COALITION AGAINST TYPHOID a program of the Sabin Vaccine Institute

Development of a Low-Cost Environmental Surveillance Method for Effective Typhoid Fever Control

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Child Health Research Foundation (CHRF)

Bangladesh বাংলাদেশ

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Typhoid is a big problem

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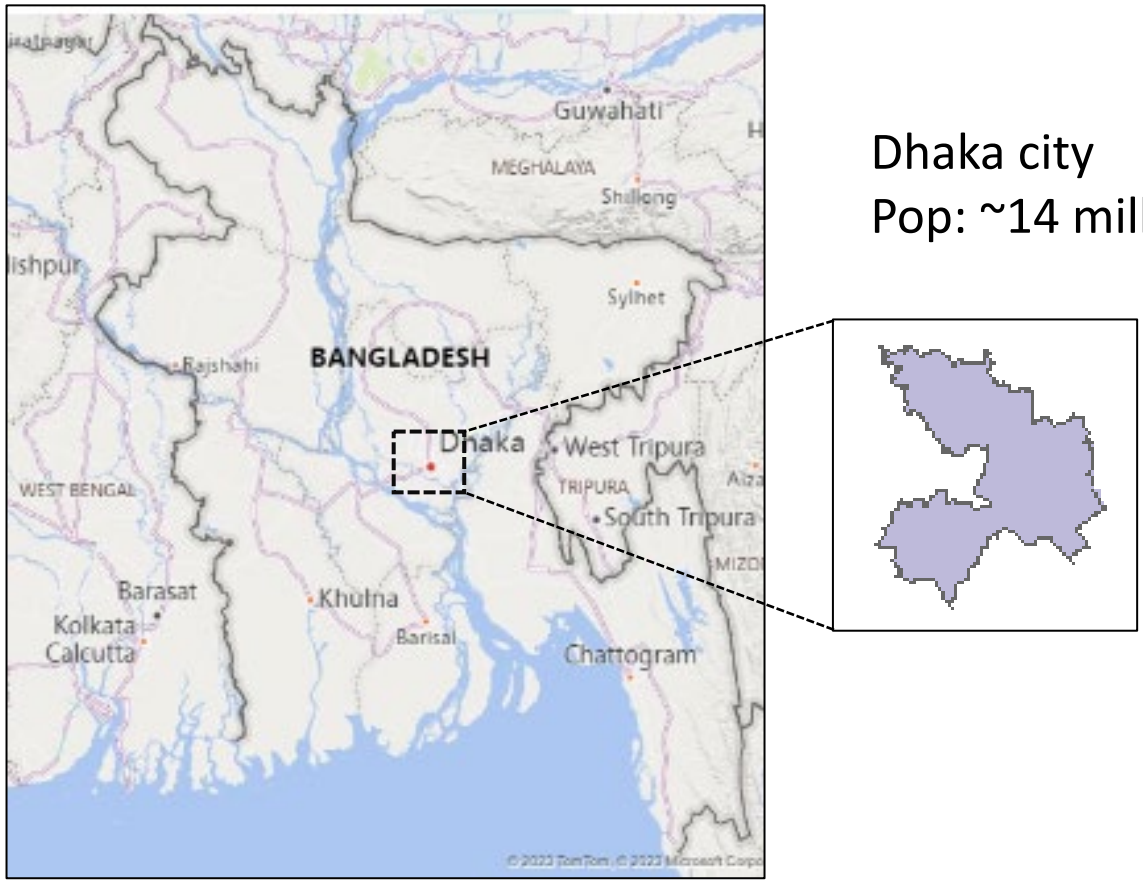


Dhaka city
Pop: ~14 million

Surveillance for Enteric Fever in Asia Project

- 913 per 100 000 person per year in Dhaka
  - 242 per 100 000 person-years were hospitalized
- Highest incidence was among 2-4 years old children

To utilize the vaccines effectively, we need accurate data

Blood Culture surveillance

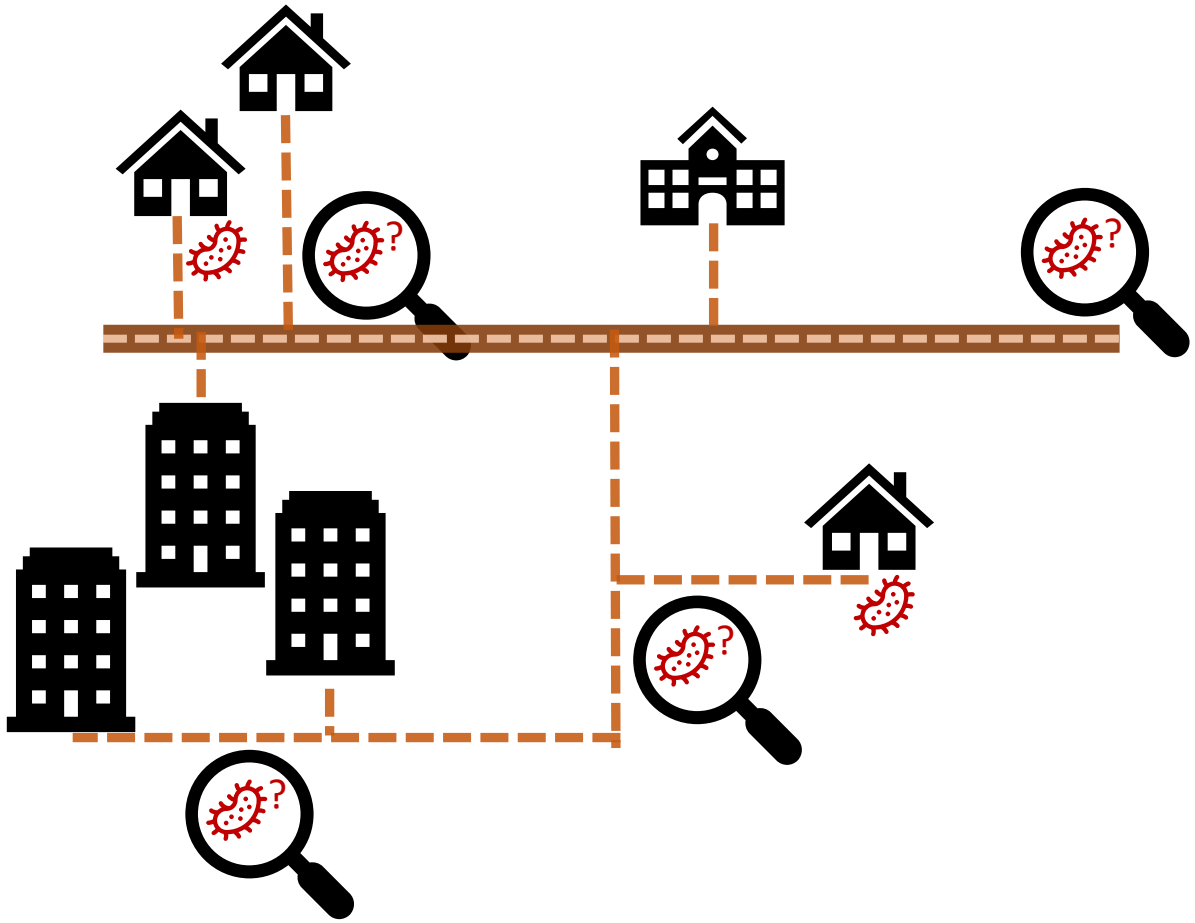
- Sensitivity is ~ 60%
- Can be costly for majority of the patients
- Requires human enrollment

Considering *Salmonella* Typhi is a human restricted pathogen, shed in stool and transmitted by contaminated water

Might environmental surveillance be a supplementary method to estimate burden of *Salmonella* Typhi?

Environmental Surveillance

Typhoid cases shed *S.* Typhi in stool



Test environmental water samples for *S.* Typhi DNA

Presence of *S.* Typhi DNA correlates with Typhoid burden

Am. J. Trop. Med. Hyg., 100(1), 2019, pp. 43–46
doi:10.4269/ajtmh.18-0428

Evaluating PCR-Based Detection of *Salmonella* Typhi and Paratyphi A in the Environment as an Enteric Fever Surveillance Tool

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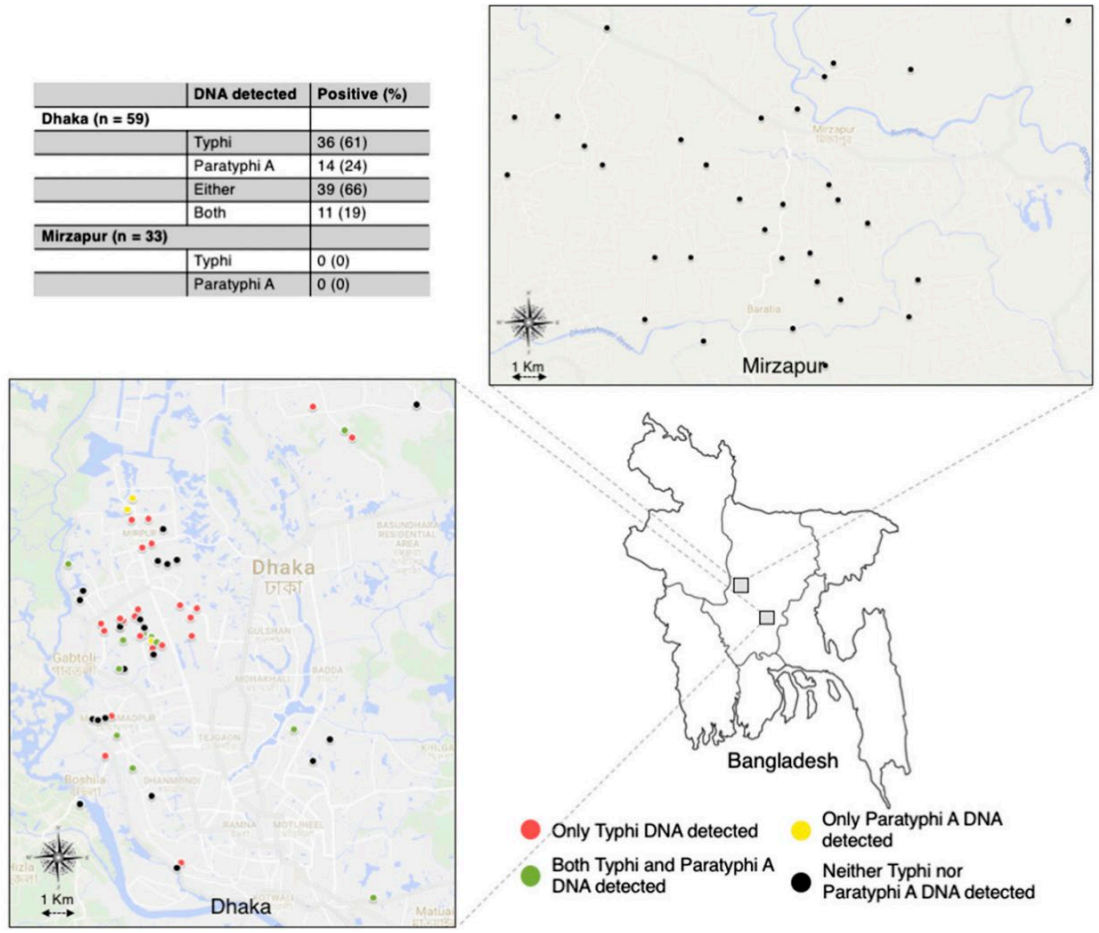
Abstract. With prequalification of a typhoid conjugate vaccine by the World Health Organization, countries are deciding whether and at what geographic scale to provide the vaccine. Optimal local data to clarify typhoid risk are expensive and often unavailable. To determine whether quantitative polymerase chain reaction (qPCR) can be used as a tool to detect typhoidal *Salmonella* DNA in the environment and approximate the burden of enteric fever, we tested water samples from urban Dhaka, where enteric fever burden is high, and rural Mirzapur, where enteric fever burden is low and sporadic. Sixty-six percent (38/59) of the water sources of Dhaka were contaminated with typhoidal *Salmonella* DNA, in contrast to none of 33 samples of Mirzapur. If these results can be replicated in larger scale in Bangladesh and other enteric fever endemic areas, drinking water testing could become a low-cost approach to determine the presence of typhoidal *Salmonella* in the environment that can, in turn, guide informed-design of blood culture-based surveillance and thus assist policy decisions on investing to control typhoid.

Enteric fever, caused by infection with *Salmonella* Typhi or Paratyphi A, B, or C (typhoidal *Salmonella*), is among the most common bacterial causes of morbidity worldwide, with the greatest burden occurring in low- and middle-income countries (LMICs).[1] However, estimates of enteric fever incidence suffer from coarse geographical and temporal resolution, because of a lack of surveillance systems for these diseases. This paucity of incidence data is in part because traditional surveillance requires population-based surveillance, which is resource intensive, requiring both robust laboratory infrastructure and population-based clinical data collection with substantial numbers of participants. Consequently, very few

environmental, water-based surveillance strategy could help fill this knowledge gap by leveraging the important role that water has in *Salmonella* Typhi/Paratyphi A transmission.[5–8] If areas with high detectable levels of typhoid in the water supply overlap with areas of typhoid disease, then sampling water could be utilized as a preliminary surveillance proxy that can guide informed selection of geographical locations for blood culture surveillance and assist policy decisions on investing to control typhoid.

There are no established methods to reliably isolate *Salmonella* Typhi/Paratyphi A from water. Recently, a quantitative real-time PCR (qPCR)–based method was used to demonstrate the

	DNA detected	Positive (%)
Dhaka (n = 59)		
	Typhi	36 (61)
	Paratyphi A	14 (24)
	Either	39 (66)
	Both	11 (19)
Mirzapur (n = 33)		
	Typhi	0 (0)
	Paratyphi A	0 (0)



Saha *et al,* AJTMH. 2019

Limitations behind PCR-based molecular diagnostics

- Do not prove if viable bacteria is present
- No information on AMR
- Large amounts of water is needed

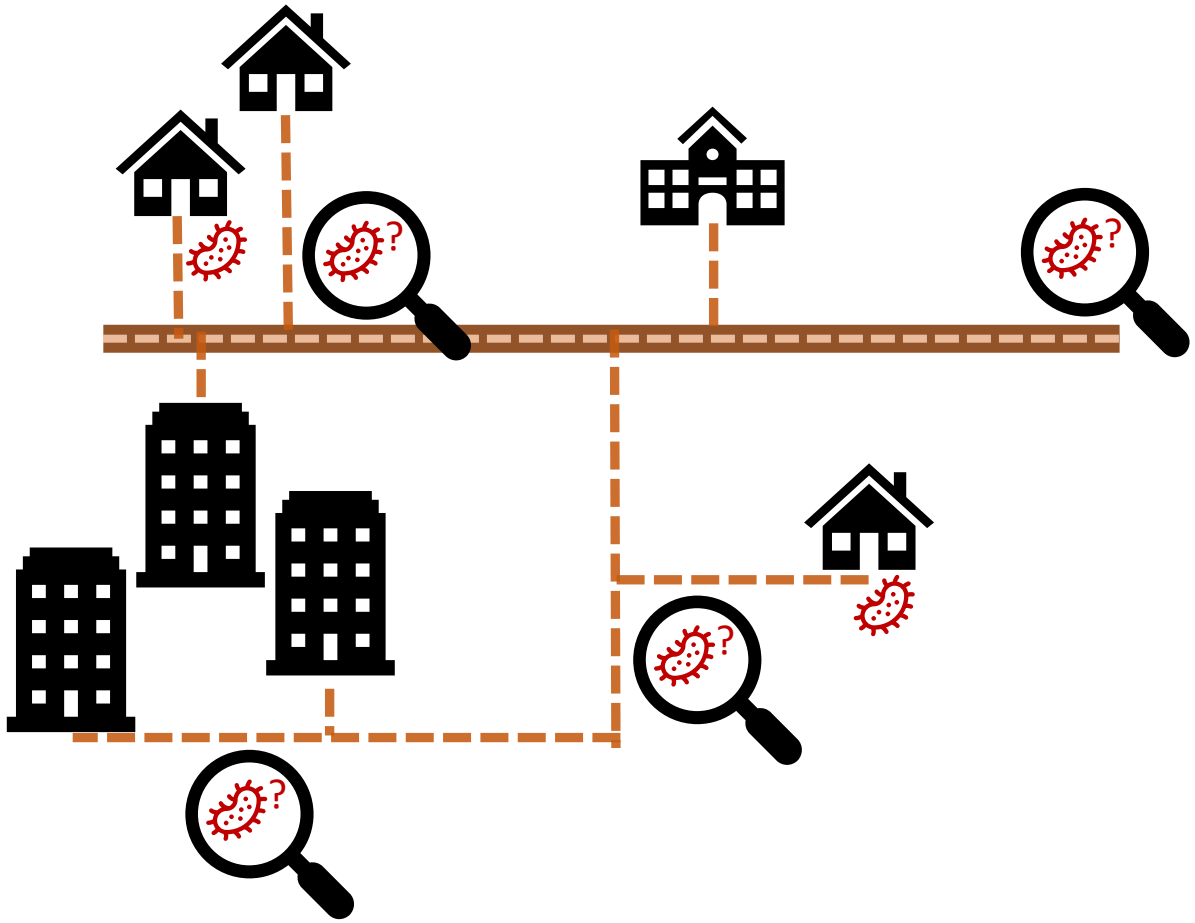
  Filtration of large amounts of water is cumbersome, hard to scale

- Requires molecular set up

  PCR, maintenance, technical skills, etc

Environmental Surveillance

Typhoid cases shed *S.* Typhi in stool

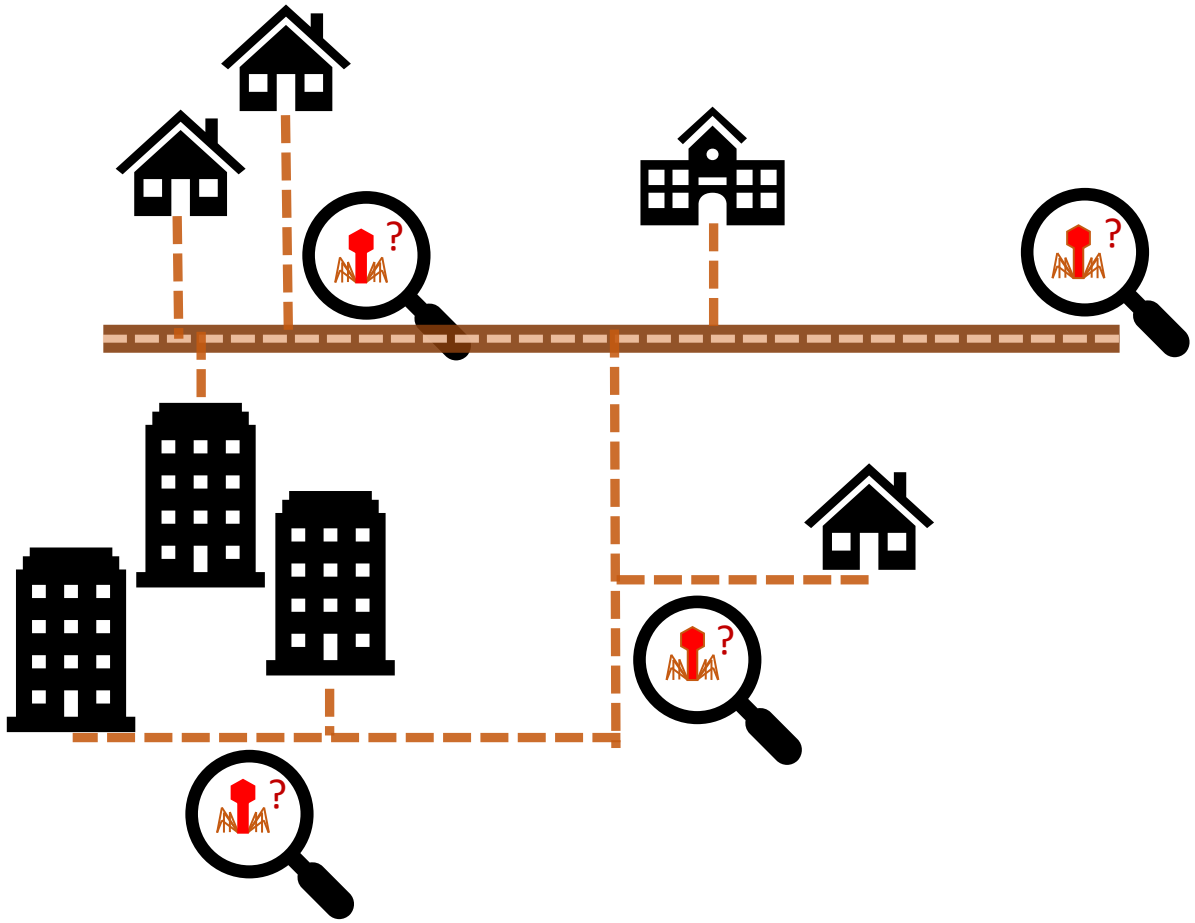


Test environmental water samples for *S.* Typhi DNA

Environmental Surveillance

Dr. Jason Andrews
Stanford University, USA

Typhoid cases shed *S.* Typhi in stool

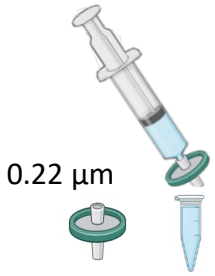


Test environmental water samples for *S.* Typhi Phages

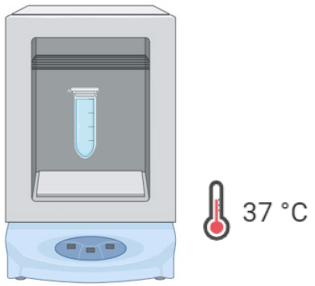
Funded by BMGF

Sample collection & phage detection

Collect environmental water
(Sewage, lakes, rivers, ponds, stagnant water)

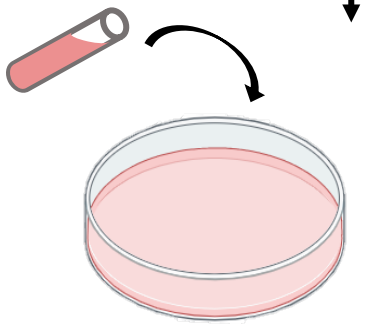


Filtration to remove
bacteria and large particles

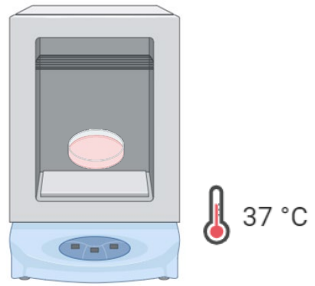


Incubation with **BRD948**
for phage enrichment

Spin to remove debris



DLA method to lawn



Overnight incubation

Dilute to isolate phages
(check plaque morphology)

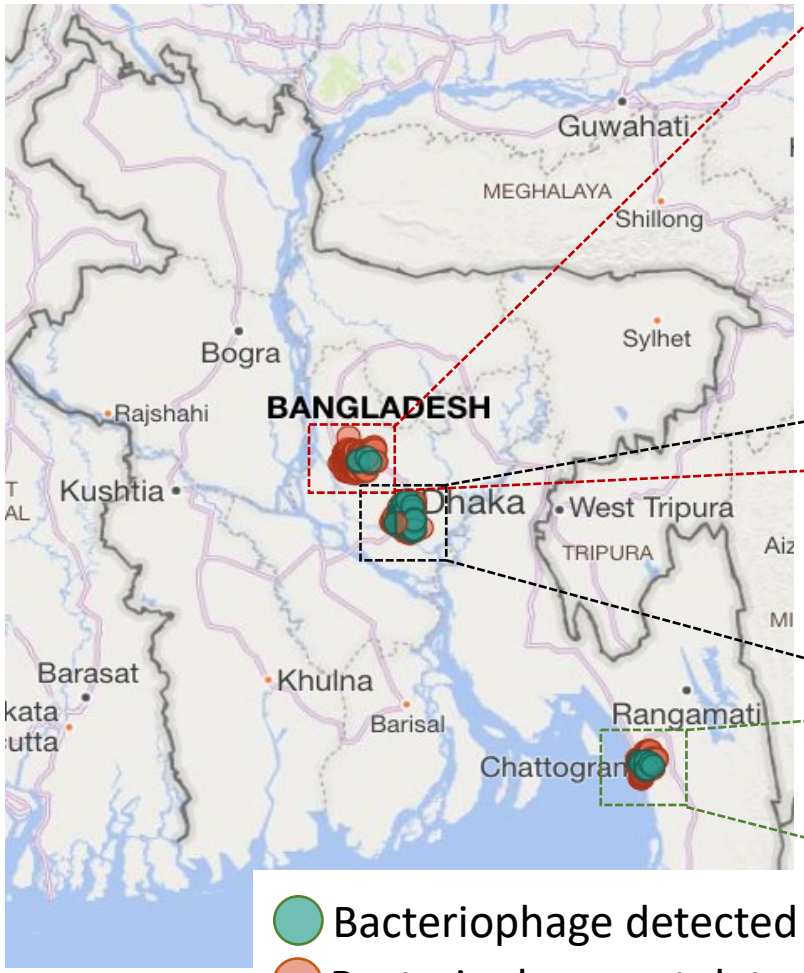
***S.* Typhi BRD948 = laboratory strain (Dr. Andrews Lab)**

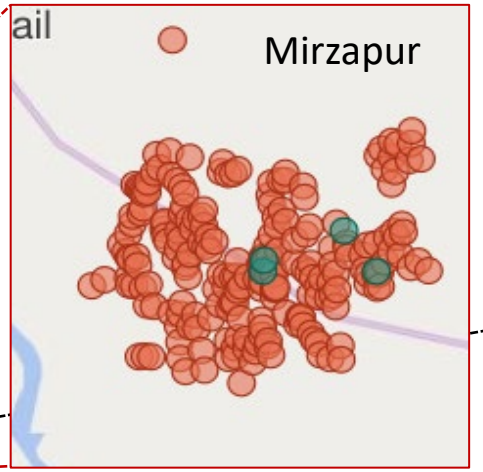
Hooda & Islam *et al* MedRxiv 2023,
Shrestha & Da Silva *et al* MedRxiv 2023

Isolation of phages

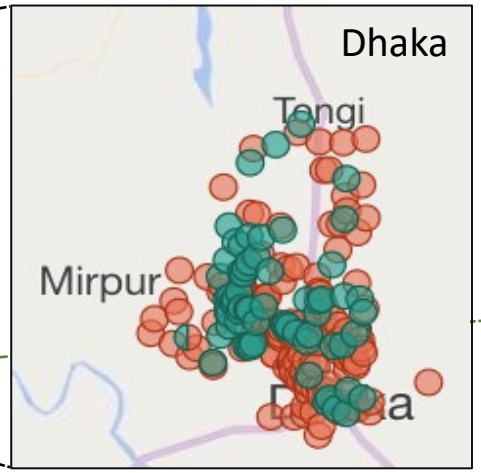
	Dhaka			Mirzapur			Chittagong			Total		
	N	Total Collection	Phages	N	Total Collection	Phages	N	Total Collection	Phages	N	Total Collection	Phages
Total	**67**	**212**	**83**	**4**	**316**	**5**	**23**	**275**	**25**	**94**	**803**	**113**

Typhi phages correlated with typhoid burden

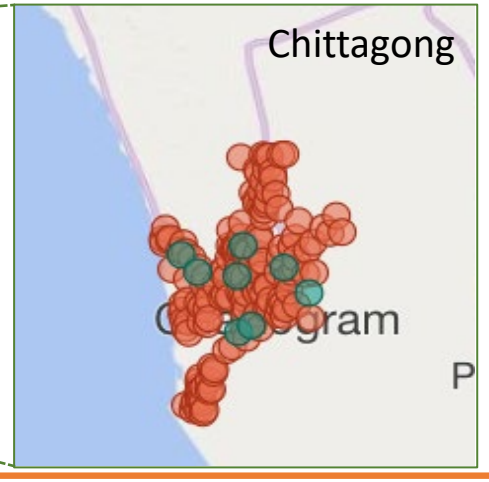




Blood culture +% = 0.1%
(n = 3,788)
Phage +% = 1.3%
(n = 316)



Blood culture +% = 4.7%
(n = 4,620)
Phage +% = 31.8%
(n = 212)



Blood culture +% = 0.6%
(n = 2,789)
Phage +% = 8.4%
(n = 275)

Bacteriophage detected
Bacteriophage not detected

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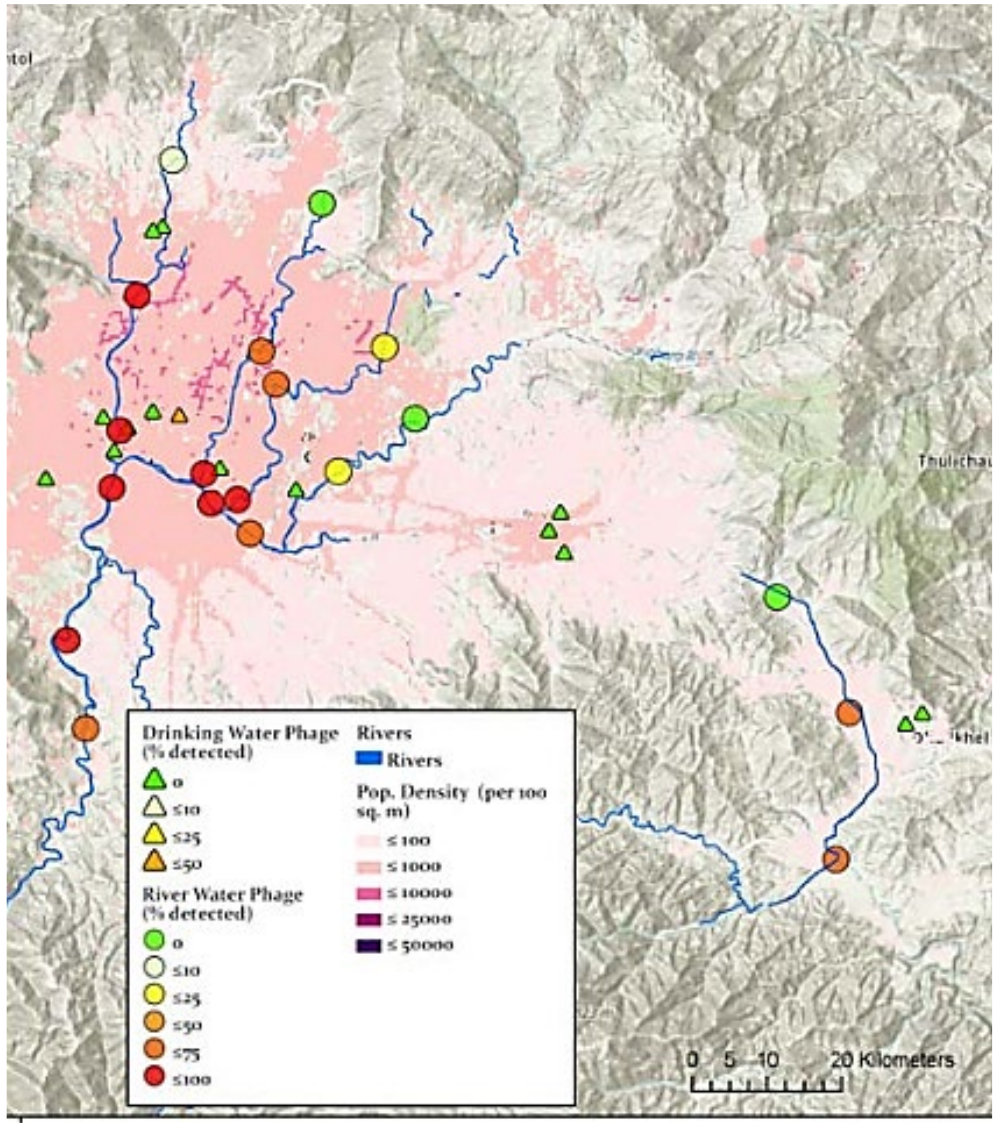
Phages in neighboring countries

Detection of *Salmonella* Typhi bacteriophages in surface waters as a scalable approach to environmental surveillance

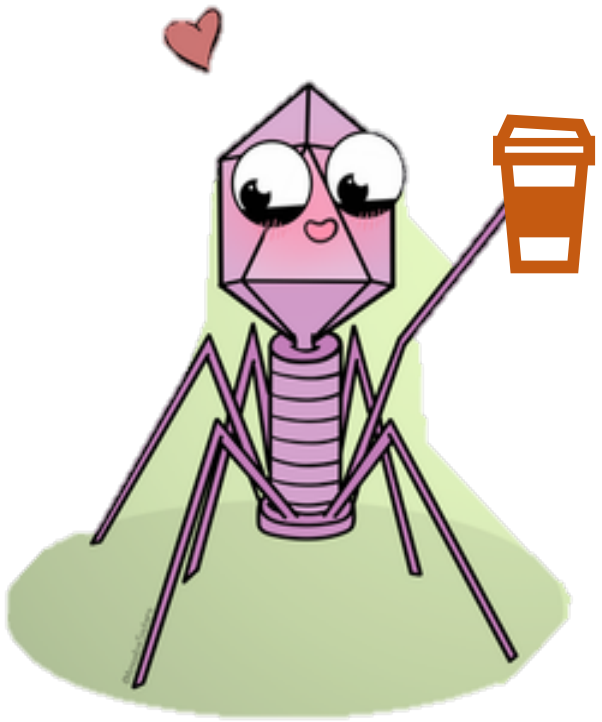
Sneha Shrestha, Kesia Esther Da Silva, Jivan Shakya, Alexander T. Yu, Nishan Katuwal, Rajeev Shrestha, Mudita Shakya, Sabin Bikram Shahi, Shiva Ram Naga, Christopher LeBoa, Kristen Aiemjoy, Isaac I. Bogoch, Senjuti Saha, Dipesh Tamrakar, Jason R. Andrews



Shrestha & Da Silva *et al* MedRxiv 2023

Phages have diverse killing spectra

Takeaway



Credit: ameobasisters

- Good correlation between phage positivity vs clinical cases in an area
- Cost effective
  - Less than 10 mL of sewage water required
  - Less resources required
- Phages can be a rapid and low-cost surveillance tool

Future directions

- Expand the surveillance throughout Bangladesh
- Studying Typhi Dynamics in the environment
- Study the role of phages in the spread of drug-resistant *S.* Typhi

Mapping typhoid fever in Bangladesh using environmental surveillance

Al Amin, Abstract: 07

Exploring Diversity and Environmental Dynamics of *Salmonella* Typhi and its Bacteriophages

Rathindranath Kabiraj, Abstract: 48

Environmental surveillance to unravel the spatiotemporal dynamics of *Salmonella* Typhi bacteriophages in Dhaka

Sadnane Hussain Pranto, Abstract: 93

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Thank You!!

Jason Andrews, Stanford
Kesia D. Silva, Stanford