The Journal of Infectious Diseases

MAJOR ARTICLE

Environmental surveillance for *Salmonella* Typhi and its association with typhoid fever incidence in India and Malawi

Christopher B. Uzzell[1*], Dilip Abraham[2*], Jonathan Rigby[3*], Catherine M. Troman[1*], Satheesh Nair[4], Nicola Elviss[5], Lalithambigai Kathiresan[6], Rajan Srinivasan[6], Veeraraghavan Balaji[7], Nicolette A. Zhou[8], John Scott Meschke[8], Jacob John[6], Gagandeep Kang[2], Nicholas Feasey[3,9], Venkata Raghava Mohan[6], Nicholas C. Grassly[1]

[1]Department of Infectious Disease Epidemiology, Imperial College London, London, UK; [2]Division of Gastrointestinal Sciences, Christian Medical College, Vellore, India; [3]Malawi-Liverpool Wellcome Programme, Kamuzu University of Health Sciences, Blantyre, Malawi; [4]Gastrointestinal Bacteria Reference Unit, UK Health Security Agency, Colindale, London, UK; [5]Science Group, UK Health Security Agency, London, UK; [6]Department of Community Health, Christian Medical College, Vellore, India ; [7]Division of Antimicrobial Resistance and Surveillance, Christian Medical College, Vellore, India ; [8]Department of Environmental and Occupational Health Sciences, School of Public Health, University of Washington, Seattle, USA; [9]Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK

Background Environmental surveillance (ES) for *Salmonella* Typhi potentially offers a low-cost tool to identify communities with a high burden of typhoid fever.

Methods We developed standardised protocols for typhoid ES, including sampling site selection, validation, characterisation; grab or trap sample collection, concentration; and quantitative PCR targeting *Salmonella* genes (*ttr*, *staG* and *tviB*) and a marker of human faecal contamination (HF183). ES was implemented over 12-months in a historically high typhoid fever incidence setting (Vellore, India) and a lower incidence setting (Blantyre, Malawi) during 2021-2022.

---

*joint first authors
Corresponding author: Nicholas Grassly, Sir Michael Uren Building, White City Campus, 80–92 Wood Lane, London W12 0BZ, United Kingdom. E-mail: n.grassly@imperial.ac.uk
Alternative corresponding author: Christopher Uzzell, address as above, e-mail: c.uzzell@imperial.ac.uk

Results *S.* Typhi prevalence in ES samples was higher in Vellore compared with Blantyre; 39/520 (7.5%, 95% Confidence Interval 4.4-12.4%) vs. 11/533 (2.1%, 1.1-4.0%) in grab and 79/517 (15.3%, 9.8-23.0%) vs. 23/594 (3.9%, 1.9-7.9%) in trap samples. Detection was clustered by ES site and correlated with site catchment population in Vellore but not Blantyre. Incidence of culture-confirmed typhoid in local hospitals was low during the study and zero some months in Vellore despite *S.* Typhi detection in ES.

Conclusions ES describes the prevalence and distribution of *S.* Typhi even in the absence of typhoid cases and could inform vaccine introduction. Expanded implementation and comparison with clinical and serological surveillance will further establish its public health utility.

Keywords: Salmonella Typhi, typhoid fever, typhoid-paratyphoid vaccines, wastewater surveillance, India, Malawi

BACKGROUND

Over the last 30 years, the global incidence of typhoid fever, caused by *Salmonella enterica* serovar Typhi (*S.* Typhi), has halved because of improvements in water and sanitation and widespread use of effective antibiotics. However, there were still an estimated 10.9 million cases and 117,000 deaths globally in 2017, mainly in Africa and south and southeast Asia [1]. Moreover, reductions in disease incidence are at risk of being reversed with the emergence of extensively resistance (XDR) *S.* Typhi in Asia and increasing detection of strains with reduced susceptibility to the commonly used oral antibiotic, azithromycin [2, 3].

Fortunately, two highly effective typhoid conjugate vaccines (TCVs) have recently been prequalified for global use by the World Health Organisation (WHO) (in 2017 and 2020 respectively) [4, 5]. WHO recommends the introduction of TCV to control typhoid fever, prioritizing countries with the highest burden of disease or a high burden of antimicrobial resistant *S.* Typhi, with national decisions on the preferred vaccination strategy (universal, risk-based or phased) based on local surveillance data [6].

The gold standard for surveillance of typhoid fever is blood culture of suspected cases but such data are severely limited, reflecting the cost and challenges with establishing and sustaining blood culture capabilities in low-resource settings [7]. *S.* Typhi is, however, shed in faeces and spread by faecal contamination of food and water [8]. Environmental surveillance (ES), based on testing sewage and wastewater for *S.* Typhi, has therefore been suggested as a low-cost surveillance tool to identify *S.* Typhi circulation and inform national decision-making about the introduction of TCV, and where phased introduction is necessary, enabling highest risk areas to be targeted first [9, 10].

Previous methods employed to detect *S.* Typhi in the environment have varied considerably, including ES site selection, and sample collection, concentration and laboratory processing [11].

Alternative methods vary in sensitivity to detect *S.* Typhi, making it difficult to interpret different levels of detection in the environment [12]. Several studies have used PCR or culture to detect *S.* Typhi in ES wastewater samples in areas where disease is common [13-16]. However, the relationship between the prevalence and abundance of *S.* Typhi detection in ES samples and the incidence of typhoid fever in the local community is unknown.

We developed a set of standardised protocols for typhoid ES, covering each stage of implementation from ES sample site selection, through sample collection to laboratory processing and PCR strategy [17]. We evaluated these protocols, including two alternative sampling methods, over 12 months in Vellore, India and Blantyre, Malawi. Here we report a comparison of *S.* Typhi detection in ES samples in the two settings, and on the relationship with clinical disease incidence, to evaluate the potential utility of ES as a low-cost tool for typhoid surveillance.

METHODS

Study design

We compared the prevalence of *S.* Typhi in ES samples in a very high typhoid fever incidence community (Vellore, India) with that in a lower incidence community (Blantyre, Malawi). The study areas in Vellore and Blantyre were selected to cover the major urban populations served by hospital-based typhoid disease surveillance. A total of 141,800 people were living in the Vellore study area (16 km^2) served by the Christian Medical College Hospital and its two satellite facilities. The Blantyre study area was considerably larger (214 km^2) with 489,900 people served by the Queen Elizabeth Central Hospital. To identify a significant difference in the prevalence of *S.* Typhi between the two communities, we estimated that we would require monthly samples from approximately 40 ES sites in each community over one year. Full details of the study design, study areas and sample size calculations are provided in the published study protocol [17]. Study protocols covering grab and Moore swab sampling, swab construction, DNA extraction and qPCR are available at https://www.protocols.io/workspaces/typhoides.

ES site selection

Candidate ES sites (sampling points) were systematically identified at wastewater confluence points using remote geographic information systems analysis based on environmental data [17]. In brief, geospatially referenced 'blue-line' data on sewage drainage channels (Vellore) and river systems (Vellore and Blantyre) were integrated with high-resolution digital elevation models to identify candidate ES sites, delineate hydrological catchments and estimate their associated populations using the High-Resolution Settlement Layer [18]. Sites were selected to represent a range of catchment population sizes across the study area. Once identified, candidate sites were

visited by the field team and their suitability for inclusion assessed based on accessibility, safety, and adequate flow (sample site questionnaire).

Sample collection and processing

Monthly grab and trap (Moore swab) samples were collected for one year beginning in May 2021 at each ES site according to the study protocols [12, 17]. Moore swabs were deployed 48 hours prior to collection. Grab samples (1000 ml) were then collected at the same time as the Moore swabs, with a recommended collection time between 6am and 10am. Moore swabs were incubated overnight in universal pre-enrichment (UPE) broth before filtration (0.45µm) and storage of the filter disc at -20C prior to DNA extraction. Grab samples were filtered (0.45µm) after pre-filtering through a coffee filter, with the filters eluted in Ringer's lactate and the eluate centrifuged to generate a pellet for storage at -20C. Personal protective equipment was worn during sample collection and infection control procedures followed to minimise risks to personnel and the potential for sample contamination.

DNA extraction and PCR strategy

DNA was extracted using the QIAamp Powerfecal Pro kit (Qiagen), eluted into 50µl and stored at -20°C. Multiplex quantitative PCR (qPCR) with primers and probes targeting 3 genes (*ttr*, *staG* (STY0201) and *tviB*) was used to detect *S.* Typhi. Samples with all three targets present were considered positive for *S.* Typhi based on their demonstrated amplification in *Salmonella* species (*ttr*), Typhi and non-typhoidal *Samonella* serovars (*staG*) and Typhi alone (*tviB*) [19]. Samples positive for *ttr* and either *staG* or *tviB* alone were re-tested in a singleplex qPCR for the negative target to improve the sensitivity of *S.* Typhi detection. We also conducted a duplex qPCR for HF183, a marker gene from a human-restricted Bacteroides [20], and an extraction/PCR positive control (Cy®5-QXL®670, Eurogentec) that was spiked into samples before extraction. Genome copy numbers were estimated based on standard curves generated using gBlocks™ DNA fragments for each target (Integrated DNA Technologies). The limit of detection (LOD) was estimated as the concentration that gave a positive result 95% of the time and genome copies calculated for samples above this threshold. Samples with a Ct value below the upper 95% confidence interval of the LOD or 40, whichever was lower, but above the point estimate for the LOD, were scored positive and given a genome copy number equal to the LOD.

Typhoid fever hospital surveillance

The number of blood culture confirmed typhoid fever cases reported from tertiary and secondary hospitals serving the study population (including inpatients and outpatients, see [17]) were recorded during the period of ES. Patient age, date of culture, and location of residence were extracted for the analysis. These were compared with previous estimates of typhoid fever incidence in the study areas based on community surveillance (Vellore) and hospital cases (with and without adjustment for healthcare seeking and culture provision/sensitivity; Vellore and

4

Blantyre) [21-23]. In Blantyre only, enhanced passive surveillance was conducted during the period of ES in three primary health care centres, including blood culture for children with febrile illness lasting ≥3 days [24].

Statistical analysis

The proportion of ES samples positive for *S*. Typhi (*ttr*, *staG* and *tviB*) was calculated at each location for each sample type and 95% confidence intervals calculated using mixed-effects logistic regression without covariates and a Gaussian random effect on the intercept to allow for repeat testing at each site. The intraclass correlation coefficient (ICC) was calculated from the maximum likelihood estimates of the fixed effect (intercept) and the variance of the random effect using Monte Carlo simulation [25]. Mixed-effects logistic and linear regression analysis was used to assess the association between detection of the different gene targets. Comparison of genome copy numbers for each target was based on rank correlation without correction for repeated measures because the relatively small number of samples positive for 2 or more targets did not allow the random effect by ES site to be estimated. The association of monthly ES detections in the study areas and clinical case numbers was assessed using binomial regression. Analysis was implemented in the R Statistical Programming language using the survey and ICCbin packages [26].

The crude incidence of culture confirmed typhoid fever per 100,000 person years of observation in each location was calculated based on the number of cases in each age group recorded during the study period and the estimated population size within the study area. Exact 95% confidence intervals were calculated based on the Poisson distribution. We did not estimate incidence in the community from these numbers using multipliers to adjust for healthcare seeking and culture availability [27]. This is because of the absence of contemporaneous healthcare utilization data and the likely large, uncertain adjustments that would be necessary during the COVID-19 pandemic when culture testing of febrile cases was severely limited.

Ethical approvals

The study protocol was reviewed by the ethics committee at Christian Medical College, Vellore (IRB 11170 (OBSERVE)) and College of Medicine Research Ethics Committee in Blantyre (COMREC P.07/0/3089).

RESULTS

ES sites selected and sample collection

A total of 40 and 43 ES sites, stratified into 3 location-specific catchment population sizes, were selected in Vellore and Blantyre, respectively (Table 1). Estimated catchment populations varied

for both locations, ranging from 14 to 320,441 in Vellore and 1,247 to 106,905 in Blantyre, with the catchments of 3 ES sites in Vellore extending beyond the targeted study area.

In Vellore, 520 grab samples and 517 Moore swabs were collected between 3 May 2021 and 29 April 2022, corresponding to 12 monthly samples from all 40 ES sites with Moore swabs missing at just 3 sampling timepoints (across 2 sites). In Blantyre, 533 grab samples and 594 Moore swabs were collected between 4 May 2021 and 22 May 2022, with more variable sampling frequency reflecting intermittent challenges with access to sites (median 13 (range: 4 to 23) grab samples and 14 (range 3 to 48) Moore swabs from 43 ES sites).

Detection of S. Typhi in ES samples

In Vellore, 39 (7.5% [95% Confidence Interval 4.4-12.4%]) grab samples and 79 (15.3% [9.8-23.0%]) Moore swabs tested positive for *S.* Typhi. Rates of detection were lower in Blantyre with 11 (2.1% [1.1-4.0%]) grab samples and 23 (3.9% [1.9-7.9%]) Moore swabs positive. The ICC was 0.11 and 0.29 for grab samples and Moore swabs in Vellore, and 0.02 and 0.06 respectively in Blantyre. This indicates a moderate degree of clustering of detection of *S.* Typhi at specific ES sites in Vellore, as indicated on the map (Figure 1). The detection of *S.* Typhi in grab samples and Moore swabs was correlated with the estimated catchment population size for each ES site in Vellore (fixed effect of log number of people in mixed effects logistic regression was 0.622 and 0.944 respectively, p-values 0.031 and 0.005). In Blantyre there was no significant association with estimated catchment population (p-values = 0.760 and 0.445 respectively). The higher prevalence of detection of *S.* Typhi in Moore swabs compared with grab samples was significant in Vellore but not Blantyre (mixed effects regression p-values <0.001 and 0.158 respectively).

Detection of the *staG* gene target was correlated with the detection of the *tviB* target (phi coefficients of 0.71 and 0.61 for grab samples and Moore swabs respectively in Vellore (mixed-effect logistic regression test p-values <0.001); 0.66 and 0.75 respectively in Blantyre (p-values <0.001; Table 2)). Detection of the pan-*Salmonella* target *ttr* showed a modest association with detection of either *staG* or *tviB* (phi coefficients of 0.35 and 0.29 for these two targets in grab samples and 0.14 and 0.12 respectively in Moore swabs in Vellore, 0.61 and 0.47 in grab samples and 0.70 and 0.57 in Moore swabs in Blantyre; mixed-effect logistic regression all p-values <0.05 except for the association of *ttr* with *staG* in grab samples in Vellore). Among those samples that were positive for all three targets, genome copy numbers inferred from the *tviB* and *staG* targets were significantly correlated (rank correlation coefficients 0.542 and 0.478, p-values < 0.001 for grab samples and Moore swabs respectively in Vellore, 0.818 and 0.542, p-values 0.004 and 0.009, in Blantyre; Supplementary Figure 1). Overall mean log genome copy number inferred for *Salmonella* species based on the *ttr* target were higher for Moore swabs compared with grab samples in Vellore (linear mixed effects regression, p-value <0.001; Table 2). There were no significant differences for the *staG* or *tviB* targets (p-values 0.052 and 0.824

respectively). Fewer samples were positive in Blantyre and there were no significant differences in the copy numbers by sample type for any target.

In Vellore, 514/520 (98.8%) grab samples and 515/517 (99.6%) Moore swabs were positive for the human-restricted Bacteroides HF183. In Blantyre, 345/533 (64.7%) grab samples and 279/594 (47.0%) Moore swabs were positive for HF183. Among those samples positive for HF183 in Blantyre, the prevalence of *S*. Typhi was 11/345 (3.2% [95% CI: 1.7-6.0%]) and 19/279 (6.8% [3.4-13.2%]) for grab samples and Moore swabs respectively. Among these sample types negative for HF183, prevalence of *S*. Typhi was 0/188 (0.0% [0.0-1.9%]) and 4/317 (1.3% [0.5-3.3%]) respectively (p-values for comparison with samples positive for HF183 in mixed effects logistic regression were 0.923 and 0.010 respectively). In Vellore, no samples negative for HF183 were positive for *S. Typhi* (0/8). The copy number of HF183 in ES samples from Blantyre was significantly higher among those that tested positive for *S*. Typhi (linear mixed-effects model p-value = 0.001 for grab samples and <0.001 for Moore swabs).

Monthly prevalence of *S*. Typhi in ES samples varied during the study period in both locations (Figure 2). In Vellore, peak detection was observed in October and November 2021 for grab samples (27.5%) and Moore swabs (24.4%), respectively. In Blantyre, detection amongst Moore swabs was highest between May-August 2021 (3-11%) whilst grab sample positivity remained low throughout the study (<5%).

Incidence of culture-confirmed typhoid fever

Overall typhoid fever case numbers reported in hospitals serving the study areas during the period of ES were low (Table 3). Crude incidence of typhoid fever in Vellore and Blantyre was 7.8 (95% CI: 3.9-13.9) and 18.4 (14.8-22.6) per 100,000 people per year respectively. The highest age-specific incidence was among children 0-4 years old (49.5 and 45.6 per 100,000 person years in Vellore and Blantyre respectively). Inclusion of typhoid fever cases identified at primary health care clinics through enhanced passive surveillance in Blantyre increased overall incidence to 24.9 (20.7-29.7) per 100,000 person years, with peak incidence among children 5-9 years old (58.0 per 100,000 person years). Incidence of cases did not show a clear trend over time in Vellore or Blantyre (Figure 2). The monthly proportion of grab samples and Moore swabs positive for *S*. Typhi was not associated with the monthly incidence of typhoid fever in Vellore (binomial regression, p-values = 0.649 and 0.513 respectively). In Blantyre, the monthly proportion of Moore swabs positive showed a negative association with typhoid fever incidence (p=0.008 or 0.003 without or with cases from primary health care enhanced surveillance respectively) but there was no association for grab samples (p=0.705 or 0.841).

DISCUSSION

We present results from a comprehensive implementation of environmental surveillance of *Salmonella* Typhi in two cities with contrasting characteristics. *S.* Typhi was detected in multiple samples collected in Vellore, India and Blantyre, Malawi during 2021-2022 indicating continuous, local circulation. Prevalence in the environment was higher in Vellore compared with Blantyre, consistent with the higher incidence of clinical disease estimated in recent surveillance studies in these two locations. In Vellore, annual incidence was approximately 2,000 cases per 100,000 children aged 0-14 years during 2017–2020 compared with approximately 700 during 2016–2018 in Blantyre, after adjusting for blood culture sensitivity, the probability of receiving a blood culture and healthcare seeking [21, 22]. Prevalence in Vellore remained higher after adjusting estimates to include only samples with evidence for human faecal contamination (HF183).

During the study period, the number of typhoid cases reported in hospitals serving both study areas was substantially lower compared with previous years, particularly in Vellore where just 11 cases were reported compared with 98 during 2015 (meaning incidence was unexpectedly lower than Blantyre) [23, 28]. This may reflect genuine changes in transmission but also the impact of COVID-19 on healthcare seeking and provision of blood culture to febrile individuals. In the absence of contemporaneous healthcare utilisation surveys and data on blood culture provision it is not possible to estimate the magnitude of this impact or whether COVID-19 has influenced *S.* Typhi transmission. The low case numbers reported during our study period meant we had limited power to detect an association between monthly ES detection of *S.* Typhi, and in Blantyre clinical incidence unexpectedly showed a modest negative association with *S.* Typhi detection in Moore swabs, but not grab samples. This may reflect differential effects of rainfall or other seasonal factors on ES sensitivity, or variability in healthcare seeking and testing during the single year of surveillance.

The detection of *S.* Typhi in Vellore showed moderate clustering by ES site with a higher prevalence of detection in the west and in northern sites draining from this community (Figure 1). ES sites with larger catchment populations in Vellore were also more likely to have *S.* Typhi detected. Overall prevalence of *S.* Typhi was lower in Blantyre and there was no evidence for clustering or association with catchment population.

Three gene targets were chosen to identify *S.* Typhi in ES samples. In brief, *ttr* is a pan-*Salmonella enterica* gene, *tviB* is specific to *S.* Typhi and *S.* Paratyphi C, and *staG* is highly sensitive for *S.* Typhi but also found in non-typhoidal *Salmonella* serovars [19]. It is therefore reasonable to assume that co-occurrence of all three targets indicates the presence of *S.* Typhi. Although in theory an ES sample could be positive if it contained a mixture of *S.* Paratyphi C and non-typhoidal *Salmonella*, this is unlikely given the rarity of *S.* Paratyphi C [29].

Moore swabs deployed for 48 hours were more likely to result in the detection of *S.* Typhi compared with grab samples. The continued immersion of the swabs is likely to facilitate trapping of *S.* Typhi, which may be intermittently present in wastewater. Enrichment in UPE may also contribute to the enhanced sensitivity of the Moore swab protocol, which complicates interpretation of the inferred genome copy number. Grab samples have a theoretical advantage of allowing quantification of bacterial load by sample volume, but variable flow rates because of dilution by rainfall, uncertainty in catchment population and more limited sensitivity suggest trap is preferable to grab samples for *S.* Typhi ES.

Our study was limited by the lack of information about community incidence of typhoid fever during the ES period and by inclusion of just two communities. This highlights the limitations of clinical surveillance, even in areas with support for hospital-based surveillance, because case numbers are strongly affected by healthcare seeking behaviour, resources available for blood culture and presumptive treatment with antibiotics. Estimates based on passive clinical surveillance need to be adjusted using imprecise and large multipliers, substantially inflating estimated incidence and the associated uncertainty (e.g. 8.3 times in Malawi [27]).). Triangulation of environmental and clinical surveillance with estimates of the incidence of infection based on seroprevalence surveys using *S.* Typhi-specific antigens may help decrease this uncertainty [30]. ES and serological surveillance capture asymptomatic as well as symptomatic infection and are independent of healthcare seeking. This contributes to the sensitivity of these surveillance mechanisms but may complicate comparison with clinical disease incidence and their interpretation by public health decision-makers. Further work on the public health communication and utility of these novel surveillance mechanisms is needed.

Sensitivity of typhoid ES is likely to be affected by the characteristics of the wastewater network. Despite standardisation of the site selection protocol, samples in Blantyre were by necessity collected from the river network or defunct sewage treatment works, whilst in Vellore they were mainly collected from open drains. This may result in differences in the probability of detecting faecal shedding of *S.* Typhi even after adjusting for human faecal contamination using HF183. Further work to identify determinants of ES site sensitivity based on site characteristics and water quality parameters is ongoing (cf. [31]). The more frequent detection of *S.* Typhi at sites with larger catchment populations in Vellore suggests that existing polio ES sites, selected to represent populations >100,000 and implemented in many countries, may be leveraged to implement typhoid ES.

In conclusion, our data supports the use of ES as an unbiased, low-cost tool to establish the local incidence of typhoid fever and to inform and monitor the introduction of TCV. Additional research sites with clinical surveillance programs (e.g., Ghana, Nigeria, Fiji, Indonesia) are now implementing typhoid ES using standardised protocols to generate further data to support a potential scale-up that would help refine the world map of typhoid burden.

FOOTNOTE PAGE

References

1.   GBD 2017 Typhoid and Paratyphoid Collaborators. The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Infect Dis **2019**; 19(4): 369-81.

2.   Klemm EJ, Shakoor S, Page AJ, et al. Emergence of an extensively drug-resistant Salmonella enterica serovar typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. mBio **2018**; 9(1): e00105-18.

3.   da Silva KE, Tanmoy AM, Pragasam AK, et al. The international and intercontinental spread and expansion of antimicrobial-resistant Salmonella Typhi: a genomic epidemiology study. Lancet Microbe **2022**; 3: e567-77.

4.   Shakya M, Voysey M, Theiss-Nyland K, et al. Efficacy of typhoid conjugate vaccine in Nepal: final results of a phase 3, randomised, controlled trial. Lancet Glob Health **2021**; 9(11): e1561-e8.

5.   Patel PD, Patel P, Liang Y, et al. Safety and efficacy of a typhoid conjugate vaccine in Malawian children. N Engl J Med **2021**; 385(12): 1104-15.

6.   World Health Organisation. Typhoid vaccines: WHO position paper - March 2018. Wkly Epi Rec **2018**; 93: 153-72.

7.   Marks F, von Kalckreuth V, Aaby P, et al. Incidence of invasive salmonella disease in sub-Saharan Africa: a multicentre population-based surveillance study. Lancet Glob Health **2017**; 5(3): e310-e23.

8.   Srinivasan M, Sindhu KN, Giri S, et al. Salmonella Typhi Shedding and Household Transmission by Children With Blood Culture-Confirmed Typhoid Fever in Vellore, South India. J Infect Dis **2021**; 224(Supplement_5): S593-S600.

9.   Hagedorn B, Zhou NA, Fagnant-Sperati CS, et al. Estimates of the cost to build a stand-alone environmental surveillance system for typhoid in low- and middle-income countries. PLOS Global Public Health **2023**; 3(1): e0001074.

10.   Bill and Melinda Gates Foundation. Target Product Profile for Salmonella Environmental Surveillance [version 1; not peer reviewed]. Available at https://doi.org/10.21955/gatesopenres.1117024.1 Accessed 18 May 2023. . Gates Open Res **2023**; 7: 73.

11.   Matrajt G, Lillis L, Meschke JS. Review of methods suitable for environmental surveillance of *Salmonella* Typhi and Paratyphi. Clin Infect Dis **2020**; 71: S79-S83.

12. Zhou NA, Ong AQW, Fagnant-Sperati CS, et al. Evaluation of sampling and concentration methods for *Salmonella enterica* serovar Typhi detection from wastewater. Am J Trop Med Hyg **2023**; 108: 482-91.

13. Saha S, Tanmoy AM, Andrews JR, et al. Evaluating PCR-based detection of *Salmonella* Typhi and Paratyphi A in the environment as an enteric fever surveillance tool. Am J Trop Med Hyg **2019**; 100(1): 43-6.

14. Liu P, Ibaraki M, Kapoor R, et al. Development of Moore Swab and Ultrafiltration Concentration and Detection Methods for *Salmonella* Typhi and *Salmonella* Paratyphi A in Wastewater and Application in Kolkata, India and Dhaka, Bangladesh. Front Microbiol **2021**: https://doi.org/10.3389/fmicb.2021.684094.

15. Amin N, Liu P, Foster T, et al. Pathogen flows from on-site sanitation systems in low-income urban neighborhoods, Dhaka: A quantitative environmental assessment. Intl J Hyg Envir Health **2020**; 230: 113619.

16. Rigby J, Elmerhebi E, Diness Y, et al. Optimized methods for detecting *Salmonella* Typhi in the environment using validated field sampling, culture and confirmatory molecular approaches. J Appl Microbiol **2022**; 132(2): 1503-17.

17. 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 **2022**; 8: 9 (https://doi.org/10.12688/wellcomeopenres.7687.1).

18. Facebook Connectivity Lab and Center for International Earth Science Information Network. High Resolution Settlement Layer. https://data.humdata.org/organization/facebook. **2019**.

19. Nair S, Patel V, Hickey T, et al. Real-time PCR assay for differentiation of typhoidal and m non-typhoidal *Salmonella*. J Clin Microbiol **2019**; 57(8): 9.

20. Seurinck S, Defoirdt T, Verstraete W, Siciliano SD. Detection and quantification of the human-specific HF183 Bacteroides 16S rRNA genetic marker with real-time PCR for assessment of human faecal pollution in freshwater. Environ Microbiol **2005**; 7(2): 249-59.

21. John J, Bavdekar A, Rongsen-Chandola T, et al. Burden of typhoid and paratyphoid fever in India. New Engl J Med **2023**; 388(16): 1491-500.

22. Meiring JE, Shakya M, Khanam F, et al. Burden of enteric fever at three urban sites in Africa and Asia: a multicentre population-based study. Lancet Glob Health **2021**; 9(12): e1688-e96.

23. Balaji V, Kapil A, Shastri J, et al. Longitudinal typhoid fever trends in India from 2000 to 2015. Am J Trop Med Hyg **2018**; 99(Suppl. 3): 34-40.

24. Patel P, Liang Y, Meiring J, et al. Efficacy of typhoid conjugate vaccine: final analysis of a four-year, randomised controlled trial in Malawian children. Available at https://ssrn.com/abstract=4411421. Accessed 10 May 2023. **2023**.

25. Goldstein H, Browne W, Rasbash J. Partitioning Variation in Multilevel Models. Understanding Statistics **2002**; 1(4): 223-31.

26. Lumley T. survey: analysis of complex survey samples. R package version 4.1.1. **2022**.

27. Phillips MT, Meiring JE, Voysey M, et al. A Bayesian approach for estimating typhoid fever incidence from large-scale facility-based passive surveillance data. Stat Med **2020**; 40: 5853-70.

28. Gauld JS, Olgemoeller F, Heinz E, et al. Spatial and genomic data to characterize endemic typhoid transmission. Clin Infect Dis **2022**; 74(11): 1993-2000.

29. Als D, Radhakrishnan A, Arora, P., et al. Global trends in typhoidal Salmonellosis: a systematic review. Am J Trop Med Hyg **2018**; 99: 10-9.

30.   Aiemjoy K, Seidman JC, Saha S, et al. Estimating typhoid incidence from community-based serosurveys: a multicohort study. Lancet Microbe **2022**; 3(8): e578-e87.

31.   Hamisu AW, Blake IM, Sume G, et al. Characterizing environmental surveillance sites in Nigeria and their sensitivity to detect poliovirus and other enteroviruses. J Infect Dis **2020**.

Table 1 ES site characteristics

Vellore	Catchment Classification		
	Small	Medium	Large
Number of sites	14	12	14
Median Population	233	1,125	28,036
(range)	(14 - 566)	(750 - 4,725)	(7,119 - 320,441)
Median Catchment Area (km²)	0.04	0.29	1.82
(range)	(0.01 - 0.14)	(0.1 - 144.8)	(0.09 - 177.9)
Median Population Density	8,121	5,076	7,535
(range)	(3,939 - 12,932)	(220 - 11,785)	(401 - 337,525)

Blantyre	Catchment Classification		
	Small	Medium	Large
Number of sites	15	14	14
Median Population	5,961	17,451	80,935
(range)	(1,247 - 8,073)	(10,508 - 50,225)	(58,422 - 106,905)
Median Catchment Area (km²)	0.8	2.6	18
(range)	(0.2 - 1.6)	(1.1 - 7.3)	(1.9 - 33.8)
Median Population Density	6,720	6,232	4,941
(range)	(4,098 - 17,694)	(3,560 - 14,523)	(3,029 - 30,059)

Table 2 Combinations of *Salmonella* Typhi qPCR targets positive for grab samples and Moore swabs in Vellore and Blantyre and the mean genome copy number

						Mean $\log_{10}$ genome copy number (standard error)		
Location	sample type	*ttr*	*staG*	*tviB*	count	*ttr*	*staG*	*tviB*
Vellore	grab	neg	neg	neg	259			

Location	Method				n			
		neg	neg	pos	3			0.3 (0)
		pos	neg	neg	194	1.03 (0.03)		
		pos	neg	pos	8	1.22 (0.2)		0.73 (0.07)
		pos	pos	neg	17	1 (0.09)	0.7 (0.12)	
		pos	pos	pos	39	1.14 (0.08)	0.75 (0.07)	0.87 (0.09)
	Moore Swab	neg	neg	neg	37			
		neg	pos	neg	1		0.34 (-)	
		neg	pos	pos	1		0.82 (-)	0.84 (-)
		pos	neg	neg	326	1.74 (0.04)		
		pos	neg	pos	19	2.28 (0.17)		0.41 (0.06)
		pos	pos	neg	54	2.4 (0.09)	0.89 (0.08)	
		pos	pos	pos	79	2.37 (0.07)	0.91 (0.06)	0.91 (0.07)
Blantyre	grab	neg	neg	neg	486			
		neg	neg	pos	3			1.43 (0.21)
		pos	neg	neg	25	1.31 (0.1)		
		pos	neg	pos	2	1.11 (0.26)		1.99 (0.98)
		pos	pos	neg	6	1.67 (0.2)	2.62 (0.16)	
		pos	pos	pos	11	1.97 (0.21)	2.9 (0.17)	2.22 (0.13)
	Moore Swab	neg	neg	neg	526			
		neg	neg	pos	1			1.62 (-)
		neg	pos	neg	1		2.6 (-)	
		pos	neg	neg	30	1.19 (0.08)		
		pos	neg	pos	1	1.21 (-)		1.11 (-)
		pos	pos	neg	12	1.7 (0.15)	2.58 (0.09)	
		pos	pos	pos	23	1.8 (0.11)	2.5 (0.19)	1.72 (0.16)

Table 3 Crude incidence of culture-confirmed typhoid fever in Vellore and Blantyre, May 2021 - April 2022

Location	Age group (years)	Number of cases	Population	Crude incidence per 100,000 person years, ML estimate (95% Confidence Interval)
Vellore				
	0-4	4	8,082	49.5 (13.5 - 126.7)
	5-9	3	9,772	30.7 (6.3 - 89.7)
	10-14	0	10,430	0.0 (0.0 - 35.4)
	15-29	4	35,728	11.2 (3.1 - 28.7)
	30+	0	77,788	0.0 (0.0 - 4.7)
	Total	11	141,800	7.8 (3.9 - 13.9)
Blantyre*				
	0-4	30, 34	65,772	45.6 (30.8 – 65.1), 51.7 (35.8 – 72.2)
	5-9	19, 40	68,946	27.6 (16.6 – 43.0), 58.0 (41.4 – 79.0)
	10-14	14, 21	70,395	19.9 (10.9 – 33.4), 29.8 (18.5 – 45.6)
	15-29	22	140,398	15.7 (9.8 – 23.7)
	30+	5	144,397	3.5 (1.1- 8.1)
	Total	90, 122	489,908	18.4 (14.8 – 22.6), 24.9 (20.7 – 29.7)

*In Blantyre, enhanced passive surveillance of children with febrile illness was conducted at three primary health care centres serving the study area. Case numbers and incidence rates are shown without and with these additional paediatric cases (separated by a comma).

FIGURE LEGENDS

Figure 1 Location of ES sites and the prevalence of *S.* Typhi detection. ES sites are plotted as circles for Vellore (A,B) and Blantyre (C,D) with the colour indicating prevalence over the study period and results shown separately for grab samples (A,C) and Moore swabs (B,D). The radius of the circles is proportional to the estimated catchment population. Maps were created using QGIS v3.22.4.
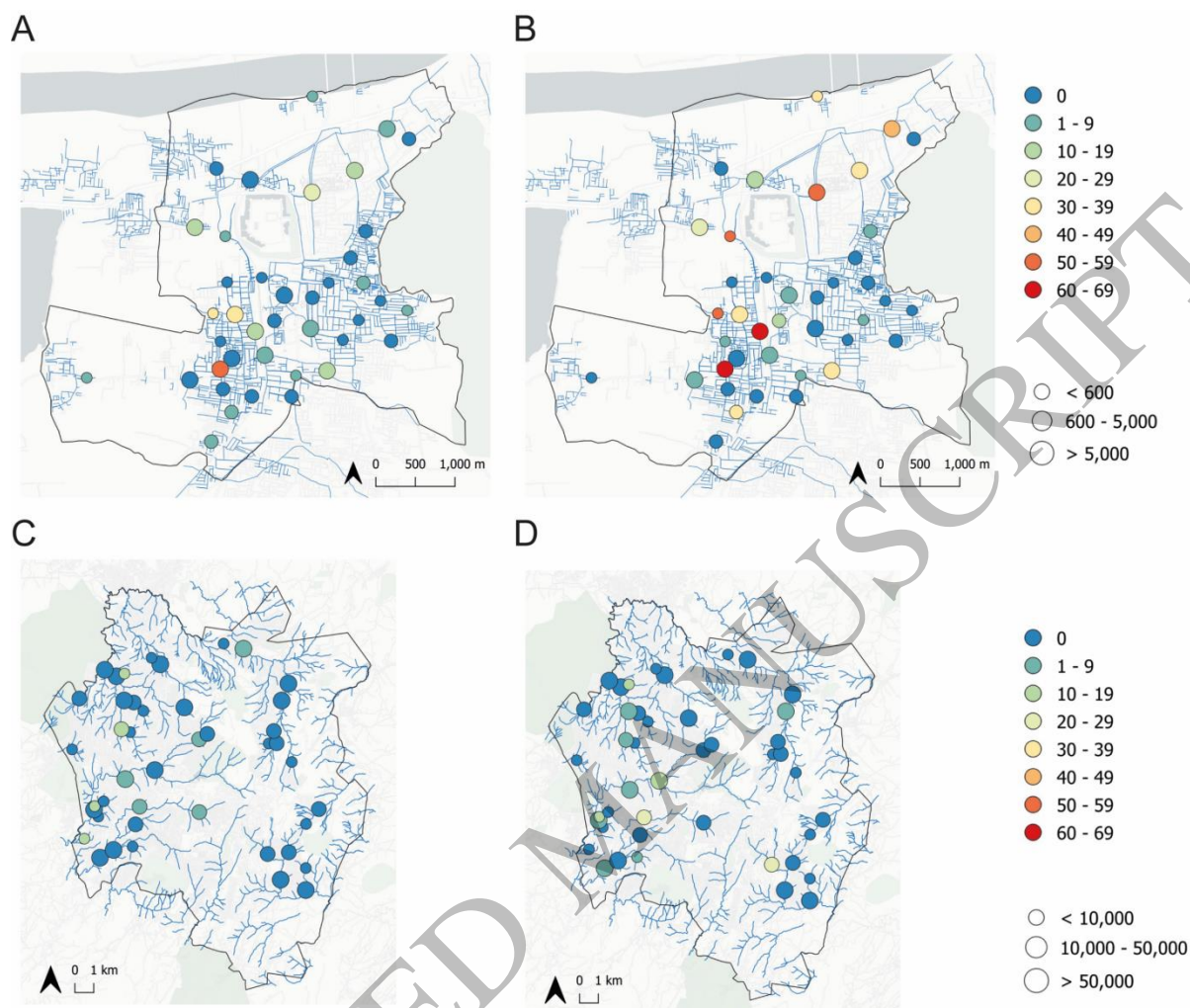
Figure 2 Monthly detection of *S.* Typhi in ES samples and the incidence of clinical cases reported from the study areas. The prevalence of *S.* Typhi across ES sites each month is shown for Vellore (A) and Blantyre (B) for each sample type. The error bars indicate 95% confidence intervals. The monthly number of blood culture confirmed typhoid fever cases reported among inpatients and outpatients resident in the study areas from hospitals in (C) Vellore and (D) Blantyre. In Blantyre, additional cases detected through enhanced passive surveillance at three primary health care centres are also shown (gray).