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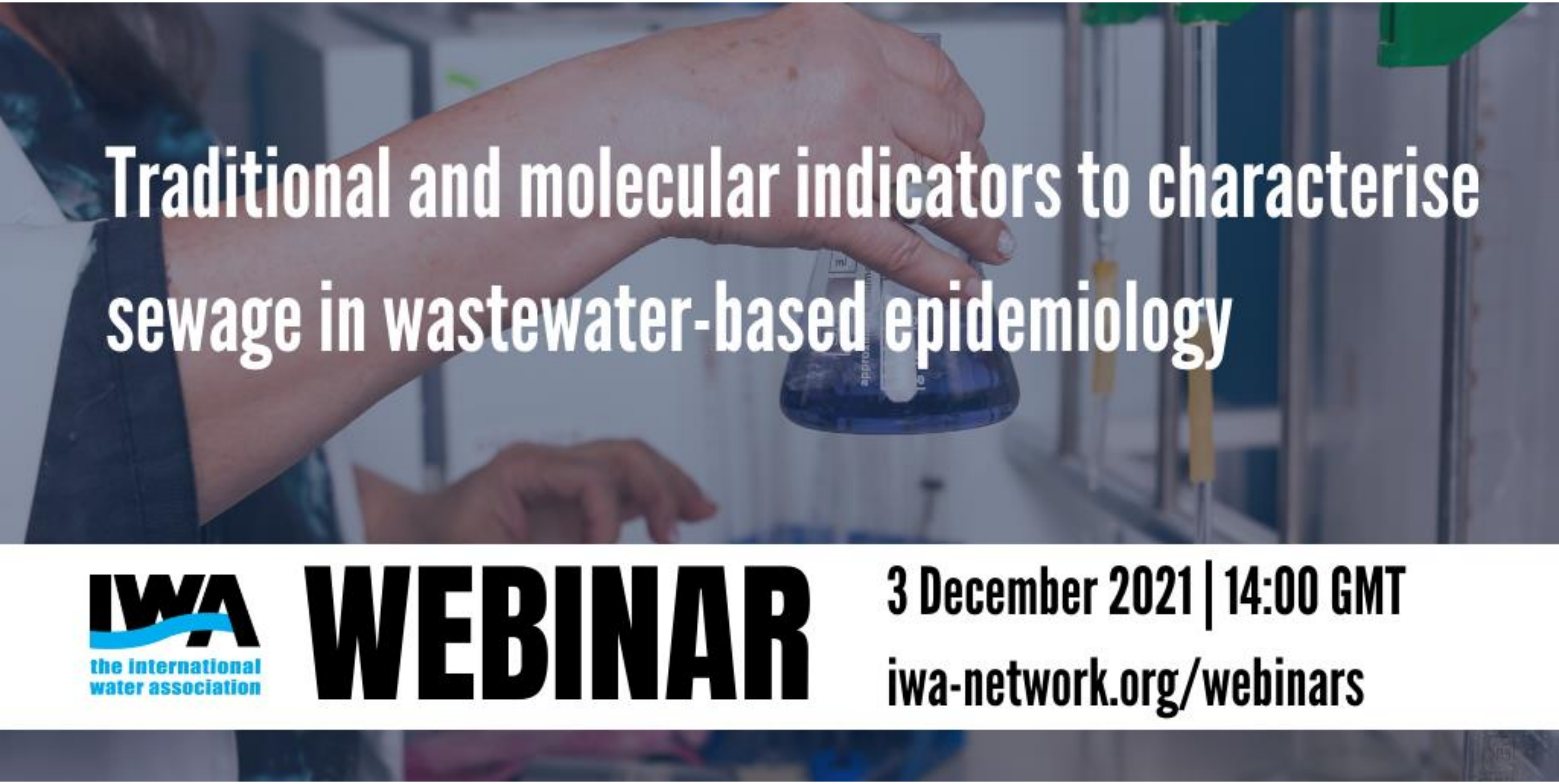

Traditional and molecular indicators to characterise sewage in wastewater-based epidemiology

the international water association

WEBINAR

3 December 2021 | 14:00 GMT

iwa-network.org/webinars

inspiring change

WEBINAR INFORMATION

- This webinar will be **recorded and made available "on-demand"** on the IWA website.
- Following the webinar, you will be sent a **post-webinar email** with the on-demand recording, presentation slides, and other 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'.*

HEALTH-RELATED WATER MICROBIOLOGY SPECIALIST GROUP

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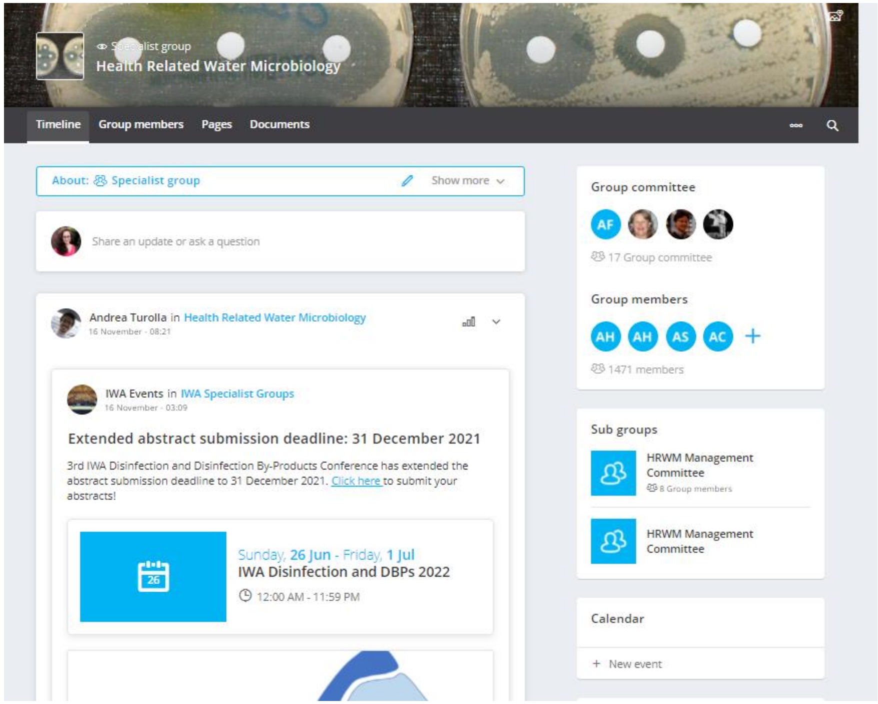

https://iwa-connect.org/group/health-related-water-microbiology/timeline

The HRWM Specialist group is composed of members who are concerned with all aspects of health-related water microbiology and carry out applied and fundamental research on water and microorganisms as it impacts public health.

Visit the website and learn more about events, publications, and news about water-related diseases: https://hrwm-watermicro.com/

Join the HRWM group on Facebook and LinkedIn!

the international water association HEALTH-RELATED WATER MICROBIOLOGY

MODERATORS AND PANELISTS

Andreas H. Farnleitner
ICC Water & Health, TU Wien and KL Krems, Austria

Joan B. Rose
Michigan State University

Joy Kwanrawee Sirikanchana
Chulabhorn Research Institute, Thailand

Anicet R. Blanch
University of Barcelona, Spain

Orin Shanks
US EPA, USA

Mats Leifels
Nanyang Technological University, Singapore

Marlene Wolfe
Emory University, USA

Gertjan Medema
KWR Water Research Institute, the Netherlands

René Mayer
TU Wien and Karl Landsteiner University of Health Sciences

Sarah M. Essert
University Hospital Bonn, Germany

INTRODUCTION - 1st HRWM Webinar Mini-Symposium

Traditional and Molecular Indicators to Support Wastewater Based Epidemiology

Organizers & Moderators
Joan B. Rose, Michigan State University, USA
Kwanrawee Joy Sirikanchana, Chulabhorn Research Institute, THA
Anicet R. Blanch, University of Barcelona, ESP
Andreas H. Farnleitner, TU Wien + KL Krems, ICC Water&Health, AUT

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HRWM WEBINAR MINI-SYMPOSIA 21-22
"THE IDEA"

- 20th Water Micro, 2019
- 21st Water Micro, **2023** (Darwin, AUS)

- **Bridging activities 21-22**
  - Webinar Mini-Symposium
  - Several on-line events
  - Active member participation

- **Call for submissions**
  - Selection by organizing committee
  - **Short presentations** (5´)
  - **Accepted submissions** shown/archived on HRWM website

Water Micro 2019, Vienna, AUT

The 20th IWA International Symposium on **Health-Related Water Microbiology**, Vienna, Sep 2019

HRWM WEBINAR MINI-SYMPOSIA
further planned topics

- **Traditional and Molecular Indicators to Support Wastewater Based Epidemiology** (2021)
- **Water Reuse & Risk Assessment**
  → joint with SG Water Reuse (2022)
- **Disaster management, preparedness & WASH**
  → other SG´s to be involved (2022)
- **Recreational water quality**
  → related to the recently launched WHO guideline (2022)

TRADITIONAL AND MOLECULAR INDICATORS TO SUPPORT WBE – TOPIC

- **Overview on current international activities**:

  → **Fecal indicators** (**FI**) and **microbial source tracking** (**MST**) markers to support **waste water based epidemiology** (WBE)

  → **FI** & **MST** to support **data interpretation**, data **comparability** and **quality control** concerning monitoring/surveillance of waste water

- **Presentations/discussions of FI & MST (bacterial, viral, others):**

  → tracking of human sewage pollution (disting. from other sources)

  → characterisation of unknown sewage effluents

  → normalisation of WBE data (SARS-CoV-2 and others)

  → determination of microbial treatment efficacies

- **Including cultivation-based & molecular diagnostics**

PROGRAMME & SPEAKERS

Time: 90 minutes

- **WELCOME AND INTRODUCTION**, Andreas H. Farnleitner & Joan B. Rose
- **INVITED MINI-KEYNOTE** - *Human Wastewater Surveillance: A New Opportunity for Microbial Source Tracking,* Orin Shanks (USEPA)
- Q&A with audience
- **PART I** - *Indicators/MST to support viral targeted WBE* (moderators: Joy & Blanch)
  - Normalisation of SARS-CoV-2 for sewage surveillance, ***Gertjan Medema***
  - Application of MST markers to normalize shedding rates in a campus monitoring program in Singapore, ***Mats Leifels***
  - Estimating relative abundance of two SARS-CoV-2 variants in wastewater settled solids, ***Marlene Wolfe***
- Q&A with audience
- **PART II** - *Indicators/MST to support bacterial targeted WBE* (moderators: Blanch & Joy)
  - Antibiotics and resistance genes as indicators for multidrug resistance bacteria?, ***Sarah Essert***
  - High persistence of traditional and molecular fecal indicators support proportional auto-sampling of sewage, ***René Mayer***
- Q&A with audience
- **FINAL DISCUSSION & CLOSING** (moderated by Andreas H. Farnleitner & Joan B. Rose)
- **CLOSING** (Joan B. Rose)

EXPANSION OF THE GLOBAL WATER PATHOGENS PROJECT NETWORK AND ACTIVITIES

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- *Mission: advance environmental surveillance of sewage to inform local and global efforts for monitoring and supporting public health measures to combat disease*
- GWPP has built an online platform housing the new book *Sanitation and Disease in the 21st Century* includes 115 chapters, 391 data tables, 7336 scientific resources, 392 glossary terms and a GWPP network of over 250 people from 52 countries.
- Includes the Knowledge to Practice (K2P):Decision support tools [mapping and technology apps]
- Now housing the global data center for SARS-CoV2 *https://sphere.waterpathogens.org/*

WASTEWATERSPHERE

GWPP | K2P SAFE SANITATION

HRWM MINI-SYMPOSIUM
MINI-KEYNOTE PRESENTATION

the international water association

Human Wastewater Surveillance: A New Opportunity for Microbial Source Tracking

Speaker:

Orin Shanks, US EPA

Moderators:
Joan B. Rose (lead)
Andreas H. Farnleitner (assist)

United States Environmental Protection Agency

HEALTH-RELATED WATER MICROBIOLOGY

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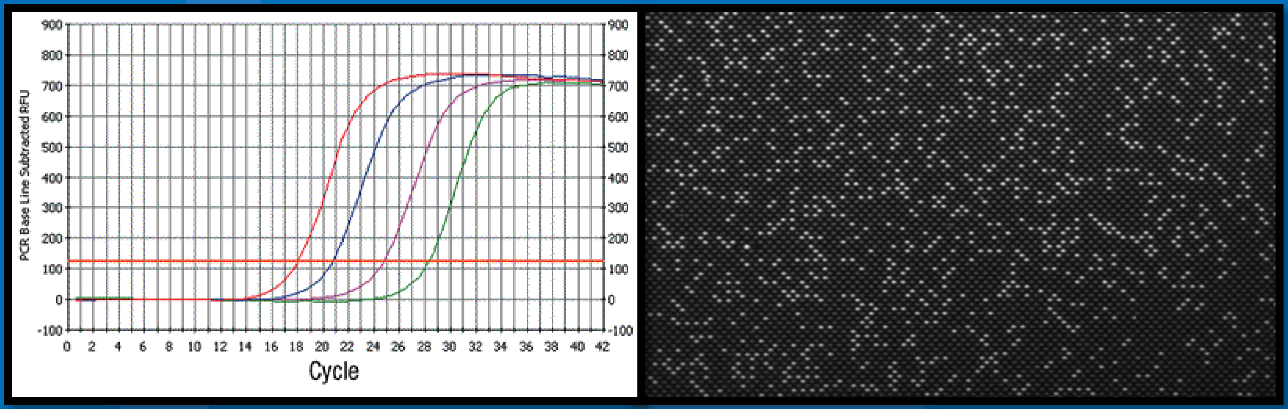
Human Wastewater Surveillance: A New Opportunity for Microbial Source Tracking

Orin C. Shanks

HRWM SG Webinar Mini-Symposia Series 2021-2022

Part I: Traditional and Molecular Indicators to Support Wastewater Based Epidemiology



Office of Research and Development
Center for Environmental Measurement and Modeling

United States Environmental Protection Agency

HEALTH-RELATED WATER MICROBIOLOGY

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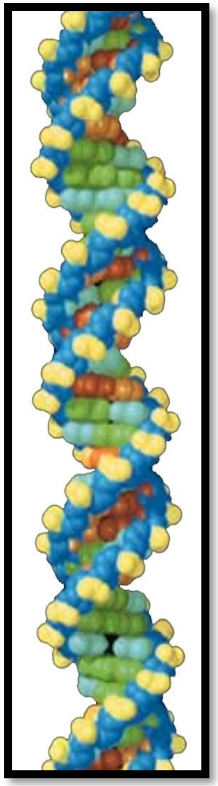
Presentation Overview



1. **Wastewater Surveillance Overview**
2. **Current MST Use Scenarios**
3. **Trends in United States**
4. **Some Observations**

Disclaimer: *The views expressed in this presentation are those of the author and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.*

Wastewater Surveillance




- **Premise:** Circulation of public health target of interest shed in population waste is linked to occurrence in wastewater

- **Chief Uses:**
  - Early detection
  - Monitor occurrence dynamics
  - Identify new or variant targets
  - Inform community response

Key Benefits:

Pooled sample:
-All individuals in community
-Healthy and non-healthy
-Children and adults

Rapidly test large population with few samples

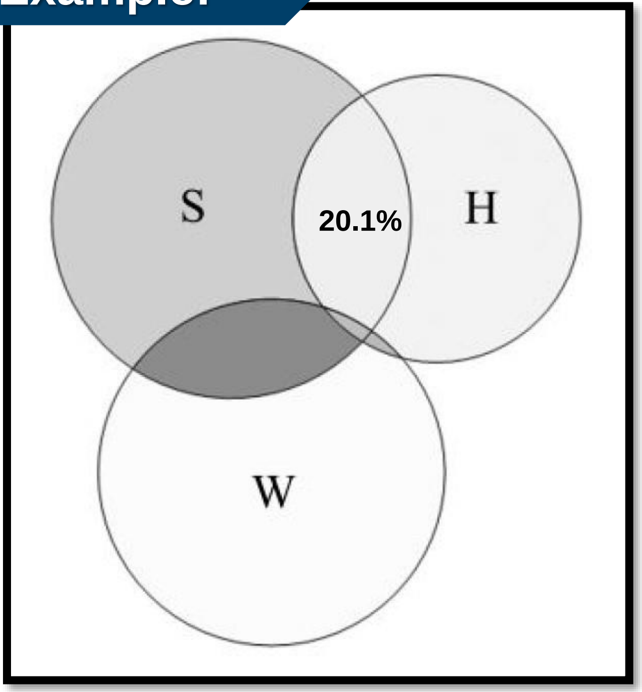
Wastewater Composition

- **Complex mixture:**
  - Mostly water
  - Suspended and dissolved organic and inorganic solids
  - Pooled human waste (feces, urine)
  - Diverse microbiome

- **Bacterial population structure:**
  - Human waste makes up:
    - 4.3% to 28.7%
  - Majority of bacteria associated with the environment

(McLellan et al. 2010; Shanks et al. 2013)

Example:

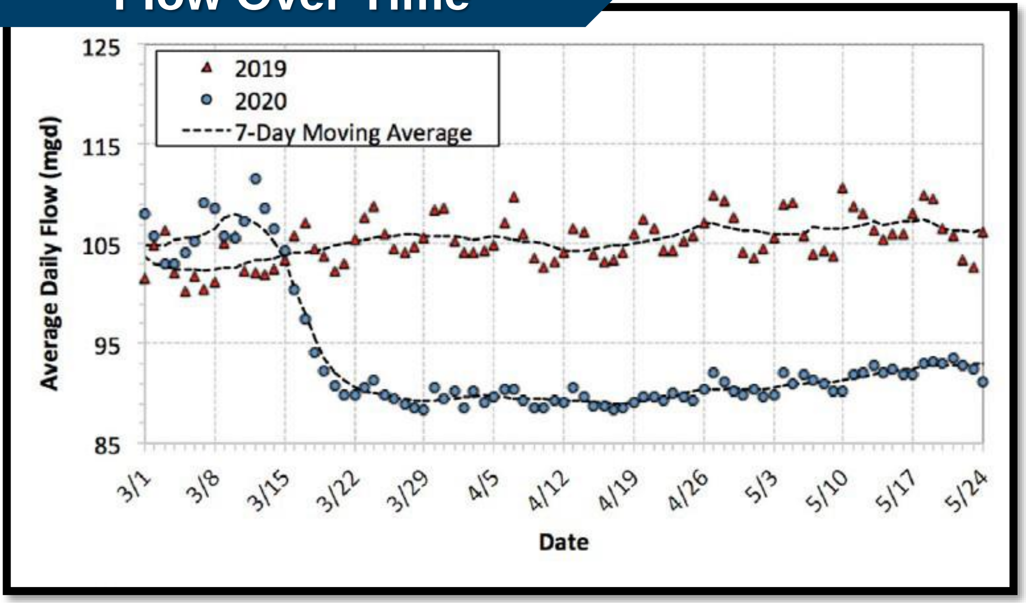


Venn diagram of OTU overlap between sewage (S), human feces (H), and surface water (W). (McLellan et al. 2010)

Multiple Factors Influence Human Waste Levels

- **Population flux:**
  - Tourism, special events, etc.
- **Variable flow:**
  - Groundwater infiltration
  - Stormwater runoff
  - Industrial discharges
  - Household water usage

Example: Average Daily Flow Over Time



Gerrity *et al.* (2021)

Wastewater Surveillance Challenges Due to Variable Human Waste Levels

1. **Human waste level is unknown prior to testing**
2. **Human waste level can vary on temporal and spatial scales**
3. **Variability may confound interpretation of sewage surveillance trends**

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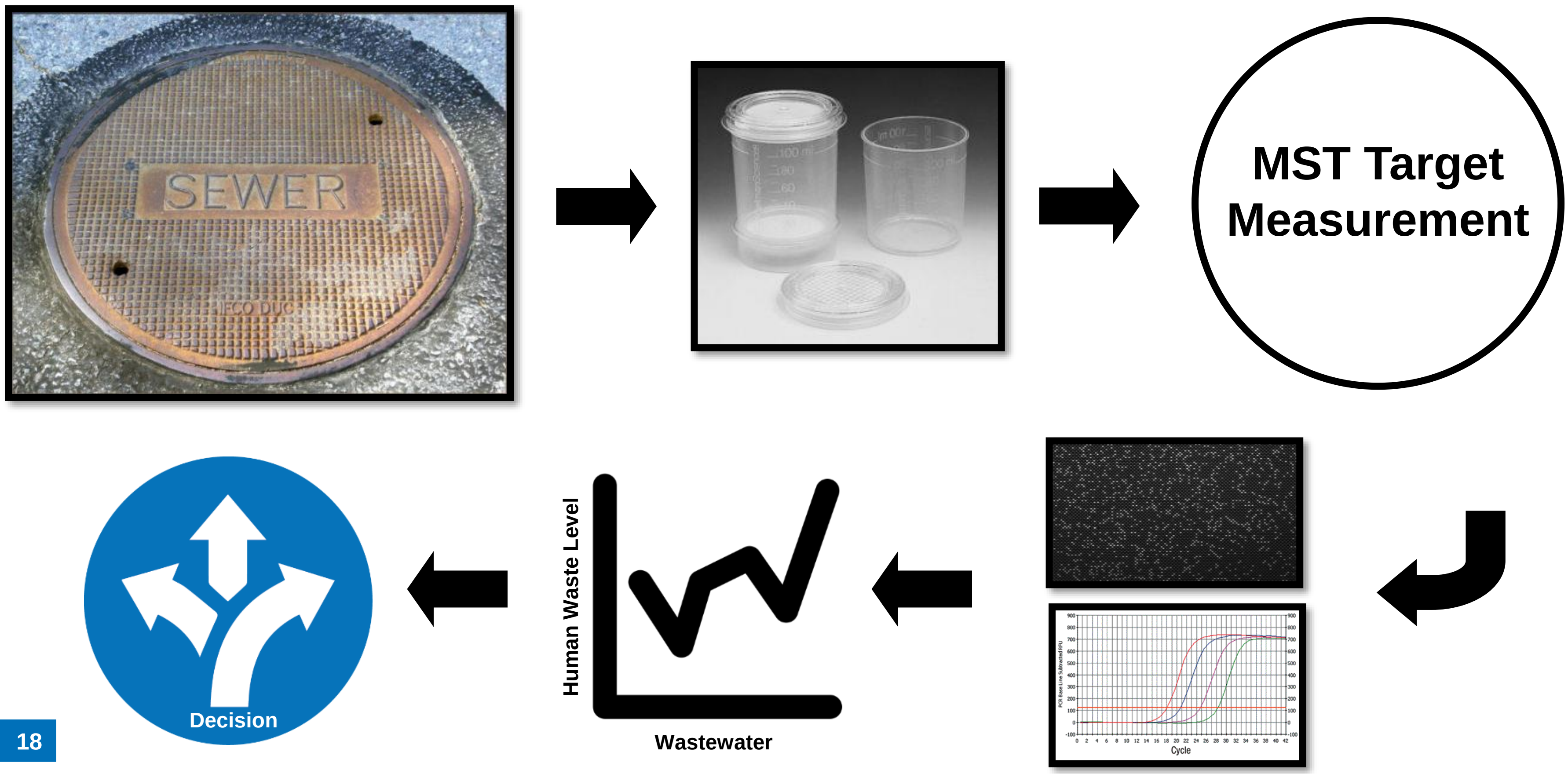
A Microbial Source Tracking Opportunity



MST Use Scenario #1:
Quantify human waste level in sample

- **Human fecal waste level:**
  - Fecal strength:

    = (MST concentration)

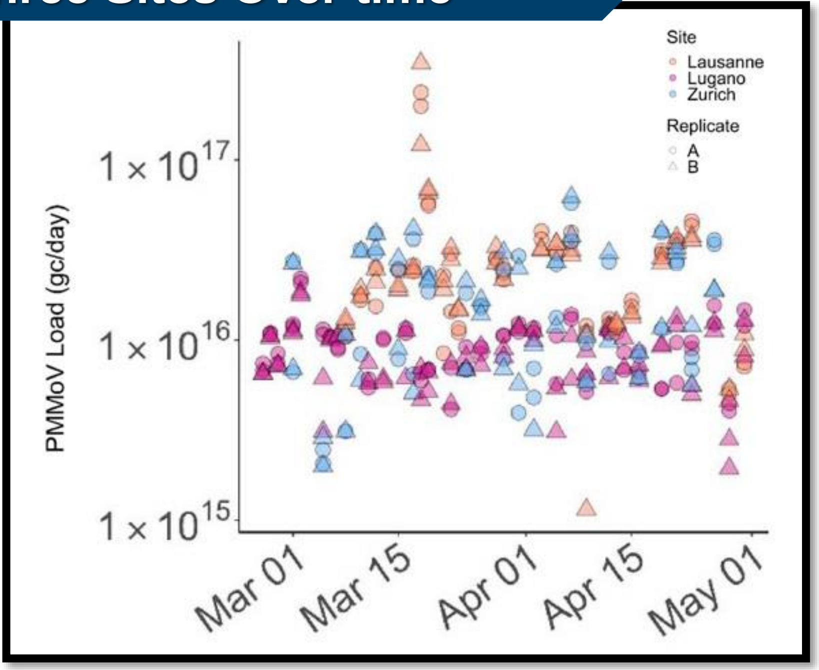
  - Fecal load:

    = (MST concentration) x (flow)

  - Per capita fecal load:

    $= \frac{\text{(MST concentration x flow)}}{\text{population served}}$

Example: Fecal Load at Three Sites Over time



Fernandez-Cassi *et al.* (2021)

MST Use Scenario #2:
Surveillance target internal process control

- **Surrogate to monitor surveillance target extraction and quantification**
- **Confirm presence of fecal waste**
- **Not a substitute for surveillance target recovery and detection controls**

MST Use Scenario #3:

Human fecal normalization control to describe spatial and temporal trends of surveillance target

- **Human fecal normalization control:**
  - Identifier "…specific to human feces that can be measured in wastewater to estimate human fecal content." (CDC 2021)
- **Surveillance target concentration normalized to:**
  - Fecal strength
  - Fecal load
  - Per capita fecal load
- **Compare measurements within or between sites**

Example:

$$= \frac{\text{Surveillance Target Concentration}}{\text{Fecal Strength}}$$

MST Use Scenario #4:

Human fecal normalization control to help with prediction of a community infection trend

- **Common community infection metrics:**
  - Cases, test positivity (rate or counts)
- **Initial studies with wastewater and settled solids**
- **Utility influenced by infection metric, site, and data quality**

MST Usage in the United States:
National Wastewater Surveillance System (NWSS) database

- **Public, commercial, and academic data**
- **Usage statistics: (November 8, 2021)**
  - 29 labs
  - 27,122 wastewater samples
  - 58.6% of labs use MST
  - 15,604 samples with MST data (57.5%)

Assay	Count	% Usage
PMMoV	12,966	83.1%
F+ RNA Coliphage	2,263	14.5%
CrAssphage	1,413	9.1%
HF183	299	1.9%

Note: Some labs report use of more than 1 MST assay

https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/testing-methods.html

Human Wastewater Surveillance MST "Toolbox"

Target	Example	Measurement Target
Bacteria	*Bacteroidales*	16S rRNA and non-ribosomal
	Lachnospiraceae	16S rRNA
	Bifidobacteria	16S rRNA
Archaea	Methanogen	Non-ribosomal
Virus	Pepper mild mottle virus	Variable (including cultivation)
	Bacteriophage	
	Polyomavirus	
Animal	Human	mtDNA

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MST Method Selection:
What method attributes are most important?

- **Attribute priority may differ from surface water applications:**
  - Human waste is likely dominant source
  - Typically present at high concentrations
- **May vary by:**
  - Usage scenario
  - Surveillance target of interest
  - Geographic location

MST Target Fecal Shedding:

How well does the MST target represent the community of interest?

- **Limited data on shedding across age groups**
- **Additional research may be necessary**

Example: PMMoV

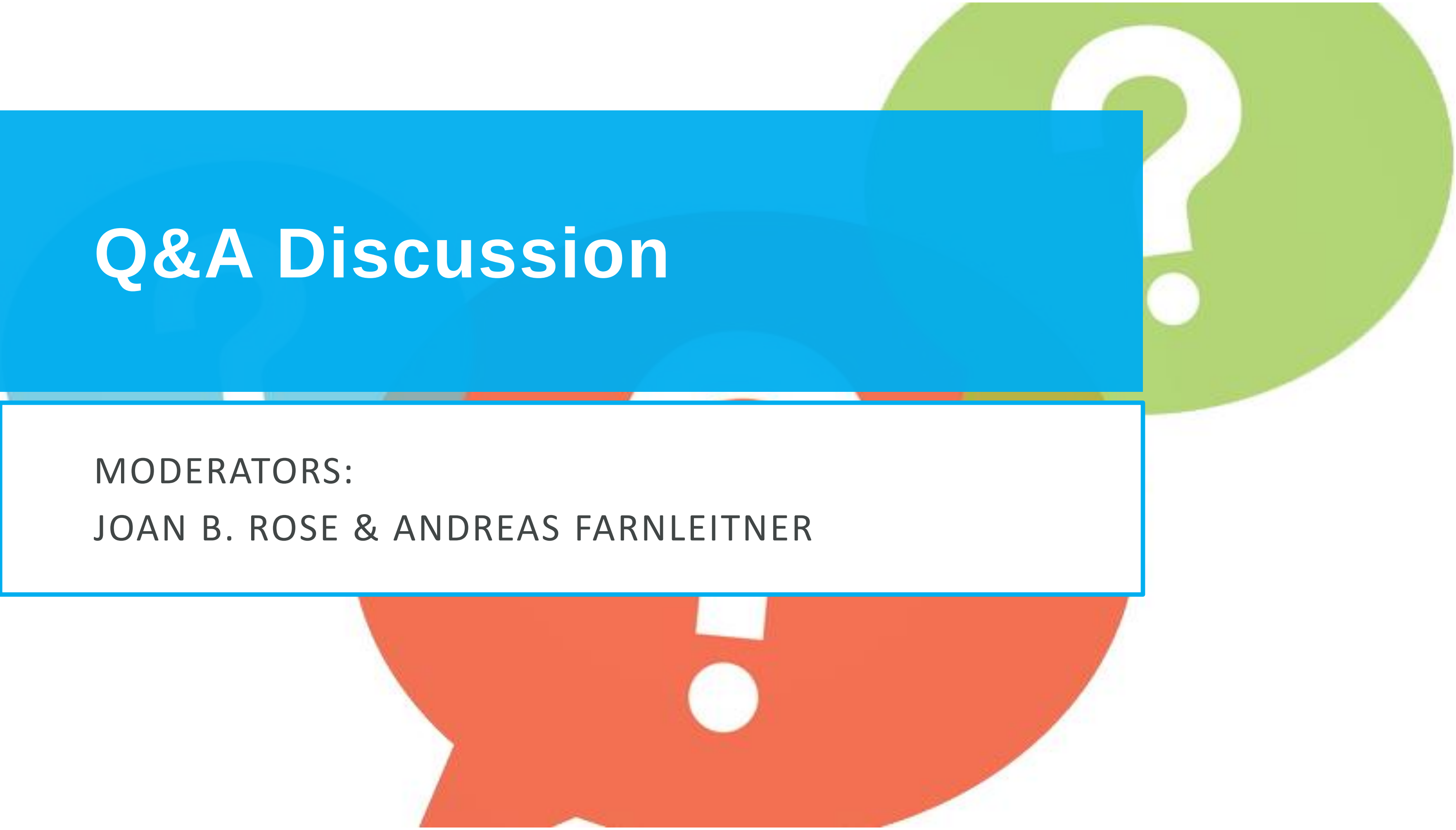
Plant pathogen that infects wide range of pepper species

Occurrence in human waste is diet dependent

Human feces detection 4.5% to 95% across studies (Katajima et al. 2018)

Possible age-related shedding trend:
-15x less detection in children (< 18 yr)
(Colson et al. 2010)

Under 18 yr is 24% of U.S. population
(U.S. 2010 Census)



Q&A Discussion

MODERATORS:

JOAN B. ROSE & ANDREAS FARNLEITNER

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Indicators and MST* marker to support viral targeted WBE**

Moderators:
Kwanrawee Joy Sirikanchana (lead)
Anicet R. Blanch (assist)

* Microbial Source Tracking

** Waste Water Based Epidemiology

Normalisation of SARS-CoV-2 for sewage surveillance

GERTJAN MEDEMA
KWR, NETHERLANDS

KWR

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HIGHLIGHTS/LEARNING OBJECTIVES

- SARS-CoV-2 concentrations in wastewater need to be normalized:
  - for reliable observation of short-term trends in COVID-19 circulation
- CrAssphage shedding is highly variable per person, but CrAssphage loads in wastewater of a population >5000 are very constant.
- Normalisation method:
  - Flow normalisation is preferred (with EC check)
  - CrAssphage normalisation is a suitable alternative in absence of flow data

ROTTERDAM DISTRICT 3 TRENDS

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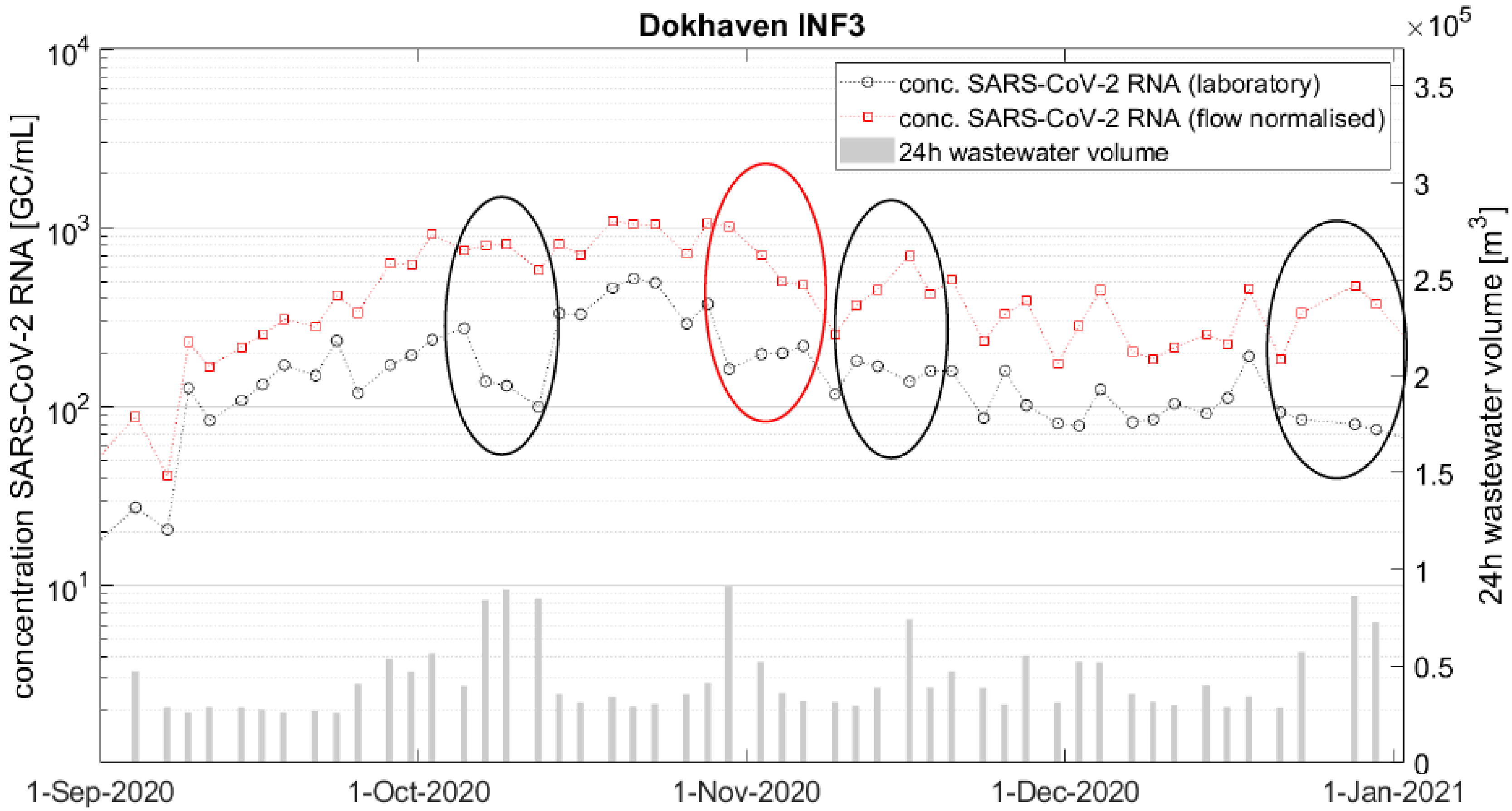
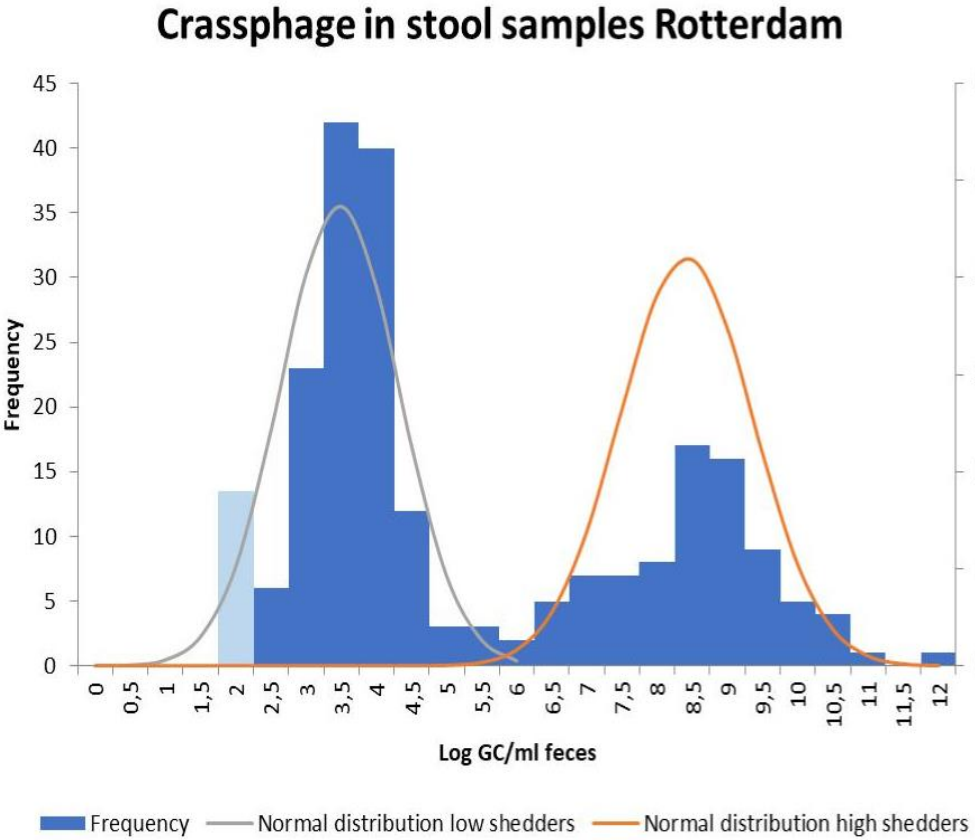
- Short term trend normalised ≠ unnormalised



CRASSPHAGE IN STOOL (N=221)

- Stool samples adults
- 95.5% detected

- Bimodal distribution
- 66.1% 3.5±0.8 LOG GC/ml
- 33.9% 8.4±1.0 LOG GC/ml



CRASSPHAGE IN WASTEWATER

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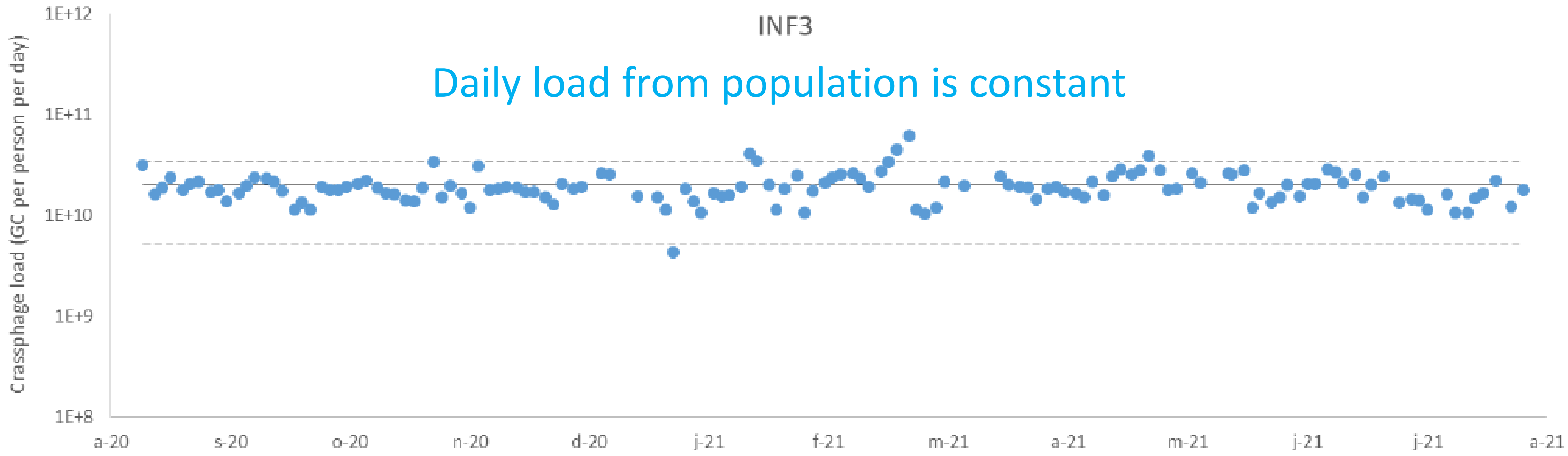
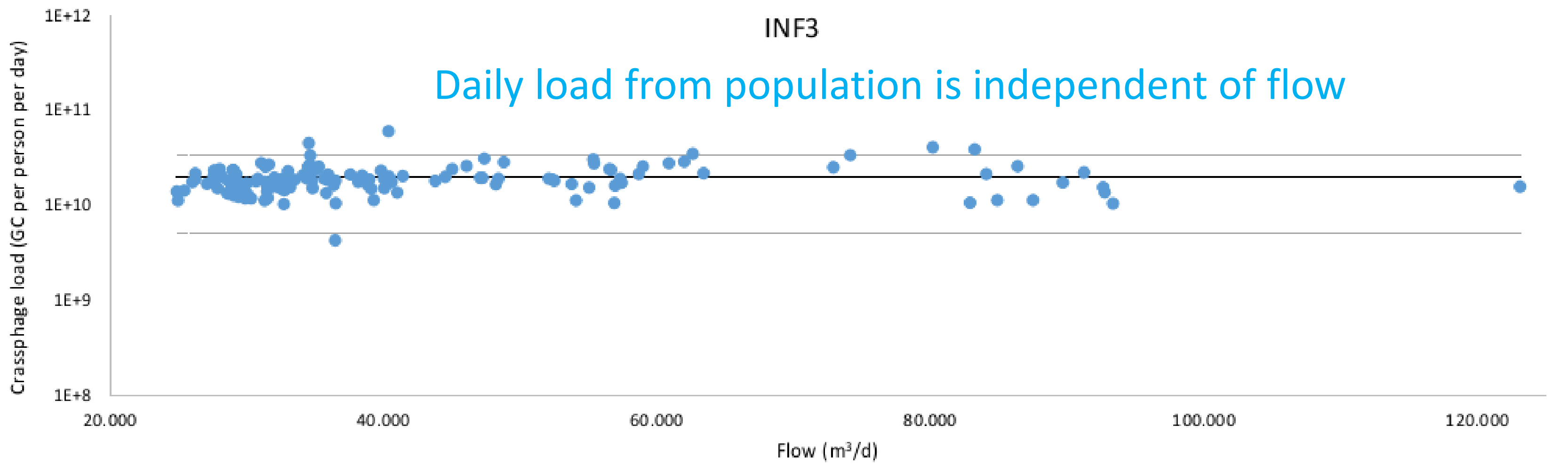
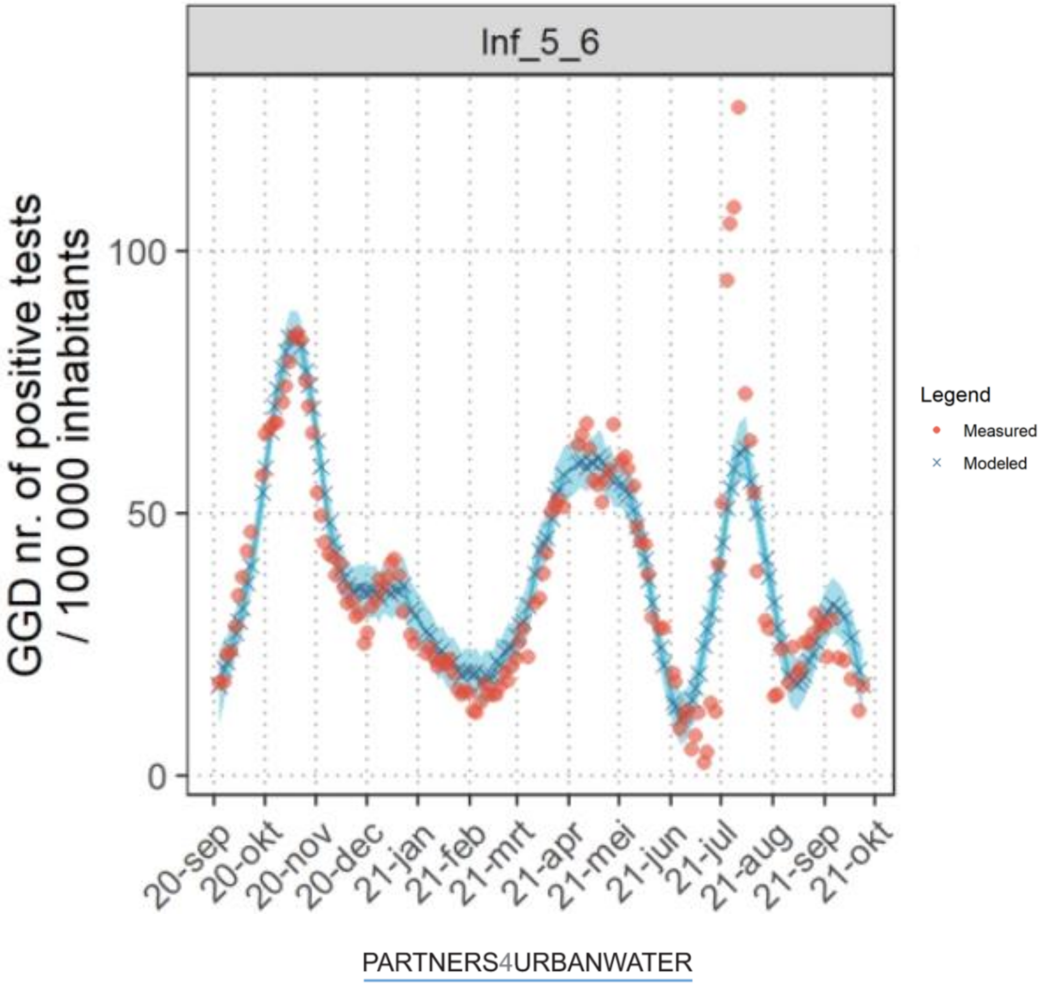




RELATION BETWEEN CONCENTRATION IN WASTEWATER AND NEWLY REPORTED CASES



- Normalisation of wastewater concentration wit flow (with EC)

  Langeveld et al, 2021

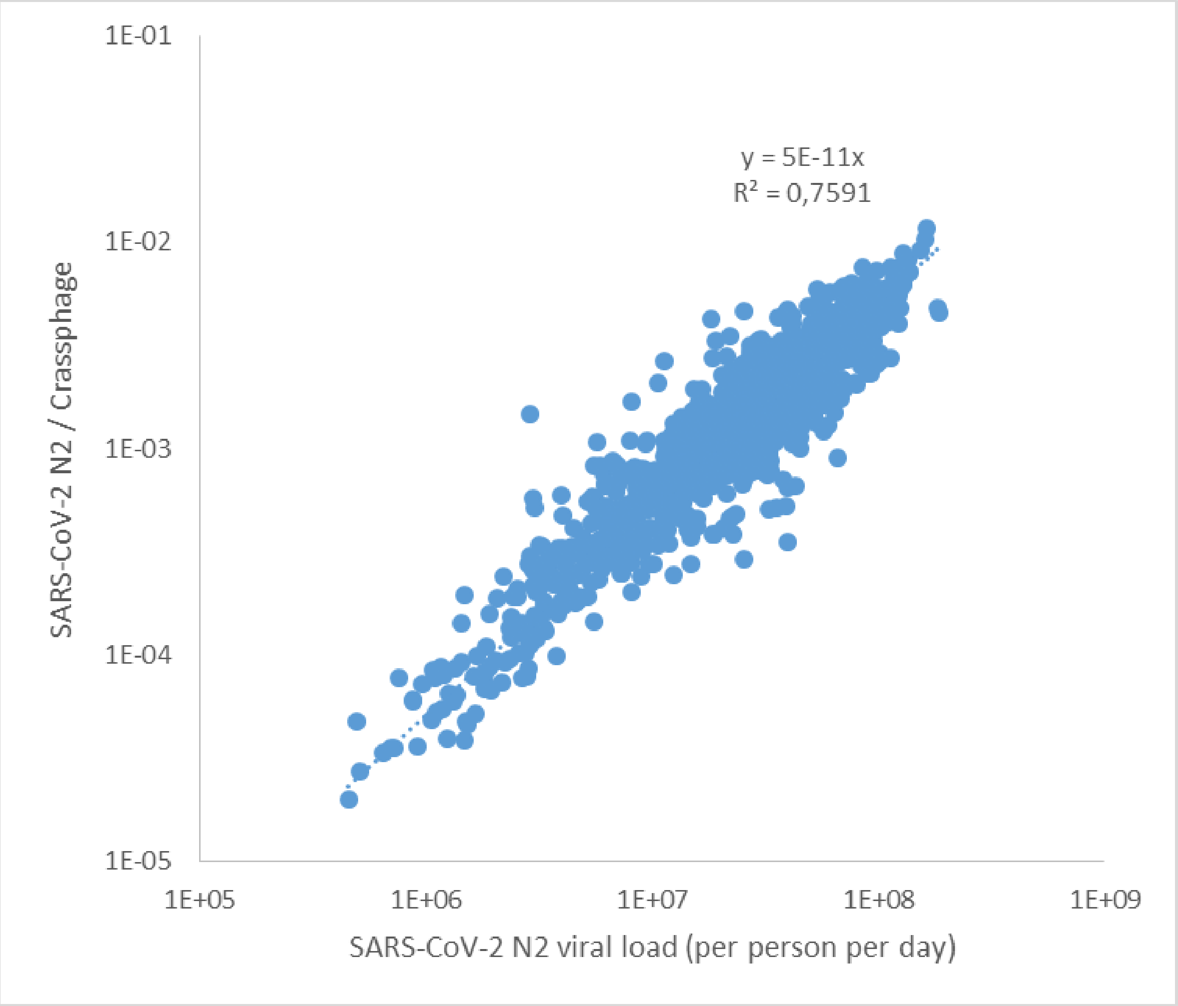
- Normalisation of newly reported cases
  For test delay (to symptom onset day)
  For test behaviour (#tests per 100,000)

  De Graaf et al, 2021

- Wastewater = newly reported cases
- Also with Alpha and Delta
- Also with vaccination
- Except for July 2021 (nightlife if tested)

CRASSPHAGE VS FLOW NORMALISATION

- Good correlation
- More variability
- Flow preferred normaliser
- No flow data?
  (passive samplers)
  → CrAssphage as normaliser



HIGHLIGHTS/LEARNING OBJECTIVES

- SARS-CoV-2 concentrations in wastewater need to be normalized:
  - to compare monitoring sites with different proportions of extraneous water in the sewer network
  - for reliable observation of short-term trends in COVID-19 circulation

- CrAssphage shedding is highly variable per person, but CrAssphage loads in wastewater of a population >5000 are very constant. Dominated by high-shedders, less suitable for small populations.

- Normalisation method:
  - Flow normalisation is preferred (with EC check)
  - CrAssphage normalisation is a suitable alternative in absence of flow data (passive samplers)

ACKNOWLEDGEMENTS

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Erasmus MC

Miranda de Graaf, Marion Koopmans, Evelien de Schepper, Izquierdo Lara, Claudia Schapendonk, Patrick Bindels, Johan van der Lei, Margreet Vos

RIJNMOND GEZOND DATA BASE

GGD Rotterdam-Rijnmond

Ewout Fanoy

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KWR

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PARTNERS4URBANWATER
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waterschap Hollandse Delta

Olaf Duin

Hoogheemraadschap van Delfland

Mariska Ronteltap

Hoogheemraadschap van Schieland en de Krimpenerwaard

Nick Ivens

waternet

Marco Dignum, Alex Veltman, Alice Fermont, Jan Peter van der Hoek

HOOGHEEMRAADSCHAP DE STICHTSE RIJNLANDEN

Mark Stevens

TOPSECTOR WATER & MARITIEM

Application of MST markers to normalize shedding rates in a campus monitoring program in Singapore

MATS LEIFELS
SCELSE, SINGAPORE

SCELSE
Singapore Centre for Environmental Life Sciences Engineering

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SOME FACTS ON SINGAPORE

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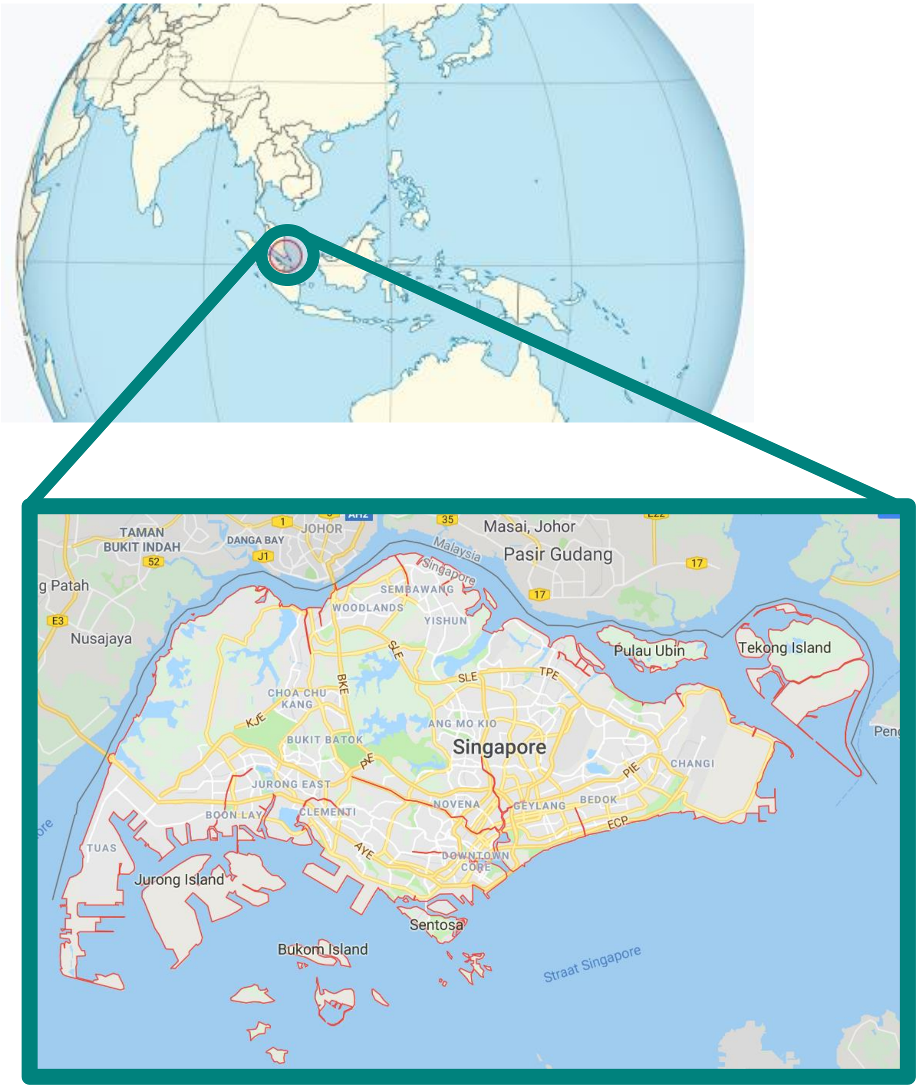

- **130** km^2 (23% increase due to land reclamation since 1950s)
- **5,703,600** inhabitants in 2019 (**second** highest **population density** with **7,804** per km^2)
- **4 Wastewater Reclamation Plants (WRP)**
- **Water supply** relies on ***four national taps***:
    1. **Imported** water from **Malaysia**
    2. Urban **rainwater** catchments
    3. (**reclaimed**) NEWater
    4. Desalinated seawater

SEWAGE MONITORING AT NTU

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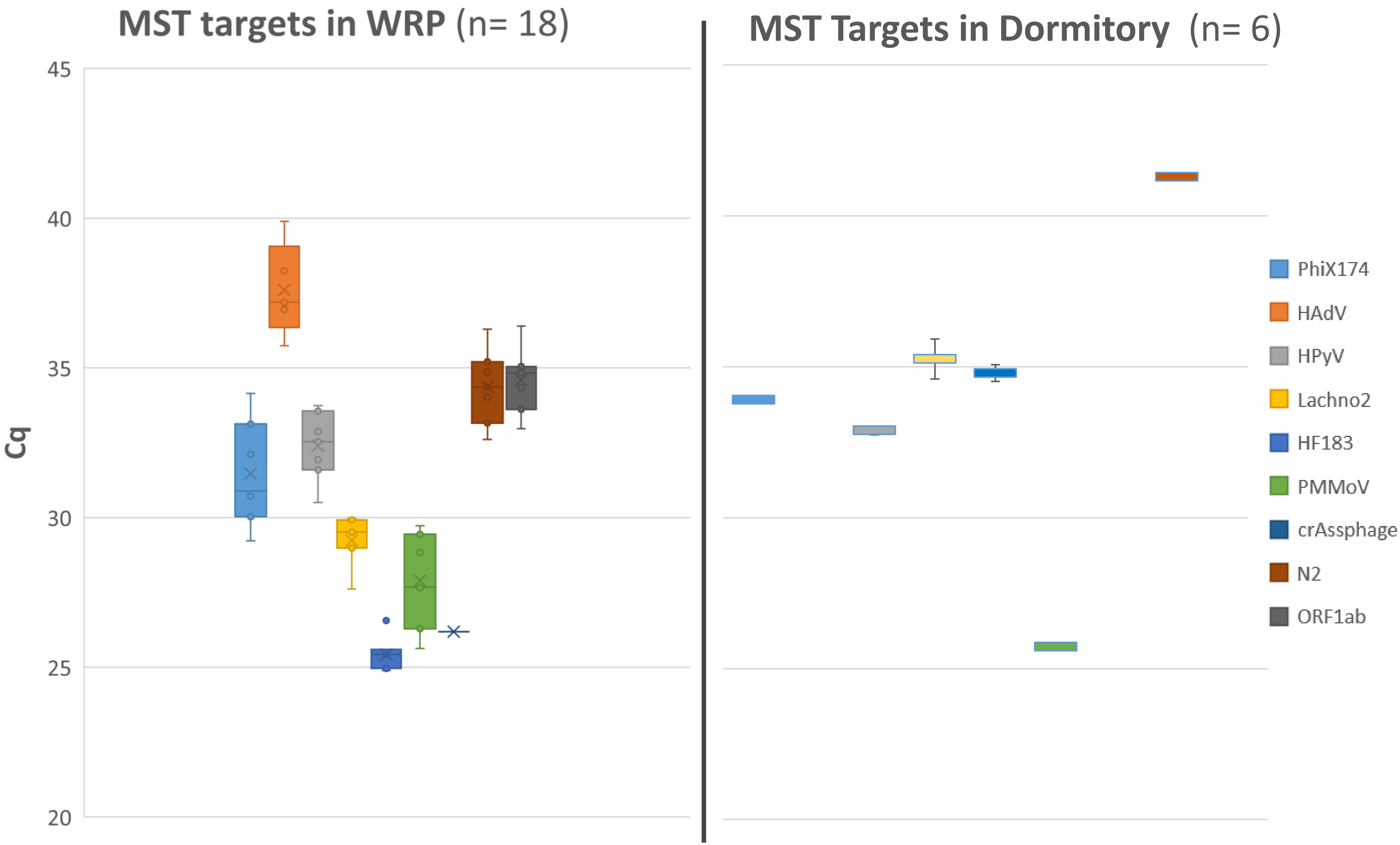
Campus Monitoring:

- **45 autosampler** on campus (**downstream** of student **halls**)
- **Biweekly** sampling (3 x 8 h **composites**)
- Sample **concentration** via **PEG** precipitation or **Amicon** Filtration

Molecular Assays:

SARS-CoV-2:	N1 / N2 gene (Halls) ORF1ab gene (WRP)
Process control:	PMMoV
Bacteriophage:	crAssphage PhiX 174
Enteric Virus:	HAdV HPyV
General Marker:	Lachno2 HF183 (BacR287)

PRELIMINARY RESULTS



NEXT STEPS:

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- **Validate** the **quantitative** Assays (once Genblocks arrive…)
- **Longitudinal** analysis of **fecal indicator concentration** in representative **WRP** / **Hall**
- **Microcosm** to determine the **decay rate** of fecal **indicators** at **4° - 30°C** within 30 - 60 days
- **Correlate occurrence** of fecal **indicators** with **presence** / **absence** of **SARS-CoV-2** in **Hall** and **WRP** (retrospectively for **Pandemic** and **Endemic** conditions)
- **Normalize** wastewater **volume** to **inhabitant** in Halls
    - during **term** period and **vacation**
    - during **dry** and **rainy season**

ACKNOWLEDGMENTS

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Dr.
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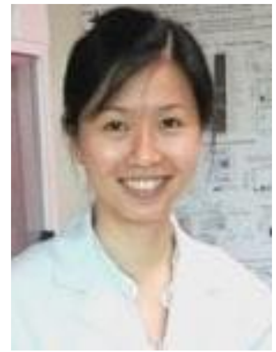
Dr.
Cheng Dan

Ms.
Kim Se Yeon (Sera)

SMART
Singapore-MIT Alliance for Research and Technology



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Xiaoqiong Gu

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Federica Armas

Mr.
Francis Chandra

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NANYANG TECHNOLOGICAL UNIVERSITY SINGAPORE

NUS National University of Singapore

Ministry of Education
SINGAPORE

PRIME MINISTER'S OFFICE
SINGAPORE

National Environment Agency
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Estimating relative abundance of two SARS-CoV-2 variants in wastewater settled solids

MARLENE WOLFE
EMORY UNIVERSITY, USA

EMORY UNIVERSITY
Stanford University
UNIVERSITY OF MICHIGAN

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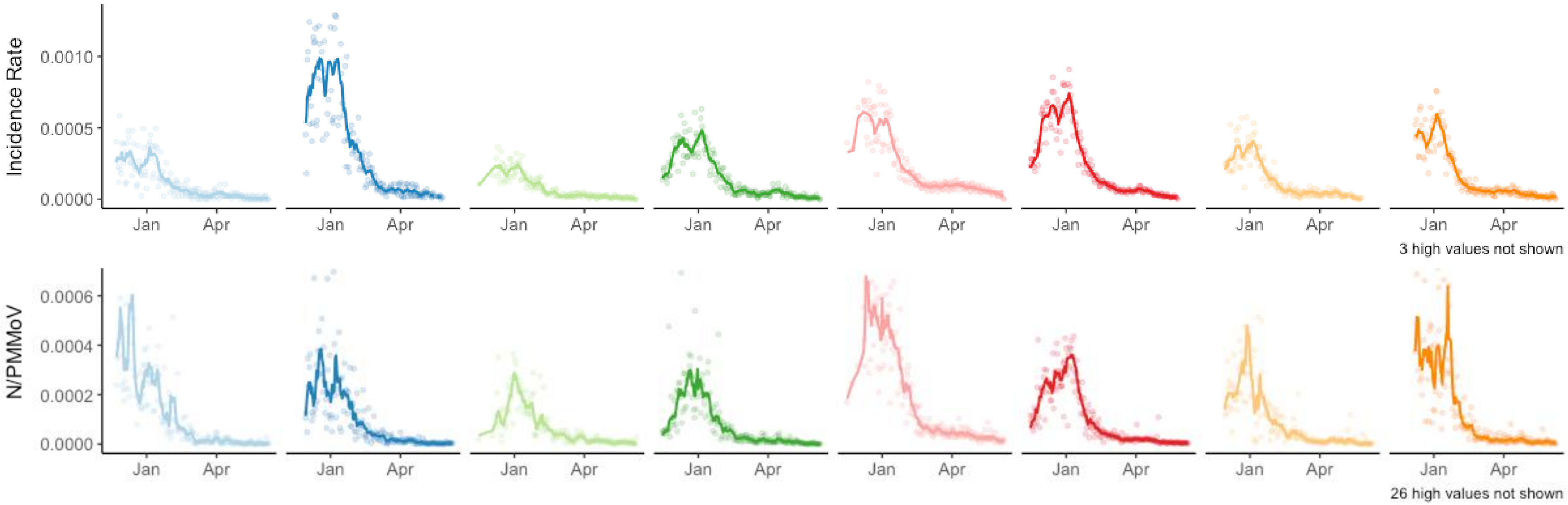
HIGH THROUGHPUT ANALYSIS OF WASTEWATER SOLIDS



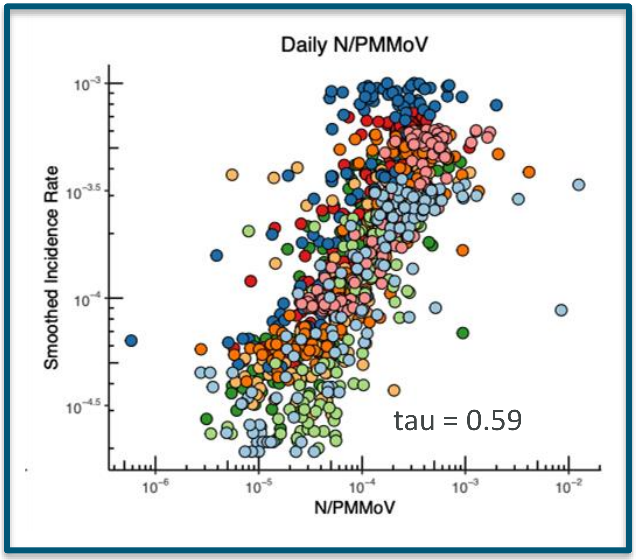
- Direct extraction of RNA from wastewater **solids** and analysis by RT-ddPCR
- **Significant, strong association** between wastewater and incidence rates
- Average estimated minimum incidence rate: **1.4 cases / 100,000 people**

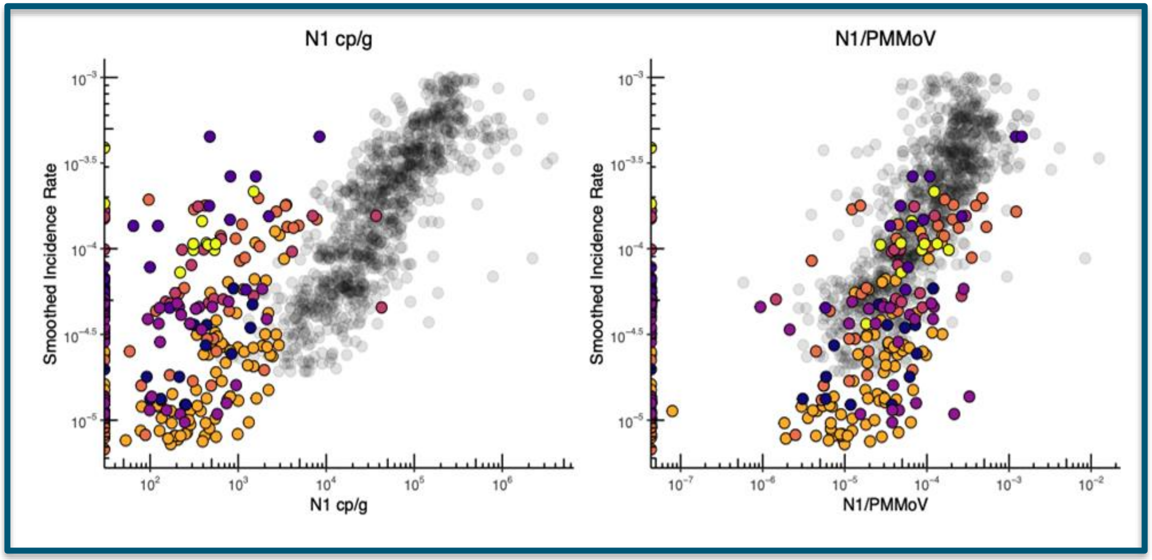
Wolfe, et al (mSystems, 2021)

STRONG ASSOCIATION WITH CLINICAL DATA COMPARABLE WHEN NORMALIZED BY PMMOV

High-throughput data

Other solids data (over high-throughput data in grey)



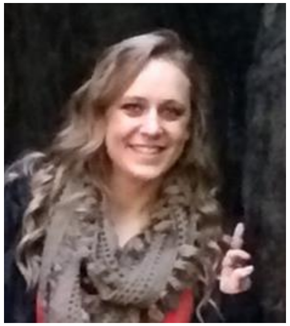


- **Different methods** used for two datasets – both utilizing wastewater solids
- Normalizing by PMMoV allows for **comparison of incidence rate** across plants from wastewater data

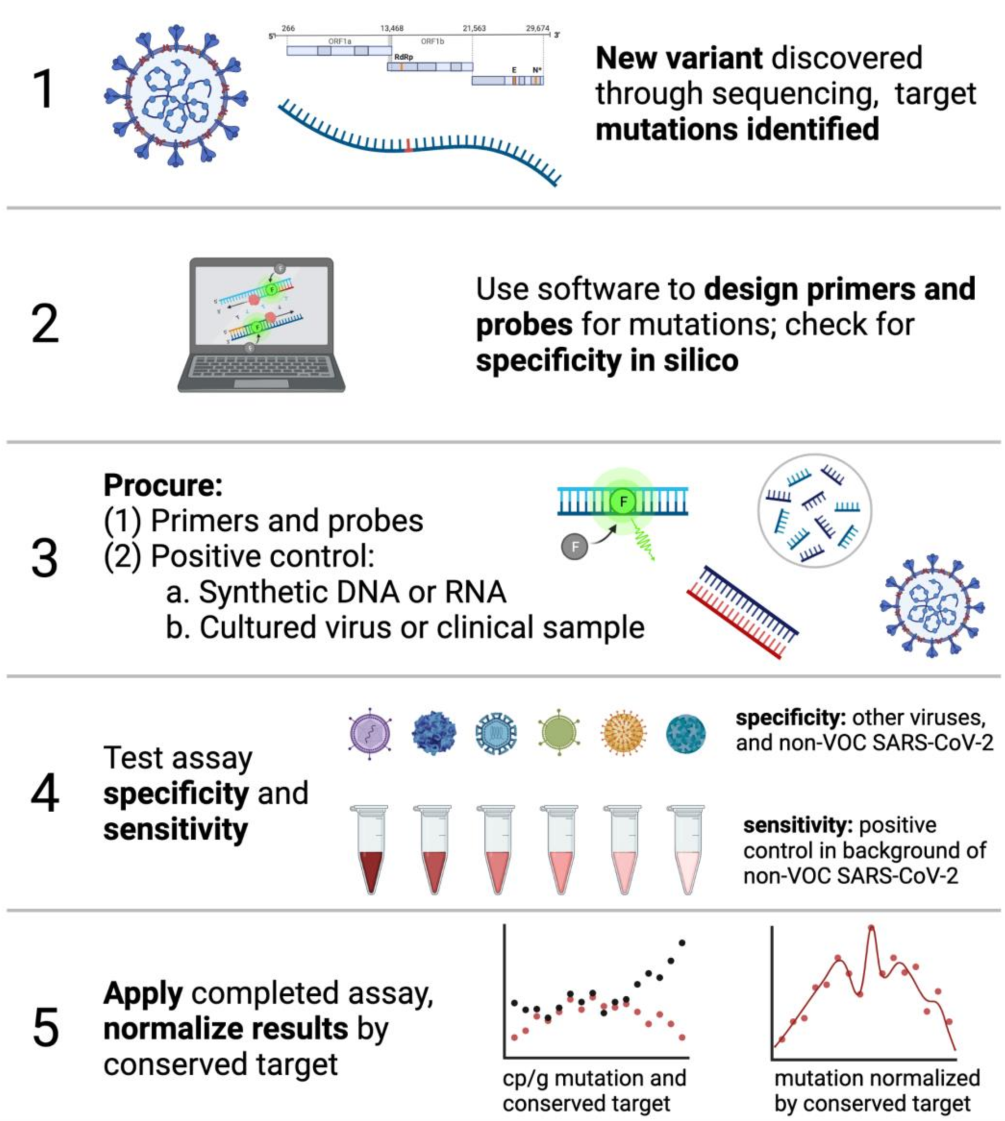
WASTEWATER TARGETED MUTATION ASSAYS

- Less expensive, quick turnaround
- Estimates for low abundance variants (< 5%)
- Assay design and testing required

Yu & Hughes, et al (preprint, 2021)

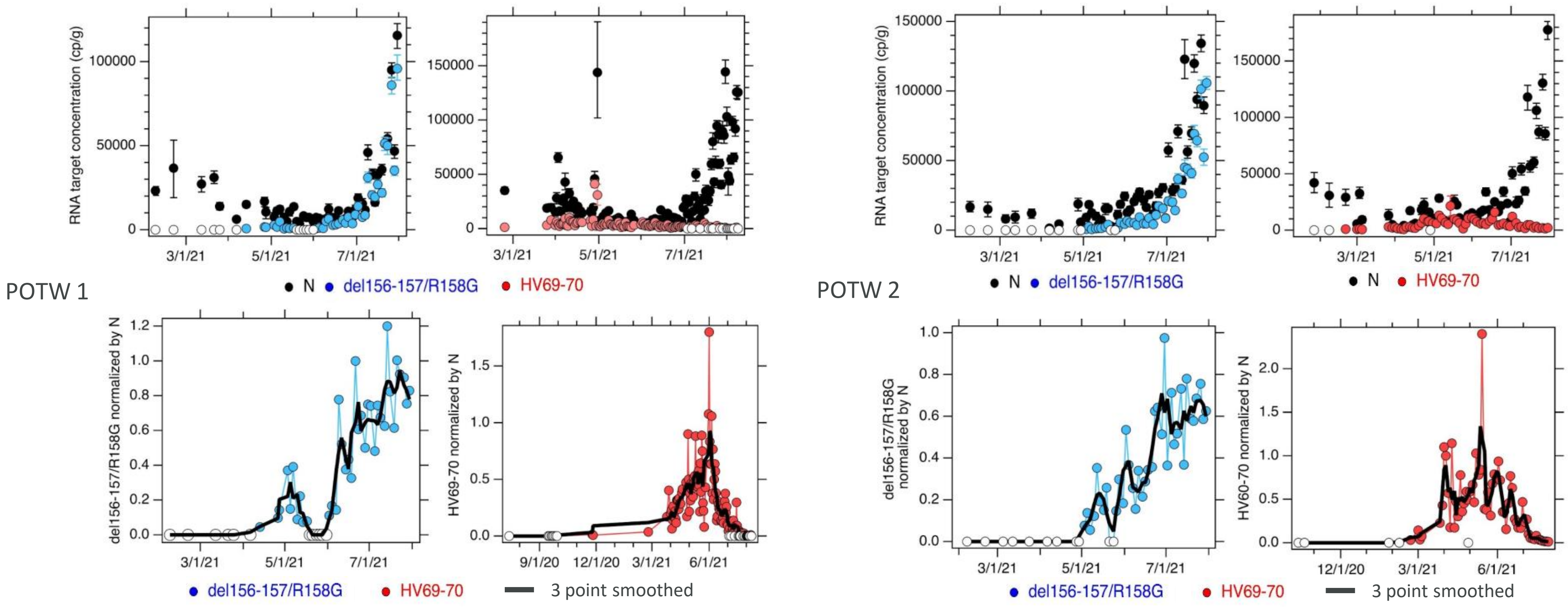


Bridgette Hughes

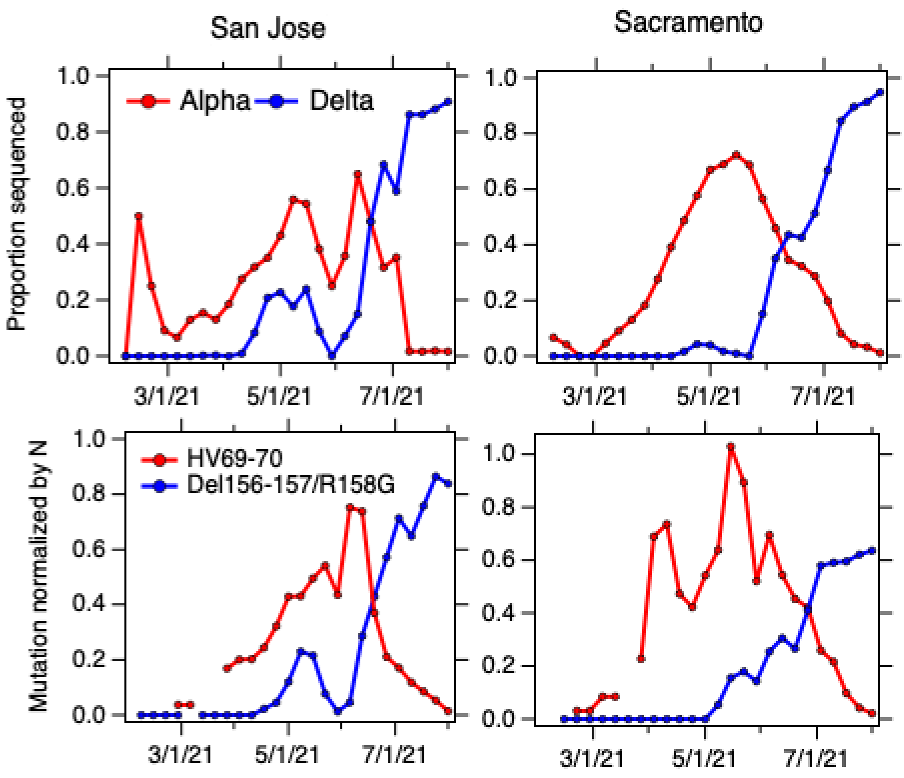


MONITORING SHOWS APPEARANCE AND DISAPPEARANCE OF MULTIPLE VARIANTS

- Assays specific and sensitive for mutations indicating Alpha, Beta, Gamma, Delta, and Mu
- Alpha and Delta mutations detected at high frequencies



VARIANT ESTIMATES ARE HIGHLY ASSOCIATED WITH CLINICAL SEQUENCING ESTIMATES



- Mutation gene ratios from wastewater were **strongly correlated** to the corresponding estimates of case isolate sequencing
  - r_p= 0.82 (SJ) and 0.88 (SAC) for Alpha
  - r_p= 0.97 (both SJ and SAC) for Delta
- Wastewater monitoring can provide **early indication** of community changes in variant circulation

ACKNOWLEDGMENTS

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and more

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Q&A Discussion

MODERATOR:

KWANRAWEE JOY SIRIKANCHANA (LEAD)

ANICET R. BLANCH (ASSIST)

Indicators and MST* marker to support bacterial targeted WBE**

Moderators:
Anicet R. Blanch (lead)
Kwanrawee Joy Sirikanchana (assist)

* Microbial Source Tracking

** Waste Water Based Epidemiology

ANTIBIOTICS AND RESISTANCE GENES AS INDICATORS FOR MULTIDRUG RESISTANT BACTERIA?

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ihph
UNIVERSITÄT BONN

IWA the international water association
HEALTH-RELATED WATER MICROBIOLOGY

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BACKGROUND OF THIS STUDY

- Part of the collaborative research project “HyReKA”:

  Biological and hygienic-medical relevance and control of antibiotic-resistant pathogens in clinical, agricultural and municipal wastewater and their relevance in raw water

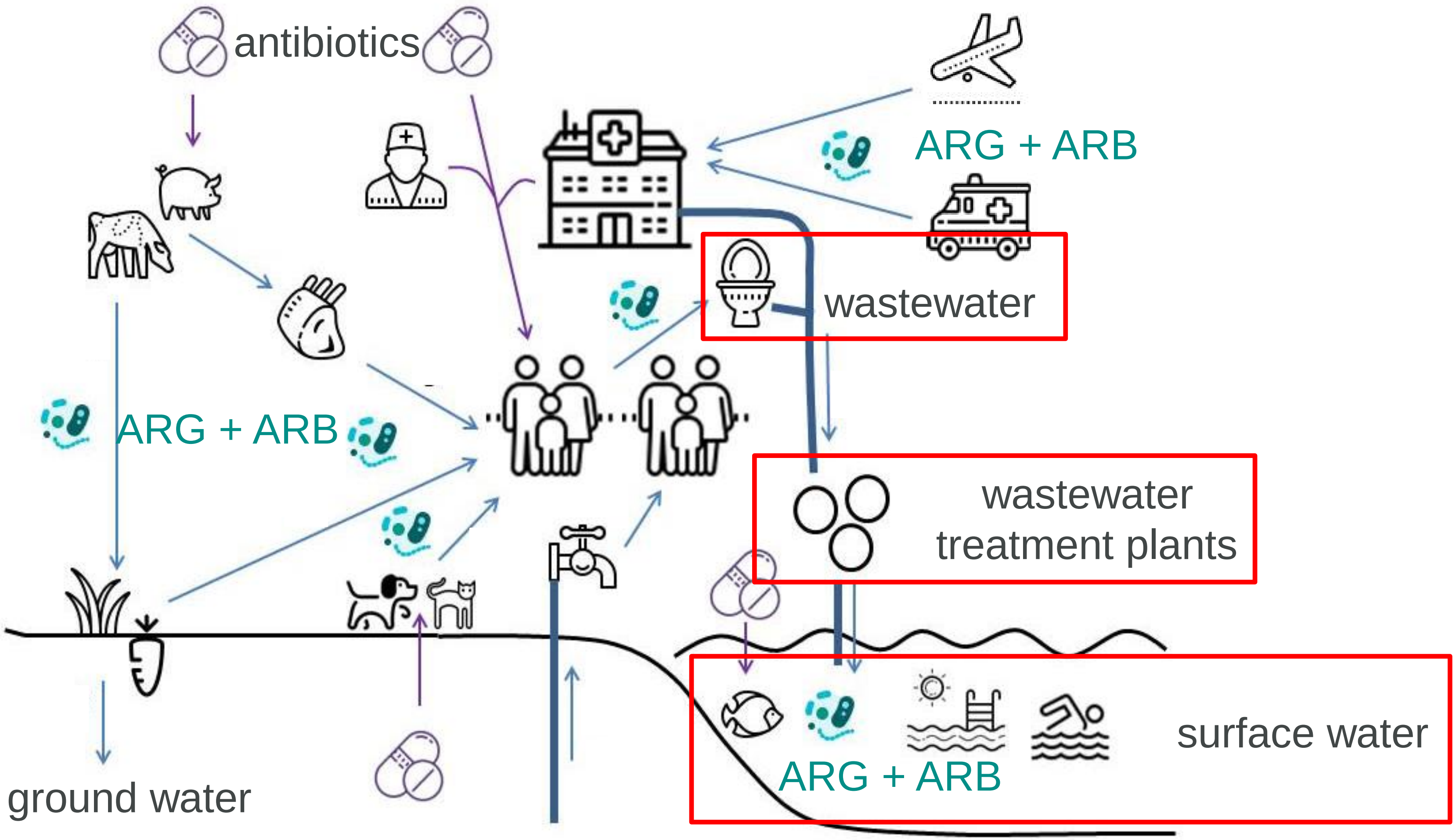
  - Funded by the Federal Ministry of Education and Research of Germany, (FKZ 02WRS1377)

Main research question of this study (Voigt, Zacharias et al., 2020)

- Are there any positive associations between the occurrence of antibiotics, antibiotic resistance genes and antibiotic-resistant bacteria in wastewater?

SPREAD OF RESISTANCES

→ Antibiotic resistant genes (ARG) and resistant bacteria (ARB)



METHODS

municipal wastewater

clinically influenced wastewater

Sample origin

Samples

Cultural detection of ARB

ESBL-producing
- *Klebsiella*
- *Enterobacter*
- *Citrobacter*
- *Pseudomonas aeruginosa*

Klebsiella, *Enterobacter*, *Citrobacter* } KEC

Target organisms

Sample processing

- Culture + MALDI TOF
- qPCR
- LC+ Mass Spectometry

Sample processing

- ARB
- ARG
- AB

Concentrations

explorative multivariate data analysis

→ Relations (Odds ratio)

Statistical analysis

RESULTS: ODDS RATIO + RELATIONS

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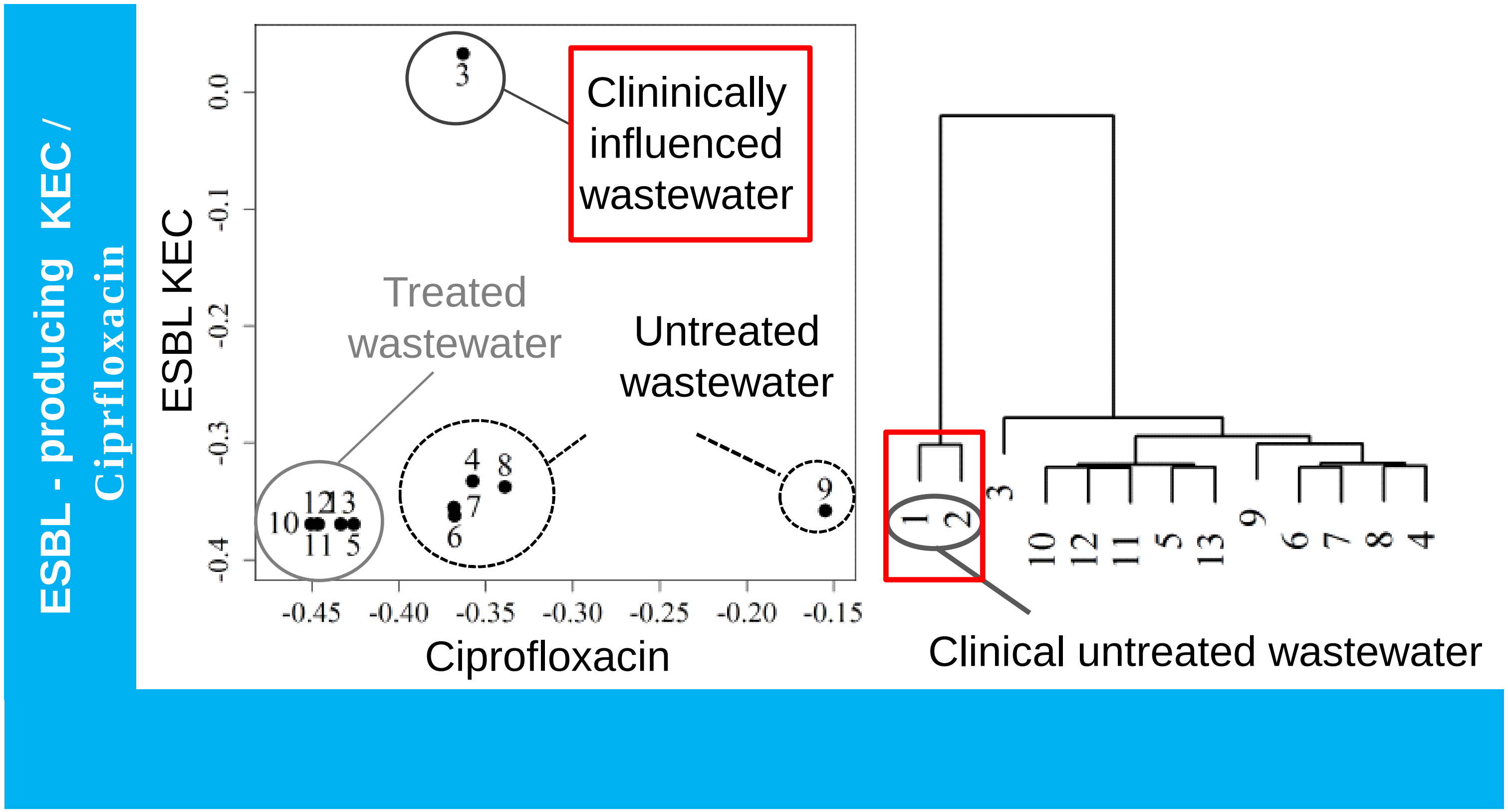
RELATION OF THE ABUNDANCES OF ANTIBIOTICS + RESISTANT BACTERIA

Resistant Bacteria	Antibiotics	n	Odds ratio	KI	p-value	Clinical Plausibility
ESBL KEC	Erythromycin	178	3.62	1.2/10.7	0.020	no
	Ciprofloxacin	**186**	**4.29**	**1.6/11.2**	**0.003**	**yes**
	Amoxicillin	202	4.67	1.1/20.4	0.041	no
	SMX.N4.Acetyl	152	12.75	2.8/58.1	0.001	no
***P. aeruginosa* 3GCR**	Vancomycin	202	2.53	1.3/5.0	0.008	no
	Ciprofloxacin	**186**	**2.75**	**1.1/7.0**	**0.034**	**yes**
	Metronidazol	178	3.17	1.5/6.7	0.003	no
	Ceftazidim	**202**	**3.20**	**1.5/6.7**	**0.002**	**yes**
	Moxifloxacin	178	3.91	1.8/8.5	0.001	no
	Meropenem	**202**	**5.00**	**2.3/10.8**	**0.000**	**yes**
	Linezolid	178	5.05	2.2/11.6	0.000	no
	Ampicillin	202	9.41	3.5/25.5	0.000	no
	Flucloxacillin	202	20.79	2.4/183.2	0.006	no
***Pseudomonas* spp. 3GCR**	Ampicillin	202	3.35	1.1/10.4	0.037	no

RESULTS: ODDS RATIO + RELATIONS

RELATION OF ABUNDANCES OF CIPROFLOXACIN + RESISTANT BACTERIA



Voigt, Zacharias et al., 2020

RESULTS: ODDS RATIO + RELATIONS

Research question of this study:

- Are there any associations between the occurrence of antibiotics, antibiotic resistance genes and antibtiotic-resistant bacteria in wastewater?

- positive associations between antibiotics and resistant bacteria
- Ciprofloxacin → good indicator of ESBL-producing bacteria
- Positive relationship of meropenem to carbapenemase genes
- Clinical wastewater differs to municipal wastewater:

  P. aeruginosa, resistant against 3rd gen. cephalosporins

  → mainly in clinical wastewater.

High persistence of traditional and molecular fecal indicators support proportional auto-sampling of sewage

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ICC Water & Health
www.waterandhealth.at

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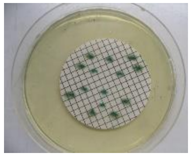
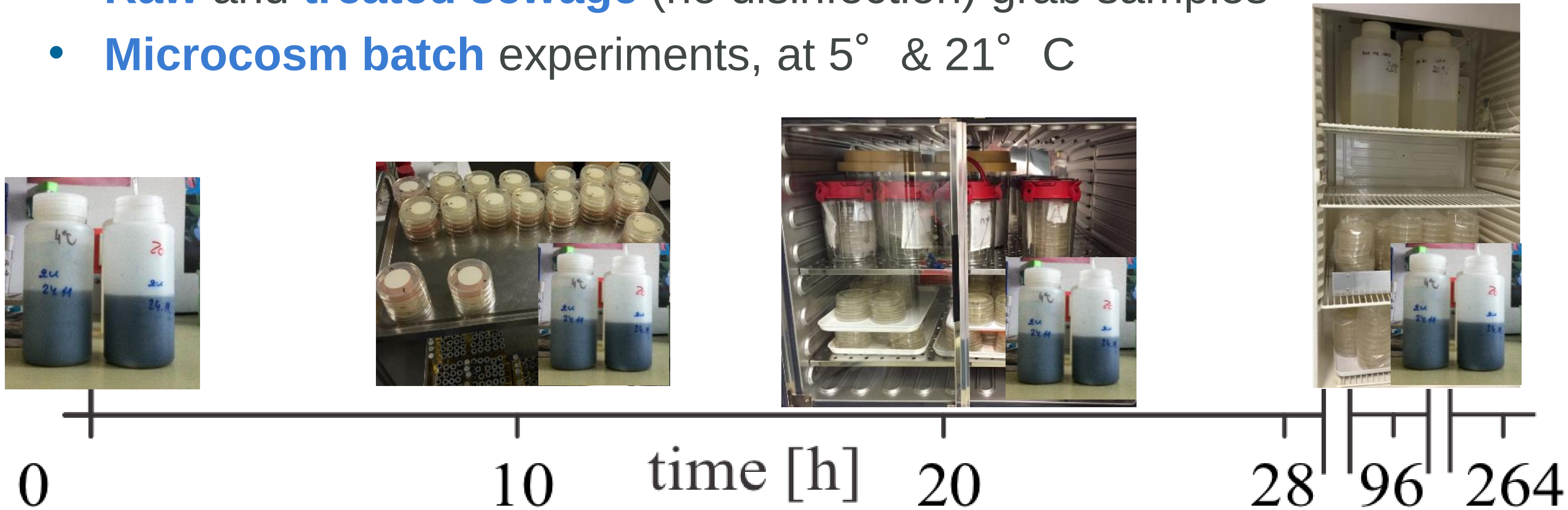
AIM & METHODS

Does persistence of traditional and molecular bacterial fecal indicators in sewage support automated sampling?

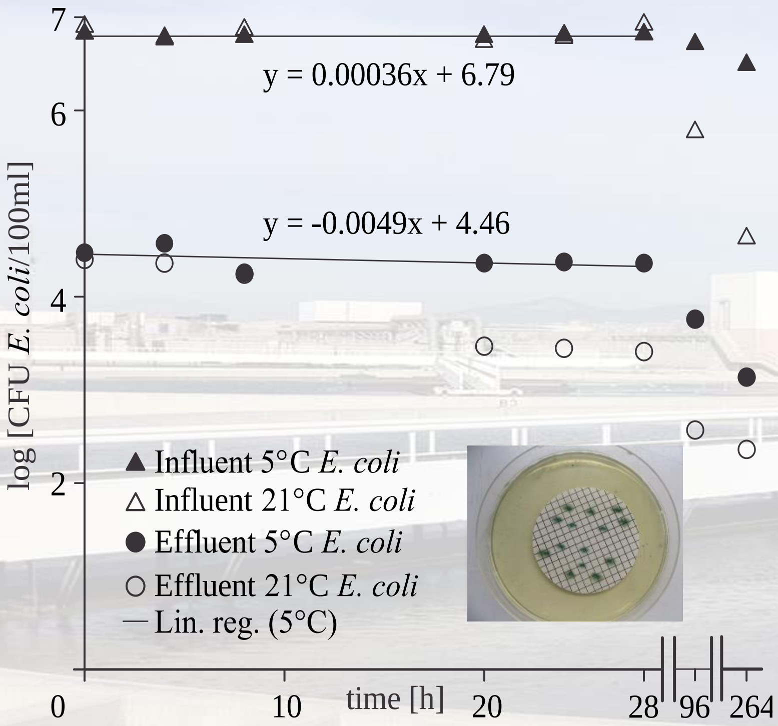
Study design:



- ***Escherichia coli*** - vegetative (cultivation, ISO 16649-1)
- ***Clostridium perfringens*** - spores (cultivation, ISO 14189)
- **BacHum** (Kildare et al.), **HF183** (Haugland et al.)
  - human MST genetic target (qPCR)
- **3 WWTPs** (20.000-140.000 P.E.), covering seasonal differences
- **Raw** and **treated sewage** (no disinfection) grab samples
- **Microcosm batch** experiments, at 5° & 21° C



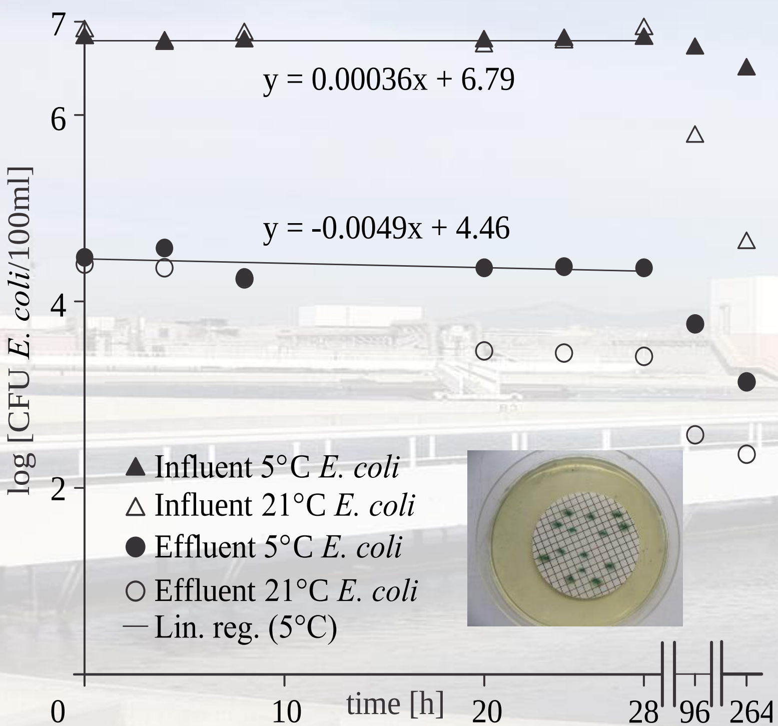
BATCH-CULTURE TIME SERIES
E.coli &

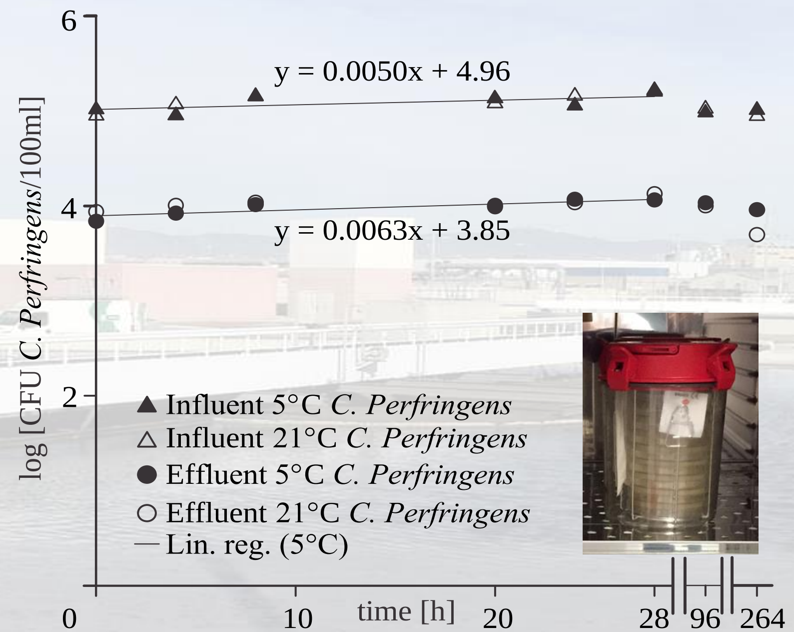


→ **No signifcant concentration change for at least 36 hours at 5°C influent/effluent.**

Automated Sampling Procedures Supported by High Persistence of Bacterial Fecal Indicators and Bacteroidetes Genetic Microbial Source Tracking Markers in Municipal Wastewater during Short-Term Storage at 5°C (2017) Mayer RE, Vierheilig J, Egle L, Reischer GH, Saracevic E, Mach RL, Kirschner AKT, Zessner M, Sommer R, and Farnleitner AH. Appl & Environ Microbiol **81** (15): 5134-5143

BATCH-CULTURE TIME SERIES
E.coli & *C.perfringens*





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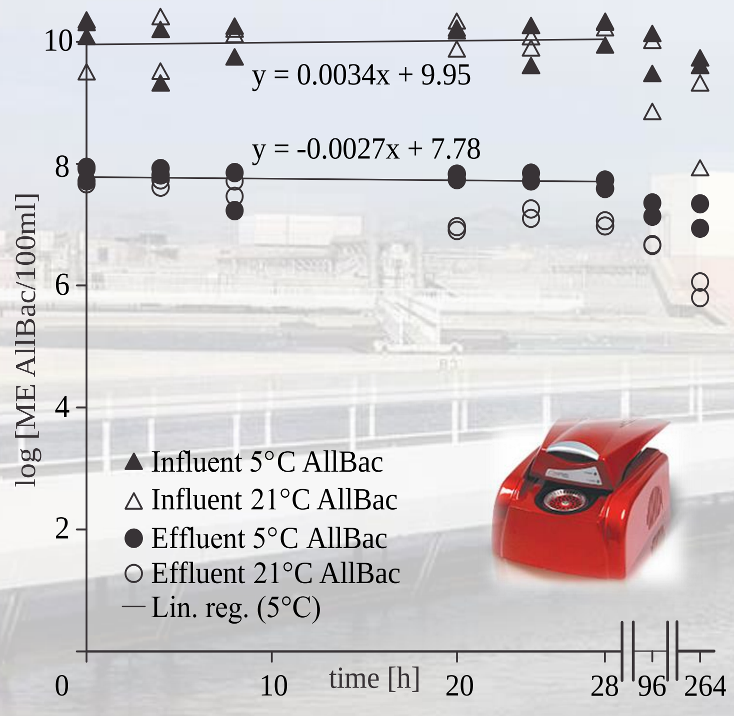
BATCH-CULTURE TIME SERIES:
Allbac & (genetic marker)



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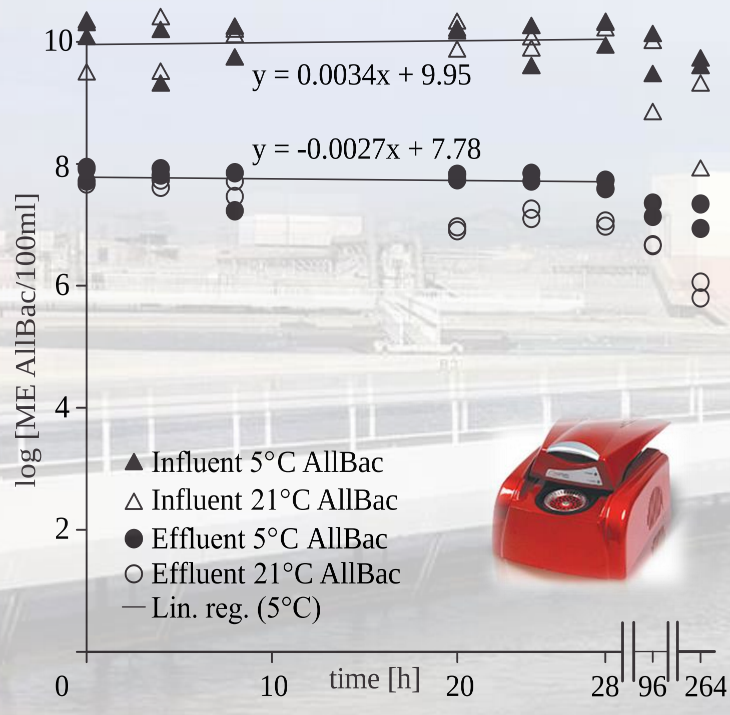

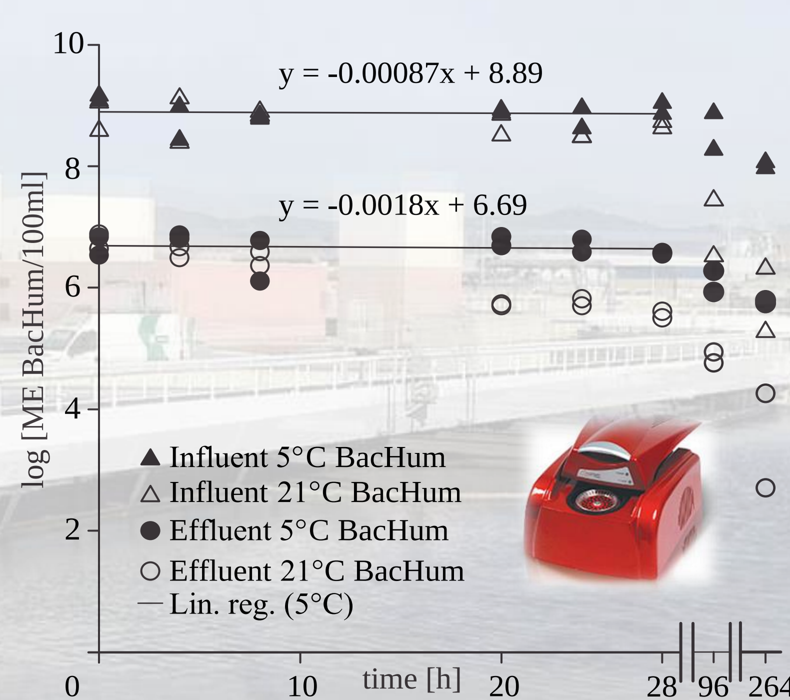

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Automated sampling: elucidating human specific indicator loads per day and connected inhabitant in raw and treated sewage

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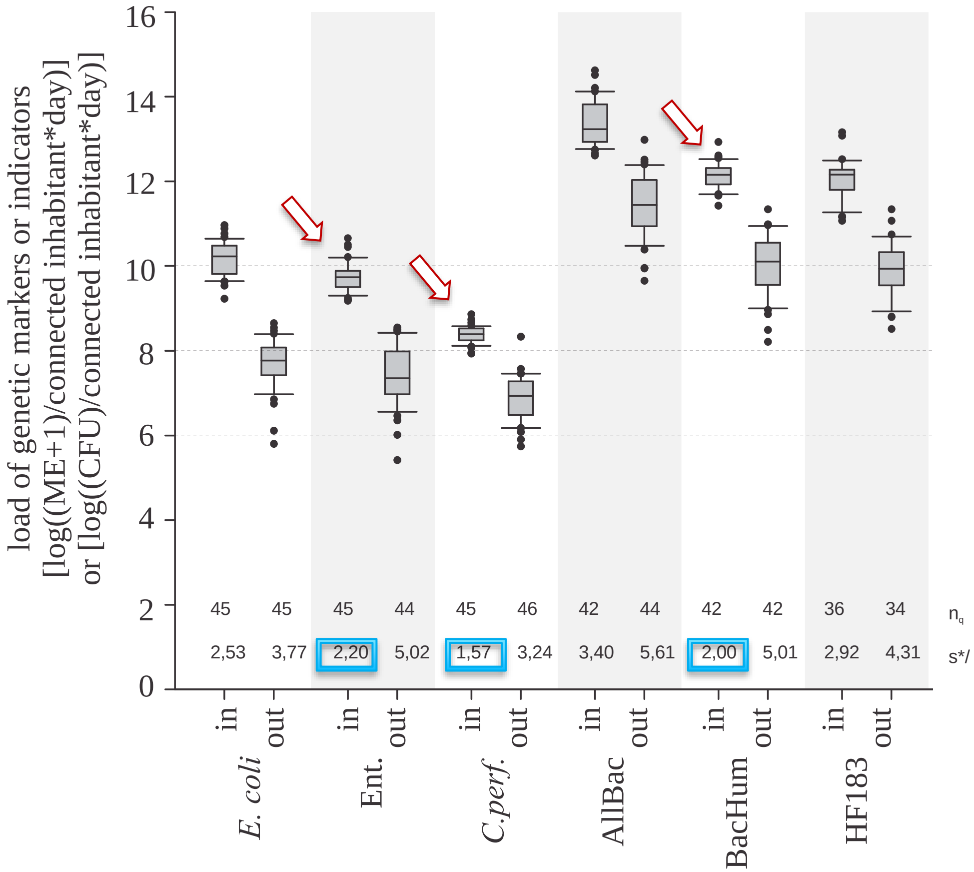
5°C, flow proportional 24 hour composite samples



Occurrence of human-associated *Bacteroidetes* genetic source tracking marker in raw and treated wastewater of municipal and domestic origin and comparison to standard and alternative indicators of faecal pollution (2018) Mayer RE, Bofill-Mas S, Egle L, Reischer GH, Schade, M., Fernandez-Cassi X, Mach RL, Kirschner, A, Brunner, K, Gaisbauer M, Piringer H, Blaschke A. P, Girones R, Zessner M, Sommer R and Farnleitner AH **Water Research**, **90:2**65-276

CONCLUSION

In contrast to expectations:

- Cultivation-based fecal indicator bacteria → high persistence
- Genetic bacterial MST marker → high persistence 36h 5°C
- Support flow-proportional auto-sampling

Support of:

- WWTP performance characteristics (e.g. log-red.)
- Health-related water quality investigation
- Waste water based epidemiology

REFERENCES

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Automated Sampling Procedures Supported by High Persistence of Bacterial Fecal Indicators and Bacteroidetes Genetic Microbial Source Tracking Markers in Municipal Wastewater during Short-Term Storage at 5°C
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All Operators of investigated WWTPs

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Q&A Discussion

MODERATOR:

ANICET R. BLANCH (LEAD)

KWANRAWEE JOY SIRIKANCHANA (ASSIST)

the international water association

The broader view: further challenges & perspectives

Moderators:
Andreas H. Farnleiter (lead)
Joan B. Rose (assist)

HRWM MINI-SYMPOSIUM - FINAL CLOSURE

Closure of the 1st HRWM - IWA Mini Symposium

Traditional and Molecular Indicators to Support Wastewater Based Epidemiology

Jointly organized by GWPP

Moderators:
Joan B. Rose (lead)
Andreas H. Farnleiter (assist)

HRWM WEBINAR MINI-SYMPOSIA
further planned topics

the international water association

- **Traditional and Molecular Indicators to Support Wastewater Based Epidemiology** (2021)
- **Water Reuse & Risk Assessment**
  → joint with SG Water Reuse (2022)
- **Disaster management, preparedness & WASH**
  → other SG´s to be involved (2022)
- **Recreational water quality**
  → related to the recently launched WHO guideline (2022)

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- **IWA support** (Rachna Sarkari & Samuela Guida)
- Andreas H. Farnleitner, Joan B. Rose, Anicet R. Blanch, and Joy Kwanrawee Sirikanchana) for the suggested topic and the organising the program of our first webinar-mini symposium and serving as moderators & mini symposium committee
- Orin Shanks for delivering the keynote
- The speakers and colleagues submitting contributions

LOOKING FORWARD TO OUR NEXT WEBINAR MINI SYMPOSIUM and YOUR CONTRIBUTIONS

Please check out our SG HRWM Website: https://hrwm-watermicro.com/

UPCOMING IWA WEBINAR

https://iwa-network.org/learn/intensifying-biological-treatment-through-selection-processes/

Learn more at

http://www.iwa-network.org/iwa-learn/