IMPACT OF A VI-POLYSACCHARIDE CONJUGATE VACCINE IN PREVENTING TYPHOID FEVER IN AN ENDEMIC SETTING, LALITPUR, NEPAL

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Declaration and Contribution

The work presented in this thesis was performed by the author and are solely the authors own work.

A large team from Oxford Vaccine Group, University of Oxford, Oxford University Clinical Research Unit, Patan Hospital and Patan Academy of Health Sciences has been involved from the design and delivery of this trial. Professor Sir Andrew J Pollard, Professor. Merryn Voysey, Dr. Katherine Theiss-Nyland, Professor. Kathleen M Neuzil and the author designed the study in collaboration with the larger TyVAC consortium. Dr. Merryn Voysey and Dr. Xinxue Liu designed the study analysis plan. Professor Sir Andrew J Pollard, Professor Dr. Buddha Basnyat and Professor Dr. Shrijana Shrestha provided the study oversight. The author, along with Rachel Colin-Jones and Sarah Kelly led the implementation of the study. Dr. Dikshya Pant, Dr. Nicola Smith and Dr. Peter O'Reilly led the clinical team that ensured clinical support to the participants. Susan Tonks monitored the study and Olga Mazur and Yama Farooq led data management for the study. The field coordination for the study was supported by Mr. Anup Adhikari and team at Nepal Family Development Foundation. The local core team the oversaw the study activities including public engagement, vaccine logistics, data management, quality assurance and clinical staff coordination included Ashata Dahal, Naheeda Haque, Archana Maharjan, Anisha Pradhan, Manij Joshi and Dr. Suchita Shrestha. The delivery of the trial included a team of over 100 staff including Medical Officers, Nurses, Health Assistants,

CMAs and Community Health Workers (THPs). The author conducted the statistical analysis with oversight provided by Professor. Merryn Voysey. The on-site administrative support for the study was provided by the administrative staff at Oxford University Clinical Research Unit, Patan Hospital and Patan Academy of Health Sciences.

Blood cultures for the study were processed by the Microbiology Laboratory at Patan Hospital. Dr. Sabina Dongol provided laboratory oversight. The on-site sample management was coordinated by Rajendra Shrestha and Bijaya Karanjit. The ELISAs tests were coordinated by and the data of the ELISAs were generated by Dr. Jennifer Hill, Dr. Jenny Clarke, Lisa Stockdale, Elizabeth Jones and the OVG lab team. Simon Evans from the Oxford University Hospitals NHS Foundation Trust Microbiology Department coordinated the antimicrobial susceptibility tests.

Abstract

Globally, an estimated 11 to 21 million cases of febrile illness and 117,000 to 161,000 deaths are attributed to typhoid fever each year. Typhoid fever is prevalent mainly in low-resource settings with poor water, and inadequate sanitation and hygiene facilities, with South Asia accounting for a large proportion of the burden. Children bear a substantial burden of the disease. Increasing antimicrobial resistance threatens to limit treatment options for typhoid fever. The WHO, in 2018, recommended the use of a new generation vaccine, typhoid conjugate vaccine (TCV), for the control of typhoid fever in endemic regions; however, data from typhoid-endemic settings were lacking. A randomized controlled trial was therefore conducted in Lalitpur, Nepal, to assess the impact of TCV.

In this phase III participant-observer-blind randomized controlled trial, children aged 9 months to under-16 years old, were individually randomized (1:1) to either receive the TCV (Typbar-TCV, Bharat Biotech) or a capsular group A meningococcal conjugate vaccine (MenA) as control, and were followed-up for two years.

The protective efficacy of TCV against blood culture-confirmed typhoid fever at two years was 79.0% (95% confidence interval (CI): 61.9%, 88.5%; P<0.001). Administration of TCV reduced the incidence of typhoid fever (72 (95% CI: 38,123) cases per 100,000 person-

years) compared with the control group (Incidence (MenA group): 342 (95% CI 262, 438) cases per 100,000 person-years).

The results of this trial represent one of the first findings confirming that TCV is efficacious in a setting of high endemicity. As a direct result of the findings of this trial and with GAVI support, TCV introduction has been implemented in policy in Nepal. TCV is now routinely administered to children at 15 months of age as part of the national immunization schedule. In this thesis, I describe the design, implementation and results of the trial conducted in Nepal.

Table of Contents

List of Figures

List of Tables

Abbreviations

AEFI: Adverse Event Following Immunization

AESI: Adverse Event of Special Interest

CHIM: Controlled Human Infection Model

CI: Confidence Interval

CoP: Correlates of protection

CLT: Control

CRF: Case report forms

CRP: C-Reactive Protein

DALY: Disability-Adjusted Life Years

DSMB: Data Safety and Monitoring Board

DTaP: Diphtheria, tetanus and acellular pertussis (Vaccine)

DTP: Diphtheria, tetanus and pertussis (Vaccine)

ELISA: Enzyme-Linked Immunosorbent Assay

EPI: Expanded Program on Immunization

ESBL: Extended Spectrum Beta Lactamase

EU: ELISA units

FBS: Fetal Bovine Serum

GAVI: Global Vaccine Alliance

GACVS: Global Advisory Committee on Vaccine Safety

GBD: Global Burden of Disease

GMT: Geometric Mean Titres

IHME: Institute for Health Metrics and Evaluation

IQR: Interquartile range

IRR: Incidence rate ratio

LMC: Lalitpur Metropolitan City

LMIC: Low- and Middle-Income Countries

LPS: Lipopolysaccharide

MIC: Minimum Inhibitory Concentration

MDR: Multidrug Resistant

MenA: Meningococcal serogroup A Conjugate Vaccine

MedDRA: Medical Dictionary for Regulatory Activities

MMR: Measles, mumps and rubella (vaccine)

NIBSC: National Institute for Biological Standards and Control

NTS: Non-Typhoidal Salmonella

OUCRU-PH: Oxford University Clinical Research Unit, Patan Hospital

QRDR: Quinolone-Resistance-Determining Regions

RCT: Randomized Controlled Trial

RDT: Rapid Diagnostic Test

SAE: Serious Adverse Event

SAGE: Strategic Advisory Group of Experts

SAR: Serious Adverse Reaction

SEAP: Surveillance of Enteric Fever in Asia Project

SEFI: Surveillance of Enteric Fever in India

SETA: Severe Typhoid Fever Surveillance in Africa

SD: Standard Deviation

SNP: Single-Nucleotide Polymorphisms

STD: Standard

STRATAA: Strategic Typhoid Alliance Across Africa and Asia

SDG: Sustainable Development Goal

TCV: Typhoid Conjugate Vaccine

THP: Tole Health Promoter

TIP: Typhoid Intestinal Perforation

TSAP: Typhoid Fever Surveillance in Africa Program

TyVAC: Typhoid Vaccine Acceleration Consortium

UI: Uncertainty Interval

UNICEF: United Nations Children's Fund

Vi-CRM197: Vi-polysaccharide derived from Citrobacter conjugated to CRM197

VI-DT: Vi-polysaccharide conjugated to diphtheria toxoid

Vi-PS: Vi-polysaccharide

Vi-rEPA: Vi-polysaccharide conjugated to recombinant Pseudomonas aeruginosa exoprotein A

Vi-TT: Vi- polysaccharide conjugated to tetanus toxoid

VE: Vaccine Efficacy

WASH: Water, Sanitation and Hygiene

WBC: White Blood Cell

WHIC: Ward Health Implementation Committee

WHO: World Health Organization

WHO-IPD: World Health Organization -immunization Preventable Diseases

XDR: Extensively Drug Resistant

1. Introduction

1.1 Introduction

Typhoid fever is an acute systemic infection caused by *Salmonella enterica* serovar Typhi. *Salmonella* serovars Paratyphi A, and occasionally B and C cause a similar systemic illness. The term enteric fever refers to both typhoid and paratyphoid fever.

1.2 Salmonella

Salmonella are Gram negative facultative anaerobic bacilli belonging to the *Enterobacteriaceae* family. The genus *Salmonella* is divided in to two species, *S. bongori* and *S. enterica*[1]. *S. enterica* is divided into 6 subspecies, of which all human and animal infections are caused by *S. enterica* subspecies *enterica*. The subspecies enterica is split into typhoidal and non-typhoidal based on the disease syndrome. Typhoidal *Salmonella* grow only in humans and can cause typhoid and paratyphoid fevers. Non-typhoidal *Salmonella* (NTS) strains cause non-invasive, self-limiting gastroenteritis, but some can invade the blood stream leading to bacteremia, and commonly includes *S. enterica* serotype Enteritidis and *S.* Typhimurium.

Analysis of surface antigen or serotype analysis and determination of biochemical properties are commonly used to differentiate the *Salmonella* strains. Combined with the

biochemical properties, the Kauffman-White serological scheme is used to classify Salmonella according to serotypes or serovars. These have been described in detail in sections 1.7.4 and 1.7.5.

1.3 Transmission of Typhoid Fever

S. Typhi is a human-restricted pathogen, and therefore not a zoonosis. It is transmitted by the feco-oral route through ingestion of food and water contaminated by human feces.

Transmission of typhoid fever is divided into two broad groups[2]. The terms short-cycle and long-cycle are used to denote direct and indirect transmission respectively. Contamination of food and water by fecal shedding in the immediate environment, resulting in direct transmission due to inadequate hygiene and sanitation measures, is defined as the short- cycle transmission. Contamination of the broader environment such as pollution of water supply by human feces that then contaminate food and water is defined as the long- transmission cycle. Breaking the transmission cycles is necessary for the control of typhoid fever.

There is evidence from multiple studies showing unimproved or unsafe water is the cause of transmission of typhoid fever. Mogasale and colleagues conducted a systematic review and meta-analysis to measure the risk of typhoid fever associated with drinking

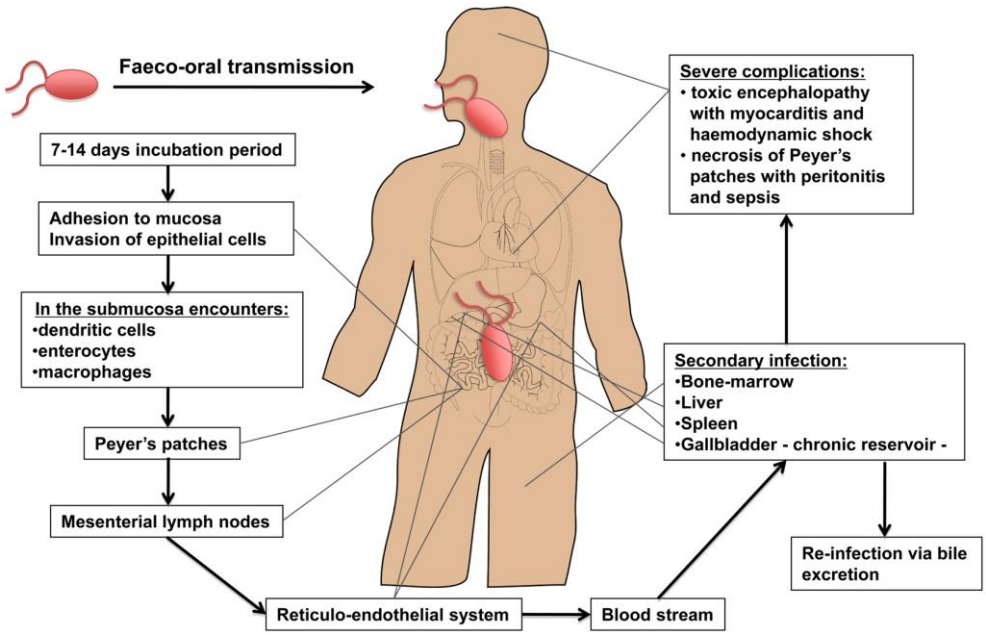
unimproved water or microbiologically unsafe water[3]. Analysis of 12 cases-control studies found that the odds of typhoid fever among those exposed to unimproved water or microbiologically unsafe water ranged from 1.06 to 9.26 with a case weighted mean of 2.44 (95% Confidence Interval (CI): 1.65 – 3.59). A matched case-control study conducted by Oxford University Clinical Research Unit at Patan Hospital (OUCRU-PH), Lalitpur, Nepal, a setting endemic to typhoid fever, including 103 culture-positive enteric fever patients and 294 community-based controls found that drinking stone spout water contaminated with sewage, drinking contaminated stored water and general lack of sanitary hygiene such as the lack of toilets or the lack of water to flush toilets was associated with typhoid fever[4]. Drinking piped water close to open drainage and with intermittent water supply[5], drinking untreated/ unchlorinated water[6], consumption of raw food irrigated with sewage water[7] have all been associated with typhoid fever from other studies in endemic areas. Similarly, hygiene practices including lack of use of soap for handwashing, lack of toilet in the household, crowded households and sharing food have also been identified as risk factors for typhoid fever[8], [9].

1.4 Pathogenesis

After ingestion of fecally contaminated food or water, the disease manifests in a susceptible host (Figure 1). The length of the incubation period and the risk of infection depend on the ingested dose of the bacteria[10]. Challenge studies in volunteers have shown that the minimum infectious dose of *S.* Typhi is as low as 1000 bacteria[11]. Once *S.* Typhi survive the gastric acid barrier, the bacteria access the submucosa in the terminal ileum, via direct penetration into the epithelial cells or via the M (microfold) cells that

serves as the antigen presenting cells. *S.* Typhi exploits the M cells as a route for host invasion[12]. The local mucosal inflammatory response is not normally triggered. *S.* Typhi then invades and leads to the hypertrophy of the intestinal lymphoid follicles, Peyer's patches, via recruitment of mononuclear cells and lymphocytes. Under normal circumstances, the presence of a foreign body in host cells would lead to activation of host cell immune response that would lead to enzymatic degradation of the bacteria[13]. However, *S.* Typhi is capable of intracellular survival and replication. *S.* Typhi contained within the macrophages disseminate from the Peyer's patches through a silent primary bacteremia via lymphatic system and blood stream and replicate in the reticuloendothelial system [14,12]. Eventually, the bacteria reside in the tissue macrophages in the liver, spleen and bone marrow, resulting in secondary bacteremia manifesting in the onset of symptoms and the clinical disease.

Figure 1. The dissemination of *S.* Typhi during systemic infection



Reproduced under CC BY from *de Jong et al*, Host–Pathogen Interaction in Invasive Salmonellosis. PLoS Pathogen 2012; 8(10): e1002933. https://doi.org/10.1371/journal.ppat.1002933

1.5 Clinical Typhoid Fever

1.5.1 Clinical Manifestation of Uncomplicated Typhoid Fever

The incubation period for a *S*. Typhi infection is usually 8 to 14 days, but ranges from 3 to 60 days depending on the number of organisms ingested and as identified from human experimental studies and outbreaks with well-defined point studies[14]. The onset of bacteremia is marked by fever and malaise. Fever is initially low grade but progressively rises to high grade fever in the first week of illness, and has been described as a step-ladder pattern fever. Flu-like symptoms, chills and headache are common symptoms. Patients also present with other non-specific symptoms including malaise, anorexia, abdominal pain, dry cough, myalgia and diarrhea or constipation. Physical signs may include a coated tongue, abdominal tenderness along with hepatomegaly and/or splenomegaly. Relative bradycardia may be present. Rose spots have been reported, but usually are not seen in dark-skinned patients.

1.5.2 Complications of Typhoid Fever

Left untreated or without proper treatment, it can lead to gastrointestinal hemorrhage, typhoid intestinal perforation (TIP), hepatitis, cholecystitis, myocarditis, shock, encephalopathy, anemia and even death[15]. Gastrointestinal hemorrhage, and TIP have been further described below.

1.5.2.1 Gastrointestinal Hemorrhage

Gastrointestinal hemorrhage occurs due to erosion of a necrotic Peyer's patch overlying an enteric vessel[14]. It can occur in up to 10% of the patients, bleeding is light and usually resolves without the need of a transfusion. However, in 1-2%, there can be significant bleeding if a large enteric vessel is eroded and can be fatal[14].

1.5.2.2 Typhoid Intestinal Perforation (TIP)

TIP is the most serious complication, occurs in 1 – 3 % of hospitalized patients and requires surgical intervention[14,16]. It usually occurs in the Peyer's patches of the terminal ileum and occurs by the third week of the onset of the illness when left untreated or the treatment is inadequate[16]. A study conducted in 27 hospitalized patients with a clinical diagnosis of gastrointestinal perforation secondary to suspected typhoid fever found that abdominal pain with signs of peritonitis, fever, headache and vomiting with raised white cells counts and neutrophil leukocytosis were the most common presentations[17]. A recent study from India surveilling nontraumatic ileal perforations found that enteric fever was the most common cause[16]. Among those identified as enteric ileal perforation, the case fatality rate was 7.1%[16].

1.6 Relapses and Carriage

Relapses can occur if *S.* Typhi persists in the reticuloendothelial system. Relapses occur in 5 - 10% of patients and usually occur 2 to 3 weeks after defervescence[14]. The *S.* Typhi isolate has the same antibiotic susceptibility pattern as in the first episode.

After an acute illness, some patients become carriers. Carriage is divided into three time periods: convalescent, temporary and chronic[18]. Convalescent carriers shed *S*. Typhi in stool from three weeks to three months after an acute illness[2], [18]. Temporary carriers shed *S.* Typhi between three to twelve months after illness. *S*. Typhi are not fully cleared from the body within one year of acute illness in around 2 – 5% of typhoid patients. Those who shed the bacilli for more than one year are known as chronic carriers[14]. However, up to 25% of chronic carriers have no history of previous disease[14]. In chronic carriage, *S.* Typhi penetrates the intestinal epithelial barrier, successfully evades the innate immune mechanism and colonizes in the gall bladder[18]. Carriers intermittently shed the bacteria in stool and into the environment. The role of convalescent, temporary and chronic carriers as reservoirs and their role in transmission and driving new infections is not fully elucidated. However, it is suggested that the transmission is mostly driven by recent infections than by chronic carriers based on epidemiological studies showing disease transmission to household contacts by recently infected patients[18].

Chronic carriage develops following a gall bladder disease, hence likelihood of becoming a chronic carrier increases with age[19]. Chronic carriage in cholecystectomy patients from

a study conducted by OUCRU-PH showed that strains of *Salmonella* isolates from the gallbladder were different from the strains causing acute infection[20]. While over 80% nalidixic resistance was observed in invasive *Salmonella* isolates from febrile patients with typhoid fever, only one of 48 *Salmonella* isolated from gallbladder was resistant to nalidixic acid. The results suggest that chronic carriers act as reservoirs that maintain the local strains in endemic regions and are likely to have limited role in acute infection. In addition, the OUCRU-PH study identified a 5.4% carriage rate of invasive Salmonella in a population of 404 patients[21].

1.7 Diagnosis

1.7.1 Blood Culture

Isolation of *S.* Typhi from blood culture is the gold standard for the diagnosis of typhoid fever. Blood culture sensitivity was estimated to be 61% (95% CI 52 – 70) according to a systematic review[22]. There is heterogenicity in the sensitivity of blood cultures. The blood volume, which is directly associated with the number of bacteria, is an important factor to obtain culture positive results and can explain some of the variation in culture positivity. A 2018 systematic review and meta-analysis explored the relationship between blood volume and culture sensitivity[23]. The study found a positive relation between sample volume and blood culture sensitivity with marginal gain in sensitivity with increase in blood volume. The blood culture sensitivity increased by 3% (95% CI, 1% - 6%) for each additional milliliter of cultured blood (between 1 and 10 mL). The mean blood culture sensitivity was 0.51 (95% CI, 0.44 – 0.57) for a 2 mL blood sample culture

and 0.65 (95% CI, 0.58 – 0.70) for a 10 mL blood sample culture, highlighting the importance of adequate blood volume for the growth of *S.* Typhi in blood culture.

Bacterial quantification showed that the counts of bacteria peaks in the first week of illness and decreases with the increase in the duration of the illness, so blood sample for culture collected early on in the illness yields more positive results[24]. The 2018 systematic review and meta-analysis reported that blood cultures performed after the first week of illness were 31% (95% CI, 19% – 41%) less sensitive compared to cultures performed during the first week[23].

Bacterial counts were higher in infections caused by multi-drug resistant S. Typhi and less in infection caused by susceptible S. Typhi[24]. Blood cultures taken from patients who had used antimicrobials prior to the culture were 34% (95% CI, 4 – 54%) less sensitive than blood cultures taken from patients with no prior antimicrobial use[24]. This suggests that prior antibiotic use decreases the sensitivity of the blood culture by inhibiting the growth of the bacteria[25].

1.7.2 Bone Marrow Culture and Cultures from Other Sites

Bone marrow has a higher concentration of bacteria (approximately ten times higher concentration than in blood) and is more sensitive irrespective of the duration of the illness[25]. A systematic review reported bone marrow sensitivity of 96% (95% CI 93 - 99)[22].

But, despite higher sensitivity, sampling the bone marrow is a relatively invasive procedure and not routinely done.

S. Typhi can also be isolated from stool and urine. There are also reports of culture from rectal swabs, duodenal strings and rose spots. The cultures from these various sites are less invasive but have low sensitivity. Isolates from stool, urine and duodenal stings may not necessarily be an acute typhoid infection, but chronic carriage with an acute infection of other cause[25].

Table 1. Colony Characteristics of *Salmonella* Typhi.

Culture Media	**Colony Characteristics**
Blood Agar	Non-hemolytic smooth white colonies
MacConkey Agar	Lactose non-fermenting smooth colonies
SS *(Salmonella-Shigella)* Agar	Lactose non-fermenting colonies with black centres
Xylose-lysine-desoxycholate Agar	Transparent red colonies with black centres
Hektoen enteric agar	Transparent green colonies with black centres
Bismuth sulfite agar	Black colonies

Adapted from World Health Organization (WHO) background documents: The diagnosis, treatment and prevention of typhoid fever.

1.7.3 Identification of *S.* Typhi from Culture

All *Salmonella* species including *S.* Typhi grow well on routinely used laboratory media[26]. Colony characteristics of *S*. Typhi varies with different culture media used and are described in Table 1.

1.7.4 Biochemical Identification

Colony growths in the culture media are screened using various media/ tests[26]. The suspected *Salmonella* growth has specific biochemical properties (Table 2). Further serotyping is required to confirm the identification.

Table 2. Biochemical Properties of *Salmonella.*

Organism	Kligler iron agar				Motility, indole urea			Citrate
	Slant	Butt	H_2S	Gas	Motility	Indole	Urea	
S. Typhi	Alk	Acid	Wk+	-	+	-	-	-
S. Paratyphi	Alk	Acid	-	+	+	-	-	-
Other *Salmonella* species	Alk	Acid	V	V	+	-	-	V

Alk = alkaline, Wk = weak, V= variable result.

Adapted from WHO background documents: The diagnosis, treatment and prevention of typhoid fever.

Kliglers Iron Agar is used as a differential media to differentiate gram-negative intestinal bacteria based on their fermentative ability for glucose and lactose; and for their ability to produce hydrogen sulphide (H_2S). Fermentation of glucose is indicated by a yellow butt. Fermentation of lactose is indicated by a yellow slant and H_2S production is indicated by a blackening in the butt. All Salmonella including *S*. Typhi ferment glucose producing acid, but do not ferment lactose showing an alkaline reaction. *S*. Typhi is anaerogenic and does not produce indole or urea.

1.7.5 Serological Identification

Salmonella is classified by serotype based on three major antigenic determinants: 1) the O or the somatic antigen; 2) the H or the flagellar antigen; and, 3) the K or capsular antigen[13]. The O antigen is the cell wall lipopolysaccharide (LPS). The H antigen is the bacterial flagellar protein that is involved in the host immune defenses. It can be in one of two phases and can switch between phases. The K antigen is a capsular polysaccharide and is the least common antigen found in the serotypes of *Salmonella*. The Vi or the virulence antigen is a special subtype of K antigen found in *S.* Typhi and protects S. Typhi from host defense mechanisms.

The Kauffmann -White classification classifies *Salmonella* based on the identification of O and H antigen (Table 3). The O antigen is identified using the slide agglutination test with group-specific antiserum followed by O- factor antiserum. The Vi antigen in *S.* Typhi prevents agglutination with O antiserum. The culture is boiled for 10 minutes which

results in removal of Vi antigen and allows agglutination. The H antigen is determined using the tube agglutination test. Phase 1 H antigen is specific and shared only by a few species which makes it sufficient to identify *Salmonella* Typhi and Paratyphi.

Table 3. Kauffmann – White Scheme for serological classification of *Salmonella.*

Serogroup	Specific O (LPS) antigen factor	Serotype (Serovar)	O antigen	Capsular Antigen	H Antigen	
					Phase 1	Phase 2
D1	9	Typhi	9, 12	Vi	d	-
A	2	Paratyphi A	1, 2, 12	-	a	[1,5]
B	4	Paratyphi B	1, 4, [5], 12	-	b	1, 2
C1	7	Paratyphi C	6, 7	Vi	c	1, 5
B	4	Typhimurim	1, 4, [5], 12	-	i	1,2
D1	9	Enteritidis	1, 9, 12	-	g,m	1,7

Antigens in parentheses are either weak or absent in some isolates.

Adapted from WHO background documents: The diagnosis, treatment and prevention of typhoid fever.

1.7.6 Widal Agglutination Test

The Widal agglutination test, developed in the 1800s, is still used to diagnose typhoid fever in developing countries. The test is based on detection of antibody against the H and O antigens of *S.* Typhi [27]. A four-fold rise in the antibody titre demonstrated by an acute and a convalescent phase serum sample usually taken about 10 days apart indicates a positive result[28]. In practice, however, a single test is used, which has no diagnostic significance. The test on its own is insufficient for the diagnosis of typhoid

fever because of test variability and a lack of reproducibility [27]. Further, there is cross-reactivity with other Enterobacteriaceae and other infectious agents such as malaria leading to false positive results[28]. In an endemic setting, there is multiple exposure to *S.* Typhi over time which can result in a positive agglutinin test. The baseline titre of Widal agglutination is not known so it is not possible to diagnose typhoid fever based on a single test. Similarly, previous immunization against typhoid fever can also lead to a positive test. Reliance of the Widal test not only leads to overdiagnosis of typhoid fever in endemic settings but also misuse and overuse of antibiotics against the disease.

1.7.7 Other Rapid Diagnostic Test

Rapid diagnostic tests (RDTs) used as a part of clinical algorithms are easy to use and can deliver quick results, and have the potential to aid diagnosis and treatment of typhoid fever, especially in low resource settings that lack blood culture facilities. RDTs are based on lateral flow, flow-through, agglutination to solid phase methods and may detect antigens or antibodies. Currently available point of care diagnostic tests includes TUBEX, Typhidot, and Test-it Typhoid. TUBEX tests for antibodies against *S.* Typhi lipopolysaccharide antigen by the ability of the antigen to inhibit binding between an indicator antibody-bound particle and a magnetic antigen-bound particle[29]. The Typhidot test is a dot Enzyme-Linked Immunosorbent Assay (ELISA) test that measures both IgM and IgG antibodies against the outer membrane protein of *Salmonella* Typhi[30]. The Test-it Typhoid test detects IgM antibodies against *S.* Typhi lipopolysaccharide antigen using a lateral flow assay.

A meta-analysis including studies evaluating the RDTs showed that the sensitivity and specificity for TUBEX was 78% (95% CI 71% – 85%) and 79% (95% CI 70%– 87%) respectively; for Typhidot test was 84% (95% CI 73% to 91%) and 79% (95% CI 70% to 87%) respectively; and for Test-It Typhoid was 69% (95% CI 59% to 78%) and 90% (95% CI 78% to 93%) respectively[31]. These tests lack sensitivity and specificity, which can lead to an overdiagnosis of typhoid fever and overuse and misuse of antimicrobials[32].

1.7.8 Molecular Typing

S. Typhi is highly clonal and has limited genetic variation. Insights into the *S.* Typhi genome and evolution of phenotypic traits of *S.* Typhi has helped improve the understanding of the pathogen. Roumagnac et al, in 2006, sequenced 199 gene fragments of *S.* Typhi and proposed a genotyping scheme based on 88 unique single-nucleotide polymorphisms (SNPs) that enabled classification of *S.* Typhi into 85 distinct haplotypes[33]. This followed a classification system proposed by Wong et al., which is derived from whole genome sequencing of a global collection of over 1800 distinct isolates[34]. Based on 68 SNPs, the *S.* Typhi are divided into four clusters, 16 clades and 49 subclades[35]. For instance, H58 subclade, according to the Roumagnac et al. scheme, is designated as genotype 4.3.1.

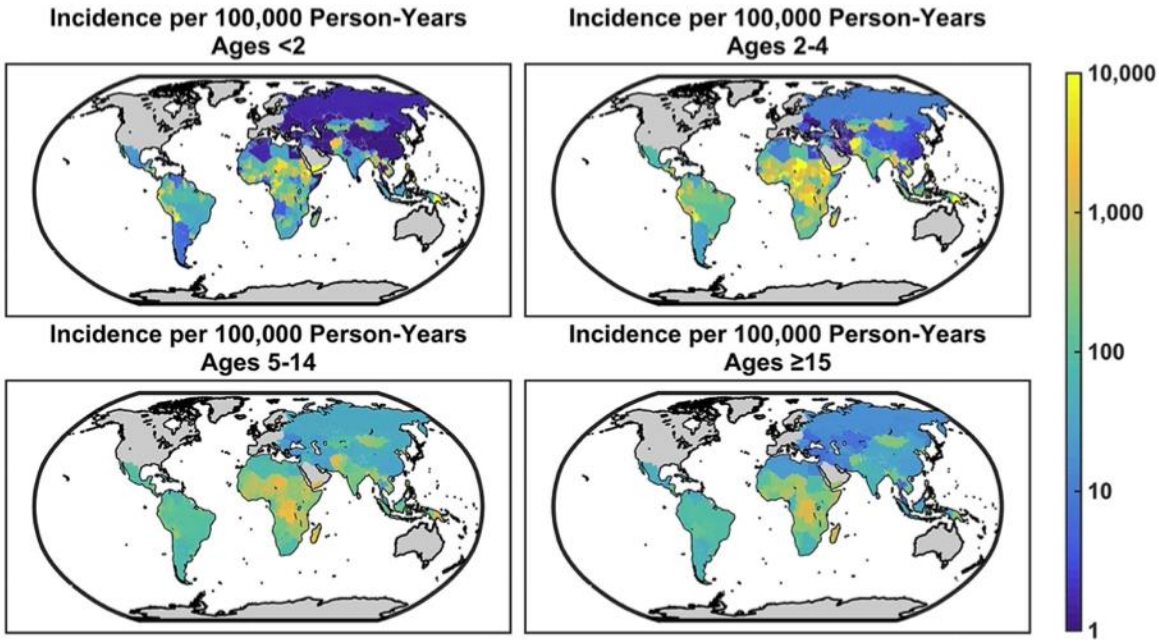
S. Paratyphi A, although of a different serogroup, appear to be closely related to *S.* Typhi as indicated by shared gene content[36]. *S.* Paratyphi A has less diverse gene content than *S.* Typhi indicating that *S.* Paratyphi A is of more recent origin.

1.8 The Burden of Typhoid Fever

Understanding typhoid fever burden is crucial for planning interventions to control the disease. Earlier data on the burden of typhoid fever mostly came from vaccine trials. These are inaccurate estimates of the burden given that trial sites are likely chosen for the high burden of the disease. There is limited surveillance in endemic settings to give accurate estimates of the disease. However, in the recent years, there have been improvements in the number and quality of surveillance studies to generate quality data on the burden of disease[37]. The Strategic Typhoid Alliance Across Africa and Asia (STRATAA) conducted a multicomponent epidemiological study combined with novel laboratory methods in urban sites in Nepal, Bangladesh and Malawi[38]. The study has been detailed in Section 1.8.2 Burden of Typhoid Fever in Nepal. The Surveillance of Enteric Fever in Asia Project (SEAP) was established to generate additional burden data from urban and periurban sites in South Asia[39]. Recognizing the heterogenicity of typhoid fever and the lack of representative data, the Surveillance of Enteric Fever in India (SEFI) was established to generate nationally representative data for India[40]. Similarly, the Typhoid Fever Surveillance in Africa Program (TSAP) followed by the Severe Typhoid Fever Surveillance in Africa (SETA) program has generated data on incidence and characterized typhoid fever across ten sub-Saharan countries[41,42]. The data from these

studies have been crucial for informing policy makers about the typhoid burden and urgent need for intervention.

Figure 2. Age-specific Incidence of Typhoid Fever per 100,000 person-years.



Reproduced under CC BY from *Antillón et al.* The burden of typhoid fever in low- and middle-income countries: A meta-regression approach. PLoS Negl Trop Dis 2017; 11: 1–21.

Additionally, recent advances in model-based estimates of the burden of enteric fever have improved understanding the global and regional burden of the disease. However, caution is required in interpreting the data given the heterogenicity of the disease in different regions, between countries within a region, and within a country itself and the differences in the methods used to estimate burden in the different studies[43,44,45]. For instance, Mogasale et al estimated the burden of typhoid fever in low- and middle-income countries (LMICs) in 2010 was 11.9 million (95% CI 9.9 – 14.7) after adjusting for water-related risks[43]. In their 2017 review, Antillon et al included indicators of

environmental characteristics and socioeconomic development and estimated there were 17.8 million cases of typhoid fever in LMICs each year[44].

The Institute for Health Metrics and Evaluation (IHME) estimated 9.24 million (95% uncertainty interval (UI) 5.94 – 14.1) cases of typhoid fever and 110,000 deaths (95% UI 52,800 – 191,000) globally in 2019[46]. Morbidity due to typhoid fever was considerable; it accounted for 8.05 (95% UI 3.86 – 13.9) million disability-adjusted life years (DALYs) in 2019. The rate of typhoid fever had decreased by 29.5% (95% UI 32.2 – 26.4) from 2010 to 2019, likely due to improvements in economy, infrastructure, food handling practices and increased access to antibiotics. However, the burden of typhoid fever is still substantial for a non-chronic disease that is preventable and easily treatable in an outpatient setting.

1.8.1 Burden of Typhoid Fever in Children

The incidence of typhoid fever is high in children, although the burden across the different age groups varies between studies. The Global Burden of Disease (GBD) 2017 study estimated that 55.9% (95% uncertainty interval (UI) 50.3 – 61.6) of the disease occurred in children younger than 15 years with the highest incidence in the 5-9 year age group[47]. Another model-based study estimated a high incidence in the 2–4 year age group and a relatively lower incidence in the under-2 year and over-15 year age group[44]. It is suggested that typhoid fever in children is represented by a sigmoid curve, where there is a low incidence in the neonatal period and the incidence increases with age[2]. The

low incidence in the younger age group could be a result of interplay between multiple factors. Passive immunity acquired from mothers potentially protect neonates and infants[2]. Similarly, neonates and infants are less likely to be exposed to contaminated food and water due to breastfeeding. In addition, diagnostic blood cultures are not routinely sought in the youngest age group in some settings. Coupled with difficulties obtaining adequate blood volumes in small children, and higher risk of contamination, this may result in under-diagnosis in this age group.

Studies also suggest that the heterogenicity in typhoid burden in children is affected by the overall burden. In the GBD 2017 study, the incidence of typhoid fever was higher in children in high incidence regions while the incidence was spread across the age groups in low incidence regions[47]. On the other hand, Antillon et al. suggested a high peak in the 2-4 year age group in high incidence regions and a smaller peak in children aged 5-14 years in low incidence regions[44]. The differences by burden likely relates to the differences in the force of infection (measured by the population typhoid incidence[2]) and resultant immunity developed against the disease. The differences are also a result of the differences in methodology and what estimates are being calculated.

1.8.2 Burden of Typhoid Fever in Nepal

There is limited data on the burden on typhoid fever in Nepal. Typhoid fever is a common diagnosis in outpatient settings in Nepal[48,49]. Earlier data demonstrating the burden of typhoid fever in Nepal is limited to culture data from hospitals. Retrospective analysis of

microbiological data collected by OUCRU-PH over 23 years at Patan Hospital in Lalitpur Sub-Metropolitan City, a tertiary level hospital in Kathmandu, Nepal and the primary site for this thesis, showed that *Salmonella* accounted for 65.4% of all bacteria positive blood cultures and that *S.* Typhi was the dominant serovar constituting 68.5% of all *Salmonella* isolates[50]. Similarly, another prospective study in the same hospital found that *Salmonella* is the leading cause of blood stream infections among pediatric out-patients with fever[51]. The reports of high typhoid burden were consistent across other hospitals in Kathmandu[48]. Although these microbiological data support demonstration of burden of typhoid fever, the data do not represent the true burden of typhoid fever.

As a part of the STRATAA programme, the burden of typhoid fever was estimated in an open cohort of around 100,000 population in a demarcated area of Lalitpur[38]. A census was conducted at the beginning of the study, an update at year one and a final census at two years. Participants within the census area were encouraged to attend the designated healthcare facility at Patan Hospital if they developed a fever and blood cultures were collected for all febrile participants that met the criteria for blood culture. Two healthcare utilization surveys were conducted to estimate the probability that the participants would seek healthcare in the designated healthcare facility. Of the blood cultures done, 6.9% were positive for pathogenic bacteria and 93.7% of the pathogenic bacteria isolated were *S.* Typhi. Across all ages, the crude incidence was 74 (95% credible interval 62 -87) per 100,000 person-years[52]. The adjusted incidence was 1014 (650 – 1698) per 100,000 person-years. The observed incidence was adjusted for the probability of healthcare-seeking, probability of receiving a blood culture, and blood culture

sensitivity, with largest adjustment for health care seeking behavior. Incidence rates were highest in the 5 – 9-year age group.

Burden data from outside urban areas are sparse. A prospective study conducted in 4 rural and peri-urban sites in Nepal found that 2.5% of all patients with acute febrile illness and 62% of all bacteria positive blood cultures were positive for *Salmonella*[49]. The majority of the participants (81.9%) were enrolled from a periurban hospital. The proportion of culture-confirmed enteric fever was much lower than in the urban areas. Although low blood culture sensitivity is a factor, this does not explain the lower rates compared to urban areas. There is no data on the proportion of participants receiving a blood culture and little is known about the health care seeking behavior in the rural hospitals. The study shows that typhoid exists is rural areas as well and more research is needed to determine true burden.

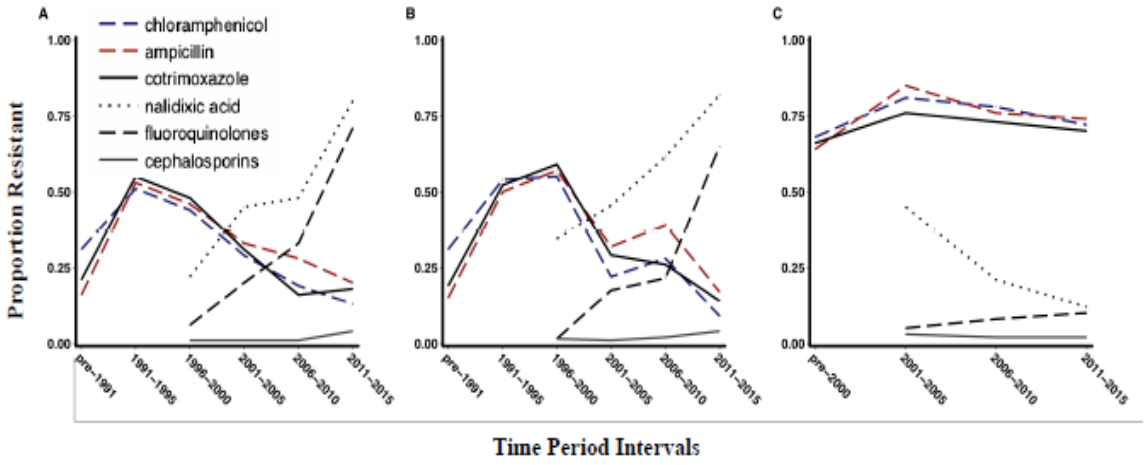
1.9 Treatment and Antibiotic Resistance

Uncomplicated typhoid fever is usually treated empirically on an out-patient basis with a single antimicrobial. The choice of antimicrobial depends on the local susceptibility pattern and the severity of the disease. Effective antimicrobial treatment shortens the duration of illness and reduces the risks of complications.

Chloramphenicol, after its discovery in the 1940s, became the first antimicrobial to be widely used for treatment of typhoid fever. By the 1980s, chloramphenicol, ampicillin and trimethoprim/sulfamethoxazole were used as first line of antimicrobials against typhoid fever. Consequently, mortality due to typhoid fever decreased from 10 – 30% in the pre-antibiotic era to around 1%[53]. Multidrug resistant (MDR) strains of *S.* Typhi with combined resistance to the first line microbials emerged in South and South-east Asia in the early 1980s and has since spread globally[53]. Resistance in MDR strains is usually plasmid mediated and is associated with resistance genes such as catA, sul1, sul2, dfrA, blaTEM-1, strA, strB, tetA, tetB, tetC, and tetD[54].

With the emergence of resistance to the first line antibiotics, fluoroquinolones such as ciprofloxacin and ofloxacin were used for the treatment MDR typhoid. In line with the increasing use in fluoroquinolones, decreased ciprofloxacin susceptibility emerged followed by increased resistance to nalidixic acid and fluoroquinolones. Fluoroquinolone resistance in *S.* Typhi is associated with single point mutations in the chromosomal quinolone-resistance-determining regions (QRDR) in the *gyrA, gyrB*, *parC* and *parE* genes, and fluoroquinolone resistance conferring plasmids containing qnrB2, qnrB4 and qnrS1 genes[53]. Triple mutations with single nucleotide polymorphism (SNP) in *gyrA* and *parC* associated with high levels of ciprofloxacin resistance are commonly seen in the H58 clonal population.

Figure 3. Antimicrobial non-susceptible trends of S. Typhi over time. A) Global trends, B) Asian trends, C) African trends.



Reproduced under CC BY from Britto CD, Wong VK, Dougan G, et al. A systematic review of antimicrobial resistance in *Salmonella enterica* serovar Typhi, the etiological agent of typhoid. PLoS Negl Trop Dis. 2018 Oct;12(10):e0006779. https://doi.org/10.1371/journal.pntd.0006779.g002

Resistance to fluoroquinolones has led to increase use of third generation cephalosporins such as oral cefixime and parenteral ceftriaxone and macrolide azithromycin for the treatment of typhoid fever. Although widespread resistance to third generation cephalosporins has not yet been reported, there are sporadic reports of extended spectrum beta lactamase (ESBL) producing S. Typhi[55]. Resistance is usually plasmid mediated and has been associated with ESBL genes including genes encoding TEM, SHV, PER, and CTX-M enzymes, and Amp- C[56,57]. More recently, in 2016, a large outbreak of extensively drug resistant (XDR) *S*. Typhi clone, resistant to chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, fluoroquinolones and third-generation cephalosporins, was reported from Sindh Province, Pakistan[58]. Azithromycin is effective against cephalosporin non-susceptible typhoid fever. Resistance to azithromycin has been

sporadically reported[59, 60]. Azithromycin resistance is associated with ereA, msrD, and msrA genes[54]. Newer antimicrobials such as carbapenems (meropenem, faropenem) and tigecycline, are reserved for more resistant strains of the infection.

Antimicrobial resistance in S. Typhi continues to evolve and the emergence of resistance to newer antimicrobials is imminent. In line with the increasing use in fluoroquinolones, resistance to nalidixic acid and fluoroquinolones increased accounting from 20% isolates in 2001 – 2005 to 65% in 2011 – 2015[53]. With the emergence of fluoroquinolone non-susceptible *S.* Typhi and use of third generation cephalosporins, resistance has risen from 1.5% in between 2006–2010 to 4% in 2011–2015[53]. With the switch to newer antimicrobials and decline in use of first line antimicrobials, MDR typhoid is decreasing in Asia and accounted for less than 20% of the isolates by 2010 – 2015, and can be used in settings with fluoroquinolone and cephalosporin resistance [53]. For example, a randomized controlled trial (RCT) conducted by OUCRU-PH in 2011 showed the effectiveness of chloramphenicol in the treatment of uncomplicated enteric fever[61]. Interestingly, in Africa, cases of MDR *S.* Typhi are still prevalent and non-susceptibility to fluoroquinolones and third generation cephalosporins is still low[53].

The spread of resistant strains of *S.* Typhi is of concern, and is increasingly a threat to typhoid control. Whole genome sequencing of *S.* Typhi has shown that the H58 subclade which is prevalent in Asia and associated with multiple drug resistance and non-susceptibility to fluoroquinolones, has spread across Africa and Oceania displacing other

lineages[34,62]. XDR *S.* Typhi originating in Pakistan, has also been reported in travelers and poses a threat regionally as well as globally[63].

1.10 Prevention and Control of Typhoid Fever

In the background of high morbidity and mortality associated with typhoid fever, and the increasing antimicrobial resistance that is limiting treatment options, it is becoming increasingly important to control the disease through prevention rather than just treatment.

1.10.1 Water, Sanitation and Hygiene (WASH)

Efforts to improve water management, sanitation and hygiene in the western industrialized counties have significantly reduced illness and death caused by typhoid fever and other waterborne and feco-orally transmitted diseases. Access to safe drinking water, adequate sanitation, safe food handling practices and personal hygiene is essential for the long- term solution to prevent and control typhoid fever.

The targets for WASH related United Nations sustainable development goals for 2015 – 2030 include ensuring "universal and equitable access to safe and affordable drinking water" and ensuring "access to adequate and equitable sanitation and hygiene for all and ending open defecation"[64]. However, 3 out of 10 people globally still lack access to safe drinking water services, 6 out of 10 people still lack access to safely managed sanitation

facilities and 2 out of 5 people still do not have access to basic handwashing facilities with soap and water[64]. Political commitment and substantial cost are required to deliver improvements in water and sanitation services, and even if available are expensive and require long time to deliver. The World Bank, the United Nations Children's Fund (UNICEF) and the World Health Organization (WHO) estimated that merely providing the basic WASH services to the unserved would cost $28.4 billion per year from 2015 to 2030, and that three times more investment would be required to achieve the WASH related sustainable development goal (SDG) targets[65]. This is a mammoth task and relying only on WASH measures to control typhoid fever universally is not likely to be successful.

1.10.2 Vaccination as a prevention measure

Since the discovery and immunity demonstrated by inoculation with the first vaccine by Edward Jenner in 1796, vaccines have been used as an effective measure to prevent infectious disease. The role of childhood immunization programmes worldwide in preventing, controlling and eliminating infectious diseases that were once endemic attest to the importance of vaccination.

Attempts to develop vaccines against typhoid fever have been recorded as early as 1896[66]. However, until recent years, use of typhoid vaccines were limited to travelers to typhoid-endemic countries. There have been huge advances in more recent years in the development of vaccines against typhoid fever and attempts to overcome the limitation of the previous ones.

1.10.2.1 Inactivated Whole Cell vaccine

Inactivated whole cell vaccines were first introduced in the 1800s; however, it was not until 1960 that the efficacy of the vaccine was established when the WHO sponsored a series of large scale field trials in Yugoslavia, USSR, Poland and Guyana to assess the efficacy of the vaccine[67,68,69]. Two doses of the vaccine had a 73% (95% CI 65% - 80%) protective efficacy over a three-year period[69]. The vaccine was however associated with high reactogenicity. Fever and systemic reactions were reported in 9 – 34% and work/ school absenteeism was reported in 2 – 17% of the vaccine recipients. The vaccine, although licensed, is no longer available for use.

1.10.2.2 Ty21a

Ty21a is an orally administered live-attenuated vaccine that contains *S*. Typhi strain Ty2, in which multiple genes, including the genes responsible for the production of the Vi polysaccharide, have been mutated. Oral vaccines can elicit local mucosal immunological response as well as cell-mediated and systemic immunological response[70].

The vaccine was first licensed for use in the 1980's in two forms: an enteric coated capsule and a liquid form. The enteric coated capsule is registered for use for adults and for children 6 years and older[69]. It is given in three doses every other day except in North America and Canada, where it is given as a four-dose regimen. It elicits protection from 10 to 14 days after the third dose[71]. The three-dose regimen is based on a randomized

placebo-controlled trial conducted in Santiago, Chile, in which the enteric coated tablets provided 67% (95% CI 47 – 79%) protective efficacy over a three-year period[71]. On additional surveillance of the study cohort, three doses of the Ty21a enteric-coated capsules (every other day schedule) provided 62% protection over seven years of follow-up[72,73]. The four dose regimen was based on a comparative efficacy trial assessing the efficacy of two, three and four dose of Ty21a, which showed a dose-response relationship between the dose and incidence of typhoid fever, and with the lowest incidence in the four-dose group[74]. According to the 2018 Cochrane review, three doses of Ty21a had a cumulative efficacy of 50% (95% CI 35% to 61%) based on 4 trials with 125,239 paticipants[69].

The enteric capsule could not be taken by younger children because it was difficult to swallow. The liquid formulation, consisting of the lyophilized powder for liquid suspension, can be taken by children 2 years and above. The liquid formulation was more protective than the enteric coated capsules[73,75], but has stopped being manufactured in recent years. In a double blinded trial conducted in Indonesia comparing the two formulations, the liquid formulation was 53% efficacious while the enteric capsule was 43% efficacious over a 30 month period[76].

In the trial conducted in Indonesia, adverse events following immunization was assessed in 460 individuals in the capsule formulation, liquid formulation and placebo group after each dose of the vaccine, stratified by age and immunization group[76]. No serious side

effects were noted in participants in either of the groups. The controlled trial conducted in Alexandria, Egypt observed minimal general and digestive reactions which were comparable with the placebo group[77]. The 2018 Cochrane review based on five Ty21a individual and cluster randomized trial also reported that fever was more common in the Ty21a group than in the placebo group, but other adverse reaction including probably vomiting, diarrhea, nausea, abdominal pain, headache, and rash were comparable to the placebo group[69].

Despite being efficacious and safe, the multiple doses (with proper storage facility) required to elicit protection means that the use of the vaccine and the impact they can have for public health use for disease control in is limited.

1.10.2.3 Vi polysaccharide vaccine

The Vi-polysaccharide vaccine, first commercially made available in the 1980s, is a subunit vaccine containing purified Vi capsular antigen of *S.* Typhi. The Vi capsular polysaccharide, also known as the virulence factor is the protective antigen of *S.* Typhi. The Vi antigen physically prevents antibodies from binding to the O antigen[78]. It is also associated with inhibition of complement activation, resistance to complement-mediated lysis and phagocytosis allowing *S.* Typhi to survive in blood and cause infection[78]. The Vi-polysaccharide vaccine produces Vi specific serum antibody which activates complement against *S.* Typhi. However, being a polysaccharide vaccine, it is T-cell independent and

does not induce a booster response upon re-injection, has a short duration of immunity and produces a poor immunogenic response in young children.

It is given as a single parenteral injection and elicits protection seven days after the vaccination with the maximum protection achieved after 28 days. Revaccination is recommended every three years for individuals who remain at risk of infection. The vaccine has been approved for use for adult and children two years and above.

The protective efficacy was of the Vi polysaccharide vaccine was assessed in two highly endemic countries, Nepal and South Africa, in the 1980s. Acharya et al. conducted a double-blinded RCT involving 6907 individuals aged 5 – 44 years[79]. The RCT was conducted in Kathmandu, Nepal where the participants were given intramuscular injections of the Vi polysaccharide vaccine or a pneumococcal vaccine (control). Blood cultures were taken from participants who had an oral temperature of 37.8 degrees C or higher for three consecutive days. The efficacy of the Vi polysaccharide vaccine was 72% in the culture-positive cases 17 months after vaccination. The efficacy was 80% in the clinically suspected cases defined by fever for 3 days or more, accompanied by bradycardia and splenomegaly. The efficacy was 75% in the two groups combined[79].

In the study in South Africa, 11,384 children aged 5 – 16 years were enrolled in a double-blind randomized controlled trial[80]. Participants received either the Vi polysaccharide vaccine or a meningococcal A + C control vaccine. Blood culture proven cases of typhoid

fever were recorded. The vaccine efficacy after 21 months of follow-up was 60% including all cases detected post-vaccination, and 81% from 6 weeks after vaccination allowing for 2-3 weeks of incubation and a further 2-3 weeks for seroconversion[80]. After 3 years of surveillance, the vaccine efficacy was 55% and not significantly different in each year of the trial showing persistent protection[81].

Similarly, in placebo-controlled vaccine trials in Jiangsu Province and Guangxi Province, China assessing the efficacy of a locally produced Vi-polysaccharide, the protective efficacy was 71% over 12 months and 69% over a 19 month period[82]. On surveillance over 6 years, the study showed that the vaccine offered protection for at least 2 years[83].

Two cluster randomized trials were conducted in Kolkata, India and Karachi, Pakistan. Sur et al., in the phase 4 cluster randomized effectiveness trial in slum-dwelling residents in Kolkata, reported a protective effectiveness of 61% (95% CI: 41% to 75%)[84]. The protective effectiveness was 80% (95% CI, 53% to 91%) among children under-5 years, and 56% (95% CI, 18% to 77%) among children 5 to 14 years. The indirect protection among the unvaccinated residents in the vaccine cluster was of 44% (95% CI: 2% to 69%). The trial results suggested a high level of protection in children less than 5 years and showed that the vaccine conferred herd immunity to the non-vaccinees. In contrast, Khan et al., in a cluster randomized trial in Karachi, reported an adjusted total protective effectiveness of 31% (95%CI:−28% to 63%), and an indirect protective effectiveness of −10% (95% CI:−116% to 44%) in the non-vaccinated population[85]. The study did not show

any protection among children 2 – 5 years of age (VE −38% (95%CI: −192% to 35%)) and showed similar protection to the Kolkata study in children 5- 16 years of age (VE 57% (95%CI: 6% to 81%)). The differences in the effectiveness were likely due to the different study designs and populations. The entire population above 2 years was vaccinated in Kolkata while only children between 2 and 16 years were vaccinated in Karachi. The resultant higher vaccine coverage in Kolkata may explain the differences in the vaccine effectiveness. There was likely lower circulation of *S.* Typhi and reduced exposure in the vaccinated clusters leading to lower incidence. Overall, based on the above studies, 2018 Cochrane review showed a pooled vaccine efficacy of 69% (95% CI: 63% to 74%) at year 1 and 59% (95 %CI: 45% to 69%) at year 2[69].

The Vi polysaccharide vaccine has a good safety profile and is well tolerated[69,78]. No related serious adverse events were reported in any of the trials. Pain at injection site, erythema and induration are the most frequently reported adverse reactions but are mild to moderate in severity and transient[78]. No significant differences were found in the incidence of fever and erythema between the vaccine and control[69].

Table 4. Typhoid Conjugate Vaccines: Currently Licensed and in Development

Vaccine	Developer/ Manufacturer	Concentration of Vi antigen	Number of Doses	Vaccine Efficacy (95% CI)	Status	Recommended/Targeted Age Group for Vaccination (if known)
Vi-rEPA	National Institutes of Health, USA	25ug/0.5ml	-		Technology transferred to Lanzhou Institute of Biological Products, China	-
Vi-rEPA	Lanzhou Institute of Biological Products, China	25ug/0.5ml	-	91.5% (77.1 – 96.6) at 27 months 89% (76 – 96.9) at 48 months	Phase 3 randomized double-blind controlled trial conducted in China	>2 years of age
Vi-TT - PedaTyph™	Bio-Med (P) Limited, India	5ug/0.5ml	1-2 doses	100% (97.6 -100) at 12 months	Licensed in India	Over 3 months of age* (Safety data available for over 6 months)
Vi-TT - Typbar TCV™	Bharat Biotech International Ltd, India	25ug/0.5ml	1 dose	54.6% (26.8 -71.8) at 1 month challenge	WHO prequalified, 2017	From 6 months of age to 45 years of age
Vi-TT – ZyVAC TCV	Calida Healthcare Ltd, India	25ug/0.5ml	1 dose		Licensed in India	From 6 months of age
Vi-CRM197	GSK Vaccines Institute for Global Health	5ug/0.5ml	-		Technology transferred to Biological E Litd, India	
Vi-CRM197	Biological E Ltd, India	5ug/0.5ml	1 dose		WHO prequalified, 2020	From 6 months of age

Vi-CRM197	Eubiologics, Republic of Korea	5ug/0.5ml			Preclinical/ Phase 1 trial	Children < 2 years of age, and adults
Vi-DT	International Vaccine Institute, South Korea	25ug/0.5ml			Technology transferred to SK Biosciences, South Korea, PT Bio Farma, Indonesia, and Incepta Vaccines, Bangladesh	Children < 2 years of age, and adults
Vi-DT	SK Biosciences, South Korea	25ug/0.5ml			Phase 3 trial	Children < 2 years of age, and adults
Vi-DT	PT Bio Farma, Indonesia	25ug/0.5ml			Plans underway for phase 3 trial	
Vi-DT	Incepta Vaccines, Bangladesh				Preclinical	Children < 2 years of age, and adults

Vi-rEPA: Vi polysaccharide derived from S. Typhi conjugated to recombinant exoprotein A of Pseudomonas aeruginosa Vi polysaccharide derived from S. Typhi conjugated to tetanus toxoid

Vi-TT: Vi polysaccharide derived from S. Typhi conjugated to tetanus toxoid

Vi-CRM197: Vi polysaccharide derived from Citrobacter freundii conjugated to cross-reactive material 197, a nontoxic mutated form of diphtheria toxin.

Vi-DT: Vi polysaccharide derived from S. Typhi conjugated to diphtheria toxoid

1.10.2.4 Vi conjugate vaccine

The immunogenic properties of polysaccharide vaccines are significantly improved by conjugating the polysaccharide antigen to a carrier protein. This was first demonstrated by the development of *Haemophilus influenza* type b conjugate vaccines[86] and other conjugate vaccines, including the *N. meningitidis* serogroup C and multivalent *S. pneumoniae* conjugate vaccines, followed thereafter[87]. The Vi conjugate vaccines have been described in the sections below and summarized in Table 4.

1.10.2.4.1 **Vi-rEPA**

Vi-rEPA was a prototype typhoid conjugate vaccine produced by conjugating a recombinant exoprotein A from *Pseudomonas* aeruginosa to the Vi polysaccharide and was first developed at the US National Institutes of Health[88]. This vaccine was later produced by Lanzhou Institute of Biological Products, Lanzhou, China, and evaluated in a large phase 3 trial in Vietnam in 1997[89]. The study reported the highest vaccine efficacy for any typhoid vaccine; importantly demonstrated immunogenic response in young children, and persistence of antibodies 46 months post-vaccination; and was safe, reporting no serious adverse effects. Licensure and wider public health use of the vaccine has however been delayed[89].

In the double-blinded randomized controlled trial, 11,091 children two to five years old from 16 communes in Dong Thap Province received two injections of Vi-rEPA or a saline placebo six weeks apart[90]. Health workers visited the children on a weekly basis, took

history and measured axillary temperature of the children. Any child with a temperature of 37.5 or higher for 3 or more days was referred to the health station for a blood draw. Samples were drawn for blood culture and serological testing. Diagnosis of typhoid fever was made based on isolation of *S.* Typhi from blood culture. The vaccine efficacy was 91.5% (95% CI: 77.1%, 96.6%) after 27 months of surveillance. After the planned unbinding, passive surveillance was conducted for an additional 19 months[91]. Over the entire 46-month period, the vaccine efficacy was 89% (95% CI: 76%, 96.9%).

To evaluate the immunogenicity of the vaccine paired serum samples were obtained from 76 children before the first vaccine and four weeks after the second dose of the vaccine[90]. Further blood samples were collected from four randomly selected children from each commune each month after the second vaccine. Anti-Vi IgG antibodies increased by at least a factor of 10 in all participants that received Vi-rEPA. The anti-Vi IgG level was approximately 35 times higher than in the placebo group six months after the second dose of vaccine, and 19 times higher 2 years after the second dose of the vaccine. On age stratification, children four to five years of age had higher levels of antibody than children two to three years of age at all three time points – 6 months, one year and two years post-vaccination – although not significantly different. Age related persistence of antibodies was reported 46 months into the study[91].

In a separate study conducted in Vietnam, the safety, immunogenicity and compatibility of Vi-rEPA to concurrent routine vaccines was evaluated in infants[92]. A total of 301

infants were enrolled to receive the routine Expanded Program on Immunization (EPI) vaccines alone or with Vi-rEPA or *Haemophilus* influenzae type b-tetanus toxoid conjugate (Hib-TT) vaccine at 2, 4, and 6 months and Vi-rEPA or Hib-TT alone at 12 months. The vaccine was safe and no serious adverse events were reported. The vaccine induced protective anti-Vi IgG antibodies and was compatible with the EPI vaccines. However, high levels of maternal anti-VI antibodies had a suppressive effect on the vaccine induced anti-Vi antibodies.

1.10.2.4.2 **PedaTyph**

PedaTyph is a typhoid Vi conjugate vaccine produced by conjugating tetanus toxoid protein with 5ug of purified Vi capsular polysaccharide of *S.* Typhi (Strain Ty2) and was the first TCV to be licensed for use in India. A randomized comparative trial was conducted in 400 children three months to five years of age in India, 200 of which received one dose of the vaccine and another 200 received 2 doses of the vaccine 8 weeks apart [93]. Serum samples were assessed for anti-Vi IgG antibody at baseline and at 8 weeks. The vaccine was immunogenic with 83% of the participants having a seroconversion ($\geq$ 4-fold increase over pre-immunization titre) at eight weeks post-vaccination. Following up the study cohort of 400 children, 40 children who received one or two doses of the vaccine were called back at 30 months post-vaccination to assess the booster effect of second dose of the conjugate vaccine[94]. The children in two doses group had higher antibody titers compared to that single dose group; however, the difference was not significant. The authors also reported 10 non-vaccinated children that were "recalled"; however, this unvaccinated group was not described in the previous paper

and the selection criteria for these children were unclear. The anti-Vi IgG titre was significantly higher in the vaccinated children compared with the unvaccinated children, irrespective of the number of doses. The vaccine was reported to be safe with fever as the most common adverse event and no serious adverse events.

An open label randomized controlled post-marketing surveillance of Pedatyph, as described by the authors, was conducted in children aged 6 months to 12 years in Kolkata, India[95]. The study however appears to be a quasi-randomized trial where the allocation does not appear to be truly random. Twelve participating schools were randomized as clusters. The siblings and neighborhood children aged 6 months to 3 years who do not attend school were included in the test or control group based on which group the school going child was enrolled in. A total of 905 children in the test group received two doses of Pedatyph vaccine 6 weeks apart and 860 children in the control group only received the vaccines as per the national guidelines. The children were followed up via active surveillance that included weekly phone calls and monthly school visits. In 12 months of surveillance, 11 participants had culture positive typhoid fever, all in the control group. The study reported 100% (95% CI: 97.65%, 100%) vaccine efficacy.

The vaccine is recommended by the manufacturers as a two-dose vaccine given 4 – 8 weeks apart, and a booster every 10 years in children 2 years and above. For children 3 months to 2 years, the vaccine is recommended as a two-dose vaccine given 4 – 8 weeks apart, followed by a booster at 24 to 30 months, and every 10 year thereafter. The is no

evidence available to support the recommendation. The WHO did not consider the vaccine for pre-qualification citing the limited evidence and non-robust study design as described above[96].

1.10.2.4.3 Typbar-TCV™

Typbar-TCV is a conjugate vaccine produced by conjugating tetanus toxoid carrier protein to 25 µg of Vi polysaccharide and was WHO-prequalified in December 2017[96]. The vaccine licensure was supported by the immunogenicity and safety results of a phase 3 trial conducted by the manufacturers (Bharat Biotech) in India[97]. Participants aged 2 – 45 years were enrolled in a double-blinded trial, where participants either received Typbar-TCV or a typhoid polysaccharide vaccine (Typbar). Participants were followed up on day 42±2 days and 90±2 days post- vaccination, and on day 720 or 540, depending upon site to assess long-term immunogenicity. The anti-Vi IgG antibodies were measured by ELISA using the VaccZyme commercial kits. Typbar-TCV elicited a significantly stronger immunogenic response with 97.3% Typbar-TCV participants having seroconversion (defined as 4-fold rise of anti-Vi IgG over baseline) compared to the 93.1% Typbar participants (P=0.01) at 42 days post-vaccination. Six weeks after a single dose of the vaccine, the geometric mean titres (GMTs) of anti-Vi IgG antibody was 1292.5 EU/ml (95% CI: 1152.9–1448.9, N=332) in the Typbar-TCV group and 411.1 EU/ml (95% CI: 358.9–470.9, N=305) in the Typbar group. A second dose of the respective vaccines was given at day 720. The GMTs 6 weeks after the second dose of the vaccine was 1680.6 EU/ml (95% CI: 1498.3–1885.1, N=174) in the Typbar-TCV group versus 475.0 EU/ml (95% CI: 339.9–663.6, N=50) in the Typbar group. At both instances, Typbar-TCV elicited a

significantly higher anti-Vi IgG titre than Typbar. Both vaccines had similar rates of adverse events with fever being most commonly reported.

Infants and toddlers 6–23 months of age were enrolled in an open-label trial, parallel to the double blinded trial[97]. The participants received a single dose of Typbar-TCV. The polysaccharide vaccine is not approved in this age group and was not use a comparator. Typbar-TCV elicited immunogenic response in this age group with seroconversion in approximately 98% of the children and GMT of anti-Vi IgG antibody titre of 1937.4 EU/ml (95% CI: 1785.0–2102.9, N=307)).

The vaccine efficacy was assessed using a controlled human infection model[98]. The vaccine efficacy demonstrated in this study aided the WHO-prequalification on the vaccine. In the phase 2b observer and participant blinded randomized controlled trial conducted at Oxford, 112 healthy adult volunteers aged 18 to 60 years with no history of typhoid vaccination, infection or prolonged residence in a typhoid-endemic region were enrolled and randomly assigned (1:1:1) to receive control meningococcal ACWY-CRM conjugate vaccine, Vi-polysaccharide vaccine or the Vi-tetanus toxoid conjugate. Following vaccination, participants filled out an online diary for 7 days to monitor the local and systemic adverse events and were clinically assessed on days 1, 3, 7, and 10. One-month post-vaccination, the participants were orally challenged with 1-5x10^4 colony forming units of *S.* Typhi Quailes strain. Typhoid fever was diagnosed if a *S.* Typhi positive

blood culture was collected more than 72 hours post-challenge or there was a fever of 38^{o}C or higher persisting for 12 hours or longer.

The criteria for typhoid fever diagnosis was met by 77% (N=24/31) participants in the control group, 35% (N=13/35) participants in the Vi-PS group and 35% (N=12/37) participants in the Vi-TT group. The vaccine efficacy for Vi-PS and Vi-TT were similar; 52.0% (95% CI 23.2 – 70.0) for Vi-PS and 54.6% (95% CI 26.8 – 71.8) for Vi-TT. The authors noted that the efficacy of Vi-PS was less than observed in field trials. This was likely because the participants in the challenge study were healthy typhoid-naïve adults. Additionally, the study used a composite definition capturing all microbiological as well as clinical cases, identifying otherwise mild or asymptomatic cases that would have gone undetected in a field setting. The protective efficacy of both Vi-PS and Vi-TT were likely to be higher in an endemic setting. Using a field definition of fever 38.0 C or higher followed by bacteremia, the vaccine efficacy post-hoc was estimated to 87.1%, similar to the prototype typhoid conjugate vaccine, Vi-rEPA, previously described.

The randomized controlled trial, conducted as a part of a larger consortium, described in this thesis assessed the vaccine efficacy of this vaccine in an endemic setting.

1.10.2.4.4 **Vi-CRM$_{197}$**

Vi-CRM$_{197}$, developed by the Novartis Vaccines Institute for Global Health, Siena, Italy, is a conjugate vaccine containing purified Vi polysaccharide, derived from Citrobacter, conjugated to mutant nontoxic diphtheria toxin carrier protein, CRM$_{197}$. Phase I and II safety, immunogenicity and dosing trials were conducted in healthy adults aged 18 to 40 years in Antwerp, Belgium[99].

In the Phase I trial, 50 participants were randomized 1:1 to receive Vi-CRM$_{197}$ 25 μg or Vi-PS[99]. In the Phase II trial, 88 participants were randomized 1:1:1:1 to receive a single dose of one of three Vi-CRM$_{197}$ concentrations (1.25 μg, 5.0 μg and 12.5 μg respectively) or the Vi-PS. Overall, the vaccine was well tolerated and the adverse reactions did not show a dose-dependent relation. Vi-CRM$_{197}$ was immunogenic. A dose of 5 μg Vi-CRM$_{197}$ was significantly more immunogenic than 25 μg of Vi-PS and was chosen for further development.

Two Phase II, observer-blind, age de-escalation, randomized controlled trials were conducted at two sites in Pakistan and India and in one site in Phillippines[100]. Four age groups were enrolled. Adults aged 18 – 45 years were enrolled in Karachi, Pakistan, and in VaduBudruk, India, and children aged 24 – 59 months, older infants aged 9 – 12 months and infants aged 6 – 8 weeks were enrolled in Pakistan and Manila, Philippines. Participants were randomized in a 1:1 ratio to receive the Vi-CRM$_{197}$ or the comparator vaccine (Vi-PS in adults and children or the 13-valent pneumococcal conjugate vaccine in

infants). Adults received one dose of the vaccine, children and older infants received 2 doses of the vaccine 8 weeks apart and infants received 3 doses, 4 weeks apart.

Vi-CRM_{197} induced a strong immunogenic response in adults, children and older infants demonstrated by the high geometric mean titres and seroconversion rates[100]. A second dose of the vaccine, however, did not induce a booster response in children and older infants. In the youngest age group, the overall immune response was lower. In adults and children, the antibody titre dropped significantly 6 months after the last dose of vaccine, with titres similar to the comparator polysaccharide group. In infants, the antibody titres fell to pre-vaccination concentration in the Pakistani cohort while it remained significantly higher than baseline in the Filipino cohort 6 months after the third dose.

Vi-CRM_{197} was safe and well tolerated across all age groups[100]. Co-administration of the vaccine with the EPI schedule vaccines was not associated with increased reactogenicity.

The technology was transferred to Biological E (Hyderabad, India) for further development[101]. A multicenter single blind randomized controlled Phase II/III trial was conducted to demonstrate the non-inferiority of immunogenicity of the vaccine to the licensed comparator (Typbar-TCV). The vaccine was WHO-prequalified in December 2020 becoming the second typhoid conjugate to receive prequalification after the trial in India showed that the vaccine's safety and immunogenicity profile were comparable to Typbar-TCV [102].

1.10.2.5 Other Typhoid Vaccines in the Pipeline

1.10.2.5.1 ZyVAC-TCV

ZyVAC-TCV, a Vi Capsular Polysaccharide Tetanus Toxoid Conjugate Vaccine, manufactured by Cadila Healthcare Limited, India, has recently been licensed for use since 2017[101]. A single-blind randomized active-controlled non-inferiority phase II/III 1:1 randomized trial was conducted by the manufacturers in healthy individuals 6 months to 45 years to assess the immunogenicity and safety of the vaccine compared to Typbar-TCV[103,104]. Blood samples were collected at baseline and 6 weeks post vaccination. Anti-Vi IgG antibody titres were measured using the VaccZyme ELISA kits. Seroconversion was defined as four-fold or higher increase in antibody titre compared with baseline. Of the 117 participants in the study group that completed the study procedures, 94.8% (96.6% in adults and 93.1% in children) had seroconversion while 91.6% (91.7% in adults and 91.5% in children) of the 119 participants in the active control group had seroconversion and was deemed non-inferior to Typbar-TCV[101,103].

1.10.2.5.2 **Vi-DT**

Vi-DT is a candidate TCV developed by the International Vaccine Institute, Seoul, Republic of Korea, where the Vi polysaccharide is conjugated to diphtheria toxoid as a carrier protein[105]. The technology was transferred to SK Bioscience (South Korea), PT Bio Farma (Indonesia) and Incepta Vaccines (Bangladesh). A phase I safety and immunogenicity trial was conducted by SK Bioscience in healthy Filipino adults and children aged 2 – 45

years[106].Two doses of the test and comparator vaccine were given 4 weeks apart. No serious adverse events were reported. Pain and tenderness were the most common solicited adverse events irrespective of age and dose. All participants who received Vi-DT had a seroconversion (defined as 4-fold rise in anti-Vi antibody titers compared to baseline) 28 days post first dose and post second dose. The Vi-DT group had a four-fold higher geometric mean titre, with no further increases in GMT after the second dose. Vi-DT was also found to be safe and immunogenic in a phase I trial conducted by PT Bio Farma in Indonesia[107]. Further safety and immunogenicity of Vi-DT was assessed in 6 – 23 month old participants in a randomized , observer-blind , phase II clinical trial in the Philippines[108]. Participants received either a single dose of Vi-DT, two dose of Vi-DT or a comparator vaccine. No serious adverse events were related to Vi-DT. There was 100% seroconversion 4 weeks after the first dose of Vi-DT and 98.2% seroconversion 4 weeks after the second dose of the vaccine. Anti Vi-IgG titre was significantly higher in the Vi-DT group in all post-vaccination visits. Similar results were noted in the Phase II trial in 6 to 24 month old Indonesian children[109].

After the results of the Phase I and Phase II trials in the Philippines demonstrating the safety and immunogenicity of Vi-DT given at a dose of 25 μg in individuals aged 6 months to 45 years, an observer-blinded randomized controlled Phase III study was conducted in Nepal to assess the non-inferiority of a single dose of Vi-DT compared to Typbar-TCV by measuring the seroconversion rates of anti-Vi IgG titres 4 weeks after a single dose of vaccine[110]. In the trial, 1800 participants from 4 different sites, aged 6 months to 45 years were randomized to one of four study groups (A-D), where groups A – C received a

single dose of the Vi-Dt vaccines from one of three good manufacturing practice lots and group D received a single dose of Typbar-TCV[110]. Safety of the vaccine was assessed from solicited adverse events recorded 7 days post-vaccination and unsolicited adverse events recorded up to 4 weeks post-vaccination. VI-DT was safe with similar rates of adverse events reported across the four vaccine groups. The Vi-DT vaccine was immunogenic and non-inferior to Typbar-TV at 4 weeks post vaccination.

1.10.2.6 Accelerating the Introduction of Typhoid Conjugate Vaccine

In the background of high burden of typhoid fever and increasing antimicrobial resistance, vaccination can be a way forward to control the disease and antimicrobial resistance associated with it. With the recent emergence and outbreak of XDR *S.* Typhi strain, there is renewed focus on vaccines. The WHO Strategic Advisory Group of Experts (SAGE) in 2008 had recommended the use of Vi polysaccharide vaccines and the Ty21a vaccines for the control of typhoid fever. However, public health use of the vaccines was limited. In 2017, the WHO SAGE re-emphasized the importance of vaccination for the control of typhoid fever and in addition to the previous vaccines, recommended the use of typhoid conjugate vaccines.

Global Vaccine Alliance (GAVI) plays an important role in ensuring access and affordability of new vaccines to low- and-middle-income countries[111]. In 2008, GAVI included typhoid vaccines in their investment plan, but showed preference for a conjugate vaccine over the polysaccharide vaccine and Ty21a recommended by the

WHO, although TCV was still in the pipeline. TCVs were anticipated to be safe for administration in infants, have a stronger immunogenic response and a longer duration of protection. However, due to setback in the development of TCV, it was not until November 2017 that GAVI approved opening of a funding window for TCV impending WHO prequalification of TCV. TCV (Bharat Biotech) was subsequently pre-qualified in January 2018.

The Typhoid Vaccine Acceleration Consortium (TyVAC) was formed in 2016 with the aim to aid Gavi-eligible countries to accelerate the introduction of TCV[112]. The consortium is a partnership between the Center for Vaccine Development and Global Health at the University of Maryland, the Oxford Vaccine Group at the University of Oxford and PATH, an international non-profit organization. Two individually randomized controlled trials in Nepal and Malawi and a cluster randomized trial in Bangladesh were conducted as a part of the consortium to contribute to data in support of the use of TCVs. The trial, described in this thesis, describes the Nepal trial (TyVAC-Nepal).

The aim of this thesis is to assess the impact of TCV in endemic Lalitpur.

The objectives of this thesis are to:

1. To determine the efficacy of TCV in preventing blood culture-confirmed symptomatic infection caused by *Salmonella* Typhi

2. To determine the immunogenicity of TCV and persistence of antibodies induced by TCV.

3. To determine the safety of TCV.

4. To determine the age and sex differences in vaccine efficacy, immunogenicity and persistence of antibodies.

5. To determine the incidence of paratyphoid infection in the vaccinated population.

2. General Methods

Methods described in this chapter have been published in:

1) Theiss-Nyland K, Shakya M, Colin-Jones R, et al (2019) Assessing the Impact of a Vi-polysaccharide Conjugate Vaccine in Preventing Typhoid Infections Among Nepalese Children: A Protocol for a Phase III, Randomized Control Trial. *Clin Infect Dis*. 68(Supplement_2): S67–S73. doi:10.1093/cid/ciy1106[113].

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2) Colin-Jones R, Shakya M, Voysey M, et al. Logistics of Implementing a Large-scale Typhoid Vaccine Trial in Kathmandu, Nepal. *Clin Infect Dis*. 2019;68(Supplement_2): S138–S145. doi:10.1093/cid/ciy1125[114].

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2.1 Introduction

Phase III trials are typically conducted to assess vaccine efficacy[115]. The term vaccine efficacy usually refers to the direct vaccine effect, which is the protection the vaccine confers to the vaccinees as a result of the direct immunizing effect of the vaccine[115,116]. Indirect vaccine effect is the protection conferred to the non-vaccinees in the same population as the vaccinees as a result of proximity to the vaccinees, and is estimated by comparing the incidence in the non-vaccinees in the vaccinated cluster with the incidence in the non-vaccinees in the control cluster[115,116]. Total vaccine effect refers to combined direct and indirect vaccine effect estimated by comparing the incidence in vaccinees in the vaccinated cluster with the incidence in the non-vaccinees in the control cluster[115,116]. Overall vaccine effect refers to the protection of the entire population as a result of vaccine administration in a target group and compares the cluster level incidence in the vaccinated and control cluster including all individuals who received or did not receive the vaccine[115,116].

Two major trial designs have been described in the literature for determining the efficacy of vaccines. Individually randomized controlled trials randomize individual participants to the experimental vaccine group or a comparator group[115,117,112]. Randomization ensures that the groups being compared are similar to each other in all manners except for the study intervention. Individually randomized controlled trials are used to measure the direct effects of the vaccine. The measured and unmeasured confounders are well-balanced due to randomization. A smaller sample size is required compared with other trial designs. Blinding is also easier to maintain in individually randomized controlled

trials with an exception when the side effects of the vaccines are distinctive[117]. This trial design has previously been used to determine efficacy of Vi-rEPA in children in Vietnam[90].

Cluster randomized controlled trials, can measure the direct, as well as the indirect and the total effects of the vaccine[115,117,112]. Cluster randomized controlled trials randomize a cluster of individuals as a unit (usually based on geographical proximity) to the experimental vaccine group or a comparator group. Cluster randomized controlled trials require a large sample size and maintaining blinding is more difficult. In a cluster randomized controlled trials, each cluster needs to be well defined and independent of one another. A single cluster receives the same treatment and is hence easier administratively and avoids contamination by participants accidentally receiving the wrong vaccine. However, there is a risk of contamination between clusters due to movement of people in between clusters. Buffer zones between clusters or a “fried egg” design can be used to reduce the impact of contamination between clusters[118]. This design has successfully been used to determine the efficacy of the Vi polysaccharide vaccine in India and Pakistan[85,84].

Choice of vaccine trial design requires consideration of the question the trial is trying to address or the vaccine effects the trial aims to estimate. For typhoid fever, direct protection conferred to the vaccinees, indirect protection offered to the non-vaccinees, or the control group, and protection offered at a population level are all important questions to address from a public health point of view. The choice of trial design in TyVAC also depended on what was feasible in the study sites with the resources, the

available team, and their experiences. Three complementary trials, were conducted in three typhoid endemic settings as a part of TyVAC. Two individually randomized controlled trials were conducted in Nepal and Malawi to assess direct protection[119], and a cluster randomized controlled trial was conducted in Bangladesh to additionally assess the indirect, total and overall protection[120]. In this chapter, the methods of the individually randomized controlled trial conducted in Lalitpur, Nepal are described. The trial is referred to as TyVAC-Nepal in this thesis.

2.2 Clinical Trial Design

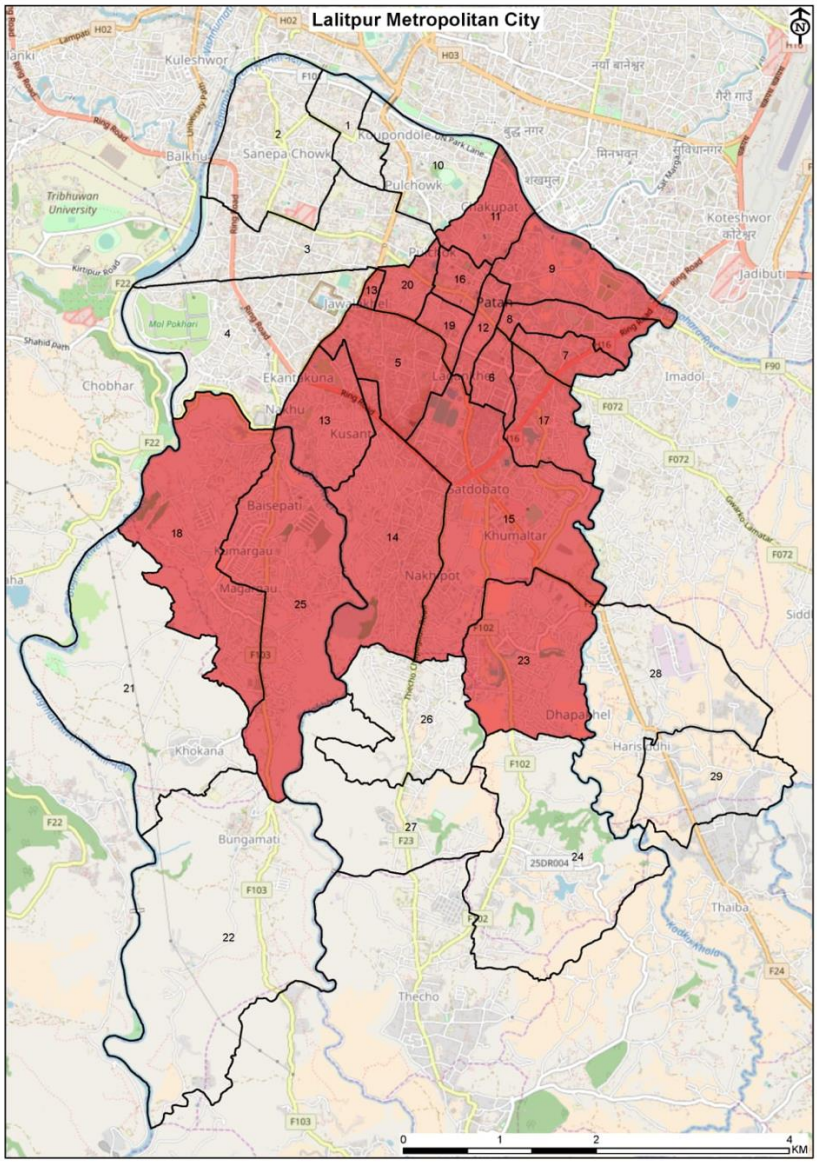
2.2.1 Study Design

This study was a Phase III, participant- and observer-blinded, 1:1 individually randomized controlled trial designed to determine the efficacy of TCV against blood culture-confirmed typhoid fever over a two-year period in children. An immunogenicity sub-study was built into the trial is detailed below (Section 2.3.1.3 Vaccination) and in Chapter 5.

2.2.2 Study Setting

The study was conducted in Lalitpur Metropolitan City (LMC), one of the three major cities in Kathmandu valley, Nepal and the headquarters for Lalitpur district. The gross national income per capita of Nepal in 2019, according to the World Bank was $1090. In 2010, 25.2% of the population were living below the national poverty line. The life expectancy at birth was 70.8 years and the under-five mortality rate were 30.8 in 2019.

Figure 4. Map showing the study area



Adapted from Lalitpur Metropolitan City, Geographical Resource Map showing Administrative Boundary. (https://lalitpurmun.gov.np/sites/lalitpurmun.gov.np/files/ward_1_29%20%28OSM%29.png)

*Highlighted 17 wards of the total 29 wards (ward no. 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 25) of Lalitpur Metropolitan City were included in the trial. Wards 5, 6, 7, 8, 9, 11, 12, 15, 16, 17, 19, 20 were previously a part of typhoid surveillance (STRATAA).

According to the population census in 2011, LMC had a population of 284,922 individual of which 48.78 % were female. There were 70,256 households and an average household size of 4.06 persons. The 20 – 24-year age group made up the largest proportion of the

population. Children below 15 years made up 22% of the total population. There is a wide ethnic diversity in LMC with the local ethnic group, the Newars, representing the largest population group. A total of 86% of the population could read and write. Around 66% of the households used piped water for drinking and over 90% of the household had flush toilets with access to the sewerage system or a septic tank.

The metropolitan city is divided into 29 wards and covers 36 square kilometers of geographical area. A ward is the smallest local unit within a municipality. A total of 17 wards from LMC were included in the trial. Figure 4 shows the wards where the trial was conducted, of which 12 wards that were previously a part of a typhoid surveillance study, STRATAA, were included[38]. Additional adjacent wards were chosen based on proximity to the study hospital, Patan hospital, and permissions from the respective wards.

2.2.3 Regulatory Approvals

The study was approved by the Oxford Tropical Research Ethics Committee and the Nepal Health Research Committee. The trial was conducted in accordance to the principles of the Declaration of Helsinki and in keeping with the principles of International Council for Harmonization Good Clinical Practice. An independent data and safety monitoring board oversaw data safety for the trial. The trial is registered with the ISRCTN registry, ISRCTN43385161.

2.2.4 Trial Participants

Children aged 9 months to under-16 years living in the defined catchment area (17 wards as highlighted in Figure 4) in Lalitpur Metropolitan City were eligible to participate in the study. The age range was chosen as there is a high burden of typhoid in the particular age group[52]. The lower age limit was selected as TCV could potentially be incorporated in the routine childhood vaccination schedule at 9 months.

2.2.5 Inclusion Criteria

Participants meeting all of following criteria were eligible to be considered for enrollment:

1. Parent/ legal guardian was willing and competent to provide informed consent. Participant aged 7 years and older was willing to give assent.
2. Aged 9 months to under-16 years at the time of vaccination.
3. In good health on the day of the vaccination.
4. Parent/ legal guardian confirmed that the child was willing and would be able to comply with the study requirements including follow-up contact.
5. Lived in the study catchment area at the time of the vaccination.

2.2.6 Exclusion Criteria

Participants were not enrolled if any of the following criteria applied:

1. Participant had knowingly received a typhoid vaccine in the last three years.

2. Participant had known allergy to any of the vaccine components.

3. Participant or parent/guardian had any medical or social reason that would prevent the participant from complying to the study requirements as judged by a medical professional.

4. Participant and parent/guardian were planning to move away from the study catchment area within the next 6 months.

2.2.7 Temporary Exclusion Criteria

Participants were temporarily excluded from the being vaccinated if any of the following criteria applied:

1. Participant had reported fever within the last 24 hours prior to vaccination.

2. There was history of use of anti-pyretics within 4 hours prior to vaccination.

Any participant meeting the above criteria were temporarily excluded for until 48 hours after the fever subsided. The participants were reassessed to confirm that the temporary exclusion criteria no longer existed before proceeding with further trial procedures. Although children with a mild illness, including mild fever, can get vaccines such participants were temporarily excluded. Fever in itself is an adverse event following immunization (AEFIs) (described in Section 2.3.2.1 Adverse Event Following Immunization (AEFI)) and was recorded in the study. Participants were only vaccinated after defervescence to ensure fever as an AEFI was correctly captured.

2.2.8 Vaccines

2.2.8.1 TCV

Typhoid conjugate vaccine, Typbar-TCV, manufactured by Bharat Biotech, Hyderabad, India was used as the interventional vaccine. The vaccine was available as a 2.5ml 5-dose vial with each 0.5 ml of vaccine dose contained 25mcg of purified Vi-Capsular Polysaccharide of S. Typhi Ty2 strain conjugated to tetanus toxoid.

2.2.8.2 MenA Vaccine

Meningococcal Group A conjugate vaccine, MenAfriVac, against *Neisseria meningitidis* serogroup A, manufactured by Serum Institute of India PVT Ltd, was used as the control vaccine. The vaccine was chosen as the control vaccine as: 1) It is a single dose intramuscular vaccine making the administration regime the same as with the intervention vaccine; 2) The vaccine does not provide any direct protection against typhoid fever; and 3) The vaccine provides some additional health benefits to the trial participants. The vaccine is not given to children as a part of the routine immunization schedule of Nepal and group A meningococcus is the most common serotype causing bacterial meningitis in Nepal.

The vaccine was available in two formulations; a standard 10 mcg/0.5 ml dose for individuals > 1 year of age and a standard 5 mcg/ 0.5ml dose for children 9 – 24 months of age. In the trial, children under 1 year of age were given the 5 mcg/0.5ml dose, and children > 1 year of age were given the 10 mcg/0.5 ml dose. The vaccine was available as

a 10-dose presentation consisting of a vial containing a lyophilised powder of meningococcal group A polysaccharide conjugated to tetanus toxoid protein and an ampoule of diluent. The lyophilised powder was reconstituted just before use with the contents of one ampoule of diluent to obtain 10 doses of the final vaccine.

2.2.9 Randomization and Masking

The participants were randomized on a 1:1 ratio to either receive the intervention vaccine, typhoid conjugate vaccine (TCV), or the control vaccine, meningococcal serogroup A conjugate vaccine (MenA), using stratified block randomization with randomly varying block sizes from 6-12. The participants were stratified by age: 9 months to under-5 years of age or $\geq$ 5 years to under-16 years of age.

In addition, for the immunogenicity sub-study, of the participants who gave additional consent for blood draw, 1500 were randomly selected for blood collection. Blood sampling randomization was done on a 2:1 basis (1000 TCV and 500 MenA) and was stratified by age (< 5 years and $\geq$ 5 years).

2.3 Study Procedures: Delivery of the Trial

The study procedures are described below. Table 5 shows the planned study procedures.

2.3.1 Vaccine Campaign and Delivery

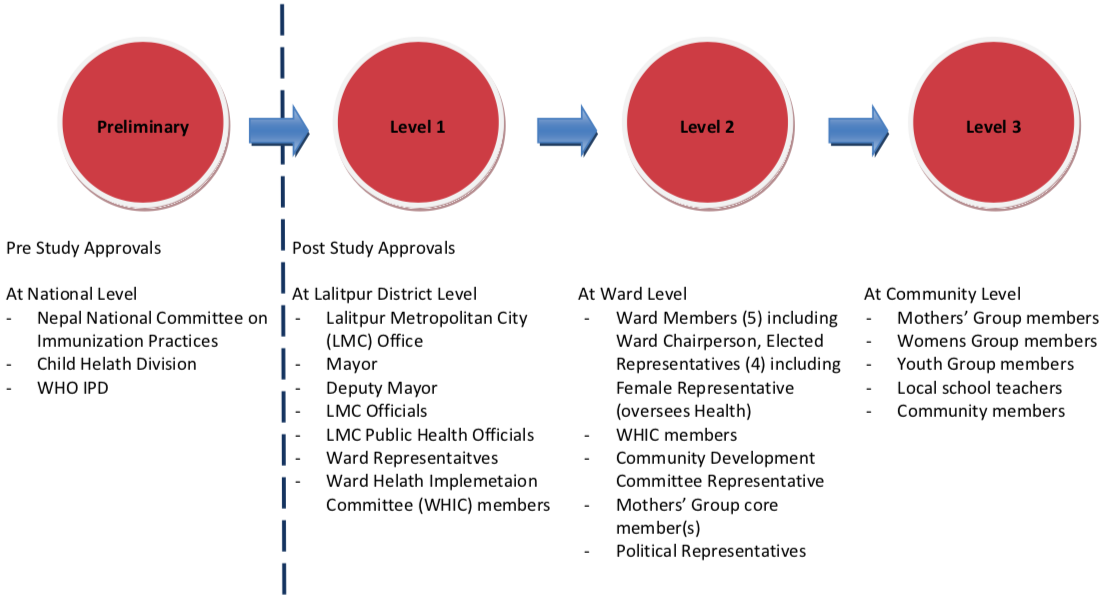
2.3.1.1 Public Engagement

Public engagement was key to running the large-scale vaccine trial in the community. A tiered approach to public engagement was taken (Figure 5). Preliminarily, study endorsements were received from national level stakeholders including the National Advisory Committee on Immunization, the Child Health Division (now Family Welfare Division) and the WHO IPD and was key for receiving local ethical approvals and approvals from local government.

In the first level, following ethical approval from the national ethical committee, series of meetings were held at a district level involving the Lalitpur metropolitan city (LMC) office, including the mayor and the deputy mayor, as well as the LMC public health officials, elected ward representatives, including the ward chairperson of each ward, and ward health implementation committee (WHIC) of each ward. The WHIC oversees all health-related activities in the community including running vitamin A supplementation and deworming tablet distribution campaigns in their respective wards and managing the ward run clinics. The first level meetings included detailed presentation about the purpose of the study and study procedures followed by vigorous discussions. A trial (commonly translated as an experiment in Nepali) at a community level that involves children and a relative new vaccine, and in which the administered vaccine would be unknown to the participants and study staff was an unfamiliar concept. Time was given to fully explain all aspects of the research to key stakeholders, and answer any questions that arose. After these discussions, permission was granted to proceed further.

With approvals from the district level, each ward was approached individually. Ward members, WHIC members, community representatives, core members of mothers' groups and political representatives were involved at this stage. Permissions were sought to run the study in their wards, to approach the community members, to set up vaccination clinics and for coordination to run vaccination clinics in their respective wards. As in the first level, series of meetings were held in each ward and involved discussions about the study objectives and procedures.

Figure 5. Tiered approach to public engagement



Reproduced under CC BY from Colin-Jones R, Shakya M, Voysey M, et al. **Logistics of Implementing a Large-scale Typhoid Vaccine Trial in Kathmandu, Nepal.** *Clin Infect Dis*. 2019;68(Supplement_2): S138–S145. doi:10.1093/cid/ciy1125.

Lastly, different community groups were engaged to disseminate information about the study. Meetings were setup with individual mothers' group, women's groups, youth groups, local schools, and clusters of communities in each ward. Presentations were followed by discussions and interactive sessions to answer queries about the study. The attendees were requested to disseminate the information in their communities. Flyers detailing the study were also distributed to relay information about the study.

Public engagement is a continuous process and was maintained beyond the vaccination phase of the trial, at the different levels. Meetings were conducted at the first level and second level to inform them about the study progress, to share the results of the study and to get feedback about the study from the stakeholders and the community. Level 3 public engagement activities were conducted to keep the community members informed about the study, and answer any queries and alleviate their concerns about the study. Informative sessions about current and relevant health topics such as fever, diarrhea, pneumonia etc. were also conducted in the level 3 public engagement to build rapport and maintain relation with the communities.

2.3.1.2 Recruitment of Participants

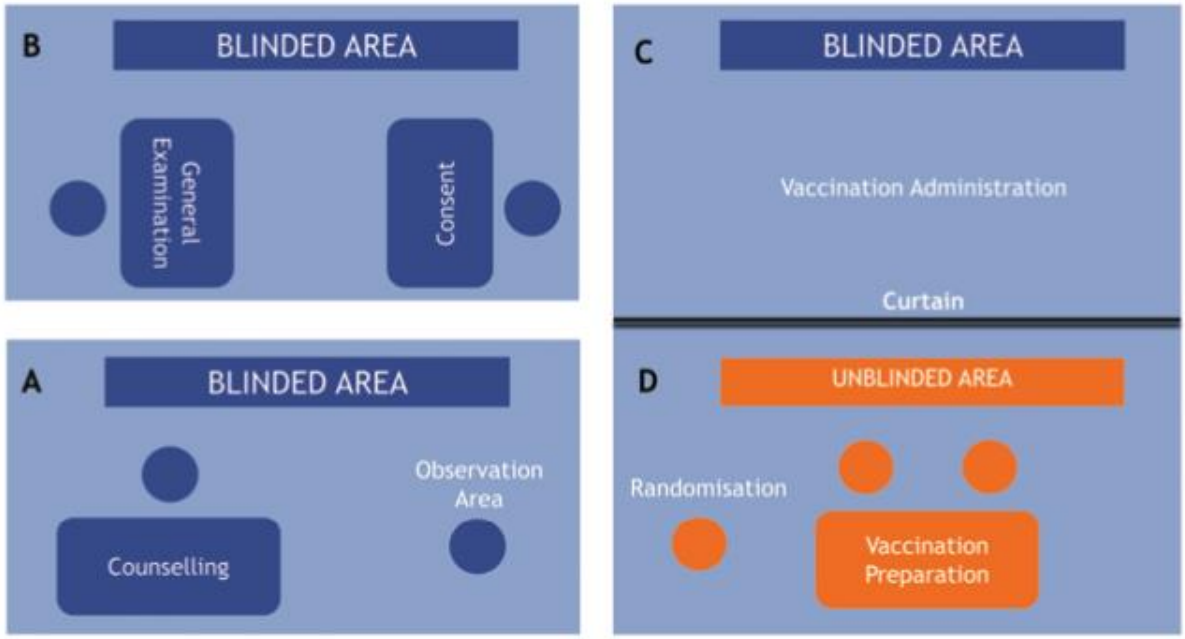
Following approvals and information dissemination at the different levels, field staff visited households and invited parents/guardians to vaccination clinics. Tole Health Promoters (THPs), who are ward-based health volunteers and are involved in routine vaccination campaigns and health related activities in their respective wards, were

employed as field staff in coordination with the individual wards as they had good knowledge and good rapport with the community. Each ward is divided into smaller clusters that is used to run routine health campaigns. The same clusters were adopted to approach households. The field staff went door-to-door to cover each household in each cluster in the ward. The home visits were made in days leading to the opening of the vaccination clinic in the wards and throughout the period the vaccination clinics were open in the particular wards.

2.3.1.3 Vaccination

Vaccine clinics were opened in a step-wise manner beginning with a single ward and slowly expanding to run up to 7 – 8 vaccination clinics at a time ensuring that enrollment picked up at a good pace and side-by-side there was adequate oversight of the clinics. One to two clinics were established in each ward depending on the size of the wards. In the larger wards where the vaccination clinics were geographically distant and less accessible to the participants, mobile clinics were also run. Clinics were run from 7 AM in the morning to capture school -going participants, up to 2 PM on weekdays and up to 5 PM on Saturdays and public holidays to boost enrollment.

Figure 6. Clinic set-up and maintaining blinding.



Reproduced under CC BY from Colin-Jones R, Shakya M, Voysey M, et al. **Logistics of Implementing a Large-scale Typhoid Vaccine Trial in Kathmandu, Nepal.** *Clin Infect Dis*. 2019;68(Supplement_2):S138–S145. doi:10.1093/cid/ciy1125.

The vaccination clinics were set-up as depicted in Figure 6. Vaccination clinic sites were chosen ensuring that the clinics were easily accessible, working electricity and had adequate space to conduct the study procedures. Each set-up had a blinded area hidden behind curtain to ensure blinding of the study would be maintained.

Figure 7 A – G shows the vaccination procedures. In the first stage, the participants and guardians were welcomed by a counselor who read/ ensured that the parents/ guardians read the participant information sheet, and counselled them about the study. The counselor took verbal consent to take the child's temperature, measured the axillary temperature and noted it down. Any history of use of anti-pyretics was also taken at this stage. All participants were given a study diary with a unique identifier for future reference. The study diary included information about what to expect in the various

study visits. The participants then moved on to a consent taker, who explained the study in detail, allowing time for questions and took written informed parental consent and assent from child 7 years and older. If the parent/ guardian were illiterate, a witness was present throughout the consent process to verify the process. Following consent, the participants were screened and had a general examination to ensure eligibility. Study doctors were consulted if there were any confusions about the study eligibility. Height and weight measurements were taken for children under 5 years of age. The participants were then seen by an unblinded team. The participants were randomized both for the main study and the immunogenicity sub-study, for those consenting for this component, using an offline mobile application built for the purpose. Participants consenting and randomized for a blood draw, had a blood draw prior to vaccination. The methods of the immunogenicity sub-study are detailed in Chapter 5.

All participants were observed for 15 minutes post-vaccination to identify any immediate adverse events.

An electronic direct data entry database on REDCap, a secure web application, with inbuilt real-time data validation was used for data capture (REDCap 9.5.22 - © 2021 Vanderbilt University). Electronic data capture was essential to be able to logistically manage the volume of data and to minimize missing data and data errors. None of the clinics had internet. Portable local servers accessible through Intranet were set up in each clinic, and at the end of the day, the local servers were transported to a central facility with secure internet, and the data were uploaded onto the main server.

Figure 7. A – G: Study procedures for vaccination of participants

A

B

C

D

E

F

G

H

Source: Bill & Melinda Gates Foundation/ Sam Reinders

A. Tole Health Promoters (THPs) visited homes to inform about the study and invite potential participants and their guardians to vaccination clinics. B. Counselling: Parents/guardians of potential participants were informed about the study. C. Counsellor took axillary temperature of participant after verbal consent. D. Consent taking: A trained and qualified nurse took written, informed consent from parents/ guardians. Assent was taken from participants 7 years and older. E. Height and weight of children under 5 years was measured. F. Participant receiving vaccine by the unblinded vaccination staff. G. Blood draw for immunogenicity component of the study. H. Vaccinated participants pose for a photo while they are observed for post-vaccination immediate adverse events.

2.3.2 Follow-Up Contact of Participants

2.3.2.1 Adverse Event Following Immunization (AEFI)

All participants were contacted via phone calls by the study communication team 7 days post-vaccination, with a permissible window of 6 – 8 days, with the aim to identify if the participants had any adverse events following vaccination. Data were collected were reported by the participant parents/ guardians and were subjective. Data on the following adverse reactions were documented: pain, swelling, redness, fever, vomiting, diarrhea, reduced activity, persistent crying, eating less, increased irritability and general feeling. The adverse reactions were graded as none, mild, moderate and severe, with an exception of fever that was reported as a yes and no, and general feeling that was reported as well and unwell. If it was identified that a participant had a serious adverse event (described in Section 2.3.2.2), the study doctor was notified and safety reporting procedures were followed.

2.3.2.2 Three-Monthly Phone Calls

All participant parents/guardians were contacted by the study communication team on a three-monthly basis following vaccination. Data were collected on fever occurrences and

durations, visits to hospitals, clinics or pharmacies, treatment seeking behavior, and use of antibiotics.

The calls aimed to actively surveil and identify participants for:

- **Active surveillance for typhoid fever**

Any participant that reported fever and visit to health care facilities, including self-reported suspected or culture-confirmed typhoid fever, were further followed up to identify cases of culture-confirmed typhoid fever that were not captured in the passive surveillance. The aim was to avoid missing culture positive cases. Trained study nurses visited the participants or invited to the participants/guardians to the passive surveillance clinics, as convenient for the participants/guardians, and reviewed the medical records to confirm culture-positive typhoid fever. These confirmed cases were also included in the study analyses. Additionally, the study participants were also reminded to visit the study clinics.

- **Serious adverse events (SAEs)**

SAEs were defined as any untoward medical occurrence in a participant to whom a medicinal product was administered that resulted in death, was life-threatening, required inpatient hospitalisation or prolonged existing hospitalisation, resulted in persistent or significant disability/incapacity or resulted in congenital anomaly or birth defect, including occurrences which were not necessarily caused by or related to the vaccine. Serious adverse reactions (SARs) were defined any adverse event that is both

serious and is believed with reasonable probability to be due to one of the trial treatments based on the information provided.

Study doctors followed up SAEs identified during the three-monthly follow-up calls. All SAEs that occurred in the first 30 days post-vaccination were reported to the Data Safety and Monitoring Board (DSMB), the principal and chief investigator within 24 hours of the study team becoming aware of the event. A more detailed report was sent within 48 hours after the initial report. After the first 30 days, all SAEs were recorded but only the SARs were reported.

2.3.2.3 Quarterly Home Visits

THPs visited the homes of participants every three months throughout the study period. The visit aimed to maintain contact with the participants, to remind them to visit the passive surveillance clinics if the participants are unwell, and to identify missed cases of typhoid fever that could not be contacted in the quarterly calls. Medical records of these participants were later reviewed by trained study staff. Any queries and concerns of the participants were also identified and addressed in these visits or escalated to the senior study team as necessary.

2.3.3 Passive Surveillance for Blood-Culture Confirmed Typhoid Fever

A hospital-based clinic at the study hospital, Patan hospital, and 18 community-based passive surveillance clinics were established. There are a large number of pharmacies,

private clinics and private hospitals in the study area that are accessible the participants. Healthcare utilization surveys in a previously conducted study in the same setting showed that this led to only limited cases being captured in the surveillance site[52]. Hence, in order to ensure a study surveillance facility was easily accessible to the study participants and a larger proportion of the cases could be captured, community-based facilities were also setup.

Patan hospital is a 450 bedded tertiary care hospital in Lalitpur that provides treatment to around 320,000 outpatients and 20,000 inpatients every year. A large proportion of the patients attending the hospital are from within Lalitpur and adjacent cities. Qualified study doctors ran the hospital-based clinic with support from nurses and health assistants.

The community clinics were set-up in the ward clinics. The ward clinics are local body run clinics in each ward that primarily function as routine vaccination clinics for children, and maternal child health clinics and are equipped with basic primary health care facility infrastructure. The community clinics were set up in close coordination with the wards. Qualified study doctors ran the passive surveillance clinics along with a triage assistant to support the doctors. A sample transport system, following appropriate safety guidelines, was setup between the community clinics and Patan hospital to ensure samples were safely transported to the hospital and timely reports were transported back to the clinical team.

Parents/guardians were encouraged to bring their children to the clinics if they were ill. Study doctors attended to all patients visiting the study clinics and managed the patients in consultation with the study pediatrician as required. Any participant presenting with a current temperature of ≥38°C and/or a self-reported fever of ≥2 days were consented for blood culture. Blood cultures are the gold standard for diagnosis of typhoid fever and was used to measure the primary outcome of the study, blood culture-confirmed symptomatic infection caused by *S.* Typhi. Written informed consent was taken from parents/guardian and assent was taken for any child 7 years or older. All participant data were directly entered onto REDCap. If direct data entry was not possible due to internet problems, data were entered onto paper case report forms (CRFs) and entered onto REDCap from the source documents.

The study participants were incentivized to attend the study clinics. Attendance to the study clinics, investigations and treatments were paid for by the study. Visits to the emergency at Patan hospital or in-patient admission to Patan hospital were partially or completely reimbursed depending on the diagnosis. All culture-confirmed typhoid fever cases were followed up two weeks after the fever presentation to record the outcome of the illness.

2.3.4 Unblinding and End of Study Vaccination

Unblinding and vaccination clinics were also run in a step-wise manner as at the beginning of the study. Participants were informed through phone calls, public engagements and THP home visits. In brief, in the last visit, the participants were

unblinded and offered the alternate vaccine, i.e. those who received TCV at the beginning of the trial were offered MenA vaccine and those who had received the MenA vaccine were offered TCV.

At the first stage, participants were verified by their participant IDs. The study nurse explained the process for the visit and identified which study arm the participants were in and whether they were due for a blood sample as a part of the immunogenicity sub study. In addition, those who were under 5 years at the first visit were also due for height and weight measurements. The participants were unblinded, offered the additional vaccine and consented for the receipt of the vaccine. At the second stage, participants had their temperatures taken, and height and weight measurements taken if they were due. Lastly, as in the first visit, 24-month immunogenicity samples were drawn, if due, and participants were given the alternate vaccine. All participants were observed for 15 minutes for immediate adverse events.

Table 5. Schedule of planned procedures

Visit	1	2	3	4	5	6	7	8	9	10	11(a)
Day	0	7	28	90	180	270	365	455	545	635	730
Permissible time window (days)		+7/-1	+/-4	+/-14	+/- 28	+/-28	+/-56	+/-56	+/-56	+/-56	+/-90
Screening	X										
Consent (b)	X										X
Medical history and general examination	X										
Height and weight (c)	X										X
Randomisation	X										
Blood collection (d)	X		X						X		X
Vaccination	X										
Follow-up contact (e)		X**		X	X	X	X	X	X	X	X
Passive Surveillance (f)	X	X	X	X	X	X	X	X	X	X	X
Public Engagement	X	X	X	X	X	X	X	X	X	X	X
Unblinding and receipt of vaccine (g)											X

a) All of the visit 11 activities occurred simultaneously, except for the follow-up contact.

b) Written informed consent was taken at the start of the study. Written informed consent was also taken when offering the alternate vaccine at the end of the 2-year study period.

c) Only children under 5 years of age at the time of enrolment

d) Blood sampling for immunogenicity in a subset of approximately 1000 TCV, and 500 control participants.

- Blood draw on day 0 was before vaccination; blood draw at day 730 was at some time as unblinding and vaccine documentation.

e) Follow-up contact included:

- Ensure participant and family still lives in area and happy to continue with study
- Enquire re: work and school absenteeism
- Record mortality and morbidity in participant, including fever
- Reminder to attend study passive surveillance clinics if they develop fever of ≥2 days
- **: At 7 days full AEFI reporting was collected

f) Passive surveillance was conducted throughout the study period.

g) Either TCV or MenA vaccine was offered at the end of the trial after unblinding depending on which vaccine the child initially received.

2.4 Statistical Analysis

Statistical analyses conducted for each of the outcomes are described under 'Statistical Analysis" in the respective chapters.

2.4.1 Sample Size Calculation

The study was powered for the primary objective. Sample size calculations assumed that the overall incidence of typhoid fever was 85 cases per 100,000 persons in the overall population, with a higher incidence in children under 16 years. Age specific incidence rates were determined using the age distribution of typhoid cases in Kathmandu from published estimates and from site specific surveillance data[52]. The direct effect of vaccination was assumed to be 75% and the indirect effect to be 25% based on mathematical modelling. A 25% loss to follow up per year due to moving out of the area was accounted for based on typhoid surveillance data from the study catchment area[52]. A sample size of 17,395 participants would be needed based on the above assumption. However, to allow for further variation in the assumption, the sample size was increased to 20,000 children. Under these assumptions, 45 cases of typhoid fever (approximately 36 cases in the control arm and 9 cases in the TCV arm) were expected over the two year follow up period.

The study also had 80% power to detect a five-fold increase in SAEs. This study assumed that the the background rate of a specific rare but serious adverse event (SAE) is

approximately 30 per 100,000 individuals, as seen with intussusception in the rotavirus vaccine trials. The study was not powered to compare the immunogenicity of the vaccines.

2.4.2 Analysis Software

Statistical analysis was conducted using Stata version 15.1 (StataCorp, Texas, USA).

3. Safety of Typhoid Conjugate Vaccine

The results presented in this chapter is published in:

1. Shakya M, Colin-Jones R, Theiss-Nyland K, et al. Phase 3 efficacy analysis of a typhoid conjugate vaccine trial in Nepal. N Engl J Med 2019; 381:2209-2218.

This article was published under the terms of the Creative Commons Attribution License (CC BY All analyses, tables, and figures for the paper were produced by Merryn Voysey. Mila Shakya wrote the first draft of the paper. Mila Shakya conducted all the analyses, produced all tables and figures for this thesis. Co-authors have given permission to include the material in this thesis.

2. Shakya M, Voysey M, Theiss-Nyland K, *et al.* Efficacy of typhoid conjugate vaccine in Nepal: final results of a phase 3, randomized, controlled trial. *Lancet Glob Heal* 2021; **9**: e1561–8.

This article was published under the terms of the Creative Commons Attribution License (CC BY). All text, analyses, tables, and figures were produced by Mila Shakya. Co-authors have given permission to include the material in this thesis.

3.1 Introduction

Vaccines have played a crucial role in reducing morbidity and mortality due to infectious diseases worldwide. Nonetheless, vaccines are biological products that are introduced into the human body and can potentially cause short-term adverse reactions. All injectable vaccines may cause local reactions at the site of injection, such as pain, swelling, redness, and all vaccines maybe associated with fever and other systematic reactions. These reactions usually resolve within a week after vaccination. Anaphylaxis, post-vaccination, can occur in rare occasions.

Vaccine safety is extremely important as vaccines are usually given to otherwise healthy individuals and more traditionally to infants and young children through national immunization programmes. Further vaccine safety is key to ensure public confidence in the vaccine and consequently for the long-term success of vaccination programmes. All vaccine candidates, before they are licensed and widely used in vaccination programmes, go through rigorous stages of clinical trials to detect adverse events associated with the vaccine along with assessment of vaccine efficacy and immunogenicity. Clinical trials assess vaccine safety in a relatively small sample and rare adverse events that occur in one in a few thousand may not be detected until the vaccines are more widely used.

The TCV used as the intervention vaccine in TyVAC-Nepal has been reported to be safe in children above 6 months and in adults prior to this trial. Six hundred and fifty-four participants, aged 2 - 45 years, were enrolled in a double blinded RCT and 327 participants, aged 6 – 23 months, were enrolled in a prelicensure open-label trial

conducted by the manufacturers in India[97]. TCV was compared with the polysaccharide typhoid vaccine in the double-blind RCT. The polysaccharide vaccine could not be use as the comparator vaccine in children less than 2 years since the vaccine is only licensed for 2 years and above, and no other comparator or placebo was used in this age group as recommended by the national regulatory body. Overall, the adverse reactions, local and systemic, reported were uncommon in both the RCT and the open label trial, and occurred at similar rates in both groups in the RCT. Similarly, no safety concerns were raised in a randomized controlled, phase 2b trial conducted in 112 adult participants in Oxford[121]. However, these studies were conducted in a small number of participants, and more importantly the trial in India, which is was the only trial that included child participants, was conducted in a relatively small number of children. The TCV was licensed in India in 2013 and is available for public use in India. However, limited post-marketing safety monitoring data was available, and so the safety of TCV in larger population is yet to be established. In TyVAC-Nepal, TCV was administered to 10,005 children aged 9 months to under 16 years of age. The main aim of this chapter is to investigate the safety outcomes associated with TCV.

The Global Advisory Committee on Vaccine Safety (GACVS), which provides the WHO independent advice on vaccine-related safety issues, in 2018, reviewed the safety of the TCV vaccine[122]. In the review, the passive surveillance following introduction of TCV into routine immunization in Navi Mumbai reported that a large number of thrombocytopenia cases were observed. Forty-three cases of thrombocytopenia were observed among the children receiving TCV, and 299 cases were observed among unvaccinated children with no statistically significant difference between the two groups.

It was suggested that the thrombocytopenia was due to the ongoing dengue infection and was unrelated to the TCV campaign. Concerns over thrombocytopenia were also raised by the Adverse Events Following Immunization (AEFI) Committee in Nepal. Thrombocytopenia following vaccine administration has been reported with several childhood vaccines including measles, mumps and rubella (MMR) vaccine; diphtheria, tetanus and pertussis (DTP) vaccine; diphtheria, tetanus and acellular pertussis (DTaP) vaccine; varicella vaccine; hepatitis A vaccine, and hepatitis B vaccine, and is considered to be immune related[123]. Thus, thrombocytopenia was explored as an adverse event of interest in the trial dataset and is also reported in this chapter.

3.2 Methods

3.2.1 Adverse Event Following Immunization (AEFIs)

The method for collecting data on solicited AEFIs has been described in Chapter 2, Section 2.3.2.1. 'Adverse Event Following Immunization (AEFI)'. Data on 11 solicited adverse reaction were collected: pain, swelling, redness, fever, vomiting, diarrhea, reduced activity, persistent crying, eating less, increased irritability, and generally unwell. Fever and feeling generally unwell were formatted as a yes and no question. Other adverse reactions were categorized as no symptoms (none), mild, moderate and severe. The classification with the definitions of the of the grades of the solicited adverse reactions is detailed in the Appendix 1.

3.2.2 SAEs

The method for collecting data on SAEs has been described in Chapter 2, Section 2.3.2.2 under the heading 'Serious adverse events (SAEs)'. All SAEs were coded according to Medical Dictionary for Regulatory Activities (MedDRA) by designated trained study doctors[124].

3.2.3 Thrombocytopenia

Thrombocytopenia was assessed from the passive surveillance dataset. The method for collecting passive surveillance data has been described in Chapter 3, Section 2.3.3 under the heading 'Passive Surveillance for blood-culture confirmed typhoid fever'. All consenting participants who reported fever ≥2 days, and/ or had a measured fever ≥38°C had their blood drawn for blood cultures. Blood counts, including platelet counts, were also sent for the participants when needed for further clinical assessment. Platelet count of less the 150,000/uL were defined as thrombocytopenia. Platelet count of participants who presented to the passive surveillance clinics within three months post-vaccination, met the criteria for passive surveillance and had blood samples sent for investigation were included in the analyses.

3.2.4 Statistical Analysis

Counts and percentages were reported for the solicited adverse reactions. Chi-square tests were used to compare the TCV and MenA group, and the younger children and the

older children. Fishers Exact test was used when the expected frequency was less than five in any cell.

Phi coefficient or Pearson's Phi coefficient **(ϕ)** was calculated to measure the effect size for comparison between TCV and MenA group. Phi coefficient is a measure of strength of association between two binary nominal variable, is typically used following a Chi-square test and values range from -1 to 1[125,126]. A value below 0 is interpreted as negative association between variables; 0 is interpreted as no association between the two variables; and, a value above 1 is interpreted as positive association between variables. For positive association, 0.01 to 0.19 is interpreted as no or negligible association; 0.2 to 0.39 as weak association; 0.4 to 0.69 as medium association; and, 0.70 to 1.0 as strong positive association.

Spearman's correlation coefficient, r_s, was calculated to measure the strength of relationship between the reactogenicity and the age groups. Spearman's correlation coefficient is used to measure the correlation or effect size of interval or ordinal data[126]. The values range from -1 to +1 where values 0.00 – 0.19 is interpreted as very weak correlation; 0.20 – 0.39 as weak; 0.40 – 0.59 as moderate; 0.60 – 0.79 as strong and 0.80 – 1.0 as very strong correlation.

Counts were reported for SAEs at 28 days and 6 months post-vaccination.

3.3 Results

3.3.1 Solicited AEFIs

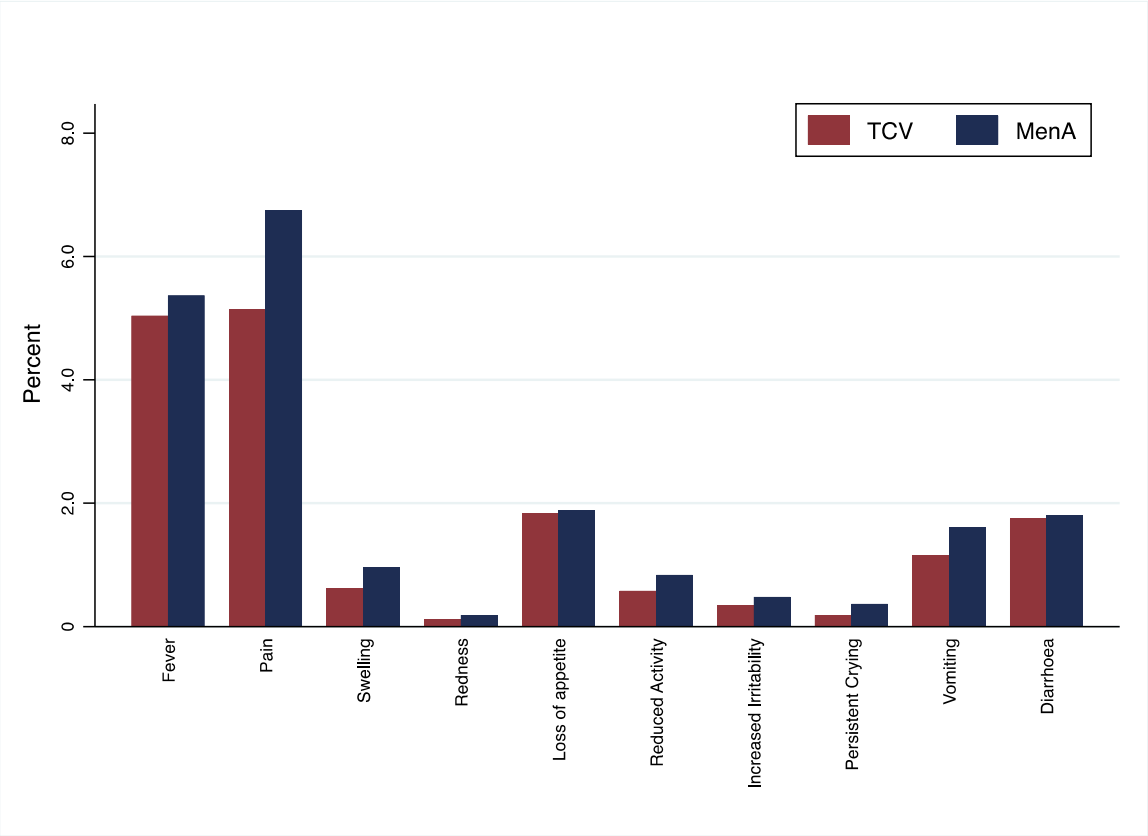
Of the 20,019 participants enrolled in the trial, 18,743 (93.6%) were assessed for adverse reactions 7 days post-vaccination. Of those assessed, 18,670 (9341/9380 in the TCV group and 9329/9363 in the MenA group) reported at least one adverse reaction.

Table 6 and Figure 8 show the solicited adverse reactions within 7 days post-vaccination by vaccine group. There were statistically significantly higher reports of pain, swelling, vomiting, reduced activity and persistent crying in the MenA group; however, the effect size was negligible ($\phi < 0.19$). Overall, pain, fever, and feeling generally unwell were most commonly reported. In total, 6.7% of the participants (6.4% in the TCV group and 7.1% in the MenA vaccine group) were reportedly unwell after vaccination. A total of 5.9% of the participants (5.2% of TCV group and 6.7% of MenA vaccine group) had pain at the site of vaccination among which, the pain was mild in 92.5% of the participants (94.4% of TCV group and 91.1% of MenA vaccine group). Redness and swelling at the site of vaccination was seen in $\leq$ 1% of the participants. Fever was present, as reported by parents or guardians, in 5.2% of the participants (5.0% of TCV group and 5.4% of MenA vaccine group). Diarrhea and vomiting were reported in 1.7% and 1.4% of the participants in the TCV group and MenA group respectively (vomiting in 1.2% of the TCV group and 1.6% of the MenA vaccine group, and diarrhea in 1.7% of the TCV group and 1.8% of the MenA vaccine group). A total of 1.8% of the children were reportedly eating less than usual (1.8% of the TCV group and 1.9% of the MenA vaccine group). In total, 6.7% of the participants (6.4% in the TCV group and 7.1% in the MenA vaccine group) were

reportedly unwell after vaccination. All other reactions were rare and occurred in fewer than 1% of the children (Table 6).

Table 7 shows the solicited reactogenicity in the TCV group stratified by age. All adverse reactions at day 7 were reported significantly more often in younger children except redness, which was reported in very few participants. There were no significant differences in severity for those with reported symptoms between the two age groups.

Figure 8. Solicited Adverse Reactions Within 7 Days After Vaccination



TCV = Typhoid conjugate vaccine. MenA = Group A meningococcal vaccine (control)

Table 6. Solicited Adverse Reactions within 7 Days after Vaccination (Reported and Classified by the Participant)

Adverse Reaction	All Participants (N=18,743)	TCV (N=9380)	MenA vaccine (N=9363)	P-value[γ] (φ)
Pain				
None	17,628(94.1)	8897 (94.9)	8731 (93.3)	
Mild	1,031 (5.5)	455 (4.9)	576 (6.1)	0.000
Moderate	79 (0.4)	26 (0.3)	53 (0.6)	(0.0341)
Severe	4 (<0.1)	1 (<0.1)	3 (<0.1)	
Swelling				
None	18,594 (99.2)	9321 (99.4)	9273 (99.0)	0.008
Mild	134 (0.7)	53 (0.6)	81 (0.9)	(0.0194)
Moderate	14 (0.1)	5 (0.1)	9 (0.1)	
Redness				
None	18,713 (99.8)	9368 (99.9)	9345 (99.8)	0.192
Mild	134 (0.7)	10 (0.1)	14 (0.9)	(0.0095)
Moderate	5 (<0.1)	1 (<0.1)	4 (<0.1)	
Fever				
No	17,766 (94.8)	8906 (95.0)	8860 (94.6)	0.311
Yes	976 (5.2)	473 (5.0)	503 (5.4)	(0.0074)
Vomiting				
None	18,483 (98.6)	9271 (98.9)	9212 (98.4)	0.007
Mild	206 (1.1)	84 (0.9)	122 (1.3)	(0.0171)
Moderate	48 (0.3)	23 (0.5)	25 (0.3)	
Severe	5 (<0.1)	1 (<0.1)	4 (<0.1)	
Diarrhea				
None	18,409 (98.2)	9215 (98.3)	9194 (98.2)	
Mild	247 (1.3)	126 (1.3)	121 (1.3)	0.770
Moderate	81 (0.4)	37 (0.4)	44 (0.5)	(0.0171)
Severe	5 (<0.1)	1 (<0.1)	4 (<0.1)	
Reduced activity				
None	18,609 (99.3)	9325 (99.4)	9284 (99.2)	
Mild	112 (0.6)	43 (0.5)	69 (0.7)	0.029
Moderate	19 (0.1)	11 (0.1)	8 (0.1)	(0.0160)
Severe	2 (<0.1)	0	2 (<0.1)	
Persistent crying				

None	18,689 (99.7)	9361 (99.8)	9328 (99.6)	
Mild	42 (0.2)	15 (0.2)	27 (0.3)	0.019
Moderate	9 (0.1)	3 (<0.1)	6 (0.1)	(0.0171)
Severe	2 (<0.1)	0	2 (<0.1)	
Eating less				
None	18, 393 (98.1)	9206 (98.2)	9187 (98.1)	
Mild	288 (1.5)	144 (1.5)	144 (1.5)	0.859
Moderate	58 (0.3)	29 (0.3)	29 (0.3)	(0.0013)
Severe	3 (<0.1)	0	3 (<0.1)	
Increased irritability				
None	18,665 (99.6)	9347 (99.7)	9318 (99.5)	
Mild	66 (0.4)	26 (0.3)	40 (0.4)	0.136
Moderate	10 (0.1)	6 (0.1)	4 (<0.1)	(0.0109)
Severe	1 (<0.1)	0	1 (<0.1)	
General feeling				
Well	17, 478 (93.3)	8779 (93.6)	8699 (92.9)	0.058
Unwell	1,264 (6.7)	600 (6.4)	664 (7.1)	(0.0138)

TCV = Typhoid conjugate vaccine. MenA = Group A meningococcal vaccine (control)

* Fever or a feverish feeling in a participant was reported by the parents or guardians of the participant. No temperature readings were taken. Percentages may not total 100 because of rounding.

ɣ P-value for comparison between those with and without symptoms.

Φ Phi coefficient is a measure of strength of association. Values 0.01 to 0.19 is interpreted as no or negligible association; 0.2 to 0.39 as weak association; 0.4 to 0.69 as medium association; and, 0.70 to 1.0 as strong positive association.

Table 7. Solicited Adverse Reactions within 7 days after Vaccination in the TCV group, by Age Group (Reported and Classified by the Participants).

Adverse Reaction	Younger children (<5 years) (N=2620)	Older children (>=5 years) (N=6759)	P-value[∝] (Severity)	P-value[γ]	r_s [σ] (P-value)
Pain					
None	2561 (97.8)	6336 (93.7)			
Mild	56 (2.14)	399 (5.9)		0.000	0.0916
Moderate	3 (0.1)	23 (0.3)	1.000		(0.0000)
Severe	0 (0.0)	1 (<0.1)			
Swelling					
None	2611 (99.7)	6710 (99.3)			
Mild	7 (0.3)	46 (0.7)		0.034	0.0218
Moderate	2 (0.1	3 (<0.1)	0.168		(0.0345)
Redness					
None	2615 (99.8)	6753 (99.9)			
Mild	4 (0.2)	6 (0.1)		0.194	-0.0134
Moderate	1 (<0.1)	0 (0.0)	0.455		(0.1951)
Fever*					
No	2415 (92.2)	6491 (96.0)		0.000	-0.0791
Yes	205 (7.8)	268 (4.0)			(0.0000)
Vomiting					
None	2551 (97.4)	6720 (99.4)			
Mild	50 (1.9)	34 (0.5)		0.000	-0.0865
Moderate	18 (0.7)	5 (0.07)	0.170		(0.0000)
Severe	1 (<0.1)	0 0.0)			
Diarrhea					
None	2524 (96.3)	6691 (99.0)			
Mild	72 (2.8)	54 (0.8)		0.000	-0.0910
Moderate	23 (0.9)	14 (0.2)	0.827		(0.0000)
Severe	1 (<0.1)	0 (0.0)			
Reduced activity					
None	2589 (98.8)	6736 (99.7)			
Mild	22 (0.8)	21 (0.3)		0.000	-0.0500
Moderate	9 (0.3)	2 (0.3)	0.092		(0.0000)

Persistent crying					
None	2605 (99.4)	6756 (99.9)	1.000	0.000	-0.0541 (0.0000)
Mild	12 (0.5)	3 (<0.1)			
Moderate	3 (0.1)	0 (0.0)			
Eating less					
None	2521 (96.2)	6685 (98.9)	0.099	0.000	-0.0895 (0.0000)
Mild	78 (3.0)	66 (1.0)			
Moderate	21 (0.8)	29 (0.3)			
Increased irritability					
None	2596 (99.1)	6751 (99.9)	1.000	0.000	-0.0614 (0.0000)
Mild	196 (0.7)	7 (0.1)			
Moderate	56 (0.2)	1 (<0.1)			
General feeling					
Well	2361(90.1)	6418 (95.0)		0.000	-0.0888 (0.0000)
Unwell	9.8 (9.9)	341 (5.1)			

TCV = Typhoid conjugate vaccine. MenA = Group A meningococcal vaccine (control)

* Fever or a feverish feeling in a participant was reported by the parents or guardians of the participant. No temperature readings were taken. Percentages may not total 100 because of rounding.

ɣ P-value for comparison between those with and without symptoms.

Spearman's correlation coefficient, r_s, is used to measure the correlation or effect size of interval or ordinal data. The values range from -1 to +1 where values 0.00 – 0.19 is interpreted as very weak correlation; 0.20 – 0.39 as weak; 0.40 – 0.59 as moderate; 0.60 – 0.79 as strong and 0.80 – 1.0 as very strong correlation.

3.3.2 Serious Adverse Events (SAEs)

In the first 28 days after vaccination, there were 18 SAEs in 17 participants (7 participants in the TCV group and 10 in the MenA vaccine group) (Table 8). Most of the SAEs were moderate in severity. One SAE in the MenA vaccine group was deemed to be related to the vaccine by the study pediatrician. The participant, a 13-month-old female, had high grade fever within 24 hours after vaccination, was admitted and given antipyretics. The fever subsided after 12 hours and the results of the laboratory tests were within normal limits. The participant was discharged without an alternative diagnosis.

Table 8. Serious Adverse Events within 28 Days and 6 Months after Vaccination.

Variable	TCV (N=10,005)	Men A Vaccine (N=10,014)	Total (N=10,019)
Serious adverse events within 28 days after vaccination			
Number of participants with serious adverse events	7	10	17
Number of serious adverse events	7	11	18
Severity of events†			
Mild	1	1	2
Moderate	5	7	12
Severe	1	3	4
Related to trial medication			
Not related	7	10	17
Related		1	1
Serious adverse events within 6 months after vaccination			
Number of participants with serious adverse events	59**	63	121
Number of serious adverse events	62**	71	133
Severity of events†			
Mild	1	4	5
Moderate	59	63	122
Severe	1	4	5
Related to trial medication			
Not related	61	70	131
Related		1	1

TCV = Typhoid conjugate vaccine. MenA = Group A meningococcal vaccine (control)
Serious adverse events were defined as outcomes that led to hospitalization or that were life-threatening or resulted in disability, incapacity, or death. Serious adverse events were observed by the investigator or members of the trial team or were reported by the parents or guardians of the participants by telephone contact.
** Details of one participant could not be ascertained. Parent/guardian unwilling to give details.
† Severity was classified by the clinician according to the intensity of the specific event as reported by parents or guardians of the participants.

Table 9. MedDRA coded serious adverse events (SAEs) occurring within 28 days and 6 months of vaccination.

MedDRA System Organ Class and Preferred Terms		TCV	MenA	All
Serious adverse events within 28 days after vaccination				
Gastrointestinal disorders	Gastrointestinal disorders	2	2	4
General disorders and administration site conditions	Pyrexia	1	3	4
Infections and infestations	Gastroenteritis	1	3	4
	Pneumonia/ LRTI	3	3	6
	URTI/ Viral infections	1	1	2
Metabolic and nutrition disorders	Dehydration	1	1	2
Nervous system disorders	Febrile convulsion	2	2	4
Investigations	Oxygen saturation decreased	0	1	1
Respiratory, thoracic and mediastinal disorders	Pneumothorax and pleural effusion	0	1	1
Serious adverse events within 6 months after vaccination				
Congenital, familial and genetic disorders	Choledochal cyst	0	2	2
Gastrointestinal disorders	Gastrointestinal disorders	9	9	18
General disorders and administration site conditions	Pyrexia	14	11	25
Infections and infestations	Appendicitis/appendicectomy	2	1	3
	Bacterial infection	1	1	2
	Gastroenteritis	8	8	16
	Pneumonia/ LRTI	21	11	32
	Typhoid fever	1	2	3
	URTI/ Viral infections	5	7	12
	Urinary tract infection	3	7	10
Investigations	Oxygen saturation decreased	0	1	1
Metabolic and nutrition disorders	Pneumothorax and pleural effusion	5	4	9
	Hyponatremia	1	2	3
	Hypoglycemia	0	1	1
Musculoskeletal and connective tissue disorders	Musculoskeletal and connective tissue disorders	1	2	3
Nervous system disorders	Febrile convulsion	5	8	13
	Loss of consciousness	1	0	1
	Neurological disorder	1	0	1
	Seizure	1	2	3
Respiratory, thoracic and mediastinal disorders	Bronchospasm	4	7	11
Skin and subcutaneous tissue disorders	Skin and subcutaneous tissue disorders	2	1	3

Table 9 details the MedDRA coded SAEs that occurred in the first 28 days and 6 months post-vaccination. The SAEs were comparable between the two groups. There were 18 SAEs in 17 participants in the first 28 days, of which pneumonia/ lower respiratory tract infection was most commonly diagnosed (N=6/18) followed by gastrointestinal disorders (N=4/18), pyrexia (N=4/18) and febrile convulsion (N=4/18). There were 133 SAEs in 121 participants in the first 6 months post-vaccination. Pneumonia/ lower respiratory tract infection was the most common (N=32/132) followed by pyrexia (N=22/132) and gastrointestinal disorders (N=18/132).

There were 8 deaths (3 in the TCV group and 5 in the MenA group) throughout the study period (Table 10). Details of the death could not be determined for one of the participants. All other deaths were considered to be unrelated to the vaccine.

Table 10. Causes of Deaths in the Study Cohort

Vaccine Arm	Age at enrollment (years)	Time after vaccination (months)	Relation to vaccination	Diagnosis
MenA vaccine	3.1	7	Unrelated	Multiple organ dysfunction syndrome with sever pneumonia with staphylococcal sepsis
MenA vaccine	8.1	14	Unrelated	Acute kidney injury with pulmonary edema
MenA vaccine	1.3	14	Unrelated	Pneumonia
MenA vaccine	13.6	16	Unrelated	Known intestinal malignancy
TCV	13.8	16	Unrelated	? Homicide, details not provided by the family
MenA vaccine	8.1	18	Unrelated	Head injury
TCV	0.8	23	Unrelated	Fall from height
TCV	1.6	NA	NA	Suspected death, no information available from family

TCV = Typhoid conjugate vaccine. MenA = Group A meningococcal vaccine (control)

3.3.3 Thrombocytopenia

Of the total fever presentations for passive surveillance, there were 623 presentations within the first 3 months post-vaccination. Blood samples were collected from 73.84% (N=460/623) of the presentations in the first 3 months post-vaccination. Platelet counts were available for 422 out of the 460 cases where blood samples were collected. The median (IQR) platelet count was 275 thousand/uL (224 – 333). The mean platelet count (95% CI) was 287 thousand/uL (279 – 296).

Two participants reported platelet count less than 150 thousand/uL. Both participants had received the control vaccine and presented 76- and 89-days post-vaccination. Four participants reported a platelet count of 150 thousand/uL of which one participant had received TCV. The participant in the TCV group had presented 7 days post-vaccination and had blood culture-confirmed paratyphoid fever.

3.4 Discussion

3.4.1 Reactogenicity

Over the study period, a large pool of safety data was collected. Reactogenicity data of over 18,000 children on the first 7 days post-vaccination was collected, of which over 9000 children received TCV.

Overall, the solicited adverse reactions were reported in a small proportion of the vaccinated children. Swelling, redness, reduced activity, persistent crying and increase

irritability were reported in less than one percent of the participants, which were of mild severity, all of which were reported in a lesser proportion in TCV recipients compared to the MenA recipients. Vomiting, diarrhea and eating less than usual was reported in less than two percent of the participants, which were again mostly mild in severity and reported in a lesser proportion in TCV recipients. General feeling of unwellness was most commonly reported, but was reported in a smaller proportion of the TCV recipients compared to the MenA vaccine. Fever was reported in 5% of the TCV, but again there were a smaller number of fever cases than in the MenA group. The MenA vaccine, MenAfriVac, is a WHO-prequalified vaccine and a commonly used meningococcal conjugate vaccine especially in the meningitis belt in sub-Saharan Africa. The above suggest that the TCV is safe and the reported adverse reactions are small enough not to raise any concern about the safety of the vaccine with the policy makers.

There were significant differences in pain, swelling, vomiting, activity and crying between the TCV and MenA group. All of the above mentioned solicited adverse reactions were reported more in the MenA group. However, although statistically significant differences were seen in above-mentioned self-reported adverse reactions, these were due to the large sample size. With a large sample size, it is possible to reach statistical significance unless the effect size is zero, and the statistically significant differences may not have clinical significance[127]. For instance, pain was reported in 5.1% of the participants in the TCV group and 6.7% in the MenA group, and the differences were significant. When quantifying the size of the differences, it was negligible with a phi coefficient value of 0.0341. This suggests that there is no clinically meaningful difference in pain levels between the two vaccines received despite the significant p-value.

There were significant differences in the solicited adverse reaction between the younger and older children for all adverse reactions except redness which was virtually unreported. Although the differences were significant, the correlation of the adverse reactions with the age groups was very weak. There was a very weak positive correlation between age groups and pain and swelling, indicating that the adverse reactions were more likely to be reported by older children then younger children. Practically, older children are more likely to be able to verbalize pain and swelling. There was a very weak negative correlation with the rest of the reported adverse reactions indicating that they were more reported in younger children than older children. The adverse reactions are reported by the parents/guardian and the younger children are more likely to receive more care due to which they were more likely to be more perceptive to report milder disease for the younger children than the older children. Due to the very weak correlation, these differences in reported adverse reactions by age group do not warrant any concern from the perspective of vaccine safety.

The local reactogenicity results were consistent with the finding of the pre-licensure trial in India. Safety data of 340 participants, which include adults and children, showed pain in 3.6%, swelling in 1.6% and redness in 0% of the participants[97]. The reports of fever were also very similar; 5% of the participants in TyVAC-Nepal reported fever, while 4.3% of the participants in the trial in India reported fever. In the trial in India, the safety data was collected via phone calls on day 2, 3 and 8 and the participant/ parent maintained a daily diary card received on the next study contact.

Similarly, AEFI has been reported following a mass immunization campaign with TCV in children 6 months to 10 years of age following a large outbreak of extensively drug-resistant typhoid fever in Hyderabad city of Sindh, Pakistan[128]. Level 3 Brighton criteria was used to ascertain the AEFI. The study documented self-reported AEFI through a hotline and active surveillance of a subset of participants. A total of 207,000 children were vaccinated. The participants were provided with a vaccination card with printed hotline number, and were asked to contact the number if the child developed any illness within 14 days post-vaccination to collect self-reported data. The study only reported 66 self-reported AEFI (0.03%) in the total vaccinated children among which fever (0.02%) was most commonly reported followed by local reactogenicity (0.01%) and cough/cold (<0.01%). A subset of age-stratified 7139 vaccinated children were selected using systematic random sampling, provided vaccination card with hotline number, as well a weekly diary for daily record of the child's health condition. Research medical officers visited the households on days 7 and 14 following vaccination, collected the weekly diaries and interviewed parents to ascertain any AEFIs. A total of 433 AEFI (6.1%) were reported in the subset of actively surveilled children, where fever (2.89%) was commonly reported, followed by local reactogenicity (1.9%) and diarrhea (0.5%) and these were also the most commonly reported AEFIs overall. Self-report was voluntary and hence under-reported in the study. As in TyVAC-Nepal, the campaign in Sindh also concluded that TCV was safe. It is difficult to make direct comparisons with the TyVAC-Nepal results due to the different data collection methods. However, a possible explanation for the relatively low AEFI in the actively surveilled group could be that the participants and their parents and guardians were aware of which vaccine they received and the circumstances under

which they were receiving the vaccine which made them more accepting to the milder AEFI and less likely to report them.

Navi Mumbai, India introduced TCV in public sector in 2018, and surveilled for AEFI passively following the government's AEFI surveillance guidelines and actively via phone calls in 7% of vaccine recipients from each vaccination site on a daily basis to obtain a geographic and temporally representative sample[129]. Fever and swelling were most reported in the government's passive surveillance system, while pain (26%), swelling (7.5%) and fever (7.4%) were most frequently reported in active surveillance. While underreporting is plausible with the passive surveillance for AEFI, the reports of pain swelling and fever are much higher compared to TyVAC-Nepal. However, since these are subjective and the populations are different, it is difficult to comment on them.

5.4.2 SAEs

The SAEs reported in TyVAC-Nepal were comparable between the TCV and control group, and were consistent with common pediatric events. Respiratory tract illnesses, gastrointestinal diseases including diarrheal diseases and central nervous system disease, predominantly febrile convulsions have been reported as major causes of morbidity in descriptive studies in Nepal[130,131]. Similarly, SAEs were documented in Hyderabad, Pakistan, but all SAES occurred within expected frequencies and consistent with existing literature in the populations[128]. TyVAC-Malawi also reported that SAEs were similar in both the TCV and control group (MenA) and that none of the SAEs were considered related to the vaccine[132].

In Navi Mumbai, adverse events of special interest (AESI) were predefined and monitored to assess casual association with TCV. AESIs, including anaphylaxis, Guillain-Barré syndrome, aseptic meningitis, encephalitis, myelitis, acute disseminated encephalomyelitis, seizures, thrombocytopenia, and sudden unexplained death were selected based on literature showing rare but serious AEFIs that are known to have occurred following administration of other conjugate vaccines and for which Brighton Collaboration case definitions have been validated[133]. AESIs were surveilled in children of vaccinated age group (9 months - 14 years) in selected hospitals irrespective of their vaccination status one week before the vaccination campaign and up to 42 days after the last day of the campaign. The vaccination campaign was conducted from July to August 2018. Thrombocytopenia and seizures were most commonly reported but was not significantly higher in the vaccinated group and alternative diagnoses were established. Thrombocytopenia was linked to the ongoing dengue infection, however the study team did note that "future evaluation with laboratory testing would be beneficial to corroborate [the] dengue infection"[133].

The above show that there were no SAEs of concern and provides further reassurance that TCV is safe.

5.4.3 Thrombocytopenia

The ad-hoc analysis in TyVAC-Nepal did not show any evidence of thrombocytopenia in children after receiving TCV. No case of thrombocytopenia were identified in the TCV

group. Only one participant in the TCV group who had culture-confirmed paratyphoid fever had platelet count of 150,000/uL. There are limitations in the methods opted to explore thrombocytopenia. The surveillance for TyVAC-Nepal was not setup to identify cases of thrombocytopenia, and used the data from passive surveillance. The study doctors attending the participants were not actively looking for cases of thrombocytopenia. A child with thrombocytopenia of immune origin is otherwise healthy and presents with skin bruises, petechia or mucosal bleeding, has no lymphadenopathy or organomegaly and full blood counts reveal isolated thrombocytopenia. Febrile presentations, ,including viral infections, as in the passive surveillance can present with thrombocytopenia[129]. The diagnosis of idiopathic thrombocytopenia is a diagnosis of exclusion. It would be possible to establish a temporal relation to vaccination, but more difficult to establish a causal relationship to vaccination[123]. And so, even though multiple vaccines have been associated with thrombocytopenia, clear evidence linking vaccination with thrombocytopenia has only been generated for MMR vaccine[129]. It is likely that thrombocytopenia documented in Navi Mumbai was indeed due to dengue infection. Thrombocytopenia is described as a common sign of dengue infection[133].

3.4.4 Limitations and Future Directions

Although, there were no adverse events of concern in TyVAC-Nepal, possible rare adverse events may be missed. As countries, including Nepal, start to roll-out the typhoid conjugate vaccine in their national immunization programs, pharmacovigilance will be important to recognize potential adverse events, and assess for a causal association between the vaccine administration and the adverse event[134].

Safety of the study vaccine alongside routine immunizations was not evaluated in this study but is an important for consideration of incorporating the vaccine into national programs. TCV safety when co-administered with yellow fever vaccine at 9 months of age and meningococcal type A conjugate vaccine (MCV-A) at 15 months was studied in a double-blind randomized controlled trial in Burkina Faso[135]. Children receive yellow fever vaccine at 9 months visit and meningococcal type A conjugate vaccine (MCV-A) at 15 months, and receive measles-rubella (MR) vaccine at 9 and 15 months according to the Burkina Faso immunization schedule. A total of 250 children, 100 children aged 9-11 months and 150 children agreed 15-23 months were enrolled at their 9-month and 15-month vaccination visit respectively. Children enrolled at the 9 month visit were randomized 1:1 to receive TCV or control inactivated polio vaccine (IPV) along with the routine MR and yellow fever vaccine[136]. Children enrolled at the 15 months visit were randomly allocated 1:1:1 to receive TCV plus control vaccine (IPV) and MCV-A 28 days later; TCV and MCV-A; or MCV-A and control vaccine[137]. All participants received the MR vaccine. Solicited adverse events were assessed at 0, 3- and 7-days post-vaccination, and adverse events were assessed for 28 days and 6 months post-vaccination[135]. The solicited symptoms were similar in the TCV and the control group indicating the TCV was safe when co-administered with MCV-A, MR or yellow fever vaccines[136,137]. There were no vaccine-related SAEs.

TyVAC-Nepal study was conducted in children 9 months to under-16-year of age. TCV, in the Nepali population, is planned for co-administration at 15 months alongside second dose of MR vaccine. It will be important to assess the safety of the vaccine when co-

administered with routine MR vaccine in Nepal as well. The WHO Global Advisory Committee on Vaccine Safety (GACVS) has also "[recommended] examination of concomitant administration with other vaccines , such as that against measles, mumps and rubella (MMR), in large-scale campaigns with the currently available TCV"[122].

Data collected from TyVAC-Nepal, along with data from other TyVAC trials and from campaigns in Hyderabad, Pakistan and Navi Mumbai, India, were reviewed by GACVS that informed WHO SAGE's position on TCV[122]. GACVS committee concluded that "the safety profile of the Typbar-TCV^{TM} vaccine [was] reassuring". However, the GACVS also recommended "robust monitoring of safety (as for any new vaccine) …… to detect any signals that require further investigation." Further evaluations of the vaccine will also be necessary in specialized populations such as malnourished children and pregnant women.

3.5 Summary

In TyVAC-Nepal, a robust surveillance system was setup to identify AEFI 7 days post-vaccination and SAEs up to 6 months post-vaccination. In summary, TCV was safe in children 9 months to under-16 years and did not flag any unexpected safety signals in the study population.

4. Efficacy of Typhoid Conjugate Vaccine

The results presented in this chapter are published in:

1. Shakya M, Colin-Jones R, Theiss-Nyland K, et al. Phase 3 efficacy analysis of a typhoid conjugate vaccine trial in Nepal. N Engl J Med 2019; 381:2209-2218[138].

This article was published under the terms of the Creative Commons Attribution License (CC BY). All analyses, tables, and figures for the paper were produced by Merryn Voysey. Mila Shakya wrote the first draft of the paper. Mila Shakya conducted all the analyses, produced tables and figures for this thesis. Co-authors have given permission to include the material in this thesis.

2. Shakya M, Voysey M, Theiss-Nyland K, *et al.* Efficacy of typhoid conjugate vaccine in Nepal: final results of a phase 3, randomised, controlled trial. *Lancet Glob Heal* 2021; **9**: e1561–8.[139]

This article was published under the terms of the Creative Commons Attribution License (CC BY). All text, analyses, tables, and figures were produced by Mila Shakya. Co-authors have given permission to include the material in this thesis.

This work was also presented in the following presentations:

1. Randomized controlled trial of conjugate typhoid vaccine in Kathmandu, Nepal. Oral Presentation. World Congress of Mountain Medicine, Kathmandu, Nepal, Nov 2018.
2. Efficacy of typhoid conjugate vaccine in Nepal. Oral presentation., 11th International Conference on Typhoid and Other Invasive Salmonelloses, Vietnam, March 2019.
3. Efficacy of Typhoid Conjugate Vaccine in Nepal: A Participant-Observer-Blind Phase III Randomized Controlled Trial. Poster Presentation. ASTMH Virtual Meeting, Nov 2020.
4. Typhoid Vaccine Study-Nepal. Oral presentation. UK Pediatric Vaccine Group Meeting, December 2020.
5. Vaccines: a potential for typhoid control. Oral presentation (Symposium). ASM Microbe 2021, June 2021.

6. Efficacy of Typhoid Conjugate Vaccine in Nepal: A participant-observer-blind phase II randomized controlled trial. Oral presentation (Symposium). 2021 International Conference on Typhoid & Other Invasive Salmonelloses. Oct 2021

7. Efficacy of Typhoid Conjugate Vaccine in Children (9 months to under-16 years in Nepal: A participant-observer-blind phase II randomized controlled trial. Oral presentation. 2021 International Conference on Typhoid & Other Invasive Salmonelloses. Oct 2021

4.1 Introduction

This chapter describes the results of the primary objective of TyVAC-Nepal, the vaccine efficacy of TCV against culture-confirmed typhoid fever. Demonstration of protective efficacy is crucial to support decision making for the wider use of the vaccine. TyVAC-Nepal, as a part of the TyVAC consortium, was conducted to generate evidence of protective efficacy in an endemic setting. In addition, this chapter explores the efficacy of vaccine against clinical and self-reported outcomes of fever and typhoid fever.

4.2 Methods

The methods have been described in Chapter 2, section 2.2 ‘Clinical Trial Design’.

4.2.1 Statistical Analysis

Cleaned data were exported from REDCap on 2020/04/03. The statistical analysis plan was signed off by the chief investigator on 2019/02/21.

4.2.1.1 Blood Culture Confirmed Typhoid Fever

The incidence of typhoid fever in each group was calculated as the total number blood culture positive cases divided by the total number of person-years of follow-up in each group. The follow-up time was calculated as the last date of contact minus the date of randomization. The last date of contact was any study contact including follow up phone

calls and passive surveillance visits. Follow-up time of 0.5 days was considered for participants who could not be followed up beyond the vaccination visit.

Incidence rate ratio (IRR) was calculated as the ratio of the incidence in the TCV group compared to the control group. Vaccine efficacy (VE) was calculated as (1 – IRR) x 100%, where IRR is the incidence rate ratio (TCV: control).

The cumulative incidence of typhoid was summarized using the Kaplan-Meier method. For any participant with more than one blood culture confirmed typhoid fever, only the first event was used in the Kaplan-Meier analysis but all events were included in incidence estimates.

Any blood culture positive cases which occurred within the first two weeks of vaccination was excluded from the primary analysis as the protection elicited by the vaccine does not start until two weeks after the vaccine is administered.

Subgroup analyses for the primary outcome was conducted using adjusted Poisson models with interaction effects to assess the simultaneous effect of the independent variable on the outcome.

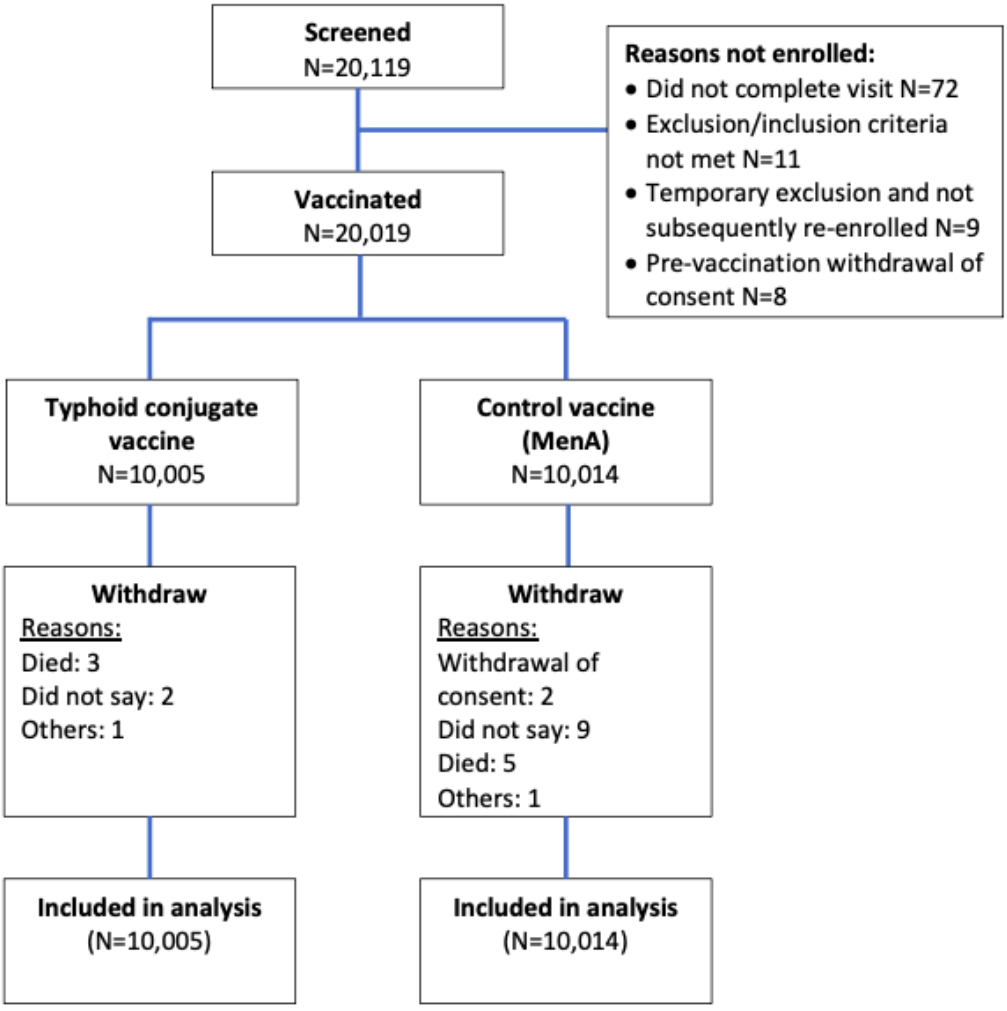
4.2.1.2 Other Outcomes

Counts of fever presenting to clinics, and fever and typhoid fever self-reported from phone calls were presented for each group. Counts of hospitalization reported from

phone calls and SAEs were presented for each group. Analysis for vaccine efficacy was the same as the analysis for the primary outcome.

Duration of fever recorded in the clinics, and self-reported via follow-up contact were analyzed using Wilcoxon rank sum test. Duration of hospitalization in each group were compared using Wilcoxon rank sum test.

Figure 9. Consort Diagram



4.3 Results

Participant were enrolled between December 6, 2017 and April 9, 2019. Of the 20,119 children that were screened, 20,019 children were enrolled in the study (Figure 9). A total of 72 participants did not complete the visit, 11 did not meet the inclusion/exclusion criteria, 9 were temporarily excluded but did not return subsequently and 8 withdrew consent prior to vaccination. Of those vaccinated, 10,005 received TCV while 10,014 received MenA vaccine, and were included in the analyses.

Table 11. Demographic Characteristics

Variable	TCV (N=10,005)	MenA vaccine (N=10,014)	Total (N=20,019)
Sex, N (%)			
Female	4,899 (48.97)	4,856 (48.49)	9,755 (48.73)
Male	5,106 (51.03)	5,158 (51.51)	10,264 (51.27)
Age at enrollment (years)			
Mean (SD)	7.9 (4.1)	7.8 (4.0)	7.9 (4.1)
Median (Range)	7.7 (0.8 – 16.1)	7.7 (0.7 – 16.1)	7.7 (0.7 – 16.1) *
< 2 years N (%)	671 (6.71)	719 (7.18)	1,390 (6.94)
2 – 5 years N (%)	2,990 (29.89)	2,964 (29.60)	5,954 (29.74)
5 years and above N (%)	6,344 (63.41)	6,331 (63.22)	12,675 (63.31)
Self-reported history of typhoid fever**			
N (%)	345 (3.5%)	395 (4.0%)	740 (3.7%)

*6 participants are outside the age range for eligibility (9 months to 15 year + 364 days)
**Self-reported history of typhoid infection prior to the beginning of the study, reported at baseline intake.
TCV = Typhoid Conjugate Vaccine. MenA = group A meningococcal vaccine (control)

4.3.1 Participant Demographics

Table 11 shows the demographic characteristics of the participants. A total of 48.97% of the participants in the TCV group were female, while 48.49% in the MenA group were

female. The mean age at enrollment was 7.9 (standard deviation (SD) 4.1) and 7.8 (SD 4.0) years for the TCV group and MenA group respectively. The median (range) age was 7.7 (0.7 – 16.1) years. There were 6 participants who were outside of the age range of eligibility. A total of 6.94% of the participants (6.71% in TCV group and 7.18% in MenA group) were under 2 years of age, 29.74% (29.89% in TCV group and 29.60% in MenA group) were 2 to under-5 years of age, and 63.31% (63.41% in TCV group and 63.22% in MenA group) were 5 years and above. A total of 3.5% of the participants in the TCV group and 4.0% in the MenA group self-reported a history of typhoid fever before the start of the study. Overall, the baseline characteristics of the participants in the TCV and MenA group were well-balanced.

4.3.2 Blood Culture Confirmed Typhoid Fever and Vaccine Efficacy

Between December 6, 2017 and April 9, 2020, 17,545 presentations to the fever clinics were screened. A total of 4795 participants met the case definition for blood culture collection of which blood cultures were collected from 71.57% (N=3432). There were 76 cases of blood culture-confirmed typhoid fever throughout the surveillance period. S. Typhi positive blood cultures were identified in 1.70% (N=59) of the total cultures sent. After review of 3068 medical records, an additional 17 culture positive cases were identified from medical record review.

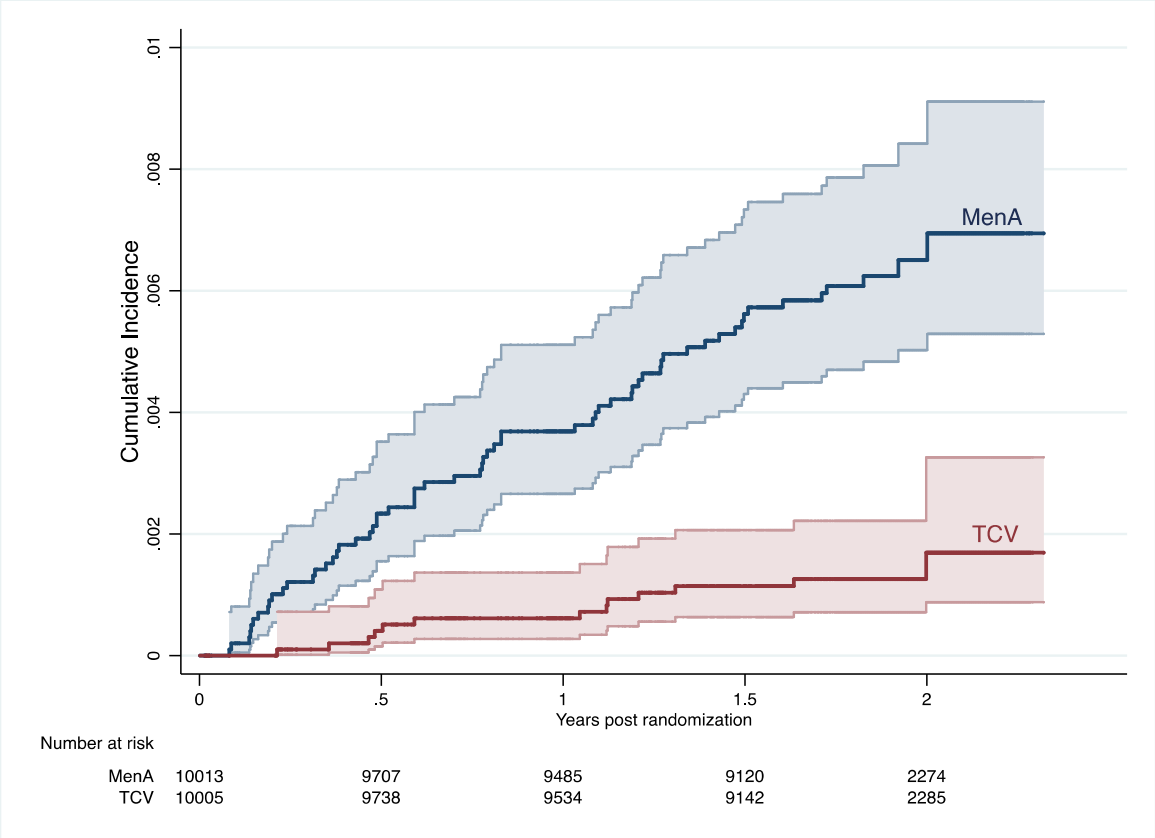
One participant in the MenA group had blood culture positive typhoid fever twice approximately 3 months apart. One case in the MenA group occurred within 14 days of

vaccination and was excluded from the analyses. There was 18,145 person-years of follow-up in the TCV group and 18,154 person-years of follow-up in the MenA group.

There were 13 blood culture-positive typhoid fever cases in the TCV group (0.13% of the total participants in the TCV group) and 62 cases in the MenA group (0.62% of the total participants in the MenA group) (Table 12). Of the culture positive cases in the MenA group, 75.8% (N=47) were detected from fever clinics and 24.2% (N=15) were detected through active follow-up and medical record review. Of the culture positive cases in the TCV group, 84.6% (N=11) were detected from fever clinics and 15.4% (N=2) were detected through active follow-up and medical record review. The incidence of typhoid fever in the TCV group was 72 (38,123) per 100,000 person-years while the incidence was 342 (262, 438) per 100,000 person-years in the MenA group. The overall protective efficacy of TCV was 79.0% (95% CI, 61.9%, 88.5%; $p < 0.001$). One participant had two episodes of culture-confirmed typhoid fever. After excluding the second episode of culture-confirmed typhoid fever, the vaccine efficacy was 78.7% (95% CI, 61.2%, 88.3%; $p < 0.001$) (Table 12). Figure 10 shows the Kaplan-Meier estimates of the cumulative incidence of blood culture confirmed typhoid fever along with the 95% confidence interval. The curve represents one episode of culture-positive typhoid fever per participant, and the first episode for the participant who had culture-positive typhoid fever twice. The curves, along with the confidence intervals, show progressive and substantial divergence starting early on in the trial period, and there is no overlap of the curves clearly illustrating the efficacy of the vaccine. At the end of the two years, 2,274

participants in the MenA group and 2,285 participants in the TCV group were still in the at-risk population in this analysis.

Figure 10. Kaplan-Meier Estimates of the Cumulative Incidence of Blood Culture Confirmed Typhoid Fever, According to Trial Group.

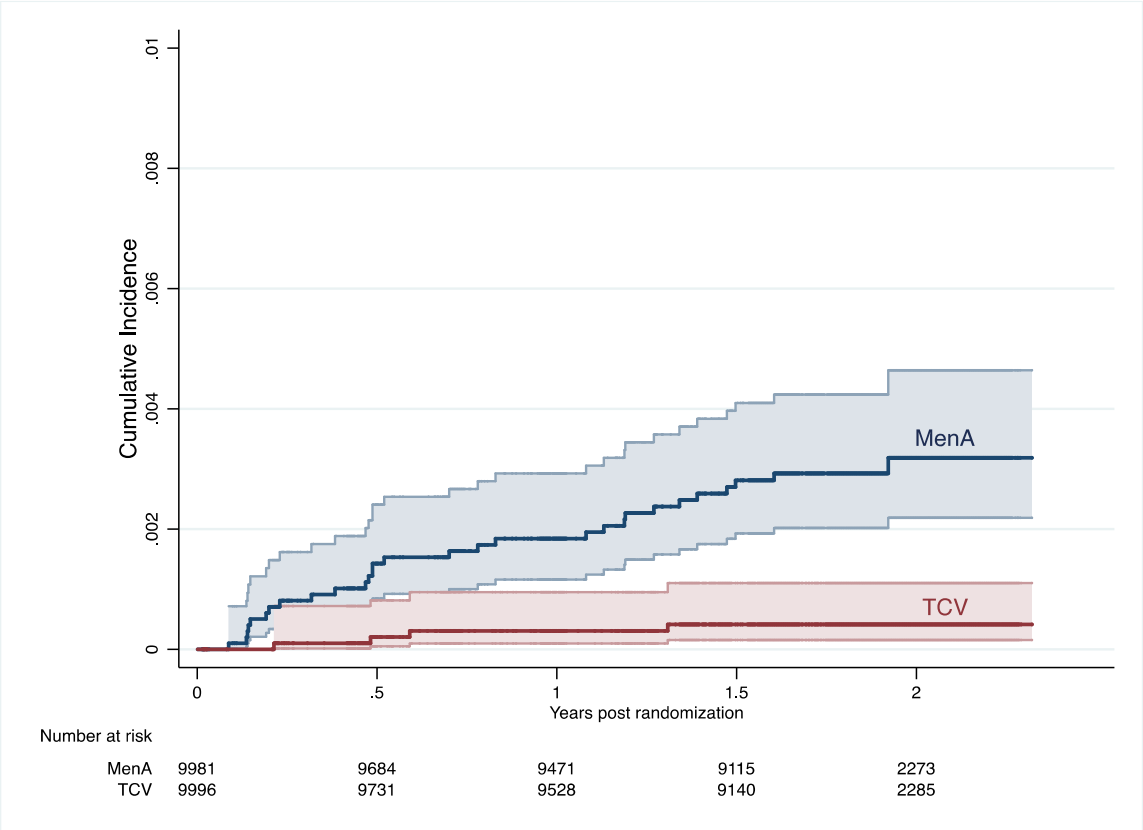


TCV = Typhoid Conjugate Vaccine. MenA = group A meningococcal vaccine (control).

There were 34 cases that presented to the fever clinics with at least 3 days of fever prior to blood culture. The vaccine efficacy was 86.7% (95% CI, 62.2%, 95.3%, $p < 0.001$) in participants who had at least 3 days of fever (Table 12). Figure 11 shows the Kaplan-Meier estimates of the cumulative incidence of blood culture confirmed typhoid fever of the participants presenting to the fever clinic with 3 or more days of fever, along with the

95% confidence interval. The curve represents one episode of culture-positive typhoid fever per participant. The curves, along with the confidence intervals, show progressive and divergence illustrating the efficacy of the vaccine.

Figure 11. Kaplan-Meier Estimates of the Cumulative Incidence of Blood Culture Confirmed Typhoid Fever with 3 days of Fever at Presentation in the Fever Clinic, According to Trial Group.



TCV = Typhoid Conjugate Vaccine. MenA = group A meningococcal vaccine (control).

Table 12. Occurrence of Blood Culture–Confirmed Typhoid Fever and Protective Efficacy of Typhoid Conjugate Vaccine (TCV).

Outcome	**TCV (N=10005)**	**Incidence per 100,000 person-years (95% CI)**	**Men A vaccine (N=10014)**	**Incidence per 100,000 person-years (95% CI)**	**Vaccine Efficacy (95% CI)**	**p value**
Person-years of follow-up [a]	18145		18154			
Blood culture-confirmed typhoid fever in first 14 days after vaccination			1			
Blood culture-confirmed typhoid fever after 14 days [b]	**13**	**72 (38, 123)**	**62**	**342 (262, 438)**	**79.0% (61.9%, 88.5%)**	< 0.001
Detected through fever clinics	*11 (84.6%)*		*47 (75.8%)*			
Detected through active follow-up and medical record review	*2 (15.4%)*		*15 (24.2%)*			
Blood culture-confirmed typhoid fever after 14 days (one case per person)	13	72 (38,123)	61	336 (258, 432)	78.7% (61.2%, 88.3%)	< 0.001
Blood culture-confirmed typhoid fever in those with at least 3 days of fever prior to blood culture (fever clinics) [c]	4	22 (6, 57)	30	166 (112, 237)	86.7% (62.2%, 95.3%)	< 0.001

a. Participants with no follow-up contact contribute half a day follow-up in calculations.
b. For all reported culture-positive cases reported from medical records review, isolates were checked, when available, to reconfirm diagnostic results.
c. From fever clinic cases only; 17 cases detected through medical records review were excluded as data on number of days of fever was not available.
TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine

Table 13. Protective Efficacy of Typhoid Conjugate Vaccine (TCV) by duration since vaccination.

Outcome	TCV (N=10005)	Incidence per 100,000 person-years (95% CI)	Men A vaccine (N=10014)	Incidence per 100,000 person-years (95% CI)	Vaccine Efficacy (95% CI)	p value (interaction)
Blood culture-confirmed typhoid fever after 14 days						
12 months or less since vaccination	6	61 (23, 134)	36	370 (259, 512)	83.4% (60.5%, 93.0%)	0.430
More than 12 months since vaccination	7	83 (34, 172)	26	310 (202, 454)	73.0% (37.9%, 88.3%)	

TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine

There were 42 blood culture-confirmed typhoid fever cases in the first twelve months post-vaccination, 6 in the TCV group and 36 in the MenA group (Table 13). The incidence of typhoid fever in the first twelve months was 370 (95% CI: 259, 512) per 100,000 person-years in the MenA group and 61 (95% CI: 23, 134) per 100,000 person-years in the TCV group. The protective efficacy of TCV was 83.4% (95% CI: 60.5%, 93.0%) in the first 12 months post-vaccination follow-up. There were 33 blood culture-confirmed typhoid fever cases after the first twelve months post-vaccination, 7 in the TCV group and 26 in the MenA group. The incidence of typhoid fever after the first twelve months was 310 (202, 454) per 100,000 person-years in the MenA group and 83 (34, 172) per 100,000 person-years in the TCV group. The vaccine efficacy was 73.0% (95% CI, 37.9 – 88.3) after the first-year post-vaccination. There were no significant differences in the vaccine efficacy in the first year and after the first year (P=0.430 for the difference in VE between year 1 and year 2) (Table 13).

4.3.3. Fever Presentations and Protective Efficacy

Table 14 shows the protective efficacy of TCV against fever presentations to the study clinics and against self-reported fevers and self-reported suspected or confirmed typhoid fever. There were 2390 episodes of fever of any duration documented in the TCV group and 2408 episodes of fever documented in the MenA groups from the study clinics. The protective efficacy of TCV against any duration of fever was 0.7% (95% CI -5.1%, 6.2%; P=0.8093). Similarly, the protective efficacy of TCV against fever for $\geq$2 days, and/or a temperature of 38 degrees C and $\geq$3 days, and/or a temperature of 38 degrees C were 0.5% (95% CI -5.3%, 6.1%; P=0.8529) and -1.1% (95% CI -7.7%, 5.0%; P=0.7259)

respectively. Of the fever presentations, 417 episodes in the TCV group and 415 in the MenA group were clinically suspected to be typhoid fever by the attending physician (VE -0.5%; 95% CI -15.4%, 12.4%; P=0.9386).

Table 14. Fever Presentations and Protective Efficacy of Typhoid Conjugate Vaccine (TCV).

Outcome	Number of fevers in TCV group (N=10005 participants)	Number of fevers in Men A group (N=10014 participants)	Vaccine Efficacy (95% CI)	P-value
Fever presentations at clinics/hospital				
Number of presentations with fever of any duration	2390	2408	0.7% (-5.1%, 6.2%)	0.8093
Number of fevers of ≥2 days, and/or a temperature of 38 degrees C at presentation	2352	2366	0.5% (-5.3%, 6.1%)	0.8529
Number of fevers of ≥3 days, and/or a temperature of 38 degrees C at presentation	1980	1968	-1.1% (-7.7%, 5.0%)	0.7259
Clinically suspected typhoid at fever presentation (clinician recorded)	417	415	-0.5% (-15.4%, 12.4%)	0.9386
Self-reported fevers from follow up phone calls				
Number of self-reported fevers via phone or follow up contact	4816	4766	-1.1% (-5.35%, 2.9%)	0.5914
Number of self-reported fevers that did not result in visit to health care provider	1258	1267	0.6% (-7.5%, 8.2%)	0.8684
Number of self-reported fevers that resulted in pharmacy visit	759	780	2.6% (-7.7%, 12.0%)	0.5998

Duration of self-reported fever Median days per fever episode [IQR] *Number of days of self-reported fever per person*	4305 fevers 3 [2, 4] N=3915 persons with fever N=2886 Sum= 15,748 4 [2, 7]	4287 fevers 3 [2, 4] N=3922 persons with fever N=2863 Sum=15,778 4 [2, 7]		
Self-reported suspected/confirmed typhoid fevers from phone call follow up				
Number of clinically suspected typhoid fevers - self-reported	96	120	19.9% (-5.6%, 39.4%)	0.1039
Number of clinically confirmed typhoid fevers - self-reported	31	71	56.3% (32.5%, 72.3%)	< 0.001
Duration of self-reported typhoid fever, median days per fever episode [IQR] *Total number of days of self-reported confirmed typhoid fever*	31 6 [4,8] 218	68 7.5 [5, 11.5] 624		0.0084

TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine

4.3.4. Self-reported Fevers and Protective Efficacy

The participant parent/guardian reported 4816 episodes of fever in the TCV group and 4766 episodes in the MenA group (VE -1.1%; 95% CI -5.35%, 2.9%; P=0.5914) (Table 14). Duration of fever was reported in 4305 fevers from 3915 persons in the TCV group and 4287 fevers from 3922 persons in the MenA groups. The median duration of fever was 3 (IQR 2,4) days in both groups. Of the self-reported fevers, 96 in the TCV group and 120 in the MenA group were clinically suspected typhoid fevers (VE 19.9%; 95% CI -5.6%, 39.4%; P=0.1039), and 31 in the TCV group and 71 in the MenA group were clinically diagnosed typhoid fevers (VE 56.3%; 95% CI 32.5%, 72.3%; P<0.001). The median duration of fever

per episode of self-reported typhoid fever was 6 (IQR 4,8) days in the TCV group and 7.5 (IQR 5, 11.5) days in the MenA group (P=0.0084).

Table 15. Hospitalizations and Protective Efficacy of Typhoid Conjugate Vaccine (TCV).

Outcome	TCV (N=10005)	Men A (N=10014)	Vaccine Efficacy (95% CI)	P value
Hospitalisations				
Hospitalisation (all cause) *[self-report from phone call follow up]*	149	141	-5.7% (-34.1%, 16.6%)	0.6359
Length of hospital stay (all cause) *[self-report from phone call follow up]*	138 5 [3,7]	127 5 [3,7]		0.3339
Length of hospital stay (all cause) *[SAE]*	178 4[3,7]	194 5[3,7]		0.2926
Hospitalisation for fever [SAE]	148	139	-9.1% (-37.7%, 13.6%)	0.4517
Hospitalisation for typhoid (suspected or clinical diagnosis) *[SAE]*	16	18	11.1% (-84.8%, 57.6%)	0.7370
Hospitalisation for typhoid (confirmed Blood culture positive+) *[SAEs]*	2	7	71.4% (-50.1%, 97.1%)	0.1095
Death (all cause)	3	5	40.0% (-208.6%, 90.6%)	0.5083

TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine
SAE= Serious adverse event.

4.3.5. Hospitalization and Vaccine Efficacy

A total of 290 hospitalizations, 149 in the TCV group and 141 in the MenA group, were self-reported from phone call follow up with a median hospital stay of 5 (IQR 3, 7) days in both groups (Table 15). The vaccine did not show any efficacy against all cause hospitalization (VE -5.7%; 95% CI -34.1%, 16.6%; P=0.6359). The length of all-cause

hospital stay reported from SAEs was 4 (IQR 3, 7) days for the TCV group and 5 (IQR 3, 7) days for the MenA group.

There were 148 hospitalization for fever reported from SAEs in the TCV group, of which 16 were suspected or clinical typhoid and 2 were blood culture confirmed typhoid. Similarly, 139 hospitalization for fever reported from SAEs in the MenA group of which 18 were suspected or clinical typhoid, of which 7 were blood culture confirmed typhoid. The vaccine did not show efficacy against any of these outcomes.

A total 8 all cause deaths, 3 in the TCV group and 5 in the MenA group, in the study duration and the vaccine efficacy was 40.0% (95% CI -208.6%, 90.6%; P=0.5083).

4.4 Discussion

4.4.1. TCV and Culture-Confirmed Typhoid Fever

TyVAC-Nepal was a large-scale field-based evaluation of the vaccine efficacy of the typhoid conjugate vaccine in a typhoid endemic setting. A single dose of the vaccine was efficacious and was significantly associated with a 79.0% reduction in the incidence of typhoid fever. The interim analysis of the same study had consistent results showing a 81.6% (95% CI 58.8–91.8, P<0.001) efficacy of the vaccine in first 15 months post-vaccination[138].

A total of 76 blood cultures were positive for S. Typhi, and 75 were included in the per-protocol analysis. The study was powered to detect 45 cases of typhoid fever over a two-year period. The number was met in the interim analysis of the study, so the two-year analysis had higher number of cases than required for the full power.

The vaccine efficacy in TyVAC-Nepal was comparable to that of studies of the same conjugate vaccine conducted at other endemic regions. An individually randomized control trial in over 28,000 children 9 months to 12 years of age conducted in Blantyre, Malawi as a part of the TyVAC consortium (TyVAC-Malawi), showed an overall vaccine efficacy of 83.7% (95% CI 68.1 – 91.6, P<0.001) 18 months post-vaccination[132]. A participant- and observer-blinded parallel cluster randomized trial in Dhaka, Bangladesh, also conducted as a part of the TyVAC consortium (TyVAC-Bangladesh), demonstrated a total vaccine protection of 85% (95% CI 76 – 91, P<0.001)[116]. The trial vaccinated over 60,000 children between the ages of 9 month to under-16 years of age. Both the trials in Malawi and Bangladesh used the same criteria for blood cultures as the trial in Nepal. The similar results across the different sites in different populations are reassuring and substantiates the findings of our study that the TCV is efficacious.

The vaccine efficacy in TyVAC-Nepal was also comparable to that of the human challenge model (VE: 87·1% (95% CI 47·2 to 96·9)[98]. However, the participants of the challenge study were of an older age group (16- 80 years) compared to our study population and

the vaccination target population. The efficacy in the Oxford volunteers was estimated post-hoc and was based on an endpoint of persistent fever of 38·0 degree C or higher followed by positive blood culture. The endpoint represents the clinical presentation of typhoid fever in our setting and in this study, which makes our results comparable.

The results were also similar to that of the prototype conjugate vaccine, Vi-rEPA. In the double-blinded placebo-controlled trial, 13,776 children between two to five years of age were enrolled. The intervention arm received two doses of Vi-rEPA 6 weeks apart. The vaccine efficacy was 91.5% (95% CI 77.1 – 96.6, P<0.001) after active surveillance for 27 months. Unlike this trial where the surveillance was mostly passive and active follow-up phone calls were made to capture missed cases, in the Vi-rEPA trial, the children were visited on a weekly basis and had their history taken and temperatures measured ensuring no cases were missed. Any child with at least 3 days of fever with a temperature of 37.5 C or higher were referred for a blood culture.

4.4.2. Study Enrollment Criteria

The criteria for blood cultures in TyVAC-Nepal was defined as subjective fever for 2 or more days and/or a temperature of 38 degree C. The WHO recommends 3 days of fever out of seven consecutive days as the threshold for taking blood cultures in routine typhoid surveillance programmes[140]. The 3-day cut-off was set by the WHO to reduce the number of non-specific and self-resolving infections in the surveillance. A 2-day criteria increases the sensitivity of the diagnosis, but there is loss of specificity. The large number

of non-specific illnesses that would be captured using a 2 -day criteria can overwhelm routine surveillance systems. However, from a trial perspective, 3-day criteria would reduce the number of cases detected and therefore reduce the power of the study and so the 2-day criteria were used. As seen in previous studies, over-the-counter antimicrobial use is widespread in the region[52]. Growth of organisms in blood culture is less likely after the use of antibiotics. In a surveillance study conducted in the same area, it was seen that parents were more likely to seek health care advice for febrile children early into the illness, either in the hospitals, or more commonly in the local pharmacies[52]. In order to capture all these cases and to prevent losing sensitivity of blood cultures due early use of antibiotics, a lower threshold for blood cultures was used.

Arguably, the study definition for blood culture could have identified milder self-limiting cases that may have been missed in the field settings. Hence, we also assessed the vaccine efficacy using the WHO recommended surveillance cut-off. We restricted the analysis to culture confirmed cases obtained via passive surveillance through fever clinics. Cases identified from active surveillance followed up medical record reviews were not included as the duration of fever at the time of the presentation could not be ascertained in these cases. The confidence intervals using the 2-day and 3-day cut-off overlap showing that the vaccine efficacy using either of the two definitions is not dissimilar.

4.4.3. Duration of TCV Protection

The efficacy of TCV in the first 12 months and thereafter did not show a significant difference. This suggests that the vaccine offers non-waning protection over the first two years after vaccination. Findings of TyVAC-Nepal as well other studies in the high burden regions of this TCV (Typbar-TCV) however, till date, only show the short-term vaccine efficacy[139, 132,116] . Vi-rEPA, the conjugate vaccine prototype, demonstrated a persistent vaccine efficacy at 46 months (VE 89.0%; 95% CI 76.0% – 96.9%)[91]. Importantly, however, two doses of Vi-rEPA were given to the children 6 weeks apart which could have boosted the response of the vaccine. Additional data on medium- and long-term efficacy of the vaccine will be important to assess the duration of protection this vaccine offers. This is important from a policy point of view as well. It will also be important to determine whether boosting will be needed to confer long term protection from the vaccine, or the single dose in itself will give long-term protection.

4.4.4. TCV and Typhoid Incidence

TyVAC-Nepal shows a high incidence of typhoid fever in the control group highlighting the high burden of the disease in the study site. An incidence of over 100 per 100,000 person-years is defined as high burden of typhoid fever [141]. The results emphasize that the administration of the vaccine considerably decreases the burden of typhoid fever in the population.

In a typhoid surveillance study (STRATAA) conducted in 12 of the 17 wards where the trial was conducted, showed an overall crude incidence of 74 (95% CI 62-87) cases per 100,000 person-years across all age groups[52]. The incidence is well below the incidence of the 342 cases per 100,000-person years detected in children in this study. The surveillance study relied entirely on passive surveillance while in this study all participants were followed up via phone calls to detect missed cases and additional culture- confirmed typhoid fever were detected after a through medical record review. Almost a fourth of the culture-confirmed cases (22.6%) in this trial were detected though medical record review. The two studies clearly demonstrate how detection bias can affect the incidence estimates. In the typhoid surveillance study, after adjusted for under-detection of cases by including inflation factors to account for the probability of having a positive blood culture if the person has typhoid, probability of having a blood culture drawn in those who have symptoms, and the probability of seeking healthcare, the incidence was 1,014 (95% credible intervals 650 – 1696) cases per 100,000-person years[52]. These factors can also affect the detection of cases in the trial and is discussed below in the study limitations.

4.4.5. TCV and Fever Presentations

TCV did not have any impact on all-cause fevers or clinically suspected typhoid fever, whether seen at the study clinics or self-reported. The results are not unexpected. Fever is a common presentation, especially in children, and one of the most common reason for seeking healthcare in a low resource setting[142]. For many infections causing fever, there are no localizing features which makes it difficult to come down to a diagnosis

strictly based on history and examination of patients. Further, typhoid fever has a non-specific clinical presentation, is one of the most common cause of undifferentiated febrile illnesses, and hence a common differential diagnosis in the study setting.

TCV was efficacious against self-reported clinically diagnosed typhoid fever, but not against self-reported clinically suspected cases. Self-reported clinically suspected typhoid fever included cases where the parents/guardians reported that the child sought healthcare, either with a healthcare provider or at a pharmacy, and the child was suspected of having typhoid fever. Self-reported clinically confirmed typhoid fever included cases where the parents/guardians reported that the child had a blood culture done, the child was diagnosed of having typhoid fever but the blood culture report was not necessarily available at the time of the self-report. The vaccine efficacy in the self-reported clinically confirmed group, although showing significant reduction in the disease, was lower than for the culture-confirmed cases. It is because not all self-reported clinically confirmed typhoid cases were typhoid fever highlighting the importance of confirmation of blood culture positive cases in the medical record review. All cases reporting suspected or confirmed typhoid fever were followed up by a medical record review. There were cases were confirmed cases presented Widal test reports, hematological reports and culture reports with growths other than *Salmonella* or culture reports with no growths. Nevertheless, clinically diagnosed group was smaller likely because the clinically suspected group had been narrowed down.

The duration of fever in the TCV group among those clinically confirmed with typhoid fever was shorter than in the MenA group (6 days vs. 7.5 days; P=0.0084). Prolonged disease is generally associated with severity that may lead to serious complications and even death. This suggests that the severity of typhoid fever may be reduced after TCV administration. Further, a shorter duration means a quicker clinical improvement which is important from the perspective of the participant's well-being and also means a lesser health loss. Typhoid fever alone was responsible for 8332 years lived with disability (YLDs, which quantifies non-fatal health loss) in 2017[143]. Data required for the duration of fever in the fever presentations at the hospital/clinics was not collected. This data could have further supported the association between TCV and duration of fever.

4.4.6. TCV and Hospitalizations

TCV did not have any impact on all-cause hospitalization or hospitalization due to fever. The results are expected for all-cause hospitalization and hospitalization due to fever – TCV is unlikely to have an impact on causes directly not associated with typhoid fever. The study did not find that the TCV impacted hospitalization due to clinically suspected/ diagnosed typhoid fever or culture-confirmed typhoid fever. As discussed above, typhoid fever is a common diagnosis for undifferentiated fevers in the study setting and not all clinically diagnosed cases turn out to be culture-confirmed cases. Further, most cases of typhoid fever and febrile illnesses that fall under the differential for typhoid fever are usually treated in an out-patient basis. In total, there were only nine cases of culture-confirmed typhoid fever cases that were hospitalized and the sample size was too small to detect the impact of TCV of hospitalizations.

To date, there are no prospective studies that have reported the impact of TCV on hospitalization. However, a cost-effectiveness analysis of TCV in five endemic LMICs estimated that up to 13 (95% credible interval 3,40) hospitalizations per 100,000 could be averted with routine TCV introduction at 9 months and up to 37 (95% credible interval 7, 155) hospitalization per 100,000 could be averted with routine vaccination at 9 months and campaign for up to 15 years in a setting (Kolkata, India) with 72 (95% credible interval 14, 202) hospitalization per 100,000[144]. The study also reported considerable uncertainty around the probability of hospitalization. Another modelling analysis reported that the probability of hospital admissions had the largest impact in determining the optimal vaccination strategy in Gavi-eligible countries highlighting the importance of the data in helping countries make decision around TCV introduction.[145]

4.4.7. Limitations

4.4.7.1. Limitation in Capturing Culture- Confirmed Typhoid Fever Cases

Blood culture of all febrile participants was crucial for the measurement of the primary study outcome. However, there are systematic problems in capturing all cases. Firstly, culture proven typhoid fever is difficult to measure. Blood cultures are the gold standard for diagnosis of typhoid fever, but only 61% (95% CI 52 – 70%) of all blood cultures are positive in those with typhoid fever[22]. Secondly, blood culture volume plays a role in the positivity of the blood culture[23]. Drawing an adequate volume of blood from children, especially younger children, can be difficult.

Thirdly, consent for blood culture in children in the study setting was difficult, and culture positive typhoid cases could be missed in individuals who did not have a blood culture. As a general clinical practice, pediatricians request blood investigations only after four days of fever, unless there are clear clinical indications for a blood draw. Viral fevers are common in children, so antipyretics are prescribed and a blood culture is only sent if the fever does not subside over 4 days. So, blood draw early in the illness is not routinely done. Further, culturally, drawing blood is associated with causing increased weakness. Hence parents declined blood cultures early in the illness, preferred to wait and watch and usually requested empirical treatment first.

An exploratory analysis of the first year of passive surveillance data of TyVAC-Nepal showed that only 68% of all fever presentations gave consent for a blood draw and parents/guardians of younger children were more reluctant to have a blood draw[146]. Further, the analyses showed that clinical suspicion of typhoid fever was a strong predictor of a positive blood culture. However, the analyses also showed that one third of the positive cultures were obtained from participants with alternative diagnoses. Additionally, there were fever presentations that did not meet the fever criteria, and were asked to follow-up on a later date. It is possible that all participants did not come for a follow-up and sought treatment elsewhere. This is again common in the study setting where people opt for immediate antibiotic treatment. The above indicate that positive cases could also be missed in those that did not get a blood culture done.

Lastly, not all fevers presented to the passive surveillance clinics. However, all measures were taken to ensure that the study participants visit the study team once they have a febrile episode. Services were provided to ensure that the study provided easy access to the doctors and for treatment. However, Lalitpur is a bustling metropolis with multiple private hospitals and clinics and numerous pharmacies. A lot of the locals visit pharmacies for over-the-counter remedies and attend private hospitals and clinics. The study had put in place regular three-monthly follow-ups and medical record reviews to capture culture positive cases. But, potential culture positive cases could still have been missed due to use of over-the counter antibiotics, tendency of prescribing antibiotics without investigations, particularly blood cultures, and lost records and reports as demonstrated by the high number of self-reported fever and large proportion of those with self-reported fever that did not visit a health care facility or visited pharmacies. This highlights the challenge of running a surveillance in the study setting.

The above highlights that the incidence estimates presented here are crude rates, and that the true incidence of typhoid fever is likely to be much higher. Although, under-estimation of incidence is likely, this does not affect the vaccine efficacy estimates. Being a blinded randomized trial, the vaccine allocated to the participants is random and the study clinicians as well as the participants are unaware of the vaccine received, and hence the collection of blood samples for culture is independent of the vaccination status. However, missed cases means the power for detecting a significant vaccine effect is lowered.

4.4.7.2. Other Limitations

In TyVAC-Nepal, we only included participants that reportedly had not received any typhoid vaccine in the past three years. The participants were requested not receive typhoid vaccines throughout the study period. Literacy around healthcare is limited in the population. Most parents/guardians are unaware of the details of the treatments/ vaccinations their children receive and usually follow directions of attending doctors. They also have tendencies to lose their vaccination records. There is a small probability that the participants may have unknowingly received typhoid vaccines outside of the study. Typhoid vaccines are not routinely prescribed by doctors, are administered with an additional cost, and are relatively expensive, so the likelihood is low.

4.5. Summary

In summary, a single dose of TCV was efficacious against culture-positive typhoid fever in children 9 months to under-16 years. There was evidence that TCV was protective against clinically confirmed typhoid fever and shortened the duration of the fever in the same group.

5. Immunogenicity of TCV

The results presented in this chapter is published in:

1. Shakya M, Colin-Jones R, Theiss-Nyland K, et al. Phase 3 efficacy analysis of a typhoid conjugate vaccine trial in Nepal. N Engl J Med 2019; 381:2209-2218[138].

This article was published under the terms of the Creative Commons Attribution License (CC BY). All analyses, tables, and figures for the paper were produced by Merryn Voysey. Mila Shakya wrote the first draft of the paper. Mila Shakya conducted all the analyses, produced tables and figures for this thesis. Co-authors have given permission to include the material in this thesis.

2. Shakya M, Voysey M, Theiss-Nyland K, *et al.* Efficacy of typhoid conjugate vaccine in Nepal: final results of a phase 3, randomised, controlled trial. *Lancet Glob Heal* 2021; **9**: e1561–8[139].

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This work was also presented in the following presentations:

1. Typhoid Vaccine Study-Nepal. Oral presentation. UK Pediatric Vaccine Group Meeting, December 2020.
2. Vaccines: a potential for typhoid control. Oral presentation (Symposium). ASM Microbe 2021, June 2021.
3. Immunogenicity of Typhoid Conjugate Vaccine in Nepal: A Participant-Observer-Blind Phase II Randomized Controlled Trial. Oral Presentation. 2021 International Conference on Typhoid & Other Invasive Salmonelloses. Oct 2021

5.1 Introduction

Immunogenicity of a vaccine is defined as "the capacity of a vaccine to elicit a measurable immune response"[147]. During the vaccine development, the magnitude of the immune response, including 'functional' antibody response (e.g. antibody that neutralizes viruses or toxins; antibody that mediates bactericidal activity or opsonophagocytosis) is measured. Humoral immune responses (e.g. antigen-specific antibody concentrations or antibody titres typically using enzyme linked immunosorbent assays (ELISAs)) to vaccination are routinely measured in serum/ plasma, while cellular immune responses (e.g. percentages of sensitized T-cells) are measured in blood. In immunogenicity trials, when immunological correlates of protection (CoP) are established, the seroprotection rate or the percentage of participants that achieve antibody level at or above the CoP is used to establish immunogenicity of a vaccine. When there is no established CoP, immunogenicity is usually established by the measure of a humoral immune response[147]. For examples, a $\geq$ four-fold increase in antigen-specific antibodies is indicated as an increase in antibodies in response to the vaccine and is used to measure the antibody response[148]. The percentage of subjects that show a four-fold rise has been used a measure for antibody response for typhoid vaccines.

A CoP is "a type and amount of immunological response that correlates with vaccine-induced protection against infection (including asymptomatic infection), disease, hospital admission, death etc. and that is considered predictive of clinical efficacy"[147]. Established CoPs have been based on measure of functional or total IgG antibody, such as

measurement of antibody against diphtheria and tetanus toxoids. Identification of a CoP can help accelerate the development and licensure of vaccines based on immunogenicity and without the need for large efficacy trials. For typhoid vaccines, no widely accepted CoP has been identified. The Vi polysaccharide capsule, which is the main virulence factor for *S*. Typhi, can confer bacterial resistance allowing the bacteria to evade the protection of the body and to disseminate systematically causing the disease[12]. The capsular polysaccharide has hence been the target component in vaccine development and anti-Vi IgG has been suggested as a CoP for Vi- vaccines. Klugman et al. assessed the correlation of anti-Vi IgG antibodies and vaccine efficacy in a Vi polysaccharide vaccine trial in South Africa and proposed a threshold of protection of 0.6-1.2 µg/mL[81]. The radioimmunoassay method used to measure the anti-Vi IgG levels in the study is no longer use. While it is possible to replicate the method, it is not possible to bridge back to the trial without access to the original sera and hence cannot be assessed in newer trials. The Vi-rEPA trial in Vietnam estimated a 3.5 ELISA units anti-Vi IgG protective level based on the geometric mean of the 2-3 years age group at six weeks post-vaccination. This protective level was equivalent to 4.3 µg /ml of anti-Vi IgG [149]. Although, this level of protection was meant to serve as a global reference for evaluation of Vi conjugate vaccines, lack of reproducibility of the enzyme-linked immunosorbent assay (ELISA) meant generalizability and interpretation of the threshold in other trials was not possible. Further, unavailability of sera from the original study means that the comparison of results with newer tests is not possible.

The typhoid conjugate vaccine assessed in TyVAC-Nepal, with Vi polysaccharide conjugated to a nontoxic tetanus toxoid carrier protein, has been shown to elicit a strong IgG response in studies in India and the UK[97,98]. Anti-Vi IgA response to TCV has more recently been studied in controlled human infection model (CHIM) studies at Oxford[150].

This chapter aims to assess the overall immunogenicity of TCV in the TyVAC-Nepal trial participants. This chapter describes the overall IgA and IgG response to TCV. The antibody response across the age groups and in between the different sexes have been explored in Chapter 6.

5.2 Methods

To assess the immunogenicity of TCV compared to the MenA vaccine group, sample collection was planned at baseline prior to vaccination (Day 0), at 28 days (Day 28 +/-4 days), at 18 months (Day 545 +/- 56 days) and at 2 years (Day 730 +/- 90 days) after vaccination. Randomization and vaccination were performed as described in Chapter 2, Section 2.1.

5.2.1 Sample Collection, Handling, Storage and Transport:

Blood samples were collected from the participants using the standard aseptic non-touch technique and transferred into an EDTA containing tube. The samples were placed in a

cool box to maintain a temperature of 2-8°C, and were transferred to Patan Hospital laboratory within 4 – 6 hours of collection.

The samples were centrifuged at 3000 rpm for 10 min to separate the cells from the plasma. The plasma was aspirated using a Pasteur pipette, put in prelabelled cryovials and stored at -20°C at Patan Hospital. The plasma samples were shipped to Oxford Vaccine Group (OVG), University of Oxford on dry ice, where they were stored at -80°C until further use.

5.2.2 Anti-Vi IgG Antibody Detection

Vi IgG titres were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (VaccZyme®, The Binding Site, Birmingham UK) according to the manufacturer's guidelines. The kit consists:

1. Typhi Vi Coated wells
2. Typhi Vi IgG Calibrators with diluted human serum with anti-Typhi Vi antibody (concentrations 7.4, 22.2, 66.7, 200 and 600U/ml)
3. High, medium and low Typhi Vi IgG controls.
4. Type V Sample diluent
5. Typhi Vi IgG Conjugate containing purified peroxidase labelled antibody
6. TMB substrate
7. Stop solution containing 3M phosphoric acid.

Briefly, 10uL of each sample was diluted with 1000uL of sample diluent. 100uL of each calibrator, control and diluted (1:100) samples were added to the wells (plate layout in Table 16) and the plates were incubated at room temperature for 30 minutes. After incubation, the wells were washed three times with 250-350uL of wash buffer per well and dried on absorbent paper. Then, 100uL of conjugate was added to each well and further incubated for 30 minutes at room temperature. After further washing, 100uL of TMB substrate was added to each well, incubated at room temperature in the dark for 30 minutes and the stop solution was added to stop the reaction. The optical density values were measured at 450nM using an automated plate reader and were used to calculate the concentration of antibody.

Table 16. Anti-Vi IgG Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1 600	STD1 600	SPL1	SPL5	SPL9	SPL13	SPL17	SPL21	SPL25	SPL29	SPL33	SPL37
B	STD2 200	STD2 200	SPL1	SPL5	SPL9	SPL13	SPL17	SPL21	SPL25	SPL29	SPL33	SPL37
C	STD3 66.7	STD3 66.7	SPL2	SPL6	SPL10	SPL14	SPL18	SPL22	SPL26	SPL30	SPL34	SPL38
D	STD4 22.2	STD4 22.2	SPL2	SPL6	SPL10	SPL14	SPL18	SPL22	SPL26	SPL30	SPL34	SPL38
E	STD5 7.4	STD5 7.4	SPL3	SPL7	SPL11	SPL15	SPL19	SPL23	SPL27	SPL31	SPL35	SPL39
F	LoQC	LoQC	SPL3	SPL7	SPL11	SPL15	SPL19	SPL23	SPL27	SPL31	SPL35	SPL39
G	HiQC	HiQC	SPL4	SPL8	SPL12	SPL16	SPL20	SPL24	SPL28	SPL32	SPL36	SPL40
H	BLK	BLK	SPL4	SPL8	SPL12	SPL16	SPL20	SPL24	SPL28	SPL32	SPL36	SPL40

STD=Standard (Typhi Vi IgG Calibrators with diluted human serum with anti-Typhi Vi antibody of concentrations 7.4, 22.2, 66.7, 200 and 600U/ml); LoQC=Low Typhi Vi IgG controls; HiQC= High Typhi Vi IgG controls; BLK=Blank; SPL=Sample

The first step plates passed if the controlled fell within the ranges specified in the QC certificate of the kit as follows:

Quality Control	Target	Lower limit (EU)	Upper limit (EU)
HiQC	146.5	117.2	175.8
LoQC	30.4	24.3	36.5

5.2.3 Anti-Vi IgA Antibody Detection

Anti-Vi IgA was assessed with Vi-coated plates and reagents supplied by The Binding Site using a protocol adapted from the commercial VaccZyme® assay.

In place of the 5 S. Typhi Vi IgG calibrators in the kit, a standard curve was generated using the 1st International Standard (IS) for anti-typhoid capsular Vi polysaccharide IgG (human) National Institute for Biological Standards and Control (NIBSC) code 16/138. STD 100 was prepared using 1 in 60 of the IS 16/138 reconstituted in 1 ml distilled water according to the NIBSC product sheet, and aliquoted into appropriate volumes and frozen at -80C. 10 µl of the IS 16/138 was added to 590 µl of Type V sample diluent to prepare STD 100. To make STD 50, STD 25, STD 12.25, STD 6.125 and STD 3.125, 1 in 2 serial dilutions of the STD 100 were prepared, in each case moving 300 µl of standard into 300 µl Type V sample diluent.

In place of Typhi Vi IgG high control and Typhi Vi IgG low control from the kit, quality controls were produced by NIBSC. For Control 1 (CTL1), 1/100 of H (CS628 H); for CLT2, 1/100 of J (CS628 J); and for CLT3, 1/100 of K (CS628 K) were used.

Typhi Vi IgG Conjugate from the kit was replaced by an IgA Conjugate. During the Pre-Assay Steps Conjugate Diluent was prepared using 7.23g NaCl + 100ml Fetal Bovine

Serum (FBS) in 500ml dH_20., and further dH_20 was added to make up to 1 litre. For running 1 – 2 plates, 4 µl Sheep anti human IgA (ref AP010, The Binding Site) was added in 24 ml of Conjugate Diluent (1/6000) and volumes were scaled up for two or more plates as necessary.

The samples, followed by CLT 1-3 and by STDs 100 – 3.125 were dispensed on to the wells as displayed in Table 17. The assay methods were followed as with Anti-Vi IgG. With an exception, the wells were incubated with TMB for 20 minutes.

Table 17. Anti-Vi IgA Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1 100	STD1 100	CLT2	SPL3	SPL7	SPL11	SPL15	SPL19	SPL23	SPL27	SPL31	SPL35
B	STD2 50	STD2 50	CLT2	SPL3	SPL7	SPL11	SPL15	SPL19	SPL23	SPL27	SPL31	SPL35
C	STD3 25	STD3 25	CLT3	SPL4	SPL8	SPL12	SPL16	SPL20	SPL24	SPL28	SPL32	SPL36
D	STD4 12.5	STD4 12.5	CLT3	SPL4	SPL8	SPL12	SPL16	SPL20	SPL24	SPL28	SPL32	SPL36
E	STD5 6.25	STD5 6.25	SPL1	SPL5	SPL9	SPL13	SPL17	SPL21	SPL25	SPL29	SPL33	SPL37
F	STD5 3.125	STD5 3.125	SPL1	SPL5	SPL9	SPL13	SPL17	SPL21	SPL25	SPL29	SPL33	SPL37
G	BLK	BLK	SPL2	SPL6	SPL10	SPL14	SPL18	SPL22	SPL26	SPL30	SPL34	SPL38
H	CLT1	CLT1	SPL2	SPL6	SPL10	SPL14	SPL18	SPL22	SPL26	SPL30	SPL34	SPL38

STD=Standard (Typhi Vi IgG Calibrators with diluted human serum with anti-Typhi Vi antibody of concentrations 3.125, 6.25, 12.5, 25, 50 and 100U/ml); BLK=Blank; CLT=Control; SPL=Sample

For the analysis of Vi IgA, the first step plates passed if the quality controls fell within the following values:

Quality Control	Lower limit (EU)	Upper limit (EU)
CTL1	49.0622	73.5933
CTL2	23.65484	35.48227
CTL3	5.468222	8.202333

5.2.4 Statistical Analysis

Total anti-Vi IgA titres less than the lower limit of detection of 3.125 EU/mL were assigned a value of 1.56 EU/mL Total anti-Vi IgG titres less than the lower limit of detection of 7·4 EU/mL were assigned a value of 3·7 EU/mL. A four-fold rise in the antibody titre was defined as seroconversion.

Counts and percentage of participants with level above lower limit of quantification of the assay was calculated. Seroconversion at day 28 and 18 months and fold increase in titre at day 28 and 10 months from baseline were calculated where paired samples were available. Descriptive statistics was generated for each vaccine group and at each timepoint for IgA and IgG titres.

5.3 Results

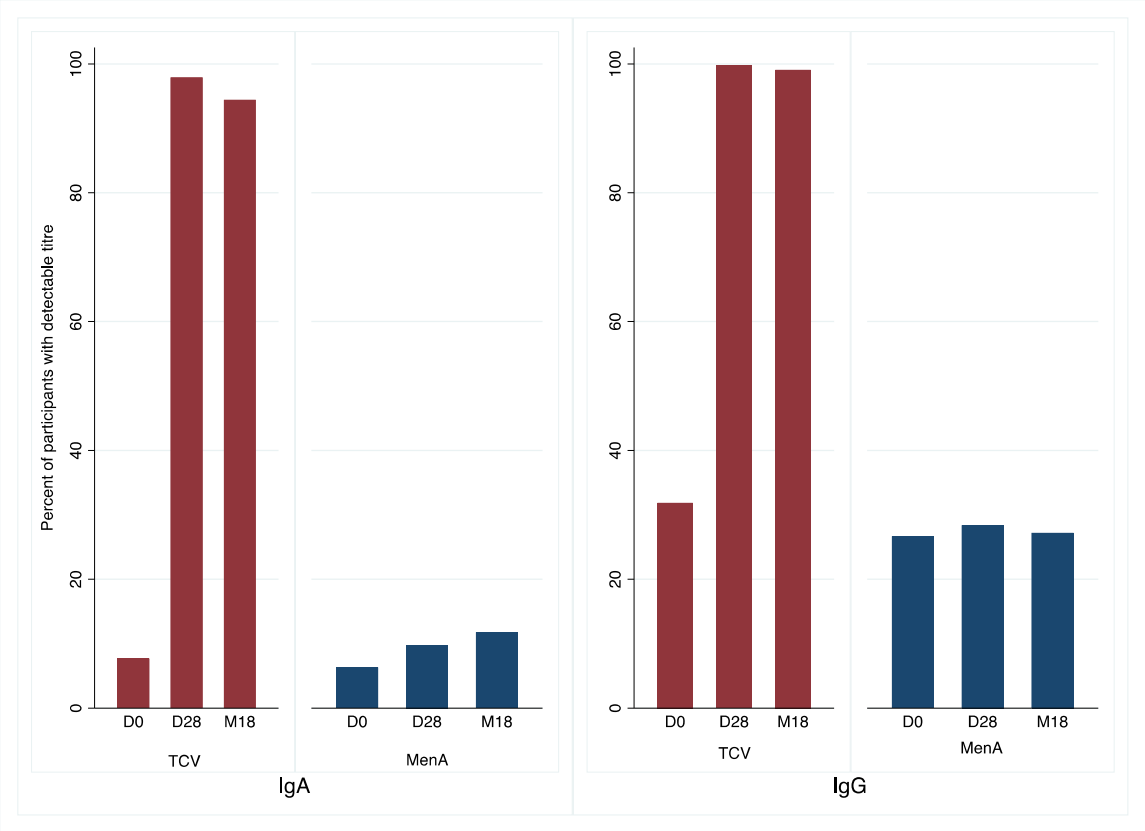
A total of 1408 participants were randomized to the immunogenicity component of the study. In total, 1386 participants provided at least one blood sample.

5.3.1 Anti-Vi IgA Results

At baseline, 56 of 638 participants in the TCV group (8.20%) and 25 of 380 participants in the MenA group (6.58%) had detectable anti-Vi IgA antibody levels (Table 18; Fig 12). The

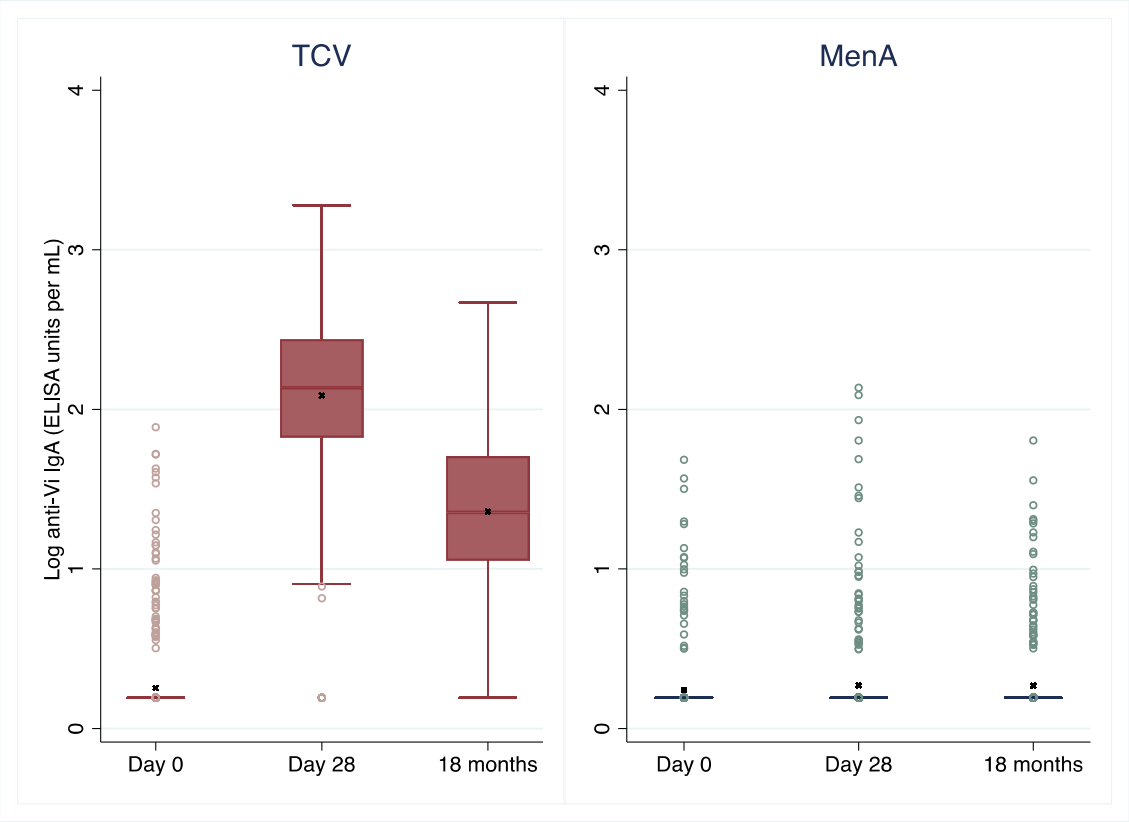
geometric mean IgA level was 1.79 ELISA Units (EU)/mL (95% CI 1.72 – 1.87) in the TCV group and 1.74 EU/mL (95% CI 1.67 – 1.83) in the MenA group (Table 18; Fig. 13).

Figure 12. Percentage of Participants with Detectable Anti-Vi IgA Titre and Detectable Anti-Vi IgG Titre at Baseline, 28 Days and 18 months post-vaccination.



TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine

Figure 13. Anti-Vi IgA Levels at Day 0, Day 28- and 18-Months Post-Vaccination, All Ages



TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine
Boxplot showing the distribution of the anti-Vi IgA levels. The black 'x' shows the mean value.
The lower limit of quantification for IgA was 3.125 EU per millilitre. Values below this limit were substituted with 1.56 EU per millilitre for the analysis.

At 28 days post-vaccination, 670 of 683 participants in the TCV group (98.10%) and 38 of 380 participants in the MenA group (10.00%) had detectable anti-Vi IgA antibody levels. The geometric mean IgA levels increased to 122.04 EU/mL (95% CI 111.83 – 133.17) in the TCV group, with a mean 120.25 (95% CI 110.59 – 129.90) fold increase in the titre. The geometric mean IgA levels was 1.87 EU/mL (95% CI 1.75 – 1.99) in the MenA group with a mean 1.79 (95% CI 1.09 – 2.49) fold increase in the titre. Seroconversion or a four-fold rise in antibody titre 28 days after vaccination was 97.2% in the TCV group and 1.58% in the MenA group.

At 18 months post-vaccination, the 601 of 639 participants in the TCV group (94.05%) and 41 of 358 participants in the MenA group (11.45%) had detectable anti-Vi IgA antibody levels. The geometric mean IgA titre decreased to 22.86 EU/mL (95% CI 20.81 – 25.12) in the TCV group. The mean IgA titre was 27.50-fold (95% CI 24.54 – 30.46) higher than at baseline, and 479 of 539 participants (88.87%) still had a four-fold rise in titre compared with baseline. The mean geometric titre was 1.85 EU/mL (95% CI 1.75 – 1.96) in the MenA group with the participants having a mean 1.27-fold increase (95% CI 1.10 – 1.43) in titre than at baseline and 6 of 299 participants (2.01%) having a four-fold rise in titre compared with baseline.

Table 18. Vi IgA and Vi IgG Levels in the Immunogenicity Cohort

Trial Group	Day 0	Day 28	Month 18	Period from Day 0 – Day 28	Period from Day 0 – Month 18
				4-fold rise	4-fold rise
Anti-Vi IgA Level					
TCV					
Level above lower limit of quantification of the assay – no. of participants/ total no. (%)*	56/683 (8.20)	670/683 (98.10)	601/639 (94.05)	664/683 (97.2)	479/539 (88.87)
Geometric mean concentration (95% CI) – EU/ml	1.79 (1.72 – 1.87)	122.04 (111.83 – 133.17)	22.86 (20.81 – 25.12)	120.25 (110.59 – 129.90) fold increase	27.50 (24.54 – 30.46) fold increase
Median (IQR)	1.56 (1.56 – 1.56)	136.54 (66.74 – 273.81)	22.60 (11.30 – 50.65)		
MenA vaccine					
Level above lower limit of quantification of the assay – no. of participants/ total no. (%)*	25/380 (6.58)	38/380 (10.00)	41/358 (11.45)	6/380 (1.58)	6/299 (2.01)
Geometric mean concentration (95% CI) – EU/ml	1.74 (1.67 – 1.83)	1.87 (1.75 – 1.99)	1.85 (1.75 – 1.96)	1.79 (1.09 – 2.49) fold increase	1.27 (1.10 – 1.43) fold increase
Median (IQR)	1.56 (1.56 – 1.56)	1.56 (1.56 – 1.56)	1.56 (1.56 – 1.56)		
Anti-Vi IgG Level					

TCV					
Level above lower limit of quantification of the assay – no. of participants/ total no. (%)**	268/849 (31.57)	708/709 (99.86)	633/639 (99.06)	677/683 (99.1)	573/601 (95.34)
Geometric mean concentration (95% CI) – EU/ml	7.21 (6.69 – 7.11)	2037.90 (1904.64–2180.48)	241.29 (220.23 – 264.36)	501.34 (463.67 – 539.01) fold increase	67.39 (60.39 – 74.39) fold increase
Median (IQR)	3.7 (3.7–13.4)	2220.7 (1298.6–3725.7)	241.29 (220.24– 264.36)		
MenA vaccine					
Level above lower limit of quantification of the assay – no. of participants/ total no. (%)**	112/460 (26.52)	112/388 (28.87)	96/358 (26.82)	8/380 (2.1)	10/331 (3.02)
Geometric mean concentration (95% CI) – EU/ml	6.48 (5.89–7.13)	6.98 (6.20–7.85)	6.57 (5.87 – 7.35)	5.59 (-0.95 – 12.14) fold increase	1.52 (1.00 – 2.05) fold increase
Median (IQR)	3.7 (3.7–8.9)	3.7 (3.7–10.5)	3.7 (3.7–9.40)		

TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine

*The lower limit of quantification was 3.125 EU per millilitre. Values below this limit were substituted with 1.56 EU per millilitre for the analysis.

**The lower limit of quantification was 7.4 EU per millilitre. Values below this limit were substituted with 3.7 EU per millilitre for the analysis.

5.3.1 Anti-Vi IgG Results

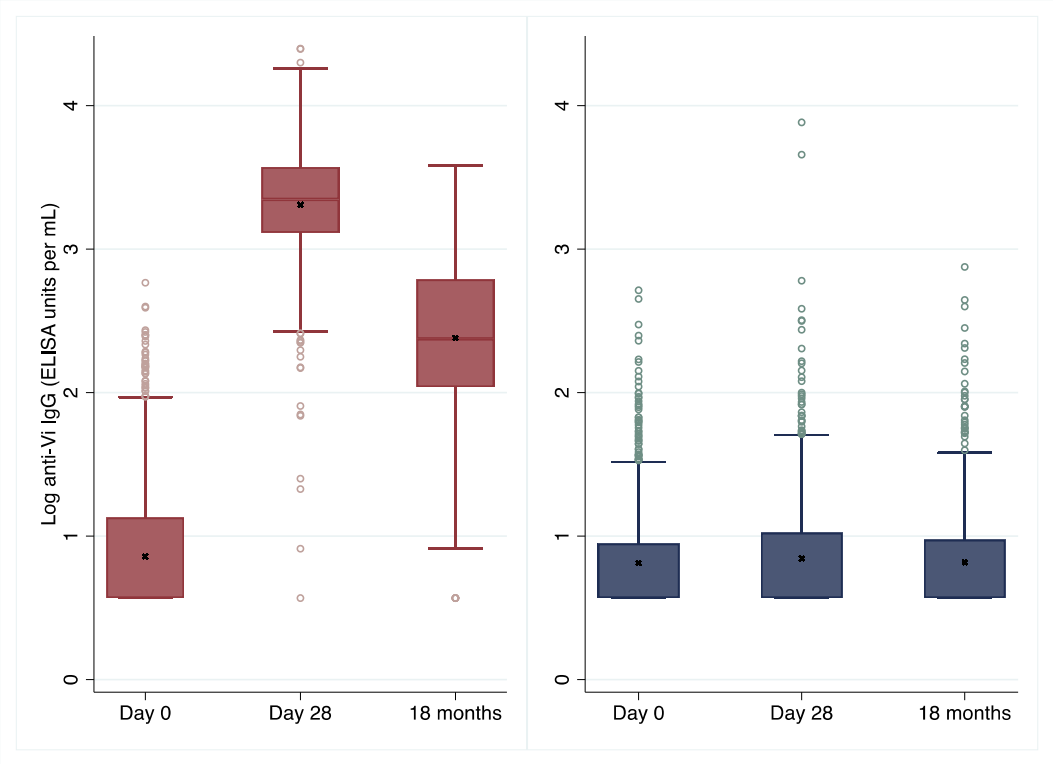
At baseline, 268 of 849 participants in the TCV group (31.57%) and 112 of 460 participants in the MenA group (26.52%) had detectable anti-Vi IgG antibody levels (Table 18; Fig 13). The geometric mean IgG level was 7.21 EU/mL (95% CI 6.69 – 7.11) in the TCV group and 6.48 EU/mL (95% CI 5.89–7.13) in the MenA group (Table 18; Fig. 14).

At 28 days post-vaccination, 708 of 709 participants in the TCV group (99.86%) and 112 of 388 participants in the MenA group (28.87%) had detectable anti-Vi IgG antibody levels. The geometric mean IgG levels increased to 2037.90 EU/mL (95% CI 1904.64–2180.48) in the TCV group and there was a mean 501.34 (95% CI 463.67 – 539.01) fold increase fold increase in the titre from baseline. The geometric mean IgG levels was 6.98 EU/mL (95% CI 6.20–7.85) in the MenA group with a mean 0.59 (95% CI -0.95 – 12.14) fold increase in the titre from baseline. In total, 99.1% of the participants (N=677/683) in the TCV group had a seroconversion, while 2.1% of the participants (N= 8/380) had a seroconversion in the MenA group.

At 18 months post-vaccination, the 573 of 601 participants in the TCV group (95.34%) and 96 of 358 participants in the MenA group (26.82%) had detectable anti-Vi IgG antibody levels. The geometric mean IgG titre decreased to 2037.90 EU/mL (95% CI 1904.64–2180.48) in the TCV group, and the mean IgG titre was 67.39 (95% CI 60.39 – 74.39) fold higher than at baseline. A total of 573 of 601 participants (95.34%) still had a four-fold rise in titre compared with baseline. The mean geometric titre was 6.57 EU/mL (95% CI 5.87 –

7.35) in the MenA group with the participants having a mean 1.52 (95% CI 1.00 – 2.05) fold increase in titre from the baseline and 10 of 331 participants (3.02%) having a four-fold rise in titre compared with baseline.

Figure 14. Anti-Vi IgG Levels at Day 0, Day 28- and 18-Months Post-Vaccination, All Ages.



TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine
Boxplot showing the distribution of the anti-Vi IgG levels. The black 'x' shows the mean value.
The lower limit of quantification for IgG was 7.4 EU per millilitre. Values below this limit were substituted with 3.7 EU per millilitre for the analysis.

5.4 Discussion

5.4.1 TCV and IgA response

TCV elicited a strong IgA response. TCV induced a high IgA antibody titre and fold increase in IgA antibody 28 days post-vaccination and high seroconversion was

maintained at 18 months post-vaccination. To date, there are no other studies based in endemic settings that report the IgA response to TCV.

The strong IgA response is of interest because a CHIM study looking into the relationship of vaccine-elicited antibodies and protection against typhoid fever suggested that the Vi IgA may serve as a surrogate of protection[150]. This Oxford study analyzed the humoral responses from participants who received either the Vi-PS vaccine or the TCV vaccine and determined the avidity, overall magnitude and vaccine- induced fold-change in magnitude of Vi IgA and IgG subclass antibodies from before immunization to day of challenge. In the study, participants were randomized to TCV, Vi-PS or control group and were challenged with *S.* Typhi 28 days post-vaccination[98]. Participants with a positive blood culture and/ or prolonged fever of $\geq$38C for 12 hours or more were defined as diagnosed, while those that did not meet the case definition of diagnosed were defined as protected. Of the different antibody types, the Vi IgA antibodies were most induced in response to the Vi polysaccharide in both vaccine groups. In the TCV group, the median IgA concentration in those diagnosed with typhoid fever was 595 µg/mL while the concentration was 2119 µg/mL in those protected from typhoid fever. Of the IgA antibodies, the IgA1 subclass had the highest fold change. And although there were no significant associations of protection in protected individuals in TCV recipients, trends of higher total IgA fold-change, IgA1 fold change and IgA2 avidity were observed. Protective threshold of Vi IgA, however, was not identified.

A follow-up study from the same group further investigated the samples from the CHIM using a systems serology approach to identify potential correlates of protection[151]. The Vi-PS and TCV groups were combined to compare the humoral responses between the diagnosed and protected participants. Those protected from typhoid infection, had a significantly higher fold increase in total IgA titre baseline to days 28, 118, and 208 compared with diagnosed participants. No significant differences were seen in absolute antibody titres between the diagnosed and protected groups when correcting for multiple testing, and no significant differences were seen in the Vi-specific measures when the vaccine groups were assessed separately. Higher fold increase in IgA response was seen in the protected individuals in the TCV group before correction for multiple testing.

Although IgA has been identified as a biomarker for protection against typhoid fever in the CHIM studies, the mechanism by which it confers protection is not known. IgA plays a strong role in mucosal immunity and likely provide protection against the enteric pathogen at the site of infection[150]. Jin et al. postulated that high concentrations of Vi IgA in local mucosal sites such as the lamina propria, Peyers patches or the efferent lymphatics could be responsible for opsonizing Vi expressing extracellular *S.* Typhi and thus preventing the infection[152]. This preliminary evidence from the CHIMs suggesting correlation between IgA and protection will need to be further investigated.

5.4.2 TCV and IgG Response

TCV elicited a strong IgG response. The robust IgG response as demonstrated by high GMT at 28 days post-vaccination and that was maintained at 18 months and high proportion of responders as indicated by seroconversion at the same time points.

The IgG response has long been used as a measure of immune response to typhoid vaccines and has also use to determine the immunogenicity of the TCV used in this study as well. The anti Vi-IgG titre in the RCT conducted by Bharat Biotech, India showed a GMT (95% CI) of 10.4 EU/mL (9.6 – 11.3) at baseline, 1292.5 EU/mL (1153 – 1149) at Day 42 and 92.8 EU/mL (81 – 106) at 18 months post-TCV vaccination. The anti-Vi IgG antibodies were assessed by ELISA using the VAccZyme commercial kit as in TyVAC-Nepal. India is a typhoid endemic country like Nepal, and the results reflect the vaccine response in a high burden setting. The results were consistent with the geometric mean concentrations of anti-Vi IgG in TyVAC-Nepal, showing the vaccine elicits a good response. Overall, 97.3% on the TCV recipients in the Indian trial seroconverted, while 99.1% in TyVAC-Nepal seroconverted.

In the CHIM study conducted at Oxford, 100% of the participants who received TCV seroconverted by day 28. The geometric mean titre at 28 days post-vaccination was 562.9 EU/mL (396.9 – 798.8) compared with 2037.90 EU/mL (1904.64–2180.48) in TyVAC-Nepal. The comparative lower total antibody titre could be attributed to lack of exposure to typhoid fever in the CHIM participants. Multiple natural exposures to

typhoid in participants in TyVAC-Nepal may mean that the participants have pre-existing immunity and can probably explain the high titres likely due to a boost in antibody maturation response post-vaccination. The different age groups and ethnicities are other probable reasons for the differences seen.

Measurement of anti-Vi IgG in participants receiving Vi-PS has been described in the introduction and it is difficult to make a direct comparison of the response to TCV in TyVAC-Nepal due to the different assays used in the Vi-PS trials. The trial in India, however, shows that TCV elicited stronger anti-Vi IgG response that Vi-PS at 42 days and 720 days post-vaccination. The day 28 GMT was also significantly higher in the TCV group, compared to the Vi-PS groups in the Oxford CHIM.

TyVAC-Bangladesh, a cluster randomized trial conducted in Mirpur, Bangladesh as a part of Typhoid Vaccine Acceleration Consortium (described in a previous chapter), also assessed the immunogenicity of TCV in children[116]. Approximately 1500 children aged 9 months to under-16 years were enrolled in an immunogenicity sub-cohort in a 2:1 ratio (TCV: MenA). Blood samples were drawn at the same time-points as in TyVAC-Nepal, and anti-Vi IgG titres were measured using the VaccZyme, The Binding Site kit. At 28 days post-vaccination, 99.6% of the participants in the TCV group had seroconverted, with a median titre of 3222.4 (IQR, 1757-5471.6) ELISA units/ml and 693 (IQR, 366-1198) fold increase from baseline, all significantly different from the MenA group. The results demonstrate a robust immune response consistent with the findings in TyVAC-Nepal.

Similar, the IgG response of TCV vaccine co-administered with EPI vaccines was assessed in children 9 – 11 months of age (at the routine 9 month visit) and 15 - 23 months of age (at the routine 15 month vaccination visit) in a double-blind randomized controlled trial conducted in Burkina Faso[135,136,137]. The study reported a robust immune response in children 9 – 11 months of age with at least a four-fold rise in anti-Vi IgG titre in 99.6% among those two received TCV with IPV and 96% among those two received TCV with MenA[136]; as well as a robust immune response in 15 - 23 months of age with at least a four-fold rise in anti-Vi IgG titre in 93.6% among those who received TCV with IPV and 96.0% seroconversion among those who received TCV with MenA[137]. TCV did not intervene with the immune response of MenA vaccine, yellow fever vaccine or the MR vaccine. These findings support the results generated in TyVAC-Nepal. Importantly, in the context of a Sub-Saharan country with a concurrent risk of meningococcal A disease that administers MenA vaccine as a part of the routine immunization, this study also generated evidence that simultaneous administration of TCV and MenA did not interfere with the immune responses to either vaccines. Same applies for the yellow fever vaccine. As the GAVI eligible countries start introduction of TCV in their routine immunization schedules, it will be important to generate evidence of non-interference with other EPI schedule vaccines as well. For instance, in Nepal, the TCV has been approved for introduction at 15 months along with the MR vaccine. It will be important to assess the immunogenicity of TCV and MR when co-administers as a part of the routine immunization schedule in the Nepali context.

Using the systems serology approach in the Oxford CHIM, it was presented that TCV protection was associated with Vi IgG1 avidity, whilst Vi-PS protection was associated with all subclasses of IgG[151]. The analyses also found that TCV induced higher titres of Vi IgG that Vi-PS, and that IgG was associated with reduced severity of the disease suggesting Vi IgG as a correlate of protection for typhoid fever disease. However, the definition of typhoid fever in the CHIM studies differed from that of TyVAC-Nepal due to which the results cannot be generalized. Additional studies are needed to determine if any of these responses relate differ in the endemic setting.

The recently WHO-prequalified conjugate vaccine, TYPHIBEV (Vi-CRM197), has demonstrated that the immune response comparable to the TCV used in this trial[102]. TYPHIBEV has reported a protective IgG seroconversion in 95.59% in individuals 6 months to under-64 years of age[153]. Published scientific paper detailing the results are however not available. Similarly, Vi-DT (IVI and SK Biosciences) has also demonstrated comparable immune response to TCV in an observer-blind, non-inferiority phase III trial in Nepal[110]. The anti-Vi IgG seroconversion rate in all age strata was 99.3% (97.5% CI 98.61 – 99.68) in the Vi-DT vaccine group and 98.88% (97.5% CI 97.10 – 99.57) in the Typbar-TCV group, showing that the Vi-DT vaccine was non-inferior to Typbar-TCV. The results from these trials are promising. With TYPHIBEV achieving WHO-prequalification and Vi-DT demonstrating results to support further development of the vaccine, this is a step towards ensuring there is a good vaccine supply to meet demands.

The anti-Vi IgG antibody levels has been used to assess protection against typhoid fever for the Vi-polysaccharide vaccine. In the Disease of Most Impoverished Program vaccine effectiveness trials conducted in Karachi, Pakistan and Kolkata, India, overall 86.7% of the children aged 2 to 16 years of age receiving the Vi polysaccharide vaccine had a 4-fold rise in antibody titre at 6 weeks post-vaccination[154]. The percentage of participants with a sustained 4-fold rise in titre dropped to 39.1% at two years post-vaccination. The geometric mean titre was 1428.3 EU/mL at 6 weeks post-vaccination and 262.6 EU/mL at 2 years post-vaccination. In the study the anti-Vi IgG was measured using ELISA technique using the GSK ELISA standards. Direct comparisons to TyVAC-Nepal is not possible due to the different ELISA methods. However, the fold increase is comparable to that of TCV 6 weeks post-vaccination. As expected with a Vi polysaccharide vaccine, the proportion of participants with 4-fold increase after 2 years is low compared to that of TCV. The antibody titres also appear to be higher for TCV at each time point. This highlights the T cell independent mechanism of antibody production in response to a polysaccharide vaccine which results in waning antibody over time, and the need for conjugate vaccines for a stronger immunogenic response and more sustained protection. Revaccination with Vi-polysaccharide vaccines every three years is hence recommended to ensure sustained protection against typhoid fever.

5.4.3 Limitations and Future Direction

TyVAC-Nepal results show that TCV has a robust immunogenic response and that the TCV is efficacious. The two results align with each other. However, it is not possible to from the study results to say if those with the high antibody response are those who were

protected from typhoid fever. Only one participant who received TCV and was enrolled in the immunogenicity component of the study had culture positive typhoid fever. The number is insignificant to say whether there is difference in the immunogenic response in those that were protected in comparison to those that developed the clinical disease.

Further given that there are no established correlates of protection for typhoid fever, it is difficult to say that the high IgG or high IgA response led to protection from typhoid fever. It is plausible that high antibodies are produced in response to the vaccine, but the mechanism of protection from typhoid fever is different. As discussed above, the Oxford CHIM studies have suggested that high total IgA titre is associated with protection from the disease. However, a CoP identified from a single trial may not be applicable to other populations and may differ from in other populations/ ethnicities. It will be important to replicate these results in other settings.

Traditionally, vaccine studies assess immunogenic response in a subset of participants. Collecting blood samples from all or most participants can be logistically challenging and is usually also directed by funds available for the study. It is important to collect post-vaccination samples from all or a substantial subset of participants from the vaccination and control groups to be able to analyze the relationship between the immunological parameters and protection against disease[147]. In a recent randomized efficacy trial of the ChAdOx1 nCoV-19 (AZD1222) vaccine in UK, blood samples were drawn from all study participants and the relationship between the immune response to the vaccine and

protection against clinical outcomes was assessed[155]. Four immune markers associated protection against symptomatic infection were identified which could potentially be used to assess immunogenicity of future vaccines and avoid the need to do large efficacy trials. Assessing large sample size for potential CoPs may be the way forward for other vaccines as well.

Whilst assessing humoral immunity through assays remains the standard for evaluating the response of vaccines, these assays only measure a single humoral response of a vaccine. There is growing evidence that mechanisms other than antibody mediated neutralization also lead to protection from disease[156]. The Fc portion of the antibodies interact with other immune components and effector cells to neutralize the pathogen via multiple mechanisms including antibody dependent cellular cytotoxicity (ADCC), antibody dependent cellular phagocytosis (ADCP), antibody dependent complement deposition (ADCD). The systems serology approach aims to comprehensively capture the antibody features and functions to determine the key mechanisms by which vaccines confer protection[156]. One or more of these markers combined can synergistically protect from disease and can be used to evaluate vaccines. Using the systems serology approach, the same Oxford group investigated Vi-specific correlates of protection using the samples obtained from the S. Typhi CHIM study[151]. As described above, IgA quantity and avidity correlated with protection from infection and IgG1 avidity correlated with reduced disease severity. Other antibody-mediated functional activities were also associated with vaccine induced protection. In the participants that received TCV, antibody dependent neutrophil phagocytosis was also associated with protection. The results suggest that

although threshold of protection from neutralizing antibodies are traditionally explored to denote protection from disease, evaluation of vaccine correlates of protection shows that human immune response is far more complex, and needs to be explored to understand protection from disease.

5.5 Summary

In summary, TCV administration was associated with a robust IgA and IgG response. The robust seroconversion rate aligns with the vaccine efficacy which is reassuring, but needs further study.

6. Age and Sex Differences in Response to TCV

The results presented in this chapter is published in:

1. Shakya M, Voysey M, Theiss-Nyland K, *et al.* Efficacy of typhoid conjugate vaccine in Nepal: final results of a phase 3, randomised, controlled trial. *Lancet Glob Heal* 2021; **9**: e1561–8[139].

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This work was also presented in the following presentations:

1. Effects of Age and Sex on the Immune Response to Typhoid Conjugate Vaccine in a Phase III Randomized Controlled Trial in Nepal. Poster Presentation. 2021 International Conference on Typhoid & Other Invasive Salmonelloses. Oct 2021

6.1 Introduction

There is evidence that age and biological sex influences immune response. Lower vaccine responses have been documented at the extreme of ages. The vaccine type, the age at priming, the interval between vaccine doses and the age at which the final dose is given for multidose vaccines influence the antibody response, all of which are important considerations for ensuring infants are adequately protected with vaccines.[157,158] There is a weak B cell response in early life and an age-dependent increase in antibody response. Infants respond poorly to T cell-independent polysaccharides which are present in vaccines against bacteria including *Neisseria*, *Pneumococcus* and *Salmonella* Typhi[157]. The polysaccharide antigen in polysaccharide vaccines cross-links to the B cell receptor, stimulating the B cells to differentiate to plasma cells leading to the production of antibodies[159]. New memory B cells are not produced in response to a polysaccharide vaccine and there is a lack an anamnestic response to further doses of the vaccine. Polysaccharide vaccines stimulate the splenic marginal zone B cells. The marginal zone only matures at about 18 months to 2 years of age, due to which polysaccharide vaccines are poorly immunogenic in children under-2 years[159,160].

Development of protein polysaccharide conjugate vaccines, in which bacterial polysaccharide is chemically conjugated to a protein carrier, allows immunogenic response even in the youngest age groups[159]. Conjugate vaccines, as in the case of TCV where the Vi polysaccharide antigen is conjugated to the tetanus toxoid carrier protein, induce a T-cell dependent immune response. The carrier protein is internalized and

processed by the polysaccharide specific B cell and presented within the antigen-blinding cleft of MHC class II molecules to the carrier protein-specific helper T cells leading to the production of the polysaccharide-specific plasma cells and polysaccharide-specific memory B cells[158,159]. Although the T cells induced by the carrier protein provide help to the B cells, polysaccharide specific T cells are not induced.

Even with conjugate vaccines, the antibody response may still be lower in the youngest age groups due to immaturity of the immune system[157]. In adults, antibody titre is maintained by long lived antibody-secreting plasma cells present in bone marrow niche[157]. In the youngest age group, the bone marrow niche that support B cells are limited. The signals to maintain a pool of plasma cells is low leading to low persistence of response in early infancy in comparison to adults and older children. To prevent decay of antibody titre, repeated doses of vaccines may be necessary as with many EPI schedule vaccines[157]. Similarly, protective antibody titres can be achieved early on with early priming followed by early boosting[157].

Maternal antibodies form complexes with antigens presented through vaccination interfering with antibody production[157]. The above means that the adult-like patterns of antibody responses to conventional vaccines in early life are not elicited until up to two or more years of life[161]. Influence of age has also been noted in other ages[162]. For example, GMTs following meningococcal conjugate vaccine have been documented to be higher in children over 10 years than in younger children.

Biological sex is defined as “differential organization of chromosomes, reproductive organs, and sex steroid levels” and is different from gender which is a construct defined by social and cultural determinants[163]. Biological sex affects the functions of the immune system and there is increasing evidence that there are sex differences in innate and adaptive immune responses. For instance, in the innate immune system, macrophages and neutrophils phagocytic capacity is higher in adult females, whilst macrophages pro-inflammatory cytokine production is higher in adult males[163]. In the adaptive immune system, females have greater T cell proliferation, increased cytotoxic activity and higher antibody production. Studies investigating neonatal immunity using cord blood samples suggest that males develop a more robust innate immunity in early life. Sex differences have also been noted in antibody response to vaccines with a propensity for greater responses in females[163]. For example, higher vaccine response has been documented to multiple vaccines in female infants and children such as higher geometric mean titres (GMTs), seroconversion rates and seroprotection rates of hepatitis A vaccine , higher GMTs to hepatitis B vaccine, higher GMTs to *Hemophilus influenza* type b vaccine , higher GMTs to 13-valent pneumococcal conjugate vaccine vaccines[162]. Higher seroconversion rates to measles vaccine and higher seroprotection rates to tetanus toxoid, however, have been documented in male infants and children[162].

Age and sex are important intrinsic host factors that can influence responses to vaccines. Age and sex influences are important from a programmatic side. WHO SAGE, in March 2018, recommended routine uses of TCV in children from 6 months of age. This has been

based on the robust immune response demonstrated in the trial conducted in India by the manufacturers. With the Gavi funding to support TCV introduction, countries have started to rollout TCV in their national immunization schedules. Pakistan, Liberia and Zimbabwe have rolled out TCV in children 9 months to 15 years in national campaign and integrated it into routine immunization making the vaccine available to all 9-month old. In Nepal, the government has planned a nationwide catch-up campaign in all children 15 months to 15 years of age, followed by integration into the routine immunization schedule at 15 months. Influence of age and sex on responses to TCV vaccine, if apparent, will need to be factored in the vaccine programme. This chapter explores the potential impact of age and sex in efficacy and immunogenicity of TCV.

6.2 Methods

The methods for passive surveillance and detection of blood culture-positive typhoid fever have been described in Chapter 2, section 2.3.3. The statistical analysis for calculation of VE and cumulative incidence analysis is described in Chapter 3, section 3.2.1.1. The VE and cumulative incidence were further calculated according age groups (less than 2 years, 2 – 4 years and 5 years and above); and, according to the sex. Subgroup analyses of the primary outcome were conducted using Poisson models with vaccine-by-age group interaction effects and vaccine-by-sex interaction effects.

The methods for immunogenicity sample collection, and antibody detection have been described in Chapter 4.0 Immunogenicity of Typhoid Conjugate Vaccine, Section 4.2

Methods. Statistical analysis was conducted according to age categories and according to the sex. Kruskal Wallis test for the differences in the age categories and Mann Whitney-U test for the sex differences.

Table 19. Age and Sex breakdown of Study Participants.

Variable	Vaccinated N (%)	Case definition met for blood culture N(%)**	Cultures done N (%)*
TCV			
Sex			
Female	4,899 (48.97)	1107 (46.32)	782 (70.64)
Male	5,106 (51.03)	1283 (53.68)	937 (73.03)
Age at enrollment (years)			
< 2 years	671 (6.71)	350 (14.64)	245 (70.00)
2 – 4 years	2,990 (29.89)	655 (27.41)	501 (76.49)
5 years and above	6,344 (63.41)	1385 (57.95)	973 (70.25)
MenA vaccine			
Sex			
Female	4,856 (48.49)	1055 (43.81)	734 (69.57)
Male	5,158 (51.51)	1353 (56.19)	1353 (56.19)
Age at enrollment (years)			
< 2 years	719 (7.18)	350 (14.54)	244 (69.71)
2 – 4 years	2,964 (29.60)	622 (25.83)	446 (71.70)
5 years and above	6,331 (63.22)	1436 (59.63)	1024 (71.31)

TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine

*Percentage of febrile cases that had blood cultures taken.

** N represents the fever presentations that met the study definition and not the individual participants. A participant may present one or more time and is not reflected in the data presented.

6.3 Results

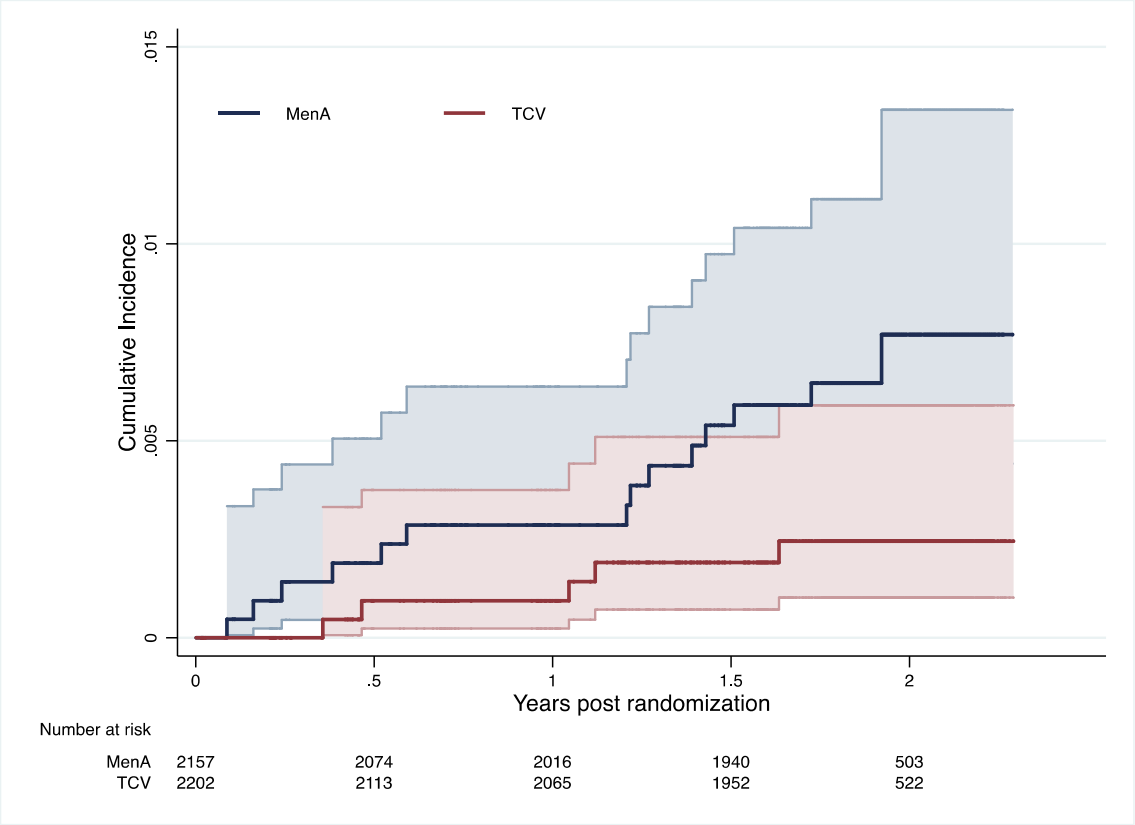
Table 19 details the age and sex breakdown of the participants according to vaccine groups. The proportion of participants in each group, the proportion of participants that

met the case definition for blood culture and the proportion that had blood taken for blood culture in the two vaccine groups were comparable.

6.3.1 Efficacy of TCV and Age

In total, 2 participants aged < 2 years, both in the TCV group; 19 participants aged 2 – 4 years (TCV=5 and MenA=14); and 53 participants aged 5 years and above had culture-positive typhoid fever (Table 20). The incidence of typhoid fever in the < 2 years in the TCV group was 167 (95% CI 20, 604) per 100,000 person-years. The protective efficacy in this youngest age group could not be computed. The incidence of typhoid fever in those aged 2 - 4 years in the MenA group was 362 (95% CI 198, 607) per 100,000 person-years and the incidence in the TCV group was 127 (95% CI 41, 296) per 100,000 person-years. The vaccine efficacy was 64.9% (95% CI 2.5%, 87.3%) in this age group. The incidence of typhoid fever in those 5 years and older in the MenA group was 369 (95% CI 272, 489) per 100,000 person-years and in the TCV group was 46 (95% CI 17, 100) per 100,000 person-years. The vaccine efficacy was 87.5% (95% CI 70.8%, 94.5%) in this age group. Vaccine efficacy was not significantly different by age group, when comparing those aged 2-4 years and those aged 5 and older (P value (interaction) = 0.127).

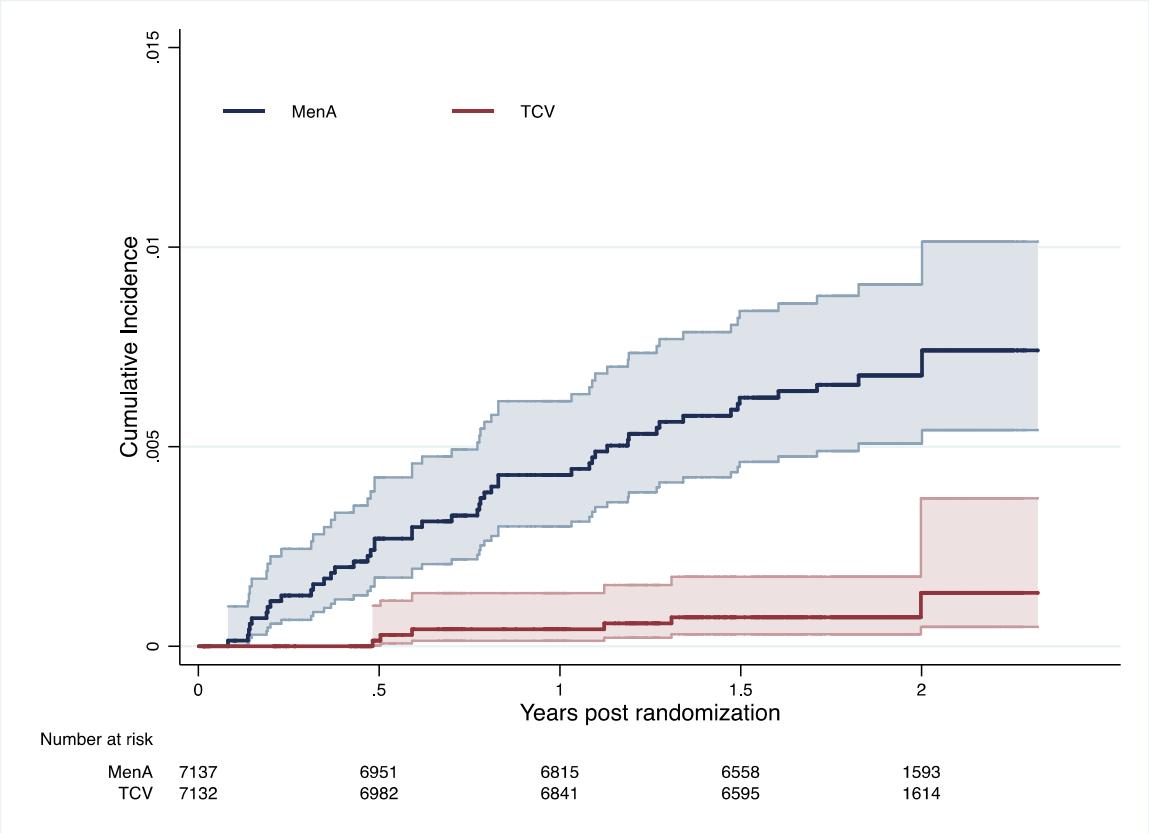
Figure 15. Kaplan-Meier Estimates of the Cumulative Incidence of Blood Culture Confirmed Typhoid Fever in 2 – 4 – Year Age Group, According to Trial Group.



TCV = Typhoid Conjugate Vaccine. MenA = group A meningococcal vaccine (control).

Figures 15 and 16 show the Kaplan-Meier estimates of the cumulative incidence of blood culture confirmed typhoid fever along with the 95% confidence interval according to the age categories. The curves progressively diverge in the 2-4-year age group but confidence intervals for the two vaccine groups overlap due to the small number of cases available for analysis. The curves, along with the confidence intervals, show progressive and substantial divergence starting early on in the trial period, and there is no overlap of the curves in the 5 years and above age group due to the larger sample size.

Figure 16. Kaplan-Meier Estimates of the Cumulative Incidence of Blood Culture Confirmed Typhoid Fever in Above 5 – Year Age Group, According to Trial Group.



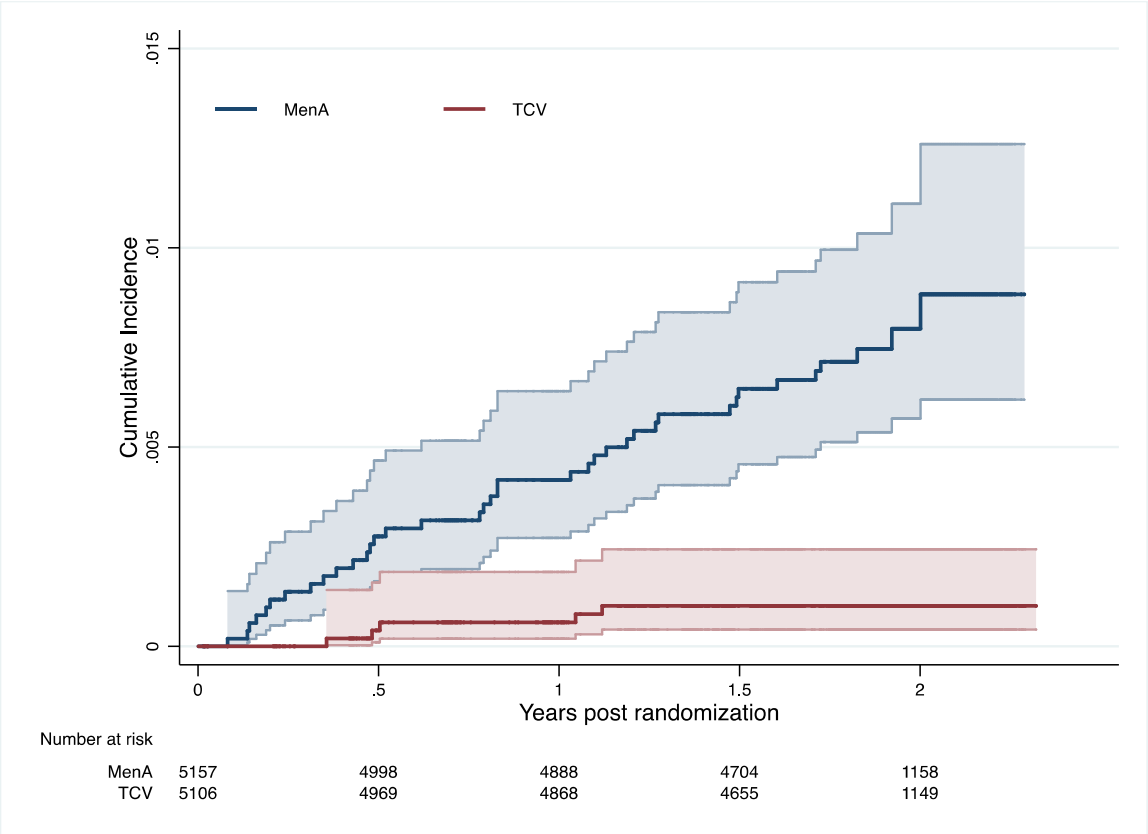
TCV = Typhoid Conjugate Vaccine. MenA = group A meningococcal vaccine (control).

6.3.2 Efficacy of TCV and Sex

Of the total 75 culture-positive typhoid fever cases, 44/75 (58.7%) occurred in male participants (Table 20). Among the male participants, there were 39 cases in the MenA group with an incidence of 417 (297, 570) per 100,000 person-years and 5 cases in the TCV group with an incidence of 54 (18, 126) per 100,000 person-years. The vaccine efficacy was 87.0% (67.1%, 94.9%). Among the female participants, there were 23 cases in the MenA group with an incidence of 262 (166, 392) per 100,000 person-years and 8 cases in the TCV group with an incidence of 90 (39, 180) per 100,000 person-years. The

vaccine efficacy was 65.6% (23.1%, 84.6%). Vaccine efficacy did not significantly differ by sex (P (interaction) = 0.120).

Figure 17. Kaplan-Meier Estimates of the Cumulative Incidence of Blood Culture Confirmed Typhoid Fever in Males, According to Trial Group.

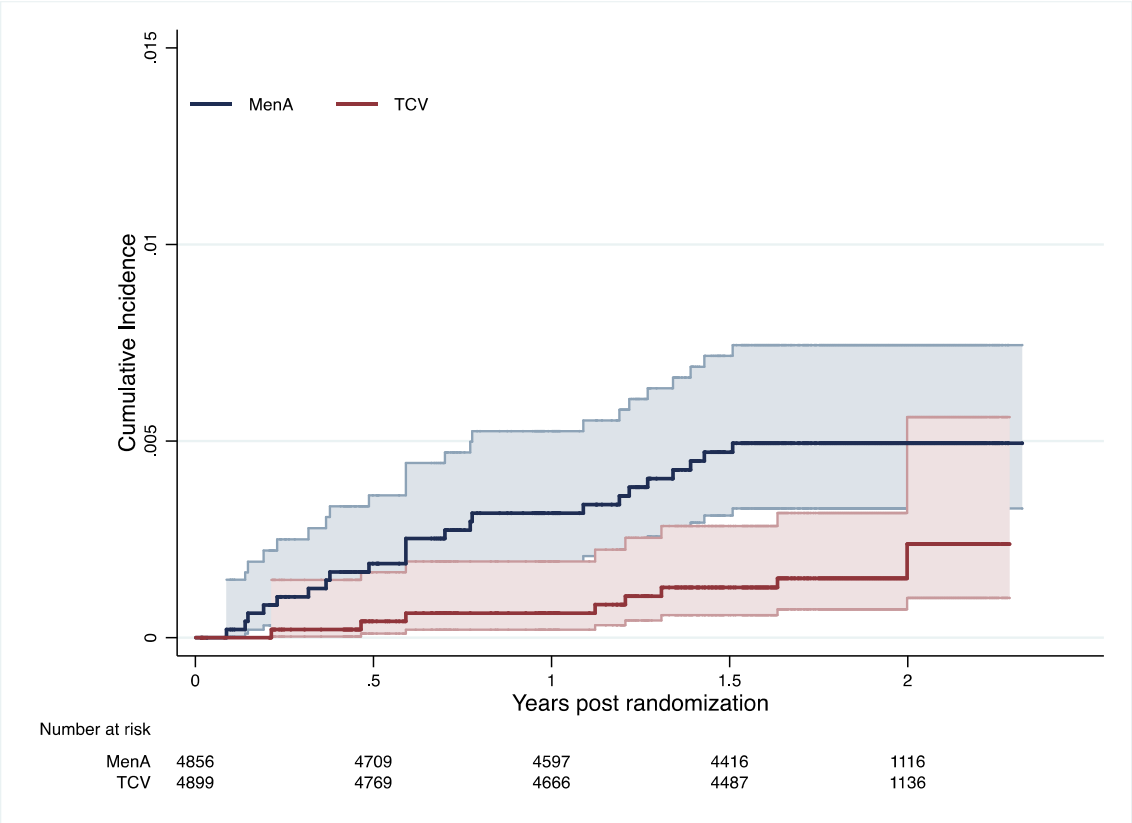


TCV = Typhoid Conjugate Vaccine. MenA = group A meningococcal vaccine (control).

Figures 17 and 18 show the Kaplan-Meier estimates of the cumulative incidence of blood-culture confirmed typhoid fever along with the 95% confidence interval according to the sex. The curves, along with the confidence intervals, show progressive and substantial divergence starting early on in the trial period in males with no overlap

between the curves (Figure 17). The curves progressively diverge in females, with no overlap between the curves, but an overlap between the confidence intervals (Figure 18).

Figure 18. Kaplan-Meier Estimates of the Cumulative Incidence of Blood Culture Confirmed Typhoid Fever in Females, According to Trial Group.



TCV = Typhoid Conjugate Vaccine. MenA = group A meningococcal vaccine (control).

Table 20. Subgroup Comparison of Protective Efficacy of Typhoid Conjugate Vaccine (TCV).

Outcome	TCV (N=10005)	Incidence per 100,000 person-years (95% CI)	Men A vaccine (N=10014)	Incidence per 100,000 person-years (95% CI)	Vaccine Efficacy (95% CI)	P value (interaction)
Blood culture-confirmed typhoid fever after 14 days						
Age < 2 years	2	167 (20, 604)	0			
Age 2- 4 years	5	127 (41, 296)	14	362 (198, 607)	64.9% (2.5%, 87.3%)	0.127
Age 5 years and above	6	46 (17, 100)	47	369 (272, 489)	87.5% (70.8%, 94.5%)	
Male	5	54 (18, 126)	39	417 (297, 570)	87.0% (67.1%, 94.9%)	0.120
Female	8	90 (39, 180)	23	262 (166, 392)	65.6% (23.1%, 84.6%)	

TCV = Typhoid Conjugate Vaccine. MenA = group A meningococcal vaccine (control).

6.3.3 Immunogenicity and Age

Table 21 shows the breakdown of the number of participants randomized for a blood draw and the number of participants from whom blood samples were drawn for immunogenicity at each visit.

Table 21, figure 19 – 22 shows the Anti-Vi IgA and IgG responses according to age group. At baseline, 1.82% (N=1/55), 0.00% (N=0/225), and 13.65% (N=55/403) of those less than 5 years, 5-<10 years and 10 years and above respectively had detectable anti VI-IgA titres in the TCV group. By day 28, in the TCV group, 95% (N=52/55), 98% (N=221/225), and 97% (N=391/403) participants had seroconverted to Vi IgA (for those less than 5 years, 5-<10 years and 10 years and above respectively), while by 18 months these percentages had dropped to 60% (N=25/42), 88% (N=160/182) and 93% (N=294/315) respectively. The geometric mean concentration and fold increase in the geometric mean concentration were significantly different across the three age categories. The geometric mean titre increase by 47.44 (95% CI 34.38 – 60.50), 110.84 (95% CI 97.17 – 124.51), and 135.43 (95% CI 121.35 – 149.51) fold from baseline to 28 days post-vaccination in less than 5 years, 5-<10 years and 10 years and above respectively (P<0.001). The fold increase from baseline decreased to 6.22 (95% CI 4.30 – 8.13), 21.04 (95% CI 17.55 – 24.55) and 34.07 (95% CI 29.58 – 38.55) at 18 months post -vaccination in less than 5 years, 5-<10 years and 10 years and above respectively (P<0.001). In the MenA group, the geometric mean IgA concentration across the three age groups remained consistently low, with significantly different titres between the age groups and higher titres in the oldest age groups.

Table 21. Anti-Vi IgA and IgG Levels at Baseline, 28 Days, and 18 Months after Randomization in the Immunogenicity Cohort, by Age Group.

Trial Group	Day 0	Day 28	Month 18	Period from Day 0 – Day 28	Period from Day 0 – Month 18	Day 0	Day 28	Month 18	Period from Day 0 – Day 28	Period from Day 0 – Month 18	Day 0	Day 28	Month 18	Period from Day 0 – Day 28	Period from Day 0 – Month 18	P-value†
Age Category	Less than 5 years					5 – <10 years					10 years and above					
				4-fold rise	4-fold rise				4-fold rise	4-fold rise				4-fold rise	4-fold rise	
Anti-Vi IgA Level																
TCV																
Level above lower limit of quantification of the assay – no. of participants / total no. (%)*	1/55 (1.82)	52/55 (94.55)	56/76 (73.68)	52/55 (94.55)	25/42 (59.52)	0/225 (0.00)	221/225 (98.22)	209/220 (95.00)	221/225 (98.22)	160/182 (87.91)	55/403 (13.65)	397/403 (98.51)	336/343 (97.96)	391/403 (97.02)	294/315 (93.33)	
Geometric mean concentration (95% CI) – EU/ml	1.66 (1.47– 1.89)	46.77 (34.54 – 63.33)	6.02 (4.83 – 7.51)	47.44 (34.38 – 60.50) fold increase	6.22 (4.30 – 8.13) fold increase	1.56 (1.56 – 1.56)	112.0 (97.19 – 129 .08)	18.20 (15.81 – 20.95)	110.84 (97.17 – 124.51) fold increase	21.04 (17.55- 24.55) fold increase	1.96 (1.84 – 2.09)	145.92 (130.45 – 163.23)	35.56 (31.67 – 39.93)	135.43 (121.35 – 149.51) fold increase	34.07 (29.58 – 38.55) fold increase	D0<0.001 D28<0.001 M18<0.001 D0-D28

																< 0.001 D0-M18 <0.001
Median (IQR)	1.56 (1.56 – 1.56)	55.17 (28.11 – 87.87)	7.35 (1.56 – 12.52)			1.56 (1.56 – 1.56)	132.08 (66.34 – 219.73)	19.89 (9.25 – 39.92)			1.56 (1.56 – 1.56)	166.87 (80.07 – 327.66)	36.58 (17.57 – 74.60)			
MenA vaccine																
Level above lower limit of quantification of the assay – no. of participants / total no. (%)*	1/46 (2.17)	2/46 (4.35)	2/57 (3.51)	0/46 (0.00)	1/35 (2.86)	3/116 (2.59)	8/116 (6.90)	6/110 (5.45)	3/116 (2.59)	2/93 (2.15)	21/218 (9.63)	28/218 (12.84)	33/191 (17.28)	3/218 (1.38)	3/171 (1.75)	
Geometric mean concentration (95% CI) – EU/ml	1.61 (1.51 – 1.72)	1.65 (1.51 – 1.79)	1.65 (1.51 – 1.81)	1.03 (0.98 – 1.09) fold increase	1.32 (0.75- 1.90) fold increase	1.64 (1.55 – 1.74)	1.84 (1.62 – 2.10)	1.70 (1.58 – 1.85)	2.59 (0.73 – 4.45) fold increase	1.26 (0.90- 1.62) fold increase	1.83 (1.70 – 1.97)	1.93 (1.77 – 2.11)	2.02 (1.84 – 2.21)	1.52 (0.79 – 2.25) fold increase	1.25 (1.08 – 1.44) fold increase	D0=0.0225 D28=0.1002 M18=0.0012 D0-D28 =0.8942 D0-M18 =0.1865

Median (IQR)	1.56 (1.56 – 1.56)	1.56 (1.56 – 1.56)	1.56 (1.56 – 1.56			1.56 (1.56 – 1.56)	1.56 (1.56 – 1.56)	1.56 (1.56 – 1.56			1.56 (1.56 – 1.56)	1.56 (1.56 – 1.56)	1.56 (1.56 – 1.56			
Anti-Vi IgG Level																
TCV																
Level above lower limit of quantification of the assay – no. of participants / total no. (%)**	8/99 (8.08)	66/67 (98.51)	75/76 (98.68)	54/55 (98.18)	55/58 (94.83)	44/288 (15.28)	234/234 (100.00)	216/220 (98.18)	225/225 (100.00)	202/209 (96.65)	216/462 (46.75)	408/408 (100.00)	342/343 (99.71)	398/403 (98.76)	316/334 (94.61)	
Geometric mean concentration (95% CI) – EU/ml	4.45 (3.92 – 5.06)	1488.31 (1160.83 - 1908.17)	81.00 (67.23 – 97.58)	525.20 (403.81 – 646.58) fold increase	28.65 (22.63 – 34.68) fold increase	4.71 (4.38 – 5.08)	2093.13 (1878.03 – 2332.87)	182.29 (156.74 – 212.00)	619.83 (556.98 – 682.67) fold increase	69.39 (58.37 – 80.41) fold increase	10.42 (9.26 – 11.70)	2113.18 (1929.68 – 2314.18)	367.87 (328.92 – 411.43)	431.94 (382.09 – 481.79) fold increase	72.87 (62.50 – 83.25) fold increase	D0 < 0.001 D28=0.0073 M18 < 0.001 D0-D28 < 0.001 D0-M18 <0.001
Median (IQR)	3.7 (3.7 – 3.7)	1619.37 (1018.63 –	89.95 (48.73 – 135.29)			3.7 (3.7 – 3.7)	2227.37 (1353.86 – 3752.75)	173.55 (92.34 – 408.76)			3.7 (3.7 – 27.72)	2342.88 (1327.70 – 3902.43)	382.20 (161.97 – 793.64)			

		2766.08)														
MenA vaccine																
Level above lower limit of quantification of the assay – no. of participants / total no. (%)**	7/66 (10.61)	6/49 (12.24)	6/57 (10.53)	0/46 (0.00)	1/47 (2.13)	18/144 (12.50)	19/121 (15.70)	11/110 (10.00)	5/116 (4.31)	3/98 (3.06)	97/250 (38.80)	87/218 (39.91)	79/191 (41.36)	3/218 (1.38)	6/186 (3.23)	
Geometric mean concentration (95% CI) – EU/ml	4.64 (3.88 – 5.57)	4.45 (3.82 – 5.19)	4.51 (3.75 – 5.43)	1.00 (0.90 – 1.10) fold increase	2.60 (-0.62 – 5.83) fold increase	4.50 (4.09 – 4.98)	5.42 (4.37 – 6.71)	4.70 (4.03 – 5.48)	15.54 (-6.01 – 37.09) fold increase	1.64 (0.87 – 2.45) fold increase	8.73 (7.51-10.15)	8.88 (7.54 – 10.47)	8.90 (7.49 – 10.59)	1.27 (0.91 – 1.64) fold increase	1.19 (0.93 – 1.45) fold increase	D0 < 0.001 D28 < 0.001 M18 < 0.001 D0-D28= 0.0572 D0-M18= 0.1461
Median (IQR)	3.7 (3.7 – 3.7)	3.7 (3.7 – 3.7)	3.7 (3.7 – 3.7)			3.7 (3.7 – 3.7)	3.7 (3.7 – 3.7)	3.7 (3.7 – 3.7)			3.7 (3.7 – 22.68)	3.7 (3.7 – 22.68)	3.7 (3.7 – 21.26)			

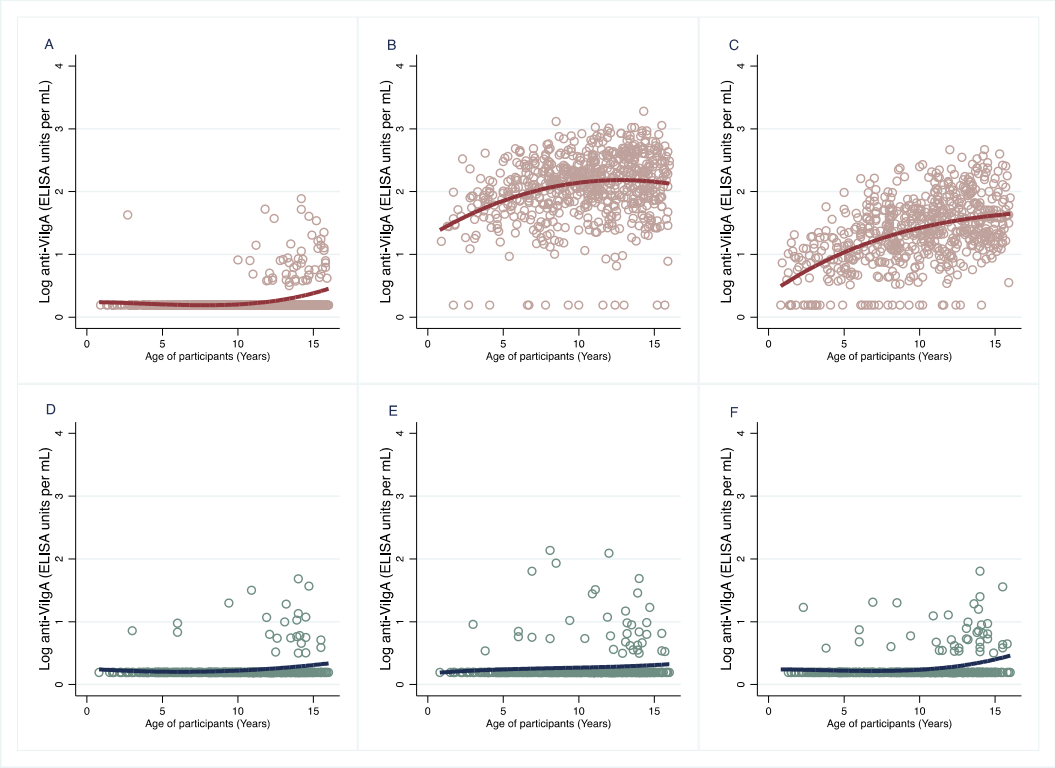
TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine

† Kruskal Wallis test

*The lower limit of quantification was 3.125 EU per millilitre. Values below this limit were substituted with 1.56 EU per millilitre for the analysis.

**The lower limit of quantification was 7.4 EU per millilitre. Values below this limit were substituted with 3.7 EU per millilitre for the analysis.

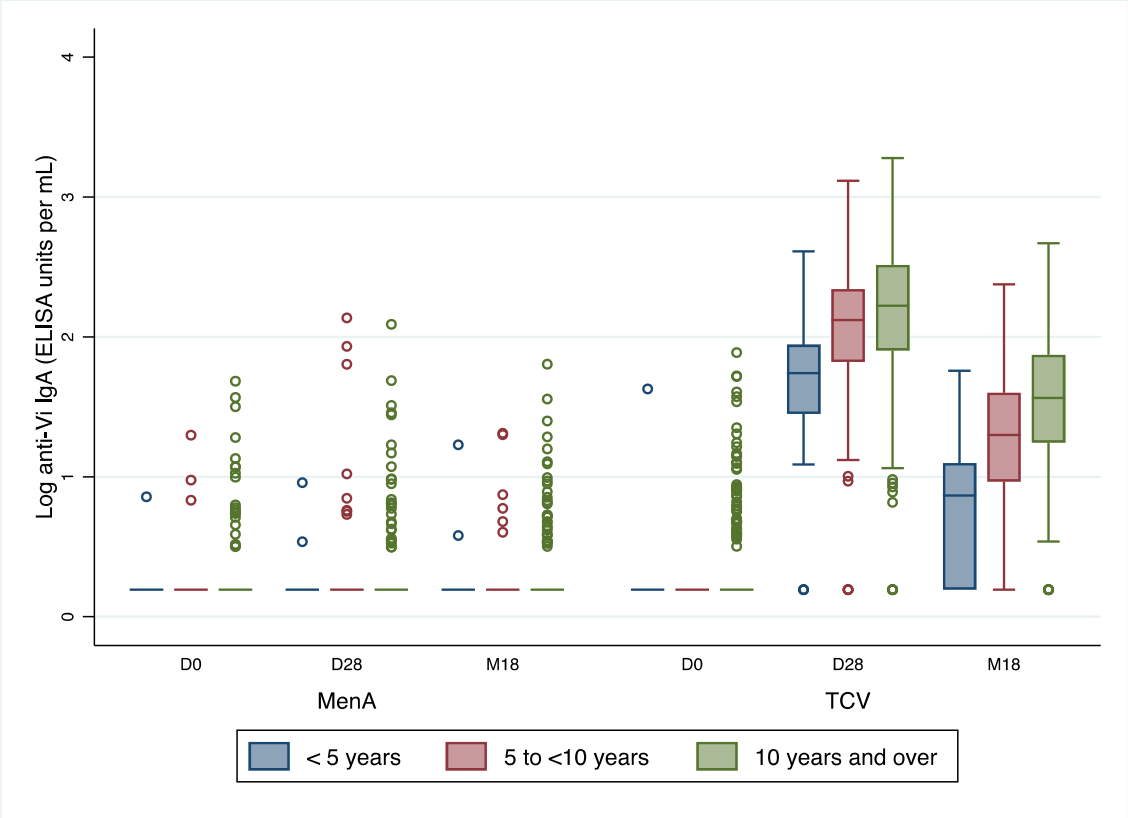
Figure 19. Anti Vi-IgA Response by Age, According to Trial Group



A: Anti-Vi IgA response at Day 0 in the TCV group. B: Anti-Vi IgA response at Day 28 in the TCV group. C: Anti-Vi IgA response at 18 months in the TCV group. D: Anti-Vi IgA response at Day 0 in the MenA group. E: Anti-Vi IgA response at Day 28 in the MenA group. F: Anti-Vi IgA response at 18 months in the MenA group.

Each circle represents the participant's observed anti Vi-IgA titre. The solid line is a fitted smooth curve for the observed data points.

Figure 20. Boxplot of Anti-Vi IgA Response at Baseline, Day 28- and 18-Months Post-Vaccination, According to Age Category.

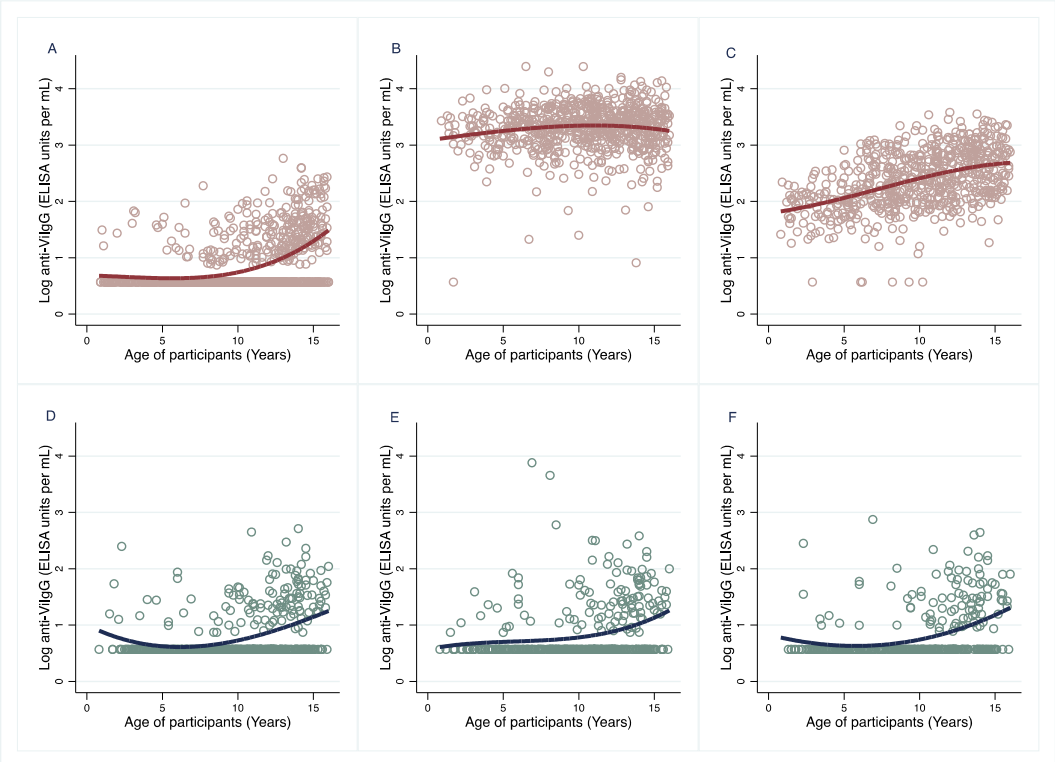


TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine
The lower limit of quantification for IgA was 3.125 EU per millilitre. Values below this limit were substituted with 1.56 EU per millilitre for the analysis.

Similarly, at baseline, 8.08% (N=8/99), 15.28% (44/288), and 46.75% (216/462) of those less than 5 years, 5-<10 years and 10 years and above respectively had detectable anti VI-IgG titres in the TCV group. The proportion with Vi-IgG seroconversion at day 28 was 98% (N=54/55), 100% (N=225/225), and 99% (N=343/343) participants in the TCV group for those less than 5 years, 5-<10 years and 10 years and above respectively, and remained high at 95% (55/58), 97% (N=202/209), and 95% (N=316/334) by 18 months. The geometric mean titre increase by 525.20 (95% CI 403.81 – 646.58), 619.83 (95% CI 556.98 – 682.67), and 431.94 (95% CI 382.69 – 481.79) fold from baseline to 28 days post-

vaccination in less than 5 years, 5-<10 years and 10 years and above respectively (P<0.001). The fold increase from baseline decreased to 28.65 (95% CI 22.63 – 34.68), 69.39 (95% CI 58.37 – 80.41) and 72.877 (95% CI 62.50 – 83.25) at 18 months post - vaccination in less than 5 years, 5-<10 years and 10 years and above respectively (P<0.001). In the MenA group, the geometric mean IgG concentration across the three age groups remained consistently low, although with higher titres in the oldest age groups.

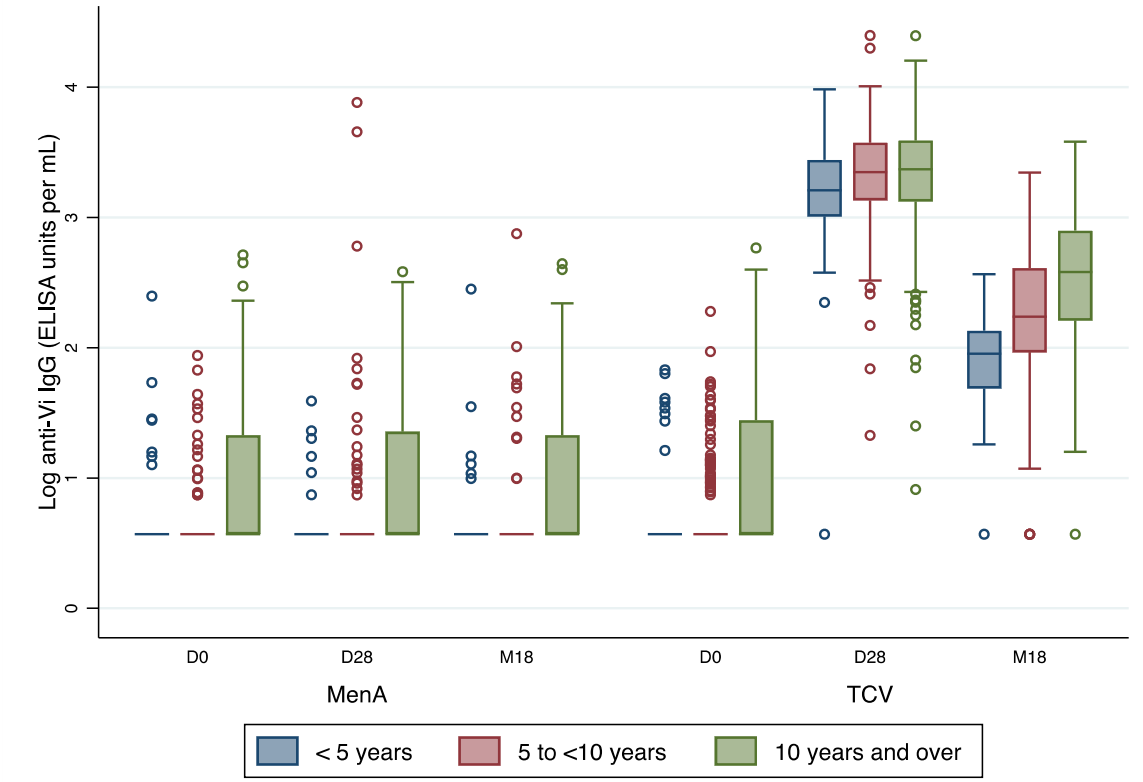
Figure 21. Anti Vi-IgG Response by Age, According to Trial Group



A: Anti-Vi IgG response at Day 0 in the TCV group. B: Anti-Vi IgG response at Day 28 in the TCV group. C: Anti-Vi IgG response at 18 months in the TCV group. D: Anti-Vi IgG response at Day 0 in the MenA group. E: Anti-Vi IgG response at Day 28 in the MenA group. F: Anti-Vi IgG response at 18 months in the MenA group.

Each circle represents the participant's observed anti Vi-IgG titre. The solid line is a fitted smooth curve for the observed data points.

Figure 22. Boxplot of Anti-Vi IgG Response at Baseline, Day 28- and 18-Months Post-Vaccination, According to Age Category.



TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine
The lower limit of quantification for IgG was 7.4 EU per millilitre. Values below this limit were substituted with 3.7 EU per millilitre for the analysis.

Table 22. shows the anti-Vi IgA and IgG responses in the under-5-year age group subdivided into under-2 and 2 to 4 years. At baseline, 0.00% (N=0/8) of those less than 2 years and 2.13% (1/47) 2 - 4 years had detectable anti VI-IgA titres in the TCV group. By day 28, in the TCV group, 87.50% (N=7/8) participants less than 2 years and 95.74% (N=45/47) participants 2 - 4 years had seroconverted to Vi IgA, and by 18 months these percentages had dropped to 57.89% (N=11/19) in the less than 2 years and 78.95% (N=45/57) in the 2-4 years. The geometric mean concentration was significantly higher in

the 2 – 4-year olds at 28 days and 18 months post-vaccination. The fold increase from baseline to day 28 was significantly higher in the 2 -4-year olds while no differences were seen at 18 months. In the MenA group, the geometric mean IgA concentration remained consistently low in both age groups.

Similarly, at baseline, 8.33% (N=2/24) of those less than 2 years and 8.00% (55.58) of those 2 - 4 years had detectable anti VI-IgG titres in the TCV group. By day 28, in the TCV group, 90.00% (N=9/10) participants less than 2 years and 100% (N=57/57) participants 2 - 4 years had seroconverted to Vi IgG, and by 18 months these percentages remained high at 100% (N=19/19) in the less than 2 years and 100% (N=57/57) in the 2-4 years. There were no differences between the two age groups in the mean geometric concentration or the fold increase. In the MenA group, the geometric mean IgG concentration remained consistently low in both age groups.

Table 22. Anti-Vi IgA and IgG Levels at Baseline, 28 Days, and 18 Months after Randomization in the Immunogenicity Cohort in the under-5-year age group.

Trial Group	Day 0	Day 28	Month 18	Period from Day 0 – Day 28	Period from Day 0 – Month 18	Day 0	Day 28	Month 18	Period from Day 0 – Day 28	Period from Day 0 – Month 18	P-value
Age Category	Less than 2 years					2 -4 years					
				4-fold rise	**4-fold rise**				**4-fold rise**	**4-fold rise**	
Anti-Vi IgA Level											
TCV											
Level above lower limit of quantification of the assay – no. of participants/ total no. (%)*	0/8 (0.00)	7/8 (87.50)	11/19 (57.89))	7/8 (87.50)	4/7 (57.14)	1/47 (2.13)	45/47 (95.74)	45/57 (78.95)	45/47 (95.74)	21/35 (60.00)	
Geometric mean concentration (95% CI) – EU/ml	1.56 (1.56 – 1.56)	22.38 (8.28 – 60.48)	4.04 (2.59 – 6.31)	21.48 (6.34 – 36.61) fold increase	4.96 (1.11 – 8.81) fold increase	1.67 (1.45 – 1.92)	53.02 (38.70 – 72.66)	6.88 (5.351 – 8.86)	51.86 (37.03 – 66.70) fold increase	6.47 (4.23-8.70) fold increase	D0=0.9239 D28=0.0248 M18=0.0438 D0-D28= 0.0256 D0-M18= 0.5776
Median (IQR)	1.56 (1.56 – 1.56)	28.62 (21.97 – 33.66)	3.94 (1.56 – 9.29)			1.56 (1.56 – 1.56)	63.88 (36.14 – 95.77)	8.17 (3.95 – 12.88)			

MenA vaccine										
Level above lower limit of quantification of the assay – no. of participants/ total no. (%)*	0/5 (0.00)	0/5 (0.00)	0/5 (0.00)	0/5 (0.00)	0/3 (0.00)	1/41 (2.44)	2/41 (4.88)	2/52 (3.85)	0/41 (0.00)	1/32 (3.12)	
Geometric mean concentration (95% CI) – EU/ml	1.56 (1.56 – 1.56)	1.56 (1.56 – 1.56)	1.56 (1.56 – 1.56)	1.00 (- - -) fold increase	1.00 (- - -) fold increase	1.62 (1.50 – 1.75)	1.66 (1.51 – 1.82)	1.66 (1.51 – 1.83)	1.03 (0.98 – 1.10) fold increase	1.35 (0.72-1.98) fold increase	D0=0.9297 D28=0.8599 M18=0.8878 D0-D28= 0.8599 D0-M18= 0.8597
Median (IQR)	1.56 (1.56 – 1.56)	1.56 (1.56 – 1.56)	1.56 (1.56 – 1.56)			1.56 (1.56 – 1.56)	1.56 (1.56 – 1.56)	1.56 (1.56 – 1.56)			
Anti-Vi IgG Level										
TCV										
Level above lower limit of quantification of the assay – no. of participants/ total no. (%)**	2/24 (8.33)	9/10 (90.00)	19/19 (100.00)	7/8 (87.50)	12/12 (100.00)	55/58 (8.00)	57/57 (100.00)	56/57 (98.25)	47/47 (100.00)	43/46 (93.48)	
Geometric mean concentration (95% CI) – EU/ml	4.30 (3.45– 5.35)	1074.78 (252.59 - 4573.23)	77.15 (55.02 – 108.18)	494.14 (262.51 – 725.77) fold increase	25.64 (14.99 – 36.29) fold increase	4.50 (3.85 – 5.26)	1575.77 (1296.04 – 1915.89)	82.32 (65.61 – 103.29)	530.48 (391.27 – 669.69) fold increase	29.44 (22.21 – 36.67) fold increase	D0=0.9870 D28=0.5380 M18=0.6356

											D0 – D28= 0.7203 D0 – M18= 0.7881
Median (IQR)	3.7 (3.7 – 3.7)	1906.73 (1470.48 – 2702.10)	88.68 (49.52– 127.98)			3.7 (3.7 – 3.7)	1557.62 (982.98 – 2766.08)	91.21 (47.95 – 136.98)			
MenA vaccine											
Level above lower limit of quantification of the assay – no. of participants/ total no. (%)**	2/5 (40.00)	1/6 (16.67)	0/5 (0.00)	0/5 (0.00)	0/3 (0.00)	5/61 (8.20)	5/43 (11.63)	6/52 (11.54)	0/41 (0.00)	1/44 (2.27)	
Geometric mean concentration (95% CI) – EU/ml	8.46 (1.88 – 38.16)	4.16 (3.08 – 5.60)	3.7 (3.7 – 3.7)	0.71 (0.18 – 1.23) fold increase	0.43 (-0.80 – 1.67) fold increase	4.42 (3.72-5.26)	4.49 (3.78 – 5.34)	4.60 (3.75 – 5.63)	1.04 (0.94 – 1.14) fold increase	2.75 (-0.70 – 6.20) fold increase	D0=0.2303 D28= 0.9029 M18=0.6722 D0 – D28= 0.1799 D0 – M18= 0.0709
Median (IQR)	3.7 (3.7 – 15.82)	3.7 (3.7 – 3.7)	3.7 (3.7 – 3.7)			3.7 (3.7 – 3.7)	3.7 (3.7 – 3.7)	3.7 (3.7 – 3.7)			

TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine

† Kruskal Wallis test

*The lower limit of quantification was 3.125 EU per millilitre. Values below this limit were substituted with 1.56 EU per millilitre for the analysis.

**The lower limit of quantification was 7.4 EU per millilitre. Values below this limit were substituted with 3.7 EU per millilitre for the analysis.

Table 23. Anti-Vi IgA and IgG Levels at Baseline, 28 Days and 18 Months after Randomization in the Immunogenicity Cohort, by Sex.

Trial Group	Day 0	Day 28	18 months	Period from Day 0 – Day 28	Period from Day 0 – 18 months	Day 0	Day 28	18 months	Period from Day 0 – Day 28	Period from Day 0 – 18 months	P-value†
Sex	Male					Female					
				4-fold rise	**4-fold rise**				**4-fold rise**	**4-fold rise**	
Anti-Vi IgA Level											
TCV											
Level above lower limit of quantification of the assay – no. of participants/ total no. (%)*	25/356 (7.02)	349/356 (98.03)	311/336 (92.56)	348/356 (97.75)	242/277 (87.36)	31/327 (9.48)	321/327 (98.17)	290/303 (95.71)	316/327 (96.64)	237/262 (90.46)	
Geometric mean concentration (95% CI) – EU/ml	1.73 (1.66 – 1.81)	112.62 (99.72 – 127.19)	19.70 (17.30 – 22.43)	113.38 (101.27 – 124.49) fold increase	25.20 (21.28 – 29.12) fold increase	1.87 (1.74 – 1.99)	133.18 (117.49 – 150.97)	26.96 (23.56 – 30.86)	127.72 (112.43 – 143.01) fold increase	29.93 (25.46 – 34.40) fold increase	D0=0.0.2098 D28=0.0611 M18=0.0016 D0-D28= 0.2516 D0-M18= 0.0331
Median (IQR)	1.56 (1.56 – 1.56)	130.69 (63.06 – 263.66)	20.63 (9.38 – 45.19)			1.56 (1.56 – 1.56)	148.04 (72.13 – 278.13)	27.14 (12.67 – 57.30)			
MenA vaccine											
Level above lower limit of quantification of the assay – no. of	9/192 (4.69)	16/192 (8.33)	15/181 (8.29)	2/192 (1.04)	4/151 (2.65)	16/188 (8.51)	22/188 (11.70)	26/177 (14.69)	4/188 (2.13)	2/148 (1.35)	

participants/ total no. (%)*											
Geometric mean concentration (95% CI) – EU/ml	1.67 (1.59 – 1.74)	1.74 (1.64 – 1.85)	1.79 (1.66 – 1.93)	1.28 (0.86 – 1.69) fold increase	1.30 (1.05 – 1.55) fold increase	1.83 (1.68 – 1.98)	2.01 (1.79 – 2.25)	1.92 (1.77 – 2.09)	2.31 (0.95 – 3.66) fold increase	1.23 (1.12 – 1.45) fold increase	D0=0.1243 D28=0.2117 M18=0.0676 D0-D28= 0.7385 D0-M18= 0.6716
Median (IQR)	1.56 (1.56 – 1.56)	1.56 (1.56 – 1.56)	1.56 (1.56 – 1.56)			1.56 (1.56 – 1.56)	1.56 (1.56 – 1.56)	1.56 (1.56 – 1.56)			
Anti-Vi IgG Level											
TCV											
Level above lower limit of quantification of the assay – no. of participants/ total no. (%)**	136/450 (30.22)	372/373 (99.73)	332/336 (98.81)	352/356 (98.88)	302/314 (96.18)	132/399 (33.08)	336/336 (100.00)	301/303 (99.34)	325/327 (99.39)	271/287 (94.43)	
Geometric mean concentration (95% CI) – EU/ml	7.08 (6.39 – 7.83)	1920.49 (1747.09 – 2111.10)	213.62 (188.23 – 242.43)	482.33 (431.03 – 533.63) fold increase	60.80 (52.26 – 69.35) fold increase	7.37 (6.59 – 8.23)	2176.67 (1976.24 – 2397.42)	276.19 (242.30 – 314.82)	522.04 (466.42 – 577.65) fold increase	74.60 (63.31 – 85.89) fold increase	D0=0.5106 D28=0.1021 M18=0.0080 D0 – D28= 0.3166 D0 – M18= 0.0305
Median (IQR)	3.7	2156.67	200.32			3.7	2311.84	267.72			

	(3.7 – 13.43)	(1269.61 – 3606.48)	(97.51 – 528.86)			(3.7 – 13.47)	(1327.70 – 3886.78)	(123.72 – 670.13)			
MenA vaccine											
Level above lower limit of quantification of the assay – no. of participants/ total no. (%)**	62/236 (26.27)	56/196 (28.57)	41/181 (22.65)	3/192 (1.56)	4/166 (2.41)	60/244 (26.79)	56/192 (29.17)	55/177 (31.07)	5/188 (2.66)	6/165 (3.64)	
Geometric mean concentration (95% CI) – EU/ml	6.18 (5.46 – 6.99)	6.50 (5.58 – 7.57)	6.01 (5.17 – 6.98)	2.56 (-0.14 – 5.26) fold increase	1.29 (0.89 – 1.69) fold increase	6.82 (5.88 – 7.90)	7.50 (6.25 – 9.00)	7.19 (6.08 – 8.51)	8.69 (-4.30 – 21.69) fold increase	1.76 (0.79 – 2.73) fold increase	D0=0.6142 D28= 0.5547 M18=0.0736 D0 – D28= 0.8198 D0 – M18= 0.8458
Median (IQR)	3.7 (3.7 – 8.53)	3.7 (3.7 – 9.47)	3.7 (3.7 – 3.7)			3.7 (3.7 – 9.41)	3.7 (3.7 – 14.12)	3.7 (3.7 – 11.91)			

TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine

† Mann-Whitney U test

*The lower limit of quantification was 3.125 EU per millilitre. Values below this limit were substituted with 1.56 EU per millilitre for the analysis.

**The lower limit of quantification was 7.4 EU per millilitre. Values below this limit were substituted with 3.7 EU per millilitre for the analysis.

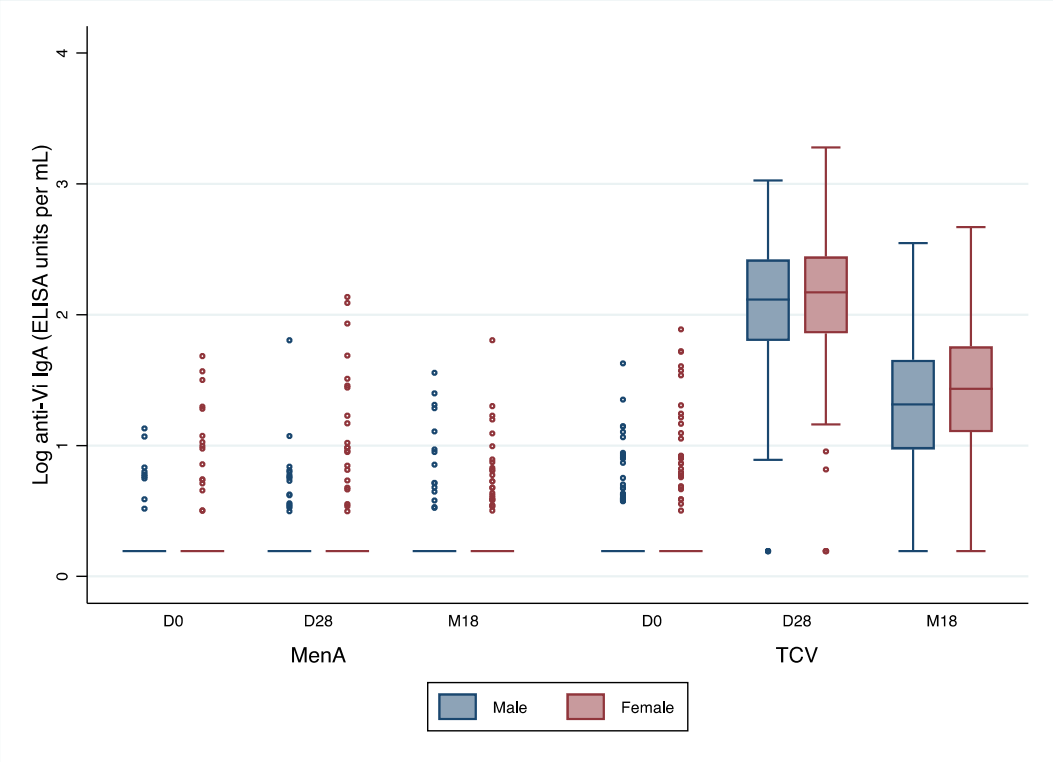
6.3.4 Immunogenicity and Sex

Table 23, figure 24 – 25 shows the Anti-Vi IgA and IgG responses according to sex. At baseline, 7.02% (N=25/356) males and 9.48% (N=31/327) females had detectable anti VI-IgA titres in the TCV group. By day 28, in the TCV group, 97.75% (N=348/356) males and 96.64% (N=316/327) female had seroconverted to Vi IgA. By 18 months, these percentages had decreased to 87.36% (N= 242/277) in males and 90.46% (N=237/262) in females. The geometric mean titre increase by 113.38 (95% CI 101.27 – 124.490)-fold from baseline to 28 days post-vaccination in males and 127.72 (95% CI 112.43 – 143.01)-fold in females (P<0.2516). The fold increase from baseline decreased to 25.20 (95% CI 21.28 – 29.12)-fold in males and 29.93 (95% CI 25.46 – 34.40)-fold at 18 months post - vaccination (P<0.0331). The geometric mean IgA concentration at 18 months was significantly higher is females (Males:19.70 (17.30 – 22.43) EU/mL, Females: 26.96 (23.56 – 30.86) EU/mL; P=0.0080). In the MenA group, the geometric mean IgA concentration remained consistently low in both sexes with no differences between the sexes.

Similarly, at baseline, 30.22% (N=136/450) males and 33.08% (N=132/399) females had detectable anti VI-IgG titres in the TCV group. By day 28, in the TCV group, 98.88% (N=352/356) males and 99.39% (N=325/327) female had seroconverted to Vi IgG. At 18 months, these percentages remained consistently high at 96.18% (N= 302/314) in males and 94.43% (N=271/287) in females. The geometric mean titre increase by 482.33 (95% CI 31.03 – 533.63)-fold from baseline to 28 days post-vaccination in males and 522.04 (95% CI 466.42 – 577.65)- fold in females (P<0.3166). The fold increase from baseline decreased to 60.80 (95% CI 52.26 – 69.35) -fold in males and 74.60 (95% CI 63.31 – 85.89)

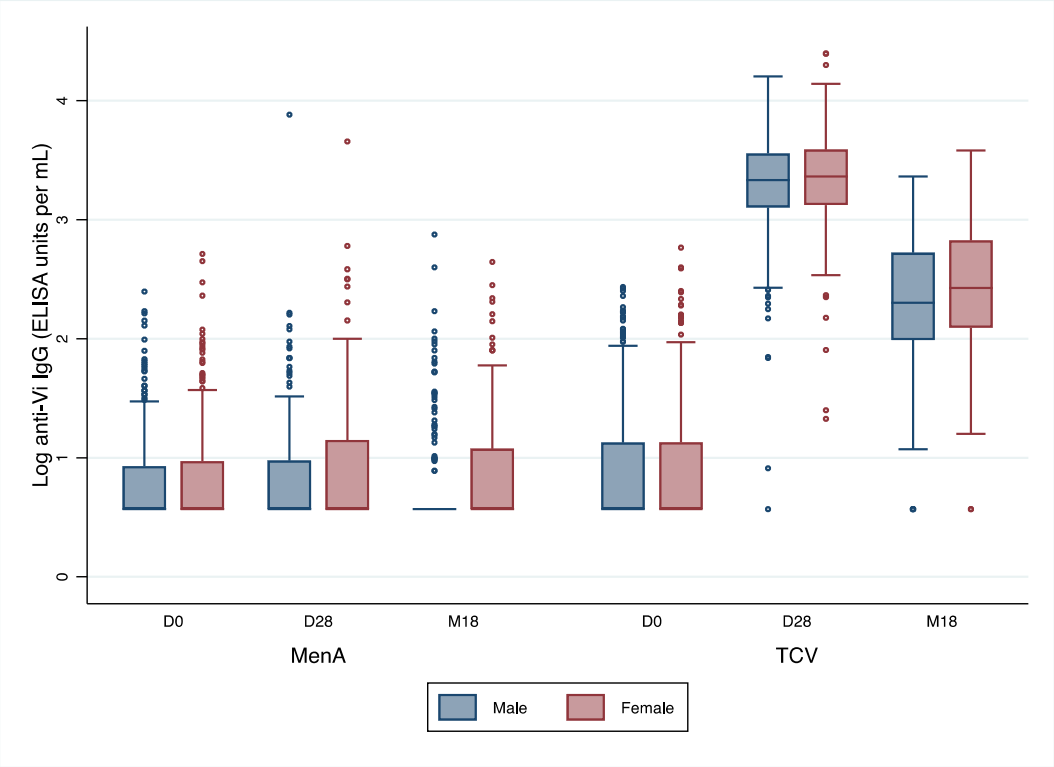
-fold at 18 months post -vaccination (P<0.0305). The geometric mean IgG concentration at 18 months was significantly higher is females (Males: 213.62 (95% CI 188.23 – 242.43) EU/mL, Females: 276.19 (95% CI 242.30 – 314.82) EU/mL; P=0.0080). In the MenA group, the geometric mean IgG concentration remained consistently low in both sexes with no differences between the sexes.

Figure 24. Boxplot of Anti-Vi IgA Response at Baseline, Day 28- and 18-Months Post-Vaccination, According to Sex.



TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine
The lower limit of quantification for IgA was 3.125 EU per millilitre. Values below this limit were substituted with 1.56 EU per millilitre for the analysis.

Figure 25. Boxplot of Anti-Vi IgG Response at Baseline, Day 28- and 18-Months Post-Vaccination, According to Sex.



TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine
The lower limit of quantification for IgG was 7.4 EU per millilitre. Values below this limit were substituted with 3.7 EU per millilitre for the analysis.

6.4 Discussion

6.4.1 Vaccine Efficacy, Immunogenic Response to TCV and Age

There was no evidence of a difference in vaccine efficacy in children 2 – 4 years and 5 years and above. In the youngest age group under-2-years, there were only two cases of culture-positive typhoid, which could either mean there is a low burden of typhoid fever in this age group or that the cases could not be captured in this age group.

The youngest age group represented 6.9% of the study cohort, so the study was underpowered to make efficacy and incidence estimates in this age group. Whilst it is plausible that the burden of typhoid fever is low in this age group, lack of data on typhoid occurrence in children under-2 years has been cited as a problem to estimate the true incidence in this age group[2]. Potential protection from maternal antibodies, possibility of less severe disease in children and lack of exposure to contaminated food and water may explain low typhoid incidence in infants and neonates[2]. However, there are systematic problems that could explain the low number of cases captured. There were a total of 1390 children under 2 years and there were 700 presentations meeting the enrollment criteria in this age group. Young children have multiple fever episodes over a period of time, and this is not reflected in the number of fever presentations documented in the study. In routine practice in our setting, symptomatic treatment of fever is given and blood investigations including cultures are not requested by pediatricians early in the illness. Parents/guardians also request medication/ symptomatic treatment and prefer to have blood cultures taken from their children only if the illness prolonged. This is more so for the youngest age group. Further, in the study area, the usage of alternative health care facilities including pharmacies, private hospitals and clinics is high. Underuse of blood cultures in infants and young children, difficulty in drawing adequate blood volumes for culture and higher risk of contamination in cultures in the young have all been cited causes for underestimation of typhoid in the youngest age group[2]. In an exploratory analysis of the first year fever presentation data of TyVAC-Nepal, age as a continuous variable was assessed as a predictor for having blood drawn for culture in those with eligible fevers presenting to study clinics[146]. Older children were significantly more likely to have blood collected for culture ($P<0.0001$). Blood samples were collected

in 78% of the fever presentations in children 10 years and above, while 65% of fever presentations in children less than 5 years had blood culture taken. The study also found that older children were significantly more likely to have a positive culture compared to the older children (P=0.0009).

The causes for underestimation of typhoid in the youngest age group mentioned above also holds true to some extent in the 2-4-year age group. In this age group, whilst the KM curve were divergent, the confidence intervals were wide and overlapping suggesting that the true incidence is likely to be higher. Overall, the 2-4-year age group represented 29.7% of the enrolled participants. It is also possible that the analysis had less statistical power in this age group due to the small sample size.

TyVAC-Bangladesh and TyVAC-Malawi also assessed the efficacy of TCV in children following the same criterions for blood culture[116]. In TyVAC-Bangladesh, 61,587 children aged 9 months to under-16 years were enrolled in a cluster randomized trial. The total vaccine efficacy was 81% (95% CI 39 to 94%; P=0.0052) in the under-2-year age group; 80% (95% CI 62 to 89%; P<0.0001) in the 2-4-year age group; and 88% (95% CI 78 to 93; P<0.0001) in the above-5-year age group. The vaccine efficacy in the 2-4 year and 5-year and above age group is consistent with the vaccine efficacy in the same age group in TyVAC-Nepal as demonstrated by similar efficacy estimates and overlapping confidence intervals. Further, TyVAC-Bangladesh was able to capture cases in the youngest age group (4 in the TCV group and 23 in the control group) and the finding show that TCV is

equally efficacious in the under-2-year age group. Bangladesh has a higher incidence of typhoid fever which could explain the higher number of cases captured across the age categories. Being a cluster-randomized trial, TyVAC-Bangladesh estimates the total protection which tend to be higher compared to the direct protection in individually randomized trials.

In TyVAC-Malawi, 28,130 children aged 9 months to 12 years were enrolled in an individually randomized trial. The vaccine efficacy of 74.7% (95% CI 31.8 – 90.4) was reported in the under-5-year age group (n(control)=20; n(TCV)=5) and a vaccine efficacy of 88.0% (95% CI 69.7 - 95.3%) in the 5 – 12-year age group (n(control)=42; n(TCV)=7) [132]. The study did not report efficacy of the vaccine separately in the under-2-year age group. Nonetheless, the study again showed that the vaccine was efficacious across the age groups and further supported the use of the vaccine in the all children 9 months and above, irrespective of the age. The vaccine efficacy in TyVAC-Nepal is much lower than reported in TyVAC-Malawi when combining the under-2-year age group and 2-4-year age group (VE= 49.9% (95% CI -24.0% – 79.8%)) and is likely an effect of the low numbers in the under-2-year-olds in Nepal.

High efficacy of TCV was also reported in the cohort study done in Hyderabad, Pakistan to assess the effectiveness of TCV against culture-confirmed typhoid fever in a setting of extensively drug-resistant typhoid fever outbreak[164]. The vaccine effectiveness was 94.5% (95% CI 91.5 – 96.6%) in the under-5-year age group and 95.2% (95% CI 92.9 –

97.0) in the 5 year and above age group. The vaccine effectiveness in much higher than that reported from Nepal, Bangladesh and Malawi. The study was conducted in the setting of an extensively drug-resistant outbreak. The authors reported that the attendance for blood cultures was higher among the unvaccinated compared with the vaccinated group. Whilst this could be a result of reduction of disease in the vaccinated, it could also be that the vaccinated felt more confident and were less likely to attend healthcare facilities. On the flip side, the unvaccinated could also be more likely to seek healthcare in the setting of the outbreak. The study may hence overestimate the vaccine efficacy for a typhoid endemic setting.

As discussed in Chapter 5 in the limitations section, it is not possible to correlate the high antibody response with protection from typhoid fever. However, the all-age efficacy and immunogenicity results corresponded to each other. In the age breakdown, while the efficacy in children above 2 years were not dissimilar, the immunological response, both IgA and IgG, are significantly different across the age categories in the TCV group with the highest mean titres and fold increase in children 10 years and over. In the MenA group, the antibody titres were also higher in the older children. The higher titres were more prominent for IgG than IgA. The higher titres in the older children in the MenA group likely reflects the naturally acquired antibody due to multiple exposure to typhoid fever over time. There is a higher force of infection in the older children and the differences seen in the IgG seems to reflect this. In the TCV group, the differences in the antibody titre across the age groups could be a result of a boosting response to the naturally acquired antibody.

Literature suggests that young infants have lower responses to vaccines than older children and adults[157]. There may be a presence of passively acquired antibody acquired via placental transfer of maternal antibodies to the infant which can interfere with the immune response in an infant. This is dependent of the concentration of the maternal antibody. However, the passively acquired antibody decays rapidly and by 6 months of age, only a third of the babies have a significant maternal antibody concentration[157]. In TyVAC-Nepal, children 9 months and older were vaccinated. It is unlikely that lower antibody response was a result of maternal antibody interference in this age group.

On the other hand, it is also possible that there are differences in the rate of decay of antibodies in the different ages, with a faster decay of antibodies in the youngest age group. It has been suggested that the bone marrow niche don't produce or express one or more survival factors required for supporting the survival of vaccine induced plasma cells in the youngest age[161]. In TyVAC-Nepal, it appears that the antibody titre, especially IgA decayed faster in children under-5 years. In the breakdown of the under-5 years, it seems that the IgA decay is faster in the under-2 years than in the 2-4 years. IgA has been suggested as a correlate of protection against typhoid fever[150]. If there is truly a faster decay of IgA antibodies in the youngest age group, it can be problematic in the programmatic implementation of the vaccine. Currently, the WHO has recommended the TCV as a single dose of vaccine, and countries have incorporated the vaccine at 9 months or 15 months in their immunization schedule. Children may need a booster dose for adequate protection against the disease. However, the number of children in the

youngest age group is very low to confirm the faster decay of antibodies. As a part of TyVAC-Nepal, the response to a booster dose of TCV is being studied in a small cohort of 100 children. Children received an initial dose of TCV at their 9- or 12-months vaccination visit to Patan Hospital, followed by a booster dose given at 15 months. Blood samples are being collected at baseline, at one month after the initial vaccination, at 15 months visit and at one month after the 15-month visit. Anti-Vi IgG and anti-Vi IgA antibodies will be measured, and the results can provide early insights into whether a booster dose may indeed be needed in the youngest age group.

The results from TyVAC-Nepal are similar to that of the pre-licensure double blinded RCT conducted by Bharat Biotech in 2 - 45 year age group, 97.3% (95% CI 94.8 – 96.6) of the participants in the TCV group had a IgG seroconversion 42 days post-vaccination and the four-fold rise in titre from baseline was maintained in 74.1% (95% CI 68.2 – 79.2) of the participants at 24 months post-vaccination[97]. In the 2- 4-year age group the IgG seroconversion at 42 days and 2 years post-vaccination was 99.0% (95% CI 94.0 – 99.9) and 76.8% (95% CI 64.1 – 86.0) respectively. Similarly, the IgG seroconversion in the 5 – 15-year age group was 99.3% (95% CI 95.8 – 99.9) at 42 days and 74.6% (95% CI 65.8 – 81.7) at 24 months post-vaccination. Similarly, in the open label trial conducted in the 6 – 23 months alongside the RCT, the study reported a 98.1% (95% CI 95.7 – 99.2) seroconversion at day 42 which dropped to 59.5% (95% CI 52.9 – 65.8) at year 2, which looked comparable when the age groups were divided in to 6 – 11 months and 12 – 23 months. Anti-Vi IgA was not measured.

TyVAC-Bangladesh, however, has reported a robust IgG response across all age groups[116]. The burden of typhoid fever in Bangladesh, including in children under-5 years, is reportedly much higher and the consistently good response across the age categories may represent the anamnestic response to the background exposure to the disease. However, the number of participants in the under-2 years (N=195) and in the 2-4 years (N=561) is higher in TyVAC-Bangladesh and the results may represent a larger sample. IgA response has not been reported. Assuming IgG correlates to disease and IgA correlated to protection, it will be important to look at the IgA response in this population as well.

Looking into the data available for the recently WHO-prequalified TYPHIBEV (Vi-CRM197), age-breakdown of the immune response is available from the phase 2 trials conducted in south and southeast Asia[100]. The study had reported that one dose of Vi-CRM197 significantly increase the anti-Vi IgG titres one month after the first vaccination across all age groups (adults 18 – 45 years, children 24 – 59 months, older infants 9 – 13 months and infants 6 – 8 weeks). In-house ELISA at the Novartis Vaccines and Diagnostics Clinical Serology Department was used to measure the antibody titres, due to which it is difficult to compare the results with TyVAC-Nepal. The study has given two doses to children and older infants and three doses to infants. No published study results for Biological E's TYPHIBEV is available, but the manufacturers report 96.90%, 95.12% and 94.05% seroconversion rates in 6 months - <2 years, 2 - <18 years and 18 - <64 years respectively using WHO defined threshold of $\geq$ 4.3ug/mL for anti-Vi IgG antibody titres[153].

It will be important to monitor TYPHIBEV's effectiveness and immunogenicity as the vaccine is rolled out.

Although Vi-DT demonstrated a non-inferior seroconversion at one-month post-vaccination compared with TCV, some difference in the GMTs were noted. In this non-inferiority trial conducted across four sites in individuals aged 6 months to 45 years in Nepal, participants were randomly assigned to one of for groups[110]. Groups A-C received Vi-DT from one of three good manufacturing practice lots and group D received TCV. For immunogenicity analysis, blood samples were drawn at baseline, 28 days (4 weeks) post-vaccination and day 168 (24 week) post vaccination. Anti-Vi IgG was measured using an in-house assay. The seroconversion rates of both vaccine groups at 4 weeks post-vaccination, across the different age groups were comparable. The all-age anti-Vi IgG titre was lower in the TCV group compared with Vi-DT (GMT(Vi-DT): 384.22 IU/mL (95% CI 350.85, 420.76) vs GMT(TCV): 447.93 IU/mL (95% CI 423.76, 473.47); p=0.0053). In age breakdown, the anti-Vi IgG titre in adults appear significantly higher in the Vi-DT group compared with the TCV group (GMT(Vi-DT): 416.70 IU/mL [95% CI 370.27, 468.94] vs GMT(TCV): 290.98 IU/mL [95% CI 246.21, 341.11]; p=0.004). The anti-Vi IgG titre in children aged 6 months to under-2 years were lower in the TCV group, although the differences were not significant (GMT(Vi-DT): 505.39 IU/mL [95% CI 468.07,545.68] vs GMT(TCV): 421.74 IU/mL [95% CI 354.69, 501.45]; p=0.0663). The titres in the 2 to under-18 years age group were comparable (GMT(Vi-DT): 419.06 IU/mL [95% CI 384.66, 456.52] vs GMT(TCV): 450.74 IU/mL [95% CI 396.56, 12.33] p=0.3514). At 24 weeks post-vaccination, however, the titres was significantly lower in the Vi-DT vaccine group

compared with TCV group (GMT(Vi-DT); 88·07 IU/mL (95% CI 83·04–93·41) vs GMT(TCV): 99·31 IU/mL (95% CI 91·07–108·29); p=0·0168). The differences were a result of the significantly lower GMT in the 6 months – 2 years age groups, where the titre 24 weeks post-vaccination was 50·17 IU/mL (95% CI 47·07–53·48) in the Vi-DT vaccine groups A – C and 73·41 IU/mL (95% CI 65·52–82·26) in the TCV vaccine group (p<0·0001). The results suggest a possible difference in the rate of decay of antibodies between Vi-DT and TCV, with a faster decay of antibodies in the Vi-DT group. It may also be true that there were more sub-clinical infections in the TCV group due to having lower antibody to start with, and that those sub-clinical infections have boosted the antibody levels at 24 weeks, but cannot be determined without looking at individual data. Thus, despite promising results showing overall non-inferiority of Vi-DT, it will be important to further study the differences observed and determine longer term protection offered by Vi-DT.

The Disease of Most Impoverished Program looked into the immune response to the Vi - polysaccharide vaccine in children under-5-year-old and 5 to under-16-year old. At 28 days post-vaccination, there was a significantly higher GMT and fold titre of antibody compared to the control group in both age groups, although the titres and fold increase were higher in the 5 to under-16-year olds. However, at 2 years post-vaccination, the GMC and fold-increase were no longer significant for the under-5-year old. The pattern of higher titre in older children than younger children is comparable to that seen with TCV in TyVAC-Nepal. It is plausible that the pre-existing antibodies in response to prior natural exposure were boosted in response to the vaccine in the above 5-year-old.

6.4.2 Vaccine Efficacy, Immunogenic Response to TCV and Sex

TCV was efficacious in both males and females. The point estimate of vaccine efficacy in males was higher than in females. However, the confidence interval of the efficacy estimates overlaps and the p value for the interaction effect is not significantly different implying that the vaccine efficacy in males and females are not dissimilar. The efficacy of TCV by sex has not been reported in other studies to make comparisons.

In addition, the immune response in males and females were similar at baseline and at Day 28 suggesting there are no sex-related differences in the response to the vaccine. Significant differences were noted at 18 months post-vaccination. Both anti-Vi IgA and anti-Vi IgG mean geometric titre as well as the fold increase in titre were significantly higher in females suggesting that the antibody decay in females could be slower. Alternatively, it is possible that some female participants enrolled in the immunogenicity cohort were exposed to typhoid fever in between the two time periods resulting in increases in antibody titres. There were 12 males and 16 females who had higher IgA titre at 18 months than at day 28. There were 3 males and 4 females who had higher IgG titre at 18 months than at day 28. For both IgA and IgG, there were no longer differences in the geometric mean titre at 18 months after excluding participants who had higher titres at 18 months than at day 28 (P(IgA)=0.5462 and P(IgG)=0.7237) suggesting that the differences seen was an artefact, and that there are no true differences in the antibody decay in males and females.

Fewer cases of typhoid fever were reported in the females than in males in the control group. Assuming that the extrinsic and intrinsic factors that result in infection and disease are the same for both males and females, it would be expected that the number of cases would be similar in both sexes. The point estimate of incidence in females was hence lower in females than in males. The confidence interval for females was wider reflecting the lower number of cases captured. However, the confidence interval for the incidence estimates overlap suggesting that the incidence in not significantly different between males and females. Interestingly, the proportion of fever presentations in males was higher than in females, compared to the proportions enrolled, and the differences were significant (P(chi)=0.000). The differences seen could reflect the behavioral factors where by males are more likely to exposed to the pathogen in the environment. Literature also shows that males have a higher prevalence of infections in comparison to females[165]. However, it could be a gender influence by which health seeking behavior for males is higher than in females. There have been reports where male children are given priority over females including in use of health services[165]. Gender difference have also been noted in receipt of vaccines including in vaccine uptake in children, although not evident in TyVAC-Nepal[165].

6.5 Summary

Vaccine efficacy in children 2-4 years and 5 years and over, and by sex were similar. TCV was immunogenic in children 5 years and over. Immunogenic response in youngest age

group is impacted by the low sample size and needs further assessment. No differences in immunology were seen by sex.

7. Clinical and Laboratory Profile of Study Participants

7.1 Introduction

All participants, presenting with fever, in TyVAC-Nepal attended an out-patient setting in the study hospital or 17 community-based fever clinics. This exploratory chapter describes the clinical and laboratory findings of the febrile participants enrolled for passive surveillance in TyVAC-Nepal.

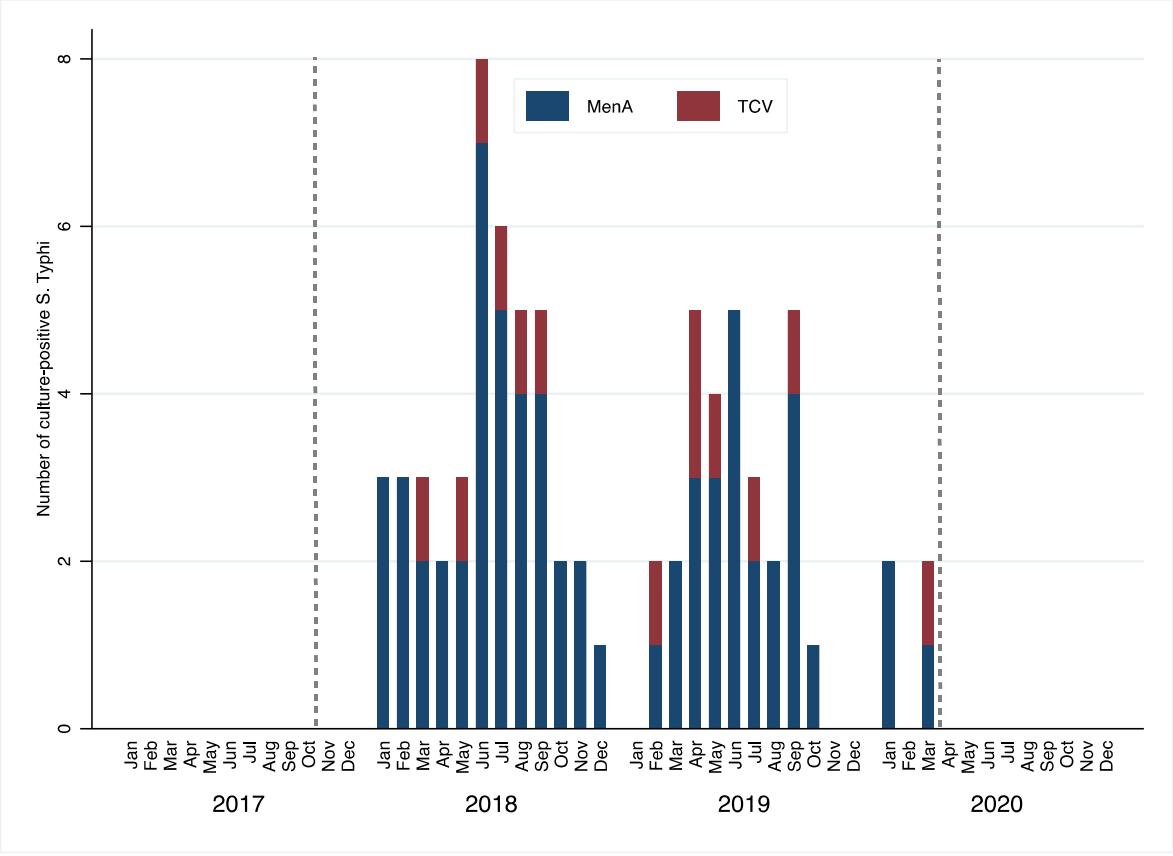
7.2 Methods

The method for the passive surveillance is described in Chapter 2, section 2.3. Any participant visiting the study clinics and meeting the fever criteria of ≥2 days of fever and/ or participant presenting with a current temperature of ≥38 degree C were consented for blood cultures. Depending on the clinical judgement of the study doctors, routine blood investigations including complete blood counts, C-reactive protein were also sent. These investigations aided the study doctors to diagnose and make a treatment plan for the participants.

Blood cultures were done using an automated system (BD BACTEC Blood Culture System [Becton-Dickinson, Franklin Lakes, NJ, USA) at the Patan Hospital microbiology lab. If

there was growth, organisms were identified using colony morphology, Gram staining, standard biochemical tests and anti-sera tests for the identification of *Salmonella*. Antimicrobial susceptibility was done using the disc diffusion method as per the Clinical and Laboratory Standard Institute (CLSI) guidelines. All isolates were stored at -70°C and sent to Oxford Vaccine Group, University of Oxford. All isolates were sent for repeat antibiotic sensitivity at the John Radcliffe Hospital lab following the EUCAST guidelines and the test results have been presented here in this chapter.

Figure 26. The distribution of S. Typhi positive blood cultures in the study period (Nov 2017 – Mar 2020)



The dotted line indicates the study period.

TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine

7.3 Results

Over the study period, a total of 3,434 participants consented for a blood draw of which 60 culture were positive for S. Typhi. Figure 26 shows the distribution of the *S.* Typhi positive blood cultures in the study period. Although a distinct seasonal pattern is not prominent over the two complete years of surveillance, April/ May through September saw the highest number of culture positive cases of typhoid fever.

Table 23. Symptom profile of participants enrolled in passive surveillance at initial presentation to clinic.

Symptom*	**TCV N=418 (%)**	**MenA N=410 (%)**	**Total N=828 (%)**
Gastrointestinal *(Symptoms include abdominal pain, nausea, vomiting, diarrhea)*	230 (55.0)	203 (49.5)	433 (52.3)
Upper respiratory *(Symptoms include sore throat, coryza, ear pain)*	237 (56.7)	245 (59.8)	482 (58.2)
Lower respiratory *(Symptoms include cough, wheeze, tachypnoea)*	110 (26.3)	83 (20.2)	193 (23.3)
Urinary *(Symptoms include dysuria, urinary frequency, new bed-wetting)*	15 (3.6)	12 (2.9)	27 (3.3)
Neurological (*Symptoms include headache, photophobia, neck-stiffness)*	25 (6.0)	25 (6.1)	50 (6.0)
Orthopedic *(Symptoms include joint pain or swelling, limping, refusal to walk)*	2 (0.5)	1 (0.2)	3 (0.4)
Skin related *(Symptoms include rash, new lesion)*	4 (1.0)	5 (1.2)	9 (1.1)
General *(Symptoms include aches, fatigue, malaise, unsettled/ crying)*	44 (10.5)	35 (8.5)	79 (9.5)

TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine

Table 24. Symptom profile of culture-confirmed typhoid fever cases at initial presentation to clinic.

Symptom*	TCV (N=11)	MenA (N=49)	Total
Gastrointestinal *(Symptoms include abdominal pain, nausea, vomiting, diarrhea)*	7 (63.6)	20 (40.8)	27 (45.0)
Upper respiratory *(Symptoms include sore throat, coryza, ear pain)*	7 (63.6)	25 (51.0)	32 (53.3)
Lower respiratory *(Symptoms include cough, wheeze, tachypnoea)*	3 (27.3)	9 (18.4)	12 (20.0)
Urinary *(Symptoms include dysuria, urinary frequency, new bed-wetting)*	0 (0.0)	2 (4.1)	2 (3.3)
Neurological (*Symptoms include headache, photophobia, neck-stiffness)*	1 (9.1)	3 (6.1)	4 (6.7)
Orthopedic *(Symptoms include joint pain or swelling, limping, refusal to walk)*	0 (0.0)	0 (0.0)	0 (0.0)
Skin related *(Symptoms include rash, new lesion)*	0 (0.0)	0 (0.0)	0 (0.0)
General *(Symptoms include aches, fatigue, malaise, unsettled/ crying)*	1 (9.1)	5 (10.2)	6 (10.0)

TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine

*A participant may present with one or more symptoms.

Tables 23 and 24 show the symptom profile of suspected and culture-confirmed typhoid fever cases at initial presentation respectively. Overall, gastrointestinal, upper and lower respiratory symptoms were the most common reasons for presentation among those enrolled in the passive surveillance. Gastrointestinal, upper and lower respiratory symptoms were also most common presenting symptoms among those that had culture-positive typhoid fever.

Table 25 shows the diagnoses given to all passive surveillance participants. Upper respiratory tract infection (N=2714, 56.6%) followed by possible typhoid fever (N=828, 17.3%), non-specific viral illnesses (N=431, 9%) and lower respiratory tract infections (N=369, 7.7%) were the most common diagnoses. Table 26 shows the suspected diagnosis for the culture-confirmed typhoid fever cases given by the attending medical officers at the initial presentation. A participant may have been given one or more differential diagnosis. Of the total 60 culture-confirmed typhoid fever cases detected from the passive surveillance, 38 (TCV=5; MenA=33) were suspected of having possible typhoid fever. There were 17 (TCV=5; MenA=12) participants who were suspected of having upper respiratory tract infection, of which 8 were also suspected of having typhoid fever. Other diagnoses included lower respiratory tract infection, urinary tract infection, non-specific viral illness, acute tonsillitis and acute gastroenteritis.

Table 25. Suspected Diagnosis for Participants with Suspected Typhoid Fever enrolled to Passive Surveillance at Initial Presentation to Clinic.

Suspected Diagnosis*	**N (4792)**	**%**
Possible Typhoid Fever	828	17.3
Upper Respiratory Tract Infection	2714	56.6
Lower Respiratory Tract Infection	368	7.7
Urinary Tract Infection	143	3.0
Meningitis/ Encephalitis	6	0.1
Orthopedic Infection	3	<0.1
Skin/ Soft Tissue Infection	31	0.7
Non-specific Viral Illness	431	9
Other Diagnosis	886	18.5

*A participant may have one or more suspected diagnosis

Table 26. Suspected Diagnosis for Culture-Confirmed Typhoid Fever Cases at Initial Presentation to Clinic.

Suspected Diagnosis	TCV (N=11)	MenA (N=49)	Total (N=60)
Possible Typhoid Fever	5	33	38
Upper Respiratory Tract Infection*	5	12	17
Lower Respiratory Tract Infection	0	1	1
Urinary Tract Infection**	0	3	3
Non-specific Viral Illness***	1	4	5
Acute Tonsillitis	1	2	3
Acute Gastroenteritis	0	1	1

TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine
*Eight participants were also suspected of having possible typhoid fever
**All 3 participants were also suspected of possible typhoid fever
***One participant was also suspected of possible typhoid fever

Table 27. Hematological Characteristics of Study Participants Enrolled in the Passive Surveillance.

	All PS enrollees		Typhoid suspected		Culture-confirmed typhoid fever	
	N	**Median (IQR)**	**N**	**Median (IQR)**	**N**	**Median (IQR)**
Hemoglobin (g/dl)	3298	12.6 (11.8–13.4)	687	12.6 (11.9-13.4)	57	12.6 (11.9 – 13.1)
Anemia (Hb<12g/dl)	*971*	*29.4%*	*200*	*29.1%*	*16*	*28.1%*
WBC count thousand/ul	3299	8.1 (5.9 – 11.3)	690	7.6 (5.3 – 10.7)	58	6 (5.2 – 8.3)
Leukopenia (WBC<5x10^3/ul)	*486*	*14.7%*	*143*	*20.7%*	*12*	*20.7%*
Leukocytosis (WBC>15x10^3/ul)	*370*	*11.2%*	*68*	*9.9%*	*0*	-
Neutrophils (%)	3299	70 (58 – 78)	690	70 (58 – 79)	58	65 (60 – 73)
Lymphocytes (%)	3299	30 (21 – 40)	689	30 (20 – 40)	58	33 (26 – 40)
Platelet count (thousand/ul)	3292	277 (224 – 332)	686	269 (220 – 322)	55	247.5 (189 – 295)
Thrombocytopenia (Platelets<150x10^3/ul)	*40*	*1.2%*	*13*	*1.9%*	*0*	-
CRP (mg/L)	3285	5 (1 – 23)	647	9 (1 – 27)	47	26 (12 – 46)

The median (IQR) hematological findings of the participants enrolled in the passive surveillance, participants suspected of having typhoid fever and participants with culture-positive typhoid fever are presented in Table 27. The three groups were non-exclusive of each other. Overall, the hemoglobin count for all febrile enrollees, participants with suspected typhoid fever and participants with culture positive typhoid fever were very similar. The white blood cell (WBC) count was within the normal parameters for all three participant groups, but participants with culture-positive typhoid fever had the lowest WBC count. The differential WBC count, namely the neutrophils and lymphocytes were similar for all participant groups with overlapping confidence intervals, but with the culture-confirmed participants having comparatively lower neutrophil count and higher lymphocyte count. The platelet count was also comparatively lowest in the culture-confirmed group. The CRP was raised in the typhoid suspected group and highest in the culture-confirmed group.

Table 28. Hematological Characteristics of Culture-Confirmed Participants, by Vaccine Group.

	Culture confirmed typhoid fever		P-value
	TCV	MenA	
Median (IQR) Hemoglobin (g/dl)	12.1 (10.7 – 12.7)	12.6 (12 – 13.3)	NS
Median (IQR) WBC count (thousand/ul)	6 (4.8 – 8)	5.9 (5.2 – 8.5)	NS
Median (IQR) Neutrophils (%)	60 (50 – 70)	66 (60 – 74)	NS
Median (IQR) Lymphocytes (%)	40 (30 – 48)	32 (26 – 38)	NS

Median (IQR) Platelet count (thousand/ul)	258.5 (180 – 332)	242 (193.5 – 294.5)	NS
Median (IQR) CRP (mg/L)	56 (27.5 – 64.7)	20.3 (10.1 – 41)	0.0092

TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine

Table 28 breaks down the hematological findings in the culture-confirmed typhoid fever participants by the vaccine group. The median hemoglobin count, the WBC count, the differential WBC count and platelet count were similar between both groups. The CRP count was significantly higher in the TCV group (P-value=0.0092).

Table 29. Antibiotic susceptibility profile for *S.* Typhi isolates.

	TCV		**MenA**	
	Susceptible	**Non-susceptible**	**Susceptible**	**Non-susceptible**
Amoxycillin	11 (100%)		53 (100%)	
Chloramphenicol	11 (100%)		53 (100%)	
Trimethoprim-sulfamethoxazole	11 (100%)		53 (100%)	
Ceftriaxone	11 (100%)		53 (100%)	
Ciprofloxacin	1 (9%)	10* (91%)	10 (19%)	43* (81%)
MIC 50 (mg/L)	*0.25*			
MIC 90 (mg/L)	*0.38*			
Range	*0.008 – 1.5*			
Azithromycin	10 (91%)	1** (9%)	52 (98%)	1** (2%)
MIC 50 (mg/L)	*8.00*			
MIC 90 (mg/L)	*16.00*			
Range	*2.00 – 24.00*			

*MIC>0.06mg/L; **MIC>16mg/L

TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine

MIC = Minimum inhibitory concentration. MIC50/90=concentration at which 50% and 90% of the organisms, respectively, are inhibited.

The antibiotic susceptibility profile was available for 64 culture-proven typhoid fever cases (Table 29). All isolates were susceptible to the first line antibiotics. All isolates were susceptible to ceftriaxone. Ten of 11 isolates (91%) in the TCV group and 81% isolates in the MenA group were non-susceptible to ciprofloxacin. The MICs of the isolates ranged from 0.008 – 1.5 mg/L. Two isolates, one in each vaccine group, were non-susceptible to azithromycin. Both isolates had intermediate susceptibility to azithromycin. The MICs of the isolates ranged from 2.00 – 24.00 mg/L.

7.4 Discussion

7.4.1 Clinical and Hematological findings in the passive surveillance cohort

Typhoid fever is one of the most common differentials for fever and most common cause of culture-proven blood stream infection in children and adolescents in the out-patient setting in Nepal[51]. Consistent with this, typhoid fever was the second most common differential for fever after upper respiratory tract infections in children enrolled in TyVAC-Nepal passive surveillance. In TyVAC-Nepal, individual symptoms of participants were not recorded, but symptoms were grouped according to the systems and reported. Gastrointestinal symptoms and respiratory symptoms were most commonly reported consistent with the most common diagnoses given to participants at initial presentation. An outpatient study in children in Nepal has similarly reported gastrointestinal symptoms namely abdominal pain, nausea, vomiting and diarrhea and respiratory symptom namely cough as common symptoms in participants with culture-confirmed enteric fever[51].

However, in young children, fever may also present as the only symptom, making diagnosis more challenging[166],[167].

Mild leukopenia, mild anemia and mild thrombocytopenia have been commonly described in complete blood counts in typhoid fever[166],[167]. This is transient and thought be a result of typhoidal invasion of the bone marrow[167]. Leukopenia is a result of the apoptosis of *Salmonella* infected macrophages. In children, there may be normal white cell count or leukocytosis, while children under-5 years are 4 times as more likely have leukocytosis[167]. The above-mentioned outpatient study among children in Nepal reported a mean hematocrit of 36% (equivalent to hemoglobin 12 gm/dl) and a median total leukocyte count of 7250 (IQR 6000 – 9050) per mm^3 which is similar to that reported in TyVAC-Nepal[51]. A systematic review assessing the clinical and laboratory findings of enteric fever in different age groups reported that anemia and leukocytosis were most commonly reported in children under-5 years compared with older children and adults[168]. Anemia was reported in about 69%, leukopenia in 43% and leukocytosis in around 30% of participants under-18 years in the same review, which is much higher compared to the TyVAC-Nepal cohort. For a large proportion of the culture-confirmed typhoid fever participants in TyVAC-Nepal, these parameters were within normal limits. One of the reasons could be that the participants in TyVAC-Nepal were encouraged to present early on in the illness, so the severity of the infection was lower among the participants. The systematic review included admitted cases of enteric fever which are likely to have more severe infection and more hematological derangements. In TyVAC-Nepal, we did not measure the severity of the infection, but if we consider admission as a

proxy of severe typhoid fever, none of participants that presented in the out-patient setting of the study fever clinic were admitted. Again, the reason for low admissions could be early presentation.

C-reactive protein is described to be raised in typhoid fever[166]. In TyVAC-Nepal, CRP was highest in the culture-confirmed typhoid fever group compared to the overall passive surveillance enrollee group and suspected typhoid fever group, but more interestingly significantly higher in the participants who received TCV. CRP is an acute-phase inflammatory protein primarily produced by liver hepatocytes and also by macrophages and lymphocytes, and is expressed in response to inflammatory conditions including infection[169]. There is also increasing evidence that CRP plays an important role in host response to infection[169]. A model of typhoid fever using transgenic mice expressing human CRP showed that CRP was protective against *Salmonella enterica* serovar Typhimurium demonstrated by early clearance of the bacteria and reduced dissemination of the bacteria to the liver and spleen in early infection[170]. This suggests that CRP produced in response to *S.* Typhi may have a role in protection. The higher CRP in TCV vaccinated participants may also suggest higher expression of CRP in vaccinated individuals and possibly a role in protection. However, the higher CRP in the TCV may also mean that vaccinated individuals have milder disease and it takes longer for them to present to the hospital allowing longer time for the rise in CRP. Further study is needed to determine the relation of high CRP in the TCV recipients.

7.4.2 Antimicrobial susceptibility in the Culture-Confirmed participants

Most of the *S.* Typhi isolates from TyVAC-Nepal were resistant to fluoroquinolones. This is of importance since fluoroquinolones are widely and incorrectly still used for the empirical treatment of typhoid fever in Nepal. Global antimicrobial resistance seen in *Salmonella* continues to evolve with resistance seen against the first line antimicrobial (chloramphenicol, ampicillin and trimethoprim/sulfamethoxazole) in the late 1980s followed by resistance to fluoroquinolones in the 1990s. Multidrug resistance (resistance against the first line antibiotics) was common in 1990s in South Asia with predominance of H58 carrying the resistant genes[171]. In Nepal, the MDR H58 *S.* Typhi has been replaced by non-MDR H58 strains with mutations associated with reduced susceptibility to fluoroquinolones and more recently by triple mutant H58 S. Typhi resulting in fluoroquinolone resistance[171]. In the gatifloxacin versus ceftriaxone open-label randomized controlled trial conducted by Oxford University Clinical Research Unit Nepal (OUCRU-PH), based at Patan Hospital, Nepal, the trial was halted by the DSMB due to gatifloxacin treatment failure in participants with culture-positive typhoid fever[172]. Fluoroquinolone-resistant subclade of H58 Salmonella Typhi was identified[173]. Prior to the trial, although decreased susceptibility to fluoroquinolones such as ciprofloxacin had been reported, gatifloxacin was still effective[174,175].

All S. Typhi isolates were sensitive to the first line antimicrobials. This is likely due to the disuse of the first line antimicrobials. The first line antimicrobials may be re-introduced for the treatment of fluoroquinolone resistant *S.* Typhi as shown by a study done by the OUCRU-PH at Patan Hospital has shown[172]. It is possible that Salmonella can again

acquire resistance after reintroduction. Co-trimoxazole or trimethoprim-sulfamethoxazole has reported been successfully for the treatment of enteric fever according to a cases report[176]. However, in a double-blind randomized placebo-controlled trial of trimethoprim-sulfamethoxazole versus azithromycin conducted by OUCRU-PH for the treatment of undifferentiated febrile illness, at Patan Hospital, Nepal, there were significantly more relapse in the trimethoprim-sulfamethoxazole group in culture-positive typhoid fever participants [177]. Trimethoprim-sulfamethoxazole was prescribed for 7 days unlike the previous 10-day schedule which may account for the high relapses.

Azithromycin was used as the drug of choice for treatment of typhoid fever in TyVAC-Nepal. Three isolates, although not resistant, showed reduced susceptibility to azithromycin. Azithromycin is widely used for the treatment typhoid fever in South Asia. With increased use of azithromycin, this may cause selective pressure leading to emergence of azithromycin resistant *Salmonella*[178]. Azithromycin resistance has been reported in typhoidal *Salmonella* strains from neighboring India and Bangladesh[179],[180]. Azithromycin resistant *S.* Typhi has also recently been reported by OUCRU-PH from Patan Hospital, the study hospital for TyVAC-Nepal[178]. Three azithromycin resistant *Salmonella* Typhi strains genetically identical and belonging to the H58 lineage with MIC>256 mg/L were identified. One of the three was successfully followed-up, had persistent fever after 7 days of treatment and had to be administered intravenous third generation cephalosporin, ceftriaxone[178]. The interpretation of the *in vitro* azithromycin susceptibility in relation to the clinical response of the drug is, however, not well

understood. This is because azithromycin acts intracellularly inhibiting bacterial protein synthesis, and so the *in vivo* effectiveness on the drug does not correspond with the *in vitro* resistance of the drug[181].

Of note, all strains of S. Typhi were sensitive to ceftriaxone. Since 2016, large scale of ceftriaxone resistant *S.* Typhi have been identified in Sindh, Pakistan. Classified as extensively drug resistant S. Typhi, the strains were resistant to the first line antimicrobials as well as fluoroquinolones and third generation cephalosporins including intravenous ceftriaxone[58]. The strain limits the treatment options, has potential for spread regionally and internationally and highlights the need for effective vaccines and improvements in diagnosis and treatment, as a part of an integrated control strategy, to curb antimicrobial resistant typhoid fever[182].

7.5 Summary

Overall, the clinical and hematological findings are consistent with existing literature. Most strains of S. Typhi were resistant to fluoroquinolones. No strains were resistant to ceftriaxone.

8. Paratyphoid Fever in the TCV Cohort

The results presented in this chapter are published in:

1. Shakya M, Voysey M, Theiss-Nyland K, *et al.* Efficacy of typhoid conjugate vaccine in Nepal: final results of a phase 3, randomised, controlled trial. *Lancet Glob Heal* 2021; **9**: e1561–8[139].

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8.1 Introduction

Paratyphoid fever is caused by *Salmonella enterica* serovar Paratyphi A, B or C. *S.* Paratyphi A is the most common serovar. Paratyphoid fever causes a febrile illness that cannot be clinically differentiated from typhoid fever[183]. *S.* Paratyphi, however, is antigenically very different from *S.* Typhi. *S.* Paratyphi A and B express the O antigens O-1, 2, 12 and O-1, 4, 5, 12, respectively and the flagellar antigen H type a and b respectively, and do not express the capsular Vi antigen. Infection from *S.* Paratyphi C is rare and the organism expresses the Vi antigen, O antigens O-6, 7 and flagellar antigen H type c.

In 2000, it was estimated that *S.* Paratyphi caused around 5 million cases, around 0.25 cases for every typhoid fever cases[141]. Paratyphoid fever is seen mainly in South Asia and China[166]. In South Asia, a 2014 systemic review estimated that the incidence of S. Paratyphi A ranged from 40 to 80 cases per 100,000 person-years[184]; however, there is a lack of data which makes assessing true burden problematic.

Vaccines against paratyphoid fever are not currently available. An inactivated whole-cell parenteral typhoid vaccine consisting of killed strains of *S.* Typhi, *S.* Paratyphi A and *S.* Paratyphi B, also known as TAB vaccine, was previously used targeting both typhoid and paratyphoid fever but has been discontinued due to high reactogenicity[185]. Several paratyphoid and typhoid-paratyphoid bivalent vaccine candidates are under development[186,185]. O:2-TT, a conjugate vaccine with O-specific polysaccharide (O:2)

conjugated to tetanus toxoid, developed by the US National Institutes of Health (NIH) completed phase I and II trials. Additional phase II trials were conducted by Chengdu and Lanzhou Institutes of Biological Products in China, after technology transfer from NIH[186]. O:2-CRM_{197}, consisting of O-specific polysaccharide conjugated to a non-mutant form of diphtheria toxin, was developed by Novartis Vaccines Institute for Global Health (NVGH). The vaccine candidate is intended for a bivalent vaccine with Vi-CRM_{197} and the technology has been transferred to Biological E Ltd., India for further development. Similarly, a live attenuated oral vaccine candidate, CVD1902 was tested in a phase I trial Center for Vaccine Development at the University of Maryland Baltimore (UMB), and is now being tested in a human challenge study in Oxford, and is eventually to be combined with CVD 909 as a bivalent vaccine.

While there are no vaccines against paratyphoid fever, there is evidence that typhoid vaccines elicit some degree of cross-protection and cross-reactive immune response against paratyphoid fever. The live oral Ty21a vaccine was first demonstrated to induce cellular immunity against S. Paratyphi A and B in the 1980s[187]. Other studies have shown cross-reactive intestinal humoral responses against *S.* Paratyphi A and *S.* Paratyphi B demonstrated by plasmablasts or antibody secreting cells, with stronger response against the latter[188,189]. Randomized controlled trials conducted in Chile showed that Ty21a induced significant protection against *S.* Paratyphi B (VE 49%, 95% CI 8 – 73%; P=0.019)[189]. There were very few cases of *S.* Paratyphi A, due to which protective efficacy against *S*. Paratyphi A could not be estimated in the Chilean trials. However, a double-blind randomized trial in Indonesia suggested that Ty21a did not confer any protection

against *S.* Paratyphi A[76]. The cross-reactivity to *S.* Paratyphi is suggested to be due to the shared O-12 antigen structure in *S.* Typhi and *S.* Paratyphi[190]. Similarly, the Vi-polysaccharide vaccine has shown to elicit a cross-reactive plasmablast activity against *S.* Paratyphi[191]. The reaction was higher against *S.* Paratyphi C and significantly lower in *S.* Paratyphi A and B. The Vi capsular antigen is expressed by *S.* Paratyphi C, which is likely to have elicited the response., but there are low rates of disease due to *S.* Paratyphi C to warrant a need for a vaccine. The Vi polysaccharide vaccine contains traces of typhoid lipopolysaccharide with O-9,12 antigens, which explain the weak immune responses against *S.* Paratyphi A and B[190]. There is, however, no very good evidence of protection.

This chapter explores the incidence of paratyphoid fever in the study cohort.

8.2 Methods

The methods for passive surveillance have been described in Chapter 2, section 2.1. Blood cultures were drawn for participants meeting fever criteria of 2 or more days of fever and/ or a temperature of 38 degree C or higher. Culture-confirmed paratyphoid fever cases have been included in the analyses. The incidence of paratyphoid fever was calculated as the total number blood culture positive cases divided by the total number of person-years of follow-up in each group. Person-time was calculated as described in Chapter 2, section 3.2.1.1.

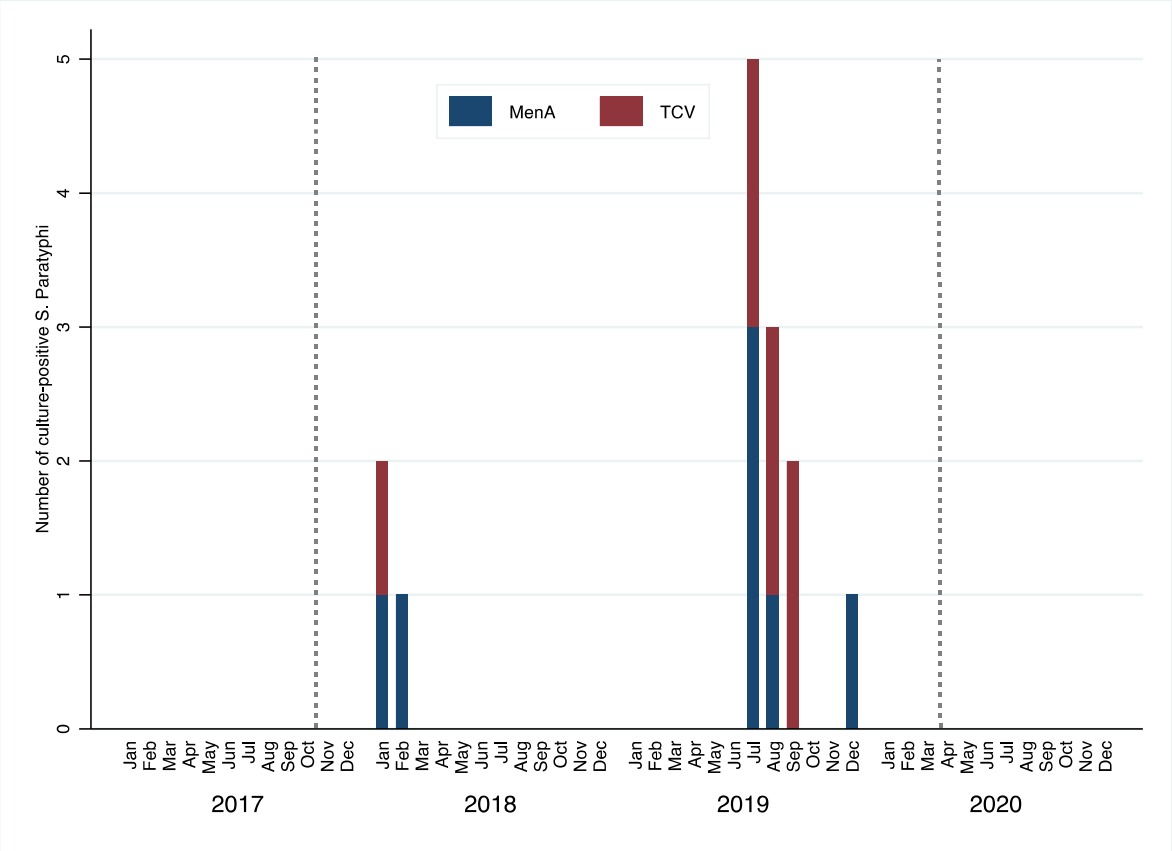
Incidence rate ratio (IRR) was calculated as the ratio of the incidence of paratyphoid fever in the TCV arm compared to the control arm. Vaccine efficacy (VE) was calculated as (1 – IRR) x 100%, where IRR is the incidence rate ratio (TCV: control).

The cumulative incidence of paratyphoid fever was summarized using the Kaplan-Meier method.

8.3 Results

A total of 14 culture-positive paratyphoid fever cases were identified over the two-year surveillance period (Table. 7.1). All cases were caused by *S.* Paratyphi A. There were 8 cases of culture-confirmed paratyphoid fever cases in the TCV group and 6 cases in the MenA group. One case in the TCV group occurred within the first 14 days of vaccination and was excluded from the analyses. Figure 27 shows the monthly distribution of *S.* Paratyphi A over the study period. There was no consistent pattern for the incidence of paratyphoid fever, although more cases were detected in the second year of the surveillance.

Figure 27. The distribution of S. Paratyphi A positive blood cultures in the study period (Nov 2017 – Mar 2020)

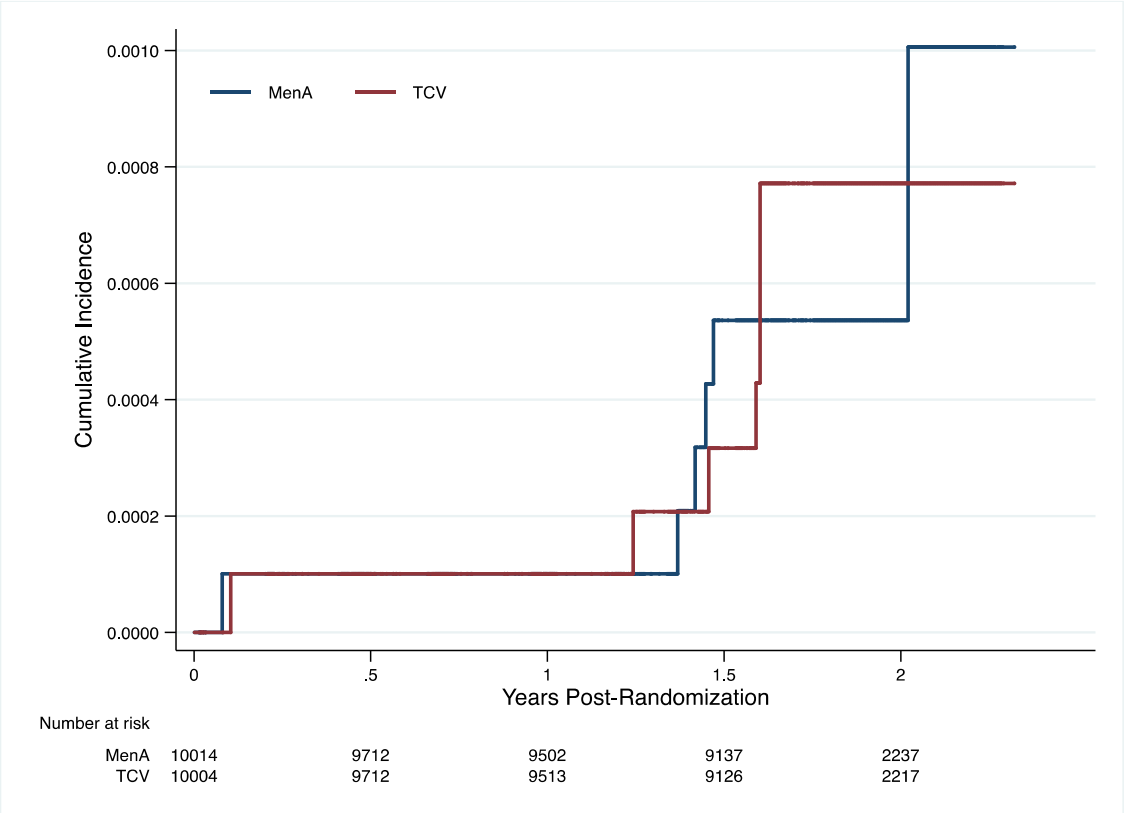


The dotted line indicates the study period.
TCV = Typhoid Conjugate Vaccine. MenA = group A meningococcal vaccine (control).

The incidence of paratyphoid fever in the TCV group was 39 (95% CI 15, 79) cases per 100,000 person-years, while the incidence was 33 (95% CI 12,72) cases per 100,000 person-years in the MenA group (Table 30). The vaccine did not demonstrate any protection against paratyphoid fever (VE -16.7% (95% CI -247.4%, 60.8%)). The Kaplan-Meier curve for TCV and MenA were similar with overlapping curves (Fig 28).

Table 31 shows the symptom profile of the culture-positive paratyphoid fever cases at the time of their initial presentation. Upper respiratory symptoms were most commonly reported by the participants. Of the culture-positive paratyphoid fever cases, most participants were suspected of having upper respiratory tract infections (TCV: N=5; MenA: N=3). Three participants were suspected of having possible typhoid fever (TCV: N=2; MenA: N=1), and one participant was suspected of having an ear infection.

Figure 28. Kaplan-Meier Estimates of the Cumulative Incidence of Blood Culture Confirmed Paratyphoid Fever, According to Trial Group



TCV = Typhoid Conjugate Vaccine. MenA = group A meningococcal vaccine (control).

Table 30. Occurrence of Blood Culture–Confirmed Paratyphoid Fever and Protective Efficacy of Typhoid Conjugate Vaccine (TCV).

Outcome	TCV (N=10005)	Incidence per 100,000 person-years (95% CI)	Men A vaccine (N=10014)	Incidence per 100,000 person-years (95% CI)	Vaccine Efficacy (95% CI)	p value
Person-years of follow-up [a]	18145		18154			
Blood culture-confirmed paratyphoid fever in first 14 days after vaccination	1					
Blood culture-confirmed paratyphoid fever after 14 days	7	39 (15, 79)	6	33 (12,72)	-16.7% (-247.4%, 60.8%)	0.781
Detected through fever clinics	7		4			
Detected through active follow-up and medical record review	0		2			

a. Participants with no follow-up contact contribute half a day follow-up in calculations.
TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine

Table 31. Symptom profile of culture-confirmed paratyphoid fever cases at initial presentation to clinic.

Symptom*	TCV (N=8)	MenA (N=4)	Total
Gastrointestinal *(Symptoms include abdominal pain, nausea, vomiting, diarrhea)*	1	0	1
Upper respiratory *(Symptoms include sore throat, coryza, ear pain)*	4	3	7
Lower respiratory *(Symptoms include cough, wheeze, tachypnoea)*	1	1	2
Urinary *(Symptoms include dysuria, urinary frequency, new bed-wetting)*	0	0	0
Neurological (*Symptoms include headache, photophobia, neck-stiffness)*	1	1	2
Orthopedic *(Symptoms include joint pain or swelling, limping, refusal to walk)*	0	0	0
Skin related *(Symptoms include rash, new lesion)*	0	0	0
General *(Symptoms include aches, fatigue, malaise, unsettled/ crying)*	2	1	3

TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine

Table 32. Antibiotic susceptibility profile for S. Paratyphi isolates

	MenA (N=4)		TCV (N=7)	
	Susceptible	**Non-susceptible**	**Susceptible**	**Non-susceptible**
Amoxycillin	4 (100%)		6 (86%)*	
Chloramphenicol	4 (100%)		7 (100%)	
Trimethoprim-sulfamethoxazole	4 (100%)		7 (100%)	
Cefotaxime	4 (100%)		7 (100%)	
Ciprofloxacin		4 (100%)		7 (100%)
Azithromycin	4 (100%)		7 (100%)	

TCV = Typhoid conjugate vaccine. Men A = Group A meningococcal vaccine
*Report not available for one.

Of the isolates available for antimicrobial susceptibility testing (TCV: N=7; MenA: N=4) (Table 32), all isolates were non-susceptible to ciprofloxacin. There were no multidrug resistant strains. No strains were resistant to ceftriaxone.

8.4 Discussion

As expected, TCV does not elicit any cross-protection against paratyphoid fever. TCV was not efficacious against paratyphoid fever. The TCV contains the Vi antigen which absent in *S.* Paratyphi A hence unlikely to protect against the disease. Although some degree of immunity has been reported to Vi vaccines previously, these are not to a degree of any clinical significance. TyVAC-Bangladesh also reported no evidence of protection against paratyphoid fever[116].

Overall, the total paratyphoid cases represented around 16% (n=14/90) of the total enteric fever cases detected in the vaccinated cohort. Other studies in Nepal have reported 17 - 44.3% *S.* Paratyphi cases of total enteric fever cases [192,193, 50,194]. *S.* Paratyphi A was the most common serovar reported across the studies as in TyVAC-Nepal. *S.* Paratyphi A causes around 20% of all enteric fever cases globally[141].

Paratyphoid fever is described to cause symptoms indistinguishable from typhoid fever and of similar severity [183], and it has been seen from an exploratory analysis of TyVAC-Nepal that those with a clinical diagnosis of typhoid fever are more likely to have blood drawn for culture and more likely to have a positive blood culture result[146]. The analysis

also showed that URTI was the most common alternative diagnosis. This hold true for paratyphoid fever as well, where a large proportion of culture-positive paratyphoid fever cases presented with upper respiratory symptoms and were suspected of having an upper respiratory tract infection.

There are reports that the proportion of S. Paratyphi infections among enteric fever cases is increasing, though not evident in TyVAC-Nepal[195,196]. There were more cases of culture-positive paratyphoid fever in the second year of the study period, but the numbers are low and likely coincidental than a direct effect of TCV vaccination. In a prospective epidemiological study (STRATAA) conducted in an overlapping study area in Lalitpur between August 2016 – August 2018, paratyphoid cases represented 7% of the total enteric fever cases and a crude incidence of 6 cases (95% CI 3 – 10) per 100,000 person-years[52]. The incidence in TyVAC-Nepal is higher than in STRATAA, but it is not possible to say whether this is an actual increase. There was enhanced surveillance in TyVAC-Nepal which likely explains the differences. The rates reported in STRATAA appear lower than rates reported in other studies in Nepal suggesting that the incidence was under-estimated in the study. Further, a 23-year retrospective analysis of blood cultures done at Patan Hospital from 1992 to 2014, reported that the proportion of *S.* Paratyphi A significantly increased over the time period while proportion of *S.* Typhi decreased[50]. Increase in *S.* Paratyphi have been reported from China[197], India[198] and Cambodia[199]. While the cause of the increase in paratyphoid fever is not known, the interplay between various "environmental, ecological or epidemiological factors" may play a role[50,200]. Use of Vi vaccines may also contribute in the potential serovar shift, although there is no

documented evidence for this. Vi vaccines, as is the TCV used in TyVAC-Nepal, significantly reduce the incidence of *S.* Typhi. *S.* Paratyphi A does not contain the Vi polysaccharide, the target antigen for Vi vaccines, and hence are not impacted by the Vi vaccines. The decrease in the *S.* Typhi burden, in the long run, may result in a reciprocal increase in the *S.* Paratyphi A in the environment and hence increase infection from *S.* Paratyphi A. With the rollout of TCV in countries across the world, including Nepal, and in the absence of vaccines targeting paratyphoid fever, strong surveillance is needed monitor the burden of paratyphoid fever. *S.* Paratyphi A has been reported to be more prone to developing resistance to antimicrobials and resistance profiles will need to be monitored as well[200]. Higher minimum inhibitory concentration have consistently been reported in S. Paratyphi A than *S.* Typhi in Nepal[201].

Paratyphoid vaccines alone are not likely to be integrated into national immunization programmes because of the relatively lower burden of the disease. However, bivalent vaccines, as described above, can potentially be used to tackle the dual burden of typhoid and paratyphoid fever, in countries like Nepal where both diseases are endemic[186].

8.5 Summary

TCV did not elicit protection against paratyphoid fever as expected. There is no evidence of an increase in paratyphoid fever incidence.

9. Discussion

9.1 Introduction

This thesis describes a large-scale clinical trial, TyVAC-Nepal, conducted in the urban Lalitpur, Nepal with the main aim to assess the impact of TCV in children in the setting of high endemicity, and with an overarching aim to inform TCV policy to help accelerate the introduction of TCV to help control typhoid fever in high burden regions.

9.2 Summary of Pertinent Findings

9.2.1 TCV is Safe

This is the first study, as a part of the TyVAC consortium (along with TyVAC-Bangladesh and TyVAC-Malawi), to report the safety of TCV (Typbar-TCV, Bharat Biotech) in a large population in an endemic setting. In the solicited adverse events, reported from 18,743 participants from Nepal, 7 days post-vaccination occurred at similar rates to the control, meningococcal A conjugate vaccine (MenAfriVac, Serum Institute of India). The results suggest that the occurrence of reactogenicity is within the acceptable limits and comparable to that of vaccines widely used in national immunization programmes.

Serious adverse events recorded over the first 6 months post-vaccination also show that TCV is safe. All serious adverse events were hospitalizations for common causes of childhood illnesses and occurred at rates similar to usual hospital admissions in the

paediatric age group. The adverse events and serious adverse events data were a part of data reviewed by the GACVS[122]. The GACVS concluded that the "safety profile of the Typbar-TCV vaccine [was] reassuring" and there were "no signals of serious adverse events."

The national AEFI committee had raised concerns of thrombocytopenia due to reports of high occurrence of thrombocytopenia in Navi Mumbai. The GACVS had concluded that the thrombocytopenia was a result of the concurrent dengue infection in Navi Mumbai, but the national AEFI committee of Nepal had still flagged it. Although formal data and sample collection was not planned in TyVAC-Nepal, post-hoc analyses conducted in TyVAC-Nepal participants presenting for passive surveillance showed that thrombocytopenia was not a signal of concern.

TyVAC- Bangladesh and TyVAC- Malawi also reported a reassuring safety profile for TCV[116,132]. The TCV is planned for introduction at 9 months or at 15 months as a part of national immunization programmes. Safety of TCV co-administered with MR or yellow fever vaccine has been reported from a phase II trial in Burkina Faso[137]. More data on safety of TCV administered concomitantly with vaccines in routine immunization schedules, such as the JE, MR and yellow fever vaccines, are needed.

9.2.2 TCV is immunogenic

The results from this study show that overall, TCV (Typbar-TCV, Bharat Biotech) induces a strong immune response 28 days post-vaccination which is sustained at 18 months post-vaccination. The study measured the IgG and IgA response to the vaccine. The IgG response has previously been evaluated for typhoid vaccines, including this TCV[97,98]. The IgA response was evaluated in a human challenge model of TCV in Oxford but the report in this thesis is the first to evaluate the IgA response in an endemic setting[98].

In an age stratified evaluation of the vaccine response, both IgG and IgA response were lowest in the under-5-year age group and highest in the 10 – 15-year age group. The older children are likely to have had multiple exposures to typhoid fever driving a higher anamnestic vaccine response. On the other hand, it is also possible that the immune response is weaker in younger children and/or the decay of antibodies is more rapid. This could be due to the lack of bone marrow niches for long lived plasma cells in the youngest age group or the differences in the memory cells across different ages. There was a more rapid decay in the IgA response compared with IgG response in the youngest age group. Further evaluation of vaccine responses, especially in the youngest age group is needed. Lower immune responses in the youngest age group, if confirmed, could be overcome with booster doses of the vaccine as discussed in section 9.5.2.

9.2.3. TCV is efficacious

This is the first study to report the efficacy of TCV (Typbar-TCV, Bharat Biotech) in an endemic setting. Efficacy from a human challenge model in a typhoid naïve adult population and seroefficacy estimates from plasma samples in an Indian controlled trial cohort were the only efficacy data available before this[98,202]. The interim efficacy analysis conducted at 15-months post-vaccination reported the efficacy of the vaccine as the 45 cases of typhoid fever were observed before the planned 2-year follow-up. The vaccine efficacy was 81.6% at 15 months and 79% at two years of complete follow-up as reported in this thesis. There was a significant decrease in the incidence of typhoid fever in the participants who received TCV compared with those who received the control vaccine showing that the vaccine can have a considerable impact on the burden of typhoid fever in children. The vaccine efficacy at year 1 and year 2 shows that TCV shows no statistically significant waning of protection up to two years post-vaccination. It will be important to ascertain the longer-term protection offered by TCV – this has been discussed in the sections 9.4.1 and 9.4.2 below.

9.3 Limitations

9.3.1 Limitations in the Surveillance

In order to capture all typhoid positive cases, it was crucial to ensure that a robust surveillance was set up. The study took multiple measures. Firstly, surveillance clinics were set up in each ward to ensure that the doctors and clinical services were accessible to the participants. The clinics were setup in coordination with the local authorities and

when possible within the structure of the wards, to ensure the participants were already aware of the location. Secondly, the participants were incentivized. All participants were seen by the doctors, free of cost irrespective of the reason of attendance. For febrile participants, blood cultures and all related investigations and treatment costs were paid by the study. Thirdly, the participants were followed up every three months via phones calls to remind them of the passive surveillance services available to the participants. Fourthly, information about the passive surveillance clinics was also relayed by THPs who visited the participants' homes every three months. Fifthly, public engagement activities especially targeting study participants and guardians were conducted in all wards throughout the study period.

Despite these measures, there were still participants who visited facilities other than that provided by the study. Lalitpur, being a busy metropolis, has pharmacies, private clinics, private hospitals and specialist doctors within reach making it easy for participants to seek care elsewhere. Acknowledging this, the study was also collecting data on fever, health care visits, investigations sought and treatments provided every three months via phone calls. Any participant reporting fever, visiting health care facilities other than that provided by the study, having investigations done and reporting typhoid fever were followed up in person, medical records were reviewed to capture all culture positive typhoid fevers. However, with antibiotics available without prescriptions in the study setting, there were participants who self-treated. The medical records are not maintained within healthcare facilities and are given the participants. There is poor record keeping, and here were cases where participants had lost records and cases were

recorded information were inadequate. Potential typhoid cases could have been missed due to these reasons leading to underestimation of incidence of cases and decrease in power to determine the vaccine effect.

The criteria for blood culture in the study was 2 or more days of fever and/ or a temperature of 38 degrees C. In general practice, blood investigations are not sent early on in the illness unless there is a definitive indication. There is no blanket protocol of when blood cultures are sent. It is subjective. So, for participants who visited other health care facilities, it is likely that blood cultures were not sent in all cases, who would have otherwise met the study criteria for blood culture and would have been approached for blood culture.

9.3.2 Lack of Data in the Youngest Age Group

As discussed previously in chapter 6, in the results of age stratified analysis, in children under-5 years and children under-2 years the results are inconclusive or need further evaluations due to the low sample size in these age groups.

The vaccine efficacy in children under-2 years could not be determined as there weren't enough cases. Only two cases of culture-positive typhoid cases were captured. One of the reasons being that there was a small cohort of children under-2 years to begin with. Other reasons could be related to surveillance and capturing cases. Younger children

develop multiple febrile episodes compared with older children, but as many cultures were not done. It is possible that guardians of younger children are more likely seek specialized care for their children. Parents of younger children are also more hesitant for multiple blood draws for their children, and doctors are more cautious in requesting blood samples from the youngest age groups.

The immunogenic response in children under-5 years appeared lowered than in older children. As discussed above, although it is possible that the immunogenic response is lower is younger children, but the low number of total samples collected and low number of paired samples available for analysis could possibly also play a role.

9.4 Contributions to Typhoid Fever Research and Policy

The results from this study support the WHO's recommendation that TCVs should be used typhoid endemic countries. TCV has also been recommended as the preferred vaccine in countries with a high burden of antimicrobial resistant *S.* Typhi. The WHO has encouraged countries to include the vaccine in routine immunization of children with a catch-up campaign for up to 15 years of age where feasible.

In Nepal, the TyVAC-Nepal study team has been actively involved in supporting the introduction of TCV in the country. The TyVAC-Nepal study team involved the policy makers in the country from the incipient stage of the study. The study was initiated in the

Nepal with endorsement from the national NITAG and support from the Child Heath Division (now Family Welfare Division) and the local WHO. The national level stakeholders were updated with the study results over the course of the study period. The final results of the study were presented to the NITAG in early 2020 which supported and was followed by Nepal's GAVI application for TCV in October 2020. The Government of Nepal conducted a nationwide TCV campaign for children 15 months to 15 years in April 2022. TCV was then available as a part Fof routine immunization at 15 months. Nepal is the first country to introduce Biological E's TCV, TYPHIBEV, in routine immunization.

9.5 Future Directions

Worldwide, countries are adopting TCV for typhoid control since GAVI announced financial support to introduce TCV in late 2017. Pakistan was the first country to introduce TCV into routine immunization program in November 2019 following the XDR typhoid outbreak that began in November 2016. Pakistan had earlier used the vaccine in response to the outbreak in Sindh Province since April 2019.

Liberia introduced TCV into its national immunization schedule in April 2021, becoming the first African country to introduce TCV. Zimbabwe followed in May 2021. Nepal has followed in April 2022. There are still gaps in our knowledge of typhoid fever and TCV protection that need to be addressed.

9.5.1 Long-term Protection

Studies on typhoid conjugate vaccine published so far, including TCV studied in TyVAC-Nepal, report the short-term protection from TCV. The duration of protection offered by TCV is not known, although theoretically conjugate vaccines offer long term protection. Currently the WHO recommends TCV as a single dose. Countries have introduced TCV into their immunization programmes at 9 months and 15 months. The burden of typhoid fever is high in children and adolescents and plateaus in adulthood, which makes it important to determine how long the protection from TCV lasts.

TyVAC-Nepal completed 2 years of surveillance in April 2020. With disruption due to the COVID 19 pandemic in 2020 and 2021, surveillance of the population continues as TyVOID-Nepal, providing unique opportunity to understand the persistence of TCV protection. At the end of TyVAC-Nepal, following the unblinding, the alternate vaccine (TCV to those who initially received MenA and MenA to those who received TCV) was offered to all participants. Due to the pandemic, there are three separate cohorts of children who have received TCV: one TCV cohort that initially received the vaccine in 2017/2018 and two late cohorts in 2020 and 2021. TyVOID-Nepal aims to assess the relative medium-term efficacy of TCV by comparing typhoid fever incidence between the initial and the two late cohorts. These results will be important to understand long term protection.

9.5.2 Need for Booster

Following on the duration of protection offered by TCV, the subsequent question is whether a booster is required. As described above, the protection offered by the vaccine and the immunogenic response to the vaccine in younger children and older children differ. What appears to be a better response in older children is probably a boosting response to natural exposure. Nonetheless, this brings up the question whether younger children, especially those less than 2 years, who have less exposure to typhoid in the environment need boosting to sustain protection.

As a part of TyVAC-Nepal, and not included in this thesis, a sub-study in 100 children aims to assess response to a booster dose of TCV. Children presenting to the study hospital, Patan Hospital, for the 9 months and 12 months routine immunization, were given a dose of TCV and boosted at the 15 months immunization visit. Blood samples were collected at baseline, at one-month post vaccination, at the 15 months visit prior to boosting and one-month post-booster. Anti Vi-IgG and IgA antibodies will be measured. The results of the study will be able to shed light on whether the response in the youngest age group is indeed low or an artefact of low sample size, and whether a boosting dose helps produce an adequate response.

So far, there are no publicly available data on the safety of two doses of TCV. Safety of two doses of TCV also needs to be further studied, especially if a booster is deemed necessary. The timing of the booster is a subsequent important question that will need to

be addressed. Currently, TCV is scheduled at 15months in the national immunization program (NIP) along with MR vaccine. It will be important to determine whether the children need to be boosted and to assess what is programmatically feasible. For the current EPI schedule vaccines in Nepal, studies have shown that almost 25% of children are not fully immunized; for the vaccines requiring multiple dose, coverage was lowest for the third dose with the first dose having 10% greater coverage that the third dose; and, timely coverage for second and third dose is lower than that of the first dose of the same vaccine[87,203]. Although, there are multiple reasons, this highlights that need to appropriately time the booster. Introduction of TCV in Nepal at 15 months has been planned to synergistically increase the coverage of MR2 (based on personal communications with WHO-IPD Nepal). A TCV booster, if needed, can be given to preschool or school-going children. There is no scheduled visit for pre-school children, and capturing preschool children and getting adequate coverage in this age group would be challenging. Boosting school-aged children may ensure better coverage through school-based vaccinations. Nonetheless, boosting in this age group for longer term protection, will need to be studied.

9.5.3 Cost-Effectiveness of TCV Vaccine

In our study setting, the minimum costs of diagnosis and treatment of uncomplicated typhoid fever in an outpatient setting is around NPR 1500 (~USD 12.5). In our experiences, the cost for hospital admission increases by approximately 5 - 7 times for an average 7-day admission. Stakeholders increasingly demand cost-effectiveness of adding a new vaccine into national immunization. For TCV, model-based cost-effectiveness data

are available. Cost-effectiveness analysis has shown that TCV will be cost-effective in GAVI eligible high burden countries for up to a price of $2 per dose. For medium burden countries, it depends on willingness to pay. Nepal has decided to incorporate TCV to the national immunization schedule. However, with increasing number of vaccines being added to immunization schedules, and the limited budget, it will be important to continuously demonstrate that the vaccination programme is important. In the medium and long term, it will be important to generate cost-effectiveness evidence for catch-up campaigns and boosters, if needed, and for currently GAVI eligible countries, including Nepal, when they transition from being GAVI eligible to being self-financing.

9.5.4 Evaluating Vaccine Rollout

Clinical trials such as TyVAC-Nepal measure the efficacy of the vaccine showing how much the vaccine lowers the risk of developing the disease among those who receive the vaccine compared with those who received the control. The efficacy of TCV was 79% in TyVAC-Nepal showing that those who received TCV are at 79% lower risk of developing typhoid fever compared with those who do not receive TCV. This does not necessarily reflect what happens in the real-world setting. While clinical trials are designed to maximize the internal validity, these tend to be at an expense of external validity or generalizability, and may not be a representation of the population.

Once a vaccine is given to a population, multiple factors can affect how effective the vaccine is. Vaccine effectiveness measures how well a vaccine works in a real-life setting,

and as with any vaccine, now that TCV is being rolled out in countries, it will be important to evaluate the effectiveness of the vaccine. With TYPHIBEV being introduced in Nepal, there is opportunity to evaluate the effectiveness of the vaccine in a real-world setting.

9.5.5 Eliminating Typhoid Fever

With the availability of the typhoid conjugate vaccine which is safe, efficacious and immunogenic, and can be given to young children, we have moved a step towards better typhoid control. The end goal in the control of typhoid fever is elimination, and possibly eradication. Elimination of a disease is defined as "reduction to zero of the incidences of a specified disease as result of deliberate efforts" requiring continued efforts[204]. Eradication means a "permanent reduction" of the incidence worldwide that no longer needs intervention measures[204]. For elimination of typhoid fever, disease transmission needs to be interrupted, cases of typhoid fever needs to be accurately identified, effective treatment of typhoid fever needs to be available and the disease reservoirs – the chronic carriers- of typhoid fever need to be identified and treated[205].

With the WHO recommendation and GAVI support, as mentioned above, some countries have started introduction of TCV in their routine immunization. Multiple studies, including TyVAC-Nepal, have shown the benefits of prioritizing TCV introduction. Whist TCV has shown to be highly effective at an individual level, the indirect protection of TCV when limiting the vaccination to children up to 15 years is low. This has been demonstrated in the TyVAC-Bangladesh study where the indirect protection was 19%.

These results suggest that children are not the drivers of infection, and to control transmission of typhoid fever vaccinating children may not be enough. Cost-effectiveness of vaccinating adults although not known is likely to be low.

Nonetheless, with the programmatic introduction of TCV in countries including Nepal, this is a step towards elimination of disease. However, vaccination alone cannot achieve this goal. With the improvements in WASH measures, rates of typhoid fever drastically reduced in Western Europe and North America, with reported cases mostly being imported from high burden countries[206]. This highlights the importance of implementing the WASH strategies including access to safe drinking water and food and improved sanitation and sewage disposal for this feco-orally transmitted disease.

Vaccination will need to be implemented hand in hand with improvements in diagnosis and appropriate treatment of typhoid fever. The current gold standard for diagnosis of typhoid fever, blood culture, only has a sensitivity of 40 – 60 % and requires specialized microbiological facilities and personnel to identify the bacterium which is not widely available in low-resource settings. Efforts need to continue to improve diagnostic tools. Similarly, timely and adequate treatment of typhoid fever with appropriate antimicrobials needs to be re-enforced.

The biggest hurdle for the eradication of typhoid fever is the presence of chronic carriers. There is no environmental reservoir for *Salmonella* and the bacterium can persist in the

gallbladder, maintain a reservoir of infection. The asymptomatic chronic carriers intermittently shed the bacteria maintaining the transmission cycle. The current gold standard for detection of carriers is stool culture, but lacks sensitivity. Treatment of chronic carriers is possible with antimicrobials, but with increasing antimicrobial resistance, this may complicate the treatment of carriers. It is hence also crucial to fill gaps in diagnosis and treatment of chronic carriers to address long term control of typhoid fever.

9.6. Conclusion

TyVAC-Nepal has shown that TCV is a safe, efficacious and immunogenic conjugate vaccine that can be given to young children. The results from this study supports the inclusion of TCV for the control of typhoid fever in endemic settings, and has led to TCV rollout in Nepal. Whist it is debatable whether typhoid fever can be eliminated or eradicated in the medium- or long term, TCV can play a critical role in reduction of typhoid fever in the near future. Vaccination efforts need to be coupled with the ongoing efforts for improving water and sanitation practices, proper diagnosis and treatment of typhoid fever, tackling antimicrobial resistance and identification and treatment of asymptomatic carriers to achieve the goal of typhoid elimination and should be synergised with efforts for control of other water borne and diarrhoeal disease.

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Appendix

Appendix 1. Classification of solicited adverse reactions in the first 7 days post-vaccination.

CRF Question	Options
How did your child feel following the vaccination?	Well/ Unwell/ Died
Has your child had any fever since the vaccination?	Mild/ Moderate/ Severe
Pain	Mild/ Moderate/ Severe
Swelling	Mild/ Moderate/ Severe
Redness	Mild/ Moderate/ Severe
Has the child been eating less than usual?	Mild (Eating less than normal for 1-2 feeds) Moderate (Missed 1-2 meals completely) Severe (Refused most or all meals)
Has the child been less active than usual?	Mild (Less interested in surrounding, toys etc.) Moderate (No interest in above and sleeping through meals) Severe (Sleeping most of the time)
Has the child been more irritable than usual?	Mild (Continuously irritable for less than 1 hour) Moderate (Continuously irritable for 1 to less than 3 hours) Severe (Continuously irritable for 3 or more hours)
Has the child cried persistently?	Mild (Cried continuously for less than 1 hour) Moderate (Cried continuously for 1 to less than 3 hour) Severe (Cried continuously for 3 or more hours)
Has the child been sick?	Mild (1-2 episodes without interfering with routine) Moderate (Several episodes & cannot keep any food down) Severe (Frequent episodes & taking nothing by mouth)
Has the child had diarrhea?	Mild (more loose stools than usual) Moderate (Frequent runny stools without much solid material) Severe (Multiple liquid stools without much solid material)