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THE STRATAA STUDY PROTOCOL: A PROGRAMME TO ASSESS THE BURDEN OF ENTERIC FEVER IN BANGLADESH, MALAWI AND NEPAL USING PROSPECTIVE POPULATION CENSUS, PASSIVE SURVEILLANCE, SEROLOGICAL STUDIES AND HEALTHCARE UTILISATION SURVEYS

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1 **THE STRATAA STUDY PROTOCOL: A PROGRAMME TO ASSESS THE**
2 **BURDEN OF ENTERIC FEVER IN BANGLADESH, MALAWI AND NEPAL USING**
3 **PROSPECTIVE POPULATION CENSUS, PASSIVE SURVEILLANCE,**
4 **SEROLOGICAL STUDIES AND HEALTHCARE UTILISATION SURVEYS**

6 **Short title: The STRATAA study**

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3 70 **ABSTRACT**
4

5 71 **Introduction**
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7 72 Invasive infections caused by *Salmonella enterica* serovar Typhi and Paratyphi A are
8
9 73 estimated to account for 12-27 million febrile illness episodes worldwide annually.
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11 74 Determining the true burden of typhoidal *Salmonellae* infections is hindered by lack
12
13 75 of population-based studies and adequate laboratory diagnostics.
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15 76 The STRATAA (Strategic Typhoid alliance across Africa and Asia) study takes a
16
17 77 systematic approach to measuring the age-stratified burden of clinical and subclinical
18
19 78 disease caused by typhoidal *Salmonellae* infections at three high-incidence urban
20
21 79 sites in Africa and Asia. We aim to explore the natural history of *Salmonella*
22
23 80 transmission in endemic settings, addressing key uncertainties relating to the
24
25 81 epidemiology of enteric fever identified through mathematical models, and enabling
26
27 82 optimisation of vaccine strategies.
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29 83 **Methods/Design**
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31 84 Using census-defined denominator populations of $\geq 100,000$ individuals at sites in
32
33 85 Malawi, Bangladesh and Nepal, the primary outcome is to characterize the burden of
34
35 86 enteric fever in these populations over a 24-month period. During passive
36
37 87 surveillance, clinical and household data, and laboratory samples will be collected
38
39 88 from febrile individuals. In parallel, healthcare utilization and water, sanitation and
40
41 89 hygiene surveys will be performed to characterise healthcare-seeking behaviour and
42
43 90 assess potential routes of transmission. The rates of both undiagnosed and
44
45 91 subclinical exposure to typhoidal *Salmonellae* (seroincidence), identification of
46
47 92 chronic carriage, and population seroprevalence of typhoid infection will be assessed
48
49 93 through, age-stratified serosurveys performed at each site. Secondary attack rates
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51 94 will be estimated among household contacts of acute enteric fever cases and
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53 95 possible chronic carriers.
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56 96 **Discussion**
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3 97 With this network of field-sites, laboratories and clinical investigators, the
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5 98 STRATAA study will address the current gaps in our knowledge regarding the
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7 99 epidemiology of typhoidal *Salmonellae* in endemic regions. These data used in
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9 100 conjunction with mathematical models, will be a timely precursor to the design
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11 101 and instigation of comprehensive enteric fever control programmes through the
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13 102 optimal deployment of new generation enteric fever vaccines.
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18 **STRENGTHS AND LIMITATIONS**

19 105 - Through the use of recent dynamic typhoid transmission mathematical models,
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21 106 specific data gaps have been identified which this protocol design aims to directly
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23 107 address.

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25 108 - We have designed a comprehensive multicomponent epidemiological approach,
26
27 109 nesting passive surveillance, serosurveillance and healthcare utilisation surveys
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29 110 within a demographic census population, to accurately determine the age-stratified
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31 111 burden of enteric fever.

32
33 112 - This programme utilises key field sites in Africa and Asia encompassing a range of
34
35 113 differing epidemiological settings, whilst also supporting the development of local
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37 114 research infrastructure and international collaboration.

38
39 115 - This programme combines traditional epidemiological methods with cutting-
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41 116 edge laboratory methods for the diagnosis of febrile illness and investigation of the
42
43 117 host and pathogen genetics and antimicrobial resistance determinants.

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45 118 - With a study of this size ensuring implementation of standardised clinical
46
47 119 definitions, data collection and sample processing is challenging.

48
49 120 - Practical limitations include the sharing and standardisation of clinical definitions,
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51 121 data and sample collection methods and laboratory assays, based on the facilities
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53 122 and staff available and community requirements at each site.

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55 123 - The number of field-sites included in this current protocol is limited to three; all of
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57 124 these sites are densely populated urban settings with likely high-incidence of enteric
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3 125 fever transmission. The degree to which our data may be extrapolated to other
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5 126 settings and countries remains to be explored.
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7 127 - Enteric fever epidemiology is in a constant state of flux due to factors including
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9 128 population movement, alterations to water and sanitation provision and seasonal
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11 129 variation. How these factors may play a role in accurately measuring the burden of
12
13 130 enteric fever over a two-year study period provides some uncertainty to sample size
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15 131 estimates and data collection procedures.
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20 133 **Registration:** ISRCTN 12131979

21 134 **Ethics References:** Oxford (Oxford Tropical Research Ethics Committee 39-15)

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23 135 Bangladesh (icddr,b Institutional Review Board PR-15119)

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25 136 Malawi (National Health Sciences Research Committee

26
27 137 15/5/1599)

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29 138 Nepal (Nepal Health Research Council 306/2015)

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33 140 **Keywords:**

34
35 141 Enteric fever, vaccination programme, infection transmission, *Salmonella* Typhi,

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37 142 *Salmonella* Paratyphi A, serosurveillance, seroepidemiology, healthcare utilisation,

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39 143 resource-limited settings; diagnosis; febrile illness; Africa, Asia.
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3 144 **BACKGROUND**

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5 145 *Salmonella enterica* serovars Typhi (S. Typhi) and Paratyphi A (S. Paratyphi A), are
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7 146 human-restricted pathogens transmitted by faeco-oral ingestion. The ensuing
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9 147 disease, enteric fever (or 'typhoid fever'), is a non-specific febrile illness which affects
10
11 148 an estimated 12-27 million people worldwide each year, resulting in 129,000–
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13 149 223,000 deaths.^{1–3} Despite a dramatic reduction in incidence over the last century in
14
15 150 most high-income countries, continuing inadequate access to clean water and
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17 151 increasing inter-continental spread of multiply antibiotic-resistant strains hamper
18
19 152 disease control efforts especially in resource-limited settings.^{3–5} The current burden
20
21 153 of disease is highest among children and young adults in South and Southeast Asia^{1–}
22
23 154 ³ but is increasingly recognized in sub-Saharan Africa.⁶

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27 156 Recently, a new generation of Vi- conjugate enteric fever vaccines, suitable for use in
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29 157 infants and providing longer lasting protection than those previously licensed,^{7–10}
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31 158 have become available. Determining how and where interventions such as
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33 159 vaccination may be best deployed is difficult due to a lack of population-based
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35 160 incidence studies and inaccurate diagnostic tests.^{1–3} Improving disease burden
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37 161 estimates and providing data on the epidemiology and transmission of S. Typhi and
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39 162 S. Paratyphi A to inform mathematical models^{11–13} could improve the evidence
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41 163 necessary to design comprehensive and effective disease control programmes.¹⁴

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45 165 **Burden of disease and diagnostics**

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47 166 Recent meta-analyses examining global causes of morbidity and mortality estimate
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49 167 that a significant burden of disease worldwide, and especially in South and
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51 168 Southeast Asia, may be attributed to enteric fever.^{15–17} The margin of error on these
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53 169 estimates is wide, however; much of the uncertainty regarding the burden of disease
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55 170 caused by typhoidal *Salmonella* is due to the unavailability of accurate diagnostics or
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57 171 misclassification of non-specific disease presentations, in addition to a general lack

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3 172 of data.¹⁸ Availability of antibiotics from local pharmacies without prescription,
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5 173 frequent misdiagnosis as malaria, dengue or other febrile illnesses, or the avoidance
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7 174 of hospital due to fear or expense are all likely to result in an underestimate of the
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9 175 true numbers of cases.¹⁹ In contrast, the widespread use of suboptimal diagnostic
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11 176 tests such as the Widal test in areas where exposure to similar bacteria in the
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13 177 environment occurs may lead to inaccurate over-diagnosis of the condition.²⁰
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15 178 Previous studies and systematic reviews have attempted to address this inaccuracy
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17 179 by calculating the imprecision associated with insensitive blood culture methods, and
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19 180 applying this correction to blood culture confirmed case numbers.^{1,2}
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22
23 182 In addition to a better understanding of the disease burden attributable to typhoidal
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25 183 *Salmonellae*, improved diagnostics and biomarkers are required to improve individual
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27 184 case management.²¹ The current gold-standard diagnostic test for
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29 185 typhoid/paratyphoid is blood culture; while this test provides the causative isolate,
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31 186 thereby allowing susceptibility testing and further typing methods to be performed,
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33 187 most laboratories in resource-limited settings lack the infrastructure to perform these
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35 188 assays. Even in ideal conditions, blood culture requires a significant volume of blood
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37 189 to enhance diagnostic yield, and is relatively insensitive even in highly-controlled
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39 190 human challenge settings where sensitivity reaches 80%.²² Highly sensitive and
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41 191 specific diagnostic tests are a fundamental requirement for surveillance and vaccine
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43 192 efficacy studies, however, both for measuring the number of cases
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45 193 avoided/prevented, but also for reassuring policy makers and funders that vaccine
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47 194 implementation is worth investment. While several rapid serological diagnostic tests
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49 195 have recently been developed, these still rely on a few selected antigens, which are
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51 196 known to be non-specific in endemic communities.^{23,24} Newer diagnostic modalities
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53 197 are in development, however, which have utilised serum banks from multiple
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55 198 countries and samples collected from controlled human infection models to identify
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57 199 putative novel antigens for serological assay and further high sensitivity approaches
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3 200 including metabolomics and functional genomics.^{5,25-27} In addition, several of these
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5 201 newer approaches have demonstrated potential for identifying individuals with
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7 202 probable chronic carriage. Identification and treatment of chronic carriers in a
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9 203 population would significantly improve our understanding of disease dynamics and
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11 204 allow targeted treatment strategies to reduce community transmission of infection.
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15 206 Furthermore, whilst utilising these new diagnostic tests in comprehensive passive
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17 207 surveillance or even active surveillance programmes will identify acute cases of
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19 208 typhoid disease, the incidence rate of sub-clinical infection and exposure is unknown.

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21 209 These data are likely essential to understanding pathogen transmission as well as
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23 210 the development and maintenance of immunity against clinical disease¹² and are
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25 211 likely only to be obtainable through the use of seroepidemiological methods.

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27 212 Previous seroepidemiological studies have examined the cross-sectional age-
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29 213 stratified prevalence of antibodies to the *S. Typhi* flagellar (H) antigen in Santiago,
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31 214 Chile²⁸, and serum bactericidal antibody in Kathmandu, Nepal.²⁹ Also, high antibody
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33 215 titres to the Vi (Vi capsular polysaccharide; virulence factor) antigen of *S. Typhi*
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35 216 measured by agglutination assay have been used to estimate chronic carrier
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37 217 frequency in population-based studies,³⁰⁻³² although these studies were not
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39 218 universally successful at identifying chronic carriers.³³ Improvements in serological
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41 219 assays and the discovery of a newer generation of diagnostic antigens³⁴ suggest that
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43 220 population-based serosurveillance studies, especially using longitudinal
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45 221 measurements, may provide key data regarding the incidence of subclinical infection
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47 222 with the bacterial agents of enteric fever and the prevalence of chronic carriers.
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51 52 224 **Transmission**

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54 225 Closely related to the estimate of incidence is the contribution of different typhoid
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56 226 states to ongoing transmission within a community. Acute cases of enteric fever,
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58 227 individuals with subclinical infection, as well as chronic carriers likely all contribute to
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3 228 the transmission and maintenance of *S. Typhi* and *S. Paratyphi A* in endemic areas,
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5 229 but not to the same degree. This is an essential area of further research, particularly
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7 230 given the negative impact transmission from chronic carriers could have on the
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9 231 indirect protection afforded by typhoid vaccines.¹² The importance of 'short cycle'
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11 232 transmission via contaminated food and water in the immediate environment versus
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13 233 'long cycle' transmission via the contamination of community water sources is also
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15 234 an area of direct relevance in the control of typhoid fever, primarily through non-
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17 235 vaccine public health interventions including water and sanitation hygiene (WASH)
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19 236 improvements.
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23 238 **Bacterial genome variation**

25 239 The enteric fever agents *S. Typhi* and *S. Paratyphi A*, while genetically distinct from
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27 240 other serovars of *Salmonella enterica*, exhibit low genetic diversity and whole
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29 241 genome sequence (WGS) analysis is required to uncover patterns of genetic
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31 242 relatedness between isolates.³⁵⁻³⁷ These techniques have been used previously to
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33 243 track the development and spread of antibiotic resistance on a global scale,⁶ and
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35 244 have shown potential to accurately track transmission links between individuals,
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37 245 specifically within affected households, in addition to the broader community.³⁸ One
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39 246 use of this high-resolution typing previously, has been to determine whether isolates
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41 247 obtained from those with acute infection are distinct from those found in chronic gall-
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43 248 bladder carriers.³⁹
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47 250 **Host susceptibility**

49 251 While environmental risk factors for enteric fever acquisition are thought to be
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51 252 relatively well understood, recent evidence has emerged implicating genetic factors
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53 253 in host susceptibility to infection.^{35,36} Many early typhoid genetic susceptibility studies
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55 254 reporting candidate genes were limited by small sample sizes and choice of controls,
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57 255 a recent large-scale genome-wide association study however, has identified a
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3 256 specific locus (HLA-DRB1*405) which confers 5-fold protection against developing
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5 257 typhoid fever.³⁵ How genetic variation at this site influences protection against
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7 258 typhoid is still under investigation, but may result in functional differences in MHC
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9 259 class II amino acid sequences; in turn, this may influence *S. Typhi* epitope selection
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11 260 and antigen presentation or the scale and format of the host's T-cell response.
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14 15 262 **Vaccines**

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17 263 Vaccines currently available for typhoid are either oral live attenuated bacterial
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19 264 strains or are derived from the *S. Typhi* polysaccharide capsule, called Vi; there are
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21 265 no licensed vaccines against *S. Paratyphi A* or non-typhoidal salmonellae (NTS).
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23 266 Despite recommendations for their use, these vaccines have only sparsely been
24
25 267 introduced in high prevalence settings.^{7,40} The reasons for this are unclear, but likely
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27 268 include failure of prioritisation and a short fall in advocacy, in addition to technical
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29 269 deficiencies of the vaccines themselves. One common reason cited is the T-cell
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31 270 independent nature of the immune response to the Vi polysaccharide vaccine (ViPS);
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33 271 this renders the vaccine of little use in young children below the age of two years. In
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35 272 addition, this feature means that the immune response does not boost, requiring
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37 273 repeat vaccination to be administered every 3-5 years. As with other thymus-
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39 274 independent vaccines, to overcome this problem the Vi capsule may be chemically
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41 275 conjugated to a protein carrier,⁸ as has successfully been done with conjugation of Vi
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43 276 to *Pseudomonas aeruginosa* exotoxin A.⁹ Despite demonstrating good
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45 277 immunogenicity and efficacy in field trials,^{10,41} the vaccine is still unlicensed. Newer
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47 278 Vi-conjugate vaccines are in development and have progressed to assessment in
48
49 279 field-trials,^{42,43} and the Oxford human typhoid challenge model.⁴⁴ Recent data from
50
51 280 the challenge models suggests that the Vi response elicited by the vaccine is high
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53 281 and likely to be protective, at least in naïve healthy adult volunteers with no immunity
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55 282 or those with previous history of infection.⁴⁴ Furthermore, a recent study in Indian
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57 283 schoolchildren found a vaccine efficacy of 100% (95% confidence interval, 97.6 to
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3 284 100%) after a single dose of Vi conjugated to tetanus toxoid during the first year of
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5 285 follow-up.⁴³
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9 287 The key issue remaining, once safe, well-tolerated, immunogenic vaccines become
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11 288 widely available, is how best to implement them in endemic settings. Currently
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13 289 available surveillance data from most regions is insufficient to demonstrate the age-
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15 290 band with the highest incidence of enteric fever.⁴⁵ Designing vaccination strategies to
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17 291 cover the years during which children are most at risk and generating indirect
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19 292 protection by preventing infection among those age groups driving transmission
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21 293 could be facilitated through the use of well-informed mathematical modelling.
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25 295 **THE STRATAA STUDY: RATIONALE AND AIMS**

27 296 The STRATAA (Strategic Typhoid alliance across Africa and Asia) study draws
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29 297 together an international team of investigators, field sites, laboratories and research
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31 298 institutes to address many of the key outstanding questions described above
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33 299 regarding the burden of enteric fever and *Salmonella* exposure in endemic regions,
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35 300 the mechanisms of susceptibility and infection transmission. These have been
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37 301 identified as key uncertainties in mathematical models.¹¹⁻¹³ By conducting field
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39 302 studies of the epidemiology and burden of enteric fever across three sites with
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41 303 distinct epidemiological profiles and applying state-of-the-art molecular methods, the
42
43 304 goal of the STRATAA study is to collect the data needed to enhance our
44
45 305 understanding of pathogen transmission, exposure and susceptibility. These data
46
47 306 can then be used to rigorously parameterize and validate models for the transmission
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49 307 dynamics of the agents of enteric fever, such that these models can then be used to
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51 308 evaluate different vaccination strategies and, importantly, help predict the expected
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53 309 impact resulting from the direct and indirect effects of vaccine introduction. These
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55 310 and other overarching objectives of the STRATAA study are listed in **Table 1**.

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312 **Table 1. Overarching objectives of the STRATAA study.**

Primary	To characterise the burden of enteric fever at three urban sites in Africa and Asia
Secondary	Assess the burden/incidence of enteric fever
	Assess the seroincidence of infection
	Assess host factors affecting burden/incidence/transmission of enteric fever
	Assess effect of pathogen genetics on burden/incidence/transmission of enteric fever
	Develop diagnostic tools for rapid and consistent typhoid diagnosis
	Develop transmission modelling and modelling of vaccine introduction impact
Tertiary Objectives	Strengthen research capacity in enteric fever endemic regions
	Provide data to appropriate institutes and governments to advocate vaccine implementation
	To characterise the burden of invasive NTS and other invasive pathogens at an urban site in Malawi, Africa

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314 To deliver on these objectives, the following studies will be performed in parallel at
315 each of the three chosen field sites, starting in May 2016 with activities continuing
316 until October 2018. Firstly, in a well-defined population catchment of
317 approximately 100,000 individuals, a detailed demographic census survey will be
318 undertaken. Secondly, in healthcare facilities utilised by the census population (as
319 confirmed through a healthcare utilisation survey) prospective passive surveillance to
320 detect cases of enteric fever will be performed. Thirdly, a seroincidence study will be

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3 321 conducted in an age-stratified sample of the census population at each site,
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5 322 collecting blood samples at intervals to estimate the rate of seroconversion to
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7 323 typhoidal *Salmonella* antigens and hence the rate of subclinical/asymptomatic
8
9 324 exposure to these bacteria in the general population.
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13 326 To meet the further objectives, which include evaluating diagnostic tests, identifying
14
15 327 antimicrobial resistance patterns, exploring host susceptibility and bacterial
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17 328 virulence/genomic variation, biological samples, specimens and metadata will be
18
19 329 collected during these individual studies. Data will be pooled to inform transmission
20
21 330 dynamic and health economic models that can be used to help design future vaccine
22
23 331 effectiveness studies and evaluate vaccine delivery strategies for widespread
24
25 332 deployment.
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28 334 **STUDY SITES**

29 335 With known high incidence throughout South and Southeast Asia and a recent
30
31 336 increase in reported cases from sub-Saharan Africa^{46,47} three sites were selected
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33 337 from across Africa and Asia based on known high rates of enteric fever and the
34
35 338 research capacity to deliver a study of this size and logistical complexity. The three
36
37 339 sites differ in their epidemiological profiles and history of enteric fever incidence.
38

39 340

40 341 ***Dhaka, Bangladesh***

41 342 Mirpur is an area located within Dhaka Metropolitan area, Bangladesh (**Figure 1A**),
42
43 343 situated 7km north-west of the International Centre for Diarrhoeal Disease Research,
44
45 344 Bangladesh (icddr,b) main campus. The icddr,b manages two hospitals in Dhaka
46
47 345 city, one in the main campus in Dhaka (the main hospital) and another in the Mirpur
48
49 346 area known as MTC (Mirpur Treatment Centre) comprising a 50-bed inpatient facility.
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51 347 As part of this programme, these and other health facilities (ten or more total) will be
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53 348 kept under careful surveillance for enteric fever in the census area. The total
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3 349 catchment area for the field site for the STRATAA study is 10.79 km² with a total
4
5 350 population of about 603,658 at a density of 55946/km². Approximately 98% of
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7 351 residents have access to tap water supplied by the municipality while the remainder
8
9 352 use wells, hand pumps and other sources such as ponds and rivers in the study
10
11 353 area.

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13 354
14
15 355 Previous data from blood culture surveillance revealed a high incidence of disease
16
17 356 within Dhaka, with the burden particularly high in children under 5 years of age
18
19 357 (estimated at 18.7 episodes/1000 person-years in this age group). The current S.
20
21 358 Typhi:Paratyphi ratio is approximately 5:1. Typhoid fever is reported throughout the
22
23 359 year but peaks during the monsoon season.^{48,49} Of note, 15% of S. Typhi isolates are
24
25 360 multi-drug resistant (MDR) and around 97% isolates are resistant to nalidixic acid⁵⁰;
26
27 361 extended-spectrum beta-lactamase (ESBL) producing organisms have also been
28
29 362 isolated from enteric fever patients in this setting.
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31 363

32 33 364 ***Blantyre, Malawi***

34
35 365 Ndirande is a large urban township on the outskirts of Blantyre city, Malawi, 6km
36
37 366 from the main referral hospital (**Figure 1B**). It has a young population of around
38
39 367 100,000 people spread over 6.77km². It is serviced by one health clinic staffed by
40
41 368 clinical officers. It has high reported rates of typhoid fever along with a population
42
43 369 HIV prevalence of around 18%.⁵¹ Queen Elizabeth Central Hospital (QECH) in
44
45 370 Blantyre, Malawi, is the government-funded hospital for Blantyre district, serving a
46
47 371 local population of 1.3 million persons and provides tertiary care to southern Malawi.
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49 372

50
51 373 NTS have previously been the commonest cause of invasive bloodstream infections
52
53 374 in Blantyre,⁵² but since 2011 there has been a rapid increase in the number of S.
54
55 375 Typhi cases seen at QECH, from approximately 14/year between 1998 and 2010 to
56
57 376 782 in 2014.⁴⁶ Much of this increase has been due to the emergence of the H58
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3 377 clone and is associated with an MDR phenotype^{47,53}. This outbreak appears
4
5 378 unrelated to HIV-infection, but has resulted in high rates of mortality (2.5% adult and
6
7 379 paediatric) despite the availability of fluoroquinolone antibiotics.⁴⁷ In this setting,
8
9 380 enteric fever is seasonal, with the peak number of cases seen at the end of the wet
10
11 381 season and during the early dry season when the prevalence of malnutrition is also
12
13 382 highest.

14 15 383 16 17 384 **Patan, Nepal**

18
19 385 Patan is located within the Lalitpur Sub-Metropolitan City (LSMC) within the
20
21 386 Kathmandu Valley, Nepal (**Figure 1C**). The population is generally poor, with most
22
23 387 living in overcrowded conditions and obtaining their water from stone spouts or
24
25 388 sunken wells. Patan Hospital is a 318-bed government hospital providing emergency
26
27 389 and elective outpatient and inpatient services to this area. The local catchment
28
29 390 population of the hospital is approximately 200,000 people in about 20 km², with a
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31 391 population density of 8,000/km²; there is a high rate of immigration for employment,
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33 392 particularly young males from rural areas. Enteric fever is frequently managed in the
34
35 393 outpatient clinic at Patan Hospital, which has approximately 200,000 outpatient visits
36
37 394 annually.

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41 396 Uptake of typhoid Vi vaccination is limited and natural exposure/subclinical infection
42
43 397 is common.²⁹ Approximately 400 culture confirmed cases of enteric fever are
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45 398 diagnosed at Patan Hospital each year, with a peak during the monsoon months. The
46
47 399 current *S. Typhi*:*Paratyphi A* ratio is approximately 1:1.⁵⁴ Antimicrobial resistance is
48
49 400 more commonly observed in *S. Paratyphi A* isolates; however, emergence of
50
51 401 fluoroquinolone resistant *S. Typhi* isolates has also been recently identified,⁵⁵
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53 402 whereas MDR strains of either serovar are rare.³⁸

54 55 403 56 57 58 404 **POPULATION CENSUS**

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2
3 405 In order to accurately calculate an incidence rate of typhoid fever for each of the
4
5 406 study sites, a demographic census will be performed at baseline and repeated at 2
6
7 407 years. The objective of the census is to identify/characterise the source population
8
9 408 corresponding to the catchment areas for the passive surveillance sites described
10
11 409 below, and estimate the person-time under surveillance.
12

13 410
14
15 411 Census data will be updated with births, deaths and migrations every six months in
16
17 412 Dhaka and Patan, and at two years in Blantyre. The number of participants for the
18
19 413 demographic census survey in each site needed to produce a two-sided 95%
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21 414 confidence interval with a precision (half-width) of 50% for the anticipated typhoid
22
23 415 incidence rate detected through passive clinical surveillance has been calculated
24
25 416 (**Table 2**). The necessary catchment population size is driven by the size required to
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27 417 estimate the expected incidence rate in the 0-4 year-old age group, which is
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29 418 estimated to be approximately 10% of the total population in each site.
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421 **Table 2. Sample size required for the target populations in the three sites to**
 422 **estimate annual, blood culture-confirmed typhoid incidence in passive clinical**
 423 **surveillance.**

Age groups	Anticipated typhoid incidence per 1000 persons*	Precision or half-width	Sample-size required
0-4 years	1.5	0.75	10,125
5-14 years	1.0	0.5	15,119
>14 years	0.5	0.25	29,801

424 * Assumed age-specific incidence rates (based on data from Dhaka, Bangladesh,
 425 Delhi, India, and Dong Thap, Vietnam).

426
 427 The census will take place within a demarcated geographic area that is a known
 428 catchment population for the surveillance sites (**Figure 1**). In total, at least 100,000
 429 individuals will be enumerated from $\geq 20,000$ households. The head/key informant
 430 within the household will provide written informed consent to take part in the study.
 431 Information on all residents within the household at the time of the census will be
 432 gathered from the head of the household/key informant.

433
 434 A household is defined as individuals living in the same dwelling or compound and
 435 sharing food from the same kitchen. A household member is considered to have
 436 migrated out if s/he has left the household and does not intend to come back within
 437 six months of the time s/he left. A person is considered to have migrated in if s/he
 438 was not previously included in the list of household members and intends to live in
 439 the household for the next six months. At enrolment, the head of each household will

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2
3 440 be made aware of the passive surveillance component of the study and encouraged
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5 441 to use the field-site facilities capturing acute febrile illnesses, including acute enteric
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7 442 fever cases. The characterised census population will form the sampling frame for
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9 443 the further components (passive surveillance, healthcare utilisation/WASH surveys
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11 444 and serosurvey) described below.
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15 446 **PASSIVE SURVEILLANCE**

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17 447 The passive surveillance component of STRATAA is designed to capture cases of
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19 448 febrile disease occurring in each of the three census populations. Patients presenting
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21 449 to any of the clinical surveillance sites with a history of subjective fever >72 hours or
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23 450 objective fever >38.5 °C on presentation will be approached for enrolment.
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28 452 An index case will be defined as an individual with a blood culture result confirming
29
30 453 infection with *S. Typhi* or *S. Paratyphi A* (or NTS in Malawi), and whose household is
31
32 454 included in the census survey. These cases will be used to calculate the disease
33
34 455 incidence in each of the three census sites. Consenting individuals will have samples
35
36 456 of blood, urine and stool collected to determine a diagnosis of *S. Typhi*, *S. Paratyphi*
37
38 457 *A* or NTS and to provide material for the further diagnostic and genetic aims of the
39
40 458 study (**Supplemental Table 1**). Patients not resident in the census area will be
41
42 459 enrolled as additional cases for the laboratory and genetic components of the
43
44 460 programme if written consent is provided.
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48 462 **HEALTHCARE UTILISATION AND WATER, SANITATION AND HYGIENE**

49 463 **SURVEYS**

50
51 464 To characterise the healthcare-seeking behaviour of individuals living in the census
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53 465 areas, at least 735 households will be randomly selected from the census area at
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55 466 each site to participate in a healthcare utilisation survey. Data will be collected from
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57 467 the head of the household/key informant to describe the actual and hypothetical
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3 468 usage of healthcare facilities for febrile episodes. The aim of this component is to
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5 469 estimate the percent of cases (fulfilling the fever case definition) in each age stratum
6
7 470 (0-4 years, 5-14 years and >14 years of age) who would or would not seek attention
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9 471 at one of the designated passive surveillance health facilities. Additional data
10
11 472 regarding sanitation and hygiene facilities and usage will also be collected. Annual
12
13 473 data collection periods will coincide with the peak typhoid season at each of the three
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15 474 sites.

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17 47518
19 476 **SEROSURVEYS**

20
21 477 To estimate the seroincidence and seroprevalence of clinical (both formally
22
23 478 diagnosed and undiagnosed cases) and subclinical infection or exposure to *S. Typhi*
24
25 479 and *S. Paratyphi*, systematic serosurveillance will be performed at each site. Blood
26
27 480 samples will be collected at baseline and 3 months later, with sample collection
28
29 481 initiated in an on-going basis over the course of one year (**Table 3**). To identify
30
31 482 participants for the serosurvey, an age-stratified approach will be used to randomly
32
33 483 select individuals from the census population from each age group. Suitable
34
35 484 participants will be enrolled by field workers; where the individual identified is not
36
37 485 available, a household member in the same age group will be selected where
38
39 486 possible.

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43 488 Seroincidence (indicative of recent infection) will be calculated by measuring the rate
44
45 489 of seroconversion to anti-H(d) (anti-flagellin) IgG and other acute phase antibodies
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47 490 between both time points, with the denominator consisting only of individuals
48
49 491 sampled twice and seronegative at the first time point.⁵⁶

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51 492

52
53 493 **Table 3. Sample size calculations for the serological surveys to estimate age-**
54
55 494 **specific rates of high titres of serum IgG anti-H(d) antibodies.** Assumes age-

495 stratified individual sampling; also accounts for detection of 1% chronic carriage rate
 496 in ≥ 10 year olds.

	Anticipate d sero- incidence (%)	Number of initial samples	Anticipat ed number of events	No. of follow- up samples *	No. of events detected	Binomial [95%	Exact CI]	Probabili ty of observin g 0 events
0-4 yrs	0.2	2500	5	2000	4	0.0005	0.0051	0.0182
5-9 yrs	0.4	1300	5.2	1040	4	0.0010	0.0098	0.0155
10-14 yrs	0.8	800	6.4	640	5	0.0025	0.0181	0.0059
>14 yrs	0.2	3900	7.8	3120	6	0.0007	0.0042	0.0019
TOTAL		8500	24.2	6800	19			

497 *Assuming 5% migration and 15% refusal

498

499 The seroprevalence of anti-Vi IgG will also be measured at baseline to identify
 500 individuals who could be potential chronic carriers. Previous studies have used high
 501 anti-Vi IgG antibody levels in serum as a marker of chronic carriage, and our aim is
 502 to use a recently validated/approved anti-Vi ELISA method to determine possible
 503 rates of chronic carriage across the three populations.^{32,57,58} In order to validate this
 504 method and to identify possible chronic carriers in the population (in addition to
 505 identifying whether there may be specific host genetic risk factors for chronic
 506 carriage), those individuals with a 'high' serum anti-Vi IgG titre will be re-approached
 507 and asked to provide two stool samples within 48 hours. With the agreement of
 508 relevant local ethics committees, identified chronic carriers (confirmed high anti-Vi
 509 IgG serum sample with a positive stool culture) will be treated with antibiotics for
 510 chronic carriage and clearance of bacterial shedding will be confirmed.

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5 512 **HOUSEHOLD CONTACTS**

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7 513 To further investigate possible transmission links, household contacts of index cases
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9 514 presenting with blood-culture confirmed *S. Typhi* or *S. Paratyphi A* infection will be
10
11 515 identified through the census data collection and approached to take part in this
12
13 516 component of the study. Up to five members of the household, with consent, will be
14
15 517 asked to provide a blood and stool sample at the time of discharge of the index case,
16
17 518 a further stool sample at one month and repeat serology at six months
18
19 519 (**Supplemental Table 1**), and asked about the occurrence of symptoms. These
20
21 520 samples will be investigated to identify those with subclinical infection or possible
22
23 521 chronic carriers. Secondary attack rates for the occurrence of symptomatic infection
24
25 522 will be estimated. If serology suggests chronic carriage in a household contact,
26
27 523 additional blood and stool samples will be collected to confirm this, and antimicrobial
28
29 524 treatment to eradicate carriage will be proposed.

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31 525

32
33 526 Those individuals who are identified as shedding bacteria but without high serum
34
35 527 anti-Vi IgG levels will be followed up at a one-year interval to repeat stool culture.
36
37 528 Repeat blood samples will be collected from household contacts approximately 3
38
39 529 months after initial sampling to explore whether rates of seroconversion are higher in
40
41 530 these individuals compared with those in the general population. At least 73
42
43 531 households of index cases are expected to be enrolled in this component of the
44
45 532 study.

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49 534 In a similar approach to the acute cases of typhoid infection, where chronic carriers
50
51 535 are identified from the serosurveys, household transmission studies will be
52
53 536 performed among household contacts of possible chronic carriers (i.e. those with a
54
55 537 'high' serum anti-Vi IgG titre). A one-time attempt will be made to enroll up to five
56
57 538 members of the household, sampling serology and stool looking for evidence of
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3 539 typhoid infection. This will provide data on secondary attack rates for both acute and
4
5 540 chronic typhoid states.

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8 9 542 **DATA MANAGEMENT AND ANALYSIS**

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11 543 Census and serosurvey data collection forms will be developed through a structured
12
13 544 iterative process and then implemented using Open Data Kit⁵⁹, a system enabling
14
15 545 electronic mobile data collection, with customizations by Nafundi, USA, on Android-
16
17 546 based tablets. Each household within the census will be assigned a unique study ID
18
19 547 and geo-located using GPS where possible; individuals will be given a member
20
21 548 number within the household. This information will be collected by local enumerators
22
23 549 over a one- to four-month period via these forms and adapted to the three
24
25 550 geographic settings. Data will be uploaded onto MySQL databases, where SQL
26
27 551 routines will be run nightly to enforce data cleaning on critical variables beyond
28
29 552 ODK's validation routines. Daily anonymized data will be backed up from the three
30
31 553 sites centrally. For the passive surveillance and household contacts studies a
32
33 554 combination of tablet and paper based case report forms will be used to capture the
34
35 555 data. Data from paper forms will be transcribed onto electronic databases using
36
37 556 Open Clinica⁶⁰. Database reports and descriptive analyses will be generated weekly.
38
39 557 To assess efficiency and quality of data capture, the volume, accuracy and time of
40
41 558 data collection can be quantified.

42
43 559 The distribution and burden of enteric illness is likely to vary between countries and
44
45 560 thus analyses will be conducted separately for each country. Where data are
46
47 561 combined across countries, an adjustment for country differences will be included in
48
49 562 statistical models.

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51 563

52 53 564 **ETHICS**

54
55 565 Written informed consent will be obtained from the head of each household (as the
56
57 566 "key informant") on behalf of the entire household in the demographic census and
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3 567 healthcare utilisation surveys. In each of the other components, individual written
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5 568 informed consent will be obtained from individuals over the age of 18 or by a
6
7 569 parent/guardian from individuals below this age with additional assent sought from
8
9 570 those between 11-17 years old.

10
11 571 This protocol has received ethics approval from the Oxford Tropical Research Ethics
12
13 572 Committee, the Malawian National Health Sciences Research Committee and
14
15 573 University of Malawi Research Ethics Committee, the Nepal Health Research
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17 574 Council and the icddr, b Institutional Review Board.

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21 576 **DISSEMINATION**

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23 577 We hope to make the results from these studies widely available and plan to
24
25 578 disseminate our analyses in international peer-reviewed journals. Investigators will
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27 579 be involved in reviewing drafts of the manuscripts, abstracts, press releases and any
28
29 580 other publications arising from the study. Furthermore, data from these studies will
30
31 581 also be used in the submission of post-doctoral theses.

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35 583 **COMMUNITY PUBLIC ENGAGEMENT**

36
37 584 A collection of specific activities engaging the local population with both the subject
38
39 585 of typhoid fever and the activities of the study have been carried out. In Malawi for
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41 586 example, community leaders have been informed and consulted on certain aspects
42
43 587 of the study, shown the proposed activities of study teams and given tours of
44
45 588 research facilities. There has been engagement with various forms of media
46
47 589 disseminating information on the importance of typhoid and study aims. In Nepal,
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49 590 field staff have been given detailed information to communicate to the local
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51 591 populations.

52
53 592 Further activities are planned to ensure the local populations are informed and
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55 593 engaged.

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3 595 **DISCUSSION**

4 596 The STRATAA study is a comprehensive multicentre study aiming to improve
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6 597 understanding of typhoidal *Salmonella* infection in high-risk endemic populations.
7
8 598 This study has been designed to answer key questions and data gaps identified
9
10 599 through an innovative application of recent mathematical modelling. These include
11
12 600 measuring the burden of age-stratified disease, identifying the relative contribution of
13
14 601 asymptomatic/subclinical *Salmonella* infection/exposure, and estimating the
15
16 602 contribution to ongoing transmission from the chronic carrier state. These data will
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18 603 inform further modelling required to develop and optimise disease control and
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20 604 prevention strategies that will eventually lead to disease elimination.
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3 605 **COMPETING INTERESTS**

4
5 606 AJP chairs the UK Department of Health's (DH) Joint Committee on Vaccination and
6
7 607 Immunisation (JCVI) and the European Medicines Agency (EMA) Scientific Advisory
8
9 608 Group on Vaccines and is a member of WHO's SAGE. The views expressed in this
10
11 609 manuscript do not necessarily reflect those of JCVI, DH, EMA or WHO. AJP has
12
13 610 previously conducted clinical trials on behalf of the University of Oxford funded by
14
15 611 vaccine manufacturers but has no personal financial interests.
16

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18
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20
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26
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28
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30
31 619 designing the study, writing this manuscript or the decision to submit.
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34 620

35 621 **Authors contributions**

36
37 622 The manuscript was drafted by TD and JM. All authors read, critically revised and
38
39 623 approved the final manuscript.
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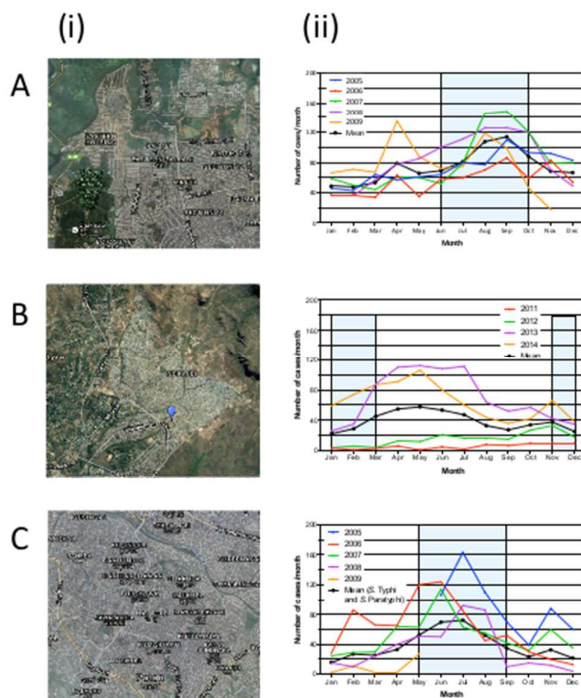
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792 **Figure Legends**

793 **Figure 1. Description of STRATAA study field sites, demonstrating (i) the**
 794 **location of the three sampling sites in (A) Mirpur (Dhaka, Bangladesh), (B)**
 795 **Ndirande (Blantyre, Malawi) and (C) Patan (Kathmandu, Nepal), (ii) the**
 796 **historical number of typhoid cases detected per month at each site (blue box**
 797 **marks the annual monsoon season), and (iii) examples of the local terrain.**

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800 Supplemental Table 1. Overview of STRATAA Study Procedures.

Study component	CENSUS							HEALTHCARE UTILISATION		PASSIVE SURVEILLANCE								SEROSURVEY				
								Peak seasons during census	All cases				Household contacts of index cases				Participants		Household contacts of possible carriers			
									Cases within census area*													
Sample size (individuals unless specified)	≥100 000 per site							>700 households		Convenience				≥73 households				8500	6800	Members of ≥50 households		
Time (months, unless specified)	0	4	8	12	16	20	24	1st	2nd	0	Day 8	1	~6	12	0	1	~3	~12	0	**	3	6
Consent	X							X		X					X		X ^{§§}		X			x
Demographic Information	X	X [#]	X [#]	X [#]	X [#]	X [#]	X															
Clinical information										X	X	X	X	X	X							
Questionnaire								X	X													
Blood sample										X [@]			X		X		X [§]		X [@]		X	X
Stool sample										X		X	X	(X)	X	X	2X [€]	(3X)		2X [€]		X

Urine sample									X@									X@			
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802 * Case within census area = index if blood-culture confirmed typhoid diagnosis, resident in census area and household members consent
803 to participation.

804 # Births, deaths and migrations updated every 4 months in Nepal & Bangladesh

805 ~ approximately - dependent on timeframe of availability for the Vi ELISA result

806 \$ Host genetics collected/used for laboratory studies if either stool or serum positive or both

807 § separate consent for host genetics

808 @ additional blood (RNA + plasma) and urine samples (subset of 100 with typhoid (culture positive), 100 with clinically suspected typhoid
809 (culture negative), 100 with alternate bacteraemia; and 100 healthy controls (from the serosurvey) from adults 16-40yrs who give
810 consent for genetic assays to be performed.

811 € Participants identified as having high anti-Vi IgG only - 2 stool samples collected 48 hours apart once ELISA result known to
812 identify/confirm carriage

813 ** When result known

814 Total blood volumes: **Passive survey** ≤16 mL adults (>16 years), ≤7 mL children (<16 years); **Serosurvey** ≤8 mL adults (>16 years), ≤7 mL
815 children. Cell pellets stored for host genetics.

BMJ Open

THE STRATAA STUDY PROTOCOL: A PROGRAMME TO ASSESS THE BURDEN OF ENTERIC FEVER IN BANGLADESH, MALAWI AND NEPAL USING PROSPECTIVE POPULATION CENSUS, PASSIVE SURVEILLANCE, SEROLOGICAL STUDIES AND HEALTHCARE UTILISATION SURVEYS

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1 **THE STRATAA STUDY PROTOCOL: A PROGRAMME TO ASSESS THE**
2 **BURDEN OF ENTERIC FEVER IN BANGLADESH, MALAWI AND NEPAL USING**
3 **PROSPECTIVE POPULATION CENSUS, PASSIVE SURVEILLANCE,**
4 **SEROLOGICAL STUDIES AND HEALTHCARE UTILISATION SURVEYS**

6 **Short title: The STRATAA study**

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3 70 **ABSTRACT**
4

5 71 **Introduction**
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7 72 Invasive infections caused by *Salmonella enterica* serovar Typhi and Paratyphi A are
8
9 73 estimated to account for 12-27 million febrile illness episodes worldwide annually.
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11 74 Determining the true burden of typhoidal *Salmonellae* infections is hindered by lack
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13 75 of population-based studies and adequate laboratory diagnostics.
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15 76 The STRATAA (Strategic Typhoid alliance across Africa and Asia) study takes a
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17 77 systematic approach to measuring the age-stratified burden of clinical and subclinical
18
19 78 disease caused by typhoidal *Salmonellae* infections at three high-incidence urban
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21 79 sites in Africa and Asia. We aim to explore the natural history of *Salmonella*
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23 80 transmission in endemic settings, addressing key uncertainties relating to the
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25 81 epidemiology of enteric fever identified through mathematical models, and enabling
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27 82 optimisation of vaccine strategies.
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29 83 **Methods/Design**
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31 84 Using census-defined denominator populations of $\geq 100,000$ individuals at sites in
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33 85 Malawi, Bangladesh and Nepal, the primary outcome is to characterize the burden of
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35 86 enteric fever in these populations over a 24-month period. During passive
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37 87 surveillance, clinical and household data, and laboratory samples will be collected
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39 88 from febrile individuals. In parallel, healthcare utilization and water, sanitation and
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41 89 hygiene surveys will be performed to characterise healthcare-seeking behaviour and
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43 90 assess potential routes of transmission. The rates of both undiagnosed and
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45 91 subclinical exposure to typhoidal *Salmonellae* (seroincidence), identification of
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47 92 chronic carriage, and population seroprevalence of typhoid infection will be assessed
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49 93 through, age-stratified serosurveys performed at each site. Secondary attack rates
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51 94 will be estimated among household contacts of acute enteric fever cases and
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53 95 possible chronic carriers.
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55 96 **Ethics and Dissemination**
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3 97 This protocol has been ethically approved by the Oxford Tropical Research Ethics
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5 98 Committee, the icddr, Institutional Review Board, the Malawian National Health
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7 99 Sciences Research Committee and College of Medicine Research Ethics Committee
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9 100 and Nepal Health Research Council. The study is being conducted in accordance
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11 101 with the principles of the Declaration of Helsinki and Good Clinical Practice. Informed
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13 102 consent is obtained before study enrolment. Results will be submitted to international
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15 103 peer-reviewed journals and presented at international conferences.
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19 105 **STRENGTHS AND LIMITATIONS**

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21 106 - The study is designed with a comprehensive multicomponent epidemiological
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23 107 approach, nesting passive surveillance, serosurveillance and healthcare utilisation
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25 108 surveys within a demographic census population, to accurately determine the age-
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27 109 stratified burden of enteric fever.
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29 110 - The diversity of field sites in Africa and Asia, will provide data on typhoid
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31 111 epidemiology and transmission from a range of differing epidemiological settings.
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33 112 - The combination of a traditional epidemiological approach with novel laboratory
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35 113 methods for the diagnosis of febrile illness and investigation of the host and
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37 114 pathogen genetics and antimicrobial resistance determinants provides a unique
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39 115 platform to study this disease.
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41 116 - Practical limitations include the sharing and standardisation of clinical definitions,
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43 117 data and sample collection methods and laboratory assays, based on the facilities
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45 118 and staff available and community requirements at each site.
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47 119 - The number of field-sites included in this current protocol is limited to three; all of
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49 120 these sites are densely populated urban settings with likely high-incidence of enteric
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51 121 fever transmission. The degree to which our data may be extrapolated to other
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53 122 settings and countries remains to be explored.
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3 125 **Registration:** ISRCTN 12131979
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5 126 **Ethics References:** Oxford (Oxford Tropical Research Ethics Committee 39-15)
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7 127 Bangladesh (icddr,b Institutional Review Board PR-15119)
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9 128 Malawi (National Health Sciences Research Committee
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11 129 15/5/1599)
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13 130 Nepal (Nepal Health Research Council 306/2015)
14
15 131
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17 132 **Keywords:**
18
19 133 Enteric fever, vaccination programme, infection transmission, *Salmonella* Typhi,
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21 134 *Salmonella* Paratyphi A, serosurveillance, seroepidemiology, healthcare utilisation,
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23 135 resource-limited settings; diagnosis; febrile illness; Africa, Asia.
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3 136 **BACKGROUND**

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5 137 *Salmonella enterica* serovars Typhi (S. Typhi) and Paratyphi A (S. Paratyphi A), are
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7 138 human-restricted pathogens transmitted by faeco-oral ingestion. The ensuing
8
9 139 disease, enteric fever (or 'typhoid fever'), is a non-specific febrile illness which affects
10
11 140 an estimated 12-27 million people worldwide each year, resulting in 129,000–
12
13 141 223,000 deaths.^{1–3} Despite a dramatic reduction in incidence over the last century in
14
15 142 most high-income countries, continuing inadequate access to clean water and
16
17 143 increasing inter-continental spread of multiply antibiotic-resistant strains hamper
18
19 144 disease control efforts especially in resource-limited settings.^{3–5} The current burden
20
21 145 of disease is highest among children and young adults in South and Southeast Asia^{1–}
22
23 146 ³ but is increasingly recognized in sub-Saharan Africa.⁶

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25 147
26
27 148 Recently, a new generation of Vi- conjugate enteric fever vaccines, suitable for use in
28
29 149 infants and providing longer lasting protection than those previously licensed,^{7–10}
30
31 150 have become available. Determining how and where interventions such as
32
33 151 vaccination may be best deployed is difficult due to a lack of population-based
34
35 152 incidence studies and inaccurate diagnostic tests.^{1–3} Improving disease burden
36
37 153 estimates and providing data on the epidemiology and transmission of S. Typhi and
38
39 154 S. Paratyphi A to inform mathematical models^{11–13} could improve the evidence
40
41 155 necessary to design comprehensive and effective disease control programmes.¹⁴

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45 157 **Burden of disease and diagnostics**

46
47 158 Recent meta-analyses examining global causes of morbidity and mortality estimate
48
49 159 that a significant burden of disease worldwide, and especially in South and
50
51 160 Southeast Asia, may be attributed to enteric fever.^{15–17} The margin of error on these
52
53 161 estimates is wide, however; much of the uncertainty regarding the burden of disease
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55 162 caused by typhoidal *Salmonella* is due to the unavailability of accurate diagnostics or
56
57 163 misclassification of non-specific disease presentations, in addition to a general lack
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1
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3 164 of data.¹⁸ Availability of antibiotics from local pharmacies without prescription,
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5 165 frequent misdiagnosis as malaria, dengue or other febrile illnesses, or the avoidance
6
7 166 of hospital due to fear or expense are all likely to result in an underestimate of the
8
9 167 true numbers of cases.¹⁹ In contrast, the widespread use of suboptimal diagnostic
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11 168 tests such as the Widal test in areas where exposure to similar bacteria in the
12
13 169 environment occurs may lead to inaccurate over-diagnosis of the condition.²⁰
14
15 170 Previous studies and systematic reviews have attempted to address this inaccuracy
16
17 171 by calculating the imprecision associated with insensitive blood culture methods, and
18
19 172 applying this correction to blood culture confirmed case numbers.^{1,2}
20
21 173
22
23 174 In addition to a better understanding of the disease burden attributable to typhoidal
24
25 175 *Salmonellae*, improved diagnostics and biomarkers are required to improve individual
26
27 176 case management.²¹ The current gold-standard diagnostic test for
28
29 177 typhoid/paratyphoid is blood culture; while this test provides the causative isolate,
30
31 178 thereby allowing susceptibility testing and further typing methods to be performed,
32
33 179 most laboratories in resource-limited settings lack the infrastructure to perform these
34
35 180 assays. Even in ideal conditions, blood culture requires a significant volume of blood
36
37 181 to enhance diagnostic yield, and is relatively insensitive even in highly-controlled
38
39 182 human challenge settings where sensitivity reaches 80%.²² Highly sensitive and
40
41 183 specific diagnostic tests are a fundamental requirement for surveillance and vaccine
42
43 184 efficacy studies, however, both for measuring the number of cases
44
45 185 avoided/prevented, but also for reassuring policy makers and funders that vaccine
46
47 186 implementation is worth investment. While several rapid serological diagnostic tests
48
49 187 have recently been developed, these still rely on a few selected antigens, which are
50
51 188 known to be non-specific in endemic communities.^{23,24} Newer diagnostic modalities
52
53 189 are in development, however, which have utilised serum banks from multiple
54
55 190 countries and samples collected from controlled human infection models to identify
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57 191 putative novel antigens for serological assay and further high sensitivity approaches
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1
2
3 192 including metabolomics and functional genomics.^{5,25-27} In addition, several of these
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5 193 newer approaches have demonstrated potential for identifying individuals with
6
7 194 probable chronic carriage. Identification and treatment of chronic carriers in a
8
9 195 population would significantly improve our understanding of disease dynamics and
10
11 196 allow targeted treatment strategies to reduce community transmission of infection.
12

13
14
15 198 Furthermore, whilst utilising these new diagnostic tests in comprehensive passive
16
17 199 surveillance or even active surveillance programmes will identify acute cases of
18
19 200 typhoid disease, the incidence rate of sub-clinical infection and exposure is unknown.
20
21 201 These data are likely essential to understanding pathogen transmission as well as
22
23 202 the development and maintenance of immunity against clinical disease¹² and are
24
25 203 likely only to be obtainable through the use of seroepidemiological methods.

26
27 204 Previous seroepidemiological studies have examined the cross-sectional age-
28
29 205 stratified prevalence of antibodies to the *S. Typhi* flagellar (H) antigen in Santiago,
30
31 206 Chile²⁸, and serum bactericidal antibody in Kathmandu, Nepal.²⁹ Also, high antibody
32
33 207 titres to the Vi (Vi capsular polysaccharide; virulence factor) antigen of *S. Typhi*
34
35 208 measured by agglutination assay have been used to estimate chronic carrier
36
37 209 frequency in population-based studies,³⁰⁻³² although these studies were not
38
39 210 universally successful at identifying chronic carriers.³³ Improvements in serological
40
41 211 assays and the discovery of a newer generation of diagnostic antigens³⁴ suggest that
42
43 212 population-based serosurveillance studies, especially using longitudinal
44
45 213 measurements, may provide key data regarding the incidence of subclinical infection
46
47 214 with the bacterial agents of enteric fever and the prevalence of chronic carriers.

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50 215

51 216 **Transmission**

52
53 217 Closely related to the estimate of incidence is the contribution of different typhoid
54
55 218 states to ongoing transmission within a community. Acute cases of enteric fever,
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57 219 individuals with subclinical infection, as well as chronic carriers likely all contribute to
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1
2
3 220 the transmission and maintenance of *S. Typhi* and *S. Paratyphi A* in endemic areas,
4
5 221 but not to the same degree. This is an essential area of further research, particularly
6
7 222 given the negative impact transmission from chronic carriers could have on the
8
9 223 indirect protection afforded by typhoid vaccines.¹² The importance of 'short cycle'
10
11 224 transmission via contaminated food and water in the immediate environment versus
12
13 225 'long cycle' transmission via the contamination of community water sources is also
14
15 226 an area of direct relevance in the control of typhoid fever, primarily through non-
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17 227 vaccine public health interventions including water and sanitation hygiene (WASH)
18
19 228 improvements.
20

21 229

23 230 **Bacterial genome variation**

25 231 The enteric fever agents *S. Typhi* and *S. Paratyphi A*, while genetically distinct from
26
27 232 other serovars of *Salmonella enterica*, exhibit low genetic diversity and whole
28
29 233 genome sequence (WGS) analysis is required to uncover patterns of genetic
30
31 234 relatedness between isolates.³⁵⁻³⁷ These techniques have been used previously to
32
33 235 track the development and spread of antibiotic resistance on a global scale,⁶ and
34
35 236 have shown potential to accurately track transmission links between individuals,
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37 237 specifically within affected households, in addition to the broader community.³⁸ One
38
39 238 use of this high-resolution typing previously, has been to determine whether isolates
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41 239 obtained from those with acute infection are distinct from those found in chronic gall-
42
43 240 bladder carriers.³⁹

44 241

47 242 **Host susceptibility**

49 243 While environmental risk factors for enteric fever acquisition are thought to be
50
51 244 relatively well understood, recent evidence has emerged implicating genetic factors
52
53 245 in host susceptibility to infection.^{35,36} Many early typhoid genetic susceptibility studies
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55 246 reporting candidate genes were limited by small sample sizes and choice of controls,
56
57 247 a recent large-scale genome-wide association study however, has identified a
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3 248 specific locus (HLA-DRB1*405) which confers 5-fold protection against developing
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5 249 typhoid fever.³⁵ How genetic variation at this site influences protection against
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7 250 typhoid is still under investigation, but may result in functional differences in MHC
8
9 251 class II amino acid sequences; in turn, this may influence *S. Typhi* epitope selection
10
11 252 and antigen presentation or the scale and format of the host's T-cell response.
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13 253

14 254 **Vaccines**

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16
17 255 Vaccines currently available for typhoid are either oral live attenuated bacterial
18
19 256 strains or are derived from the *S. Typhi* polysaccharide capsule, called Vi; there are
20
21 257 no licensed vaccines against *S. Paratyphi A* or non-typhoidal salmonellae (NTS).
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23 258 Despite recommendations for their use, these vaccines have only sparsely been
24
25 259 introduced in high prevalence settings.^{7,40} The reasons for this are unclear, but likely
26
27 260 include failure of prioritisation and a short fall in advocacy, in addition to technical
28
29 261 deficiencies of the vaccines themselves. One common reason cited is the T-cell
30
31 262 independent nature of the immune response to the Vi polysaccharide vaccine (ViPS);
32
33 263 this renders the vaccine of little use in young children below the age of two years. In
34
35 264 addition, this feature means that the immune response does not boost, requiring
36
37 265 repeat vaccination to be administered every 3-5 years. As with other thymus-
38
39 266 independent vaccines, to overcome this problem the Vi capsule may be chemically
40
41 267 conjugated to a protein carrier,⁸ as has successfully been done with conjugation of Vi
42
43 268 to *Pseudomonas aeruginosa* exotoxin A.⁹ Despite demonstrating good
44
45 269 immunogenicity and efficacy in field trials,^{10,41} the vaccine is still unlicensed. Newer
46
47 270 Vi-conjugate vaccines are in development and have progressed to assessment in
48
49 271 field-trials,^{42,43} and the Oxford human typhoid challenge model.⁴⁴ Recent data from
50
51 272 the challenge models suggests that the Vi response elicited by the vaccine is high
52
53 273 and likely to be protective, at least in naïve healthy adult volunteers with no immunity
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55 274 or those with previous history of infection.⁴⁴ Furthermore, a recent study in Indian
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57 275 schoolchildren found a vaccine efficacy of 100% (95% confidence interval, 97.6 to
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3 276 100%) after a single dose of Vi conjugated to tetanus toxoid during the first year of
4
5 277 follow-up.⁴³
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9 279 The key issue remaining, once safe, well-tolerated, immunogenic vaccines become
10
11 280 widely available, is how best to implement them in endemic settings. Currently
12
13 281 available surveillance data from most regions is insufficient to demonstrate the age-
14
15 282 band with the highest incidence of enteric fever.⁴⁵ Designing vaccination strategies to
16
17 283 cover the years during which children are most at risk and generating indirect
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19 284 protection by preventing infection among those age groups driving transmission
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21 285 could be facilitated through the use of well-informed mathematical modelling.
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23 286

24 25 287 **THE STRATAA STUDY: RATIONALE AND AIMS**

26
27 288 The STRATAA (Strategic Typhoid alliance across Africa and Asia) study draws
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29 289 together an international team of investigators, field sites, laboratories and research
30
31 290 institutes to address many of the key outstanding questions described above
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33 291 regarding the burden of enteric fever and *Salmonella* exposure in endemic regions,
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35 292 the mechanisms of susceptibility and infection transmission. These have been
36
37 293 identified as key uncertainties in mathematical models.¹¹⁻¹³ By conducting field
38
39 294 studies of the epidemiology and burden of enteric fever across three sites with
40
41 295 distinct epidemiological profiles and applying state-of-the-art molecular methods, the
42
43 296 goal of the STRATAA study is to collect the data needed to enhance our
44
45 297 understanding of pathogen transmission, exposure and susceptibility. These data
46
47 298 can then be used to rigorously parameterize and validate models for the transmission
48
49 299 dynamics of the agents of enteric fever, such that these models can then be used to
50
51 300 evaluate different vaccination strategies and, importantly, help predict the expected
52
53 301 impact resulting from the direct and indirect effects of vaccine introduction. These
54
55 302 and other overarching objectives of the STRATAA study are listed in **Table 1**.
56
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58 303

304 **Table 1. Overarching objectives of the STRATAA study.**

Primary	To characterise the burden of enteric fever at three urban sites in Africa and Asia
Secondary	Assess the burden/incidence of enteric fever
	Assess the seroincidence of infection
	Assess host factors affecting burden/incidence/transmission of enteric fever
	Assess effect of pathogen genetics on burden/incidence/transmission of enteric fever
	Develop diagnostic tools for rapid and consistent typhoid diagnosis
	Develop transmission modelling and modelling of vaccine introduction impact
Tertiary Objectives	Strengthen research capacity in enteric fever endemic regions
	Provide data to appropriate institutes and governments to advocate vaccine implementation
	To characterise the burden of invasive NTS and other invasive pathogens at an urban site in Malawi, Africa

305

306 To deliver on these objectives, the following studies will be performed in parallel at
 307 each of the three chosen field sites, starting in May 2016 with activities continuing
 308 until October 2018. Firstly, in a well-defined population catchment of
 309 approximately 100,000 individuals, a detailed demographic census survey will be
 310 undertaken. Secondly, in healthcare facilities utilised by the census population (as
 311 confirmed through a healthcare utilisation survey) prospective passive surveillance to
 312 detect cases of enteric fever will be performed. Thirdly, a seroincidence study will be

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3 313 conducted in an age-stratified sample of the census population at each site,
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5 314 collecting blood samples at intervals to estimate the rate of seroconversion to
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7 315 typhoidal *Salmonella* antigens and hence the rate of subclinical/asymptomatic
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9 316 exposure to these bacteria in the general population.
10

11 317
12
13 318 To meet the further objectives, which include evaluating diagnostic tests, identifying
14
15 319 antimicrobial resistance patterns, exploring host susceptibility and bacterial
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17 320 virulence/genomic variation, biological samples, specimens and metadata will be
18
19 321 collected during these individual studies. Data will be pooled to inform transmission
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21 322 dynamic and health economic models that can be used to help design future vaccine
22
23 323 effectiveness studies and evaluate vaccine delivery strategies for widespread
24
25 324 deployment.
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29 326 **STUDY SITES**

30
31 327 With known high incidence throughout South and Southeast Asia and a recent
32
33 328 increase in reported cases from sub-Saharan Africa^{46,47} three sites were selected
34
35 329 from across Africa and Asia based on known high rates of enteric fever and the
36
37 330 research capacity to deliver a study of this size and logistical complexity. The three
38
39 331 sites differ in their epidemiological profiles and history of enteric fever incidence.
40

41 332

42 333 ***Dhaka, Bangladesh***

43
44 334 Mirpur is an area located within Dhaka Metropolitan area, Bangladesh (**Figure 1A**),
45
46 335 situated 7km north-west of the International Centre for Diarrhoeal Disease Research,
47
48 336 Bangladesh (icddr,b) main campus. The icddr,b manages two hospitals in Dhaka
49
50 337 city, one in the main campus in Dhaka (the main hospital) and another in the Mirpur
51
52 338 area known as MTC (Mirpur Treatment Centre) comprising a 50-bed inpatient facility.
53
54 339 As part of this programme, these and other health facilities (ten or more total) will be
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56 340 kept under careful surveillance for enteric fever in the census area. The total
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3 341 catchment area for the field site for the STRATAA study is 10.79 km² with a total
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5 342 population of about 603,658 at a density of 55946/km². Approximately 98% of
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7 343 residents have access to tap water supplied by the municipality while the remainder
8
9 344 use wells, hand pumps and other sources such as ponds and rivers in the study
10
11 345 area.

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13 346
14
15 347 Previous data from blood culture surveillance revealed a high incidence of disease
16
17 348 within Dhaka, with the burden particularly high in children under 5 years of age
18
19 349 (estimated at 18.7 episodes/1000 person-years in this age group). The current S.
20
21 350 Typhi:Paratyphi ratio is approximately 5:1. Typhoid fever is reported throughout the
22
23 351 year but peaks during the monsoon season.^{48,49} Of note, 15% of S. Typhi isolates are
24
25 352 multi-drug resistant (MDR) and around 97% isolates are resistant to nalidixic acid⁵⁰;
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27 353 extended-spectrum beta-lactamase (ESBL) producing organisms have also been
28
29 354 isolated from enteric fever patients in this setting.

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31 355
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33 356 ***Blantyre, Malawi***

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35 357 Ndirande is a large urban township on the outskirts of Blantyre city, Malawi, 6km
36
37 358 from the main referral hospital (**Figure 1B**). It has a young population of around
38
39 359 100,000 people spread over 6.77km². It is serviced by one health clinic staffed by
40
41 360 clinical officers. It has high reported rates of typhoid fever along with a population
42
43 361 HIV prevalence of around 18%.⁵¹ Queen Elizabeth Central Hospital (QECH) in
44
45 362 Blantyre, Malawi, is the government-funded hospital for Blantyre district, serving a
46
47 363 local population of 1.3 million persons and provides tertiary care to southern Malawi.

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49 364
50
51 365 NTS have previously been the commonest cause of invasive bloodstream infections
52
53 366 in Blantyre,⁵² but since 2011 there has been a rapid increase in the number of S.
54
55 367 Typhi cases seen at QECH, from approximately 14/year between 1998 and 2010 to
56
57 368 782 in 2014.⁴⁶ Much of this increase has been due to the emergence of the H58

1
2
3 369 clone and is associated with an MDR phenotype^{47,53}. This outbreak appears
4
5 370 unrelated to HIV-infection, but has resulted in high rates of mortality (2.5% adult and
6
7 371 paediatric) despite the availability of fluoroquinolone antibiotics.⁴⁷ In this setting,
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9 372 enteric fever is seasonal, with the peak number of cases seen at the end of the wet
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11 373 season and during the early dry season when the prevalence of malnutrition is also
12
13 374 highest.

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17 376 ***Patan, Nepal***

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19 377 Patan is located within the Lalitpur Sub-Metropolitan City (LSMC) within the
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21 378 Kathmandu Valley, Nepal (**Figure 1C**). The population is generally poor, with most
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23 379 living in overcrowded conditions and obtaining their water from stone spouts or
24
25 380 sunken wells. Patan Hospital is a 318-bed government hospital providing emergency
26
27 381 and elective outpatient and inpatient services to this area. The local catchment
28
29 382 population of the hospital is approximately 200,000 people in about 20 km², with a
30
31 383 population density of 8,000/km²; there is a high rate of immigration for employment,
32
33 384 particularly young males from rural areas. Enteric fever is frequently managed in the
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35 385 outpatient clinic at Patan Hospital, which has approximately 200,000 outpatient visits
36
37 386 annually.

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41 388 Uptake of typhoid Vi vaccination is limited and natural exposure/subclinical infection
42
43 389 is common.²⁹ Approximately 400 culture confirmed cases of enteric fever are
44
45 390 diagnosed at Patan Hospital each year, with a peak during the monsoon months. The
46
47 391 current *S. Typhi*:*Paratyphi A* ratio is approximately 1:1.⁵⁴ Antimicrobial resistance is
48
49 392 more commonly observed in *S. Paratyphi A* isolates; however, emergence of
50
51 393 fluoroquinolone resistant *S. Typhi* isolates has also been recently identified,⁵⁵
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53 394 whereas MDR strains of either serovar are rare.³⁸

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3 396 **METHODS**
4

5 397 **POPULATION CENSUS**
6

7 398 In order to accurately calculate an incidence rate of typhoid fever for each of the
8
9 399 study sites, a demographic census will be performed at baseline and repeated at 2
10
11 400 years. The objective of the census is to identify/characterise the source population
12
13 401 corresponding to the catchment areas for the passive surveillance sites described
14
15 402 below, and estimate the person-time under surveillance.
16

17 403

18
19 404 Census data will be updated with births, deaths and migrations every six months in
20
21 405 Dhaka and Patan, and at two years in Blantyre. The number of participants for the
22
23 406 demographic census survey in each site needed to produce a two-sided 95%
24
25 407 confidence interval with a precision (half-width) of 50% for the anticipated typhoid
26
27 408 incidence rate detected through passive clinical surveillance has been calculated
28
29 409 (**Table 2**). The necessary catchment population size is driven by the size required to
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31 410 estimate the expected incidence rate in the 0-4 year-old age group, which is
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33 411 estimated to be approximately 10% of the total population in each site.
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414 **Table 2. Sample size required for the target populations in the three sites to**
 415 **estimate annual, blood culture-confirmed typhoid incidence in passive clinical**
 416 **surveillance.**

Age groups	Anticipated typhoid incidence per 1000 persons*	Precision or half-width	Sample-size required
0-4 years	1.5	0.75	10,125
5-14 years	1.0	0.5	15,119
>14 years	0.5	0.25	29,801

417 * Assumed age-specific incidence rates (based on data from Dhaka, Bangladesh,
 418 Delhi, India, and Dong Thap, Vietnam).

419
 420 The census will take place within a demarcated geographic area that is a known
 421 catchment population for the surveillance sites (**Figure 1**). In total, at least 100,000
 422 individuals will be enumerated from $\geq 20,000$ households. The head/key informant
 423 within the household will provide written informed consent to take part in the study.
 424 Information on all residents within the household at the time of the census will be
 425 gathered from the head of the household/key informant.

426
 427 A household is defined as individuals living in the same dwelling or compound and
 428 sharing food from the same kitchen. A household member is considered to have
 429 migrated out if s/he has left the household and does not intend to come back within
 430 six months of the time s/he left. A person is considered to have migrated in if s/he
 431 was not previously included in the list of household members and intends to live in
 432 the household for the next six months. At enrolment, the head of each household will

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2
3 433 be made aware of the passive surveillance component of the study and encouraged
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5 434 to use the field-site facilities capturing acute febrile illnesses, including acute enteric
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7 435 fever cases. The characterised census population will form the sampling frame for
8
9 436 the further components (passive surveillance, healthcare utilisation/WASH surveys
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11 437 and serosurvey) described below.
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15 439 **PASSIVE SURVEILLANCE**

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17 440 The passive surveillance component of STRATAA is designed to capture cases of
18
19 441 febrile disease occurring in each of the three census populations. Patients presenting
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21 442 to any of the clinical surveillance sites with a history of subjective fever >72 hours or
22
23 443 objective fever >38.5 °C on presentation will be approached for enrolment.
24

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27 445 An index case will be defined as an individual with a blood culture result confirming
28
29 446 infection with *S. Typhi* or *S. Paratyphi A* (or NTS in Malawi), and whose household is
30
31 447 included in the census survey. These cases will be used to calculate the disease
32
33 448 incidence in each of the three census sites. Consenting individuals will have samples
34
35 449 of blood, urine and stool collected to determine a diagnosis of *S. Typhi*, *S. Paratyphi*
36
37 450 *A* or NTS and to provide material for the further diagnostic and genetic aims of the
38
39 451 study (**Supplemental Table 1**). Patients not resident in the census area will be
40
41 452 enrolled as additional cases for the laboratory and genetic components of the
42
43 453 programme if written consent is provided.
44

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47 455 **HEALTHCARE UTILISATION AND WATER, SANITATION AND HYGIENE**

48 456 **SURVEYS**

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51 457 To characterise the healthcare-seeking behaviour of individuals living in the census
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53 458 areas, at least 735 households will be randomly selected from the census area at
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55 459 each site to participate in a healthcare utilisation survey. Data will be collected from
56
57 460 the head of the household/key informant to describe the actual and hypothetical
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3 461 usage of healthcare facilities for febrile episodes. The aim of this component is to
4
5 462 estimate the percent of cases (fulfilling the fever case definition) in each age stratum
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7 463 (0-4 years, 5-14 years and >14 years of age) who would or would not seek attention
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9 464 at one of the designated passive surveillance health facilities. Additional data
10
11 465 regarding sanitation and hygiene facilities and usage will also be collected. Annual
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13 466 data collection periods will coincide with the peak typhoid season at each of the three
14
15 467 sites.
16
17 468

19 469 **SEROSURVEYS**

20
21 470 To estimate the seroincidence and seroprevalence of clinical (both formally
22
23 471 diagnosed and undiagnosed cases) and subclinical infection or exposure to *S. Typhi*
24
25 472 and *S. Paratyphi*, systematic serosurveillance will be performed at each site. Blood
26
27 473 samples will be collected at baseline and 3 months later, with sample collection
28
29 474 initiated in an on-going basis over the course of one year (**Table 3**). To identify
30
31 475 participants for the serosurvey, an age-stratified approach will be used to randomly
32
33 476 select individuals from the census population from each age group. Suitable
34
35 477 participants will be enrolled by field workers; where the individual identified is not
36
37 478 available, a household member in the same age group will be selected. Where this is
38
39 479 not possible further households will be randomised into this component to ensure
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41 480 adequate numbers of individuals in the different age groups.
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46 482 Seroincidence (indicative of recent infection) will be calculated by measuring the rate
47
48 483 of seroconversion to anti-H(d) (anti-flagellin) IgG and other acute phase antibodies
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50 484 between both time points, with the denominator consisting only of individuals
51
52 485 sampled twice and seronegative at the first time point.⁵⁶
53
54 486

55
56 487 **Table 3. Sample size calculations for the serological surveys to estimate age-**
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58 488 **specific rates of high titres of serum IgG anti-H(d) antibodies.** Assumes age-

489 stratified individual sampling; also accounts for detection of 1% chronic carriage rate
 490 in ≥ 10 year olds.

	Anticipate d sero- incidence (%)	Number of initial samples	Anticipat ed number of events	No. of follow- up samples *	No. of events detected	Binomial [95%	Exact CI]	Probabili ty of observin g 0 events
0-4 yrs	0.2	2500	5	2000	4	0.0005	0.0051	0.0182
5-9 yrs	0.4	1300	5.2	1040	4	0.0010	0.0098	0.0155
10-14 yrs	0.8	800	6.4	640	5	0.0025	0.0181	0.0059
>14 yrs	0.2	3900	7.8	3120	6	0.0007	0.0042	0.0019
TOTAL		8500	24.2	6800	19			

491 *Assuming 5% migration and 15% refusal

492

493 The seroprevalence of anti-Vi IgG will also be measured at baseline to identify
 494 individuals who could be potential chronic carriers. Previous studies have used high
 495 anti-Vi IgG antibody levels in serum as a marker of chronic carriage, and our aim is
 496 to use a recently validated/approved anti-Vi ELISA method to determine possible
 497 rates of chronic carriage across the three populations.^{32,57,58} In order to validate this
 498 method and to identify possible chronic carriers in the population (in addition to
 499 identifying whether there may be specific host genetic risk factors for chronic
 500 carriage), those individuals with a 'high' serum anti-Vi IgG titre will be re-approached
 501 and asked to provide two stool samples within 48 hours. With the agreement of
 502 relevant local ethics committees, identified chronic carriers (confirmed high anti-Vi
 503 IgG serum sample with a positive stool culture) will be treated with antibiotics for
 504 chronic carriage and clearance of bacterial shedding will be confirmed.

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3 5054
5 506 **HOUSEHOLD CONTACTS**

6
7 507 To further investigate possible transmission links, household contacts of index cases
8
9 508 presenting with blood-culture confirmed *S. Typhi* or *S. Paratyphi A* infection will be
10
11 509 identified through the census data collection and approached to take part in this
12
13 510 component of the study. Up to five members of the household, with consent, will be
14
15 511 asked to provide a blood and stool sample at the time of discharge of the index case,
16
17 512 a further stool sample at one month and repeat serology at six months
18
19 513 (**Supplemental Table 1**), and asked about the occurrence of symptoms. These
20
21 514 samples will be investigated to identify those with subclinical infection or possible
22
23 515 chronic carriers. Secondary attack rates for the occurrence of symptomatic infection
24
25 516 will be estimated. If serology suggests chronic carriage in a household contact,
26
27 517 additional blood and stool samples will be collected to confirm this, and antimicrobial
28
29 518 treatment to eradicate carriage will be proposed.

30
31 519

32
33 520 Those individuals who are identified as shedding bacteria but without high serum
34
35 521 anti-Vi IgG levels will be followed up at a one-year interval to repeat stool culture.
36
37 522 Repeat blood samples will be collected from household contacts approximately 3
38
39 523 months after initial sampling to explore whether rates of seroconversion are higher in
40
41 524 these individuals compared with those in the general population. At least 73
42
43 525 households of index cases are expected to be enrolled in this component of the
44
45 526 study.

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49 528 In a similar approach to the acute cases of typhoid infection, where chronic carriers
50
51 529 are identified from the serosurveys, household transmission studies will be
52
53 530 performed among household contacts of possible chronic carriers (i.e. those with a
54
55 531 'high' serum anti-Vi IgG titre). A one-time attempt will be made to enroll up to five
56
57 532 members of the household, sampling serology and stool looking for evidence of
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3 533 typhoid infection. This will provide data on secondary attack rates for both acute and
4
5 534 chronic typhoid states.

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7 535

8 9 536 **DATA MANAGEMENT AND ANALYSIS**

10
11 537 Census and serosurvey data collection forms will be developed through a structured
12
13 538 iterative process and then implemented using Open Data Kit⁵⁹, a system enabling
14
15 539 electronic mobile data collection, with customizations by Nafundi, USA, on Android-
16
17 540 based tablets. Each household within the census will be assigned a unique study ID
18
19 541 and geo-located using GPS where possible; individuals will be given a member
20
21 542 number within the household. This information will be collected by local enumerators
22
23 543 over a one- to four-month period via these forms and adapted to the three
24
25 544 geographic settings. Data will be uploaded onto MySQL databases, where SQL
26
27 545 routines will be run nightly to enforce data cleaning on critical variables beyond
28
29 546 ODK's validation routines. Daily anonymized data will be backed up from the three
30
31 547 sites centrally. For the passive surveillance and household contacts studies a
32
33 548 combination of tablet and paper based case report forms will be used to capture the
34
35 549 data. Data from paper forms will be transcribed onto electronic databases using
36
37 550 Open Clinica⁶⁰. Database reports and descriptive analyses will be generated weekly.
38
39 551 To assess efficiency and quality of data capture, the volume, accuracy and time of
40
41 552 data collection can be quantified.

42
43 553 The distribution and burden of enteric illness is likely to vary between countries and
44
45 554 thus analyses will be conducted separately for each country. Where data are
46
47 555 combined across countries, an adjustment for country differences will be included in
48
49 556 statistical models.

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52 53 558 **ETHICS**

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55 559 Written informed consent will be obtained from the head of each household (as the
56
57 560 "key informant") on behalf of the entire household in the demographic census and
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2
3 561 healthcare utilisation surveys. In each of the other components, individual written
4
5 562 informed consent will be obtained from individuals over the age of 18 or by a
6
7 563 parent/guardian from individuals below this age with additional assent sought from
8
9 564 those between 11-17 years old.

10
11 565 This protocol has received ethics approval from the Oxford Tropical Research Ethics
12
13 566 Committee, the Malawian National Health Sciences Research Committee and
14
15 567 University of Malawi Research Ethics Committee, the Nepal Health Research
16
17 568 Council and the icddr, b Institutional Review Board.

19 569

21 570 **DISSEMINATION**

22
23 571 We hope to make the results from these studies widely available and plan to
24
25 572 disseminate our analyses in international peer-reviewed journals. Investigators will
26
27 573 be involved in reviewing drafts of the manuscripts, abstracts, press releases and any
28
29 574 other publications arising from the study. Furthermore, data from these studies will
30
31 575 also be used in the submission of post-doctoral theses.

33 576

35 577 **COMMUNITY PUBLIC ENGAGEMENT**

36
37 578 A collection of specific activities engaging the local population with both the subject
38
39 579 of typhoid fever and the activities of the study have been carried out. In Malawi for
40
41 580 example, community leaders have been informed and consulted on certain aspects
42
43 581 of the study, shown the proposed activities of study teams and given tours of
44
45 582 research facilities. There has been engagement with various forms of media
46
47 583 disseminating information on the importance of typhoid and study aims. In Nepal,
48
49 584 field staff have been given detailed information to communicate to the local
50
51 585 populations.

52
53 586 Further activities are planned to ensure the local populations are informed and
54
55 587 engaged.

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3 589 **DISCUSSION**
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5 590 The STRATAA study is a comprehensive multicentre study aiming to improve
6
7 591 understanding of typhoidal *Salmonella* infection in high-risk endemic populations.
8
9 592 This study has been designed to answer key questions and data gaps identified
10
11 593 through an innovative application of recent mathematical modelling. These include
12
13 594 measuring the burden of age-stratified disease, identifying the relative contribution of
14
15 595 asymptomatic/subclinical *Salmonella* infection/exposure, and estimating the
16
17 596 contribution to ongoing transmission from the chronic carrier state. These data will
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19 597 inform further modelling required to develop and optimise disease control and
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21 598 prevention strategies that will eventually lead to disease elimination.
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3 599 **COMPETING INTERESTS**

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5 600 AJP chairs the UK Department of Health's (DH) Joint Committee on Vaccination and
6
7 601 Immunisation (JCVI) and the European Medicines Agency (EMA) Scientific Advisory
8
9 602 Group on Vaccines and is a member of WHO's SAGE. The views expressed in this
10
11 603 manuscript do not necessarily reflect those of JCVI, DH, EMA or WHO. AJP has
12
13 604 previously conducted clinical trials on behalf of the University of Oxford funded by
14
15 605 vaccine manufacturers but has no personal financial interests.
16

17 606

18
19 607 **FUNDING**

20
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22
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24
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26
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28
29 612 Research Unit in Vietnam are supported by the Wellcome Trust with Major Overseas
30
31 613 Programme core awards. Neither funding body had any role in designing the study,
32
33 614 writing this manuscript or the decision to submit.
34

35 615

36
37 616 **Authors contributions**

38
39 617 SB, BB, JDC, GD, CD, MAG, RSH, VEP, FQ, KZ, SD, KH and AJP contributed to the
40
41 618 conception and design of the study. ST drafted the protocol of the study. This
42
43 619 manuscript was drafted by TD and JM. SB, BB, JDC, GD, SD, CD, MAG, KH, RSH,
44
45 620 VEP, FQ, ST, KZ, MAK, FK, MS, DT and AJP read and critically revised the protocol
46
47 621 and this manuscript prior to submission.

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Supplemental Table 1. Overview of STRATAA Study Procedures.

Study component	CENSUS							HEALTHCARE UTILISATION		PASSIVE SURVEILLANCE								SEROSURVEY					
								Peak seasons during census		All cases				Household contacts of index cases				Participants		Household contacts of possible carriers			
										Cases within census area*													
Sample size (individuals unless specified)	≥100 000 per site							>700 households		Convenience				≥73 households				8500	6800	Members of ≥50 households			
Time (months, unless specified)	0	4	8	12	16	20	24	1st	2nd	0	Day 8	1	~6	12	0	1	~3	~12	0	**	3	6	
Consent	X							X		X					X		X ^{\$\$}		X				x
Demographic Information	X	X [#]	X [#]	X [#]	X [#]	X [#]	X																
Clinical information										X	X	X	X	X	X								
Questionnaire								X	X														
Blood sample										X [@]			X		X		X ^{\$}		X [@]			X	X
Stool sample										X		X	X	(X)	X	X	2X [€]	(3X)		2X [€]			X
Urine sample										X [@]									X [@]				

Supplemental Table 1. Overview of STRATAA Study Procedures.

* Case within census area = index if blood-culture confirmed typhoid diagnosis, resident in census area and household members consent to participation.

Births, deaths and migrations updated every 4 months in Nepal & Bangladesh

~ approximately - dependent on timeframe of availability for the Vi ELISA result

\$ Host genetics collected/used for laboratory studies if either stool or serum positive or both

§ separate consent for host genetics

@ additional blood (RNA + plasma) and urine samples (subset of 100 with typhoid (culture positive), 100 with clinically suspected typhoid (culture negative), 100 with alternate bacteraemia; and 100 healthy controls (from the serosurvey) from adults 16-40yrs who give consent for genetic assays to be performed.

€ Participants identified as having high anti-Vi IgG only - 2 stool samples collected 48 hours apart once ELISA result known to identify/confirm carriage

** When result known

Total blood volumes: **Passive survey** ≤16 mL adults (>16 years), ≤7 mL children (<16 years); **Serosurvey** ≤8 mL adults (>16 years), ≤7 mL children. Cell pellets stored for host genetics.

BMJ Open

THE STRATAA STUDY PROTOCOL: A PROGRAMME TO ASSESS THE BURDEN OF ENTERIC FEVER IN BANGLADESH, MALAWI AND NEPAL USING PROSPECTIVE POPULATION CENSUS, PASSIVE SURVEILLANCE, SEROLOGICAL STUDIES AND HEALTHCARE UTILISATION SURVEYS

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1 **THE STRATAA STUDY PROTOCOL: A PROGRAMME TO ASSESS THE**
2 **BURDEN OF ENTERIC FEVER IN BANGLADESH, MALAWI AND NEPAL USING**
3 **PROSPECTIVE POPULATION CENSUS, PASSIVE SURVEILLANCE,**
4 **SEROLOGICAL STUDIES AND HEALTHCARE UTILISATION SURVEYS**

6 **Short title: The STRATAA study**

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3 70 **ABSTRACT**
4

5 71 **Introduction**
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7 72 Invasive infections caused by *Salmonella enterica* serovar Typhi and Paratyphi A are
8
9 73 estimated to account for 12-27 million febrile illness episodes worldwide annually.
10

11 74 Determining the true burden of typhoidal *Salmonellae* infections is hindered by lack
12
13 75 of population-based studies and adequate laboratory diagnostics.
14

15 76 The STRATAA (Strategic Typhoid alliance across Africa and Asia) study takes a
16
17 77 systematic approach to measuring the age-stratified burden of clinical and subclinical
18
19 78 disease caused by typhoidal *Salmonellae* infections at three high-incidence urban
20
21 79 sites in Africa and Asia. We aim to explore the natural history of *Salmonella*
22
23 80 transmission in endemic settings, addressing key uncertainties relating to the
24
25 81 epidemiology of enteric fever identified through mathematical models, and enabling
26
27 82 optimisation of vaccine strategies.
28

29 83 **Methods/Design**
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31 84 Using census-defined denominator populations of $\geq 100,000$ individuals at sites in
32
33 85 Malawi, Bangladesh and Nepal, the primary outcome is to characterize the burden of
34
35 86 enteric fever in these populations over a 24-month period. During passive
36
37 87 surveillance, clinical and household data, and laboratory samples will be collected
38
39 88 from febrile individuals. In parallel, healthcare utilization and water, sanitation and
40
41 89 hygiene surveys will be performed to characterise healthcare-seeking behaviour and
42
43 90 assess potential routes of transmission. The rates of both undiagnosed and
44
45 91 subclinical exposure to typhoidal *Salmonellae* (seroincidence), identification of
46
47 92 chronic carriage, and population seroprevalence of typhoid infection will be assessed
48
49 93 through, age-stratified serosurveys performed at each site. Secondary attack rates
50
51 94 will be estimated among household contacts of acute enteric fever cases and
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53 95 possible chronic carriers.
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56 96 **Ethics and Dissemination**
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3 97 This protocol has been ethically approved by the Oxford Tropical Research Ethics
4
5 98 Committee, the icddr,b Institutional Review Board, the Malawian National Health
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7 99 Sciences Research Committee and College of Medicine Research Ethics Committee
8
9 100 and Nepal Health Research Council. The study is being conducted in accordance
10
11 101 with the principles of the Declaration of Helsinki and Good Clinical Practice. Informed
12
13 102 consent is obtained before study enrolment. Results will be submitted to international
14
15 103 peer-reviewed journals and presented at international conferences.
16
17 104

19 105 **STRENGTHS AND LIMITATIONS**

20
21 106 - The study is designed with a comprehensive multicomponent epidemiological
22
23 107 approach, nesting passive surveillance, serosurveillance and healthcare utilisation
24
25 108 surveys within a demographic census population, to accurately determine the age-
26
27 109 stratified burden of enteric fever.
28
29 110 - The diversity of field sites in Africa and Asia, will provide data on typhoid
30
31 111 epidemiology and transmission from a range of differing epidemiological settings.
32
33 112 - The combination of a traditional epidemiological approach with novel laboratory
34
35 113 methods for the diagnosis of febrile illness and investigation of the host and
36
37 114 pathogen genetics and antimicrobial resistance determinants provides a unique
38
39 115 platform to study this disease.
40
41 116 - Practical limitations include the sharing and standardisation of clinical definitions,
42
43 117 data and sample collection methods and laboratory assays, based on the facilities
44
45 118 and staff available and community requirements at each site.
46
47 119 - The number of field-sites included in this current protocol is limited to three; all of
48
49 120 these sites are densely populated urban settings with likely high-incidence of enteric
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51 121 fever transmission. The degree to which our data may be extrapolated to other
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53 122 settings and countries remains to be explored.
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3 125 **Registration:** ISRCTN 12131979
4
5 126 **Ethics References:** Oxford (Oxford Tropical Research Ethics Committee 39-15)
6
7 127 Bangladesh (icddr,b Institutional Review Board PR-15119)
8
9 128 Malawi (National Health Sciences Research Committee
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11 129 15/5/1599)
12
13 130 Nepal (Nepal Health Research Council 306/2015)
14
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16
17 132 **Keywords:**
18
19 133 Enteric fever, vaccination programme, infection transmission, *Salmonella* Typhi,
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21 134 *Salmonella* Paratyphi A, serosurveillance, seroepidemiology, healthcare utilisation,
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23 135 resource-limited settings; diagnosis; febrile illness; Africa, Asia.
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3 136 **BACKGROUND**

4 137 *Salmonella enterica* serovars Typhi (S. Typhi) and Paratyphi A (S. Paratyphi A), are
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6
7 138 human-restricted pathogens transmitted by faeco-oral ingestion. The ensuing
8
9 139 disease, enteric fever (or 'typhoid fever'), is a non-specific febrile illness which affects
10
11 140 an estimated 12-27 million people worldwide each year, resulting in 129,000–
12
13 141 223,000 deaths.^{1–3} Despite a dramatic reduction in incidence over the last century in
14
15 142 most high-income countries, continuing inadequate access to clean water and
16
17 143 increasing inter-continental spread of multiply antibiotic-resistant strains hamper
18
19 144 disease control efforts especially in resource-limited settings.^{3–5} The current burden
20
21 145 of disease is highest among children and young adults in South and Southeast Asia^{1–}
22
23 146 ³ but is increasingly recognized in sub-Saharan Africa.⁶

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25 147
26
27 148 Recently, a new generation of Vi- conjugate enteric fever vaccines, suitable for use in
28
29 149 infants and providing longer lasting protection than those previously licensed,^{7–10}
30
31 150 have become available. Determining how and where interventions such as
32
33 151 vaccination may be best deployed is difficult due to a lack of population-based
34
35 152 incidence studies and inaccurate diagnostic tests.^{1–3} Improving disease burden
36
37 153 estimates and providing data on the epidemiology and transmission of S. Typhi and
38
39 154 S. Paratyphi A to inform mathematical models^{11–13} could improve the evidence
40
41 155 necessary to design comprehensive and effective disease control programmes.¹⁴

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45 157 **Burden of disease and diagnostics**

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47 158 Recent meta-analyses examining global causes of morbidity and mortality estimate
48
49 159 that a significant burden of disease worldwide, and especially in South and
50
51 160 Southeast Asia, may be attributed to enteric fever.^{15–17} The margin of error on these
52
53 161 estimates is wide, however; much of the uncertainty regarding the burden of disease
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55 162 caused by typhoidal *Salmonella* is due to the unavailability of accurate diagnostics or
56
57 163 misclassification of non-specific disease presentations, in addition to a general lack
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3 164 of data.¹⁸ Availability of antibiotics from local pharmacies without prescription,
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5 165 frequent misdiagnosis as malaria, dengue or other febrile illnesses, or the avoidance
6
7 166 of hospital due to fear or expense are all likely to result in an underestimate of the
8
9 167 true numbers of cases.¹⁹ In contrast, the widespread use of suboptimal diagnostic
10
11 168 tests such as the Widal test in areas where exposure to similar bacteria in the
12
13 169 environment occurs may lead to inaccurate over-diagnosis of the condition.²⁰
14
15 170 Previous studies and systematic reviews have attempted to address this inaccuracy
16
17 171 by calculating the imprecision associated with insensitive blood culture methods, and
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19 172 applying this correction to blood culture confirmed case numbers.^{1,2}
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21 173
22
23 174 In addition to a better understanding of the disease burden attributable to typhoidal
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25 175 *Salmonellae*, improved diagnostics and biomarkers are required to improve individual
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27 176 case management.²¹ The current gold-standard diagnostic test for
28
29 177 typhoid/paratyphoid is blood culture; while this test provides the causative isolate,
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31 178 thereby allowing susceptibility testing and further typing methods to be performed,
32
33 179 most laboratories in resource-limited settings lack the infrastructure to perform these
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35 180 assays. Even in ideal conditions, blood culture requires a significant volume of blood
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37 181 to enhance diagnostic yield, and is relatively insensitive even in highly-controlled
38
39 182 human challenge settings where sensitivity reaches 80%.²² Highly sensitive and
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41 183 specific diagnostic tests are a fundamental requirement for surveillance and vaccine
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43 184 efficacy studies, however, both for measuring the number of cases
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45 185 avoided/prevented, but also for reassuring policy makers and funders that vaccine
46
47 186 implementation is worth investment. While several rapid serological diagnostic tests
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49 187 have recently been developed, these still rely on a few selected antigens, which are
50
51 188 known to be non-specific in endemic communities.^{23,24} Newer diagnostic modalities
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53 189 are in development, however, which have utilised serum banks from multiple
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56 190 countries and samples collected from controlled human infection models to identify
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58 191 putative novel antigens for serological assay and further high sensitivity approaches
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3 192 including metabolomics and functional genomics.^{5,25-27} In addition, several of these
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5 193 newer approaches have demonstrated potential for identifying individuals with
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7 194 probable chronic carriage. Identification and treatment of chronic carriers in a
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9 195 population would significantly improve our understanding of disease dynamics and
10
11 196 allow targeted treatment strategies to reduce community transmission of infection.
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15 198 Furthermore, whilst utilising these new diagnostic tests in comprehensive passive
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17 199 surveillance or even active surveillance programmes will identify acute cases of
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19 200 typhoid disease, the incidence rate of sub-clinical infection and exposure is unknown.

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21 201 These data are likely essential to understanding pathogen transmission as well as
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23 202 the development and maintenance of immunity against clinical disease¹² and are
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25 203 likely only to be obtainable through the use of seroepidemiological methods.

26
27 204 Previous seroepidemiological studies have examined the cross-sectional age-
28
29 205 stratified prevalence of antibodies to the *S. Typhi* flagellar (H) antigen in Santiago,
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31 206 Chile²⁸, and serum bactericidal antibody in Kathmandu, Nepal.²⁹ Also, high antibody
32
33 207 titres to the Vi (Vi capsular polysaccharide; virulence factor) antigen of *S. Typhi*
34
35 208 measured by agglutination assay have been used to estimate chronic carrier
36
37 209 frequency in population-based studies,³⁰⁻³² although these studies were not
38
39 210 universally successful at identifying chronic carriers.³³ Improvements in serological
40
41 211 assays and the discovery of a newer generation of diagnostic antigens³⁴ suggest that
42
43 212 population-based serosurveillance studies, especially using longitudinal
44
45 213 measurements, may provide key data regarding the incidence of subclinical infection
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47 214 with the bacterial agents of enteric fever and the prevalence of chronic carriers.

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51 216 **Transmission**

52
53 217 Closely related to the estimate of incidence is the contribution of different typhoid
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55 218 states to ongoing transmission within a community. Acute cases of enteric fever,
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57 219 individuals with subclinical infection, as well as chronic carriers likely all contribute to
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3 220 the transmission and maintenance of *S. Typhi* and *S. Paratyphi A* in endemic areas,
4
5 221 but not to the same degree. This is an essential area of further research, particularly
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7 222 given the negative impact transmission from chronic carriers could have on the
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9 223 indirect protection afforded by typhoid vaccines.¹² The importance of 'short cycle'
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11 224 transmission via contaminated food and water in the immediate environment versus
12
13 225 'long cycle' transmission via the contamination of community water sources is also
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15 226 an area of direct relevance in the control of typhoid fever, primarily through non-
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17 227 vaccine public health interventions including water and sanitation hygiene (WASH)
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19 228 improvements.
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21 229

23 230 **Bacterial genome variation**

25 231 The enteric fever agents *S. Typhi* and *S. Paratyphi A*, while genetically distinct from
26
27 232 other serovars of *Salmonella enterica*, exhibit low genetic diversity and whole
28
29 233 genome sequence (WGS) analysis is required to uncover patterns of genetic
30
31 234 relatedness between isolates.³⁵⁻³⁷ These techniques have been used previously to
32
33 235 track the development and spread of antibiotic resistance on a global scale,⁶ and
34
35 236 have shown potential to accurately track transmission links between individuals,
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37 237 specifically within affected households, in addition to the broader community.³⁸ One
38
39 238 use of this high-resolution typing previously, has been to determine whether isolates
40
41 239 obtained from those with acute infection are distinct from those found in chronic gall-
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43 240 bladder carriers.³⁹

45 241

48 242 **Host susceptibility**

50 243 While environmental risk factors for enteric fever acquisition are thought to be
51
52 244 relatively well understood, recent evidence has emerged implicating genetic factors
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54 245 in host susceptibility to infection.^{35,36} Many early typhoid genetic susceptibility studies
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56 246 reporting candidate genes were limited by small sample sizes and choice of controls,
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58 247 a recent large-scale genome-wide association study however, has identified a

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2
3 248 specific locus (HLA-DRB1*405) which confers 5-fold protection against developing
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5 249 typhoid fever.³⁵ How genetic variation at this site influences protection against
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7 250 typhoid is still under investigation, but may result in functional differences in MHC
8
9 251 class II amino acid sequences; in turn, this may influence *S. Typhi* epitope selection
10
11 252 and antigen presentation or the scale and format of the host's T-cell response.
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13 253

14 254 **Vaccines**

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17 255 Vaccines currently available for typhoid are either oral live attenuated bacterial
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19 256 strains or are derived from the *S. Typhi* polysaccharide capsule, called Vi; there are
20
21 257 no licensed vaccines against *S. Paratyphi* A or non-typhoidal salmonellae (NTS).
22
23 258 Despite recommendations for their use, these vaccines have only sparsely been
24
25 259 introduced in high prevalence settings.^{7,40} The reasons for this are unclear, but likely
26
27 260 include failure of prioritisation and a short fall in advocacy, in addition to technical
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29 261 deficiencies of the vaccines themselves. One common reason cited is the T-cell
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31 262 independent nature of the immune response to the Vi polysaccharide vaccine (ViPS);
32
33 263 this renders the vaccine of little use in young children below the age of two years. In
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35 264 addition, this feature means that the immune response does not boost, requiring
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37 265 repeat vaccination to be administered every 3-5 years. As with other thymus-
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39 266 independent vaccines, to overcome this problem the Vi capsule may be chemically
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41 267 conjugated to a protein carrier,⁸ as has successfully been done with conjugation of Vi
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43 268 to *Pseudomonas aeruginosa* exotoxin A.⁹ Despite demonstrating good
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45 269 immunogenicity and efficacy in field trials,^{10,41} the vaccine is still unlicensed. Newer
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47 270 Vi-conjugate vaccines are in development and have progressed to assessment in
48
49 271 field-trials,^{42,43} and the Oxford human typhoid challenge model.⁴⁴ Recent data from
50
51 272 the challenge models suggests that the Vi response elicited by the vaccine is high
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53 273 and likely to be protective, at least in naïve healthy adult volunteers with no immunity
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55 274 or those with previous history of infection.⁴⁴ Furthermore, a recent study in Indian
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57 275 schoolchildren found a vaccine efficacy of 100% (95% confidence interval, 97.6 to
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3 276 100%) after a single dose of Vi conjugated to tetanus toxoid during the first year of
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5 277 follow-up.⁴³
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7 278
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9 279 The key issue remaining, once safe, well-tolerated, immunogenic vaccines become
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11 280 widely available, is how best to implement them in endemic settings. Currently
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13 281 available surveillance data from most regions is insufficient to demonstrate the age-
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15 282 band with the highest incidence of enteric fever.⁴⁵ Designing vaccination strategies to
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17 283 cover the years during which children are most at risk and generating indirect
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19 284 protection by preventing infection among those age groups driving transmission
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21 285 could be facilitated through the use of well-informed mathematical modelling.
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23 286

24 25 287 **THE STRATAA STUDY: RATIONALE AND AIMS**

26
27 288 The STRATAA (Strategic Typhoid alliance across Africa and Asia) study draws
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29 289 together an international team of investigators, field sites, laboratories and research
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31 290 institutes to address many of the key outstanding questions described above
32
33 291 regarding the burden of enteric fever and *Salmonella* exposure in endemic regions,
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35 292 the mechanisms of susceptibility and infection transmission. These have been
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37 293 identified as key uncertainties in mathematical models.¹¹⁻¹³ By conducting field
38
39 294 studies of the epidemiology and burden of enteric fever across three sites with
40
41 295 distinct epidemiological profiles and applying state-of-the-art molecular methods, the
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43 296 goal of the STRATAA study is to collect the data needed to enhance our
44
45 297 understanding of pathogen transmission, exposure and susceptibility. These data
46
47 298 can then be used to rigorously parameterize and validate models for the transmission
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49 299 dynamics of the agents of enteric fever, such that these models can then be used to
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51 300 evaluate different vaccination strategies and, importantly, help predict the expected
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53 301 impact resulting from the direct and indirect effects of vaccine introduction. These
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55 302 and other overarching objectives of the STRATAA study are listed in **Table 1**.
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304 **Table 1. Overarching objectives of the STRATAA study.**

Primary	To characterise the burden of enteric fever at three urban sites in Africa and Asia
Secondary	Assess the burden/incidence of enteric fever
	Assess the seroincidence of infection
	Assess host factors affecting burden/incidence/transmission of enteric fever
	Assess effect of pathogen genetics on burden/incidence/transmission of enteric fever
	Develop diagnostic tools for rapid and consistent typhoid diagnosis
	Develop transmission modelling and modelling of vaccine introduction impact
Tertiary Objectives	Strengthen research capacity in enteric fever endemic regions
	Provide data to appropriate institutes and governments to advocate vaccine implementation
	To characterise the burden of invasive NTS and other invasive pathogens at an urban site in Malawi, Africa

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306 To deliver on these objectives, the following studies will be performed in parallel at
 307 each of the three chosen field sites, starting in May 2016 with activities continuing
 308 until October 2018. Firstly, in a well-defined population catchment of
 309 approximately 100,000 individuals, a detailed demographic census survey will be
 310 undertaken. Secondly, in healthcare facilities utilised by the census population (as
 311 confirmed through a healthcare utilisation survey) prospective passive surveillance to
 312 detect cases of enteric fever will be performed. Thirdly, a seroincidence study will be

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3 313 conducted in an age-stratified sample of the census population at each site,
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5 314 collecting blood samples at intervals to estimate the rate of seroconversion to
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7 315 typhoidal *Salmonella* antigens and hence the rate of subclinical/asymptomatic
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9 316 exposure to these bacteria in the general population.
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11 317
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13 318 To meet the further objectives, which include evaluating diagnostic tests, identifying
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15 319 antimicrobial resistance patterns, exploring host susceptibility and bacterial
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17 320 virulence/genomic variation, biological samples, specimens and metadata will be
18
19 321 collected during these individual studies. Data will be pooled to inform transmission
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21 322 dynamic and health economic models that can be used to help design future vaccine
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23 323 effectiveness studies and evaluate vaccine delivery strategies for widespread
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25 324 deployment.
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27 325

28 326 **STUDY SITES**

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31 327 With known high incidence throughout South and Southeast Asia and a recent
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33 328 increase in reported cases from sub-Saharan Africa^{46,47} three sites were selected
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35 329 from across Africa and Asia based on known high rates of enteric fever and the
36
37 330 research capacity to deliver a study of this size and logistical complexity. The three
38
39 331 sites differ in their epidemiological profiles and history of enteric fever incidence.
40

41 332

42 333 ***Dhaka, Bangladesh***

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44 334 Mirpur is an area located within Dhaka Metropolitan area, Bangladesh (**Figure 1A**),
45
46 335 situated 7km north-west of the International Centre for Diarrhoeal Disease Research,
47
48 336 Bangladesh (icddr,b) main campus. The icddr,b manages two hospitals in Dhaka
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50 337 city, one in the main campus in Dhaka (the main hospital) and another in the Mirpur
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52 338 area known as MTC (Mirpur Treatment Centre) comprising a 50-bed inpatient facility.
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54 339 As part of this programme, these and other health facilities (ten or more total) will be
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56 340 kept under careful surveillance for enteric fever in the census area. The total
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3 341 catchment area for the field site for the STRATAA study is 10.79 km² with a total
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5 342 population of about 603,658 at a density of 55946/km². Approximately 98% of
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7 343 residents have access to tap water supplied by the municipality while the remainder
8
9 344 use wells, hand pumps and other sources such as ponds and rivers in the study
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11 345 area.

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14
15 347 Previous data from blood culture surveillance revealed a high incidence of disease
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17 348 within Dhaka, with the burden particularly high in children under 5 years of age
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19 349 (estimated at 18.7 episodes/1000 person-years in this age group). The current S.
20
21 350 Typhi:Paratyphi ratio is approximately 5:1. Typhoid fever is reported throughout the
22
23 351 year but peaks during the monsoon season.^{48,49} Of note, 15% of S. Typhi isolates are
24
25 352 multi-drug resistant (MDR) and around 97% isolates are resistant to nalidixic acid⁵⁰;
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27 353 extended-spectrum beta-lactamase (ESBL) producing organisms have also been
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29 354 isolated from enteric fever patients in this setting.

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34 356 ***Blantyre, Malawi***

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36 357 Ndirande is a large urban township on the outskirts of Blantyre city, Malawi, 6km
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38 358 from the main referral hospital (**Figure 1B**). It has a young population of around
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40 359 100,000 people spread over 6.77km². It is serviced by one health clinic staffed by
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42 360 clinical officers. It has high reported rates of typhoid fever along with a population
43
44 361 HIV prevalence of around 18%.⁵¹ Queen Elizabeth Central Hospital (QECH) in
45
46 362 Blantyre, Malawi, is the government-funded hospital for Blantyre district, serving a
47
48 363 local population of 1.3 million persons and provides tertiary care to southern Malawi.

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52 365 NTS have previously been the commonest cause of invasive bloodstream infections
53
54 366 in Blantyre,⁵² but since 2011 there has been a rapid increase in the number of S.
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56 367 Typhi cases seen at QECH, from approximately 14/year between 1998 and 2010 to
57
58 368 782 in 2014.⁴⁶ Much of this increase has been due to the emergence of the H58

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3 369 clone and is associated with an MDR phenotype^{47,53}. This outbreak appears
4
5 370 unrelated to HIV-infection, but has resulted in high rates of mortality (2.5% adult and
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7 371 paediatric) despite the availability of fluoroquinolone antibiotics.⁴⁷ In this setting,
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9 372 enteric fever is seasonal, with the peak number of cases seen at the end of the wet
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11 373 season and during the early dry season when the prevalence of malnutrition is also
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13 374 highest.

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17 376 ***Patan, Nepal***

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19 377 Patan is located within the Lalitpur Sub-Metropolitan City (LSMC) within the
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21 378 Kathmandu Valley, Nepal (**Figure 1C**). The population is generally poor, with most
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23 379 living in overcrowded conditions and obtaining their water from stone spouts or
24
25 380 sunken wells. Patan Hospital is a 318-bed government hospital providing emergency
26
27 381 and elective outpatient and inpatient services to this area. The local catchment
28
29 382 population of the hospital is approximately 200,000 people in about 20 km², with a
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31 383 population density of 8,000/km²; there is a high rate of immigration for employment,
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33 384 particularly young males from rural areas. Enteric fever is frequently managed in the
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35 385 outpatient clinic at Patan Hospital, which has approximately 200,000 outpatient visits
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37 386 annually.

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41 388 Uptake of typhoid Vi vaccination is limited and natural exposure/subclinical infection
42
43 389 is common.²⁹ Approximately 400 culture confirmed cases of enteric fever are
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45 390 diagnosed at Patan Hospital each year, with a peak during the monsoon months. The
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47 391 current *S. Typhi*:*Paratyphi A* ratio is approximately 1:1.⁵⁴ Antimicrobial resistance is
48
49 392 more commonly observed in *S. Paratyphi A* isolates; however, emergence of
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51 393 fluoroquinolone resistant *S. Typhi* isolates has also been recently identified,⁵⁵
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53 394 whereas MDR strains of either serovar are rare.³⁸

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3 396 **METHODS**
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5 397 **POPULATION CENSUS**
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7 398 In order to accurately calculate an incidence rate of typhoid fever for each of the
8
9 399 study sites, a demographic census will be performed at baseline and repeated at 2
10
11 400 years. The objective of the census is to identify/characterise the source population
12
13 401 corresponding to the catchment areas for the passive surveillance sites described
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15 402 below, and estimate the person-time under surveillance.
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17 403

18
19 404 Census data will be updated with births, deaths and migrations every six months in
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21 405 Dhaka and Patan, and at two years in Blantyre. The number of participants for the
22
23 406 demographic census survey in each site needed to produce a two-sided 95%
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25 407 confidence interval with a precision (half-width) of 50% for the anticipated typhoid
26
27 408 incidence rate detected through passive clinical surveillance has been calculated
28
29 409 (**Table 2**). The necessary catchment population size is driven by the size required to
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31 410 estimate the expected incidence rate in the 0-4 year-old age group, which is
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33 411 estimated to be approximately 10% of the total population in each site.
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414 **Table 2. Sample size required for the target populations in the three sites to**
 415 **estimate annual, blood culture-confirmed typhoid incidence in passive clinical**
 416 **surveillance.**

Age groups	Anticipated typhoid incidence per 1000 persons*	Precision or half-width	Sample-size required
0-4 years	1.5	0.75	10,125
5-14 years	1.0	0.5	15,119
>14 years	0.5	0.25	29,801

417 * Assumed age-specific incidence rates (based on data from Dhaka, Bangladesh,
 418 Delhi, India, and Dong Thap, Vietnam).

419
 420 The census will take place within a demarcated geographic area that is a known
 421 catchment population for the surveillance sites (**Figure 1**). In total, at least 100,000
 422 individuals will be enumerated from $\geq 20,000$ households. The head/key informant
 423 within the household will provide written informed consent to take part in the study.
 424 Information on all residents within the household at the time of the census will be
 425 gathered from the head of the household/key informant.

426
 427 A household is defined as individuals living in the same dwelling or compound and
 428 sharing food from the same kitchen. A household member is considered to have
 429 migrated out if s/he has left the household and does not intend to come back within
 430 six months of the time s/he left. A person is considered to have migrated in if s/he
 431 was not previously included in the list of household members and intends to live in
 432 the household for the next six months. At enrolment, the head of each household will

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3 433 be made aware of the passive surveillance component of the study and encouraged
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5 434 to use the field-site facilities capturing acute febrile illnesses, including acute enteric
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7 435 fever cases. The characterised census population will form the sampling frame for
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9 436 the further components (passive surveillance, healthcare utilisation/WASH surveys
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11 437 and serosurvey) described below.
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15 439 **PASSIVE SURVEILLANCE**

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17 440 The passive surveillance component of STRATAA is designed to capture cases of
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19 441 febrile disease occurring in each of the three census populations. Patients presenting
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21 442 to any of the clinical surveillance sites with a history of subjective fever >72 hours or
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23 443 objective fever >38.5 °C on presentation will be approached for enrolment.
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27 445 An index case will be defined as an individual with a blood culture result confirming
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29 446 infection with *S. Typhi* or *S. Paratyphi A* (or NTS in Malawi), and whose household is
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31 447 included in the census survey. These cases will be used to calculate the disease
32
33 448 incidence in each of the three census sites. Consenting individuals will have samples
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35 449 of blood, urine and stool collected to determine a diagnosis of *S. Typhi*, *S. Paratyphi*
36
37 450 *A* or NTS and to provide material for the further diagnostic and genetic aims of the
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39 451 study (**Supplemental Table 1**). Patients not resident in the census area will be
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41 452 enrolled as additional cases for the laboratory and genetic components of the
42
43 453 programme if written consent is provided.
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47 455 **HEALTHCARE UTILISATION AND WATER, SANITATION AND HYGIENE**

48 456 **SURVEYS**

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50
51 457 To characterise the healthcare-seeking behaviour of individuals living in the census
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53 458 areas, at least 735 households will be randomly selected from the census area at
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55 459 each site to participate in a healthcare utilisation survey. Data will be collected from
56
57 460 the head of the household/key informant to describe the actual and hypothetical
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3 461 usage of healthcare facilities for febrile episodes. The aim of this component is to
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5 462 estimate the percent of cases (fulfilling the fever case definition) in each age stratum
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7 463 (0-4 years, 5-14 years and >14 years of age) who would or would not seek attention
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9 464 at one of the designated passive surveillance health facilities. Additional data
10
11 465 regarding sanitation and hygiene facilities and usage will also be collected. Annual
12
13 466 data collection periods will coincide with the peak typhoid season at each of the three
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15 467 sites.
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19 469 **SEROSURVEYS**

20
21 470 To estimate the seroincidence and seroprevalence of clinical (both formally
22
23 471 diagnosed and undiagnosed cases) and subclinical infection or exposure to *S. Typhi*
24
25 472 and *S. Paratyphi*, systematic serosurveillance will be performed at each site. Blood
26
27 473 samples will be collected at baseline and 3 months later, with sample collection
28
29 474 initiated in an on-going basis over the course of one year (**Table 3**). To identify
30
31 475 participants for the serosurvey, an age-stratified approach will be used to randomly
32
33 476 select individuals from the census population from each age group. Suitable
34
35 477 participants will be enrolled by field workers; where the individual identified is not
36
37 478 available, a household member in the same age group will be selected. Where this is
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39 479 not possible further households will be randomised into this component to ensure
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41 480 adequate numbers of individuals in the different age groups.
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45
46 482 Seroincidence (indicative of recent infection) will be calculated by measuring the rate
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48 483 of seroconversion to anti-H(d) (anti-flagellin) IgG and other acute phase antibodies
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50 484 between both time points, with the denominator consisting only of individuals
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52 485 sampled twice and seronegative at the first time point.⁵⁶
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54 486

55
56 487 **Table 3. Sample size calculations for the serological surveys to estimate age-**
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58 488 **specific rates of high titres of serum IgG anti-H(d) antibodies.** Assumes age-

489 stratified individual sampling; also accounts for detection of 1% chronic carriage rate
 490 in ≥ 10 year olds.

	Anticipate d sero- incidence (%)	Number of initial samples	Anticipat ed number of events	No. of follow- up samples *	No. of events detected	Binomial [95%	Exact CI]	Probabili ty of observin g 0 events
0-4 yrs	0.2	2500	5	2000	4	0.0005	0.0051	0.0182
5-9 yrs	0.4	1300	5.2	1040	4	0.0010	0.0098	0.0155
10-14 yrs	0.8	800	6.4	640	5	0.0025	0.0181	0.0059
>14 yrs	0.2	3900	7.8	3120	6	0.0007	0.0042	0.0019
TOTAL		8500	24.2	6800	19			

491 *Assuming 5% migration and 15% refusal

492

493 The seroprevalence of anti-Vi IgG will also be measured at baseline to identify
 494 individuals who could be potential chronic carriers. Previous studies have used high
 495 anti-Vi IgG antibody levels in serum as a marker of chronic carriage, and our aim is
 496 to use a recently validated/approved anti-Vi ELISA method to determine possible
 497 rates of chronic carriage across the three populations.^{32,57,58} In order to validate this
 498 method and to identify possible chronic carriers in the population (in addition to
 499 identifying whether there may be specific host genetic risk factors for chronic
 500 carriage), those individuals with a 'high' serum anti-Vi IgG titre will be re-approached
 501 and asked to provide two stool samples within 48 hours. With the agreement of
 502 relevant local ethics committees, identified chronic carriers (confirmed high anti-Vi
 503 IgG serum sample with a positive stool culture) will be treated with antibiotics for
 504 chronic carriage and clearance of bacterial shedding will be confirmed.

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5 506 **HOUSEHOLD CONTACTS**

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7 507 To further investigate possible transmission links, household contacts of index cases
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9 508 presenting with blood-culture confirmed *S. Typhi* or *S. Paratyphi A* infection will be
10
11 509 identified through the census data collection and approached to take part in this
12
13 510 component of the study. Up to five members of the household, with consent, will be
14
15 511 asked to provide a blood and stool sample at the time of discharge of the index case,
16
17 512 a further stool sample at one month and repeat serology at six months
18
19 513 (**Supplemental Table 1**), and asked about the occurrence of symptoms. These
20
21 514 samples will be investigated to identify those with subclinical infection or possible
22
23 515 chronic carriers. Secondary attack rates for the occurrence of symptomatic infection
24
25 516 will be estimated. If serology suggests chronic carriage in a household contact,
26
27 517 additional blood and stool samples will be collected to confirm this, and antimicrobial
28
29 518 treatment to eradicate carriage will be proposed.

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32
33 520 Those individuals who are identified as shedding bacteria but without high serum
34
35 521 anti-Vi IgG levels will be followed up at a one-year interval to repeat stool culture.
36
37 522 Repeat blood samples will be collected from household contacts approximately 3
38
39 523 months after initial sampling to explore whether rates of seroconversion are higher in
40
41 524 these individuals compared with those in the general population. At least 73
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43 525 households of index cases are expected to be enrolled in this component of the
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45 526 study.

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49 528 In a similar approach to the acute cases of typhoid infection, where chronic carriers
50
51 529 are identified from the serosurveys, household transmission studies will be
52
53 530 performed among household contacts of possible chronic carriers (i.e. those with a
54
55 531 'high' serum anti-Vi IgG titre). A one-time attempt will be made to enroll up to five
56
57 532 members of the household, sampling serology and stool looking for evidence of
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3 533 typhoid infection. This will provide data on secondary attack rates for both acute and
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5 534 chronic typhoid states.

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8 9 536 **DATA MANAGEMENT AND ANALYSIS**

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11 537 Census and serosurvey data collection forms will be developed through a structured
12
13 538 iterative process and then implemented using Open Data Kit⁵⁹, a system enabling
14
15 539 electronic mobile data collection, with customizations by Nafundi, USA, on Android-
16
17 540 based tablets. Each household within the census will be assigned a unique study ID
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19 541 and geo-located using GPS where possible; individuals will be given a member
20
21 542 number within the household. This information will be collected by local enumerators
22
23 543 over a one- to four-month period via these forms and adapted to the three
24
25 544 geographic settings. Data will be uploaded onto MySQL databases, where SQL
26
27 545 routines will be run nightly to enforce data cleaning on critical variables beyond
28
29 546 ODK's validation routines. Daily anonymized data will be backed up from the three
30
31 547 sites centrally. For the passive surveillance and household contacts studies a
32
33 548 combination of tablet and paper based case report forms will be used to capture the
34
35 549 data. Data from paper forms will be transcribed onto electronic databases using
36
37 550 Open Clinica⁶⁰. Database reports and descriptive analyses will be generated weekly.
38
39 551 To assess efficiency and quality of data capture, the volume, accuracy and time of
40
41 552 data collection can be quantified.

42
43 553 The distribution and burden of enteric illness is likely to vary between countries and
44
45 554 thus analyses will be conducted separately for each country. Where data are
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47 555 combined across countries, an adjustment for country differences will be included in
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49 556 statistical models.

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52 53 558 **ETHICS**

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55 559 Written informed consent will be obtained from the head of each household (as the
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57 560 "key informant") on behalf of the entire household in the demographic census and
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3 561 healthcare utilisation surveys. In each of the other components, individual written
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5 562 informed consent will be obtained from individuals over the age of 18 or by a
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7 563 parent/guardian from individuals below this age with additional assent sought from
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9 564 those between 11-17 years old.

10
11 565 This protocol has received ethics approval from the Oxford Tropical Research Ethics
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13 566 Committee, the Malawian National Health Sciences Research Committee and
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15 567 University of Malawi Research Ethics Committee, the Nepal Health Research
16
17 568 Council and the icddr, b Institutional Review Board.

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21 570 **DISSEMINATION**

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23 571 We hope to make the results from these studies widely available and plan to
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25 572 disseminate our analyses in international peer-reviewed journals. Investigators will
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27 573 be involved in reviewing drafts of the manuscripts, abstracts, press releases and any
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29 574 other publications arising from the study. Furthermore, data from these studies will
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31 575 also be used in the submission of post-doctoral theses.

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35 577 **COMMUNITY PUBLIC ENGAGEMENT**

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37 578 A collection of specific activities engaging the local population with both the subject
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39 579 of typhoid fever and the activities of the study have been carried out. In Malawi for
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41 580 example, community leaders have been informed and consulted on certain aspects
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43 581 of the study, shown the proposed activities of study teams and given tours of
44
45 582 research facilities. There has been engagement with various forms of media
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47 583 disseminating information on the importance of typhoid and study aims. In Nepal,
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49 584 field staff have been given detailed information to communicate to the local
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51 585 populations.

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53 586 Further activities are planned to ensure the local populations are informed and
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55 587 engaged.

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3 589 **DISCUSSION**

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5 590 The STRATAA study is a comprehensive multicentre study aiming to improve
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7 591 understanding of typhoidal *Salmonella* infection in high-risk endemic populations.
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9 592 This study has been designed to answer key questions and data gaps identified
10
11 593 through an innovative application of recent mathematical modelling. These include
12
13 594 measuring the burden of age-stratified disease, identifying the relative contribution of
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15 595 asymptomatic/subclinical *Salmonella* infection/exposure, and estimating the
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17 596 contribution to ongoing transmission from the chronic carrier state. These data will
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19 597 inform further modelling required to develop and optimise disease control and
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21 598 prevention strategies that will eventually lead to disease elimination.
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25 600 **Figure Legends**

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27 601 **Figure 1. Description of STRATAA study field sites, demonstrating (i) the**
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29 602 **location of the three sampling sites in (A) Mirpur (Dhaka, Bangladesh), (B)**
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31 603 **Ndirande (Blantyre, Malawi) and (C) Patan (Kathmandu, Nepal), (ii) the**
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33 604 **historical number of typhoid cases detected per month at each site (*blue box***
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35 605 **marks the annual monsoon season).**
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3 607 **COMPETING INTERESTS**

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5 608 AJP chairs the UK Department of Health's (DH) Joint Committee on Vaccination and
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7 609 Immunisation (JCVI) and the European Medicines Agency (EMA) Scientific Advisory
8
9 610 Group on Vaccines and is a member of WHO's SAGE. The views expressed in this
10
11 611 manuscript do not necessarily reflect those of JCVI, DH, EMA or WHO. AJP has
12
13 612 previously conducted clinical trials on behalf of the University of Oxford funded by
14
15 613 vaccine manufacturers but has no personal financial interests.
16

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22
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24
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26
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28
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30
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32
33 621 Programme core awards. Neither funding body had any role in designing the study,
34
35 622 writing this manuscript or the decision to submit.

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37 623

38
39 624 **Authors contributions**

40
41 625 SB, BB, JDC, GD, CD, MAG, RSH, VEP, FQ, KZ, SD, KH and AJP contributed to the
42
43 626 conception and design of the study. ST drafted the protocol of the study. This
44
45 627 manuscript was drafted by TD and JM. SB, BB, JDC, GD, SD, CD, MAG, KH, RSH,
46
47 628 VEP, FQ, ST, KZ, MAK, FK, MS, DT and AJP read and critically revised the protocol
48
49 629 and this manuscript prior to submission.

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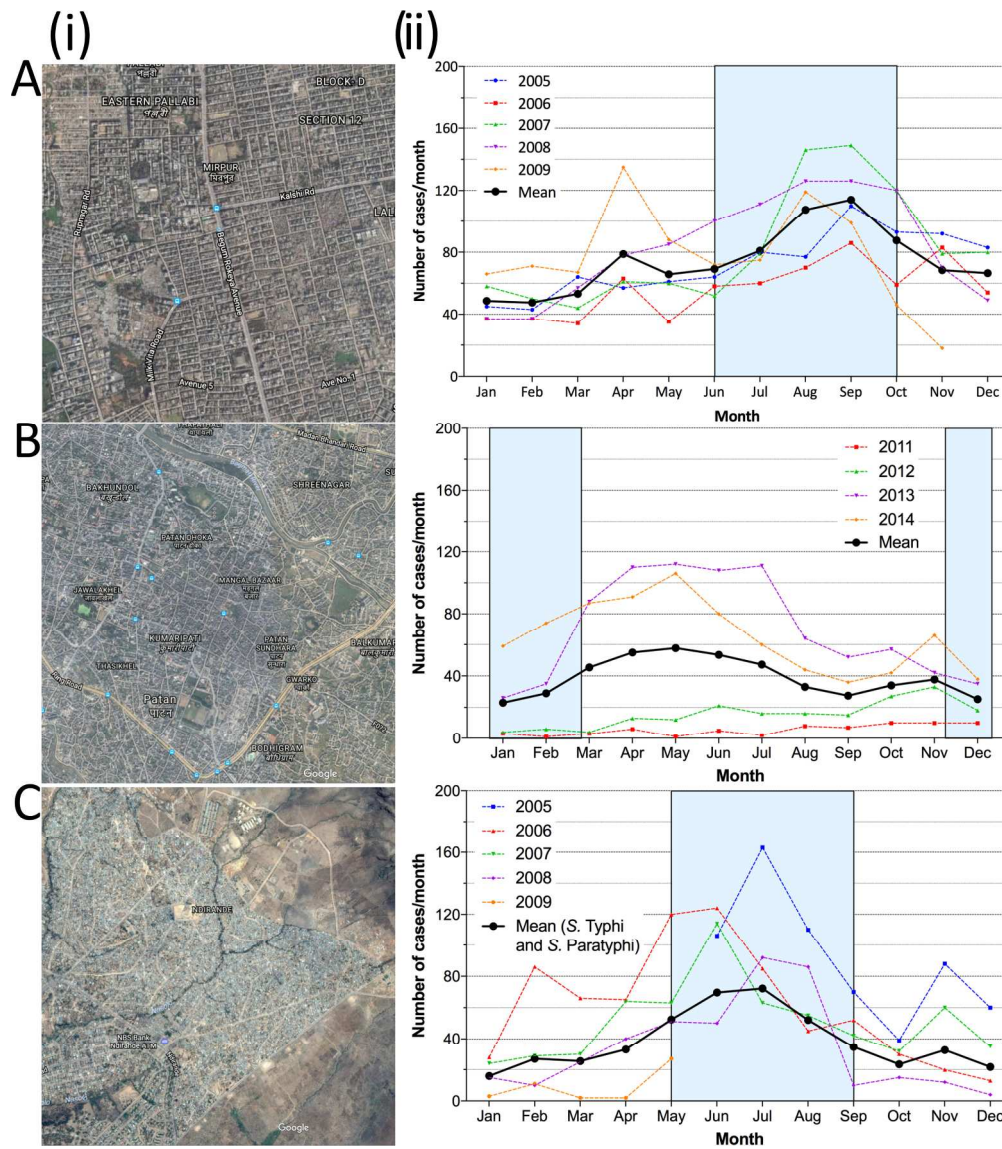


Figure 1. Description of STRATAA study field sites, demonstrating (i) the location of the three sampling sites in (A) Mirpur (Dhaka, Bangladesh), (B) Ndirande (Blantyre, Malawi) and (C) Patan (Kathmandu, Nepal), (ii) the historical number of typhoid cases detected per month at each site (blue box marks the annual monsoon season).

204x231mm (300 x 300 DPI)

Supplemental Table 1. Overview of STRATAA Study Procedures.

Study component	CENSUS							HEALTHCARE UTILISATION		PASSIVE SURVEILLANCE								SEROSURVEY					
								Peak seasons during census		All cases				Household contacts of index cases				Participants		Household contacts of possible carriers			
										Cases within census area*													
Sample size (individuals unless specified)	≥100 000 per site							>700 households		Convenience				≥73 households				8500	6800	Members of ≥50 households			
Time (months, unless specified)	0	4	8	12	16	20	24	1st	2nd	0	Day 8	1	~6	12	0	1	~3	~12	0	**	3	6	
Consent	X							X		X					X		X ^{\$\$}		X				x
Demographic Information	X	X [#]	X [#]	X [#]	X [#]	X [#]	X																
Clinical information										X	X	X	X	X	X								
Questionnaire								X	X														
Blood sample										X [@]			X		X		X ^{\$}		X [@]			X	X
Stool sample										X		X	X	(X)	X	X	2X [€]	(3X)			2X [€]		X
Urine sample										X [@]									X [@]				

Supplemental Table 1. Overview of STRATAA Study Procedures.

* Case within census area = index if blood-culture confirmed typhoid diagnosis, resident in census area and household members consent to participation.

Births, deaths and migrations updated every 4 months in Nepal & Bangladesh

~ approximately - dependent on timeframe of availability for the Vi ELISA result

\$ Host genetics collected/used for laboratory studies if either stool or serum positive or both

§ separate consent for host genetics

@ additional blood (RNA + plasma) and urine samples (subset of 100 with typhoid (culture positive), 100 with clinically suspected typhoid (culture negative), 100 with alternate bacteraemia; and 100 healthy controls (from the serosurvey) from adults 16-40yrs who give consent for genetic assays to be performed.

€ Participants identified as having high anti-Vi IgG only - 2 stool samples collected 48 hours apart once ELISA result known to identify/confirm carriage

** When result known

Total blood volumes: **Passive survey** ≤16 mL adults (>16 years), ≤7 mL children (<16 years); **Serosurvey** ≤8 mL adults (>16 years), ≤7 mL children. Cell pellets stored for host genetics.