Aspects of disease burden and risk factors for bacterial disease in low- and middle-income countries



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Aspects of disease burden and risk factors for invasive bacterial disease in low- and middle-income countries

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Abstract

Bacterial infections cause substantial morbidity and mortality, particularly in low- and middle-income countries. Strained healthcare systems, limited access to clean water, and fragmented hygiene and sanitary infrastructure increase risk of disease transmission. However, effective vaccines have been developed and can be deployed strategically to prevent disease. This thesis sets out to measure aspects of cholera and typhoid disease burden and to evaluate how natural infection and WASH affect disease risk in endemic settings. The analyses are performed on historical datasets of clinical studies of cholera and typhoid vaccines that were conducted in Bangladesh and India, respectively. Using a vaccine probe analytical approach, I assessed whether there was evidence of cholera in diarrhoeal cases that tested negative for cholera by conventional faecal culture and provided evidence that cholera cultures are highly sensitive. Secondly, I developed a clinical definition for typhoid fever that satisfied the criteria of being predictive of culture-confirmed typhoid fever and being protected against by an effective typhoid vaccine. Thirdly, I measured the degree to which natural typhoid protected against a subsequent typhoid fever episode and found little evidence for the development of natural immunity. Finally, I evaluated which components of household WASH in a typhoid endemic

setting were associated with reduction of typhoid risk and asked whether WASH and vaccine interventions acted synergistically to control typhoid.

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Finally, I could not imagine completing this work without the love and support of my family. In particular, I am truly humbled by my mother, who has patiently reminded me that through faith, perseverance, and the grace of God, there is nothing that cannot be achieved. You have been the most important source of strength and purpose for me during the last four years. Thank you.

Declaration

I, Justin Im, confirm that the work presented in this thesis is my own and was conducted at the International Vaccine Institute in Seoul, South Korea. Where information or work has been derived from other sources, I confirm that this has been indicated in the thesis. This thesis has not been submitted for a degree or other qualification to this or any other university.

Statistical analysis has been conducted with support from Faisal Ahmmed, icddr,b, Bangladesh.

COVID-19 Statement

The SARS-CoV-2 (COVID-19) pandemic resulted in restrictions on international travel beginning in March 2020. This impacted my ability to complete the data collection and analyses originally included in my DPhil thesis proposal. In October 2020, based on guidelines published by Oxford University in *Guidance on PGR student assessment in the pandemic context MSD,* and given the uncertainty of when international travel would be permitted, my supervisors and I made the decision to redefine the scope and content of my thesis. The work presented here was evaluated for eligibility according to University guidelines, was approved by my thesis supervisors and Oxford University faculty counsellor, and was completed within the period of enrolment in the DPhil research degree programme.

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Abbreviations

AG	Andersen-Gill
AUC	Area under curve
AWD	Acute watery diarrhea
BC	Blood culture
BMGF	Bill & Melinda Gates Foundation
BS-WC	B subunit combined with inactivated bacterial whole cells
CI	Confidence interval
CP	Complexity parameter
CRT	Cluster randomized trial
DRC	Democratic Republic of Congo
DTP	Diphtheria, tetanus, pertussis
EADII	Extended analysis of past datasets of cholera and typhoid, icddr,b and IVI
EPI	Expanded Program on Immunization
GBD	Global burden of disease
GTFCC	Global Task Force on Cholera Controlfeik
HDSS	Health and Demographic Surveillance System
HepA	Hepatitis A
Hib	*Haemophilus influenzae B*
HR	Hazard ratio
icddr,b	International Centre for Diarrheal Disease Research, Bangladesh
IR	Incidence rate
LMIC	Low- and middle-income country
LOESS	locally estimated scatterplot smoothing
OCV	Oral cholera vaccine

ORT	Oral rehydration therapy
PCR	Polymerase chain reaction
PE	Protective efficacy Protective effectiveness
PY	Person-years
ROC	Receiver operating characteristic
S. Typhi	*Salmonella enterica* serovar Typhi
SNP	Single nucleotide polymorphism
TCV	Typhoid conjugate vaccine
V. cholerae	*Vibrio cholerae*
VE	Vaccine efficacy Vaccine effectiveness
Vi-TT	Vi-tetanus toxoid
ViPS	Vi polysaccharide
VPDI	Vaccine-preventable disease incidence
WASH	Water, sanitation, and hygiene
WC	Whole cell
WC-only	Whole cell only
WHO	World Health Organization

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Chapter 1 Introduction

Bacterial and viral pathogens that cause disease of epidemic and pandemic potential have been recorded throughout human history. Up until the 19th century, humans, apart from protection afforded by their adaptive and innate immune systems, were relatively unarmed against the threat of emergent pathogens and societies had few tools to prevent transmission. By the mid-1800s, acceptance of the germ theory of diseases, substantiated by the combined works of Louis Pasteur and Robert Koch, and early understanding of how microorganisms were transmitted, credited to observations by John Snow, produced the first principles of modern public health programs to combat disease. By the end of the century, several important vaccines, against smallpox, rabies, and cholera, became available and major discoveries broke ground for effective vaccines against typhoid, tuberculosis, tetanus, diptheria, and yellow fever in the early 20th century. Simultaneously, technology and infrastructure allowing for the separation of drinking water from human waste appeared in many advanced economies. The pairing of these technologies, combined with upgraded standards for personal hygiene, drove massive alleviation from death and suffering due to infectious disease.

1.1. The context for bacterial disease in low- and middle-income settings

Despite major advancements in our understanding of the spread of microorganisms and development of tools to prevent disease, a large part of the world is still afflicted by deadly infections. In low-income countries, of the top ten most common causes of death, six are communicable diseases (1). These include neonatal conditions, lower respiratory infections, diarrhoeal disease, malaria, tuberculosis, and HIV/AIDS. By contrast, in upper-middle-income countries, only one of ten leading causes of death is by an infectious disease – lower respiratory infections (2). Although diarrhoeal deaths have decreased drastically in the past decade, particularly in young children, globally there are still an estimated 1.7 million deaths that occur each year (2), an overwhelming majority of these occurring in sub-Saharan Africa and South Asia, where diarrhoeal disease are still the fifth most common cause of death (2).

In lower- and middle-income countries (LMICs), the current state of clean water infrastructure and waste disposal systems resembles that of affluent economies in the 19th century. In low-income countries, only 29% of the population has access to safely managed drinking water (3) and even fewer (18%) have access to safely managed sanitation facilities (3). Basic handwashing facilities are available to less than a third of the population (3). Rapid urbanization has resulted in overcrowding and the expansion of urban slums. Globally, over 4 billion people, more than half of the world's population, live in urban settings, and one-third of urban dwellers live in slums (4). In these settings, overwrought waste management systems increase the risk of human waste seeping into water sources used for consumption, daily use, and food preparation. In addition, hundreds of millions of urban residents rely on subsistence or small-

scale commercial urban agriculture or rearing of livestock for sale (5, 6). In a survey of urban centres in East Africa, 17-36% of urban populations grow crops and/or keep livestock (7). Lacking sanitary facilities and clean water supplies, compounded by unhygienic behaviours, exacerbates the risk of transmission of infectious agents from their animal or environmental reservoirs to humans. Moreover, increased mobility of populations, facilitated by expanding road and rail networks and fuelled by outward radiating social and commercial networks, has expediated the spread of microorganisms. In this thesis, I focus on the infections caused by bacteria, although viruses, fungi and parasites also proliferate in the same context.

Of bacterial aetiologies identified in a Global Burden of Disease (GBD) diarrhoeal study (2), Shigella diarrhoea was found to cause the most deaths overall (212 438 deaths; 2.9 deaths per 100 000 person years) with a particularly high burden in children under 5 years (63 713 deaths; 10.1 deaths per 100 000 person years). *V. cholerae* accounted for 107 290 deaths overall (1.5 deaths per 100 000 person years) and 52 232 deaths in children under 5 years (8.3 deaths per 100 000 person years). Non-typhoid *Salmonella* spp, *Campylobacter* spp, and *Cryptosporidum* spp also caused significant diarrhoeal mortality. Estimates of attributable fractions of death and disease due to bacterial infections in LMICs are incomplete and likely underestimates due to absent confirmatory testing in health care settings and incomplete reporting practices.

Many of the diseases that afflict populations in LMICs can be effectively treated with modern medicine, vaccines, and therapeutics. However, access to quality health care and appropriate treatment is often obstructed by cost or availability. In the world's poorest countries, diagnostic tools to differentiate the causes of common syndromes and the technical expertise to apply them are beyond the scope of routine care. Incentives to seeking healthcare are complicated by the opportunity cost of lost wages, childcare, and transportation. Healthcare systems have, however, improved drastically even in the poorest and most remote regions, and antibiotics, which are both affordable and widely available, significantly improved health outcomes for a broad scope of diseases.

Despite the relief provided by effective antibiotics, which were introduced in the early twentieth century, new threats to human health have emerged. Bacterial and viral pathogens first developed mechanisms of resistance to antimicrobials shortly after they became available (8) and continue to evolve in response to prevalent antimicrobial treatment regimens. Globally, bacterial antimicrobial resistance (AMR) was estimated to cause 1.27 million (95% UI: 0.911, 1.71) deaths in 2019, with the highest burden in western Sub-Saharan Africa (27.3 deaths per 100 000; 95% UI: 20.9, 35.3) (9). The bacterial pathogens contributing the most to resistance-associated death are *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa,* which were responsible for a combined 929 000 (95% UI: 660 000, 1 270 000) deaths (9).

The emergence of sweeping AMR has renewed the urgency to develop new solutions to combat evolving microorganisms. Vaccines are one of the most effective tools in the global health arsenal of interventions to combat infectious disease. Since the end of the eighteenth century, vaccines that protect against a range of 25 diseases are now available, and vaccines against 15 additional disease targets are under development (10). The success of vaccines in preventing death and disease is clear, as demonstrated by the eradication of smallpox (11), elimination of polio (12), and suppression of measles, diphtheria, mumps, and rubella to vanishing numbers in most of the world (13, 14). With the development of new technologies, analytic approaches, and standards for safety and effectiveness, the quality, utility, and affordability of life-saving vaccines has continued to increase at a rapid pace.

In this thesis, I investigate aspects of disease burden, natural immunity, effects of water, sanitation, and hygiene (WASH), and vaccine protection in the context of two bacterial infectious diseases: cholera diarrhoea and typhoid fever. I make use of datasets derived from field studies of vaccines against these disease targets and that were conducted in settings that were endemic for each disease, respectively. In this chapter I provide a brief overview of cholera and typhoid disease epidemiology, discuss components of WASH intervention and natural immunity, describe a methodological approach applied in subsequent chapters, and introduce the datasets exploited during analysis.

1.2. Cholera

Cholera is a diarrhoeal disease that can cause severe illness and death through rapid dehydration. There are over 200 serogroups of *Vibrio cholerae* (*V. cholerae),* a Gram-negative rod-shaped, non-invasive bacterium, of which only two serogroups – toxigenic 01 and 0139 – cause disease. Serogroups are different based on the polysaccharide O antigen. Serogroup 01 is further divided into two biotypes, El Tor and classical, and each biotype can be further categorized as Ogawa, Inaba, or Hikojima serotypes. In cholera endemic countries alone, cholera diarrhoea occurs in over 2.9 million individuals annually causing over 95,000 deaths (15, 16). Once ingested, disease is caused by toxigenic serogroups of the bacterium *V. cholerae*, which is transmitted through contaminated water sources and typically affects settings with poor water and sanitation infrastructure. Seven major cholera pandemics have been recorded to date. Historically, outbreaks of cholera have decimated populations leading to millions of cases and hundreds of thousands of deaths. Through the course of a devastating cholera outbreak in London, England in 1854, investigation into the cause, a contaminated water source, and possible solution to the outbreak, restricting access to that water source, led by English physician John Snow, resulted in the establishment of some of the first principles of epidemiology. This breakthrough paved the way for evidence-based approaches to public health policy.

Although the biology, pathology, and epidemiology of cholera, and effective preventive strategies, including vaccines, are now very well understood, the disease persists in vulnerable settings today. Cholera is still reported in high numbers from large regions within Sub-Saharan Africa, South Asia, and South-

East Asia, and 69 countries worldwide remain cholera endemic (15). In October 2010, following a catastrophic earthquake that shook the Caribbean, a cholera outbreak swept through Haiti causing 820,000 reported cholera episodes and 10,000 deaths (17). More recently, supplanting the Haiti outbreak as the worst outbreak in recent history is the ongoing endemic in Yemen, where, as of May 2020, there were 1.4 million cholera cases reported and a case fatality of 0.11% (18). This epidemic was permitted by devastation caused by ongoing armed conflicts within the country which has left health care systems overrun and dysfunctional (19).

Cholera disease is characterized by acute, watery diarrhoea (AWD) which can be compared to rice water in appearance. Disease is frequently accompanied by vomiting, leading to rapid dehydration which can cause death in severe instances. The infection progresses rapidly and can cause death within 24 hours of infection depending on the infectious dose, immune status, and timely administration of effective treatments. Bacterium are transmitted from infected patients through faeces and vomitus through direct faecal-oral routes or through ingestion of contaminated water or food. The standard diagnostic for cholera is microbiological culture of an acute stool specimen and isolation of *V. cholerae.* Enrichment of stool samples in alkaline peptone water is common for the promotion of *V. cholerae* growth and inhibition of growth of background microorganisms. The diagnostic sensitivity of standard culture techniques has not been challenged, although a recent report provides counter evidence using modern molecular techniques (20). In Chapter 2 I evaluate the notion of

substantial misclassified culture-negative cholera due to insensitivity of cholera culture in a cholera-endemic setting.

Vaccines against cholera have been under development since 1885. The first vaccine was a whole-cell injectable vaccine, although this formulation was found to be reactogenic and generated limited protective efficacy against disease. The most recent generation of cholera vaccine, an oral vaccine, is designed on the understanding that protection against cholera primarily depends on stimulation of local mucosal immunity in the intestine. Oral cholera vaccines (OCVs), which are composed of killed or live cells, have been developed since the 1980s, and two vaccines are currently available. Dukarol® is a killed whole cell monovalent (01) vaccine with a recombinant B subunit of cholera toxin, and Shanchol™, Euvichol®, and mORCVAX™ are the same vaccine formulation based on a killed modified whole cell bivalent (01 and 0139) vaccine without B subunit. OCVs have been stockpiled by the WHO since 2013 in order to respond rapidly to outbreak situations and control cholera epidemics. In 2021, over 15 million doses of OCV were disseminated in Niger, Nigeria, Cameroon, Ethiopia, Nepal and Bangladesh. The recognition of cholera as a major preventable disease that continues to disproportionately affect low-income countries and widens economic inequity has led to the Declaration to Ending Cholera, a commitment to achieve cholera mortality reduction of 90% by 2030, issued by the Global Task Force on Cholera Control (GTFCC) in 2017 (21). This pledge outlines a cholera control strategy, a commitment to invest in the use of available tools, including OCV and improvements in WASH, and a mission to garner national and international political support.

1.3 Typhoid fever

Typhoid fever is an important cause of enteric disease especially among children in LMICs. Global burden of disease studies have estimated that 14.3 million cases of typhoid fever occur annually leading to approximately 145 000 to 161 000 deaths (22). Case-fatality rates have dropped to <1% since the era of antibiotics ushered in a series of highly effective treatment options (22, 23), however, the emergence of multi-resistant strains (24-26) and the discovery of lineages with resistance characteristics to all available antibiotics (27) indicates that the imminent threat of typhoid to global health cannot be underestimated or ignored.

Typhoid fever is caused by an infection with *Salmonella enterica* serovar Typhi (*S.* Typhi), a Gram-negative bacterium, typically transmitted through contaminated water or food sources. The only known reservoir of *S.* Typhi is humans. Symptomatic infection often includes fever, abdominal pain, nausea, and vomiting, and presents with varying disease severity. Severe disease may include persistent high fever, abdominal discomfort, malaise, and headache. If the infection is not treated, intestinal perforation may occur and can lead to severe outcomes, including death.

Differentiating typhoid fever from other febrile and gastrointestinal illnesses is challenging. Empiric diagnosis of typhoid fever requires a reasonable level of clinical suspicion, particularly when other common causes of fever, such as

malaria, exist. I evaluate clinical characteristics associated with typhoid fever in an endemic population in Kolkata, India in Chapter 3. Without timely diagnosis and appropriate treatment, infected individuals may develop serious complications beyond the second week of illness (28). Recent reviews have estimated that approximately one quarter of typhoid infections are accompanied by one or more clinical complications (29, 30).

The standard diagnostic test for typhoid is blood culture, although this method has limited sensitivity, reported between 40% to 87% (28), associated with a variety of inputs including blood volume (31), age of patient, duration of symptoms, pre-treatment with Ab, duration of illness, and quality of microbiology laboratory and technicians (28, 32-37). Each of these variables weakens the certainty of the test outcome and thereby impacts our understanding of disease burden, particularly where surveillance infrastructure is incomplete and laboratory capacity is weak. In addition, low and variable diagnostic sensitivity impairs the ability to evaluate the protective effect against confirmed typhoid clinical endpoints in vaccine field studies.

Vaccines against typhoid were developed as early as 1896 using heat or chemicals to kill and thus inactivate *S.* Typhi cells. These vaccines were licensed and used in England and Germany, however they were also accompanied by significant side effects, including local inflammation, pain, systemic fever, malaise, and other disease-like symptoms, and are no longer considered suitable for public use and are no longer produced (38, 39). More recently, subunit vaccines were constructed around the capsular Vi

polysaccharide (ViPS) antigen. In 1986, Typhim Vi® (Sanofi Pasteur, Lyon, France) and Typherix® (GlaxoSmithKline, Brentford, UK) were developed and demonstrated strong protection against typhoid during trials in Nepal, South Africa and China (40-42). These vaccines, however, are limited because they are non-immunogenic in children below two years of age. Additionally, many *S.* Typhi strains do not express the ViPS or lose their Vi antigen thus rendering these vaccines ineffective (43, 44). Vi-negative *S.* Typhi isolates, though less infective than Vi-positive isolates, can cause a disease indistinguishable from the one caused by Vi-positive isolates (43) and are prevalent in typhoid endemic settings.

To improve on the killed whole cell vaccine, a vaccine based on a live attenuated *Salmonella* strain was considered. The Ty21a strain was identified as a suitable candidate and the first live oral attenuated vaccine was developed by Berna Biotech (now Crucell, Leiden, Netherlands) and is sold commercially as Vivotif®. However, this highly mutated strain lacks the Vi antigen and is highly attenuated, therefore, despite evidence for strong protective effects, to obtain sufficient immunity, a high inoculum is required. This vaccine is therefore recommended for use only in ages 5 years and above.

The struggle of early stage typhoid vaccines in providing high levels of durable protection to younger children has fuelled development of an optimized typhoid vaccine. Polysaccharide vaccines do not produce memory B cells and stimulate short-lived antibody production with no evidence of affinity maturation. The latest

typhoid vaccines are based on a polysaccharide antigen conjugated to a known entity that would stimulate cell-mediated immunity.

In 2018, the first typhoid conjugate vaccine (TCV) was prequalified by the World Health Organization (WHO), Typbar-TCV® (Bharat Biotech India Ltd, Hyderabad), and in 2020, the second TCV, TYPHIBEV® (Biological E Limited, India), was prequalified, although there are several additional conjugate vaccines licensed and commercially available. The development of TCVs is important since this vaccine has demonstrated longer duration of protection than the ViPS and Ty21a vaccines and confers protection in young children (45). Since its prequalification, TCV has been administered to 30 million children globally, and Pakistan was the first country to introduce the vaccine as part of routine immunization in 2019 (46).

1.4 Protection against disease through immunization

The WHO estimates that 2-3 million deaths are prevented each year due to current immunization programs (47). Vaccines confer protection against infection and/or disease by exploiting the immune system's ability to respond to and remember foreign antigens. An effective and safe vaccine will stimulate the adaptive immune system to produce antibodies (humoral immunity) and T cells (cellular immunity). The specificity, strength, and duration of protection offered by such immunity depends on various factors related to both the host – age, maternal antibody levels, prior antigen exposure – and the vaccine – schedule and dosage. Some vaccines may reduce asymptomatic infection, colonization,

and further transmission, thereby providing herd immunity. These features of vaccine-induced immunity are important for designing effective public health programs. In addition to vaccine-induced immunity, natural infection-derived immunity is also important, especially in endemic settings. Exposure to indigenous pathogens in immunocompetent individuals will stimulate an immune response and, at best, may confer life-long protection against future disease. Understanding pathogen-specific responses and degrees of protection conferred by prior infection are crucial to assessing disease risk and optimizing vaccine programs.

Studies conducted in cholera endemic areas have shown that infection with *V. cholerae* results in long-lasting immunity (48-50). A cholera episode in individuals residing in Matlab, Bangladesh resulted in 90% protection against subsequent disease during a ten-year period (1968-1977) (49). A direct demonstration of natural immunity was provided by a human challenge study that showed complete protection up until three years, the longest interval tested (48). The prevalence of prior exposure is relevant to cholera vaccine strategies. In Dhaka, Bangladesh, a highly-endemic cholera setting, it has been shown that significant protection can be achieved after a single dose of OCV in adults and children above 5 years, whereas children below 5 years require a two-dose regimen for the vaccine to be protective (51). Single-dose campaigns have major programmatic and economic advantages over multi-dose campaigns, and therefore it is important to understand the underlying disease burden and characterize the effects of natural infection-immunity in each setting prior to vaccination.

There are few studies that evaluate naturally occurring immunity following typhoid infection, and immune correlates of protection are not yet well established. There are two studies that have measured protection conferred by an episode of typhoid against subsequent infection. One study reported moderate levels of protection in a human challenge study of adult volunteers (52) and the other described the rate of recurrent typhoid in an army battalion during two successive typhoid outbreaks (53). These studies are not recent and were conducted on narrow population subsets in non-endemic settings. In Chapter 4, I address the question of protective association by initial typhoid episode against subsequent typhoid in a typhoid-endemic population followed-up over four years.

1.5 Disease control through improvements in water, sanitation, and hygiene

Clean water reduces both the frequency and magnitude of exposure to circulating pathogens in a population. Reduced inoculum reduces risk of infection and risk of disease and, thereby, optimizes additive protective measures, such as vaccines and hygienic behaviour. Effective water filtration and sanitation facilities are costly and the capital investment currently necessary for radical development is not available for most communities within LMICs.

Recurrent outbreaks of cholera in London during the mid-nineteenth century resulted in thousands of deaths (54). In 1858, the stench of open sewage emitted from Thames River flowing through London, England was so foul that

parliament quickly approved scoping bills to modernize the sewage systems across multiple jurisdictions (55, 56). Over 15 years, a network of 100 miles of underground pipes were installed to connect sewage collected all over the city and pumping stations were built to divert waste water far from the city (55). Since then, there have been no cholera outbreaks in London. This approach to cholera control has been replicated in high-income countries and wealthy pockets of low-income settings, globally. However, this solution has not been implemented throughout low-income countries. In addition to immense initial capital investment, substantial upgrades to water and sanitation infrastructure require steady political commitment. Municipalities must have the technical capacity to manage these systems through revenue-generating schemes that sustain resources for maintenance and expansion. Unfortunately, urban centres that are in most need of these upgrades have large informal economies and are further strained by rapid population growth.

In communities where massive infrastructure development is unlikely in the short- or even mid-term, household-level strategies towards to improve WASH and minimize the risk of infectious disease transmission can be employed. These approaches, however, are interim solutions and convincing evidence for sustained success is lacking. Treatment of drinking water at point-of-consumption has been shown to be effective in protecting against cholera and all cause diarrhoea when evaluated in a trial setting (57-59). Similarly, households can install private pit latrines to contain faecal and urinary waste. Thirdly, households can improve handwashing behaviour, such as by increasing frequency and insisting on use of soap. Although the ability to enforce and

ascertain quality of handwashing is low, several studies have identified handwashing to be effective at preventing cholera (60, 61) and typhoid (62).

Despite demonstrated effectiveness of these component approaches to improve household-level WASH, there are intrinsic challenges that limit their efficacy and threaten their sustainability. Household water treatment requires proactive effort and correct treatment techniques. Treatment methods may cost money and/or time, and therefore uptake of water treatment interventions may be low (63, 64). Toilets and pit latrines are only one component of a comprehensive sanitation system. Without a supporting network of closed sewers that contain waste and maintain separation from the environment, latrines will not necessarily prevent contamination of water sources, especially in densely populated urban areas and flood-prone climates. For example, although 99% of the population in Dhaka, Bangladesh has access to a toilet, 98% of the untreated wastewater seeps back into the environment through a corrupted and leaky sewage system (65). Finally, effective hand-washing practices require running water and soap. Running water is often lacking, especially in areas where risk of disease is high. In Bangladesh, 36% of government hospitals do not have running water (66) and only 21% of schools have designated handwashing stations with running water and soap (67). Effective handwashing behaviour is even more scarce. Even in high-income countries, compliance with recommended hand hygiene in healthcare workers is low (65%), with the lowest level of compliance, ironically, in physicians (33%) (68).

Although transformative improvements in WASH in LMICs will take time, the combination of incremental improvements in household-level WASH and individual behaviours with parallel administration of effective vaccines has a measurable impact on disease prevention, including against cholera and typhoid. However, the degree to which WASH modifies the effect of cholera and typhoid vaccines, respectively, has not yet been measured. In Chapter 5 and 6, I investigate this question in a typhoid endemic setting where a typhoid vaccine was introduced.

1.6 Vaccine probe studies

The concept of using a vaccine intervention to interrogate, or 'probe', a dataset for implicit outcomes that are subtle, but of no less public health importance, has been introduced only recently. The first study to formally use a vaccine probe approach was of a randomized control trial of a *Haemophilus influenzae B* (Hib) conjugate vaccine published in 2004 (69). Whereas vaccine studies traditionally focus on assessing vaccine efficacy or effectiveness (VE), vaccine probe studies characterize disease (70). A vaccine probe approach can be implemented in the context of a randomized controlled trial of a vaccine with known efficacy. The underlying principle of a probe design is that the relative difference in disease incidence of a target disease, for which the vaccine under investigation is protective, between vaccinated and unvaccinated individuals is attributable to the vaccine-specific pathogen. This is referred to as the vaccine-preventable disease incidence (VPDI) and indicates the incidence of excess disease that

would be otherwise prevented by a vaccine administered in that particular setting.

One advantage of a vaccine probe design is the direct appeal to public health value. Typically, outcomes of vaccine studies characterize the efficacy or effectiveness of the vaccine, and these measures are given in terms of the relative risk of disease outcome between intervention groups. These metrics characterize the vaccine itself, and while providing important information, messaging around public health utility can be cloudy. For example, the effect size of a vaccine is highly dependent on the underlying frequency or risk of the disease for which the vaccine is designed to prevent. A vaccine of low effectiveness against a commonly occurring disease can still prevent, in absolute terms, substantial morbidity and mortality within a population. On the other hand, a vaccine with high protective efficacy might be considered to be of high public health value, but if the targeted disease is rare, attributing public health value is less straight forward, especially for policy-makers balancing public health priorities with finite resources. Vaccine probe studies can demonstrate a vaccine's public health value in simplistic and direct terms, and as the analyses presented in Chapter 2 and 3 set out to demonstrate, the approach is straight forward, broadly applicable, and can extend the value of existing and available datasets beyond their primary intended purpose.

1.6.1 Principles and design

The validity of a vaccine probe study depends on several important assumptions. Firstly, the vaccine under investigation must have a known estimate of vaccine effectiveness. This will enable direct correlation of disease incidence difference between vaccinated and non-vaccinated individuals to the vaccine. For diseases where diagnostics have limited sensitivity, a probe design can help to determine the aetiological fraction of a particular disease syndrome, since a vaccine should protect against its target disease irrespective of the diagnostic's ability to correctly identify the causative agent. This provides a programmatic advantage as it is easier to measure the difference in syndromic cases compared to confirmed disease between two populations. Finally, this analytical approach can be applied retrospectively to historical datasets to extract additional information and generate novel insights. Clinical trial datasets typically include meticulously collected patient metadata and demographic data and are, therefore, promising resources.

1.6.2 First applications of vaccine probe studies

Hib infection can cause bacteraemia, pneumonia, and acute bacterial meningitis. In the evaluation of a Hib conjugate vaccine in a trial in Gambia, the vaccine showed protective efficacy of 95% against culture-confirmed invasive Hib disease and 21% against all-cause radiologically confirmed pneumonia (71). This evidence supported the introduction of Hib vaccine introduction into the Expanded Program on Immunization (EPI) in Africa in 1997. In Asia, the decision to introduce Hib into the EPI program was not immediate. Until this point, most studies of Hib disease in Asia had reported low incidences.

However, it was unclear as to whether Hib burden was low, or whether case detection was incomplete. It was understood at the time that in Asia that most Hib pneumonia infections were non-bacteraemic and therefore Hib infections were difficult to measure in typical surveillance systems that excluded radiological examinations. To evaluate uncertainty of Hib burden in Asia, 55,000 children in Lombok, Indonesia were enrolled into a randomized study comparing the rates of pneumonia between recipients of a diphtheria, tetanus, pertussis (DTP) vaccine or a DTP-PRP-T (Hib conjugate) vaccine (72). This study did not measure the efficacy of the vaccine, rather, it compared the rates of clinical outcomes between the two vaccine groups, and the findings revealed that the incidence of vaccine-preventable clinical pneumonia was substantial, 1561 clinical pneumonia cases per 100 000 person-years (95% CI: 270, 2853), despite lack of direct evidence of Hib pneumonia burden in the region. Studies that have used an established vaccine as a probe have been further applied to *Streptococcus pneumonia*, influenza, and rotavirus.

1.6.3 Challenges and limitations

There are limiting assumptions that must be considered when applying a vaccine probe design. The first is that since probe studies evaluate the difference in disease syndrome, the vaccine is assumed to provide protection only against infections caused by the target disease. Cross-protection offered by the vaccine against other organisms leads to an overestimation of VPDI. For example, rotavirus vaccine may provide protection against other viral gastroenteritis infections, such as adenovirus and sappovirus (73), therefore the

reduction of diarrhoeal disease in the vaccinated group cannot be attributed to the reduction of rotavirus disease only. Secondly, in order to estimate aetiological fraction, the vaccine should have a known efficacy demonstrated in prior trials. Since effectiveness of a vaccine varies according to multiple factors, if vaccine effectiveness is unknown, it will be difficult to interpret findings with certainty. A nested efficacy study may be necessary for interpretation of a vaccine probe analysis if vaccine efficacy is unknown. Thirdly, since vaccine probe studies are typically applied to clinical studies of vaccines, the analysis is subject to the same design challenges as the trials themselves, such as selection bias, confounding variables, and secular trends. Finally, in many clinical evaluations of vaccines, the primary outcome is a function of relative time to occurrence of first clinical event. If a pathogen causes recurrent vaccine-preventable episodes, this approach will lead to an underestimation of disease burden using a vaccine probe approach. Alternatively, investigators may consider recurrent disease episodes in the analysis, making provisions for periods of immunity during and following each episode and including assumptions of susceptibility to recurrent infection following recovery.

1.7 Extended analysis of past datasets of cholera and typhoid

The International Centre for Diarrheal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh and the International Vaccine Institute (IVI), Seoul, South Korea have independently and collaboratively conducted cholera and typhoid epidemiological studies and vaccine trials in Asia and Africa. Over several decades, each institute has generated a rich archive of study datasets, however,

these datasets have had limited utility beyond their original stated purpose. Many of these datasets were made available for reanalysis through a grant by the Bill & Melinda Gates Foundation (BMGF), funded under the program *Extended analysis of past datasets of cholera and typhoid, icddr,b and IVI* (EADII), awarded to both institutions in 2018 (OPP1171432) and renewed in 2020 (INV-025386). This program assigned epidemiologists and statisticians to revisit and re-evaluate dormant datasets to extract new insights using novel approaches and statistical methods. The analyses in Chapters 2 to 6 are performed on longitudinal data collected from populations undergoing OCV and typhoid ViPS vaccine studies previously conducted by icddr,b and IVI.

1.7.1 Clinical trial of oral cholera vaccine in Matlab, Bangladesh, 1985

A clinical evaluation of oral cholera vaccine was conducted in Matlab, Bangladesh by the icddr,b beginning in 1985 and main findings are published by Clemens JD., *et al*. in Lancet, 1990 (74). Matlab is located just outside Dhaka, Bangladesh, and was a cholera hotspot during the time of the study. Since 1966, a Health and Demographic Surveillance System (HDSS), which consists of regular cross-sectional censuses and longitudinal registration of vital events, has been maintained in the study area (75). There were approximately 190 000 residents in Matlab at the time of the trial, of whom 124 035 were age-eligible for participation in the study. The study design was an individually randomized, placebo-controlled trial. Children from 2 to15 years of age and women 15 years or older were randomized to receive a single dose of one of three vaccines: 1) cholera toxin B subunit combined with inactivated bacterial whole cells (BS-WC);

2) inactivated whole cells only (WC-only); or 3) placebo (76). In total, 89 596 individuals received at least one dose of a study agent and were followed up by diarrhoeal and demographic surveillance for 3 years. After three years of follow-up, the cumulative protective efficacy of the completed three-dose regiment in all age groups was 50% for BS-WC and 52% for WC-only, although protection was notably higher in persons older than 5 years compared to children 2-5 years for BS-WC (63% versus 26%) as well as for WC-only (68% versus 23%) (76). Protection by BS-WC during year 1 and during year 2 of follow-up was similar, but protection measured in year 3 declined (76). Similar findings were reported for the WC-only vaccine.

1.7.2 Cluster-randomized effectiveness trial of Vi typhoid vaccine in India, 2004

A clinical evaluation of ViPS typhoid vaccine was conducted in an urban slum site in Kolkata City, the capital of West Bengal, India in 2004 and primary results are published by Sur D. *et al.,* in New England Journal of Medicine, 2009 (77). Kolkata had an estimated population of six million at the time of the trial. A census of 66 458 people was conducted two years prior to the onset of the trial, and individual and household level demographic and socioeconomic data information were subsequently updated at regular intervals in a demographic surveillance system that continued through the vaccine trial. A close-out census was conducted at the end of surveillance, two years after vaccination. The study design was a cluster-randomized trial (CRT). Residents aged 2 years and older in 80 clusters were randomly allocated at a one-to-one ratio to single-dose Vi-

vaccine (Typherix®, GlaxoSmithKline) or inactivated hepatitis A (HepA) vaccine (Havrix®, GlaxoSmithKline). Typhoid fever surveillance was carried out in the study population during a 2-year lead-in period before vaccination and continued for two years following vaccination. Subjects from the study area who presented at one of five study clinics with a history of fever for at least three days were enrolled by a study physician. Subjects' clinical histories and physical findings were systematically captured during enrolment. After obtaining verbal informed consent, a 5-ml blood specimen was collected by venepuncture, and blood was examined by microbiological culture using an automated system (Becton Dickinson, New Jersey, USA) and bacterial identification through conventional biochemical and serologic methods (78). A total of 18 869 participants received Vi-vaccine and 18 804 received HepA vaccine. After two years of surveillance, the total Vi-vaccine protection against typhoid fever (comparing the incidence of typhoid in Vi vaccinees versus HepA vaccinees) was 61%; indirect protection (comparing the incidence of typhoid in non-vaccinees in the Vi clusters versus non-vaccinees in the HepA clusters) was 44%; and overall protection (comparing the incidence of typhoid in all members of the Vi clusters versus all members of the HepA clusters) was 57% (77).

1.8 Overview of the following chapters

In Chapter 2 and 3 I present a reanalysis of the OCV Matlab trial and the ViPS Kolkata trial using a vaccine probe approach. In the OCV study, I assessed the protective impact of whole-cell-only OCV against acute watery diarrhoea that was culture-negative for cholera, the hypothesis being, if culture diagnostics for

OCV are insensitive, there should be a measurable degree of protection against culture-negative AWD (Chapter 2). Subsequently, I employed the typhoid ViPS vaccine as a vaccine probe to delineate criteria for culture negative typhoid fever, relying on the assumption that if culture diagnostics for typhoid are roughly 50% sensitive, then a clinical profile associated with typhoid should be present in approximately equivalent numbers in both culture positive and negative patients. To validate this assumption, I assessed whether culture-negative clinical typhoid cases were susceptible to protection by ViPS vaccine (Chapter 3).

Recurrent episodes of typhoid fever can occur in individuals, particularly those living in typhoid endemic regions. Natural typhoid infection should, in theory, offer a degree of protection against future typhoid, however, this protection has only been empirically evaluated in a limited number of studies, none of which were conducted recently nor in the context of a carefully controlled clinical trial. In Chapter 4, I evaluate evidence for protection conferred by a confirmed typhoid episode against subsequent typhoid in a typhoid endemic population by observing the frequency of recurrent typhoid episodes in a cohort. In Chapter 5 and 6, I evaluate the relative risk of typhoid associated with residence in a household with characteristics of poor WASH as well as the impact on typhoid risk associated with living in a neighbourhood with varying prevalence of households with poor WASH. As WASH intervention is recommended alongside typhoid vaccination, I also evaluate if characteristics of household-level WASH modify the protective effects of an effective typhoid vaccine.

Chapter 2 Use of oral cholera vaccine as a vaccine probe to determine the burden of culture-negative cholera

The work described in this chapter has been published during the course of my DPhil in *PLOS Neglected Tropical Diseases*.

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doi:10.1371/journal.pntd.0007179

Abstract

Analyses of stool from patients with acute watery diarrhoea (AWD) using sensitive molecular diagnostics have challenged whether faecal microbiological cultures have sufficiently high sensitivity for cholera diagnosis. If true, these findings imply that current estimates of the global burden of cholera, which rely largely on culture-confirmation, may be underestimates. A vaccine probe study was conducted to evaluate this possibility, assessing whether an effective killed OCV tested in a field trial in a cholera-endemic population conferred protection against cholera culture-negative AWD, with the assumption that if cultures are indeed insensitive, OCV protection in such cases should be detectable. I re-analysed the data of a Phase III individually-randomized placebo-controlled efficacy trial of killed OCVs conducted in Matlab, Bangladesh in 1985. The protective efficacy (PE) of a killed whole cell-only (WC-only) OCV against first-episodes of cholera culture-negative AWD was calculated during two years of

post-dosing follow-up. In secondary analyses, PE against cholera culture-negative AWD by age at vaccination, season of onset, and disease severity was evaluated. In this trial 50 770 people received at least 2 complete doses of either WC-only OCV or placebo, and 791 first episodes of AWD were reported during the follow-up period, of which 365 were culture-positive for *Vibrio cholerae* 01. Of the 426 culture-negative AWD episodes, 215 occurred in the WC group and 211 occurred in the placebo group. The adjusted PE was -1.7% (95% CI: -23.0, 13.9, p=0.859). No measurable PE of OCV was observed against all or severe cholera culture-negative AWD when measured overall or by age and season subgroups. These results suggest that faecal cultures from patients with AWD were highly sensitive for cholera episodes that were etiologically attributable to this pathogen.

2.1 Introduction

An estimated 2.9 million cases and 95 000 deaths occur each year due to cholera, caused primarily by *V. cholerae* O1, in endemic countries (15). Until now, microbiological culture of stool has provided an accepted gold standard for diagnosing cholera in patients with diarrhoea. These cultures, particularly when pre-treated with alkaline peptone water overnight enrichment, have been regarded as having very high diagnostic sensitivity, as well as high diagnostic specificity. However, one influential paper produced by researchers at the icddr,b has questioned the notion that conventional faecal cultures have high sensitivity in diagnosing cholera by suggesting that cultures miss an appreciable fraction of cholera cases (20).

A study conducted in Dhaka, Bangladesh, where cholera is endemic, reported that conventional cultures identified *V. cholerae* 01 in stool in only 86 (66%) of 131 patients with clinically suspected cholera identified during a seasonal cholera outbreak who were diagnosed as cholera positive by at least one of a panel of alternative diagnostic tests, consisting of culture, multiplex polymerase chain reaction (PCR), and direct florescent antibody tests (20). The authors postulated that periodic failure of culture methods to isolate *V. cholerae* from stool may be caused by bacterial inactivation by *in vivo* vibriolytic action of the phages and/or prevention by host-induced mechanisms. One implication of this conclusion is that the current estimates of cholera disease burden in local settings as well as globally, that are based on culture-confirmed cholera, would effectively be significant underestimates, and that a reassessment of past and recent cholera studies may be needed to improve public health policy on cholera control measures in countries affected by the disease and to provide a more accurate depiction of the effectiveness of vaccines against cholera.

It can then be reasoned that if conventional faecal cultures for cholera do indeed have only moderate diagnostic sensitivity, and if culture-negative cholera represented an appreciable fraction of cases of AWD, the clinical syndrome of cholera, inactivated OCVs, which are effective against culture-confirmed cholera (51, 74, 79, 80), should also exhibit detectable efficacy against cholera culture-negative AWD. In this sense, OCVs could be used as a probe to evaluate the hypothesis that conventional diarrhoeal cultures are an insensitive tool for the diagnosis of cholera. The analyses of OCV field trials can be used as a probe

not only to better define the burden of cholera and the overall impact of OCVs, but also to delineate clinical criteria for culture negative cholera cases.

In order to evaluate this possibility, I reanalysed a Phase III efficacy trial of inactivated OCVs in Matlab, Bangladesh to assess whether conventional cultures do indeed under-ascertain disease burden of cholera, and if so, by how much.

2.2. Methods

2.3.1 Trial and population

The original vaccine trial was conducted in rural Bangladesh at Matlab, where icddr,b has been maintaining a field research centre since 1963. The cumulative protective efficacy of the completed three-dose regimen in all age groups was 50% for BS-WC and 52% for WC-only after three years of follow-up. Protection was notably higher in persons older than 5 years (63%) compared to children 2-5 years (26%) for BS-WC as well as for WC-only (68% versus 23%) (76).

2.3.2 Analytical approach

In order to evaluate whether the use of conventional faecal cultures to define cholera underestimated the true incidence of cholera in the Matlab trial, I evaluated vaccine-associated protection of at least two complete doses of the WC-only OCV against AWD that was culture-negative for cholera. Protection

conferred by two doses (cumulative three-year PE=39%) was found to be similar to protection conferred by three doses (cumulative three-year PE=52%), therefore two-dose recipients were included in the analysis.

One necessary assumption for this vaccine probe analytical approach is that the protection by this vaccine against culture-negative cholera is equivalent to the vaccine's protection against culture-positive cholera. The subsequent analysis comparing protection of the vaccine against two different clinical outcomes, different only by the isolation of vibrios from stool sample by microbiological culture, is predicated on this assumption. As such, I did not evaluate protection by BS-WC in this analysis because this vaccine, in contrast to WC-only vaccine, cross-protects against heat labile toxin (LT)- producing enterotoxigenic *Escherichia coli* diarrhoea, a cause of AWD for which conventional cholera cultures detecting cholera would be negative (81). The post-vaccination follow-up selected for this analysis was two years, an interval in which the WC-only OCV demonstrated considerable protective efficacy against culture-confirmed cholera (74, 81).

A diarrhoea visit was defined as a patient visit to the study health centre in which the patient reported three or more loose or liquid stools or one to two or an indeterminate number of loose stools with at least two objective signs of dehydration on initial physical examination. Signs of dehydration included feeble or absent pulse, tenting of skin, sunken eyes, or dry mucous membranes. Diarrhoeal visits were concatenated into diarrhoeal episodes when the date of onset of symptoms for one visit was seven or fewer days after the date of

discharge for the previous visit. The onset of an episode was the onset of first component visit of the episode. AWD episodes were diarrhoeal episodes for which no stool with visible blood was reported. Cholera culture-negative AWD episodes were those for which *V. cholerae* O1 was not isolated from any faecal culture. Cholera culture-positive AWD episodes required at least one culture during the episode that was positive for *V. cholerae* O1. Such episodes were considered to be severe if, at the time of any of the component visits for treatment, an absent or feeble pulse was noted and at least one additional objective sign of dehydration (listed above) was observed and documented.

In the vaccine probe analysis, I measured the difference in the disease incidence of cholera culture-negative AWD in WC-only OCV recipients compared to placebo recipients. In the primary analysis, the overall occurrence of first episodes of cholera culture-negative AWD was compared between subjects who had received at least two complete doses of inactivated WC-only vaccine or placebo, as earlier analyses had demonstrated PE to be equivalent for recipients of two and three doses and the vaccine was demonstrably protective against cholera during this follow-up interval (74). In secondary analyses, I evaluated vaccine protection against cholera culture-positive AWD and cholera culture-negative AWD stratified separately by age at vaccination, season of onset, and disease severity. In these analyses, which were undertaken to address the possibility that vaccine protection might be unmasked when analysed for the older age group, during the cholera season, or against severe cholera, age was categorized as under five years versus five years and older, seasonality was classified as cholera season (April-May and October-

November) versus other, and cholera was classified as severe or non-severe, as defined earlier. The age group cut-off was selected based on the age strata assessed in the original trial and cholera season was determined based on discussion with authors of the original trial report who were part of the icddr,b team conducting the trial and who had extensive experience conducting cholera surveillance in the study site.

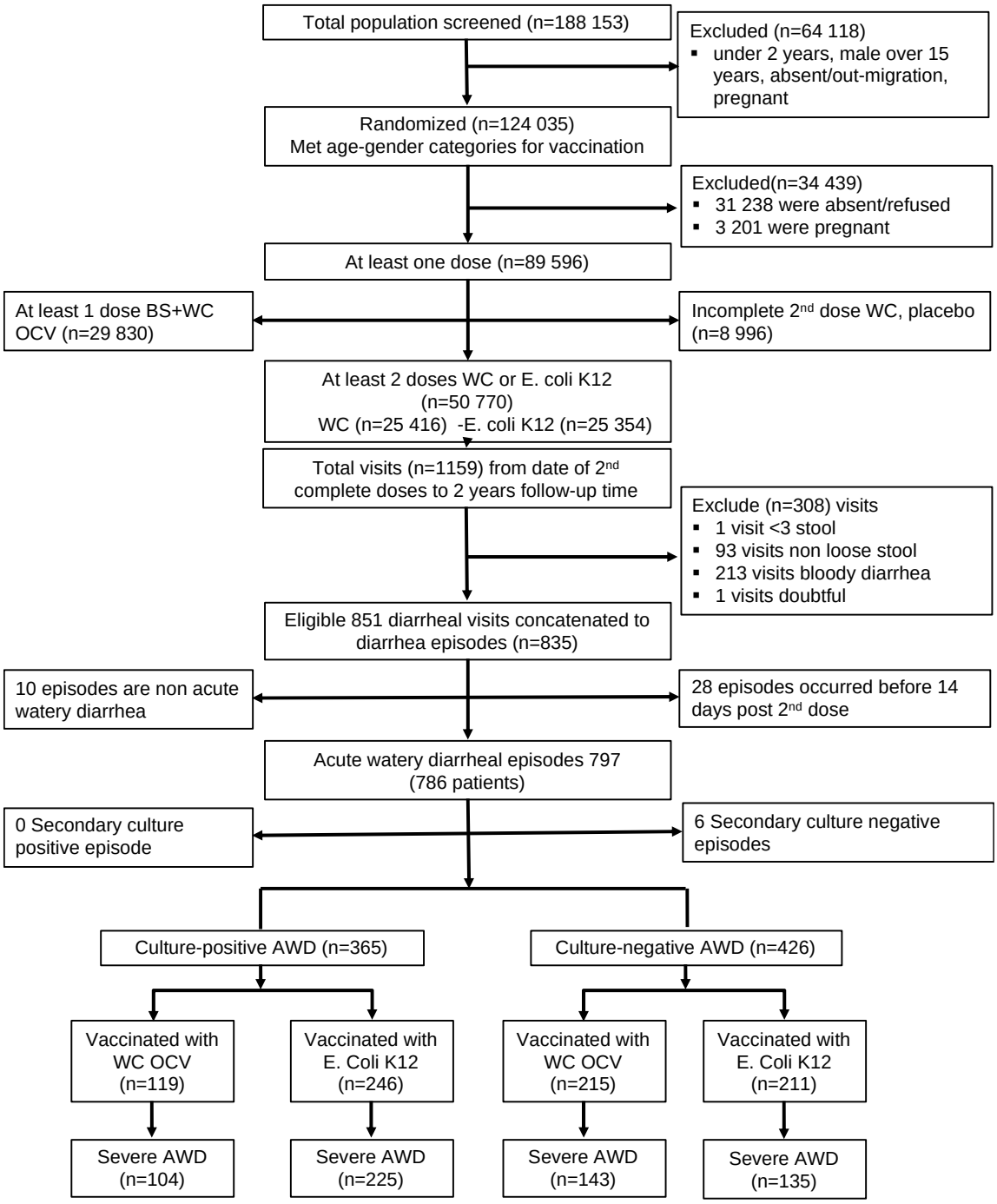
Vaccine PE was measured against first episodes of cholera culture-positive and cholera culture-negative AWD in Cox proportional hazard regression models, in which time to event was measured in relation to receipt of the second dose. Deaths, out-migrations, and study completion 730 days after the second dose were right-censoring events. In the analysis of cholera seasonality, the events that occurred during the cholera season were counted in the numerator and the events that occurred outside of the cholera season were censored at the time of event. In these models, vaccination was expressed dichotomously as vaccine versus placebo. The models were controlled for potentially confounding variables; the variables selected were independently associated with time to event at p-value <0.10 (two-tailed) in a backward selection algorithm. To evaluate heterogeneity of vaccine protection among different subgroups (age <5 and ≥5 years), interaction terms between vaccination and subgroup variables in these models were evaluated. Before including any variable as an independent variable in the model, it was first determined whether the proportional hazard assumption was fulfilled for the variable. There was no violation of the assumption for variables included in this model. The hazard ratio (HR) for the outcome was estimated by exponentiating the coefficient for the vaccination

variable in the model; the 95% confidence interval for the HR was estimated using the standard error of the coefficient. The margin of statistical significance was considered as $p<0.05$ (two-tailed).

2.4 Results

In this trial 25 416 individuals were vaccinated with at least two doses of WC-only OCV, and 25 354 received at least two doses of placebo. Of the 50 770 people vaccinated with either WC-only OCV or placebo, 791 first episodes of AWD from 786 patients during two years of follow-up among which 365 were culture-positive for *V. cholerae* O1. Of the remaining 426 first episodes of culture-negative AWD, 215 (51%) occurred in recipients vaccinated with WC and 211 (50%) occurred in placebo recipients (Figure 2-1). A majority of the cases, 373 (88%), occurred in individuals ≥5 years of age. The proportion of severe AWD occurring among culture-positive AWD was 309/365 (85%) and the proportion of severe AWD occurring among culture-negative AWD was 278/426 (65%).

Figure 2-1 CONSORT diagram showing cholera culture-negative AWD episodes in trial participants through 2 years of follow-up



The overall occurrence of cholera culture-negative AWD did not differ statistically between vaccinees and placebo recipients (adjusted PE= -1.7%; 95% CI: -23.0, 15.9, p=0.859) (Table 2-1). In contrast, the occurrence of cholera

culture-confirmed AWD differed significantly between the two groups (adjusted PE= 51.7; 95% CI: 39.9, 61.2, p<0.001). When evaluating the incidence of cholera culture-negative AWD stratified by age subgroups, vaccine protection was not detected, adjusted PE=-47.9 (95% CI: -156.4, 14.7, p=0.164) for <5 years and adjusted PE=3.6 (95% CI: -18.0, 21.3, p=0.720) for ≥5 years, nor was it detected when evaluated in the cholera season only, adjusted PE=12.2 (95% CI: -18.3, 34.9, p=0.393) (Table 2-1). Finally, comparison of the incidence of severe cholera culture-negative AWD in vaccinees versus placebo recipients failed to detect protective efficacy of the OCV among all individuals (adjusted PE = -5.9%; 95%CI: -34.0, 16.3%, p=0.633), in different age subgroups, and in the cholera season (Table 2-1). To examine the possibility that specificity of cholera culture is different during the cholera and non-cholera season, a secondary analysis of children ≥5 years, for whom OCV was protective, during the cholera season was conducted. Protection was also not detected for AWD or severe AWD under these conditions (Table 2-1).

Table 2-1Incidence rates and protective effect of cholera culture-negative and -positive AWD and culture-negative severe AWD between vaccine and placebo groups stratified by age group and cholera season

	WC OCV				*E. coli* K12				Protective effect (PE)			
	n	Cases	Person-days	IR per 10^5 person-days (95% CI)	n	Cases	Person-days	IR per 10^5 person-days (95% CI)	Crude PE (95% CI)	p-value	Adj.* PE (95% CI)	p-value
Cholera negative AWD												
All ages	25416	215	18459505	1.2 (1.0, 1.3)	25354	211	18409762	1.2 (1.0, 1.3)	-1.6 (-22.9, 16.0)	0.868	-1.7 (-23.0, 15.9)	0.859
<5 years	2864	32	2071704	1.5 (1.1, 2.2)	2772	21	2011696	1.0 (0.7, 1.6)	-47.9 (-156.4, 14.7)	0.164	-47.9 (-156.4, 14.7)	0.164
≥5 years	22552	183	16387801	1.1 (1.0, 1.3)	22582	190	16398066	1.2 (1.0, 1.3)	3.6 (-18.1, 21.3)	0.722	3.6 (-18.0, 21.3)	0.720
Cholera season†	25416	81	18459505	0.4 (0.4, 0.6)	25354	92	18409762	0.5 (0.4, 0.6)	12.2 (-18.4, 34.9)	0.394	12.2 (-18.3, 34.9)	0.393
≥5 years and cholera season†	22552	71	16387801	0.4 (0.3, 0.6)	22582	83	16398066	0.5 (0.4, 0.6)	14.4 (-17.5,37.6)	0.337	14.4 (-17.5,37.7)	0.336

(Table 2-1 continued on next page)

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	WC OCV				*E. coli* K12				Protective effect (PE)			
	n	Cases	Person-days	IR per 10^5 person-days (95% CI)	n	Cases	Person-days	IR per 10^5 person-days (95% CI)	Crude PE (95% CI)	p-value	Adj.* PE (95% CI)	p-value
Cholera negative severe AWD												
All ages	25416	143	18490342	0.8 (0.7, 0.9)	25354	135	18449112	0.7 (0.6, 0.9)	-5.7 (-33.7, 16.5)	0.645	-5.9 (-34.0, 16.3)	0.633
<5 years	2864	11	2080386	0.5 (0.3, 1.0)	2772	9	2018038	0.5 (0.2, 0.9)	-18.6 (-186.1, 50.9)	0.705	-21.1 (-192.2, 49.8)	0.670
≥5 years	22552	132	16409956	0.80 (0.7, 1.0)	22582	126	16431074	0.8 (0.6, 0.9)	-4.9 (-33.9, 17.8)	0.701	-4.5 (-33.4, 18.1)	0.721
Cholera season†	25416	49	18490342	0.3 (0.2, 0.4)	25354	52	18449112	0.3 (0.2, 0.4)	6.0 (-38.9, 36.4)	0.757	6.1 (-38.7, 36.4)	0.752
≥5 years and cholera season†	22552	46	16409956	0.3 (0.2, 0.4)	22582	50	16431074	0.30 (0.23,0.40)	7.9 (-37.5,38.3)	0.688	8.1 (-37.1,38.5)	0.678

(Table 2-1 continued on next page)

(Table 2-1 continued from previous page)

	WC OCV				*E. coli* K12				Protective effect (PE)			
	n	Cases	Person-days	IR per 10^5 person-days (95% CI)	n	Cases	Person-days	IR per 10^5 person-days (95% CI)	Crude PE (95% CI)	p-value	Adj.* PE (95% CI)	p-value
Cholera positive AWD												
All ages	25416	119	18497878	0.6 (0.5, 0.8)	25354	246	18416140	1.3 (1.2, 1.5)	51.8 (40.1, 61.3)	<0.001	51.7 (39.9, 61.2)	<0.001
<5 years	2864	48	2066164	2.3 (1.7, 3.1)	2772	49	2004104	2.4 (1.8, 3.3)	5.0 (-41.5, 36.2)	0.802	4.6 (-42.1, 35.9)	0.818
≥5 years	22552	71	16431714	0.4 (0.3, 0.6)	22582	197	16412036	1.2 (1.0, 1.4)	64.0 (52.8, 72.6)	<0.001	63.9 (52.6, 72.5)	<0.001
Cholera season†	25416	42	18497878	0.2 (0.2, 0.3)	25354	103	18416140	0.6 (0.5, 0.7)	59.4 (41.9, 71.6)	<0.001	59.2 (41.6, 71.5)	<0.001

WC: Killed whole cell-only vaccine; OCV: oral cholera vaccine; AWD: acute watery diarrhoea,

CI: confidence interval

*All ages models are adjusted by religion, age, and sex; age-specific models are adjusted by religion and sex

†Cholera season defined as April-May and October-November

2.5 Discussion

I used OCV as a vaccine probe to evaluate whether there was an undetected burden of culture-negative cholera diarrhoea, which was otherwise compatible with culture-positive cholera diarrhoea, in a cholera endemic setting in Bangladesh. During the first two years of follow-up in a placebo-controlled, randomized trial in Matlab, this analysis failed to detect OCV protection against all episodes of cholera culture-negative AWD when evaluated overall, by age groups, for cholera season, or by disease severity. In contrast, the analyses showed 51.7% PE against culture confirmed cholera in patients with AWD during the same interval of follow-up. As previously postulated (20), if there was an appreciable fraction of true cholera cases that were not detected by conventional stool culture techniques, it would be expected that it would be possible to measure protection of OCV against these misclassified cholera cases.

There are several important limitations that must be considered when interpreting these findings. Only patients with diarrhoea severe enough to seek care at a health facility were captured in the surveillance and included in the analysis, thus these findings may not pertain to less severe cases of diarrhoea. Diarrhoea is common, particularly in the slum setting under investigation. Although cholera is recognized within this population as a deadly and preventable illness, factors impacting the decision of caretakers to seek health care, including knowledge or education, financial barriers, distance to healthcare facility, or reliance on alternative sources of health care exist. The availability

and encouraged use of oral rehydration therapy (ORT) at home, a campaign that the icddr,b was actively participating in throughout Bangladesh between 1980 and 1990, may have enabled management of mild cholera AWD at home (82). However, since assignment of the interventions was random in this study, systematic differences in healthcare seeking behaviour between intervention groups contributing to confounding of outcomes is unlikely. Nonetheless, if the sensitivity of a cholera culture was associated with increasing severity of cholera disease, then exclusion of mild forms of cholera in this analysis would result in overestimates of cholera culture sensitivity and increase the likelihood of detecting protection against culture negative AWD.

The study was conducted in a cholera endemic region where it is likely that prior exposure to *V. cholerae* conferred a degree of pre-existing natural immunity to cholera in both OCV and placebo groups, potentially diminishing the measurable effect size of vaccine protection against culture-positive and culture-negative cholera in this population. However, in this analysis there was strong evidence for OCV protection against culture-positive cholera, although the estimate of protection may have been conservative due to existing immunity in the population. Moreover, one would expect natural immunity to reduce the faecal shedding of ingested cholera vibrios, which would tend to increase rather than decrease the diagnostic sensitivity of conventional cultures.

Further, this analysis was performed on data collected over 3 decades ago which calls into question present day generalizability. At the time of the trial, the prevailing circulating strains were both El Tor and classical biotypes (83). Since

then, variants of the El Tor biotype have emerged and become predominant in Bangladesh and many other cholera-endemic areas (84). Similarly, the population under study was in a cholera endemic setting where cholera culture was performed systematically, therefore the results of this study cannot be generalized for populations lacking pre-existing natural immunity to cholera and where cholera culture diagnostics are not routine.

Finally, the validity of these conclusions hinges on the assumption that the OCV under study was protective against culture-positive and culture-negative cholera. While there is no reason to doubt this assumption, there is no direct evidence to support it.

On the other hand, the study had several strengths. The data were obtained from a prospective, placebo-controlled, individually randomized trial with comprehensive and systematic surveillance for all episodes of diarrhoea in the study population. Faecal cultures were conducted at a well-established high-calibre diagnostic laboratory with technical support from the icddr,b, a global leader in research related to diarrhoeal disease. Importantly, Matlab has a well-functioning demographic surveillance system, and patients in this study were accurately identified when they presented for care at the heath facilities. This analysis only included registered individuals in the Matlab demographic surveillance who had verifiably ingested vaccine or placebo, and therefore the probability of intervention misclassification was low. Note that non-differential misclassification of patient identities would have acted to diminish measured

vaccine PE. Also arguing against such misclassification was the concurrent demonstration of vaccine PE against culture-proven cholera.

Finally, this analysis was adequately powered to detect vaccine protection in cholera culture-negative AWD. As shown in Figure 2-1, the surveillance detected a total of 791 first episodes of AWD, of which 365 were culture-positive for cholera and 426 were culture-negative. If, as reported by Alam et al., the diagnostic sensitivity of conventional faecal cultures for cholera is 66%, we would expect 189 (44%) of the 426 culture-negative AWD cases to be cholera. For an OCV that was 51.7% protective against cholera in the same setting and for the same duration of follow-up, a level of OCV protection against culture-negative AWD of 30% would have been detected. However, the upper boundary of the 95% confidence interval for measured PE in the primary analysis (16%) excluded this value.

This vaccine probe study was designed to evaluate whether there was an appreciable fraction of cholera culture-negative cases of AWD in which patient symptoms could be aetiologically attributed to infection by *V. cholerae* O1. It is well documented that isolation of cholera vibrios from faecal specimens may not be sufficient per se to incriminate the isolated vibrios as the cause of the patient's diarrhoea (85). In the earlier Bangladesh study that reported low diagnostic sensitivity of conventional microbiological cultures, diagnoses of cholera were based on a panel of multiple diagnostic tests, including very sensitive molecular methods (20). It is possible that some of cholera culture-negative cholera cases designated as cholera by the alternative tests in this

study were due to false positive isolations. However, it is also possible that in many of the cases where faecal shedding of *V. cholerae* O1 was detected, vibrios may have been present but not the aetiology of symptoms.

The contention that conventional culture methods do not capture all cholera cases has implications for cholera global burden estimates, which are already thought to be an underestimation due to the incomplete diagnostic testing and reporting of cholera cases in many settings. However, it is important to consider that identification of *V. cholerae* O1 in the stool does not always confirm the aetiologic role of the isolated organisms in causing a patient's diarrhoeal symptoms, and that sophisticated diagnostic technologies, in some cases, may overstate the fraction of diarrhoeal disease caused by cholera. While the findings of this study support the use of conventional faecal cultures to diagnose cholera, contemporary studies in endemic as well as non-endemic settings are needed to examine the validity of these findings.

Chapter 3 Developing a clinical definition for typhoid fever

The work described in this chapter has been published in The *American Journal of Tropical Medicine and Hygiene*.

Islam MT, Im J, Ahmmed F, et al. Use of Typhoid Vi-Polysaccharide Vaccine as a Vaccine Probe to Delineate Clinical Criteria for Typhoid Fever. Am J Trop Med Hyg. 2020;103(2):665-671. doi:10.4269/ajtmh.19-0968

Abstract

Blood cultures detect only 50% of typhoid fever cases. There is need for validated clinical criteria to define cases that are blood culture-negative, both to help direct empiric antibiotic treatment and to better evaluate the magnitude of protection conferred by typhoid vaccines. To derive and validate a clinical rule for defining blood culture (BC)-negative typhoid fever, in a cluster-randomized effectiveness trial of ViPS typhoid vaccine in Kolkata, India, 14 797 episodes of fever lasting at least 3 days during 4 years of comprehensive, BC-based surveillance of 70 865 persons were assessed. A recursive partitioning algorithm was used to develop a decision rule to predict BC-proven typhoid cases with a diagnostic specificity of between 97% to 98%. To validate this rule as a definition for BC-negative typhoid fever, a vaccine probe analysis was utilized and ViPS vaccine-associated protection against the defined culture-negative syndromes was measured. In a training subset of individuals, 2 rules were generated for identifying clinical typhoid: Rule-1: patients aged < 15 years with prolonged fever accompanied by a measured body temperature ≥ 100°F,

headache, and nausea; Rule-2: patients aged ≥ 15 years with prolonged fever accompanied by nausea, palpable liver, but without constipation. The adjusted protective efficacy of ViPS against clinical typhoid defined by these rules in persons ≥ 2 years of age in a separate validation subset was 33% (95% CI: 4%-53%). This study demonstrates that by using recursive partioning decision tree modelling and a novel vaccine probe design, a validated clinical rule for predicting BC-negative typhoid fever can been developed. If validated in other settings, this rule may be useful to guide clinical care and to enhance typhoid vaccine evaluations.

3.1 Introduction

Typhoid fever is a poverty-related disease with a high disease burden estimated at 14.3 million cases and approximately 200 000 deaths per year globally (22). Typhoid disproportionately affects low- and middle-income countries (28, 86). Unfortunately, the standard diagnostic test, microbiological culture of venous blood, is only about 50% sensitive in detecting typhoid fever (31, 87-89). This diagnostic is frequently unavailable in LMICs, and where they are implemented, there are myriad issues impacting standards of quality. Moreover, there are no alternative reliable point-of-care diagnostic tests nor well validated rules for defining blood culture-negative typhoid using combinations of presenting clinical signs and symptoms to guide empiric antibiotic therapy. The lack of a highly sensitive diagnostic also impacts on the ability to evaluate the preventive impact of typhoid vaccines in clinical studies (90, 91). The need for more selective targeting of patients for antibiotic therapy is underscored by growing antibiotic resistance and the need for antibiotic stewardship (92-94). Similarly, the need for more comprehensive assessments of prevented typhoid cases is highlighted by the advent of improved typhoid vaccines now under clinical evaluation (95-98).

One of the challenges confronting development of non-BC based criteria for typhoid fever is the absence of a gold standard diagnostic test for such cases. For several other invasive bacterial diseases, including invasive Hib and *Streptococcus pneumonia*, vaccine probe studies have been used to define pathogen-attributable disease syndromes in which cultures of normally sterile

fluids are negative (70, 72, 99-101). The probe design, which relies on detecting vaccine efficacy against these culture-negative clinical syndromes, can produce an estimate of the aetiological fraction of a disease syndrome due to the pathogen targeted by vaccine, effectively overcoming challenges in diagnostic insensitivity (70). However, this approach only works when a clinical syndrome can be defined for which the proportion of culture negative disease caused by the target pathogen is sufficiently high. If low, measured vaccine protection will be “washed out” by cases not caused by the target pathogen.

In an earlier CRT of typhoid ViPS-vaccine it was possible to detect both direct and herd protection of Vi against BC-proven typhoid (70). However, it was not possible to detect significant Vi protection, either direct or herd, against BC-negative cases presenting with fever of at least three days duration, suggesting that more specific clinical criteria for defining the target syndrome than prolonged fever per se was needed. However, these clinical criteria were lacking. In this analysis, I present a novel approach for defining clinical criteria for culture-negative, clinical typhoid leveraging a vaccine probe approach within the earlier CRT of the ViPS vaccine.

3.2 Methods

3.2.1 Trial and population

The study was conducted in an urban slum site in Kolkata City, the capital of West Bengal, India. Typhoid fever surveillance was carried out in the study

population during a two-year lead-in period before vaccination and continued for two years following vaccination. Subjects from the study area who presented at one of five study clinics with a history of fever for at least three days were enrolled by a study physician. Subjects' clinical histories and physical findings were systematically captured during enrolment. After obtaining verbal informed consent, a 5-ml blood specimen was collected by venipuncture for microbiological culture using an automated system (Becton Dickinson) and bacterial identification through conventional biochemical and serologic methods (78). The CRT was initiated in 2004. Residents aged two years and older in eighty clusters were randomly allocated at a one-to-one ratio to Vi-vaccine (Typherix®, GlaxoSmithKline) or inactivated HepA vaccine (Havrix®, GlaxoSmithKline). A total of 18 869 participants received Vi-vaccine and 18 804 received HepA vaccine. After two years of surveillance, the total Vi-vaccine protection against typhoid fever (comparing the incidence of typhoid, defined as a febrile episode where typhoid was confirmed by blood culture, in Vi vaccinees versus HepA vaccinees) was 61%; indirect protection (comparing the incidence of typhoid in non-vaccinees in the Vi clusters versus non-vaccinees in the HepA clusters) and overall protection (comparing the incidence of typhoid in all members of the Vi clusters versus all members of the HepA clusters) were 44% and 57%, respectively (77).

3.2.2 Study design and analytical approach

For this analysis, I re-evaluated a CRT of ViPS vaccine launched in 2004, as well as two years of antecedent typhoid surveillance in the vaccine trial

population, in an urban slum in Kolkata, India (77). To derive an empirically validated clinical rule for BC-negative, clinical typhoid fever, there were three conditions that had to be met:

1) the clinical features for the rule should discriminate between episodes of prolonged fever that are BC-positive for S. Typhi and those that are negative for S. Typhi;
2) the diagnostic specificity of the rule should yield a number of BC-negative clinical typhoid episodes approximately equal to the number of BC-positive episodes in the same population (since BCs are known to be about 50% sensitive in detecting typhoid fever (102-106)); and
3) a rule meeting the first two conditions should identify BC-negative episodes against which there is detectable vaccine efficacy by ViPS vaccine in the CRT, thereby using ViPS vaccine as a probe.

To derive a clinical rule with the desired level of diagnostic specificity, I used recursive partitioning, a classification algorithm that considers candidate individual variables for the rule conjointly to arrive at a rule with desired levels of specificity and sensitivity (107). The main advantage of this approach over other machine learning classification algorithms is that it produces an easily visualized decision tree allowing for simplistic interpretation of subject classification.

All prolonged febrile episodes from the surveillance leading up to and during the cluster randomized trial were assembled for analysis. A febrile visit was defined as an individual visit by a study area resident to a study clinic presenting with a history of fever for at least 3 days. A febrile episode was defined as all component febrile visits in which the onset of symptoms for a subsequent visit

was less than 14 days from the date of discharge for the previous visit. A BC-proven typhoid episode was considered to be a febrile episode in which *S.* Typhi was isolated from at least one blood culture. For this analysis, where multiple fever visits comprised a febrile episode, the date of onset of illness was considered as the date of onset of symptoms for the first component visit of the episode. From these episodes, analysis included individuals for whom data for all clinical parameters under consideration were available (Figure 3-1). The clinical parameters under consideration are listed in Table 3-1.

3.2.3 Statistical analysis

I employed a machine learning algorithm to create a decision tree constructed via binary recursive partitioning (31). The decision tree was designed to predict whether a febrile episode was culture positive typhoid fever, using 22 clinical signs and symptoms and epidemiological characteristics recorded at the time of initial presentation for care for the episode. Each sign and symptom were first converted into a dichotomous variable. For the machine learning modelling we selected as candidate variables only those that had an association with typhoid with p-value ≤0.25 in bivariate logistic regression analyses.

Because predictive rules may overestimate predictive performance when tested on the data used to derive the rules, all individuals who resided in the study area during the four years of follow-up were split at random into two approximately equal sized groups for development (training data set) and validation (validation data set) of the clinical predictive rule, respectively. For development and testing

of the rule, all febrile episodes of all patients presenting with at least three days of fever in the training and validation groups for whom complete clinical data were available were considered. The recursive partitioning algorithm was initially run for candidate clinical predictive variables in the training set episodes, specifying an alternative loss function that penalized a false negative prediction 10 times more heavily than a false positive prediction. Due to unbalanced class data, the minimum number of observations was set as 30 for the non-terminal nodes of the decision tree and 10 for the terminal nodes of the decision tree. For cross-validation, the training set was randomly partitioned into ten parts, one part was used in estimating cross-validation error (32).

For these analyses of the training dataset, decision trees were constructed at several different levels, and the tree with the lowest level, and thus the simplest clinical algorithm, that still met the three performance criteria, specified above, was chosen. To find the optimal tree in the training set, the model was pruned by the minimal complexity parameter (CP) corresponding to minimum error with at least 5 tree rules. A Receiver Operating Characteristic (ROC) Curve was constructed for the selected rule, evaluating the area under the ROC curve as described elsewhere (108, 109) and the curve was also used to identify a cut-off probability that corresponded to the highest sensitivity for cut-offs giving 97% to 98% specificity. To measure the PE of ViPS vaccine against culture negative typhoid, ViPS vaccine recipients were contrasted with HepA vaccine recipients for the occurrence of all episodes of culture-negative fever meeting the criteria of the clinical prediction rule, using the Andersen-Gill (AG) generalized Cox proportional hazard regression model after verifying that the proportionality

assumptions were fulfilled (35). The model was adjusted for age of the individual and living in a household that used a designated place for waste disposal. The HR for culture negative typhoid fever was estimated by exponentiation of the coefficient of receipt of ViPS in the model, and the 95% confidence interval (CI) for the HR was estimated using a robust sandwich method. The PE of ViPS vaccination against BC-negative typhoid fever defined by our clinical rule was estimated as [1-HR]×100%. The margin of statistical significance was considered to be $p<.05$ (two-tailed). The analysis was performed using “rpart” package for decision tree modelling, “caTools” for random selection, “rpart.plot” package for tree plotting, “pROC” packages for ROC curve (36), “survival” for AG Cox model (37) and “dplyr” package for data management under R-Studio (38). Once having conducted these developmental analyses in the training data set, the sensitivity and specificity of the rule in predicting BC-positive typhoid fever and in defining a BC-negative syndrome against which ViPS protected was assessed in the validation dataset.

3.3 Results

From March 2003 to January 2007 a cumulative population of 70 865 individuals was included in the surveillance. During vaccination, which took place between November and December 2004, 67 163 age-eligible individuals were randomized to receive either ViPS vaccine or HepA vaccine (Figure 3-1). In total, 18 869 individuals received the ViPS vaccine and 18 804 individuals received the HepA vaccine. Total protection by ViPS against culture-confirmed typhoid was 61% (95% CI: 41, 75; $p<0.001$) (23). A total of 14 905 febrile visits,

comprising 14 797 fever episodes, in 11 255 individuals were observed between 19 March 2003 and 28 January 2007, from which 323 BC-proven typhoid cases were recorded. For analysis, 515 fever episodes, including nine typhoid cases, were excluded due to incompatibility with fever criterion (i.e. fever duration less than 3 days) or incomplete clinical metadata (Figure 3-1). In the analysis, 14 282 fever episodes and 314 BC proven typhoid cases were included. From bivariate logistic regression analysis, 17 of 22 recorded clinical and epidemiological features had a significant association ($p \leq 0.25$) with confirmed typhoid. These include: age <15 years, typhoid season (April to June and September to November), high temperature (≥100°F by axillary measurement), abdominal pain, constipation, dehydration, diarrhoea, abdominal distension, drowsiness, headache, nausea, palpable liver, palpable spleen, abdominal tenderness, heart rate >80/ minute, thirst and vomiting (Table 3-1). BC-confirmed typhoid was not observed among individuals exhibiting abdominal peritoneal signs or seizure.

Figure 3-1 CONSORT diagram showing culture confirmed typhoid fever in ViPS and HepA clusters

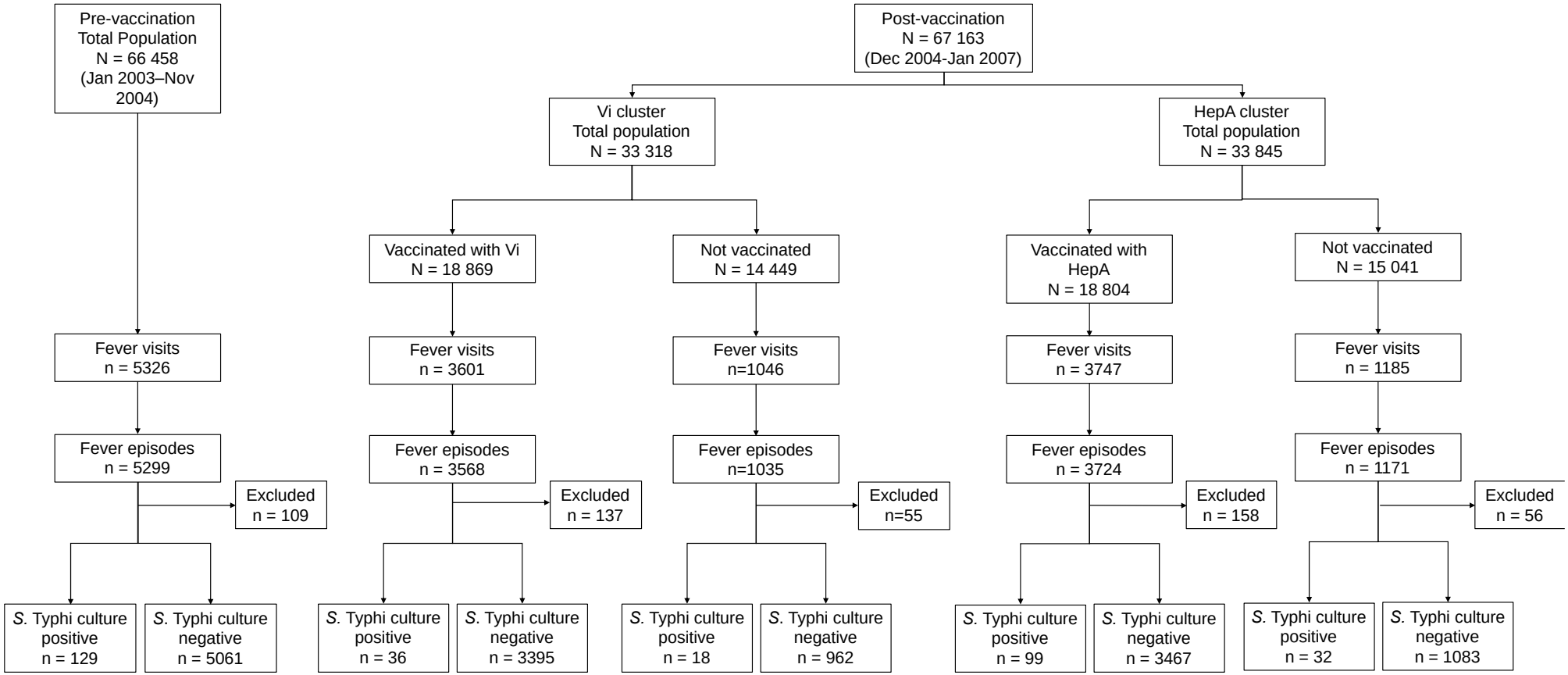


Table 3-1 Bivariate relationships† between candidate clinical and epidemiological features and typhoid fever

Feature	Culture-negative fever episode (%) n=13 968	Culture-positive typhoid fever episode (%) n=314	p-value
Age (<15 years)‡	4742 (33.9)	191 (60.8)	<0.001
Gender (male)	7180 (52.1)	159 (51.8)	0.919
Typhoid season§	7573 (54.2)	201 (64.0)	0.001
Fever duration (>7 days)‖	1238 (8.9)	28 (8.9)	0.973
High temperature (≥100°F)¶	7012 (50.2)	205 (65.3)	<0.001
Abdominal pain	1711 (12.2)	71 (22.6)	<0.001
Constipation	1752 (12.5)	47 (15.0)	0.201
Dehydration	13 134 (94)	284 (90.4)	0.009
Diarrhoea	374 (2.7)	12 (3.8)	0.219
Abdominal distension	709 (5.1)	24 (7.6)	0.043
Drowsiness	110 (0.8)	8 (2.5)	0.001
Headache	8015 (57.4)	195 (62.1)	0.095
Ileus sign	5 (0)	0 (0)	0.967
Nausea	3312 (23.7)	95 (30.3)	0.007
Palpable liver	577 (4.1)	22 (7)	0.013
Peritoneal signs	6 (0)	0 (0)	0.964
Seizure	19 (0.1)	0 (0)	0.972
Palpable spleen	267 (1.9)	9 (2.9)	0.228
Abdominal tenderness	1079 (7.7)	36 (11.5)	0.015
Heart rate >80 bpm	12 054 (86.3)	299 (95.2)	<0.001
Thirsty	3455 (24.7)	117 (37.3)	<0.001
Vomiting	1341 (9.6)	42 (13.4)	0.026

†Assessed with logistic regression

‡Age was considered at date of fever onset

§Typhoid seasons defined as April to June and September to November

‖By reported clinical history

¶Axillary, oral, or rectal temperature measurement

Assembly of training and validation datasets

In the training dataset, 7195 febrile episodes occurring in 5462 individuals were randomly selected. Among the episodes occurring after vaccination, 1748 episodes were reported in ViPS recipients and 1799 episodes were reported in HepA vaccine recipients. In the validation dataset, 7087 febrile episodes occurring in 5462 individuals were randomly selected, of which, following vaccination, 1683 episodes were reported in ViPS recipients and 1767 episodes were reported in HepA vaccine recipients (Table 3-2).

Table 3-2 Population assembly for training and validation sets

			Population	Full study period		Before vaccination		After vaccination	
			N	Fever episodes (subjects)	Typhoid cases	Fever episodes	Typhoid cases	Fever episodes	Typhoid cases
Full Set	**Before vaccination**		3702	102	4	102	4		
	Vi Cluster	Vaccinated	18869	5438	91	2007	55	3431	36
		Not vaccinated	14449	1459	26	479	8	980	18
	HepA Cluster	HepA vaccinated	18804	5639	147	2073	48	3566	99
		Not vaccinated	15041	1644	46	529	14	1115	32
		Total	70865	14282 (10924)	314	5190	129	9092	185
Training Set	**Before vaccination**		1851	56	2	56	2		
	Vi Cluster	Vaccinated	9436	2752	41	1004	21	1748	20
		Not vaccinated	7197	732	11	217	4	515	7
	HepA Cluster	HepA vaccinated	9404	2846	77	1047	31	1799	46
		Not vaccinated	7540	809	26	257	11	552	15
		Total	35428	7195 (5462)	157	2581	69	4614	88
Validation Set	**Before vaccination**		1851	46	2	46	2		
	Vi Cluster	Vi vaccinated	9433	2686	50	1003	34	1683	16
		Not vaccinated	7252	707	13	262	4	465	11

HepA Cluster	HepA vaccinated	9400	2793	70	1026	17	1767	53
	Not vaccinated	7501	816	20	272	3	563	17
	Total	35437	7087 (5462)	157	2609	60	4478	97

Note: For randomized individuals who did not receive a vaccine, vaccine date was set as the median date of vaccination of vaccinees in the cluster of residence.

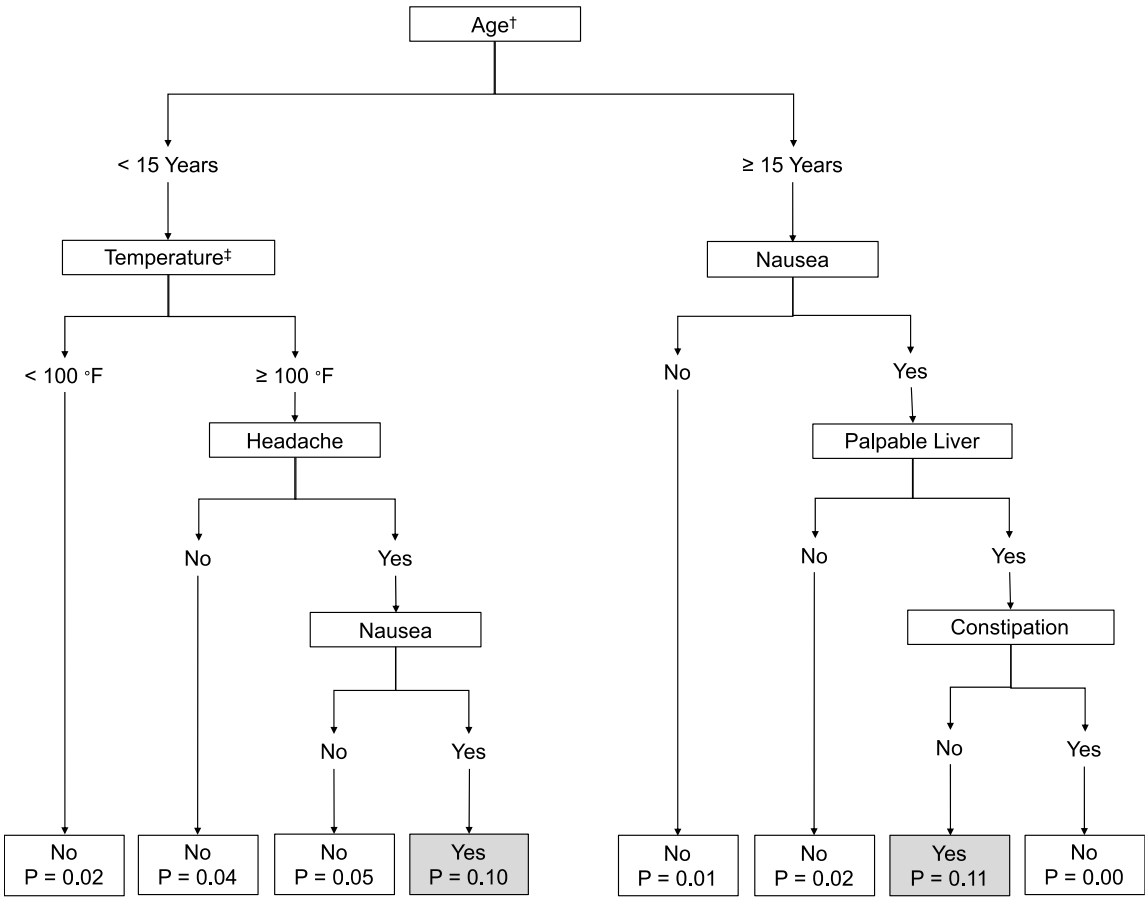
Analysis of training dataset

The training set was used to build a training model to predict BC-proven typhoid. The model was tuned by varying cut-off probabilities starting from 0% to 50% incremented by 1% using the grid-search technique (110). A predefined specificity of between 97% and 98% was matched at 6%-10% cut-off probabilities. A decision tree with depth of four – meaning four levels of bifurcation – was selected as the optimal model since it was able to provide a sufficiently simple rule for application in clinical diagnosis and yielded specified probability cut-off range of 9%. Based on these parameters, two rules were identified for defining BC-proven typhoid in the population (Figure 3-2).

Rule 1: patients aged <15 years with high temperature (≥100°F), headache and nausea

Rule 2: patients aged ≥15 years with nausea, palpable liver and without constipation

Figure 3-2 Depth 4 decision tree specifying clinical and epidemiological criteria for prediction of BC-positive typhoid.



Note: prediction (represented by terminal node) cut-off probability (P) is set as 0.09

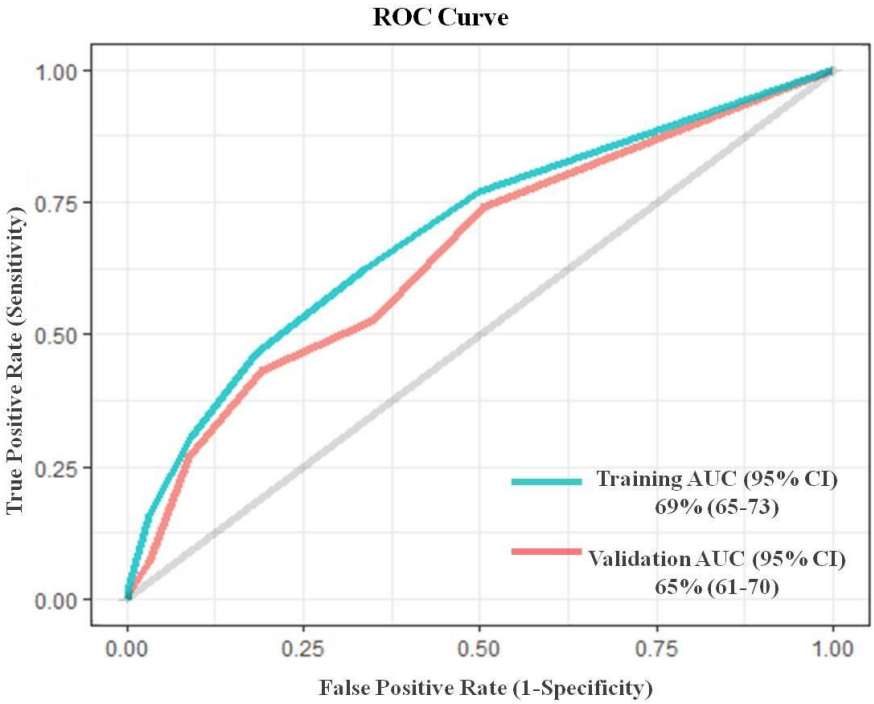
†Age was considered at date of fever onset

‡Axillary, oral, or rectal temperature measurement

Overall, the accuracy of the training model was 95% with 16% sensitivity and 97% specificity, and the area under curve (AUC) showed significant difference from the 50% random line, measured as 69% (95% CI: 65, 73) (Figure 3-3). When these criteria were used to define BC-negative syndromes, the adjusted PE of the ViPS vaccine was 31% (95% CI: 2, 52, p=0.040) for all age groups (Table 3-3). Evaluations of the validation dataset yielded similar results. Overall accuracy of the validation model was 95% with 97% specificity. Sensitivity of the

model decreased from 16.0% to 7.0%; however, the AUC from the ROC curve remained significant at 65% (95% CI: 61, 70) (Figure 3-3). The vaccine PE of ViPS vaccine against BC-negative syndromes meeting the derived clinical criteria in the validation set was 33% (95% CI: 4, 53 p=0.027) for all age groups. By comparison, PE of ViPS vaccine against all fever episodes was 0% (95% CI: -7, 7) and 1% (95% CI: -6, 8) in the training and validation datasets, respectively (Table 3-4).

Figure 3-3 ROC curve for different cut-off values for defining typhoid in the training and validation datasets



AUC: Areas under the curve

Table 3-3 Protective effectiveness (PE) of Vi-PS vaccine against clinical typhoid as defined by Rules 1 and Rule 2

	Vi Vaccine Group				**HepA Vaccine Group**				**Protective Effectiveness (PE)**			
	N	case†	PY	IR per 10^5 PY	N	case†	PY	IR per 10^5 PY	Crude PE	p-value	Adj. PE	p-value
Training Set												
All age	9436	51	18345	278	9404	77	18266	422	34 (6, 54)	0.021	31 (2, 52)‡	0.040
2-14 years	2657	49	5143	953	2847	74	5511	1343	29 (-2, 51)	0.062	29 (-2, 50)§	0.063
≥15 years	6779	2	13202	15	6557	3	12754	24	36 (-285, 89)	0.630	36 (-285, 89)§	0.630
Validation Set												
All age	9433	52	18320	284	9400	79	18242	433	34 (7, 54)	0.018	33 (4, 53)‡	0.027
2-14 years	2722	49	5284	927	2832	75	5486	1367	32 (3, 53)	0.035	32 (3, 53)§	0.033
≥15 years	6711	3	13035	23	6568	4	12756	31	27 (-227, 84)	0.684	27 (-227, 84)§	0.684

Note: Age is measured at date of vaccination

PY= Person years; IR= Incidence rate; PE= (1-HR) ×100

†Based on clinical typhoid endpoint, defined as either Rule 1 (patients aged <15 years with high temperature (≥100°F), headache and nausea) or Rule 2 (Rule 2: patients aged ≥15 years with nausea, palpable liver and without constipation)

‡Protective effectiveness is adjusted for age and individual living in a household using a specific place for waste disposal

§Protective effectiveness is adjusted for individual living in a household using a specific place for waste disposal

Table 3-4 Protective effectiveness (PE) of Vi-PS vaccine against all fever episodes

	Vi Vaccine Group				HepA Vaccine Group				Protective Efficacy (PE)			
	N	Fever episodes	PY	IR per 10^5 PY	N	Fever episodes	PY	IR per 10^5 PY	Crude PE	p-value	Adj. PE	p-value
Training Set												
All age	9436	1685	16961	9934	9404	1709	16884	10122	2 (-5, 8)	0.584	0 (-7, 7)†	0.933
2-14 yrs	2657	725	4600	15762	2847	764	4949	15437	-2 (-13, 8)	0.702	-2 (-13, 8)‡	0.682
15+ yrs	6779	960	12362	7766	6557	945	11935	7918	2 (-7, 10)	0.661	2 (-7, 11)‡	0.652
Validation Set												
All age	9433	1641	16912	9703	9400	1677	16913	9915	2 (-5, 9)	0.544	1 (-6, 8)[1†]	0.752
2-14 yrs	2722	711	4678	15200	2832	732	4964	14748	-3 (-14, 7)	0.573	-4 (-15, 7)‡	0.485
15+ yrs	6711	930	12234	7602	6568	945	11950	7908	4 (-5, 12)	0.398	4 (-5, 12)‡	0.394

Note: Age is measured at date of vaccination

PY = Person years; IR = Incidence rate; PE = (1-HR) × 100

† Protective effectiveness is adjusted for age and individual living in a household using a specific place for waste disposal

‡ Protective effectiveness is adjusted for individual living in a household using a specific place for waste disposal

3.4 Discussion

I this analysis I have demonstrated that it is possible to use clinical, demographic, and epidemiological variables to describe a clinical syndrome for typhoid in a manner which maintains acceptable diagnostic specificity for BC-proven typhoid. The methodology used yielded a plausible number of clinical typhoid cases in the BC-negative group, based on the expected number of typhoid cases that would have otherwise been misclassified using conventional blood culture. Finally, the clinical definitions developed in this analysis demarcates a group of BC-negative, persistent febrile episodes against which ViPS vaccine was protective.

Several studies have investigated the clinical symptoms predictive of proven typhoid fever, although these studies assessed disparate populations and age groups, include varying ranges of clinical factors, and employed different methods to substantiate their findings. Despite this heterogeneity, several notable variables are stated to have a high positive predictive value for culture-confirmed typhoid or to be statistically associated with culture-confirmed typhoid by logistic regression across multiple studies. These clinical variables include fever (111-115), relative bradycardia (116-118), coated tongue (117, 118), positive Widal test (115, 118), vomiting (111-114), diarrhoea (111-114), palpable spleen (111-115), and palpable liver (113-115, 119). A clinical trial of ViPS vaccine in Nepal identified culture negative patients with clinical suspicion of typhoid fever, defined as prolonged fever of three consecutive days, heart rate ≥80 beats per minute, and palpable spleen, for whom ViPS vaccine was 80% efficacious; however, specificity or sensitivity of these criteria were not reported

(41). When these criteria for culture-negative typhoid from the Nepal trial were examined in this dataset, there were four episodes in HepA vaccinees versus zero episodes in ViPS recipients.

These findings should be interpreted in light of several important limitations. Firstly, reporting of clinical signs and symptoms may have included errors due to limitations in the accurate recording of medical histories provided by patients and the exams done by clinicians under hectic outpatient clinic conditions in crowded study facilities in urban slums. However, these findings do provide a realistic picture of diagnostic rules that can be practically applied in such settings, which are common in typhoid-endemic populations in the developing world. Secondly, point-of-care diagnostic laboratory results, such as data from the Widal test, blood counts, liver and kidney function tests, and inflammation markers, were not considered, as these were not uniformly available at the time of presentation in the clinics. Again, the absence of availability of such data for clinical decision-making at the time of patient presentation may be the rule not the exception in typhoid endemic settings. While including results from simple routine laboratory tests may have increased the discriminatory power of the decision tree, the applicability of the clinical criteria for predicting typhoid in low resource settings would decrease. Thirdly, post-presentation clinical information was not included. Information collected on subsequent days following initial screening, such as lab test results mentioned above, results from repeat blood cultures, updated status of clinical variables evaluated at enrolment, would not be applicable to the needs of clinicians making decisions on initial presentation of febrile patients, but might be of interest for other purposes, such as classifying

BC-negative typhoid endpoints for a typhoid vaccine efficacy trial. Fourthly, the analysis contained a mix of vaccinated and unvaccinated febrile patients. It is possible that the immunological state conferred by vaccination, or prior exposure to *S.* typhi through natural infection, may modify the clinical manifestation of a typhoid infection. In future analyses it would be interesting to consider the applicability of the clinical prediction rule to these two types of patients separately. Although, for this analysis that employed a vaccine probe assessment to validate the definition of clinical typhoid, it was necessary to implement this study design in the context of a typhoid vaccine intervention study. Fifthly, it is possible that the findings are idiosyncratic to the population and epidemiological setting for the study. Further assessments in disparate populations with varying degrees of natural exposure, healthcare seeking behaviour patterns, and clinical evaluation practices are required to determine if clinical predictors generated in this analysis might be generalizable from one setting to another. Finally, the level of protective effectiveness observed in the ViPS probe analysis in the training and validation sets, while statistically significant and consistent between the two sets, was lower than the 61% protection by ViPS against BC-proven typhoid in the Kolkata trial (77). This could mean that the rules for clinical typhoid were insufficiently specific, that ViPS protection is indeed lower against BC-negative typhoid fever, or both.

Several factors strengthen the findings. Firstly, the data was generated from a large, prospective, population-based cohort. The population was followed and systematically evaluated for febrile episodes and for typhoid using well-validated microbiological methods in experienced clinical laboratories. Secondly, the

accumulated data were sufficient in size to make use of independent cohorts to both derive and test the clinical prediction rule. Moreover, validation of the derived rule was strengthened by demanding high diagnostic specificity and utilizing the results of a vaccine probe analysis. Finally, vaccine protection against all persistent fever episodes was not detected, demonstrating the discriminatory value of the predictive rule.

In the absence of a sensitive typhoid point-of-care diagnostic, a reliable and highly specific clinical rule for predicting typhoid, irrespective of culture positivity, will be useful to guide healthcare workers practicing in endemic, low-resource settings (120). With a new series of Phase III and IV trials being undertaken in Bangladesh, Nepal, Malawi, Ghana and Democratic Republic of Congo (DRC) to evaluate new generation, Vi-protein conjugate vaccines (98, 121-123), a vaccine probe approach should be considered in future analyses to confirm and extend these findings.

As is the case for OCVs, policy-relevant cost-effectiveness analyses of vaccines against typhoid fever are highly sensitive to the assumed disease burden of typhoid. While it is known that blood cultures have limited sensitivity in the detection of typhoid, the level of their diagnostic sensitivity depends on many variables, including the severity and duration of the disease, the volume of blood drawn, patient age, and prior receipt of antibiotics. Because use of typhoid vaccines as probes to define the burden of disease, an approach used for other diseases such as rotavirus, invasive pneumococcal and Hib disease, may overcome several of the limitations of using assumed levels of diagnostic

sensitivity of uncertain accuracy, the proposed analyses have great relevance for policy of use typhoid conjugate vaccines.

Chapter 4 Protection conferred by typhoid fever against recurrent typhoid fever in urban Kolkata

The work described in this chapter has been published during the course of my DPhil in *PLOS Neglected Tropical Diseases*.

Im J, Islam MT, Kim DR, et al. Protection conferred by typhoid fever against recurrent typhoid fever in urban Kolkata. PLoS Negl Trop Dis. 2020;14(8):e0008530. Published 2020 Aug 17. doi:10.1371/journal.pntd.0008530

Abstract

Protection conferred by a first documented visit for clinical care of typhoid fever against recurrent typhoid fever prompting a visit was evaluated. This study takes advantage of multi-year follow-up of a population with endemic typhoid participating in a cluster-randomized control trial of ViPS typhoid vaccine in Kolkata, India. A population of 70 566 individuals, of whom 37 673 were vaccinated with one dose of either ViPS vaccine or a control (HepA) vaccine, was observed for four years. Surveillance detected 315 first typhoid visits, among whom 4 developed subsequent typhoid, 3 due to reinfection, defined using genomic criteria and corresponding to -124% (95% CI: -599, 28) protection by the initial illness. Point estimates of protection conferred by an initial illness were negative or negligible in both vaccinated and non-vaccinated subjects, though confidence intervals around the point estimates were wide. These data provide little support for a strong protective immunizing effect of

clinically treated typhoid illness, though modest levels of protection cannot be excluded.

4.1 Introduction

Global burden of disease studies have estimated that 14.3 million cases of typhoid fever occur annually (22), and case-fatality rates have dropped to <1% since the era of antibiotics ushered in a series of highly effective treatment options (22, 23). Better understanding of the genetic elements conferring resistance against antibiotics in emergent *S.* Typhi lineages has been made possible by whole-genome sequencing, and the potential for resistant clades to spread locally and globally has been well-documented (124-126). Consequently, antimicrobial therapy as a means to limit severe disease and death may become critically compromised. Although the typhoid endgame will ultimately be shaped by radical infrastructural improvements enabling widespread and lasting access to clean water and improved standards of sanitation, strategic use of existing vaccines, including typhoid conjugate vaccine, must be made a priority as a shorter-term solution (127).

The parenteral Vi capsular polysaccharide vaccine and the oral live-attenuated Ty21a strain are safe and effective but have been supplanted by a promising, parenteral Vi-tetanus toxoid conjugate vaccine (Vi-TT), which the WHO prequalified in 2018 and recommended for use in children 6 months to adults 45 years of age in endemic regions (127). This conjugate vaccine has several documented and potential advantages over earlier vaccines, including extending protection to younger children and inducing long-lasting protection in all age groups (95-97).

The Vi-TT vaccine has the potential to dramatically reduce the burden of typhoid fever in vulnerable populations. However, the optimal strategy for vaccine introduction will be greatly assisted by predictions of dynamic transmission models of typhoid incidence that capture the interplay between natural and vaccine-induced protection. Such predictions will allow policymakers to make rational decisions on such issues as age at vaccination, the need for catch up vaccination, and the frequency of boosting. To date, predictions by these models have been limited by uncertainty about several assumptions, including the protective effect of natural typhoid infection against subsequent typhoid infection in typhoid-endemic settings (128, 129). In this chapter I present an evaluation of the protection conferred by an initial clinically treated typhoid fever illness against subsequent typhoid fever due to reinfection, taking advantage of the multi-year follow-up of a population with endemic typhoid participating in a cluster-randomized control trial of Vi capsular polysaccharide typhoid vaccine.

4.2 Methods

4.2.1 Trial and population

The evaluation was conducted on a study from urban slums of Kolkata, India, where a cluster-randomized trial of ViPS vaccine against typhoid was conducted. Two years before the onset of the trial, in January 2003, a population of approximately 60 000 persons was censused, recording individual and household level demographic and socioeconomic data. This information was subsequently updated at regular intervals in a demographic surveillance system

that continued through the vaccine trial, culminating in a close-out census at the end of surveillance, two years after vaccination (129). Typhoid fever surveillance commenced in the study population from the onset of the two-year lead-in period before vaccination and continued for two years following vaccination.

4.2.2 Study design and analytical approach

Defining typhoid relapse and reinfections

For this analysis, a typhoid *relapse* was defined as a second typhoid visit for a given individual caused by the infecting strain from the first visit of the same individual and can be understood as a continuation of the first infection. A typhoid *reinfection* was defined as an independent typhoid visit for a given individual resulting from a new infection from an external source, not a continuation of the first infection of that individual. Because there is no gold standard for distinguishing relapses from reinfections in all instances of recurrent typhoid visits, in this analysis typhoid reinfection was considered simultaneously in several alternative ways.

The primary approach was based on genomic criteria. *S.* Typhi isolates cultured from the first and all recurrent visits were subjected to whole genome sequencing and single nucleotide polymorphism (SNP) analysis as previously described (77). Primary analysis considered a recurrent typhoid visit to be a reinfection if there were >5 pairwise SNP differences between isolates from the two visits, a definition that has previously been used to distinguish relapse from reinfection for invasive non-typhoidal salmonellosis (130). Secondary analyses

considered two alternative criteria for defining recurrent typhoid as reinfections. First, I considered the susceptibility pattern across 11 tested antibiotics (chloramphenicol, ampicillin, amoxicillin, trimethoprim-sulfamethoxazole, ciprofloxacin, ceftriaxone, nalidixic acid, tetracycline, ofloxacin, aztreonam, amikacin), where the detection of at least one discordant susceptibility result between isolated pairs indicated infection with a new strain. Second, a recurrent typhoid visit was considered to be a reinfection if a period of >30 days intervened between the date of the first typhoid visit and the date of onset of the next typhoid visit. Each definition was considered irrespective of classification in alternative definitions. It should be noted that each of these definitions gave a conservative measure of the risk of reinfection, since second typhoid attacks not meeting the most stringent definition above, given by genomic criterion, could be caused by reinfection of genetically identical *S.* Typhi strains, given that *S.* Typhi evolution is very slow, less than 1 SNP per year (125, 131).

4.2.3 Statistical analysis

The primary analysis, which considered the entire dynamic cohort followed from 1 January 2003 until 31 December 2006, estimated the HR for the incidence rate of recurrent typhoid due to reinfection, defined by genetic criteria as stated above, in persons in whom an earlier typhoid visit was observed (Cohort 2), relative to the incidence of a first typhoid visit in persons without an earlier detected visit (Cohort 1). Secondary analyses were conducted by modifying the classification criteria for reinfection. An HR <1 reflected a protective relationship.

For Cohort 1, the incidence of first typhoid visits was measured for the study population residing in the study area at the study start, 1 January 2003, as well as for persons who moved into the area via in-migration or birth thereafter. Person-days of follow-up for each member of Cohort 1 were calculated from zero time (1 January 2003, or date of entry into the cohort via birth or in-migration, if after 1 January 2003) until the date of onset of the first typhoid visit date, or one of the right censoring events of out-migration, death or the end of follow-up (31 December 2006), whichever came first. For Cohort 2, the incidence of recurrent typhoid due to reinfection was measured among those in whom a first visit was recorded, with zero time for follow-up beginning on the visit date for the first visit, and continuing until the date of onset of a reinfection visit, or right censoring event, whichever came first (Figure 4-1A).

To estimate the HRs relating the rate of recurrent typhoid due to reinfection to the rate of a first typhoid visit in subgroups of ViPS vaccinated versus non-vaccinated individuals, a different strategy for defining Cohort 1 and Cohort 2 was employed, since vaccination took place between 27 November 2004 and 31 December 2004. Among ViPS vaccinees, for Cohort 1, zero time was taken as the date of dosing, with follow-up continuing until the date of onset of the first typhoid visit or the first right censoring event, whichever came first. Cohort 2 measured the incidence of recurrent typhoid due to reinfection among ViPS vaccinees in whom a prior typhoid visit was detected, with zero time taken as the date of the first visit and follow-up continuing until the date of onset of a typhoid reinfection visit, or a right censoring event, whichever came first. For the non-Vi vaccinated group, followed during a secular interval comparable to Vi

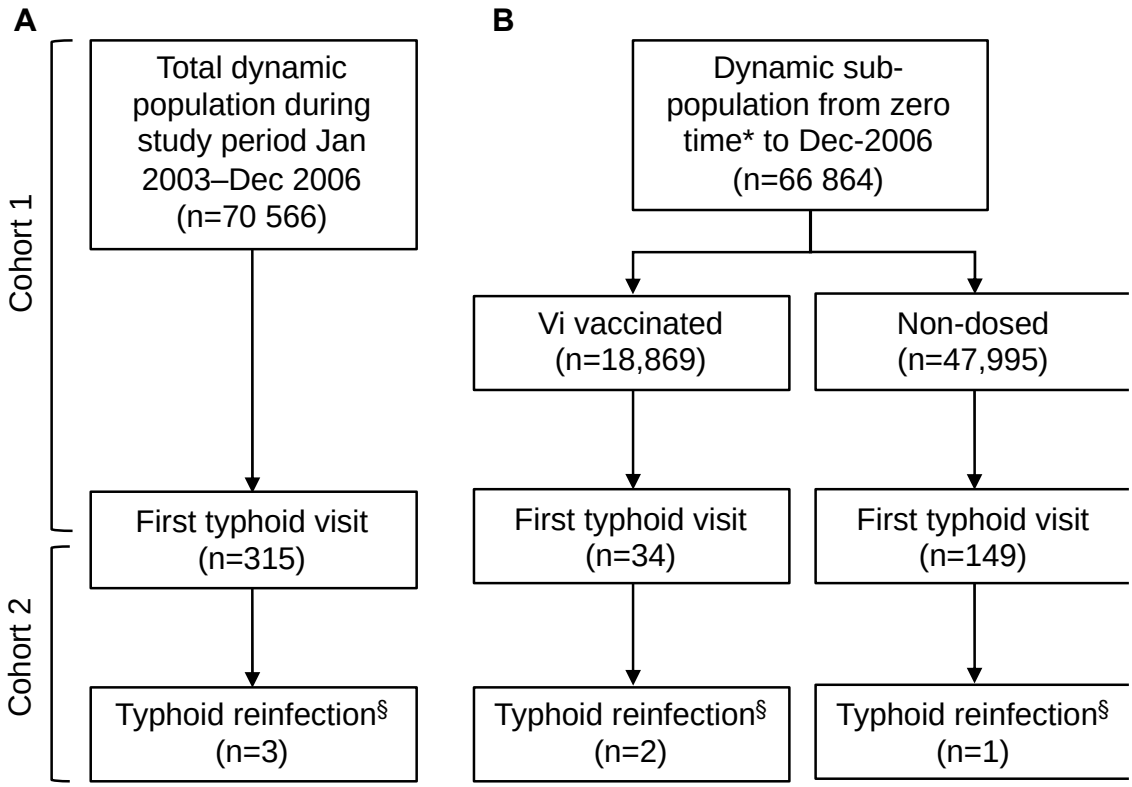
vaccinees, Cohort 1 zero time was the date of vaccination with HepA vaccine, or, among non-dosed individuals, the median date of dosing with either vaccine for other members of the same cluster in the trial if residing in the cluster at the time of vaccination, otherwise birth date or date of migration into the cluster. Zero time for Cohort 2 was the date of the first typhoid visit. Follow-up continued until the date of onset of the first visit (for Cohort 1), the date of onset of the recurrent visit due to reinfection (for Cohort 2), or a right censoring event, whichever came first (Figure 4-1B).

For these analyses, a generalized Cox model (AG model), which allow for recurrent events along the timeline for an individual, was fitted to estimate HRs, crude and adjusted for potential covariates, and 95% confidence intervals (132). The AG model is able to evaluate subsequent events which are mediated through time-varying covariates and gives an output indicating the occurrence intensity of a recurrent event. For the primary analysis, the AG HR was adjusted for ViPS vaccination status (vaccinated or non-vaccinated) as a time varying covariate and age at zero time, which were independently associated with time to event at p-value <0.10. Vaccine protection was expressed as (1 – adjusted HR) X 100%. For the subgroup analyses, models were fit for Vi vaccinees and non-vaccinees separately. A p-value <0.05 (two-tailed) was considered as the margin of statistical significance, and all modelling was done using the PHREG procedure in SAS/STAT software (SAS Institute Inc., Cary, NC, USA).

4.3 Results

There were 70 566 individuals residing in the study area at any point in time from the study start date to study end (Figure 4-1A). A total of 315 first typhoid visits were detected in Cohort 1 during the study period, four of which were followed by a second typhoid visit (Figure 4-1A). Of 315 first typhoid visits, 313 were discharged as outpatients and two were admitted for a duration of 20 and 28 days, respectively. All second typhoid visits were discharged as outpatients. Second typhoid visits occurred in patients ranging from 10 to 33 years of age. Three of four isolate pairs belonged to the H58 haplotype. By SNP criterion, three second typhoid visits were considered to be reinfections with a different strain (Table 4-1). One second visit fit the reinfection criterion based on antibiotic susceptibility and another based on intervening period definition (Table 4-1). None of the second visits were classified as reinfection by all defining criteria and one infection pair was not classified as reinfection according to any criteria.

Figure 4-1 CONSORT diagram showing culture-positive S. Typhi first and second visits in A) the total population during the study period of January 1, 2003 until December 31, 2006, and B) sub-populations of Vi-vaccinated and non-dosed individuals from zero time to December 31, 2006



Note: In- and out-migratory populations are not reflected in this diagram.

*Given as date of vaccination occurring between 27 November 2004 and 31 December 2004

§ Based on pairwise SNP difference >5

Table 4-1 Classification of recurrent typhoid pairings as due to reinfection based on genomic, antibiotic susceptibility, and intervening period criteria.

ID	NCBI/ENA accession	Haplotype	SNPs§ (No. pairwise SNP differences)	Antibiotic susceptibility‖ (No. discordant pairs)	Intervening period¶ (No. days)
C03551	ERR279346	H58	Reinfection (11)	Relapse (0)	Reinfection (49)
C03891	ERR279347	H58			
C03495	ERR279348	H58	Reinfection (10)	Reinfection (1)	Relapse (19)
C03634	ERR279349	H58			
E02889	ERR279350	H58	Reinfection (9)	Relapse (0)	Relapse (30)
E02990	ERR279351	H58			
E01240	ERR279352	H14	Relapse (5)	Relapse (0)	Relapse (14)
E01303	ERR279353	H14			

§ Pairwise SNP difference >5

‖ Discordance in antibiotic susceptibility between infection pair >0

¶ Intervening period >30 days

The crude incidence of first visits during the entire surveillance period was 0.36 per 100 000 person-days and the crude incidence of all second visits was 0.95 per 100 000 person-days (Table 4-2). In adjusted analyses, individuals detected with a first typhoid visit were two times more likely to have a subsequent typhoid visit compared to individuals without a prior typhoid visit detected during the study period (Adjusted Protection: -200% (95% CI: -708, -12)) (Table 4-2). Using the primary genomic definition of reinfection resulted in an adjusted protection effect conferred by a first typhoid visit of -124% (95% CI: -599, 28) (Table 4-2). Significant protection conferred by first typhoid visit against reinfection, after adjustment for potential confounders, was not detected regardless of which of the three criteria was used to define reinfection, although the antibiotic

susceptibility and intervening period criteria gave an estimate that was positive, but low, with wide confidence limits (Adjusted Protection: 26% (95% CI: -427, 90) and 26% (95% CI: -429, 90), respectively) (Table 4-2).

Table 4-2 Protective effect of a first typhoid visit against recurrent typhoid due to reinfection in the total population (n=70 566)

	No. of individuals	No. of typhoid visits	Person-days †	IR per 100,000 person-days	Crude HR ‡	Adjusted Protective Effect (95% CI) ‡
Cohort 1	70566	315	86369890	0.36	Ref	Ref
All recurrent typhoid visits						
Cohort 2	315	4	420965	0.95	5.4 (2.0, 14.4)	-200% (-708, -12)
Recurrent typhoid due to reinfection defined by SNP difference§						
Cohort 2	315	3	421740	0.71	4.0 (1.3, 12.5)	-124% (-599, 28)
Recurrent typhoid due to reinfection defined by antibiotic susceptibility‖						
Cohort 2	315	1	422584	0.24	1.3 (0.2, 9.5)	26% (-429, 90)
Recurrent typhoid due to reinfection defined by intervening time¶						
Cohort 2	315	1	422653	0.24	1.3 (0.2, 9.5)	26% (-427, 90)

†In Cohort 1, person-days calculated from study start date or date when person entered the study to end date, which is defined as date of study end, out-migration, or onset of the first typhoid visit. In Cohort 2, person-days are calculated from the date of first typhoid visit to the date of study end, out-migration, death, or onset of the subsequent typhoid visit.

‡ Fitted a generalized proportional hazard model (AG model). The number of previous infections (0 or 1) was included in the model as a dummy variable; the reference group was the group without a previously detected typhoid visit. The risk was adjusted for Vi vaccination status as a time varying covariate and age at zero time.

§ Pairwise SNP difference >5

‖ Discordance in antibiotic susceptibility between infection pair >0

¶ Intervening period >30 days

Among the four second typhoid visits, two occurred in the Vi vaccine arm and one was in the non-ViPS vaccine arm (includes HepA vaccinated and non-dosed) (Figure 4-1B). One second typhoid visit (E01303) occurred before the period of vaccination and was therefore excluded from vaccination subgroup analyses. Analyses of these subgroups estimated that the crude incidence of first typhoid visit was 0.25 and 0.45 per 100 000 person-days in the ViPS vaccinated group (Table 4-3) and non-ViPS vaccinated group (Table 4-4), respectively. We identified a very high HR of reinfection (by genomic criterion) in the ViPS vaccinated group with evidence that those with prior typhoid visits were at increased risk for typhoid relative to those without a previously detected typhoid (Adjusted Protection: -3549% (95% CI: -15473, -755)) (Table 4-3). In the non-ViPS vaccinated group all point estimates for reinfection reflected no protection by an initial typhoid visit (Adjusted Protection: -88% (95% CI: -1246, 74)), although the wide confidence interval did not exclude a moderate level of protection (Table 4-4).

Table 4-3 Protective effect of a first typhoid visit against recurrent typhoid due to reinfection in the ViPS vaccinated population (n=18 869)

	No. of individuals	No. of typhoid visits	Person-days †	IR per 10^5 person-days	Crude HR‡	Adjusted Protective Effect (95% CI)‡
Cohort 1	18869	34	13705796	0.25	Ref	Ref
All recurrent typhoid visits						
Cohort 2	34	2	24624	8.12	74.1 (17.5, 313.2)	-3549% (-15473, -755)
Recurrent typhoid due to reinfection defined by SNP difference§						
Cohort 2	34	2	24624	8.12	74.1 (17.5, 313.2)	-3549% (-15473, -755)
Recurrent typhoid due to reinfection defined by antibiotic susceptibility‖						
Cohort 2	34	0	25468	0	--	--
Recurrent typhoid due to reinfection defined by intervening time¶						
Cohort 2	34	1	24909	4.01	35.6 (4.8, 262.8)	-1640% (-12835, -134)

†In Cohort 1, person-days calculated from zero time (date of vaccination) to end date, which is defined as date of study end, out-migration, or onset of the first typhoid visit. In Cohort 2, person-days are calculated from the date of first typhoid visit to the date of study end, out-migration, death, or onset of the subsequent typhoid visit.

‡ Fitted a generalized proportional hazard model (AG model). The number of previous infections (0 or 1) was included in the model as a dummy variable; the reference group was the group with no previously detected typhoid visit. The hazard ratio was adjusted for age at date of entry.

§ Pairwise SNP difference >5

‖ Discordance in antibiotic susceptibility between infection pair >0

¶ Intervening period >30 days

Table 4-4 Protective effect of a first typhoid visit against recurrent typhoid due to reinfection in the non-ViPS vaccinated population including in-migrants post-vaccination (n=47 995)

	No. of individuals	No. of typhoid visits	Person-days †	Incidence per 100,000 person-days	Crude HR‡	Adjusted Protective Efficacy (95% CI)‡
Cohort 1	47995	149	32868952	0.45	Ref	Ref
All recurrent typhoid visits						
Cohort 2	149	1	108912	0.92	4.2 (0.6, 30.1)	-88% (-1246, 74)
Recurrent typhoid due to reinfection defined by strain§						
Cohort 2	149	1	108912	0.92	4.2 (0.6, 30.1)	-88% (-1246, 74)
Recurrent typhoid due to reinfection defined by antibiotic susceptibility‖						
Cohort 2	149	1	108912	0.92	4.2 (0.6, 30.1)	-88% (-1246, 74)
Recurrent typhoid due to reinfection defined by interval¶						
Cohort 2	147	0	109540	0	--	--

†In Cohort 1, person-days calculated from zero time (date of vaccination with placebo or for non-dosed individuals, the median zero time for the cluster of residence at the time of vaccination, or date of entry for in-migrants post-vaccination) to end date, which is defined as date of study end, out-migration, or onset of the first typhoid visit. In Cohort 2, person-days are calculated from the date of first typhoid visit to the date of study end, out-migration, death, or onset of the subsequent typhoid visit.

‡ Fitted a generalized proportional hazard model (AG model). The number of previous infections (0 or 1) was included in the model as a dummy variable; the reference group was the group with no previously detected typhoid visit. The hazard ratio was adjusted for age at date of entry.

§ Pairwise SNP difference >5

‖ Discordance in antibiotic susceptibility between infection pair >0

¶ Intervening period >30 days

4.4 Discussion

In this analysis, I assessed the level of protection associated with typhoid illnesses in patients seeking clinical care against a subsequent typhoid visit due to reinfection. Point estimates failed to find a significant level of protection by an initial typhoid illness against typhoid illnesses defined as reinfections using multiple alternative definitions of reinfection. Moreover, the analyses suggested the relationship was different for those who had earlier received ViPS vaccine and those who had not, with the former group exhibiting negative protective relationships. The 95% confidence interval upper boundaries were below 0%, not only suggesting that there was an absence of protection but that the risk of subsequent typhoid reinfection was actually higher in those with an initial infection. In contrast, non-recipients of ViPS, who also had negative values for point estimates of adjusted protection, displayed very wide confidence intervals whose upper boundaries did not exclude moderate protection by an initial visit.

In aggregate, these findings are compatible with previous reports that a natural typhoid infection confers, at best, moderate or incomplete protective effects against subsequent attack (52, 53). It was also observed that the hazard ratios for reinfection were higher in ViPS vaccinated compared to non-vaccinated individuals. These data suggest that persons who develop typhoid fever despite earlier ViPS vaccination represent a subgroup of non-responders who are especially vulnerable to typhoid, for immune or non-immune reasons. The earlier studies evaluated risk of typhoid in adults from non-endemic settings who were exposed to typhoid during sequential, concentrated outbreaks occurring in a military unit (53) or in the setting of an experimental typhoid volunteer challenge

study (52). These studies did not address the issue of greatest relevance to deployment of new generation vaccines to populations experiencing endemic typhoid: what level of natural protection is conferred by typhoid infection against recurrent infection in a typhoid-endemic population of children and adults followed over a period of several years. This issue which is evaluated in this analysis, is a key gap in evidence for constructing dynamic transmission models designed to predict the population impact of alternative introduction strategies for new generation typhoid vaccines into populations with endemic typhoid, who account for the vast majority of the world's typhoid morbidity and mortality.

It is important to discuss the study's limitations. First, there was no gold standard criterion for differentiating typhoid due to reinfection from typhoid due to relapse. However, the consistency of these findings despite use of multiple alternative definitions for reinfection supports their credibility. In this evaluation, findings based on genomic criteria, which were more discriminatory, were likely more credible. Secondly, the two groups under comparison were not randomly allocated or concurrently followed, namely those with previously detected typhoid and those who had not yet been shown to have typhoid. Therefore, those who developed typhoid fever may have been at systematically higher risk of typhoid or were more likely to seek medical care for fever. This may be especially true of those who were vaccine failures, and may not have been completely controlled for in our multivariable models. Counterbalancing this potential bias, however, is the fact that all of the criteria for defining typhoid reinfection were likely underestimates, making the estimates of increased risk conservative, since identical organisms may have caused secondary attacks,

given that the low rate of nucleotide substitution in *S.* Typhi would have corresponded to an accumulation of very few SNPs throughout the study (125, 131). As well, when controlling for typhoid seasonality to adjust for unequal exposure to typhoid, the results were unaffected. Additionally, this approach of comparing disease incidence in naturally infected versus non-infected individuals in cohort studies has been successfully used to determine protection naturally conferred by infection in other infectious diseases such as cholera (50) and rotavirus (133).

Thirdly, the number of typhoid reinfections, regardless of the defining criteria, were low in number, and the resulting estimates of protection had very wide confidence intervals. However, confidence intervals for most of the estimates exclude even moderate levels of protection. Fourthly, some typhoid visits that were classified as first visits in our surveillance may in fact have been reinfections, as an artifact of the limited time period for our surveillance, though exclusion of these cases would have lowered the incidence of first visits, again making the analyses conservative. Fifthly, the findings reflect the protection conferred by clinical typhoid, excluding asymptomatic or mildly symptomatic typhoid infections, though it seems counterintuitive that these milder typhoid infections could confer greater protection than clinical typhoid severe enough to prompt patients to seek care. Sixthly, all confirmed typhoid cases were treated with antibiotics according to WHO guidelines, considering antibiotic-susceptibility results. Treatment may have reduced the course of infection thereby impacting immunological development and diminishing the measurable protective effect of natural infection. Lastly, these findings reflect the protection conferred by the

particular *S.* Typhi strains circulating in the population under observation during the period of study. Importantly, the analysis does not make provisions for undetected typhoid due to limitations of diagnostic sensitivity of blood cultures, although presumably sensitivity is not impacted by the serial order of visits occurring in an individual.

The biological plausibility of these findings is supported by several observations. Supportive evidence includes the intrinsic ability of *S.* Typhi to down-regulate its own virulence genes to facilitate evasion of human immune signalling pathways (134) and to disrupt host transcription regulation effectively disabling components of the host immune response (135). Additionally, host characteristics, such as genetic determinants (136, 137), prior pathogen exposure (138), and baseline immune cell presence (139) can lead to a muting of immune responses against *S.* Typhi invasion. In contrast, a murine model using *S.* Typhimurium has shown that immunity developed after infection does indeed offer protection from a second attack, though this relationship may be specific to the murine host or to the non-typhoid Salmonella pathogen under study (140). In a human challenge model, elicited immunity provided, at best, only moderate protection against second attack (52).

In view of the lack of other contemporary studies of natural protection conferred by typhoid disease in human populations with endemic typhoid disease, these analyses should be considered when parameterizing dynamic transmission models of typhoid fever for the purpose of designing programs for and predicting the impact of alternative introduction strategies for Vi conjugate vaccines in

endemic settings. Protection conferred by natural typhoid disease against recurrent disease due to typhoid reinfection was not detected in the cohort analyses. However, in view of the limitations of the study stated above, additional studies of the protective impact of typhoid fever are needed.

Chapter 5 Protective association of aspects of water, sanitation, and hygiene against typhoid fever in an urban slum

The work described in this chapter has been published during the course of my DPhil in *Clinical Infectious Diseases*.

Im J, Islam MT, Ahmmed F, et al. Can Existing Improvements of Water, Sanitation, and Hygiene (WASH) in Urban Slums Reduce the Burden of Typhoid Fever in These Settings? Clin Infect Dis. 2021;72(11):e720-e726. doi:10.1093/cid/ciaa1429

Abstract

Sustained investment in WASH has lagged in resource-poor settings; incremental WASH improvements may, nonetheless, prevent diseases such as typhoid in disease-endemic populations. Using prospective data from a large cohort in urban Kolkata, India, I evaluated whether baseline WASH variables predicted typhoid risk in a subpopulation (n=28 470) used as a training dataset. A machine learning algorithm was applied to the training subset to create a composite, dichotomous (“Better”, “Not Better”) WASH variable based on four variables, and sensitivity and specificity of this variable was evaluated in a validation subset (n=28 470). Using Cox regression models, the dataset was interrogated for whether residents of Better WASH households experienced lower typhoid risk after controlling for potential confounding variables. Virtual clusters (radius 50m) surrounding each household were overlaid and used to evaluate whether Better WASH prevalence modified typhoid risk in central

household members. Better WASH was associated with protection in analyses of all households (HR=0.57, 95% CI: 0.37, 0.90, p=0.015). This protection was evident in persons ≥5 years at baseline (HR=0.47, 95% CI: 0.34, 0.93, p=0.005) and was suggestive, though not statistically significant, in younger age groups (HR=0.61, 95% CI: 0.27, 1.38, p=0.235). The level of surrounding household Better WASH coverage was also associated with protection (HR=0.988, 95% CI: 0.979, 0.996, p=0.004, for each percent coverage increase). However, collinearity between household WASH and WASH coverage prevented assessment of their independent predictive contributions. In this typhoid-endemic setting, natural variation in household WASH was associated with typhoid risk. If replicated elsewhere, these findings suggest that WASH improvements short of major infrastructural investments may enhance typhoid control.

5.1 Introduction

Major infrastructural improvements in clean municipal water and sanitary treatment and disposal of human waste have eliminated typhoid fever, an infection caused by *S.* Typhi, as a significant public health problem in affluent countries. However, typhoid continues to affect populations of LMICs (141-143). Because resources in LMICs are generally too limited to enable the major infrastructural improvements achieved by more affluent countries in the foreseeable future, it may be questioned whether incremental improvements in water quality, hygiene and sanitation that are feasible and affordable in these settings will be sufficient to have a measurable impact on typhoid burden. One

way of approaching this problem is to evaluate whether natural variations in the level of household WASH that are already present in poor, typhoid-endemic settings, and thus are affordable and feasible, impart a measurable impact on the risk of typhoid fever in those settings. In this paper, I reanalyse longitudinal data from a Kolkata slum population under comprehensive surveillance for typhoid to address this question.

5.2 Methods

5.2.1 Development of a composite WASH variable predictive of typhoid risk

Selection of WASH variables for analysis

I analysed whether variables characterizing the level of household WASH, ascertained in the baseline census, predicted the risk of typhoid fever in the baseline closed cohort over the ensuing four years of clinical surveillance for typhoid fever. In the census, which was an enumerative household survey conducted on the study population at the beginning of the surveillance period capturing socio-demographic information as well as variables related to infectious disease risk, there were 5 non-binary household variables characterizing household source of drinking water, treatment of water for daily use, site of defecation, handwashing practice after defecation, and waste disposal location. The categories of each of these variables were grouped into two classifications reflecting Better or Not Better WASH based on substantive judgements and the distribution of the population according to the different categories, but without prior knowledge of the rates of typhoid associated with

each component category. The baseline population was then divided at random into two equal sized, mutually exclusive subsets, a “training” population and a “validation” population, and four binary WASH variables that were associated with the hazard of typhoid fever in Cox regression models at $p<0.1$ were selected for inclusion in the model, after first ascertaining whether the variables met the proportional hazards assumption. These variables were household source of drinking water (private tap, private well, or private pump versus other); household daily water treatment (filtered or boiled versus other), site of defecation (private or shared flush toilet versus other), and handwashing after defecation (always with soap versus other).

5.2.2. Development of the WASH decision tree to predict typhoid in the training subpopulation

To create an overall binary composite variable for household WASH predicting typhoid in household members, a machine learning algorithm was used to develop a decision tree constructed with recursive partitioning (107). The decision tree was designed to predict development of typhoid fever among baseline household members over 4 years of follow-up, using 4 dichotomous household WASH variables independently associated with typhoid hazard. The algorithm for the training population was initially run while specifying a default loss function of 1:1 for the ratio of costs of false negative and false positive classifications and requiring that each terminal node have at least 300 observations. For cross-validation, the training population was randomly partitioned into 10 parts; 1 part was used in estimating cross-validation error. To

find the optimal typhoid prediction tree in the training population, the model was pruned by the minimal CP corresponding to a minimum error with at least 2 terminal nodes in the tree. An ROC curve was constructed for the selected rule, and the AUC was estimated (108, 109). Maximization of the Youden index was used to select the cut-off probability for the ROC demarcating the overall binary WASH variable into Better versus Not Better household WASH in relation to the risk of typhoid in inhabitants of the household.

5.2.3. Protection conferred by household-level WASH status and neighbourhood WASH coverage against typhoid fever

Because analysis of predictive rules developed in a training population may overestimate the performance of the rule if analysed in the same subpopulation, the rule was assessed in a separate validation population. Procedures for analysis of the validation subpopulation were the same as for the training subpopulation. After confirming that the predictive rule for Better WASH performed similarly in the training and validation populations with respect to sensitivity (the proportion individuals developing typhoid who lived in households with Not Better WASH) and specificity (the proportion of individuals not developing typhoid who lived in households with Better WASH) in predicting typhoid, I undertook further analyses in the total population. First, because the first two years of surveillance occurred in a population that had not been vaccinated with ViPS, whereas the second two years occurred during the ViPS trial, I evaluated whether the predictive rule performed similarly with respect to sensitivity and specificity before and during the ViPS trial. Second, protection

against typhoid associated with Better WASH in the household was evaluated in the total population. To measure the protective association between Better WASH in the household and the subsequent risk of typhoid fever in household inhabitants, Cox proportional hazard regression models were used, after first evaluating proportionality assumptions for independent variables. To evaluate independent variables not fulfilling proportionality assumptions, the variables were included as interaction variables with time of follow-up. Models were adjusted for age in years with time interaction, number of household members with time interaction, and monthly household expenditure (in Indian rupees), all of which were associated at $p<0.05$ with the hazard of typhoid in bivariate Cox models. The HR for culture positive typhoid fever was estimated by exponentiation of the coefficient of living in a household with Better WASH in the model, and the 95% CI for the HR was estimated using a robust sandwich method where each household was assumed as a cluster in the model. Cox models were also used to evaluate the protective association between the percentage of Better WASH households ascertained by GIS in the baseline census as located within a 50m radius of the central household under analysis, on the one hand, and the rate of typhoid in the total population during the ensuing four years, on the other. The analysis was performed using “rpart” package for decision tree modelling, “rpart.plot” package for tree plotting, “pROC” packages for ROC curve, “survival” for Cox model and “dplyr” package for data management under R-Studio analytical software (144-146). In all statistical analyses, $p<0.05$ (two-tailed) was taken as the margin of statistical significance.

5.3 Results

Characteristics of the baseline population

During the first census, 10 944 households and 56 940 residents were enumerated and included in the study closed cohort (Figure 5-1). During the follow-up period, 276 incident cases of typhoid were identified (132 cases per 100 000 person-years), from which 4 were recurrent secondary cases and excluded from analysis. Of 272 typhoid episodes analysed, 113 occurred before the trial and 159 during the trial. The total population, matched on individuals, was randomly partitioned into equal sized training and validation subsets with 28 470 individuals in each and 5415, and 5529 households respectively. The mean age was 27.8 years in both populations; and the proportion of males in each population was 53.8% and 54.2%, respectively. Reported typhoid cases were also distributed evenly in the two populations, 136 cases each. The distribution of individuals residing in Better WASH households versus Not Better WASH households before vaccination was 11 154 (19.6%) versus 45 786 (80.4%), respectively. This proportion did not change after vaccination, 10 494 (19.6%) and 42 977 (80.4%) in Better WASH versus Not Better WASH households (Figure 5-1).

Figure 5-1 Flow diagram showing population at first census and incident typhoid cases before and after vaccination classified by residence in Better or Not Better WASH household

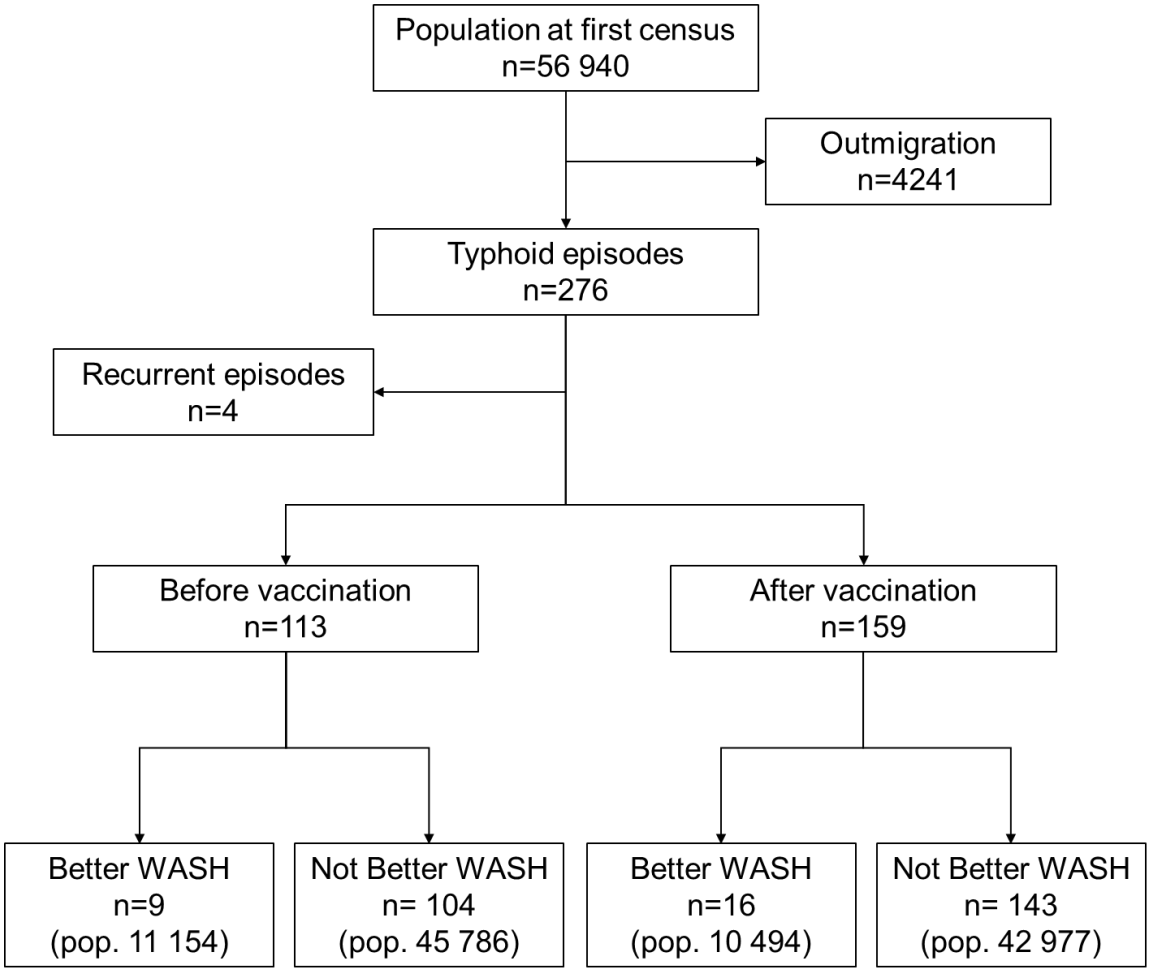
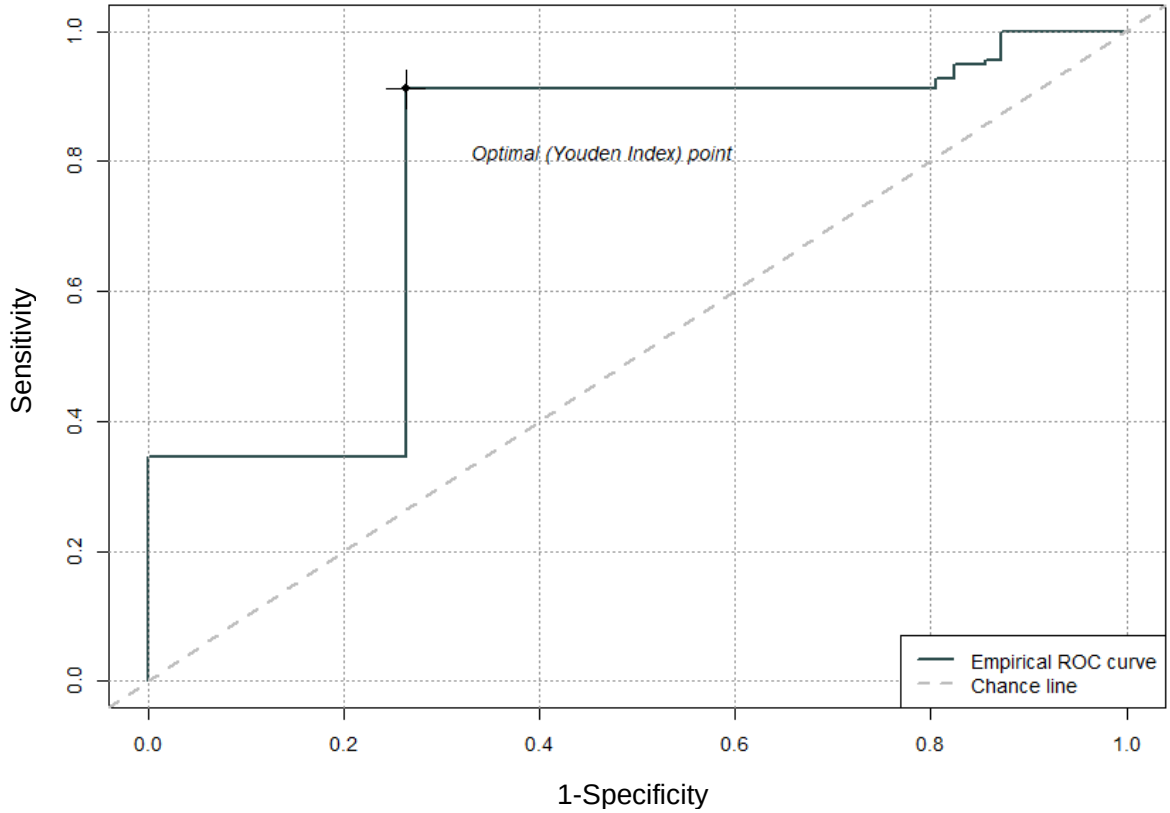


Figure 5-2: Receiver Operator Characteristic (ROC) curve for defining cut-off probability for defining dichotomous composite WASH variable



Note: AUC = 58% (95% CI: 54-61); best cut-off using the Youden Index is 0.004343

Development and evaluation of the WASH decision tree to predict typhoid

Bivariate analysis of individual WASH variables and the risk of typhoid in the training population showed an association for private or shared flush toilet (HR=0.3, 95% CI: 0.1, 1.0, p=0.041), source of drinking water from private tap, well, or pump (HR=0.3, 95% CI: 0.1, 0.7, p=0.005), and water treatment by filter or boiling for daily use (HR=0.5, 95% CI: 0.2, 1.1, p=0.081) (Table 5-1). There was some evidence for association with always hand washing with soap after defecation (HR=0.7, 95% CI: 0.5, 1.1, p=0.097) and no evidence for association with waste disposal practice (p=0.958).

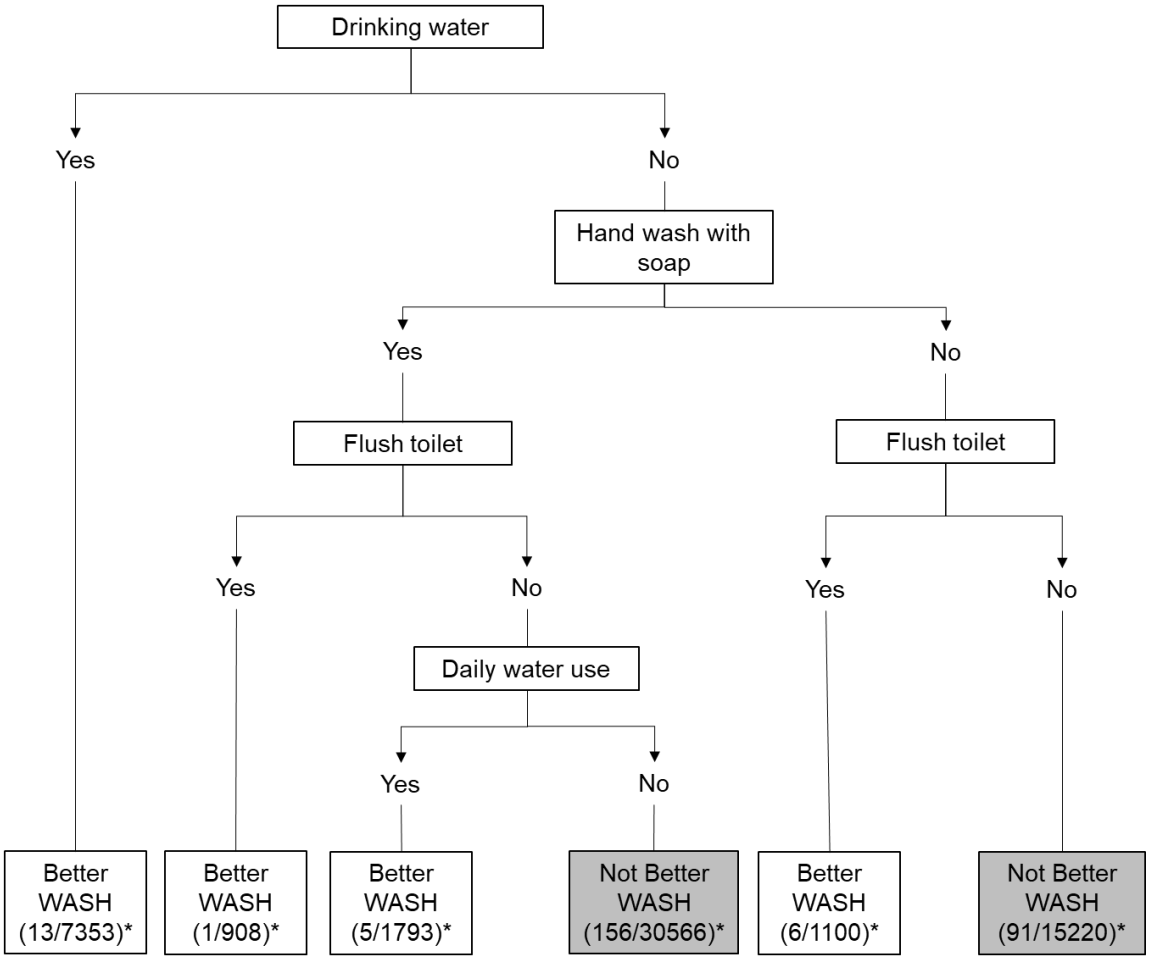
Table 5-1 Bivariate analysis of WASH variable and typhoid risk in the training population

WASH variable	**Yes**				**No**				**HRs (95% CI)**	
	n	Typhoid	PY	IR per 10^5 PY	n	Typhoid	PY	IR per 10^5 PY	Crude HRs	p-value
Site of defecation: *Private or shared flush toilet*	1956	3	7090	42	26514	133	96063	138	0.3 (0.1, 1.0)	0.041
Main source of drinking water: *Private tap, well, or pump*	3655	6	13288	45	24815	130	89865	145	0.3 (0.1, 0.7)	0.005
Treatment of water for daily use: *Filtered or boiled*	2189	5	8008	62	26281	131	95146	138	0.5 (0.2, 1.1)	0.081
Hand wash practice after defecation: *Always with soap*	19286	83	69926	119	9184	53	33227	160	0.7 (0.5, 1.1)	0.097
Waste disposal location: *Specific*	28067	134	101693	132	403	2	1461	137	1.0 (0.3, 3.8)	0.958

Note: p-values are adjusted using robust standard error; each household is treated as a cluster

PY = person years, IR = incidence rate

Figure 5-3 Decision tree for categorization of Better and Not Better WASH based on 4 dichotomous WASH variables



*(Typhoid episodes/household population)

In the training population, the 4 WASH variables associated with risk of typhoid ($p<0.10$) were included in a decision tree model for binary classification of household-level WASH. The optimal cut-off probability maximized the Youden Index (0.004343) applied to an ROC curve that had an AUC=58% (95% CI: 54, 61) (Figure 5-2). The resulting decision tree delineated 2 rules for Not Better WASH and 4 rules for Better WASH (Figure 5-3). The dominant bifurcation in the model was primary source of drinking water for the household, where private ownership of a tap, well or pump, regardless of other WASH characteristics

indicated Better household WASH (Figure 5-3). The sensitivity and specificity of the classification criterion to predict typhoid given by the model was 91.2% (95% CI: 85.1, 95.4) and 19.5% (95% CI: 19.0, 19.9), respectively, in the training set, and evaluation of the rule in the validation population yielded similar results, 90.4% (95% CI: 84.2, 94.8) and 19.8% (95.5 CI: 19.3, 20.3). We therefore applied the rule to the total population. Evaluation of the composite variable in the total population before the trial and during the trail resulted in a sensitivity and specificity of 92.0% (95% CI: 85.4, 96.3) and 19.6% (95% CI: 19.3, 19.9), respectively, before and 89.9% (95% CI: 84.2, 94.1) and 19.6% (95% CI: 19.3, 19.9) during the trial.

Table 5-2 Household-level protection of Better WASH in the total population

Age (years)	Better WASH				Not Better WASH				HR (95% CI)			
	N	Typhoid episodes	PY	IR per 10^5 PY	N	Typhoid episodes	PY	IR per 10^5 PY	Crude HR	p-value	Adj. HR	p-value
All	11154	25	40598	62	45786	247	165842	149	0.41 (0.27, 0.63)	<0.001	0.57 (0.37, 0.90)*	0.015
0 to <5	708	7	2599	269	3693	58	13407	433	0.62 (0.28, 1.35)	0.224	0.61 (0.27, 1.38)†	0.235
≥5	10446	18	37998	47	42093	189	152435	124	0.38 (0.24, 0.62)	<0.001	0.56 (0.34, 0.93)‡	0.024

Note: p-values are adjusted using robust standard error; each household is treated as a cluster

PY = person years, IR = incidence rate

* HR is adjusted by age in years, age in years with time interaction, number of household members, number of household members with time interaction, average household expenditure

† HR is adjusted by age in years, number of household members, number of household members with time interaction, average household expenditure

‡ HR is adjusted by age in years, number of household members, average household expenditure

Table 5-3 Household-level protection of WASH stratified by WASH coverage in the community

WASH Coverage (%)	Better Wash				Not Better Wash				HR (95% CI)			
	n	Typhoid episodes	PY	IR per 10^5 PY	n	Typhoid episodes	PY	IR per 10^5 PY	Crude HR	p-value	Adj. HR	p-value
0 to ≤34	5589	15	20367	74	41070	226	148752	152	0.48 (0.29, 0.81)	0.006	0.63 (0.37, 1.07)*	0.085
>34 to 100	5565	10	20231	49	4716	21	17090	123	0.40 (0.18, 0.91)	0.029	0.52 (0.21, 1.28)†	0.154

Note: p-values are adjusted using robust standard error; each household is treated as a cluster

PY = person years, IR = incidence rate

* HR is adjusted by age in years, age in years with time interaction, number of household members, average household expenditure

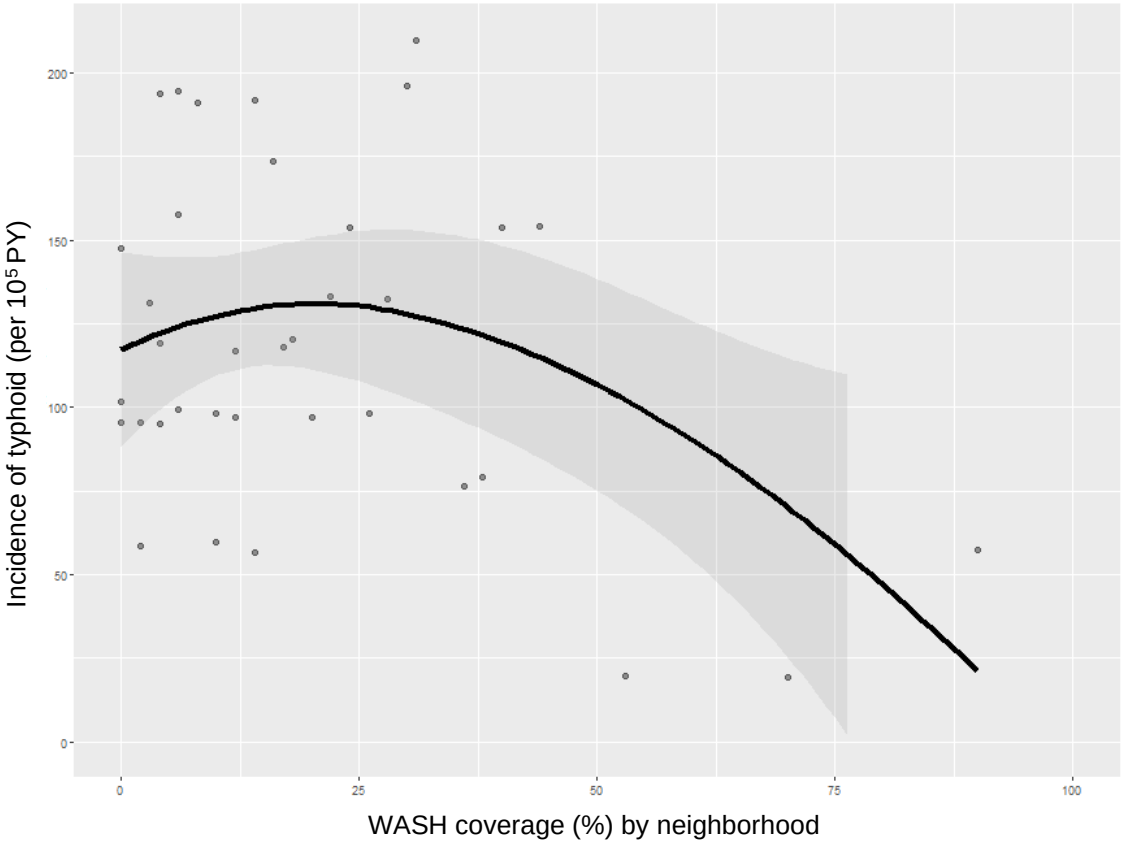
† HR is adjusted by age in years, number of household members, average household expenditure

Evaluation of the protective association between WASH and the rate of typhoid

In the total population, in comparison with residence in a Not Better WASH household, residence in a Better WASH household was associated with 59% (HR=0.41, 95% CI: 0.27, 0.63, p<0.001) crude reduction in hazard for culture confirmed typhoid overall and a 43% (HR=0.57 (95% CI: 0.37, 0.90, p=0.015) hazard reduction in the adjusted model (Table 5-2). When stratified by age at baseline, both the younger and older age groups residing in “better” WASH households experienced a reduction in typhoid hazard, 39% (HR=0.61, 95% CI: 0.27, 1.38, p=0.235) and 44% (HR=0.56 95% CI: 0.34, 0.93, p=0.005), respectively; the association was statistically significant only in the older age group, likely due to the lower number of typhoid cases in the younger age group. I next stratified the population into high versus low levels of Better WASH in households surrounding the central household within 50m virtual clusters, taking the cut point (34%) as the prevalence of Better WASH at which there was a noticeable decrease in the incidence of typhoid in the central households (Figure 5-4). The data failed to show that the level of Better WASH coverage in the community surrounding a central household under analysis modified the protective association between the level of household WASH and the risk of typhoid among members in the central household (Table 5-3). Next, I measured the association between the level of surrounding households with Better WASH and the risk of typhoid in members of the central household in a model that controlled for potentially confounding variables (age, household size, and household expenditure). In this analysis, I found that the association was significant (HR=0.988, 95% CI: 0.979-0.996, p=0.004, for each percent increase of coverage). However, it was not possible to evaluate the independent

protective associations with typhoid fever for household WASH on the one hand and level of neighbourhood WASH on the other hand due to collinearity between these two variables.

Figure 5-4 Relationship between neighbourhood WASH coverage and risk of typhoid



Note: Typhoid incidences were calculated for 40 equal subgroups of WASH coverage; a smooth line was drawn using a locally estimated scatterplot smoothing (LOESS) function

5.4 Discussion

I used recursive partitioning to develop a dichotomous classification rule for household-level Better WASH based on 4 independent non-binary WASH variables collected at baseline. When applied to the study population, residence

in a Better WASH household was associated with a moderate decrease in risk for contracting typhoid during the study period. When analysed separately by age group, the protective association was significant for the older age group and suggestive for the younger age group. The neighbourhood level of Better WASH coverage appeared to be associated with the risk of typhoid among members of central households, however it did not modify the association between the level of WASH in the central household and the risk of typhoid in household members.

The findings of this study must be considered in light of several important limitations. Firstly, the construction of a composite WASH variable to classify households relied on very simply ascertained WASH variables that were collected at baseline only as potential confounders for analysis of the vaccine trial. More in depth characterization of WASH variables might have resulted in a more powerfully predictive WASH composite variable and created the opportunity for further assessment of independent contributions of WASH component variables. For example, although it is likely that drinking water from a household's private tap, well, or pump was microbiologically superior to that from other sources, I did not have direct data on this issue. Secondly, WASH variables were ascertained at baseline and the population was followed for four years. Although the population was quite stable, it is possible that the WASH status of households changed over time. However, such misclassification bias, if random, would have made the analyses conservative. Thirdly, I used a machine learning algorithm that created a dichotomous composite WASH variable. Although useful, particularly in classifying the level of WASH coverage in

surrounding households, this dichotomization may have resulted in some loss of information about the variables, again making our analyses conservative. Fourthly, the analysis of the protective association of household WASH and the risk of typhoid by age was limited by sparse data in the younger age stratum. Fifthly, I partitioned the training and validation sets by individuals rather than households, with the result that there were some shared households in the training and validation sets. However, sensitivity and specificity values for the composite variable were nearly identical in the training and validation subsets when I excluded households that were shared by the two subpopulations. Finally, the period of follow-up for the study included periods before and during a ViPS vaccine trial. However, as indicated in the results, separate analyses of the predictive rule during these periods revealed consistent results.

Despite these potential limitations, several factors strengthened our findings. First, the analyses utilized data prospectively collected in the context of a clinical trial for which population information was collected systematically and typhoid ascertainment was done in a comprehensive manner and was confirmed by blood culture positivity. Secondly, the population was relatively stable over time, and thus patterns of migration were unlikely to affect the assessments. And lastly, the dataset was large enough to both train and validate the model in mutually exclusive populations to minimize overfitting.

These results indicate that improvements to WASH short of major municipal infrastructure developments might have an appreciable effect on the risk of typhoid in urban settings of South Asia. The results reinforce the notion that

household transmission was an important contributor to disease transmission in this urban setting, and moreover, that household source of drinking water appears to be a dominant determinant of typhoid risk, although the effect of drinking water source can be modified by a variety of other WASH variables. This evaluation also suggests that the effect of WASH in the household was not modified by the level of WASH in the surrounding neighbourhood.

The ability of this analysis to demonstrate that household WASH may influence the risk of typhoid in household residents encourages future studies of this topic. It would be helpful for future studies to characterize WASH in a more comprehensive fashion, including WASH behaviours, and that cohort studies of this topic monitor and analyse changes in household WASH that occur during follow-up. As well, because of the theoretical likelihood that protection conferred by newer generation typhoid vaccines may be modified by the intensity of the typhoid inoculum to which vaccinees are exposed, it will be of great interest to examine the interactions between levels of household WASH and typhoid vaccine protection in future vaccine evaluations.

Chapter 6 Prevention of typhoid fever by existing improvements of WASH and the use of Vi polysaccharide typhoid vaccine in poor urban slums. Results from a cluster-randomized trial.

The work described in this chapter has been published during the course of my DPhil in *American Journal of Tropical Medicine and Hygiene*.

Im J, Khanam K, Ahmmed F, et al. Prevention of Typhoid Fever by Existing Improvements in Household Water, Sanitation, and Hygiene, and the Use of the Vi Polysaccharide Typhoid Vaccine in Poor Urban Slums: Results from a Cluster-Randomized Trial. Am J Trop Med Hyg. [Accepted for publication]

Abstract

Modest improvements in household WASH and typhoid vaccination can reduce typhoid risk in endemic settings. However, empiric evaluation of their combined impact is lacking. 62 756 persons residing in 80 clusters in a Kolkata slum were randomly allocated 1:1 to either typhoid ViPS vaccine or HepA vaccine. Surveillance was conducted for two years prior to and two years following vaccination. Households were classified as having Better or Not Better WASH and calculated the prevalence of Better WASH households in clusters using previously validated criteria. I evaluated the protection by Better household WASH, Better household WASH prevalence, and ViPS vaccination against typhoid in all cluster members present at baseline using Cox proportional hazard models. Overall, ViPS vaccination was associated with a 55% (95%CI: 35, 69; p<0.001) reduction of typhoid risk and was similar regardless of Better WASH in

the residence. Living in a Better WASH household was associated with typhoid risk reduction of 31% (95%CI: -16, 59; p=0.16) overall; the reduction was 48% (95%CI: -1, 73; p=0.05) in HepA clusters, 6% (95% CI: -82, 51; p=0.85) in ViPS clusters, and 57% (95% CI: 15, 78; p<0.05) in the population during the two years preceding the trial. These findings demonstrate a preventive association of Better household WASH in non-ViPS population, but, unexpectedly, an absence of additional protection to ViPS by Better WASH in the ViPS population. This analysis highlights the importance of assessing the combination of WASH in conjunction with typhoid vaccines and has implications for the evaluation of new generation typhoid conjugate vaccines.

6.1 Introduction

Typhoid infection occurs through ingestion of contaminated food or water, and transmission can be high in areas with insufficient clean water infrastructure and limited access to sanitary facilities. We know that major improvements in municipal WASH have drastically reduced infectious disease transmission, including typhoid, and prevented disease in affluent countries (147). As well, empiric evidence from a Kolkata urban slum has demonstrated that improvements in household WASH occurring naturally, without a specific intervention, were predictive of reduced typhoid risk (148). In addition, several effective typhoid vaccines are now licensed and available, including two that are WHO-prequalified, one a vaccine consisting of ViPS, and the other ViPS chemically conjugated to tetanus toxoid (149). Recognizing that current recommendations for control of typhoid in LMICs emphasize use of both effective typhoid vaccines and improvement of WASH (127), I investigated whether protection by an effective typhoid vaccine acted independently of protection conferred by better existing WASH and if these independent effects were complementary when implemented together in an urban slum where typhoid is endemic. A CRT of an effective typhoid vaccine, ViPS, conducted in an urban slum of Kolkata, India, provided a unique platform to address these questions.

6.2 Methods

6.2.1 Trial and population

I evaluated a cluster-randomized, controlled trial of typhoid ViPS vaccine in an urban slum in Kolkata, India, conducted between 2004 and 2006 (77). Demographic information and household WASH characteristics were collected from the trial population during a census conducted just prior to vaccination (baseline). All 62 756 individuals residing in the study area at baseline were included in the analysis. Individuals migrating into the study area and births occurring during the two-year surveillance period were excluded. Eighty clusters were randomly assigned into two groups of 40 clusters and received either a single-dose ViPS typhoid vaccine (Typherix®, GlaxoSmithKline) or a single-dose inactivated HepA vaccine (Havrix®, GlaxoSmithKline), administered to participants aged 24 months and older. For this randomization, clusters were stratified by ward, size of population 18 years or younger, and size of population older than 18 years. Vaccination occurred between 27 November and 31 December 2004. Of 61 280 age eligible individuals, 37 673 were vaccinated – 18 869 in the ViPS vaccine arm and 18 804 in the HepA arm. The trial was masked at four levels – participant, care provider, investigator, and outcomes assessors.

6.2.3 Clinical surveillance

Passive surveillance for typhoid fever was conducted for two years following vaccination. In addition, as reported elsewhere, identical methods of demographic, WASH, and clinical surveillance were conducted in the 57 949

residents of all ages of the study area, none of whom had received either study vaccine, during the two years prior to the inception of the vaccine trial (148). Residents of the study area presenting to one of five study clinics with a febrile episode received a blood culture. A febrile episode was defined as history of fever for at least three days, and sequential visits, where date of discharge and subsequent date of fever onset were separated by 14 or fewer days, were considered as a single episode. Typhoid fever was defined as a febrile episode in which *S.* Typhi was isolated from one or more blood cultures, and a home visit conducted within seven days after discharge confirmed that the person whose name was given at the surveillance site had indeed sought care on the date of presentation.

6.2.4 Definition of WASH

Characteristics of household WASH were collected during a baseline census of the trial population. Using these data, I developed a composite dichotomous (Better, Not Better) household WASH variable comprising several WASH features that predicted typhoid risk independent of household socioeconomic status (Chapter 5). A Better WASH household was defined based on criteria using the following variables: having a drinking water source from private tap, well, or pump; always handwashing with soap after defecation; having a private or shared flush toilet; and use of filtered or boiled water for daily household use (Figure 5-3).

6.2.5 Analytical approach

In this analysis I evaluated overall ViPS vaccine protection against typhoid by comparing rates of blood culture-confirmed typhoid fever among all residents, vaccinated or not, of ViPS clusters versus all residents of HepA clusters. Correspondingly, I evaluated protection associated with Better WASH in the household by comparing rates of typhoid in all members of Better WASH households versus Not Better WASH households. Finally, I assessed protection associated with the prevalence of Better WASH households in the clusters, given as the proportion of households with Better WASH among all households stratified into lower (0-33rd percentile), middle (34-66 percentile), and upper (67-100th percentile) tertiles, by comparing rates of typhoid in members of households in clusters with lower prevalence versus those in clusters with higher prevalence. To further explore these associations, I undertook analyses of overall ViPS protection of individuals by Better versus Not Better WASH of the households in which they lived, as well as by the prevalence of Better WASH households of the clusters in which they resided, and evaluated evidence for a modifying effect of WASH on Vi protection and vice versa. And to further evaluate the impact on typhoid of living in a Better versus not Better WASH household, as well as the impact of living in a cluster with a higher versus lower prevalence of Better WASH in the household, I examined these associations separately for the clusters assigned to ViPS and those assigned to HepA.

6.2.6 Statistical analysis

Simple associations of typhoid risk between groups were assessed using a Chi-squared test for heterogeneity. To measure the protection by ViPS vaccine in models, a Cox proportional-hazard regression analysis was used after evaluating proportionality assumptions for independent variables. Zero time for follow-up was considered as the day of vaccination for vaccine recipients and the median day of vaccination in the cluster of residence for non-vaccinees. For individuals present at zero-time, follow-up continued for two years until the study end date on 30 December 2006, the first typhoid episode, outmigration, or death, whichever occurred first. As described earlier, typhoid surveillance also was performed for the population in the trial area during the two years leading up to the trial. Classification of WASH of the households during this lead-in period was performed in a manner identical to that used for the trial, using information from a baseline census conducted before inception of surveillance, and follow-up of this closed cohort was conducted in the same manner as for follow-up of subjects assembled at the onset of the trial. In individuals with multiple typhoid episodes, only the first episode was considered in analysis. Models were adjusted for the variables used to define strata for randomization. Additional covariates (age, sex, Hindu religion, household population size, household monthly expenditure, distance to the nearest treatment centre [above median]) were selected with a forward stepwise algorithm, based on a significance level of $p<0.05$. An interaction term between household WASH and ViPS cluster assignment and Better WASH prevalence and ViPS cluster assignment, respectively, was introduced into models to evaluate the presence of modifying effects. Protective effect of ViPS, Better WASH in the household, and prevalence of Better WASH in households in the cluster was calculated as

(1-HR) × 100%, where the HR was estimated by exponentiation of the coefficient for the relevant variable in the model, and the 95% CI for the HR was estimated using a robust sandwich method to account for the design effect of cluster randomization for the trial population. The analysis was performed using the “survival” package for the Cox model, “car” package for collinearity and “dplyr” package for data management in R-Studio analytical software (144-146).

6.3 Results

6.3.1 Assembly of the trial population

In total, 62 756 individuals, of whom 61 280 were age-eligible to receive vaccine, residing in 80 clusters were present at the baseline census just before inception of the trial, and clusters were randomly assigned with equal probability to receive either ViPS (n=31 075) or control (n=31 681) (Figure 6-2). There were 178 confirmed typhoid episodes recorded during follow-up, 50 cases in ViPS clusters and 128 cases in HepA clusters. Within ViPS clusters, 10 cases occurred in Better WASH households and 40 cases in Not Better WASH households, and in HepA clusters, 9 cases occurred in Better WASH households and 119 cases in Not Better WASH households (Figure 6-2). With the exception of Better WASH in the household, the treatment arms were well balanced with respect to the baseline characteristics, although several variables showed statistically significant differences due to the large sample size for the study (Table 6-1).

Figure 6-1. CONSORT diagram showing population at first census and incident typhoid cases in ViPS and HepA vaccine clusters classified by residence in Better or Not Better WASH households

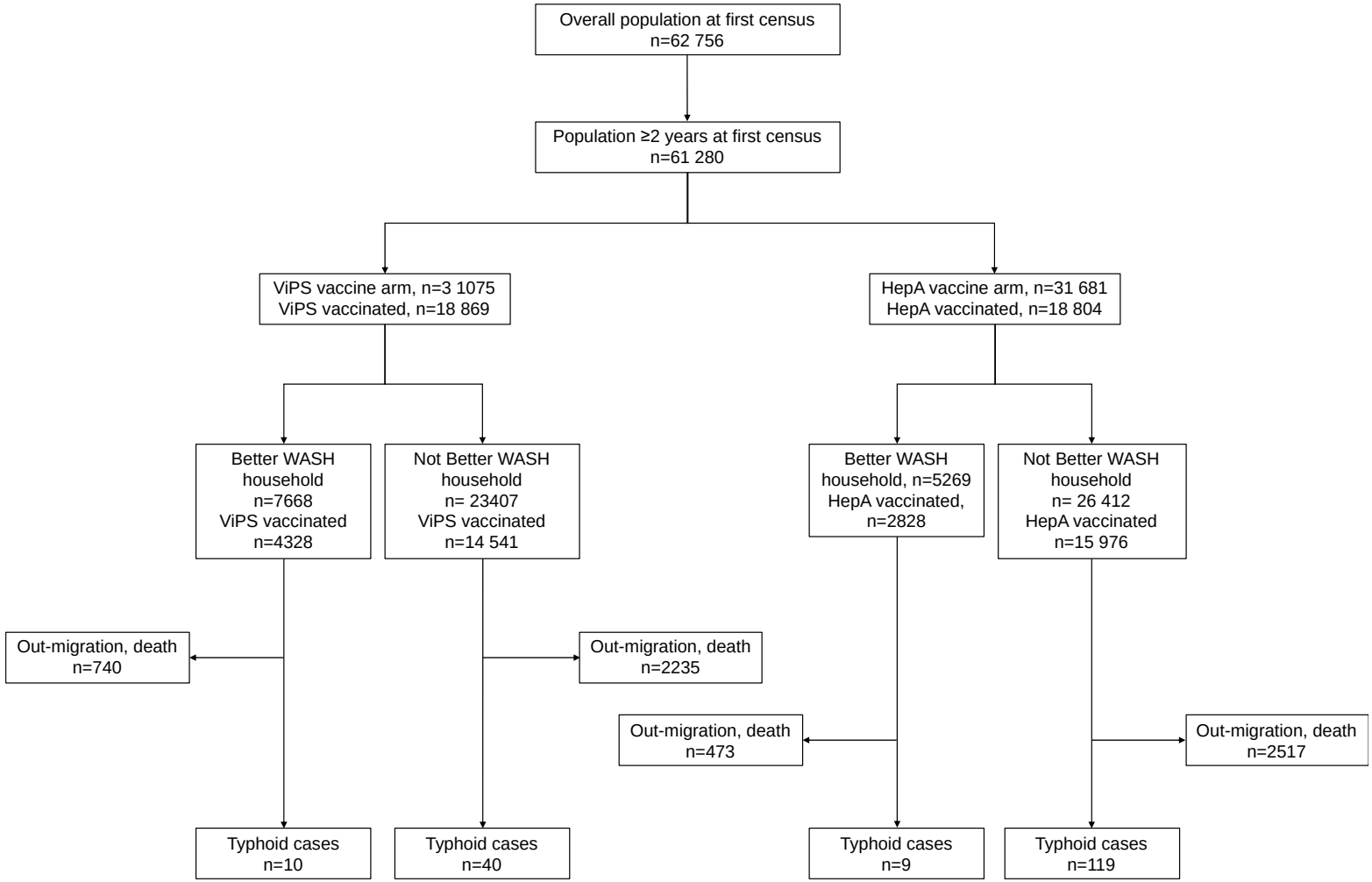


Table 6-1 Baseline comparability between ViPS and HepA clusters for analyses of overall vaccine protection by ViPS

	HepA clusters	**ViPS clusters**	
	Mean ± SD / n (%)	Mean ± SD / n (%)	p-value
Better WASH households	1044 (18)	1455 (26)	<0.001
Age*	28.1±18.2	28.7±18.3	<0.001
Male	17111 (54)	16680 (54)	0.406
Hindu religion	16878 (53)	18794 (61)	<0.001
Household members	7.2±3.8	7.3±4	0.001
Expenditure† (local currency)	3504.5±2432.3	3531.1±2206.6	0.152
Treatment centre distance‡ > median	16516 (52)	14859 (48)	<0.001

*Age in years measured at baseline

†Expenditure calculated as average monthly expenditure in Indian rupees.

‡Measured in meters as the minimum straight-line distance from household to closest study centre

6.3.2 Overall preventive impact of residence in the ViPS vaccine clusters, membership in Better WASH households, and residence in a household in clusters with higher WASH prevalence

Vaccine coverage was 61% (18 869/31 075) in ViPS clusters and 59% (18 804/31 681) in HepA clusters. The prevalence of Better WASH households was 26% (1455/5685) in ViPS clusters and 18% (1044/5824) in HepA clusters (Table 6-1). The incidence of typhoid fever in members of ViPS clusters was 0.84/1000 PY and in HepA clusters was 2.12/1000 PY ($p<0.001$) (Table 6-2). Residence in ViPS clusters was associated with an overall adjusted PE of 55% (95% CI: 35, 69; $p<0.001$) against typhoid (Table 6-2). The incidence of typhoid fever in members of Better WASH households was 0.77/1000 PY and in Not Better WASH households incidence was 1.67/1000 PY ($p=0.001$). Residence in a Better WASH household was associated with an overall adjusted PE of 31% (95%CI: -16, 59; $p=0.16$) among residents of all the clusters (Table 6-2). Evidence for interaction between household WASH and ViPS was not observed ($p=0.331$).

The incidence of typhoid fever in members of households in the lower tertile of Better WASH household prevalence was 2.07/1000 PY, in the middle tertile incidence was 1.41/1000 PY, and in the upper tertile incidence was 0.99/1000 PY ($p<0.001$). Relative to the lower tertile of Better WASH household prevalence, residence in the middle tertile was associated with an adjusted PE of 17% (95% CI: -32, 48; $p=0.43$), and residence in the upper tertile was associated with an adjusted PE of -19% (95% CI: -96, 27; $p=0.49$). In models

that expressed Better WASH prevalence on a continuous scale, each 1% increase in Better WASH prevalence was associated with an adjusted PE of -0.02% (95% CI: -1.43, 1.37; p=0.98) (Table 6-2). Evidence for interaction between Better WASH coverage and ViPS was not observed (p=0.222).

6.3.2 Incidence of typhoid by vaccination and household WASH status

Typhoid incidence was lowest in members of ViPS clusters residing in households with Better WASH (0.68/1000 PY), highest in members of HepA clusters residing in households with Not Better WASH (2.37/1000 PY), and intermediate in members of ViPS clusters residing in Not Better WASH households (0.89/1000 PY) and in members of HepA clusters residing in Better WASH households (0.89/1000 PY, p<0.001 for heterogeneity of incidence in the four groups) (Table 6-3).

6.3.3Protection by vaccine assignment in the cluster of residence according to Better WASH in the household and prevalence of Better WASH in the clusters

In members of Better WASH households, residence in ViPS clusters was associated with an adjusted PE of 58% (95% CI: 14, 79; p<0.05). Similarly, in members of Not Better WASH households, residence in ViPS clusters was associated with an adjusted PE of 61% (95% CI: 44, 73; p<0.001) (Table 6-3). Residence in the ViPS clusters was associated with an adjusted PE of 67% (95% CI: 27, 85; p<0.01) for clusters in the lowest tertile of Better WASH

prevalence; 48% (95% CI: 6, 71; p<0.05) in the middle tertile; and 50% (95% CI: 10, 73; p<0.05) in the highest tertile (Table 6-4).

6.3.4 Protective impact of Better WASH in the household and prevalence of 6.3.4 Better WASH in the clusters according to vaccine assignment of the cluster of residence

In ViPS clusters, residence in a Better WASH household failed to show a protective association with the risk of typhoid (adjusted PE of 6%; 95% CI: -82, 51; p=0.85) (Table 6-3), and this absence of a protective association was seen both for recipients of ViPS (adjusted PE 16%; 95% CI: -79, 61; p=0.65) and non-recipients of ViPS (adjusted PE of -3%; 95% CI: -222, 67; p=0.96). In contrast, in HepA clusters, residence in a Better WASH household was associated with an adjusted PE of 48% (95% CI: -1, 73; p=0.05) (Table 6-3).

In ViPS clusters, relative to residence in the lower tertile of Better WASH household prevalence, residence in the middle tertile was associated with an adjusted PE of -50% (95%CI: -177, 19; p=0.20), and residence in the upper tertile was associated with an adjusted PE of -55% (95% CI: -303, 40; p=0.37) (Table 6-4). In HepA clusters, relative to residence in the lower tertile of Better WASH household prevalence, residence in the middle tertile was associated with an adjusted PE of 25% (95% CI: -45, 61; p=0.39), and residence in the upper tertile was associated with an adjusted PE of -5% (95% CI: -86, 41; p=0.87) (Table 6-4). In models that expressed Better WASH prevalence on a continuous scale, in ViPS clusters, each 1% increase in Better WASH

prevalence was associated with an adjusted PE of -0.58% (95% CI: -2.50, 1.30; p=0.55), while in HepA clusters, each 1% increase was associated with an adjusted PE of 0.96% (95% CI: -1.00, 2.88; p=0.33) (Table 6-4). The presence of collinearity prevented simultaneous assessment of household WASH and household WASH prevalence.

6.3.5 Further evaluation of the association of Better WASH in the household and typhoid risk in a population not vaccinated with ViPS

Because the association between Better WASH in household and the risk of typhoid in household residents in the HepA clusters was of borderline (p=0.05) statistical significance, I assessed the relationship in the 57 949 non-vaccinated residents of the study area followed during the two years before the inception of the vaccine trial. These residents were followed with the same procedures and with the same data collection instruments (including the same definition of Better WASH in the household) as those used during the trial. During this lead-in period, the incidence of typhoid fever in members of Better WASH households was 0.46/1000 PY and in Not Better households, incidence was 1.36/1000 PY ($p<0.05$). After adjustment for potential confounding variables, a 57% reduction of typhoid risk was observed in residents of households with Better WASH compared with residents in households with Not Better WASH (95%CI: 15, 78; $p<0.05$).

Table 6-1 Protective effectiveness of ViPS vaccine, Better household WASH, and Better household WASH prevalence against typhoid

	N	PY	Typhoid episodes	IR (per 10^4 PY) ‡	Protective effectiveness (PE)	
					Crude PE (95% CI)	Adj. § PE (95% CI)
ViPS vaccine cluster	31 075	59 397	50	0.84	60%* (39, 74)	55%* (35, 69)‖
HepA vaccine cluster	31 681	60 387	128	2.12		
Better WASH households	12 937	24 724	19	0.77	54%† (22, 73)	31% (-16, 59)¶
Not Better WASH households	49 819	95 059	159	1.67		
Better WASH prevalence (lower tertile)**	20 486	39 063	81	2.07	Ref.	Ref.
Better WASH prevalence (middle tertile)**	21 164	40 306	57	1.41	32% (-17, 60)	17% (-32, 48)¶
Better WASH prevalence (upper tertile)**	21 106	40 414	40	0.99	52%† (19, 72)	-19% (-96, 27)¶

*p-value<0.001; †p-value<0.01

‡IR, incidence rate; PY, person years

§All models were adjusted for cluster stratification variables (ward [Ward 29, Ward 30]; number of residents ≤18 years [<200 persons, ≥200 persons]; number of residents >18 years [<500 persons, ≥500 persons]), design effect and selected covariates

‖Model adjusted for covariates: household WASH, age, Hindu religion, longer distance to the nearest treatment centre than median

¶Model adjusted for covariates: vaccine assignment, age, Hindu religion, longer distance to the nearest treatment centre than median

**Lower (<6% prevalence); middle (6-26% prevalence); upper (≥26% prevalence)

Note: When evaluated in models including ViPS, WASH measured as either household WASH or Better WASH coverage, stratifying variables and covariates, there was no statistical evidence for interaction between household WASH and ViPS cluster (p=0.331) or between Better household WASH coverage and ViPS cluster (p=0.222). In models that expressed Better WASH prevalence on a continuous scale, each 1% increase in Better WASH prevalence was associated with a preventive impact against typhoid of 1.73% (95% CI: 0.32, 3.12; p=0.02) (crude) and -0.02% (95% CI: -1.43, 1.37; p=0.98) (adjusted)¶

Table 6-2 Protective effectiveness of ViPS and Better household WASH stratified by WASH and vaccine status

	ViPS cluster			Hep A cluster			ViPS Protective effectiveness (PE)	
	N	PY	Typhoid (IR per 10^4 PY)[§]	N	PY	Typhoid (IR per 10^4 PY)[§]	Crude PE (95% CI)	Adj.[†] PE (95% CI)
Not Better WASH	23407	44755	40 (0.9)	26412	50304	119 (2.4)	62%* (44, 75)	61%* (44, 73)[¶]
Better WASH	7668	14642	10 (0.7)	5269	10082	9 (0.9)	23% (-108, 72)	58%[‡] (14, 79)[**]
Better WASH PE (95%CI)	Crude PE = 24% (-62, 64)			Crude PE = 62%[†] (30, 80)				
	Adj. PE = 6% (-82, 51)[††]			Adj. PE = 48% (-1, 73)[¶]				

*p-value<0.001; [†]p-value<0.01; [‡]p-value<0.05

[§]IR, incidence rate; PY, person years

[‖]All models were adjusted for cluster stratification variables (ward [Ward 29, Ward 30]; number of residents ≤18 years [<200 persons, ≥200 persons]; number of residents >18 years [<500 persons, ≥500 persons]), design effect and selected covariates

[¶]Model adjusted for covariates: age, household population size, longer distance to the nearest treatment centre than median

[**]Model adjusted for covariates: age, religion, longer distance to the nearest treatment centre than median

[††]Model adjusted for covariates: age, household monthly expenditure

Table 6-3 Protective effectiveness of ViPS and Better household WASH prevalence stratified by WASH prevalence tertile and vaccine status

	ViPS cluster			Hep A cluster			ViPS Protective effectiveness (PE)	
WASH Prevalence	N	PY	Typhoid (IR per 1000PY)[§]	N	PY	Typhoid (IR per 1000PY)[§]	Crude PE (95%CI)	Adj.[‖] PE (95%CI)
Lower tertile[¶]	7673	14612	9 (0.6)	12813	24452	72 (2.9)	79%* (62, 89)	67%[†] (27, 85)**
Middle tertile[¶]	9615	18394	19 (1.0)	11549	21912	38 (1.7)	40% (-19, 70)	48%[‡] (6, 71)[††]
Upper tertile[¶]	13787	26391	22 (0.8)	7319	14023	18 (1.3)	35% (-27, 67)	50%[‡] (10, 73)[‡‡]
Middle vs. Lower PE, (95% CI)	Crude PE = -68% (-231, 15)			Crude PE =41% (-9, 68)				
	Adj. PE = -50% (-177,19)[‡‡]			Adj. PE =25% (-45, 61)[§§]				
Upper vs. Lower PE, (95% CI)	Crude PE = -35% (-175, 33)			Crude PE =56%[†] (24, 75)				
	Adj. PE = -55% (-303, 40)[‡‡]			Adj. PE =-5% (-86, 41)[§§]				

*p-value<0.001; [†]p-value<0.01; [‡]p-value<0.05

[§]IR, incidence rate; PY, person years;

[‖]All models were adjusted for cluster stratification variables (ward [Ward 29, Ward 30]; number of residents ≤18 years [<200 persons, ≥200 persons]; number of residents >18 years [<500 persons, ≥500 persons]), design effect and selected covariates

[¶]Lower (<6% prevalence); middle (6-26% prevalence); upper (≥26% prevalence)

**Model adjusted for covariates: age, Hindu religion, household population size, longer distance to the nearest treatment centre than median

[††]Model adjusted for covariates: age, household population size, household monthly expenditure

‡‡Model adjusted for covariates: age, household monthly expenditure

§§Model adjusted for covariates: age, household population size, longer distance to the nearest treatment centre than median

Note: In models that expressed Better WASH prevalence on a continuous scale, in the ViPS clusters, each 1% increase in Better WASH prevalence was associated with a preventive impact against typhoid of -0.06% (95%CI: -1.59, 1.45; p=.94) (crude) and -0.58% (95% CI: -2.50, 1.30; p=0.55) (adjusted)‡‡. In HepA clusters, each 1% increase in Better WASH prevalence was associated with a preventive impact against typhoid of 2.52% (95% CI: 0.93, 4.08; p<0.01) (crude) and 0.96% (95%CI: -1.00, 2.88; p=0.3) (adjusted)§§.

6.4 Discussion

In this trial, living in a cluster vaccinated with ViPS was associated with protection against typhoid, and the level of protection was similar regardless of whether or not the individual lived in a household with Better WASH. Conversely, living in a Better WASH household was associated with suggestively reduced typhoid risk among members of all the clusters, but this reduction was only evident in clusters that received HepA, a finding consistent with protection observed in members of households with Better WASH in the population followed during two years of surveillance preceding the trial. A higher prevalence of Better WASH households in the cluster was not associated with a reduced typhoid risk overall in all clusters, or in the ViPS and HepA clusters considered separately.

Before discussing the implications of these findings, it is important to consider the limitations of our study. First, the variable classifying households as having Better or Not Better WASH was constructed using simple variables collected at baseline, which may not have fully captured, or might not even have served as proxies for the WASH behaviours and facilities responsible for the reduction of risk of typhoid in the household. As a result, the household WASH classification variable may have underestimated the true impact of household WASH against typhoid. Second, the definition that I used for Better WASH may be idiosyncratic to the population under study and may not be generalizable to other settings. Nonetheless, the findings support the concept that Better WASH in the household occurring spontaneously without external intervention was associated

with a reduced risk of typhoid in the slums participating in the trial. Third, although the trial cluster-randomized vaccination, it did not randomize WASH. However, I controlled for potentially confounding variables in our analyses. Fourth, due to collinearity of household-level WASH and neighbourhood WASH prevalence, it was not possible to evaluate the independent protection associated with these two variables when both were considered simultaneously in our analyses. Finally, these findings, while elucidating the separate and joint impacts of ViPS and WASH in the setting for the study, may or may not apply to other settings that have different WASH practices or to different typhoid vaccines.

Conversely, these analyses also have several important strengths. The analyses were conducted on data collected in the context of a prospective CRT of a well-defined population under comprehensive follow up for typhoid. The definition of Better household WASH was developed and validated in the same study population, as is detailed in Chapter 5. As well, typhoid was detected with clear clinical criteria including blood culture confirmation, and diagnoses were made without knowledge of either vaccination or household WASH status.

This analysis produced two unexpected observations. First, the protective association of Better household WASH was absent in clusters assigned to ViPS, suggesting no additional benefit of WASH in a vaccinated population. That living in a household with Better WASH was not associated with a lower risk of typhoid in clusters targeted for ViPS, but was in those targeted for HepA, might be related to the observation that ViPS was earlier shown to confer substantial herd

protection in this trial (PE=44%, 95% CI: 2, 69) (77), reflecting a reduction of the intensity of typhoid transmission in the ViPS clusters. It is possible that the reduction of typhoid transmission in the Better WASH households resulted in less of a reduction of typhoid disease incidence in the lower transmission settings of the ViPS clusters due to the shape of the curve relating the size of the ingested inoculum of typhoid and the risk of typhoid disease (52). However, as the interaction term between ViPS and WASH variables in this analysis was not significant, lack of statistical heterogeneity indicates that apparent differences in typhoid risk in the vaccine groups may rather be due to random variation and small sample size and not the modification of WASH effects by vaccination status. Second, protection associated with ViPS was similar irrespective of household WASH status. I anticipated that the protective association of Better household WASH, mediated by reducing exposure to the infectious agent within the household, would enhance the protective effects of the vaccine. However, this observation may be consistent with the similar levels of ViPS protection recorded in randomized trials done in Nepal, South Africa, and China (40, 41, 77), sites with different rates of typhoid infection, possibly mimicking settings where exposure to typhoid varies due to the presence or absence of improved WASH.

In aggregate, these findings suggest that minor improvements in WASH, which already existed within this poor urban slum study setting and are therefore affordable and attainable, were associated with a substantial reduction in the risk of typhoid in the population that had not received an effective typhoid vaccine. However, these improvements failed to further reduce the risk of

typhoid in a population that had received an effective typhoid vaccine. While these observations do not argue against possible combined protection against endemic typhoid by well-designed WASH interventions and effective typhoid vaccines, the data do not support the assertion that naturally occurring improvements of household WASH added to the protection by ViPS vaccine in this urban slum setting. Although ViPS is no longer the primary recommended vaccine for developing countries, the unexpected results underscore the importance of evaluating the combined and independent effects of WASH and effective typhoid vaccines, in particular Vi conjugate vaccines, which are now approved and recommended for use in endemic settings.

Chapter 7 General Discussion

V. cholerae and *S*. Typhi bacteria are ancient organisms and are known to have caused disease in humans for many centuries. The biology, epidemiology, and pathophysiology of these organisms are well studied. Multiple generations of vaccines against both targets have been developed and assessed for safety and efficacy, and these agents are now widely used across the world in endemic settings. The analyses presented in the preceding chapters exploit datasets generated in field studies of vaccines against both cholera and typhoid. At minimum, these analyses demonstrate the continued utility of systematically collected data from populations where robust demographic data is available. Given the high cost of conducting clinical studies, extending analyses of archived datasets beyond the scope of the trial's stated objectives presents interesting and inexpensive research opportunities.

7.1 Use of a vaccine probe design to evaluate conventional diagnostics

In the assessment of cholera culture diagnostic sensitivity, discussed in Chapter 2, OCV was used as a probe to determine if there was an appreciable burden of cholera culture-negative diarrhoea that were otherwise compatible with clinical cholera (i.e. acute watery diarrhoea). Cholera clinical disease manifestation is specific and consistent across broad population groups, and although stool culture diagnostics have not been a source of great contention up until now, sensitive molecular techniques that detect fragments of pathogenic DNA have challenged conventional microbiological techniques to detect presence of

cholera in stool. Typically, presence of pathogenic biological material in a biological specimen is evidence to implicate that organism as the cause of disease. However, in disease endemic areas wherein the population has high and extended exposure to circulating pathogens, the likelihood of asymptomatic infection and chronic carriage is increased. Advanced techniques such as multiplex serological assays capture a snapshot of previous exposures based on existing antibodies. These assays may, in some cases, over-attribute disease aetiology based on past exposure. The vaccine probe design is an important public health tool to evaluate *disease*, and in the case of the analysis in Chapter 2, decipher whether the detection of fragments of residual vibrios in culture-negative diarrhoeal patients contributed to diarrhoeal illness. In the analysis there was no evidence for cholera diarrhoea that was not identified by culture. Since estimates of global burden and the measure of vaccine protection against cholera rely on culture diagnostics for cholera confirmation, this outcome supports the integrity of past findings.

One advantage of a vaccine probe study design is the ability to quantify the number of cases prevented by a vaccine intervention. Similarly, assuming that a vaccine of known effectiveness does not offer protection against other pathogens apart from the vaccine target, then the reduction of a particular disease syndrome associated with an infectious disease can be attributed to the vaccine under investigation. This is particularly useful when diagnostic tests are insensitive or unavailable. The analysis described in Chapter 3 uses a ViPS vaccine against typhoid to confirm a burden of typhoid fever that was not detected by culture diagnostics. In this study, although vaccine protection

against clinical typhoid was lower compared to culture-confirmed typhoid, measured protection, per se, indicated that development of a predictive profile for typhoid fever based on clinical assessments is possible, albeit in this case lacking specificity. In LMIC settings where febrile presentation is common and the ability to discriminate between different prevalent fever aetiologies of disease is low, a tool such as this clinical rule for predicting typhoid that can be employed at point-of-care is valuable.

7.2 Protection against typhoid by natural infection and typhoid vaccines

A vaccine induces protection against its target by mimicking its natural interaction with the human immune system. Unlike cholera, for which it is well-established that natural infections confer substantial protective immunity in endemic settings (48-50), an observation that has motivated development of live OCVs, there is limited evidence on the natural protection conferred by typhoid infections against future typhoid in endemic settings. This knowledge gap is important. From a policy perspective, predictions from dynamic transmission models of typhoid, including predictions of typhoid vaccine impact, depend on accurate estimates of the degree of such natural protection. Accurate predictions by such models are critical to informing policy about typhoid vaccine introduction. In Chapter 3, I investigated the protective properties of an initial typhoid infection against subsequent typhoid during four years of observation in Kolkata, India. The data did not suggest a high degree of protection conferred by a natural infection. There are several plausible explanations as to why a natural infection does not confer protection against future typhoid disease. Evidence in

the literature suggests several mechanisms of action including gene regulation (135), different baseline levels of regulatory T cells based on previous exposure to other *Enterobacteraciae* (138), and host genomic factors (135, 137). Evidence from a challenge study where previously infected individuals were subject to a secondary typhoid challenge suggested also that there was little to no degree of protection conferred by previous exposure, although the typhi strains used were modified from wild-type (150). Several groups claim that *Salmonella* infection actively suppresses immunity, although from an immunological perspective in humans it is difficult to prove this observation (151, 152). Despite these aligned observations, failure of natural protection does not necessarily imply that vaccines targeting typhoid will be ineffective; as described earlier, there are multiple effective vaccines that make use of different *S.* typhi epitopes in existence. However, vaccination programmes, particularly with recently approved TCVs, may consider modifying dosing approaches in highly-endemic settings.

7.3 Combined approaches using WASH and vaccination to combatting infectious disease

Although proponents of WASH and vaccines will each contend their relative importance to public health, neither camp will dispute that each intervention is independently effective at reducing the risk of infectious disease transmission. The WHO recommends the combined implementation of WASH and vaccine for both cholera and typhoid control programs (153, 154). However, it was not always believed that the two interventions would act complementarily. For

example, in cholera endemic countries, it was thought that the introduction of OCV may have a negative impact on national implementation of ORTs and distract from ongoing WASH programs. Additionally, protection afforded by an effective vaccine may interfere with incentives to uphold good hygiene practices and any political momentum towards major investment in improving WASH infrastructure would be lost. These concerns have long been dismissed. The relationship between WASH and typhoid fever, however, has not been as clearly illustrated. Understanding of risk factors contributing to typhoid fever risk and adherence to preventative WASH behaviours was disappointingly low in Malawi and Fiji, typhoid endemic countries that experience frequent outbreaks (155, 156). Nonetheless, the theory that WASH advancements will reduce exposure to the inoculum and thus enhance the ability of vaccines to prevent infection and disease has already influenced typhoid control programs.

To my knowledge, the analysis performed in Chapter 6 is the first attempt to evaluate the synergistic effects of WASH and ViPS vaccine administered together in a typhoid endemic community. I found household WASH to reduce typhoid risk, but surprisingly, only in residents of clusters that were not assigned to ViPS vaccine. This suggests that while improved household WASH is independently associated with reduced typhoid risk, the marginal benefits of WASH do not add to protection offered by vaccine, as was initially expected.

These findings should not be interpreted as a challenge to combined approaches of WASH and vaccination, but they suggest that additional studies using highly specific definitions of WASH should be undertaken. Importantly, this

analysis did not attempt to measure the external benefits of WASH improvement on health metrics apart from clinical typhoid fever, therefore the public health value of these approaches to control typhoid cannot be interpreted in isolation.

Institution of major water and sanitation infrastructure improvements, as well as enforcement of hygienic standards, have largely eliminated indigenous typhoid and cholera transmission in the industrialized world. However, in the low-income settings, improvements in WASH attainable in the near future fall far short of the major improvements implemented in industrialized countries. At the same time, natural variation in WASH quality and coverage between households in communities in low-income settings can be sufficient to impact the transmission of typhoid. These findings provide an indication of impact on infectious diseases that can be expected with similar WASH improvements that can be feasibly implemented in the near future.

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