Investigating the Burden of Acute and Chronic Typhoid Fever and the Relative Contribution to Ongoing Transmission of *Salmonella* Typhi in Three Urban Sites in Africa and Asia.

James Edward Meiring

St Hilda's College

University of Oxford

A thesis submitted for the degree of

Doctor of Philosophy

Paediatrics

Supervisors: Professor Andrew J Pollard & Professor Melita A Gordon

Abstract - Investigating the Burden of Acute and Chronic Typhoid Fever and the Relative Contribution to Ongoing Transmission of *Salmonella* Typhi in Three Urban Sites in Africa and Asia.

Enteric fever is a serious public health concern in many low- and middle-income countries. However, numerous data gaps exist concerning the epidemiology and transmission of *Salmonella* Typhi and Paratyphi A, the causative agents of enteric fever, in different global contexts. Facility-based passive surveillance for enteric fever underestimates true incidence of disease as not all cases of infection can be captured. In typhoid endemic sites, where febrile illness is a common clinical syndrome, there are many healthcare facilities that individuals utilise when they develop fever. In addition, blood-culture, the current gold-standard for typhoid diagnosis, is not collected on all individuals who seek healthcare within a healthcare facility. Blood-culture also has a low sensitivity for diagnosis and is affected by prior antimicrobial usage and the volume of blood collected. A proportion of acutely infected individuals develop chronic carriage of *Salmonella* Typhi with the pathogen resident in the gall bladder. These individuals continue to shed the bacteria in their stool contributing to ongoing transmission within their community. It is not known what the prevalence of these chronic carriers are in endemic sites and the relative contribution of transmission from carriers compared with acutely infected individuals.

This thesis provides data from a comprehensive surveillance study designed to answer some of these data gaps. A demographic census was used to enumerate an open cohort of approximately 100,000 individuals in each of three urban sites in Africa (Malawi) and Asia (Nepal and Bangladesh). Facility-based passive surveillance was conducted for two years with blood culture collection for febrile illness. Healthcare utilisation surveys carried out in a sample of households enabled adjustment of incidence estimates for healthcare-seeking behaviour. A community-based serological survey was performed enrolling over 15,000 randomly selected individuals, stratified by age, with paired blood samples collected three months apart throughout a 12-month period to provide data on serologically defined infections and to identify the prevalence of chronic carriers.

Between November 2016 and December 2018, 423, 618 participants were enrolled in the demographic census across the three sites. From this population, 625 *S.* Typhi and 107 *S.* Paratyphi A isolates were cultured from the blood of 11,529 febrile patients. Multi-drug resistance was observed in 44% and fluoroquinolone resistance in 61% of *S.* Typhi isolates. The overall crude incidence of blood culture confirmed *S.* Typhi per 100,000 person-years of observation was 58 (95% confidence interval (CI): 48-70) in Malawi, 74 (95% CI: 62-87) in Nepal, and 161 (95% CI: 145-179) in Bangladesh. Adjusted incidence (with adjustment made for healthcare-seeking behaviour, blood culture collection of eligible individuals and blood culture sensitivity) was highest in the 5-9-year age group in all three sites, with overall adjusted incidence per 100,000 person-years of observation of 482 (95% CI: 398-578) in Malawi, 947 (95% CI: 801-1,111) in Nepal and 1,054 (95% CI: 948-1,169) in Bangladesh. Incidence rates per 100,000 person-years estimated from seroconversion episodes in serological surveillance were 3382 (95% CI: 2845-3991) in Malawi, 4326 (95% CI: 3753-4962) in Nepal and 3157 (95% CI: 2786-3565) in Bangladesh, suggesting higher rates of infection than captured through passive surveillance. Only one individual in Bangladesh was defined as a chronic carrier, with the highest rate of stool shedding identified in acutely infected individuals at the time of bacteraemia.

The design of this study applying multiple surveillance methodologies, and adjustment for known factors that lead to underestimation, has enabled a comprehensive estimation of incidence demonstrating *S.* Typhi to be a major cause of febrile illness particularly among the youngest children in the populations studied, with *S.* Paratyphi A contributing to disease incidence in the Asian sites. High rates of disease in combination with the observed high rates of antimicrobial resistance, necessitate multiple intervention strategies to achieve global control of these pathogens through development in water and sanitation infrastructure, the introduction of efficacious typhoid conjugate vaccines and the development of vaccines for *S.* Paratyphi A.

Publications

The following list of publications represent work I have been involved with during the time of my DPhil. The majority relate to the program of enteric fever surveillance performed in three countries, the data from which form the content of this thesis. In addition, I have been involved in several other projects including a phase 2b typhoid vaccine trial and a series of phase 3 typhoid vaccine trials that were run concurrently in the three surveillance sites.

1. **Meiring JE**, Shakya M, Khanam F on behalf of the STRATAA Consortium. Burden of enteric fever in three urban sites in Africa and Asia: a multicentre population-based study with 626,219 person-years of observation. Planned submission to The Lancet April 2020.

2. Carey ME, MacWright WR, Im J, **Meiring JE** et al. SEFI, SETA and STRATAA enteric fever studies: A review of methodological similarlities and differences. Clinical Infectious Diseases (2020 – Accepted for Publication. In Press)

3. **Meiring JE**, Giubilini A, Savulescu et al. Generating the Evidence for Typhoid Vaccine Introduction: Considerations for global disease burden estimates and vaccine testing through human challenge. Clinical Infectious Diseases. 2019 October; S395-401.

4. Pitzer V, **Meiring JE** et al. The Invisible Burden: Diagnosing and Combatting Typhoid in Asia And Africa. Clinical Infectious Diseases. 2019 October; S402-407.

5. **Meiring JE**, Laurens M, Patel P on behalf of the TyVAC Consortium. TyVAC Malawi: a phase III randomized, double-blind, controlled trial of the clinical efficacy of typhoid conjugate vaccine among children age 9 months through 12 years in Blantyre, Malawi: study protocol for a randomized controlled trial. Clinical Infectious Diseases. 2019 March; Pages S50–S58.

6. **Meiring JE**, Sambukansi R, Moyo E on behalf of the TyVAC Consortium. TyVAC Malawi: Community Engagement before initiation of typhoid conjugate vaccine trial in schools in two urban townships in Blantyre, Malawi – experience and lessons. Clinical Infectious Diseases. 2019 March; Pages S146–S153.

7. Patel P, Patel P, **Meiring JE** et al. Stories from the Field - On the Ground in Malawi-First Typhoid Conjugate Vaccine Study in Africa. June 2019. The American Journal of Tropical Medicine and Hygiene 100(6):1299-1300. DOI: 10.4269/ajtmh.19-0014

8. **Meiring JE**, Patel P, Patel P, Gordon MA. Typhoid Conjugate Vaccines: Making vaccine history in Africa. Expert Review of Vaccines 2018 Aug;17(8):673-676. DOI: 10.1080/14760584.2018.1496825.

9. Jin C, Gibani MM, Moore M, Juel HB, Jones E, **Meiring JE**, et al. Efficacy and immunogenicity of a Vi-tetanus toxoid conjugate vaccine in the prevention of typhoid fever using a controlled human infection model of Salmonella Typhi: a randomised controlled, phase 2b trial. Lancet 2017 Sep 28.

10. Darton TC, **Meiring JE**, Tonks S on behalf of the STRATAA Study Consortium, et al. 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. BMJ Open 2017. DOI: 10.1136/bmjopen-2017-016283

11. Kachimanga C, Jamu M, Gates T… **Meiring JE**, Kawonga R. A Typhoid Epidemic in Rural Malawi: Real-world Challenges. Annals of Global Health 2017 Feb.

12. **Meiring JE**, Gibani MM on behalf of the TyVAC Consortium Meeting Group: Vaccine effectiveness study designs: Accelerating the introduction of typhoid conjugate vaccines and reducing the global burden of enteric fever. Report from a meeting held on 26-27 October 2016, Oxford, UK. Vaccine 2017; 38

13. Thindwa D, Farooq YG, Shakya M, **Meiring JE** on behalf of the Strategic Typhoid alliance across Africa and Asia consortium. Electronic Data Capture for Large Scale Typhoid Surveillance, household contact tracing, and health utilisation survey: Strategic Typhoid Alliance across Africa and Asia. Accepted. PLoS One.

In addition to the publications listed here, I had the privilege of being part of a World Health Organisation Working group for the development of surveillance standards for invasive salmonellosis. The Working Group was responsible for writing a chapter which was included in the latest edition (2018) of the WHO Vaccine Preventable Diseases Surveillance Standards.

World Health Organization Vaccine Preventable Diseases Surveillance Standards. Co-author of guidelines for Surveillance for invasive Salmonellosis. 2018.
www.who.int/immunization/monitoring_surveillance/burden/vpd/standards/en/

Acknowledgements

The work presented here in this thesis represents the combined efforts of hundreds of individuals, over a five-year timespan, across multiple countries and continents. It would not have been possible to collect and present these important data without the efforts and expertise of these colleagues, and whilst it is not possible to acknowledge them all individualy I would like to draw special attention to certain individuals here.

Firstly, I would like to thank the greater than 400,000 participants who were involved at some level in this study from the three sites. These are the individuals and families who have to live alongside enteric fever every day. For them it is a daily reality, as they watch this disease make their children sick. For a disease that has long been eradicated from the richer countries in the world, these participants deserve more from the global community, so that interventions are put in place so that they too do not have to suffer the consequences of a lack of access to an efficacious vaccine or the provision of clean drinking water. It is to their credit that, despite the many challenges that living in these sites bring, they provided their time, information and clinical samples so that we might learn more about this important disease and contribute to the introduction of interventions which may eradicate it, not just from their own communities, but communities across their countries and continents.

I would like to express my thanks to my supervisor Professor Andrew Pollard. He provided me with my first opportunity in clinical research working on the typhoid controlled human infection model, and then enabled me to do my 'dream job' travelling through Africa and Asia studying typhoid epidemiology. The opportunities he has provided to present internationally, publish widely and collaborate with the scientific community have been unparalleled and his constant encouragement to 'keep pushing' has been much needed.

I am also very grateful for the support of my co-supervisor, Professor Melita Gordon during the three years I spent living in Blantyre, Malawi with my family. I benefitted greatly from her understanding of the local research and healthcare systems along with her scientific and medical expertise in the sub-Saharan African, Malawian context. Her dedication and passion to improving the lives of the world's poorest people through science and education is an inspiration.

I must add a note of thanks to Susan Tonks. The many hours of teleconferencing, troubleshooting and travelling have paid off. The work presented here would certainly not have been achieved without Susan's logistical, operational and administrative support and she should take huge credit and be very proud of the achievements of the STRATAA consortium.

Particlular thanks should go to the enteric fever research teams at the Malawi-Liverpool Wellcome Trust Clinical Research Programme lead by Professor Melita Gordon and Professor Robert Heyderman, the international centre of diarrhoeal disease research, Bangladesh lead by Dr Firdausi Qadri and Professor John Clemens and the Oxford University Clinical Research Unit – Nepal lead by Dr Buddha Basnyat, Dr Christiane Dolecek and Professor Stephen Baker. Additional thanks should go to junior colleagues at these great institutions, particularly Deus Thindwa, Tikhala Makhaza Jere, Maurice Mbewe, Priyanka Patel, Pratiksha Patel, Farhana Khanam, Arif Khan, Nirod Chandra Saha, Prasanta Kumar Biswas, Mila Shakya, Archana Maharjan. These large studies were planned and delivered by hundreds of research clinicians, assistants and interns, and I am grateful to them all. It has been a privilege to travel regularly to these sites. The welcome and spirit of collaboration has always been strong.

The Oxford Vaccine group and those associated with it provided me with excellent colleagues from which I learned a great deal. I am particularly indebted to the support of Meryn Voysey for coaching me through data analysis and presentation. I am also grateful to Virginia Pitzer and Maile Philips for their support in data analysis.

Personally, I am grateful to the wider family of staff and students based at the Malawi-Liverpool Wellcome Trust. It was very special to be a member of this family for three years. Malawi is an incredibly special country which I now miss deeply. It is filled with remarkable people, many of whom I will count as friends for years to come. My three young children will be shaped forever by their experiences growing up with the red soil between their toes, and I hope that one day we can all return.

I must say a special word of thanks to my parents Leslie and Joyce Meiring who have provided me with the opportunity to do many of the things I have been able to do. I am deeply grateful for their love, support, guidance and care over many years and I will always remain indebted to them both.

Finally, I must offer heartfelt thanks to my wife Ruth. She has sacrificed much so that I can follow this journey. My achievements and the credit for this thesis should be shared with her. Thank you for making home for our family wherever we have been for the last three years across three cities and 2 continents. For more reasons than I can express here, I am deeply grateful.

Contributions

The nature and complexity of this study required hundreds of members of staff across multiple sites. My role in the delivery of this study whilst significant was only made possible through the collaboration and expertise of many people. My contribution to the study and to this thesis are outlined here:

1. In conjunction with Susan Tonks, site project investigators and local research fellows I designed and trialled the case record forms used for data capture across the different aspects of the study.
2. Working closely with Susan Tonks and site teams in Malawi, Nepal and Bangladesh I performed site initiation and regular visits to keep up to date with site teams to ensure study activities were performed on time, according to protocol and approaches at all three sites were harmonised where possible.
3. Being based in Malawi I took a leadership role in the day to day running of the trial at this site, setting up the surveillance platforms, ensuring appropriate sample collection, lab processing and recording of results. The co-ordination of clinical teams to perform blood-culture surveillance in health centres, sero-surveillance in the community, household surveys for healthcare utilisation and the demographic surveillance of over 100,000 people was complex and required involvement of large staff teams.
4. I co-ordinated the collection and inventory of samples at all three sites and the numbers required for shipping to collaborating laboratories.
5. I was the joint first author on the trial protocol manuscript taking the protocol written by the study principal investigators and preparing it for publication.
6. With regards to the content of this thesis, I collated, analysed and presented the data, which is discussed in the results chapters 3, 4, 5 and 6 with the statistical support and guidance of

Merryn Voysey and Virginia Pitzer. These data are also being prepared for publication where I will be first or joint-first author with research fellows from the collaborating sites.

Table of Contents

List of Figures

List of Tables

1. Chapter 1 – Introduction: The Clinical Presentation, Burden and Prevention of Enteric Fever

1.1. Enteric Fever

1.1.1. The Pathogen

The causative organisms responsible for the clinical syndrome enteric fever; *Salmonella enterica* serovar Typhi (*S.* Typhi) and serovar Paratyphi A, B and C (*S.* Paratyphi) are found within the Enterobacteriaceae family. They are rod-shaped, gram-negative, facultative anaerobic bacteria within the Salmonella genus that cause a human-restricted invasive febrile disease.[1] The Salmonella genus is divided into two species, *Salmonella enterica* and *Salmonella bongori*, with over 2,500 serotypes within the enterica group, a number of which are also capable of causing invasive human disease, such as *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium).[2]

1.1.2. Clinical presentation

The clinical presentation of enteric fever has been well characterised, with numerous case series within the historical literature from before and after the advent of antimicrobials.[3–5] As indicated by its name, the predominant symptom is one of high fever, which is classically described as 'step-wise' manifesting in the first week of the illness.[6,7] This is commonly accompanied by a collection of non-specific symptoms including malaise, headache, anorexia, abdominal pains, constipation and diarrhoea, which are found in other febrile illnesses making the clinical diagnosis of enteric fever challenging.[8–10]

If left untreated, enteric fever can progress into a third week with a series of complications, including hepatitis, acute kidney injury, myocarditis and haematological complications.[7,11]

Historically, it has been thought that *S.* Paratyphi infections cause milder disease than *S.* Typhi[5,9,12] but more recent, large, prospective studies have shown the two pathogens to have a near identical clinical course.[13]

There can be numerous clinical findings on examination including a coated tongue, abdominal tenderness, hepatomegaly and splenomegaly.[7,11] Patients have been well described as having a relative bradycardia, or pulse-temperature dissociation.[14] Rose spots; a blanching erythematous rash containing culturable *S.* Typhi bacteria are found in 1-30% of patients, but difficult to visualise in dark skinned individuals.[15]

1.1.3. Neurological complications

Although rare, numerous neurological manifestations of enteric fever have been reported in the literature, from typhoid meningitis and encephalopathy through to neuropsychiatric conditions.[16] A large outbreak of blood-culture confirmed typhoid fever was reported from the Malawi-Mozambique border with a large burden of neurological complications and high mortality rate. Dysarthria, ataxia, upper motor neuron signs and altered mental status were identified in over 40 individuals.[17] Whilst it is rare to culture *S.* Typhi directly from the cerebrospinal fluid (CSF), it is thought the cortical irritation leading to clinical symptoms is mediated by the typhoid toxin.[18,19]

1.1.4. Intestinal perforation

Intestinal perforation (IP) is commonly reported as a sequalae of severe typhoid infection, with the primary site of perforation occurring in the terminal ileum.[20,21] Increasing rates of perforation have been documented in outbreak scenarios and countries with increasing antimicrobial resistance.[22] Recognising this, the WHO have included guidance on the surveillance of IPs, recommending all instances be recorded from countries with endemic typhoid.[23] IPs could be a sentinel event providing evidence of an outbreak.

Clinicians responding to an outbreak of blood-culture confirmed typhoid fever on the Ugandan-DRC border reported high rates of IP (43% of all blood culture confirmed individuals), with delays to healthcare seeking, and the use of non-sensitive antibiotics reported as the primary cause.[22] A systematic review for IP estimated the case-fatality-rate (CFR) at 15.4% with a mean duration of 18.4 days in hospital.[24]

1.1.5. Mortality

It has been estimated in the historical literature that typhoid had an overall CFR of 1%.[25–27] Estimating mortality for typhoid burden is challenging however, as the surveillance method used will influence the estimate. Hospital-based passive surveillance will miss milder cases of disease as patients with mild infection are less likely to attend a healthcare facility. The reporting fraction of more severe disease, or episodes of infection with complications is therefore greater, leading to an overestimation of severity. With active, community based surveillance, severity is underestimated as participants are often identified and enrolled earlier in the natural history of their infection and provided with appropriate antimicrobials sooner, interrupting the normal clinical course and preventing many of the sequalae of untreated typhoid infection.[28] A recent systematic review and meta-analysis of the CFR for typhoid fever however has aimed to bring these together, reviewing literature from 1971 to 2016, from both passive and active surveillance studies, with an estimated overall CFR of 2.49%, with a CFR of 4.49% for hospitalised patients with a range reported in the studies from 0% to 23% CFR.[29] They found an increased CFR of 6.84% for AMR strains, although this was not statistically significantly higher when compared to sensitive strains.[29] Accurate CFRs for enteric fever are important when considering vaccine introduction and evaluating the cost-effectiveness of any vaccine. In a further meta-analysis, death from enteric fever made up 86-98% of disability-adjusted life-years attributed to enteric fever.[30]

There is evidence to suggest higher CFR in outbreak scenarios,[31] and with the escalating antimicrobial resistance particularly in South Asia,[32] a return to the pre-antibiotic era, with CFRs of over 15% is a possibility.[33]

1.1.6. Transmission

Transmission of enteric fever is through the faecal-oral route[34] and therefore instances of poor personal hygiene, contaminated drinking water and food preparation and ineffective human waste removal are responsible for the ongoing transmission of enteric fever within populations.[35,36] This has

been known since the middle of the 19th Century when William Budd described the early epidemiology of several typhoid fever outbreaks in southern England.[37–39] However, despite this common epidemiological link, a number of case-control studies have identified a wide ranging, and sometimes contrasting collection of risk factors for typhoid fever acquisition in different global contexts.

A study comparing typhoid fever cases to both hospital and community controls in the Mekong Delta, Vietnam, found that recent contact with a confirmed typhoid fever case was associated with typhoid diagnosis.[40] This was also found to be the case in Jakarta, Indonesia.[41] Interestingly, this study showed a different set of risk factors for *S.* Paratyphi A infection compared with *S.* Typhi, with consumption of food from outside the home more associated.[41] Further case-control studies from Indonesia, highlighted households with a higher number of individuals residing within them, along with university education dwellings, and poor handwashing associated with infection.[42,43]

The differential epidemiology between *S.* Typhi and *S.* Paratyphi A was also demonstrated in Kathmandu, Nepal. Contact with a typhoid fever case was found to be protective, although this non-intuitive result was likely due to a lack of further information collected. *S.* Typhi was more strongly associated with a poor-quality water source for the home, where as eating street food in the two weeks preceding illness and residing within Kathmandu for less than 1 year was associated with *S.* Paratyphi A infection.[44]

Historical data from Santiago, Chile, and Karachi, Pakistan further highlight the importance of food prepared outside the home, with school lunches, roadside restaurants and specifically flavoured ices implicated in transmission.[45,46] Additionally, cases of *S.* Typhi in Karachi had a higher rate of treatment with antimicrobials two weeks prior to diagnosis. The authors hypothesise that antimicrobials lower the required infectious dose to cause systemic illness, citing animal studies from other Salmonella species.[47]

More recent data from Fiji and Malawi have shown water-based and sanitation risk factors to be important. In Fiji, unimproved sanitation facilities leading to drinking contaminated surface water, and eating unwashed produce resulted in the highest risk, whilst in Malawi the use of river water for washing cooking utensils and using multiple sources of water were found to be more common in typhoid cases.[48,49]

1.1.7. Chronic Carriage

The role of typhoid chronic carriers in the ongoing transmission of enteric fever within typhoid endemic countries remains an important knowledge gap in typhoid epidemiology, with implications for vaccine effectiveness[50] at a community level.[51] Historical data provide estimates that 2-5% of acutely infected individuals develop typhoid carriage, although this was in the era prior to fluoroquinolone antimicrobials, and more recent estimates may be less.[52] Defined as persistent shedding of *S.* Typhi bacteria at least 12 months after finishing an appropriate course of antimicrobial treatment and the resolution of symptoms following a laboratory confirmed episode of acute disease,[23] the asymptomatic carrier state has been the source of interest since the infamous 'Typhoid Mary' was incarcerated in New York City, following her role in starting multiple typhoid outbreaks in the 19th century.[53]

Epidemiological investigations through case-control studies, and ultrasound imaging in both mice and human participants, have shown the association between the carrier state, and the development of bacterial biofilm on gallstones within the gall bladder by *S.* Typhi bacteria.[54–56] This is supported by data from diverse global contexts showing prevalence of chronic carriers increase with age, and are predominantly female, risk factors which mirror the development of gall bladder pathology.[52,56,57] To establish long-term carriage, organisms must enter the biliary tract either directly by ascending through a malfunctioning sphincter of Oddi, or via the liver during systemic infection.[58]

The importance of carriage in low incidence, non-endemic settings has been shown through multiple outbreaks which have been traced to a chronic carrier often responsible for food preparation.[59]

However, the contribution of carriers to ongoing transmission within endemic sites and the diagnosis of these individuals, is problematic. Shedding of the pathogen within stool is intermittent, and at a low level, meaning detection through serial stool culture, is both programmatically difficult, and has a low sensitivity.[60] The use of serological screening, using anti-Vi antibody, has been successful in non-endemic sites[59,61] but in areas of medium to high incidence, where regular infection or exposure to the pathogen increases the Vi titre, this has had mixed results.[62–64] More recent work has been done to identify novel serological markers, transcriptomic and a metabolomic profile that could improve the prospective diagnosis of this population.[65–68]

Historically, the only treatment option available for carriage, was radical surgery with removal of the gallbladder.[69] However, a small number of clinical trials have been performed which show clearance of the pathogen from stool with prolonged antibiotic administration. High dose ampicillin and amoxicillin showed a cure rate of 75% and 90% respectively, although with only sample sizes of 12 and 10 participants.[70,71] Following an outbreak of typhoid in Aberdeen, UK in the 1960s, four individuals were treated with co-trimoxazole, with only 1 of the 4 achieving clearance of the pathogen.[72] More recently, 4 trials with a total of 57 participants showed an efficacy of 78-93% from prolonged courses (14-28days) of fluoroquinolone.[73] The high treatment success is thought to be secondary to the high concentration of antibiotic in the gut, macrophages and biliary tree. High dose fluroquinolones (e.g. ciprofloxacin 750mg BD for 28 days) have become the advised treatment of choice for carriage[74] although there are concerns regarding the appropriate choice in situations of drug resistance with azithromycin suggested as an alternative (personal correspondence).

1.2. Global Enteric Fever Incidence Estimates

1.2.1. Acute Disease

Precise estimates for the global burden of enteric fever are difficult to ascertain owing to the non-specific presentation of disease, the lack of reliable diagnostic facilities in many regions, and the insensitivity of commonly used diagnostic tests.[75,76] Additionally, the burden of typhoid disease can

fluctuate widely depending on the geographic context with the occurrence of outbreaks, epidemics and wide-scale infrastructure improvements.

Prior to the year 2000 there was also a distinct lack of data points from which to estimate a global burden.[77] The well cited systematic review conducted by Crump et al, published in 2004, compiling population-based incidence studies from 1966 to 2000 had a total of 22 studies, although notably only three from the African continent, and seven from Asia (Error! Reference source not found.).[25] Greater than 50% of the studies used the control arm of a vaccine trial to estimate incidence, and seven studies were from Eastern Europe and Russia, where there was a documented decline in typhoid incidence during the follow-up period of the trial.[78] Extrapolation between countries and regions which had no source data was based on geographical proximity and a number of socioeconomic indicators as defined by the United Nations.[79] Regions were defined as high, medium or low incidence, and then age curves were fitted to each country/region. Crude total estimates were 10,825,487 cases of *S.* Typhi, with an adjustment factor of two applied for the sensitivity of blood culture resulting in an adjusted estimate of 21,650,974. Of the 22 studies, eight also reported on *S.* Paratyphi, with a proportion of 0.25 applied to the total number of *S.* Typhi cases, resulting in adjusted global estimate of 5, 412, 744 cases.

Publication	Burden Estimate	Papers included	Surveillance Sites	Surveillance Methods
Crump et al, Bulletin WHO, 2004[25] n=22 (Year 2000) Studies published between 1966 and 2001	Unadjusted 10,825,487 Adjusted (blood culture sensitivity) 21,650,974	Wahdan et al, Bulletin WHO, 1975[80] Wahdan et al, JID, 1982[81] Klugman et al, Lancet, 1987[82] Klugman et al, Vaccine, 1996[83] Yang et al, Bull WHO, 2001[84] Sinha et al, Lancet, 1999[85] Chuttani et al, Bull WHO, 1977[86] Acharya et al, NEJM, 1987[87] Simanjuntak, Lancet, 1991[88] Lin, AJTMH, 2000[89] Lin, NEJM, 2001[90] Polish Typhoid Committee, Bull WHO, 1965[91] Hejfec et al, Bull WHO, 1965[92] Hejfec et al, Bull WHO, 1966[93] Hejfec et al, Bull WHO, 1968[94] Hejfec et al, Bull WHO, 1969[78] Yugolsav Typhoid Committee, Bull WHO, 1962[95] Yugolsav Typhoid Committee, Bull WHO, 1964[96] Ashcroft et al, Lancet, 1967[97] Black et al, Vaccine, 1990[98] Levine et al, Lancet, 1987[99] Levine et al, Lancet, 1990[100] Tapa et al, Bull WHO, 1975[101]	Africa Egypt 2 South Africa Asia China 1 India 2 Indonesia 1 Nepal 1 Vietnam 2 South America Chile 2 Guyana 1 Europe Poland 1 USSR 4 Yugoslavia 2 Oceania Tonga 1	Hospital based surveillance with active surveillance for school absenteeism 4 Hospital based passive surveillance 10 Demographic census with household active surveillance 1 Household active surveillance 6 Demographic census with facility-based passive surveillance 1

Table 1-1: Summary Table of studies included in Crump et al, Bulletin WHO, 2004 including the date of publication, continents from which surveillance was performed and surveillance methods used

In an updated global burden estimate, Buckle et al,[27] employed similar methods, dividing the world into super-regions, as defined by the Global Burden of Disease Project.[102] Restricting their search to population-based studies conducted since 1980, this yielded 25 studies and five advanced surveillance systems, providing data for 47 countries across 14 of the 21 regions. Notably, there were now 17 studies from Asia from which to estimate burden, although Africa still only contributed six, with three coming from Egypt. With these data, crude global burden was estimated to be 13,474,369 typhoid episodes each year and following an adjustment factor of two for blood culture sensitivity, 26,948,739. (Table 1-2: Summary Table of studies included in Buckle et al, Journal Glob Health , 2012 including the date of publication, continents from which surveillance was performed and surveillance methods used

)

The new studies generating incidence information were mainly from the Diseases of Most Impoverished (DOMI) programme.[103] As a template for further surveillance work to come, DOMI identified five countries where typhoid incidence was perceived as a public health problem and conducted blood-culture surveillance for febrile illness within enumerated demographic census areas, employing standardised inclusion criteria for blood-culture collection. Looking specifically at the 5-15-year age group, typhoid incidence per 100,000 person-years of observation was 24.2 in Vietnam, 29.3 in China, 180.3 in Indonesia, 412.9 in Pakistan and 493.5 in India, with the highest incidence overall in 2-4-year olds in Karachi, Pakistan.[103] Whilst enrolment for blood culture was standardised, the India and Pakistan sites, did utilise active surveillance with community health workers visiting homes on a periodic basis. Whilst this may not fully explain the differential in incidence estimates between the sites, research done since this study estimates active surveillance may increase typhoid episode detection by a factor of >5.[104]

Publication	Burden Estimate	Papers included	Surveillance Sites	Surveillance Methods
Buckle et al, J Glob Health, 2012[27] n=25 Paratyphoid (Year 2010) Studies published between 1980 and 2009	Unadjusted 13 500 000 (95% CI 9.1-17.8million) Adjusted (blood culture sensitivity) 26 900 000 (95% CI; 18.3 – 35.7million)	Black et al, Vaccine, 1990[98] Levine et al, Lancet, 1987[99] Levine et al, Lancet, 1990[100] Klugman et al, Lancet, 1987[82] Wahdan et al, JID, 1982[81] Crump et al, EID, 2003[105] Srikantiah et al, AJTMH, 2006[106] Brooks et al, EID, 2005[107] Sinha et al, Lancet, 1999[85] Chen et al, JHPN, 2007[108] Khan et al, Bull WHO, 2006[109] Ochiai et al, Bulletin WHO, 2008[103] Ochiai et al, EID, 2005[110] Acharya et al, NEJM, 1987[87] Sur, BMC Public Health, 2009[111] Sur et al, NEJM, 2009[112] Siddiqui et al, Int JID, 2006[113] Yang et al, Bull WHO, 2001[84] Lin et al, NELM, 2001[114] Lin et al, AMJTH, 2000[89] Simanjuntak et al, Lancet, 1991[88] Vollaard et al, TRSTMH, 2005[115] Breiman, PloS ONE, 2012[116]	Africa Egypt 3 South Africa 1 Kenya 2 Asia China 2 India 4 Indonesia 3 Nepal 1 Vietnam 3 Bangladesh 1 Pakistan 3 South America Chile 3	Hospital based passive surveillance 3 Demographic census with household active surveillance 3 Hospital based surveillance with active surveillance for school absenteeism 2 Demographic census with passive surveillance 4 Active Surveillance 1

Table 1-2: Summary Table of studies included in Buckle et al, Journal Glob Health , 2012 including the date of publication, continents from which surveillance was performed and surveillance methods used

A

further updated systematic review exclusively for low- and middle-income countries was published by Mogasale et al in 2014.[26] Whilst the source data included was very similar to the previous review, additional methods for adjustment were included. Through a second systematic review, countries were identified as either being 'high-risk', or 'at-risk' based on their access to improved water supply. Adjusting for an estimated blood-culture sensitivity of 61.1%, global incidence was estimated at 20,558,180 (95% CI 17 500 000 – 24 200 000) and with water-based risk adjustments applied incidence was 11,883,047 (95% CI 9 925 551 – 14 751 214). This range of global incidence was similar to previously published data. (**Error! Reference source not found.**)

Publication	Burden Estimate	Papers included	Surveillance Sites	Surveillance Methods
Mogasale et al, Lancet Global Health, 2014[26] n=23 (Year 2010) Studies published between 1990 and 2013	Unadjusted 20 558 180 (95% CI 17 500 000 – 24 200 000) Adjusted (water-based risk factors) 11 883 047 (95% CI 9 925 551 – 14 751 214)	Ochiai et al, Bulletin WHO, 2008[103] Crump et al, EID, 2003[105] Srikantiah et al, AJTMH, 2006[106] Marks et al, EID, 2010[117] Nielsen et al, PLoS One, 2012[118] Breiman et al, PLoS One, 2012[116] Thriemer et al, PLoS One, 2012[119] Klugmann et al, Vaccine, 1996[83] Yang et al, Bull WHO, 2001[84] Sinha et al, Lancet, 1999[85] Sur et al, NEJM, 2009[112] Brooks et al, EID, 2005[107] Naheed et al, Int J Inf Dis, 2010[120] Siddiqui et al, Int J Inf Dis, 2006[113] Owais et al, Paed Inf Dis J, 2010[121] Khan et al, Vaccine, 2012[122] Punjabi, J Inf Dec Ctries, 2013[123] Simanjuntak, Lancet, 1991[88] Lin, AJTMH, 2000[89] Lin, NEJM, 2001[90] Levine, Lancet, 1990[100] Black, Vaccine, 1990[98] Levine, Lancet, 1987[99]	Africa Egypt 2 Ghana 2 Kenya 2 Zanzibar South Africa Asia China 2 India 5 Indonesia 3 Pakistan 4 Vietnam 2 Bangladesh 2 South America Chile 2	Demographic census with facility-based passive surveillance 3 Enhanced facility-based surveillance (Community health workers visiting HH) 2 Demographic census with facility-based passive surveillance and HUS 2 Facility-based passive surveillance with HUS 2 Demographic census with household active surveillance 2

Table 1-3: Summary Table of studies included in Mogasale et al, Lancet Global Health, 2014. Abbreviations – HH; Households, HUS; Healthcare utilisation surveys, including the date of publication, continents from which surveillance was performed and surveillance methods used

A modelling study from Antillon et al[124] took a similar collection of published papers and looked to adjust for a wider number of factors, using new data from the Typhoid Surveillance in Africa Program (TSAP) to fit the model.[125] Countries were categorised by 12 separate possible risk-factors collected through expert opinion; population density, gross domestic product, Gini-coefficient, access to improved water, access to improved sanitation, years of education for women, percentage of roads paved, percentage of population living on less that $2/day, prevalence of stunting, number of major floods, HIV prevalence and water stress. Data from population-based surveillance studies were then used to extrapolate into countries with no data. Interestingly, the three most predictive risk-factors; percentage of roads paved, percentage of stunting and population living in extreme poverty suggest that general measures of poverty for a country or region may be more predictive of typhoid risk, than more traditional water and sanitation-based factors. With these adjustments burden for low- and middle-income countries was estimated at 17.8million episodes of typhoid per year, although the confidence interval of 6.9-48.4million suggested a large degree of uncertainty. Unusually, the highest incidence region was estimated to be Oceania followed by central Africa, rather than South Asia as has previously been the case,[25,26] this despite no data points from these regions. (Error! Reference source not found.)

Publication	Burden Estimate	Papers included	Surveillance Sites	Surveillance Methods
Antillon et al PLoS NTD, 2017 n=32 Studies published between 1990 and 2014 Year 2015	17 800 000 (95% CI 6.9-48.4million)	Breiman et al, PloS One, 2012[116] Klugman et al, Lancet, 1987[82] Marks et al, EID, 2010[117] Crump et al, EID, 2003[105] Srikantiah et al, AJTMH, 2006[106] Wahdan et al, JID, 1982[81] Siddiqui et al, Intl JID, 2006[113] Khan et al, Vaccine, 2012[122] Ochiai et al, Bull WHO, 2008[103] Owais et al, PIDJ, 2010[121] Naheed et al, Intl JID, 2010[120] Brooks et al, EID, 2005[107] Sinha et al, Lancet, 1999[85] Bahl et al, JHP, 2004[126]	Africa Kenya 2 South Africa 1 Ghana 1 Egypt 3 Asia Pakistan 3 Bangladesh 2 India 5 Nepal 1 Indonesia 3	Demographic census with active surveillance 14 Passive Surveillance 17

		Sur et al, RSTMH, 2006[127] Sur et al, NEJM, 2009[112] Archarya, NEJM, 1987[87] Simanjuntak et al, Lancet, 1991[88] Punjabi, J Inf Dec Ctries, 2013[123] Yang et al, , 2001[84] Lin et al, AJTMH, 2000[89] Lin, NEJM, 2001[114] Lanh, NEJM, 2004[128] Srikantiah, TMIH, 2007[129] Black et al, Vaccine, 1990[98] Levine et al, Lancet, 1987[99] Levine et al, Lancet, 1990[100] Olle-Goig, Bulletin PAHO, 1993[130]	China 2 Vietnam 4 Uzbekistan 1 South America Chile 3 Haiti 1	

Table 1-4: Summary Figure of studies included in Antillon et al, PloS NTD, 2017, including the date of publication, continents from which surveillance was performed and surveillance methods used

Antillon et al, also adjusted for blood-culture sensitivity and case ascertainment. With this, they adjusted for the difference between active and passive surveillance studies, either with an enumerated census or without. The recently published IHME GBD estimates for typhoid and paratyphoid made similar adjustments, with passive surveillance estimates increased by a factor of 5.8. With this review authors included published incidence estimates from 1982 to 2017, as well as including national surveillance systems from higher income countries. (Error! Reference source not found.) As with the DOMI programme previously, this review benefited from the recently published TSAP program, where 13 studies across 10 African countries were conducted, employing consistent clinical and microbiological procedures across the sites.[131] Catchment populations were defined using previous population estimates for each area. Healthcare utilisation surveys (HUS) were conducted within these populations to estimate the proportion of the population who used the study facilities where blood-culture surveillance was taking place. This platform enables the set-up of a more cost-efficient method for surveillance, as household visits are not required whilst also providing the opportunity to quantify the potential missed episodes of fever through alternative healthcare usage. It relies on the assumption that the rate of typhoid within the individuals who visit the study facility is

the same as those who go elsewhere, which introduces a degree of uncertainty into any estimate. For example, from the Burkina Faso site in the TSAP study, 13 positive blood-cultures were collected in total, from a study population of 7,574 individuals, providing a crude incidence of 145/100,000person-years of observation (PYO). However, HUS demonstrated just over a third of that population used the study facility and so incidence was adjusted to 383 (CI: 274-535) PYO. Whilst there were very high reported rates of disease from countries like Burkina Faso, TSAP also reported low rates of disease from countries that had previously reported a medium incidence, with 0 cases in Sudan, and very low-incidence in Ethiopia and South Africa, with a reduction in cases in Kenya, compared to the 2012 estimates.[116] There was also marked intra-country variation with higher rates in rural Ghana compared with an urban setting.[117]

Publication	Burden Estimate	Papers included	Surveillance Sites	Surveillance Methods
IHME, Lancet Infectious Diseases n=28 Year 2019	14.3million (95 % CI 12.5-16.3 million)	Brooks, EID, 2005[107] Dewan, PloS NTD, 2013[132] Naheed, Intl JID, 2010[120] Black, Vaccine, 1990[98] Levine, Lancet, 1987[99] Levine, Lancet, 1990[100] Ochiai, Bull WHO, 2008[103] Ochiai, EID, 2005[110] Crump, EID, 2003[105] Srikantiah, AJTMH, 2006[106] Wahdan, JID, 1982[81] Marks, Lancet Global Health, 2017[125] Nielsen, PloS One, 2012[118] Sinha, Lancet, 1999[85] Sur, BMC Public Health, 2007[111] Sur, NEJM, 2009[112] Punjabi, JIDC, 2013[123] Simanjuntak, Lancet, 1991[88] Breiman, PloS One, 2012[116] Karkey et al, PLoS One, 2010[133] Khan, Epid Inf, 2012[134] Khan, Bull WHO, 2006[109] Khan, JIDC, 2012[135] Owais, PIDJ, 2010[121] Siddiqui, IJID, 2006[113] Klugman, Vaccine, 1996[83] Lin, NEJM, 2001[114] Lin, AJTMH, 2000[89]	Africa Kenya 2 South Africa 1 Ghana 1 Egypt 3 Asia Pakistan 3 Bangladesh 2 India 5 Nepal 1 Indonesia 3 China 2 Vietnam 4 Uzbekistan 1 South America Chile 3 Haiti 1	

Table 1-5 Summary Figure of studies included in IHME, Lancet Infectious Diseases, 2019, including the date of publication, continents from which surveillance was performed and surveillance methods used.

This further illustrates the uncertainty around typhoid fever incidence and the global burden. This uncertainty presents several challenges. When considering the implementation of any intervention, either water and sanitation (WASH) improvements or vaccine introduction individual countries want relevant data to be able to make informed decisions. With the heterogeneity of disease between and even within countries, policy makers will require applicable data for their context. These systematic reviews clearly show there has been an increase in data points (**Error! Reference source not found.**) over the last thirty years, but with changing epidemiology further evidence of burden is still required.

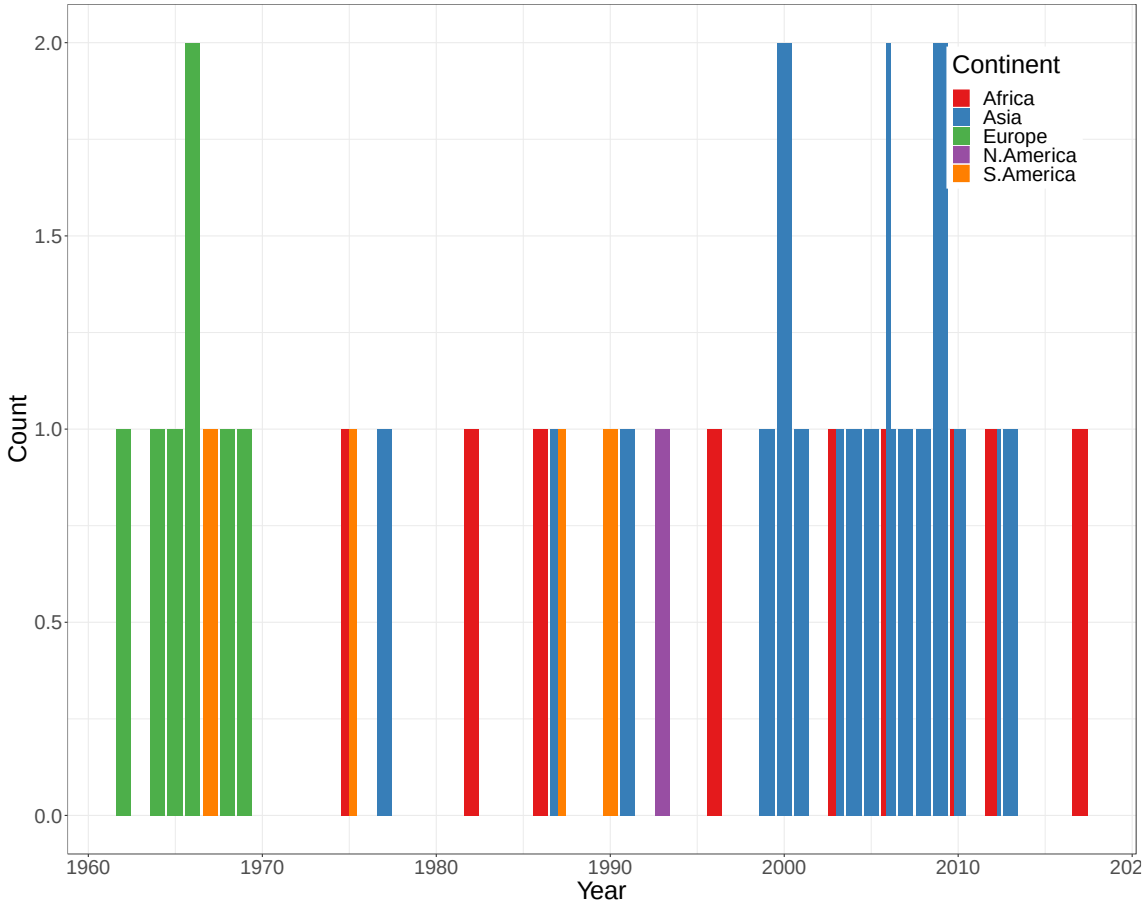


Figure 1-1: Number of burden studies conducted over time grouped by continent.

1.2.2. Antimicrobial Resistance

Whilst data on incidence is an important area of typhoid epidemiology, with increasing antimicrobial resistance (AMR), reporting on changing resistance patterns across endemic sites is also essential to fully understand the burden of disease and to inform antimicrobial prescribing guidelines and other intervention strategies. Chloramphenicol resistance was first reported in the 1952.[136] Following the common use of this antibiotic as first line treatment for enteric fever, epidemics of chloramphenicol-resistant *S.* Typhi were widely reported through the 1970s from wide-ranging geographical sites including Mexico,[137] Vietnam,[138] South Korea and Bangladesh. Following this, amoxicillin and co-trimoxazole resistance was documented, with increasing 'multi-drug' resistance (MDR) defined as resistance to all three antibiotics becoming common throughout South Asia.[139] More recently, MDR outbreaks have been documented throughout sub-Saharan Africa resulting in sharp increases in the number and severity of cases.[140] Indeed global typhoid is now largely dominated by a single MDR lineage, H58, originating in South Asia and spreading throughout the world.[141]

This propagation of MDR typhoid necessitated a switch to usage of fluroquinolones. However, increasing fluoroquinolone minimum inhibitory concentrations were documented throughout endemic sites following introduction, with resistance and treatment failure then seen.[142–146] This was demonstrated dramatically during a clinical trial in Kathmandu, Nepal, comparing the efficacy of a 2nd generation fluroquinolone gatifloxacin to intravenous ceftriaxone in the treatment of typhoid fever. The data safety and monitoring board (DSMB) stopped the trial early due to the emergence of high-level resistance to ciprofloxacin and gatifloxacin. At this stage in the trial, 26% of participants with blood-culture confirmed *S.* Typhi in the gatifloxacin arm had treatment failure, compared with only 7% in the ceftriaxone arm.[147] Interestingly, blood-culture negative enrolled participants had an improved clinical response (treatment failure 3% in gatifloxacin arm compared to 23% in the ceftriaxone arm) in the gatifloxacin arm, suggesting a common alternative aetiology, for example, scrub typhus as a cause of their fever.[148,149]

Multiple outbreaks of typhoid fever have shown the impact of a new resistance pattern of the pathogen being introduced into a population throughout both Asia and Africa.[140,150] Interestingly, in sites where the MDR drugs have been discontinued, there is a return to sensitivity with the pathogen although it has been noted that single administration of these drugs is likely to induce resistance once more.[151–153]

Depending on location current first line treatment for typhoid fever is oral azithromycin, an oral fluoroquinolone or an oral third-generation cephalosporin for uncomplicated, out-patient infection, with intravenous third-generation cephalosporin recommended for severe disease. Of great concern is the current outbreak of extensively drug-resistant (XDR) typhoid fever in Sindh, Pakistan.[33,154,155] In addition to MDR and fluroquinolone resistance, the bacteria has acquired cephalosporin resistance through the acquisition of a blaCTX-M-15 extended spectrum ß-lactamase gene.[32] There has already been evidence of international spread of this strain to both the UK and USA.[32,156] The typhoid conjugate vaccine has been introduced in to the area in attempt to reduce the spread of this highly resistant pathogen.

Infectious diseases such as tuberculosis, HIV and malaria have proven the benefits of combination treatment to prevent the development of resistance and improve cure rates. A limited number of trials have explored this approach with typhoid.[157,158] Evidence from a recent trial supports combining oral cefixime with oral azithromycin, resulting in a reduced fever clearance time (FCT) and duration of bacteraemia.[158] Whether these combinations result in prevention of resistance remains to be seen.

With escalating global AMR, salmonella is now on the WHO global priority list of antibiotic resistant bacteria for further research and the development of new antibiotics.[159] The impact of vaccine introduction may well therefore be greater than merely a reduction in the number of cases. The introduction of previous conjugate vaccines has been shown to have a positive impact on AMR[160–163] and it is hypothesised that TCVs could demonstrate a similar advantage.[164,165] With typhoid responsible for varying percentages of febrile illness in endemic sites, it acts as a driver of antibiotic

consumption both prescribed by healthcare workers, and acquired personally over-the-counter (OTC).[155,166] The increase in both number and use of private pharmacies, particularly in South Asia has been well documented.[167,168] As access to antimicrobials increases in low- and middle-income countries prevention of infection through vaccination is likely to reduce febrile illness and therefore the consumption of anti-microbials and the resulting development of AMR.[169,170] The Typhoid Vaccine Acceleration Consortium has antimicrobial usage as an exploratory endpoint in all three of its currently recruiting field trials to provide the primary data on TCVs and antibiotic consumption.[171–174]

1.3. Methods of Surveillance

Error! Reference source not found. demonstrates the steps of surveillance that can be performed for febrile illness and specifically typhoid fever in order to capture every individual infected with the pathogen and therefore gain some understanding on the burden of disease in a given community or country. The further down the pyramid the surveillance system operates the more surveillance infrastructure is required, but the more febrile illness and therefore typhoid fever the system will capture.

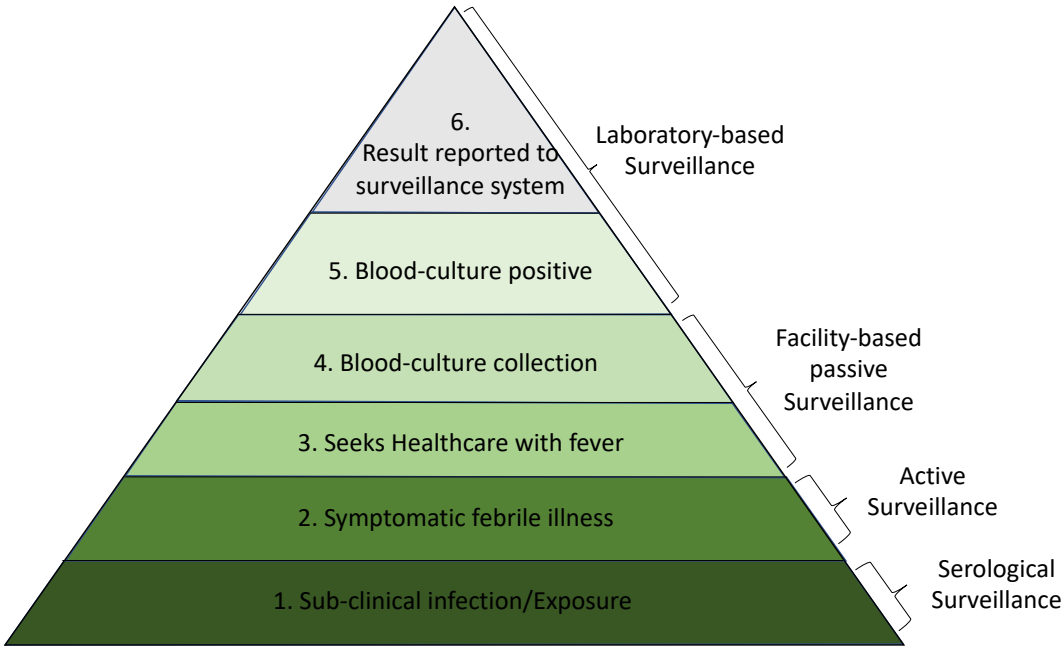


Figure 1-2 The surveillance pyramid for Typhoid Fever, adapted from Crump et al, Emerg Inf Dis, 2003[105]

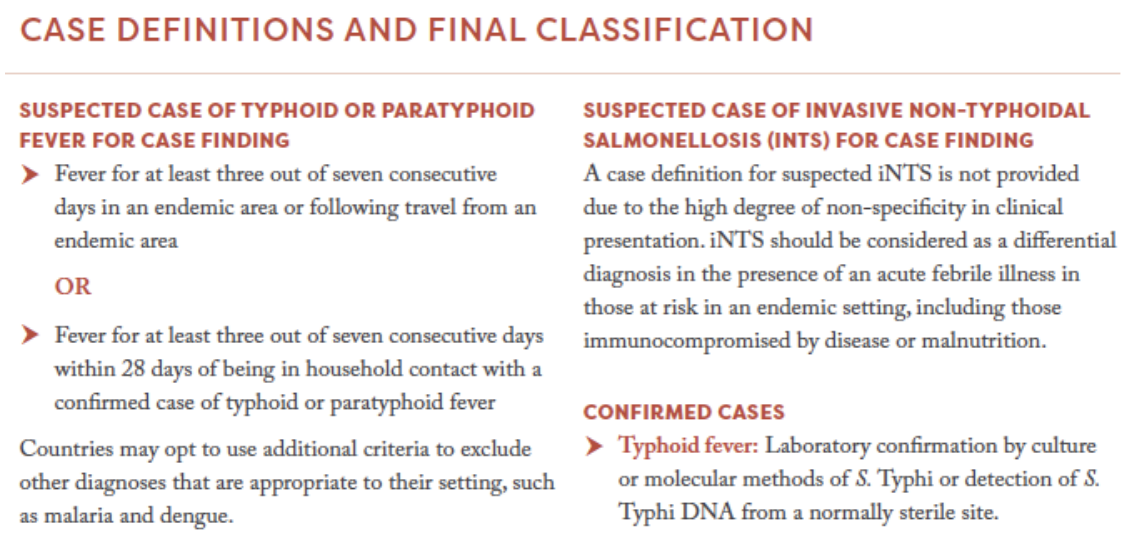
1.3.1. Laboratory-based Surveillance

Laboratory-based surveillance uses blood cultures collected at sentinel sites to report on the number of cases over time. This requires little clinical or research infrastructure and can be maintained with minimal financial and personnel costs. It also allows a country or region to monitor AMR trends longitudinally. As demonstrated by the pyramid however, it likely misses a large number of cases. It is difficult to determine the catchment area from which the number of cases originated, particularly if reference laboratories are utilised for a number of different healthcare facilities, making incidence calculations problematic and also making the monitoring of disease rates difficult as incidence could be reducing or there could be a shift in healthcare seeking behaviour. It can however be performed both retrospectively and prospectively providing useful historical data. The Surveillance of Enteric Fever in Asia Programme (SEAP) utilised this platform to report on enteric fever cases from three teaching hospitals in two cities in Pakistan. Over a three-year period, the study highlighted a reduction in MDR from 63% to 48% of the isolates with a small but important increase in ceftriaxone resistance (0.1%-0.3%). Positive isolates could also be linked back to in-patient records providing useful data on admission rates and complications.[146]

1.3.2. Facility-based Passive Surveillance

Facility-based passive surveillance requires healthcare staff within specifically identified facilities to prospectively identify individuals that meet pre-specified enrolment criteria and perform a blood culture during the clinical assessment. Further value can be added to this surveillance system if all eligible individuals attending the facility are recorded, regardless of blood-culture collection. The enrolment criteria set will affect the sensitivity and specificity of the surveillance. If the aim is to identify every case of typhoid fever presenting to the facility, a broad suspected case definition should be used as recommended in the latest WHO Vaccine Preventable Disease surveillance guidelines (WHO-VPD) for Typhoid (Error! Reference source not found.). This will require performing a lot of

blood cultures, particularly in the youngest children, as there are many alternative causes of fever in this age-group.[175] Adding additional criteria (e.g. clinical signs and symptoms) to make the culture of typhoid cases more likely may have the benefit of reducing the number of cultures, but it is likely that typhoid fever cases will be missed. Facility-based surveillance enables the recruitment of all eligible participants who attend the facility but relies on the population under surveillance using the facility. In areas with a high number of private healthcare facilities or pharmacies cases will be missed. In addition, facilities at different levels of healthcare are likely to recruit cases with different characteristics. Analysis of typhoid fever cases from Dhaka, Bangladesh, showed that surveillance at an in-patient facility recruited younger children, with more severe disease and higher rates of AMR compared to out-patient facilities.[176]



CASE DEFINITIONS AND FINAL CLASSIFICATION

SUSPECTED CASE OF TYPHOID OR PARATYPHOID FEVER FOR CASE FINDING

- Fever for at least three out of seven consecutive days in an endemic area or following travel from an endemic area

OR

- Fever for at least three out of seven consecutive days within 28 days of being in household contact with a confirmed case of typhoid or paratyphoid fever

Countries may opt to use additional criteria to exclude other diagnoses that are appropriate to their setting, such as malaria and dengue.

SUSPECTED CASE OF INVASIVE NON-TYPHOIDAL SALMONELLOSIS (INTS) FOR CASE FINDING

A case definition for suspected iNTS is not provided due to the high degree of non-specificity in clinical presentation. iNTS should be considered as a differential diagnosis in the presence of an acute febrile illness in those at risk in an endemic setting, including those immunocompromised by disease or malnutrition.

CONFIRMED CASES

- Typhoid fever: Laboratory confirmation by culture or molecular methods of *S.* Typhi or detection of *S.* Typhi DNA from a normally sterile site.

Figure1-3: Case definitions for suspected and confirmed typhoid fever. Taken from WHO Vaccine Preventable Disease Surveillance Guidelines for Typhoid Fever.[23]

To improve the capture of the maximum number of typhoid cases, healthcare utilisation surveys can be performed prior to the surveillance programme taking place, to ensure the most frequented facilities are chosen. Alternatively, these data can be used to make adjustments to the final estimated case numbers, adjusting the incidence estimates based on the proportion of individuals from within the population that use the facilities from which surveillance was performed, assuming the incidence

of disease is equal for all health facilities frequented.[177] This is a hybridised system of surveillance, and whilst uncertainty is introduced to the final estimate the resources required for set-up and long term running are less when compared with an active demographic health surveillance system (DHS).[178] Examples of this approach have been shown in the DOMI, SEAP and TSAP programmes[103,131,178,179] but also in Dhaka, Bangladesh, where an already active WHO-VPD programme for respiratory pathogens was in operation. Enrolment criteria were broadened to include a recorded temperature at enrolment or history of fever for >3days. For a modest increase in funding (27% of the total budget), an additional 1,699 blood cultures were collected with 349 enteric fever cases isolated.[180] This approach could be utilised in many low- and middle-income countries to provide missing data on burden to inform vaccine introduction decisions.

1.3.3. Active Surveillance

Active Surveillance usually occurs within a pre-enumerated demographic census. Households within that census are visited at regular timepoints by community health workers and interviewed regarding signs or symptoms for typhoid infection. If a febrile individual is identified they are encouraged to attend a health facility for blood culture collection. This has the benefit that any symptomatic individual will be captured, and a blood culture obtained. As the surveillance system is interrupting normal healthcare seeking behaviour and individuals are potentially identified earlier in the disease process, less severe cases will be identified. This is an important consideration for vaccine introduction and the cost-effectiveness of any vaccine, as the prevention of severe disease and hospitalisation are an important factor in the economic consideration for any vaccine.[30,181] Examples of active surveillance for typhoid fever was previously published data from two sites in Kenya[116] and the currently operational Surveillance of Enteric Fever in India Programme.[182]

1.3.4. Serological Surveillance

The very base of the pyramid are episodes of infection which may be sub-clinical or where the individual does not seek healthcare at all, which would not be captured by any of the surveillance

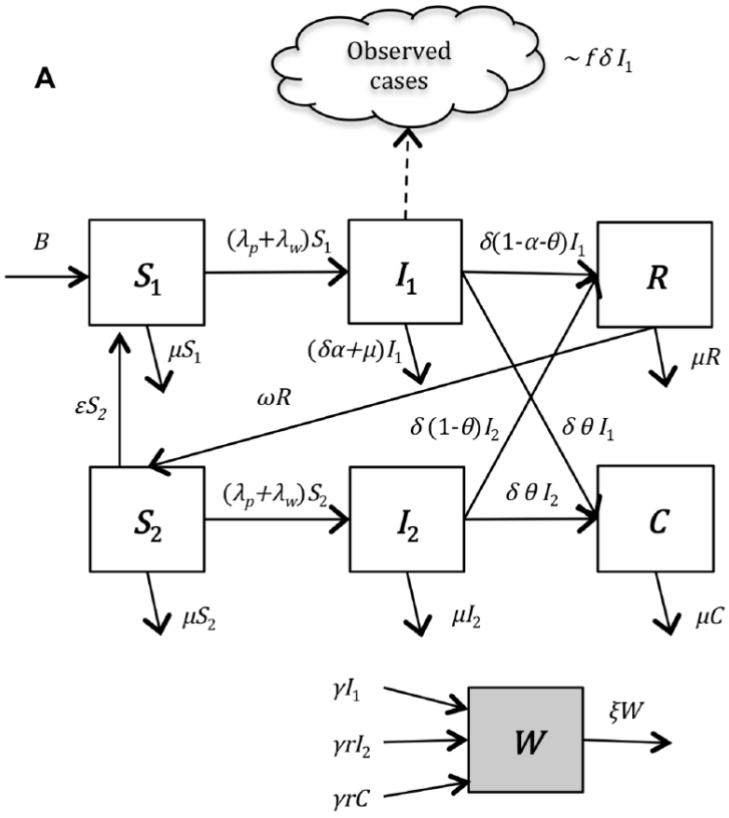
systems on the higher steps. These episodes may not be important from the perspective of severe disease or even antibiotic use, but exposure events would still lead to pathogen shedding in the stool and therefore contribution to transmission which a vaccine would potentially help to interrupt.[183] Serological surveillance to identify episodes of seroconversion has been studied for non-typhoidal salmonellae[184,185] and utilised in other areas of infectious disease surveillance[186–188] but is relatively underused in typhoid epidemiology. Whilst typhoid does lead to an antibody response, it does not lead to a reliably sustained response, or one for which a definite correlate of protection could be set to predict protection against future infection.[189]

Recent serological surveillance from Fiji using the anti-Vi IgG (Vi capsular polysaccharide; virulence factor) antibody estimated a seroprevalence of infection of 32%, one to two orders of magnitude higher than would be predicted from acute disease surveillance.

1.4. WHO Recommendations

The latest WHO-VPD guidelines were updated in 2018, and included typhoid fever for the first time.[23] In addition to setting case definitions and outlining surveillance systems that have already been discussed, there was also guidance to countries on surveillance performance indicators, along with special considerations of typhoid surveillance including carriage, outbreaks and humanitarian emergencies.

1.5. Data Gaps identified through a mathematical model



Fixed parameters			
Parameter definition	**Symbol**	**Value**	**Source**
Birth rate	B	14.9–19.2 live births per 1,000	Census data
Natural mortality rate	μ	7.2–8.2 deaths per 1,000	Census data
Duration of infectiousness	$1/\delta$	4 weeks	[36]
Fraction infected who become chronic carriers	θ	0.003–0.101 depending on age	[33]
Disease-induced mortality	α	0.001	Assumption, [1]
Duration of temporary full immunity to infection	$1/\omega$	104 weeks	Assumption*, [30]
Rate of shedding into the water supply	γ	1 infectious unit/week	Assumption†
Rate of decay of infectious particles from water supply	ξ	1/3 week^{-1}	[37]
Estimated parameters			
Parameter definition	**Symbol**	**Estimate**	
Basic reproductive number for short-cycle transmission	$R_{0,p}$	2.49	
Basic reproductive number for long-cycle transmission	$R_{0,w}$	0.28	
Amplitude of seasonal forcing (long-cycle transmission)	q	0.69	
Seasonal offset parameter (timing of seasonal peak)	ϕ	18.2 weeks	
Rate of waning immunity to clinical disease	ε	2.68×10^{-11}	
Relative infectiousness of chronic and short-term carriers	r	0.01	
Reporting fraction	f	0.0052	

Figure1-4: Salmonella *Typhi transmission dynamics from Pitzer et al.*[51] *Licensed under CC BY 4.0*[190]

Pitzer et al developed a susceptible-infected-recovered mathematical model to explore the transmission dynamics of typhoid fever and model the impact of typhoid conjugate vaccines dependent on certain variables. The model identified numerous data gaps which exist in typhoid epidemiology and added to the uncertainty of the model.

1.5.1. Age-stratified Incidence of Disease

Within the model, there is a susceptible population with no immunity to typhoid infection. At a given rate, individuals from within this population are infected, and a proportion of these are counted as observed cases. In different epidemiological contexts the peak age of infection changes dependent on the total force of infection, with a lower average age of disease in sites with a higher incidence.[25] Infection with *S.* Typhi has often been described as a disease predominantly of school aged children, and this understanding has had an impact on vaccine introduction campaigns in multiple countries.[191,192] However because diagnosis relies on blood-culture there are reasons why children under two years of age particularly may have an underestimated disease incidence. Firstly, the incidence and proportion of other infections, particularly viral infections, causing a similar presentation to typhoid fever are higher, meaning physicians may be reluctant to order blood-culture in cases of undifferentiated fever.[34] Secondly, venepuncture can be challenging in younger children with collection of adequate blood volumes limited and a higher rate of contamination.[11,193] More recently however, there has been increased evidence demonstrating high rates of disease in children under 2 years of age.[11,194] In the WHO Strategic Advisory Group of Experts meeting in October 2017, data on the incidence of disease in children less than 5 years of age was reported by combining data from previously published studies and unpublished data from four large current surveillance studies (Strategic Typhoid Alliance across Africa and Asia Programme (STRATAA),[195] Severe Typhoid in Africa Programme (SETA),[196] Surveillance of Enteric Fever in Asia Programme (SEAP)[197] and the Navi Mumbai TCV Introduction Programme (**Error! Reference source not found.**).[198]

Figure 3. Age distribution in children <2 years
Frequency plots of aggregate age distribution data by month for *S.* Typhi in children <5 years of age.

Black bars represent cases where age in months is known and gray bars represent the cases where age is known in years evenly distributed across the year [33].

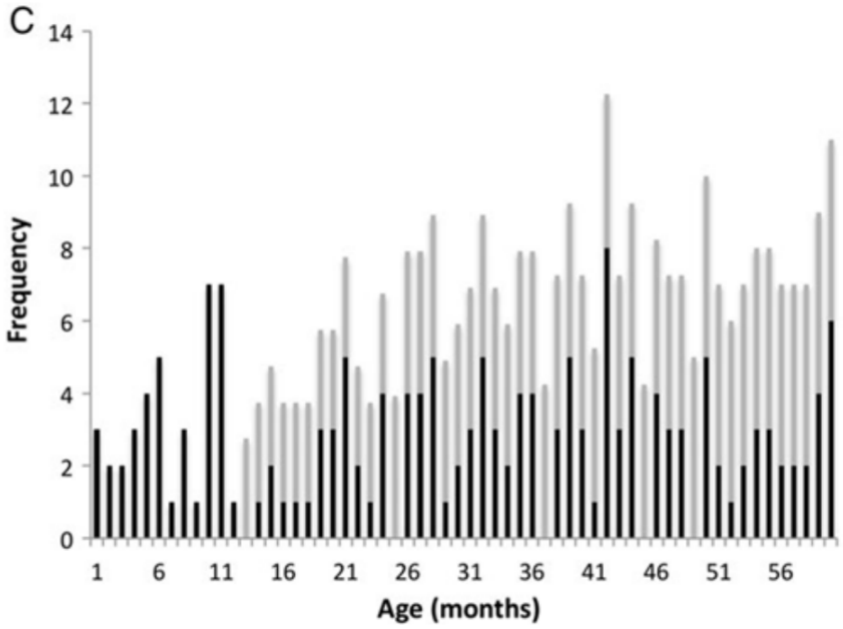


Figure1-5: Data compiled by John Crump for the Background Paper on Typhoid Vaccines for SAGE Meeting (October 2017)[199]

From studies that recruited patients of all ages, 27% of typhoid fever episodes occurred in children under 5 years of age. Of those episodes under 5 years, 29.7% occurred in children under 2 years of age, 9.9% under 1 and 2.9% under 6 months. This has important implications for vaccine programming. A reanalysis of the TSAP data showed no cases of *S.* Typhi in children under 9 months of age, with highest incidence in the 4th year of life with the conclusion that an efficacious vaccine introduced at the 9month EPI visit would have the potential to prevent all cases of disease.[200]

1.5.2. Prevalence of Carriage

Within the model a proportion of infected individuals progress to become chronic carriers of typhoid infection. The prevalence of chronic carriers and their contribution to transmission was highlighted as an important factor for the indirect protection a typhoid vaccine may confer. It was hypothesized that where the prevalence of carriers was higher, the overall protection afforded by a vaccine would be reduced. Any individual who was already in the carrier state, would not be afforded any protection from the vaccine, they would therefore continue to shed the pathogen into their environment,

contributing to ongoing transmission, and placing non-vaccinated individuals at risk of infection. Further evidence from controlled human infection (CHIM) studies that typhoid vaccination does have an impact on reducing stool shedding would add weight to this assumption.[183] The model was validated using data from two cluster randomised Vi-PS vaccine trials in Karachi, Pakistan and Kolkata, India where contrasting evidence for the indirect protection afforded by the vaccine was shown, with an overall protection of 26 % (95% CI: -21%,55%) in Karachi, and 57% in Kolkata (95% CI: 37%, 71%). Unfortunately, these two trials were not designed in the same way, with a much higher proportion of the total population vaccinated in the Kolkata trial due to vaccination of adults as well as children. This may be a more likely explanation for the differences in indirect effects seen compared to the role of carriers.

1.5.3. Shedding and Transmission

In addition to the shedding of chronic carriers, the model also contains shedding from acute cases, with person to person spread within the immediate environment, labelled as short cycle transmission. Long cycle transmission is where bacterial contamination of water supplies and the wider environment occurs and puts individuals at risk of infection. It is not known what contribution each makes to overall ongoing transmission, and indeed may differ depending on epidemiological context. Data from Kathmandu suggest contaminated water plays a predominant role in exposure to *S.* Typhi as a range of genotypes existed within geographical clusters of disease.[201]

1.6. *Salmonella* Typhi Vaccines

Understanding typhoid epidemiology in different global contexts, and recommending intervention with vaccine for certain age groups, relies on the existence of a safe and efficacious vaccine. Typhoid vaccines have been in development for over 100 years but have never been introduced into endemic countries on a global scale or for a prolonged period of time.

1.6.1. Whole-cell Vaccine

Development of enteric fever vaccines started in the late 19th century with the first heat-killed vaccines.[92] In the 1950-60s, whole cell vaccines (WCV) went through a number of efficacy trials with a meta-analysis suggesting a cumulative 3-year efficacy of 73%.[202] One WCV was introduced into the extended programme of immunisation (EPI) in Thailand for a period of 8 years following an epidemic of typhoid; blood-culture positive cases dropped from 880 to 54 annually within Bangkok over that time period.[203] Unfortunately, this vaccine caused high rates of systemic and localised side effects, leading to school absenteeism in children.[204]

1.6.2. Ty21a

In the 1970-80s, two further vaccines with moderate efficacy and an improved side-effect profile were developed. Ty21a is a live, oral, attenuated vaccine, which following promising efficacy results (87% after 5-8 doses) in the Maryland CHIM[205] went on to large-scale field trials in Egypt, Chile and Indonesia. Meta-analysis of these trials demonstrated a cumulative efficacy of 50% at 3 years.[202,206] Analysis from these trials, also demonstrated an estimated protective efficacy against *S.* Paratyphi B of 45%(95% CI: 8%, 73%).[207] Ty21a is not licensed for use in children <5 years of age due to the formulation of the vaccine in a capsule causing difficulty in administration for children. Despite this, Ty21a is recommended for use by the WHO in the appropriate age groups and is being used as a control measure in Samoa for individuals over 50-years of age in the Samoa Typhoid Fever Control Programme (Presentation at Coalition Against Typhoid Conference 2019, Prof Myron Levine).

1.6.3. Vi-PS

Vi-polysaccharide (Vi-PS) is a parenteral vaccine containing the purified capsular Vi-polysaccharide antigen. A number of Vi-PS vaccines are available either as a single vaccine (for example, Typhim Vi, Sanofi Pasteur; Typherix, GSK; Typbar Vi, Bharat Biotech; Typho Vi, BioMed; Vax-tyVi, Finlay Institute) or in combination with Hepatitis A (Sanofi Pasteur ViVaxim and ViATIM, GSK Hepatyrix), but only the Sanofi Pasteur Typhim Vi has WHO pre-qualification.[204] Combining results from individually and cluster randomised trials, efficacy at 2 years for Vi-PS was 59%[206]. Vi-PS provides no cross-protection

for *S.* Paratyphi A and B, as these pathogens do not express Vi-polysaccharide. Vi-PS is not licensed in children <2 years of age due to poor immunogenicity. Since the vaccine contains a T-cell-independent antigen, it does not induce immunological memory, and cannot be boosted with repeated vaccination.[208]

1.6.4. Vi Vaccines

However, conjugate vaccines, where the polysaccharide capsule is chemically conjugated to a protein carrier, do produce a T-cell-dependent response. Such vaccines have demonstrated high levels of protection for other pathogens, including Haemophilus influenzae type b (Hib), pneumococcal and meningococcal vaccines[209–211]. This technology has now been applied to both the Vi-antigen, with a number of TCVs in various stages of development and the O-specific polysaccharide from *S.* Paratyphi A.

1.6.5. Vi-rEPA

Conjugation of Vi-polysaccharide to a recombinant exoprotein A from *Pseudomonas aerugionosa* (Vi-rEPA) was undertaken by the National Institutes of Health to produce the first Vi-conjugate vaccine. Safety and immunogenicity was demonstrated in adults in the US, and both adults and children in Vietnam[212,213] with significantly higher anti-Vi IgG induced by Vi-rEPA compared with Vi-PS. Efficacy was then shown in Vietnamese children between 2-5 years of age with a protective effect of 91.5% at 27 months and 89% at 47 months.[90,128] Vi-rEPA is being further developed and evaluated by Lanzhou Insititue of Biological Products in China.[214]

1.6.6. Vi-DT

Conjugation of the Vi-polysaccharide to a diptheria toxoid carrier protein (Vi-DT), has been pioneered by the International Vaccine Institute (Seoul, Korea). Vi-DT has undergone phase 1 studies and further evaluation in the Philippines, which showed Vi-DT to be safe, well-tolerated and immunogenic in participants aged 2-45 years.[215] Further development is being undertaken by SK Bioscience/Bio Farma.[216]

1.6.7. Vi-CRM_{197}

Conjugation of Vi polysaccharide to a CRM197 protein, purified from *Citrobacter freundii*, was undertaken by Novartis Vaccines (now GSK vaccines) Institute for Global Health. Phase 2 immunogenicity studies have been conducted in European adults, and adults and children from India, Pakistan and the Philipines.[217,218] These demonstrated that one dose of Vi-CRM_{197} induced high levels of anti-Vi IgG in age groups down to 9 months, but that a second dose two months later did not induce a boosting of titre. Children in the 6-8-week cohort had a lower immune response compared to older children.[218] Further development is being undertaken following technology transfer to Biological-E.

1.6.8. Vi-TT

Conjugation of the Vi-polysaccharide to tetanus toxoid protein (Vi-TT) has been utilised in two separate TCVs. PedaTyph manufactured by Bio-Med is licensed in India and has been trialled in a phase 4 school-based cluster randomized study. Administered to children aged 6 months through 12 years in a two-dose schedule 6 weeks apart, a vaccine efficacy of 100% (95% CI 97.6-100%) was reported, with no typhoid cases reported among 905 vaccinated children and 11 (1.2%) among 860 unvaccinated children at 1 year of follow-up; however, few children <2 years of age were recruited.[219] Typbar-TCV (Bharat Biotech, Hyderabad, India) is now licensed for use in a number of countries and received WHO pre-qualification in 2018.[220] Phase 2 studies in India demonstrated the vaccine to be safe and immunogenic in participants aged 2-45 years. The Oxford CHIM evaluated the protective efficacy (PE) of the Typbar-TCV Vi-TT. Within this study, 112 healthy participants were enrolled, receiving either Vi-TT, Vi-PS, or a control meningococcal vaccine in a 1:1:1 double-blind, randomisation. One month following vaccination, participants received oral challenge with *S.* Typhi bacteria, and were followed up daily with temperature recording and blood-culture collection. The PE of Vi-TT was 54.6%, which was comparable to PE of Vi-PS (52%). However, with a clinical case definition of fever followed by bacteraemia, PE of Vi-TT increased to 87.1%.[221]

Tybar-TCV has now achieved WHO pre-qualification,[220] with a WHO SAGE recommendation for use of TCVs in countries with high burden of disease or high rates of antimicrobial resistance.[199] There is a commitment from Gavi to fund the introduction of TCVs into eligible countries, with field trials providing data to inform this implementation.[171,222,223]

Vaccine		Vi-rEPA		Vi-DT		Vi-CRM$_{197}$		Vi-TT (PedaTyph)	Vi-TT (Tybar-TCV)	O:2-TT		
Pathogen		*S.* Typhi		*S.* Typhi		*S.* Typhi		*S.* Typhi	*S.* Typhi	*S.* Paratyphi A		
Carrier Protein		Recombinant *Pseudomonas aerugionosa* exoprotein A		Diphtheria Toxoid carrier protein		Diphtheria toxin CRM$_{197}$ carrier protein		Tetanus Toxoid	Tetanus Toxoid	Tetanus Toxoid		
Clinical Trials (n=14)	Safety & Immunogenicity	1. 18-44years of age, USA[213] 2. 2-44years of age, Vietnam[212]		1. 2-45years of age, Philippines[215] 2. 2-40years of age, Indonesia[216]		1. 18-40years of age, Belgium[217] 2. 6weeks to 45years of age, Pakistan, India and Philippines[218]		1. 3months to 5years of age, India[224,225] 2. 6months to 12years of age, India[219]	1. 6months to 45years of age, India[226] 2. 18-60years of age, UK[221]	1. 2-44years of age, Vietnam[227]		
	Efficacy	1. 2-5years of age, Vietnam. Protective Efficacy 91.5% at 27months, 89% at 47months[90,128]		None		None		1. 6months to 12years of age, India. Protective efficacy 100%*(95% CI: 97.6%, 100%)[219]	1. 18-60years of age, UK. Protective efficacy 54.6-87.1%[221]	None		
Developer		NIH, United States	LIBP, China	IVI, Seoul, Korea	SK Bioscience/Bio Farma, Korea	GSK Vaccines for Global Health, Italy	Biological E, India	Bio Med, India	Bharat Biotech, India	NIH, United States	Changchun Institute of Biological Products	LIBP, China
Licensed								India	India, Nepal, Nigeria, Cambodia, WHO Pre-qualification			

Table 1-6: Summary table for typhoid vaccines in late stage clinical development. Abbreviations – NIH, National Institutes of Health; IVI, International Vaccine Institute; LIBP, Lanzhou Institute of Biological Products

1.7. Summary

The global control of enteric fever relies in part on an accurate understanding of the global burden of disease. Burden here includes the incidence of disease but also rates of antimicrobial resistance, complications of disease and hospitalisation. Population-based studies to estimate this burden of acute disease are limited. As has been highlighted there are also a number of surveillance designs which may provide different estimates, and no typhoid surveillance study to date has combined both clinical surveillance with serosurveillance to investigate incidence. Whilst data for acute burden is limited, studies providing estimates of chronic carriage and their relative contribution towards typhoid transmission compared with acute disease are even fewer.

These data are important for individual countries to understand what the burden of infection might be within their country to inform decisions on vaccine introduction, but also global policy makers to prioritise funding and country engagement. More recent data points for surveillance also improve the accuracy of extrapolation of disease into countries which currently do not have their own surveillance data. An accurate understanding on age of disease from different epidemiological contexts is also important for vaccine programmes.

In this thesis, I will present results from a multi-site, multi-component study aiming to bring together a comprehensive surveillance system to answer some of the key questions and data gaps surrounding typhoid epidemiology.

1.8. Thesis Rationale and Aims

Over 14 million cases of febrile illness and 135,000 deaths are attributed to enteric fever each year.[104] Predominantly caused by *Salmonella enterica* serovars Typhi (*S*. Typhi) and Paratyphi A, the current burden of enteric fever occurs in low- and middle-income countries in Asia and Africa.[228] Understanding global burden of disease is hampered by the lack of population-based epidemiological surveillance studies throughout Asia and Africa. Surveillance for enteric fever is problematic. As discussed in this chapter blood-culture is the only reliable diagnostic and is estimated to be around

60% sensitive, clinical disease is non-specific and has many parallels with other febrile diseases and within endemic countries, there are multiple healthcare providers at which to seek care.[175,193] In addition, it is estimated that 2-5% of acute infections progress to chronic carriage but due to the asymptomatic nature of this condition, identifying these individuals and understanding their role in ongoing transmission is problematic.[51]

The burden of disease attributable to symptomatic enteric fever, sub-clinical infection and the prevalence of chronic carriage in endemic settings have not previously been surveyed within the same populations. These important data from different epidemiological contexts could be used to develop vaccination strategies and define the likely impact of these strategies on the ongoing high rates of disease occurring in endemic urban areas. Identification of high incidence rates in children <5yrs, for example, could support vaccine introduction as part of the EPI schedule.

1.8.1. Strategic Typhoid Alliance across Africa and Asia

The Strategic Typhoid alliance across Africa and Asia (STRATAA) study brought together three international field sites in typhoid endemic urban centres, with other laboratories and research institutes across the world. The STRATAA study was designed to address some key questions regarding the burden of enteric fever and *Salmonella* exposure in endemic regions, the mechanisms of susceptibility and infection transmission.[195] The objectives for this thesis were nested within this study and are outlined below;

	Aims	Outcome Measures
Primary	To characterise the burden of enteric fever at three urban sites in Africa and Asia	1. Observed incidence rates of blood-culture confirmed enteric fever from facility-based passive surveillance. 2. Adjusted incidence rates of infection accounting for blood culture sensitivity, enrolment proportion and healthcare seeking behaviour. 3. Age-stratified incidence of clinical disease. 4. Seasonality of blood-culture confirmed infection. 5. Reporting fraction of typhoid to surveillance facilities through healthcare utilisation surveys. 6. Prevalence of typhoid chronic carriage determined through serological surveillance 7. Incidence of non-clinical typhoid infection determined through serological surveillance 8. Proportion of isolates from culture confirmed index cases demonstrating antibiotic resistance
Secondary	To characterise the transmission of enteric fever in endemic sites	9. Rates of stool shedding from acute, convalescent and chronic typhoid states
	To characterise the demographic and WASH based risk factors present within different epidemiological contexts	10. Description of sites through repeat demographic census 11. Description of economic and WASH risk factors through household surveys
	To characterise the healthcare seeking behaviour of individuals	12. Description of healthcare seeking behaviour through household surveys

Table 1-7: Thesis Aims and Outcome measures

2. Chapter 2 Methods

2.1. Introduction

In the following chapter is a description of the design and methodology for three parallel studies which began in May 2016 with field work finishing in December 2018. Each section of the study is described below beginning with the choice of field sites. Within these field sites a detailed demographic census of approximately 100,000 individuals was undertaken in each of the three field sites This census was updated at different time points through a two-year period to characterise migration, with all sites completing a close out census at the end of the two years. Secondly, in healthcare facilities utilised by the census population (as confirmed through healthcare utilisation surveys) prospective facility-based passive surveillance to detect cases of enteric fever has been performed, enrolling eligible febrile individuals from within the demographic census area with blood-culture collection. Thirdly, a serological surveillance study was conducted in an age-stratified sample of the census population at each site, collecting blood samples at intervals of approximately three months in order to estimate the rate of seroconversion to typhoidal *Salmonella* antigens and hence the rate of non-clinical infection in the population. In addition, serological surveillance was used to identify the prevalence of typhoid chronic carriage within these populations by identifying individuals with a high antibody titre and following up with stool culture looking for evidence of *S.* Typhi shedding. Fourthly, to understand the healthcare seeking behaviour of the populations, to describe the economic and WASH based risk factors within the population, and to enable the adjustment of crude incidence rates two healthcare utilisation surveys were performed in a subset of households. Fifthly, following the identification of both blood-culture confirmed index cases through passive surveillance and chronic carriers through serological surveillance, household contacts were followed up for evidence of infection.

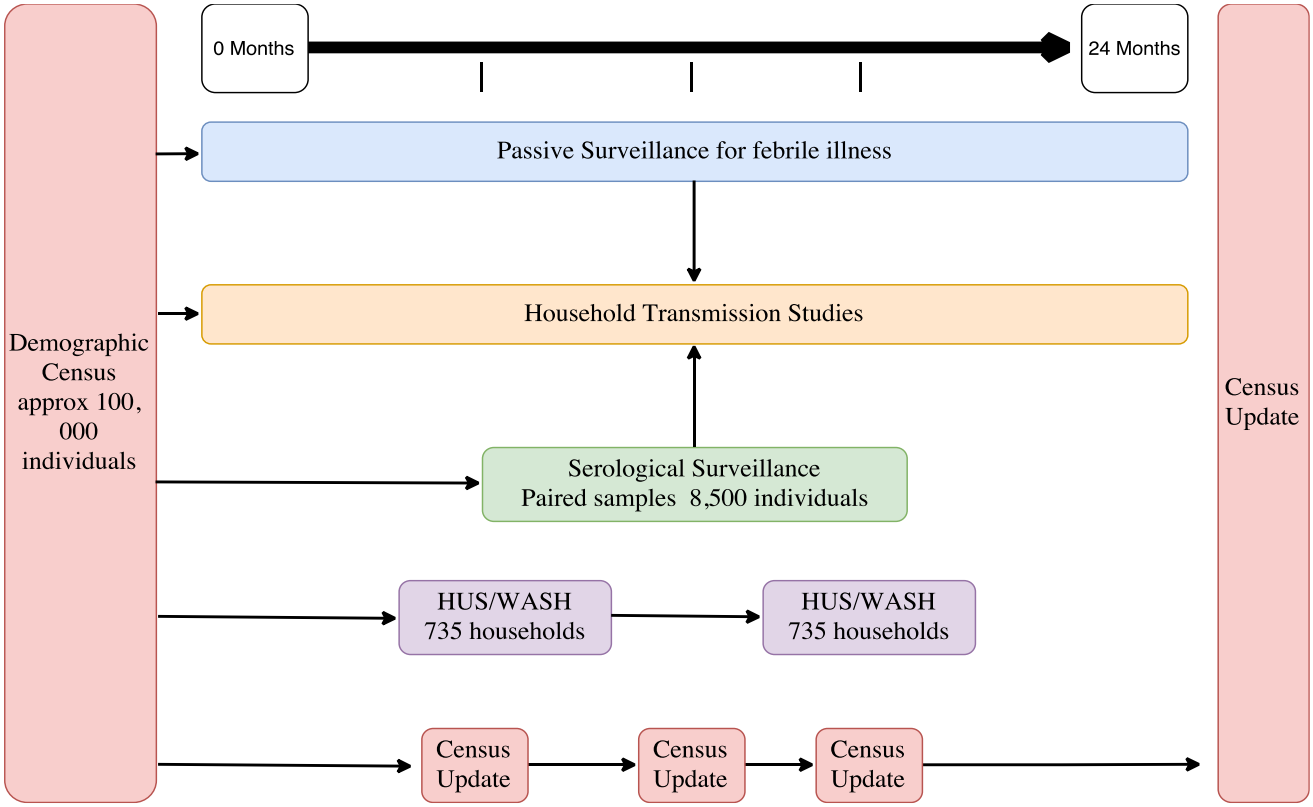


Figure2-1: Overview of the STRATAA Study

2.2. Study Sites

With known high incidence of enteric fever throughout South and Southeast Asia and a recent increase in reported cases from sub-Saharan Africa three sites were selected from across Africa and Asia based on known high rates of enteric fever and the research capacity to deliver a study of this size and logistical complexity. The three sites differ in their epidemiological profiles and history of enteric fever incidence.

Figure2-2: Study sites for the STRATAA study

2.2.1. Ndirande, Blantyre, Malawi

Ndirande is a large urban township on the outskirts of Blantyre city, Malawi, 6km from the main referral hospital. It has a young population of around 100,000 people spread over 6.77km^2. It is serviced by one health clinic staffed by clinical officers. It has high reported rates of typhoid fever along with a population HIV prevalence of around 18%. Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi, is the government-funded hospital for Blantyre district, serving a local population of 1.3 million persons and provides tertiary care to southern Malawi. Non-typhoidal salmonellae have previously been the commonest cause of invasive bloodstream infections in Blantyre but since 2011 there has been a rapid increase in the number of *S.* Typhi cases seen at QECH, from approximately 14/year between 1998 and 2010 to 782 in 2014.[194] Much of this increase has been due to the

emergence of the H58 clone and is associated with an MDR phenotype.[229] This outbreak appears unrelated to HIV-infection but has resulted in high rates of mortality (2.5% adult and paediatric) despite the availability of fluoroquinolone antibiotics. In this setting, enteric fever is seasonal, with the peak number of cases seen at the end of the wet season and during the early dry season when the prevalence of malnutrition is also highest.

2.2.2. Patan, Kathmandu, Nepal

Patan is located within the Lalitpur Sub-Metropolitan City within the Kathmandu Valley, Nepal. The population is generally poor, with most living in overcrowded conditions and obtaining their water from stone spouts or sunken wells. Patan Hospital is a 318-bed government hospital providing emergency and elective outpatient and inpatient services to this area. The local catchment population of the hospital is approximately 200,000 people in about 20 km^2, with a population density of 8,000/km^2; there is a high rate of immigration for employment, particularly young males from rural areas.[230] Enteric fever is frequently managed in the outpatient clinic at Patan Hospital, which has approximately 200,000 outpatient visits annually. Approximately 400 culture confirmed cases of enteric fever are diagnosed at Patan Hospital each year, with a peak during the monsoon months.[151] Emergence of fluoroquinolone resistant *S.* Typhi isolates has been recently identified, whereas MDR strains are now rare.[147]

2.2.3. Mirpur, Dhaka, Bangladesh

Mirpur is an area located within Dhaka Metropolitan area, Bangladesh, situated 7km north-west of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) main campus. The icddr,b manages two hospitals in Dhaka city, the central diarrhoeal hospital and another in the Mirpur area known as MTC (Mirpur Treatment Centre) comprising a 50-bed inpatient facility. In addition, they run a well-attended outpatient facility, the Mirpur Field Clinic As part of this programme, these and other health facilities (Error! Reference source not found.) will be kept under surveillance for enteric fever. The total catchment area for the field site for the STRATAA study is 10.79 km^2 with a

total population of about 603,658 at a density of 55946/km^2. Approximately 98% of residents have access to tap water supplied by the municipality while the remainder use wells, hand pumps and other sources such as ponds and rivers in the study area.

Previous data from blood culture surveillance revealed a high incidence of disease within Dhaka, with the burden particularly high in children under 5 years of age.[116,120,231] Typhoid fever is reported throughout the year but peaks during the monsoon season.[132] Of note, 15% of *S*. Typhi isolates are multi-drug resistant (MDR) and around 97% isolates are resistant to nalidixic acid; extended-spectrum beta-lactamase (ESBL) producing organisms have also been isolated from enteric fever patients in this setting.[232]

2.3. Demographic Census

In order to accurately calculate an incidence rate for typhoid fever from each of the study sites, a demographic census was performed at baseline and repeated at two years. The objective of the census is to identify and characterise the source population corresponding to the catchment areas for the passive surveillance sites described below and estimate the person-time under surveillance. Census data was updated with births, deaths and migrations twice in Dhaka at intervals of six months, once in Patan at 12 months, and at two years in Blantyre.

Site / Component	Malawi		Nepal			Bangladesh			
Census	Baseline	Final	Baseline	Update 1	Final	Baseline	Update 1	Update 2	Final
Survey period	04 Jul 2016 - 18 Oct 2016	7 Jun 2018 – 25 Sep 2018	2 Jun 2016 - 29 Jun 2016	27 Mar 2017 - 27 Jul 2017	22 May 2018 - 31 Aug 2018	1 Jun 2016 - 31 Aug 2016	18 Mar 2017 - 16 Aug 2017	22 Oct 2017 - 11 Jan 2018	7 Jun 2018 - 25 Sep 2018

Table 2-1: Dates of Demographic Census and Census Updates for the three sites

The number of participants for the demographic census survey in each site needed to produce a two-sided 95% confidence interval with a precision (half-width) of 50% for the anticipated typhoid incidence rate detected through facility-based passive surveillance is demonstrated below. The necessary catchment population size is driven by the size required to estimate the expected incidence

rate in the 0-4year-old age group, which was estimated to be approximately 10% of the total population in each site. Each site, therefore required at least 101, 250 persons total in the target population.

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

Table 2-2: Sample size required for the target populations in the three sites to estimate annual, blood culture confirmed typhoid incidence in passive surveillance

The census was performed within a demarcated geographic area that is a known catchment population for the surveillance sites. In total, approximately 100,000 individuals were enumerated from ≥20,000 households. The head/key informant within the household provided written informed consent to take part in the study. Information on all residents within the household at the time of the census were collected from the head of the household/key informant. A household was defined as individuals living in the same dwelling or compound and sharing food from the same kitchen. A household member is considered to have migrated out if s/he has left the household and does not intend to come back within six months of the time s/he left. A person is considered to have migrated in if s/he was not previously included in the list of household members and intends to live in the household for the next six months. At enrolment, the head of each household was made aware of the passive surveillance component of the study and encouraged to use the field-site facilities capturing acute febrile illnesses, including acute enteric fever cases. The characterised census population formed the sampling frame for the other components of the study (passive surveillance, healthcare utilisation/WASH surveys and serosurvey).

Household ID	|__|__|__|__|__|__|__|__|__|__|__|			
GIS ID/tablet ID	|__|__|__|__|__|__|__|__|__|__|__|			
Address				
Visit Date	*DD/MM/YYYY*	Interviewer ID/initials	|__|__|__|	
Household occupied	Yes	No (*Return at later time/date*)	Vacant (*no further involvement*)	
Study Information provided to respondent?	Yes No	Consent given	Yes	No (*no further questions*)
Respondent code *(relationship to head of household)*	1. Head of household	2. Spouse of head	3. Son/daughter of head	
	4. Spouse of son/daughter of head	5. Sibling of head	6. Spouse of sibling of head	
	7. Mother/Father of head	8. Mother/father in law of head	9. Grandchild of head	
	10. Niece/nephew of head	11. Other – please specify		

Member number	Member first name	Member surname	Member s phone number	Ethnicity / Tribe	Sex M/F	DoB DD/MM/YYYY	Age (yrs and months)	Relationship to HH Head	Mothers name if <16 years	Fathers name if <16 years	Marital status	Spouse name	Adult: > 16 number of years education received?	Child <16: Attends school?	Employment status
01				Provided by Sites	1. M 2. F						1. Single 2. Married 3. Separated 4. Divorced 5. Widow/er		2. No education 3. Some Primary 4. Finished Primary 5. Some Secondary 6. Finished Secondary 7. Technical college 8. University 9. Postgraduate	1. Yes 2. No	1. Unemployed 2. Student 3. Unpaid family worker 4. Paid domestic worker 5. Paid employee 6. Self employed 7. Other

Table 2-3: Household level census case record form

Table 2-4: Member level census case record form

2.4. Passive surveillance

The passive surveillance component of the study was designed to capture all cases of febrile illness occurring from within each of the three census populations. Participants were encouraged to attend study healthcare facilities when they developed fever. A number of community engagement activities occurred in the three sites prior to and during the surveillance period to sensitise the community to the aims and benefits of the study. All sites enrolled participants of all ages from both outpatient and inpatient facilities.

	Facility Based Passive Surveillance		
	Malawi	Nepal	Bangladesh
Survey period	1 Oct 2016 - 31 Oct 2018	1 Jan 2017 - 31 Dec 2018	1 Jan 2017 - 31 Dec 2018
Facility Type	Inpatient Queen Elizabeth Central Hospital Outpatient Ndirande Community Clinic	Inpatient Patan Hospital Outpatient Patan Hospital Adult and Paediatric Out-Patient Clinics	Inpatient Mirpur Field Clinic Mirpur Treatment Centre Outpatient Adhunik Hospital Dhaka Hospital Kalsi Shisu Hospital Radda MCH-CP Centre SHMCH

Table 2-5: Surveillance sites for the passive surveillance component of the study

In Malawi, the Ndirande clinic is the only government facility within the community and provides free healthcare to the population. Queen Elizabeth Central Hospital is approximately 6km away from Ndirande and is the primary inpatient facility for Blantyre. In Nepal, Patan Hospital is situated within the census area and provides both inpatient and outpatient care to the community. Through the STRATAA study, participants were provided with free consultation, investigations and medication to encourage participation in the study. In Bangladesh, a number of diverse study facilities were used throughout the census area to recruit eligible participants. Free healthcare and treatment were provided to participants. The majority of individuals were recruited from the icddr,b run clinic in the centre of Mirpur, which is well known to the community through many years of research activity.

At the start of the surveillance period, patients presenting to any of the clinical surveillance sites with a history of subjective fever >72 hours or objective fever >38.5 ^{o}C on presentation were approached

for enrolment. After approximately 6months of surveillance this was changed to a history of subjective fever >48 hours or objective fever >38.0 ^{o}C. This was to ensure the maximum number of typhoid cases were captured through the surveillance system.

An index case was defined as an individual with a blood culture result confirming infection with *S.* Typhi or *S*. Paratyphi (or non-typhoidal *Salmonellae* (NTS) in Malawi), and whose household was included in the census survey. Where participants could not be linked back to a census household, if they resided within the geographical census area individuals were enrolled in the surveillance component and their household was enrolled after the event if follow up could be maintained. These cases have been used to calculate the disease incidence in each of the three census sites.

Consenting individuals completed a detailed case record form and had samples of blood, urine and stool collected to determine a diagnosis of *S.* Typhi, *S.* Paratyphi A or NTS and to provide material for further diagnostic and genetic aims of the study.

2.5. Healthcare-Utilisation Surveys

To characterise the healthcare-seeking behaviour of individuals living in the census areas, approximately 735 households containing at least one child under 16 years of age were randomly selected from the demographic census at each site to participate in a healthcare utilisation survey. Data were collected from the head of the household/key informant describing the actual (if febrile illness had occurred in the previous 3months) and hypothetical usage of healthcare facilities for febrile episodes. The aim of this component was to estimate the proportion of cases (fulfilling the fever case definition) in each age stratum (0-4 years, 5-14 years and >15 years of age) who would or would not seek attention at one of the designated passive surveillance health facilities. Additional data regarding sanitation and hygiene facilities and usage were also collected. Surveys were performed twice aiming to coincide with the peak typhoid season at each of the three sites.

	Malawi	Nepal	Bangladesh

Total Households from Census 1	22,364	24,405	26,119
Households with at least one child (% of total)	11,893 (53)	13,980 (57)	19,325 (74)
Households enumerated (% of total)	1,131 (5)	1478 (6)	1198 (5)

Table 2-6: Sampling frame for selection of households for healthcare utilisation surveys, with proportion of total households in parentheses

2.6. Serological Surveillance

To estimate the seroincidence and seroprevalence of clinical (both formally diagnosed and undiagnosed cases) and subclinical infection or exposure to *S.* Typhi systematic serosurveillance was performed at each site. 8,500 participants were randomly selected in an age-stratified manner from the original demographic census. Using GPS/address data these individuals were followed up by field teams, and 1-3 ml of blood were collected twice at three-month intervals. Field teams moved throughout the entire population every three months to account for geospatial and seasonal changes in typhoid incidence. Suitable participants were enrolled by field workers; with no exclusion criteria for enrolment other than being too unwell for a blood draw. Where the individual identified was not available, a household member in the same age group was selected where possible. In addition, because the serosurvey took place more than six months after the original demographic census, no children under six months of age were randomised for enrolment. To address this, households visited that had a child younger than six months within it were also asked if the child could be enrolled.

In Malawi, to facilitate enrolment into this component, participants were offered financial remuneration for their time, roughly equivalent to $1 per visit. This is a standardised remuneration amount for a household visit containing a procedure, set centrally by the Malawi-Liverpool Wellcome Trust and approved by the local ethics committee.[233]

For this thesis, seroincidence (indicative of recent infection) will be calculated by measuring the rate of seroconversion to anti-Vi IgG antibody between both time points, with the denominator consisting only of individuals sampled twice. The sample size for this component was based on anticipated incidence rates of high anti-H(d) IgG of between 0.8 and 3.2% and adjusted for baseline prevalence of between 2 and 32%. Samples were divided into 8,500 first and 6,800 second samples assuming a 20% loss-to-follow-up rate (5% migration and 15% refusal), and were then allocated among age groups to ensure a >95% probability of detecting at least one seroconversion in each age group, and an approximately equal number of seroconversions across age groups.

Age group	Anticipated seroincidence (%)[1]	No of initial samples	Anticipated number of events	No. of follow-up samples[2]	No. of events detected	Binomial [95%	Exact CI]	Probability of observing 0 events
0-4 yrs	0.2	2500	5	2000[3]	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
>15 yrs	0.2	3900	7.8	3120	6	0.0007	0.0042	0.0019
TOTAL		**8500**	**24.2**	**6800**	**19**			

Table 2-7: Sample size calculations for the serological surveys to estimate age-specific rates of seroconversion over a three-month interval between samples, as defined by an increase in titres of serum IgG anti-H antibodies

[1]Based on three-month interval and an anticipated mean seroprevalence of 2% in 0-4year olds, 6% in 5-9year olds, 14% in 10-14year olds, and 32% in ≥15year olds.

[2]Assuming 5% migration and 15% refusal.

[3]Due to the smaller than anticipated number of individuals in this age group in Kathmandu, only 1880 initial samples (1504 follow-up samples) will be collected from 0-4year olds at this site.

Samples among participants ≥15 years old were further allocated to different age groups based on the anticipated prevalence of chronic carriers. The number of samples needed to produce a two-sided 95% confidence interval with a precision (half-width) of 50% for the anticipated seroprevalence of chronic carriers among 30-49year olds and ≥50year olds was calculated using OpenEpi Version 3.03a (http://www.openepi.com/SampleSize/SSPropor.htm) and utilise a finite population correction factor.

Age groups	Anticipated prevalence of chronic carriers (%)	Precision or half-width	Dhaka, Bangladesh		Kathmandu, Nepal		Blantyre, Malawi	
			Census population size	Number of initial samples	Census population size	Number of initial samples	Census population size	Number of initial samples

0-4 years	0.01	--	10490	2500	6090	1880	12675	2500
5-9 years	0.01	--	10198	1300	6817	1300	12785	1300
10-14 yrs	0.05	--	10624	800	7608	960	12995	800
15-29 yrs	0.5	0.4	36093	1200	33656	1440	31896	1420
30-49 yrs	1	0.5	30378	1820	31301	1820	21671	1820
50+ yrs	2	1.0	12948	880	17329	1100	5388	660
TOTAL				**8500**		**8500**		**8500**

Table 2-8: Sample size calculations for the serological surveys to estimate the age specific sero-prevalence of suspected chronic carriers, as defined by high levels of anti-Vi antibody titres

The seroprevalence of anti-Vi IgG was measured at baseline to identify individuals who could be potential chronic carriers. Previous studies have used high anti-Vi IgG antibody levels in serum as a marker of chronic carriage,[62,77] and the aim of this study is to use a recently validated anti-Vi IgG ELISA method[221] to determine rates of chronic carriage across the three populations. In order to validate this method and to identify possible chronic carriers in the population, individuals with a 'high' serum anti-Vi IgG titre were re-approached and asked to provide two consecutive stool samples within 48 hours. With the agreement of relevant local ethics committees, identified chronic carriers (confirmed high anti-Vi IgG serum sample with a positive stool culture) were treated with antibiotics for chronic carriage and clearance of bacterial shedding confirmed by three negative stool cultures collected at least one week after cessation of antibiotic therapy..

2.7. Household Contacts

To investigate possible transmission links, household contacts of index cases diagnosed with blood-culture confirmed *S.* Typhi or *S.* Paratyphi infection were identified through the census data collection and approached to take part in this component of the study. Up to five members of the household, with consent, were asked to provide a blood and stool sample within eight days of the index case being identified as blood-culture positive. A further stool sample at one month and repeat serology at six months was collected. Secondary attack rates for the occurrence of symptomatic infection will be

estimated. Approximately 73 households of index cases are expected to be enrolled in this component of the study.

In a similar approach to the acute cases of typhoid infection, where chronic carriers are identified from the serosurveys, household transmission studies will be performed among household contacts of possible chronic carriers (i.e. those with a 'high' serum anti-Vi IgG titre). A one-time attempt will be made to enrol up to five members of the household, blood for serology and stool will be collected, looking for evidence of typhoid infection. This will provide data on secondary attack rates for both acute and chronic typhoid states.

2.8. Data collection

Census and serosurvey data collection forms were developed through a structured iterative process and then implemented using Open Data Kit,[234] a system enabling electronic mobile data collection, with customizations by Nafundi, USA, on Android-based tablets. Each household within the demographic census was assigned a unique study ID and geo-located using GPS where possible; individuals were given a member number within the household. This information was collected by local enumerators over a one- to four-month period via these electronic forms and adapted to the three geographic settings. Data were uploaded onto MySQL databases, where SQL routines were run nightly to enforce data cleaning on critical variables beyond ODK's validation routines. Daily anonymized data were backed up from the three sites centrally. For the passive surveillance and household contacts studies a combination of tablet and paper-based case report forms were used to capture the data. Data from paper forms were transcribed onto electronic databases using Open Clinica.

2.9. Statistical Methods

2.9.1. Crude and adjusted Blood-culture Positive Incidence

Crude incidence rates were calculated by dividing the number of blood-culture confirmed cases by the total amount of person-time observed in each age group; 95% binomial confidence intervals (CI) were estimated. Adjusted incidence rates were estimated taking into account:

a) Data for the sensitivity of blood culture for detecting "true" typhoid cases from a systematic review and meta-analysis was used estimating blood-culture sensitivity at 59%.[193]
b) The probability that an individual who met the enrolment criteria had blood collected. This adjustment factor was estimated by recording eligible individuals who presented to the healthcare facilities but were not enrolled with blood-culture collection. In Nepal and Bangladesh, this adjustment factor was provided through data collected in country during a vaccine trial. In Malawi, these data were collected as part of the STRATAA study.
c) Healthcare-seeking behaviour. Using data from the healthcare utilisation survey on the probability of seeking care for febrile illness at one of the STRATAA facilities.

2.9.2. Seroincidence

Seroconversion was defined as a 2-fold rise in anti-Vi IgG titre from visit 1 to visit 2 and an absolute titre of $\geq$50 EU/ml for the second sample to account for small variations above the lower limit of detection for the assay (based on data from the human challenge model) after examining a variety of different cut-offs in sensitivity analyses. The number of participants with an antibody rise that met the definition for seroconversion were divided by the total number of participants in each age group only including participants with two blood tests approximately three-months apart. The mean duration of follow up time for the entire cohort was used to adjust the incidence to give an estimate of seroincidence per 100,000 years of observation. 95% binomial confidence intervals were estimated.

2.10. Ethical Approvals

Written informed consent was obtained from the head of each household (as the "key informant") on behalf of the entire household in the demographic census and healthcare utilisation surveys. In each of the other components, individual written informed consent will be obtained from individuals over

the age of 18 or by a parent/guardian from individuals below this age with additional assent sought from those between 11-17 years old. The protocol received ethical approval from the Oxford Tropical Research Ethics Committee, the Malawian National Health Sciences Research Committee and University of Malawi Research Ethics Committee, the Nepal Health Research Council and the icddr, b Institutional Review Board.

2.11. Laboratory Procedures

2.11.1. Blood Culture

Sites used their own site-specific SOPs to ensure the sterile collection and transportation of blood culture to the local microbiology lab but in general terms:

1. Allocation of blood volume to the various assays will prioritise the blood-culture bottle if attempts to obtain the full amount of blood are unsuccessful.
2. Blood will be collected in a sterile manner preferably using a closed vacutainer system, with appropriate cleaning of the site prior to collection and wearing the necessary personal protective equipment (PPE).
3. To optimise the sensitivity of the culture, blood should be collected prior to receiving anti-microbial therapy and maximal blood volumes should be taken wherever possible. The quantity of blood collected is recorded within the participants case record form.

   Children 3-5ml

   Adults 5-10ml
4. The clinical team have responsibility to label the samples correctly, with sample ID, date and time of collection recorded both on the sample and the participants CRF.
5. Transportation of the sample to the laboratory for further processing should take place as soon as possible. Blood culture bottles to be kept at room temperature prior to incubation.

Microbiological culture of blood was performed using an automated system (BD BACTEC, Becton-Dickinson, USA, or BacT/ALERT, BioMerieux, France) at all three sites following collection of a

single aerobic bottle. Cultures with a positive organism were then identified using colony morphology, gram staining, standard biochemical tests and specific antisera.[235] Antimicrobial susceptibility testing was performed by disc diffusion method following either British Society of Antimicrobial Chemotherapy (BSAC) or Clinical and Laboratory Standards Institute (CLSI) guidelines;[236] antibiotic discs used for the test were obtained from Oxoid, UK. A range of antimicrobials were tested for all isolated strains but varied depending on site, and the antimicrobials available to clinical teams. For *S.* Typhi multi-drug resistance (MDR) was defined as non-susceptibility to amoxicillin/ampicillin, chloramphenicol and co-trimoxazole. Bacteria found as part of the normal human skin and oral flora were defined as contaminants, including diptheroids, bacilli, micrococci and coagulase-negative staphylococci.

The procedure for identification and confirmation of *S.* Typhi, *S.* Paratyphi or non-typhoidal salmonella bacteraemia should follow local SOPs, and the following is intended as a guide.

1) Any bottle which triggers the fluorescence sensor due to an increase in CO_2 produced as a result of microbial growth will flag up.
2) A Gram film should be performed on all positive blood cultures according to standard methods (Public Health England 2015). On observation of gram-negative bacteria, bacterial subculture should be performed for organism identification in addition to antimicrobial susceptibility tests on the blood/broth fluid.
3) Subculture a loopful of blood/broth onto blood agar, chocolate agar, MacConkey's agar for the isolation of single colonies. Incubate the plates at 35^0C±2.
4) After overnight incubation, carry out biochemical tests for identification and antimicrobial susceptibility test depending upon colony characteristics and Gram stain.
5) Colonies presumptive of *Salmonella* should be identified using standard biochemical testing (Analytical Profile Index, Biomerieux), or MALDI-TOF where available and serotype-specific antisera to identify *S*. Typhi, *S*. Paratyphi A or NTS isolates where applicable (02, 04, 09 Vi).

2.11.2. Organism Identification

Identification of *Salmonella* serovars may be performed by colonial appearance, using biochemical methods (by identification of catabolic substrates or enzymatic activity using a commercial API method or local 'short sets') and confirmed/classified by serological agglutination reactions according to the White-Kauffmann-Le Minor scheme (Figure 2).

Colonial appearance**:** on XLD agar colonies are red and usually with a black centre, although this may be missing in non-H_2S producing strains. On other media (MacConkey and CLED agar) *Salmonella* are non-lactose fermenters.

Biochemical tests: *Salmonella* are urease NEG, oxidase NEG, indole NEG, and any colonies meeting these criteria should be assessed by serological agglutination reactions.

Serological agglutination: *Salmonella spp.* can be distinguished by their antigentic characteristics. Serological testing is based on the production of antibodies in response to exposure to bacterial antigens and these will agglutinate bacteria carrying homologous antigens. In this case antibodies produced against *Salmonella spp* are used to distinguish the serological type of possible Salmonella isolates.

1) Inspect the XLD and MacConkey plates for non-lactose fermenting colonies.
2) Carry out screening agglutination tests, using *Salmonella* poly H and poly O antisera.
3) Subculture suspected colonies on a nutrient agar plate.
4) Incubate overnight at 37˚C.
5) Visually inspect the plate for purity.
6) Carry out confirmatory agglutination tests, using poly H and poly O antisera, on discreet colonies and if positive then proceed checking agglutinations with colonies using mono-H (flagellin), mono-O and Vi antisera.
7) Agglutination of the antiserum and isolate are taken as a positive.

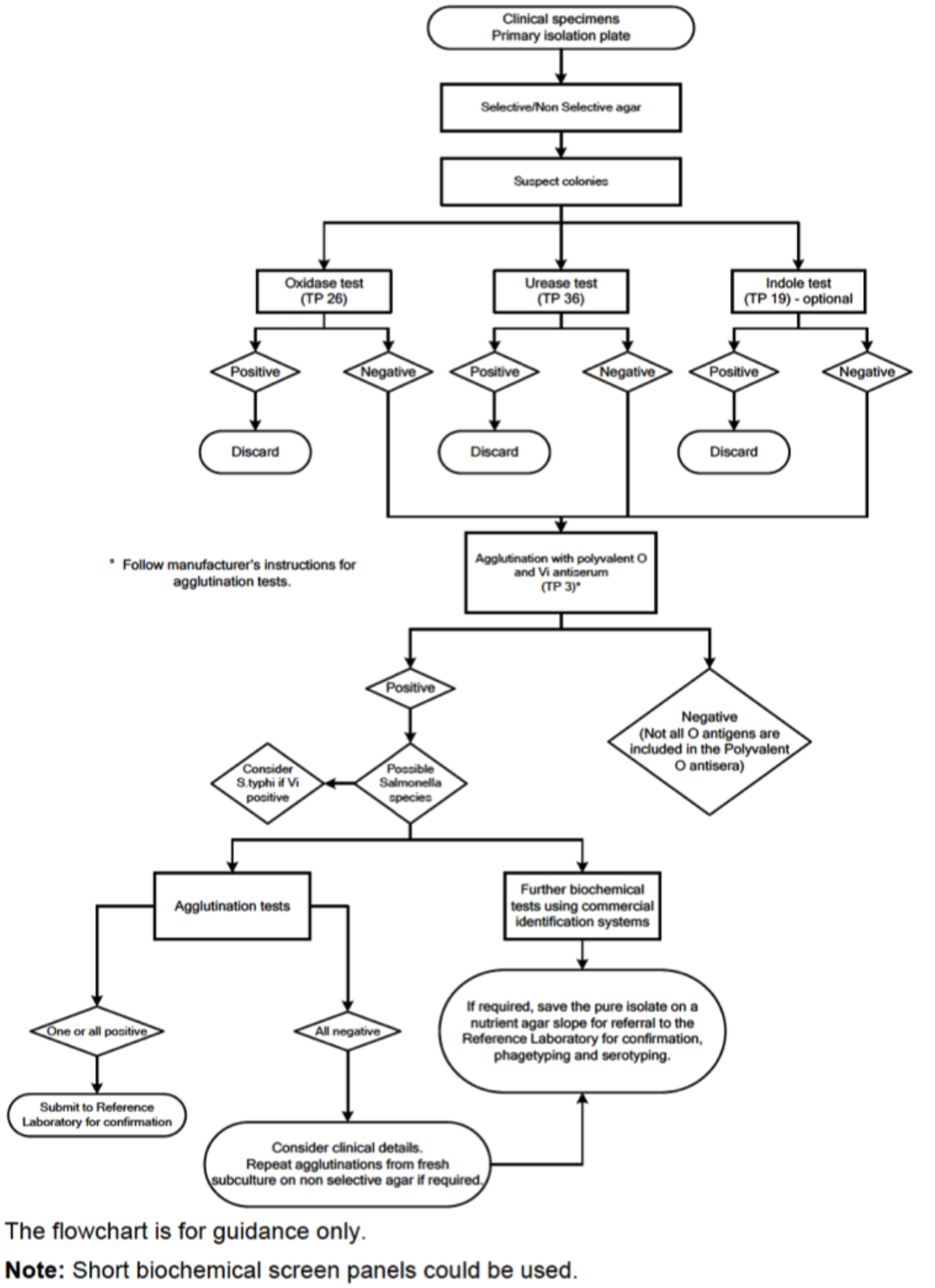


Figure2-3 Laboratory identification of *Salmonella* Typhi

2.11.3. Antimicrobial Sensitivity Testing

Antimicrobial susceptibility will be performed using the Kirby-Bauer disc diffusion method with zone size interpretation based on the most recent CLSI guidelines, i.e. using Mueller-Hinton (MH) agar with an ~0.5 McFarland standard colony suspension and incubating at 35°C ±2°C in ambient air for 16 to

18hours (CLSI 2014). Standard concentrations of antimicrobials are impregnated onto absorbent paper and are placed onto an agar plate seeded with a known concentration of bacteria. These plates are then incubated overnight at a suitable temperature and atmosphere. The antibiotic diffuses through the media radially from the disc and the presence or absence of growth around the discs is an indirect measure of the ability of that compound to inhibit the organism. The size of the zone of inhibition of growth is also influenced by the depth of the agar, since the antimicrobial diffuses in three dimensions. As a result, plates that are too shallow will produce false susceptible results as the antimicrobial compound will diffuse further than it should, creating larger zones of inhibition. Therefore, the plates must be poured to a depth of 4 mm (approximately 25 ml of liquid agar for 90 mm plates). Conversely, plates poured to a depth >4 mm will result in false resistant results. pH of the MH agar should fall between 7.2 and 7.4 at room temperature after solidification and should be tested when the media is first prepared. If the pH is <7.2 certain drugs will appear to lose potency (aminoglycosides, quinolones, macrolides), while other agents may appear to have excessive activity (tetracycline). If the pH is >7.4, the opposite results may occur.

The diameter of this zone has to be measured and compared with CLSI guidelines for zone sizes to determine whether the bacteria are sensitive, intermediate sensitive or resistant to the antibiotic. Methods to ensure the ongoing quality of the laboratory testing should already be in place. This might include the use of quality control organisms tested at regular (e.g. monthly) intervals. Individual labs should ensure that they meet necessary GCP in addition to national clinical laboratory requirements.

Compare results of zone sizes with CLSI guidelines. The recommended interpretations of results are taken as follows:

- Susceptible (S) - The “susceptible” category show zones of inhibition of bacterial growth which fall within the recommended CLSI guidelines. That implies that an infection due to the strain may be appropriately treated with the dosage of antimicrobial agent recommended for that type of infecting species, unless otherwise contradicted.

- Intermediate (I) - The intermediate category show zone sizes of inhibition of bacterial growth which fall within the recommended CLSI guidelines. This category includes isolates with antimicrobial agent MICs that approach usually attainable blood tissue levels and for which response rates may be lower than for susceptible isolates. The “intermediate” category implies clinical applicability in body sites where the drugs are physiologically concentrated (e.g., quinolones and β-lactams in urine) or when a high dosage of a drug can be used (e.g., β-lactams). The “intermediate” category also includes a “buffer zone” which should prevent small, uncontrolled technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins.
- Resistant (R) - Resistant strains show inhibition zone sizes less than the acceptable size as prescribed by CLSI. Resistant strains are not inhibited by the usually achievable systemic concentrations of the agent with normal dosage and/or fall in the range where specific microbial resistance mechanisms are likely (e.g., β-lactams) and clinical efficacy has not been reliable in treatment studies.
-

2.11.4. Stool Culture

Stool sample collection should be performed according to local SOPs where available. The exact methods used will vary between study sites. The key principles are to obtain the samples required whenever possible, to try and ensure the sample is as ‘fresh’ as possible prior to processing and culture and where possible, stool samples should be collected prior to the use of antibiotics. The administration of antibiotics should not be delayed in sick or severely unwell patients and clinical management always takes priority over study sample collection. Emphasis must be placed on infection control precautions and, where required for example, disposable gloves and transport material should be supplied with the collection container and instructions.

The following is a broad description of the collection procedure to be performed.

1) A study team member should provide sufficient explanation and support to the enrolled study participant to explain how and why a stool sample is required.
2) The study team member should supply the enrolled participant with a sterile stool container (with 'spoon'), disposable transport material (for example, opaque envelope or plastic bag) and disposable gloves.
3) Instruction to participant or their care giver:
    a. Place toilet paper/newspaper in the toilet bowl/around the latrine to prevent stool being submerged in toilet water (*adapt locally as required*). Note the time and date of sample collection on the container label.
    b. After defecation, using the 'spoon' in the container provided, fill the tube so it is approximately half full (1gram or x1 walnut sized amount).
    c. Ask the participant not to collect any toilet water or urine with the stool sample but not to worry if this is unavoidable.
    d. Screw cap tightly onto stool pot.
    e. Flush/empty/clean the toilet/latrine.
    f. Wash hands with soap and water.
    g. Place stool sample in transport material and deliver to study site laboratory as soon as possible if not performed on site.
4) Stool samples, where possible, should be kept cool if there is any delay in delivery to the laboratory.

Selenite F is enrichment medium employed for detection of pathogens in faecal specimens as the pathogens are present in a very small number in the intestinal flora. The casein peptone provides essential nitrogenous and carbon compounds. The lactose in the medium maintains the pH of medium. Selenite is reduced by bacterial growth and alkali is produced. An increase in pH lessens the toxicity of the selenite and results in overgrowth of other bacteria. The acid produced by bacteria due to lactose fermentation serves to maintain a neutral pH. Sodium phosphate maintains a stable pH and also lessens the toxicity of the selenite, thus increasing the capacity of the medium. Sodium selenite inhibits many species of gram-positive and gram-negative bacteria including enterococci and coliforms.

After enrichment, the broth will be sub-cultured onto XLD agar medium, which differentiates enteric pathogens based on xylose fermentation, lysine decarboxylation and production of hydrogen sulphide.

2.11.5. Urine Collection

The following is a broad description of the collection procedure to be performed. Where local SOPs are available these should be followed.

1) A study team member should provide sufficient explanation and support to the enrolled study participant to explain how and why a urine sample is required.
2) The study team member should supply the enrolled participant with a sterile universal container or falcon tube (complete with participant details, time and date of collection), temporary disposable transport material (for example, opaque envelope or plastic bag) and (where required) disposable gloves.
3) The participant should be asked to urinate into the universal container, after omitting the first part of the urine stream. Only ~5mL are required at this stage.
4) This sample can then be kept at 4^{o}C before delivery to the laboratory, which should occur within one hour (two-hour window between collection and terminal storage).

2.11.6. EDTA for Serology

Following inoculation of the blood culture bottle, remaining blood should be inoculated into the EDTA tube. This should be transported to the laboratory within the same day, maintained at 2-8^{O}C. Samples should be protected from shaking as much as possible during transportation to limit the haemolysis rate. The SOP from within the standardised kit should be followed, with no further dilution steps necessary. The SOP from within the kit stipulates that blood is collected in serum, but in this study, we collected EDTA samples using plasma. Prior to sample collection in the three sites, samples collected from the human challenge model were used and comparable anti-Vi IgG titres were achieved using both plasma and serum samples. This enabled plasma samples to be used for this study with confidence in the results.

VaccZyme™
Human Anti-S *typhi* Vi IgG
Enzyme Immunoassay Kit

For *in-vitro* research use

Product code: MK091.U

Product manufactured by:
The Binding Site Group Ltd, PO Box 11712, Birmingham, B14 4ZB, U.K.
Telephone: +44 (0)121 436 1000
Fax: +44 (0)121 430 7061
e-mail: info@bindingsite.co.uk

1 INTENDED USE

This assay is designed for the *in-vitro* measurement of specific IgG antibodies against the virulence factor (Vi) of *Salmonella typhi* present in human serum.

Sufficient materials are supplied to allow a maximum of 41 samples to be tested in duplicate or 89 in single, with a calibration curve and two controls.

2 BACKGROUND

Salmonella typhi is a gram-negative bacterium which causes the systemic febrile illness typhoid fever. This infectious disease is characterised by a headache and fever that increases in severity over three to four days. Other symptoms including cough, sore throat, abdominal pain and changes in behaviour can also occur. The disease can be fatal if untreated killing around 10% of the people infected[1]. Vaccination with the purified Vi capsular polysaccharide can protect adults and children > 2 years against typhoid fever[2,3].
A patient's specific antibody response to the vaccine may be evaluated by the serological determination of their IgG anti-Typhi Vi antibody levels pre- and post-vaccination using this quantitative enzyme immunoassay.
Patients with suspected immunodeficiency may be investigated by assessing their ability to respond to specific polysaccharide antigens like those present in the Typhi Vi vaccine[3].

3 PRINCIPLE OF THE ASSAY

Microwells are pre-coated with the Typhi Vi antigen. The calibrators, controls and diluted patient samples are added to the wells and antibodies recognising the Typhi Vi antigen bind during the first incubation.
After washing the wells to remove all unbound proteins, purified peroxidase labelled rabbit anti-human IgG (**g** chain specific) conjugate is added. The conjugate binds to the captured human antibody and the excess unbound conjugate is removed by a further wash step. The bound conjugate is visualised with 3,3',5,5' tetramethylbenzidine (TMB) substrate which gives a blue reaction product, the intensity of which is proportional to the concentration of antibody in the sample. Phosphoric acid is added to each well to stop the reaction. This produces a yellow end point colour, which is read at 450nm.

4 PRECAUTIONS

4.1 WARNING

All donors of human sera supplied in this kit have been serum tested and found negative for Hepatitis B surface antigen (HBsAg) and antibodies to human immunodeficiency virus (HIV 1 and HIV 2) and Hepatitis C virus. The assays used were either approved by the FDA (USA) or cleared for *in vitro* diagnostic use in the EU (Directive 98/79/EC, Annex II); however these tests cannot guarantee the absence of infective agents. Proper handling and disposal methods should be established as for all potentially infective material, including (but not limited to) users wearing suitable protective clothing at all times. Only personnel fully trained in such methods should be permitted to perform these procedures.
This product contains sodium azide and Proclin 300 and must be handled with caution; suitable gloves and other protective clothing should be worn at all times when handling this product. Do not ingest or allow contact with the skin (particularly broken skin or open wounds) or mucous membranes. If contact does occur wash with a large volume of water and seek urgent medical advice. Explosive metal azides may be formed on prolonged contact of sodium azide with lead and copper plumbing; on disposal of reagent, flush with a large volume of water to prevent azide build-up.
The buffers and serum supplied in this kit contain various enzyme inhibitors as listed below. These are hazardous and should be handled with care.

INHIBITOR	CONCENTRATION
Kathon	0.02%
Sodium Azide	0.099%
Proclin™ 300	0.045%
Bromonitrodioxane	0.002%
Methylisothiazone	0.002%

Proclin™ is a trademark of Rohm and Haas Corp. Philadelphia, PA

Kathon is an irritant and may cause sensitisation by skin contact.
The stop solution contains 3M phosphoric acid, which is an irritant. Avoid contact with skin or eyes.
Reagent spills should be cleaned up appropriately, observing local and environmental regulations.

4.2 CAUTION

This kit is for *in-vitro* research use only. Not for use in diagnostic procedures.
This product should only be used by appropriately trained personnel.
Strict adherence to the protocol is recommended. Any deviation may affect assay performance, and the results obtained. Pay attention to specific **'Notes'** and warnings throughout these Instructions for Use.
Reagents from different batch numbers of kits are **NOT** interchangeable. If large numbers of tests are performed care should be taken to ensure that all reagents are from the **SAME** batch. All strips must be taken from the same foil pouch.
Substitution of any component may lead to incorrect results.
To avoid reagent contamination, only use new or clean plastic/glassware. **Never** return unused reagents to the bottles.
Do **not** leave reagent bottles uncapped; any resulting evaporation or contamination will lead to inconsistent results.
TMB substrate must not be exposed to light or water.
Microbially contaminated, haemolysed or lipaemic serum and specimens containing particulate matter should not be used.
Inaccurate sample dilution cannot be checked, as kit controls are ready to use. The use of calibrated pipettes and appropriate internal QC samples is recommended.
When using automated assay systems, sample dilutors and other automated equipment, follow the manufacturer's instructions carefully. Particular attention should be given to the setup of the equipment and installation and connection to external services. All settings for automatic washers and readers must be followed carefully and equipment must be maintained and serviced according to the manufacturer's instructions.

4.3 STORAGE AND STABILITY

The kit should be stored at 2-8°C and should **not** be frozen. Inappropriate storage temperatures will affect the results.
Wash buffer diluted into a clean container can be stored at room temperature for a maximum of 4 weeks.
The expiry date of the kit is shown on the outer label.

5 SAMPLE COLLECTION AND STORAGE

Blood samples should be collected by venepuncture allowed to clot naturally and the serum separated.
The serum may be stored at 2-8°C for up to 48 hours prior to assay, or for prolonged storage kept undiluted at -20°C or below.
Repeated thawing and freezing should be avoided.
Serum samples should **not** be heat-inactivated, as this may give false positive results.

6 MATERIALS

6.1 MATERIALS SUPPLIED

6.1.1 Instruction leaflet: Giving full assay details.
6.1.2 QC Certificate: Indicating the expected performance of the batch.
6.1.3 Typhi Vi Coated Wells: 12 breakapart 8 well strips coated with inactivated Typhi Vi antigen. Each plate is packaged in a re-sealable foil bag containing 2 desiccant pouches.
6.1.4 Type V Sample Diluent: 2 bottles containing 50mL of buffer for sample dilution. Coloured yellow, ready to use.
6.1.5 Wash Buffer: 1 bottle containing 50mL of a 20-fold concentrated buffer for washing the wells.
6.1.6 Typhi Vi IgG Calibrators: 5 bottles each containing 1.2mL of diluted human serum, with the following concentrations of anti-Typhi Vi antibody: 7.4, 22.2, 66.7, 200 and 600 U/ml. Ready to use.
6.1.7 Typhi Vi IgG High Control: 1 bottle containing 1.2mL of diluted human serum. The expected value is given on the QC certificate. Ready to use.
6.1.8 Typhi Vi IgG Low Control: 1 bottle containing 1.2mL of diluted human serum. The expected value is given on the QC certificate. Ready to use.
6.1.9 Typhi Vi IgG Conjugate: 1 bottle containing 12mL of purified peroxidase labelled antibody to human IgG. Coloured red, ready to use.
6.1.10 TMB Substrate: 1 bottle containing 14mL TMB substrate. Ready to use.
6.1.11 Stop Solution: 1 bottle containing 14mL of 3M phosphoric acid. Ready to use.

6.2 ADDITIONAL MATERIALS AND EQUIPMENT - not supplied

6.2.1 Automatic microplate plate washer: This is recommended, however, plate washing can be performed manually.
6.2.2 Plate reader: Capable of measuring optical densities at 450nm referenced on air.
6.2.3 Distilled or deionised water: This should be of the highest quality available.
6.2.4 Calibrated micropipettes: For dispensing 1000, 100 & 10mL.
6.2.5 Multichannel pipette: Recommended for dispensing 100mL volumes of conjugate, substrate and stop solution.
6.2.6 Glass/plastic tubes: For sample dilution.

6.3 PRE-ASSAY STEPS

1. Bring the kit to room temperature
The kit is designed for room temperature operation (20-24°C).
Remove the kit from storage and stand at room temperature for approximately 60 minutes. Wells must **not** be removed from the foil bag until they have reached room temperature.
Note: The kits may be maintained at room temperature for up to 1 week.

2. Kit components
Gently mix each kit component before use.

3. Wash buffer dilution
Add 50mL of the wash buffer concentrate to 950mL of distilled water (1 in 20 dilution) into a clean container and mix. Smaller volumes can be diluted as appropriate.
Note: Diluted wash buffer can be stored at room temperature for up to 4 weeks, therefore only dilute the appropriate amount.

4. Sample dilution
Dilute 10mL of each sample with 1000mL of sample diluent and (1:100) and mix well.
Note: Diluted sample **must** be used within 8 hours.

5. Strip and frame handling
Place the required number of wells in the strip holder. Position from well A1, filling columns from left to right across the plate. When handling the plate, squeeze the long edges of the frame to prevent the wells falling out.
Note: Return unused wells to the foil bag immediately with the two desiccant pouches and re-seal tightly to minimise exposure to moisture.
Take care not to puncture or tear the foil bag, see below.
WARNING: Exposure of wells to moisture or contamination by dust or other particulate matter will result in antigen degradation, leading to poor assay precision and potentially false results.

7 ASSAY METHOD

Maintain the same dispensing sequence throughout the assay.
1. Sample addition
Dispense 100mL of each calibrator, control and diluted (1:100) sample into the appropriate wells of the plate provided.
Note: Samples must be added to the plate as quickly as possible to minimise assay drift, and the timer started after the addition of the **last** sample.
Incubate at room temperature for 30 minutes.

2. Washing
The washing procedure is critical and requires special attention. An improperly washed plate will give inaccurate results, with poor precision and high backgrounds.
After incubation remove the plate and wash wells 3 times with 250-350mL wash buffer per well. Wash the plate either by using an automatic plate washer or manually as indicated below. After the final automated wash, invert the plate and tap the wells dry on absorbent paper.

Plates can be washed manually as follows:
a. Flick out the contents of the plate into a sink.
b. Tap the wells dry on absorbent paper.
c. Fill each well with 250-350mL of wash buffer using a multichannel pipette.
d. Gently shake the plate on a flat surface.
e. Repeat a-d twice.
f. Repeat a and b.

3. Conjugate addition
Dispense 100mL of conjugate into each well, blot the top of the wells with a tissue to remove any splashes.
Note: To avoid contamination, never return excess conjugate to the reagent bottle.
Incubate at room temperature for 30 minutes.

4. Washing
Repeat step 2.

5. Substrate (TMB) addition
Dispense 100mL of TMB substrate into each well, blot the top of the wells with a tissue to remove any splashes.
Note: To avoid contamination never return excess TMB to the reagent bottle.
Incubate at room temperature in the dark for 30 minutes.

6. Stopping
Dispense 100mL of stop solution into each well. This causes a change in colour from blue to yellow.

7. Optical density measurement
Read the optical density (OD) of each well at 450nm on a microplate reader, within 30 minutes of stopping the reaction.

8 RESULTS AND QUALITY CONTROL

1. Quality control
In order for an assay to be valid, all the following criteria must be met:
- Calibrators and controls must be included in each run.
- The values obtained for the controls should be in the ranges specified on the QC Certificate.
- The curve shape should be similar to the calibration curve, shown on the QC Certificate.

If the above criteria are not met, the assay is invalid and the test should be repeated.

2. Calculate mean optical densities (for assays run in duplicate)
For each calibrator, control and sample calculate the mean OD of the duplicate readings. The percentage coefficient of variation (%C.V.) for each duplicate OD should be less than 15%.

3. Plot calibration curve
The calibration curve can be plotted either automatically or manually as follows by plotting the anti-Typhi Vi IgG antibody concentration on the log scale against the OD on the linear scale for each calibrator:
- Automatic - use appropriately validated software, and the curve fit that best fits the data.
- Manual - using log/linear graph paper, draw a smooth curve through the points (**not** a straight line or point to point).

4. Treatment of anomalous points
If any one point does not lie on the curve, it can be removed. If the absence of this point means that the curve has a shape dissimilar to that of the sample calibration curve, or more than one point appears to be anomalous, then the assay should be repeated.

5. Calculation of the control values
Read the level of the anti-Typhi Vi IgG antibody in the controls directly from the calibration curve. The values should fall within the ranges given on the QC Certificate.

6. Calculation of antibody levels in diluted samples
Read the level of the anti-Typhi Vi IgG antibody in the diluted samples directly from the calibration curve.
Note: The calibrator values have been adjusted by a factor of 100 to account for a 1:100 sample dilution. No further correction is required.

7. Assay calibration
The assay is calibrated against an affinity purified preparation of pooled human antiserum to Typhi Vi. The IgG concentration of the preparation was quantified using The Binding Site Human IgG ML BINDARID™ kit (RL004.3) and MININEPH™ IgG Low Level Kit (ZK004.LL). The quantity of IgG antibodies in the affinity purified preparation was confirmed against the European reference material ERM®-DA470 (Institute for Reference Materials and Measurements, Belgium). The anti-Typhi Vi units assigned to this assay are equivalent to the quantity of IgG (µg/ml) in The Binding Site's affinity purified reference material.

8. Limitations
- Pre- and post-vaccination samples should be run simultaneously.
- This kit is for *in-vitro* research use only. Not for use in diagnostic procedures.

9 MEASURING RANGE

The measuring range of the assay is 7.4 – 600 U/ml.

10 PERFORMANCE CHARACTERISTICS

10.1 PRECISION

The intra-assay precision was measured using 20 replicates of eight samples within the range of the calibration curve. The mean and % C.V. for each sample are given below:

INTRA-ASSAY PRECISION		
n=20	**Concentration (U/ml)**	**% C.V.**
Sample 1	12.1	6.3
Sample 2	38.4	4.3
Sample 3	34.2	4.1
Sample 4	69.5	4.6
Sample 5	58.9	6.5
Sample 6	127.5	3.7
Sample 7	196.8	3.2
Sample 8	343.2	3.9

The inter-assay precision was measured using eight samples tested in duplicate six times over three days. The mean and % C.V. for each sample are given below:

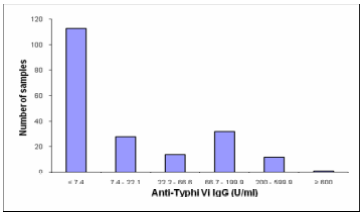
INTER-ASSAY PRECISION		
n=6	**Concentration (U/ml)**	**% C.V.**
Sample 1	13.4	12.6
Sample 2	40.1	7.0
Sample 3	41.7	9.5
Sample 4	76.6	8.9
Sample 5	57.5	7.3
Sample 6	131.2	8.0
Sample 7	200.5	9.6
Sample 8	301.8	6.1

10.2 ANALYTICAL SENSITIVITY

Assay sensitivity of 7.4 U/mL was confirmed by assaying two samples in multiple replicates with values 1.2 and 1.8 times the lowest calibrator point (7.4 U/ml). Statistical analysis by the Student's t test, confirmed that these samples were significantly different from each other ($p<0.0001$).

10.3 TYPICAL VALUES

Anti-Typhi Vi IgG antibody levels were measured in serum from 200 normal adult blood donors (of unknown vaccination and immune status). The results displayed below, are provided for illustration only, and should not be used to calculate a normal range.



3. Chapter 3 – Site Characteristics and Healthcare Seeking Behaviour

3.1. Introduction

To estimate the incidence of enteric fever at three endemic sites a demographic census was enumerated at each site to provide a denominator population from which facility-based passive surveillance could be performed. Data were collected from each household at the household and member level. Census-update was performed at different time-periods at each site, so that the overall census-population for the two-year period could remain updated.

In addition, to understand the healthcare seeking behaviour of individuals within these populations and enable adjustment of incidence estimates healthcare utilisation surveys were performed on two separate occasions within each of these populations. Data were also collected on the economic and water-based (WASH) risk factors known to be associated with high typhoid incidence.

This chapter provides the results for these three surveys and discusses the similarities and differences between these three endemic sites and the impact these factors may have on typhoid burden. The denominator population and healthcare seeking behaviour are important to the primary aims of this thesis and are required for the following outcome measures:

1. Observed incidence rates of blood-culture confirmed enteric fever from facility-based passive surveillance.
2. Adjusted incidence rates of infection accounting for blood culture sensitivity, enrolment proportion and healthcare seeking behaviour.

The household and member level data, and WASH data are also important for the following outcome measures:

3. Description of sites through repeat demographic census
4. Description of economic and WASH risk factors through household surveys

3.2. Methods

For a detailed description on the methods for the demographic census and census update, including copies of case record forms, please refer to Chapter 2, section 3. For a description of the methods for the healthcare utilisation and water and sanitation surveys please refer to Chapter 2, section 6.

3.3. Results - Demographic Census

3.3.1. Age and Gender

A total of 423,618 individuals residing in 99,033 households were enumerated in the demographic censuses, providing 626,219 person-years of observation (pyo) for febrile illness surveillance across the three sites studied. The population in Malawi was younger than the other sites with a median age of 19 years, (Interquartile Range (IQR): 9-31 years), compared with 28 years in Nepal (IQR: 17-42) and 25 years in Bangladesh (IQR: 13-37). 42%, 30% and 22% of the population was below 16-years of age, in Malawi, Nepal and Bangladesh respectively (Figure3-1). The male to female ratio was 0.51, 0.52 and 0.50, in Malawi, Nepal and Bangladesh respectively.

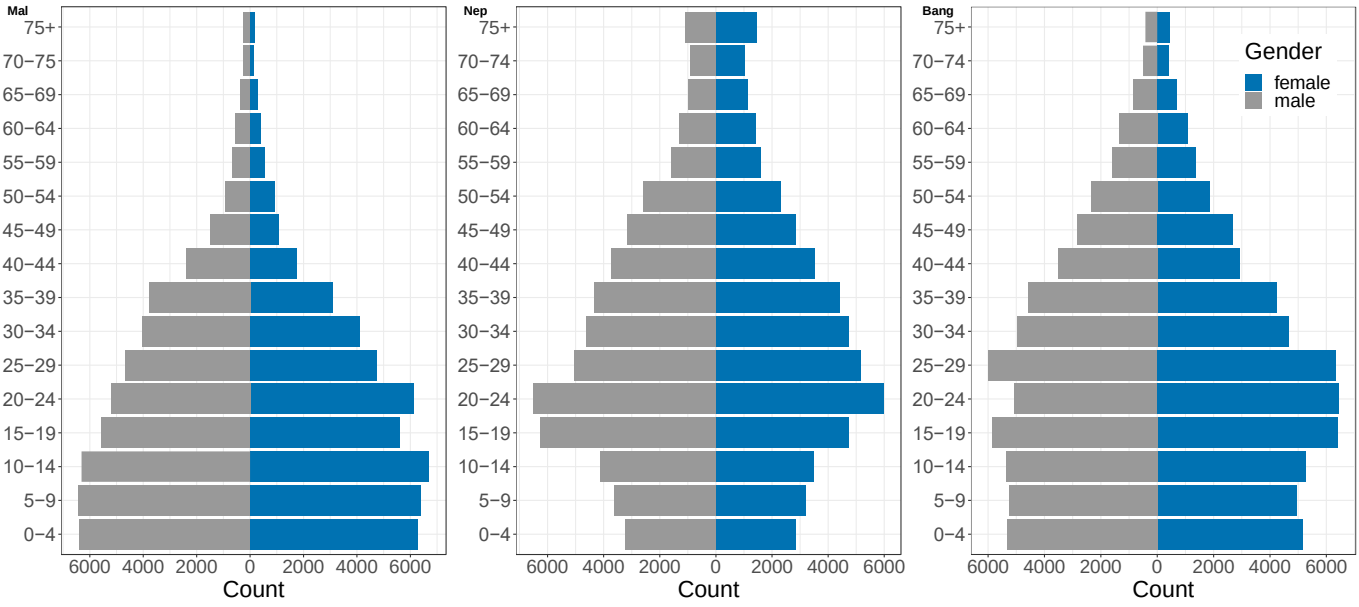


Figure3-1: Population Pyramid from the first demographic census in Malawi, Nepal and Bangladesh with the number of participants within age group shown across x-axis, split between female (blue) and male (grey)

The population pyramids show a very typical shape for urban settings in the countries where the studies were performed. In Malawi the average life expectancy is 63 years according to available WHO statistics, however it is known that as individuals get older, they leave the urban areas and return to their villages of birth, leaving a youthful population in the cities, where improved education and employment opportunities exist. In addition, the birth rate remains high at 4.5 births per woman. In Kathmandu, Nepal large numbers of individuals migrate to the city for economic reasons as they finish school[230] which would explain the higher proportion of individuals in our census from 15 years and above. Again, according to data collected from the WHO average life expectancy in Nepal is 70 years, with the birth rate of 2.1 per woman. This is reflected in our data. Bangladesh lies between Malawi and Nepal. With high rates of migration in and out of urban areas, an average life expectancy of 72 years and a birth rate of 2.1 children per woman, the population pyramid demonstrates large numbers of young children with a slight increase in those of working age.

3.3.2. Ethnicity

There was a difference in the diversity of ethnic groups reported from the census in the populations studied. From Malawi there was a diversity of tribal groups which people ascribed themselves to (Figure3-2). Whilst it is acknowledged that there is now a lot of crossover marriage in tribal groups, historically they would have had distinct migration patterns and histories. In Kathmandu the predominant tribal group were the Newar. In Bangladesh, everyone in the census self-reported as Bengali. There are a number of smaller tribal groups that live on the peripheries of Bangladesh, but these are not represented in urban Dhaka.

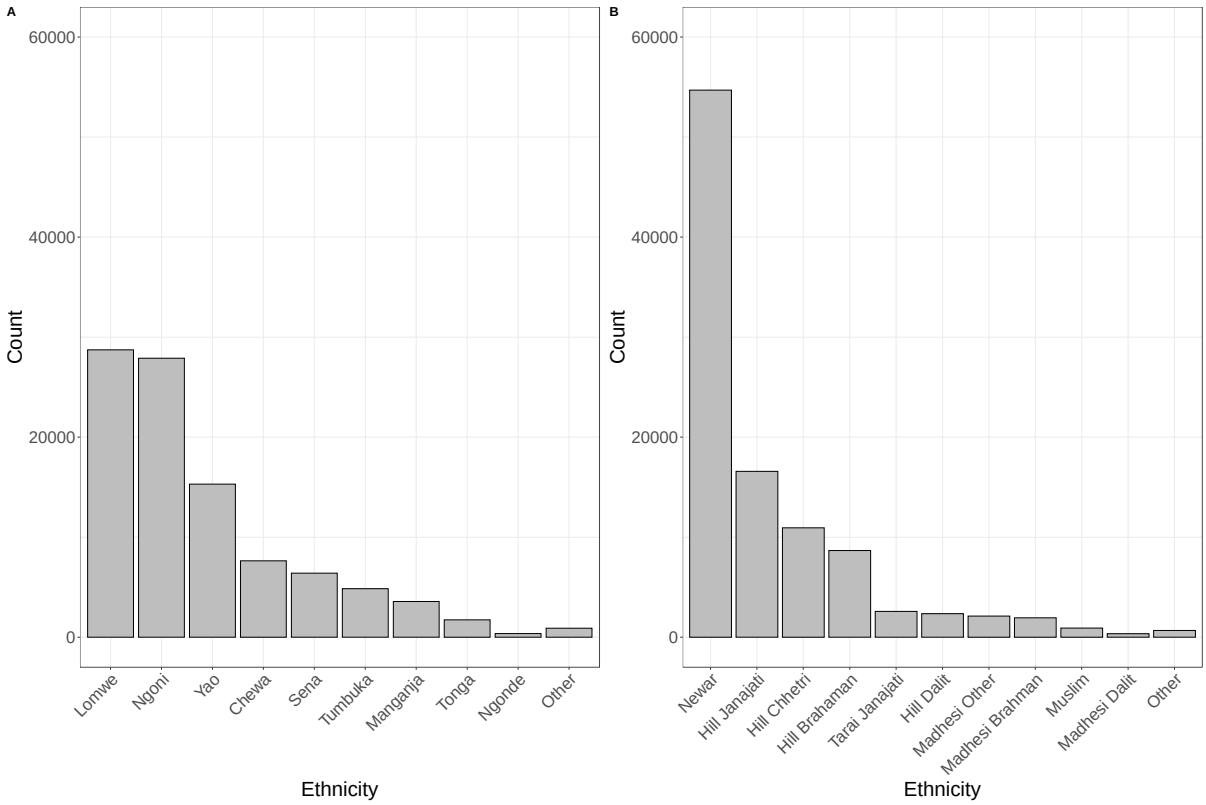


Figure3-2: Tribal/Ethnic Groups of individuals resident within each household as reported by the household head in A; Blantyre, Malawi and B; Kathmandu, Nepal

3.3.3. Household Size

Median household size was 4 (IQR: 3-6), 4 (IQR: 3-5) and 4 (IQR: 3-5) in Malawi, Nepal and Bangladesh respectively (Figure3-3). In our study a member of a household was defined as individuals eating together for greater than 4 nights a week. Previous case-control studies have shown households with more individuals living within them to be a risk factor for enteric fever.[237]

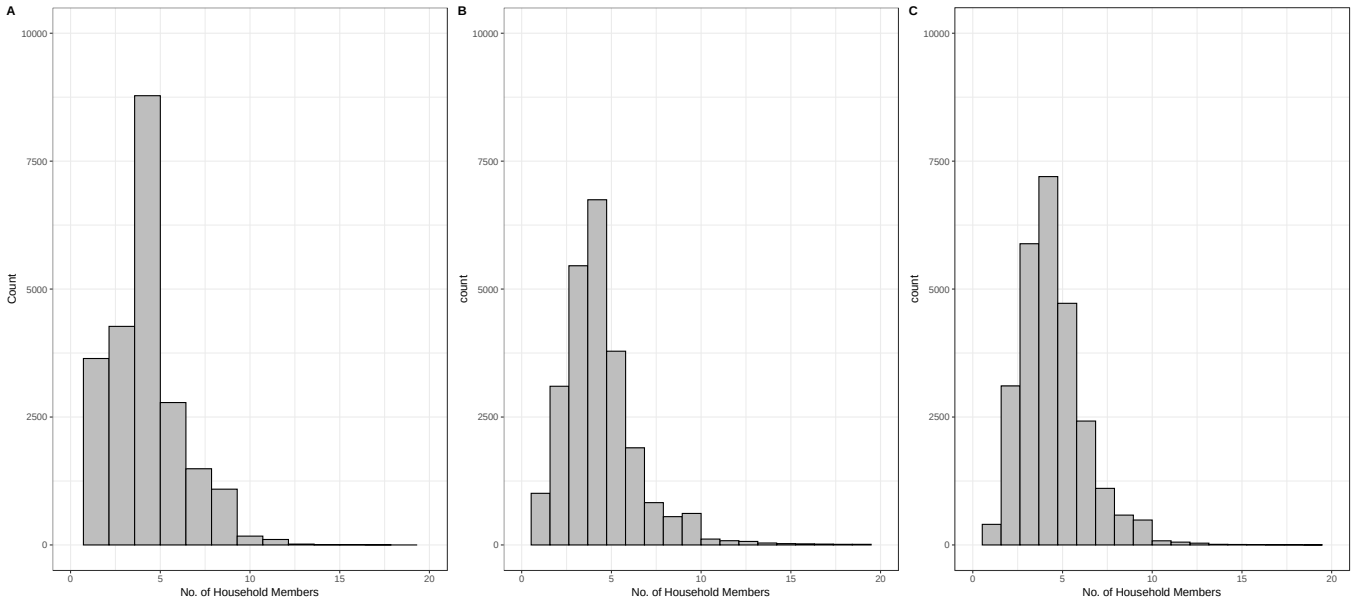


Figure3-3: Number of people resident per household as reported by household head for A; Blantyre, Malawi, B; Kathmandu, Nepal, C; Dhaka, Bangladesh

3.3.4. Education

For individuals within the households who had finished their education, we asked for the highest educational level they had achieved. In Malawi as has been well documented in much of sub-Saharan Africa there was a significant difference between the highest educational level achieved by males and females. In **Error! Reference source not found.**A, the proportion of individuals at each level is shown, with a higher proportion of females at the lower levels and higher proportion of males finishing secondary school and beyond. In order to understand this further, I gave each level a numerical score, with no education scoring a 0, increasing by 1 at each educational level up to post-graduate educational level scoring a 7. The histogram in **Error! Reference source not found.**B shows the scores by count. Females had a median score of 3 (IQR; 1-4), mean 2.69, compared to males median score 3 (IQR; 3-4), mean 3.184. This difference was statistically significant with a p-value of <0.005.

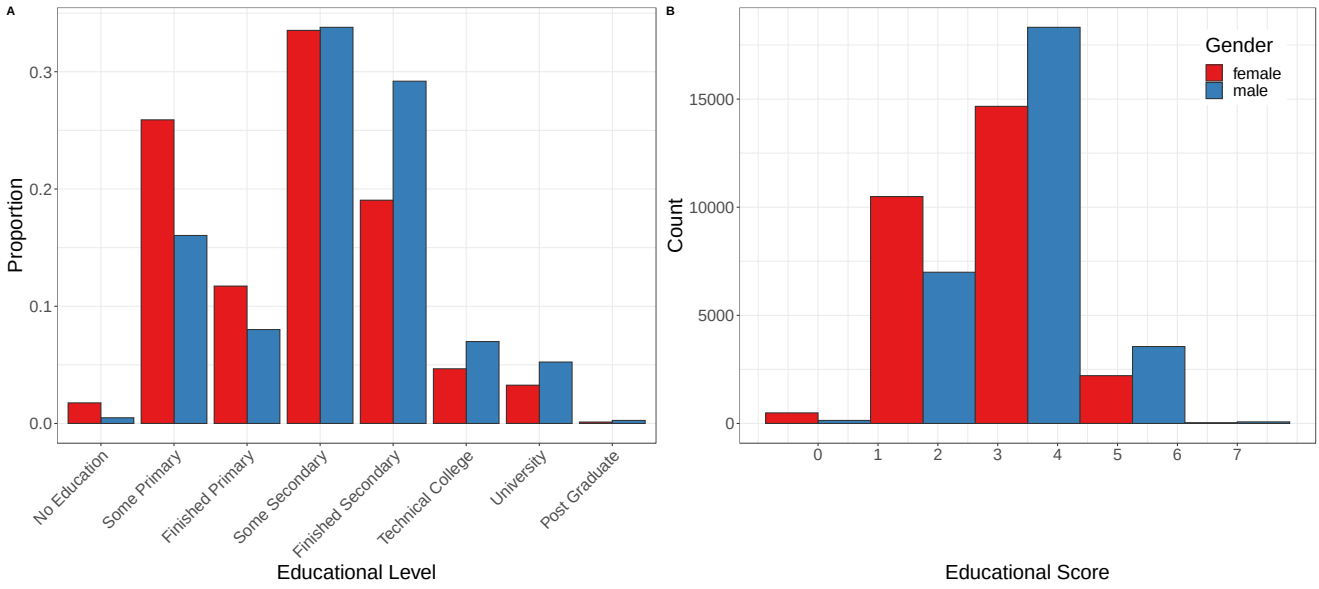


Figure3-4: From Malawi, A; The proportion of household members who have finished education at their highest educational level, B; Histogram of household members educational score with each level of education scoring an extra point

In Nepal a similar pattern is observed with female median educational score of 3 (IQR; 1-4), mean 2.6, compared with a median score for males of 3 (IQR; 2-4), mean 3.2, with p-value <0.005. There are a higher proportion of individuals at the extremes of the educational system compared with Malawi, with both a greater proportion reporting either no education or University level education.

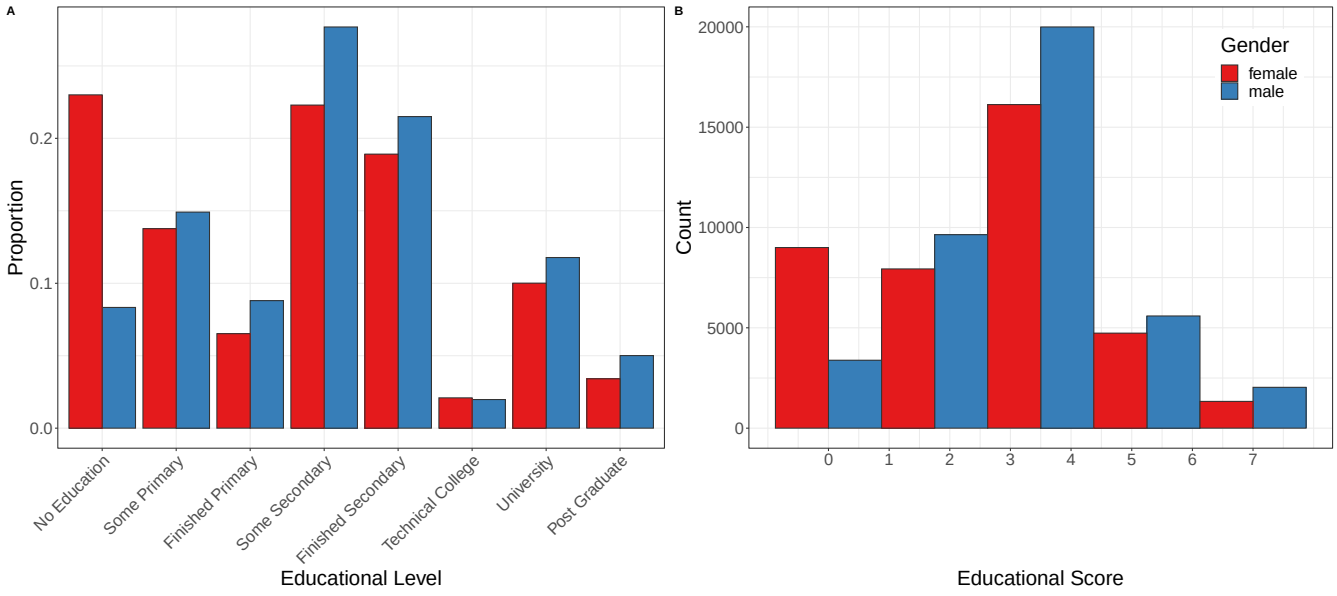


Figure3-5: From Nepal, A; The proportion of household members who have finished education at their highest educational level, B; Histogram of household members educational score with each level of education scoring an extra point.

In Bangladesh, the reported pattern was quite different with a higher proportion of both males and females reporting no education. The higher educational level was still seen in males with a median educational score of 3 (IQR; 1-4) compared with a median of 2 (IQR: 0-3) in females, p-value <0.005.

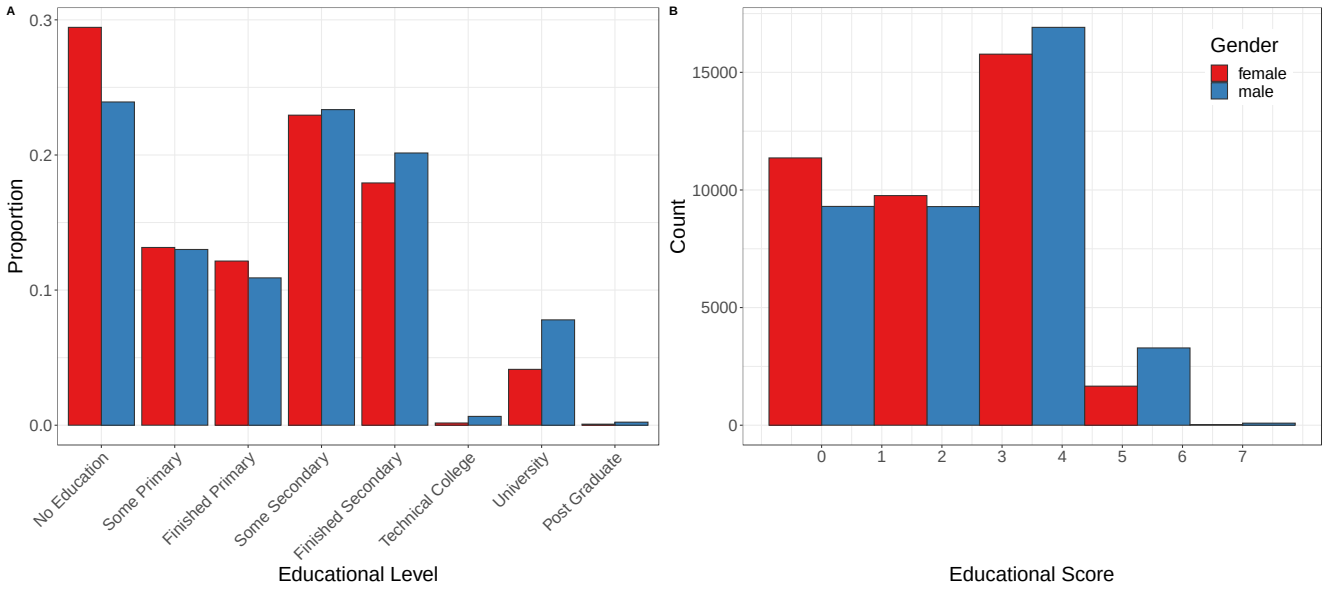


Figure3-6: From Bangladesh, A; The proportion of household members who have finished education at their highest educational level, B; Histogram of household members educational score with each level of education scoring an extra point.

3.3.5. Employment

Individuals within the demographic census who had finished school education were asked what form of employment they undertook (Figure3-7). Prior to the census taking place we consulted with the sites to ensure the categories of work were applicable to each context. This shows some similarities across the three sites with similar proportions of men and women undertaking the same tasks. Women were most likely to be unpaid family workers in Nepal and Bangladesh, however in Malawi there was an almost even split for women either working as unpaid family workers at home or self-employed, with men most commonly performing paid employment in Malawi and Nepal but self-

employed in Bangladesh.

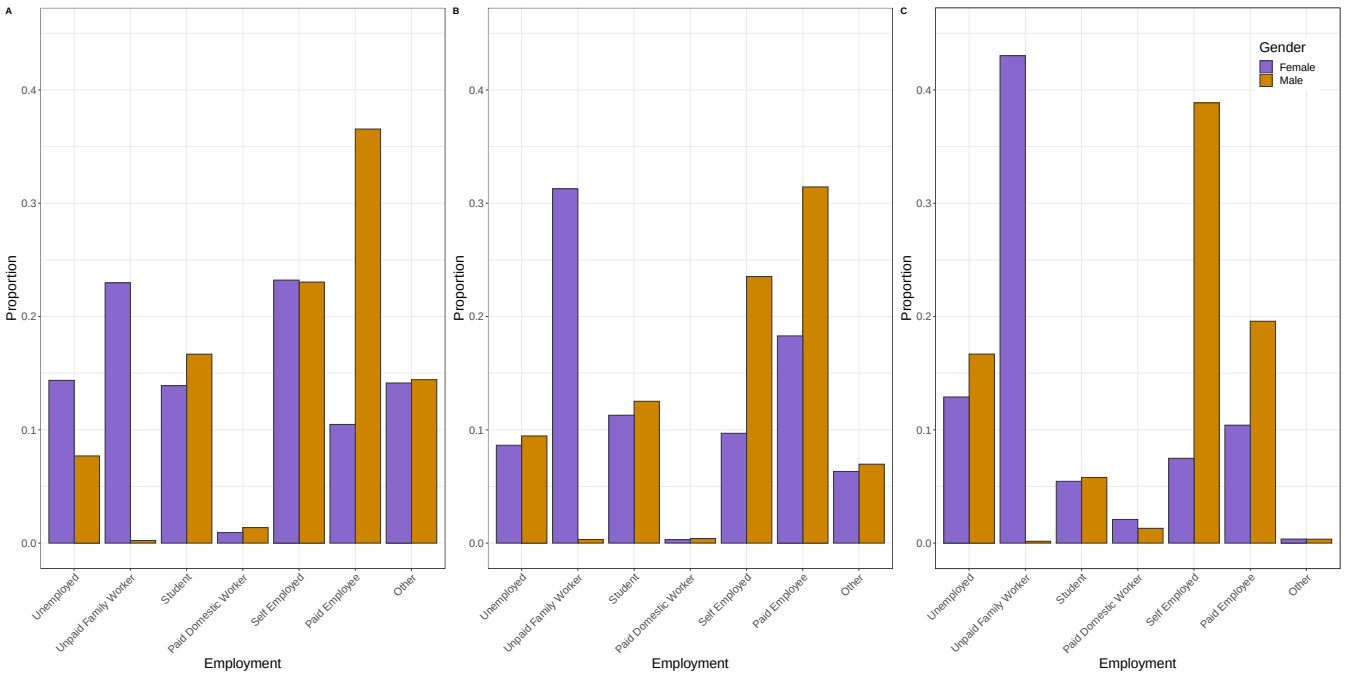


Figure3-7 Employment practices of male and female household residents who finished education in A; Blantyre, Malawi, B; Kathmandu, Nepal and C; Dhaka, Bangladesh

3.3.6. Births and Deaths

In Malawi there was a single census update recorded at the end of the passive surveillance period approximately two years after the initial census (**Error! Reference source not found.**). The number of births recorded were 3269 which results in a birth rate of 16.4 per 1000 people. In Nepal there was a census update at approximately 1 year with a final census at 2-years. In total, there were 1301 births recorded resulting in a birth rate of 6.4 births per 1000 people. In Bangladesh, there was a census update approximately every 6-months. In total, there were 2630 births recorded over the 2-year period resulting in a birth rate of 11.8 births per 1000 people. The number of deaths recorded in all 3 sites were lower with death rates of 1.5, 3.6 and 3.4, in Malawi, Nepal and Bangladesh respectively.

3.3.7. Migration

Migration rates at each of the sites were high (Figure3-8). This was calculated by determining the proportion of new individuals that had moved into households previously enumerated, and the proportion of entirely new households that were not present in the previous census, as well as the

proportion of individuals and households that had emigrated since the last census. In Malawi, the rate of immigration was 141.9 per 1000 and emigration of 150.7 with a net emigration of 8.8 per 1000 annually. In Nepal, the rate of immigration was 91.4 per 1000 people, with emigration of 134.5 per 1000 resulting in net emigration of 43.1 per 1000 people. In Bangladesh, rates of migration were even higher with over 25% of the population migrating annually. The immigration rate was 266.9 per 1000 people, with emigration of 270.3 resulting in a net emigration of 3.4 per 1000 people. The immigration of individuals from rural areas, where the incidence of *S.* Typhi is likely to be less than in the urban areas, is thought to be an important cause of on ongoing transmission of *S.* Typhi in the cities as immunologically susceptible individuals continue to relocate within the endemic zone, become infected and continuing to shed the pathogen into the environment.[230,238]

Error! Reference source not found. outlines the numbers of both individuals and households that were enumerated at each census with a breakdown by age- group. This provided the overall denominator population for the incidence and adjusted incidence estimates. Active households were the number of households that were present and enumerated at that census, with the active population being the total number of residents within those households. This was calculated at each census update by taking the total number of residents enumerated in all previous censuses, removing the deaths and emigrations and adding in the births and immigrations at the individual level, and removing the emigration and adding in the immigration at the household level. The total populations at each of the three sites remain relatively stable over the time period that this demographic census was conducted.

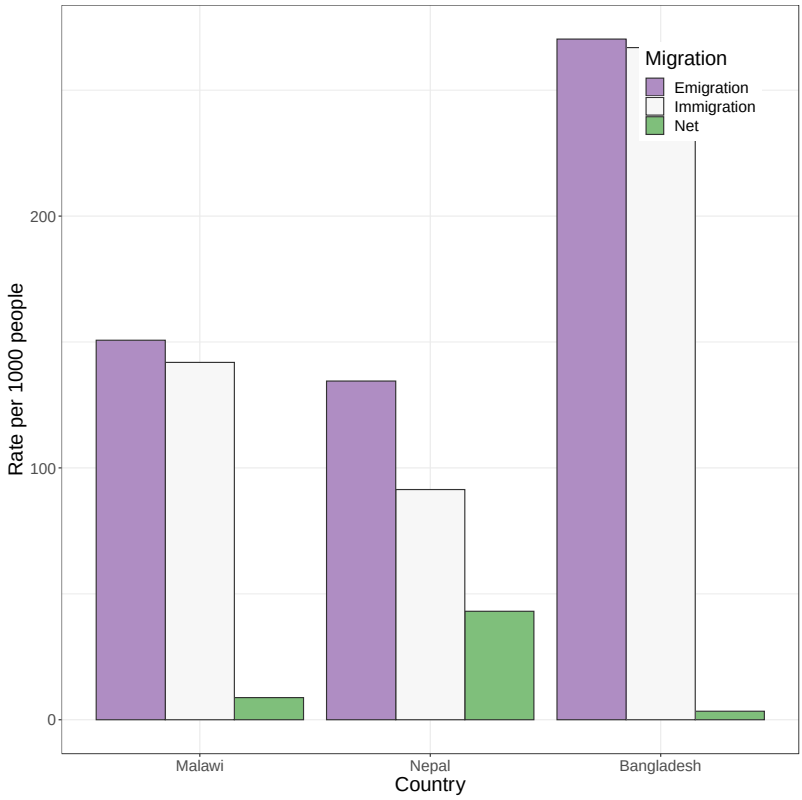


Figure3-8: Migration rates from the demographic census area per 1000 people from Malawi, Nepal and Bangladesh calculated through repeat census updates. Net migration was negative for all three sites.

Site / Census	Malawi			Nepal				Bangladesh				
	Baseline	Final	PYO	Baseline	Update 1	Final	PYO	Baseline	Update 1	Update 2	Final	PYO
Total Households	22364	29791		24405	27002	28597		26119	32042	36723	40645	
Total Population	97392	119573		101810	109791	115182		110731	132960	150506	164924	
Births	NA	3269		NA	617	684		NA	991	936	703	
Immigration	NA	6158		NA	2630	2614		NA	1732	2197	1408	
Deaths	NA	299		NA	282	456		NA	269	269	236	
Emigration	NA	7332		NA	3232	3225		NA	1541	2568	1811	
Vacant/Emigration Households	NA	6412		NA	2105	4150		NA	6032	4410	4093	
Vacant/Emigration Population	NA	22754		NA	7015	13907		NA	22455	16430	15459	
Active Households	22364	23826		24405	24897	22342		26119	26010	26281	26110	
Active Population	97392	102242	199634	101810	102590	101021	203614	110731	111418	112830	110963	222971
Age Groups												
0-4yrs	12673	16160	28833	6054	6449	6342	12563	10492	11500	11694	11362	22524
5-9yrs	12784	12576	25360	6766	6763	6701	13487	10181	10164	10251	10225	20411
10-14yrs	12991	13214	26205	7569	7665	7522	15171	10617	10665	10653	10537	21236
15-29yrs	31893	34464	66357	33380	32558	31479	62635	36097	35666	35907	35422	71546
30-49yrs	21666	20996	42662	30954	31474	31524	62634	30376	30487	30686	30593	61071
50+yrs	5385	5213	10598	17087	17430	17453	34647	12968	12936	12971	12824	25850

Table 3-1: Demographic Census and Census Update, including births, deaths, immigration and emigration for both indivuduals entering established household and entirely new housholds. In Malawi there was a single repeat census at 2 years, in Nepal there was a census update at approximately year 1 and year 2, in Bangladesh census updates were performed every 6 months for two years. Abbreviations: PYO; Person-years of observation

.

3.4. Results - Household Surveys for Water and Sanitation risk factors and Healthcare Utilisation

Using the demographic census, a randomised cohort of 735 households were visited by field workers in each of the sites to perform a detailed questionnaire capturing household level data on water, sanitation and hygiene practices (WASH), household construction, indicators of economic status and healthcare utilisation to provide data on known typhoid risk factors within each site, to inform understanding of healthcare usage and provide adjustment factors for blood-culture confirmed incidence of disease (**Error! Reference source not found.**).

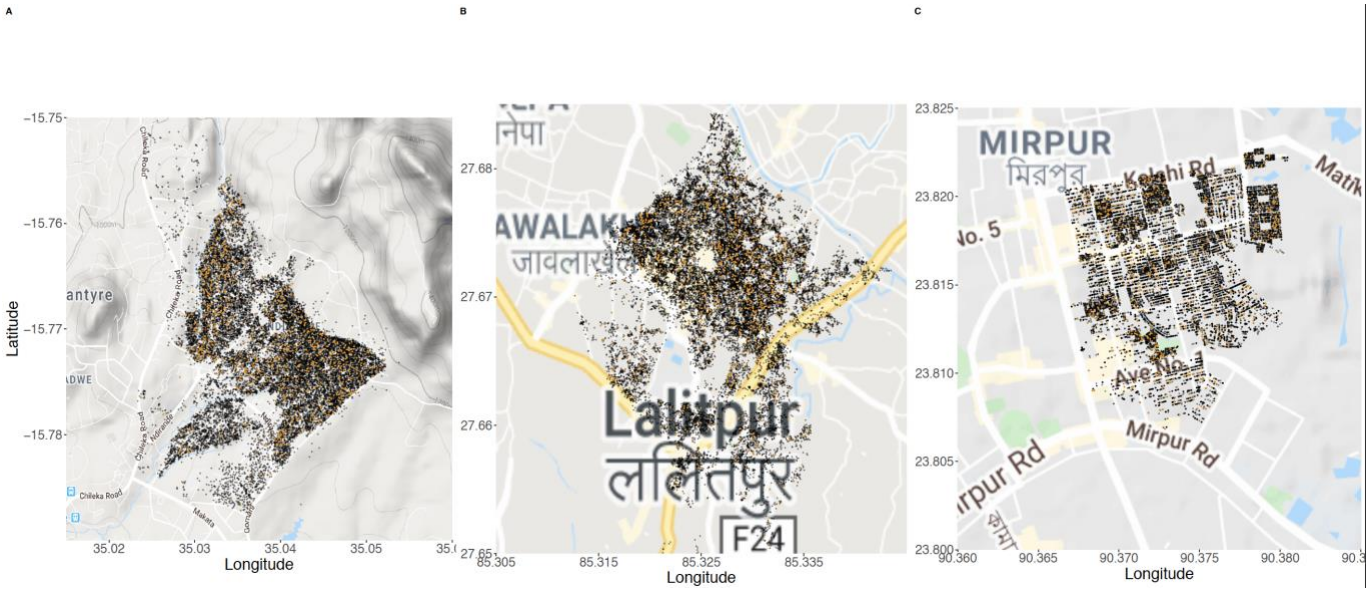


Figure 3-9: GPS located positions of households from the demographic census (black) and healthcare utilisation surveys (orange) for A; Blantyre, Malawi, B; Kathmandu, Nepal and C; Dhaka, Bangladesh

3.4.1. Water, Sanitation and Hygiene (WASH) description

The household head was asked which water source the household used for drinking water, washing utensils and bathing (Figure3-10). Respondents were asked whether they used the range of water sources always, sometimes or never. The term reservoir means water temporarily stored within the home within a closed system. This was the commonest water source in Malawi where there are significant periods of time when the piped water is turned off to entire townships due to lack of

supply, followed by communal taps, with occasional usage of communal boreholes. Water sources were used consistently despite different purposes for the water collected. In contrast, in Nepal the commonest source for drinking water was bottled water with reservoir water used for washing and bathing. Vendor usage in Nepal was also higher than the other sites, presumably from the well-publicised water tankers which operate widely throughout South-Asian cities.[239] In Bangladesh, the majority of households had access to a private tap, which they used for all purposes.

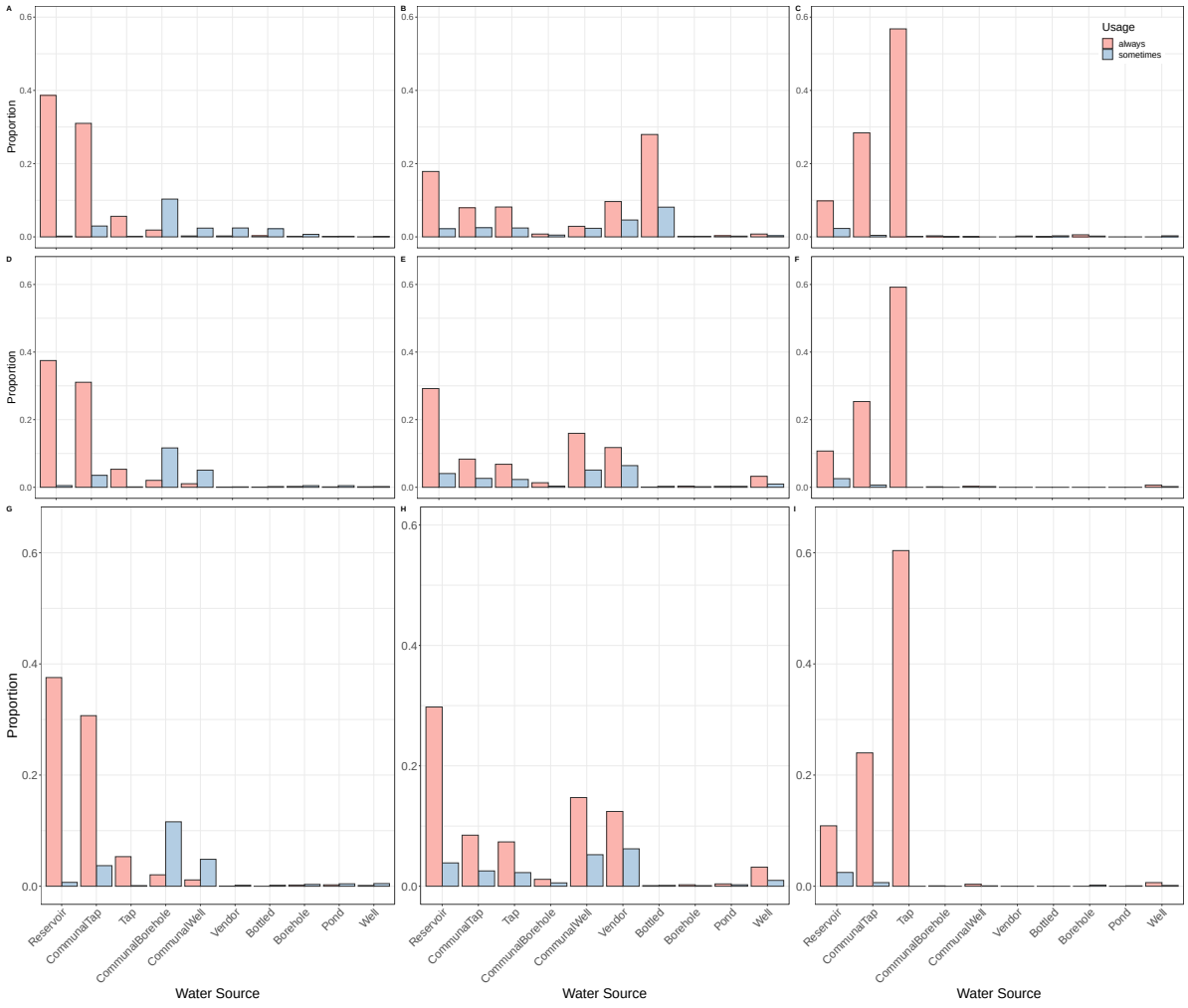


Figure3-10: Water Sources used for Drinking (A-C), Washing Utensils (D-F) and Bathing (G-I) in Malawi (Column 1), Nepal (Column 2) and Bangladesh (Column 3).

Toilet usage was different across sites with pit latrines used almost exclusively in Malawi (Figure3-11). A pit latrine is a toilet type that collects human faeces in a hole in the ground, entering through a drop hole in the floor.[240] This is one of the most basic and cheapest forms of sanitation. When latrines are

full, they are often filled in and in densely populated areas reside in close proximity to water sources. Ventilated latrines contain a single pipe which exits the pit externally to where the user sits removing unpleasant odours and preventing flies from accessing the faces and limiting spread of disease.[241]

The two Asia sites had improved toileting facilities with sanitary toilets, usually connected to a mains system which would remove the faces from the immediate geography, these are either with or without a water flush.

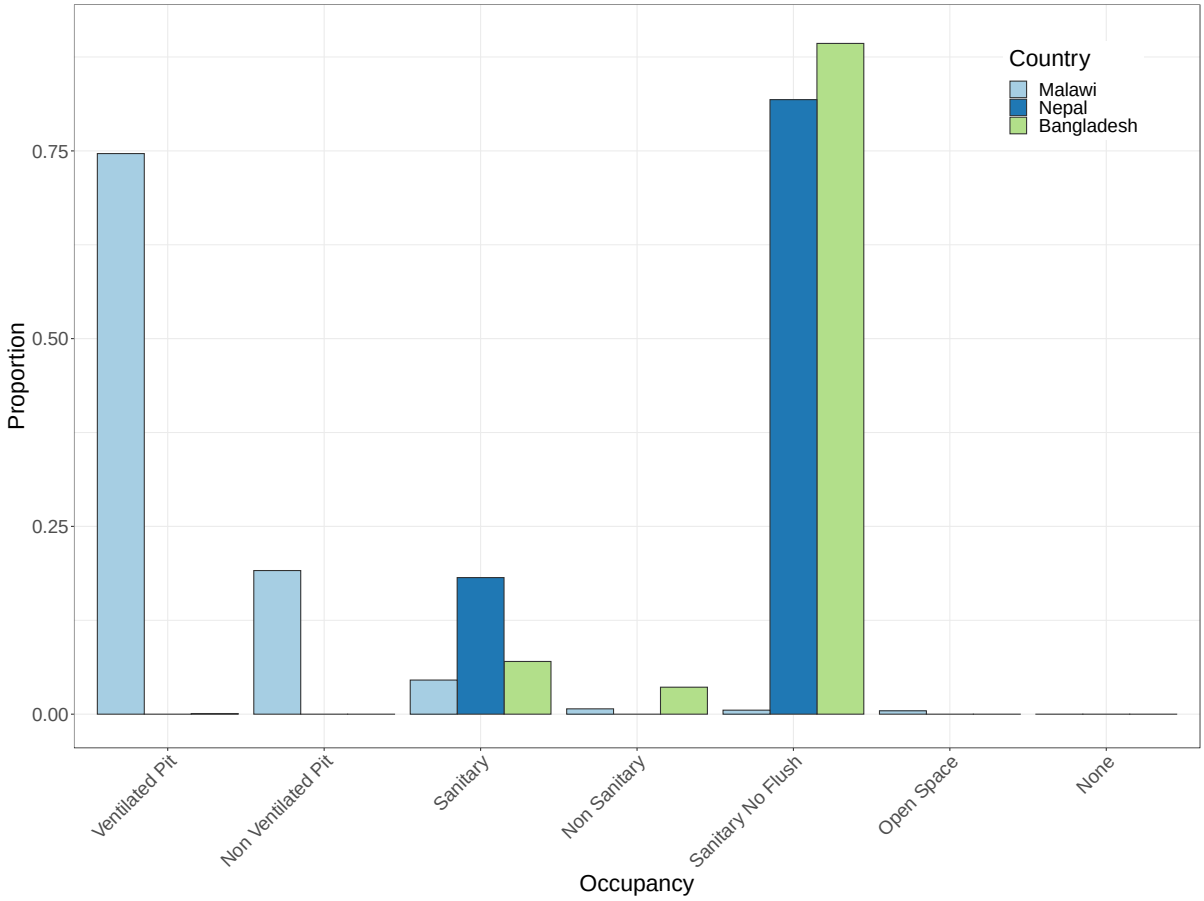


Figure3-11: Household toilet usage as reported by household head during household for Malawi, Nepal and Bangladesh.

3.4.2. Household Economic description

Questions were asked to determine the economic status of households within the census area. A higher proportion of individuals owned their own home in Nepal compared with Malawi and Bangladesh (Figure3-12). Households in Nepal also had a higher proportion of household assets when compared with Malawi and Bangladesh, specifically a higher number of devices used for transport

with more bicycles, motorbikes and cars, and more luxury items such as televisions (Figure3-13). Poverty is a known risk factor for *S.* Typhi infection and diarrhoeal disease more generally.[242] These data could be used to determine areas within the populations and identifying specific households that are at a higher risk for typhoid infection.

World bank statistics place Malawi at the bottom of the three countries studied here in terms of wealth, with a gross domestic product (GDP) per capita of $1,311, positioning it as the 6th poorest country in the world. Nepal is the next poorest with a GDP per capita of $3,089 with Bangladesh's GDP slightly higher at $4,371.[243] Our data would suggest the Nepal site is more affluent than the Bangladesh site, although our demographic area is from a highly mobile, working population who are likely to have a higher income than households in rural Nepal.

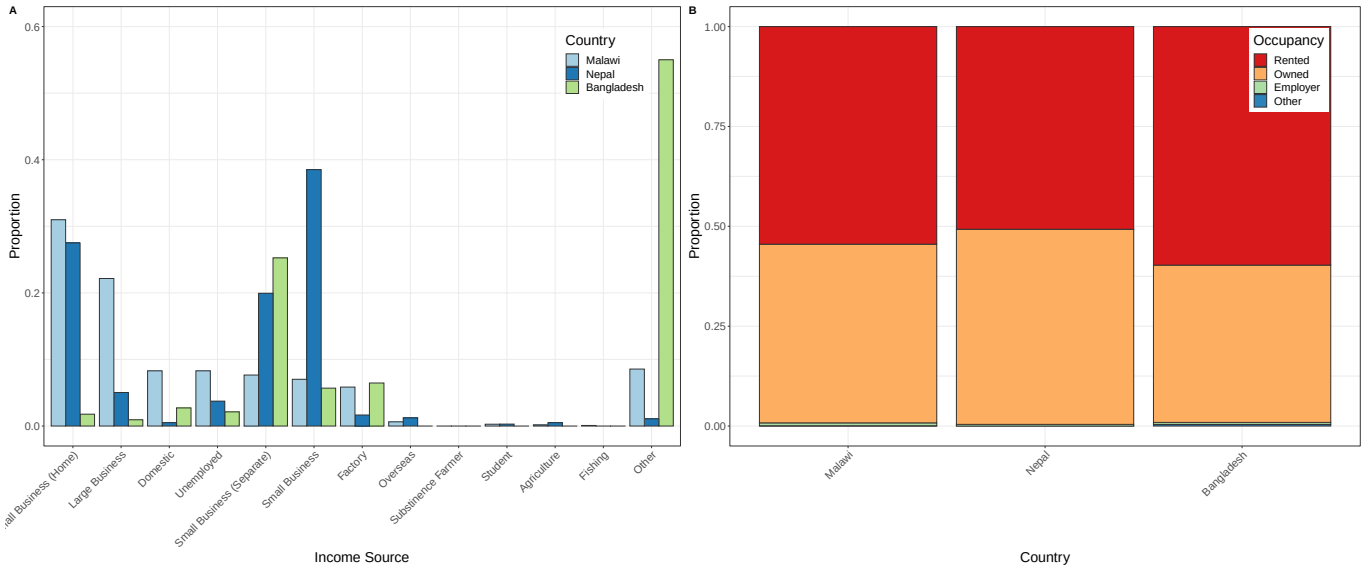


Figure3-12: A; The primary income source for the head of household, and B; the Occupancy status of the household with reference to the house they were inhabiting at the time of the survey.

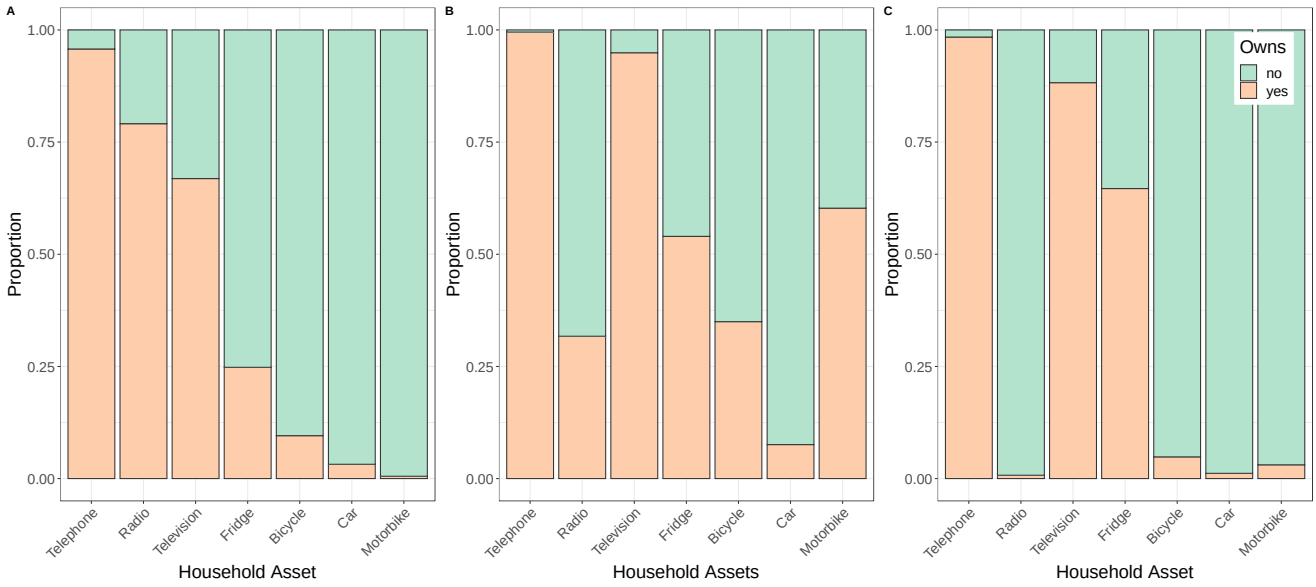


Figure3-13: Proportion of households who own certain household assets in A; Blantyre, Malawi, B; Kathmandu, Nepal and C; Dhaka, Bangladesh.

3.4.3. Healthcare Seeking Behaviour

Field teams asked the household head about whether any individual who resided within the household had been febrile for greater than or equal to three days within the last three months. Where the answer was yes, the actual healthcare seeking behaviour was recorded, outlining from a list of options (Figure3-15) which healthcare option they chose first. If the fever persisted, they were asked what they did next and so on. If there had been no fever within one of the age groups within the last three months, the hypothetical healthcare usage was recorded. The rationale for this was that recall of information is time-dependent and asking participants to remember beyond three months ago may have limited accuracy. Hypothetical-usage is to determine what they would do, if they were to have fever now.

3.5. Results - Fever Severity

The household head was also asked to grade the severity of fever as mild, moderate or severe (Figure3-14). In Malawi, 22% of respondents from within the household had an episode of fever. This was consistent across age groups (0-4years; 21%, 5-14years; 20%, >14years; 24%). Severe fever was highest in the oldest age groups, with severe and moderate fever combined highest in children under 5 years in age. In Nepal, respondents revealed a much lower rate of fever at 8% of household

members overall (0-4years; 5%, 5-14years; 9%, >14years; 9%). In Bangladesh, the overall rate of fever from respondents was 13% (0-4years; 5%, 5-14years; 9%, >14years; 9%) with the highest rate of severe fever in the youngest age group. Whilst this is a subjective measure and limited by the specific questioning from field teams and interpretation by respondents in very different cultural contexts, this pattern does reflect what was observed in the passive surveillance component of the study with higher rates of febrile children in Malawi and Bangladesh.

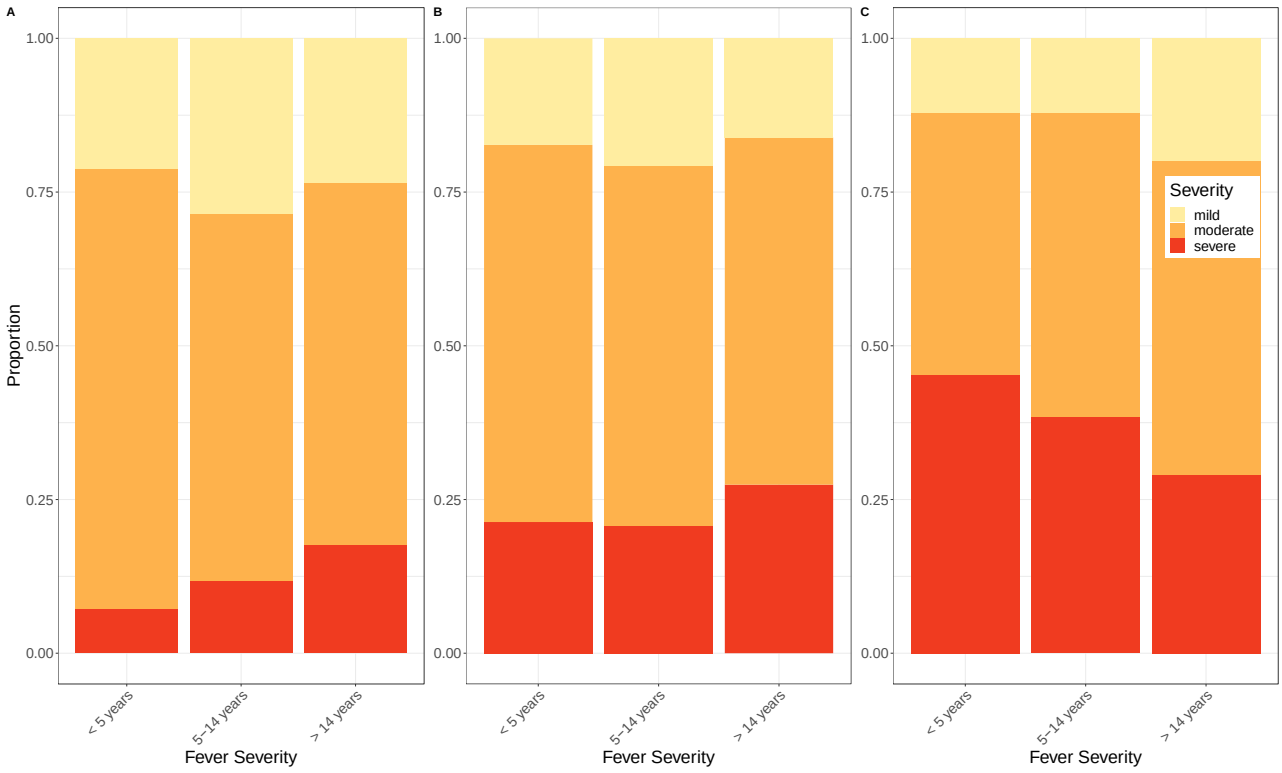


Figure3-14: Severity of fever, graded by household head for individuals who had fever for greater than or equal to 3 days in last 3 months for A; Blantyre, Malawi, B; Kathmandu, Nepal and C; Dhaka, Bangladesh.

3.6. Results - Healthcare Usage by Facility

When respondents indicated they had an episode of fever, field teams then gave them a list of options to determine their healthcare seeking behaviour, this firstly revealed some striking observations (Figure3-15) and secondly provided an estimate for the proportion of individuals who used study facilities and may have received a blood-culture during their febrile episode (Table 3-2: Proportion of individuals seeking healthcare at study facilities (95% binomial confidence intervals).

).

In Malawi, the commonest source of healthcare for fever was the government clinic which is situated within the township and offers free health care and prescriptions. This was used as first and second priority by most respondents, with nowhere and buying medication over the counter as second and third choice. Malawi had the highest usage of study facilities, with blood-culture collection for the study provided for patients in the government clinic and hospital. Within this low-income area the use of private pharmacies and clinics was relatively low where there would be a financial cost to the services provided.

In contrast, in the Asia sites the situation is very different. In Nepal the use of private clinics, leftover medication and pharmacies were all more commonly used than government healthcare facilities. The proliferation of pharmacies selling prescription-free, broad-spectrum antibiotic has been dramatic, with many facilities staffed by physicians providing advice on antimicrobial use. Most of these clinics provide antibiotics on a purely empirical basis without any formal diagnostic testing. In Nepal, this usage of alternative facilities resulted in a low proportion of individuals with fever utilising study facilities ranging from 0.11 in the 5-14-year age group to 0.21 in the 0-4-year age group. Free clinical care, diagnostic tests and prescriptions were provided for individuals within the demographic census area to try and incentivise use and the population were sensitised to study activities.

In Bangladesh a higher proportion of individuals used study facilities (0.19 – 0.33) than Nepal through a network of clinics within the population, however private pharmacies and traditional medication were the commonest. In Bangladesh, the study teams increased attendance by offering free care and prescriptions and performing regular household visits to remind the population of health facilities where blood-culture collection was being performed for the study.

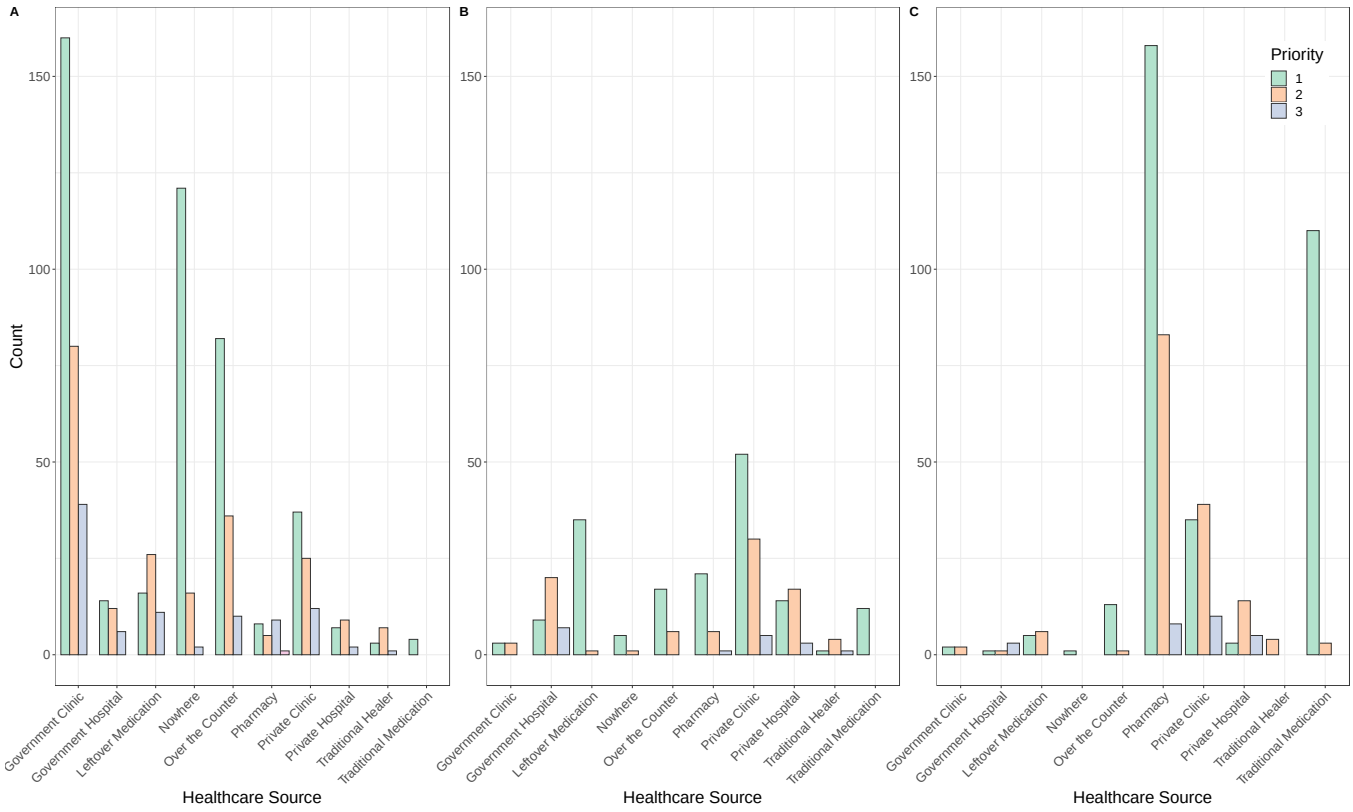


Figure3-15: Actual healthcare seeking behaviour in order of priority when respondents had fever greater than or equal to 3 days in A; Blantyre, Malawi, B; Kathmandu, Nepal and C; Dhaka, Bangladesh

Site / Age Group	Malawi	Nepal	Bangladesh
0-4years	0.59 (0.49-0.68)	0.21 (0.11-0.34)	0.32 (0.21-0.45)
5-9years	0.78 (0.68-0.87)	0.11 (0.01-0.22)	0.33 (0.24-0.46)
10-14years	0.78 (0.68-0.87)	0.11 (0.01-0.22)	0.33 (0.24-0.46)
15-29years	0.63 (0.53-0.74)	0.13 (0.02-0.25)	0.19 (0.11-0.27)
30+years	0.63 (0.53-0.74)	0.13 (0.02-0.25)	0.19 (0.11-0.27)
All	0.66 (0.60-0.72)	0.15 (0.10-0.22)	0.27 (0.22-0.33)

Table 3-2: Proportion of individuals seeking healthcare at study facilities (95% binomial confidence intervals).

3.7. Discussion

This component of the study was not designed as a case-control study to assess risk factors for individual cases of typhoid fever. However, these data have provided a wealth of information on the three sites studied, highlighting some of the similarities and differences between them.

These data show that Malawi is a low-income site with poor water and sanitation infrastructure within the township with most people relying on pit latrines and an intermittent water supply. Women still have a poorer educational level than men and the birth rate remains high. With a disease of poverty like typhoid fever, spread through the faecal-oral route these are relevant observations for an endemic site, and highlight the difficulty of long-term intervention. Improving the overall economic living standards and sanitation infrastructure for a place like Ndirande will be challenging and require a prolonged period of investment. Given that typhoid is only one of the country's most pressing health concerns alongside the ongoing HIV epidemic, high rates of tuberculosis and falciparum malaria, it is hard to see how these issues might be addressed in the short term. The site is very young, which means a high proportion of the population are at risk from typhoid infection, but with vaccine introduction and a catch-up campaign to 15 years, for example, a large proportion of the population would be protected, meaning disease rates could be impacted quickly through vaccine.

In Bangladesh, where comparatively the standard of living appears to be higher there are several risk factors for typhoid fever identified in this analysis. High migration rates from outside the city will be having an impact on the population of susceptible individuals. In addition, most residents within the census use either their own tap or a communal tap. This is municipal water piped into the wards but there is good evidence that this publicly provided water system has faecal contamination with 80% of tested water contaminated with coliforms in one study.[244,245] In Nepal, we found a similar collection of risk-factors. Whilst not presented in this chapter, both Nepal and Bangladesh have high rates of antimicrobial resistance, making typhoid fever harder to treat. The large number of healthcare facilities and greater diversity in healthcare seeking behaviour in a largely unregulated system makes

standardised diagnosis and treatment of enteric fever impossible, therefore limiting control within these populations. With vaccine introduction, there would be a reduction in the incidence of typhoid fever, and as a consequence a reduction in the incidence of febrile disease. This would have an impact on the amount and choice of antimicrobials prescribed, which in turn may have a positive impact on rates of antimicrobial resistance.[246]

Introduction of vaccine, whilst not improving any of the underlying causes presented in this chapter would have potential impact on disease rates despite them. With vaccine introduction it would have impact on the burden of disease much sooner than other long-term interventions and have the additional benefit of removing some of the burden typhoid fever causes on the healthcare system and society more generally which would enable wider investment elsewhere.

4. Chapter 4 – Passive Surveillance for febrile illness with participant characteristics, disease incidence and antimicrobial susceptibility

4.1. Introduction

To estimate the burden of enteric fever in three urban sites, facility-based passive surveillance was set up in healthcare facilities throughout the geographic census area. This chapter will describe the data from this component of the study and address the first four primary aims of this thesis, in addition to the aim on antimicrobial resistance:

1. Observed incidence rates of blood-culture confirmed enteric fever from facility-based passive surveillance.
2. Adjusted incidence rates of infection accounting for blood culture sensitivity, enrolment proportion and healthcare seeking behaviour.
3. Age-stratified incidence of clinical disease.
4. Seasonality of blood-culture confirmed infection.
5. Proportion of isolates from culture confirmed index cases demonstrating antibiotic resistance

This chapter details the enrolment of participants with blood-culture positive *S.* Typhi, *S.* Paratyphi and *S.* Typhimurium in the three sites studied, comparing their characteristics at enrolment and antimicrobial usage data. The observed incidence rates for the three sites are presented, and using data already discussed in chapter 3, adjusted incidence rates are calculated. Finally, antimicrobial susceptibility profiles are presented for all three sites.

4.2. Methods

The detailed description of the methods used for this chapter can be found in chapter 2, section 4 for the enrolment of participants through passive surveillance, and chapter 2, section 11 for the collection and processing of clinical samples.

Individuals who presented to the facilities from within the geographic area of the census were screened. Individuals who met the eligibility criteria of a recorded temperature of greater than or equal to 38^{o}C or gave a history of subjective fever for greater than or equal to two days were invited to be enrolled into the study. Clinical and demographic data were collected along with blood-culture

and EDTA. Where possible, enrolled individuals were linked back to their household from the demographic census.

4.3. Results - Blood Culture Positive Cases

During the two-year period of passive surveillance, a total of 11,529 individuals who met the fever criteria (3,726 in Malawi, 2,416 in Nepal and 5,387 in Bangladesh) were enrolled from ten healthcare facilities (two in Malawi, two in Nepal and six in Bangladesh).

Pathogenic bacteria were isolated from the blood cultures of 822 participants (7.1% of blood cultures taken): 162 (4.3%) in Malawi, 166 (6.9%) in Nepal, and 494 (9.2%) in Bangladesh. The commonest were *S.* Typhi with 624 isolates (76.2% of pathogenic bacteria isolated) (115 in Malawi, 150 in Nepal and 359 in Bangladesh), *S.* Paratyphi A with 107 isolates (12 in Nepal and 95 in Bangladesh), *S.* Typhimurium (16 in Malawi), *E. coli* (7 in Malawi, 1 in Nepal and 6 in Bangladesh) and *Streptococcus spp.* (3 in Malawi and 7 in Bangladesh). (Error! Reference source not found.)

Site / Bacteria	Malawi	Nepal	Bangladesh
No of cases (% of total)			
S. Typhi	116 (72)	150 (90)	359 (73)
S. Paratyphi A	0 (0)	12 (7)	95 (19)
S. Typhimurium	16 (10)	0 (0)	0 (0)
S. Paratyphi B	0 (0)	0 (0)	1 (0.1)
E. Coli	7 (4)	1 (0.5)	6 (1)
Streptococcus Pneumoniae	3 (2)	0 (0)	0 (0)
Salmonella Enteritidis	1 (1)	0 (0)	0 (0)
Neisseria spp	1 (1)	0 (0)	0 (0)
Staphylococcus aureus	0 (0)	3 (0.2)	0 (0)
Klebsiella pneumoniae	0 (0)	2 (0.1)	3 (0.5)
Streptococcus spp	0 (0)	0 (0)	7 (1)
Eneterococcus	0 (0)	0 (0)	5 (1)
Corynebacterium spp.	0 (0)	0 (0)	3 (0.5)

Other	0 (0)	0 (0)	11 (2)

Table 4-1: Total number of pathogenic bacteria isolated from blood cultures during the two-year surveillance period

The overall isolation rate for *S.* Typhi from blood-cultures collected was 5% for the three sites combined (3% in Malawi, 6% in Nepal and 7% in Bangladesh). *S.* Typhi was the main organism found in blood-culture causing febrile disease by a considerable margin with other *Salmonellae* organisms adding to this burden (*S.* Paratyphi A in the Asia sites and *S.* Typhimurium in Malawi). The overall contamination rate was 7% (9% in Malawi, 2% in Nepal, 10% in Bangladesh).

4.3.1. Blood Culture Positivity by Time

Blood culture positivity rates differed over time at all three sites (Error! Reference source not found.). *S.* Typhi was isolated throughout the entire two-year surveillance period, but no clear seasonal patterns were observed. All three sites experience a high period of rainfall restricted to a roughly four-month period, monsoon in the Asia sites and the rainy season in Malawi where rates of typhoid fever usually increase. There was an increase in cases during the first rainy season in Malawi and during the second monsoon in Bangladesh, but this wasn't observed in Nepal. Part of the reason for this was the changing nature of surveillance over the surveillance period. All three sites improved their surveillance and enabled more blood cultures to be collected at their surveillance facilities. This was achieved by extra staffing and physical space in the clinics so multiple individuals could be enrolled at the same time. Extra clinics from within the census area were used for blood culture collection and certain clinics stayed open into the evening and at the weekend. The enrolment criteria were also broadened to ensure any individual with a temperature or history of fever regardless of localising clinical symptoms was approached for enrolment. Finally, the history of fever was reduced from three days to two days. In Malawi and Nepal this resulted in the positivity rate gradually decreasing over the two-year period as more blood cultures were collected from febrile individuals. In

Bangladesh, this increase in the number of blood cultures collected demonstrated an increasing amount of enteric fever causing febrile illness.

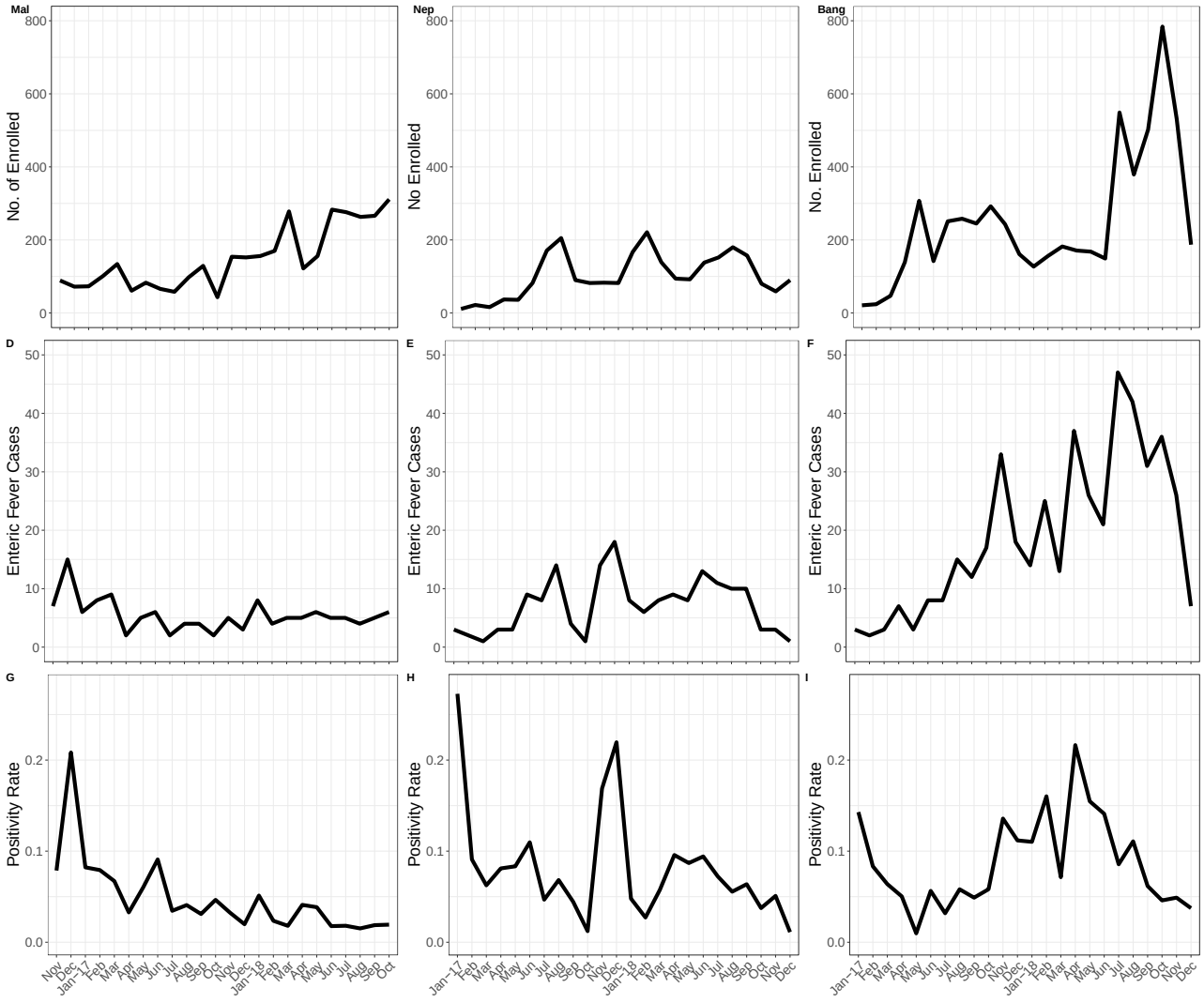


Figure 4-1: A-C Numbers of individuals enrolled from whom a blood culture was collected, D-F: Number of S. Typhi blood culture-confirmed cases, G-I: Positivity rate over the two-year surveillance period. Grey bars indicate Monsoon season.

4.3.2. Positivity by Age

In Malawi the health facilities struggled to enrol all eligible individuals. Figure4-2A demonstrates firstly, the large number of young children within this population and secondly in Figure4-2D, the large number of children in the youngest age group presenting with fever. We have already noted that the study facilities in Malawi had the highest healthcare usage for all three sites with almost 60% of children under 5 years of age visiting the Government health clinic. Malawi is a malaria endemic

country, and whilst incidence is generally on the decline, is still responsible for many cases of fever in young children. In Nepal, the situation was quite different, with a smaller proportion of young children in the demographic census area, and a much higher usage of alternative health care sources, therefore the number of young children enrolled was much smaller. In Bangladesh, the clinical teams enrolled large numbers of children and particularly in the 0-4 year and 5-9-year age group confirmed high numbers of blood culture-confirmed *S.* Typhi.

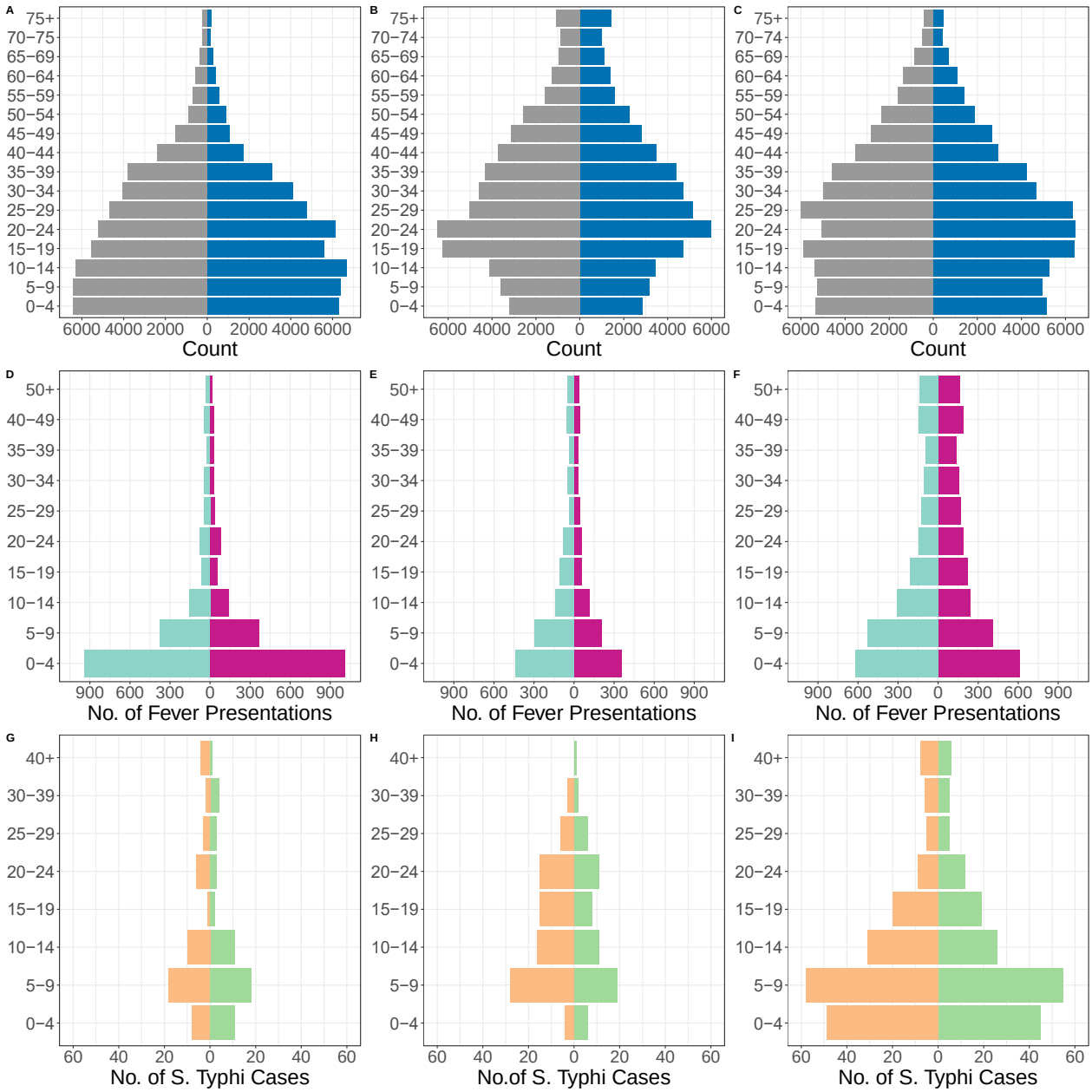


Figure4-2: Population pyramid for the A-C; Demographic Census, D-F; Passive surveillance enrolled participants and G-I; S. Typhi blood culture-confirmed cases, for Column 1; Malawi, Column 2; Nepal and Column 3; Bangladesh.

Figure4-3 demonstrates the blood culture positivity rate across the different age groups. For Malawi and Nepal, the positivity rate is low in the 0-4-year age group. Young children have more febrile illness from other aetiologies than older children and adults, namely viral respiratory tract infections, and so the contribution typhoid makes to the total amount of febrile illness should be less. Positivity rates increase through the 5-9 and 10-14-year age groups and reduce again as incidence of disease reduces in adults (). In Bangladesh, the high positivity rates throughout children of all ages possibly indicates

that despite the high number of cases cultured, the clinical teams may not have been enrolling enough cases as you would expect positivity rates to be lower. This may have had the consequence of missing clinical episodes of *S.* Typhi infection.

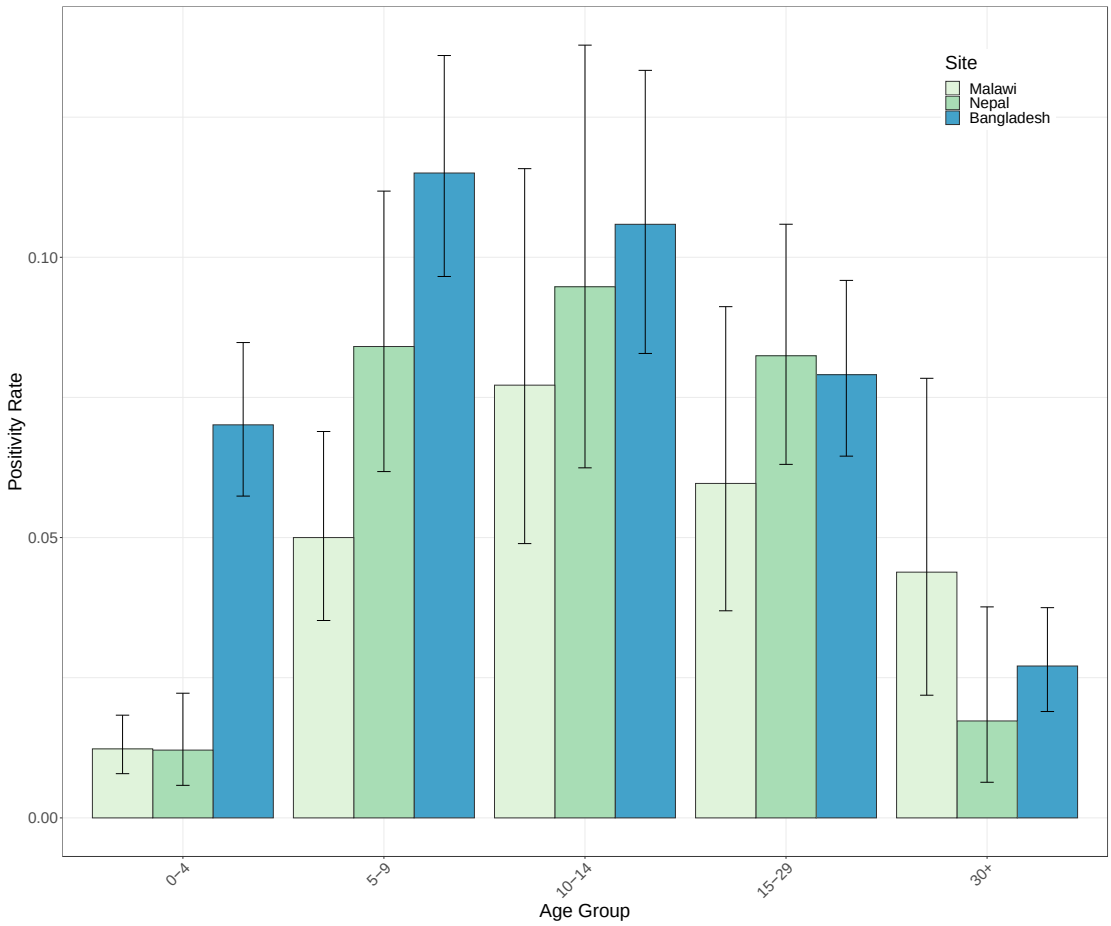


Figure4-3: Positivity Rate of S. Typhi in blood cultures collected by age-group across the three sites studied with error bars denoting the 95% confidence intervals

Site / Age Group	Malawi	Nepal	Bangladesh
0-4years	0.01 (0.01-0.02)	0.01 (0.01-0.02)	0.07 (0.06-0.08)
5-9years	0.05 (0.04-0.07)	0.08 (0.06-0.11)	0.12 (0.10-0.14)
10-14years	0.08 (0.05-0.11)	0.09 (0.06-0.14)	0.11 (0.08-0.13)
15-29years	0.06 (0.04-0.09)	0.08 (0.06-0.1)	0.08 (0.06-0.10)
30+years	0.04 (0.02-0.08)	0.02 (0.01-0.03)	0.03 (0.02-0.04)

Table 4-2: Positivity Rate of S. Typhi in blood cultures collected by age-group across the three sites studied with 95% confidence intervals in parenthesis

4.4. Results - Participant Characteristics

The median age of confirmed cases of typhoid fever was 9.9 years (IQR 6-17) in Malawi, 12.9 years (IQR 8-21) in Nepal, and 8.2 years (IQR 4-16) in Bangladesh. Cases of blood culture-positive typhoid fever were significantly younger than those with a negative blood culture in Bangladesh ($p< 0.0001$), but older in Malawi ($p< 0.0001$) and Nepal ($p< 0.0001$). As has been shown previously, there was a male predominance for culture positive cases in the Asia sites. Individuals with blood culture-confirmed typhoid fever had a significantly longer duration of fever and a higher recorded temperature at enrolment than blood culture-negative individuals at all three sites ($p< 0.0001$). Between 89-98% of all *S.* Typhi blood culture-confirmed cases were enrolled from community, out-patient clinics. Hospital admission rates were low in all three sites (7.9% in Malawi, 3.3% in Nepal and 2.5% in Bangladesh), and only Malawi reported deaths secondary to typhoid infection. Prior antimicrobial usage and prescription of antimicrobials following blood culture-collection was significantly higher in blood culture-positive individuals across all three sites (Error! Reference source not found.).

Blood culture-confirmed participants from Malawi had a longer duration of fever (median 6 days vs 4 days in Nepal and Bangladesh), a higher recorded temperature (median 38.6^{o}C vs 38.3 in Nepal and 37.7 in Bangladesh) and the highest proportion of hospitalisations compared with the other two sites, indicating a higher proportion of severe disease.

4.4.1. Physical Characteristics of Enrolled Participants

Physical characteristics of individuals enrolled through passive surveillance were not collected comprehensively at the sites. However, from the data that are available there are some notable comparisons to be made (Error! Reference source not found.). In individuals over 12 years of age from Malawi and Bangladesh, there was a higher heart rate and lower blood pressure recorded in blood culture-confirmed *S.* Typhi cases compared with culture negative individuals. This would be in-

keeping with a gram-negative bacteraemia causing a systemic inflammatory response with tachycardia and a degree of hypotension compared with other causes of non-bacterial fever that do not cause this systemic response. However, from all three sites in children less than 12 years of age there was a general trend to a lower heart rate in *S.* Typhi blood culture-confirmed cases compared with the culture negative individuals. As discussed in the introduction there is a documented phenomenon with typhoid fever of pulse-temperature dissociation, where patients have a slower heart rate than would be expected. The lack of uniformity in the cohorts studied here makes it difficult to draw any firm conclusions, and indeed, presentation may depend on age, duration of illness and overall severity of the episode more than any inherent difference in presentation in these different epidemiological contexts.

Middle upper-arm circumference was not measured in Nepal, and no clear pattern was observed in the other two sites.

Site	Malawi			Nepal			Bangladesh		
	S. Typhi	*S.* Typhimurium	Blood Culture Negative	*S.* Typhi	*S.* Paratyphi A	Blood Culture Negative	*S.* Typhi	*S.* Paratyphi A	Blood Culture Negative
Total Enrolled	116	16	3,594	150	13	2,255	359	95	4,931
Enrolled (number of patients with available data)	105	12	3,594	150	13	2,255	359	96	4,931
Community Clinic, number of patients (% of total)	93 (89)	11 (92)	3385 (94)	148 (98)	13 (100)	2,187 (97)	352 (98)	95 (100)	4,894 (99)
Hospital, number of patients (% of total)	12 (8)	1 (8)	209 (6)	2(2)	0 (0)	68 (3)	7 (2)	0 (0)	37 (1)
Participant Characteristics									
Male, number of patients (% of total)	53 (50)	5 (42)	1791 (50)	87 (58)	8 (62)	834 (57.5)	185 (51.5)	56 (59)	2429 (49.3)
Median age, years (IQR)	**9.9 (6-17) ***	**1.6 (1.4-2.9) @**	**4 (2-10) §**	**12.9 (8.4-21.2) ***	11.9 (8-24)	8.1 (3.7-20)	**8.2 (4.8-16) ***	12.4 (7.6-24.1)	12.4 (5-28)
Median recorded temperature ^{o}C (IQR)	**38.6 (38.1-39.1) ***	38.3 (37.9-38.8)	38.2 (37.7-38.8)	**38.3 (37-39) ***	37.6 (36.9-38.2)	37.7 (36.7-38.5)	**37.8 (37-38.5) ***	37.8 (36.9-38.5)	37.2 (36.5-38.2)
Median duration of fever days (IQR)	**6 (3-7) ***	3 (2.8-8.8)	3 (2-3)	**4 (3-6) ***	3 (3-5)	3 (3-5)	**4 (4-7) ***	5 (4-7)	4 (3-7)
Hospitalisation number of patients (% of total)	8 (7.9)	2 (17)	NA	5 (3.3)	0 (0)	NA	10 (2.5)	1 (1)	NA
Median duration of hospitalization, days (IQR)	##	##	NA	9.5 (8-10)	NA	NA	5.5 (3-7)	3	NA
Deaths number of patients (% of total)	2 (2)	3 (19)	NA	0 (0)	0 (0)	NA	0 (0)	0 (0)	NA
Antibiotics in prior 2weeks number of patients (% of total)	**28 (28) ***	**5 (32) @**	281 (7.8)	**47 (31) ***	3 (23)	378 (16.7)	**123 (34) ***	30 (32)	774 (15.7)
Antibiotic prescribed at visit number of patients (% of total)	82 (71)	**9 (75) ^**	2693 (74)	**120 (81) ***	**11 (85) ^**	**1276 (57) §**	**337 (94) ***	88 (93)	**2117 (43) §**

Table 4-3: Characteristics for participants enrolled through passive surveillance with blood culture collection

Abbreviations: IQR, Inter-quartile range. Statistical significance within each country is demonstrated through the following annotations (no significance testing across countries is shown here):

**S. Typhi vs Blood Culture Negative p-value < 0.0001*

^ S. Typhi vs S. Paratyphi A/Typhimurium p-value <0.05
@ S. Typhi vs S. Paratyphi A/Typhimurium p-value <0.0001
§ S. Paratyphi A/Typhimurium vs Blood Culture Negative p-value <0.05

Site	Malawi			Nepal			Bangladesh		
	S. Typhi	S. Typhimurium	Blood Culture Negative	S. Typhi	S. Paratyphi	Blood Culture Negative	S. Typhi	S. Paratyphi	Blood Culture Negative
HR (bpm)									
<1yr (IQR)	NA	115	132 (120-145)	124 (124-124)	NA	124 (115-136)	**90 (89-93) ***	110 (110-110)	100 (95-107)
1-2yrs (IQR)	120 (120-120)	120 (87-126)	130 (115-145)	90 (90-90)	NA	120 (109-133)	98 (91-100)	**85 (85-85) ^**	97 (90-100)
2-5yrs (IQR)	120 (106-128)	116 (106-128)	128 (115-141)	118 (108-149)	NA	120 (100-130)	92 (88-98)	92 (89-96)	92 (88-98)
5-12yrs (IQR)	120 (101-122)	104 (104-104)	120 (103-130)	**100 (86-108) ***	**124 (115-133) ^**	104 (94-120)	86 (82-90)	88 (84-90)	86 (82-90)
>12yrs (IQR)	**105 (96-120) ***	NA (86-90)	100 (88-114)	92 (84-100)	89 (86-90)	90 (80-100)	**76 (74-80) ***	78 (74-78)	76 (72-80)
SBP (mmHg)									
<1yr (IQR)	NA	NA	NA	NA	NA	100 (90-140)	75 (73-80)	75 (75-75)	80 (75-85)
1-2yrs (IQR)	NA	NA	NA	NA	NA	120 (109-133)	80 (75-85)	75 (75-75)	80 (72.5-87.5)
2-5yrs (IQR)	NA	NA	NA	86 (86-86)	NA	120 (100-130)	85 (80-85)	85 (76-88)	85 (75-90)
5-12yrs (IQR)	NA	NA	NA	90 (90-100)	90 (90-90)	100 (90-110)	90 (88-95)	**90 (88-95) ^**	**90 (85-95) §**
>12yrs (IQR)	111 (103-120)	NA	117 (105-129)	**100 (100-110) ***	110 (105-113)	110 (100-110)	110 (100-110)	**110 (100-110) ^**	110 (100-120)
MUAC (cm)									
<1yr (IQR)	NA	13 (13)	14 (13-15)	NA	NA	NA	13.6 (13.4-13.9)	13.3 (13.3-13.3)	13.7 (13-14.3)
1-4yrs (IQR)	15 (14-16)	14.3 (13-16)	15 (14-16)	NA	NA	NA	14.5 (13.6-15.3)	14.3(13.4-15.2)	14.4 (13.5-15)
5-9yrs (IQR)	16 (15-16.5)	17 (17)	15.5 (10-16.5)	NA	NA	NA	15.8 (14.9-17.2)	15.8 (15-17)	15.9 (14.6-17.3)
10-14yrs (IQR)	18.2 (15.5-19.1)	NA	NA	NA	NA	NA	19.7 (17.4-21.8)	19.6 (17.5-20.4)	19.5 (17.9-21.7)
Blood Culture Volume Collected (ml)									
0-4yrs	3 (3-3)	NA	3 (3-5)	3 (3-3)	NA	3 (3-3)	3 (3-3)	3 (3-3)	3 (3-3)
5-14yrs	3 (3-3)	3 (3-4.5)	3 (3-5)	3 (3-3)	3 (3-4.5)	3 (3-3)	3 (3-3)	3 (3-3)	3 (3-3)
15+yrs	5 (5-7)	5 (4.5-5)	5 (3-5)	5 (5-5)	5 (4.5-5)	5 (3-5)	5 (5-5)	5 (5-5)	5 (5-5)

Table 4-4: Physical Characteristics for participants enrolled through passive surveillance with blood culture collection

Abbreviations; IQR, Inter-quartile range; HR, Heart Rate; bpm, beats per minute; SBP, Systolic blood pressure; mmHg, millimetres of mercury; MUAC, mid-upper arm circumference; ml, millilitres.

*S. Typhi – BC Negative p-value < 0.05

^ S. Typhi – S. Paratyphi p-value <0.05

§ S. Paratyphi – BC Negative <0.05

4.5. Results - Antibiotics Consumption

At the time of enrolment to passive surveillance participants were asked if they had received antibiotics in the preceding two weeks. In all three sites, participants who went on to be blood culture positive for *S.* Typhi had a statistically significantly higher antibiotic consumption rate than blood culture negative individuals (Malawi 0.28 vs 0.08 ($p<0.0001$), Nepal 0.31 vs 0.17 ($p<0.0001$), Bangladesh 0.34 vs 0.16 ($p<0.0001$)). In addition, blood culture positive individuals in Nepal and Bangladesh were more likely to be prescribed antibiotic (Nepal 81% vs 57% ($p<0.0001$), Bangladesh 94% vs 43% ($p<0.0001$)) at the time of blood culture collection (**Error! Reference source not found.**).

The antibiotics purchased prior to seeking healthcare and prescribed from study facilities at the sites were different, reflective of local resistance patterns and availability. In Malawi more ciprofloxacin and cotrimoxazole were used. The use of ciprofloxacin in local healthcare facilities has only been present since the introduction of MDR *S.* Typhi organisms around 2010. Cotrimoxazole remains a very widely used antibiotic particularly in adults for the prevention of opportunistic infections in patients with HIV. In Nepal, azithromycin was the commonest antibiotic prescribed from the healthcare facility. This is same facility from which numerous typhoid fever treatment trials have been performed, most recently showing reduced efficacy of fluoroquinolone compared with ceftriaxone for treatment of uncomplicated *S.* Typhi infection.[147,247,248] In Bangladesh, where there have been reports of emerging azithromycin resistance,[249] cefixime was the commonest antibiotic prescribed from the healthcare facilities. (**Error! Reference source not found.** and **Error! Reference source not found.**).

Site	Malawi			Nepal			Bangladesh		
	S. Typhi	S. Typhimurium	Blood Culture Negative	S. Typhi	S. Paratyphi	Blood Culture Negative	S. Typhi	S. Paratyphi	Blood Culture Negative
Total Number Enrolled	116	16	3,594	150	13	2,255	359	95	4,931
Antibiotic Prescribed (% of Total Enrolled)									
Total	82 (71)	9 (75)	2693 (73.9)	120 (79.4)	9 (90)	1276 (56.6)	337 (93.8)	88 (92.6)	2117 (43.3)
Cefixime	2 (2)	0 (0)	7 (0.2)	3 (2)	0 (0)	13 (0.6)	228 (63.5)	64 (67.3)	985 (19.9)
Azithromycin	0 (0)	0 (0)	0 (0)	80 (53)	7 (70)	411 (18)	89 (24.8)	22 (23.1)	971 (19.7)
Ciprofloxacin	26 (22)	2 (13)	189 (5)	0 (0)	0 (0)	2 (0.1)	8 (2.2)	1 (1.1)	38 (0.7)
Co-Amoxiclav	0 (0)	0 (0)	17 (0.5)	0 (0)	0 (0)	50 (2)	4 (1.1)	0 (0)	34 (0.7)
Amoxicillin	15 (13)	2 (13)	637 (18)	7 (5)	0 (0)	673 (30)	3 (0.8)	1 (1.1)	71 (1.4)
Cefuroxime	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (0.5)	0 (0)	11 (0.2)
Ceftriaxone	4 (3)	4 (25)	67 (2)	3 (2)	0 (0)	1 (0.1)	1 (0.27)	0 (0)	4 (0.08)
Cephradine	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (0.5)	0 (0)	3 (0.06)
Cotrimoxazole	20 (17)	4 (25)	1523 (42)	0 (0)	1 (10)	20 (1)	0 (0)	0 (0)	0 (0)
Chloramphenicol	1 (1)	0 (0)	1 (0.1)	1 (1)	0 (0)	1 (0.1)	0 (0)	0 (0)	0 (0)
Ofloxacin	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	45 (2)	0 (0)	0 (0)	0 (0)
Other	4 (3)	3 (18)	901 (25)	30 (20)	1 (10)	32 (1)	0 (0)	0 (0)	20 (0.4)
None	23 (20)	1 (7)	252 (7)	23 (15)	0 (0)	960 (43)	22 (6.1)	7 (7.3)	2794 (56.6)

Table 4-5: Antibiotics prescribed at enrolment to the study after blood culture collection

Site	Malawi			Nepal			Bangladesh		
	S. Typhi	S. Typhimurium	Blood Culture Negative	S. Typhi	S. Paratyphi	Blood Culture Negative	S. Typhi	S. Paratyphi	Blood Culture Negative
Total Number Enrolled	116	16	3,594	150	13	2,255	359	95	4,931
Antibiotic Prescribed (% of Total Enrolled)									
Total	28 (28)	5 (41)	281 (8)	47 (31)	1 (10)	378 (17)	123 (34)	30 (32)	774 (16)
Cefixime	0 (0)	0 (0)	0 (0)	8 (5)	0 (0)	75 (3)	43 (12)	9 (9)	233 (5)
Azithromycin	1 (1)	0 (0)	2 (0.1)	12 (8)	0 (0)	73 (3)	37 (10)	11 (12)	276 (6)
Ciprofloxacin	0 (0)	0 (0)	10 (0.3)	1 (1)	0 (0)	8 (0.4)	26 (7)	8 (8)	139 (3)
Co-Amoxiclav	0 (0)	0 (0)	1 (0.1)	0 (0)	0 (0)	21 (1)	1 (0.3)	0 (0)	2 (0.1)
Amoxicillin	6 (5)	3 (25)	85 (2)	14 (9)	0 (0)	102 (5)	6 (2)	1 (1)	38 (1)
Combination	0 (0)	0 (0)	0 (0)	2 (1)	0 (0)	18 (1)	3 (1)	0 (0)	3 (0.1)
Cotrimoxazole	15 (13)	2 (15)	142 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Chloramphenicol	0 (0)	0 (0)	1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ofloxacin	0 (0)	0 (0)	0 (0)	4 (3)	0 (0)	24 (1)	0 (0)	0 (0)	0 (0)
Other	9 (8)	0 (0)	38 (1)	8 (5)	1 (10)	41 (2)	48 (1)	0 (0)	48 (1)
Unknown	0 (0)	0 (0)	2 (0.1)	2 (1)	0 (0)	15 (1)	32 (1)	1 (1)	32 (1)

Table 4-6: Antibiotics taken in the 2 weeks prior to seeking healthcare and enrolment at one of the study facilities

4.6. Results - Incidence rates

4.6.1. Observed Incidence Rates

The overall observed or unadjusted incidence rate of typhoid fever was 58 (95% CI: 48-70) per 100,000 person-years of observation (pyo) in Malawi, 74 (95% CI: 62-87) per 100,000 pyo in Nepal, and 161 (95% CI: 145-179) per 100,000 pyo in Bangladesh. Age-stratified unadjusted incidence rates for *S.* Typhi were highest in the 5-9-year age group in all three sites; however, in Bangladesh the incidence rates for the 4th and 5th year of life were higher at 643 (95% CI: 424 – 936) and 620 (95% CI: 405 – 908) cases per 100,000 pyo, respectively and in Malawi the incidence rate was highest in the 5th year of life at 190 (95% CI: 91-350) cases per 100,000 pyo (Error! Reference source not found.). For *S.* Paratyphi A, incidence rates were again highest in the 5-9-year age group in Bangladesh and Nepal. In Malawi, *S.* Typhimurium peaked in the second year of life, with an incidence rate of 124 (CI 45-269) per 100,000 pyo. All three sites isolated *S.* Typhi in children under 2 years of age, with the youngest case aged 10 months in Bangladesh (Error! Reference source not found.).

In estimates for global typhoid incidence, areas of the world with incidence rates of 0-10 per 100,000 pyo are considered low incidence, 10-100 per 100,000 are medium incidence, >100 per 100,000 pyo are high incidence and >500 per 100,000 are deemed very high incidence. Even before incidence adjustment we have demonstrated high rates of typhoid incidence overall in Bangladesh, with very high rates in some of the younger age groups.

Site / Age	Malawi			Nepal			Bangladesh		
	Crude cases	Population	Unadjusted IR (95% CI)	Crude cases	Population	Unadjusted IR (95% CI)	Crude cases	Population	Unadjusted IR (95% CI)
Age	***S.* Typhi**			***S.* Typhi**			***S.* Typhi**		
0-11mnths	1	3416	20 (1-112)	1	1466	34 (1-190)	3	2798	54 (11-157)
12-23mnths	1	3135	20 (1-115)	1	1201	42 (1-232)	14	2125	329 (180-553)
24-35mnths	6	2570	124 (46-271)	1	1194	41 (1-233)	24	2144	560 (359-833)
36-47mnths	6	2686	110 (41-240)	2	1164	86 (10-310)	27	2098	643 (424-936)
48-59mnths	10	2610	190 (91-350)	4	1256	159 (43-408)	26	2097	620 (405-908)
0-4yrs	24	14417	83 (53-124)	9	6282	72 (33-136)	94	11262	417 (337-511)
5-9yrs	37	12680	146 (103-201)	46	6743	341 (250-455)	113	10205	554 (456-666)
10-14yrs	23	13103	88 (56-132)	27	7585	178 (117-259)	57	10618	268 (203-348)
15-29yrs	21	33179	32 (20-48)	60	32472	92 (71-119)	70	35773	98 (76-124)
30+yrs	11	26630	21 (10-37)	6	48640	6 (2-13)	25	43460	29 (19-42)
Overall	116	100009	58 (48-70)	150	101722	74 (62-87)	359	111318	161 (145-179)
	***S.* Typhimurium**			***S.* Paratyphi A**			***S.* Paratyphi A**		
0-11mnths	4	3416	80 (22-205)	0	1466	0 (0-126)	0	2798	0 (0-66)
12-23mnths	6	3135	124 (45-269)	0	1201	0 (0-154)	1	2125	24 (1-131)
24-35mnths	2	2570	41 (5-150)	0	1194	0 (0-154)	4	2144	93 (25-239)
36-47mnths	1	2686	18 (0-103)	1	1164	43 (1-239)	3	2098	71 (15-209)
48-59mnths	2	2610	38 (5-138)	0	1256	40 (1-222)	4	2097	95 (26-244)
0-4yrs	15	14417	52 (29-86)	1	6282	8 (0-44)	12	11262	53 (28-93)
5-9yrs	1	12680	4 (0-22)	5	6743	37 (12-87)	24	10205	117 (75-175)
10-14yrs	0	13103	0 (0-14)	3	7585	20 (4-58)	15	10618	71 (40-117)
15-29yrs	0	33179	0 (0-6)	2	32472	3 (0-11)	33	35773	46 (32-65)
30+yrs	0	26630	0 (0-7)	2	48640	2 (0-7)	11	43460	13 (6-23)

Table 4-7: Observed incidence rates of blood culture-confirmed S. Typhi, S. Paratyphi and S. Typhimurium infections, with 95% Confidence intervals in parentheses, with the crude cases from passive surveillance as the enumerator and the population from the demographics surveillance

4.6.2. Adjustment factors for Observed Incidence Rates

Observed incidence rates for typhoid fever are known to be an underestimate of actual incidence due to several factors. Firstly, blood culture sensitivity is less than 100% with prior antimicrobials, blood volume collected and the timing of collection in the natural history of infection all affecting the sensitivity of the diagnostic test. A meta-analysis comparing blood culture positivity to bone marrow culture, the current gold-standard for diagnosis of acute typhoid fever, estimated the overall sensitivity of blood culture at 0.59 (95% CI: 0.54-0.64).[193]

Secondly, not all eligible individuals who attend a healthcare facility receive a blood-culture. This could be for several reasons, including a lack of acceptance of what are deemed to be invasive tests in certain contexts, the lack of availability of blood-culture testing due to limited resources, or lack of staff and space in which to perform the testing. In our study we aimed to collect a blood-culture from all individuals who presented to a study facility and who met the case definition. This was achieved in the Asia sites, with a high proportion of eligible individuals receiving a blood-culture, however, in Malawi recruitment was lower. As has already been shown in chapter 4, the healthcare seeking behaviour in Malawi was different from the Asia sites, with a higher proportion of the population attending study facilities. There was also, proportionally more febrile illness in the youngest children. Combining this with the resource-poor setting of Malawi, and the lack of resources at the government facilities to effectively enrol all eligible individuals meant only 35% of the total eligible individuals were enrolled (Table 8). Enrolment did improve over the two-year-period, as more study resources were used to set-up extra clinics and provide more capacity to enrol febrile individuals and take the blood-cultures. In addition, there was a higher refusal rate for blood-cultures in Malawi, often from mothers of young children, where having an invasive test is not a normal part of individuals experience of healthcare.

Site / Age Group	Malawi	Nepal	Bangladesh
0-4years	0.40 (0.38-0.41)	0.82 (0.62-0.91)	0.94 (0.86-0.97)

5-9years	0.38 (0.36-0.41)	0.87 (0.73-0.94)	0.97 (0.94-0.99)
10-14years	0.33 (0.29-0.36)	0.87 (0.73-0.94)	0.97 (0.94-0.99)
15-29years	0.21 (0.19-0.24)	0.91 (0.71-0.98)	0.98 (0.96-0.99)
30+years	0.20 (0.17-0.23)	0.91 (0.71-0.98)	0.98 (0.96-0.99)
All	0.35 (0.34-0.36)	0.84 (0.67-0.92)	0.96 (0.92-0.98)

Table 4-8: Proportion of individuals who received a blood culture having sought healthcare and who met the eligibility criteria. Rates from Malawi were calculated from the STRATAA study, rates for Nepal and Bangladesh were calculated from a vaccine trial recruiting from the same population

Thirdly, as was discussed in chapter 3, usage of healthcare facilities was different across the sites with different proportions of the population presenting to study facilities for a blood-culture test.

In this analysis, these three steps of adjustment have been taken into consideration and the incidence of blood-culture confirmed *S.* Typhi has been adjusted accordingly. This has been performed in a stepwise manner as outlined in Table 4-9, with each step of adjustment taking into consideration the prior adjustments. With adjustment, the overall incidence of disease is 482 (95% CI; 398-578) per 100,000 pyo in Malawi, 947 (95% CI; 801-1111) in Nepal and 1054 (95% CI; 948-1169) in Bangladesh. The 5-9-year age group remained the age group with the highest incidence across the three sites and Bangladesh continued with the highest incidence overall and the highest incidence in the youngest children. Adjusted incidence rates were 5- to 18 -fold higher across the three sites following adjustment for the sensitivity of blood culture, the probability of receiving a blood culture, and healthcare-seeking behaviour (Table 4-10). The largest overall adjustment was made in Nepal, driven largely by the small proportion of individuals who sought healthcare at the study facilities, with this most prevalent in the 5-9 and 10-14-year age group. Adjusting for the enrolment fraction in Malawi had the greatest impact on incidence in that country, whereas adjusting for the probability of seeking healthcare had greater impact in Nepal and Bangladesh (Error! Reference source not found.).

	Malawi				Nepal				Bangladesh			
	Crude Rates	BC Sensitivity	BC Test	HC Seek	Crude Rates	BC Sensitivity	BC Test	HC Seek	Crude Rates	BC Sensitivity	BC Test	HC Seek
Age Groups												
0-4 years	83 (53-124)	141 (90-210)	353 (226-525)	598 (383-889)	72 (33-136)	121 (56-230)	148 (67-281)	705 (323-1339)	417 (337-511)	707 (572-866)	752 (608-921)	2352 (1900-2878)
5-9 years	146 (103-201)	247 (174-341)	651 (458-897)	834 (587-1150)	341 (250-455)	578 (423-771)	665 (487-886)	6041 (4425-8063)	554 (456-666)	938 (733-1128)	967 (797-1163)	2932 (2416-3524)
10-14 years	88 (56-132)	149 (94-223)	451 (286-676)	578 (367-867)	191 (128-275)	324 (217-465)	372 (249-535)	3386 (2266-4860)	268 (203-348)	455 (345-589)	469 (355-608)	1421 (1076-1841)
15-29 years	32 (20-48)	54 (33-82)	255 (158-390)	405 (251-620)	92 (71-119)	157 (119-202)	172 (131-221)	1324 (1010-1704)	98 (76-124)	166 (129-210)	169 (132-214)	891 (694-1125)
30+ years	21 (10-37)	35 (17-63)	175 (87-313)	278 (139-497)	6 (2-13)	10 (4-22)	11 (4-25)	88 (32-192)	29 (19-42)	49 (32-72)	50 (32-73)	262 (169-386)
All Ages												
	58 (48-70)	98 (81-118)	328 (271-393)	482 (398-578)	74 (62-87)	125 (106-147)	142 (120-167)	947 (801-1111)	161 (145-179)	273 (246-303)	285 (256-316)	1054 (948-1169)

Table 4-9: Crude and Adjusted incidence rates with adjustments made for BC Sensitivity; blood-culture sensitivity set at 0.59 for all age groups and sites, BC Test; the proportion of eligible individuals who presented to healthcare facilities and received a blood culture (as outlined in Figure 7) and HC Seek; the proportion of individuals who sought healthcare at study facilities (outlined in Chapter 3, Table 3-2).

	Malawi			Nepal			Bangladesh		
	Minimum Crude Rate	Maximum Adjusted Rate	Ratio (Adj./Crude)	Minimum Crude Rate	Maximum Adjusted Rate	Ratio (Adj./Crude)	Minimum Crude Rate	Maximum Adjusted Rate	Ratio (adj./crude.)
Age Groups									
0-4 years	83 (53-124)	598 (383-889)	7.2	72 (33-136)	705 (323-1339)	9.8	417 (337-511)	2352 (1900-2878)	5.6
5-9 years	146 (103-201)	834 (587-1150)	5.7	341 (250-455)	6041 (4425-8063)	17.7	554 (456-666)	2932 (2416-3524)	5.3
10-14 years	88 (56-132)	578 (367-867)	6.6	191 (128-275)	3386 (2266-4860)	17.8	268 (203-348)	1421 (1076-1841)	5.3
15-29 years	32 (20-48)	405 (251-620)	12.7	92 (71-119)	1324 (1010-1704)	14.4	98 (76-124)	891 (694-1125)	9.1
30+ years	21 (10-37)	278 (139-497)	13.2	6 (2-13)	88 (32-192)	14.7	29 (19-42)	262 (169-386)	9.0
All Ages									
	58 (48-70)	482 (398-578)	8.3	74 (62-87)	947 (801-1111)	12.8	161 (145-179)	1054 (948-1169)	6.5

Table 4-10: Minimum crude incidence rates and maximum adjusted incidence rate with the ratio for adjusted: crude rates for the different age groups across the three sites

4.7. Results - Antimicrobial Susceptibility

The antimicrobial susceptibility profiles for both *S.* Typhi and *S.* Paratyphi A or *S.* Typhimurium differed at each site (**Error! Reference source not found.**) and were consistent with other published data from these countries. Fluoroquinolone non-susceptibility (FNS) was common amongst *S.* Typhi in Bangladesh (99%) and Nepal (83%), but only detected in a single isolate in Malawi (<1%). FNS was almost universal in *S*. Paratyphi A (100% in Nepal, >99% in Bangladesh). Azithromycin non-susceptibility (ANS) was detected in both Asian sites but was not assessed in Malawi. ANS was more frequent in *S.* Paratyphi A (30% in Nepal, 78% in Bangladesh) than *S*. Typhi (7% in Nepal, 3% in Bangladesh). Resistance to Ampicillin, Chloramphenicol and Cotrimoxazole, referred to as multi-drug resistant (MDR) *S.* Typhi predominated in Malawi with 98% of the isolates classified as MDR. Interestingly, in the Asia sites, where these antibiotics are no longer used for treatment of febrile

illness, there has been a decrease in the resistance towards these antibiotics with only 1% MDR in Nepal, and 39% in Bangladesh, with *S.* Paratyphi A fully susceptible in both Asia sites.

Site / Antimicrobial	Malawi			Nepal			Bangladesh		
	S. Typhi			*S.* Typhi			*S.* Typhi		
No of cases (% of total)	Susceptible	Non-susceptible	NT	Susceptible	Non-susceptible	NT	Susceptible	Non-susceptible	NT
Ampicillin	3 (2)	152 (98)	11	154 (99)	1 (1)	4	203 (57)	155 (43)	1
Chloramphenicol	3 (2)	163 (98)	0	149 (99)	2 (1)	8	188 (52)	167 (48)	4
Cotrimoxazole	3 (2)	163 (98)	0	150 (99)	2 (1)	7	190 (53)	168 (47)	1
MDR		152 (98)			1 (1)			140 (39)	
Amikacin	1 (100)	0 (0)	165	10 (91)	1 (9)	148	357 (99.5)	1 (0.5)	1
Amoxiclav	1 (100)	0 (0)	165	NA	NA	NA	321 (90)	35 (10)	3
Azithromycin	NA	NA	NA	140 (93)	10 (7)	9	348 (97)	11 (3)	0
Cefixime	NA	NA	NA	NA	NA	NA	359 (100)	0 (0)	0
Ceftriaxone	166 (100)	0 (0)	0	146 (94)	9 (6)	4	359 (100)	0 (0)	0
Ciprofloxacin	165 (99)	1 (1)	0	26 (17)	127 (83)	6	4 (1)	355 (99)	0
Nalidixic Acid	NA	NA	NA	19 (15)	134 (85)	6	15 (4)	344 (96)	0
Meropenem	NA	NA	NA	NA	NA	NA	356 (99.5)	0 (0.5)	3
	S. Typhimurium			*S.* Paratyphi A			*S.* Paratyphi A		
Ampicillin	22 (65)	12 (35)	2	14 (100)	0 (0)	0	95 (100)	0 (0)	0
Chloramphenicol	26 (72)	10 (28)	0	13 (100)	0 (0)	1	95 (100)	0 (0)	0
Cotrimoxazole	23 (64)	13 (36)	0	14 (100)	0 (0)	0	95 (100)	0 (0)	0
MDR		10 (28)							
Amikacin	NA	NA	NA	1 (100)	0 (0)	13	0 (0)	0 (0)	95
Amoxiclav	NA	NA	NA	NA	NA	NA	93 (98)	2 (2)	0
Azithromycin	NA	NA	NA	10 (70)	4 (30)	0	21 (22)	74 (78)	0
Cefixime	NA	NA	NA	NA	NA	NA	94 (100)	0 (0)	1
Ceftriaxone	36 (100)	0 (0)	0	4 (100)	0 (0)	10	95 (100)	0 (0)	0
Ciprofloxacin	27 (100)	0 (0)	0	0 (0)	14 (100)	0	1 (1)	94 (99)	0
Nalidixic Acid	NA	NA	NA	2 (15)	12 (85)		0 (0)	95 (100)	0
Meropenem	NA	NA	NA	NA	NA	NA	93 (100)	0 (0)	2

Table 4-11: Antimicrobial resistance patterns for S. Typhi, S. Paratyphi and S. Typhimurium. Abbreviations: NT, Not Tested; MDR, multi-drug resistance

4.8. Discussion

In the surveillance described here, *S.* Typhi was the primary cause of bacterial blood-stream infection in people with a prolonged fever across three different epidemiological contexts in Africa and Asia. Crude incidence rates of blood-culture-confirmed disease detected through passive surveillance were adjusted to estimate the incidence of typhoid fever occurring within the census populations using parameters measured from within the populations, indicating true rates are likely to be considerably higher than those estimated directly from blood culture, and providing an insight into the likely burden of this important disease.

In Bangladesh, prior crude incidence estimates from population-based studies in the capital city Dhaka have ranged from 53 to 390 cases per 100,000 pyo[120,132] for all age groups, with the highest crude incidence rates (of up to 1,870 per 100,000 pyo) recorded in children under 5 years of age.[107] Global Burden of Diseases 2017 estimated an adjusted incidence of 641 per 100,000 pyo in Bangladesh, reduced from 1,459 per 100,000 pyo in 1990.[104] The observed and adjusted incidence for all age groups of 161 and 1,125 per 100,000 pyo, respectively, are consistent with these prior estimates and suggest that typhoid fever incidence may not be declining as quickly as previously estimated. Incidence for *S.* Typhi was as high as 3770 per 100,000 pyo in the 5-9-year age group with the highest single year incidence being in the 4th year of life.

A prior estimate from Kathmandu, Nepal had indicated overall crude *S.* Typhi incidence to be 59 cases per 100,000 pyo, averaged over a four-year period, with a mean age of disease at 16 years.[133] While this is similar to the crude incidence of 74 per 100,000 pyo that was observed in this study, the adjusted surveillance suggests that this is likely to be an underestimate, with adjusted incidence rates of 947 per 100,000 pyo. In the surveillance from Kathmandu, the proportion of participants using alternative healthcare facilities and private pharmacies was high, particularly in the 5-9-year age group, leading to the large increase between the crude and adjusted rates. As with Dhaka, this was

the age group with the highest observed and adjusted incidence, but also the greatest degree of uncertainty in estimates due to the limited use of study facilities.

In Africa there is a greater degree of uncertainty in disease incidence due to fewer population-based studies. A recent meta-analysis estimated adjusted rates of 620 cases per 100,000 pyo for Eastern Sub-Saharan Africa and 1459 cases per 100,000 pyo for Central Sub-Saharan Africa, although with wide confidence intervals.[124] Since 2010 in Malawi, there has been an increase in the number of typhoid fever cases reported from central hospitals, with a minimum adjusted incidence estimate (accounting only for blood culture sensitivity) of 207 per 100,000 pyo at the peak of the outbreak in 2013 from Blantyre.[140] The results from this analysis detail the ongoing high incidence of disease in Blantyre, with adjusted incidence rates of 482 cases per 100,000 pyo, and the 5-9 year age group again having the highest incidence.

According to the literature, with the observed data from surveillance, this would be categorized as a medium incidence rate of disease for all age groups in Malawi and Nepal, with a high incidence detected in Bangladesh. With adjustments made for healthcare-seeking behaviour, the probability of receiving a blood culture and the sensitivity of blood culture diagnostics, overall incidence is high in Malawi and very high in Nepal and Bangladesh. These three parallel studies have demonstrated, however, that adjustment factors cannot be applied universally to observed rates of blood culture-confirmed disease across different epidemiological contexts and age groups. In the Asian sites, there was a much higher usage of alternative healthcare facilities and pharmacies, particularly within the 5-9-years age group, leading to higher inflation of incidence once adjusted, whilst in Malawi, fewer individuals who sought healthcare at study facilities received a blood culture due to high rates of febrile illness, limited healthcare infrastructure and parental refusal. Understanding these differences across the different sites enables more appropriate adjustment to be made with the observed data. The more recent global estimates of enteric fever have applied a number of different adjustment factors to the observed incidence estimates, but they have done this universally to all areas of the

globe. The data presented in this study suggests that a more localised approach needs to be taken to get closer to the true incidence.

The surveillance data were consistent with other published data on the increasing importance of *S.* Paratyphi A in both Dhaka and Kathmandu[151,250,251] with observed rates of 117 and 37 per 100, 000 pyo in the 5-9 year age group respectively. In Blantyre, there were no cases of *S.* Paratyphi A but consistent with surveillance across Africa there remains a significant burden of *S.* Typhimurium in the youngest children,[125,194,252] with an observed incidence of 117 per 100, 000 pyo in the 2nd year of life. We did not detect a significant amount of NTS bacteraemia in the adult population, as has previously been documented in case series in the continent.[253] With the widespread introduction of antiretrovirals in Malawi the rates of NTS have been in decline. Additionally, the focus of this surveillance was community-based, where a case of NTS sepsis may be less likely to present unwell and admitted cases in hospital may have been missed.

Salmonellae are on the WHO priority pathogen list due to the high rate of associated AMR and the risk this confers to human health.[254] Data from our surveillance are consistent with recently published meta-analyses on the increase in AMR amongst typhoidal *Salmonellae* and the different AMR profiles of *S.* Typhi and *S.* Paratyphi A, as well as the differences in AMR between Africa and Asia.[232,255]

Isolates from Malawi continue to be almost entirely MDR, with no FNS documented during this surveillance period. By contrast, in Bangladesh and Nepal high rates of FNS and documented ANS were observed. These data, combined with data from passive surveillance showing individuals who were *S.* Typhi culture-positive had significantly higher rates of antibiotic use prior to seeking healthcare, and higher rates of antibiotic prescription at time of visit, clearly demonstrate the escalating risk of AMR associated with global typhoid management.

Of current concern is the large and ongoing outbreak of XDR *S.* Typhi in two districts of Pakistan.[32,154,155] In addition to MDR, there is additional FQ and Cephalosporin resistance within the

majority of isolates. The data from our sites showing ANS is a concern as this would be one of the only oral agents remaining for treatment of this pathogen. To control this XDR outbreak, almost 10 million doses of typhoid conjugate vaccine have been administered in the area affected. In a sub-study, 20,000 participants were followed up for blood-culture conformed disease with a reported efficacy at 18months of 89% (Data presented at Asian Conference on Diarrhoeal Disease and Nutrition 2020).[256] This would be consistent with published data from Nepal showing a vaccine efficacy at 1 year of 82%.[257]

5. Chapter 5 – The Seroincidence of *S.* Typhi Infection

5.1. Introduction

Facility-based surveillance for clinical infection with blood culture collection to capture acute cases of typhoid fever provides data on the incidence of disease that presents to healthcare facilities. This is an established method for estimating disease burden, not just for enteric fever, but many communicable diseases.[105] It has the additional benefit of providing data on other aspects of the disease like the clinical presentation and the antimicrobial susceptibility of the pathogen. These data have been presented for the three sites studied and discussed in this thesis. However, surveillance at the facility level does not capture all cases of the disease, and importantly when considering interventions, does not estimate the amount of disease that could be prevented. Either sub-clinical infection, or infection that is missed by the surveillance system would not be captured.

To further understand the burden of typhoid fever in the three endemic sites studied and to provide another method for estimating the incidence of *Salmonella* Typhi, alongside surveillance for clinical infection at healthcare facilities a community-based serological survey was performed from healthy participants within the demographic census population. This additional approach for estimating incidence, performed in the same population and at the same time as clinical surveillance will allow comparison between the two methodologies and enable triangulation of clinical incidence rates. Serological surveillance can ascertain the incidence of exposure to the pathogen which may result in either sub-clinical infection, or infection that does not present to a healthcare facility. This will therefore capture a level of exposure and possible infection that facility-based surveillance will miss. Additionally, if seroincidence rates correlate well with observed and adjusted clinical incidence rates, seroincidence studies could be performed in sites of unknown typhoid burden to inform vaccine introduction strategies.

The data presented in this chapter addresses point number in 7 in the list of primary outcomes for this thesis outlined in chapter 1:

1. Incidence of non-clinical typhoid infection determined through serological surveillance.

5.2. Methods

Detailed methods for this component of the study can be found in chapter 2. For the design of the survey and enrolment of participants, please refer to chapter 2, section 6. For the processing of the Vi ELISA please refer to section 2.11. For the statistical analysis of the anti-Vi IgG antibody results please refer to chapter 2 section 9.2.

Briefly, for the serological surveillance component of the study, 8,500 participants were randomly selected in an age-stratified manner from the original demographic census. Using GPS/address data these individuals were followed up by field teams, and 1-3 ml of blood were collected twice, at three-month intervals. Field teams worked on a rolling programme, moving throughout the entire populations every three months to avoid bias from geospatial and seasonal changes in typhoid incidence. For this analysis anti-Vi IgG antibody, the antibody produced against Vi capsular polysaccharide, the 'virulence' factor for *S.* Typhi, was measured using a standardised ELISA assay produced by The Binding Site (See Methods). Assays were run locally at each site.

5.3. Results – Seroprevalence of anti-Vi IgG antibody

In total 7,063, 4,630 and 8,240 samples were analysed for visit 1 from Malawi, Nepal and Bangladesh respectively. From the visit 1 result the proportion of individuals with an antibody level above the lower limit of detection for the assay (7.407 EU/ml) was different across age groups and differed across the three sites. Overall, with the threshold set at 7.407 EU/ml the seropositivity rate or serprevalence was 0.21 (95% CI: 0.20-0.22) in Malawi, 0.63 (95% CI: 0.61-0.66) in Nepal and 0.31 (95% CI: 0.29-0.32) in Bangladesh. For sensitivity analysis, to determine if the patterns of seropositivity were different at a threshold of 50 EU/ml the seropositivity rate was 0.049 (95% CI: 0.04-0.05) in Malawi, 0.14 (95% CI: 0.126-0.148) in Nepal and 0.03 (95% CI: 0.027-0.034) in Bangladesh. Across all three sites, using either the lower limit of detection for the assay or a threshold of 50 EU/ml seropositivity increased with age (Figure5-1).

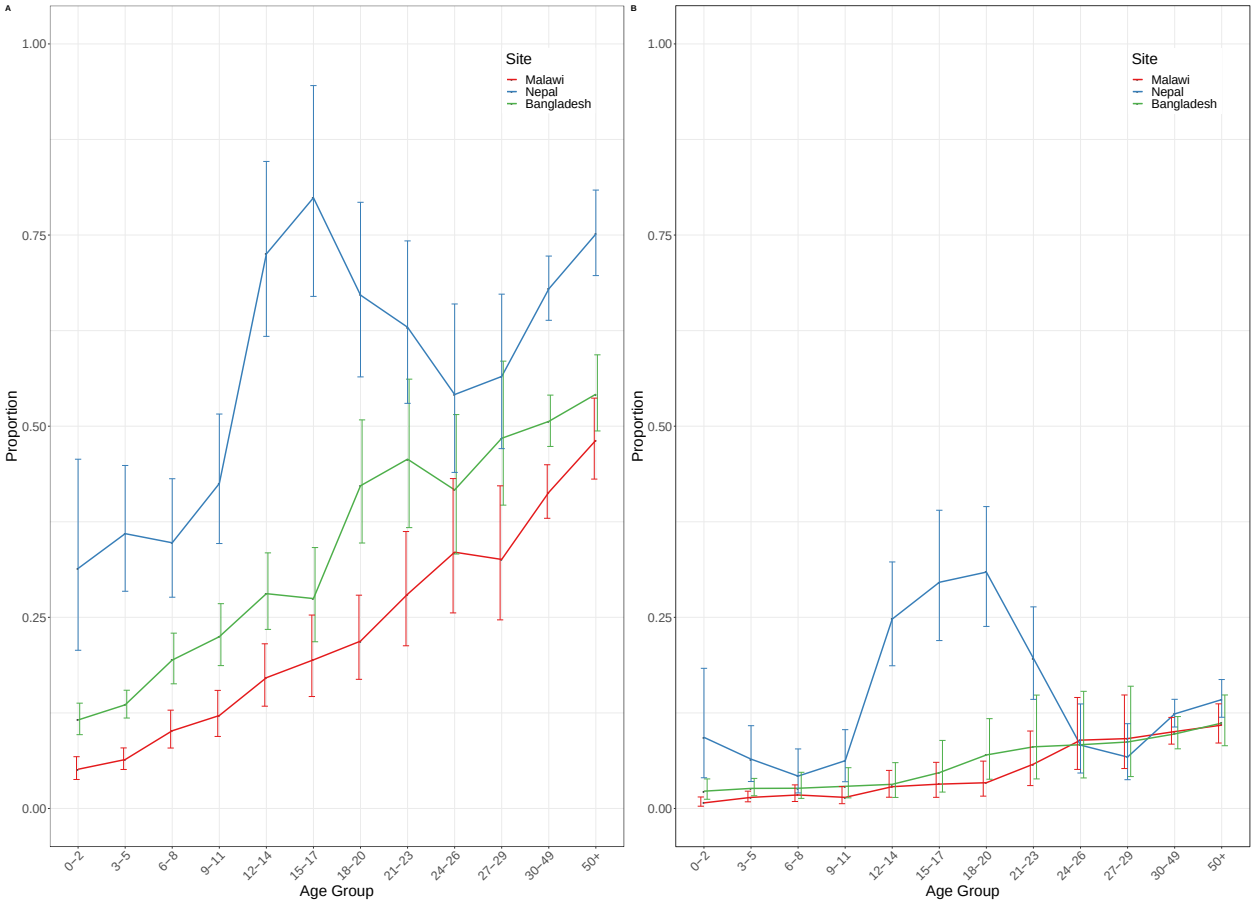


Figure5-1: Proportion of individuals with antibody titre above A; 7.704 EU/ml, the lower limit of detection for the assay and B; 50 EU/ml across the three sites. Error bars denote 95% confidence intervals.

Rates of seropositivity were similar in Malawi and Bangladesh but with higher rates demonstrated in younger children in Bangladesh. This would be consistent with the passive surveillance data collected from the sites showing a higher incidence of clinical disease in younger children in Bangladesh. This would indicate a higher force of infection at which susceptible individuals acquire infection at a younger age due to an overall earlier exposure to the pathogen. The Nepal data here show a different pattern with an increase in rates in children from 12-years of age, peaking in the 15-17-year age group. This would be consistent with a Vi-polysaccharide typhoid vaccine campaign carried out in 2012 within the school-aged children of Lalitpur, the location where this serological survey was performed. There is not a lot of information about this vaccine campaign available, but it was a school-based campaign, run by the International Vaccine Institute with funding from the Bill and Melinda Gates Foundation. It would explain the increase in antibody titre in these results observed in the exact age-group who would have been eligible for the campaign five years earlier. Participants

were asked whether they had received prior typhoid vaccine but very few individuals answered affirmatively to this question, and their Vi-IgG was not correspondingly higher.

Seropositivity, using anti-Vi IgG mirrors and provides some explanation for the age profile of acute disease at the sites studied. As the protective level of anti-Vi antibody increases with increasing age, at a population level, the age profile of acute disease incidence reduces with increasing age. Individuals are increasingly exposed to the pathogen, whether this exposure results in acute disease or sub-clinical infection, the anti-Vi-antibody increases. Whilst there is no formal correlate of protection for *S.* Typhi, these data along with randomised control trial data using Vi-based vaccines, does show that Vi antibody is likely to be protective against acute typhoid infection.

5.4. Results – Seroincidence of *S.* Typhi infection

With the addition of visit two it was possible to determine the proportion of individuals who had a rising antibody titre between visit 1 and visit 2, indicating seroconversion to the Vi-antigen and therefore likely exposure to, or infection with *S.* Typhi. Individuals who had a blood sample collected in visit 1 were followed up approximately three months later for a repeat blood draw. In total, 4,139, 4,452 and 7,040 paired samples were analysed for seroincidence from Malawi, Nepal and Bangladesh respectively.

5.5. Results - Setting a Threshold for Seroconversion

Prior to this study, no threshold at which a seroconversion episode could be said to have taken place had been published using anti-Vi IgG with this assay. In vaccine trials at day 28 post vaccination, a 4-fold-rise in antibody is usually set as the threshold for a significant immunological response.[258] The difficulty with using that approach for this analysis is that, vaccine trials usually recruit young children where the pre-vaccination IgG titre is low, as was demonstrated in our results also, and so a 4-fold rise can be demonstrated within the limits of the assay or by using dilutions. In this study, sites recruited adults, a proportion of whom already had a raised anti-Vi IgG at baseline. Dilutions of the assay were not run due to the large number of samples processed therefore a proportion of results

from visit 2 were above the upper limit of detection for the assay. This meant that a proportion of individuals did not meet the definition for a 4-fold rise in antibody titre, as the baseline titre may have been high, and the visit 2 titre was brought down to the upper limit for the assay. This resulted in participants not being included in the analysis despite having a rise in their antibody titre between the two visits.

Using a two-fold rise in antibody titre is more sensitive for individuals who have had a rise in their antibody level following exposure to the pathogen, however, it included participants where the visit one result was below the limit of detection for the assay, and who only had a small rise in absolute antibody level, but would still be included in the two-fold rise definition. It was thought that to only have an absolute rise of antibody from 7.4EU/ml to 15EU/ml for example, was too small of a change to qualify as seroconversion.

The Oxford typhoid controlled human infection model was used for a vaccine trial where typhoid naïve participants, who have not lived in typhoid endemic countries, or previously been vaccinated for typhoid were recruited.[221] On the day of vaccination, participants had their anti-Vi IgG measured. As would be expected, the majority of individuals had an undetectable titre however, a small proportion had a level up to approximately 50EU/ml (Figure5-2). There is known to be some cross-reactivity with the Vi-antigen from organisms such as *Citrobacter freundii*, which is presumed to have caused these higher levels. The single individual with a very high level >300 EU/ml is likely to have been vaccinated in the past but not recorded in medical or vaccination records.

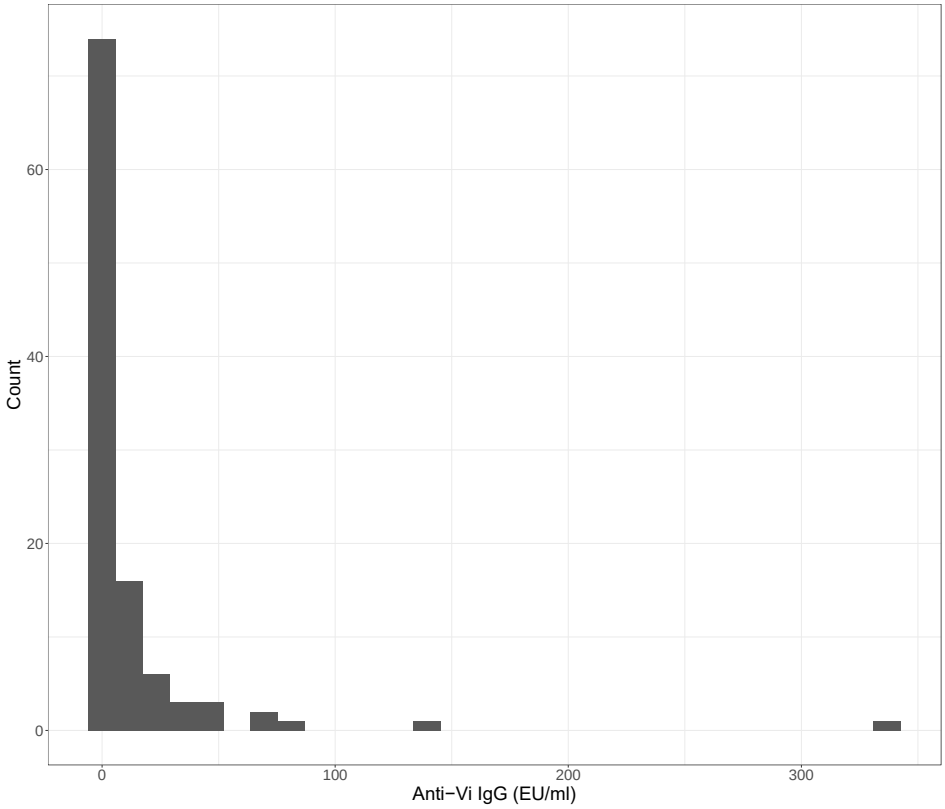


Figure5-2: Anti-Vi IgG titre from typhoid-naïve individuals recruited in Oxford, UK

Informed by these data the threshold for a seroconversion episode was defined as a two-fold rise in antibody from visit one to visit two, with an absolute antibody titre of 50 EU/ml at visit two, ensuring that individuals with an antibody rise at the lower end of the assay were excluded, but that individuals who had a higher baseline level IgG and a significant rise in titre, were still included in the analysis. This is demonstrated in the line plots seen in Figure 5-3 and 5-4 where plots A-C demonstrate the participants included in the analysis when the threshold for seroconversion is set at greater than or equal to a 2-fold-rise. In both the plot by EU/ml and the log plot, there is a predominance of individuals with only a small change in absolute antibody level. Plots D-F show the individuals included when the threshold for seroconversion is defined as greater than a four-fold-rise. This demonstrates the loss of individuals with a higher baseline IgG in visit 1, particularly from Nepal and Bangladesh, where the baseline Vi-IgG level was higher, as demonstrated previously in the seropositivity analysis. Plots G-I show the individuals included when the threshold for seroconversion is set at greater than a

2-fold-rise and absolute antibody level of 50 EU/ml at visit 2. This ensures that fewer individuals with only a small rise in antibody titre are included and individuals with a higher baseline IgG are included.

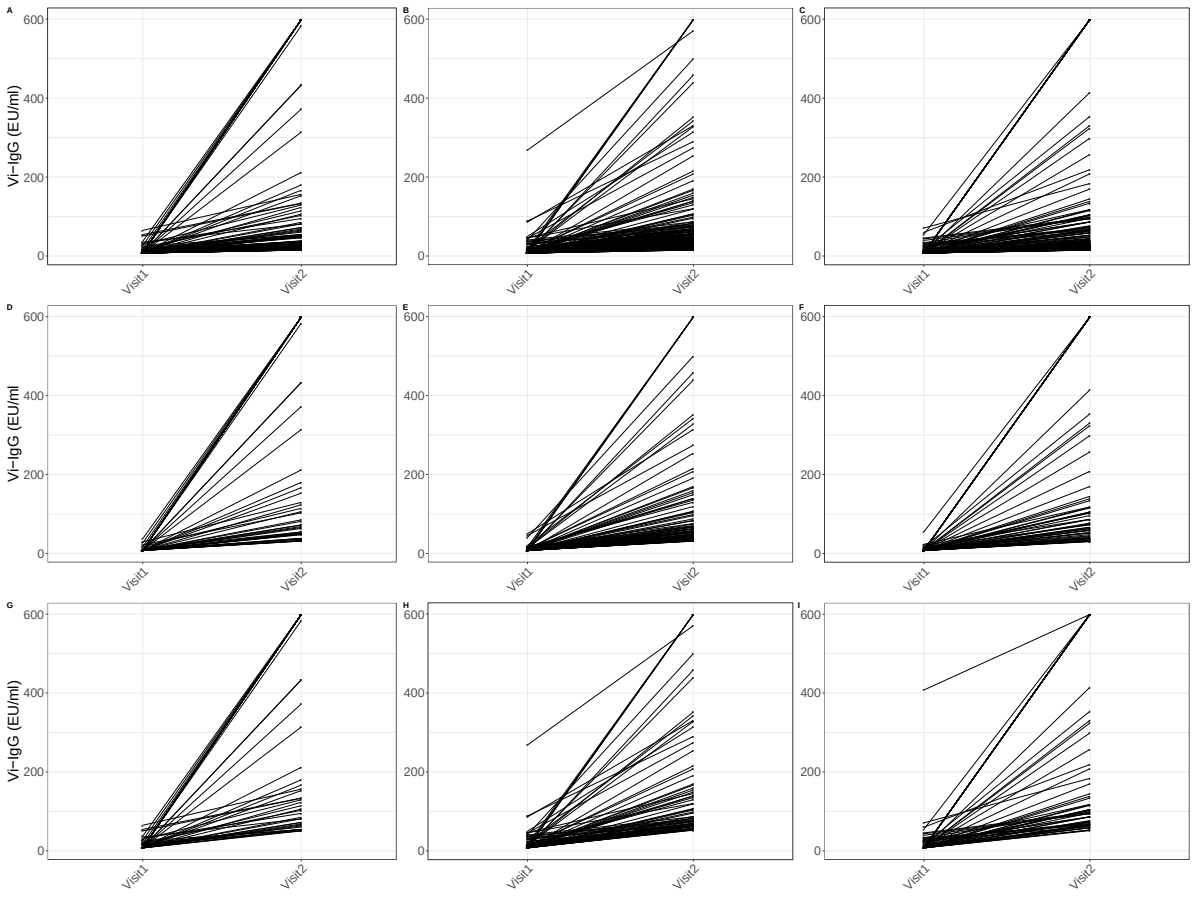


Figure5-3: Change in anti-Vi IgG titre by EU/ml for participants with two visits from the serological survey, included in the analysis if the threshold for seroconversion was A; >2-fold-rise, B; >4-fold-rise and C; >2-fold rise with an absolute value of 50 EU/ml at visit 2, from serological surveys performed in Column 1; Blantyre, Malawi, Column 2; Kathmandu, Nepal and Column 3; Dhaka, Bangladesh.

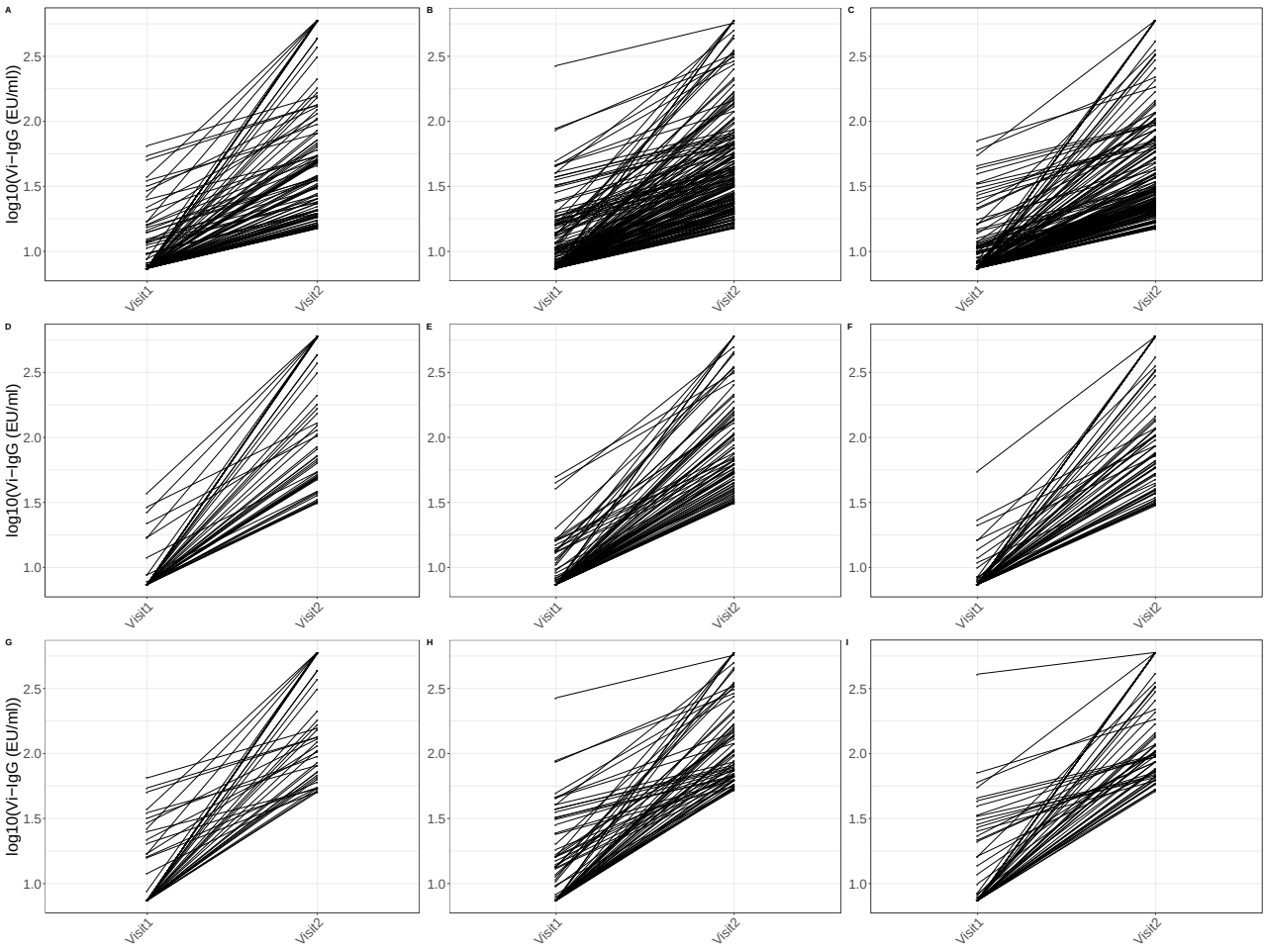


Figure5-4: Change in anti-Vi IgG titre by EU/ml on log10 scale for participants with two visits from the serological survey, included in the analysis if threshold for seroconversion was A; >2-fold-rise, B; >4-fold-rise and C; >2-fold rise with an absolute value of 50 EU/ml at visit 2, from serological surveys performed in Column 1; Blantyre, Malawi, Column 2; Kathmandu, Nepal and Column 3; Dhaka, Bangladesh.

5.6. Results - Anti-Vi IgG Fold Change

As was demonstrated looking at seroprevalence, the dynamics of Vi-IgG are different at the three sites. This was also found to be the case with seroconversion and confirmed some of the findings from the passive surveillance component of the study. Most individuals did not have a fold change as demonstrated in Figure 5-5 A-C. Figure 5-5 plots D-F show only individuals with a fold change of greater than two with an absolute anti-Vi IgG titre of 50 EU/ml in visit 2 and plots G-I show only individuals with a fold change of greater than 4. In Bangladesh, the youngest children, under five years of age, had the highest proportion of participants with a high fold change compared with Malawi where school-age children had the higher fold-change. This does reflect the pattern observed

in surveillance of acute infection, where the incidence rate in Bangladesh was higher in younger children, and highest in the 4th year of life, whereas in Malawi the highest incidence was seen later in the 5-9-year age group. In Nepal, the difference across the age-groups is less marked with individuals with a high antibody fold change seen across the age groups.

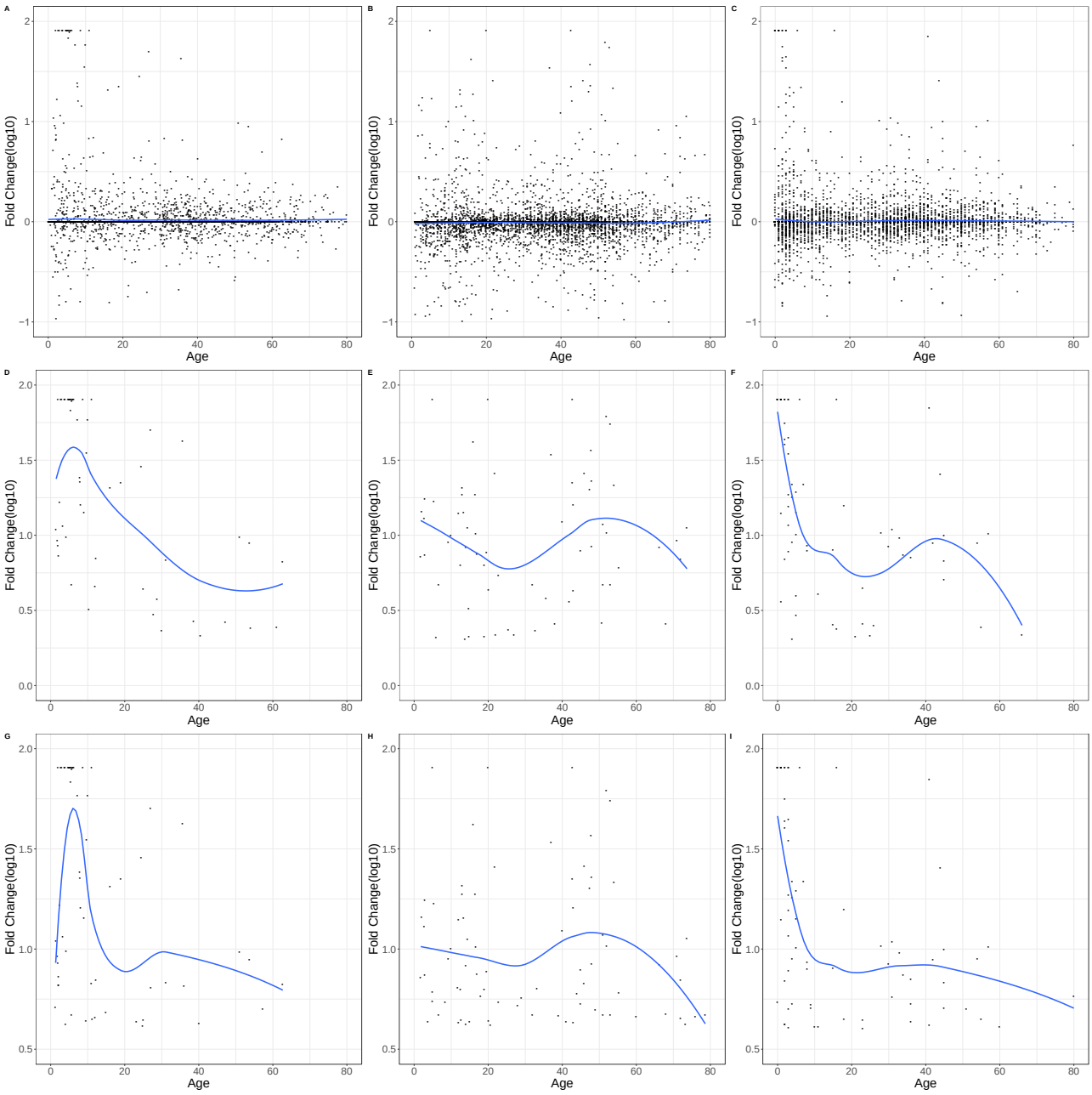


Figure5-5: Fold change for anti-Vi IgG antibody titre on the log scale for A; All participants, B; Only participants with a greater than two-fold rise plus absolute titre of 50 EU/ml from second visit and C; Only participants with a greater than four-fold rise, from serological surveys performed in Column 1; Blantyre, Malawi, Column 2; Kathmandu, Nepal and Column 3; Dhaka, Bangladesh. Blue line indicates line of best fit with shaded area, 95% Confidence Interval.

5.7. Results - Duration of Time between Visits

To calculate seroincidence per 100,000 person-years of observation to compare with the incidence from acute passive surveillance, the median time between visits was used for each site. Due to several factors at each site, follow-up time was not uniform. In Bangladesh, field teams needed to work around the month of Ramadan, where adults are not permitted to provide a blood sample. In Nepal, there are major festivals each year when many individuals leave the city to return to their home village. In Malawi, at the start of the rainy season, many individuals leave the city and return to their home village to assist with planting and harvest. Additionally, during the serosurvey there was also a period of rumours circulating throughout Southern Malawi spreading suspicion around the activity of healthcare and research activity, all field work was stopped for almost two-months preventing the field teams from collecting any samples during this time. The median duration between visits was 106 (IQR; 91-116) days, 106 (IQR; 100-116) days and 84 (IQR; 79-90) days in Malawi, Nepal and Bangladesh respectively. In order to calculate this for a full year multiplication factors were used. This resulted in multiplication factors of 3.44, 3.44 and 4.34 for Malawi, Nepal and Bangladesh respectively, to calculate an annual incidence.

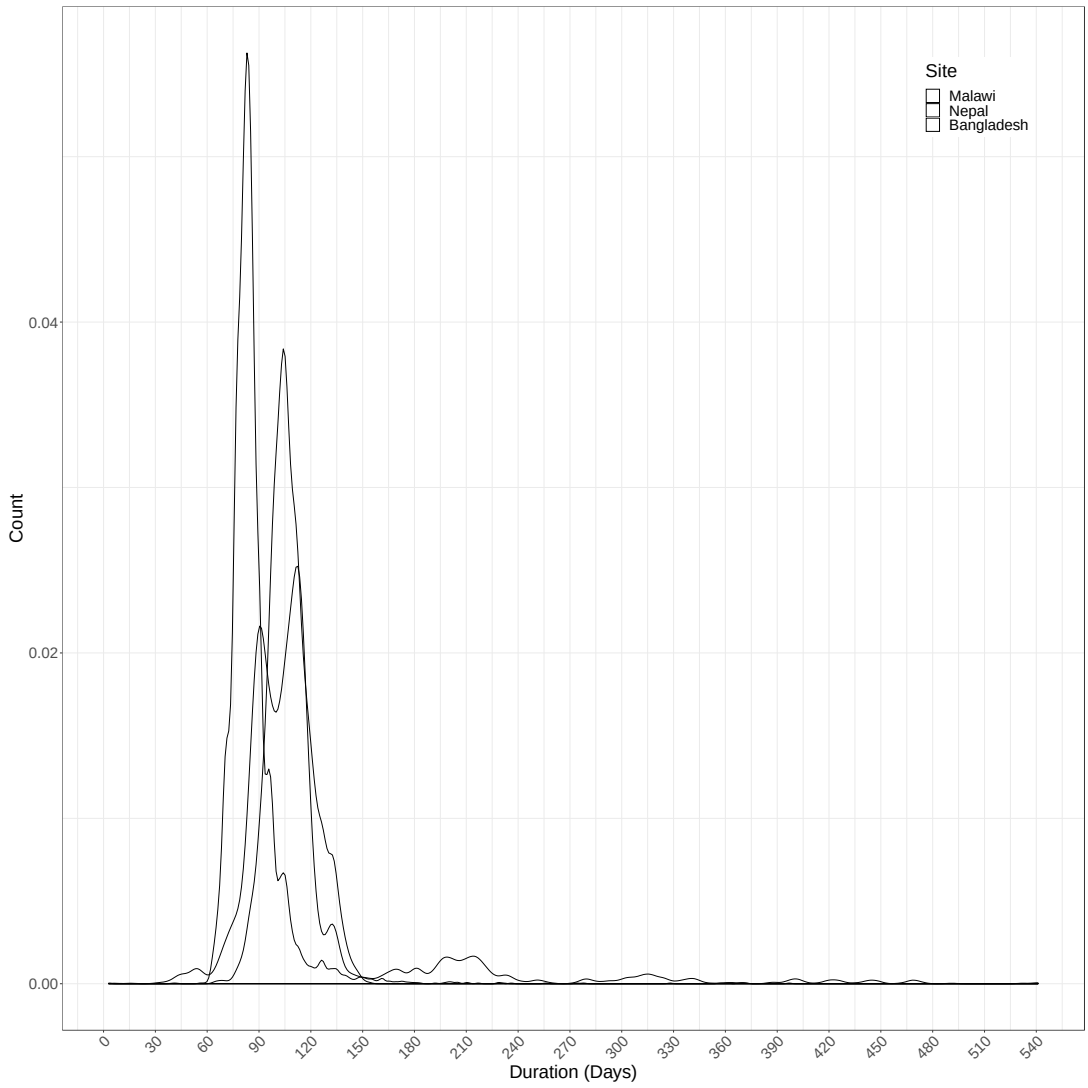


Figure5-6: Duration between Visit 1 and Visit 2 in the serosurvey in days for the three sites.

5.8. Results - Calculating Seroincidence

Overall seroincidence was highest in Nepal with 5,504 episodes (95% CI; 4299-6942) per 100,000 pyo, almost six times higher than the estimated adjusted incidence. Rates of 4,600 (95% CI; 3455-6002) and 3,956 (95% CI; 3046-5051) per 100,000 pyo in Malawi and Bangladesh were 9.5 and 3.8 times higher than adjusted incidence respectively (**Error! Reference source not found.** and **Error! Reference source not found.**). In Malawi, seroincidence estimates follow a similar pattern to those observed through passive surveillance, with a peak in incidence in the 5-9-year age group. Similar to the incidence estimates from passive surveillance, the seroincidence analysis showed high rates of exposure to *S.* Typhi in the youngest children, particularly in Bangladesh with over 6000 episodes per 100,000 pyo. Apart from the 5-9-year age group in Nepal and 10-14-year age group in Bangladesh all

seroincidence estimates exceeded those from the adjusted incidence, although both groups have overlapping confidence intervals. This means seroincidence estimates could be used as an upper limit of incidence in endemic sites. This would enable a more rapid assessment of disease burden to be implemented and provide important data for decisions on vaccine introduction.

One of the main differences between the seroincidence estimates and adjusted incidence estimates are the high seroincidence estimates in the 30+ age group, with rates of 3081, 5070 and 2772 in Malawi, Nepal and Bangladesh respectively. These are 11, 58 and 10.5 times higher than those estimated through adjusted incidence. This may indicate ongoing transmission and exposure to *S.* Typhi in this age-group, without clinical disease. This is an important consideration for vaccine introduction, as only vaccinating young children may not interrupt transmission of the pathogen at a community level and provide the herd-protection required to reduce overall incidence of disease.

	Malawi		Nepal		Bangladesh	
	Incidence Rate	95% Confidence Interval	Incidence Rate	95% Confidence Interval	Incidence Rate	95% Confidence Interval
Age Groups						
0-4 years	4437	2362 – 7587	7576	2460 – 17679	6318	4231 – 9073
5-9 years	8696	5154 – 13743	5042	1850 – 10974	3435	1570 – 6521
10-14 years	3937	1278 – 9187	9523	4567 – 17514	599	15 – 3336
15-29 years	3665	1473 – 7551	4828	4828 – 2639	5309	2743 – 9275
30+ years	3081	1538 – 5513	5070	3551 - 7020	2772	1516 – 4651
All Ages						
	4600	3455 - 6002	5504	4299 - 6942	3956	3046 - 5051

Table 5-1: Incidence rate, per 100,000 person years of observation, calculated through serological surveys, with 95% Confidence Intervals

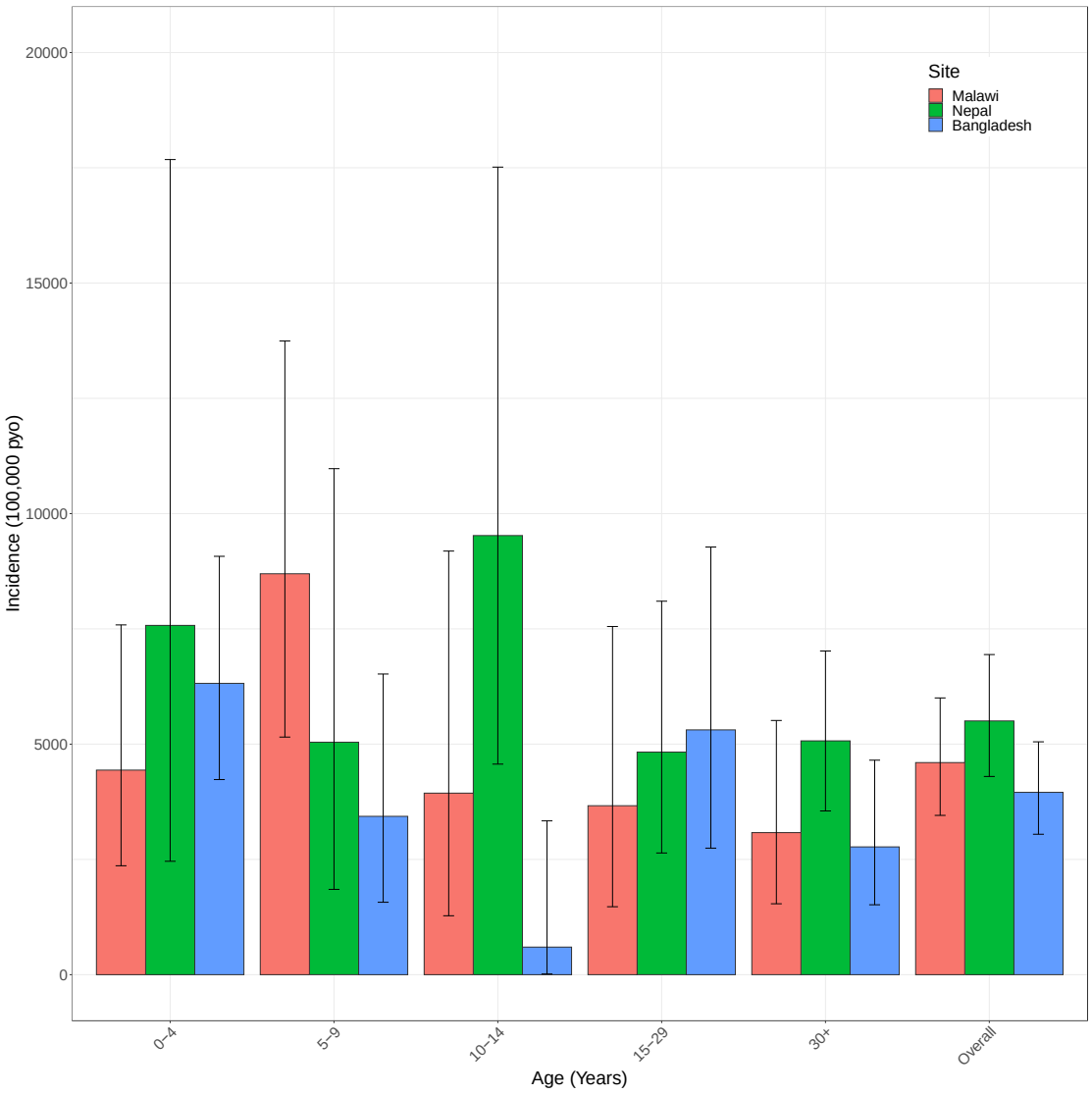


Figure 5-7: Incidence rate, per 100,000 person years of observation, calculated through serological surveys. Error bars denote the 95% confidence interval

5.9. Discussion

Measuring seroincidence provides an alternative estimate for the rate of exposure to *S.* Typhi than provided by passive blood-culture surveillance. This is the first study to use serology to calculate seroincidence for *S.* Typhi across multiple sites. Apart from the 5-9-year age group in Nepal and 10-14-year age group in Bangladesh, all the seroincidence age-group estimates either exceed or have overlapping confidence intervals with those from the adjusted incidence in passive surveillance, such that seroepidemiology could be used as an upper bound for disease rates within a certain population.

It would be expected that seroincidence would be higher than acute disease incidence, as no cases are missed through incomplete surveillance. However, determining the relevance of an antibody rise without symptoms or need for healthcare is important. A cross-sectional seroepidemiological study from Fiji used anti-Vi IgG antibody and estimated seroprevalence to be higher than predicted based on blood-culture-confirmed disease.[259] Interestingly, in the Fiji study there was serological evidence of infection in a cohort of the population (non-iTaukei ethnic groups) not known for high rates of clinical disease. In the discussion of these published data it was hypothesised this could be secondary to a genetic difference protecting that population from clinical infection or different healthcare utilisation meaning the clinical disease was never captured.

The high rates of seroconversion among older individuals, particularly in Nepal, could be evidence of ongoing exposure and transmission within these age-groups, which due to the high use of antimicrobials from private pharmacies, and/or partial immunity leading to subclinical infection, never requires healthcare facility access. A prior cross-sectional serological study in Nepal, showed that titres of anti-Vi IgG were highest in the 16-55 year age group supporting our data.[260] Additionally, modelling data looking at the drivers of transmission for *S.* Typhi in Kathmandu found there to be high rates of infection in young adult men.[230] The hypothesis therefore is that the influx of susceptible male workers migrating into Kathmandu from rural settings where the incidence of typhoid fever may be less[166], is one of the drivers of infection in this setting. This may explain the pattern observed in this analysis, with higher rates of seroconversion seen in older age-groups, as susceptible older individuals who may be less likely to seek formal healthcare are exposed to the pathogen. It would also explain the reduction in seroprevalence for Nepal only, in the 25-29 and 30-35-year age group, compared with the age cohort who may have taken part in the Vi-PS vaccine trial.

In Malawi, very high rates of disease were observed in the 5-9-year age group in serological surveillance. Prior cross-sectional data from the control arm of a Vi-polysaccharide vaccine trial from Cape Town in South Africa, which at the time of the study was endemic for typhoid fever, showed

40% of children aged 9 years of age to have a possibly protective antibody level. This would indicate high rates of exposure to *S.* Typhi in school aged children.[82]. It is unclear, why this high rate of exposure is not replicated in the passive surveillance and why it is not observed in Bangladesh, where both observed and adjusted incidence rates from passive surveillance are higher for this age group.

Due to the larger sample size from Bangladesh, the confidence intervals for the estimates are tighter compared with the other two sites. The Bangladesh seroincidence data does confirm high rates of disease in the youngest children.

There are some limitations to this element of the study. In Malawi and Nepal, recruitment to the study was logistically difficult with participants often being unwilling to either give blood in their own homes or attend a healthcare facility for a purely research-based test. Follow-up at three months also proved difficult due to high rates of migration and identifying the same individuals again in densely populated urban areas. Additionally, a Vi-TCV vaccine campaign was started within the three populations studied, and children who had received vaccine in this trial, were excluded from a second visit in the serological surveillance.

Anti-Vi IgG may not be the best serological marker to use for acute infection. A large proportion of the population have titres below the level of detection for the assay, and it may not be the most sensitive in detecting exposure. There is also known to be some cross-reactivity with non-typhoidal pathogens, such as Citrobacter and other Enterobacteriaceae which may have affected these results. This assay was chosen for the additional purpose of also detecting chronic carriers, based on prior work in Chile and because there is a standardised assay which can be used in multiple laboratories producing comparable results. Further work using a wide range of typhoid antigens is planned with the plasma collected in this study.

6. Chapter 6 – Acute, Convalescent and Chronic Stool Shedding

6.1. Introduction

Salmonella enterica serovar Typhi (*S.* Typhi) and *Salmonella enterica* serovar Paratyphi A (*S.* Paratyphi A) are enteric pathogens that are spread through the faecal-oral route. Both *S.* Typhi and *S.* Paratyphi A are human restricted pathogens without a known environmental niche. Ongoing infection within a given population therefore depends on individuals who are exposed, shedding the bacteria in their stool and contaminating either the immediate environment with 'short-cycle' person to person or household transmission or shedding the bacteria into the wider environment with 'long-cycle' transmission, contaminating water supplies.[51,183] The degree to which short-cycle and long-cycle transmission is responsible for ongoing infection within a given population is not known, and may differ depending on the epidemiological context.[44,201]

The rate at which individuals who are infected with *S.* Typhi shed bacteria in their stool is also not well understood. Acute stool shedding is defined as an individual shedding typhi bacteria in their stool up to 3 months following acute infection, with convalescent shedding >3-12 months. Chronic carriage is defined as ongoing shedding in the stool >12 months following the acute infection.[34]

This chapter will address some of these gaps in the understanding of typhoid shedding and provide data to address the outcome measures 6 and 9, which are outlined in chapter 2 of the thesis:

1. Prevalence of typhoid chronic carriage determined through serological surveillance
2. Rates of stool shedding from acute, convalescent and chronic typhoid states

In addition to performing surveillance for acute and sub-clinical infection, another aim of the study was to determine the prevalence of chronic carriers and understand their contribution to ongoing transmission of the bacteria within the populations studied. Identifying carriage is problematic as individuals are no longer symptomatic and may not recall their original acute illness. Stool sampling is logistically challenging and shedding the bacteria in the stool is sporadic over time. Using published data from Chile,[261] one approach to identifying chronic carriers within a population was through

serological screening, using the anti-Vi IgG antibody. It is well described that individuals who progress to chronic carriage have elevated levels of anti-Vi IgG antibody. As was described in chapter 6, a serological survey was performed in all three sites for the dual purpose of estimating the seroincidence of disease and identifying these individuals within the populations to determine the prevalence of carriage.

Along with identifying shedding from potential chronic carriers, participants recruited through passive surveillance were followed-up with stool collected at Day 0, Day 30 and Day 180, to investigate the rates of shedding in acute and convalescent patients.

6.2. Methods

For a detailed description on the methods for this component of the study please refer to Chapter 2, section 6 for the enrolment of participants into the serological survey. For the methods for stool collection and culture, please refer to section 3.11.4, and for processing of Vi ELISA, section 3.

6.2.1. Working Practice Document on Chronic Carriers

A working practice document was created for the three sites outlining the study definitions, testing and treatment for carriage, which was added to the study protocol and agreed on by local ethics committees. All individuals with a positive stool culture for *S.* Typhi should be given advice on personal hygiene, hand washing prior to food preparation and safe disposal of faeces. All individuals with a positive stool culture for *S.* Typhi should have history taken for food handling/childcare/patient care.

6.2.2. Definitions for Carriage, Testing and Treatment

6.2.2.1. Chronic carriers (Definitive)

For the purposes of the study we defined definitive chronic carriage as an individual excreting *S.* Typhi in the stool for longer than one year after the onset of acute typhoid fever as defined by the most up to date WHO guidelines.[262] If confirmed, all patients should be referred for ultrasound scan of the

abdomen and consideration for surgery for those with gallstones. In Malawi due to the association with both biliary and renal tract calculi empirical treatment for schistosomiasis with praziquantel 40mg/kg should be given.[263] If the *S.* Typhi tests sensitive, treatment should be ciprofloxacin 750mg BD for 28 days.[57] If evidence of fluroquinolone resistance – azithromycin (20 mg/kg per day; maximum dose, 1000 mg/day for day one, with 500mg following on) for 2 weeks. To test for cure, participants should have three consecutive negative stool cultures at least 48-hours apart and one week after antimicrobial therapy has stopped.

6.2.2.2. Chronic carriers (Presumptive)

For the purposes of the study the definition of a presumptive chronic carrier is an individual who has a high anti-Vi antibody titre as defined by analysis performed on the first 1000 samples from each site and who is excreting *S.* Typhi in the stool. Testing and treatment would be as for definitive carriers.

6.2.2.3. Convalescent carriers

For the purposes of the study the definition of a convalescent carrier is an individual who is stool culture positive discovered through routine passive surveillance who does not have a high anti-Vi antibody titre. Additional advice for food handlers/childcare providers and clinical patient care providers to follow strict hygiene advice and be followed up at 12 months to determine continued shedding and then treated as definitive chronic carriers.

6.3. Results – Setting the Threshold for 'High Vi'

Prior to starting the serological survey, it was not known what the baseline level of Vi would be within each of the populations, and how this would change with age. These two factors would determine what a 'high-Vi' result would be, and therefore what threshold to set to determine which individuals should be followed-up. After 1000 samples had been collected at each of the sites, the anti-Vi IgG titre and age-distribution was analysed in order to determine a threshold whereby participants with the highest responses would have stool collected. The rationale for setting the threshold was;

- To answer the primary research question on the prevalence of chronic carriage within the three populations.
- To sample individuals across all the age groups to investigate chronic carriage rates in relation to age.
- To choose a realistic number of participants for follow up so that field teams could meet these targets. This final point is important as stool sampling in participants homes is difficult and so field teams needed a reasonable target figure.

After the first 1000 samples had been collected, due to the timing of Ramadan in Bangladesh where fewer adults could be recruited, there was a higher proportion of young children in the Bangladesh cohort compared with Malawi and Nepal. Children have a higher proportion of anti-Vi IgG titres below the lower limit of detection for the assay. This had the effect of lowering the quantiles for Vi titre for the Bangladesh cohort and made comparisons with the other two sites problematic. Due to this, only quantiles for Vi for participants over 15 years of age were analysed for all three sites. These quantiles were then applied back across the whole cohort for all age-groups (**Error! Reference source not found.**).

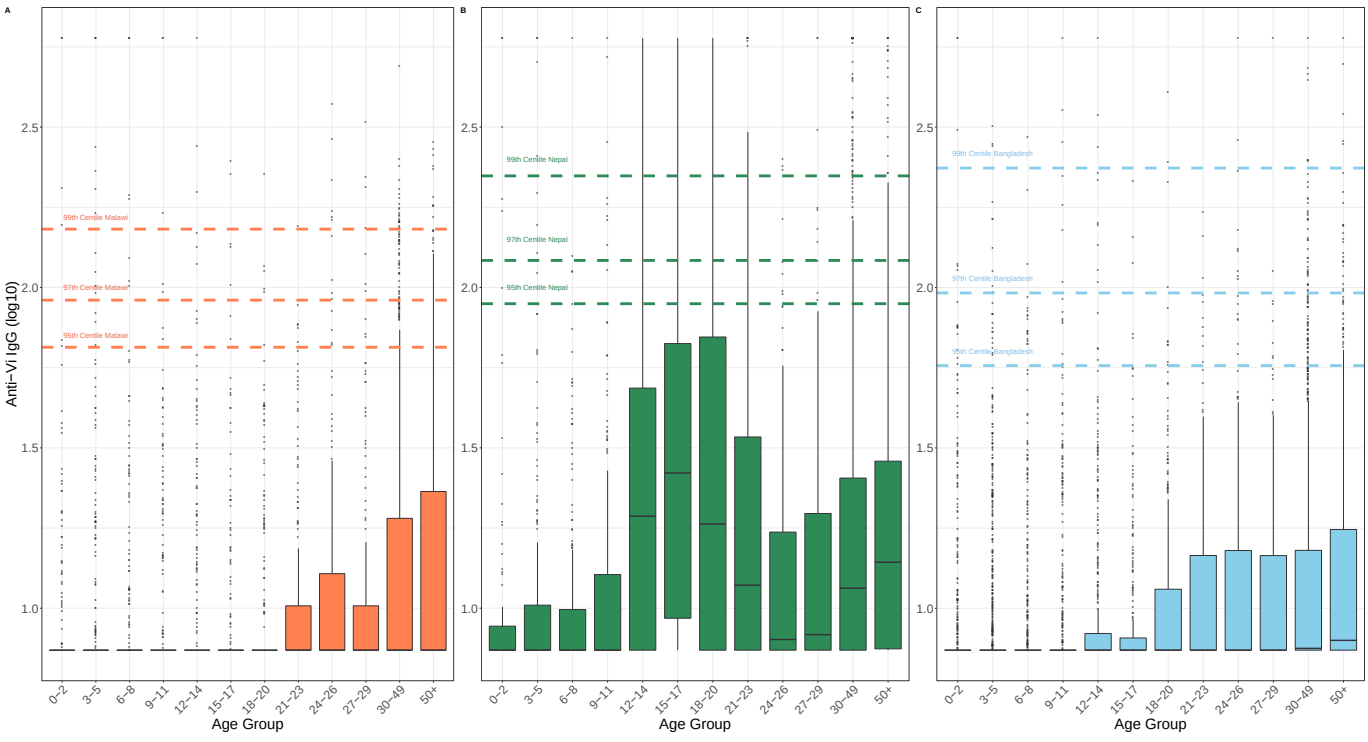


Figure6-1: Age distribution for anti-Vi IgG from the first 1000 samples from the serosurvey for A; Malawi, B; Nepal and C; Bangladesh showing the 95th, 97th and 99th centile of titres for each site.

Centiles were plotted using only data from participants >15 years of age, and then applied to the entire cohort.

The top 1%, 3% and 5% of Vi antibody levels for approximately the first 1000 samples were calculated and then the total number of participants requiring follow-up at these different thresholds was projected if the sites met their recruitment targets (Figure6-1). Bangladesh had the highest 99th centile suggesting more individuals within this population with high values. From previous data it is known that the chance of becoming a chronic carrier increases with age. To increase the sensitivity for detecting individuals at the higher age group the threshold for participants less than 15 years of age was set at the 97th centile line, reducing to the 95th centile line for participants aged over 15 years. This resulted in a projected 219, 294 and 222 participants for follow-up in Malawi, Nepal and Bangladesh respectively (**Error! Reference source not found.**). The thresholds for follow up for the three sites were;

- Malawi

Ages 0-14 – 97th Centile; 91.3 EU/ml

Ages >15 – 95th Centile; 65.1 EU/ml

- Nepal

Ages 0-14 – 97th Centile; 121.4 EU/ml

Ages >15 – 95th Centile; 89 EU/ml

- Dhaka

Ages 0-14 – 97th Centile; 96.2 EU/ml

Ages >15 – 95th Centile; 57.1 EU/ml

	Malawi			Nepal			Bangladesh		
	Planned Total	No>Centile/Total	Expected No	Planned Total	No>Centile/Total	Expected No	Planned Total	No>Centile/Total	Expected No
97th Centile									
0-4 years	2500	0.5/310	4	1880	1/132	14.2	2500	2/270	18.5

5-9 years	1300	1/184	7	1300	1/163	4	1300	1/214	3
10-14 years	800	4/121	26.4	960	8/145	53	800	1/140	5.7
95th Centile									
15-29 years	1420	1/180	7.9	1440	12/174	99.3	1200	4/84	57.1
30-49 years	1820	18/247	132.6	1820	14/294	86.7	1820	8/134	108.7
50+ years	660	6/95	41.7	1100	9/258	38.4	880	2/60	29.3
Total	8500	22/1137	**219**	8500	58/1136	**294**	8500	14/902	**222**

Table 6-1 Number of projected participants to be recruited based on Vi thresholds of 95th and 97th centile from the first 1137, 1136 and 902 samples in three populations.

6.4. Results - Participants with 'High-Vi'

In total 163, 200 and 205 participants had stool collected following a 'high-Vi' result from Malawi, Nepal and Bangladesh respectively. Two consecutive stool samples were collected from each participant within 48 hours. Figure 6-2 shows most participants with a Vi antibody titre above the threshold had a fairly static titre over the three-month period, with fewer individuals having large changes in their antibody titre, which may indicate acute exposure rather than carriage. In Malawi, younger participants had a greater change in antibody level compared with older participants in this analysis. Nepal had a greater proportion of individuals with a high response followed by Bangladesh. In this analysis when you only select out individuals with a high antibody titre, there is not an increasing titre observed with increasing age as was demonstrated in Figure 6-1 when you analyse the entire cohort.

Figure 6-2C in Bangladesh shows the only participant who was identified with *S.* Typhi in their stool across the three sites (the black line). This individual had a rising antibody titre across the two visits from 142 EU/ml to 346 EU/ml, with both visits above the set threshold. Compared with other stool-culture negative individuals at the site and at the other two sites, this participant did not have one of the highest Vi titres. The participant's characteristics were consistent with previously published data

on risk factors for being a chronic carrier. She was female, in her late thirties and was confirmed to have cholelithiasis on ultrasound scan of the biliary system.

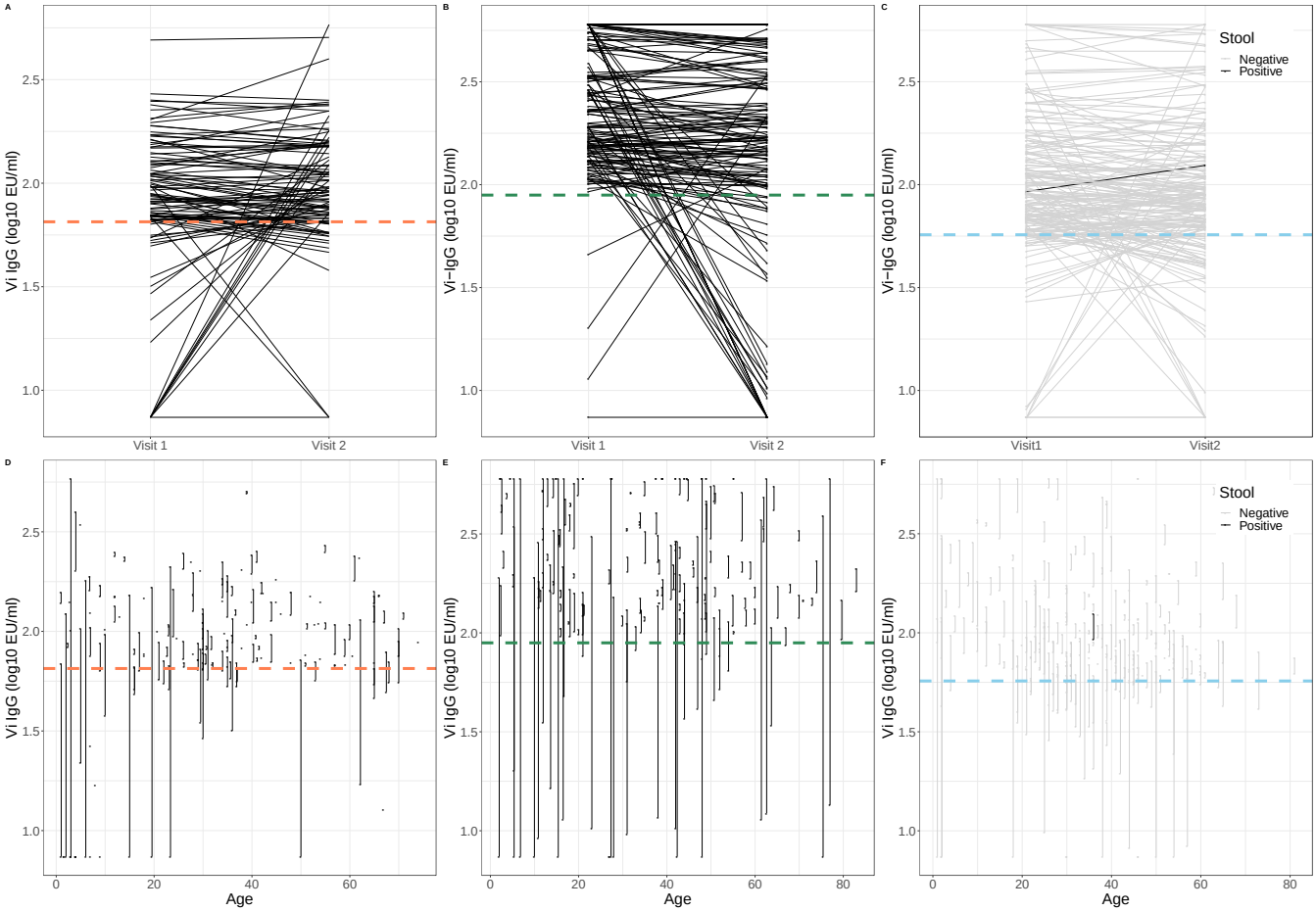


Figure 6-2: A-C; The change in antibody level between Visit 1 and Visit 2 for individuals who had a 'high-Vi' and had stool culture collected, D-F; The change in antibody level across the age-range for participants for A; Malawi, B; Nepal and C; Bangladesh. The dotted line indicates the 95th Centile for Vi IgG at each of the three sites.

6.5. Acute and convalescent stool culture results

In all three sites the highest rate of stool shedding was seen in participants who presented to a healthcare-facility with a febrile episode, who were subsequently found to be blood-culture positive for *S.* Typhi. However, there were some differences between the sites with rates of 0.34 (95% CI: 0.29-0.41) and 0.21 (95% CI: 0.12-0.35) in Bangladesh and Malawi compared with only 0.03 (95% CI: 0.008-0.08) in Nepal. Shedding of *S.* Typhi for participants with an *S.* Typhi bacteraemia was higher in Bangladesh and Nepal compared with shedding of *S.* Paratyphi A for participants with an *S.* Paratyphi A bacteraemia, (Bangladesh; 0.12 (95% CI: 0.06-0.21), Nepal; 0 (95% CI: 0-0.25)) which may support

previous data suggesting transmission routes of these two pathogens are different. In all three sites there was evidence of *S.* Typhi stool-culture positive, blood-culture negative participants. Whilst these cases were not included in the formal definition of an acute case, it is likely that these individuals would have been bacteraemic at some point in time during their illness (Figure*6-3*).

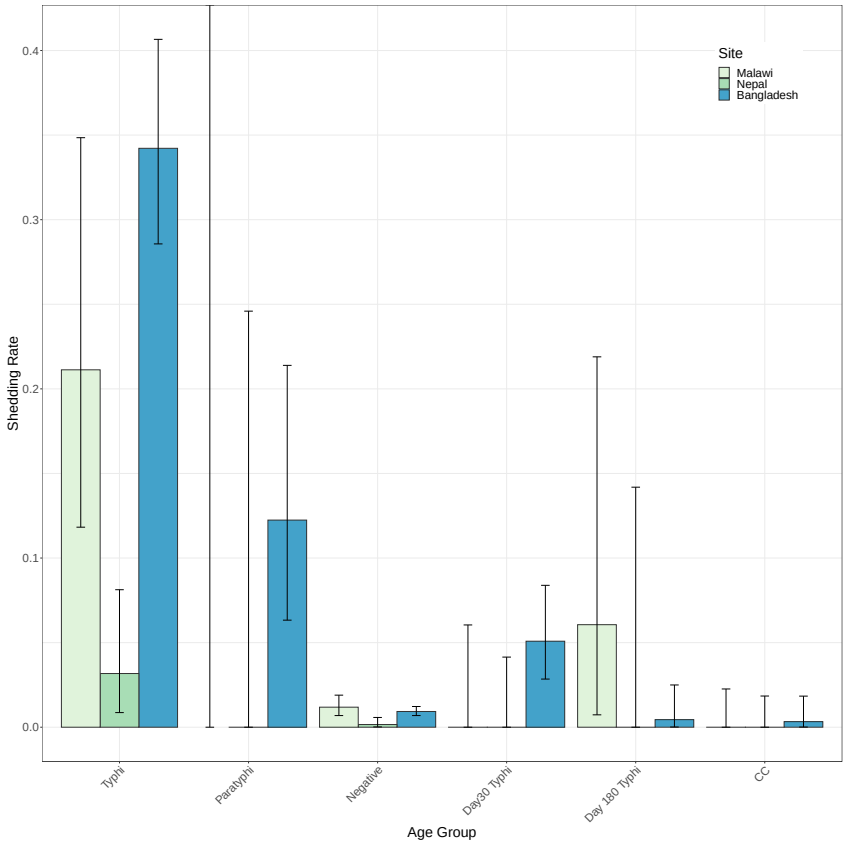


Figure6-3: Proportion of individuals with a positive stool culture for S. Typhi (or S. Paratyphi A in the Paratyphi column) for participants at; Typhi; Day 0 Passive Surveillance Enrolment S. Typhi blood culture positive, Paratyphi; Day 0 Passive Surveillance Enrolment *S.* Paratyphi A blood culture positive, Negative; Day 0 Passive Surveillance Enrolment blood culture negative, Day 30 Typhi; Day 30 follow up for participants that were *S.* Typhi blood culture positive on day 0, Day 180 Typhi; Day 180 follow up for participants that were *S.* Typhi blood culture positive on day 0, CC; Participants with anti-Vi IgG above the threshold indicating potential chronic carriage.

In both Malawi and Bangladesh, participants who had both a blood-culture and stool-culture collected when they presented with a febrile illness to a healthcare-facility showed that those who were *S.* Typhi blood-culture and stool-culture positive had a shorter duration of fever prior to attending the facility and had a lower usage of antibiotics in the two weeks prior to seeking healthcare compared with participants who were *S.* Typhi blood-culture positive but stool-culture

negative (Error! Reference source not found.). There was no significant difference in the median age or measured temperature between these two groups. This would suggest that shedding during your acute illness occurs earlier in the natural history of disease and is interrupted by antimicrobial therapy but is not more prevalent in certain age groups, or associated with more severe disease, which a higher recorded temperature might represent. This would also indicate why rates of shedding in the convalescent day 30 samples was so low, as a high majority of *S.* Typhi blood-culture positive cases were prescribed antimicrobials. The number of cases from Nepal with *S.* Typhi positive stool cultures were so few, it prevented any meaningful comparisons.

	Malawi			Bangladesh		
	BC positive SC positive	BC positive – SC negative	p-value	BC positive SC positive	BC positive SC negative	p-value
Age (Years)	11.3 (7.5-18.2)	9.9 (6.6-20)	0.65	8.22 (5.2-13.3)	8.9 (4.7-18.1)	0.27
Temperature (^{O}C)	38.4 (38-38.7)	38.6 (38-39)	0.68	38 (37-38.8)	37.8 (37-38.5)	0.46
Fever Duration (Days)	**4 (3-5)**	**6 (3-7)**	**0.17**	**5 (3-7)**	**5 (4-7)**	**0.04**
Prior Antibiotics (proportion)	**0.13**	**0.22**	**0.4**	**0.17**	**0.66**	**<0.05**

Table 6-2: Comparison of participants who are blood-culture positive (BC) and stool-culture positive (SC) with participants who are blood-culture positive but stool culture negative, with associated p-values, from passive surveillance day 0.

The age-spread of participants who shed *S.* Typhi in stool was similar to the overall age-spread of blood-culture confirmed acute disease, with young and school-aged children being the predominant age-group affected (Error! Reference source not found.). Interestingly, in both Malawi and Bangladesh there were a few very young children under 1 year of age, who were found to be stool-culture positive but blood-culture negative.

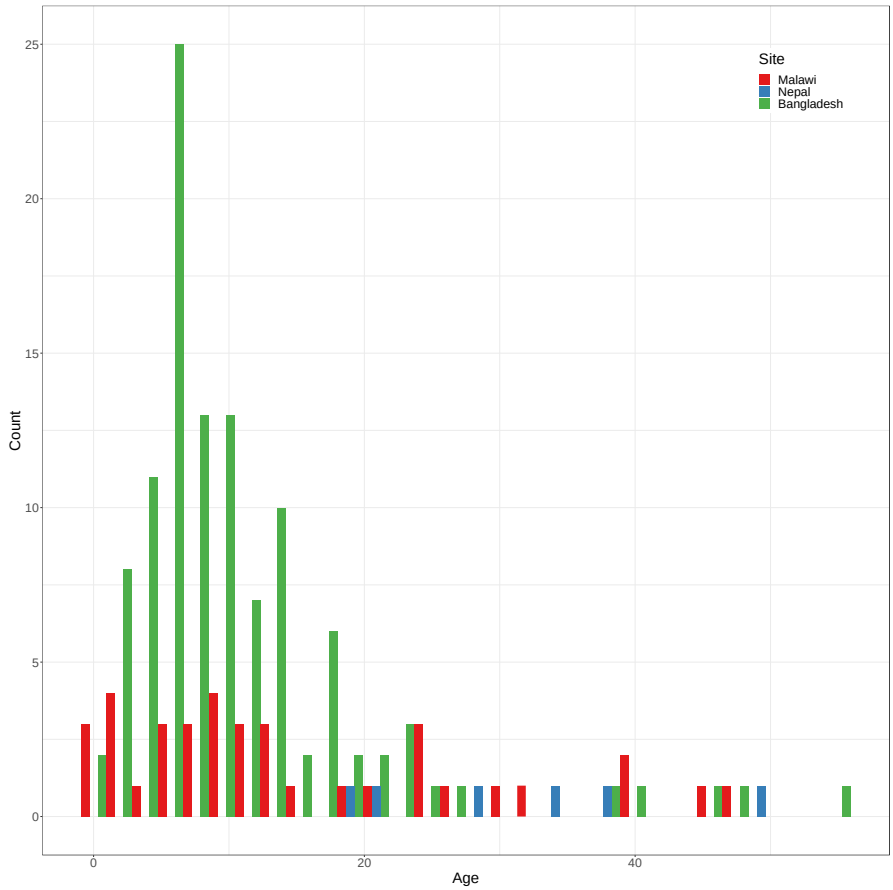


Figure 6-4: Age distribution of participants with S. Typhi positive stool-culture from Day 0 passive surveillance, either blood-culture positive or negative across three sites.

6.6. Discussion

In Malawi, Nepal and Bangladesh the rate of shedding for S. Typhi is highest in acutely infected participants compared with convalescent participants and potential chronic carriers identified through serological surveillance. Rates of shedding are also higher in S. Typhi bacteraemia, compared with *S.* Paratyphi A bacteraemia. Due to the age-profile of acute blood-culture confirmed disease, children are the predominant age-group responsible for shedding. These data across three different epidemiological contexts are consistent with previously published data and provide important insights into the contribution of transmission of *S.* Typhi and *S.* Paratyphi A in endemic sights.

Data from the Oxford controlled human infection model for *S.* Typhi show a number of similar trends to these real-world data. Firstly, rates of shedding were higher in day 1 and day 2 following challenge compared with later in the follow-up period and shedding entirely stopped after receipt of anti-microbials. Rates of shedding were higher in blood-culture confirmed cases, and blood-culture

confirmed *S.* Typhi had higher rates of shedding than blood-culture confirmed *S.* Paratyphi A.[183] A number of these observations are also confirmed in historical challenge studies performed in the 1960s.[264] Consistency with these challenge studies is important, as the Oxford data also showed that individuals who had received a typhoid vaccine, had lower rates of shedding. This suggests that in addition to providing individual protection, a vaccine may prevent shedding therefore interrupting transmission of the pathogen and providing community-level herd protection.

Through the serological surveillance and stool-culture collection only one chronic carrier was identified. This was lower than expected prior to the study being performed, and lower than has previously been identified in other published data from other sites.[52] However, this finding may be genuine for a number of reasons. Firstly, in Malawi and Nepal high rates of *S.* Typhi have only been observed since 2010 and 2002 respectively when an MDR strain of S. Typhi entered the population for the first time, and in Nepal, there was higher than expected rainfall.[31,151] This may not have provided enough exposure time to the pathogen within the populations for a significant number of these carriers to be created. Secondly, the literature provides a number of risk factors for carriage, of which cholelithiasis is primary.[57] Whilst there are no published data on this, discussion with surgical colleagues in the Queen Elizabeth Central Hospital in Blantyre suggests that the prevalence of cholelithiasis in Malawi is low. Thirdly, most studies identifying a population prevalence for chronic carriers were performed in the 1980s, prior to the introduction and widespread use of fluoroquinolones and third generation cephalosporins which we know have a higher treatment success, and prevention of carriage. As our data demonstrate, fluoroquinolones and cephalosporins are the predominant antibiotic used in these sites for both febrile illnesses broadly and typhoid fever more specifically, and so this could be having an impact on the progression from acute disease to chronic carriage within individuals acutely infected.

The data presented here in this analysis is important as it shows that not only is the burden of acute disease concentrated in young and school-aged children, but the predominant burden of stool-

shedding is also within this population. Introduction of an efficacious vaccine to children from 9months of age, that provides protection for greater than ten years could have a significant impact on the incidence of acute disease and the transmission of the pathogen through the population. Along with data from the challenge model, the cluster-randomised trial of the Vi-PS vaccine in Kolkatta, India demonstrated an overall protection of 57% within clusters that received typhoid vaccine. This also supports the hypothesis that the vaccine must interrupt transmission and provide protection for the unvaccinated individuals within the population.

Previously published data from Kathmandu, Nepal have demonstrated a different set of risk factors for acquisition of *S.* Typhi and *S.* Paratyphi A highlighting risk factors outside the home may be more important for acquiring *S.* Paratyphi A.[44] In another study, *S.* Paratyphi A was more diffusely spread across the city with less spatial clustering when compared with *S.* Typhi.[201] In this chapter, I have demonstrated the lower rate of shedding with *S.* Paratyphi A bacteraemia, which would be consistent with these data, conforming the hypothesis that short-cycle, person to person transmission may be less significant.

There are a few limitations with the results presented here. Firstly, the low number of positive stool-culture cases from Nepal from all components of the study highlights a potential problem with either the collection of or techniques used for culture of stool in the laboratory used for the study. There was a high usage of antimicrobials in Nepal prior to seeking healthcare which may partially explain the low numbers of participants with *S.* Typhi isolated from stool, but the rates are markedly lower than the other two sites.

Secondly, as discussed already, the low rate of *S.* Typhi in the stool-cultures of participants with a high-Vi antibody titre may be a genuine result. Alternatively, the design of this component of the study may be responsible. Shedding of S. Typhi in stool is likely to be sporadic and so only collecting two consecutive stool-cultures within 48 hours may not have been sufficient to capture evidence of shedding. This approach was chosen on a largely logistical basis so that a larger number of

participants could be followed up. A previous study from Vietnam where participants were followed up once a week for six weeks following a high-Vi titre for a repeat rectal swab, found no cases of S. Typhi.[62] Alternatively, it may be that anti-Vi IgG is not the best antibody to identify chronic carriage. It also has the complication that once Vi-based conjugate vaccines are introduced into a population, using Vi-antibody to identify participants would become impossible. There is a set of novel antigens that are being validated with this data set, to see if an alternative antigen could be used to better identify carriage within endemic sites.

7. Chapter 7 - Discussion

7.1. Introduction

Enteric fever is a major public health concern in many low- and middle-income countries. The global incidence of disease is estimated to be 14.3 million infections with *S.* Typhi and *S.* Paratyphi annually.[104] This is an estimated reduction in disease incidence since the year 1990, however the number of population-based burden studies from which to derive these estimates, particularly in Africa is small. Additionally, the design of these typhoid surveillance studies have been different.[177] Where incidence is estimated using only blood culture-confirmed cases from facility-based surveillance, this is likely to be an underestimate of disease burden.[265]

Antimicrobial resistance is increasing globally[255] with the emergence over time of multi-drug resistance,[266] fluroquinolone resistance,[267] azithromycin resistance[232] and now the XDR strains of typhoid circulating in Pakistan with MDR, FQ and cephalosporin resistance.[32] This escalation in anti-microbial resistance threatens to reverse some of the trends in global incidence, as untreatable typhoid fever takes communities back to a pre-antibiotic era.[155]

However, the development and introduction of an efficacious typhoid vaccine could have a major impact on the incidence of disease throughout much of the world. Through phase 2 trials in the controlled human infection model, and now phase 3 trials, efficacy data for the Vi-Typhoid conjugate vaccine is being demonstrated.[165,221,257]

For typhoid conjugate vaccines to have the largest impact, countries need a detailed understanding of the epidemiology of disease in their population, or at least a comparative population with similar geography and demographics. In this thesis I have presented data from three large epidemiological studies describing a multi-component program of surveillance, with the aim of accurately understanding the epidemiology of enteric fever in three endemic sites.

7.2. Summary of Findings

7.2.1. Site Description and Healthcare usage

The detailed description of these three endemic sites enabled a deeper understanding of the risk factors for infection within them, and the potential impact of vaccine introduction. Malawi was a low-income site, with poor infrastructure and the youngest population. The healthcare seeking behaviour was more homogenous, with most of the population using government facilities. In the two Asia sites, there was a wide variety of healthcare usage, with participants using private pharmacies and clinics, with high rates of antimicrobial use and high rates of antimicrobial resistance. Nepal and Bangladesh had high rates of migration in and out of the census area, which would likely maintain a population of susceptible individuals. The rates of migration in all three sites would support a policy of vaccine introduction at the country level, rather than a 'hot-spot' approach. Only vaccinating children within the high incidence urban areas, for example would provide limited protection for those individuals who migrate in to the higher incidence zone, and reduce the overall impact of any vaccine campaign.[171]

7.2.2. Observed and Adjusted Incidence Rates

S. Typhi was the commonest source of bacteraemia in participants with prolonged fever at the three sites in this study. Blood-culture surveillance of febrile individuals from three endemic sites for enteric fever revealed a medium to high incidence of disease based on blood culture-confirmed cases. However, with adjustments made for reasons known to cause an under-estimate in disease burden, all three sites had a high incidence of disease overall. For *S.* Typhi, 5-9-year-olds had the highest incidence as an age group in all three sites, with very high incidence in children in the 4th year of life in Bangladesh. Disease was seen in children under two years of age. Adjustment factors were not the same across the three sites, with proportion of recruitment having the largest impact in Malawi, with healthcare seeking behaviour having the largest impact in the two Asia sites. As discussed in Chapter

3 and 4 this understanding of differing adjustment factors in different epidemiological contexts is likely to lead to more refined and accurate incidence estimates, at a local, national and global level.

These findings are consistent with much of the literature on typhoid burden, showing the peak of disease in young and school-aged children.[25,104,124,228] With no cases detected in children less than 6 months of age and very few less than one year, it would support the decision to introduce an efficacious vaccine at 9 months. This would have a number of benefits. Firstly, most countries EPI schedule contains a visit at 9 months for measles vaccine. Current typhoid vaccine trials are assessing co-administration of TCV with the measles vaccine to ensure immunogenicity is not affected.[172] Assuming this is not the case, this would allow countries to introduce a new vaccine without any change to the EPI schedule, and the infrastructural investment this would require. If as has been recommended, vaccine was introduced with a catch-up campaign to 15 years of age, individuals within the age-distribution who most commonly present with disease would be largely protected.[171,181]

7.2.3. Seroincidence of Infection

The seroincidence data from the three sites, apart from within two age groups in Nepal and Bangladesh, either exceeded or had overlapping confidence intervals with the adjusted blood culture-confirmed incidence of infection. Higher rates of infection were found in the older age groups compared with acute surveillance. Despite some of the limitations of using anti-Vi IgG as a marker of acute infection, these three serosurveys have shown that using serological surveillance for estimating typhoid incidence is possible, and that seroincidence estimates could be used as an upper-bound estimate for a population incidence. This could be a valuable method for estimating disease burden in populations where the incidence of disease is not known, and decisions on vaccine introduction need to be made where the logistics of setting up blood-culture collection and processing are not possible.

The higher rate of infection estimated in the older age groups, may suggest ongoing exposure and infection with *S.* Typhi that is not captured through routine clinical surveillance. This could potentially support vaccine catch-up campaigns to 45 years of age, to prevent exposure and consequently shedding of the pathogen into the community.

7.2.4. Prevalence of Carriage

The question on the prevalence and importance of typhoid chronic carriers within endemic sites is an important one, particularly in terms of overall community protection following vaccine introduction. The data presented here potentially shows that carriage within these sites is not a significant driver of disease, as only one individual out of almost 600 followed-up was identified to be shedding *S.* Typhi in the stool. This may be due to the relatively recent introduction of *S.* Typhi into these populations, particularly in Malawi or a lack of the known risk factors for carriage acquisition, namely cholelithiasis. Previous estimates of carriage prevalence were from before the use of fluoroquinolone antimicrobials which we now know have a lower infection relapse rate, and likely prevent the development of carriage when compared with antibiotics such as co-trimoxazole and chloramphenicol.

As discussed already, anti-Vi IgG may not be the most sensitive marker for carriage, and further serological work is planned with these samples. Identifying a new serological marker will be of particular importance following the introduction of Vi-based conjugate vaccines as all immunised individuals will have persistently higher anti-Vi IgG levels. Through protein microarray analysis of enteric fever plasma samples, a number of antigens with high sero-diagnostic potential have been identified for both acute infection and chronic carriage.[67,268] Through this study we have over 15,000 paired samples from community-based serological surveillance of healthy individuals and over 5000 samples from febrile participants presenting to healthcare facilities which can be used to further evaluate these antigens.

Diagnosis of typhoid fever remains a significant limitation in the control of this disease. Due to the non-specific presentation of the disease, clinical diagnosis alone is difficult. To perform blood-cultures

requires high levels of laboratory infrastructure that are not present in many endemic sites and the sensitivity is only around 60%. Current serology-based rapid-diagnostic tests do not have the sensitivity or specificity to be used reliably.[76] The further assessment of new antigens for the diagnosis of acute disease, to use in seroincidence studies and to identify chronic carriage would be a very important development in global typhoid control.

Additionally, carriers may shed bacteria in the stool intermittently and the collection of stool in this study may not have been sufficient to capture this. As these three sites have performed vaccine trials and will be some of the first globally to apply for Gavi support to introduce vaccine, it remains to be seen the level of population protection the vaccine will provide.

7.2.5. Acute Stool Shedding

Across all three sites, and particularly in Bangladesh, the highest rate of shedding was found in participants who were blood culture-positive for *S.* Typhi compared with blood culture-negative participants and convalescent participants. Due to the age-distribution of acute disease, this was primarily school-aged children. *S.* Typhi also had a higher rate of shedding in acute disease compared with *S.* Paratyphi A. Evidence from the human challenge model suggests, that along with providing individual protection against infection, typhoid vaccine also reduces the rate of shedding.[183] These data, combined with data from a Vi-polysaccharide typhoid vaccine trial in Kolkata, demonstrating a degree of herd protection with the vaccine[112] show that typhoid conjugate vaccines may produce an overall protective efficacy when introduced to endemic communities.

7.3. Vaccine Introduction

One of the major outputs from this series of studies has been important data on disease burden informing decision making on typhoid vaccine policy at a global level. Burden data from the three studies was presented at the WHO Strategic Advisory Group of Experts meeting when typhoid conjugate vaccines were recommended for country introduction into countries with a high burden of

disease or high rates of antimicrobial resistance.[279] These data were also shared with Gavi, the vaccine alliance when they allocated funding for introduction of vaccine into Gavi-eligible countries.[222]

Starting in 2018 in all three sites, as described in Appendix 1 was a series of phase 3 typhoid conjugate vaccine trials, to test the efficacy of vaccine in children from 9months of age.[171,280] The first data from these trials has been published from the Nepal site, showing direct efficacy of TCV of 82% at 1 year.[257] The surveillance system for these trials was based upon the surveillance system set-up for the set of studies described in this thesis. The Nepal trial is a phase 3 double-blind, individually randomised trial, recruiting children from 9-months to 16-years of age, with an initial follow-up time of 2 years.[281] In Malawi, a similar trial design is being employed, with recruitment up to 12-years of age.[172,282] In Bangladesh, in order to explore the indirect effects of vaccine introduction, a cluster-randomised trial is being performed, with recruitment of children from 9-months of age to 16-years of age.[174] In addition to efficacy trials, all 3 studies also have a safety and immunogenicity sub-study.

Following efficacy data at two years, these studies will be best placed to provide longer term efficacy data out to 5-years as blood culture-surveillance continues. In addition to these data, as countries introduce vaccine into EPI schedules, it will be important to assess vaccine impact on a national level post-introduction. The three sites described here are well placed to demonstrate the impact of vaccine rollout, with well-functioning surveillance systems and detailed pre-vaccine data.

The work described in this thesis detailing accurate burden estimates utilising multiple surveillance approaches, combined with vaccine efficacy from field trials will provide policy makers with the data they require to understand more clearly the potential disease burden of *S.* Typhi, and the likely impact vaccine introduction might have.

7.4. S. Paratyphi A and *S.* Typhimurium

With the introduction of typhoid conjugate vaccines, the relative importance of *Salmonella* Paratyphi A is likely to increase. As has been shown in this thesis, there is already high rates of disease in South

Asia and there is concern that as *S.* Typhi rates decrease, *S.* Paratyphi A may fill the ecological niche.[283] The vaccine pipeline for S. Paratyphi A is further behind vaccines for S. Typhi but some are in development and clinical trials.[284] Additionally, the development of serological assays for *S.* Paratyphi A would enable serological surveys to be performed to further characterise burden of disease in different sites.

In the data presented here from Malawi and from other publications throughout sub-Saharan Africa there remains a significant incidence of infection from *Salmonella* Typhimurium, particularly in the very youngest children.[125,252,253] The risk factors for *S.* Typhimurium bacteraemia have been known for some time and include advanced HIV infection, malaria co-infection, malnutrition and anaemia.[2] As the prevalence for some of these conditions decrease, the incidence of *S.* Typhimurium has decreased also, but with an infection that has a mortality of 10-20%, the burden of disease in this part of the world remains considerable.

7.5. Conclusion

The multi-country, multi-component surveillance described in this study has demonstrated high rates of disease with *S.* Typhi and *S.* Paratyphi A in combination with observed high rates of antimicrobial resistance. This necessitates multiple intervention strategies to achieve global control of these pathogens through development of water and sanitation infrastructure, the introduction of efficacious typhoid conjugate vaccines and the development of vaccines for *S.* Paratyphi A. The necessary improvements to water and sanitation would be challenging, costly and would take time to implement. The children and families who currently live in these endemic areas, do not have the luxury of that time. The medical and scientific communities have known about this pathogen for over 100 years and it has been virtually eradicated from the high-income countries of the world. The global community now has the understanding required to introduce efficacious vaccine into these high-incidence populations to reduce the burden of this afflicting disease.

8. Bibliography

1. Dougan, G. & Baker, S. *Salmonella enterica* Serovar Typhi and the Pathogenesis of Typhoid Fever. *Annu. Rev. Microbiol.* **68**, 317–336 (2014).

2. Feasey, N. A., Dougan, G., Kingsley, R. A., Heyderman, R. S. & Gordon, M. A. Invasive non-typhoidal salmonella disease: an emerging and neglected tropical disease in Africa. *Lancet (London, England)* **379**, 2489–2499 (2012).

3. Bergh, E. T. A. M., Hussein Gasem, M., Keuter, M. & Dolmans, M. V. Outcome in three groups of patients with typhoid fever in Indonesia between 1948 and 1990. *Trop. Med. Int. Heal.* **4**, 211–215 (1999).

4. Gay, F. Typhoid fever considered as a problem of scientific medicine : Gay, Frederick P. (Frederick Parker), 1874-1939 : Free Download, Borrow, and Streaming : Internet Archive. Available at: https://archive.org/details/typhoidfevercon00gayf/page/2. (Accessed: 14th June 2019)

5. Gadeholt, H. & Madsen, S. T. Clinical Course, Complications and Mortality in Typhoid Fever as Compared with Paratyphoid B. *Acta Med. Scand.* **174**, 753–760 (2009).

6. Parry, C. M., Hien, T. T., Dougan, G., White, N. J. & Farrar, J. J. Typhoid Fever. *N. Engl. J. Med.* **347**, 1770–1782 (2002).

7. Azmatullah, A., Qamar, F. N., Thaver, D., Zaidi, A. K. & Bhutta, Z. A. Systematic review of the global epidemiology, clinical and laboratory profile of enteric fever. *J. Glob. Health* **5**, 020407 (2015).

8. STUART, B. M. & PULLEN, R. L. TYPHOID. *Arch. Intern. Med.* **78**, 629 (1946).

9. Bhan, M., Bahl, R. & Bhatnagar, S. Typhoid and paratyphoid fever. *Lancet* **366**, 749–762 (2005).

10. Bhutta, Z. A. Current concepts in the diagnosis and treatment of typhoid fever. *BMJ* **333**, 78–82 (2006).

11. Britto, C., Pollard, A. J., Voysey, M. & Blohmke, C. J. An Appraisal of the Clinical Features of Pediatric Enteric Fever: Systematic Review and Meta-analysis of the Age-Stratified Disease Occurrence. *Clin. Infect. Dis.* **64**, 1604–1611 (2017).

12. Cook, G. C. (Gordon C., Zumla, A. & Manson, P. *Manson's tropical diseases.* (Saunders/Elsevier, 2009).

13. Maskey, A. P. *et al.* Salmonella enterica Serovar Paratyphi A and S. enterica Serovar Typhi Cause Indistinguishable Clinical Syndromes in Kathmandu, Nepal. *Clin. Infect. Dis.* **42**, 1247–1253 (2006).

14. Relative bradycardia in infectious diseases. *J. Infect.* **33**, 185–191 (1996).

15. Crump, J. A., Sjölund-Karlsson, M., Gordon, M. A. & Parry, C. M. Epidemiology, Clinical Presentation, Laboratory Diagnosis, Antimicrobial Resistance, and Antimicrobial Management of Invasive Salmonella Infections. *Clin. Microbiol. Rev.* **28**, 901–37 (2015).

16. Osuntokun, B. O., Bademosi, O., Ogunremi, K. & Wright, S. G. Neuropsychiatric Manifestations

of Typhoid Fever in 959 Patients. *Arch. Neurol.* **27**, 7–13 (1972).

17. Lutterloh, E. *et al.* Multidrug-resistant typhoid fever with neurologic findings on the Malawi-Mozambique border. *Clin. Infect. Dis.* **54**, 1100–1106 (2012).

18. Britto, C. *et al.* Laboratory and Molecular Surveillance of Paediatric Typhoidal Salmonella in Nepal: Antimicrobial Resistance and Implications for Vaccine Policy. *bioRxiv* 250142 (2018). doi:10.1101/250142

19. Hoffman, S. L. *et al.* Reduction of Mortality in Chloramphenicol-Treated Severe Typhoid Fever by High-Dose Dexamethasone. *N. Engl. J. Med.* **310**, 82–88 (1984).

20. Bitar, R. & Tarpley, J. Intestinal Perforation in Typhoid Fever: A Historical and State-of-the-Art Review. *Clin. Infect. Dis.* **7**, 257–271 (1985).

21. Hosoglu, S. *et al.* Risk Factors for Enteric Perforation in Patients with Typhoid Fever. *Am. J. Epidemiol.* **160**, 46–50 (2004).

22. Neil, K. P. *et al.* A large outbreak of typhoid fever associated with a high rate of intestinal perforation in Kasese District, Uganda, 2008-2009. *Clin. Infect. Dis.* **54**, 1091–1099 (2012).

23. WHO | Vaccine Preventable Diseases Surveillance Standards. *WHO* (2018).

24. Mogasale, V. *et al.* Case fatality rate and length of hospital stay among patients with typhoid intestinal perforation in developing countries: A systematic literature review. *PLoS One* **9**, (2014).

25. Crump, J. A., Luby, S. P. & Mintz, E. D. The global burden of typhoid fever. *Bull. World Health Organ.* **82**, 346–53 (2004).

26. Mogasale, V. *et al.* Burden of typhoid fever in low-income and middle-income countries: a systematic, literature-based update with risk-factor adjustment. *Lancet. Glob. Heal.* **2**, e570-80 (2014).

27. Buckle, G. C., Walker, C. L. F. & Black, R. E. Typhoid fever and paratyphoid fever: Systematic review to estimate global morbidity and mortality for 2010. *J. Glob. Health* **2**, 010401 (2012).

28. Qamar, F. N., Azmatullah, A. & Bhutta, Z. A. Challenges in measuring complications and death due to invasive Salmonella infections. *Vaccine* **33**, C16–C20 (2015).

29. Pieters, Z., Saad, N. J., Antillón, M., Pitzer, V. E. & Bilcke, J. Case Fatality Rate of Enteric Fever in Endemic Countries: A Systematic Review and Meta-analysis. *Clin. Infect. Dis.* **67**, 628–638 (2018).

30. Antillón, M., Bilcke, J., Paltiel, A. D. & Pitzer, V. E. Cost-effectiveness analysis of typhoid conjugate vaccines in five endemic low- and middle-income settings. *Vaccine* **35**, 3506–3514 (2017).

31. Feasey, N. N. A. *et al.* Rapid Emergence of Multidrug Resistant, H58-Lineage Salmonella Typhi in Blantyre, Malawi. *PLoS Negl. Trop. Dis.* **9**, e0003748 (2015).

32. Klemm, E. J. *et al.* Emergence of an Extensively Drug-Resistant Salmonella enterica Serovar Typhi Clone Harboring a Promiscuous Plasmid Encoding Resistance to Fluoroquinolones and

Third-Generation Cephalosporins. *MBio* **9**, e00105-18 (2018).

33. Levine, M. M. & Simon, R. The Gathering Storm: Is Untreatable Typhoid Fever on the Way? *MBio* **9**, e00482-18 (2018).

34. Crump, J. A. Progress in Typhoid Fever Epidemiology. *Clin. Infect. Dis.* **68**, S4–S9 (2019).

35. Sedgwick, W. T. & Macnutt, J. S. On the Mills-Reincke Phenomenon and Hazen s Theorem Concerning the Decrease in Mortality from Diseases Other than Typhoid Fever Following the Purification of Public Water-Supplies. *J. Infect. Dis.* **7**, 489–564 (1910).

36. SOPER, G. A. THE WORK OF A CHRONIC TYPHOID GERM DISTRIBUTOR. *JAMA J. Am. Med. Assoc.* **XLVIII**, 2019 (1907).

37. Moorhead, R. William Budd and typhoid fever. *J. R. Soc. Med.* **95**, 561–4 (2002).

38. Smith, D. C. On the causes of fevers (1839) by William Budd. *Henry E. Sigerist Suppl. Bull. Hist. Med.* iii–xi, 1–164 (1984).

39. Budd, W. ON INTESTINAL FEVER. *Lancet* **74**, 131–133 (1859).

40. Luxemburger, C. *et al.* Risk factors for typhoid fever in the Mekong delta, southern Viet Nam: a case-control study. *Trans. R. Soc. Trop. Med. Hyg.* **95**, 19–23 (2001).

41. Vollaard, A. M. *et al.* Risk Factors for Typhoid and Paratyphoid Fever in Jakarta, Indonesia. *JAMA* **291**, 2607 (2004).

42. Alba, S. *et al.* Risk Factors of Typhoid Infection in the Indonesian Archipelago. *PLoS One* **11**, e0155286 (2016).

43. Velema, J. P., van Wijnen, G., Bult, P., van Naerssen, T. & Jota, S. Typhoid fever in Ujung Pandang, Indonesia - high-risk groups and high-risk behaviours. *Trop. Med. Int. Heal.* **2**, 1088–1094 (1997).

44. Karkey, A. *et al.* Differential Epidemiology of Salmonella Typhi and Paratyphi A in Kathmandu, Nepal: A Matched Case Control Investigation in a Highly Endemic Enteric Fever Setting. *PLoS Negl. Trop. Dis.* **7**, e2391 (2013).

45. Black, R. E. *et al.* Case-control study to identify risk factors for paediatric endemic typhoid fever in Santiago, Chile. *Bull. World Health Organ.* **63**, 899–904 (1985).

46. Luby, S. P. *et al.* Risk factors for typhoid fever in an endemic setting, Karachi, Pakistan. *Epidemiol. Infect.* **120**, 129–38 (1998).

47. Bohnhoff, M. & Miller, C. P. Enhanced Susceptibility to Salmonella Infection in Streptomycin-Treated Mice*. *J. Infect. Dis.* **111**, 117–127 (1962).

48. Gauld, J. S. *et al.* Domestic river water use and risk of typhoid fever: results from a case-control study in Blantyre, Malawi. *Clin. Infect. Dis.* (2019). doi:10.1093/cid/ciz405

49. Prasad, N. *et al.* Epidemiology and risk factors for typhoid fever in Central Division, Fiji, 2014–2017: A case-control study. *PLoS Negl. Trop. Dis.* **12**, e0006571 (2018).

50. Weinberg, G. A. & Szilagyi, P. G. Vaccine Epidemiology: Efficacy, Effectiveness, and the Translational Research Roadmap. *J. Infect. Dis.* **201**, 1607–1610 (2010).

51. Pitzer, V. E. *et al.* Predicting the Impact of Vaccination on the Transmission Dynamics of Typhoid in South Asia: A Mathematical Modeling Study. *PLoS Negl. Trop. Dis.* **8**, e2642 (2014).

52. Levine, M. M., Black, R. E. & Lanata, C. Precise estimation of the numbers of chronic carriers of Salmonella typhi in Santiago, Chile, an endemic area. *J. Infect. Dis.* **146**, 724–6 (1982).

53. Marineli, F., Tsoucalas, G., Karamanou, M. & Androutsos, G. Mary Mallon (1869-1938) and the history of typhoid fever. *Ann. Gastroenterol.* **26**, 132–134 (2013).

54. Schiøler, H. *et al.* Scandinavian Journal of Infectious Diseases Biliary Calculi in Chronic Salmonella Carriers and Healthy Controls: A Controlled Study Biliary Calculi in Chronic Salmonella Carriers and Healthy Controls: A Controlled Study. *Scand J Infect Dis* **15**, 17–19 (1983).

55. Crawford, R. W. *et al.* Gallstones play a significant role in Salmonella spp. gallbladder colonization and carriage. *Proc. Natl. Acad. Sci.* **107**, 4353–4358 (2010).

56. Dongol, S. *et al.* The Microbiological and Clinical Characteristics of Invasive Salmonella in Gallbladders from Cholecystectomy Patients in Kathmandu, Nepal. *PLoS One* **7**, e47342 (2012).

57. Gunn, J. S. *et al.* Salmonella chronic carriage: epidemiology, diagnosis, and gallbladder persistence. *Trends Microbiol.* **22**, 648–55 (2014).

58. Gonzalez-Escobedo, G., Marshall, J. M. & Gunn, J. S. Chronic and acute infection of the gall bladder by Salmonella Typhi: understanding the carrier state. *Nat. Rev. Microbiol.* **9**, 9–14 (2011).

59. Olsen, S. J. *et al.* Outbreaks of typhoid fever in the United States, 1960-99. *Epidemiol. Infect.* **130**, 13–21 (2003).

60. BOKKENHEUSER, V. DETECTION OF TYPHOID CARRIERS. *Am. J. Public Health Nations. Health* **54**, 477–486 (1964).

61. Lin, F. Y. *et al.* Restaurant-associated outbreak of typhoid fever in Maryland: identification of carrier facilitated by measurement of serum Vi antibodies. *J. Clin. Microbiol.* **26**, 1194–1197 (1988).

62. Gupta, A. *et al.* Evaluation of community-based serologic screening for identification of chronic Salmonella Typhi carriers in Vietnam. *Int. J. Infect. Dis.* **10**, 309–314 (2006).

63. Ferreccio, C. *et al.* [The detection of chronic Salmonella typhi carriers: a practical method applied to food handlers]. *Rev. Med. Chil.* **118**, 33–37 (1990).

64. Lanata, C. F. *et al.* Vi serology in detection of chronic Salmonella typhi carriers in an endemic area. *Lancet (London, England)* **2**, 441–3 (1983).

65. Tran Vu Thieu, N. *et al.* An evaluation of purified Salmonella Typhi protein antigens for the serological diagnosis of acute typhoid fever. *J. Infect.* **75**, 104–114 (2017).

66. Näsström, E. *et al.* Diagnostic metabolite biomarkers of chronic typhoid carriage. *PLoS Negl.*

Trop. Dis. **12**, e0006215 (2018).

67. Charles, R. C. *et al.* Identification of Immunogenic Salmonella enterica Serotype Typhi Antigens Expressed in Chronic Biliary Carriers of S. Typhi in Kathmandu, Nepal. *PLoS Negl. Trop. Dis.* **7**, e2335 (2013).

68. Thompson, L. J. *et al.* Transcriptional response in the peripheral blood of patients infected with Salmonella enterica serovar Typhi. *Proc. Natl. Acad. Sci.* **106**, 22433–22438 (2009).

69. VAICHULIS, J. A., LITTMAN, A., IVY, A. C., ZUBOWICZ, G. & KAPLAN, R. THE CHRONIC TYPHOID CARRIER. III. THERAPY WITH ANTAGONISTIC BACILLUS, ANTIBIOTICS AND SULFONAMIDES. *Ann. Intern. Med.* **33**, 361 (1950).

70. Phillips, W. E. Treatment of Chronic Typhoid Carriers With Ampicillin. *JAMA J. Am. Med. Assoc.* **217**, 913 (1971).

71. Nolan, C. M. & White, P. C. Treatment of Typhoid Carriers With Amoxicillin. *JAMA* **239**, 2352–2354 (1978).

72. Brodie, J., Macqueen, I. A. & Livingstone, D. Effect of trimethoprim-sulphamethoxazole on typhoid and salmonella carriers. *Br. Med. J.* **3**, 318–9 (1970).

73. Trujillo, I. Z. & Quiroz, C. *Fluoroquinolones in the Treatment of Typhoid Fever and the Carrier State*. *Eur. J. Clin. Microbiol: Infect. Dis* **10**, (1991).

74. Marie Ameda, I. *Guidelines for the Management of Typhoid Fever*. (2011).

75. Mogasale, V., Ramani, E., Mogasale, V. V & Park, J. What proportion of Salmonella Typhi cases are detected by blood culture? A systematic literature review. *Ann. Clin. Microbiol. Antimicrob.* **15**, 32 (2016).

76. Wijedoru, L., Mallett, S. & Parry, C. M. Rapid diagnostic tests for typhoid and paratyphoid (enteric) fever. *Cochrane database Syst. Rev.* **5**, CD008892 (2017).

77. Edelman, R. & Levine, M. M. Summary of an international workshop on typhoid fever. *Rev. Infect. Dis.* **8**, 329–349 (1986).

78. Hejfec, L. B. *et al.* A controlled field trial to evaluate the protective capacity of a single dose of acetone-killed agar-grown and heat-killed broth-grown typhoid vaccines. *Bull. World Health Organ.* **40**, 903–907 (1969).

79. *THE STATE OF THE WORLD'S CHILDREN 1996*. (1996).

80. Wahdan, M. H. *et al.* Controlled field trial of a typhoid vaccine prepared with a nonmotile mutant of Salmonella typhi Ty2. *Bull. World Health Organ.* **52**, 69–73 (1975).

81. Wahdan, M. H., Serie, C., Cerisier, Y., Sallam, S. & Germanier, R. A controlled field trial of live Salmonella typhi strain Ty 21a oral vaccine against typhoid: three-year results. *J. Infect. Dis.* **145**, 292–295 (1982).

82. Klugman, K. P. *et al.* Protective activity of Vi capsular polysaccharide vaccine against typhoid fever. *Lancet* **2**, 1165–9 (1987).

83. Klugman, K. P., Koornhof, H. J., Robbins, J. B. & Le Cam, N. N. Immunogenicity, efficacy and serological correlate of protection of Salmonella typhi Vi capsular polysaccharide vaccine three years after immunization. *Vaccine* **14**, 435–438 (1996).

84. Yang, H. H. *et al.* Efficacy trial of Vi polysaccharide vaccine against typhoid fever in south-western China. *Bull. World Health Organ.* **79**, 625–631 (2001).

85. Sinha, A. *et al.* Typhoid fever in children aged less than 5 years. *Lancet (London, England)* **354**, 734–7 (1999).

86. Chuttani, C. S., Prakash, K., Gupta, P., Grover, V. & Kumar, A. Controlled field trial of a high-dose oral killed typhoid vaccine in India. *Bull. World Health Organ.* **55**, 643–4 (1977).

87. Acharya, I. L. *et al.* Prevention of typhoid fever in Nepal with the Vi capsular polysaccharide of Salmonella typhi. A preliminary report. *N. Engl. J. Med.* **317**, 1101–1104 (1987).

88. Simanjuntak, C. H. *et al.* Oral immunisation against typhoid fever in Indonesia with Ty21a vaccine. *Lancet* **338**, 1055–1059 (1991).

89. Lin, F. Y. *et al.* The epidemiology of typhoid fever in the Dong Thap Province, Mekong Delta region of Vietnam. *Am. J. Trop. Med. Hyg.* **62**, 644–8 (2000).

90. Lin, F. Y. C. *et al.* The Efficacy of a *Salmonella typhi* Vi Conjugate Vaccine in Two-to-Five-Year-Old Children. *N. Engl. J. Med.* **344**, 1263–1269 (2001).

91. Controlled field trials and laboratory studies on the effectiveness of typhoid vaccines in Poland, 1961-64. *Bull. World Health Organ.* **34**, 211–222 (1966).

92. HEJFEC, L. B. RESULTS OF THE STUDY OF TYPHOID VACCINES IN FOUR CONTROLLED FIELD TRIALS IN THE USSR. *Bull. World Health Organ.* **32**, 1–14 (1965).

93. Hejfec, L. B. *et al. A Controlled Field Trial and Laboratory Study of Five Typhoid Vaccines in the USSR*. Bull. Org. mond. Sant* **34**, (1966).

94. Hejfec, L. B. *et al. Controlled Field Trials of Paratyphoid B Vaccine and Evaluation of the Effectiveness of a Single Administration of Typhoid Vaccine *. Bull. Org. mond. Sante* **38**, (Bull. Wid Hith Org, 1968).

95. A controlled field trial of the effectiveness of phenol and alcohol typhoid vaccines: Final report. *Bull. World Health Organ.* **26**, 357–369 (1962).

96. *A Controlled Field Trial of the Effectiveness of Acetone-dried and Inactivated and Heat-Phenol-Inactivated Typhoid Vaccines in Yugoslavia* Report YUGOSLAV TYPHOID COMMISSION*'.

97. Ashcroft, M. T. *et al.* A SEVEN-YEAR FIELD TRIAL OF TWO TYPHOID VACCINES IN GUYANA. *Lancet* **290**, 1056–1059 (1967).

98. Black, R. E. *et al.* Efficacy of one or two doses of Ty21a Salmonella typhi vaccine in enteric-coated capsules in a controlled field trial. Chilean Typhoid Committee. *Vaccine* **8**, 81–4 (1990).

99. Levine, M. M., Black, R. E., Ferreccio, C. & Germanier, R. Large-scale field trial of TY21A live oral typhoid vaccine in enteric-coated casule formulation. *Lancet* **329**, 1049–1052 (1987).

100. Levine, M. M., Ferreccio, C., Cryz, S. & Ortiz, E. Comparison of enteric-coated capsules and liquid formulation of Ty21a typhoid vaccine in randomised controlled field trial. *Lancet (London, England)* **336**, 891–4 (1990).

101. Tapa, S. & Cvjetanović, B. Controlled field trial on the effectiveness of one and two doses of acetone-inactivated and dried typhoid vaccine. *Bull. World Health Organ.* **52**, 75–80 (1975).

102. GBD 2016 Causes of Death Collaborators, M. *et al.* Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet (London, England)* **390**, 1151–1210 (2017).

103. Ochiai, R. L. *et al.* A study of typhoid fever in five Asian countries: disease burden and implications for controls. *Bull World Heal. Organ* **86**, 260–268 (2008).

104. GBD 2017 Typhoid and Paratyphoid Collaborators, J. D. *et al.* The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet. Infect. Dis.* **19**, 369–381 (2019).

105. Crump, J. A. *et al.* Estimating the incidence of typhoid fever and other febrile illnesses in developing countries. *Emerg. Infect. Dis.* **9**, 539–44 (2003).

106. Srikantiah, P. *et al.* Population-based surveillance of typhoid fever in Egypt. *Am. J. Trop. Med. Hyg.* **74**, 114–9 (2006).

107. Brooks, W. A. *et al.* Bacteremic Typhoid Fever in Children in an Urban Slum, Bangladesh. *Emerg. Infect. Dis.* **11**, 326–329 (2005).

108. Ochiai, R. L. *et al.* The use of typhoid vaccines in Asia: the DOMI experience. *Clin. Infect. Dis.* **45 Suppl 1**, S34-8 (2007).

109. Khan, M. I. *et al.* Enhanced disease surveillance through private health care sector cooperation in Karachi, Pakistan: experience from a vaccine trial. *Bull. World Health Organ.* **84**, 72 (2006).

110. Ochiai, R. L. *et al.* Salmonella paratyphi A rates, Asia. *Emerg. Infect. Dis.* **11**, 1764–6 (2005).

111. Sur, D. *et al.* Comparisons of predictors for typhoid and paratyphoid fever in Kolkata, India. *BMC Public Health* **7**, 289 (2007).

112. Sur, D. *et al.* A Cluster-Randomized Effectiveness Trial of Vi Typhoid Vaccine in India. *N. Engl. J. Med.* **361**, 335–344 (2009).

113. Siddiqui, F. J., Rabbani, F., Hasan, R., Nizami, S. Q. & Bhutta, Z. A. Typhoid fever in children: some epidemiological considerations from Karachi, Pakistan. *Int. J. Infect. Dis.* **10**, 215–222 (2006).

114. Lin, F. Y. *et al.* The efficacy of a Salmonella typhi Vi conjugate vaccine in two-to-five-year-old children. *N. Engl. J. Med.* **344**, 1263–9 (2001).

115. Vollaard, A. M. *et al.* Identification of typhoid fever and paratyphoid fever cases at presentation in outpatient clinics in Jakarta, Indonesia. *Trans. R. Soc. Trop. Med. Hyg.* **99**, 440–450 (2005).

116. Breiman, R. F. *et al.* Population-Based Incidence of Typhoid Fever in an Urban Informal

Settlement and a Rural Area in Kenya: Implications for Typhoid Vaccine Use in Africa. *PLoS One* **7**, e29119 (2012).

117. Marks, F. *et al.* Typhoid fever among children, Ghana. *Emerg. Infect. Dis.* **16**, 1796–7 (2010).

118. Nielsen, M. V. *et al.* Incidence and Characteristics of Bacteremia among Children in Rural Ghana. *PLoS One* **7**, e44063 (2012).

119. Thriemer, K. *et al.* Clinical and epidemiological features of typhoid fever in Pemba, Zanzibar: assessment of the performance of the WHO case definitions. *PLoS One* **7**, e51823 (2012).

120. Naheed, A. *et al.* Burden of typhoid and paratyphoid fever in a densely populated urban community, Dhaka, Bangladesh. *Int. J. Infect. Dis.* **14**, e93–e99 (2010).

121. Owais, A., Sultana, S., Zaman, U., Rizvi, A. & Zaidi, A. K. M. Incidence of typhoid bacteremia in infants and young children in southern coastal Pakistan. *Pediatr. Infect. Dis. J.* **29**, 1035–9 (2010).

122. Khan, M. I. *et al.* Effectiveness of Vi capsular polysaccharide typhoid vaccine among children: A cluster randomized trial in Karachi, Pakistan. *Vaccine* **30**, 5389–5395 (2012).

123. Punjabi, N. H. *et al.* Enteric fever burden in North Jakarta, Indonesia: a prospective, community-based study. *J. Infect. Dev. Ctries.* **7**, (2013).

124. Antillón, M. *et al.* The burden of typhoid fever in low- and middle-income countries: A meta-regression approach. *PLoS Negl. Trop. Dis.* **11**, e0005376 (2017).

125. Marks, F. *et al.* Incidence of invasive salmonella disease in sub-Saharan Africa: a multicentre population-based surveillance study. *Lancet Glob. Heal.* **5**, e310–e323 (2017).

126. Bahl, R. *et al.* Costs of illness due to typhoid fever in an Indian urban slum community: implications for vaccination policy. *J. Health. Popul. Nutr.* **22**, 304–10 (2004).

127. Sur, D. *et al.* The malaria and typhoid fever burden in the slums of Kolkata, India: data from a prospective community-based study. *Trans. R. Soc. Trop. Med. Hyg.* **100**, 725–733 (2006).

128. Mai Ngoc, L. *et al.* Persistent efficacy of Vi conjugate vaccine against typhoid fever in young children. *N. Engl. J. Med.* **349**, 1390–1391 (2003).

129. Srikantiah, P. *et al.* Epidemiology and risk factors for endemic typhoid fever in Uzbekistan. *Trop. Med. Int. Heal.* **12**, 838–847 (2007).

130. Ollé-Goig, J. E. & Ruiz, L. Typhoid fever in rural Haiti. *Bull. Pan Am. Health Organ.* **27**, 382–8 (1993).

131. von Kalckreuth, V. *et al.* The Typhoid Fever Surveillance in Africa Program (TSAP): Clinical, Diagnostic, and Epidemiological Methodologies. *Clin. Infect. Dis.* **62 Suppl 1**, S9–S16 (2016).

132. Dewan, A. M. *et al.* Typhoid Fever and Its Association with Environmental Factors in the Dhaka Metropolitan Area of Bangladesh: A Spatial and Time-Series Approach. *PLoS Negl. Trop. Dis.* **7**, e1998 (2013).

133. Karkey, A. *et al.* The Burden and Characteristics of Enteric Fever at a Healthcare Facility in a

Densely Populated Area of Kathmandu. *PLoS One* **5**, e13988 (2010).

134. KHAN, M. I. *et al.* Risk factors associated with typhoid fever in children aged 2–16 years in Karachi, Pakistan. *Epidemiol. Infect.* **140**, 665–672 (2012).

135. Khan, M. I. *et al.* Epidemiology, clinical presentation, and patterns of drug resistance of Salmonella Typhi in Karachi, Pakistan. *J. Infect. Dev. Ctries.* **6**, 704–14 (2012).

136. Colquhoun, J. & Weetch, R. S. RESISTANCE TO CHLORAMPHENICOL DEVELOPING DURING TREATMENT OF TYPHOID FEVER. *Lancet* **256**, 621–623 (1950).

137. Olarte, J. & Galindo, E. Salmonella typhi resistant to chloramphenicol, ampicillin, and other antimicrobial agents: strains isolated during an extensive typhoid fever epidemic in Mexico. *Antimicrob. Agents Chemother.* **4**, 597–601 (1973).

138. Butler, T., Arnold, K., Linh, N. & Pollack, M. CHLORAMPHENICOL-RESISTANT TYPHOID FEVER IN VIETNAM ASSOCIATED WITH R FACTOR. *Lancet* **302**, 983–985 (1973).

139. Mandal, B. K. Treatment of multiresistant typhoid fever. *Lancet* **336**, 1383 (1990).

140. Feasey, N. A. *et al.* Rapid emergence of multidrug resistant, H58-lineage Salmonella Typhi in Blantyre, Malawi. *PLoS Negl Trop Dis* **9**, (2015).

141. Wong, V. K. *et al.* Phylogeographical analysis of the dominant multidrug-resistant H58 clade of Salmonella Typhi identifies inter- and intracontinental transmission events. *Nat. Genet.* **47**, 632–639 (2015).

142. Umasankar, S., Wall, R. A. & Berger, J. A case of ciprofloxacin-resistant typhoid fever. *Commun. Dis. Rep. CDR Rev.* **2**, R139-40 (1992).

143. Chitnis, V., Chitnis, D., Verma, S. & Hemvani, N. Multidrug-resistant Salmonella typhi in India. *Lancet (London, England)* **354**, 514–5 (1999).

144. Dahiya, S. *et al.* Characterisation of antimicrobial resistance in Salmonellae during 2014–2015 from four centres across India: An ICMR antimicrobial resistance surveillance network report. *Indian J. Med. Microbiol.* **35**, 61 (2017).

145. Balaji, V. *et al.* Longitudinal Typhoid Fever Trends in India from 2000 to 2015. *Am. J. Trop. Med. Hyg* **99**, 34–40 (2018).

146. Qamar, F. N. *et al.* A Retrospective Study of Laboratory-Based Enteric Fever Surveillance, Pakistan, 2012–2014. *J. Infect. Dis.* **218**, S201–S205 (2018).

147. Arjyal, A. *et al.* Gatifloxacin versus ceftriaxone for uncomplicated enteric fever in Nepal: an open-label, two-centre, randomised controlled trial. *Lancet Infect. Dis.* **16**, 535–545 (2016).

148. Ryan, E. T. Troubling news from Asia about treating enteric fever: a coming storm. *Lancet Infect. Dis.* **16**, 508–509 (2016).

149. Basnyat, B. Typhoid versus typhus fever in post-earthquake Nepal. *The Lancet Global Health* **4**, e516–e517 (2016).

150. Murdoch, D. A. *et al.* Epidemic ciprofloxacin-resistant Salmonella typhi in Tajikistan. *Lancet*

(London, England) **351**, 339 (1998).

151. Zellweger, R. M. *et al.* A 23-year retrospective investigation of Salmonella Typhi and Salmonella Paratyphi isolated in a tertiary Kathmandu hospital. *PLoS Negl. Trop. Dis.* **11**, e0006051 (2017).

152. Menezes, G. A., Harish, B. N., Khan, M. A., Goessens, W. H. F. & Hays, J. P. Antimicrobial resistance trends in blood culture positive Salmonella Typhi isolates from Pondicherry, India, 2005-2009. *Clin. Microbiol. Infect.* **18**, 239–245 (2012).

153. Parry, C. M. *et al.* Multidrug resistant enteric fever in South Asia: unmet medical needs and opportunities. *BMJ* **364**, k5322 (2019).

154. Qamar, F. N. *et al.* Outbreak investigation of ceftriaxone-resistant Salmonella enterica serotype Typhi and its risk factors among the general population in Hyderabad, Pakistan: a matched case-control study. *Lancet. Infect. Dis.* **18**, 1368–1376 (2018).

155. Andrews, J. R., Qamar, F. N., Charles, R. C. & Ryan, E. T. Extensively Drug-Resistant Typhoid — Are Conjugate Vaccines Arriving Just in Time? *N. Engl. J. Med.* **379**, 1493–1495 (2018).

156. Chatham-Stephens, K. *et al.* Emergence of Extensively Drug-Resistant *Salmonella* Typhi Infections Among Travelers to or from Pakistan — United States, 2016–2018. *MMWR. Morb. Mortal. Wkly. Rep.* **68**, 11–13 (2019).

157. Parry, C. M. *et al.* Randomized controlled comparison of ofloxacin, azithromycin, and an ofloxacin-azithromycin combination for treatment of multidrug-resistant and nalidixic acid-resistant typhoid fever. *Antimicrob. Agents Chemother.* **51**, 819–25 (2007).

158. Zmora, N. *et al.* Open label comparative trial of mono versus dual antibiotic therapy for Typhoid Fever in adults. *PLoS Negl. Trop. Dis.* **12**, e0006380 (2018).

159. WHO | Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. *WHO* (2017).

160. Tomczyk, S. *et al.* Prevention of Antibiotic-Nonsusceptible Invasive Pneumococcal Disease With the 13-Valent Pneumococcal Conjugate Vaccine. *Clin. Infect. Dis.* **62**, 1119–1125 (2016).

161. Heilmann, K. P. *et al.* Decreasing Prevalence of -Lactamase Production among Respiratory Tract Isolates of Haemophilus influenzae in the United States. *Antimicrob. Agents Chemother.* **49**, 2561–2564 (2005).

162. Hargreaves, R. M., Slack, M. P. E., Howard, A. J., Anderson, E. & Ramsay, M. E. Changing patterns of invasive Haemophilus influenzae disease in England and Wales after introduction of the Hib vaccination programme. *BMJ* **312**, 160–161 (1996).

163. Hampton, L. M. *et al.* Prevention of Antibiotic-Nonsusceptible Streptococcus pneumoniae With Conjugate Vaccines. *J. Infect. Dis.* **205**, 401–411 (2012).

164. Kaufhold, S., Yaesoubi, R. & Pitzer, V. E. Predicting the Impact of Typhoid Conjugate Vaccines on Antimicrobial Resistance. *Clin. Infect. Dis.* **68**, S96–S104 (2019).

165. Andrews, J. R. *et al.* Typhoid conjugate vaccines: a new tool in the fight against antimicrobial resistance. *Lancet Infect. Dis.* **19**, e26–e30 (2019).

166. Andrews, J. R. *et al.* High Rates of Enteric Fever Diagnosis and Lower Burden of Culture-Confirmed Disease in Peri-urban and Rural Nepal. *J. Infect. Dis.* (2017). doi:10.1093/infdis/jix221

167. Thi Quynh Nhi, L. *et al.* Quantifying antimicrobial access and usage for paediatric diarrhoeal disease in an urban community setting in Asia. *J. Antimicrob. Chemother.* **73**, 2546–2554 (2018).

168. Sabde, Y. D. *et al.* Mapping private pharmacies and their characteristics in Ujjain district, Central India. *BMC Health Serv. Res.* **11**, 351 (2011).

169. Jansen, K. U., Knirsch, C. & Anderson, A. S. The role of vaccines in preventing bacterial antimicrobial resistance. *Nat. Med.* **24**, 10–19 (2018).

170. Laxminarayan, R. *et al.* Access to effective antimicrobials: a worldwide challenge. *Lancet* **387**, 168–175 (2016).

171. Meiring, J. E. *et al.* The Typhoid Vaccine Acceleration Consortium (TyVAC): Vaccine effectiveness study designs: Accelerating the introduction of typhoid conjugate vaccines and reducing the global burden of enteric fever. Report from a meeting held on 26–27 October 2016, Oxford. *Vaccine* **35**, 5081–5088 (2017).

172. Meiring, J. E. *et al.* Typhoid Vaccine Acceleration Consortium Malawi: A Phase III, Randomized, Double-blind, Controlled Trial of the Clinical Efficacy of Typhoid Conjugate Vaccine Among Children in Blantyre, Malawi. *Clin. Infect. Dis.* **68**, S50–S58 (2019).

173. Theiss-Nyland, K. *et al.* Assessing the Impact of a Vi-polysaccharide Conjugate Vaccine in Preventing Typhoid Infections Among Nepalese Children: A Protocol for a Phase III, Randomized Control Trial. *Clin. Infect. Dis.* **68**, S67–S73 (2019).

174. Theiss-Nyland, K. *et al.* Assessing the Impact of a Vi-polysaccharide Conjugate Vaccine in Preventing Typhoid Infection Among Bangladeshi Children: A Protocol for a Phase IIIb Trial. *Clin. Infect. Dis.* **68**, S74–S82 (2019).

175. Prasad, N., Murdoch, D. R., Reyburn, H. & Crump, J. A. Etiology of Severe Febrile Illness in Low- and Middle-Income Countries: A Systematic Review. *PLoS One* **10**, e0127962 (2015).

176. Saha, S. *et al.* Designing Comprehensive Public Health Surveillance for Enteric Fever in Endemic Countries: Importance of Including Different Healthcare Facilities. *J. Infect. Dis.* **218**, S227–S231 (2018).

177. Luby, S. P., Saha, S. & Andrews, J. R. Towards sustainable public health surveillance for enteric fever. *Vaccine* **33**, C3–C7 (2015).

178. Andrews, J. R. *et al.* Integrating Facility-Based Surveillance With Healthcare Utilization Surveys to Estimate Enteric Fever Incidence: Methods and Challenges. *J. Infect. Dis.* **218**, S268–S276 (2018).

179. Sur, D. *et al.* A Retrospective Review of Hospital-Based Data on Enteric Fever in India, 2014–2015. *J. Infect. Dis.* **218**, S206–S213 (2018).

180. Saha, S. *et al.* Integration of enteric fever surveillance into the WHO-coordinated Invasive Bacterial-Vaccine Preventable Diseases (IB-VPD) platform: A low cost approach to track an

increasingly important disease. *PLoS Negl. Trop. Dis.* **11**, e0005999 (2017).

181. Bilcke, J. *et al.* Cost-effectiveness of routine and campaign use of typhoid Vi-conjugate vaccine in Gavi-eligible countries: a modelling study. *Lancet. Infect. Dis.* **0**, (2019).

182. John, J., Bavdekar, A., Rongsen-Chandola, T., Dutta, S. & Kang, G. Estimating the incidence of enteric fever in children in India: a multi-site, active fever surveillance of pediatric cohorts. *BMC Public Health* **18**, 594 (2018).

183. Gibani, M. M. *et al.* The Impact of Vaccination and Prior Exposure on Stool Shedding of Salmonella Typhi and Salmonella Paratyphi in 6 Controlled Human Infection Studies. *Clin. Infect. Dis.* (2018). doi:10.1093/cid/ciy670

184. Emborg, H.-D. *et al.* Seroincidence of non-typhoid Salmonella infections: convenience vs. random community-based sampling. *Epidemiol. Infect.* **144**, 257–264 (2016).

185. Simonsen, J. *et al.* Sero-epidemiology as a tool to study the incidence of Salmonella infections in humans. *Epidemiol. Infect.* **136**, 895–902 (2008).

186. Masel, J. *et al.* Seroprevalence and Seroincidence of Herpes Simplex Virus (2006–2010), Syphilis (2006–2010), and Vaccine-Preventable Human Papillomavirus Subtypes (2000–2010) Among US Military Personnel. *Sex. Transm. Dis.* **42**, 253–258 (2015).

187. Blaylock, J. M. *et al.* The seroprevalence and seroincidence of dengue virus infection in western Kenya. *Travel Med. Infect. Dis.* **9**, 246–248 (2011).

188. Ikematsu, H., Kawai, N. & Yajima, S. A cross sectional survey measuring sero-incidence of pertussis infection among Japanese junior and senior high school students in 2013 and 2014. *Vaccine* **35**, 3859–3864 (2017).

189. Metcalf, C. J. E. *et al.* Use of serological surveys to generate key insights into the changing global landscape of infectious disease. *Lancet* **388**, 728–730 (2016).

190. Creative Commons — Attribution 4.0 International — CC BY 4.0. Available at: https://creativecommons.org/licenses/by/4.0/legalcode. (Accessed: 17th September 2019)

191. Acharya, I. L. *et al.* Prevention of Typhoid Fever in Nepal with the VI Capsular Polysaccharide of *Salmonella Typhi*. *N. Engl. J. Med.* **317**, 1101–1104 (1987).

192. DeRoeck, D. *et al.* Typhoid vaccination: the Asian experience. *Expert Rev. Vaccines* **7**, 547–560 (2008).

193. Antillon, M., Saad, N. J., Baker, S., Pollard, A. J. & Pitzer, V. E. The Relationship Between Blood Sample Volume and Diagnostic Sensitivity of Blood Culture for Typhoid and Paratyphoid Fever: A Systematic Review and Meta-Analysis. *J. Infect. Dis.* **218**, S255–S267 (2018).

194. Feasey, N. A. *et al.* Three Epidemics of Invasive Multidrug-Resistant *Salmonella* Bloodstream Infection in Blantyre, Malawi, 1998–2014. *Clin. Infect. Dis.* **61**, S363–S371 (2015).

195. Darton, T. C. *et al.* 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. *BMJ Open* **7**, e016283 (2017).

196. Park, S. E. Severe Typhoid Fever Surveillance in Africa (SETA) Program: First Data from the Six African Sites.

197. Garrett, D. The surveillance for enteric fever in Asia project (SEAP): Estimating the community burden of enteric fever. *Int. J. Infect. Dis.* **45**, 64 (2016).

198. Typhoid Conjugate Vaccine Introduction in Navi Mumbai, India - Full Text View - ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03554213. (Accessed: 17th September 2019)

199. WHO | SAGE meeting of October 2017. *WHO* (2017).

200. Jeon, H. J. *et al.* Determining the Best Immunization Strategy for Protecting African Children Against Invasive Salmonella Disease. *Clin. Infect. Dis.* (2018). doi:10.1093/cid/ciy386

201. Baker, S. *et al.* Combined high-resolution genotyping and geospatial analysis reveals modes of endemic urban typhoid fever transmission. *Open Biol.* **1**, (2011).

202. Engels, E. A., Falagas, M. E., Lau, J. & Bennish, M. L. Typhoid fever vaccines: a meta-analysis of studies on efficacy and toxicity. *BMJ* **316**, 110–6 (1998).

203. Bodhidatta, L., Taylor, D. N., Thisyakom, U. & Echeverria, P. Control of Typhoid Fever in Bangkok, Thailand, by Annual Immunization of Schoolchildren with Parenteral Typhoid Vaccine. *Clin. Infect. Dis.* **9**, 841–845 (1987).

204. WHO. Typhoid vaccines: WHO position paper. *Wkly. Epidemiol. Rec.* **83**, 49–59 (2008).

205. Hornick, R. B. *et al.* Efficacy of a live oral typhoid vaccine in human volunteers. *Dev. Biol. Stand.* **33**, 89–92 (1976).

206. Milligan, R., Paul, M., Richardson, M. & Neuberger, A. Vaccines for preventing typhoid fever. *Cochrane Database Syst. Rev.* (2018). doi:10.1002/14651858.CD001261.pub4

207. Levine, M. M. *et al.* Ty21a Live Oral Typhoid Vaccine and Prevention of Paratyphoid Fever Caused by Salmonella enterica Serovar Paratyphi B. *Clin. Infect. Dis.* **45**, S24–S28 (2007).

208. WHO. WHO | Typhoid vaccines position paper. *WHO*

209. obbins, J. B., Schneerson, R., Porter, A. & Smith, D. H. Prevention of Systemic Infections, Especially Meningitis, Caused by Haemophilus influenzae Type b. *JAMA* **276**, 1181 (1996).

210. Goldblatt, D. Conjugate vaccines. *Clin. Exp. Immunol.* **119**, 1–3 (2000).

211. Pichichero, M. E. Protein carriers of conjugate vaccines: characteristics, development, and clinical trials. *Hum. Vaccin. Immunother.* **9**, 2505–23 (2013).

212. Kossaczka, Z. *et al.* Safety and immunogenicity of Vi conjugate vaccines for typhoid fever in adults, teenagers, and 2- to 4-year-old children in Vietnam. *Infect. Immun.* **67**, 5806–5810 (1999).

213. Szu, S. C. *et al.* Laboratory and preliminary clinical characterization of Vi capsular polysaccharide-protein conjugate vaccines. *Infect. Immun.* **62**, 4440–4444 (1994).

214. Szu, S. C. Development of Vi conjugate – a new generation of typhoid vaccine. *Expert Rev. Vaccines* **12**, 1273–1286 (2013).

215. Capeding, M. R. *et al.* Safety and immunogenicity of a Vi-DT typhoid conjugate vaccine: Phase I trial in Healthy Filipino adults and children. *Vaccine* **36**, 3794–3801 (2018).

216. Safety and Immunogenicity of Vi-DT Typhoid Conjugate Vaccine (Bio Farma) in Adults and Children (Phase I) - Full Text View - ClinicalTrials.gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03109600. (Accessed: 14th November 2018)

217. van Damme, P. *et al.* Safety, immunogenicity and dose ranging of a new Vi-CRM_{197} conjugate vaccine against typhoid fever: randomized clinical testing in healthy adults. *PLoS One* **6**, e25398 (2011).

218. Bhutta, Z. A. *et al.* lmmunogenicity and safety of the Vi-CRM197 conjugate vaccine against typhoid fever in adults, children, and infants in south and southeast Asia: results from two randomised, observer-blind, age de-escalation, phase 2 trials. *Lancet Infect. Dis.* **14**, 119–129 (2014).

219. Mitra, M. *et al.* Efficacy and safety of vi-tetanus toxoid conjugated typhoid vaccine (PedaTyph™) in Indian children: School based cluster randomized study. *Hum. Vaccin. Immunother.* **12**, 939–945 (2016).

220. Burki, T. Typhoid conjugate vaccine gets WHO prequalification. *Lancet. Infect. Dis.* **18**, 258 (2018).

221. Jin, C. *et al.* Efficacy and immunogenicity of a Vi-tetanus toxoid conjugate vaccine in the prevention of typhoid fever using a controlled human infection model of Salmonella Typhi: a randomised controlled, phase 2b trial. *Lancet (London, England)* **0**, (2017).

222. Gavi, T. V. A. Millions of children set to be protected against typhoid fever - Gavi, the Vaccine Alliance. Available at: https://www.gavi.org/library/news/press-releases/2017/millions-of-children-set-to-be-protected-against-typhoid-fever/. (Accessed: 19th April 2018)

223. Meiring, J. E., Patel, P., Patel, P. & Gordon, M. A. Expert Review of Vaccines Typhoid conjugate vaccines: making vaccine history in Africa Typhoid conjugate vaccines: making vaccine history in Africa. (2018). doi:10.1080/14760584.2018.1496825

224. Chinnasami, B., Mangayarkarasi, V., Prema, A., Sadasivam, K. & Davis, M. J. Safety and Immunogenicity of Salmonella Typhi Vi conjugate vaccine (Peda Typh TM) in children upto five years. *Int. J. Sci. Res. Publ.* **3**, (2013).

225. Chinnasami, B., Sadasivam, K., Vivekanandhan, A., Arunachalam, P. & Pasupathy, S. A Study on Longevity of Immune Response after Vaccination with Salmonella Typhi Vi Conjugate Vaccine (Pedatyph) in Children. *J. Clin. Diagn. Res.* **9**, SC01-3 (2015).

226. Mohan, V. K. *et al.* Safety and Immunogenicity of a Vi Polysaccharide–Tetanus Toxoid Conjugate Vaccine (Typbar-TCV) in Healthy Infants, Children, and Adults in Typhoid Endemic Areas: A Multicenter, 2-Cohort, Open-Label, Double-Blind, Randomized Controlled Phase 3 Study. *Clin. Infect. Dis.* **61**, 393–402 (2015).

227. Konadu, E. Y. *et al.* Phase 1 and phase 2 studies of Salmonella enterica serovar paratyphi A O-specific polysaccharide-tetanus toxoid conjugates in adults, teenagers, and 2- to 4-year-old

children in Vietnam. *Infect. Immun.* **68**, 1529–34 (2000).

228. Mogasale, V. *et al.* Revisiting typhoid fever surveillance in low and middle income countries: lessons from systematic literature review of population-based longitudinal studies. *BMC Infect. Dis.* **16**, 35 (2016).

229. Pitzer, V. E. *et al.* Mathematical Modeling to Assess the Drivers of the Recent Emergence of Typhoid Fever in Blantyre, Malawi. *Clin. Infect. Dis.* **61**, S251–S258 (2015).

230. Saad, N. J. *et al.* The impact of migration and antimicrobial resistance on the transmission dynamics of typhoid fever in Kathmandu, Nepal: A mathematical modelling study. *PLoS Negl. Trop. Dis.* **11**, e0005547 (2017).

231. Saha, S. K. *et al.* Typhoid fever in Bangladesh: implications for vaccination policy. *Pediatr. Infect. Dis. J.* **20**, 521–4 (2001).

232. Tanmoy, A. M. *et al.* Salmonella enterica Serovar Typhi in Bangladesh: Exploration of Genomic Diversity and Antimicrobial Resistance. *MBio* **9**, e02112-18 (2018).

233. Gordon, S. B. *et al.* Open Peer Review A Malawi guideline for research study participant remuneration [version 2; peer review: 2 approved]. (2018). doi:10.12688/wellcomeopenres.14668.1

234. ODK. Open Data Kit. Available at: https://opendatakit.org/. (Accessed: 2nd February 2017)

235. Roth, J. R. Salmonella. in *Brenner's Encyclopedia of Genetics: Second Edition* 321–323 (Elsevier Inc., 2013). doi:10.1016/B978-0-12-374984-0.01367-X

236. *M100 Performance Standards for Antimicrobial Susceptibility Testing An informational supplement for global application developed through the Clinical and Laboratory Standards Institute consensus process*.

237. Hosoglu, S. *et al.* Risk factors for typhoid fever among adult patients in Diyarbakir, Turkey. doi:10.1017/S0950268805005583

238. Bano-Zaidi, M. *et al.* Typhoid fever outbreak with severe complications in Yucatan, Mexico. *The Lancet Global Health* **6**, e1062–e1063 (2018).

239. The Merchants of Thirst - The New York Times. Available at: https://www.nytimes.com/2020/01/11/business/drought-increasing-worldwide.html. (Accessed: 10th March 2020)

240. Nakagiri, A. *et al.* Are pit latrines in urban areas of Sub-Saharan Africa performing? A review of usage, filling, insects and odour nuisances. *BMC Public Health* **16**, 120 (2016).

241. UN-Water: WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation (JMP). Available at: http://www.unwater.org/publications/jmp/en/. (Accessed: 31st March 2017)

242. Keusch, G. T. *et al. Diarrheal Diseases*. *Disease Control Priorities in Developing Countries* (The International Bank for Reconstruction and Development / The World Bank, 2006).

243. GDP (current US$) | Data. Available at: https://data.worldbank.org/indicator/NY.GDP.MKTP.CD. (Accessed: 10th March 2020)

244. Khalid Hasan, M., Shahriar, A. & Ullah Jim, K. Water pollution in Bangladesh and its impact on public health. *Heliyon* e02145 (2017). doi:10.1016/j.heliyon.2019.e02145

245. 80% of water in Dhaka reservoirs contaminated with coliform bacteria | Dhaka Tribune. Available at: https://www.dhakatribune.com/bangladesh/dhaka/2019/07/18/water-of-80-reservoirs-in-dhaka-contaminated-with-coliform-bacteria. (Accessed: 10th March 2020)

246. Dyson, Z. A., Klemm, E. J., Palmer, S. & Dougan, G. Antibiotic Resistance and Typhoid. *Clin. Infect. Dis.* 68, S165–S170 (2019).

247. Koirala, S. *et al.* Gatifloxacin Versus Ofloxacin for the Treatment of Uncomplicated Enteric Fever in Nepal: An Open-Label, Randomized, Controlled Trial. *PLoS Negl. Trop. Dis.* 7, e2523 (2013).

248. Arjyal, A. *et al.* Gatifloxacin versus chloramphenicol for uncomplicated enteric fever: An open-label, randomised, controlled trial. *Lancet Infect. Dis.* 11, 445–454 (2011).

249. Patel, S. R., Bharti, S., Pratap, C. B. & Nath, G. Drug Resistance Pattern in the Recent Isolates of Salmonella Typhi with Special Reference to Cephalosporins and Azithromycin in the Gangetic Plain. *J. Clin. Diagn. Res.* 11, DM01–DM03 (2017).

250. Saha, S. *et al.* Epidemiology of Typhoid and Paratyphoid: Implications for Vaccine Policy. *Clin. Infect. Dis.* 68, S117–S123 (2019).

251. Saha, S. *et al.* Enteric Fever Cases in the Two Largest Pediatric Hospitals of Bangladesh: 2013–2014. *J. Infect. Dis.* 218, S195–S200 (2018).

252. Stanaway, J. D. *et al.* The global burden of non-typhoidal salmonella invasive disease: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Infect. Dis.* 0, (2019).

253. Gordon, M. A. *et al.* Epidemics of Invasive *Salmonella enterica* Serovar Enteritidis and *S. enterica* Serovar Typhimurium Infection Associated with Multidrug Resistance among Adults and Children in Malawi. *Clin. Infect. Dis.* 46, 963–969 (2008).

254. WHO publishes list of bacteria for which new antibiotics are urgently needed. Available at: https://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed. (Accessed: 12th January 2020)

255. Browne, A. J. *et al.* Drug-resistant enteric fever worldwide, 1990 to 2018: A systematic review and meta-analysis. *BMC Med.* 18, (2020).

256. Research finds new vaccine protecting children from typhoid | The Aga Khan University News. Available at: https://www.aku.edu/news/Pages/News_Details.aspx?nid=NEWS-002068. (Accessed: 11th March 2020)

257. Shakya, M. *et al.* Phase 3 Efficacy Analysis of a Typhoid Conjugate Vaccine Trial in Nepal. *N. Engl. J. Med.* 381, 2209–2218 (2019).

258. Zaccaro, D. J., Wagener, D. K., Whisnant, C. C. & Staats, H. F. Evaluation of vaccine-induced antibody responses: Impact of new technologies. *Vaccine* 31, 2756–2761 (2013).

259. Watson, C. H. *et al.* A cross-sectional seroepidemiological survey of typhoid fever in Fiji. *PLoS Negl. Trop. Dis.* 11, e0005786 (2017).

260. Pulickal, A. S. *et al.* Kinetics of the Natural, Humoral Immune Response to Salmonella enterica Serovar Typhi in Kathmandu, Nepal. *Clin. Vaccine Immunol.* **16**, 1413–1419 (2009).

261. Losonsky, G. A. *et al.* Development and evaluation of an enzyme-linked immunosorbent assay for serum Vi antibodies for detection of chronic Salmonella typhi carriers. *J. Clin. Microbiol.* **25**, 2266–2269 (1987).

262. Organization, W. H. Background document: the diagnosis, treatment and prevention of typhoid fever. (2003).

263. Hsiao, A., Toy, T., Seo, H. J. & Marks, F. Interaction between Salmonella and Schistosomiasis: A Review. *PLOS Pathog.* **12**, e1005928 (2016).

264. Hornick, R. B. *et al.* Typhoid fever: pathogenesis and immunologic control. *N. Engl. J. Med.* **283**, 686–91 (1970).

265. Crump, J. A. & Kirk, M. D. Estimating the Burden of Febrile Illnesses. *PLoS Negl. Trop. Dis.* **9**, e0004040 (2015).

266. Dutta, P. *et al.* Multi-drug resistant typhoid fever with diarrhea. *Indian Pediatr.* **34**, 891–899 (1997).

267. Parry, C. M. Typhoid Fever. *Curr. Infect. Dis. Rep.* **6**, 27–33 (2004).

268. Alam, M. M. *et al.* Identification of in vivo-induced bacterial proteins during human infection with Salmonella enterica serotype Paratyphi A. *Clin. Vaccine Immunol.* **20**, 712–719 (2013).

269. Dunstan, S. J. *et al.* Variation at HLA-DRB1 is associated with resistance to enteric fever. *Nat. Genet.* **46**, 1333–1336 (2014).

270. Holt, K. E. *et al.* High-throughput sequencing provides insights into genome variation and evolution in Salmonella Typhi. *Nat. Genet.* **40**, 987–993 (2008).

271. Holt, K. E. *et al.* High-Resolution Genotyping of the Endemic Salmonella Typhi Population during a Vi (Typhoid) Vaccination Trial in Kolkata. *PLoS Negl. Trop. Dis.* **6**, e1490 (2012).

272. Holt, K. E. *et al.* Emergence of a Globally Dominant IncHI1 Plasmid Type Associated with Multiple Drug Resistant Typhoid. *PLoS Negl. Trop. Dis.* **5**, e1245 (2011).

273. Baker, S. *et al.* High-throughput genotyping of Salmonella enterica serovar Typhi allowing geographical assignment of haplotypes and pathotypes within an urban district of Jakarta, Indonesia. *J. Clin. Microbiol.* **46**, 1741–1746 (2008).

274. Kariuki, S. *et al.* Typhoid in Kenya is associated with a dominant multidrug-resistant salmonella enterica serovar typhi haplotype that is also widespread in Southeast Asia. *J. Clin. Microbiol.* **48**, 2171–2176 (2010).

275. Wong, V. K. *et al.* Molecular Surveillance Identifies Multiple Transmissions of Typhoid in West Africa. *PLoS Negl. Trop. Dis.* **10**, e0004781 (2016).

276. Park, S. E. *et al.* The phylogeography and incidence of multi-drug resistant typhoid fever in sub-Saharan Africa. *Nat. Commun.* **9**, 5094 (2018).

277. Näsström, E. *et al. Salmonella* Typhi and *Salmonella* Paratyphi A elaborate distinct systemic metabolite signatures during enteric fever. *Elife* **3**, e56971 (2014).

278. Näsström, E. *et al.* Reproducible diagnostic metabolites in plasma from typhoid fever patients in Asia and Africa. *Elife* **6**, (2017).

279. WHO | SAGE meeting of October 2017. *WHO* (2018).

280. Meiring, J. E., Patel, P., Patel, P. & Gordon, M. A. Typhoid conjugate vaccines: making vaccine history in Africa. *Expert Rev. Vaccines* **17**, (2018).

281. Theiss-Nyland, K. *et al.* Assessing the Impact of a Vi-polysaccharide Conjugate Vaccine in Preventing Typhoid Infections Among Nepalese Children: A Protocol for a Phase III, Randomized Control Trial. *Clin. Infect. Dis.* **68**, S67–S73 (2019).

282. Meiring, J. E. *et al.* Community Engagement Before Initiation of Typhoid Conjugate Vaccine Trial in Schools in Two Urban Townships in Blantyre, Malawi: Experience and Lessons. *Clin. Infect. Dis.* **68**, S146–S153 (2019).

283. Arndt, M. B. *et al.* Estimating the Burden of Paratyphoid A in Asia and Africa. *PLoS Negl. Trop. Dis.* **8**, e2925 (2014).

284. MacLennan, C. A., Martin, L. B. & Micoli, F. Vaccines against invasive *Salmonella* disease. *Hum. Vaccin. Immunother.* **10**, 1478–1493 (2014).

285. *Community Engagement-Under the Microscope*.

286. Emanuel, E. J., Wendler, D., Killen, J. & Grady, C. What Makes Clinical Research in Developing Countries Ethical? The Benchmarks of Ethical Research. *J. Infect. Dis.* **189**, 930–937 (2004).

287. Good Participatory Practice (GPP) Guidelines | AVAC. Available at: https://www.avac.org/good-participatory-practice. (Accessed: 10th August 2018)

288. Gooding, K., Phiri, M., Petersen, I., Parker, M. & Desmond, N. Six dimensions of research trial acceptability: How much, what, when, in what circumstances, to whom and why? *Soc. Sci. Med.* (2018). doi:10.1016/J.SOCSCIMED.2018.07.040

289. Mfutso-Bengo, J., Ndebele, P. & Masiye, F. Disseminating research results to research participants and their communities. *Malawi Med. J.* **20**, 64–6 (2008).

290. Nyirenda, D. *et al.* 'We are the eyes and ears of researchers and community': understanding the role of community advisory groups in representing researchers and communities in Malawi. *Dev. World Bioeth.* (2017). doi:10.1111/dewb.12163

291. Crampin, A. C. *et al.* Hypertension and diabetes in Africa: design and implementation of a large population-based study of burden and risk factors in rural and urban Malawi. *Emerg. Themes Epidemiol.* **13**, 3 (2016).

292. Kingori, P. Experiencing everyday ethics in context: frontline data collectors perspectives and practices of bioethics. *Soc. Sci. Med.* **98**, 361–70 (2013).

293. Blantyre children to lead typhoid vaccine trial - The Nation Online. Available at: https://mwnation.com/blantyre-children-lead-typhoid-vaccine-trial/. (Accessed: 10th August

2018)

294. Malawi launches typhoid vaccine | The Times Group. Available at: https://www.times.mw/malawi-launches-typhoid-vaccine/. (Accessed: 10th August 2018)

295. Nyirenda, L. J., Sandberg, K. I. & Justice, J. When Are Health Systems Ready for New Vaccines? The Introduction of Pneumococcal Vaccine in Malawi. *Forum Dev. Stud.* **41**, 317–336 (2014).

296. Lavery, J. V. *et al.* Towards a framework for community engagement in global health research. *Trends Parasitol.* **26**, 279–283 (2010).

297. Participants in the Community Engagement and Consent Workshop , Kilifi, Kenya , March 2011, P. in the C. E. and C., Kilifi, Kenya & 2011, M. Consent and community engagement in diverse research contexts. *J. Empir. Res. Hum. Res. Ethics* **8**, 1–18 (2013).

Appendix

9. The Set Up and Community Engagement for Phase 3 Typhoid Conjugate Vaccine Trial in Blantyre, Malawi

9.1. Introduction

Whilst *S.* Typhi disease rates have decreased in developed countries with improvements in water and sanitation, as this thesis has demonstrated, disease rates continue to be high in many parts of the world. Whilst there has been some reduction in disease rates over the last 20 years globally the threat of escalating antimicrobial resistance (AMR) could reverse these trends.[32,151] Therefore, the prequalification of a new typhoid conjugate vaccine (Vi-TCV), Typbar-TCV®, by the World Health Organisation (WHO) [220] after promising efficacy results from the Oxford human challenge model [221] is an encouraging development, meaning control of the global typhoid burden may be possible through vaccination.

After WHO prequalification of Typbar-TCV®, the WHO Strategic Advisory Group of Experts on Immunization recommended Vi-TCV in countries with elevated burden of disease or increased rates of AMR.[199] Through funding from the Bill & Melinda Gates Foundation, the Typhoid Vaccine Acceleration Consortium (TyVAC) was formed to accelerate introduction of TCVs into low- and middle-income countries [171,223] and provide evidence for vaccine impact through a series of efficacy trials in endemic countries.

The TyVAC Malawi trial is a phase 3 randomized, double blind, controlled trial of the clinical efficacy of TCV in 28,000 children aged 9 months through 12 years. Children are randomized 1:1 to receive Vi-TCV or a meningitis A conjugate vaccine. Building on the platform of surveillance and much of the community engagement work already set up for the study already described, TyVAC Malawi vaccinated and followed participants, through passive surveillance for febrile illness, for up to three years. The primary outcome is blood culture-confirmed *S.* Typhi infection.

Community stakeholder engagement is increasingly recognized as essential for both ethical research practice and trial feasibility. Engagement can build trust with communities, generate awareness and understanding of the research, promote ownership among local stakeholders, and seek feedback that helps researchers ensure trial procedures are appropriate in the local setting. Engagement thus has the potential to reduce exploitation and harm, support recruitment [285–287], facilitate research activities and enable the feedback of findings to the population; however, community engagement is a complex undertaking and there are few published examples to provide lessons for future practice. Previous research in Malawi has described varied community perceptions of vaccine trials,[288] the importance of community engagement, [289] and challenges surrounding engagement strategies.[290] This chapter describes the large community and stakeholder engagement effort performed prior to and during the vaccination program, and draws on reflections from trial staff to evaluate the relative success of different engagement modalities.

9.2. Methods

9.2.1. Setting

In October 2017, approximately five months before the first vaccination, a range of engagement activities within the communities of Ndirande and Zingwangwa townships (**Error! Reference source not found.**) were conducted. Both townships are densely populated urban centres with a combined population of roughly 200,000 people. For sub-Saharan Africa, they are a typically youthful population [291] with increased rates of in-migration as people relocate from villages for economic and educational opportunities. Vaccination administration occurred at two health centres and multiple primary schools throughout the community. (Figure9-2)

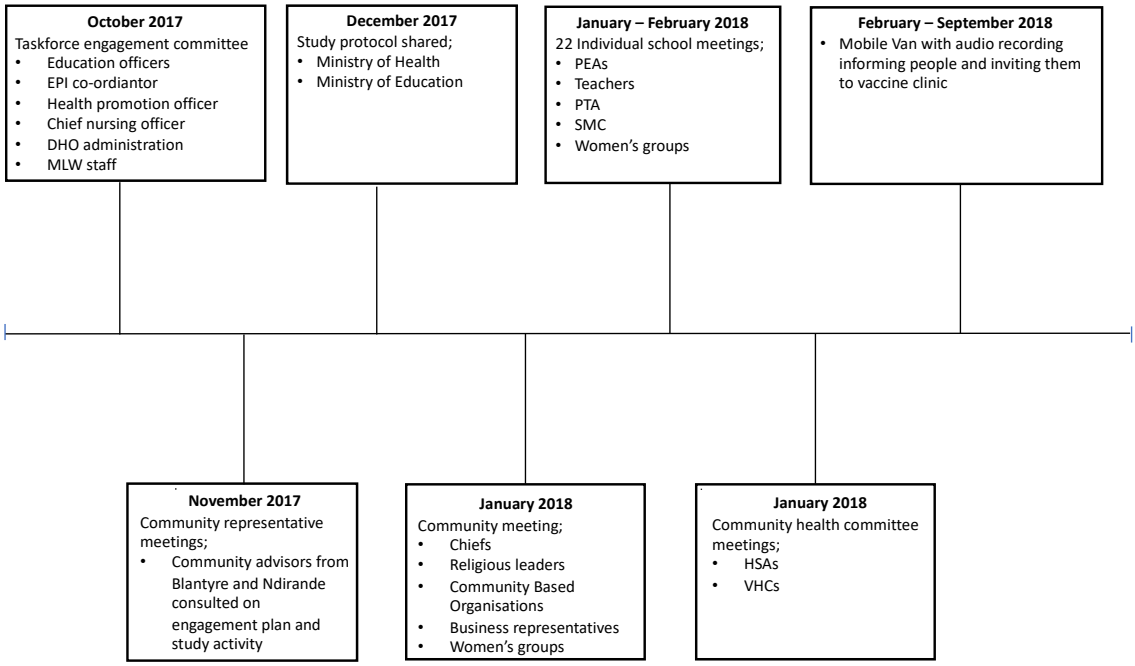


Figure 9-1: Timeline of engagement activities. (EPI; Extended Programme of Immunisation, DHO; District Health Officer, MLW; Malawi Liverpool Wellcome Trust, HSA; Health Surveillance Assistants, VHC; Village Health Committee, PEA; Primary Education Advisors, PTA; Parent Teacher Association, SMC; School Management Committee)

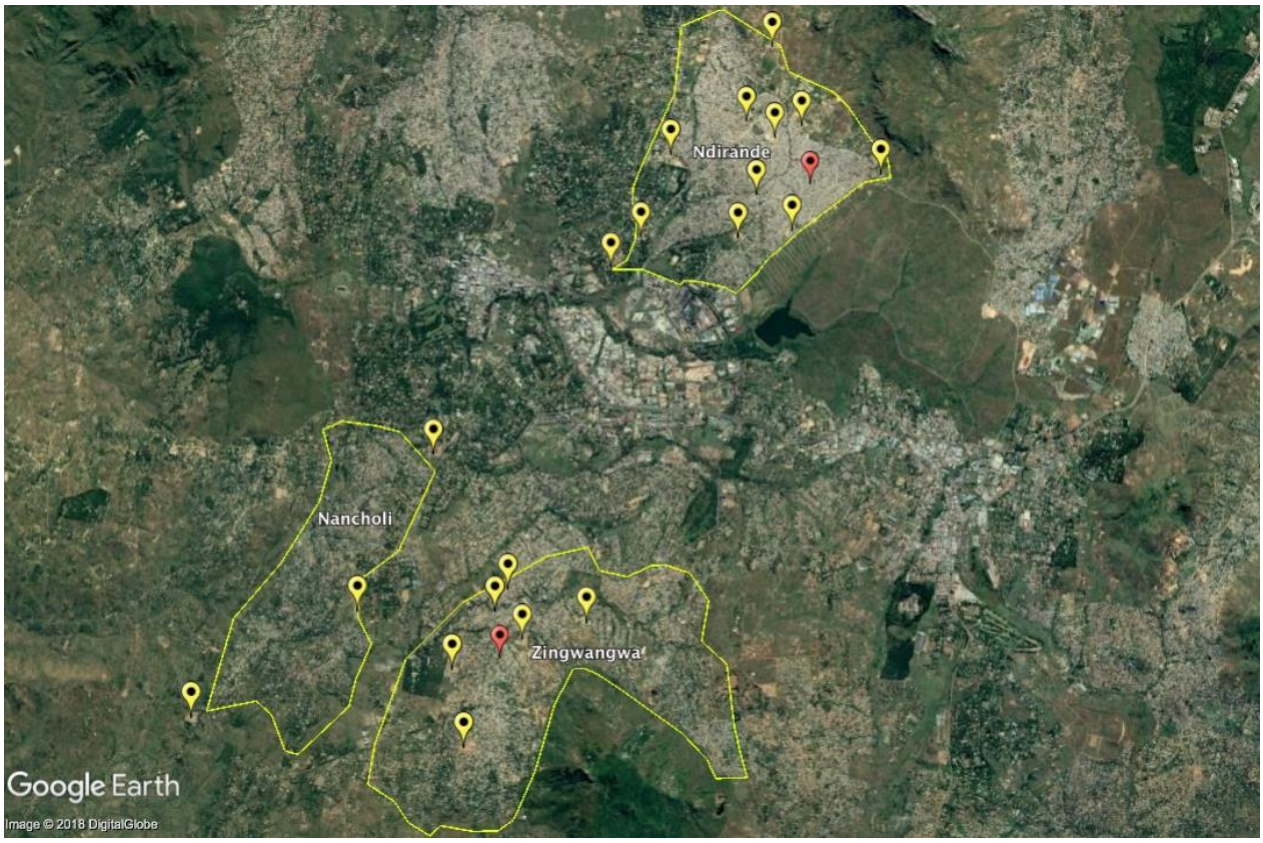


*Figure9-2:*Blantyre City with Ndirande (upper middle), Zingwangwa (lower middle) and Nancholi (lower left) townships with schools (yellow) and health centres(red) located. Source Google Earth.

9.2.2.Community Engagement Activities

9.2.2.1. Community Engagement Taskforce

Under the guidance and leadership of the Blantyre district health office (DHO), the taskforce consisted of education officers, school health and nutrition officers, Extended Programme Immunisation (EPI) officers, Health Promotion officers, the chief nursing officer, members of the Malawi TyVAC team, and DHO administration. The taskforce designed and implemented engagement activities for trial initiation. The DHO provided district-level permission to perform the study and to use designated health and educational facilities for study activities. This permission and support were essential to gain facility access and be seen as a legitimate activity by the community. The taskforce involved 20 people and cost approximately $275 USD.

9.2.2.2. Community Advisory Group (CAG) Meetings

MLW's resident group of about 20 community advisors consult on study conduct in and around Blantyre. The TyVAC team explained the trial and engagement plan and sought feedback.

9.2.2.3. Ministry of Health and Ministry of Education (MoH/MoE)

The MoH and MoE has to grant permission for study implementation in Blantyre. The Malawi TyVAC trial team provided study protocols to the ministries, answered questions, and clarified planned activities. Once permission was obtained from MoH, management and clinic staff in individual health facilities were trained on the protocol through collaboration with the DHO. Similarly, permission was sought from MoE. Because this was the first-time schools were used for a large-scale medical research trial in Malawi, and because the MoE is less connected with the national research ethics regulatory bodies, TyVAC Malawi obtained face-to-face permission from the Minister and Permanent Secretary for Education prior to regional and local engagement. Subsequent contact with individual

schools was mediated by the School Health and Nutrition Officer, who acted as the key link between health and education departments. The support of these two ministries was vital for individual school engagement. Study information was disseminated at each school via presentations, discussion groups, flyers, posters, and letters home.

9.2.2.4. Community Leader Meetings in Ndirande and Zingwangwa

Over two days, TyVAC Malawi brought together traditional village chiefs and headman, ward councillors, religious leaders, business representatives, women's groups, and community-based organizations to explain the study and answer questions. Approximately 160 community leaders were engaged in this process, costing $840 USD.

9.2.2.5. School-based Meetings

The engagement taskforce visited 22 schools to present a study overview and seek participation to establish vaccine clinics on school property. The individual meetings involved primary education advisors, teachers, parent-teacher association, school management committee members, and women's groups. The taskforce sought permission to recruit within the school, provided clarification, and responded to questions from participants. The schools became the primary location of vaccination for both school aged children and non-school aged children as Mothers often brought multiple children for vaccination. Over 200 people participated in these meetings costing approximately $8,250 USD.

9.2.2.6. Community Health Committee Meetings

Health surveillance assistants (HSAs) and village health committees are vital members of the primary health care system in Malawi. These individuals are responsible for immunisations, conducting household follow-up, and distributing health messages to the local community. Engagement with this group focused on their assistance disseminating study information and relaying questions and

feedback from the community to the study team. Time was spent ensuring HSAs understood the study protocol, eligibility criteria, and key messages. Approximately 50 people were involved in these meetings at a cost of $1,375 USD.

9.2.2.7. Mobile Van

A local musician created a jingle containing study messages and invited guardians to bring children for vaccination. This jingle played via a mobile van with large speakers on the roof that drove through the communities. The van operated in the early evening when parents were home and before night-time when vehicle security became problematic. The van campaign began two weeks before first vaccinations and continued throughout the vaccination period. Production costs and van use totalled approximately $1,925 USD.

9.2.3. Assessment methods

To obtain preliminary feedback on the benefits and challenges of different engagement activities, reflection meetings were held with trial and engagement staff throughout the vaccine campaign. To generate additional feedback, we held a focus discussion group (FDG) with frontline trial staff to better understand community reactions and recruitment challenges. Frontline staff bring particular insight to discussions of community engagement and research ethics because of direct contact with community members and participants.[292] The focus group included six frontline trial staff (including fieldworkers and research nurses responsible for approaching community members, meeting community members at trial sites, taking consent and facilitating vaccination). To support open discussion, a non-trial team member facilitated the one-hour focus group. All participating staff provided verbal consent to use focus group material within this article.

9.3. Results

Community engagement was a benefit to the trial and encouraged community uptake in a number of ways:

1. Providing feedback that helped design engagement activities and consent procedures

Discussions with the CAG and other stakeholders helped the trial team identify appropriate ways to engage the Blantyre community and select specific activities.

2. Creating awareness and increasing recruitment

Initial consultation with the CAG highlighted areas to focus. A member advised;

"The use of a mobile van with key messages would be effective in recruitment for the study. This is still a popular medium by which health, political, and commercial messages are delivered to mass audiences in Blantyre and TyVAC should take advantage of this."

The mobile van increased recruitment success (Figure 3) and as noted by a frontline trial team member;

"The van is best. Mostly they do it at night when everyone is almost home and it was very effective. Almost everyone got the information. Everyone was saying "during the night they were saying this, they were saying this, we have to go there." When the van has gone around we do have good turn up."

The community engagement resulted in a cartoon appearing in a major newspaper. The appearance of this cartoon, unsolicited and entirely unrelated to the public engagement teams, indicated engagement penetration with the general public. When the cartoonist was contacted, he confirmed that the study van campaign and other engagement efforts prompted him to write the cartoon. (Figure 4)

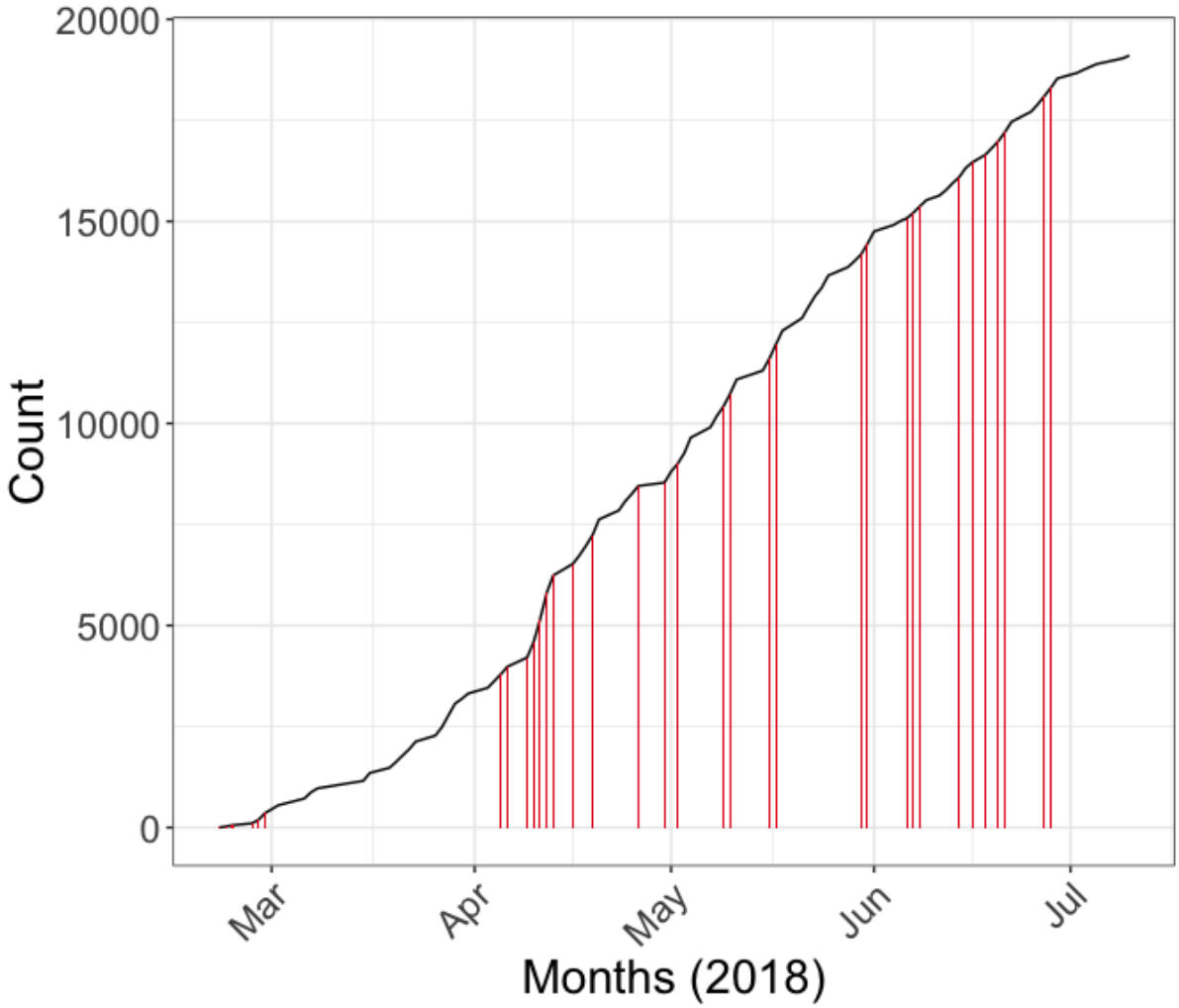


Figure9-3: Vaccine recruitment by month. Van sensitisation events are shown as red bars.

Figure9-4: National Newspaper cartoon.

Translation:
"Mothers, bring your children for research study of …..whatchamacallit….Tadi…..ugghhh…..Tafiyo"
"Typhoid fever

My brother!"
"Yes! The disease is the one mentioned by that goat like bearded man!
When I meet another person they will remind me again"

3. Opportunity to provide information and answer questions

While the van increased awareness of the study, which helped recruitment, other channels provided opportunities for the community to ask questions. A CAG member advised:

"Engagement of religious leaders within the community would be important as many parishioners continue to seek advice on health and study enrolment from these leaders".

Meetings with key stakeholders provided a venue for community members to ask questions and gain understanding of the trial. One vaccinator mentioned:

"The van will just call them that 'there is a vaccine'. But for them to accept it, there should be meetings with the core people in the community, because they can't ask questions on the van but if you meet them, they will be able to ask the questions "what is happening"".

During the community leadership and school-based meetings, there was a broad range of questions. The dialogue increased the understanding and acceptance of trial activities. (Table 9-1: Example list of questions asked during community meetings with leaders, parents, and teachers.).

Study-specific questions
What will happen to individuals recruited to the study, but relocate?
Will participants receive special/extra provisions if they visit health centres with a medical problem?
Within the 28,000 children to be vaccinated, were specific age categories or groups targeted?
How long would the immunogenicity study be conducted and what is involved?
General public health questions on typhoid transmission and control and sanitation and hygiene.
What is the safety profile of the vaccine and what effect will it have on the children who receive it?
Has the vaccine been "certified?"
Will blood samples be collected in the study?
How will the process of randomization and blinding/unblinding be performed? How will the study team know which vaccine each child has received?
Why are only children aged 9 months through 12 years being vaccinated?

What are the main side effects of the vaccine?
How will the study enrol children under five given they are not in school?
What is the difference between the two vaccines administered?

Table 9-1: Example list of questions asked during community meetings with leaders, parents, and teachers.

4. Promoting policy interest in future roll out

The trial and community engagement activities created significant interest in national and international media. The TyVAC Malawi trial team conducted interviews with multiple national newspapers, radio programmes, and television broadcasters,[293,294] further increasing community awareness of the trial and raising the topic and importance of typhoid fever and TCVs. As Malawi and other African countries consider applying for Gavi funding for vaccine introduction, these key messages will ensure discussions are well informed and TCVs are on the national health agenda.

9.4. Discussion

Delivery of messages at several levels in the community from grass roots to upper ministry, ensured individuals were aware of the trial and informed of details. Local leaders provided a channel to disseminate information and answer questions. Community trust in, and accessibility to, local leaders helped reassure the public.

"In one of the schools the headmistress was saying that some of the parents, they come to confirm – 'we heard this from our kids, we had letters, is it okay to bring our kids here to be vaccinated?' Because they trust the teachers, because they are the ones who are with their kids most of the times. So, they went there for confirmation" (Frontline trial staff, FGD)

Despite community engagement activities and community leaders sharing information and answering questions, there were still misconceptions within the community. Providing information and answering questions at enrolment was important for addressing misconceptions and helping individuals to make informed decisions. Information provided at enrolment travels through the wider community as those messages were delivered back to friends and family by participants:

"One man was told that what they [MLW] are doing with this vaccine might affect fertility; 'they are doing this so that we should not have children', but after explaining to them they said that 'okay, now we have the right information, we'll bring some more children'" (Frontline trial team member, FGD)

While a van with a loudspeaker is a relatively unsophisticated approach compared to social media campaigns and other activities employed by trial teams in other locations, it proved effective in raising awareness and suited the context of low-income, densely populated urban areas. Vans with loudspeakers are commonly used in the local community for advertising and other health campaigns, which meant the community was familiar with the approach.

Other channels proved less effective in Blantyre. Engagement with local chiefs is a common community engagement strategy, but can be problematic.[289] For this trial, chiefs played a limited role because of challenges to hold community meetings in an urban setting where people are occupied with income-generating activities:

"Chiefs here in town are very different from those in typical villages… in typical villages they really trust in chiefs, and they can get information from the chiefs, but here in town it is so hard to call for a meeting. The turn up won't be all that good. Most people will say, 'we are busy, we are busy.'" (Frontline trial team member, FGD)

As well as influencing appropriate engagement activities, the social context affected trial uptake and may have encouraged enrolment regardless of engagement activities. In particular, increased vaccine uptake exists in Malawi, with trust in the effectiveness of vaccination.[295] Combined with concern and knowledge about the severity of typhoid, this trust in vaccination encouraged trial participation:

"Most of them know how terrible typhoid is, like in areas where they are doing the study when they hear that we are vaccinating against typhoid, so some of them have relatives or children who have suffered from typhoid, so they said; 'I have to go'".

Personal experience and an awareness of typhoid severity maybe the result of several years of typhoid research and engagement in the community prior to this trial taking place, including door-to-door surveys and health centre recruitment.[140,195] A comment from a community member on social media referencing earlier MLW work highlighted:

> "The [vaccine campaign] has been done after an earlier survey in which results from blood samples taken showed there are active patients with typhoid, and they don't know it."

Other aspects of social context included reduced engagement of relatively wealthy families that prefer to attend private sector health facilities covered through health insurance schemes, rather than the government facilities where vaccine administration and follow-up visits occurred. This discouraged attendance in Zingwangwa, a wealthier area than Ndirande.

> "In Zingwangwa most of the parents were not attending just because when we tell them that after vaccinating, when the child is sick you have to go to Zingwangwa health centre. So, they say we are on MASM [insurance scheme], so we can't go there…while a lot of parents at Ndirande they go to the health centre, they go to the government facilities." (Frontline trial staff, FGD)

This chapter has summarised a series of effective methods for community engagement in two densely populated urban communities in southern Malawi. Engagement activities proved effective in raising trial awareness and mobilising community participation, as demonstrated through the recruitment rate and feedback from frontline vaccine staff. We also used feedback that enabled appropriate engagement activity design.

A range of activities that approach different community stakeholders is essential to successful community engagement. The social context is important to consider as traditional authorities have less influence than in the past, religious leadership remains influential, and online social media use grows. Use of a van to disseminate public messages to these communities was an inexpensive and

effective way to reach large numbers of people. We would recommend it in other urban areas in sub-Saharan African.

Many of these lessons are based on the trial team's perspectives. A more thorough assessment would require feedback from the community on TyVAC Malawi engagement activities, including reasons behind enrolment and people's understandings and experiences of the trial. We recognize our engagement focused primarily on informing and consulting. We did engage at a deeper level with some stakeholders, including partnerships with local and national government, for collaborative decision-making throughout the trial process.

9.5. Recommendations

The experience here confirms the value of community engagement on successful trial activities and enabling community awareness and recruitment. Based on this experience and in line with others,[296,297] recommendations for :

1. Researchers involve a wide range of stakeholders in discussions.
2. Start community engagement early and continue through the project.
3. Adequate resource allocation to support engagement activities.
4. Use of a broad range of complementary engagement activities.