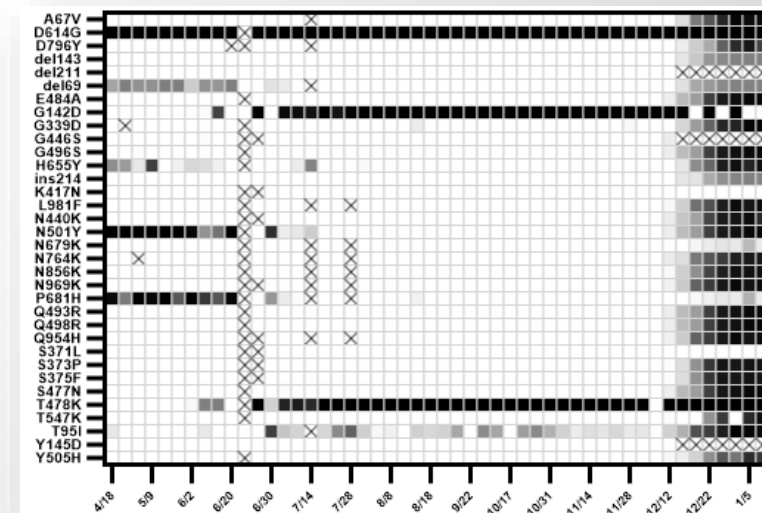
Alpha



Delta

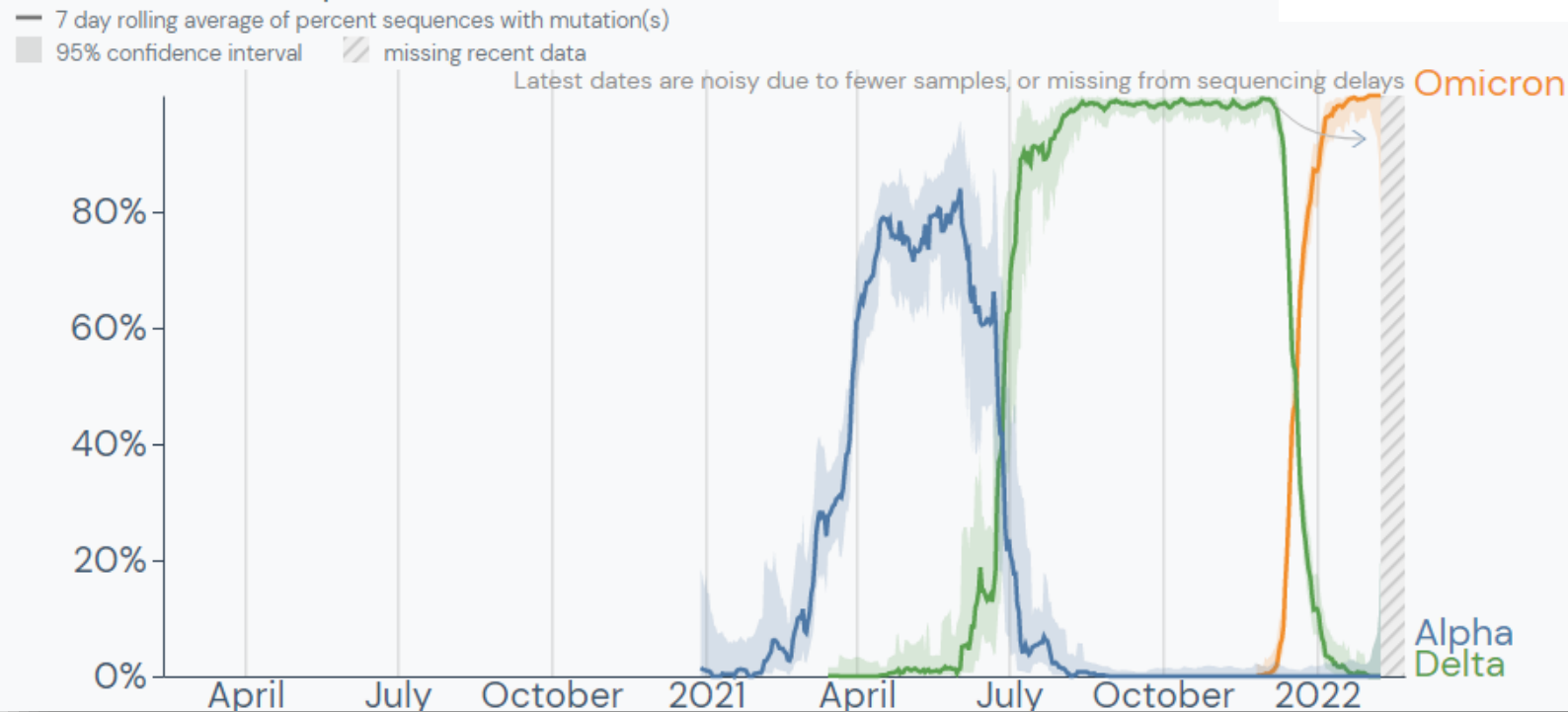


Omicron



Mutation and case prevalence over time in Ohio, United States

Outbreak.info





Omicron Sublineage BA.2 in OH

Sporadic appearance of BA.2 signatures at a couple OH WWTP

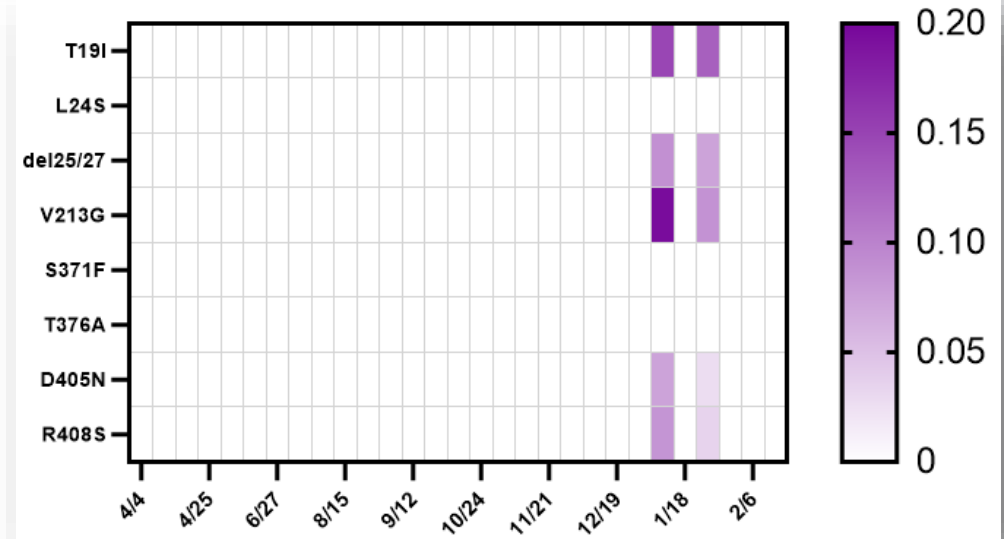
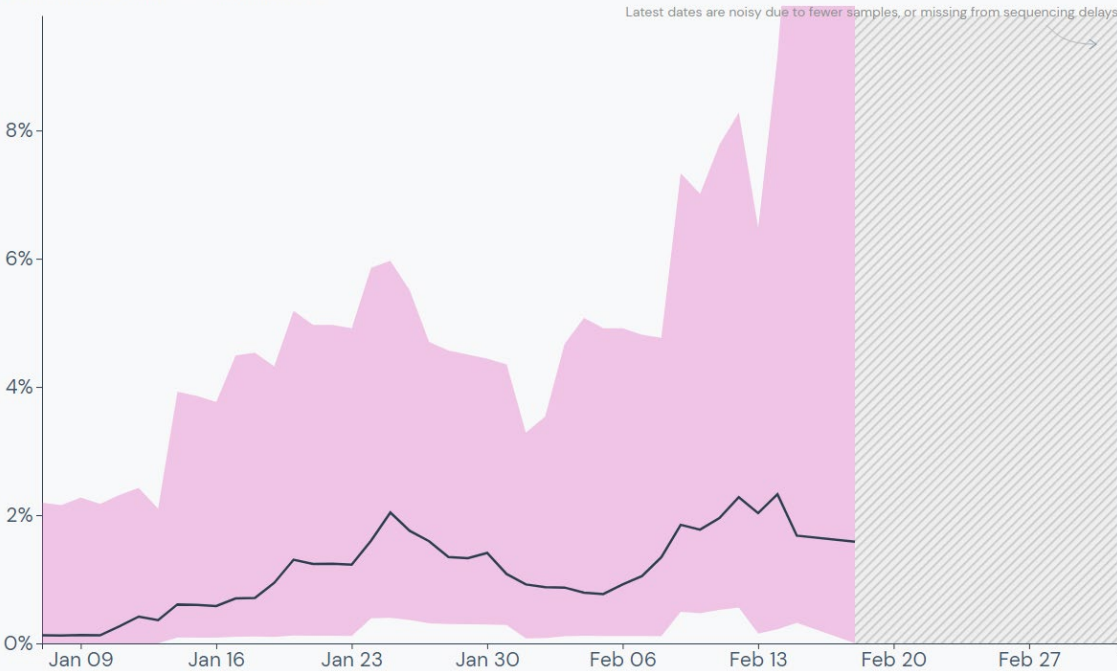
WWTP D

Average daily BA.2 prevalence in Ohio, United States

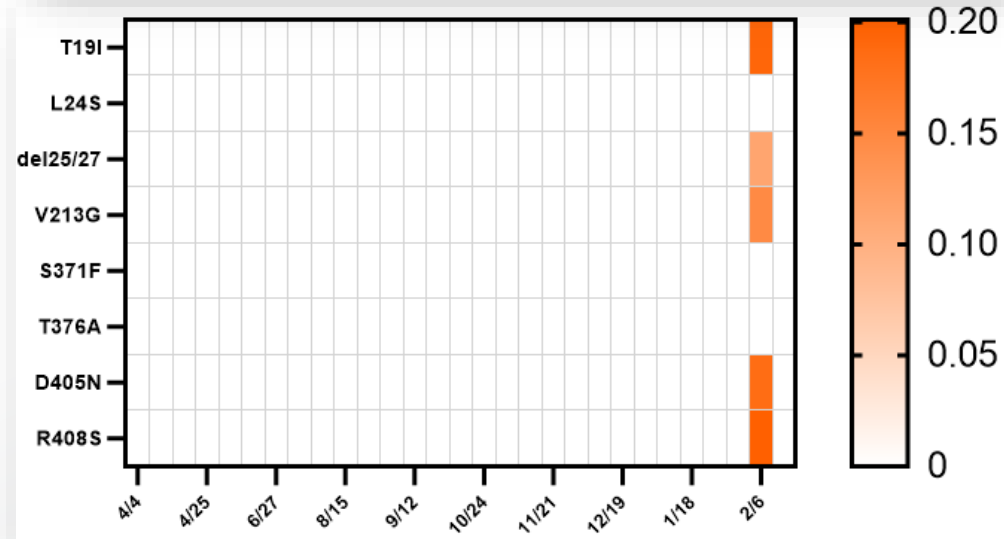
Based on reported sample collection date

— 7 day rolling average of percent of BA.2-positive sequences
■ 95% confidence interval

▨ missing recent data



WWTP E





Summary and Data Usage

- Wastewater surveillance has enabled large-scale monitoring of SARS-CoV-2 trends
- The Alpha, Delta and Omicron variant surges were observed by wastewater sequencing and reflects the confirmed case data
- Presence of minority sublineage (BA.2) was observed sporadically in wastewater
- Ohio Department of Health is using wastewater sequencing data in conjunction with clinical samples to confirm and monitor the spread of the variants across communities in Ohio
- Clinical data is not readily available from all OH locations; wastewater sequencing data was able to identify the presence of variants in these communities
- Explore other mutations in the full-length genome data set