Development and Application of Pilot *Salmonella* Typhi Environmental Surveillance Program **in Kolkata, India**

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Outline

- Kolkata background
- Sampling strategy
- Lab methods
- Environmental surveillance pilot studies
- Next steps

Collaborating Institutions

National Institute for Cholera and Enteric Diseases

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University of Brighton

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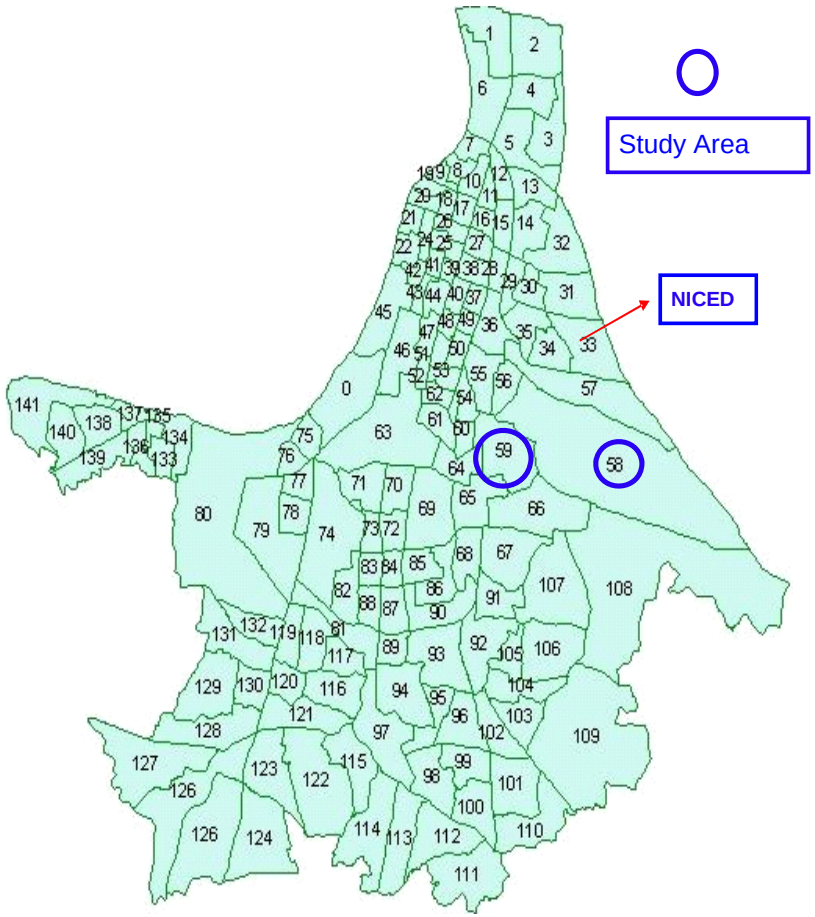
James Ebdon

Kolkata background

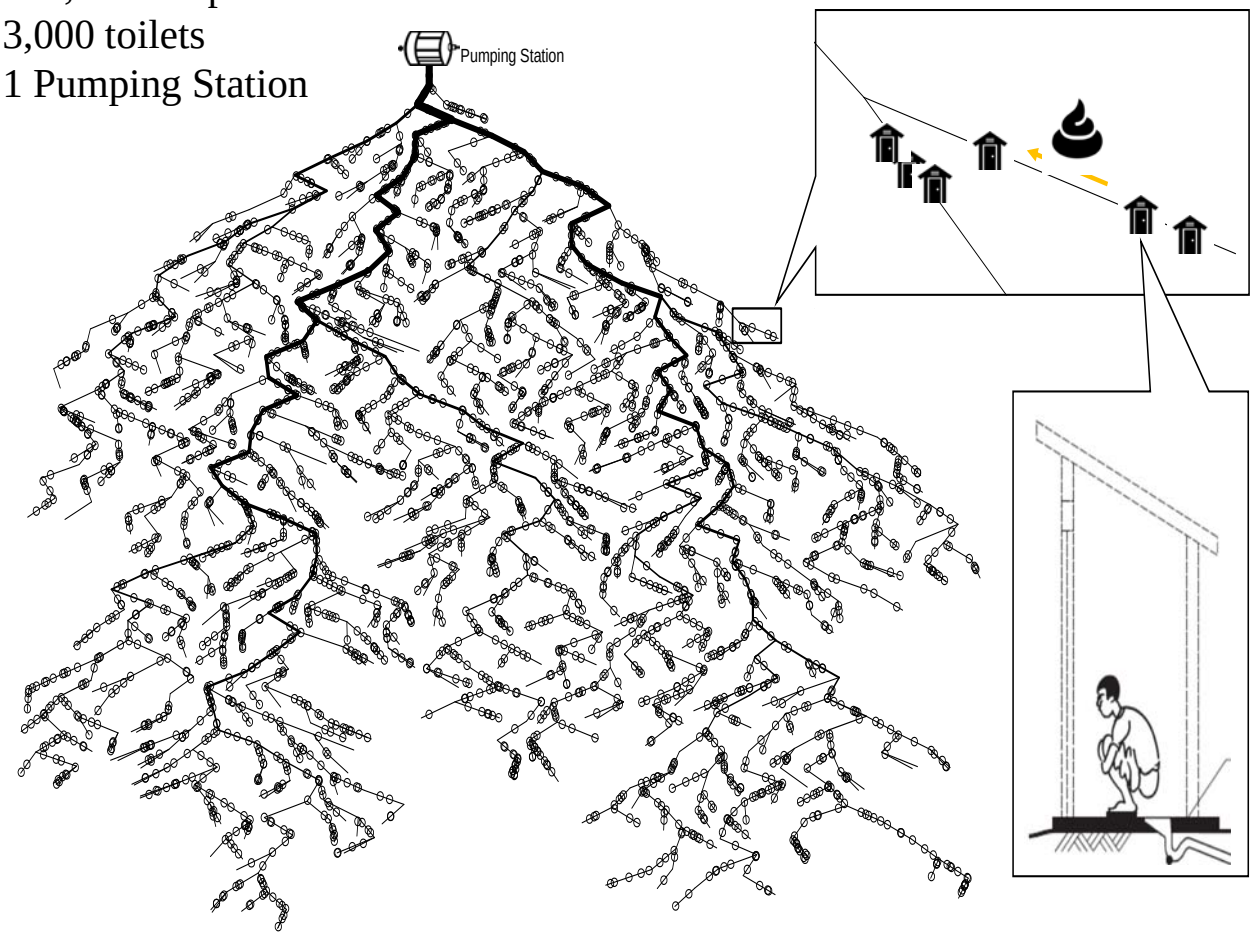


- Size of city : Area ~ 80 mi^2, population 4.5 million, divided into 144 administrative wards
- Previous estimates of incidence of:
  - Typhoid- 235/100,000 person years
  - Paratyphoid- 105/100,000 person years
- Active surveillance since November 2017
- Location: Wards 58 & 59
- Low income – both horizontal and "vertical", high population density, intermittent municipal water supply, shared pour-flush toilets, seasonal flooding
- Number of clinical cases detected by active surveillance (till Jan 2019): 55 (50 typhoid, 5 paratyphoid fever)

Modelling for Adaptive Sampling Site Allocation

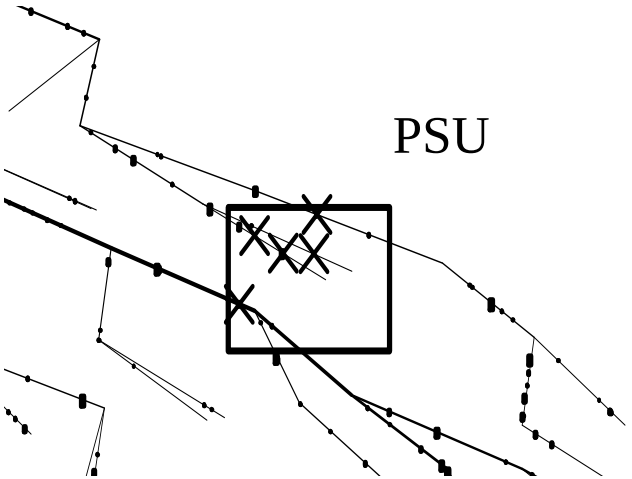
Mathematical model to simulate fecal shedding dynamics and pathogen fate in the sewage network – Please see presentation by Yuke Wang

160,000 People
3,000 toilets
1 Pumping Station



Sampling Sites:

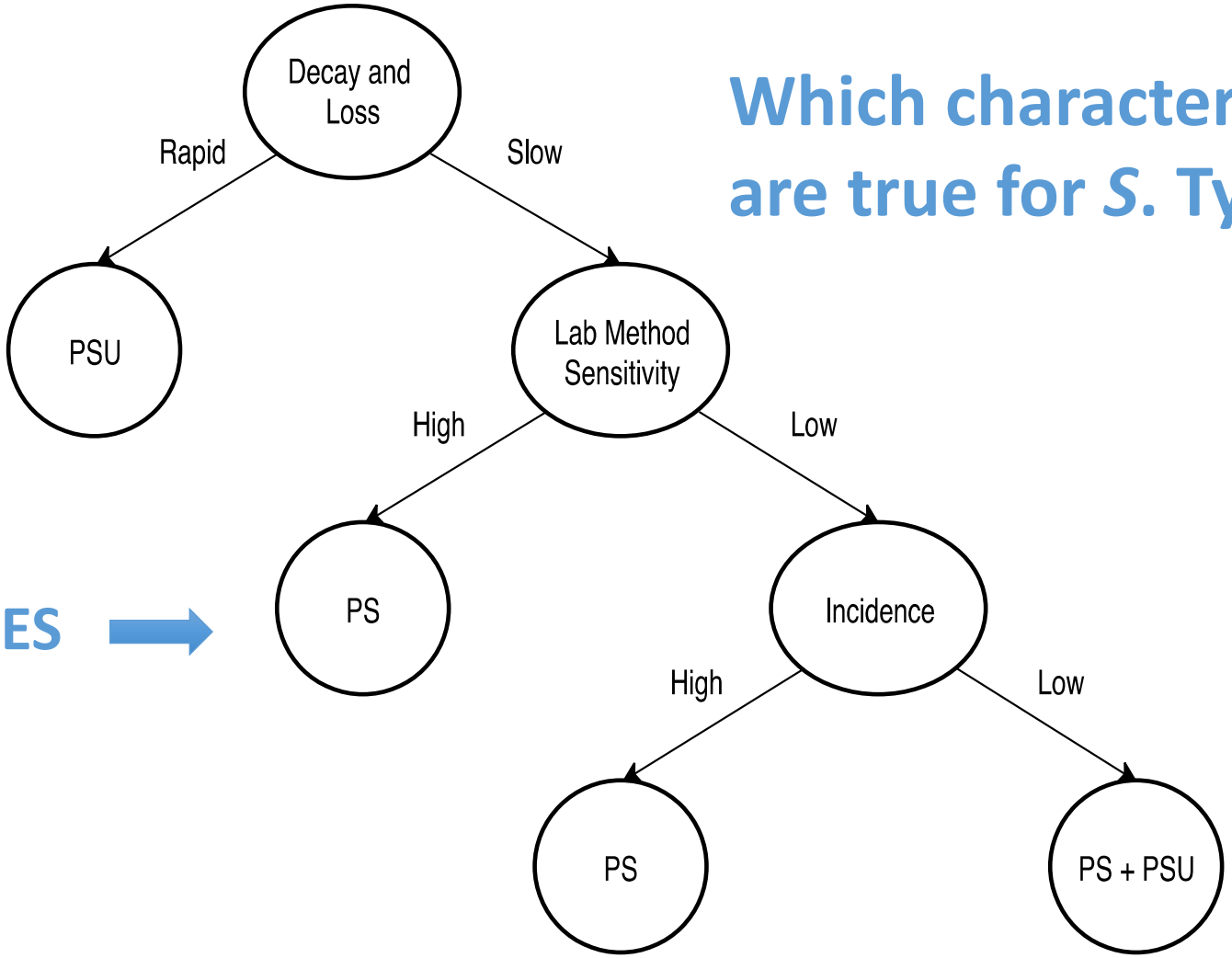
1. Pumping Station
2. Toilet
3. Primary Sampling Unit (PSU), pooled sample from shared toilets



Focused on Two Types of Sampling Locations

Sample Location	Strengths	Limitations
Sewage Pumping Stations	• Captures feces from more people • Large volume sample ~40 L has higher probability of capturing pathogen target ie. greater sensitivity	• Feces are more dilute • Greater distance and travel time from fecal source – more opportunity for pathogen die off • Large volume sample is more challenging to collect and process
Shared toilets (“Primary sampling unit” PSU)	• Closer to fecal source so less opportunity for pathogen die-off • Less dilution of feces • Smaller sample volume (500ml) is easier to collect and process	• Captures feces from fewer people • Smaller sample volume decreases probability of capturing pathogen target

Decision tree for selecting type of sampling location based on physical and epidemiological attributes of the target pathogen and detection methods



Which characteristics are true for *S*. Typhi?

Polio ES ➡

How can we ensure that the environmental surveillance system is capturing excretion from high-risk populations?

Additional questions for selecting sampling locations

1) Where do feces from young children go – into toilets? into solid waste? or elsewhere?
2) Where do feces from the poorest populations (without access to toilets) go?

Sampling Strategies for Typhoid Environmental Surveillance

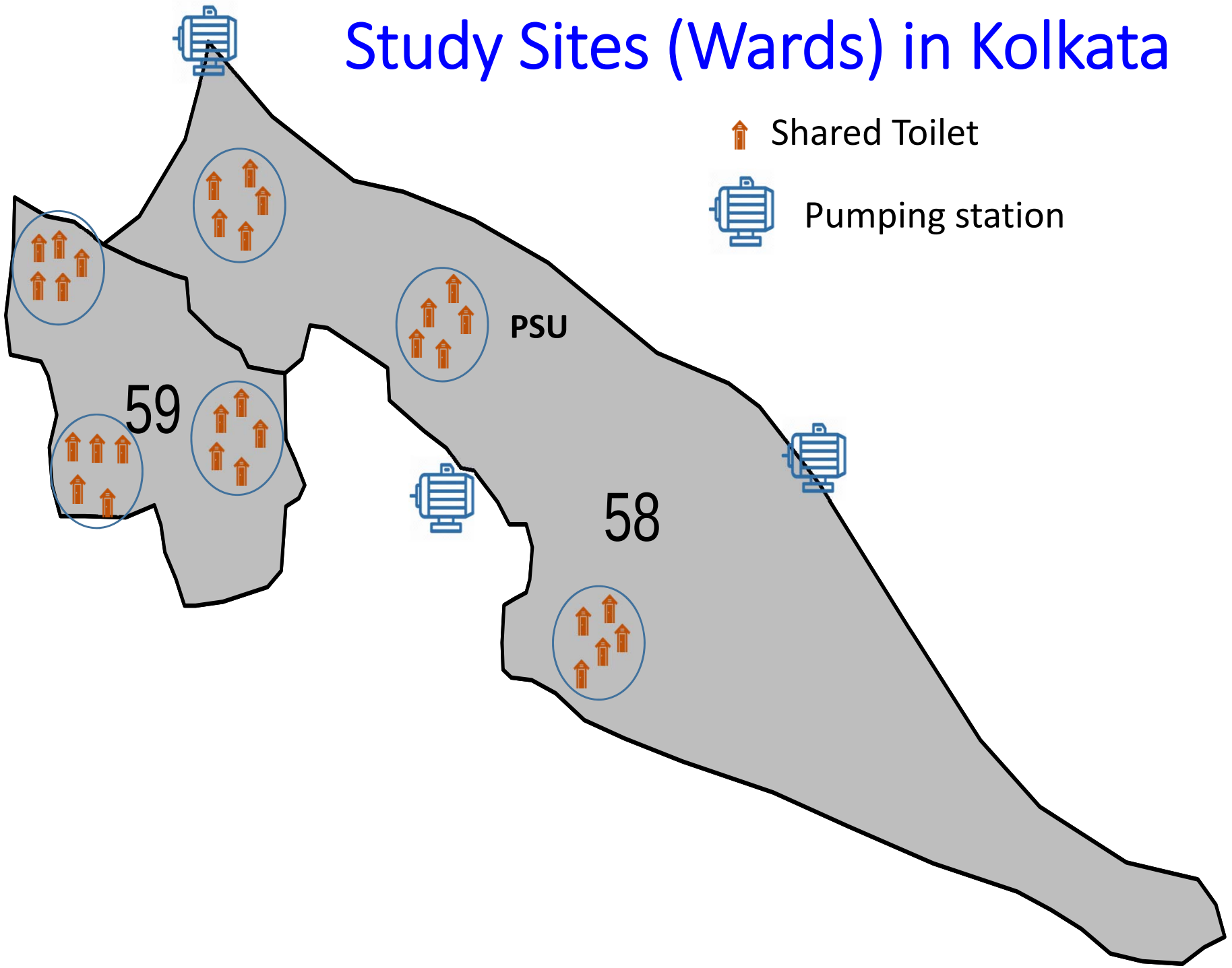
- **Grab samples** from selected sampling sites
  - Pros: Ability to collect different sample volumes; allows quantitative detection
  - Cons: Cumbersome to collect and process large volumes; longer sample processing time; comparatively expensive
- **"Trigger" samples** from households with clinical cases
  - Pros: allow correlation b/w clinical and environmental surveillance
  - Cons: Requires clinical surveillance system; Does not capture whole city or asymptomatic cases
- **Trap samples** (Moore swab) from selected sampling sites
  - Pros: comparatively easier sample collection and processing; less expensive; allows sampling from more sites
  - Cons: only presence-absence detection

Pilot ES Study in Two Wards in Kolkata, India

Collection of grab samples from selected sites

Time period	Aug- Dec 2018
Location	Wards 58 & 59
Sampling sites	• 3 Sewage Pumping Stations • 6 PSUs (shared toilets) (3/ward)
Sampling schedule	Weekly
Sample volume	• Pumping station- 40 L • PSU- 500 mL

Study Sites (Wards) in Kolkata



Environmental Surveillance informed by Clinical Surveillance

Collection of "trigger" samples from HH of clinical cases identified during active clinical surveillance

Time period	Nov 2018- present
Location	Wards 58 & 59
Sample types	Pooled sewage from shared toilets Piped drinking water
Sampling schedule	Samples collected at 3-day interval over a period of two weeks after case was identified
Sample size	12 samples/case • 6 sewage samples • 6 drinking water samples
Sample volume	• Sewage from shared toilets – 500 mL • Drinking water- 40 L from nearest public tap

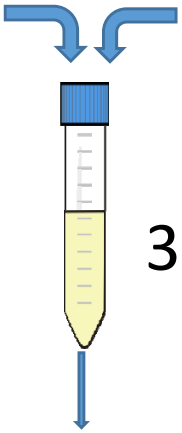
Methods: Sample Processing and Testing

- Microbial Source tracking (MST)- Screening assay for human feces using *Bacteroides* phage
- Membrane filtration for *E. coli*
- *Salmonella* Typhi and Paratyphi A
  - Ultrafiltration (UF) and/or PEG precipitation- Sample concentration
  - Quantitative real time PCR (qRTPCR)- detection of *S.* Typhi and *S.* Paratyphi A
  - Lab methods developed and validated using seeded sewage samples in Atlanta and Kolkata. Limit of detection ~10^3 cells for UF. Limit of detection for Moore Swab with enrichment ~ 10 cells

Methods: Phage for human-specific bacterial host (*Bacteroides fragilis* GB-124) and somatic coliphage

Sample Processing and Testing

1 mL test sample (Sewage)

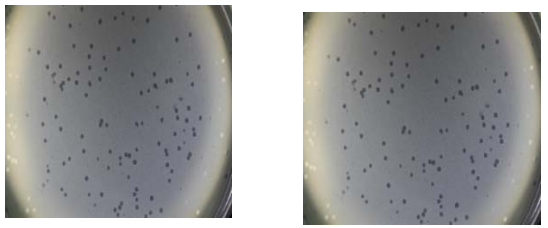


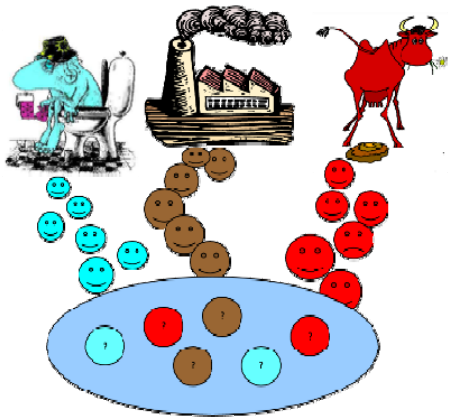
1 mL host (GB-124/WG-5)

3 mL ss BPRMA (GB-124) / ssMSA (WG-5)

Pour on BPRM/ MSA agar plate

Incubate anaerobically (BPRM)/ aerobically (MSA) at 37°C for 18-24 hr





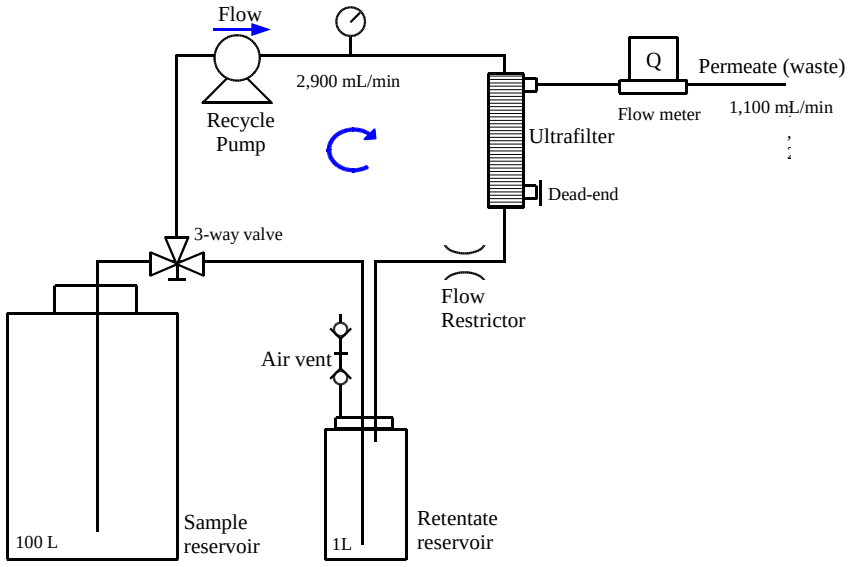
Methods: Sewage/Water Sample Concentration

Please see poster by Dr. Renuka Kapoor

Environmental sample

Sewage from shared toilets → PEG precipitation → Total DNA extraction → qRTPCR (Baker's method)

Sewage from Pumping station, Piped drinking water



Ultra-filtration → PEG precipitation → Total DNA extraction → qRTPCR (Baker's method)

Results: August 2018-February 2019

Sample type	No. tested	PCR Results
Pumping station	31	No detection
Pooled sewage from shared toilets - routine samples	56	No detection
Pooled sewage from shared toilets - Trigger samples	18	3 positive Confirmed *S*. Typhi by sequencing
Piped drinking water- Trigger samples	38	No detection

Please see poster by Dr. Goutam Chowdhury

Next Steps: City-Wide Surveillance + SaniPath Exposure Assessment

Proposed Timeline

Mar 2019

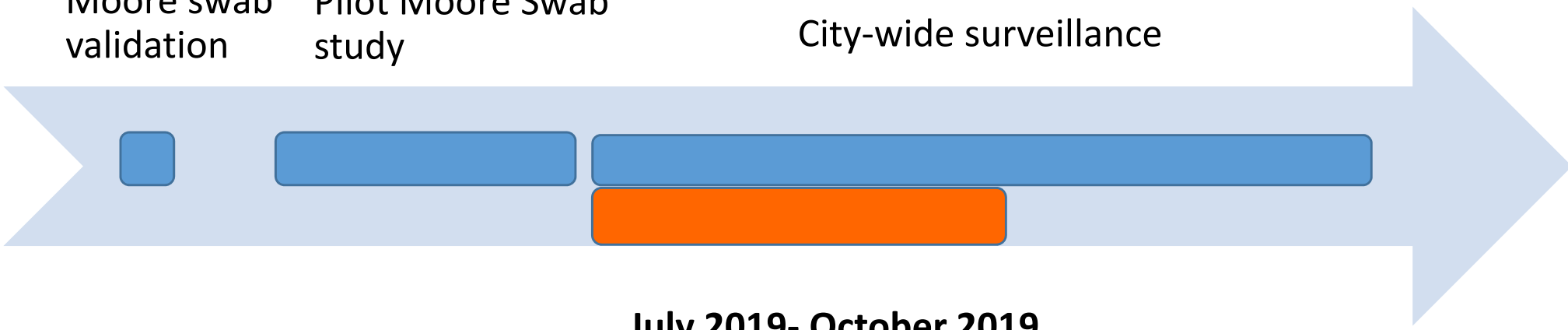
Moore swab validation

Apr-June 2019:

Pilot Moore Swab study

July 2019- June 2020

City-wide surveillance



July 2019- October 2019

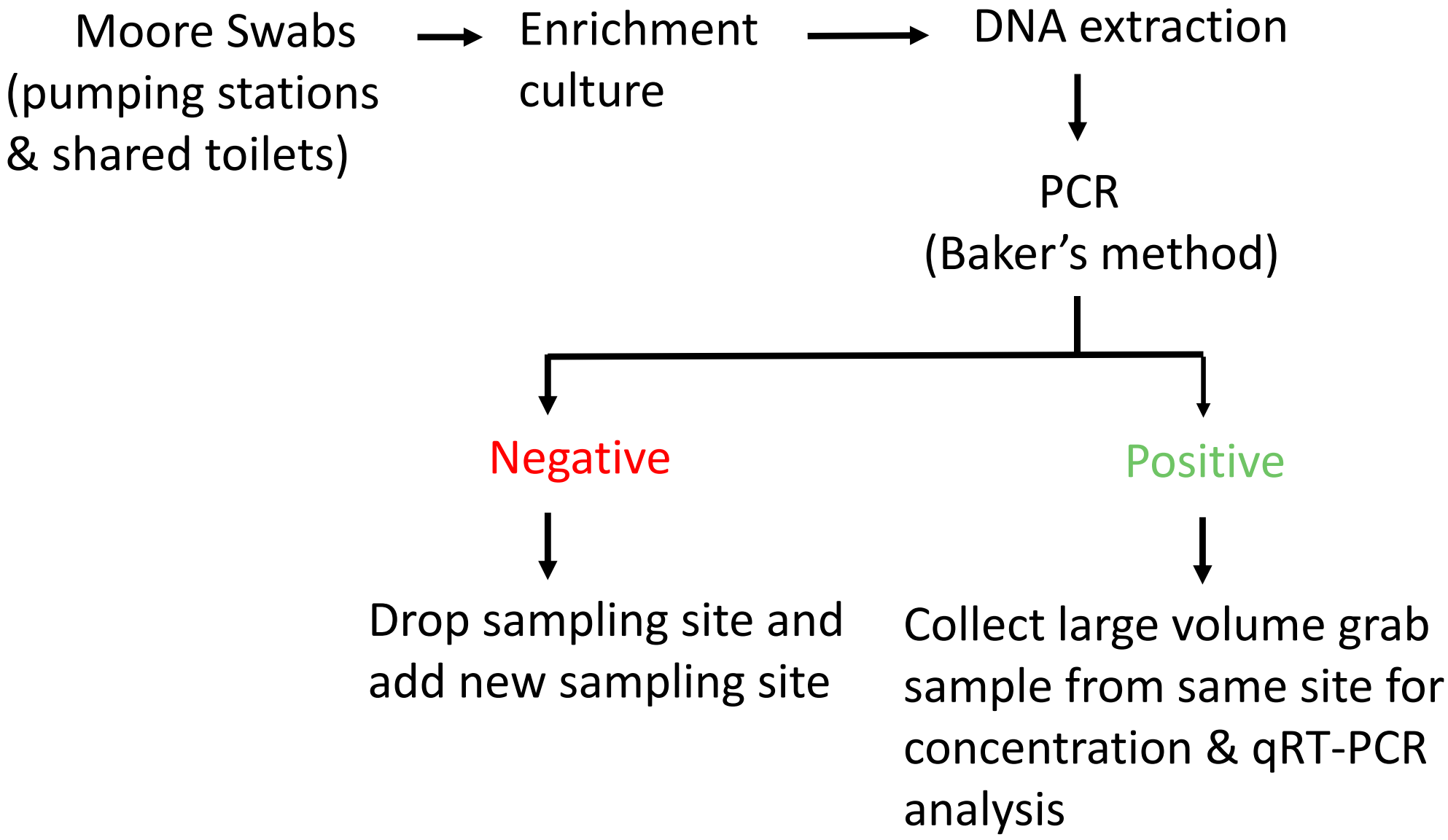
SaniPath-Typhoid Exposure Assessment

Next Steps: Moore Swab Pilot April 2019-June 2019

Sampling sites

- 5 polio environmental surveillance sites
- 25 pumping stations
- Shared latrines in Wards 58 & 59 to complement active clinical surveillance
- Weekly samples

Next Steps: Test Collection and Analyses of Moore Swabs as a Screening Method



Next Steps :
City-Wide Surveillance July 2019 - June 2020

Sampling Sites:

- 5 polio environmental surveillance sites
- 25 sewage pumping stations across city
- 20? shared latrines in Wards 58 & 59 to complement active clinical surveillance

Sampling Frequency:

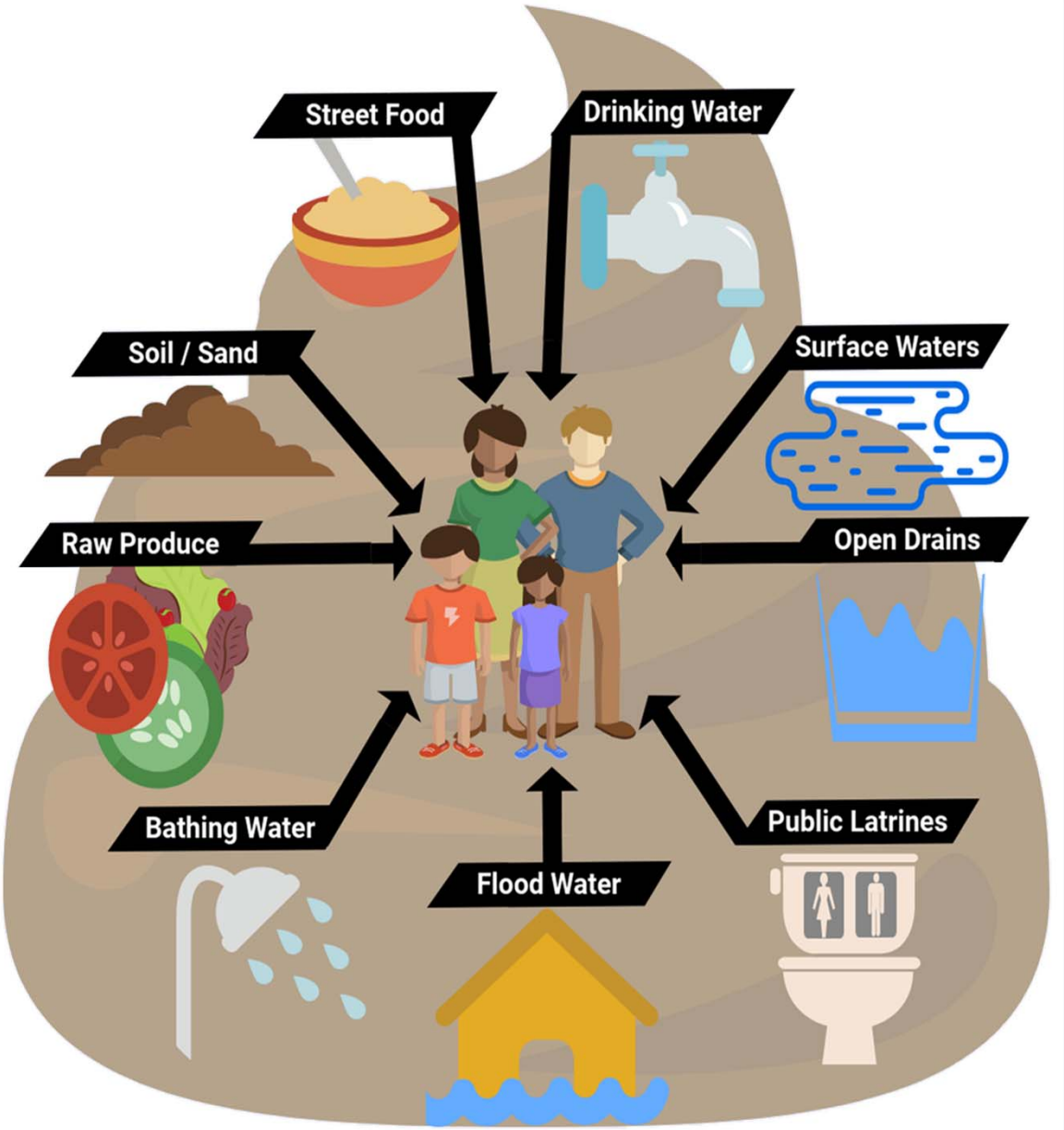
- Weekly samples?
- Moore swabs followed by large volume samples?

Lab Methods:

- qRT-PCR with Baker primers or other primers?
- Confirm % of presumptive positives by sequencing

Urban environments have multiple risks….

Which exposures pose the greatest risk for typhoid transmission?



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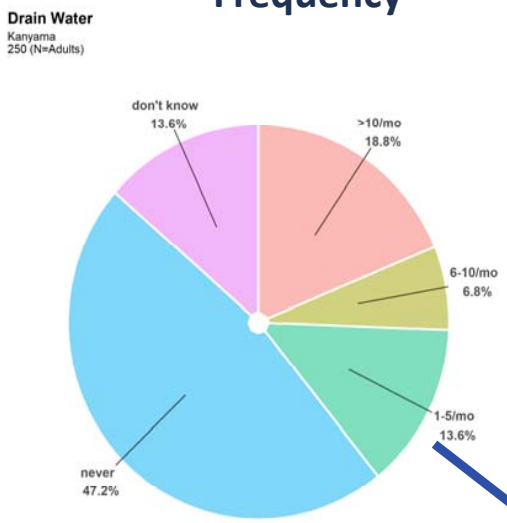
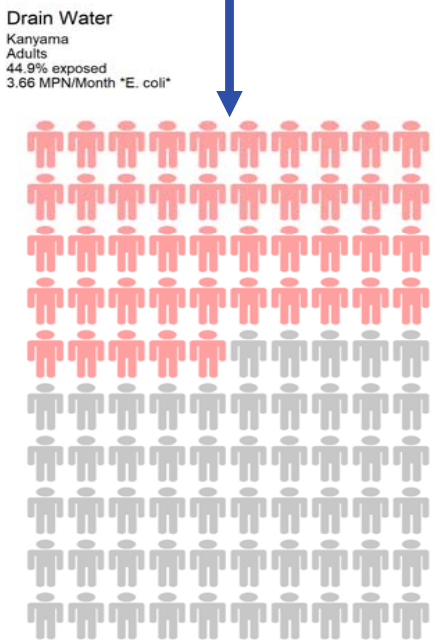
Estimating Exposure to Fecal Contamination

Behavior Frequency



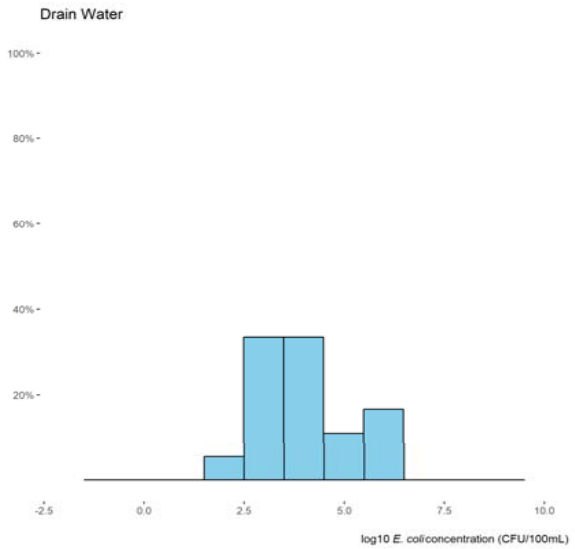
Tool uses Bayesian analysis to estimate the distribution of environmental contamination and frequency of exposure.

Other parameters: intake volumes, duration of exposure, etc.



Results are presented in a normalized and comparable unit – Dose as MPN *E. coli* **ingested** per month

Environmental Contamination



The mean dose and proportion of the population exposed are summarized from simulated distributions and displayed in risk profiles.

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Please see SaniPath.org

SaniPath-Typhoid Exposure Assessment

- Conduct structured observations, focus group discussions, GPS tracking of peak typhoid age groups to get more detailed behavior information

  eg. Street food consumption, surface water contact

- Collect relevant environmental samples and test for *E. coli*, phage markers for human feces, and *S*. Typhi and *S*. paratyphi A
- Bayesian modeling to develop city-level risk profiles for typhoid and paratyphoid and identify key transmission

Dhaka, March 2017

SaniPath

SaniPath

Thank You

For more information
visit **SaniPath.org**

@SaniPath

This study is made possible through the generous support of the Bill & Melinda Gates Foundation. Special thanks to Megan Carey.

Moving forward…

1. Continue to develop and test lab methods in Atlanta using environmental samples seeded with known amounts of *S*. Typhi and *S*. Paratyphi A
2. Test lab methods in Kolkata using seeded environmental samples
3. Pilot environmental surveillance using best lab methods on field samples in Kolkata. Attempt to sequence presumptive positives.
4. Examine field results while simultaneously working to improve lab methods
5. Repeat steps 2-4