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Wastewater surveillance in non-sewered settings

16 MAY 2024

inspiring change

AGENDA

- **Wastewater-based Epidemiological Surveillance & the IWA Cluster**
  Gertjan Medema, KWR Water Research Institute, Netherlands
- **Non-sewered settings**
  Sudhir Pillay, Water Research Commission, South Africa
- **SARS-CoV-2 surveillance in non-sewered settings in South Africa**
  Gina Pocock & Fiona Els, Waterlab & National Center for Infectious Diseases, South Africa
- **Ensuring Health Equity: Infectious Disease Surveillance in South East Asia through Non-sewered Wastewater**
  Leshan Wannigama, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Thailand
- **Wastewater-Based Multi-Pathogen Surveillance in Malawi: Lesson and possible application to One Health**
  Petros Chigwechokha, Malawi University of Science and Technology, Malawi
- **Wastewater surveillance from Vellore, India - utilities, insights and challenges**
  Dilip Abraham, Christian Medical College Vellore, India
- **Q&A Panel Discussion**
  Speakers & Moderators
- **Close**
  Gertjan Medama & Sudhir Pillay

WEBINAR INFORMATION

- This webinar will be **recorded and made available “on-demand”** on the IWA Connect Plus platform and IWA Network website, with presentation slides, and other information.
- The **speakers** are responsible for **securing copyright permissions** for any work that they will present of which they are not the legal copyright holder.
- The opinions, hypothesis, conclusions or recommendations contained in the presentations and other materials are the **sole responsibility of the speaker(s)** and do not necessarily reflect IWA opinion.

WEBINAR INFORMATION

- **‘Chat’ box**: please use this for general requests and for interactive activities.
- **‘Q&A’ box**: please use this to send questions to the panelists.
  (We will answer these during the discussions)

Please Note: *Attendees’ microphones are muted. We cannot respond to ‘Raise Hand’.*

SPEAKERS & MODERATORS

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Gertjan Medema
Netherlands

Petros Chigwechokha
Malawi

Fiona Els
South Africa

Leshan Wannigama
Thailand

Sudhir Pillay
South Africa

Gina Pocock
South Africa

Dilip Abraham
India

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Wastewater Surveillance in Non-Sewered Settings

GERTJAN MEDEMA, SPECIALIST GROUP: HEALTH-RELATED WATER MICROBIOLOGY

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IWA CLUSTER
WASTEWATER-BASED EPIDEMIOLOGICAL SURVEILLANCE

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Vision

Global network of professionals and institutions for rapid info exchange and dialogue within water and between water and public health

MULTIDISCIPLINARY IN WATER

LINKING WATER TO PUBLIC HEALTH

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Deutsches Ärzteblatt


IWA
the international water association
WEBINAR
Wastewater surveillance in non-sewered settings
16 May 2024
15:00 BST
Gertjan Medema
KWR
Netherlands
Sudhir Pillay
WRC
South Africa
Gina Pocock
Waterlab
South Africa
Fiona Els
NICD
South Africa
Petros Chigwechokha
University of Science & Technology
Malawi
Leshan Wannigama
Chulalongkorn University
Thailand
Dilip Abraham
Christian Medical College Vellore
India

GETTING TO KNOW YOU

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Dear participants,

please surf in your browser to

pollev.com/iwa

to enter the poll

so we can learn a few things

about your background

or scan this QR

IWA CLUSTER
WASTEWATER-BASED EPIDEMIOLOGICAL SURVEILLANCE

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Cluster workshop on value of wastewater surveillance for public health,
with WHO, US CDC, MoH Thailand, Ottawa Public Health

Surveillance in Non-Sewered Settlements: Non-Sewered Settings: The Why, What (and later How?)

SUDHIR PILLAY, SPECIALIST GROUP: NON-SEWERED SANITATION (NSS)

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Main photo by WaterLab PTY LTD

WHAT ARE NON-SEWERED AREAS

- Any sanitation system treating human excreta that operates **without connection to any sewer or drainage network**
- A **non-sewered sanitation** (NSS) system is a sanitation system that 1) is not connected to a networked sewer system and 2) collects, conveys, and fully treats the specific input, to allow for safe reuse or disposal of the generated output
- Where there is no sewer:
  - Greywater (washing water) discharge into toilet or environment
  - Faecal sludge can accumulate
  - On-site recycling (new generation systems)

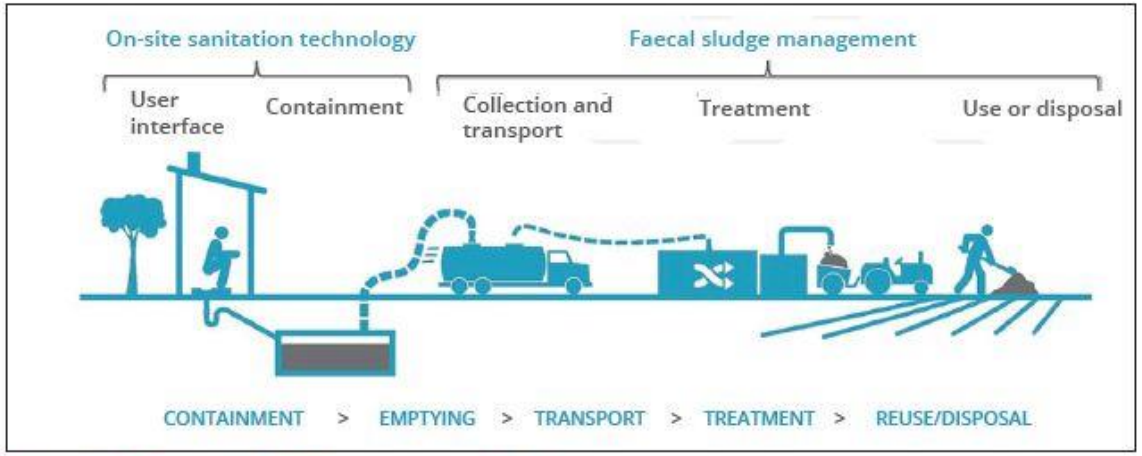

Figure 3.2: A typical FS Management System
Source: Modified from Wikipedia.org, 2020

SANITATION VALUE CHAIN

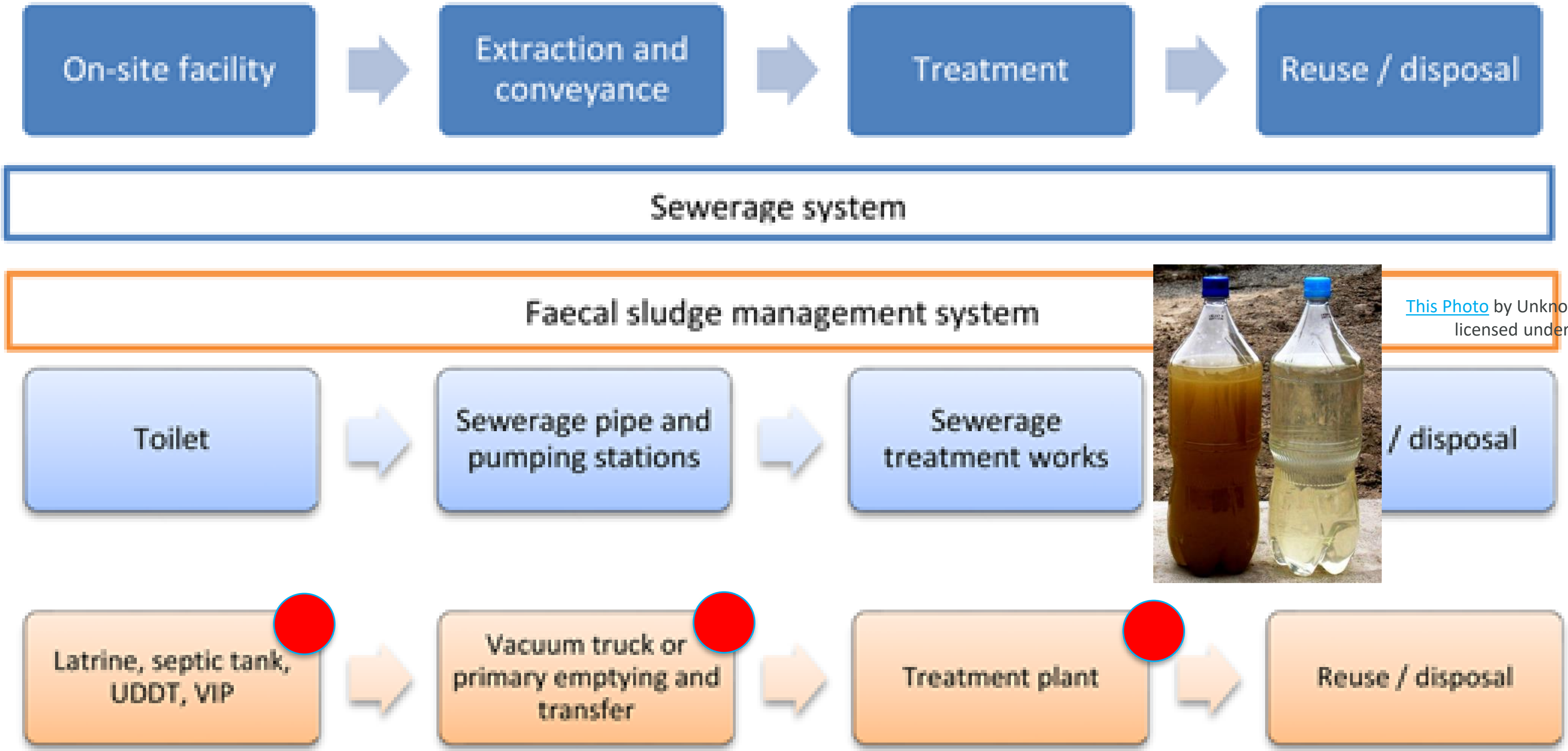

Image From: https://iwaponline.com/washdev/article/8/2/176/39027/The-cost-of-urban-sanitation-solutions-a

NSS ENVIRONMENTS – HIGHLY VARIABLE EVEN IN SAME LOCALITY

Photos by WRC in same province

SAMPLES – DIFFERENT WAYS TO COLLECT

A

B

C

D

Photos (a) Jimma University, (b) WRC, (c) University of Malawi, (d) WaterLab PTY LTD

FUNDAMENTAL DIFFERENCES IN SAMPLE COMPOSITION

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- **Heterogeneous Composition**: mixture of human excreta, water, urine, and other solid and liquid waste. Its composition can vary significantly depending on factors such as location, diet, and sanitation practices, making it challenging to establish consistent characterization parameters
- **Variable Chemical & Physical Properties**: wide range of organic and inorganic compounds, solids and water content, viscosity, density and particle size distribution
- **Pathogen Content**: usually contains a high concentration of pathogens, including bacteria, viruses, and parasites
- **Storage & Collection Variability**: wide range of systems from latrines to septic tanks, wet vs dry, different loading rates and designs
- **Regulatory & Standards Variability**: variation in designs and regulation
- **Sampling variability**: Analysis can be challenging due to its heterogeneous nature and the potential for stratification within storage systems, especially dry sanitation systems.

HIGHLY VARIABLE WASTE IN NSS SYSTEMS

	Pit latrine sludge	High strength sludge from bucket latrines and public toilets	Low strength sludge from septic tanks	Sewage – in waterborne sewerage systems
Source	Brouckaert and Foxon, 2008	**Heinss et al, 1998**		
COD (mg/l wet)	**90 000-225 000**	20 000-50 000	< 10 000	**500 – 2 000**
COD (mg/g dry)	**210-1230**	571-1429	<333	**50-200**
N as NH_4 (mg/l wet)	**9 000 (TKN)**	2 000-5 000	<1 000	**30-70**
N as NH_4 (mg/g dry)	**100 (TKN)**	60-150	<33	**3-7**
Total solids (%)	**20**	>3.5	< 3	**< 1**
Soluble solids(mg/l wet)	**220 000**	**≥30 000**	**≈ 7 000**	**200-700**

THE CHALLENGE OF NSS SURVEILLANCE

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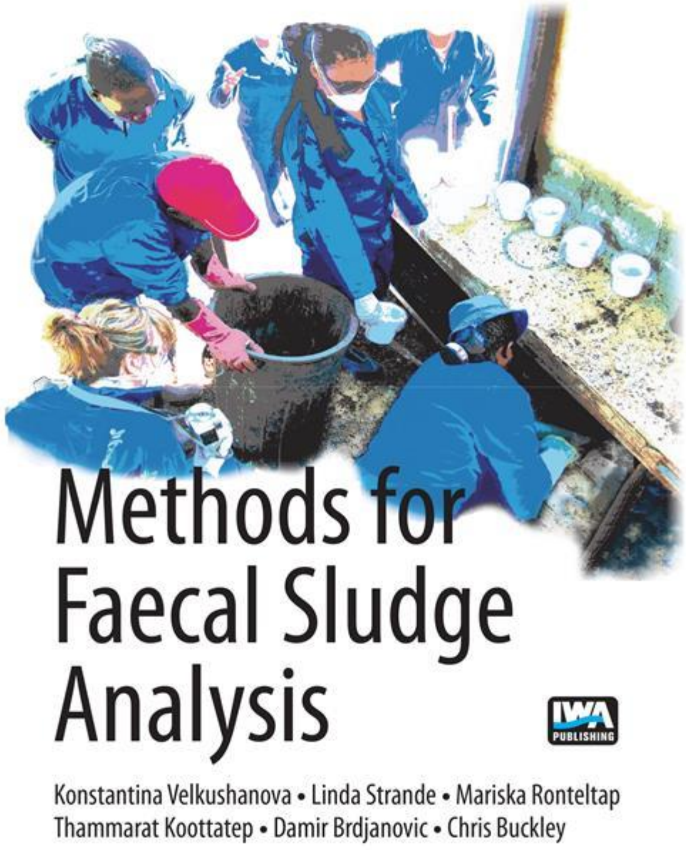
- **Variety of NSS systems**
  - Variable characteristics (not like WWTW)
  - Variable sampling sites (toilets, transport like honeysuckers, treatment wor
  - Not a regulated site
  - Lack of formalised workers for sampling (training & SOPs required)
- **NSS systems are highly variable per site & country**
  - Variable design
  - Washers vs wipers
  - Dry vs flush
  - Water ingression
- **Standards, Financing & Regulation**
  - Business case & modelling (vs clinical)
  - Long-term financing
  - Citizen-science approach? SOP for sampling, collection and analysis



Should we include into this or develop a separate technical guidance document?

HOW WOULD IT BENEFIT DEVELOPING COUNTRIES

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- Urban slums – Social Distancing difficult
- Shared water & sanitation facilities
- Accessibility & affordability to medical care
- Availability of test kits for patient testing
- Alleviate supply chain for test kits & vaccines
- COVID-19: Inequity with vaccines

SARS-CoV-2 surveillance in non-sewered settings in South Africa

DR GINA POCOCK AND FIONA ELS

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WATERLAB

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

WATER RESEARCH COMMISSION

GCRO Gauteng City-Region Observatory

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

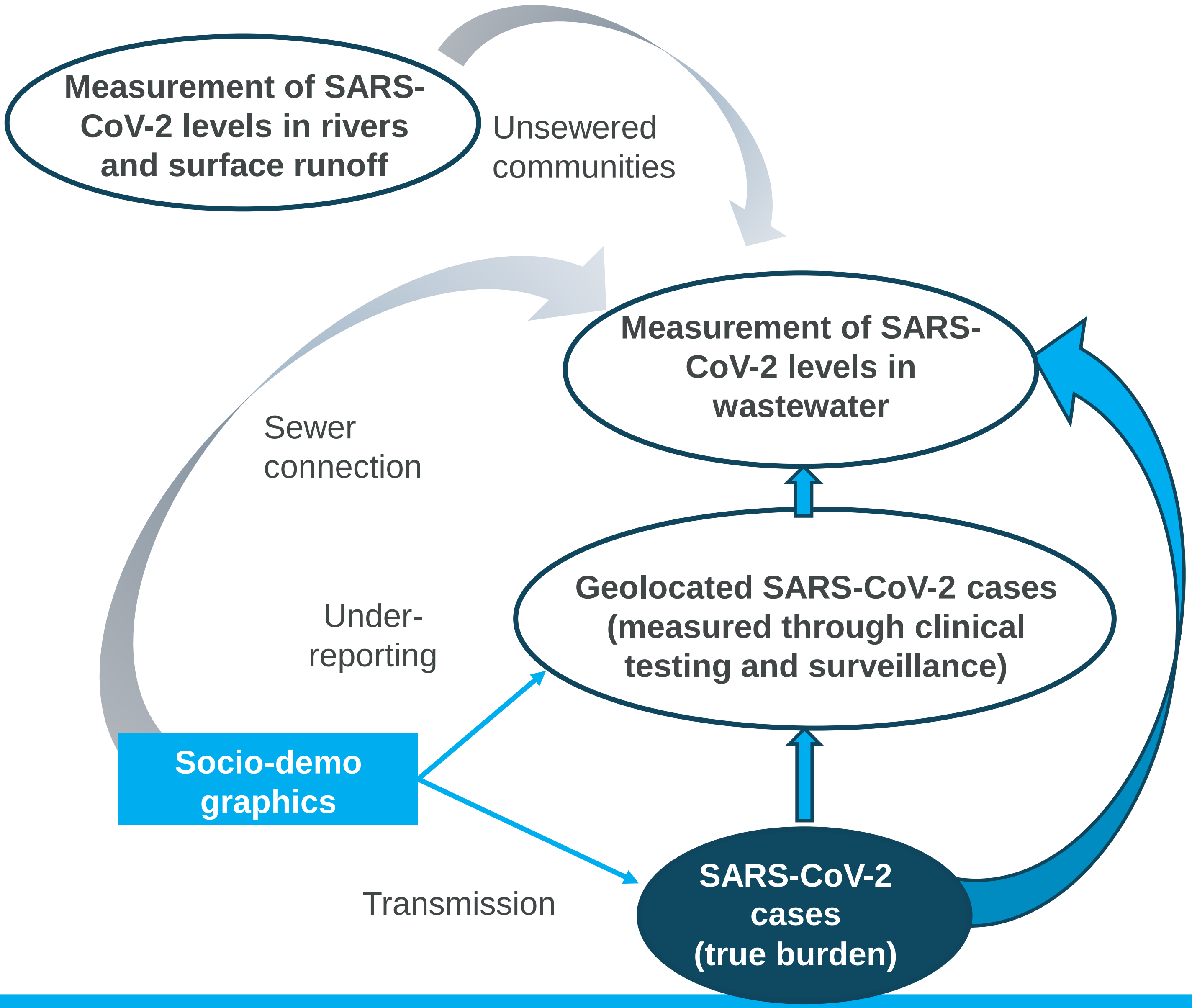
BACKGROUND

- Wastewater surveillance has recently emerged as a flexible and robust method to monitor infectious diseases
- It has been used in various countries to monitor SARS-CoV-2
- Recently started to monitor for other pathogens (influenzas, cholera, typhoid etc)
- Wastewater infrastructure need to be in place for it to work effectively

BACKGROUND

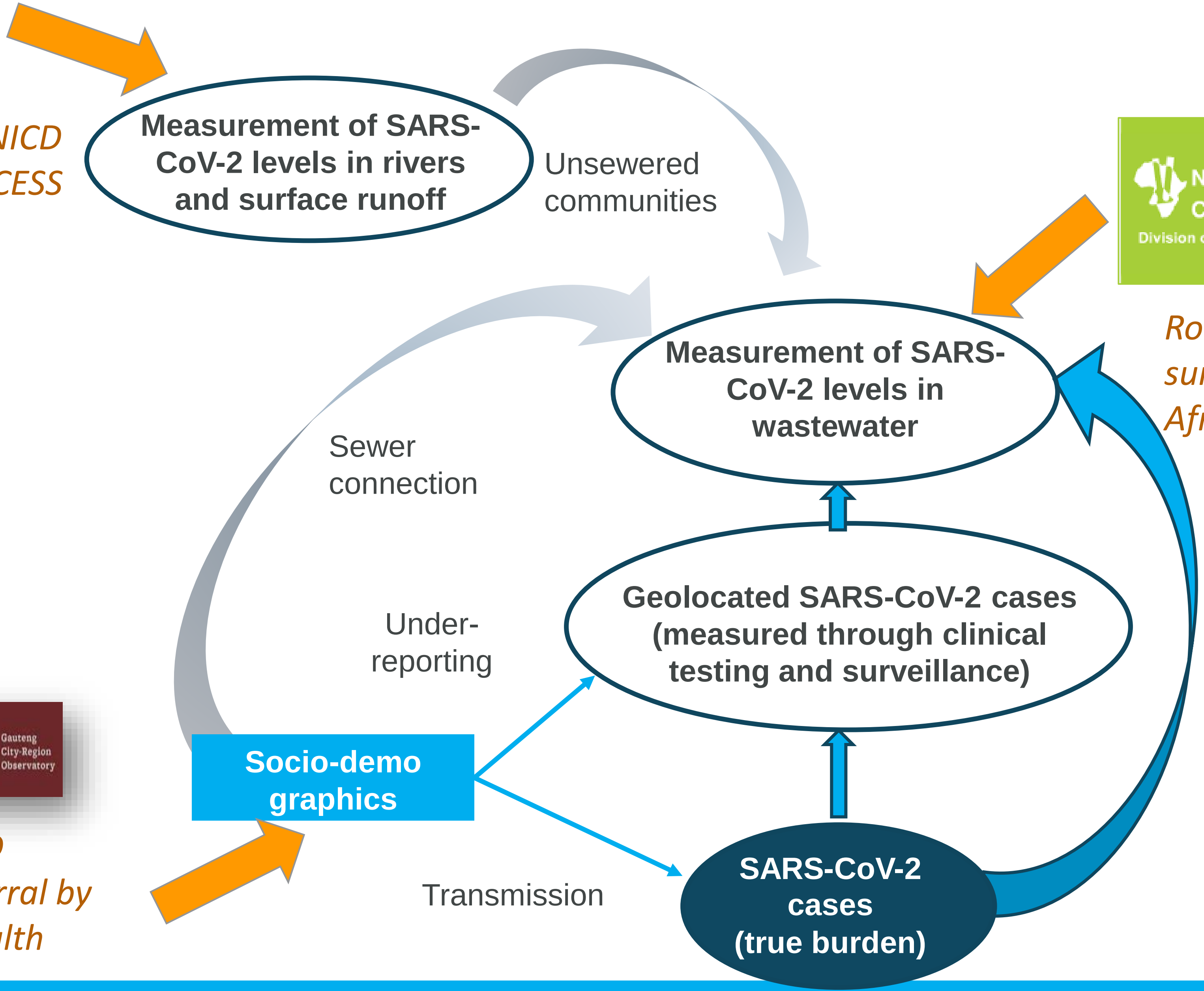
- South Africa has a functioning and representative wastewater surveillance system for SARS-CoV-2 located at sentinel site wastewater treatment plants
  - This programme has achieved success in monitoring levels and genotypes circulating in the population
- However, 43% of the population is not connected to a sewer network (Statistics SA)
  - In South African metropolitan areas 16.8% live in informal dwellings
- Utility of environmental surveillance in unsewered areas is presently unmeasured in our context and data pertaining to wastewater surveillance will have global reference

We developed a conceptual framework to help us understand what approach to take in evaluating environmental surveillance


Measurement of SARS-CoV-2 levels in rivers and surface runoff
Unsewered communities
Measurement of SARS-CoV-2 levels in wastewater
Sewer connection
Under-reporting
Geolocated SARS-CoV-2 cases (measured through clinical testing and surveillance)
Socio-demo graphics
Transmission
SARS-CoV-2 cases (true burden)

WATERLAB

Waterlab - identified by NICD researchers through SACCESS network participation



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NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

Routine wastewater surveillance in South Africa

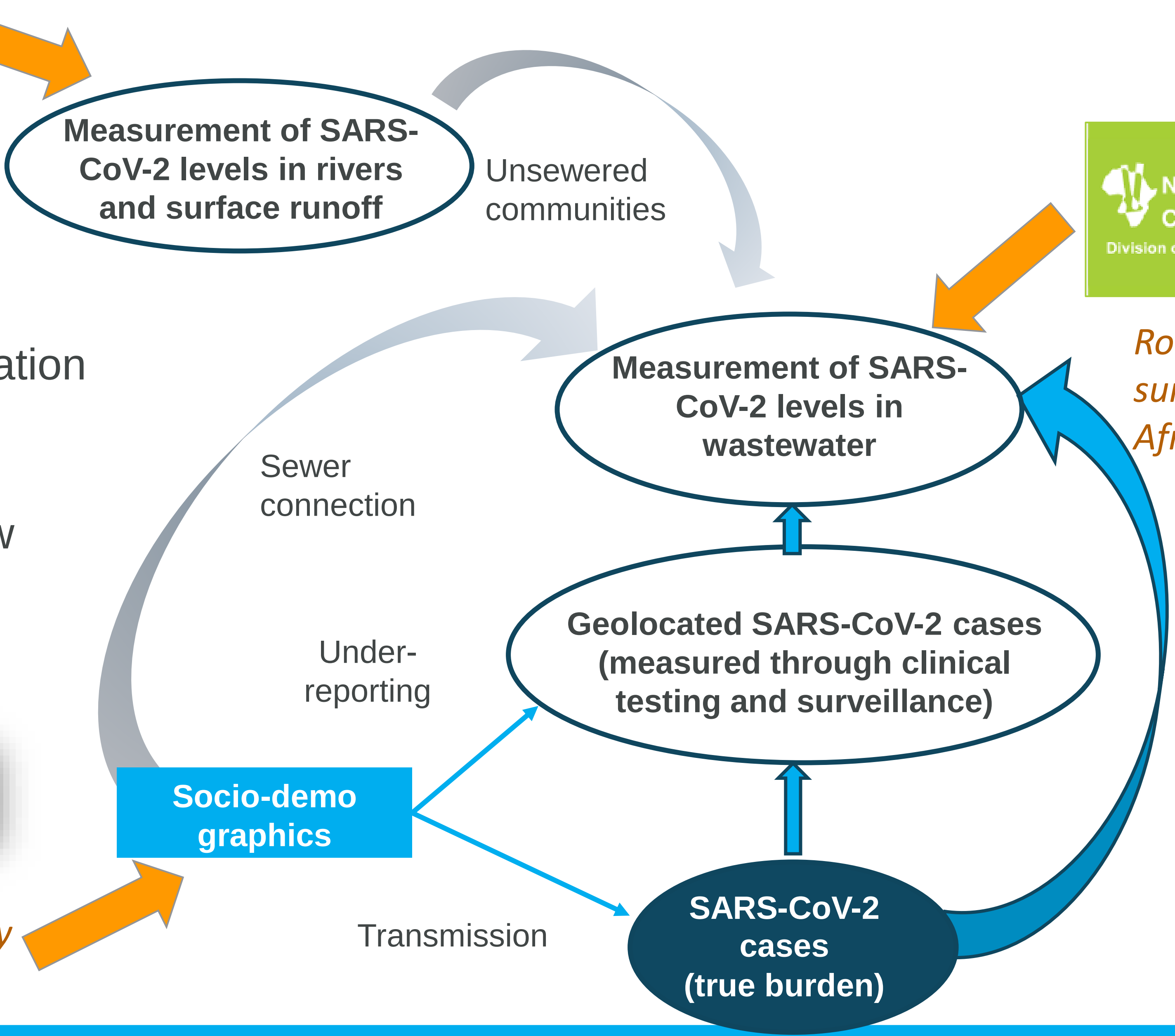
GCRO Gauteng City-Region Observatory

GCRO - identified to NICD researchers through referral by Wits School of Public Health

Waterlab - identified by NICD researchers through SACCESS network participation

- Interdisciplinary collaboration was needed
- How do we fit various datasets together to draw meaningful conclusions?

GCRO - identified to NICD researchers through referral by Wits School of Public Health



Routine wastewater surveillance in South Africa

AIMS AND OBJECTIVES

Aim:

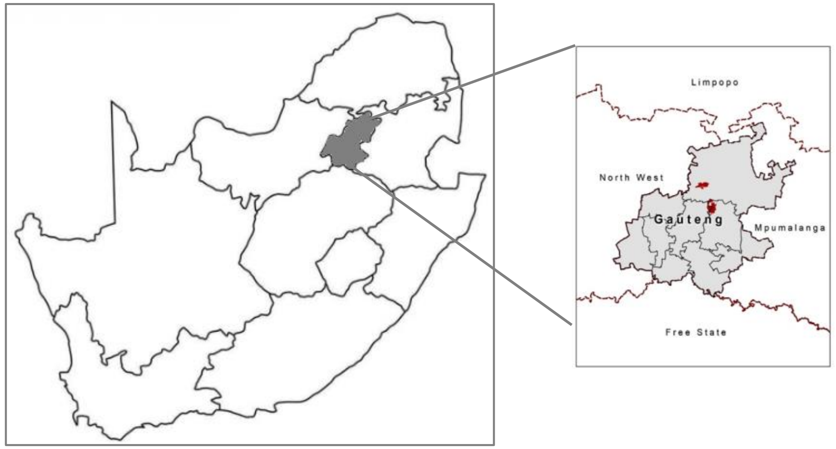
Characterise populations in non-sewered areas where wastewater samples were tested for SARS-CoV-2 to better understand the relationship with environmental surveillance

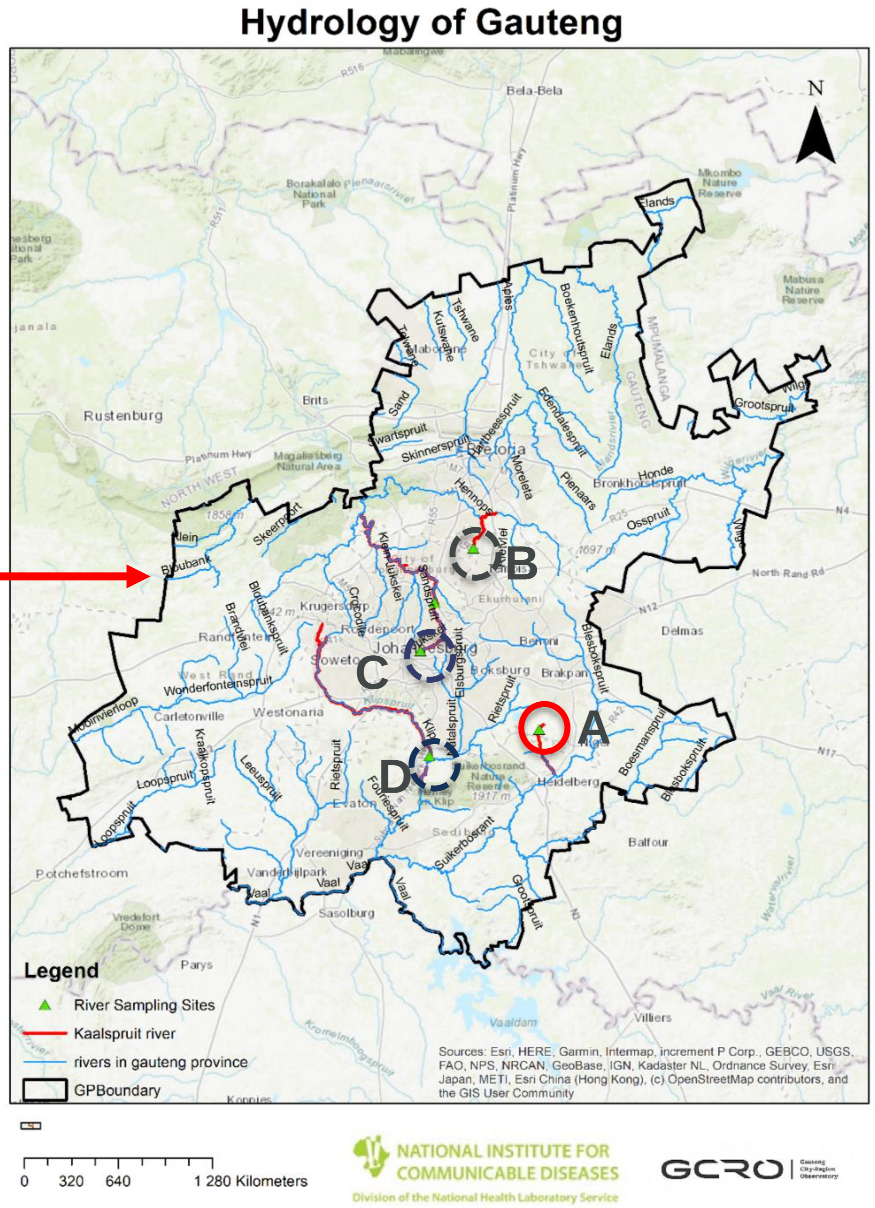
Objectives:

1. To identify sites, collect samples and quantify SARS-CoV-2 in non-sewered settings in densely populated areas of Gauteng province
2. Characterise catchment areas (topography and drainage profile), population demographics and Quality of Life at sample collection sites
3. Geolocate laboratory-confirmed SARS-CoV-2 cases to catchment areas
4. To describe relationships between environmental SARS-CoV-2 concentration, demographics and laboratory-confirmed cases

METHODS

- Descriptive study to determine causal and contributory relationships regarding incidence of communicable disease and social determinants of health

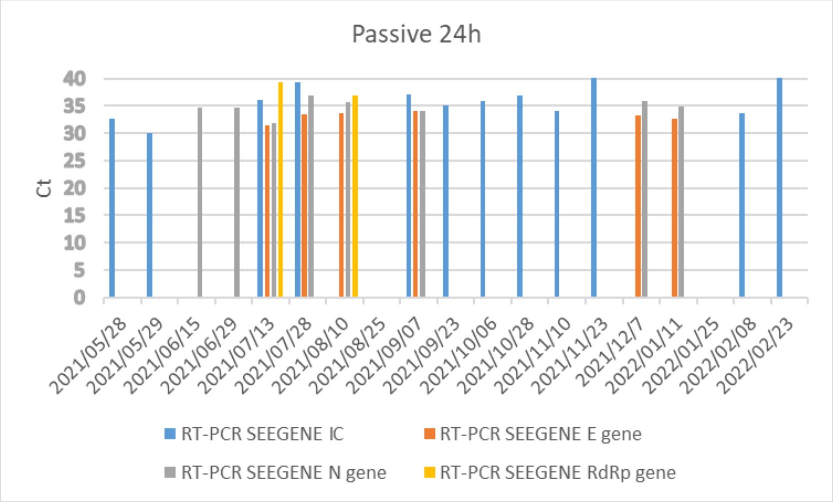


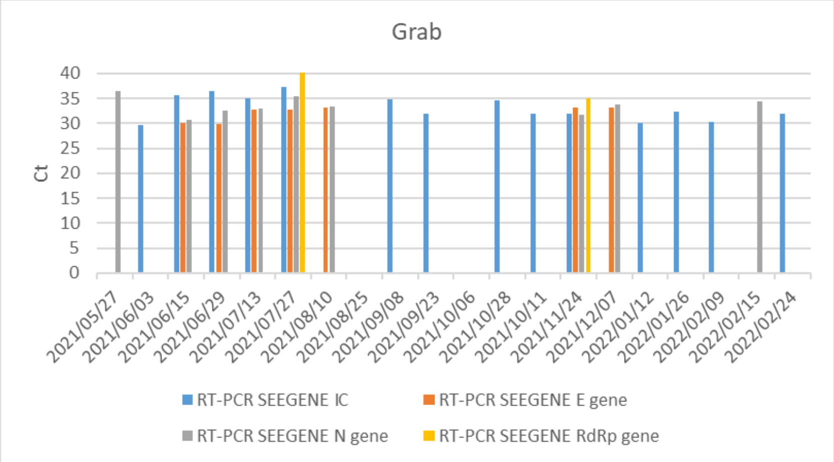


- Gauteng is one of 9 Provinces in South Africa, and has 46 rivers
- We purposely identified 4 river sampling sites within the City of Ekurhuleni and the City of Johannesburg that are impacted by untreated surface run-off from informal and unsewered communities:
  - Blesbokspruit (A), Kaalspruit (B)
  - Jukskei River (C), Klipspruit (D)

METHOD: SAMPLE TYPES

- **Sample selection**
  - **Grab Samples**
    - Identified informal settlements lacking access to sewered sanitation located in close proximity to a river
    - Rivers provide more stable sample points than greywater pools which are transient in nature
  - **Passive samples**
    - Device covered in shadecloth and anchored at sample point for a specific period, then viral nucleic acid eluted from gauze in the lab
    - Passive sampling was conducted in parallel to grab samples at a subset of sample sites





Results: Grab vs Passive, Jukskei River downstream Alexandra

Concentration	Nucleic acid extraction	SARS-CoV-2 RT-PCR & amplification	Molecular analysis platform	Limit of quantification (genome copies/ml)
Skimmed milk flocculation	QIAamp® Ultrasens® Virus kit (1mL)	Allplex™ 2019-nCoV Assay and 2019_ nCoV_N positive control plasmid	QuantStudio™ 5 Real-Time PCR System (Applied Biosystems)	16

BLESBOKSPRUIT SAMPLING SITE

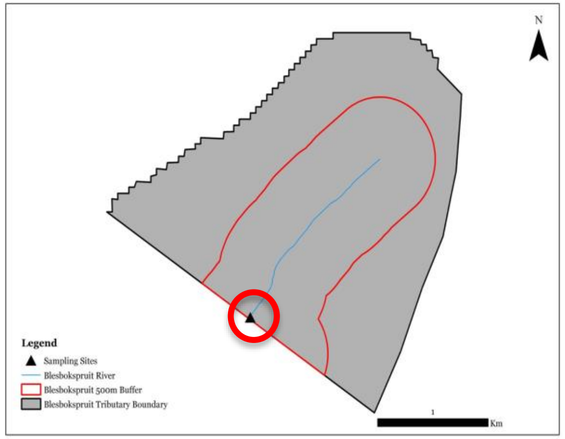


Fig 1: Buffer (red line) and catchment areas (black line) upstream of sampling site

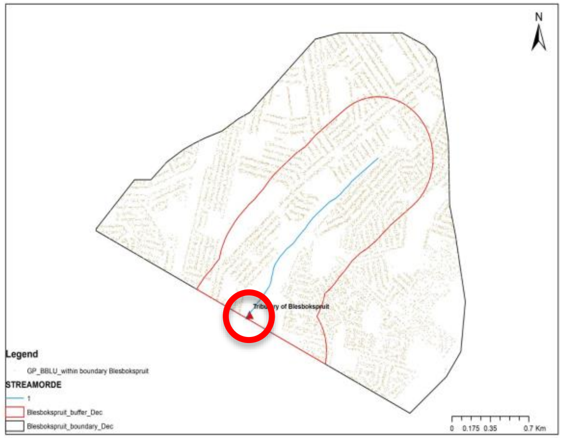


Fig 2: Building base land use upstream of sampling site

BLESBOKSPRUIT SAMPLING SITE

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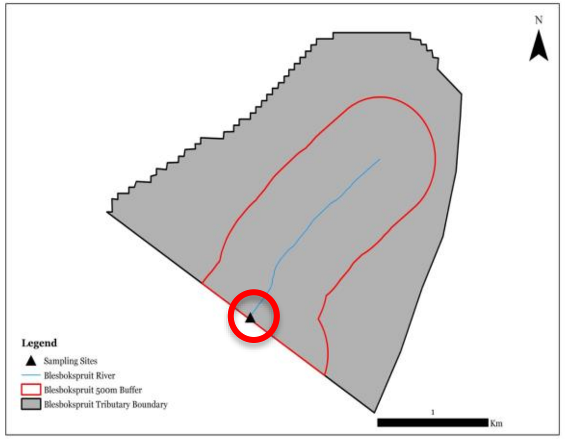

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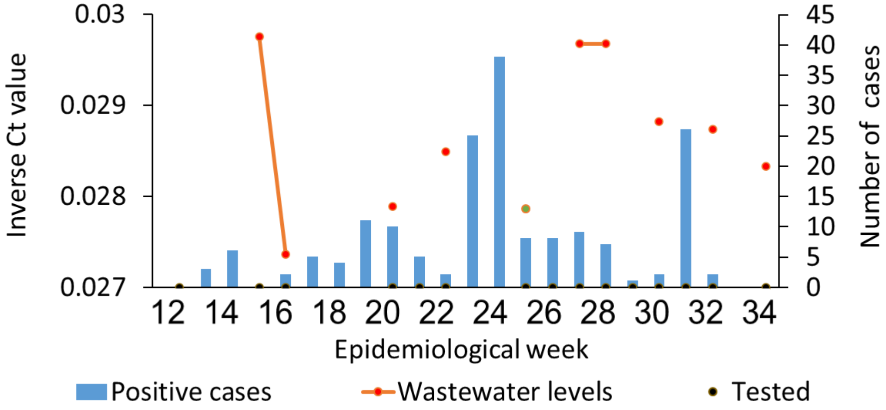
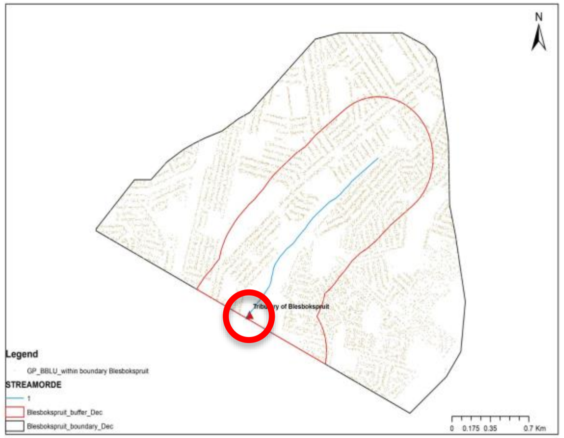


Fig 3: Geolocated SARS-CoV-2 cases in the catchment area (blue bars) and quantitative SARS-CoV-2 river results (Ct threshold), from epidemiological week 12-35, 2021.



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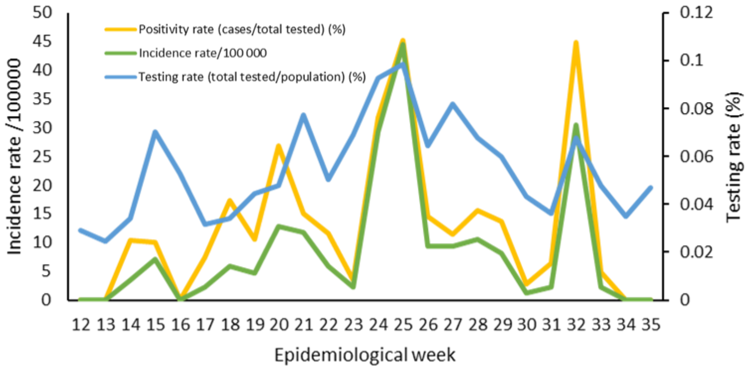
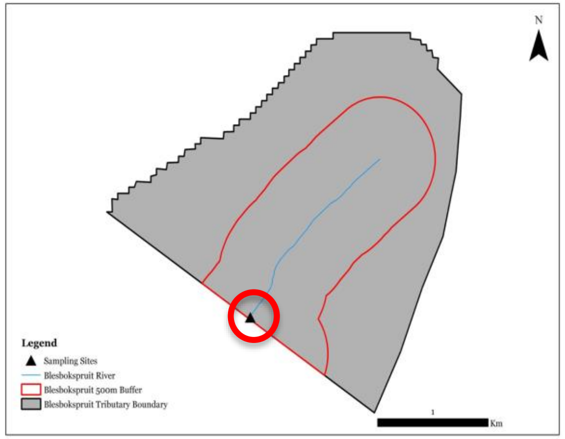


Fig 4: Positivity rate, incidence rate and testing rate of geolocated SARS-CoV-2 cases in the catchment area, from epidemiological week 12-35, 2021.

BLESBOKSPRUIT SAMPLING SITE



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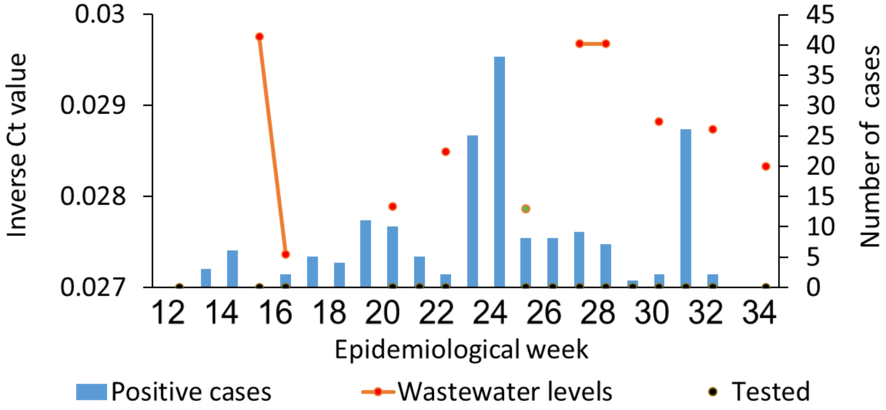

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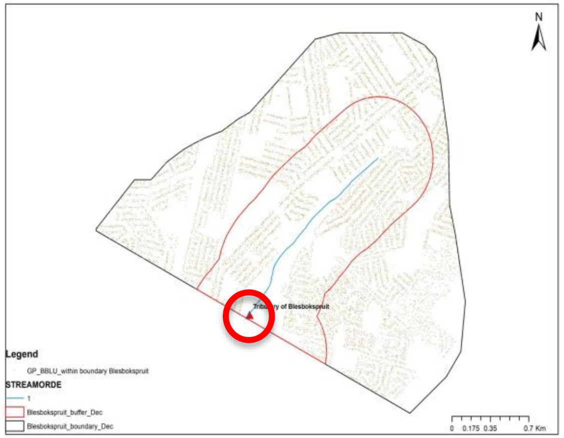

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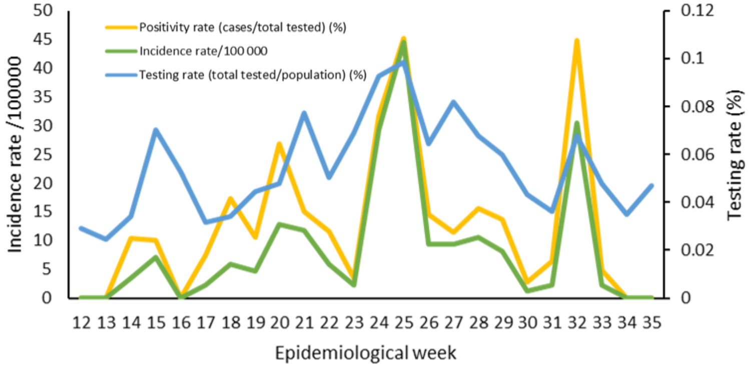

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Quality of Life Survey

	Blesbokspruit Catchment	City of Ekurhuleni
Total population	85,383	4,074,030
Population density (people/km^2)	15,580	2,063
Cumulative incidence/100 000	172	2,470
Number of respondents	47	2214
Economic:		
Income <R1600	25 (53.0%)	830 (34.5%)
No income	1 (2.2%)	35 (1.5%)
Informal dwelling	3 (6.7%)	475 (21.5%)
% ≥ 3 persons per room	50.3	35.9
Water:		
Piped, into the dwelling	24 (50.8%)	2,505 (72.5%)
Piped, into the yard only	23 (49.2%)	714 (20.7%)
Healthcare:		
Uses public health care facilities	41 (88.7%)	2,385 (69.1%)
Tried and successfully tested for COVID-19	45 (95.9%)	3,384 (98.0%)
Struggled to access healthcare	2 (4.1%)	228 (6.6%)
COVID-19 Index (% unable to protect against COVID-19)	39.7	31.7
Quality of life (Score out of 100)	55.1	61.3

INTERPRETATION AND IMPACT

- Positive results in rivers indicate that the river is being used for sanitary purposes, or there is broken infrastructure present
- Access to care is limited in informal settlements, and they have higher vulnerability,
- This could have impacts on disease transmission and severity, especially if patients don't get treatment on time
- Strengthening environmental surveillance in marginalised communities could bridge the gap for vulnerability
- When environmental surveillance indicate higher concentrations of disease (especially in vulnerable communities), additional support and services should be rendered

KEY LESSONS AND CONCLUSIONS

- Sampling logistics can be difficult and time consuming, requiring extensive support from municipalities, river action groups and community leaders
  - Transport of large volumes of water is costly, and cold chain must be maintained out of rural areas
  - Dilution during rainy season may hinder detection
- Passive sampling may overcome issues of low yield during high dilution periods, allow for easier and cheaper transport of samples, and improved consistency
  - Sample processing is much quicker, although the logistics of passive sampling are more onerous and require community assistance
- Opportunity for training and capacity building, and development of community “champions” to assist with sampling

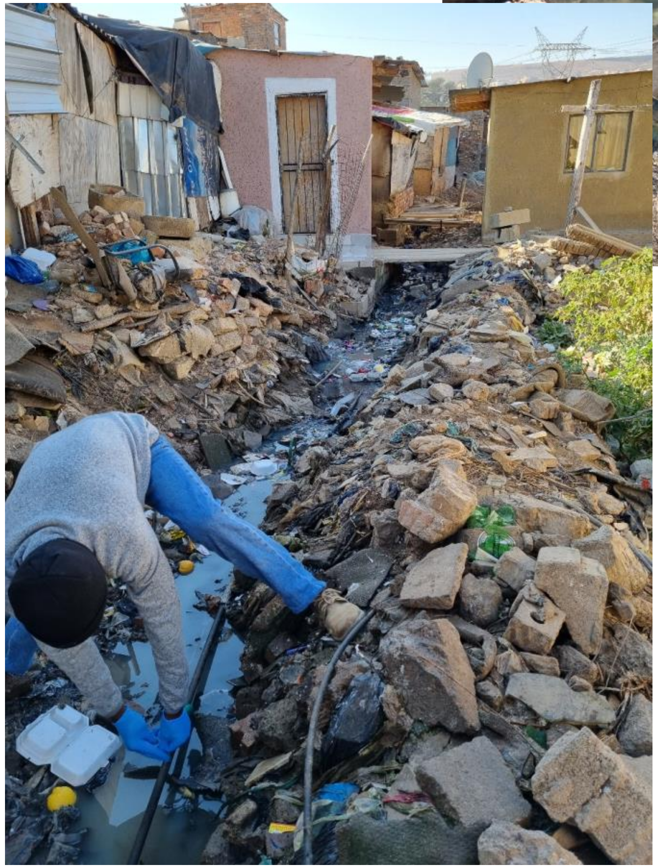
KEY LESSONS AND CONCLUSIONS

- Urban water streams represent a rich and highly relevant source of information about exposure to pathogens
  - as well as the opportunity to monitor emerging contaminants, lifestyle indicators, and antimicrobial resistance

- Environmental surveillance can better inform clinicians or public health authorities on disease burden and health service needs in marginalised communities

- Traditional clinical surveillance can be strengthened to include environmental water testing
  - Report to policy makers in real time (district health officials)
  - Report to public health officials on a dashboard

- Samples can be used for wastewater-based epidemiology for a broader scope of pathogens, using the same methods as applied for SARS-CoV-2



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CHALLENGES

- How best to start collaborations, and enter these marginalised communities?
- How do we get buy-in and support from the community
- How do we share information with the communities?
- Public health policies; How do we inform clinicians?

THANK YOU

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Ensuring Health Equity: Infectious Disease Surveillance in SouthEast Asia through Non-sewered Wastewater

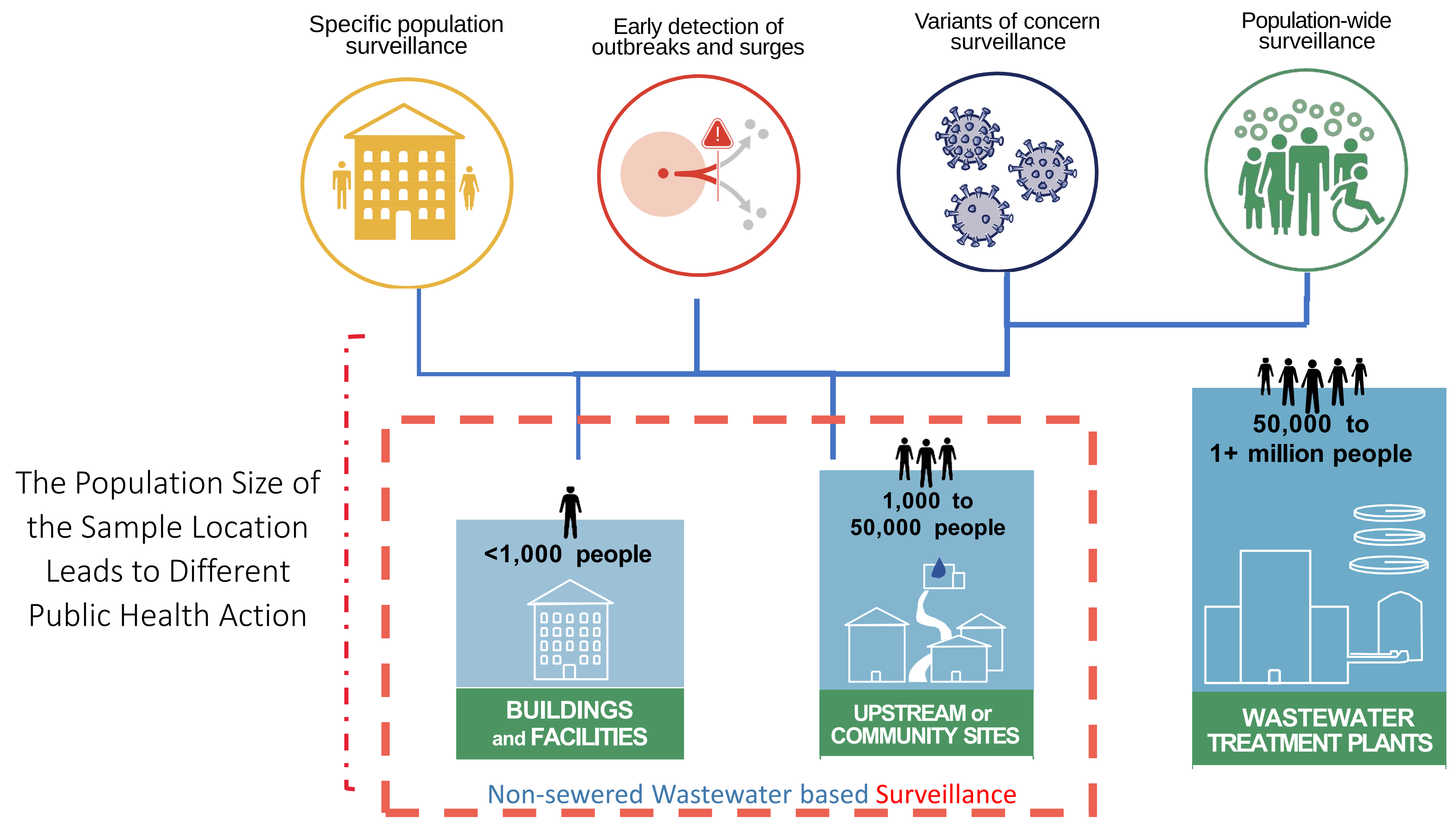
Leshan Wannigama M.D., Ph.D.

Department of Infectious Diseases and Infection Control, Yamagata Prefectural Central Hospital Yamagata, Japan

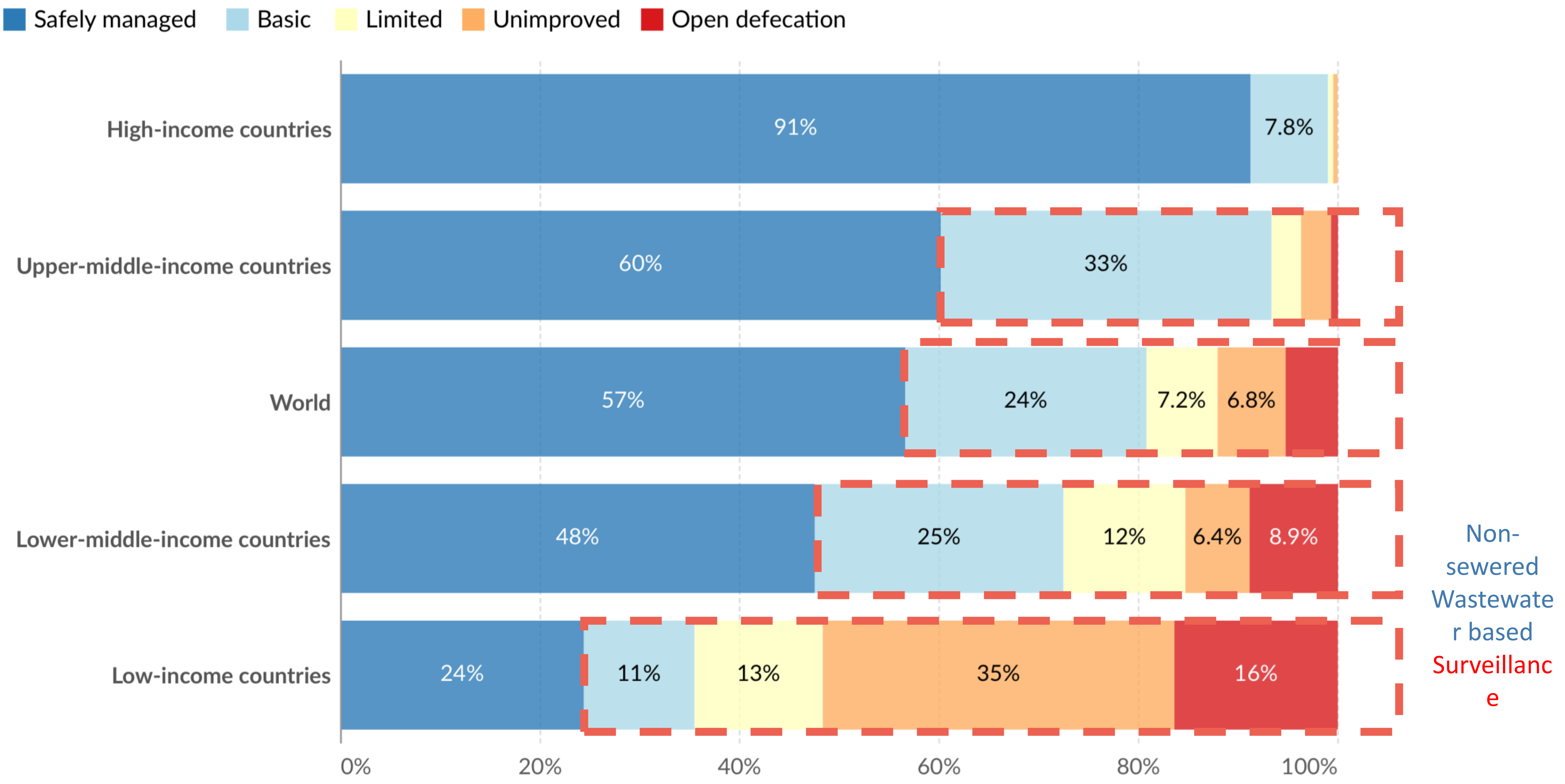
Centre of Excellence in Antimicrobial Resistance and Stewardship, Faculty of Medicine, King Chulalongkorn Memorial Hospital, Chulalongkorn University, Bangkok, Thailand

山形県立中央病院 YAMAGATA PREFECTURAL CENTRAL HOSPITAL
Chulalongkorn University จุฬาลงกรณ์มหาวิทยาลัย Pillar of the Kingdom
CHARLES DARWIN UNIVERSITY AUSTRALIA
Yamagata Prefectural University of Health Sciences
THE UNIVERSITY OF WESTERN AUSTRALIA
北里大学 KITASATO UNIVERSITY
The University Of Sheffield.
東北大学 TOHOKU UNIVERSITY
TELETHON KIDS INSTITUTE Discover. Prevent. Cure.

Four Uses of Wastewater Testing results

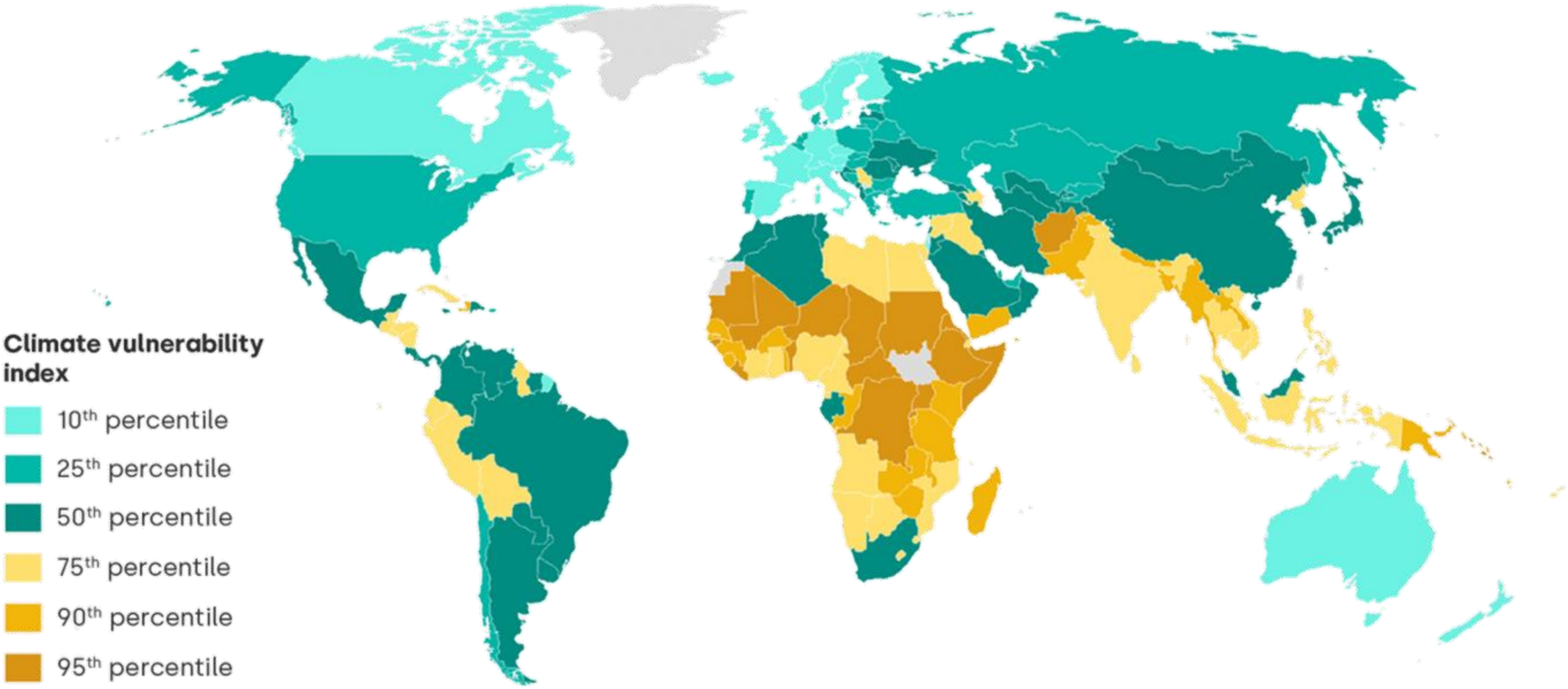


Share of the population using sanitation facilities



Data source: WHO/UNICEF Joint Monitoring Programme for Water Supply, Sanitation and Hygiene (JMP) (2024)

Regions in sub-Saharan Africa and South Asia are among the most vulnerable to climate change

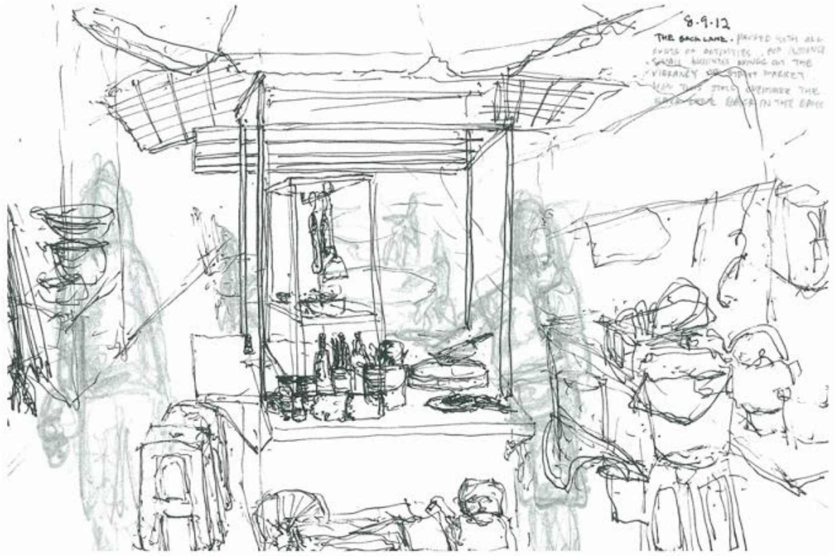


Source: Data from ND-GAIN (https://gain.nd.edu/our-work/country-index/)

Despite the potential for wastewater testing to include populations that are not monitored through individual testing or sewered testing, there are health inequalities that must be addressed in low and middle-income countries

Identifies new clusters and outbreaks, which then triggers investigation and action through Non-sewered Wastewater based Surveillance

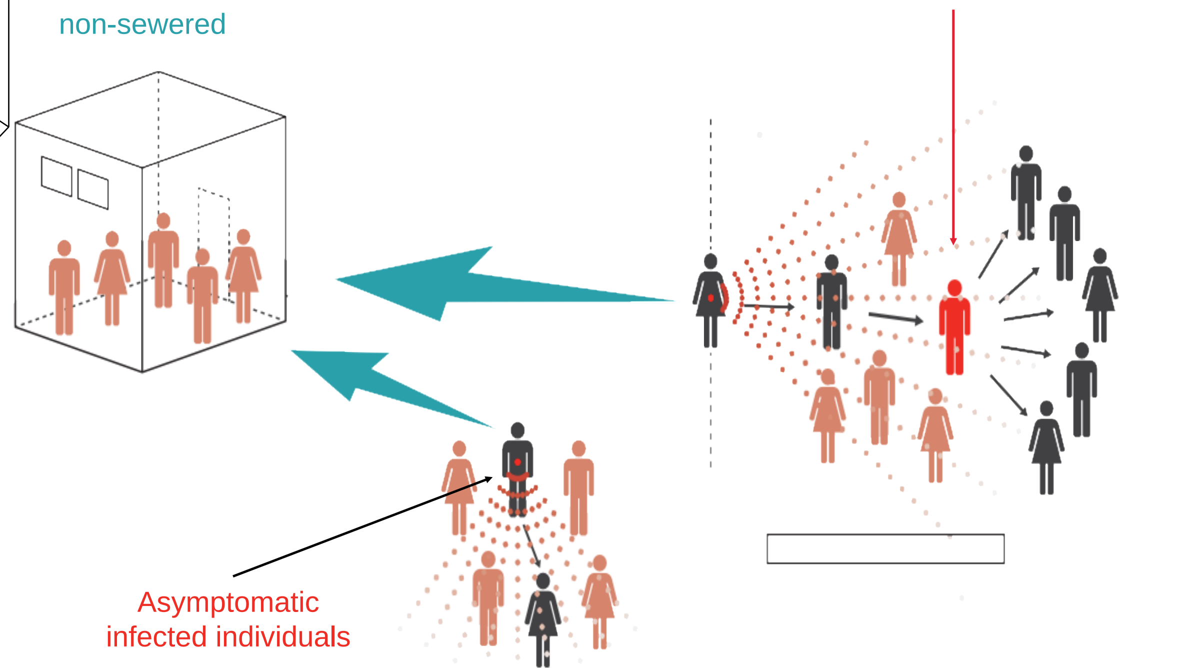
Community houses



Community markets/ shopping centers

sketch by TAN SOON CHERN

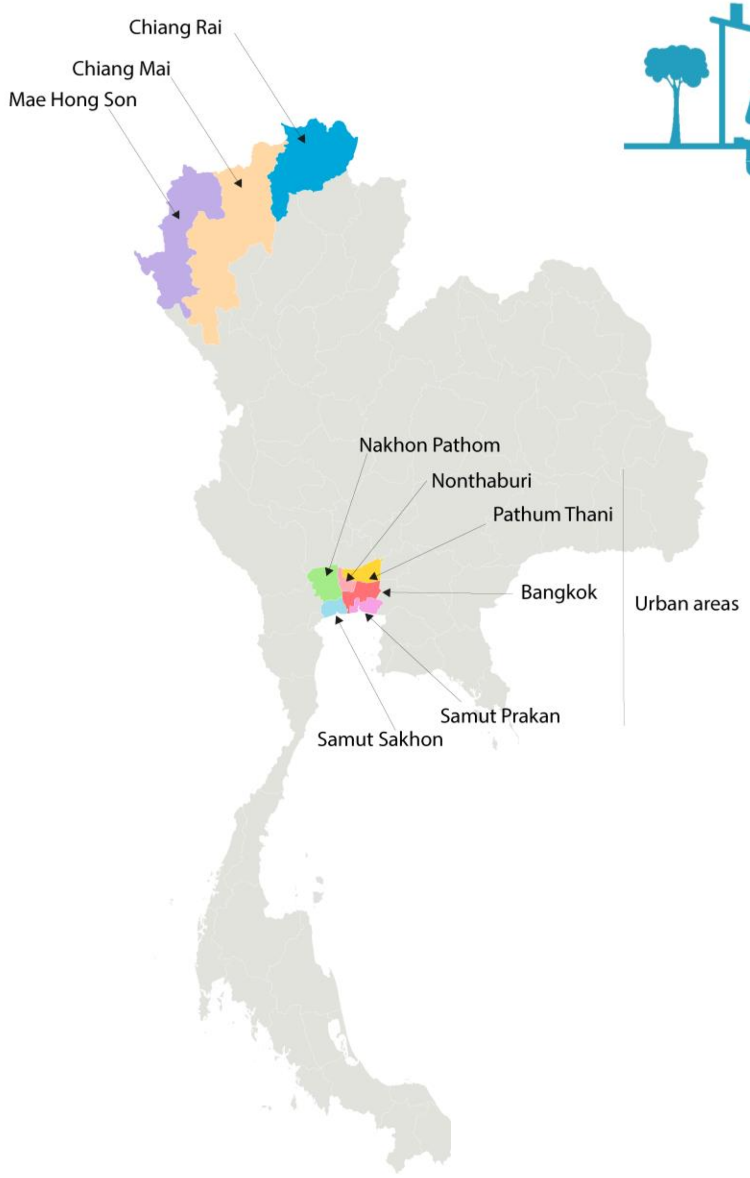
Entertainment venues



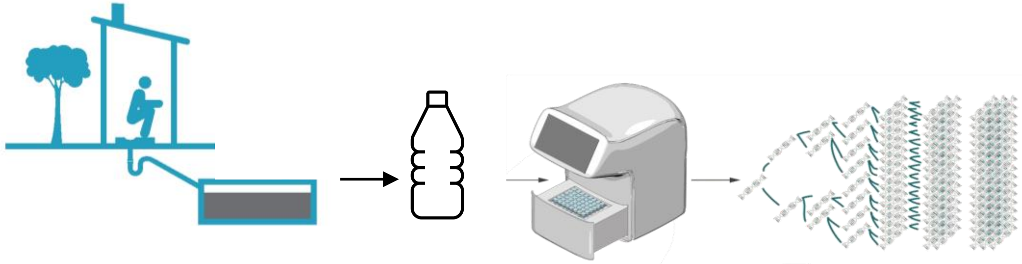
Participatory infectious disease surveillance through non-sewered wastewater in low and middle -income countries

Rural areas

- Community/food markets
- Community malls



Wastewater sampling provinces in Thailand



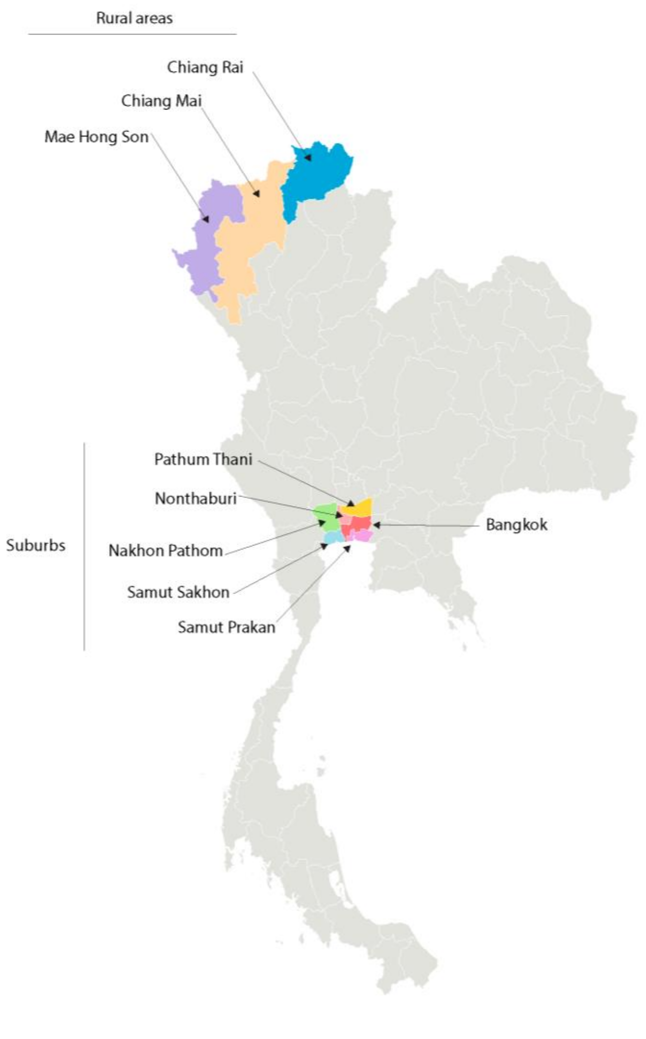
Standard grab sampling method followed by molecular detection

Urban areas

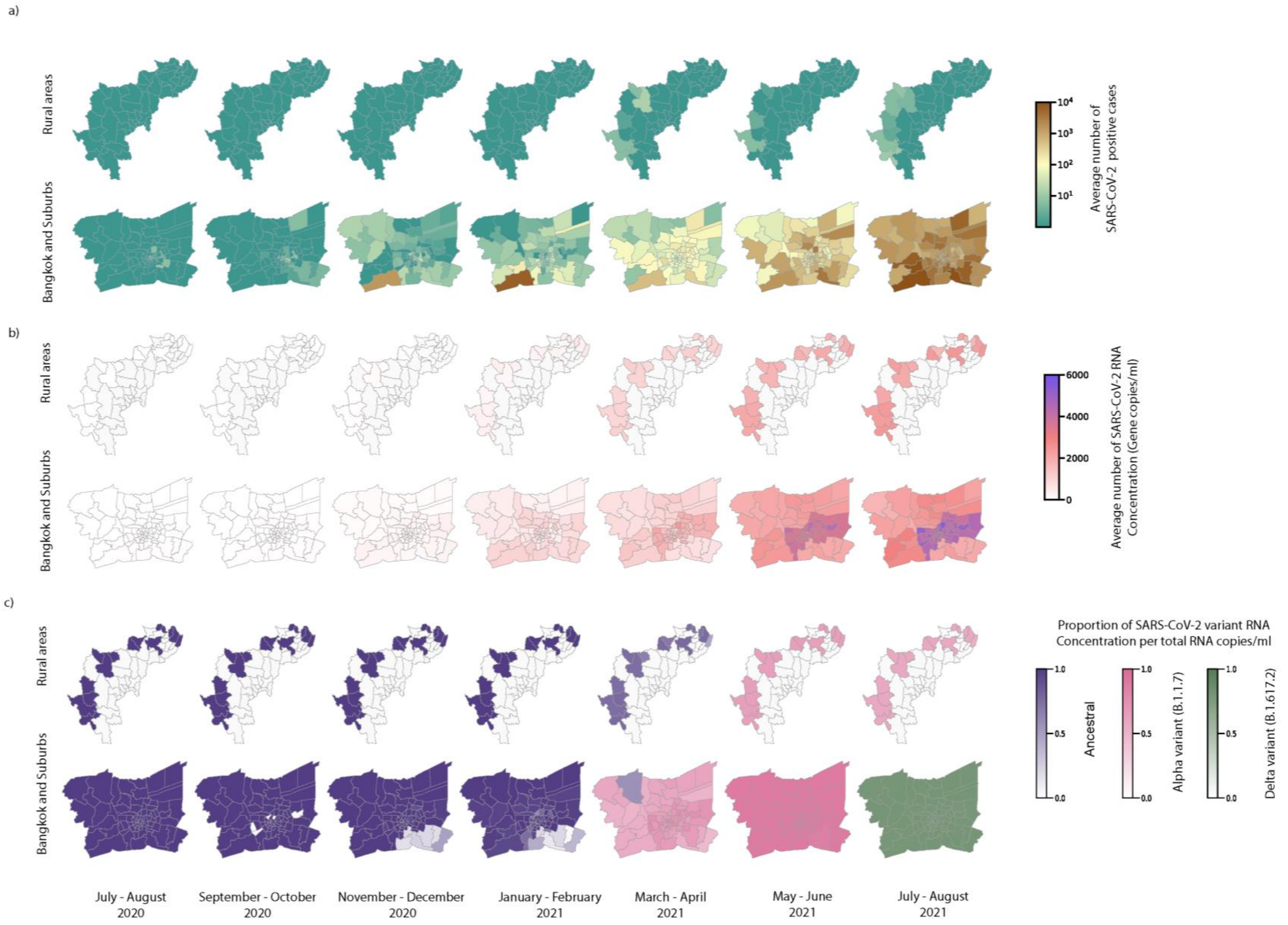
- Condominium complexes
- Cafeteria and shopping centers
- Community/food markets
- Office complexes
- Wastewater treatment plants
- Entertainment/leisure venues
- Work sites (construction camps)
- Housing complexes

In total, 1512 samples were collected from Bangkok, 2976 from five adjacent provinces, and 744 from rural provinces between July 2020 and August 2021.

Starting in November 2020, SARS-CoV-2 RNA concentrations in wastewater increased gradually, aligned with the increasing trend in daily newly reported cases in Bangkok and five surrounding provinces. Rural areas followed the same direction, even though SARS-CoV-2 RNA concentration remained relatively low compared to Bangkok and the five surrounding provinces

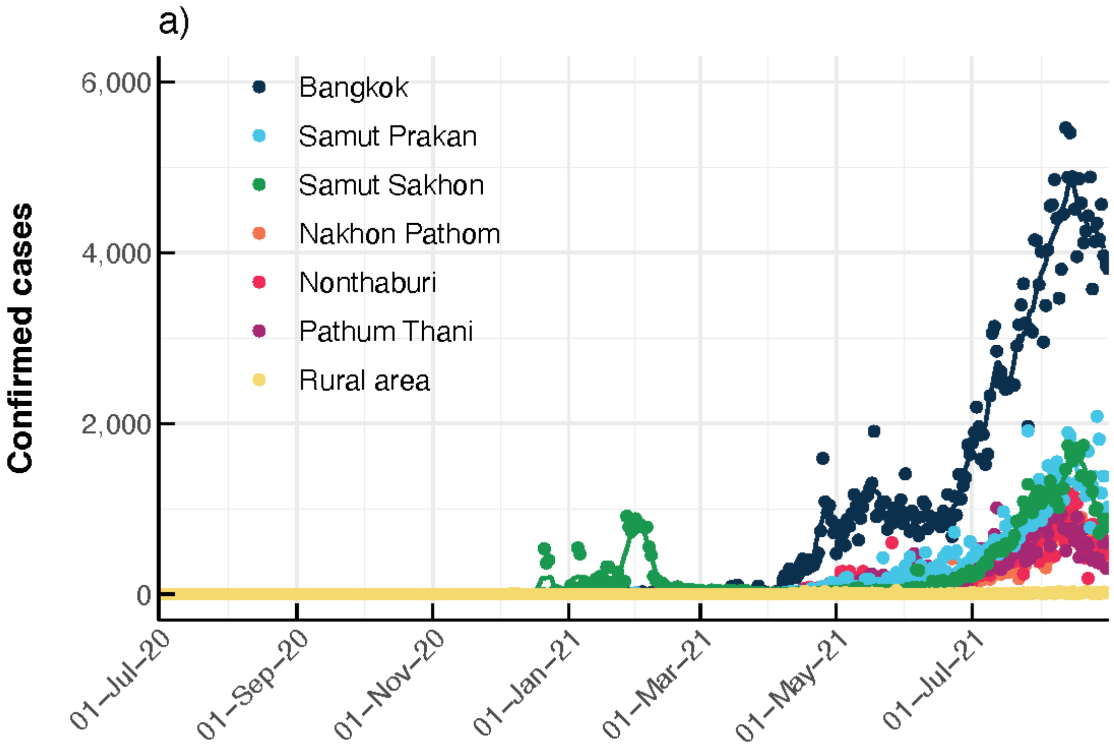


Wastewater sampling provinces in Thailand

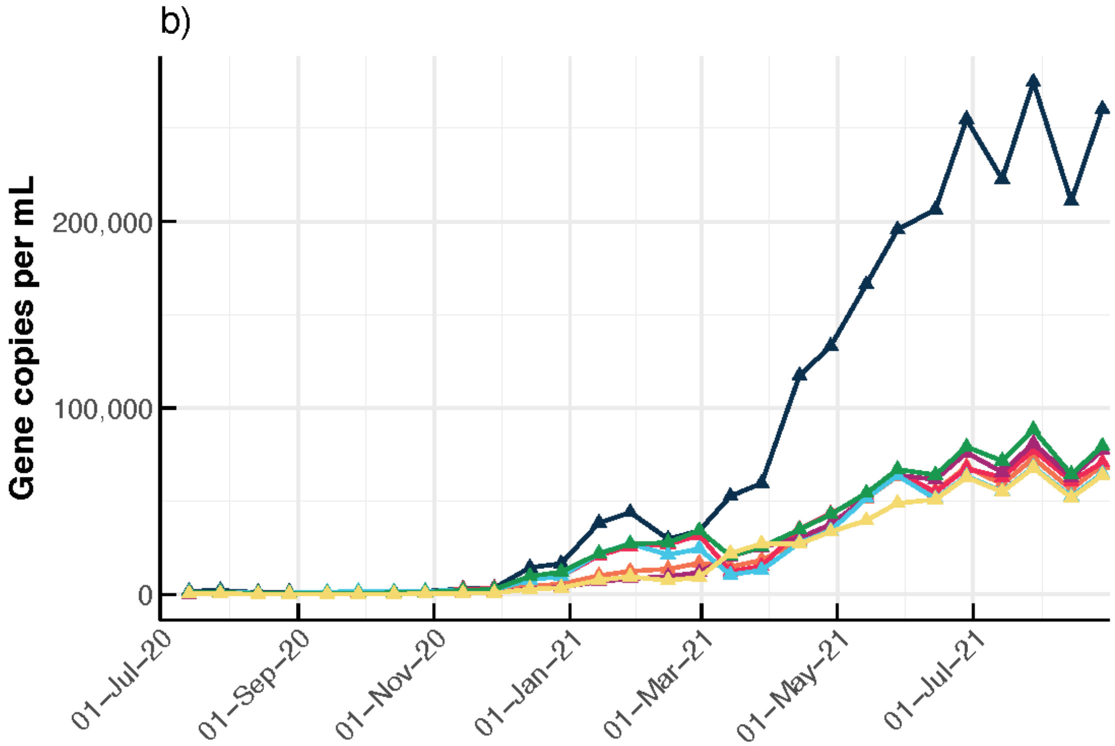


COVID-19 monitoring with sparse sampling of sewered and non-sewered wastewater in urban and rural communities. iScience. 2023 Jul 21;26(7):107019. doi: 10.1016/j.isci.2023.107019.
COVID-19 monitoring with sparse sampling of sewered and non-sewered wastewater in urban and rural communities. iScience. 2023 Jul 21;26(7):107019. doi: 10.1016/j.isci.2023.107019.

An increase in the estimated incidences based on wastewater SARS-CoV-2 RNA concentrations was observed before infected individuals were officially reported



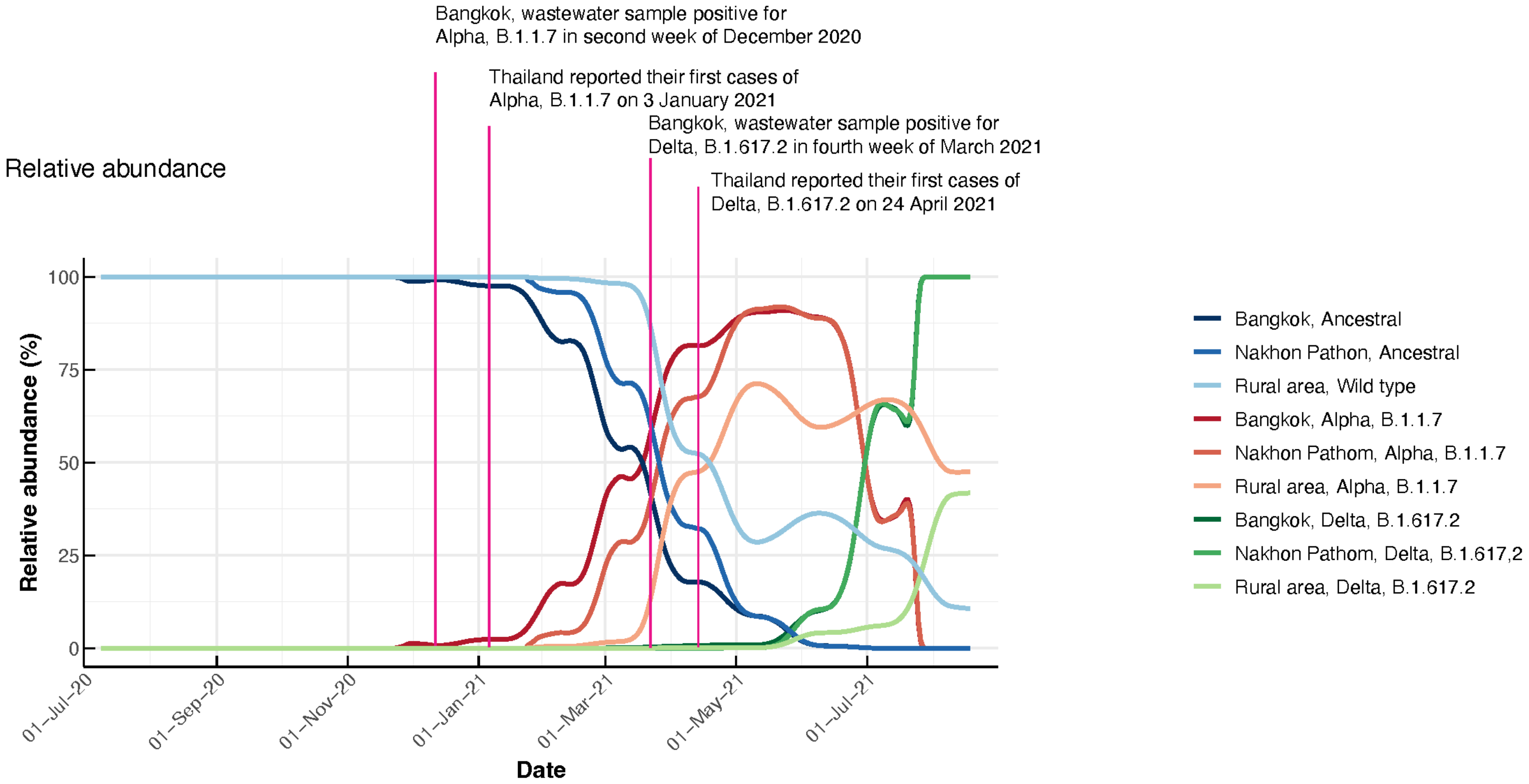
confirmed positive cases



RNA concentration in wastewater

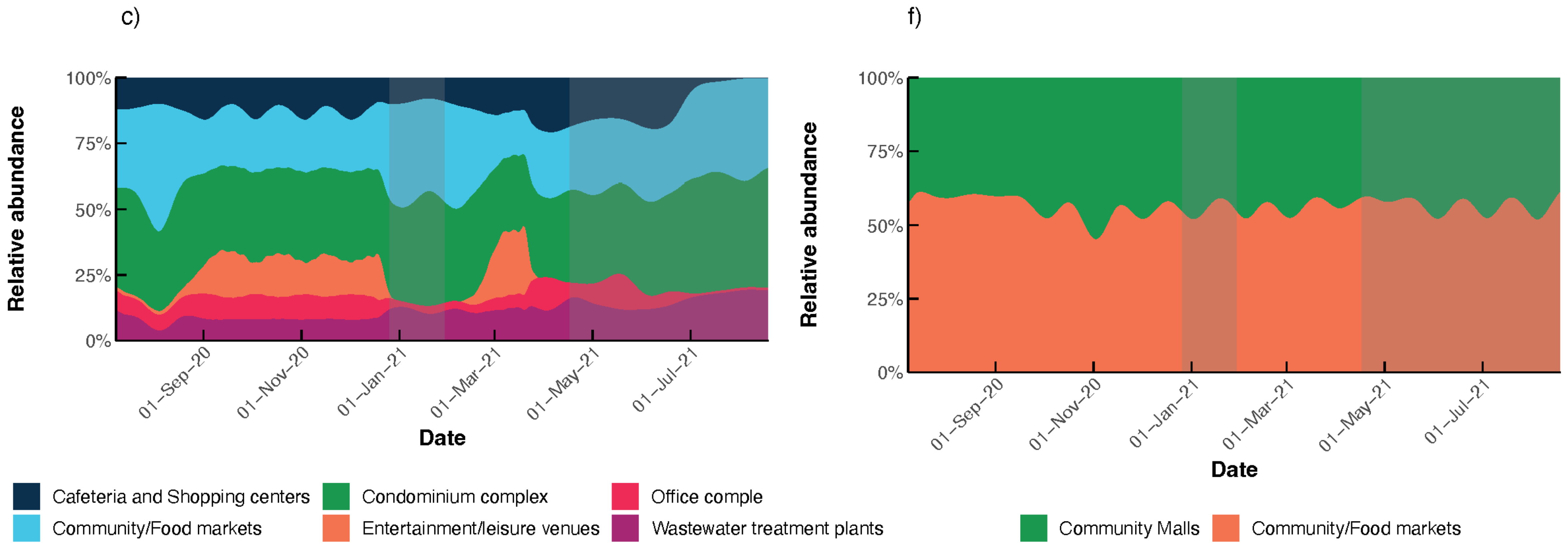
Wastewater-based estimated incidence showed a steep increase starting from the last week of November 2020. However, case-based incidences remained low until April 2021

COVID-19 monitoring with sparse sampling of sewered and non-sewered wastewater in urban and rural communities. iScience. 2023 Jul 21;26(7):107019. doi: 10.1016/j.isci.2023.107019.



The relative abundance of different SARS-CoV-2 variants in wastewater revealed that the ancestral SARS-CoV-2 was dominant until early March of 2021 in Bangkok and the five surrounding provinces and remained dominant in rural areas until mid-April 2021

COVID-19 monitoring with sparse sampling of sewered and non-sewered wastewater in urban and rural communities. iScience. 2023 Jul 21;26(7):107019. doi: 10.1016/j.isci.2023.107019.



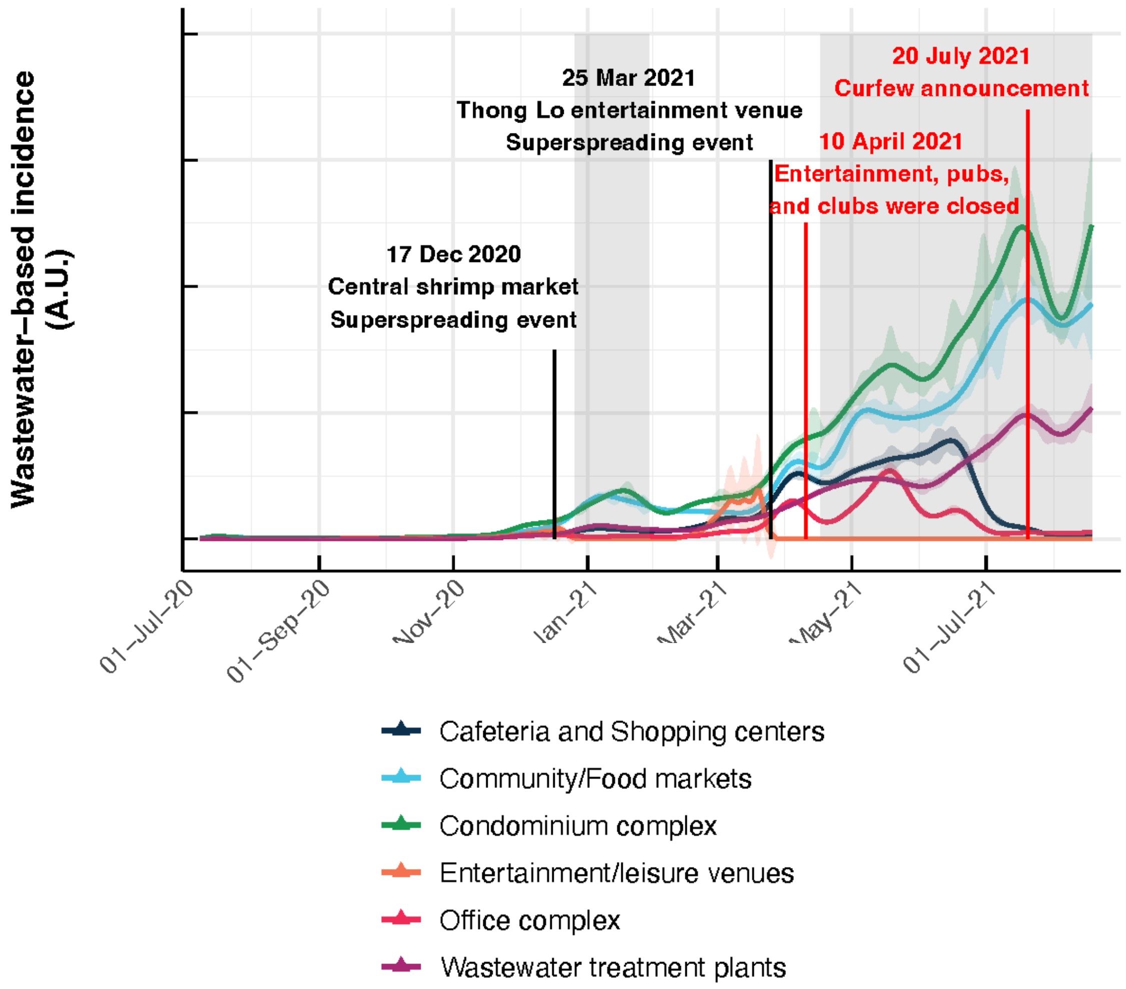
Analysis of SARS-CoV-2 viral RNA concentrations in different facilities revealed that the relative abundances of SARS-CoV-2 viral RNA concentrations in wastewater were relatively high in community/food markets and condominium complexes in Urban areas.

In rural regions, community malls and community/food markets had similar SARS-CoV-2 viral RNA concentrations in wastewater.

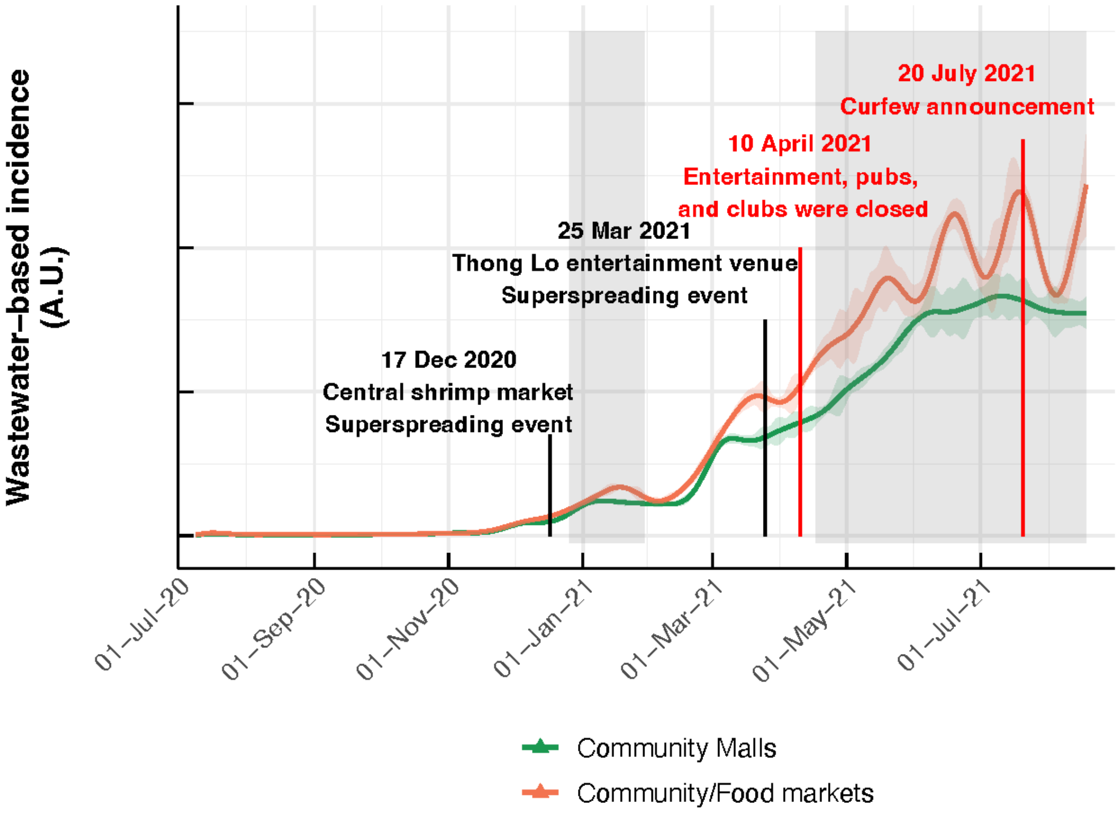
COVID-19 monitoring with sparse sampling of sewered and non-sewered wastewater in urban and rural communities. iScience. 2023 Jul 21;26(7):107019. doi: 10.1016/j.isci.2023.107019.

Estimation of infection incidences for each facility showed that community/food markets and condominium complexes tend to carry more infected people (symptomatic or asymptomatic) during the lockdown period in Bangkok

Bangkok



Rural area

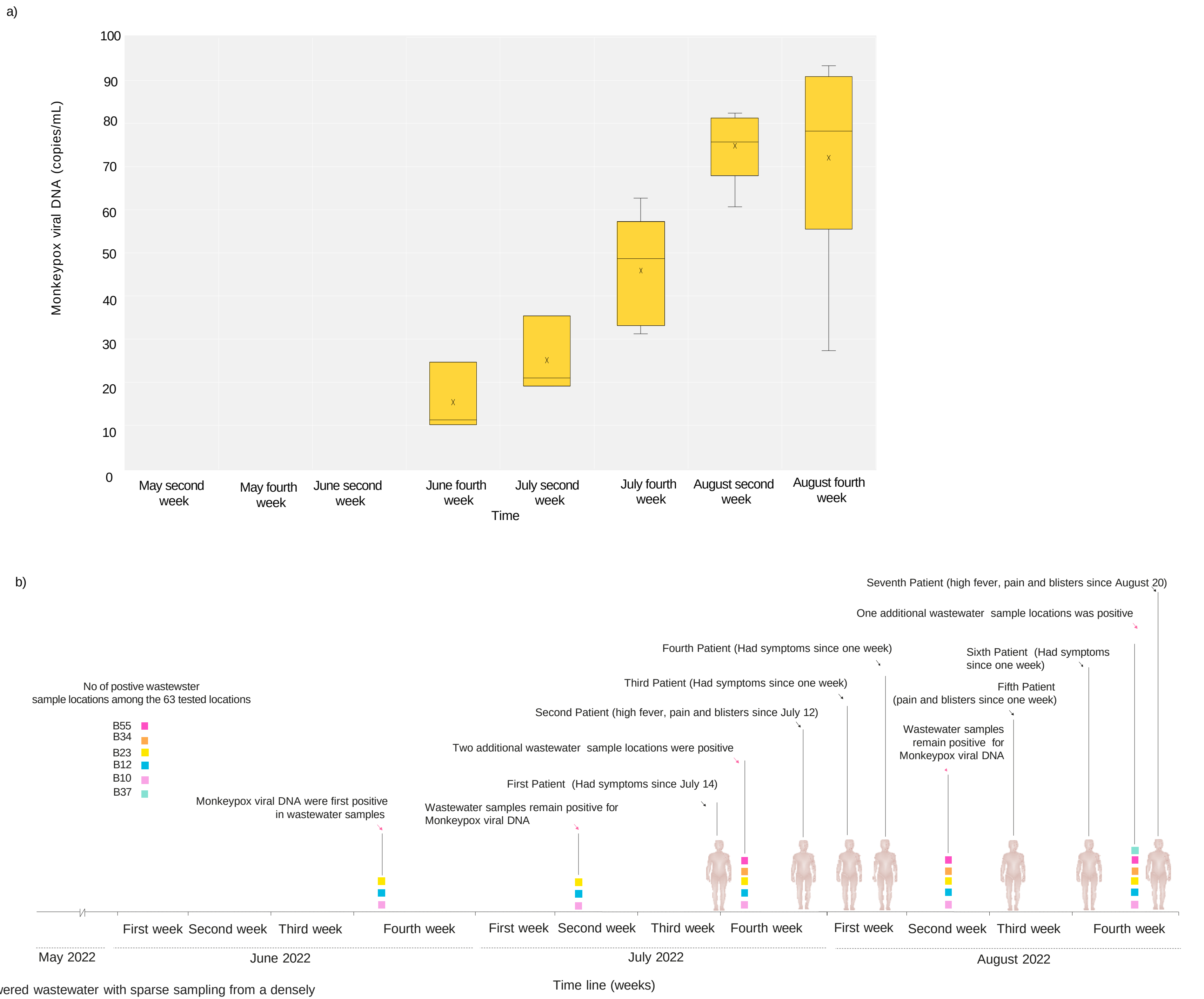


Community/food markets had higher estimated infection incidences in rural regions than community malls based on viral RNA concentrations in wastewater during the lockdown period

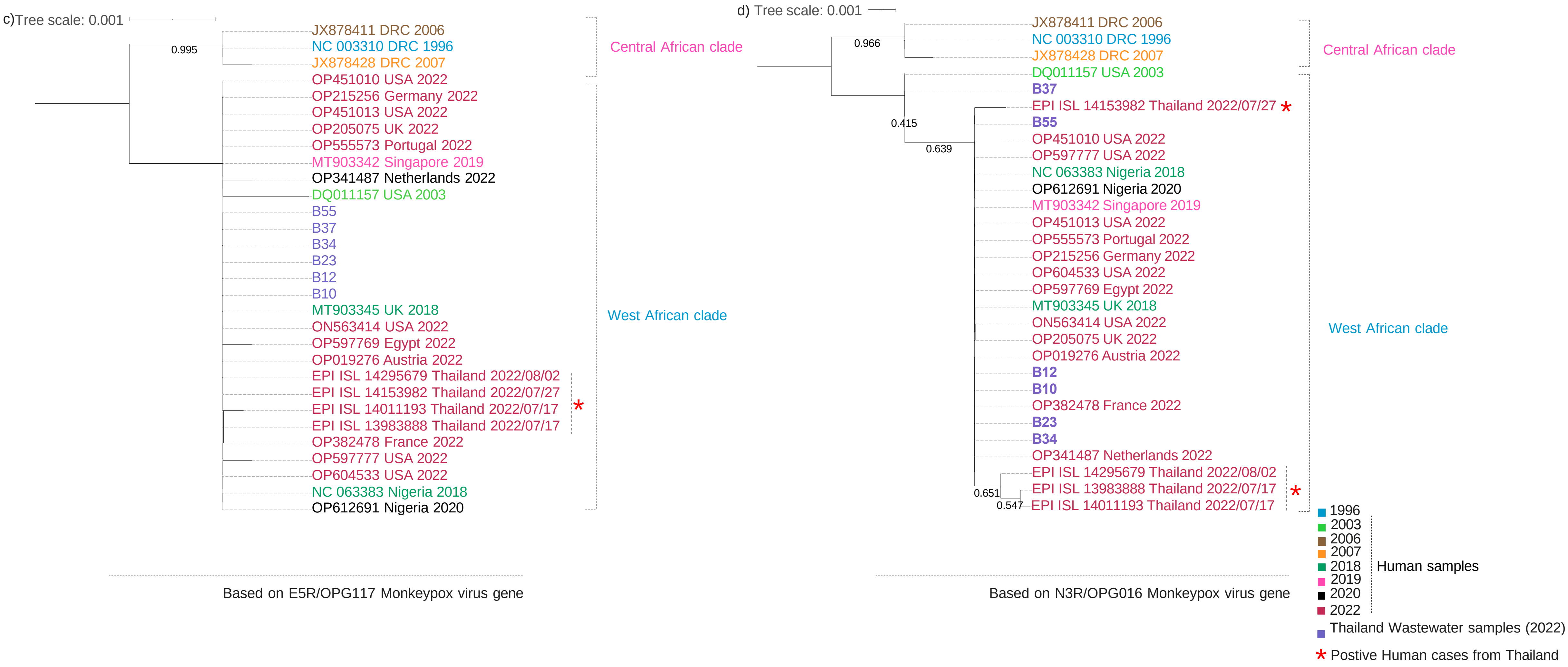
COVID-19 monitoring with sparse sampling of sewered and non-sewered wastewater in urban and rural communities. iScience. 2023 Jul 21;26(7):107019. doi: 10.1016/j.isci.2023.107019.

Multiple traces of monkeypox detected in non-sewered wastewater with sparse sampling from a densely populated metropolitan area in Asia

- First dataset on monkeypox viral DNA detection from wastewater in Thailand
- Monkeypox viral DNA tracing is possible with sparse sampling events
- Non-sewered locations showed positive signals for monkeypox DNA
- Sanger sequencing confirmed the identification of the monkeypox virus.
- Findings expand the use of wastewater surveillance in resource limited countries



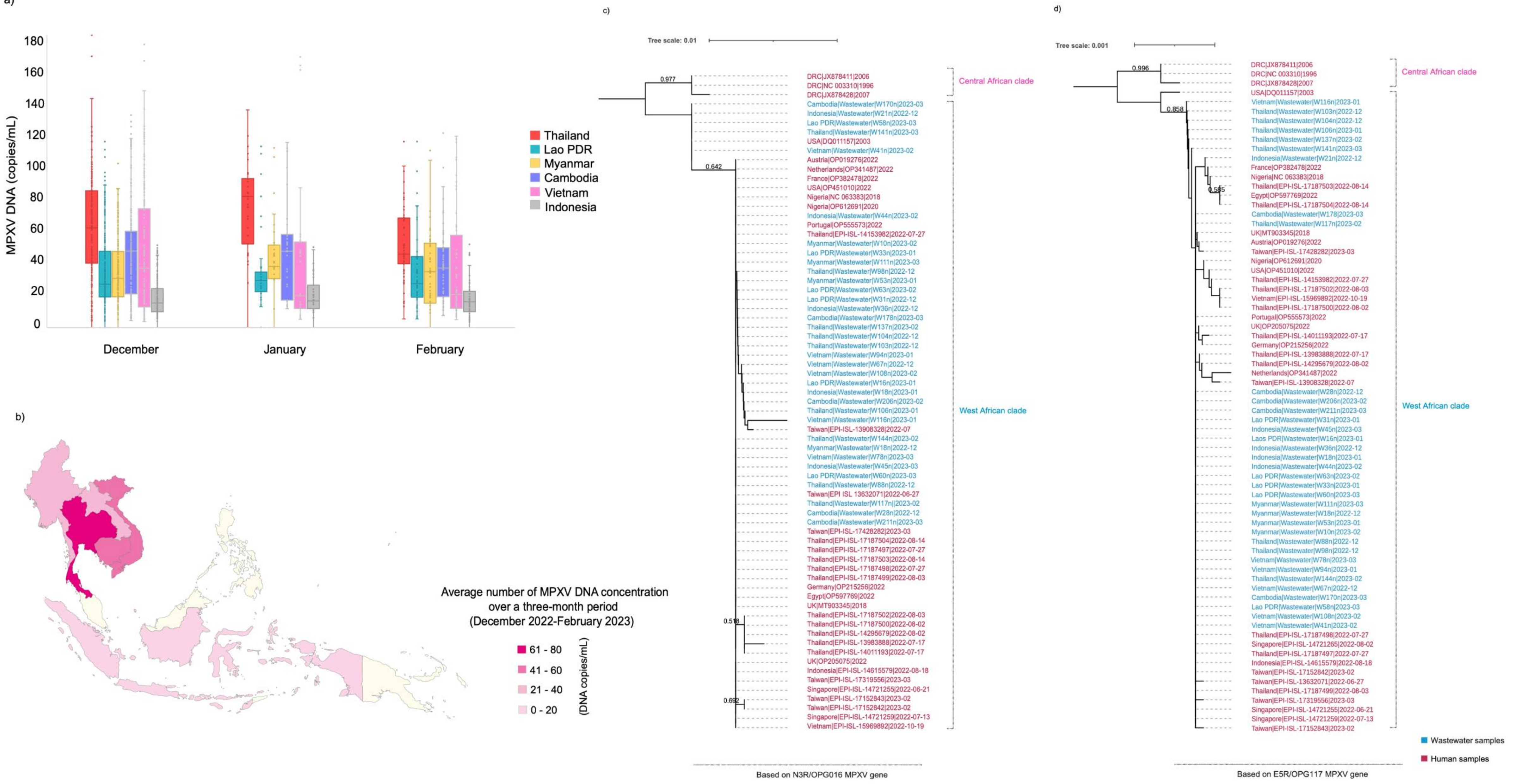
Wannigama, Dhammika Leshan, et al. "Multiple traces of monkeypox detected in non-sewered wastewater with sparse sampling from a densely populated metropolitan area in Asia." *Science of the Total Environment* 858 (2023): 159816.



Phylogenetic analysis of the sequence of c) E5R/ OPG117 and d) N3R/OPG016 gene reflect that wastewater samples are clustering with positive clinical cases from Thailand and other geographical origins connected to the monkeypox 2022 outbreak

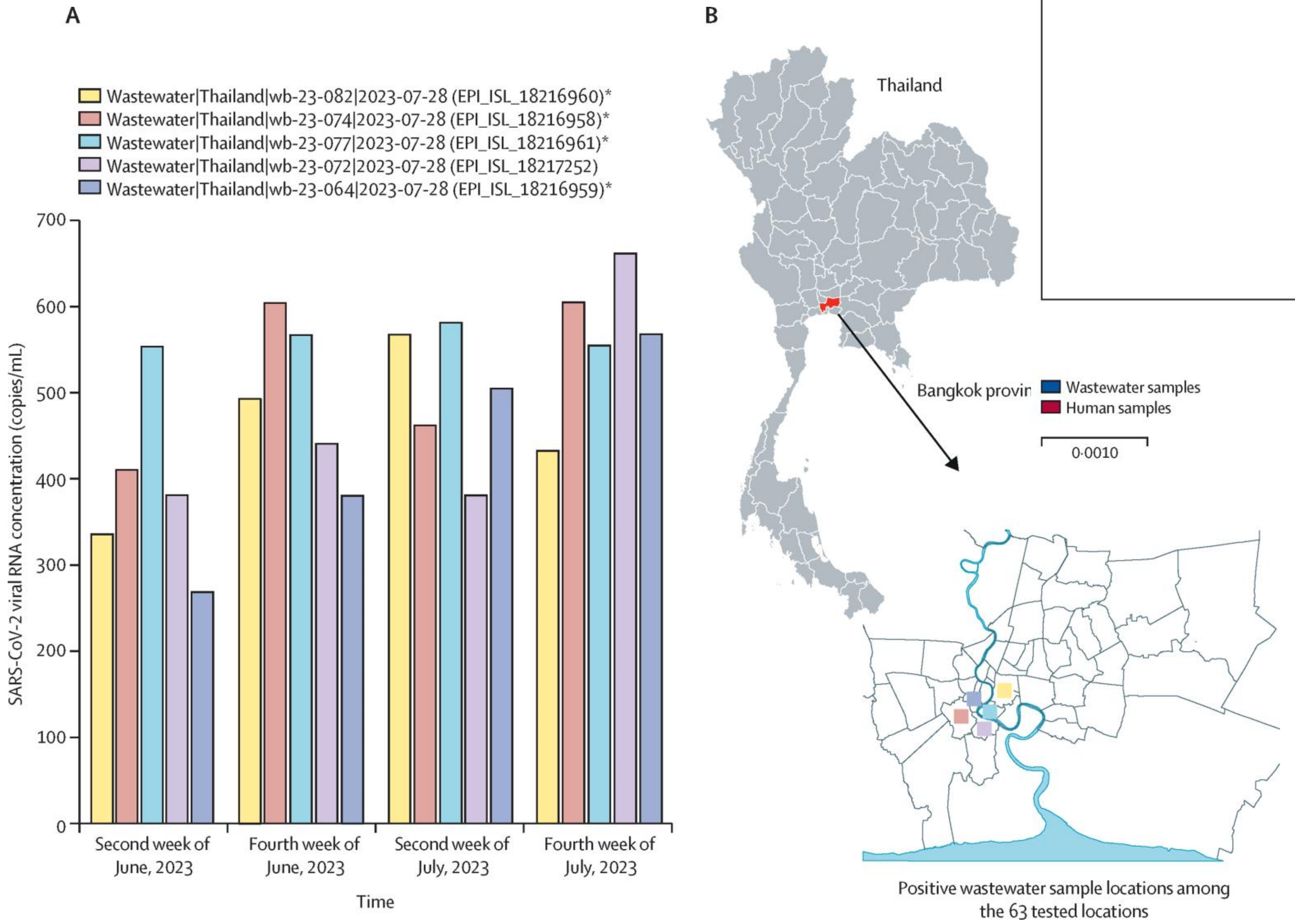
Wannigama, Dhammika Leshan, et al. "Multiple traces of monkeypox detected in non-sewered wastewater with sparse sampling from a densely populated metropolitan area in Asia." *Science of the Total Environment* 858 (2023): 159816.

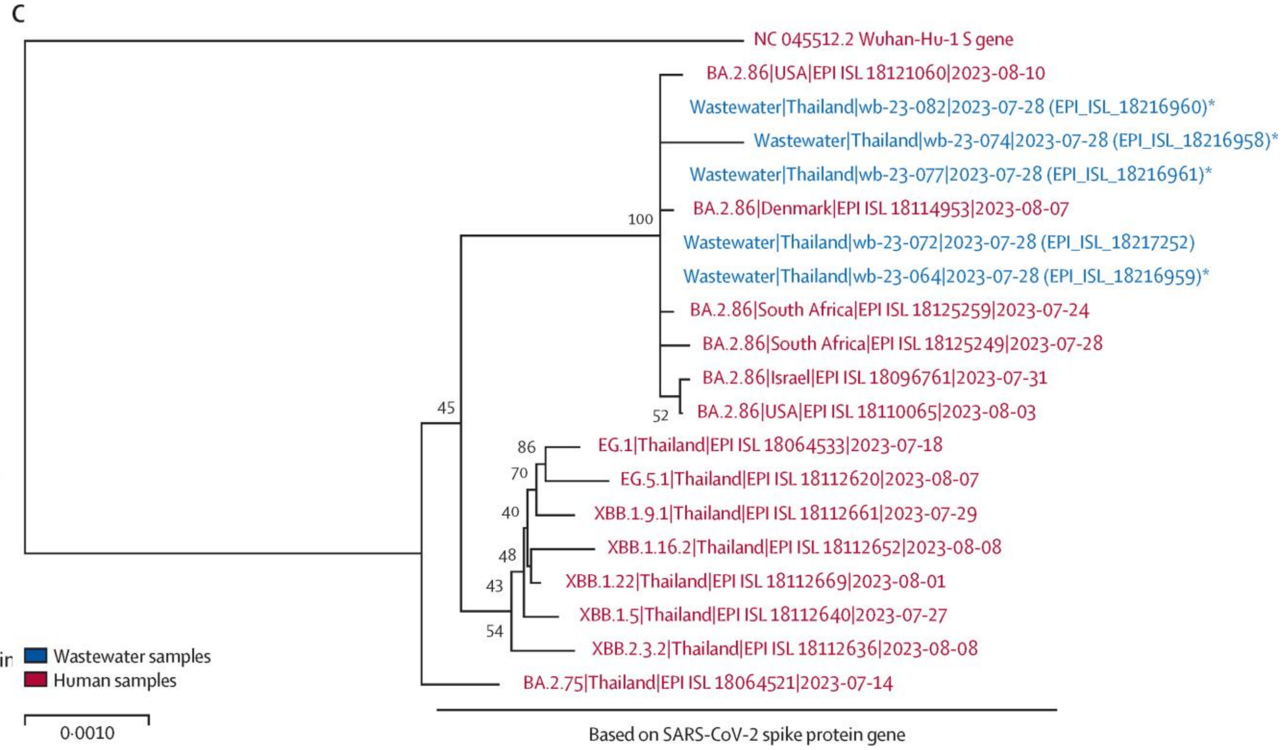
Non-sewered wastewater surveillance in six Southeast Asian countries revealed positive signals for Monkeypox virus (MPXV) DNA, indicating local transmission.



Tracing the transmission of mpox through wastewater surveillance in Southeast Asia. *Journal of travel medicine*, *30*(5), taad096. https://doi.org/10.1093/jtm/taad096

Tracing the new SARS-CoV-2 variant BA. 2.86 in the community through wastewater surveillance in Bangkok, Thailand.

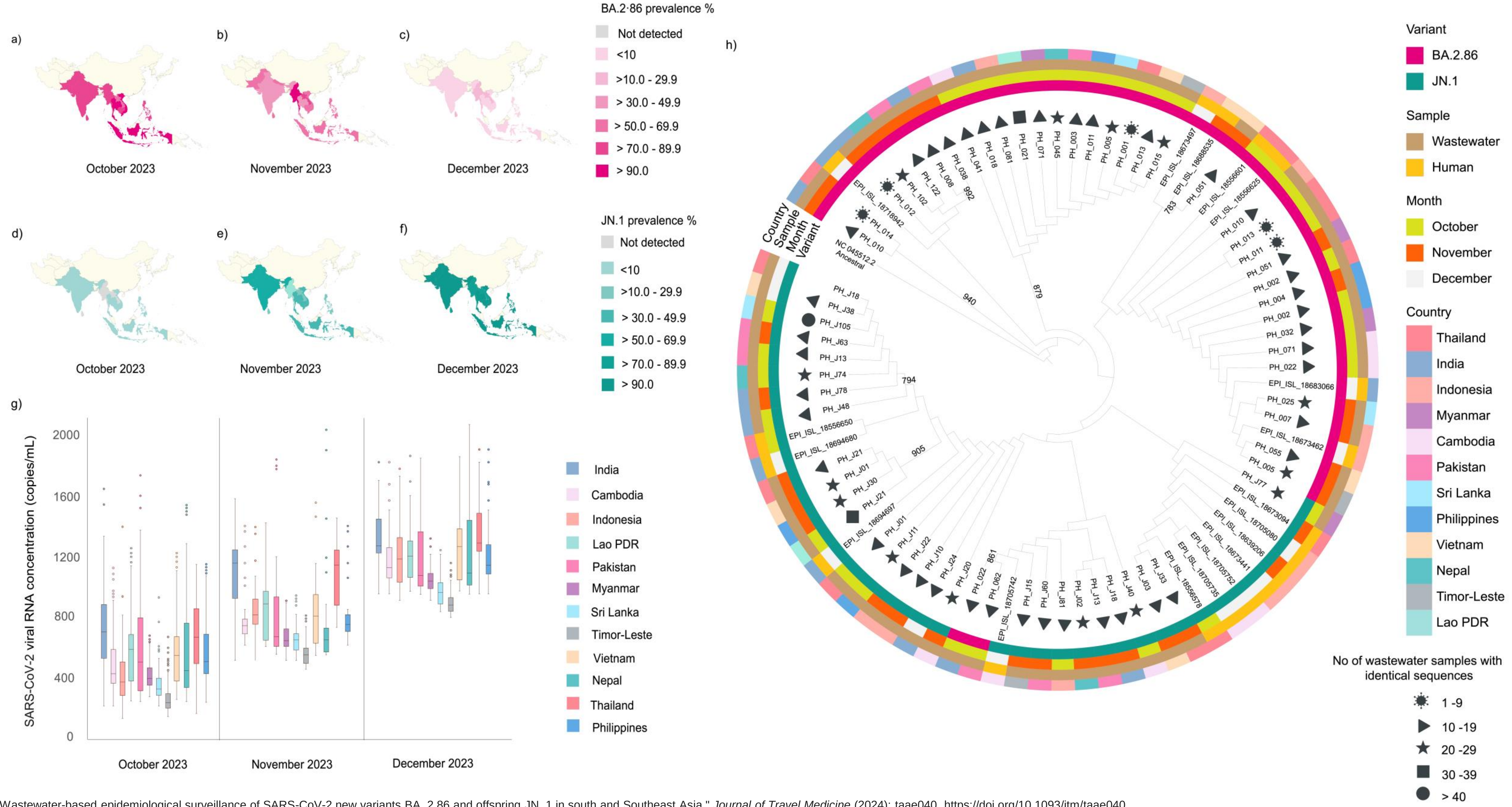




- F our identical sequences from different locations suggest parallel circulation across multiple areas.
- Furthermore, we identified two different sequences within the same short time frame and small geographical area.
- These sequences were found in different commercial or public venues with non-sewered wastewater

Tracing the new SARS-CoV-2 variant BA. 2.86 in the community through wastewater surveillance in Bangkok, Thailand. *The Lancet Infectious Diseases*, *23*(11), e464-e466.

Wastewater-based epidemiological surveillance of SARS-CoV-2 new variants BA.2.86 and offspring JN.1 in South and Southeast Asia through non-sewered wastewater



"Wastewater-based epidemiological surveillance of SARS-CoV-2 new variants BA. 2.86 and offspring JN. 1 in south and Southeast Asia." *Journal of Travel Medicine* (2024): taae040. https://doi.org/10.1093/jtm/taae040

Summary

- Participatory infectious disease surveillance through Non-sewered Wastewater can provide actionable information in more a clinically relevant timeframe
- Non-sewered Wastewater showed the distribution of infectious disease across different facilities
- We provide an equitable approach to wastewater monitoring with sparse sampling
- Also proven useful for assessing risk factors, vaccine effectiveness, and patterns of healthcare utilization while being less expensive, more flexible, and more scalable than traditional systems.
- Participatory infectious disease surveillance through Non-sewered Wastewater provides unique disease information that is not available through traditional surveillance sources
- Findings advance health equity in low-resource countries with poor sewer system

Thanks to all the members and collaborators

University of Otago, Te Whare Wānanga o Otāgo, New Zealand · Tohoku University · 山形県立中央病院 Yamagata Prefectural Central Hospital · 北里大学 Kitasato University · Chulalongkorn University · King Chulalongkorn Memorial Hospital, The Thai Red Cross Society · The University of Western Australia · QIMR Berghofer Medical Research Institute, The Future of Health · Telethon Kids Institute, Discover. Prevent. Cure. · Perth Children's Hospital

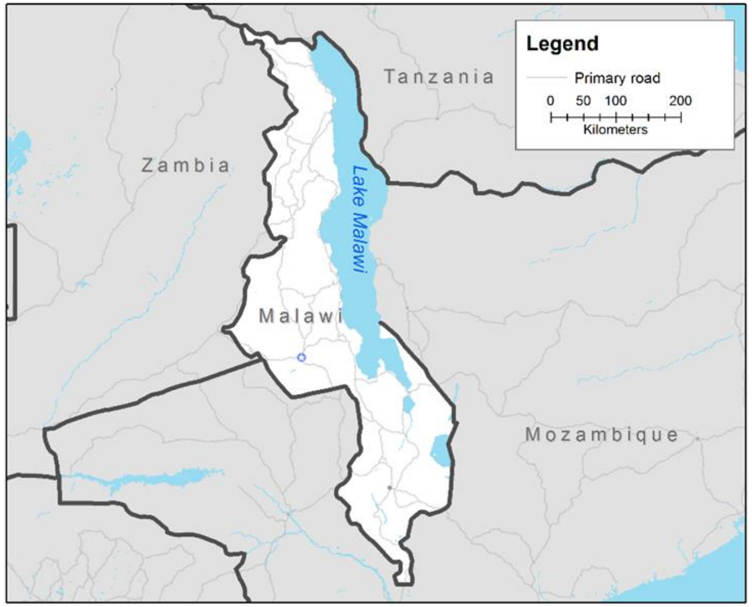
We thank all the volunteers, LGBTQIA+ community and marginalized, vulnerable indigenous communities who kindly supported with sample collection. Our team comprises individuals who self-identify as underrepresented ethnic minorities, gender minorities, members of the LGBTIQOO+1 Ally community and individuals living with disabilities.

Addressing Challenges for Establishing a Non-Sewered Wastewater Surveillance system in Malawi

PETROS CHIGWECHOKHA, MALAWI UNIVERSITY OF SCIENCE AND TECHNOLOGY (MUST)

inspiring change

SANITATION PROFILE FOR MALAWI



Key Area	Stats/Burden
Diarrheal disease burden	824 foodborne DALY per 100,000
Household drinking water (basic)	70% of the population
Household sanitation facilities (safely managed)	24% of the population
Household hygiene (basic)	8% of the population
School drinking water (basic)	78% of schools
School sanitation facilities (basic)	66% of schools
Health care facilities water (basic)	78% of facilities
Health care facilities sanitation (basic)	3% of facilities
Literacy levels	67.31%
Unemployment rate	18.5%

- **SS Africa remains behind - 7% sewered sanitation against 43% global status.**

CONTEXT

Low WBE surveillance

Most LMICs remain behind on wastewater-based pathogen surveillance and detection

92% of MW relies on on-site and NS sanitation systems.

- ❑ Fecal sludge management problems
- ❑ Pit emptying services unavailable/ expensive
- ❑ The pits are often abandoned once full

Public Health monitoring Challenges

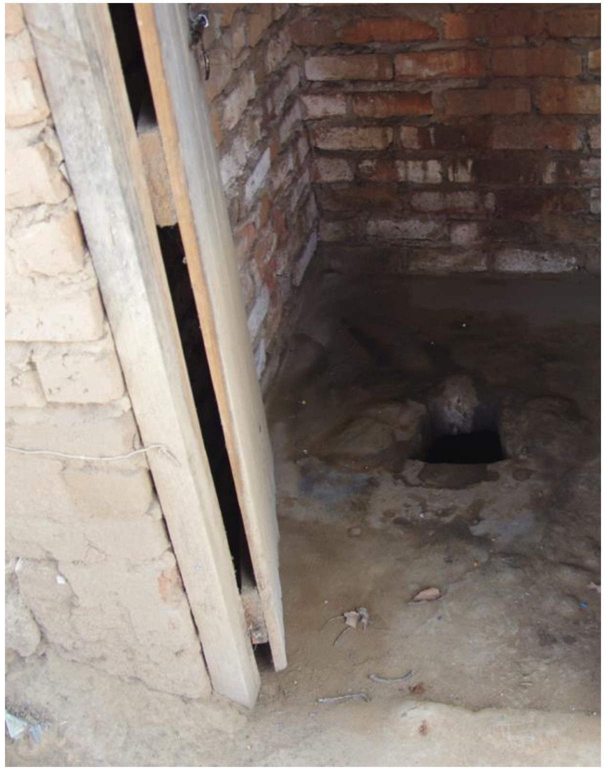
Utilization of WBE to monitor community health status difficult/ impossible.

Dependency on clinical data for community disease monitoring, often unavailable and unreliable

NSS WASTEWATER SAMPLING – NOT EASY

On-site sanitation sampling is a daunting task as opposed to centralized treatment plants

- ❑ NS sampling not a luxury – requires dedication
- ❑ Let us honor the tireless efforts of those who brave these challenging environments.




POLICY LEVEL CHALLENGES

the international water association

Policy challenges are often related to the nascency of WBE

Limited commitment from governments	Lack of awareness by policy makers

WHY??

Associated with the fact that WBE is a relatively new in most LMICs

Need promote awareness among relevant government authorities

TECHNICAL CHALLENGES IN SETTING-UP NS SURVEILLANCE

the international water association

Sampling Frame and protocol not easy

POSSIBLE SOLUTION

Need for fully functional lab for wastewater-based surveillance

Limited Equipment

Lack of BSL-2 and RT-qPCR

POSSIBLE SOLUTION

North-south partnerships and collaborations

Limited Human Capacity

(Lab and Bioinformatics)

POSSIBLE SOLUTION

Embark on Training capacity building programs

Well trained workforce is critical

Unavailability of well documented clinical data

POSSIBLE SOLUTION

Governments needs to strengthen data digitization

LOGISTICAL AND PROTOCOL CHALLENGES

Supply of Consumables

Most reagents not available in African countries

Supply chain bottlenecks

Ethical considerations

IRB approval and site approval takes time and require good planning

Operation and maintenance of laboratory

Problems with access to foreign currency for purchase of supplies

CURRENT EFFORTS

Environmental Science & Technology
pubs.acs.org/est
Viewpoint

Advancing the Use of Fecal Sludge for Timelier and Better-Quality Epidemiological Data in Low- and Middle-Income Countries for Pandemic Prevention

Petros Chigwechokha, Renée Street, and Rochelle H. Holm*

Cite This: https://doi.org/10.1021/acs.est.2c07788 Read Online

ACCESS | Metrics & More | Article Recommendations

KEYWORDS: *COVID-19, fecal sludge, low- and middle-income countries, pathogen, wastewater-based epidemiology*

The availability and accessibility of water, sanitation, and hygiene (WASH) services are key in preventing disease transmission.[1] Monitoring non-infectious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in wastewater can serve as an early indicator of changes in coronavirus disease (COVID-19) cases in a particular catchment area.[2] Fecal sludge is excreta held on site (unsewered) in sanitation systems, such as at households, in shared outhouses, and in septic tanks. These on-site sanitation systems, a positive step toward improved household WASH services, are used by 43% of the global population, mainly comprising low- and middle-income countries (LMICs).[1,3] There is an urgent need to expand decentralized (non-sewered system) surveillance as LMICs are currently being overlooked in pandemic prevention as it is currently focused on SARS-CoV-2 wastewater from centralized (sewered) systems.

Special Issue: Data Science for Advancing Environmental Science, Engineering, and Technology

ACS ES&T Water
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Viewpoint

Promoting Surveillance in Sub-Saharan Africa: Moving to Wastewater and Environmental Genomic Surveillance Requires More Attention

Rochelle H. Holm,* Petros Chigwechokha, Craig Kinnear, Anna Winters, and Renée Street

Cite This: *ACS EST Water* 2023, 3, 1994–1996 Read Online

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Laboratory infrastructure and local capacity are essential for surveillance programs to achieve the Sustainable Development Goals. Globally, morbidity and mortality due to unsafe household drinking water, sanitation, and hygiene remain major problems.[1] During the coronavirus disease 2019 (COVID-19) pandemic, more than a few water, sanitation, and hygiene (WASH) researchers pivoted to use polymerase chain reaction (PCR)-based methods to indicate community disease spread from sewered and non-sewered sanitation samples.

Need to optimize NSSS protocols to enhance wastewater sample collection, processing and analysis

Need to build infrastructural capacity that will promote NSSS.

Need to build local Human capacity in NSSS to enable detection and containment of re-emerging and novel disease threats

Need to promote research partnerships to unlock funding opportunities

WASTEWATER-BASED MULTI-PATHOGEN SURVEILLANCE IN MALAWI

GOAL:

- To build capacity for Wastewater Based surveillance as an early warning tool for various pathogens.
- To understand the relationship between sewage detection and clinical cases

Objectives

- To determine feasibility of wastewater surveillance in a low-resource laboratory setting using available equipment and existing infrastructure.
- To determine if wastewater surveillance data could be modelled with clinical data for decision making process.
- To pilot a Timelier and better-quality in-country epidemiological data sets for better pandemic preparedness in the future.

WASTEWATER-BASED MULTI-PATHOGEN SURVEILLANCE

the international water association

Current focus → Five pathogens

- *V. cholerae*
- *S. Typhi*
- Mycobacterium tuberculosis (TB)
- Measles
- SARS – CoV2

- Sample collection – August 2022
- Collection interval - Weekly
- Processing done in BSL-2
- Initial focus – PCR detection
- Switched to culture – Procurement
- PCR later done on isolated for confirmation
- Challenges in market access

IMPLEMENTATION TIMELINE

the international water association

Timeline

2 days — Days to prepare

1 day

4 days — Days to results

3 days — Quarterly confirmation

Preparation of media

Culture lab

Sample collection

Field

Culture-based methods

Nucleic Acid Extraction

Microbiology (BSL-2) and Molecular Bio Labs

Report as presence/absence

RT-PCR confirmation analysis

RT-PCR lab

Report as confirmation with paired clinical results

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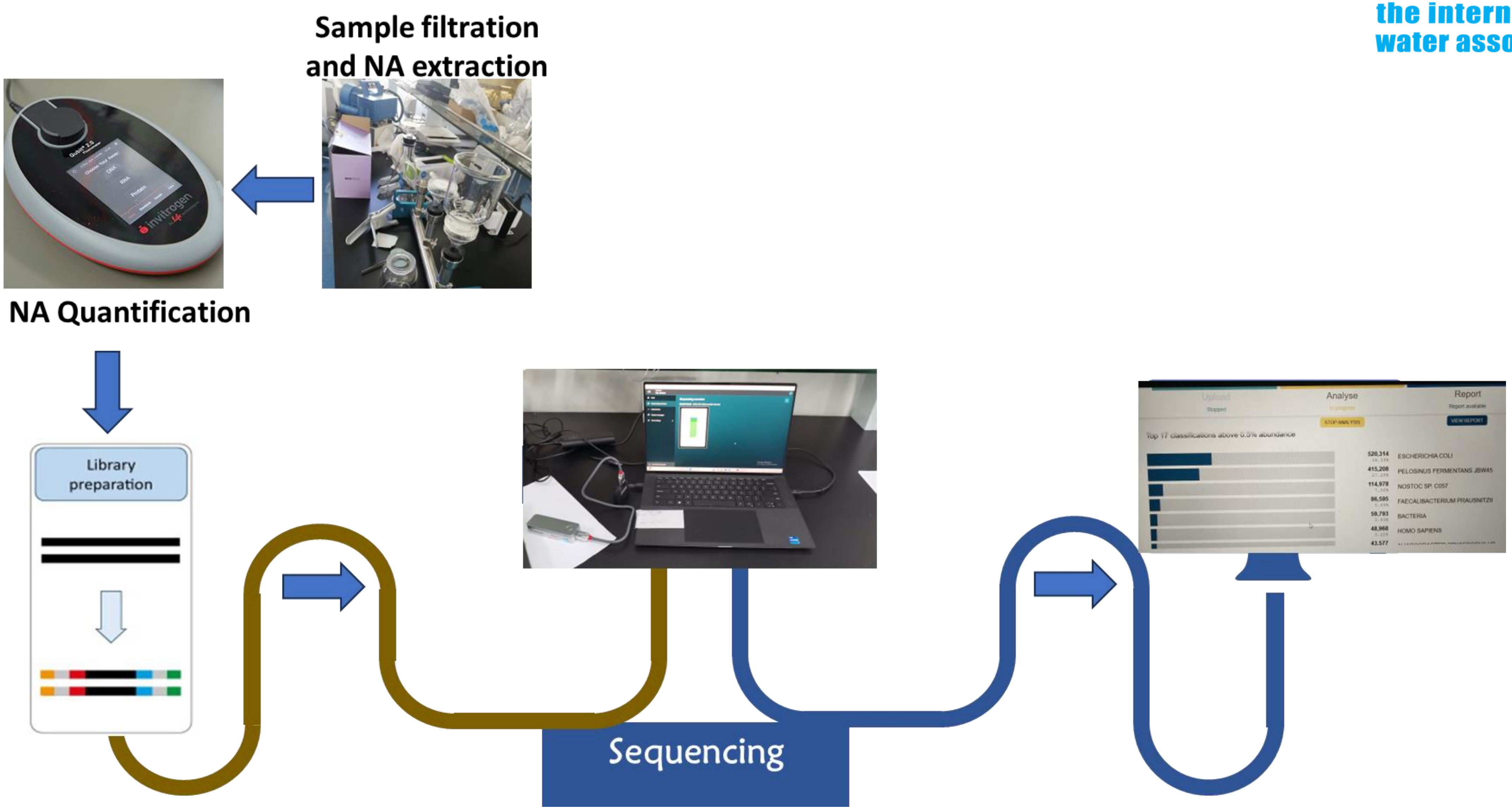
Timeline	
1 week	Identify locally relevant fecal shedding pathogens?
1 week	What is current clinical testing approach, including BSL2, 3, or 4?
2 weeks	Obtain ethical clearance for samples, set-up sanitation lab space, define quality monitors
1-12 weeks	Start culture methods → Order PCR supplies
8 weeks	Collect and analyze sanitation samples from sites with known positive clinical cases
2 weeks	Obtain clinical data
2 weeks	Pair clinical and sanitation system data for comparison for proof of concept

GENOMICS ANALYSIS



FUTURE PLANS

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To expand to more sites of sample collection as well as add other pathogens of concern to Malawi

To build a robust metagenomic sequencing capabilities for Wastewater based surveillance.

To develop a dashboard based on our weekly Lab

Train Medical microbiologists in wastewater analysis to increase the capacity in the Lab

Capacity building in Bioinformatics.

Procurement of Robust sequencing platform.

ACKNOWLEDGEMENTS

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Ruth Nyirenda

Chimwemwe Tandwe

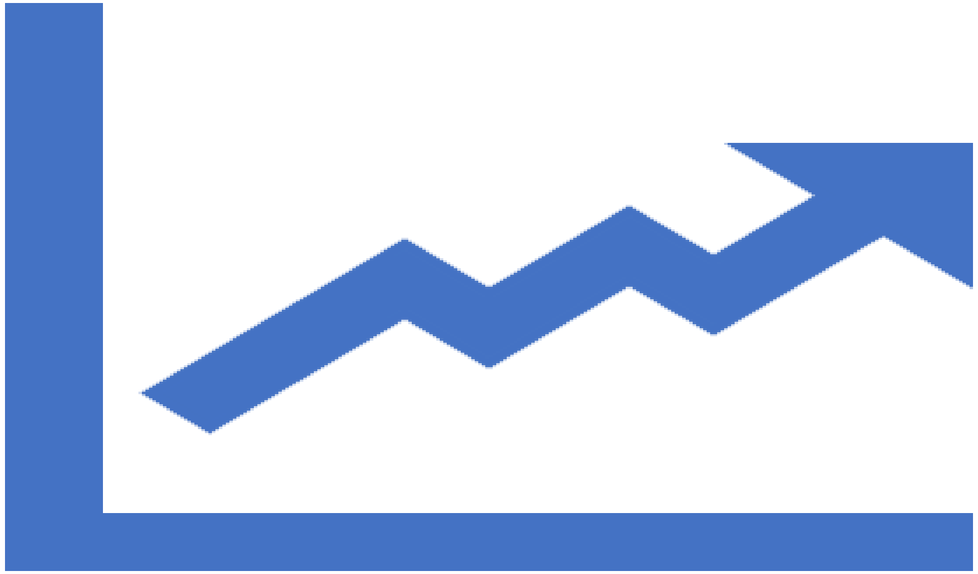
Ranken Namaumbo

Dr Rochelle Holm.

University of Louisville

Rockefeller Foundation

For the Financial support of the pilot



the international water association

Wastewater surveillance from Vellore, India - utilities, insights and challenges

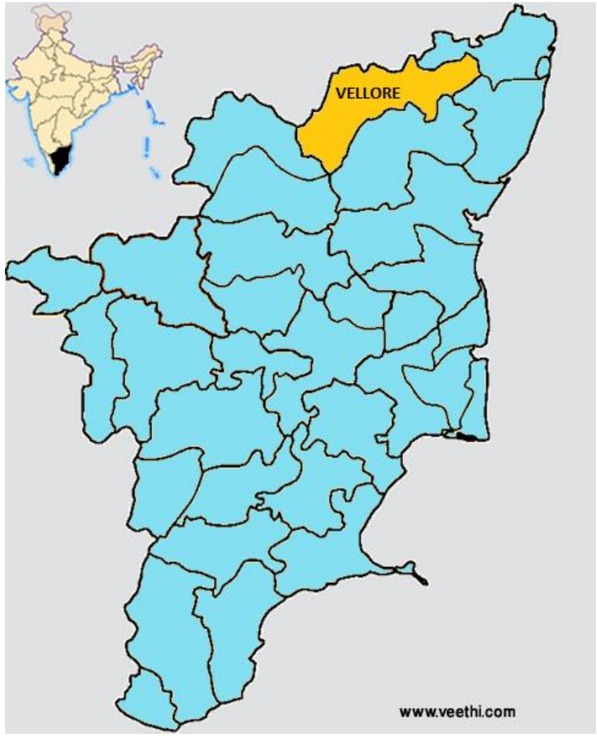
DILIP ABRAHAM, CHRISTIAN MEDICAL COLLEGE VELLORE

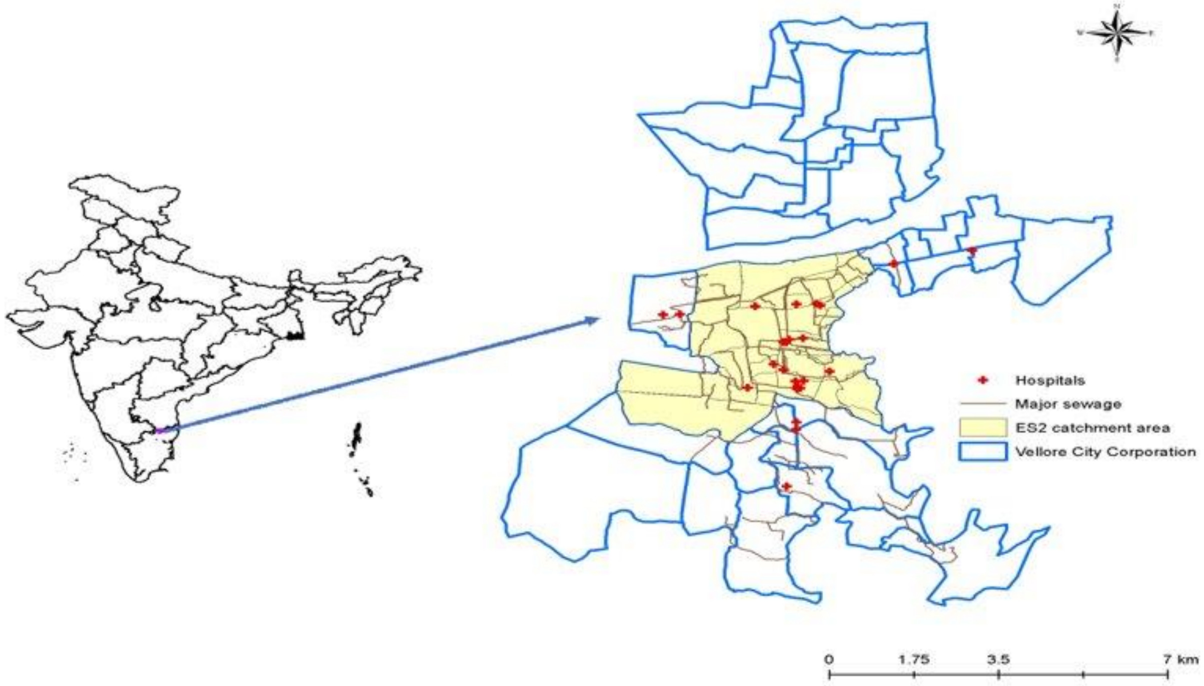
dilip.abraham@cmcvellore.ac.in

inspiring change

Study area in Vellore

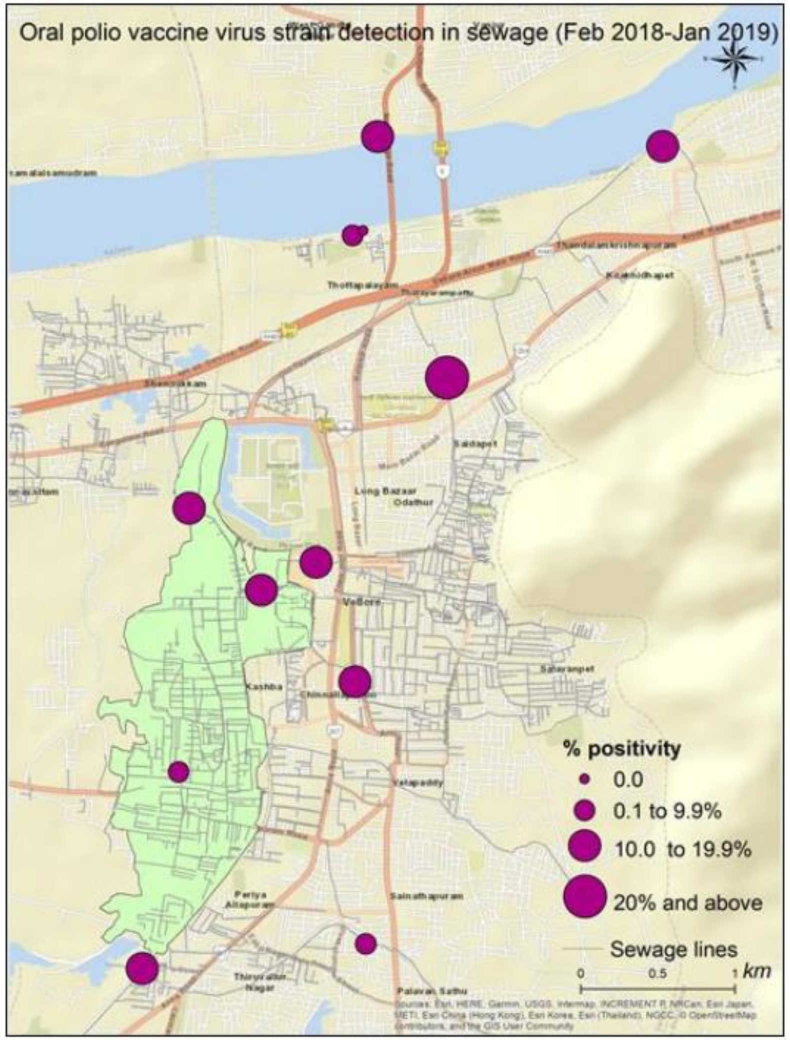
- Environmental surveillance across 24 wards of Vellore city in May 2021
- Spread over 16.25 sq.km; catchment population of 1,95,000 people
- Average population density of 26,500 / sq.km



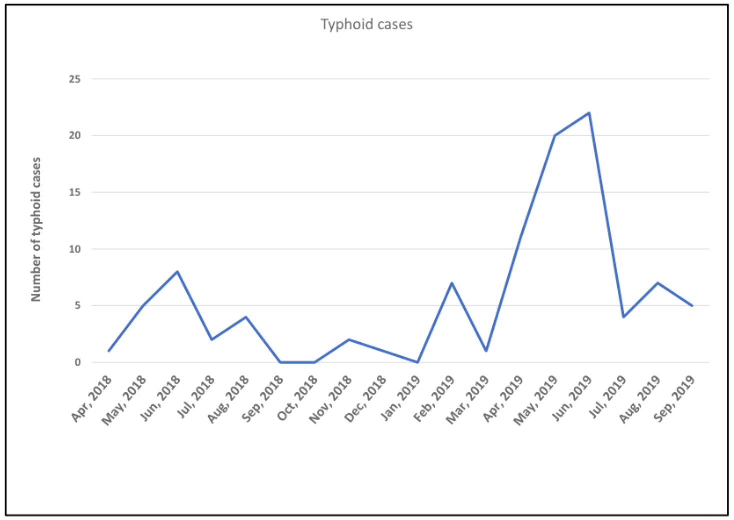


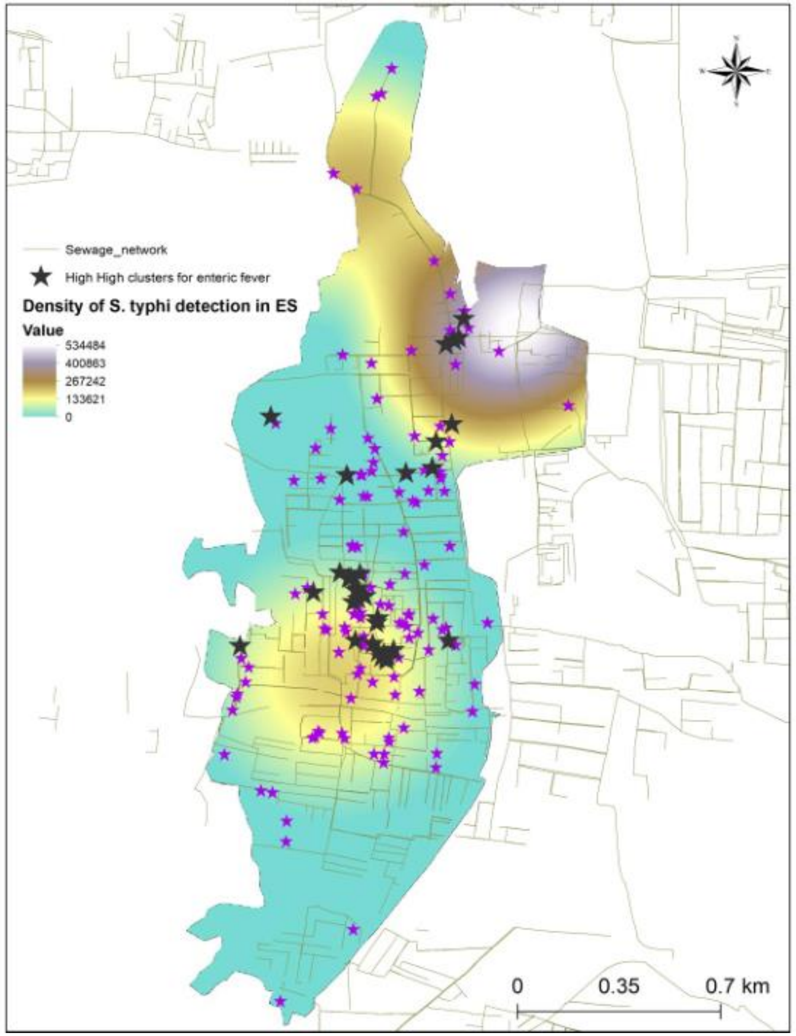
ES – 1: ENVIRONMENTAL SURVEILLANCE FOR SABIN POLIO VIRUSES, SALMONELLA TYPHI AND AMR GENES IN VELLORE (*JANUARY 2018 – JUNE 2020*)

the international water association



: Distribution of typhoid cases from April, 2018 to September, 2019





ES2 - WASTEWATER SURVEILLANCE OF *S.* TYPHI IN INDIA AND COMPARISON WITH CLINICAL/SEROLOGICAL SURVEILLANCE (2020 – 2021)

- investigate the optimal design for a typhoid/paratyphoid environmental surveillance (ES) system that will inform vaccine introduction and impact monitoring

- 1) implement typhoid ES with partners at sites conducting blood-culture surveillance over a 12-month period (India, Malawi and potentially Ghana).
- 2) ask what ES site characteristics (catchment population, local facilities, chemical and physical water-quality parameters) correlate with detection of *S.* Typhi or human-restricted control organisms that indicate faecal contamination.
- 3) investigate whether *S.* Typhi load and genetic diversity in ES samples correlate with disease incidence rates in the local population.

ES2 - SITE SELECTION

Spatial mapping of sewage network

→

Characterizing drains into three levels based on their sizes (length & width), sewage flow..

↓

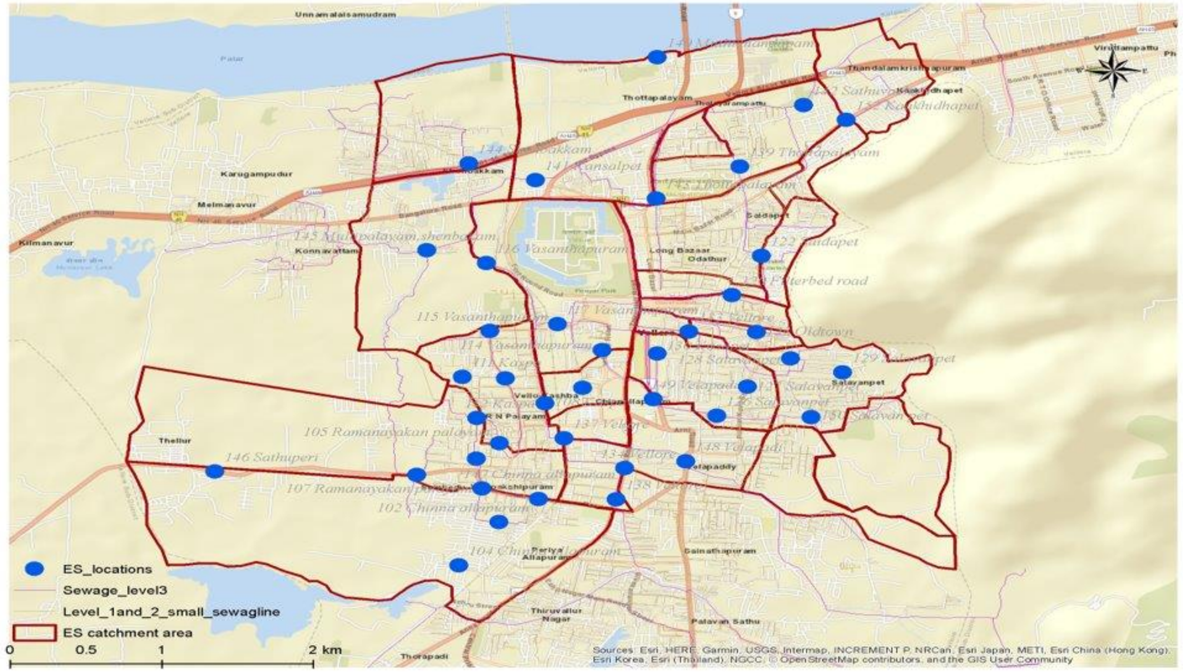
Ground truthing to identify potential sampling locations and feasibility of sampling

←

"Integration with high-resolution digital elevation models to delineate hydrological catchments, and estimate associated populations"*

↓

Selection of 40 sampling locations based on slope, catchment population and spread



*Uzzell CB, Troman CM, Rigby J *et al.* **Environmental surveillance for *Salmonella* Typhi as a tool to estimate the incidence of typhoid fever in low-income populations.** Wellcome Open Res 2023, **8**:9 (https://doi.org/10.12688/wellcomeopenres.17687.1)

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Detection of *S.* Typhi – sample processing

1. Membrane filtration

- Collection – 1 L
- Filtration
- Filter processing
- Extraction
- PCR

2. Moore Swab

- Preparation
- Deployment
- Enrichment
- Filtration
- Filter processing
- Extraction and PCR

- 2 sample processing methods employed:
  - One grab sample and one "trap" sample
- Sampling frequency:
  - Once per month
- 3rd May 2021 – 29 April 2022
  - 520 grab samples and 517 Moore swabs

Detection of *S.* Typhi & WW characteristics

Table 5

Salmonella strain (No. tested)	*ttr*	*sseJ*	*tviB*	*srfJ*	*SPC0869*	*SPA2308*	*staG*
Salmonella Typhi (556)	556	0	553	0	0	0	556
Atypical *Salmonella* Typhi (3)	3	0	0	0	0	0	3
Salmonella Paratyphi A (315)	315	0	0	0	0	315	0
Salmonella Paratyphi B (53)	53	0	0	53	0	0	0
Salmonella Paratyphi C (6)	6	6	6	0	6	0	0
*NTS Serovar (952)	952	938	0	380	19	50	41
Non-*Salmonella* spp. (7)	0	0	0	0	0	0	0

*The combination of genes present were heterogeneous, please see Supplementary Table 1 for details

Nair *et al*, 2019 – **A real-time PCR for the differentiation of typhoidal and non-typhoidal Salmonella**

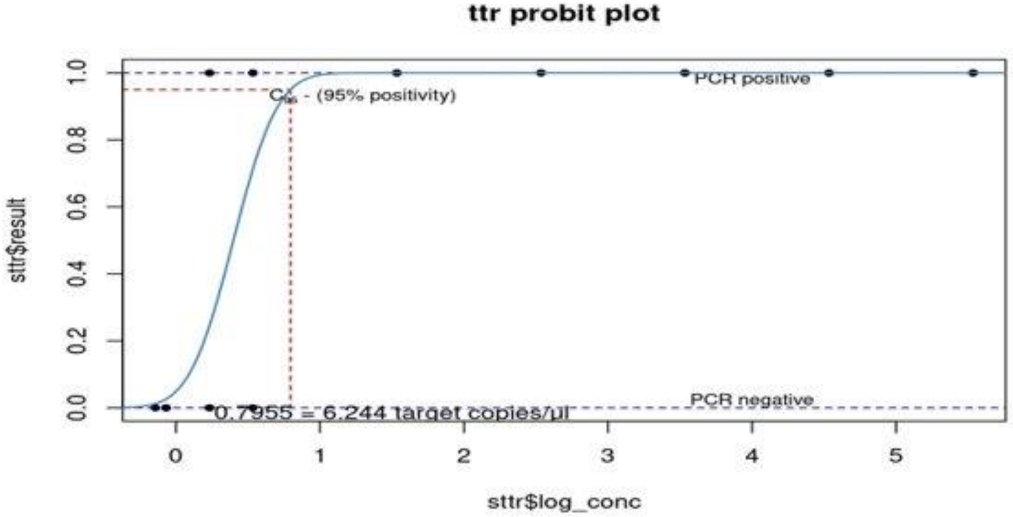
Targets

- *ttr*
- *staG*
- *tviB*
- HF-183
- IC
- Sample positive only if all Typhi targets detected
- Double positives were retested for the negative target as a singleplex PCR

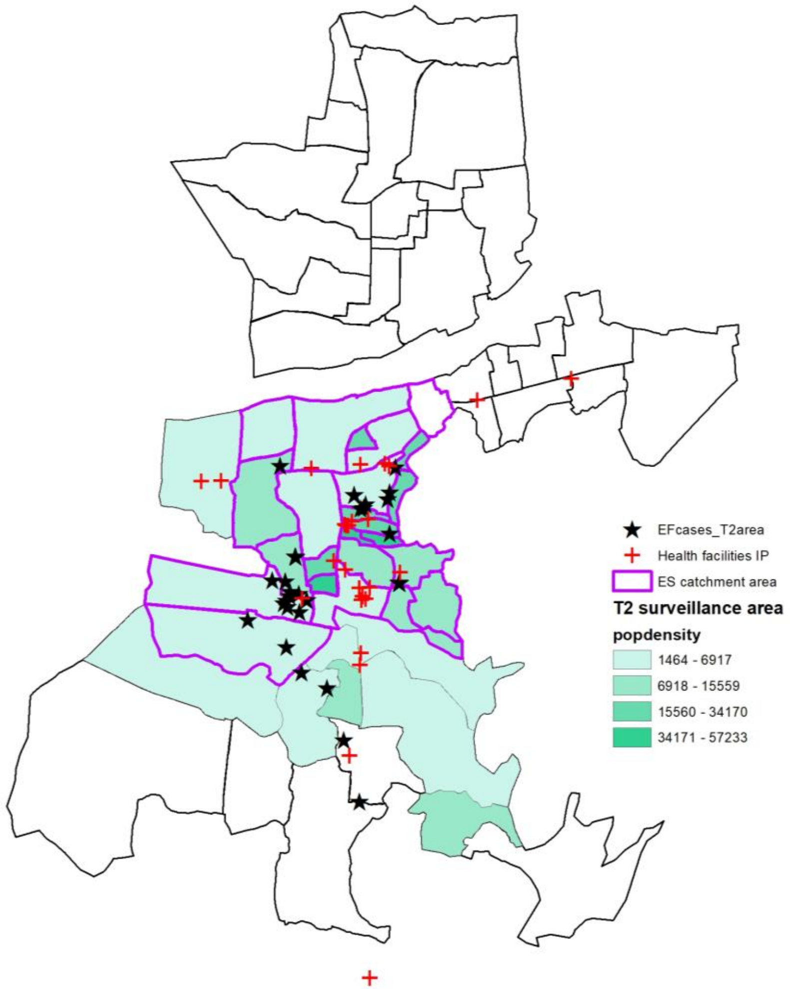
Aquaprobe AP-2000

- Temperature
- Baro
- PH
- ORP
- TDS
- Salinity
- Turbidity
- …

qPCR standardization



Clinical incidence of Typhoid



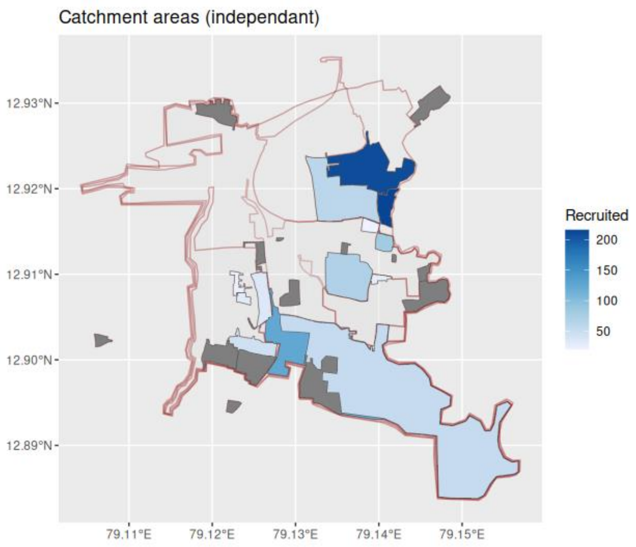
- Hospital based sentinel surveillance since April 2021
- Recruiting fever cases aged 6 months and above from defined geographical area; 1,90,000 population across 31 wards
- Of the 1108 eligible cases, 95% recruited and blood culture done in 92% of them
- 32 cases of enteric fever over one year from study area
- 28 cases (87.5%) of EF cases were from the ES catchment area
- Of the 28 cases, 17 – *Salmonella* Paratyphi "A" & 11 *S.* Typhi

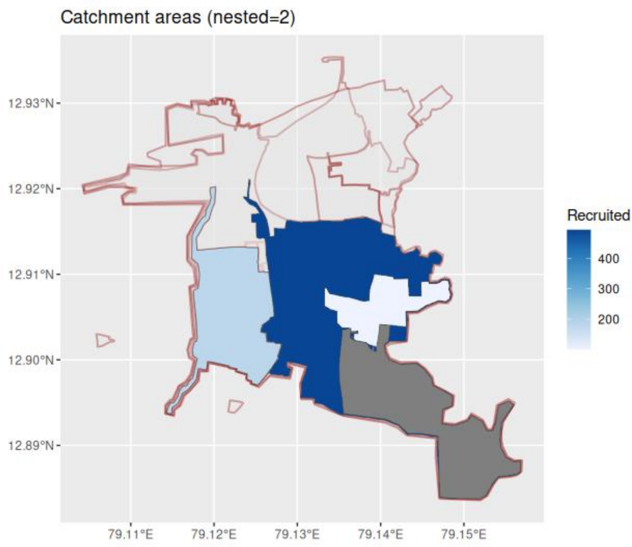
Age Group	No. of Cases	Population	Crude Incidence Per 100 000 Person Years (95% CI)
0–4	4	8082	49.5 (13.5–126.7)
5–9	3	9772	30.7 (6.3–89.7)
10–14	0	10 430	0.0 (.0–35.4)
15–29	4	35 728	11.2 (3.1–28.7)
30+	0	77 788	0.0 (.0–4.7)
Total	11	141 800	7.8 (3.9–13.9)

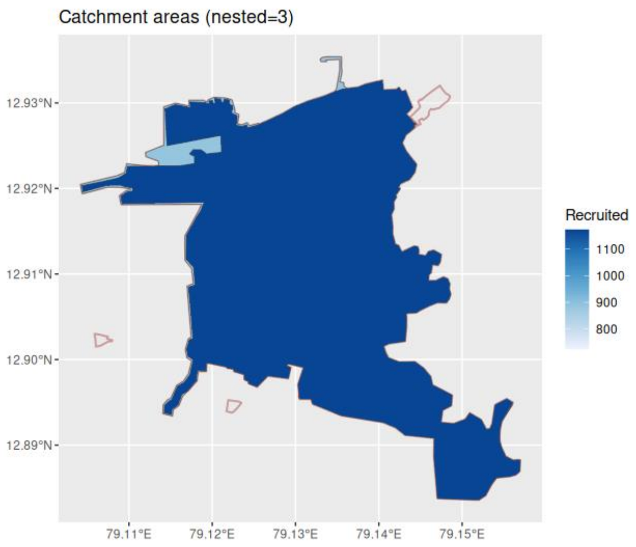
Christopher B Uzzell, Dilip Abraham, Jonathan Rigby, Catherine M Troman *et al.* **Environmental Surveillance for *Salmonella* Typhi and its Association With Typhoid Fever Incidence in India and Malawi**, *The Journal of Infectious Diseases*, 2023; https://doi.org/10.1093/infdis/jiad427

Sero-survey for HlyE IgG

- Clinical surveillance was limited due to deviation in health-seeking behaviour during the pandemic
- Carried out HlyE IgG testing for 1200 study participants from 0–15-year-old
- Based on catchment areas (CA) for ES site
- Each CA would recruit proportionate age-stratified numbers
- Correlate ES detection with sero-incidence



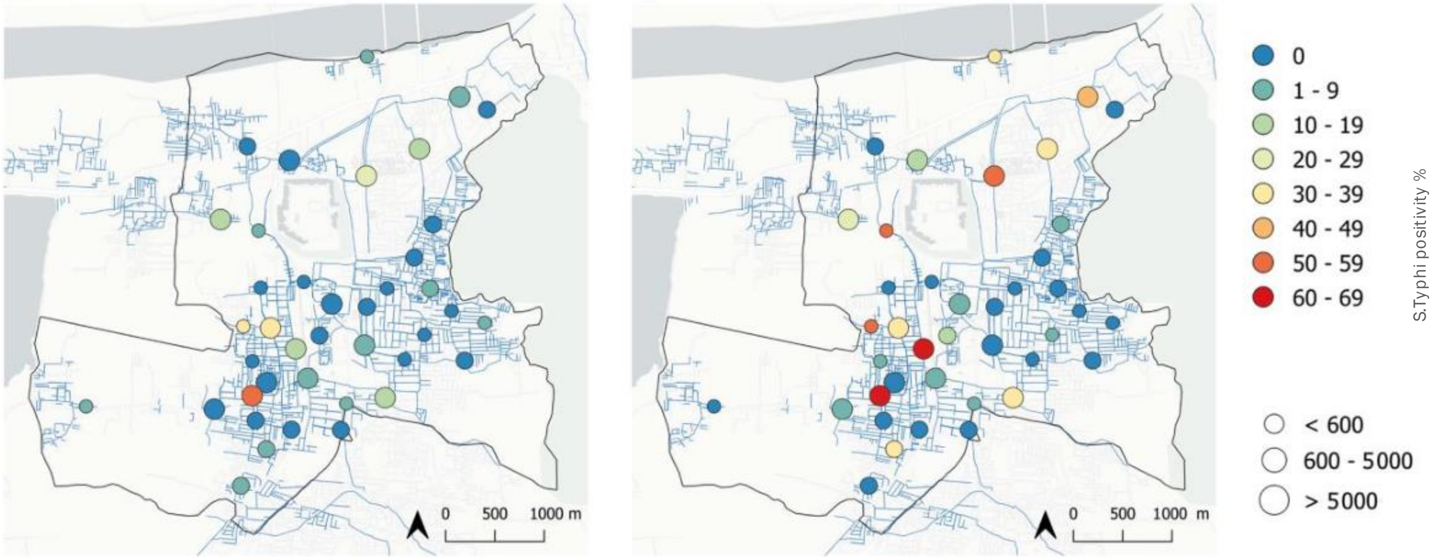


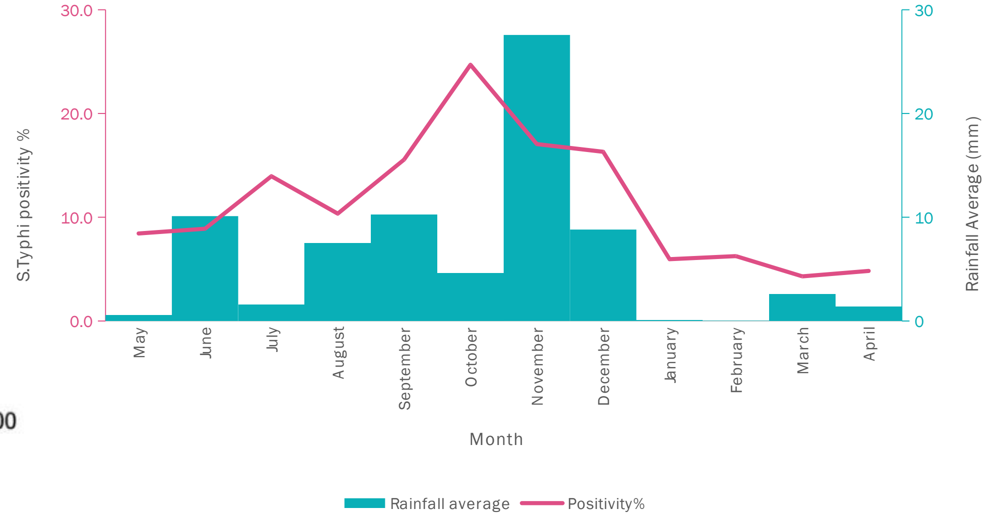


Results - ES positivity & trends

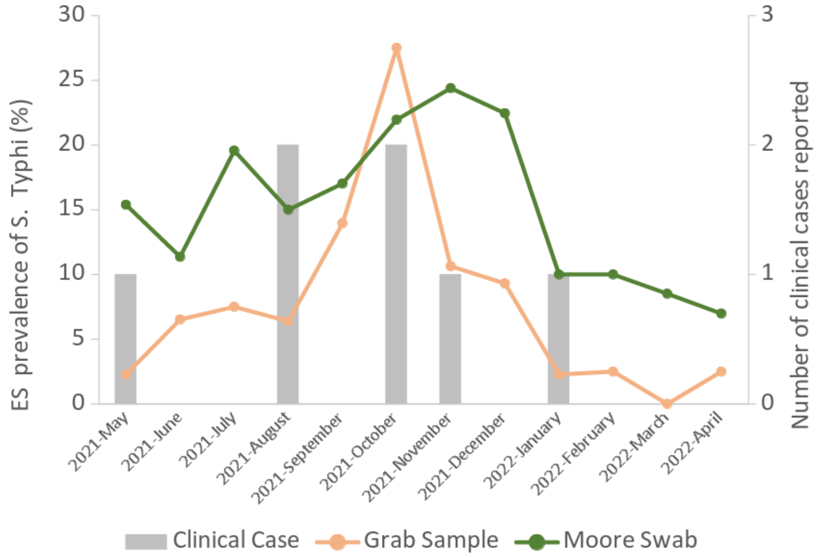
Membrane filtration

Moore swab





Type	Total samples	Positive
Membrane filtration	520	39 (7.5%)
Moore	517	79 (15.3%)
Total	1037	118 (11.4%)



Utilities, insights and challenges?

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Challenges/Watch out for

- Validate the method at your own site and train personnel
- Loss of Moore swabs – rat bites, removal by members of the local community
- Time management
- Reagent procurement and supply chain
- Data management
- qPCR validation
- Avoid freeze-thaws of DNA extracts
- Scalability and automation
- What is a positive sample?
- Normalization – MST? Flow rate?
- Communication with public health personnel on how to use this data

Insights

- Sampling frequency and methodology would need to fit with the defined use-case and setting of ES
- Data analysis is not standard and would need to be tailored for the pathogen and use-case
- Variability of detection is very high in LMIC settings
- Molecular targets should be chosen after thorough literature review and preferably with consensus in the field
- Delineation and description of catchment areas is essential
- Availability of some amount of clinical surveillance data or estimation of disease burden is essential for validation

Utility

- Estimation of disease burden - typhoid
- Predicting outbreaks – SARS-CoV-2
- Detection of novel variants/vaiantf of concern – SARS-CoV-2, polio
- Localization of source of outbreak
- Delineation and description of catchment areas is essential
- Availability of some amount of clinical surveillance data or prior estimation of disease burden is essential for validation

Q&A Discussion

MODERATOR: GERTJAN MEDEMA & SUDHIR PILLAY

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