Wastewater Surveillance for COVID-19 and Other Infectious Diseases: Case Studies in Atlanta, Georgia, Accra, Ghana, and Kolkata, India

Marlene Wolfe and Christine Moe

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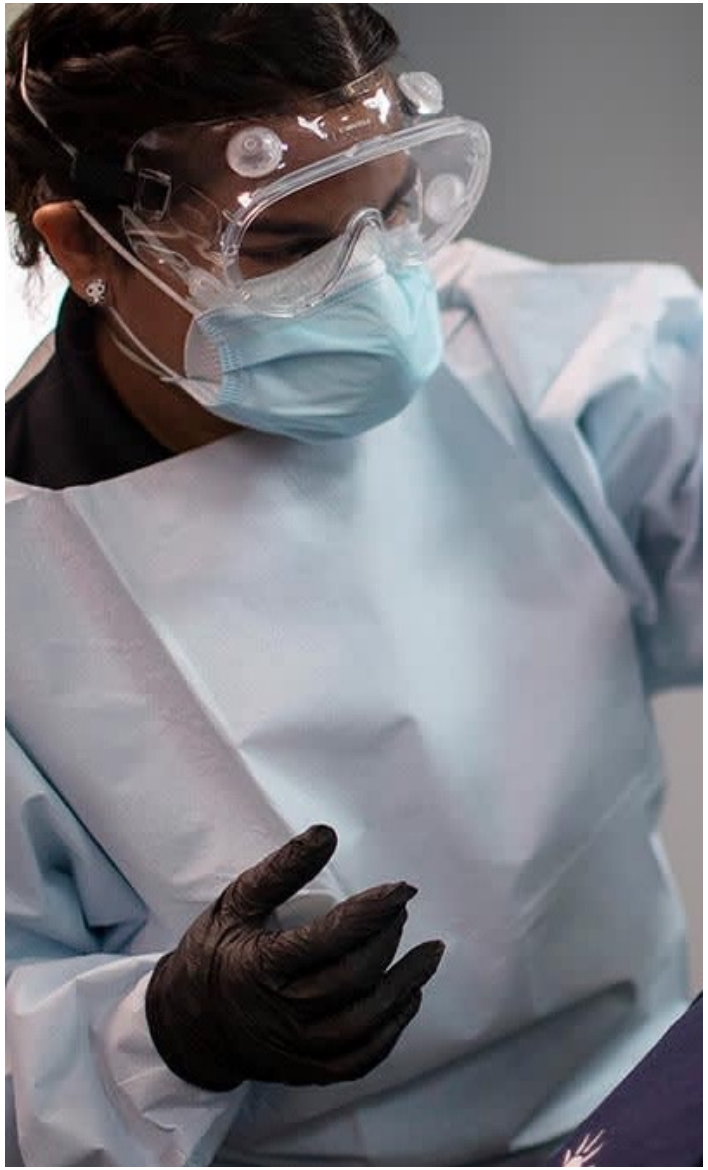

Surveillance Challenges

"Traditional" infectious disease surveillance relies on individual case identification and aggregation, which can be challenging

- Diagnostic test development
- Test availability
- Lack of test-seeking (asymptomatic and mild cases)
- Lag times for reporting
- Diseases that aren't reportable
- Similar presentation with other diseases

Wastewater Surveillance

Look to the environment!

- Wastewater is a **composite** biological sample
- Many **pathogens** are shed in feces, urine, sputum, vomit and **appear in wastewater**
- Evaluates community **with less bias** when individual testing or case identification is challenging
- Promotes **health equity** by inclusion of under-served groups

Historic Example: Polio Surveillance

- Often asymptomatic or mild
- Serious cases of acute flaccid paralysis (very rare)

- 2013-2014 outbreak in Israel detected through sewage surveillance
- No cases of acute flaccid paralysis, outbreak subsided after a vaccination campaign

Environmental surveillance sites added in polio endemic countries (Nigeria, Afghanistan, and Pakistan)

Oral Polio Vaccine Administration (UNICEF)

Epidemiology of the silent polio outbreak in Rahat, Israel, based on modeling of environmental surveillance data

Andrew F. Brouwer[a], Joseph N. S. Eisenberg[a,1], Connor D. Pomeroy[a], Lester M. Shulman[b,c], Musa Hindiyeh[b], Yossi Manor[b], Itamar Grotto[d,e], James S. Koopman[a], and Marisa C. Eisenberg[a,1]

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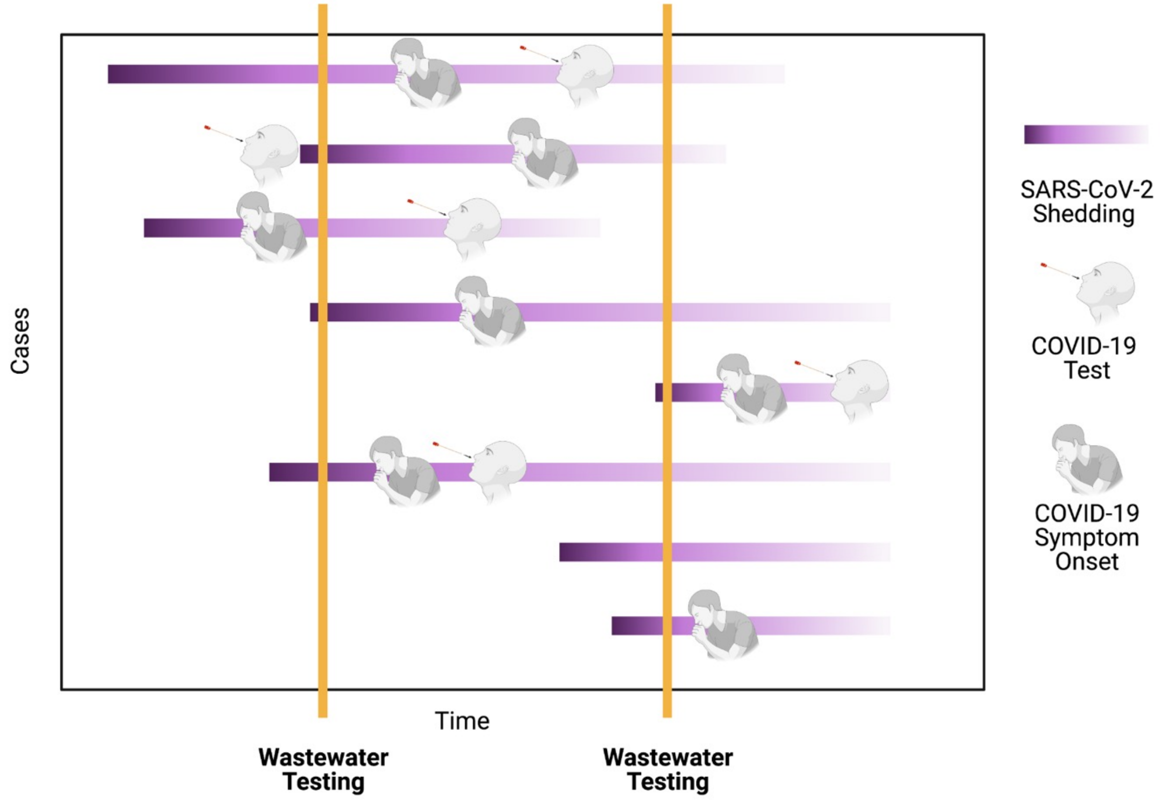
Wastewater monitoring for true infection burden

Measurements of SARS-CoV-2 ascertain COVID-19 burden in a **less biased way**

Enhance inclusion of communities that are under-represented in traditional surveillance

Especially important in low-income communities of South Atlanta where **cases are likely underestimated**



People with COVID-19 excrete the SARS-CoV-2 virus in their feces. The virus can be detected in wastewater samples.

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Wastewater monitoring is part of the public health toolbox

- Has been done for **decades**, but **accelerated** development for the past 2 years during the pandemic
- National attention on wastewater as **meaningful source of information** on the omicron surge
- Development of tests **beyond SARS-CoV-2** and variants – part of CDC future plans for National Wastewater Surveillance System
- Building **sustainable platforms** for monitoring

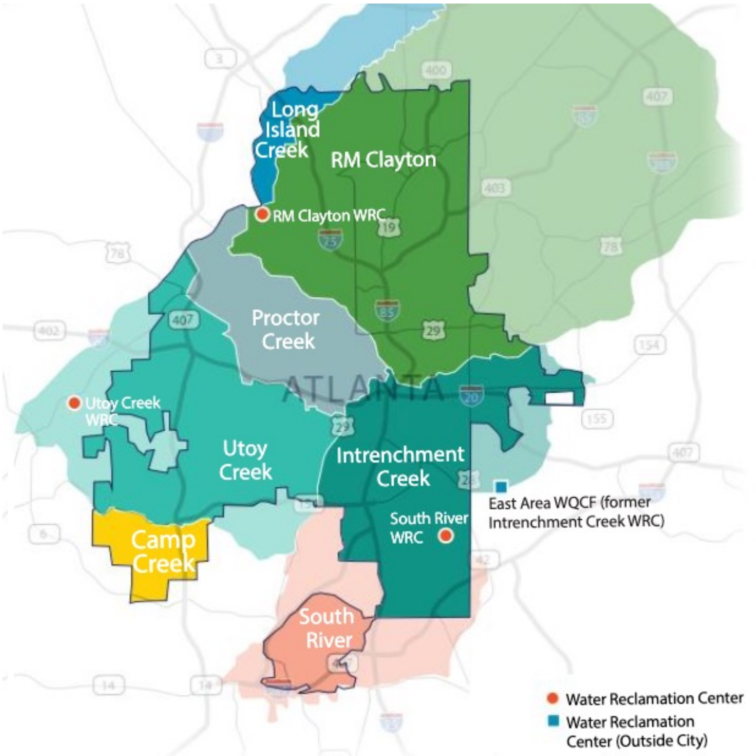
Wastewater monitoring can guide outbreak response

- Shows **trends** in COVID-19 cases over time
- Identifies introduction of **new threats**
- Shows **where** COVID-19 burden is high
- Identifies **risks** in institutions

Goal: ascertain the COVID-19 occurrence to:

1) **Complement** existing clinical surveillance

2) **Identify** and **monitor** high risk areas



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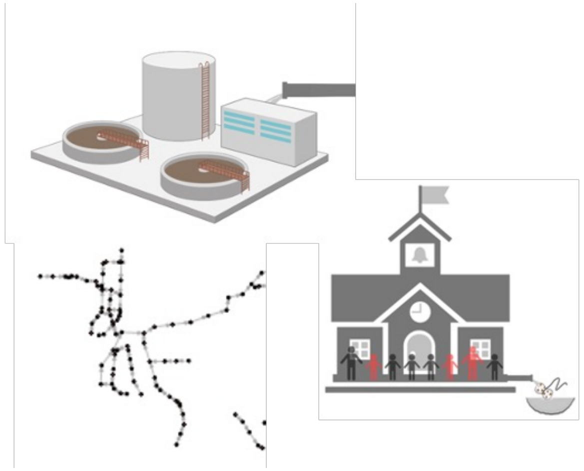
Monitoring in Atlanta

To utilize wastewater monitoring for vulnerable populations in Atlanta, we focus on:

Multi-level sampling



Passive collection

High-throughput analysis

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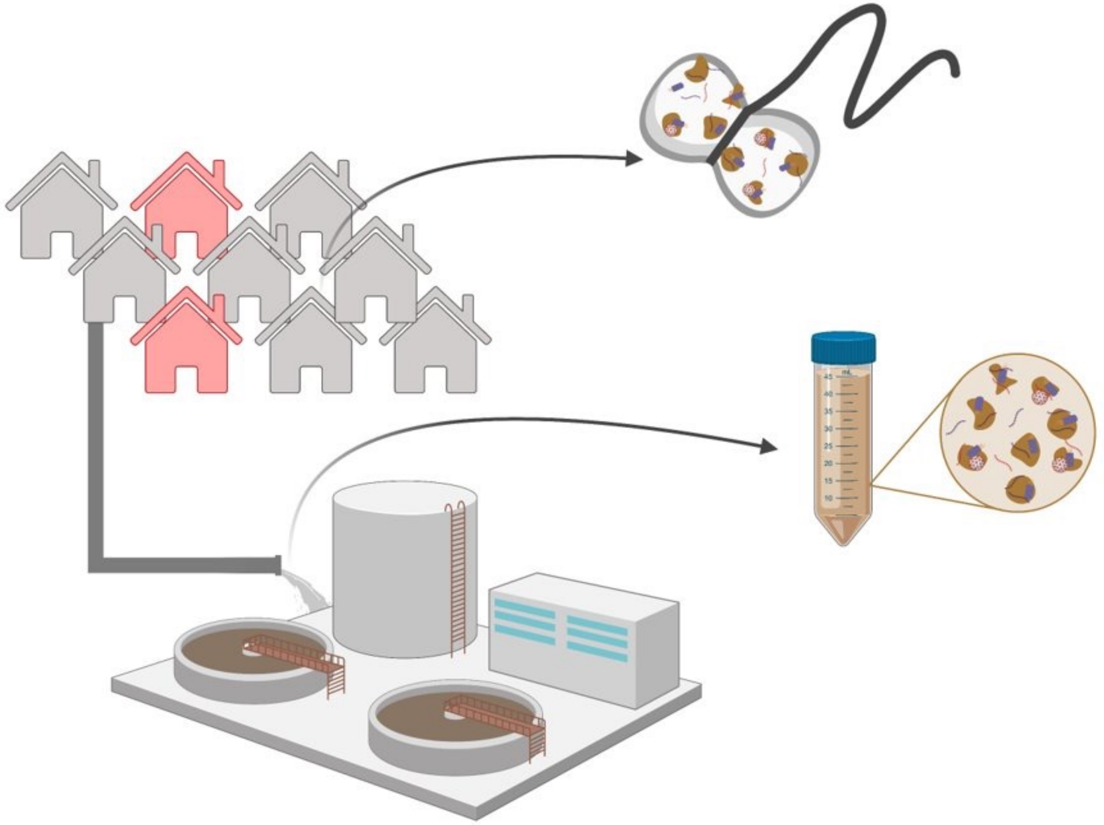
What do we mean by "multi-level" monitoring?

Sampling that captures areas that represent different:

- Population sizes
- Community characteristics
- Exposures (institutions)

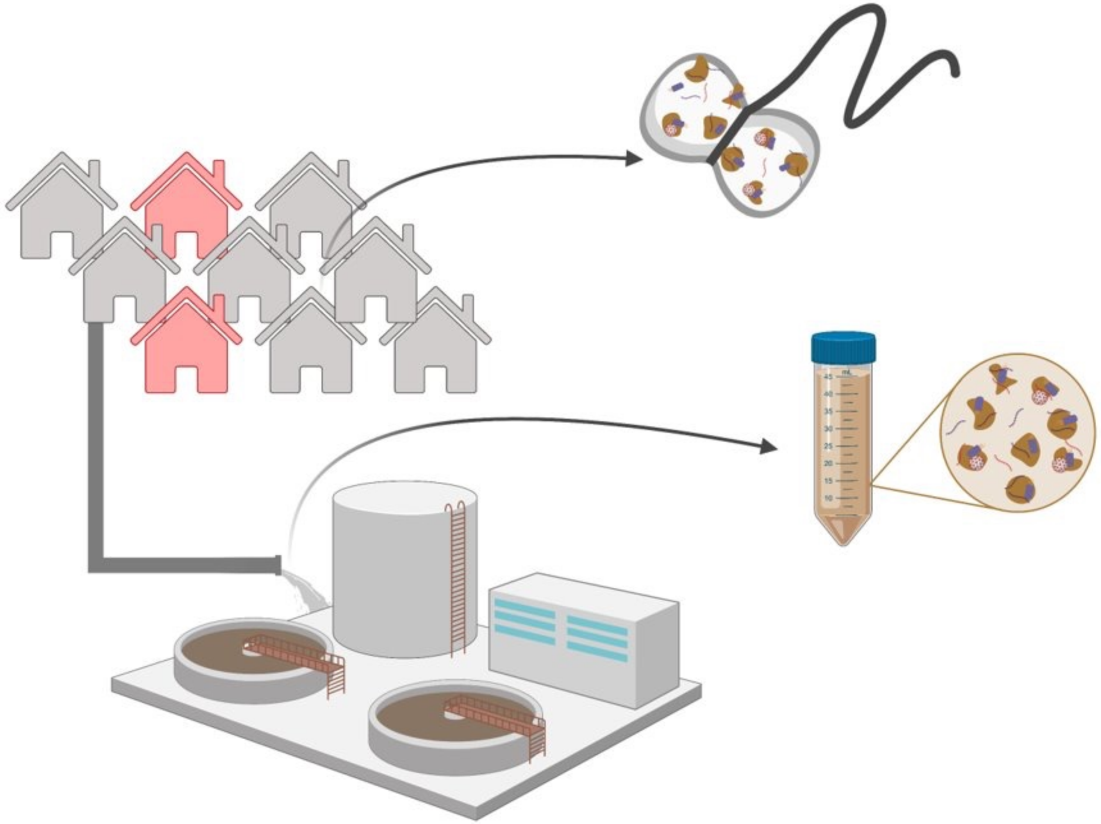


These sites are often **nested within each other** to get an early, clear picture of risk

Network Analysis for Site Selection


Yuke (Andrew) Wang

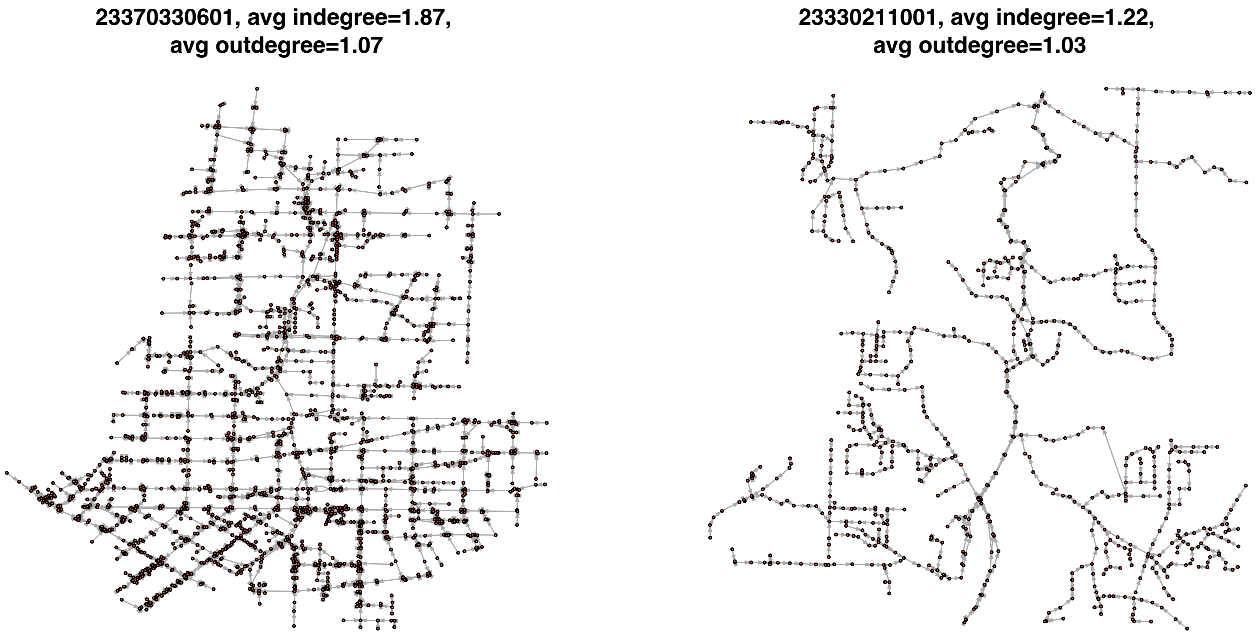
- Obtained maps of sewer network from the department of watershed management
- Utilized these maps to select sites that represent:
  - Large community sites
  - Smaller community sites
  - Institutions



Network Analysis for Site Selection

Yuke (Andrew) Wang

- Understand the topology and connectivity of sewer networks in the city
- Identify the catchment area/size of sampling sites
- Select sites that represent strategic sampling areas



(a) (b)

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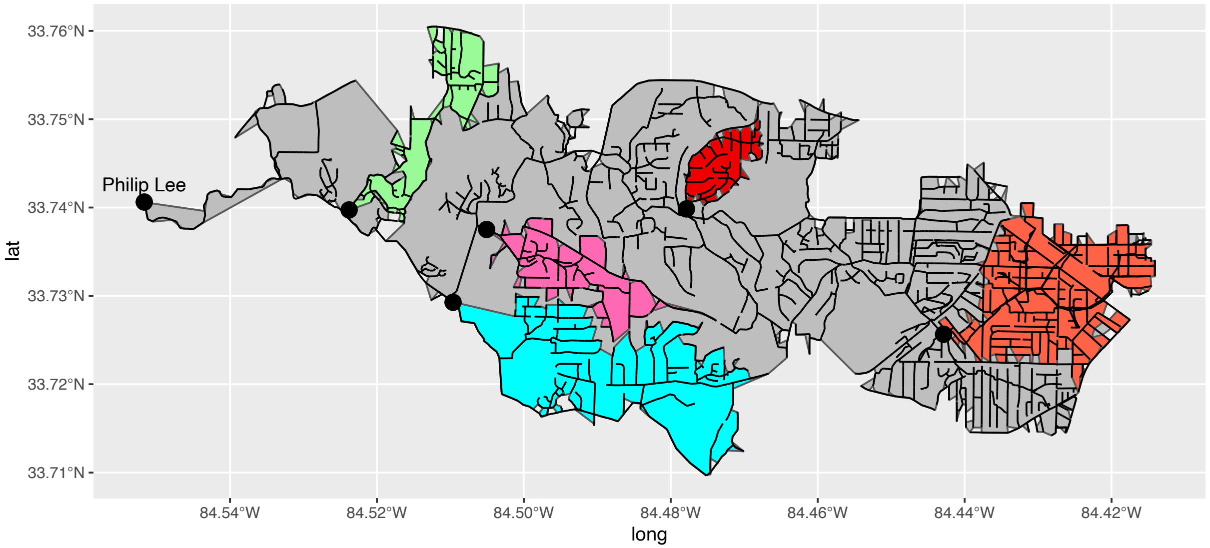
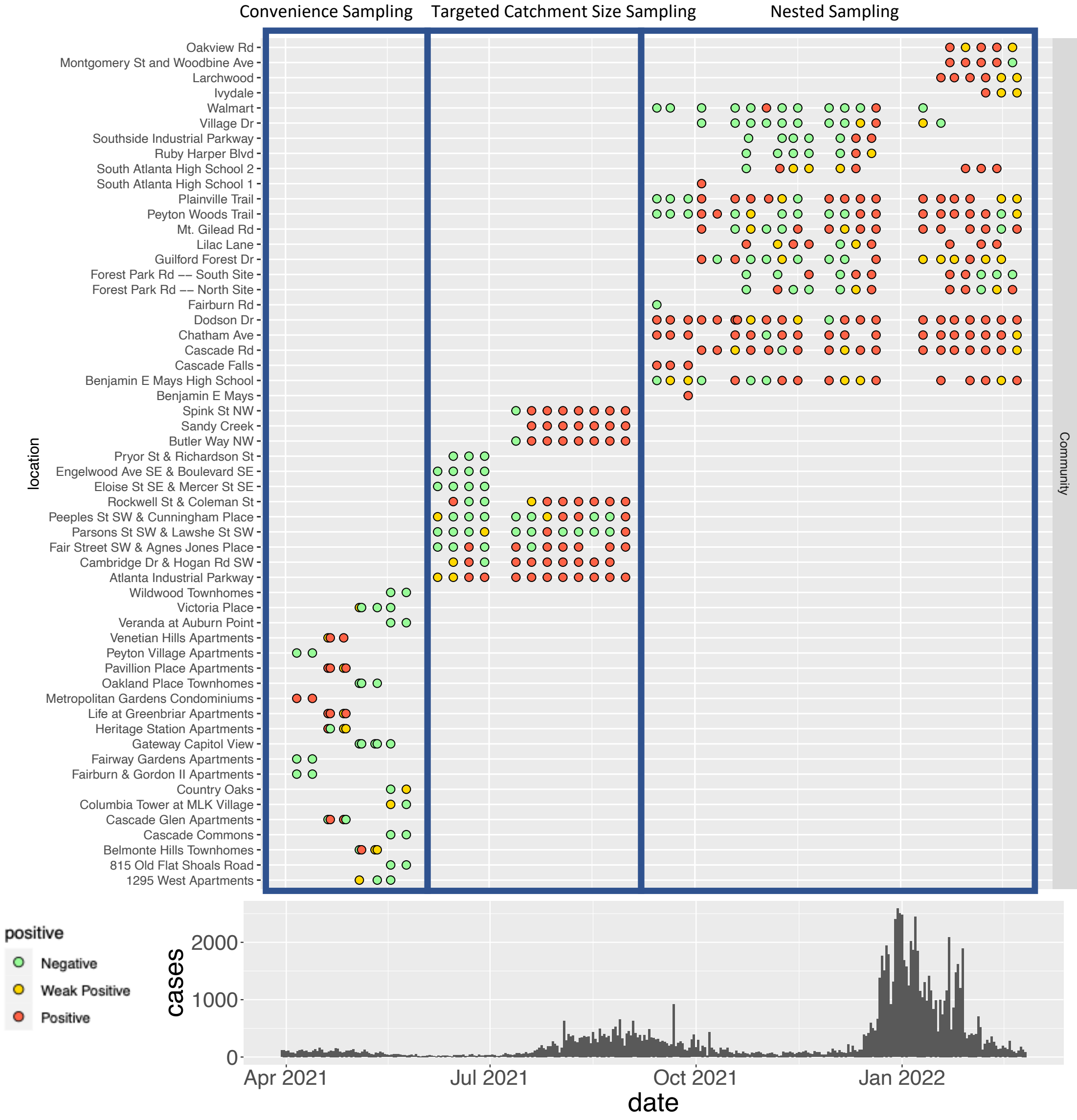
Community Site Sampling Phases

Phase 1 (Apr–May 2021):
convenience sampling

Phase 2 (Jun–Aug 2021):
targeted catchment size sampling

Phase 3 (Sep 2021–Apr 2022):
nested sampling + adaptive sampling





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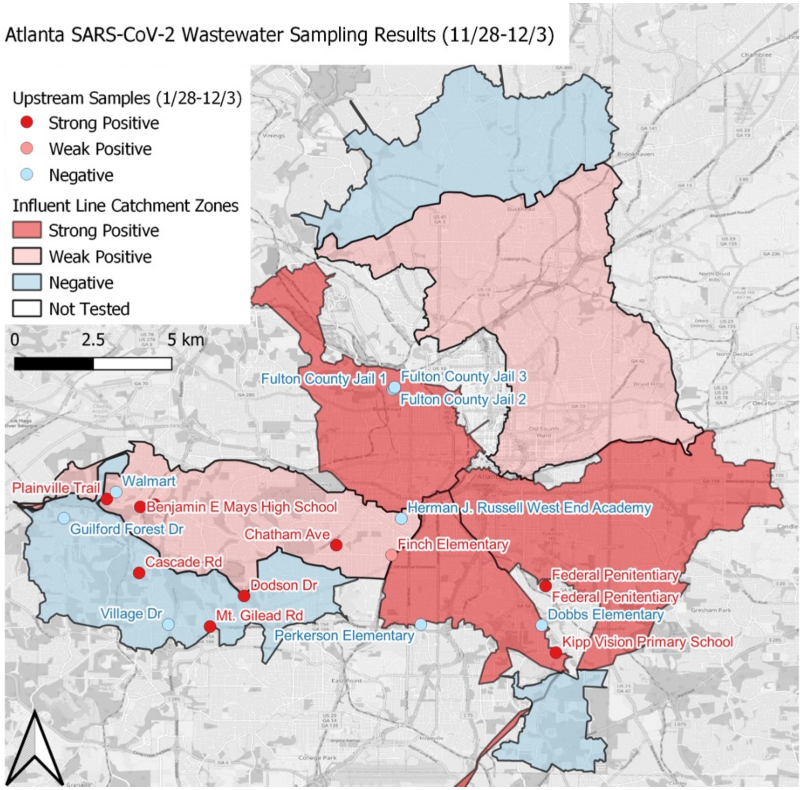
Multi-level sampling results

Sewage lines
from 3 treatment plants

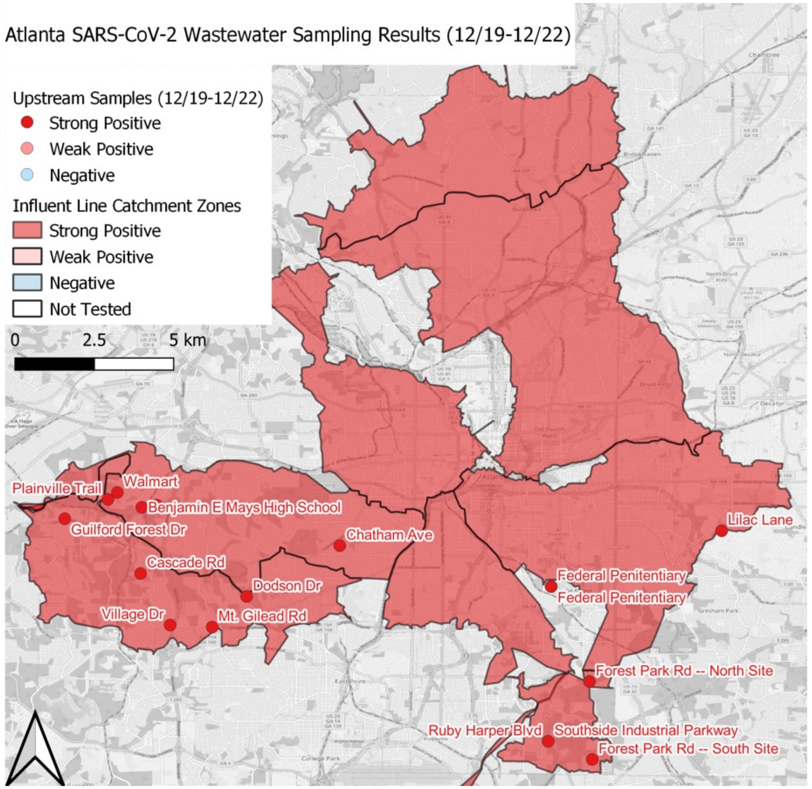
57 community sites
South Atlanta

13 Institutions
2 correctional facilities, 11 schools

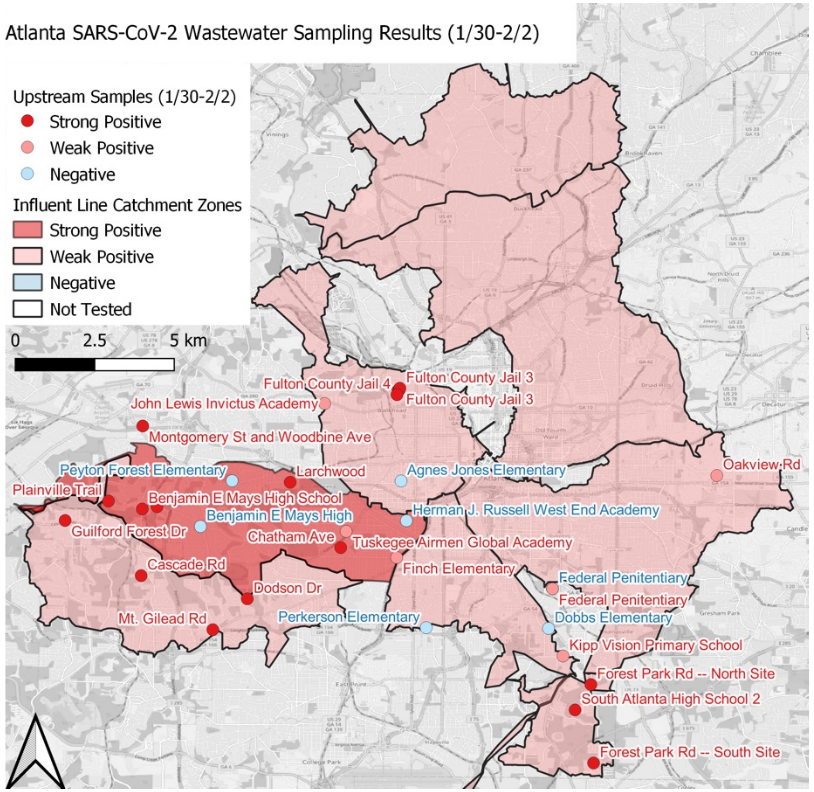
Early December 2021



Mid December 2021



Late January 2022



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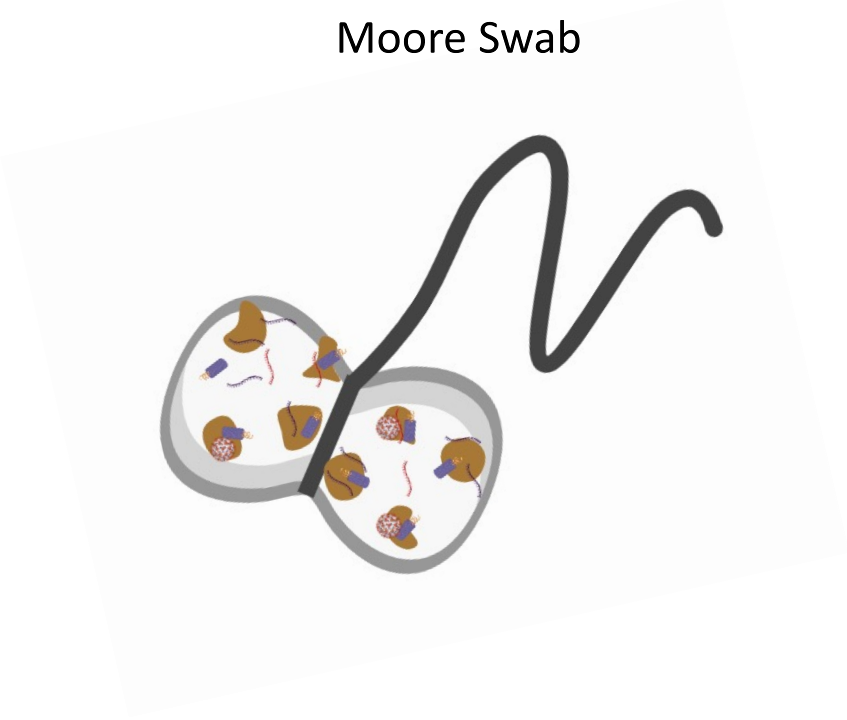
Passive Collection

- Samples can be challenging to obtain for community and institution sites
- Autosamplers are expensive, bulky, and subject to theft and tampering
- Moore swabs are cotton gauze tied with fishing line/string

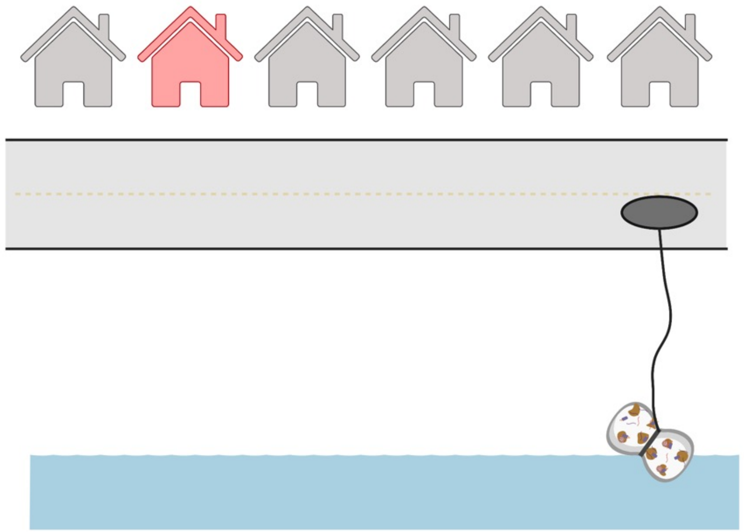
Moore Swab



Placement and Collection

Jamie VanTassell

Many passive, low-cost samplers can be placed at a lower cost and risk



Swab stays in manhole for 24 hrs

Swab massaged in elution buffer before processing

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Moore Swabs

Pengbo Liu

Passive collection of a "composite" sample

Advantages

- Low cost
- Simple to set up
- Higher sensitivity than a grab sample
- Little risk of theft or tampering

Challenges

- Results are not strictly quantitative

Table 3
RT-qPCR results for 26 matched Moore swab and grab samples from Emory University Hospital wastewater, June 2020 – February 2021.

	Moore swab[b] results		
Grab sample[c] results	+	–	Total
+	18[a]	0	18
–	6	2	8
Total	24	2	26

[a] Ct values of positive swabs were between 30.8 and 39.9. % concordance = 20/26 = 77%. Moore swab detected 24/26 = 92%. Grab sample detected 18/26 = 69%.
[b] Moore swab samples were processed by the skimmed milk method.
[c] Grab samples were processed by membrane filtration.

A sensitive, simple, and low-cost method for COVID-19 wastewater surveillance at an institutional level

Pengbo Liu *, Makoto Ibaraki, Jamie VanTassell, Kelly Geith, Matthew Cavallo, Rebecca Kann, Lizheng Guo, Christine L. Moe *

Center for Global Safe Water, Sanitation, and Hygiene, Rollins School of Public Health, Emory University, Atlanta, GA 30322, USA

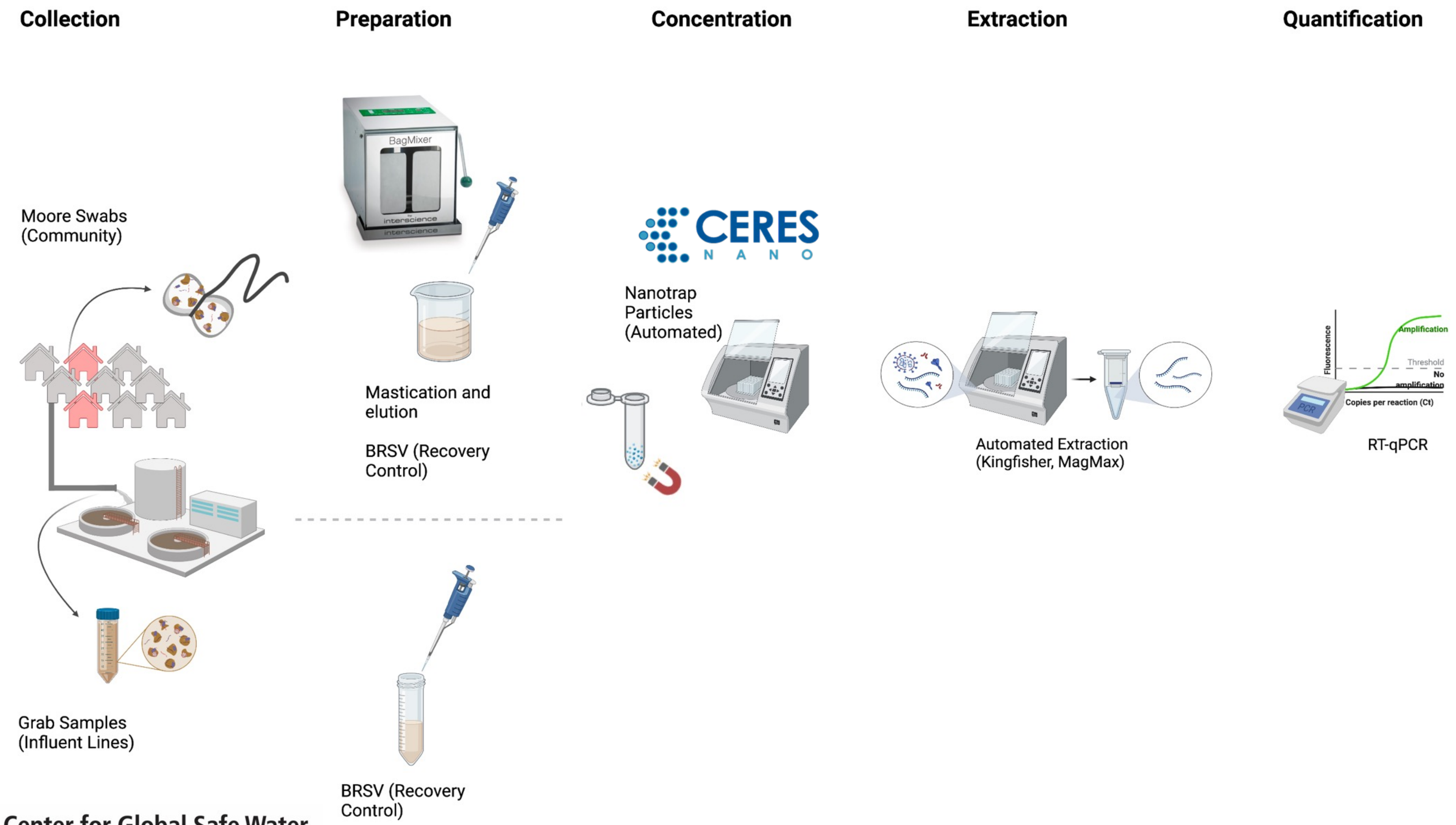
Laboratory Processing

High-throughput methods enable testing all samples at multiple levels throughout the city

Each sample undergoes:

- Sample prep (Moore swabs)
- Concentration
- RNA extraction
- Viral RNA detection and quantification by PCR

Effective and high-throughput processing



Multi-level Results

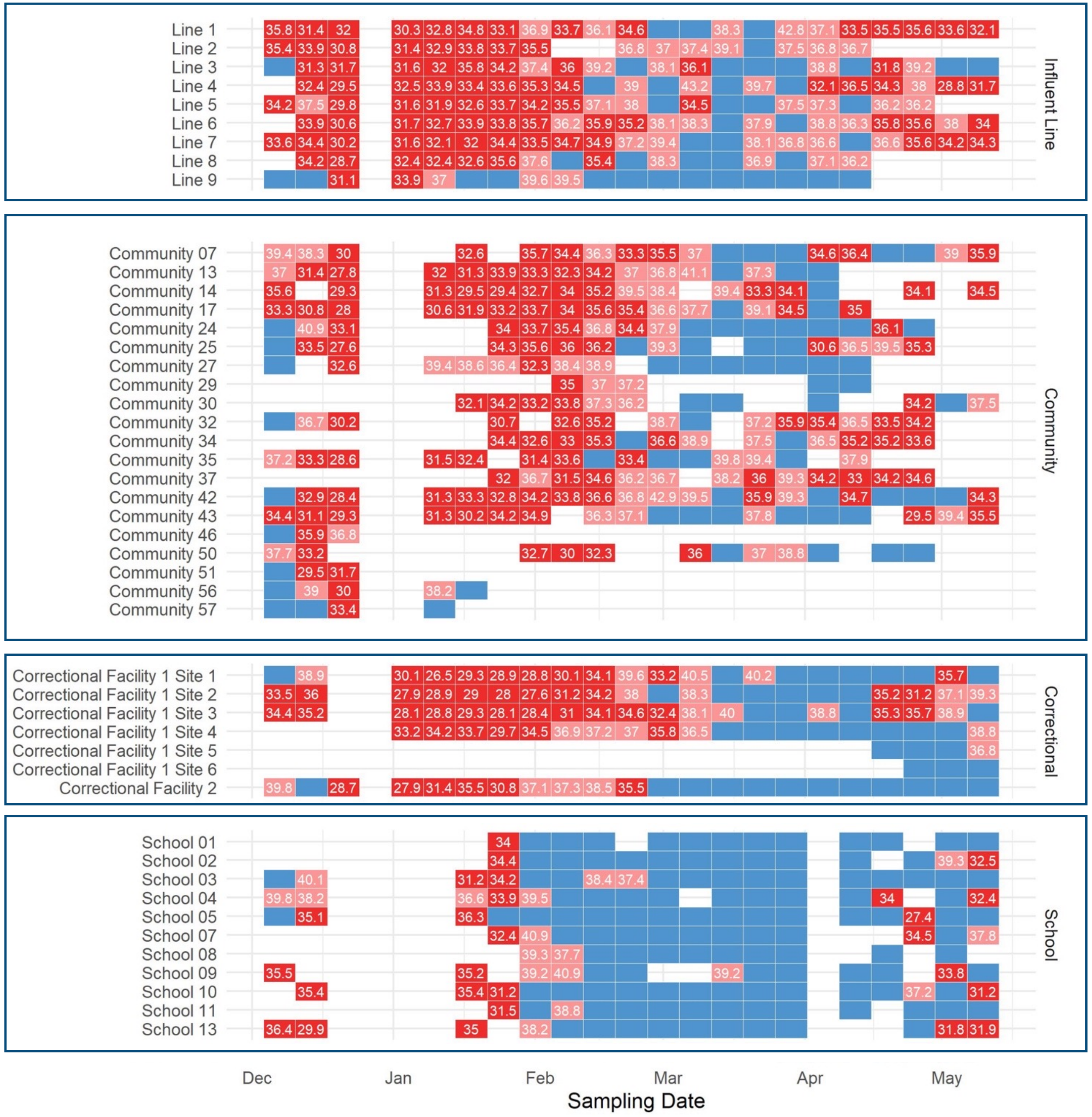
- Frequency of SARS-CoV-2 detection in wastewater samples increases with rise in COVID-19 cases
- Positive detections in community samples often precede increases in influent concentrations
- Spatial agreement between geocoded case data and results from community sites

Treatment Plants

Community Sites

Correctional Facilities

Schools



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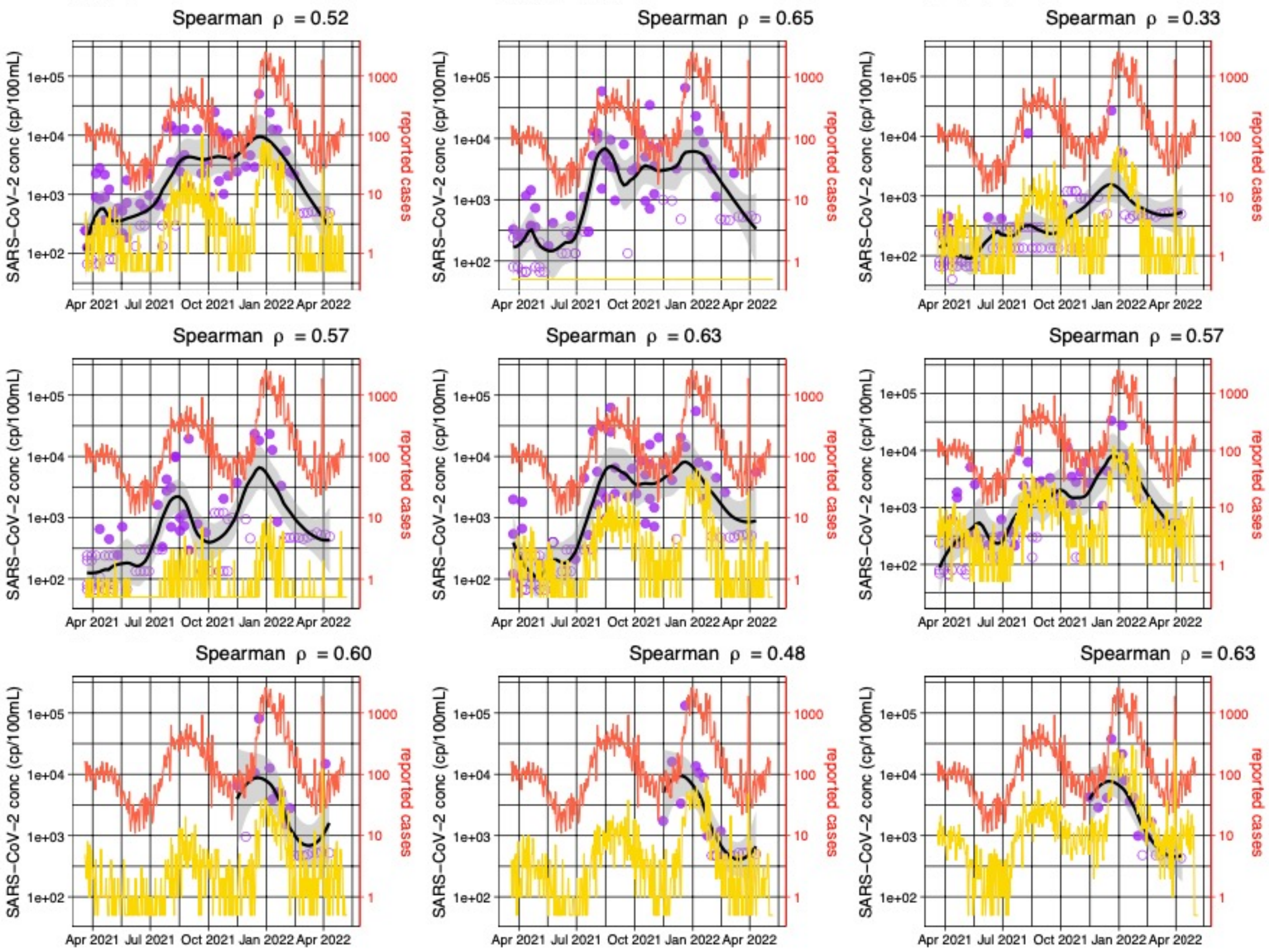
Influent Line Results

- Quantitative results from RT-qPCR
- Quantitative results reflect the pattern of clinical cases in each area of the city



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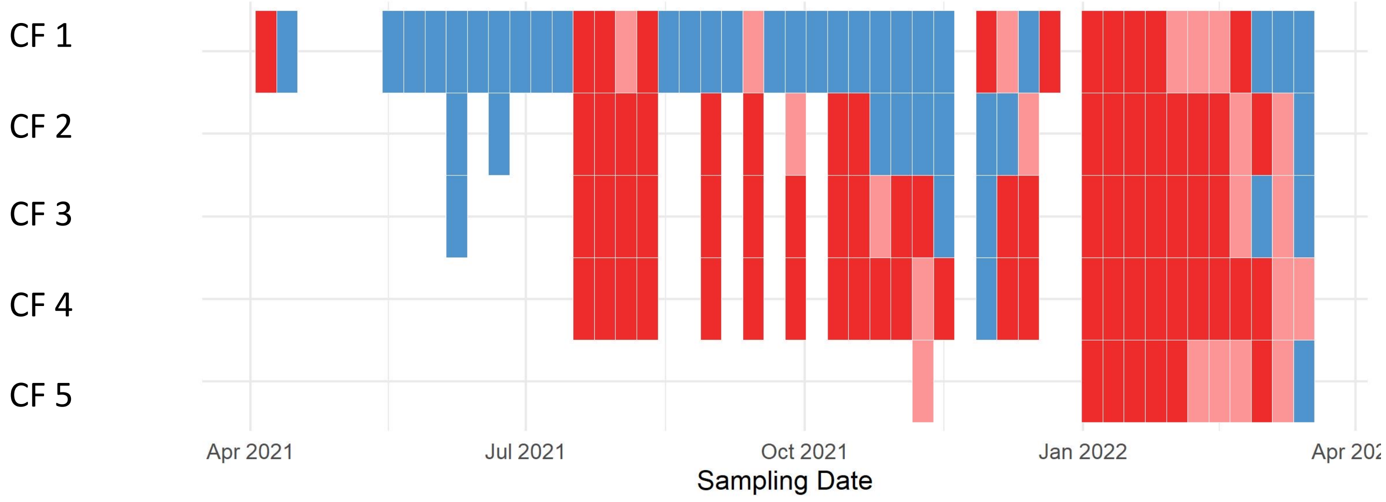
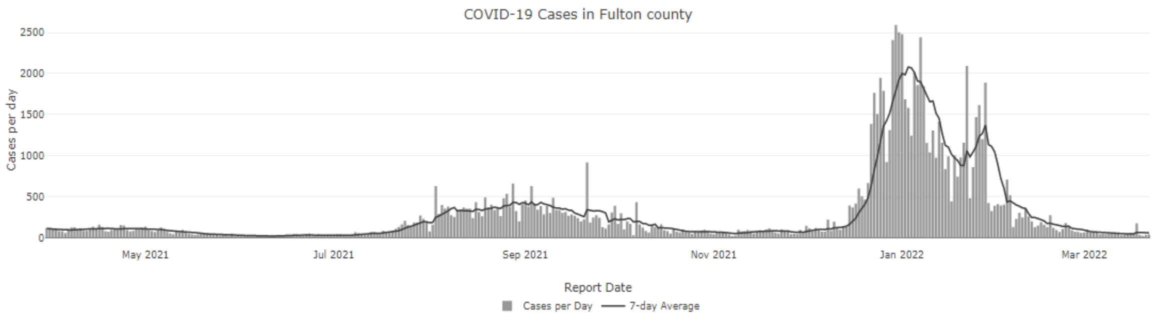
Correctional Facilities

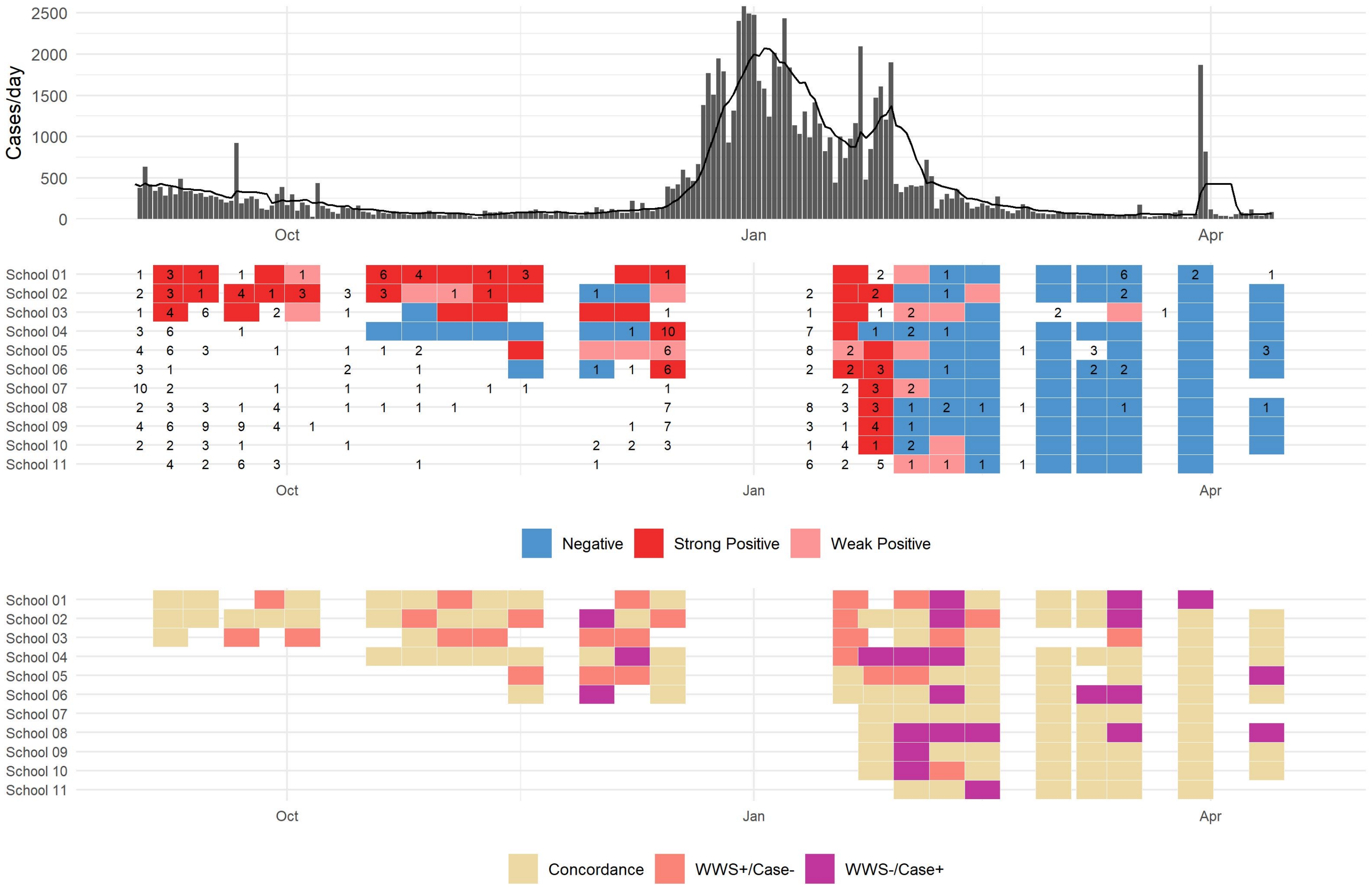
- Samples from the County Jail and Federal Penitentiary
- Weekly reports to facilities/self-swabbing study to guide testing
- Jail represents and more transient population – possible sentinel site for community





Public Schools

- SARS-CoV-2 RNA detected when no self reported cases in school
- Wastewater was occasionally negative for SARS-CoV-2 with few cases
- Schools interested in sharing data to help communicate risk and prevention events



Dissemination to Multiple Stakeholders

City Utility

Excited by results/data, but experiencing sample collection **fatigue and challenge** to scale community sample collection

Fulton County COVID-19 Area Command

Interested in "**micro-targeting"** of interventions and information based on wastewater results

Emory

County Health Department

Unfamiliar with wastewater data, **unsure** how it might change pandemic response activities

State Health Department

Originally overwhelmed, supported to apply for **CDC funding. Now launching statewater NWSS funded surveillance**

CDC

Especially interested in **community sampling model** and **institution-level** sampling

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Research Team

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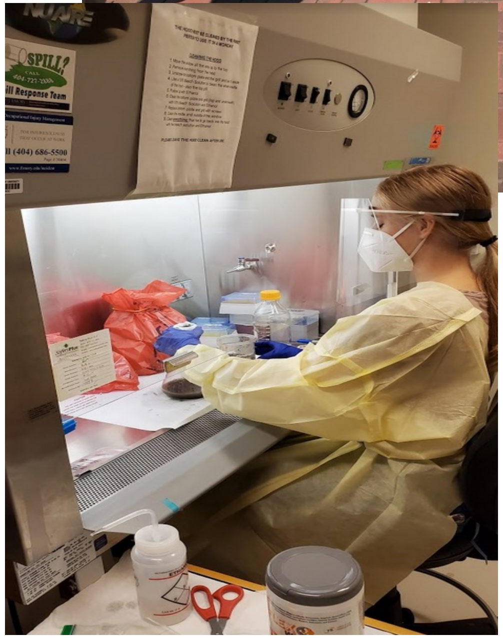

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DPH GEORGIA DEPARTMENT OF PUBLIC HEALTH

CITY OF ATLANTA DEPARTMENT OF watershed management

Wastewater Surveillance: International Case studies

COVID-19 Surveillance in Accra, Ghana

Typhoid Surveillance in Kolkata, India

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Environmental Surveillance for COVID-19 Proof of Concept for Accra, Ghana

OBJECTIVE:

- To develop a strategy for wastewater surveillance for COVID-19 in low-income urban settings with a **mix of sanitation systems**.
  - Area 1 - served primarily by a sewerage system and wastewater treatment plant
  - Area 2 - served primarily by shared public toilet facilities

RATIONALE:

- Weekly information on changes in SARS-CoV-2 RNA detection in environmental samples will be valuable to the Ghana Health Service to guide public health responses to the COVID-19 epidemic in Greater Accra Region that has **limited diagnostic testing capacity**

Study Approach

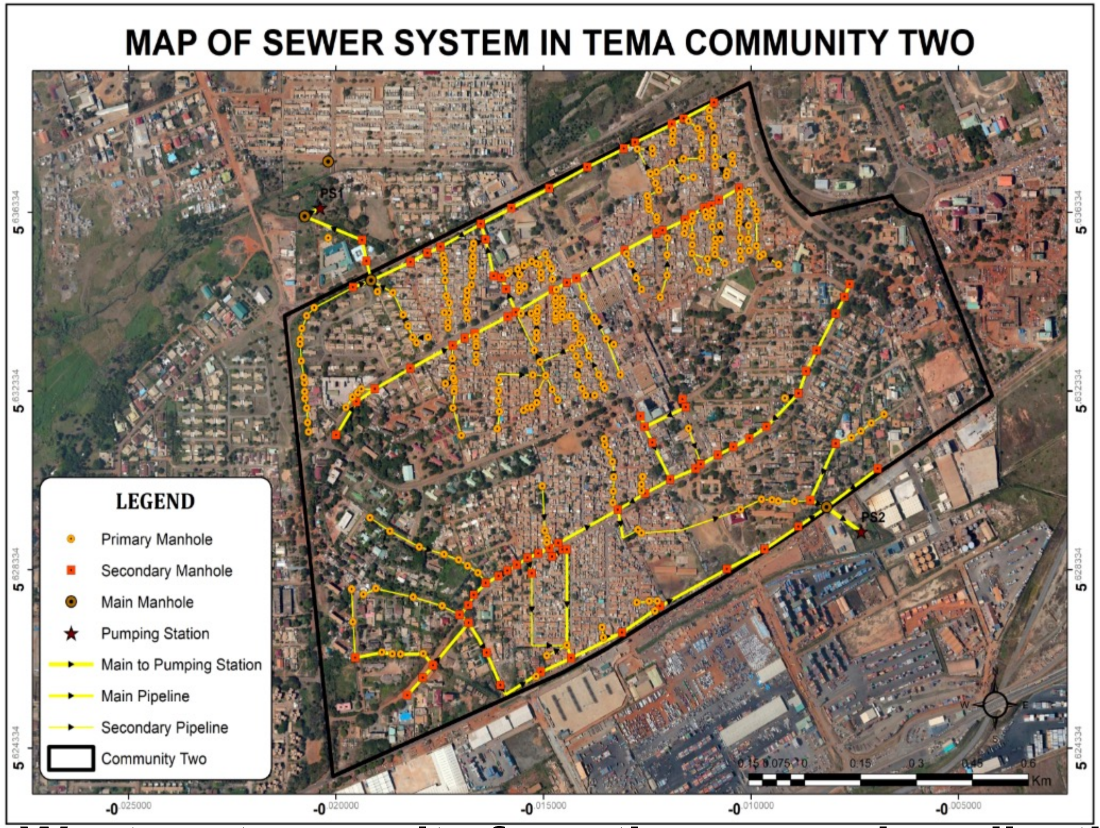
- Stakeholder engagement
  a. Integration within existing Ghana Health Service surveillance systems
  b. National and Municipal Technical Committees led by GHS
  c. Study sites selected based on identified COVID-19 hotspots with a mix of sanitation facilities
- Mapping of study sites to inform local decision making
- Capacity building to support environmental surveillance
  - Training of field and lab teams
  - Adapting methods to fit local context and available resources
- Weekly grab/Moore swab sampling from 2 study sites in Greater Accra Metropolitan Area from Jan-June 2021
  - 210 samples collected and analyzed
  - Physical parameters- pH, temperature, TDS, and turbidity
  - RT-qPCR analysis
  - Skim milk flocculation and membrane filtration concentration methods were used for processing samples
  - Primers N,E and RdRP target genes were used for qPCR analysis

Moore Swab Sample Collection in Manholes – Tema Sewerage System

Sampling from Public Toilet Septic Tanks - Osu

Tema COVID-19 Wastewater Surveillance Dashboard

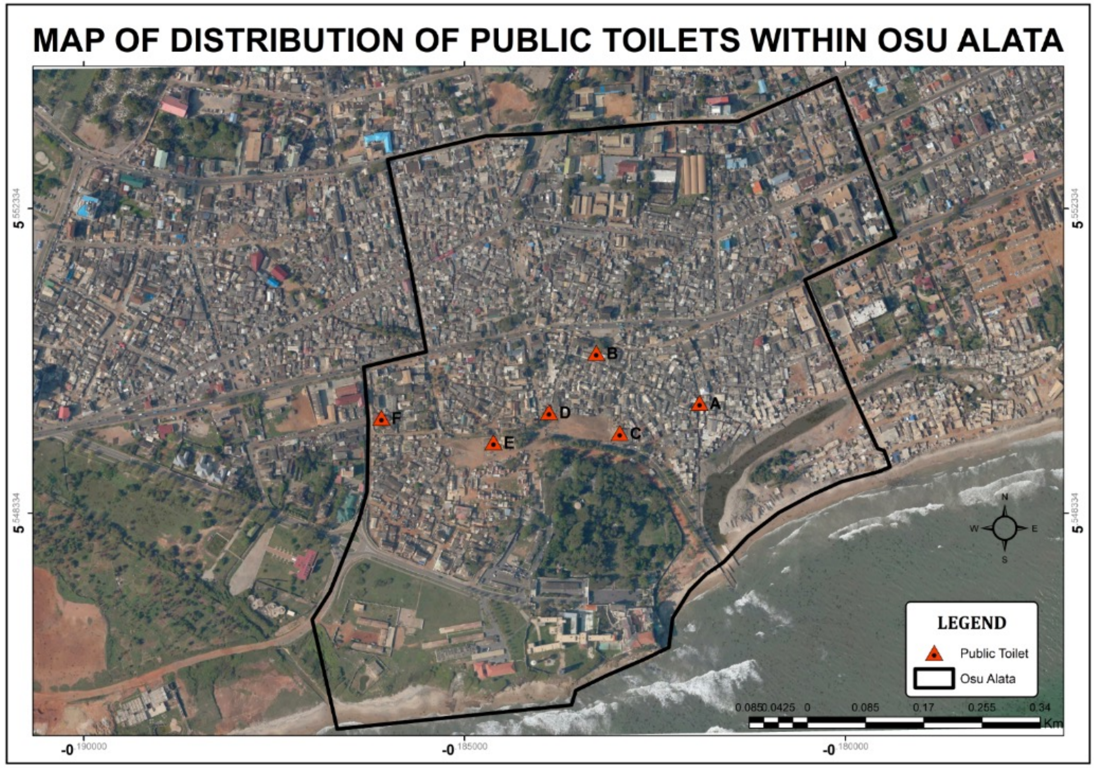
Accra,Ghana Covid-19 ≡

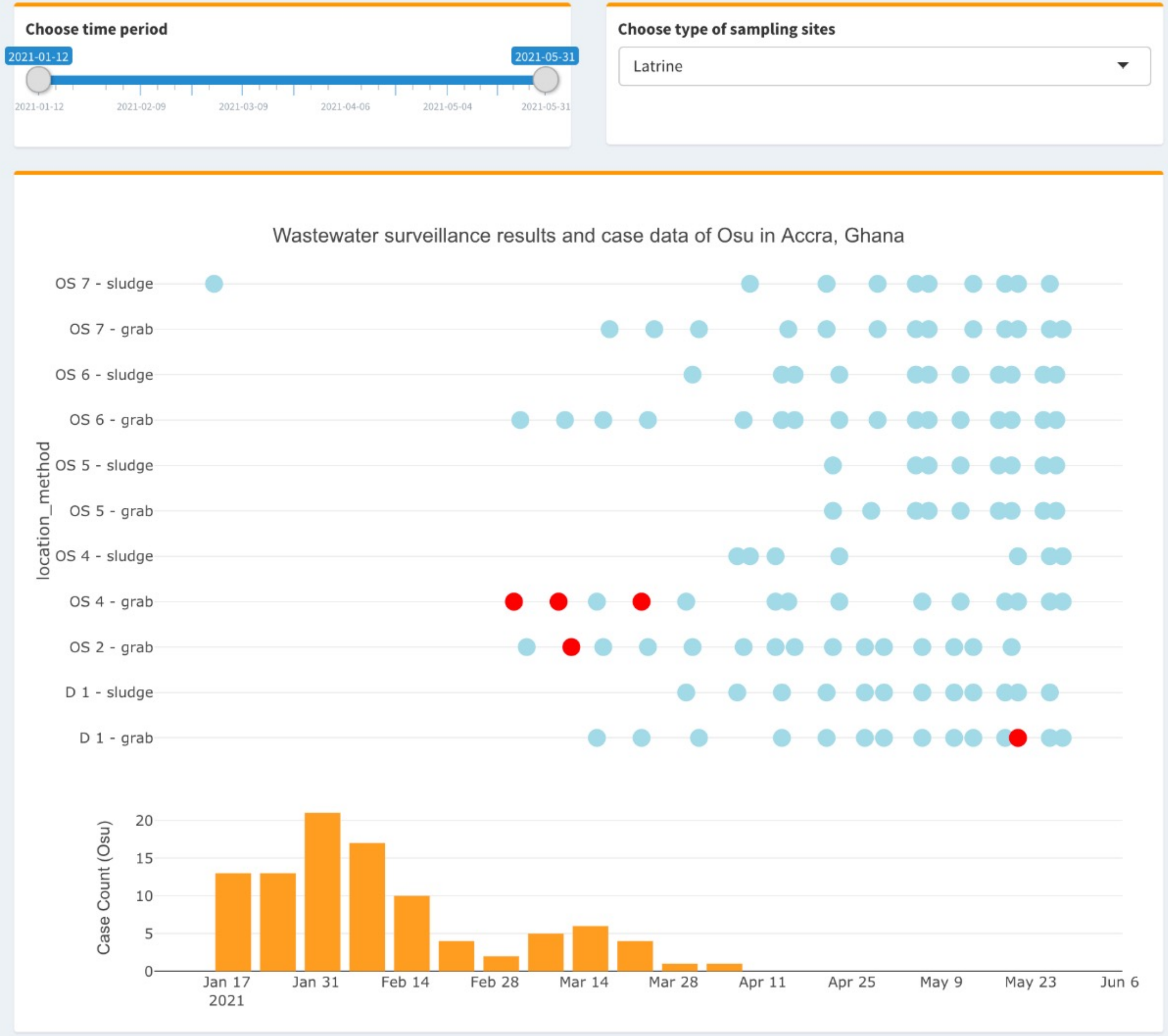


Wastewater results from three sample collection sites in Tema. Feb – May 2021.The epi curve labeled “Tema” (top curve) shows case counts for the entire City of Tema. The epi curve on the right labelled “Tema Community 2” (bottom curve) shows clinical case counts from the Community Two neighborhood of Tema city.

Osu COVID-19 Public Toilet Wastewater Surveillance Dashboard

Accra,Ghana Covid-19





Locations and wastewater results from six public toilets, February – May 2021

Stakeholder Recommendations for Next Steps

1. Conduct environmental surveillance in 6 districts where there have not been any reported COVID 19 clinical cases.
2. Investigate the impact of vaccination in Ghana Health Service (GHS) identified hot spots.
3. Use wastewater surveillance for genomic surveillance of COVID 19 variants.
4. Mainstream and scale up environmental surveillance across Ghana and West Africa.
5. Develop a data repository and environmental surveillance dashboard for decision making. Ideally, this would be integrated into the SORMAS system if possible.
6. Develop a decision making support tool to guide interpretation of environmental surveillance results for effective public dissemination.
7. Include other pathogens of national interest, such *Vibrio Cholerae* and *Salmonella* Typhi, into a national environmental surveillance program. This would also involve integrating into the existing polio environmental surveillance to form one national surveillance platform.
8. Use COVID 19 environmental surveillance for institutions and industries given recent outbreaks. This could involve monitoring critical places, such as secondary schools, universities, factories, parliament etc.

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Environmental Surveillance for Typhoid Fever in Kolkata, India

Environmental Surveillance Use Case for Typhoid Fever

Background: Want to prioritize deployment of new typhoid conjugate vaccine to countries/areas with highest need, but the true burden of typhoid fever is difficult to estimate. Typhoid fever is underestimated due to non-specific symptoms (eg. fever and systemic illness) and poor lab diagnostic tests.

Goal: Use detection of *S.* Typhi in municipal wastewater as **surveillance strategy to estimate typhoid fever burden in the population** and determine priority for vaccination campaigns

Study Design & Methods

- **Study site**: Kolkata, India
- **Sample collection**: May 2019-March 2020
- **Weekly wastewater samples** collected from 13 pumping stations
  - 28 Large volume (40L) samples
  - 199 Moore swabs

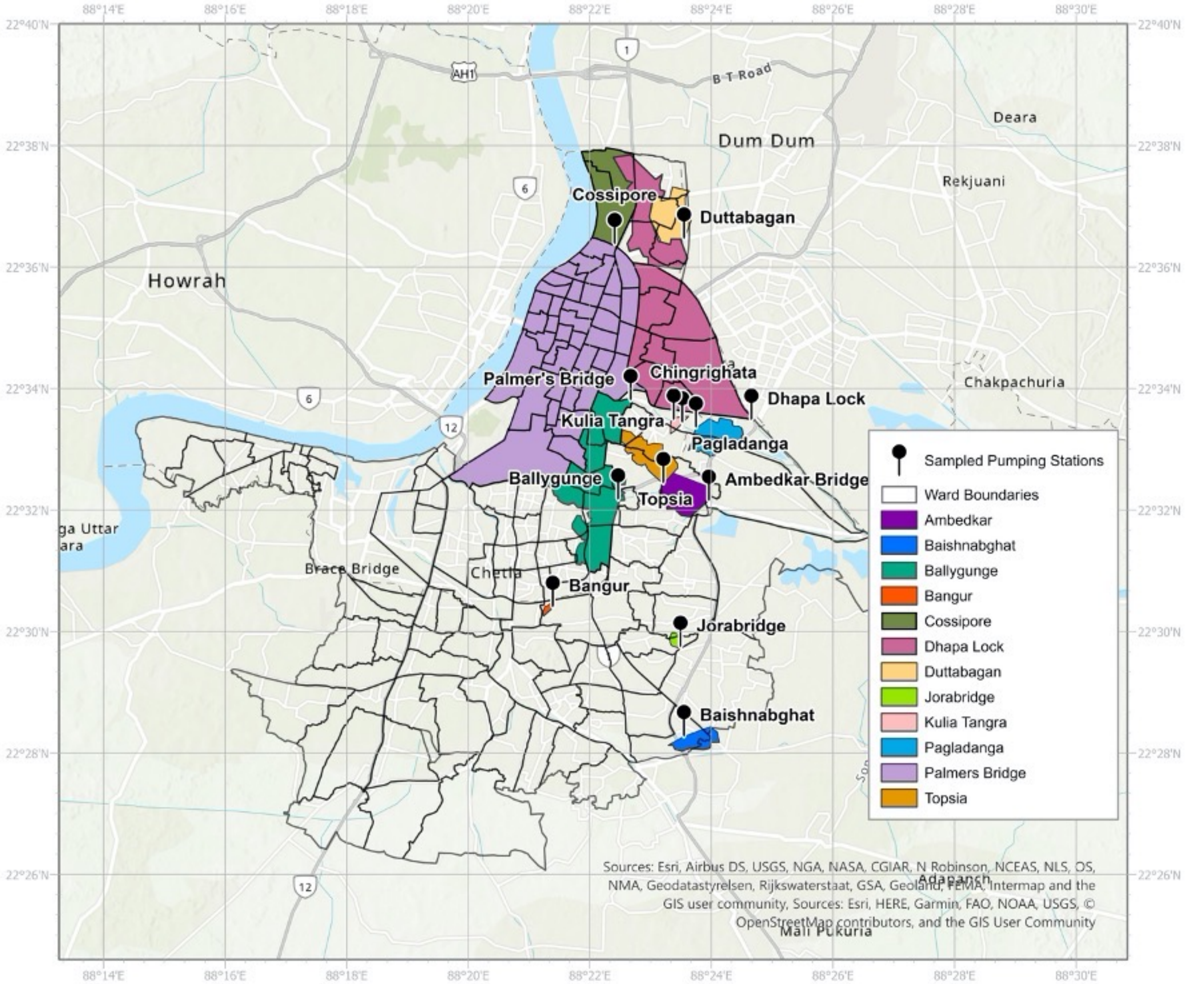
Sample Processing:

- 40 L samples: Concentrate by ultrafiltration-PEG precipitation->DNA extraction
- Grab samples & Moore swabs: overnight enrichment in UP broth –> DNA extraction
- Duplex qPCR for staG and tviB genes (Nair et al., J Clin Micro, 2019) specific for *S*. Typhi

Liu, P, M Ibaraki, R Kapoor, N Amin, A Das, M Miah, A Mukhopadhyay, M Rahman, S Dutta, CL Moe. (2021). Development of Moore Swab and Ultrafiltration Concentration and Detection Methods for Salmonella Typhi and Salmonella Paratyphi A in Wastewater and Application in Kolkata, India and Dhaka, Bangladesh. *Front Microbiol* 12: doi: 10.3389/fmicb.2021.684094

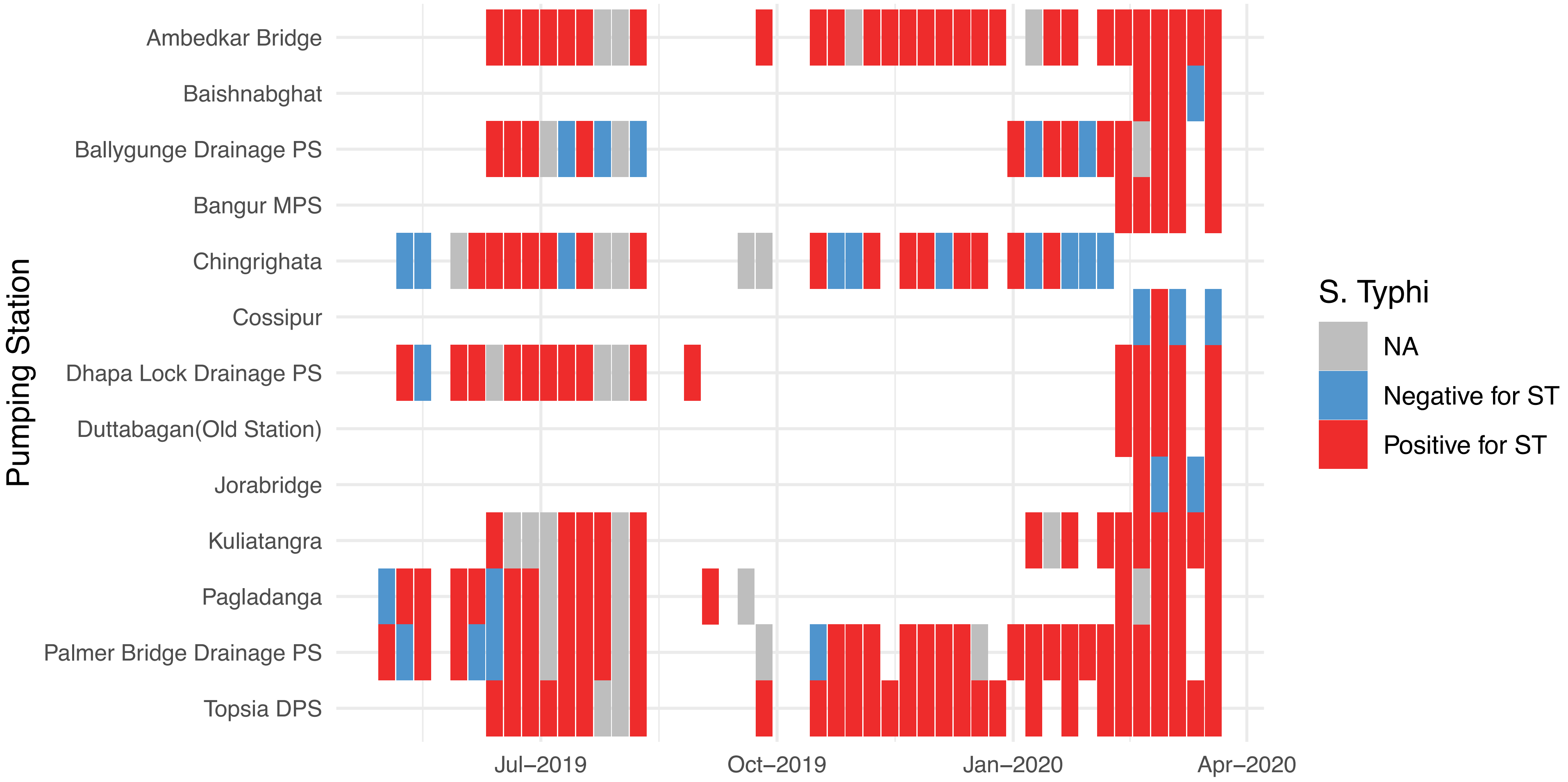
Estimating pumping station catchment areas using open source GIS tools

Pumping Station	No. Samples Collected	Geographic Catchment Area (sq km)	Population Size Estimate
Kulia Tangra	20	0.12	1,493
Bangur	5	0.06	1,525
Jorabridge	5	0.13	3,323
Baishnabghat	5	0.59	5,916
Chingrighata	22	0.78	9,405
Pagladanga	30	1.13	13,160
Topsia	31	1.19	33,542
Ambedkar	31	1.19	34,517
Duttabagan	5	1.37	49,788
Cossipore	4	3.10	95,471
Ballygunge	20	5.71	225,074
Dhapa Lock	19	12.19	378,753
Palmer's Bridge	35	20.42	890,681
Total	232	47.98	1,742,649



Sample type`	PCR	
	S. Typhi positive	Mean Ct values
Moore Swabs from Pumping stations	172/199 (86%)	28.09 (*tviB*), 29.51 (*staG*)

Moore swab results for 13 pumping stations, Kolkata, India May 2019-March 2020



Public Health Application

- Demonstrated that environmental surveillance could be used to indicate the burden of typhoid fever

- Widespread detection of *S*. Typhi at all the pumping stations throughout the entire sampling period.

- Wastewater surveillance results suggest high burden of typhoid fever in Kolkata – consistent with an estimate from 2 years of active clinical surveillance of 6000 children in two wards. (Incidence =714 per 100,000 child-years). **Results may be used to advocate for early childhood typhoid vaccination in Kolkata.**

- Moore swabs were easy to deploy, low cost, and more sensitive than other types of wastewater samples https://www.sanipath.org/

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