A TARGET PRODUCT PROFILE FOR WASTEWATER SURVEILLANCE OF *S.* TYPHI

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INTERVENTION: ENVIRONMENTAL SURVEILLANCE OF *S.* TYPHI

• What is the problem we are trying to solve? • What is the change we're seeking to affect?	Why do we need this intervention and how is it differentiated from current practice / behaviors?	Who and what does intervention require for successful use?	What are the anticipated barriers to success?
Lack of data of typhoid burden data in many countries hinders government decision making on TCV introduction. **Seeking to motivate introduction of TCV** in countries without blood culture surveillance.	**We need this intervention to develop affordable methods to generate evidence** regarding ongoing typhoid transmission. **Current practice is blood culture surveillance**, which is time- and resource-intensive.	The intervention requires **knowledge of the correlation** between a given quantity of *S.* Typhi in the environment and the burden of typhoid in a community. Presence of or planning for **Polio ES may increase willingness** to undertake sewage sampling and testing for *S.* Typhi.	Lack of infrastructure to conduct sewage surveillance Lack of transportation (cold chain) Lack of data and reporting systems to transmit data and make decisions **Unwillingness to adopt TCV in the absence of blood culture surveillance**

TARGET PRODUCT PROFILE INFORMED R&D FOR TYPHOID ES

Intended Use Case Scenario

1) **In countries considering national TCV introduction:** decision makers use ES in one or more location, possibly big cities, to understand whether ***S.* Typhi is circulating in the population**. Based on predetermined criteria, they decide whether TCV should be introduced or not.
2) **In countries considering phased introduction**, decision makers may use ES to understand whether *S.* Typhi is circulating sub-nationally. Such knowledge may allow them to **equitably** distribute TCV, targeting the vaccine to high burden areas first.

A TEST THAT IS SPECIFIC FOR *S.* TYPHI FROM SEWAGE OR HUMAN FECALLY CONTAMINATED SURFACE WATER

Variable	Minimum	Optimistic
What type of specimens will be tested?	Sewage. Also, water with high levels of human fecal contamination: i.e. contaminated surface water.	Same.
Specificity/cross reactivity	Must be specific to *S.* Typhi; must not cross-react with nucleic acid from other organisms	Must be specific to *S.* Typhi and *S.* Paratyphi A organisms; must not cross-react with nucleic acid from other organisms

Minimum should be considered as a potential go/no go decision point; Optimistic should reflect what is needed to achieve broader, deeper, quicker global health impact. https://gatesopenresearch.org/documents/7-73

STUDY LOCATIONS AND TYPHOID INCIDENCE RATES

- Compare prevalence of *S.* Typhi in ES samples over 12 months with incidence in hospital-based BC-based surveillance studies
- Sites with a variety of typhoid incidence rates chosen
  - very high (≥500/100,000)
  - high (>100 - <500/100,000)
  - medium (10 - 100/100,000)
- Serosurveys used to triangulate data during COVID-19 pandemic

Incidence Rate

Site, Country	Incidence rate in <15y, based on BC*	Context
Vellore, India	2000/100,000	**Very high** incidence. SEFI study estimate
Blantyre, Malawi	700/100,000	**High** incidence site. STRATAA study estimate
Agogo, Ghana	255/100,000	**High** incidence site. SETA study estimate
Ibadan, Nigeria	139/100,000	**High** incidence site. SETA study estimate
Northern Division, Fiji	55/100,000**	**Medium** incidence site, Ty-FIVE estimate

*Corrected for BC sensitivity, probability of getting a BC, and healthcare seeking
**Crude incidence in all ages

Posters 37, 55, 71, 120

IN SAMPLES TESTING POSITIVE FOR *S.* TYPHI, ABILITY TO DETECT AMR MARKERS ON THE GENOME COULD BE INFORMATIVE

- To inform appropriate antibiotic treatment based on antibiotic resistance signatures of *S.* Typhi from sewage
- Situational awareness regarding prevalence of macrolide resistance in *S.* Typhi, especially in regions where Azithromycin is the first-line antibiotic for treating typhoid fever

Wastewater surveillance of *S.* Typhi in India and comparison with clinical/serological surveillance

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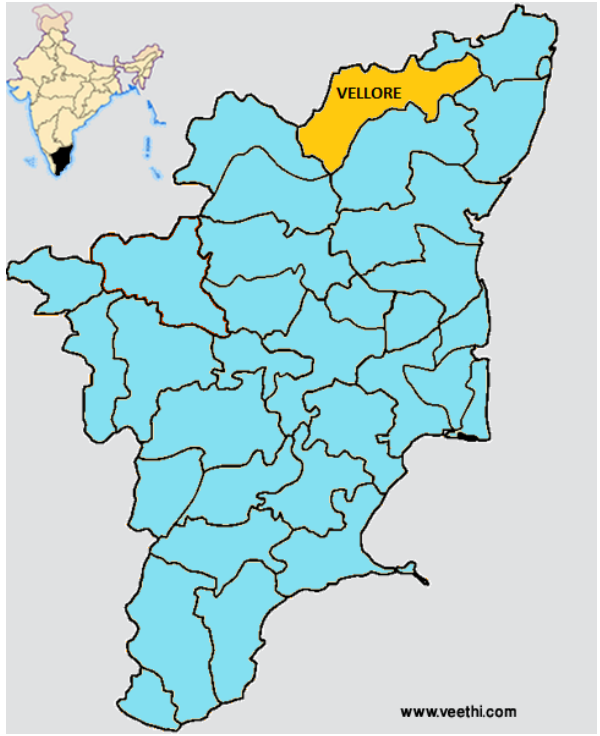
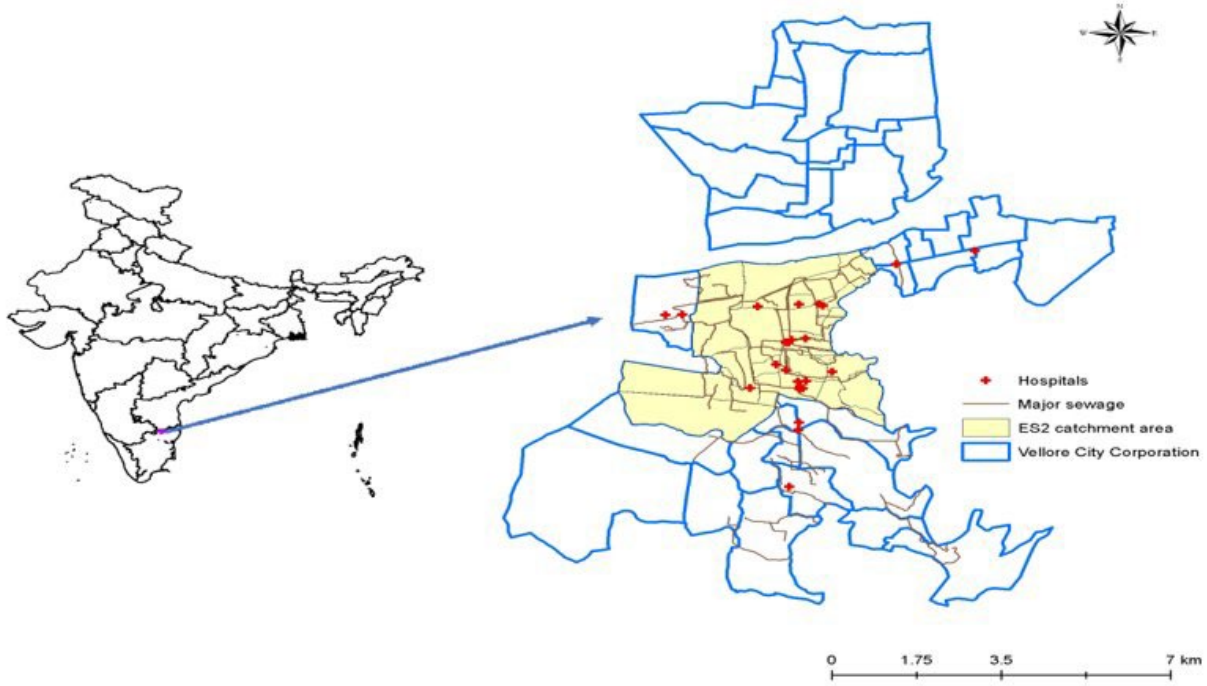
Study area in Vellore

- 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 site selection process

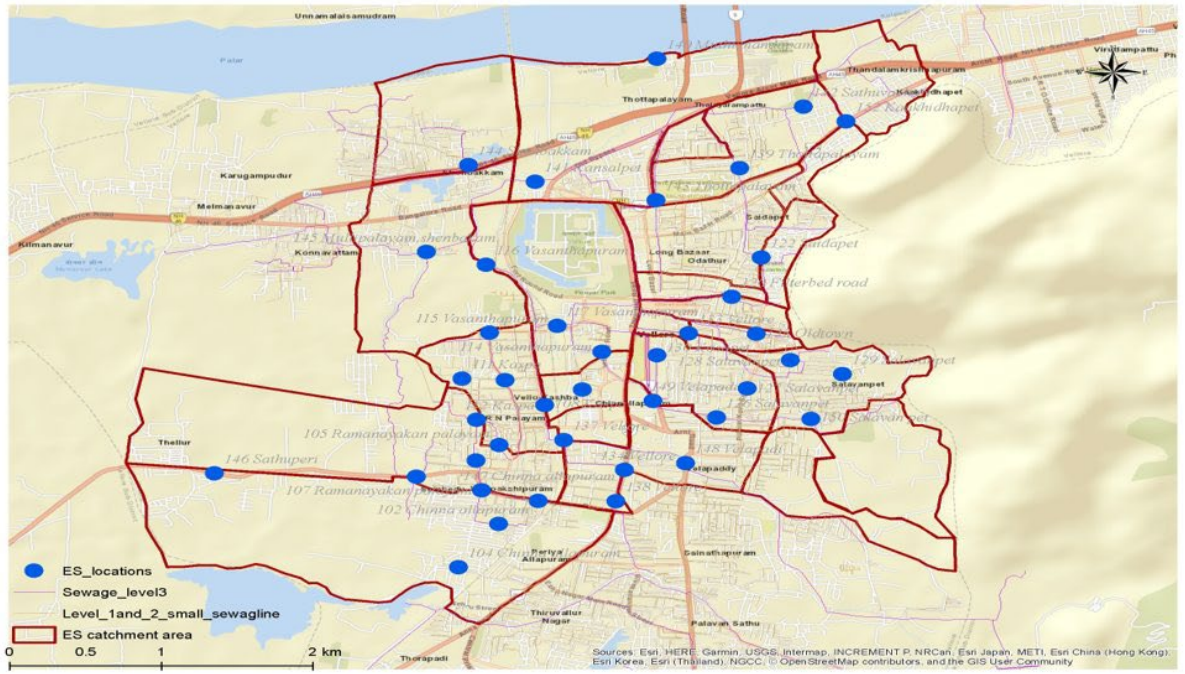
Spatial mapping of sewage network

Characterizing drains into three levels based on varying catchment populations

Ground truthing to identify potential sampling locations and feasibility of sampling

"Integration with high-resolution digital elevation models to delineate hydrological catchments"*

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)

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 (Moore swab)
- 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 h[illegible]ous, plea[illegible]plementary Table 1 for details

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

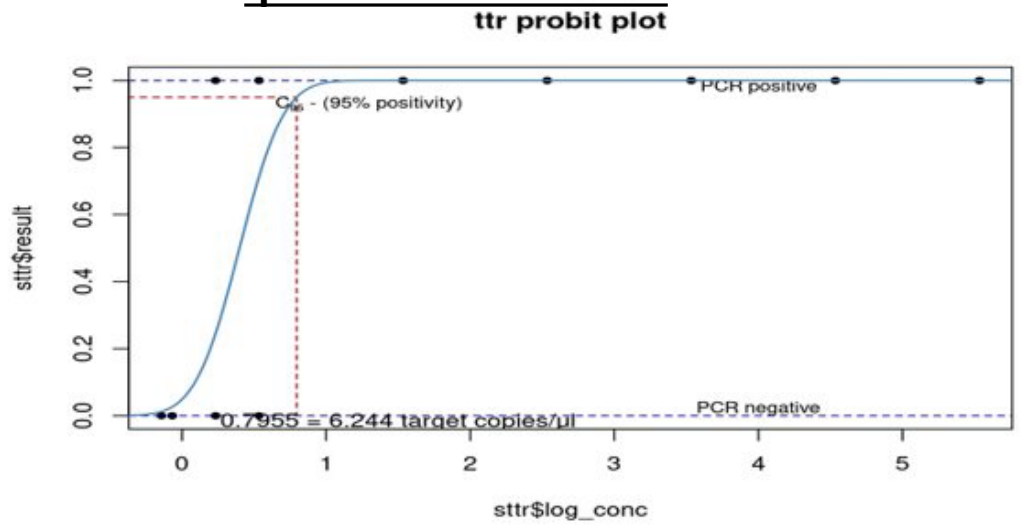
Targets

- *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

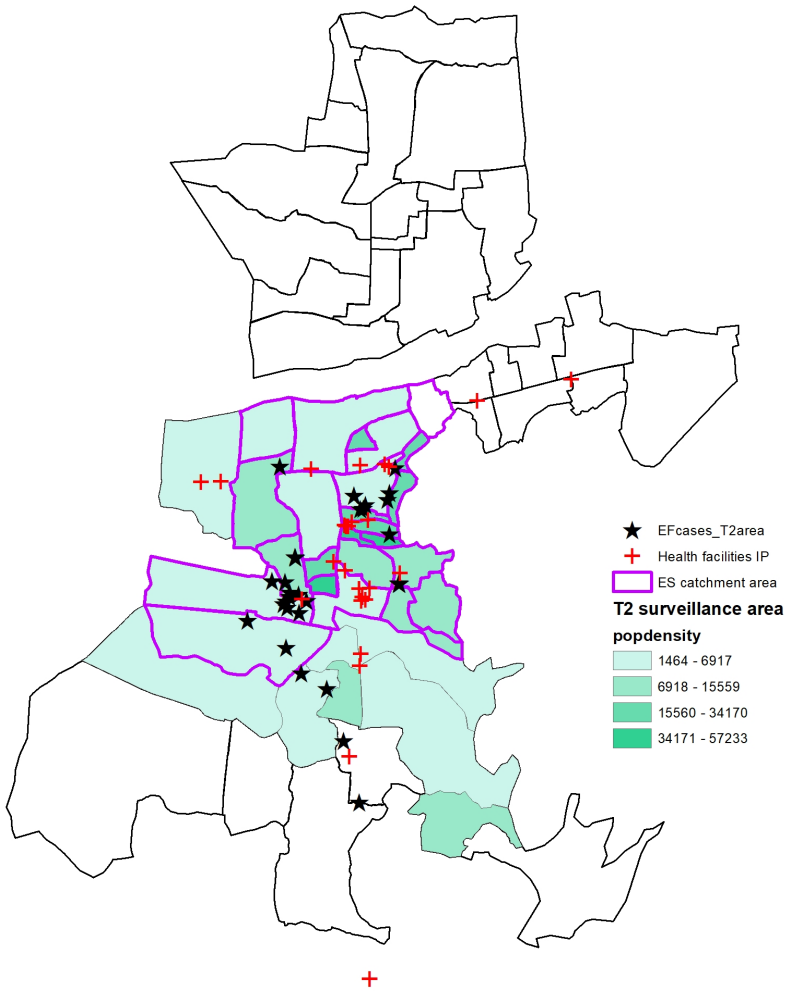
Aquaprobe AP-2000

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

qPCR standardization



Clinical incidence of Typhoid



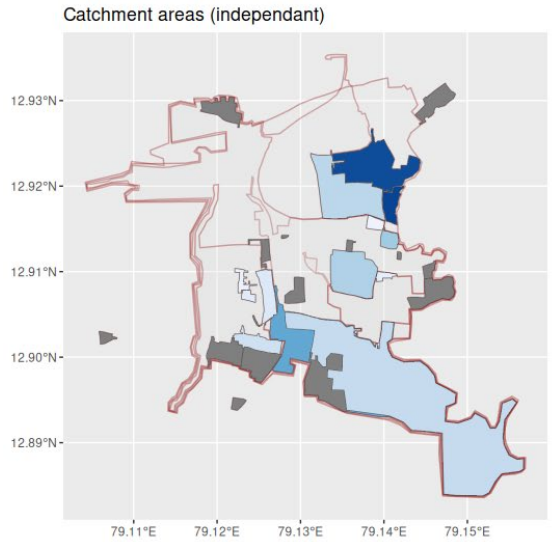
- Hospital based sentinel surveillance since April 2021
- Recruiting fever cases aged 6 months and above from defined geographical area; 1,90,000 population across 31 wards
- Of the 1108 eligible cases, 95% recruited and blood culture done in 92% of them
- 32 cases of enteric fever over one year from 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

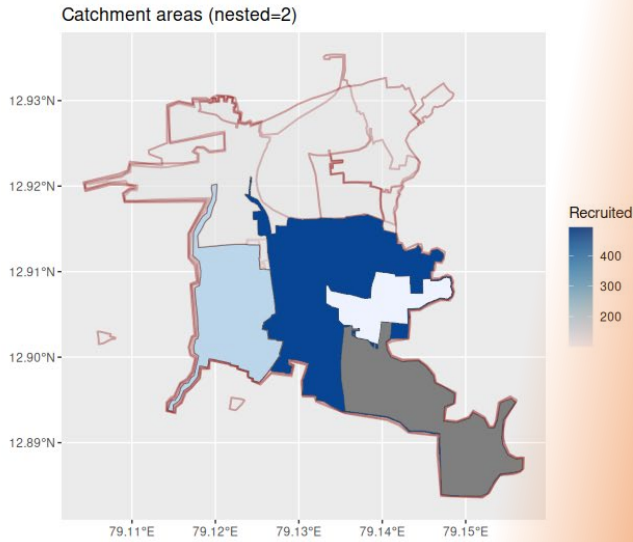
Age Group	No. of Cases	Population	Crude Incidence Per 100 000 Person Years (95% CI)
0–4	4	8082	49.5 (13.5–126.7)
5–9	3	9772	30.7 (6.3–89.7)
10–14	0	10 430	0.0 (.0–35.4)
15–29	4	35 728	11.2 (3.1–28.7)
30+	0	77 788	0.0 (.0–4.7)
Total	11	141 800	7.8 (3.9–13.9)

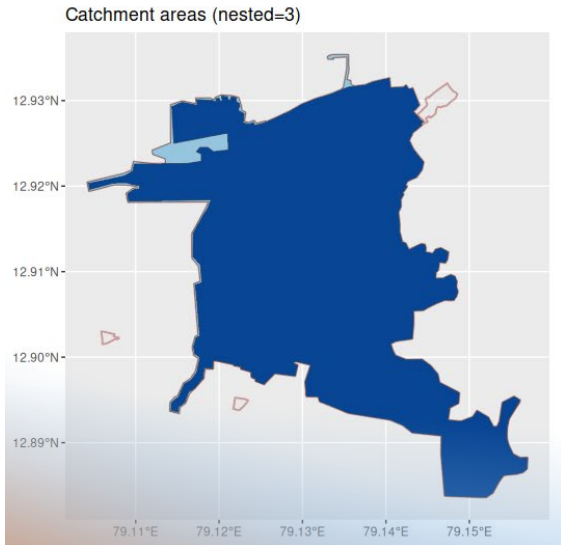
Christopher B Uzzell, Dilip Abraham, Jonathan Rigby, Catherine M Troman *et al.* **Environmental Surveillance for *Salmonella* Typhi and its Association With Typhoid Fever Incidence in India and Malawi**, *The Journal of Infectious Diseases*, 2023; https://doi.org/10.1093/infdis/jiad427

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 from study area
- Map each study participant to the ES catchment area
- Correlate ES detection with sero-incidence

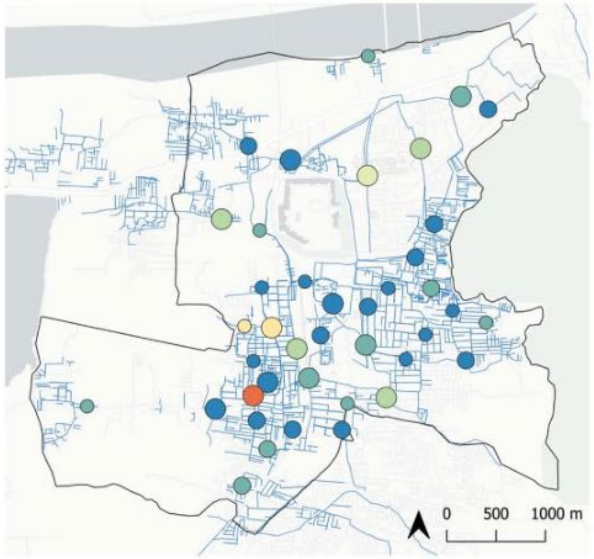




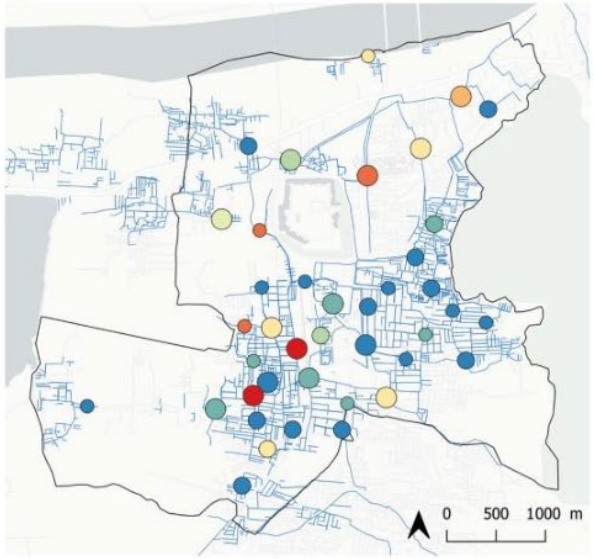


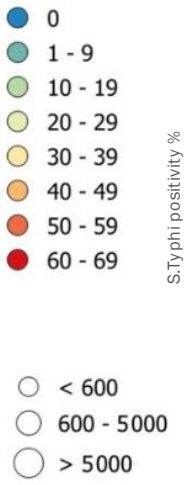
Results - ES positivity & trends

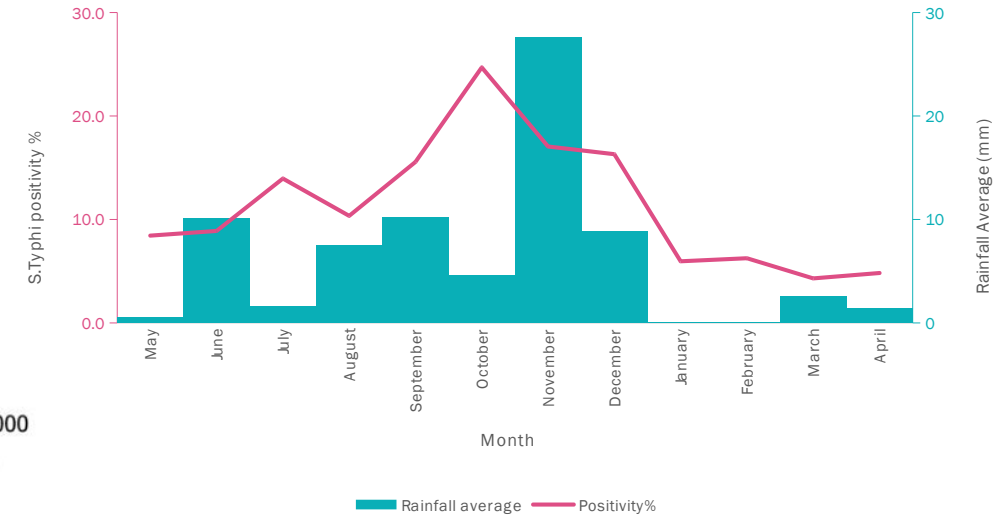
Membrane filtration



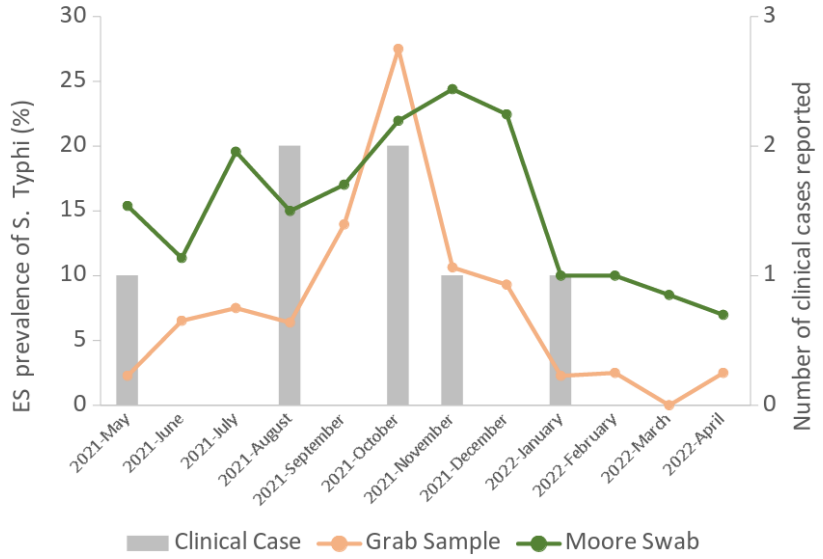
Moore swab







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



Water quality and other variables associated with the detection of S. Typhi from wastewater

Variables	Level	Unadjusted OR (P value)
Temperature		1.01 (0.91)
pH		0.51 (0.27)
ORP		1.00 (0.92)
DO		0.99 (0.89)
TDS		0.99 (0.79)
Turbidity		1.00 (0.45)
Flowspeed	Fast	Ref
	Slow	1 (empty)
	Stagnant	1.06 (0.95)
Average Rainfall		1.01 (0.33)
log hf183		1.16 (0.05)
Season	Summer	Ref
	Monsoon	5.35 (<0.01)
	Winter	2.30 (0.12)

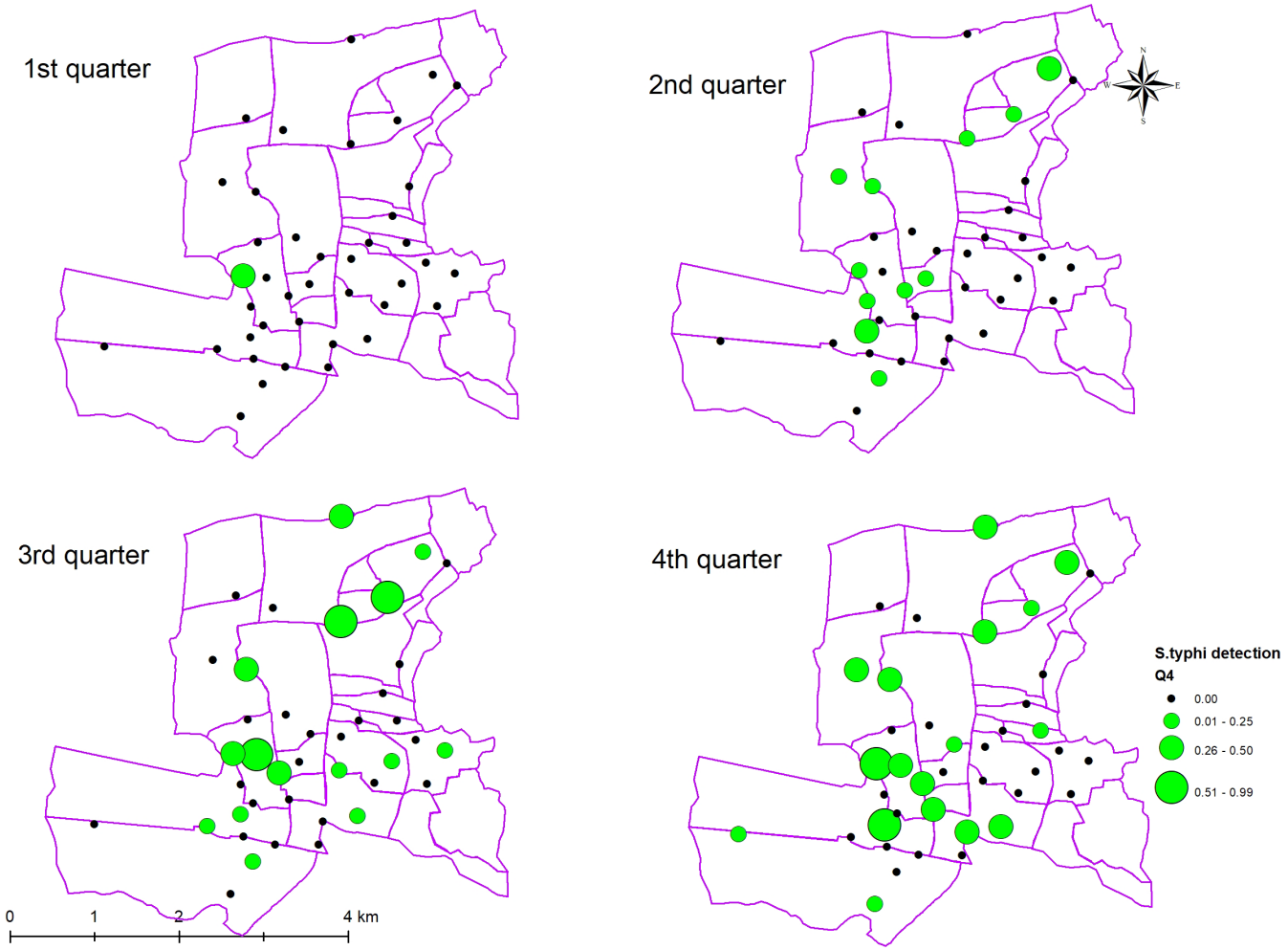
Membrane filtration

Variables	Level	Unadjusted OR (P value)	Adjusted OR (P value)
Flow speed	Fast	Ref	Ref
	Slow	0.35 (0.01)*	1.72 (0.26)
	Stagnant	1.17 (0.84)	0.68 (0.06)
Average rainfall		1.02 (0.02)*	1.03 (<0.01)
log hf183		1.22 (0.02)*	1.58 (<0.01)
Season	Summer	Ref	Ref
	Monsoon	1.94 (0.03)*	1.73 (0.16)
	Winter	1.77 (0.07)	1.32 (0.40)

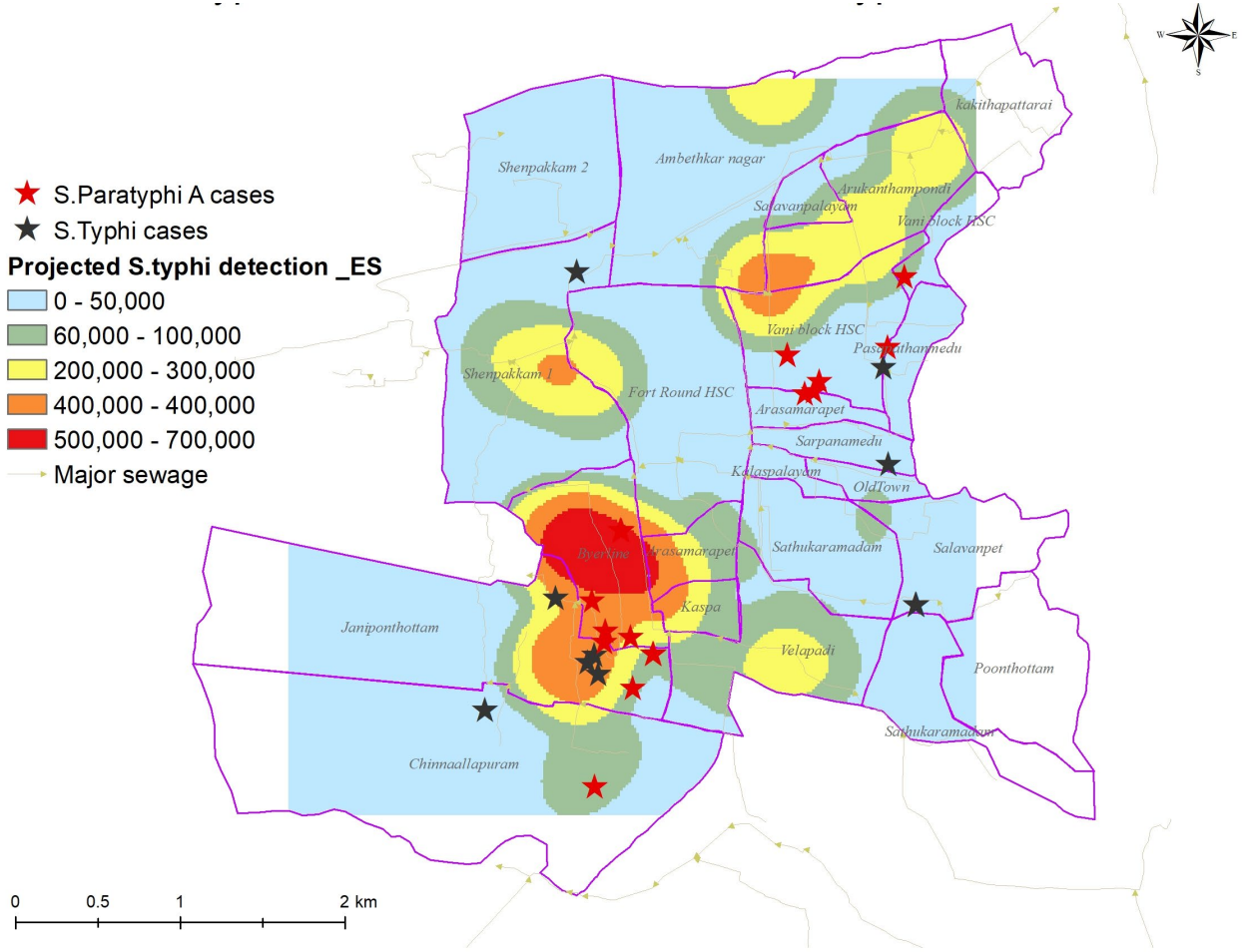
* Adjusted for flow speed, average rainfall, log hf183, Season

Moore swab

Seasonality in environmental detection

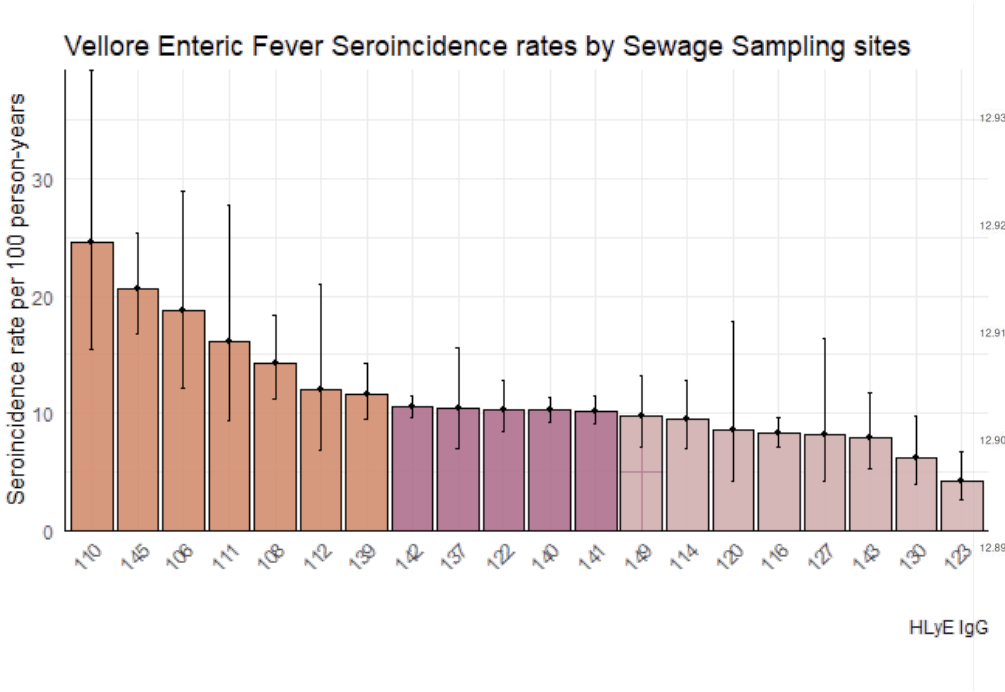


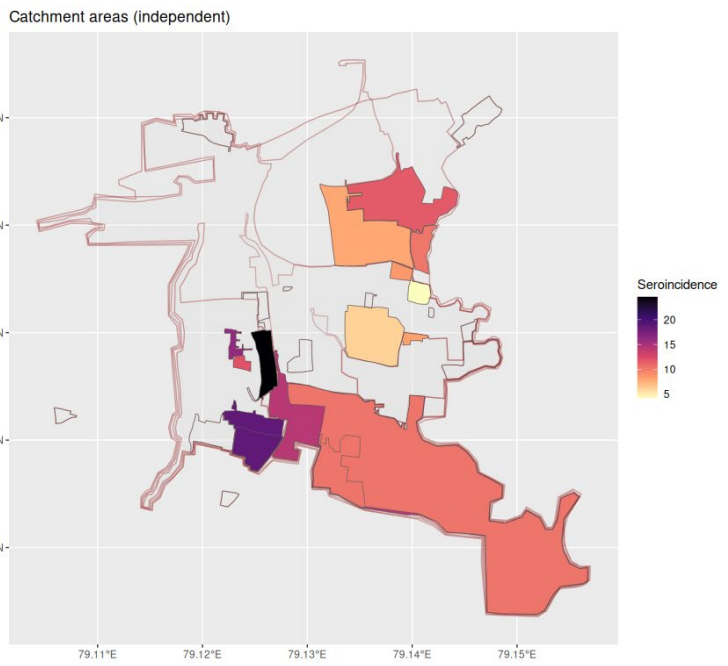
Typhoid cases and environmental *S.* Typhi detection

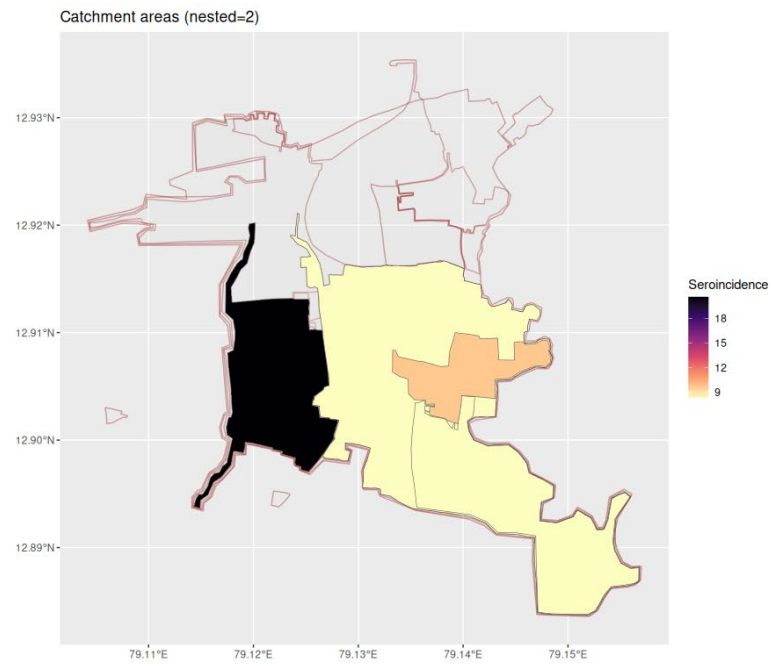


Results: Sero-incidence

- Overall sero-incidence: 10.4/100 p.y (9.9 - 10.9)







	Overall			Membrane filtration			Moore swab		
Characteristics	**OR**	**95%CI**	**P value**	**OR**	**95%CI**	**P value**	**OR**	**95CI**	**P value**
Seroincidence	1.24	1.23 – 1.24	<0.001	1.19	1.08 – 1.32	0.001	1.29	1.08 – 1.54	0.006
HF183 (log)	1.32	1.32 – 1.33	<0.001	1.06	0.80 – 1.40	0.709	1.72	1.11 – 2.69	0.016
Catchment population (log)	1.48	1.48 - 1.49	<0.001	1.13	0.67- 1.92	0.645	1.84	0.82 - 4.12	0.136
Flow Speed									
Fast	1.12	1.11 - 1.13	<0.001				0.31	0.05 – 1.91	0.205
Slow	Ref.								
Depth									
Deep	1.53	0.67 – 3.49	0.315	2.45	0.64 – 9.34	0.189	1.36	0.38 – 4.83	0.633
Medium	Ref.								
Maximum rain (week)	1.02	1.02 – 1.03	<0.001	1.02	0.98 – 1.06	0.350	1.02	0.99– 1.06	0.234

Association between ES positivity and characteristics for all catchment areas (non overlapping, Mixed effects logistic regression)

Limitations

Sero-incidence

- Survey could not be carried out comparably in all catchment areas
- Assumed that antibody responses were similar across immuno-naïve and exposed patients
- Reinfections
- Long term carriage might modify antibody responses
- Assumed a constant force of infection

Clinical surveillance

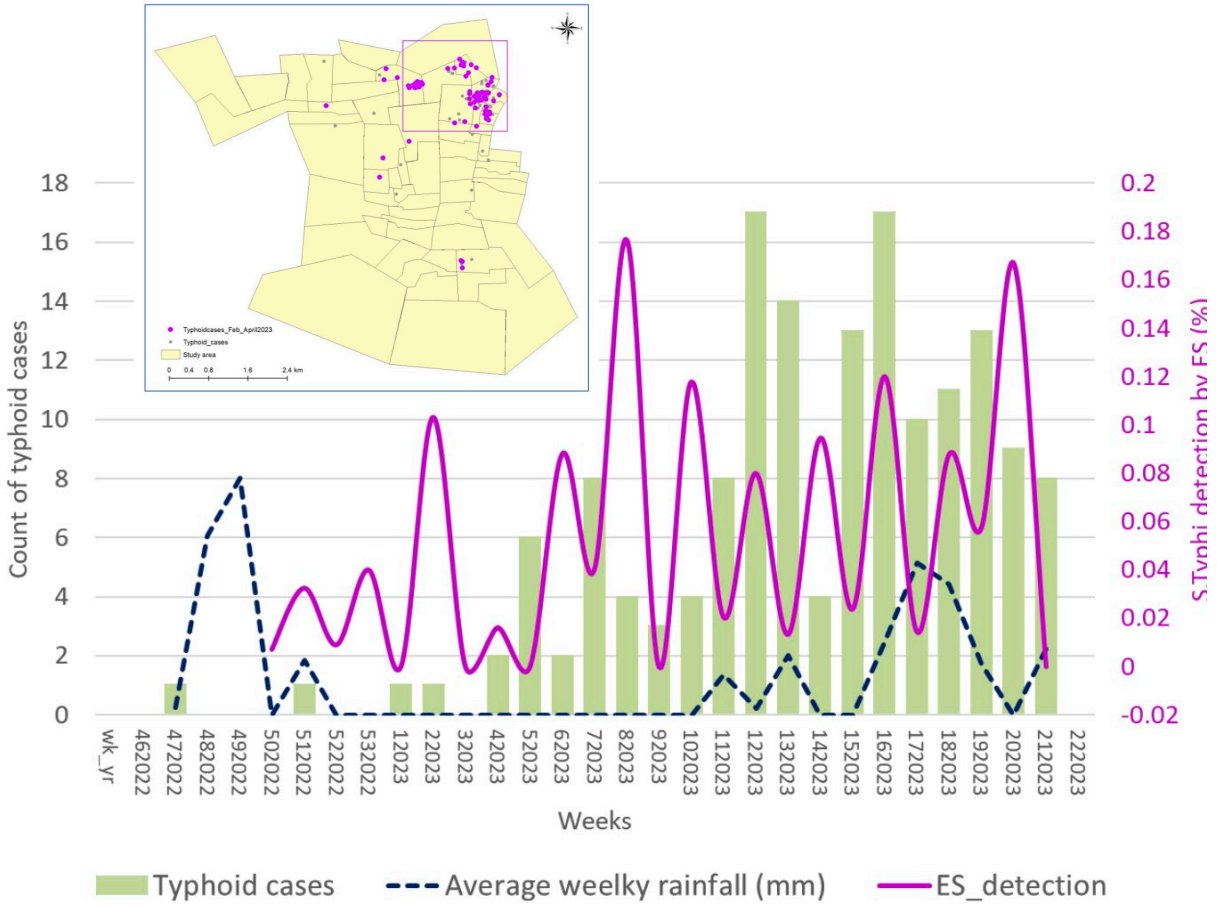
- Health-seeking behaviour changed due to effects of pandemic
- Disease rates were very low

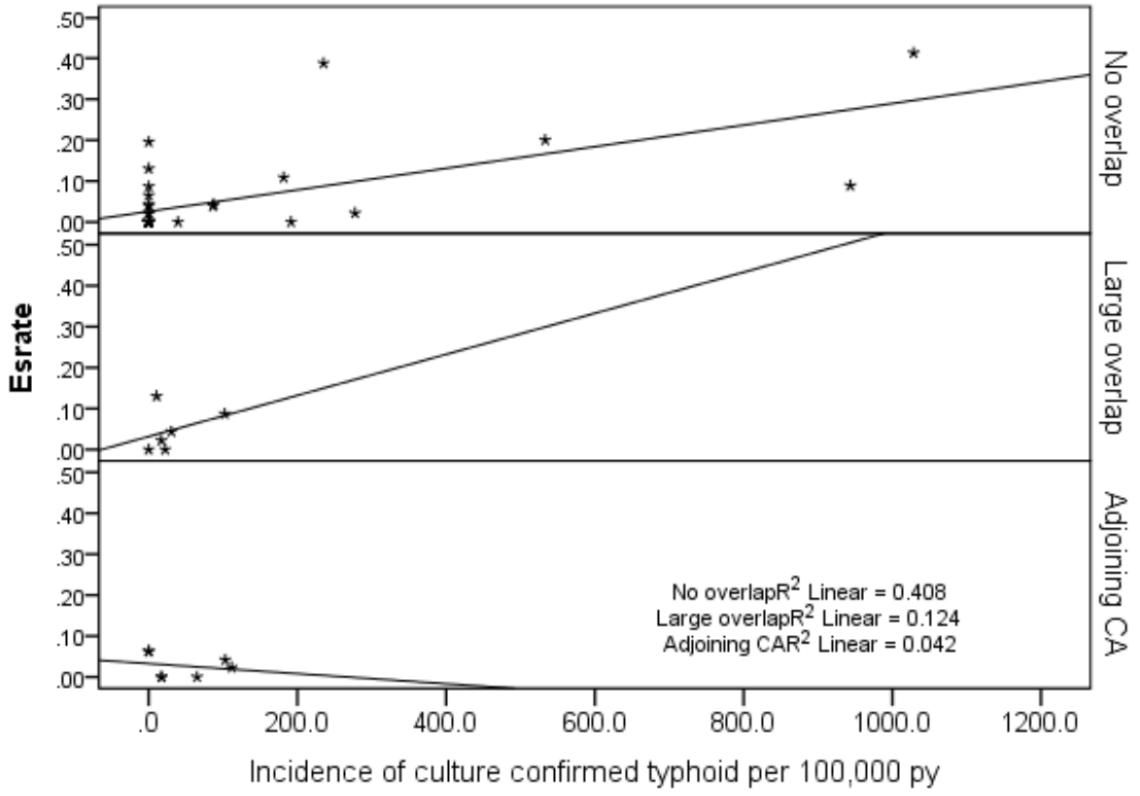
ES sampling

- Sampling frequency too low (once per month)
- *S.* Paratyphi “A” was not measured

Poster 55

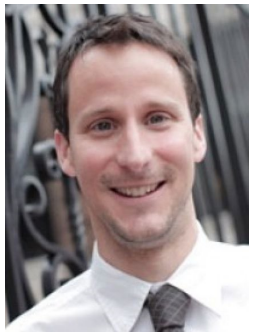
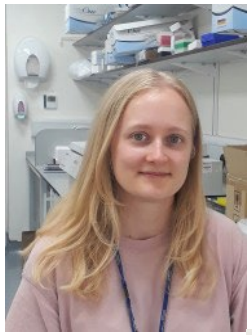
ES3 – Dec 2022 – May 2023: correlation of wastewater *S.* Typhi positivity with clinical incidence across catchments





Acknowledgements

BILL & MELINDA GATES foundation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NOT TO BE MINISTERED UNTO BUT TO MINISTER
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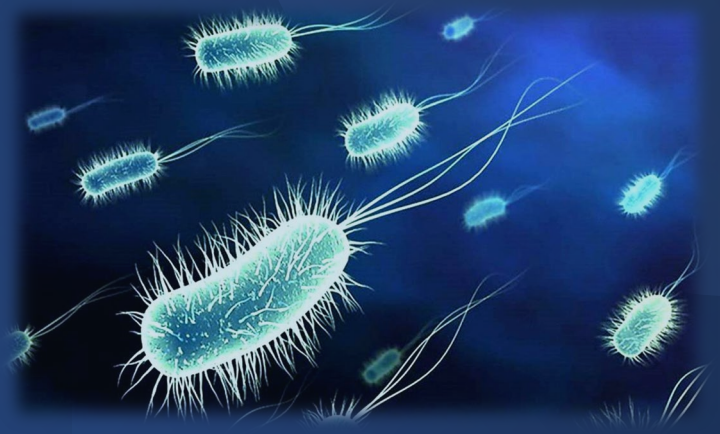
Direct sequencing of *Salmonella* Typhi in wastewater to determine AMR and genotype



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[1]Vaccine Epidemiology Research Group, Imperial College London

[2]Chrisitian Medical College, Vellore, India

[3]London School of Hygiene and Tropical Medicine

Background

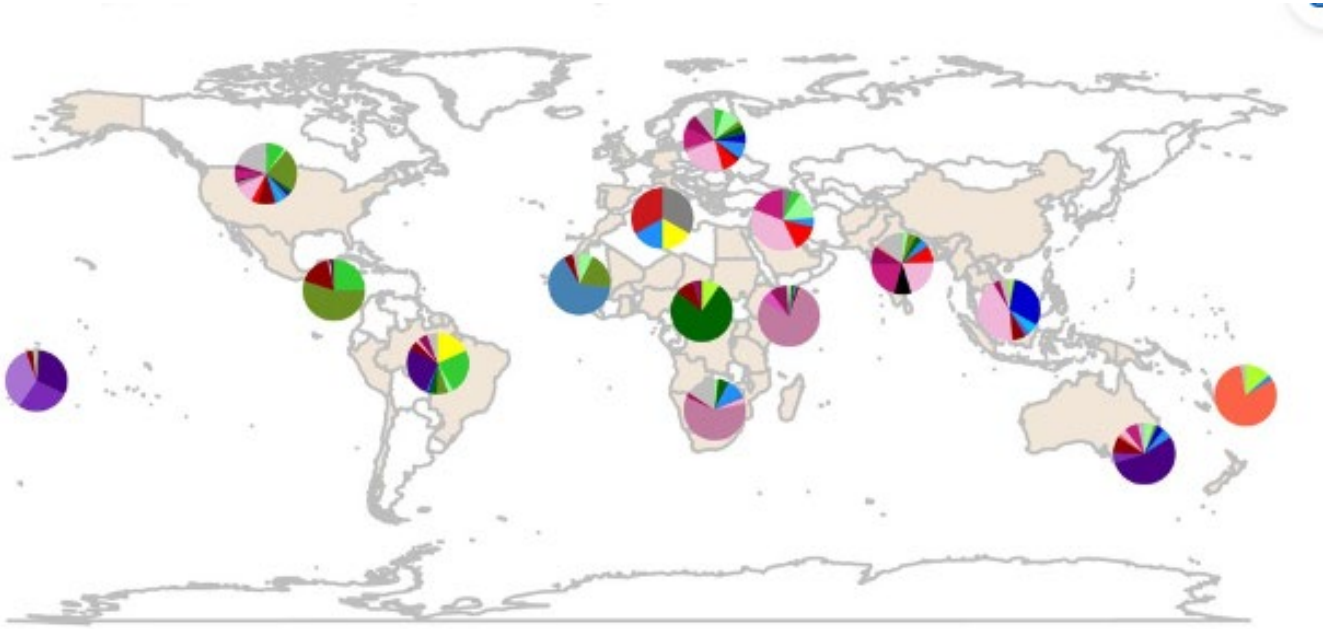
- Detection of *S.*Typhi in wastewater could help to inform estimates of the burden of Typhi in a community and help guide vaccine introduction

- Identifying the genotypes present in wastewater and their associated AMR would provide more information to help guide treatment or improve understanding of circulating strains.

- Designed an amplicon sequencing approach to try and genotype and identify AMR in *S.*Typhi in wastewater samples.

Genotyping Scheme

- Genotypes can be separated and identified by the SNPs present in the genome
- As more genomes are sequenced, more lineages have been identified
- Can use these genotypes to look at the global diversity of *Salmonella* Typhi

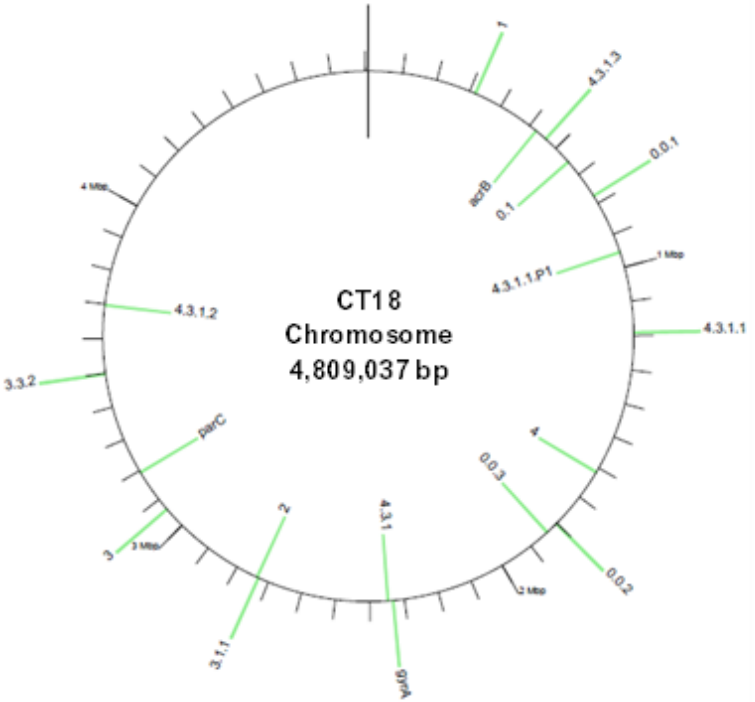


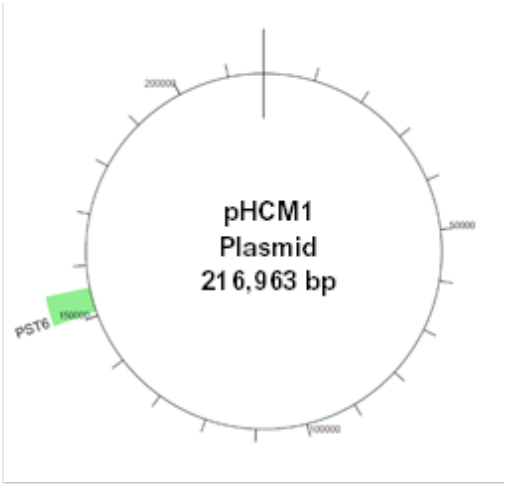
Genotype prevalence by world region
Carey *et al* 2023

Targets

15 genotyping SNPs and 4 AMR SNPs were selected

- 0.0.1, 0.0.2, 0.0.3, 0.1, 1, 2, 3, 3.1.1, 3.3.2, 4, 4.3.1, 4.3.1.1, 4.3.1.1.P1, 4.3.1.2, 4.3.1.3
- *gyrA*, *parC* (fluoroquinolone resistance)
- *acrB* (azithromycin)
- PST6 IncHI1 plasmid (multi drug resistance)





Primers target a ~5kb region around the genotype defining SNPs

- Designed using RUCS (**R**apid identification of PCR primers for **u**nique **c**ore **s**equences)

Samples

- Controls
  - Genomic DNA from CT18 and two H58 strains

- Pilot samples
  - Samples from Vellore suspected to be positive for *S.*Typhi
  - From a pilot study to look at sampling methods for *S.*Typhi ES
    - Hospital – samples taken from a hospital wastewater outflow (n=8)
    - Community – samples collected from wastewater in the community (n=8)

- Vellore ES Study samples
  - ES samples from Vellore suspected to be positive for *S.*Typhi
    - Moore swab (n=64)
    - Grab samples (n=30)

Method overview

Primers grouped into 4 pools of 4-5 primer sets

Amplified in separate reactions then pooled after quantification of the PCR product

ONT barcodes ligated for multiplexing samples and library run on a MinION Mk1B

Real-time basecalling and demultiplexing using MinKNOW, and read mapping in RAMPART

Final genotype call of mapped reads provided by GenoTyphi

barcode01

github.com/artic-network/rampart

Sample	Final_call	Final_call_support
H12106	4.3.1.1	1

github.com/typhoidgenomics/genotyphi

GenoTyphi results summary

Sample type	Final call	Final call support	Subclade	Primary Clade	Support Subclade	Support Primary Clade	Number of SNPs	Called AMR mutations
CT18 control	3	1		3			23	
H58-A control	4.3.1.1	0.95	4.3.1.1	4	0.98	0.97	23	gyrA-S83Y
H58-B control	4.3.1.1	0.96	4.3.1.1	4	0.99	0.98	23	
Community-1	3	1		3			1	
Community-2	No SNPs encountered against expected reference. Wrong reference or no SNP calls?							
Community-3	3	1		3			1	
Community-4	3	1		3			14	
Community-5	3	1		3			4	
Community-6	No SNPs encountered against expected reference. Wrong reference or no SNP calls?							
Community-7	3	1		3			9	
Community-8	No SNPs encountered against expected reference. Wrong reference or no SNP calls?							
Hospital-1	3	1		3			1	
Hospital-2	2	1		2			15	
Hospital-3	4.3.1.2	0.88	4.3.1.2	3	0.88		20	gyrA-S83Y
Hospital-4	4.3.1.1	0.48	4.3.1.1	4	0.91	0.52	22	gyrA-S83F
Hospital-5	3	1		3			11	
Hospital-6	4.3.1.1	0.12	4.3.1.1	4	0.47	0.25	19	gyrA-S83F
Hospital-7	4.3.1	0.96	4.3.1	2	0.96		20	gyrA-S83F
Hospital-8	4.3.1	0.72	4.3.1	4	1	0.72	14	

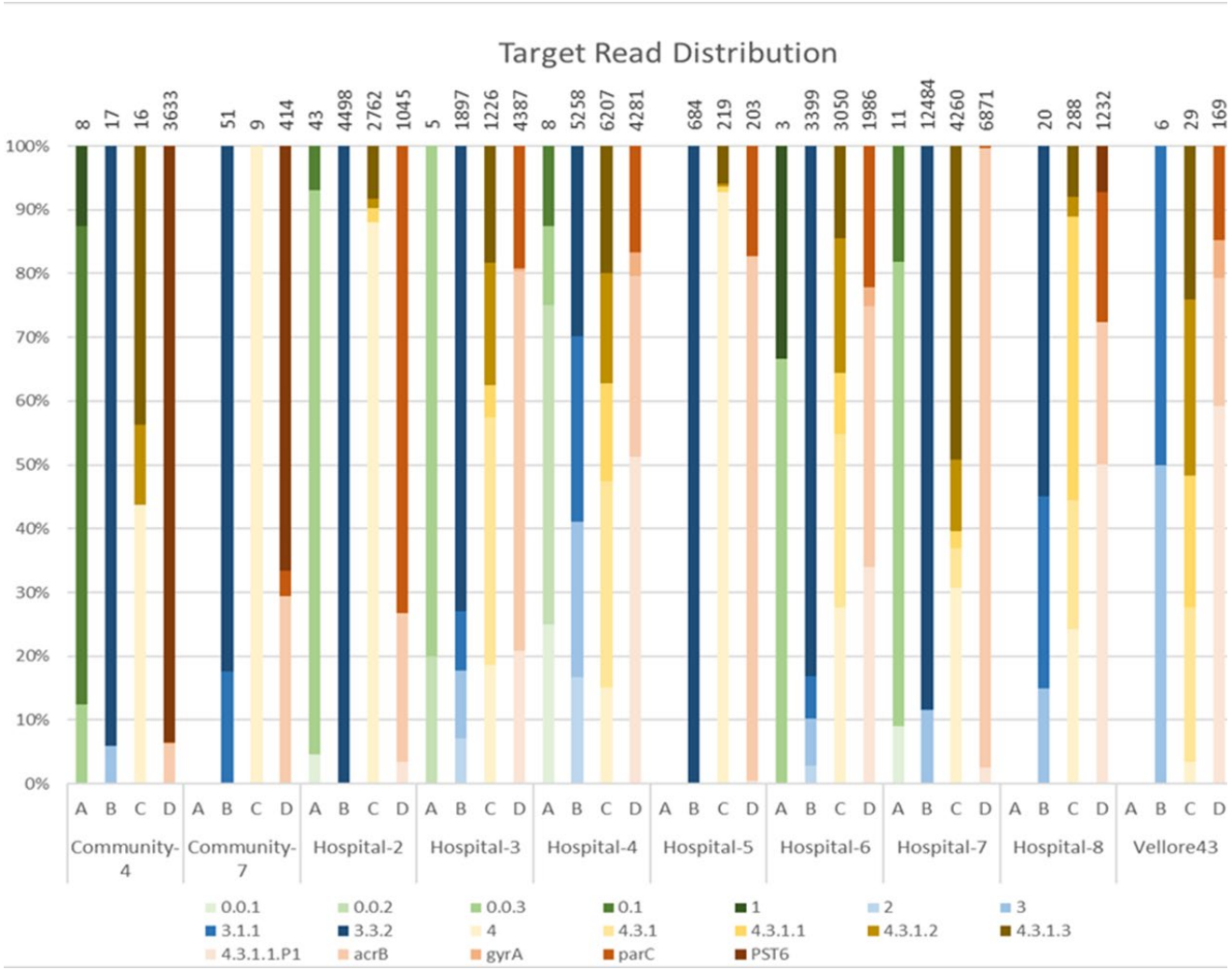
Controls and Pilot samples GenoTyphi output

Summary of GenoTyphi results from Vellore ES samples

Genotype	Number of Samples	Average support value
3	76	1
2	3	1
4.3.1.2	1	1
gyrA-S83F	1	NA
parC-E84K	1	NA
No SNPs Encountered	14	NA

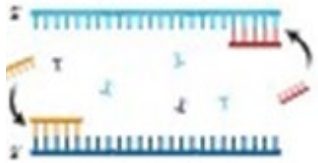
github.com/typhoidgenomics/genotyphi

Challenges



- Large number of off-target reads
  - 0-83% mapping to Typhi
- Highly variable distribution of on-target reads across the primer sets
- A lot of reads shorter than the target length

Ongoing work



Redesigned primer sets to:

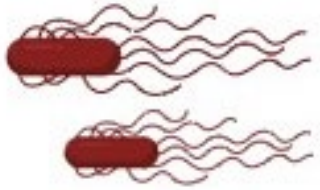
Reduce the number of primer pairs

Reduce off-target amplification

Reduce the length of the amplicons

Utilising the most recent ONT chemistry for improved raw read accuracy

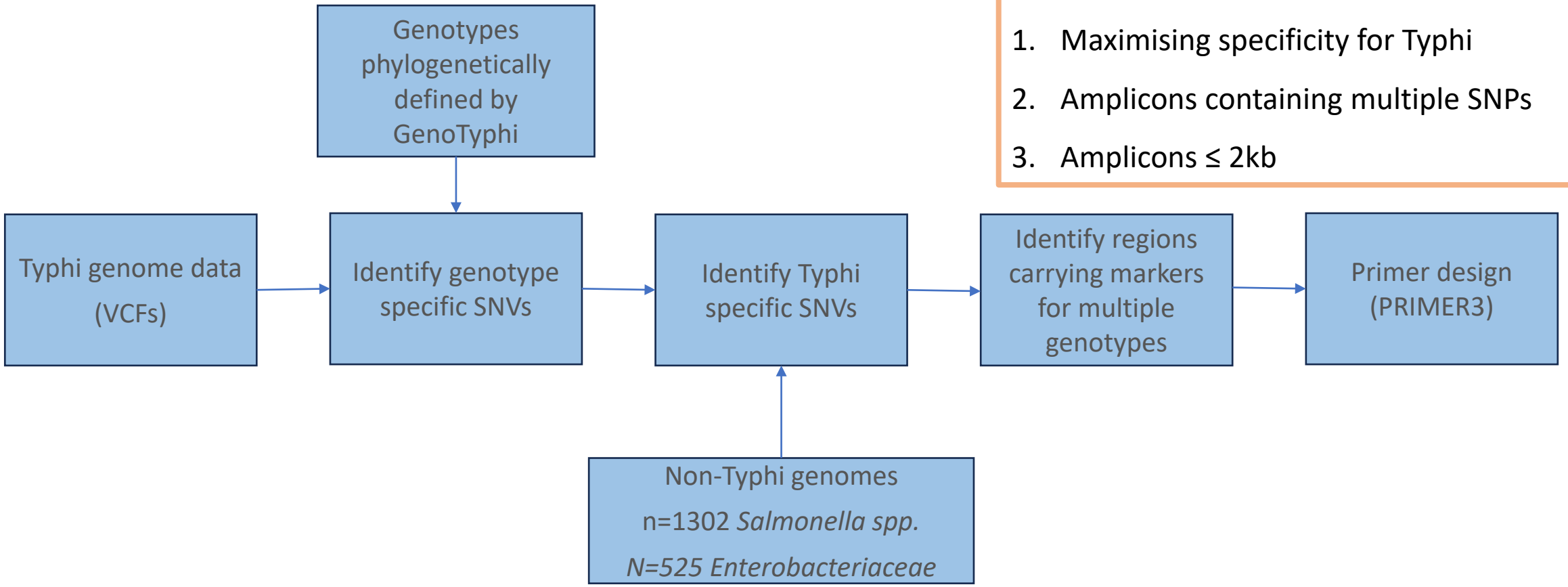
Looking to employ a more streamlined pipeline for analysing the amplicon sequence data



Testing in Vellore, India and Kumasi, Ghana

Aim to look at Paratyphi A and others in addition to *S.*Typhi

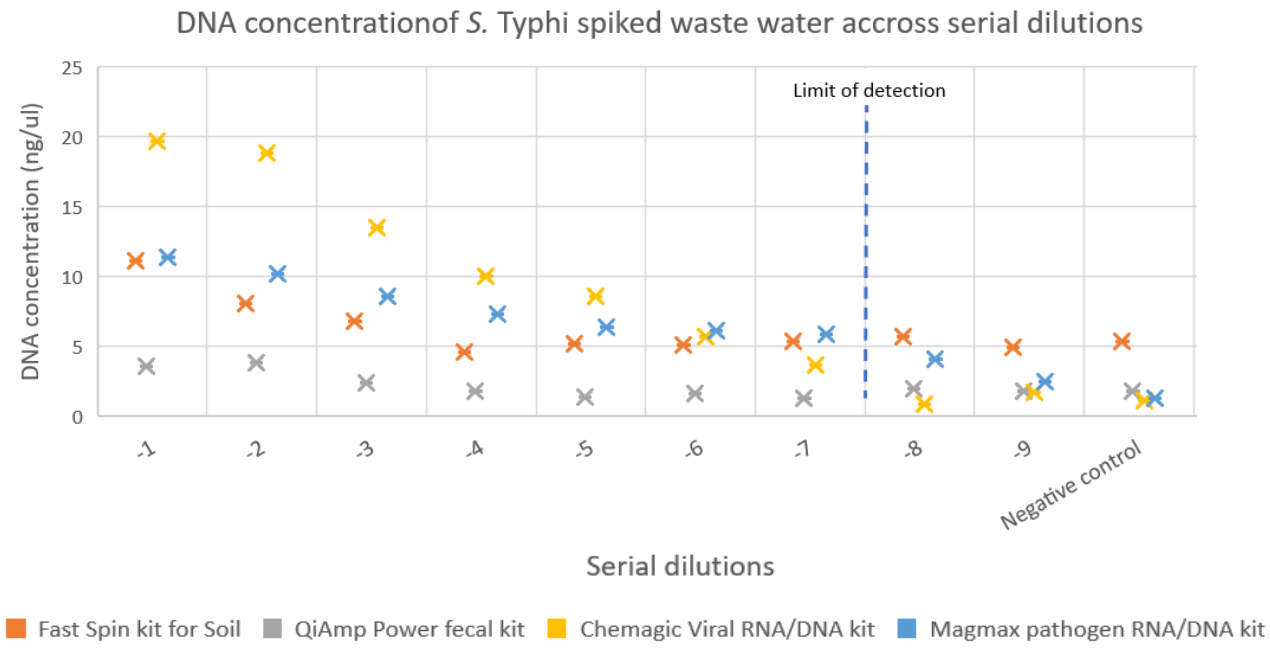
Redesigning Primers

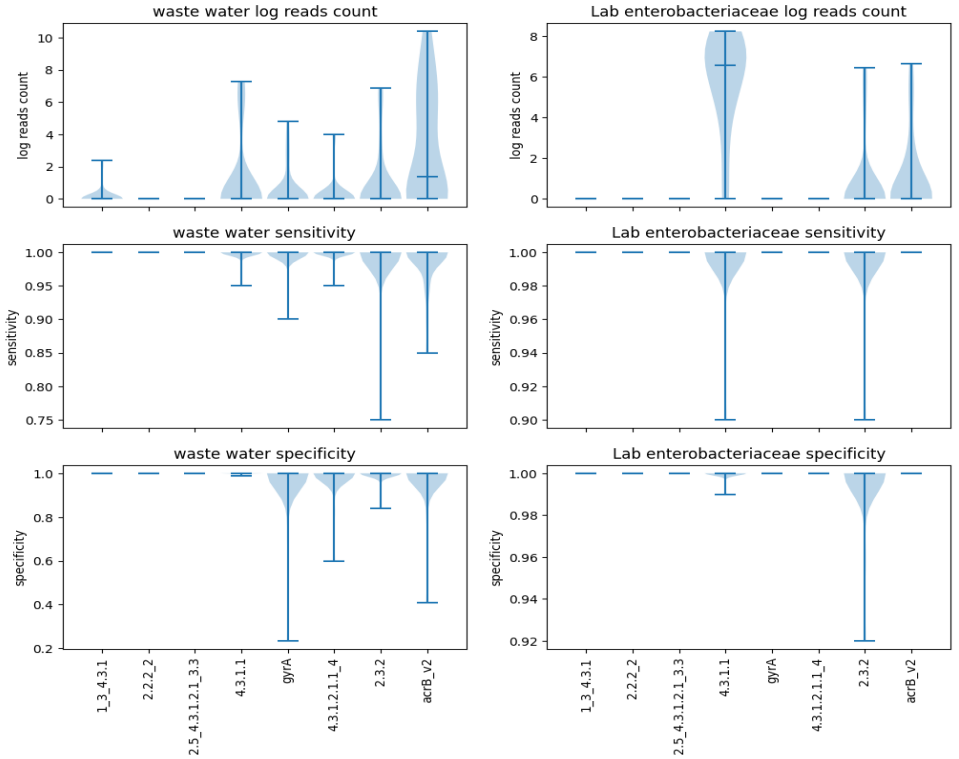


Focus on:

1. Maximising specificity for Typhi
2. Amplicons containing multiple SNPs
3. Amplicons ≤ 2kb

Primer testing





- Typhi DNA spiked into wastewater amplified well
- Some amplicons underperformed and have been redesigned (in testing)
- The PCR shows good sensitivity and specificity when run on off-target DNA samples

Next steps

CONTINUE PRIMER TESTING AND OPTIMISATION

TEST MULTIPLEX ON SAMPLES IN GHANA, INDIA, AND NIGERIA

OPTIMISE AND MAKE AVAILABLE THE ANALYSIS PIPELINE

Thank you

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