Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: 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;381:2209-18. DOI: 10.1056/NEJMoa1905047

Protocol Supplement

This supplement contains the following items:
1. Original protocol, final protocol, summary of changes.
2. Original statistical analysis plan (no changes)

Original protocol

Trial Title: **Assessing the impact of a Vi-Polysaccharide Conjugate Vaccine in preventing typhoid infections among Nepalese children – a Phase III trial**

Internal Reference Number / Short title: TyVAC Nepal: Typhoid Vaccine Study

Ethics Ref: OxTREC 17-15

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Chief Investigator Signature:	
Statistician Signature:	

There are no potential conflicts of interest.

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, host organisation, and members of the Research Ethics Committees, unless authorised to do so.

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1. KEY TRIAL CONTACTS

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2. SYNOPSIS

Trial Title	Assessing the impact of a Vi-Polysaccharide Conjugate Vaccine in preventing typhoid infection among Nepalese children – a Phase III trial	
Internal ref. no. (or short title)	TyVAC Nepal: Typhoid Vaccine Study	
Clinical Phase	Phase IV	
Trial Design	Participant- and observer-blind randomised-controlled trial	
Trial Participants	Children aged 9 months to <16 years in a defined catchment area of Kathmandu	
Planned Sample Size	20,000 children, allocated to two vaccination arms on a 1:1 ratio (10,000 each arm)	
Treatment duration	Single dose vaccination after enrolment.	
Follow up duration	Two years follow-up, post vaccination, for each participant	
Planned Trial Period	Nov 2017 – August 2020	
	Objective	**Outcome Measure**
Primary	To determine the efficacy and rate reduction of the Vi-TCV in preventing blood culture-confirmed symptomatic infection caused by S. Typhi	The Incidence of blood culture confirmed typhoid fever in each of the vaccination arms.
Secondary	To investigate safety outcomes associated with Vi-TCV vaccination, within the study population	The proportion of participants developing all adverse events within the first 7 days post-vaccination, and serious adverse events within 6 months of vaccination, as determined through self-reporting at follow-up contact
	To determine the impact of vaccination with Vi-TCV on the incidence of inpatient/outpatient admission rates for fever	Rates of participants with at least ≥2 days of subjective persistent fever, or a temperature of at least 38 degrees C, at presentation, at Patan Hospital or trial clinics in each vaccination arm, stratified by duration and severity of fever
	To measure the difference in rates of hospital and clinic presentation for febrile illness in each vaccination arm	Rates of hospital or clinic presentation with febrile illness of any duration in each vaccination arm, measured by hospital presentation logs, hospital records, trial clinic records and self-

		reporting during three monthly follow-up
	To determine Paratyphoid infection rates in each vaccination arm	Rates of blood culture confirmed Paratyphoid cases in each vaccination arm
	To measure days spent in hospital from febrile illness	Length of stay in hospital, collected from Patan hospital patient records, and parent/self-reported, in each vaccination arm
	To measure incidence of clinically-suspected enteric fever	Number of clinical diagnoses of typhoid fever, as determined by trial staff in Patan hospital outpatient clinics and trial clinic, in each vaccination arm.
Exploratory	To measure antibiotic/ antimicrobial use for inpatients/outpatients in each vaccination arm	Incidence of antibiotic/antimicrobial use in in/outpatient settings, from Patan hospital and trial clinic admission logs, and/or parent/self-reported in follow-up contact
	To measure the difference in the duration of febrile illness in each vaccination arm	Duration of fever recorded in Patan hospital and trial clinics, and parent/self-reported via follow-up contact
	To measure difference in rates of all-cause hospitalisation	Rates of hospitalization, identified through hospital admission logs and self-reporting in each vaccination arm
	To determine effect of vaccination on child growth and weight in children <5 years of age	Measurement of anthropomorphic parameters of children at baseline and at two years, in all children aged <5 years at the time of vaccination
	To determine the immunogenicity of Vi-TCV in a subset of participants, stratified by age groups.	Assay of anti-Vi IgG antibodies in blood samples collected at baseline (Day 0) and at one month (Day 28) in a subset of participants (1000 in vaccine arm; 500 in control arm)

	To determine the persistence of antibodies induced by Vi-TCV in stratified age groups.	Assay of anti-Vi IgG antibodies in blood samples collected at baseline (day 0), 18 months (day 545) and two years (day 730) in a subset of participants (1000 in vaccine arm; 500 in control arm)
	To measure the incidence of fever that does not result in medical treatment in each vaccination arm	Parent/self-reported fever at follow-up contact of participants.
	To determine rates of absenteeism from school/work in each vaccination arm	Rates of absenteeism from school or work, as applicable, as reported by parents at follow-up contact
	To measure all-cause mortality, and all-cause mortality with fever, in each vaccination arm	Rates and circumstances of mortality in each vaccination arm, recorded from hospital records and three-monthly follow-up
	To measure the rate of suspected and confirmed acute abdominal presentation in each vaccination arm	Rates of presentation to Patan hospital or trial clinics with acute abdomen
	To measure the rate of surgical intervention for acute abdominal complaints in each vaccination arm	Rates of acute abdomen surgery in each vaccination arm, and gross surgical findings
Investigational Medicinal Product	Vi polysaccharide-tetanus toxoid conjugate vaccine (Vi-TCV) Licensed Trade name: Typbar-TCV®, Bharat-Biotech	
Formulation, Dose, Route of Administration	Each 0.5ml vaccine dose contains: Purified Vi-Capsular Polysaccharide of *S.* Typhi Ty2 conjugated to Tetanus Toxoid 25µg Sodium chloride 4.5 mg Water for Injection q.s. to 0.5ml Administration by Intramuscular injection Pre-filled multi dose (2.5ml) vial	
Comparator (control)Treatment	Serogroup A meningococcal conjugate vaccine Licensed trade name: MenAfriVac™, Serum Institute of India PVT. Ltd. Dose 10µg for participants aged ≥1 year; 5µg for participants aged 9 to <12 months	

3. ABBREVIATIONS

AE	Adverse event
AEFI	Adverse event following immunisation
AR	Adverse reaction
CI	Chief Investigator
CRF	Case Report Form
CT	Clinical Trials
DMC/DMSC	Data Monitoring Committee / Data Monitoring and Safety Committee
EPI	Expanded Programme on Immunizations
GCP	Good Clinical Practice
IB	Investigators Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
IRB	Independent Review Board
NHRC	Nepal Health Research Council
OXTREC	Oxford Tropical Research Ethics Committee
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SMPC	Summary of Medicinal Product Characteristics
SOP	Standard Operating Procedure
STRATAA	Strategic Typhoid alliance across Africa & Asia
S. Typhi	*Salmonella enterica* serovar Typhi
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
Vi-PS	Vi antigen polysaccharide vaccine
Vi-TCV	Vi antigen typhoid conjugate vaccine

4. BACKGROUND AND RATIONALE

Enteric fever is a systemic illness caused by the human restricted pathogens *Salmonella enterica* serotypes Typhi (*S*. Typhi) and Paratyphi A-C. It is estimated to affect >20 million people worldwide annually, with an estimated 200,000 fatalities per annum, primarily in lower income countries with poor sanitation(1). Areas

with an incidence of >100/100,000 are considered endemic, including many countries in Africa, South Asia, South-East Asia and Central Asia(2). The burden of disease and mortality is increasingly recognised in the under 5 age group, as well as in older children and young adults(3–6). Enteric fever also remains a concern in high-income countries for travellers to endemic regions and laboratory workers (7,8).

Control of enteric fever, historically, has been established primarily through improved sanitation and infrastructure, leading to the elimination of disease as a public health problem from most developed countries. This remains the case, but there are substantial costs and difficulties implementing these measures in high typhoid incidence areas. As such, use of an effective vaccination programme targeting the highest risk populations will likely be a useful and cost-effective addition to control measures. Given the causative organisms are human-restricted, global eradication is possible and an effective vaccine will contribute to this.

4.1. Typhoid Vaccines

Currently, licensed vaccines exist only for the most prevalent serovar causing enteric fever, *S.* Typhi. The existing options are as follows:

Inactivated Whole Cell vaccine

This vaccine consists of heat-phenol-inactivated whole cell *S*. Typhi, which is injected subcutaneously in two doses four weeks apart. It had efficacy of 51-67% in controlled trials. It was associated with high degree of reactogenicity, causing fever and systemic symptoms in 9-34% of recipients, leading to school absence in 2-17% of cases (9). Due to these side-effects it has largely dropped out of mainstream use, however it is still used in several lower-income countries.

Vi Polysaccharide Vaccine (Vi-PS)

Developed in the 1980s this vaccine consists of purified Virulence factor (Vi antigen) capsular polysaccharide (Vi-PS) that forms the capsule of, and is specific to, *S.* Typhi. It elicits a T-cell independent antibody response, which means it has poor immunological memory and repeat doses do not result in an additional boosting response (10,11).

Similar to other polysaccharide vaccines, Vi-PS vaccine is poorly immunogenic and not licensed for use in children under 2 years old, presumably due to the absence of specific splenic marginal zone B-cells that are needed to produce an immunological response to polysaccharides. In clinical trials, clinical protection is non-comprehensive with protective efficacies of 64-72% (12–14). Additionally, protection is short lived, lasting only 2-3 years (15,16).

Live attenuated oral vaccine (Ty21a)

Also developed in the 1980s, this is an attenuated strain of *S*. Typhi (Ty21a) that has had many virulence genes mutated chemically, including the gene leading to failure to produce the Vi antigen. Ingestion of this strain induces local gut mucosal immunity as well as systemic antibody and cell mediated response(17,18). The strain is lyophilised and administered in either an oral enteric capsule or a liquid solution and requires 3-4 doses to induce effective protective immunity.

Clinical trials preformed in Chile and Indonesia demonstrated Ty21a vaccine had a protective efficacy of 67% and 53%, respectively (5,19). While the enteric-coated formulation is difficult to administer to young

children, the alternative liquid formulation is better tolerated but may be less immunogenic in younger children (18,20). Ty21a vaccine is not licensed for children under the age of 6 years.

Vi-rEPA Vaccine

This vaccine was developed by the US National Institute for Health (US NIH) in 1994 utilising Vi-polysaccharide conjugated with a recombinant exoprotein A from *Pseudomonas aeruginosa* (rEPA) (21). A two-dose schedule six weeks apart was shown to be highly immunogenic with a protective efficacy of 91.1% in children aged 2 to 5 years in a trial in Vietnam (22). More recently, a study has demonstrated its immunogenicity in infants (23). However, the licensure of Vi-rEPA has been delayed due to lack of regulatory precedent for the use of rEPA carrier based vaccines.

Vi antigen typhoid conjugate vaccine (Vi-TCV)

Vi-TCV (Tybar-TCV™) is a newly available vaccine developed by Bharat Biotech consisting of 25 µg of Vi polysaccharide antigen conjugated to a nontoxigenic tetanus toxoid carrier protein. Similar to other vaccines, which are designed to protect against encapsulated bacterial pathogens and are conjugated to tetanus toxoid carrier proteins, Vi-TCV induces a T-cell dependent response. It can therefore produce an immunogenic response in infants under 2 years of age and has the potential to generate durable immune response via induction of immunological memory.

A Phase III randomised controlled trial comparing Vi-TCV with Vi-PS demonstrated seroconversion to anti-Vi IgG in the 6 month to 2 year age group (24). Additionally, a comparison of the sub-groups receiving boosters of either vaccine at two years demonstrated significantly higher anti-Vi IgG titres in the Vi-TCV group compared to the Vi-PS group (titres of 1685.3 EU/ml [95% CI: 1468-1797] in Vi-TCV vs 445.6 EU/ml [95% CI: 323-615] in Vi-PS). Safety data from the same study demonstrated that Vi-TCV was well tolerated by all age groups and that there were no differences in the number or variety of adverse events reported between the vaccine arms (25).

Efficacy data are available from a recent study performed using the typhoid challenge model at the University of Oxford (26). This study measured the efficacy of single-dose Vi-TCV, Vi-PS or a control vaccine in protecting against the development of typhoid infection after oral challenge. The study was conducted in healthy, UK adult volunteers and challenge was performed 28 days after vaccination. Using a composite diagnostic endpoint of clinical and/or microbiologically confirmed typhoid fever, the Vi-TCV and Vi-PS vaccines demonstrated comparable protective efficacy of 54.6% [95% CI: 26.8 – 71.8%] and 52.0% [95%CI: 23.2-70.0%], respectively, when compared to the control vaccine (26). This calculated Vi-TCV vaccine efficacy of 54.6% likely underestimates the protective effect of Vi-TCV in endemic settings. When applying a definition of typhoid fever which more closely approximates diagnosis in health care settings, i.e. fever followed by confirmatory bacteraemia, in a post-hoc analysis the protective efficacy of Vi-TCV vaccine increased to 87.1% [95%CI: 44.2-96.9%] compared to 52.3% [95%CI: -4.2%, 78.2%] for the unconjugated Vi-PS vaccine (26).

While Tybar-TCV is licensed for use in India and Nepal, and the data from seroconversion and efficacy studies are strong, field impact studies for Vi-TCV, demonstrating a reduction in the burden of disease attributable to typhoid infections, have not yet been conducted.

4.2. Description of TyVAC

This Vi-TCV trial falls within a larger multi-institution collaboration, called The Typhoid Vaccine Acceleration Consortium (TyVAC). TyVAC is a Bill and Melinda Gates Foundation funded project to generate evidence for Vi-TCV vaccine impact, and accelerate use of Typhoid Conjugate Vaccines in countries with significant typhoid burden. Managed by University of Maryland, in collaboration with University of Oxford, and PATH international, the TyVAC programme includes vaccination trials, health economics studies, country preparedness support for routine vaccine introduction, and the collation and synthesis of typhoid research and evidence.

Three sites have been identified for parallel field impact studies; Kathmandu, Nepal; Dhaka, Bangladesh; and Blantyre, Malawi. Each represents a geographical setting where enteric fever is endemic and has a substantial local burden of disease. In each site, independent studies with differing study designs will be implemented to identify a range of impact scenarios. Between the sites, there is a range of demographic and geographic variation to give confidence in the generalisability of the study results. The trial presented here, is one of the three planned studies, which will be conducted in Kathmandu, Nepal.

4.3. Aim of the Project

This study aims to assess the impact of Vi-TCV in a field setting in order to inform and support the use of the vaccine as a control measure for enteric fever in endemic settings, and to reduce global morbidity and mortality from enteric fever. Vi-TCV has shown promise from existing studies; it can produce seroconversion in infants; it potentially produces long lasting immunity; and it is efficacious in a controlled challenge setting. As such, it is an obvious candidate to test in a field impact study.

4.4. Rationale for Kathmandu as the study site

Kathmandu is the selected trial site for the following reasons:

- Enteric fever is endemic to Nepal, with a high incidence in Kathmandu.
- Enteric fever is recognised locally as a public health concern, both within the Nepal Ministry of Health and the local community.
- The National Immunisation Technical Advisory Group (NITAG) has prioritised control of the disease;
- The Ministry of Health is receptive to impact studies and subsequent vaccination introduction;
- Bharat-Biotech's Vi-TCV (Tybar-TCV) is already licensed in Nepal;
- The Strategic Typhoid Alliance Across Africa and Asia (STRATAA), funded by Wellcome Trust and the Bill and Melinda Gates Foundation, is a typhoid surveillance study, already running in 15 wards (community districts) of Lalitpur, Kathmandu, allowing for lower costs and potential synergy (27);
- STRATAA has identified that there is a sufficiently large population of children aged 9 months to <16 years within which to conduct this vaccination trial.

4.5. Rationale for Study Design

A randomised controlled trial will be performed with a two-year follow-up to assess the protective impact of the Vi-TCV vaccine.

The study will assess the impact of vaccination of children aged 9 months to <16 years of age living in a geographically defined catchment area in Lalitpur, Kathmandu. This age range has been selected because children bear a substantial burden of the disease in both mortality and morbidity, without an effective vaccine available in the routine vaccination schedule. Therefore, this demographic group has most to gain from vaccination with the Vi-TCV and would be the primary target for any subsequent vaccination campaign.

4.5.1 Rationale for Intervention vaccine

As discussed above, the Vi-TCV (Typbar-TCV®) is the most promising vaccine candidate for control of typhoid in an endemic area for the following reasons:

- One dose schedule,
- Immunogenic in children,
- Potentially prolonged immunogenicity,
- Shown to have minimal side effects.

Vi-TCV is licensed for use in Nepal and has been submitted for WHO prequalification. While the vaccine is currently licensed in Nepal, it is not routinely available for use. The results of this randomised trial will be used to inform country decision making for introduction of Vi-TCV into the routine expanded programme on immunisation (EPI).

4.5.2 Rationale for control vaccine

In this trial, the control vaccine should have the following features:

- Identical administration regime to Vi-TCV i.e. one dose,
- No provision of any direct protection against enteric fever,
- Potentially provides some additional health benefit to the trial participants.

The Men A vaccine (MenAfriVac) is a vaccine against *Neisseria meningitidis* serogroup A and is not currently given routinely in Nepal. It is a single dose vaccine, licensed for use from 9 months of age, and provides protection against group A *N. meningitidis*, the most common serotype in Nepal.

4.6. Summary of the known and potential risks and benefits, if any, to human participants

4.6.1 Potential benefits to participants

All participants enrolled will have the benefit of receiving Vi-TCV (the intervention arm at the beginning of the study, and the control arm at the end of the study), which is likely to provide protection against typhoid. Additionally, the control vaccine can provide protection against group A meningitis, which, while not common in Nepal, can cause severe disease. There is no routine vaccination for typhoid or serogroup A meningitis in Nepal.

During the duration of the study, all participants will have access to free and accurate health assessments and diagnostics at the Patan hospital fever clinic, as well as at trial health clinics set up in the community,

for all episodes of fever occurring during the study. Participants will also have access to information about general health issues, through trial staff.

Additionally, the trial will help improve the understanding of the impact of the Vi-TCV vaccine on typhoid infection rates, and help guide future implementation of Vi-TCV vaccination programmes. Whilst participants would not necessarily benefit directly from this, a positive result from this trial could lead to implementation of a Vi-TCV vaccine programme in typhoid endemic areas, including Nepal. This would provide direct and indirect protection to both those close to the participant and those in the wider population.

4.6.2 Potential risks to the participants

The Vi-TCV vaccine has demonstrated a favourable safety record in the approximately 400 people vaccinated in a Phase III study (24) and the Oxford challenge study (26), with vaccination being well tolerated with no side effects above that shown by comparator Vi-PS vaccines. However, this is the first study involving so many participants and there may be rare adverse events hereto unidentified that may become apparent in this study. While it is not anticipated this to be the case, participants will be exposed to this potential risk. As a result, we will be conducting safety monitoring as a secondary outcome of this study.

5. OBJECTIVES AND OUTCOME MEASURES

	Objective	**Outcome Measure**
Primary	To determine the efficacy and rate reduction of the Vi-TCV in preventing blood culture-confirmed symptomatic infection caused by S. Typhi	The Incidence of blood culture confirmed typhoid fever in each of the vaccination arms.
Secondary	To investigate safety outcomes associated with Vi-TCV vaccination, within the study population	The proportion of participants developing all adverse events within the first 7 days post-vaccination, and serious adverse events within 6 months of vaccination, as determined through self-reporting at follow-up contact
	To determine the impact of vaccination with Vi-TCV on the incidence of inpatient/outpatient admission rates for fever	Rates of participants with at least ≥2 days of subjective persistent fever, or a temperature of 38 degrees C, at presentation, at Patan Hospital or trial clinics in each vaccination arm, stratified by duration and severity of fever

	To measure the difference in rates of hospital and clinic presentation for febrile illness in each vaccination arm	Rates of hospital or clinic presentation with febrile illness of any duration in each vaccination arm, measured by hospital presentation logs, hospital records, trial clinic records and self-reporting during three monthly follow-up
	To determine Paratyphoid infection rates in each vaccination arm	Rates of blood culture confirmed Paratyphoid cases in each vaccination arm
	To measure days spent in hospital from febrile illness	Length of stay in hospital, collected from Patan hospital patient records, and self-reported, in each vaccination arm
	To measure incidence of clinically-suspected enteric fever	Number of clinical diagnoses of typhoid fever, as determined by trial staff in Patan hospital outpatient clinics and trial clinic, in each vaccination arm.
Exploratory	To measure antibiotic/ antimicrobial use for inpatients/outpatients in each vaccination arm	Incidence of antibiotic/antimicrobial use in in/outpatient settings, from Patan hospital and trial clinic admission logs, and/or self-reported in follow-up contact
	To measure the difference in the duration of febrile illness in each vaccination arm	Duration of fever recorded in Patan hospital and trial clinics, and self-reported via follow-up contact
	To measure difference in rates of all-cause hospitalization	Rates of hospitalization, identified through hospital admission logs and self-reporting in each vaccination arm
	To determine effect of vaccination on child growth and weight in children <5 years of age	Measurement of anthropomorphic parameters of children at baseline and at two years, in all children aged <5 years at the time of vaccination
	To determine the immunogenicity of Vi-TCV in a subset of participants, stratified by age groups.	Assay of anti-Vi IgG antibodies in blood samples collected at baseline (Day 0) and at one month (Day 28) in a subset of participants (1000 in vaccine arm; 500 in control arm)
	To determine the persistence of antibodies induced by Vi-TCV in stratified age groups.	Assay of anti-Vi IgG antibodies in blood samples collected at baseline (day 0), 18 months (day 545), and two years (day 730) in a subset of participants (1000 in vaccine arm; 500 in control arm)

	To measure the incidence of fever that does not result in medical treatment in each vaccination arm	Self-reported fever at follow-up contact of participants.
	To determine rates of absenteeism from school/work in each vaccination arm	Rates of absenteeism from school or work, as applicable, as reported by parents at follow-up contact
	To measure all-cause mortality, and all-cause mortality with fever, in each vaccination arm	Rates and circumstances of mortality in each vaccination arm, recorded from hospital records and three-monthly follow-up
	To measure the rate of suspected and confirmed acute abdominal presentation in each vaccination arm	Rates of presentation to Patan hospital or trial clinics with acute abdomen
	To measure the rate of surgical intervention for acute abdominal complaints in each vaccination arm	Rates of acute abdomen surgery in each vaccination arm, and gross surgical findings

6. TRIAL DESIGN SUMMARY

6.1. Details of study design and procedure

This is a participant- and observer-blind, individually randomised study of the typhoid conjugate vaccine (Vi-TCV), brand name: Tybar-TCV, in Nepalese children. The population within a selected geographical catchment area of Lalitpur, Kathmandu, will be offered entry into the study. The aim is to enrol 20,000 children within the target age range (9 months to <16 years) residing in the target area. They will be randomised in a 1:1 ratio to receive Vi-TCV or control vaccine.

All participants will be contacted by telephone or in person seven (7) days after vaccination for follow-up and to record any adverse events following vaccination.

A subset of 1500 participants (1000 Vi-TCV, 500 control vaccine) will be randomised to have blood samples collected at baseline (D0), at D28, at 18 months (D545), and at two years (D730) post-vaccination to study immunogenicity.

All Children 9 months to <5 years of age at the time of enrolment will have their height and weight measured at baseline and at 2 years post-vaccination.

The parent/guardians of participants will be contacted every three months at days 90, 180, 270, 365, 455 (1 year 3 months), 545 (1 year 6 months), 635 (1 year 9 months), and 730 (2 years). This contact will consist of either a telephone call, or an in-person visit. These follow-up contacts will:

- Confirm that the participant is still willing to continue with the study,
- Ensure participant and family still lives in area
- Collect information about mortality and morbidity end points, including fever,
- Collect information about antibiotic use,
- Collect rates of school/work absenteeism (for both child and parent) in the last 3 months,
- Provide additional reminders to attend Patan Hospital or trial ward clinic sites if the participant develops fever of ≥2 days.

At two years after the initial vaccination campaign, the trial will end and all participants and trial staff will be unblinded. At this point, the control group will be offered vaccination with the Vi-TCV vaccine, and both control and intervention groups will be informed of their vaccination status and have their vaccines documented on the patient record.

Duration of participation is two years from enrolment.

The number of planned participant contacts will be as follows:

- Study participants, not enrolled in the immunogenicity sub-study: up to 11 (2 face to face, 8 to 9 separate follow-up contact)
- Subset of participants enrolled in the immunogenicity sub-study: up to 13 (4 face to face, 7 to 9 separate follow-up contact).

Patients presenting at healthcare facilities with persistent fever will have a blood sample taken for conformation of diagnosis. The samples will be processed by Patan Hospital Microbiology Laboratory and other microbiology laboratories with appropriate blood culture facilities over the two-year period of participant follow-up. Results of these blood samples will be obtained to acsertain the number of blood-culture positive cases of *S.* Typhi in the trial cohort. Participants presenting at Patan hospital with fever

will be directed to the Fever Clinic at Patan Hospital, and their data will be collected. Participants presenting at the trial ward clinics will be seen by a clinican and nurse and their data will be collected.

A trial flow chart and trial schedule can be seen in Appendix 1 and Appendix 2.

7. PARTICIPANT IDENTIFICATION

7.1. Trial participants

Children aged 9 months to <16 years (i.e., up to 15 years 364 days) who are in good health at the time of enrolment will be eligible to participate in this study. Participants will be identified as living within the defined catchment area of Lalitpur, Kathmandu, Nepal. Trial staff, including local community health volunteers, will identify households with children <16 years of age and approach them for participation.

7.2. Inclusion Criteria

The participant must satisfy all the following criteria to be eligible for enrolment:

- Parent/legal guardian is willing and competent to provide informed consent. If the participant is 12 years of age or older, assent will also be sought,
- Aged between 9 months (or eligible for measles vaccination according to local protocol) and <16 years (i.e. up to 15 years 364 days) at time of vaccination,
- In good health on the day of vaccination,
- Parent/legal guardian confirms that their child will be wiling and be able to comply with study requirements including follow-up contact, according the schedule (Appendix B),
- Live within the study catchment area at the time of vaccination.

7.3. Exclusion Criteria

The participant will not be enrolled if any of the following criteria apply:

- They have knowingly received a typhoid vaccine in the last three years,
- They have a known allergy to any of the vaccine components,
- Any medical or social reasons that will prevent the participant from conforming to the study requirements as judged by a medical professional,
- They are planning to move away from the catchment area within the next six months

7.4. Temporary exclusion criteria

Participants will be temporarily excluded from being vaccinated if, at point of vaccination, any of the following apply:

- Reported fever within 24 hours prior to vaccination,
- Use of anti-pyretics within 4 hours prior to vaccination.

If these apply, the participant will be temporarily excluded for vaccination until 48 hours has passed. A re-assessment will be conducted to ensure these temporary exclusion criteria no longer exist.

7.5. Withdrawal of Patients/Subjects

Participants' parents/legal guardians can withdraw consent at any point. The Investigator may also discontinue a participant from the trial at any time if the Investigator considers it necessary for any reason including:

- Ineligibility (either arising during the trial or retrospectively having been overlooked at screening),
- Significant protocol deviation,
- Significant non-compliance with trial requirements,
- An adverse event or disease progression resulting in the inability to continue to comply with trial procedures and follow-up,
- Loss to follow up.

Withdrawal will result in cessation of any follow-up calls, visits, or blood tests (as applicable to the subset). Once vaccines have been administered no further treatment is required in the study, so no additional action will need to take place. Participants' parents/legal guardians will have the choice when withdrawing, to withdraw from active study procedures only (follow-up calls and visits) but remain in the passive surveillance for the primary outcome, (allowing us to access their hospital records and blood test results), or withdraw from all study contact. In the case of a participant withdrawing from all study contact, we will not collect any further data of hospital presentation or blood culture results. Data and blood samples collected prior to the time of participant withdrawal will be kept and analysed as part of the study data. A participant who withdraws from the study has the option to re-engage at a future date if they choose to do so. All participants who withdraw from the study will be given information on how to re-engage with the study if they so choose. Reasons for withdrawal from the study, if known, will be recorded in the participants CRF.

8. TRIAL PROCEDURES

8.1. Recruitment

Potential participants living in Lalitpur, Kathmandu will be identified. Trial staff, already embedded within each catchment area, including community health volunteers, will systematically approach each household in the area to identify households with children aged 9 months to <16 years (i.e. up to 15 years 364 days). Identified children will be screened as per the process below.

8.2. Screening and Eligibility Assessment

Once a household which includes a potential participant is identified, the parent/legal guardian of the child will be given basic information about the trial and invited to attend the nearest study clinic if they are interested. After arrival at the study clinic, screening according to inclusion/exclusion criteria will occur. Eligible participants will then have informed consent/assent obtained. After consent is taken, a medical examination, including temperature for temporary exclusion criteria, will be conducted. Participants with a temporary exclusion criterion identified after the medical assessment will be informed of the reason and asked to return in ≥48 hours for repeat assessment and re-consent.

8.3. Informed Consent

The parent/legal guardian must personally sign and date the latest approved version of the Informed Consent form before any trial specific procedures are performed. Assent will also be sought from children 12 years of age or older, to participate in the trial.

Written and verbal versions of the Participant Information and Informed Consent/Assent will be presented to the participants and their parent/legal guardian in the local language detailing no less than: the exact nature of the trial; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participants' parent/legal guardian is free to withdraw their child from the trial at any time for any reason without prejudice to future care, without affecting their legal rights and with no obligation to give the reason for withdrawal. Participants will also be informed that they can choose to have their remaining blood samples destroyed and not maintained for future analysis at the end of the trial.

The participant and their parents/legal guardians will be allowed as much time as they wish to consider the information, within the recruitment period until 20,000 children are enrolled, and the opportunity to question the Investigator or other independent parties to decide whether they will participate in the trial. Written Informed Consent/Assent will then be obtained, with additional opt-in consents to be randomised for blood sampling and to be contacted at the end of the trial, by means of participants' parent/legal guardians dated signature or thumbprint and dated signature of the person who presented and obtained the Informed Consent. The person obtaining consent will record assent on the same consent form, for children aged 12 and older. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the participant. The original signed form will be retained at the trial site.

If the parent/guardian is illiterate then a third party may act as a witness for the parent/guardian to attest that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the parent/guardian and that informed consent was freely given by the parent/guardian. In this event, the witness will also sign and date the consent form.

8.4. Randomisation and blinding

8.4.1. Randomisation

Computer generated randomisation lists will be prepared by the study statistician. Randomisation lists will allocate participants to receive either Vi-TCV or the control vaccine on a 1:1 basis using stratified block randomisation with randomly varying block sizes from 6-12. Stratification will be by age (9 months to <5 years old or >= 5 years old to <16 years). Participant randomisation will occur at the vaccination visit, using the pre-prepared randomisation lists. It will be performed by the staff member after collection of baseline data during the same visit.

In addition, a separate randomisation list will allocate 1500 individuals to blood sampling at D0, D28, 18 months, and at 2 years for the immunogenicity study. Blood sampling randomisation will be on a 2:1 basis (1000 Vi-TCV: 500 control) and be age stratified (< 5 years and >= 5 years).

The result of participant consent, and randomisation into both the vaccine trial and immunogenicity sub-study (the blinded trial randomisation ID number), will be directly entered onto the CRF.

Participants and parents will not be informed which vaccine was administered at this time.

8.4.2 Blinding

Vaccine allocation will be observer- and parent/guardian/participant-blind, with only the vaccinating trial staff aware of which vaccination has been given. To maintain observer blinding these staff members will not be involved in subsequent study procedures in wards in which they performed vaccinations until such time as study unblinding occurs. Efforts will be made to conceal vaccination preparation and vaccine supplies from participants and their families to maintain blinding.

Circumstances may arise where unblinding is needed prior to the end of the study e.g. occurrence of a SUSAR or requirement of a medical intervention that would be influenced by knowledge of which vaccine the individual has received. In such circumstances the local principal investigator will be contacted to discuss whether urgent unblinding is warranted. If it is deemed necessary, unblinding will occur based on the recommendation of the local PI. Any event of unblinding will be fully documented in the CRF.

8.5. Vaccination Visit

Potential participants will attend the study clinic situated within each ward. After potential participants have considered the PIS, and decided to participant, inclusion/exclusion will be assessed, and written informed consent will be formally obtained with the parent/legal guardian of the participant, and assent will be sought from the eligible subjects 12 years of age or older. Basic medical history will be taken and temporary exclusion will then be checked, and if fever is identified, the participant will be asked to return in >48 hours for re-affirmation of consent.

Once consented, a member of the study team will collect demographic information (including age and address) and participant contact details. Height/length and weight will be collected for all children <5 years of age. The participant will then be allocated via the randomisation lists, described above, to one of the study arms, and if they consented, to the immunogenicity study. If the participant is randomised to immunogenicity study, a blood sample will be taken by a suitably trained staff member at the clinic.

All details will be recorded in the CRF.

Based on the randomisation, the appropriate assigned vaccine will be administered by a trained member of the study team. The site of vaccination (right or left arm or thigh) will be recorded. The participant will be considered enrolled into the study at the point that any medical procedure takes place (i.e. blood sample taken or vaccination administered).

A study card containing the name of the study, and contact details for the study team will be given to parents/legal guardians to call if they have any concerns, or if their child is admitted to hospital at any time during the duration of the study. The card will also contain instructions to attend Patan Hospital fever clinic or the study clinics in the wards, if the participant develops prolonged fever (≥2 days) at any time over the next two years.

Parents/legal guardians of participants allocated to the immunogenicity study will be given details of the follow-up appointments at D28 and D545.

8.6. Subsequent Follow-up and Visits

See Appendix A and B for trial flow chart and visit timelines.

8.6.1 Adverse Events Follow-up (Day 7, -1/+7)

Follow-up contact at 7 days post-vaccination will collect parent/legal guardian-reported information on adverse events following immunisation (AEFIs).

Information collected will include:

- Verbal reconfirmation of consent for participation in the trial,
- Report from parent/legal guardian of adverse events related to vaccination, including: pain, swelling, fever, etc., (see section 10 of the protocol for action in the event of these occurring) and use of medications following vaccination.
- Reiteration of contact details and instructions to attend Patan Hospital / Study clinic in the case of a prolonged fever (≥2 days)

8.6.2 Standard Follow-up

There will be routine participant follow-up via telephone or face-to-face contact every three months for the two-year duration of participation in the trial, for all participants enrolled in the trial. (See Appendix B for timelines.) A brief interview will be conducted by trial staff at each follow-up contact.

These interviews will collect parent/legal gaurdian-reported participant information, including:

- Confirmation that the parent/legal guardian of the participant is still willing to continue with the study,
- Ensure participant and family still lives in area,
- A record of mortality and morbidity end points, including:
    - Mortality,
    - fever occurrence and duration with and without treatment seeking behavior,
    - use of antibiotics,
    - visits to clinics, hospitals, or pharmacies (and records requested if they attended a facility other than the study sites),
- Rates of school/work absenteeism (for both child and parent) in the last 3 months,
- A reminder to attend designated health care facilities if they develop fever of ≥2 days.

The results from this follow-up contact will be recorded in the participant's CRF. Appropriately qualified trial staff within the ward clinics will review participant reports of febrile illnesses, documented during follow-up contact to make a clinical judgement of suspected typhoid, especially for those cases that did not result in medical treatment.

Text messages will be sent regularly to all participants' parents/guardians to remind all participants to visit Patan hospital or study clinics for fever of ≥2 days, for free treatment, confirmation of typhoid infection, and trial follow-up.

8.6.3 Immunogenicity Study

The subset of participants enrolled in the immunogenicity substudy will receive three additional face-to-face follow-up visits at their nearest trial clinic 28 days (D28+/-4 days), 18 months (D545 +/-56 days), and at two years (D730+/-90 days) after receiving vaccination. At these additional visits the following procedures will be performed:

- Confirmation of continued participation in the study,
- Draw of ~5ml blood for transport to, and processing at, the Patan Hospital Microbiology Laboratory,

- Confirmation of contact details and reiteration of instructions to attend Patan Hospital / trial ward clinic in the case of a prolonged fever ≥2 days (to be performed at the D28 and D545 visits only).

Effort will be made to perform the 18 month blood draw and the 18 month follow-up contact simultaneously, so as not to greatly increase the time burden of participants. The blood draw at two years post-vaccination will be performed at the same time as the study unblinding and vaccination recording.

The above information will be recorded in the participant's CRF.

8.6.4 Participant Presentation with Fever

Participants enrolled to this study who experience a fever lasting for ≥2 days, as subjectively reported by a parent/guardian, will be asked to present to Patan Hospital or trial clinics. At the hospital or clinic, medically trained staff will assess the participant. Details of this assessment, including measurement of temperature, will be recorded and the participant will receive routine standard of care management and treatment, as deemed appropriate by the assessing member of staff. Trial staff will collect information about the participant illness episode and record it in the CRF. This will include information such as temperature and duration, hospital admission, surgeries, and antibiotics prescribed, etc. The only additional study related procedure performed at this visit will be collection of an additional ~5mL blood sample, if typhoid is suspected.

Laboratory blood-culture confirmed typhoid cases will be followed-up approximately two weeks after presentation with fever, to record outcome of illness and resolution. If the illness is not resolved at that time of follow-up, an additional follow-up will occur four weeks later (6 weeks after initial presentation with fever) to record outcome of illness and resolution, including any use of antibiotics.

See Appendix C for flow chart of unscheduled procedures.

8.7. Sample Handling

Blood samples to confirm typhoid infections:

Blood samples drawn in the Patan Hospital for diagnosis and confirmation of suspected typhoid fever will be handled, stored, processed, in accordance with standard operating procedures of Patan Hospital Microbiology Laboratory. The results of these tests will be recorded in the participant CRF for use in this study.

Remaining samples will be stored at Patan Hospital for further analysis after the conclusion of the trial.

Immunogenicity study samples:

Blood samples taken for the immunogenicity study will be transported to Patan Hospital daily where they will be processed and stored by trained study staff, in accordance with standard operating procedures (SOP).

Plasma will be stored for investigation of novel diagnostic markers, for example, indications of an acute serological response indicating recent typhoid exposure, or identification of a metabolomics signature compatible with infection. DNA will also be stored, with consent, for investigation of the genetic control of immunity to vaccines and susceptibility to infectious diseases like typhoid.

The primary laboratory technique performed will be anti-Vi IgG antibody ELISA performed on the extracted plasma sample, using a commercially available assay (Vacczyme Binding Site, or other comparable assay). This assay will be performed according to the manufacturer's instructions.

As some laboratory processes are currently not available in Nepal, the samples will be shipped to laboratories in Asia, Europe, and/or the US, as necessary, for performance of assays that are unavailable in Nepal. When sample volumes are sufficient to not compromise the ability to perform necessary laboratory procedures, samples may be split so that one set of samples can be retained in Nepal, and a second set of samples can be shipped abroad. At the end of the trial, all remaining samples, in Nepal, and abroad, will be kept for future analysis.

8.8. Discontinuation/Withdrawal of Participants from Trial Treatment

Trial treatment consists of a single vaccination received at the point of enrolment into the study. It is not possible to withdraw from trial treatment after vaccination. For details of participant withdrawal from study procedures and follow-up, see section 7.5.

8.9. Catch-up vaccination

At the two-year follow-up visit, all participants will be informed which vaccination they received, and their vaccination records will be updated to reflect this. At this point, the study will become unblinded. Control vaccine participants will be invited to receive the Vi-TCV vaccine. Individuals accepting this offer will have the Vi-TCV vaccine administered by a trained member of the study team.

All participants receiving the catch-up typhoid vaccination will be given information for reporting any adverse events occurring in the week following vaccination.

8.10. Definition of End of Trial

The end of trial is the date that the last sample is processed for the purposes of this study.

9. INVESTIGATIONAL MEDICINAL PRODUCT (IMP)

9.1. IMP Description

Trial treatment

Vi polysaccharide-tetanus toxoid conjugate vaccine (Vi-TCV). Trade name: Typbar-TCV®, Bharat-Biotech.

Each 0.5ml vaccine dose contains:

- Purified Vi-Capsular Polysaccharide of *S.* Typhi Ty2 conjugated to Tetanus Toxoid 25µg
- Sodium chloride 4.5 mg
- Water for Injection q.s. to 0.5ml

The vaccine is packaged as a pre-filled 2.5ml 5-dose vial. It will be administered as an intramuscular injection in the antero-lateral thigh for younger children, or the upper arm for older children, according to local protocols.

Control vaccine

Meningococcal Group A conjugate vaccine Licensed trade name: MenAfriVac™, Serum Institute of India PVT Ltd. This vaccine is produced in two formulations; a standard 10µg/0.5 ml dose for individuals aged ≥1 year of age; and a 5µg/0.5ml single dose for individuals aged 9 – 24 months.

MenAfriVac™ is provided as a 10-dose presentation consisting of a vial and an ampoule. Each vial contains a lyophilised powder of meningococcal group A polysaccharide conjugated to tetanus toxoid protein and excipients. Each 5ml ampoule of diluent contains:

- Aluminium phosphate (≤1.25mg per single human dose) and
- Thimerosal (0.01%)

The lypohilised conjugate is reconstituted just before use with the contents of one ampoule of diluent to obtain 10 doses of the final vaccine in a white homogeneous suspension. A single dose of vaccine is equivalent to 0.5ml of the reconstituted suspension, with the doses as above depending on the formulation.

The vaccine will be administered as a deep intramuscular injection in the antero-lateral thigh for younger children, or the upper arm for older children, according to local protocols.

9.1.1 Labelling

The vaccines will be labelled by the manufacturer and not be relabelled.

9.1.2 Supply

The Vi-TCV vaccine (Typbar-TCV®) will be provided by Bharat-Biotech. The Men A control vaccine (MenAfriVac ®) will be provided by the Serum Institute of India. The Study vaccines will be shipped to the Logistics Department at the Ministry of Health in Kathmandu before distribution to Patan Hospital.

9.2. Storage of IMP

Both the intervention vaccine (TypBar), and the control vaccine (MenAfriVac) will be stored according to manufacturer specifications, at at 2° to 8° C (35 ° to 46 ° F), in a temperature monitored refrigerator at Patan Hospital, Nepal, when not in use for the daily activities. When in use for vaccination days, both vaccines will be stored in temperature monitored refrigerators or cold boxes. Fridge temperatures and cold chain transport will be audited during the vaccination campaign to ensure they are within range.

Both the intervention and control vaccine vials will be labelled with a "vaccine vial monitor"; a temperature-sensitive dot that provides an indication of the cumulative heat to which the vial has been exposed. It warns the end user when exposure to heat is likely to have degraded the vaccine beyond an acceptable level and should not be used.

Vi-TCV (TypBar)

The intervention vaccine is presented in 5 dose vials of active vaccine, ready for administration. The vaccine will be stored at 2° to 8° C, as described above. The vaccine should not be frozen, and if it has been, it should be discarded. Opened vials should be discarded 6 hours after opening.

MenA (MenAfriVac™)

The control vaccine is presented in 10 dose vials of active vaccine, and 10 dose ampoules of diluent. The vaccine will be stored at 2° to 8° C, as described above, and protected from light. The vaccine is stable and can be used when exposed up to 40°C for a period of 4 days immediately prior to reconstitution. Open

vaccine vials will be discarded 6 hours after opening. The diluent will be stored at room temperature, according to manufacturer specification, in a clinical area. The reconstituted vaccine should be protected from direct sunlight.

9.3. Accountability of the Trial Treatment

The vaccines will be shipped to a central storage facility in Nepal and passed through customs. They will then be transported to Patan Hospital and distributed to local clinics whilst maintaining the cold-chain (aiming for temperature between 2-8^{o}C), with the exception of the Meningococcal A vaccine diluent which will be kept at 25^{o}C.

The number of doses of study vaccines that are received, used and wasted will be documented daily during the trial and checked weekly.

Unused vaccines at the end of the trial may be retained for laboratory use only (such as laboratory assay development). Any recall of study vaccines required for use in the study or reporting of defective vaccines will be performed according to trial SOPs.

10. SAFETY REPORTING

10.1. Definitions

Below are the various categories of Adverse Events Following Immunization (AEFIs).

Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect.

	Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out: • in the case of a product with a marketing authorisation, in the summary of product characteristics (SmPC) for that product • in the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the trial in question.

NB: to avoid confusion or misunderstanding of the difference between the terms "serious" and "severe", the following note of clarification is provided: "Severe" is often used to describe intensity of a specific event, which may be of relatively minor medical significance. "Seriousness" is the regulatory definition supplied above.

Brighton Collaboration case definitions for anticipated outcomes will be used to standardise the identification and reporting of all AEFIs.

Any pregnancy occurring during the clinical trial and the outcome of the pregnancy should be recorded and followed up for congenital abnormality or birth defect, at which point it would fall within the definition of "serious".

A flow chart for AEFI recording and reporting can be seen in Appendix D. Descriptions of these procedures are listed below (sections 10.3 and 10.4)

10.2. Causality

A medically qualified individual must determine the relationship of each adverse event to the trial medication according to the following definitions:

Related: The adverse event follows a reasonable temporal sequence from trial medication administration. It cannot reasonably be attributed to any other cause.

Not Related: The adverse event is probably produced by the participant's clinical state or by other modes of therapy administered to the participant.

10.3. Procedures for Recording Adverse Events

From vaccination through day 7

All adverse events related to vaccination (ARs), as judged by a medically qualified investigator or the Sponsor, occurring during the first 7 days post vaccination that are observed by the Investigator or reported by the participants parent/legal guardian, will be recorded on the CRF.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to trial medication, and action taken. Follow-up information should be provided as necessary.

The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

All Serious Adverse Events (SAEs) observed by the Investigator, members of the study team or reported by the parent/guardian will be recorded on the CRF.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to trial medication, other suspect drug or device and action taken. Follow-up information should be provided as necessary.

Day 8 – 6 months

Serious Adverse Events (SAEs) observed by the Investigator, members of the study team or reported by the parent/guardian, will be recorded on the CRF.
The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to trial medication, and action taken. Follow-up information should be provided as necessary.

6 Months through end of trial
Serious Adverse Reactions (SARs), as judged by a medically qualified investigator, observed by the Investigator, members of the study team or reported by the parent/guardian, will be recorded on the CRF.
The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to trial medication, and action taken. Follow-up information should be provided as necessary.

All mortality occurring during the duration of the trial will be recorded in the CRF, and investigated by medically qualified trial staff.

Follow-up post Adverse Event
All adverse events recorded in the CRFs, as described above, for the duration of the study, from first vaccination until trial completion, will be followed by a medically qualified investigator either until resolution, or until the event is considered stable.

10.4. Reporting Procedures for Adverse Events

As per Nepal's immunisation reporting requirements all documented minor local and systemic adverse events following immunisation (AEFI), occurring within the first 7 days after vaccination, will be reported

monthly to the Lalitpur District Public Health Office (DPHO) as part of the routine report submitted by Patan Hospital. Under the guidelines minor AEFIs in this instance are defined as:

1. Local reactions - pain, swelling and redness at injection site
2. Systemic reactions - fever, irritability, malaise.

Nepal has its own system and committee for monitoring AEFIs as well as detailed reporting forms. For this study, these forms will be used for reporting all SAEs/SARs occurring during this study. English versions of these forms will be completed to ensure all Investigators are fully informed.

All Adverse events occurring within one week after vaccination will be reported to Patan Hospital, as per the immunisation reporting system described above.

All SAEs occurring within the first 30 days of vaccine administration, and SARs occurring until the end of the trial, must be reported on the English version of the AEFI reporting form titled 'AEFI form-Annex-I' to the Data Safety Monitoring Board (DSMB), the Nepali AEFI committee, the Principal Investigator (PI) in Nepal, the Chief Investigator in Oxford within 24 hours of the Site Study Team becoming aware of the event. A more detailed report form; the English version, titled 'AEFI form- Annex 2' should be completed and sent, within 48 hours of the initial report to the Principal Investigator (PI) in Nepal, and the Chief Investigator in Oxford and the other study investigators. Additional and further requested information (follow-up or corrections to the original case) will be detailed on a clean 'AEFI Form – Annex 2'. All SAEs must be reported to the trial sponsor (University of Oxford) within 7 days.

The more detailed AEFI form – Annex 2 (Nepali version) can be completed by a member of the study team and reviewed with either a member of the AEFI Committee, or their representative, within 48 hours of the initial report sent by the site study team. All reported SAEs would be reviewed at the next AEFI committee meeting. All AEFI forms must be emailed to the AEFI committee. The trial will also maintain records of reports and receipt of report.

SAEs will also be reported to the Nepal and Oxford RECs in the Annual Progress Reports.

10.5. Expectedness

Expectedness will be determined according to the Summary of Product Characteristics.

10.6. SUSAR Reporting

All SUSARs will be reported by the Nepal and Oxford CI's to the relevant Competent Authority and to the REC and other parties as applicable. For fatal and life-threatening SUSARS, this will be done no later than 7 calendar days after the Sponsor or delegate is first aware of the reaction. Any additional relevant information will be reported within 8 calendar days of the initial report. All other SUSARs will be reported within 15 calendar days.

Principal Investigators will be informed of all SUSARs for the relevant IMP for all studies with the same Sponsor or funder, investigating the same IMP, whether or not the event occurred in the current trial.

10.7. Safety Monitoring Committee

An independent safety monitoring committee will be assembled to oversee data safety monitoring for the TyVAC typhoid vaccine trials conducted in Nepal, Bangladesh and Malawi. This committee will include physician representatives from each country, as well as an independent statistician.

10.8. Development Safety Update Reports

A pharmacovigilance safety report will be submitted, annually in accordance with the Nepal regulations and guidelines.

11. STATISTICS

11.1. Description of Statistical Methods

The primary endpoint will be blood culture positive typhoid fever obtained from enhanced passive surveillance in Patan hospital and study clinics.

The incidence of typhoid will be estimated as the number of cases divided by the total number of person-years of follow up. Incidence will be presented with 95% confidence intervals for each group and overall. Incidence rate ratio (IRR) will be computed as the ratio of the incidence in the Vi-TCV arm compared to the control arm.

Vaccine efficacy (VE) will be calculated as (1 – IRR) x 100%, where IRR is the incidence rate ratio (Vi-TCV: control).

The cumulative incidence of typhoid will be summarised using the Kaplan-Meier method. Participants will be censored in the analysis at the time of last known residence in or near the surveillance area, at the last known contact time or at the 2 year final visit. Statistical significance will be determined as a p value from a log-rank test of less than 0.05.

Subgroup analyses will include:

- age (< 5 years and >= 5 years)
- age (< 2 years and >= 2 years)
- male vs female

A fully detailed statistical analysis plan will be prepared and signed off by the Chief Investigator prior to conducting any data analyses.

11.2. The Number of Participants

Sample size calculations are based on the following assumptions:

1. An overall incidence of typhoid fever of 85 cases per year, per 100,000 persons in the entire population, with higher incidence rates in children under 16 years.
2. Age specific incidence rates were determined from the age distribution of typhoid cases, which is specific to Kathmandu, from published estimates and from site specific surveillance data.

3. A direct effect of vaccination of 75% and an indirect effect of 25% based on mathematical modelling.
4. 25% loss to follow up per year due to moving out of the area, based on current surveillance data from Patan.

Table 2. Sample Sizes under Varying Assumptions in Nepal (80% power, 5% alpha).

Overall incidence in the entire population without vaccination Per 100,000py	Direct effect of vaccine	Total number to enroll
100 cases py	80%	12,475
85 cases py	80%	14,677
100 cases py	75%	14,785
85 cases py	75%	17,395

The above assumptions are conservative; however, to allow for further variation in the assumptions, the total sample size has been increased to 20,000 children (10,000 in each vaccination arm).

Over the two year follow-up of the trial it is expected to see approximately 36 cases of typhoid in the control arm and 9 cases in the Vi-TCV arm.

Investigation of safety outcomes:

If the background rate of a specific rare but serious adverse event (SAE) is ~30/100,000 individuals, (as seen with intussusception in the rotavirus vaccine trials), this study has 80% power to detect a five-fold increase in this SAE.

11.3. Procedure for Accounting for Missing, Unused, and Spurious Data.

All available data will be included in the analysis

11.4. Inclusion in Analysis

All vaccinated participants will be included in the analysis.

11.5. Procedures for Reporting any Deviation(s) from the Original Statistical Plan

A detailed statistical analysis plan will be prepared and signed off by the Chief Investigator prior to unblinding of final study data.

12. DATA MANAGEMENT

12.1. Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in confidential conditions. On all trial-specific documents, other than the signed consent, the participant will be referred to by the trial participant number/code, not by name.

12.2. Access to Data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

12.3. Data Recording and Record Keeping

Hand-held tablets will be used to collect and record all data in trial CRFs. All CRF and randomisation data will be collected off-line and uploaded to a secure server on a regular basis, when tablets are brought back to the central office, and reliable internet is available.

CRFs will be designed and maintained on REDCap, a secure web application for building and managing online surveys and databases. REDCap will be validated by data management and IT staff within Oxford University. The CRFs will be designed and maintained by a dedicated trail data manager, and quality control checks will be performed on a regular basis.

All participants will be identified by a unique trial specific number and/or codes, this will not include any identifiable information. Individual names, addresses, and any other identifying detail will not be included in any trial data electronic file. CRFs will capture participant medical information from Patan hospital and trial clinic records, including but not limited to type of illness, severity, duration of illness, and treatment prescribed. Blood culture confirmed typhoid infections will be recorded in the CRFs. The results of three-monthly follow-up contact will be captured in the CRFs, including but not limited to previous illnesses occurring since last contact with or without medical treatment, and type of treatment sought, if any.

Trial staff will have access to REDCap via unique usernames and passwords. Each trial staff member will have an appropriate level of access to CRFs and collected data, according to their roles and responsibilities within the trial.

All participant data will be stored and maintained on servers within Nepal for the duration of the trial. Anonymised data will be uploaded to a central database in the UK. At the end of the trial, all individually identifiably data will be removed, and fully anonymised data will be retained for further analysis.

13. QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures.

Monitoring will be performed by representatives of the sponsor and according to the principles of ICH GCP. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following a risk based monitoring plan, the monitors will verify that the clinical study is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

14. SERIOUS BREACHES

A serious breach is defined as "A breach of GCP or the trial protocol which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the subjects of the trial; or

(b) the scientific value of the trial".

In the event that a serious breach is suspected the Sponsor must be contacted within one (1) working day. In collaboration with the CI, the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee and Regulatory authority within seven (7) calendar days.

15. ETHICAL AND REGULATORY CONSIDERATIONS

15.1. Declaration of Helsinki

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki.

15.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this trial is conducted in accordance with relevant regulations and with Good Clinical Practice.

15.3. Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to the appropriate Research Ethics Committees (REC) and regulatory authorities in Nepal and Oxford and Patan Hospital for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

15.4. Reporting

The CI shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the RECs (Nepal and Oxford), host organisation and Sponsor. In addition, an End of Trial notification and final report will be submitted to the RECs, host organisation and Sponsor.

15.5. Participant Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant ID number on all trial documents and any electronic database. All documents will be stored securely and will only be accessible by trial staff and authorised personnel. The trial will comply with the UK Data Protection Act, which requires data to be anonymised as soon as it is practical to do so and local regulations.

15.6. Expenses and Benefits

There will not be any payments or reimbursements made to participants, as incentive for participant recruitment. It is anticipated that provision of vaccination will be enough incentive to reach the necessary

sample size. Local hospitals, clinics, and vaccination points are being used to deliver all trial components, which will not add additional travel or expense to participants and their families.

The trial will cover the costs of standard care treatment for participants presenting with fever (≥2 days) as part of the trial, including the cost of test, antibiotics and/or other prescribed medications, and in-patient hospital stays and care, if medically necessary.

If participant presentation to hospital and ward trial clinics is less than expected, community health volunteers working in the trial area may be provided nominal incentives for referring participants with febrile illness to the trial health clinics and Patan hospital.

15.7. Other Ethical Considerations

All efforts will be made to conduct the research in a way that is sensitive to the Nepali culture and the social values. Nepali trial staff will be present at all times during the consent process, and the participant study related materials (information sheet, consent forms, etc) will be printed in Nepali.

Children aged 9 months – 15 years have been selected because children bear a substantial burden of the disease in both mortality and morbidity, without an effective vaccine available. Therefore, this demographic group has most to gain from vaccination with the Vi-TCV and would be the primary target for any subsequent vaccination campaign.

The meningococcal group A vaccine was selected as the control vaccine to ensure that the control group is receiving a beneficial intervention. The control vaccine will provide protection against group A meningitis, which is currently the most common serotype in Nepal, and can cause severe disease.

Samples and data collected may be shared with other researchers in Europe, Asia, and/or America, as some of the assays and analyses cannot be done in Nepal. Only anonymised samples and data will be sent outside of the research site. At the end of the study, all remaining samples in Nepal will be kept for storage in the Patan Hospital Microbiology Laboratory, as required by the Nepal Health Research Council (NHRC). All remaining samples overseas will be kept for storage under the oversight of Oxford University. All samples will be kept for a minimum of 10 years after the end of the trial. New and better tests may become available in the future. Storage of these samples may also allow important future research to be done without needing to take new samples from Nepalese children.

Potential participants or their parents/legal guardians will be notified that they will be able to refuse to have the relevant biological samples stored, without this otherwise influencing participation in the study or the clinical care of their child. They will also be informed that should they no longer wish for their samples to be retained they may request their destruction.

16. FINANCE AND INSURANCE

16.1. Funding

This study is funded by the Bill and Melinda Gates Foundation to the University of Maryland, Baltimore, with the University of Oxford as a collaborating institution.

16.2. Insurance

The University of Oxford has a specialist insurance policy in place that would operate in the event of any participant suffering harm as a result of their involvement in the research.

17. PUBLICATION POLICY

The investigators will co-ordinate dissemination of data from this study. All publications, including manuscripts, abstracts, oral/slide presentations, and book chapters, etc., based on data from this study will be reviewed by each sub-investigator prior to submission. Authors will acknowledge that the study was funded by BMGF. In accordance with BMGF, all publications related to this study will be open access. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

18. REFERENCES

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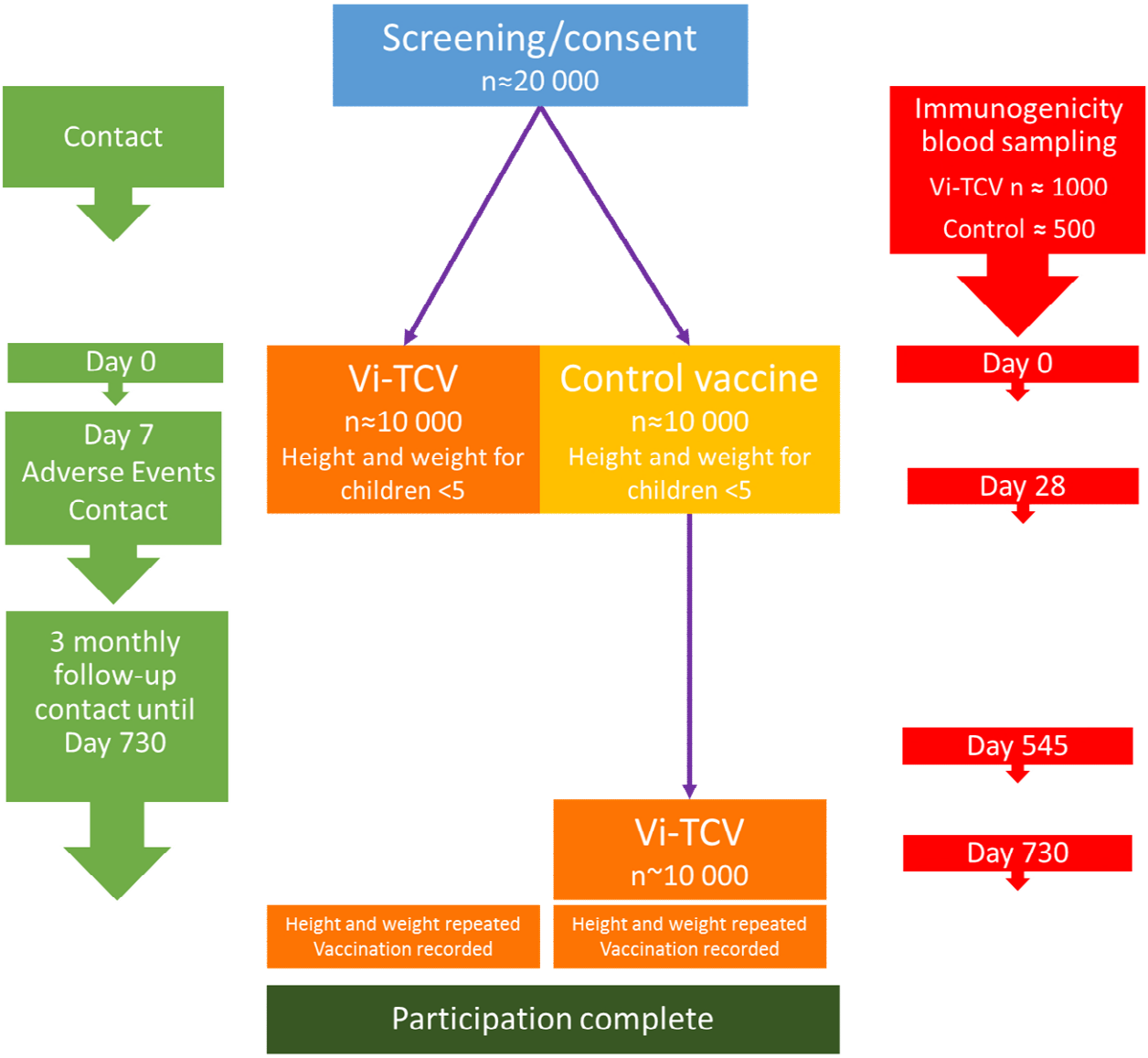
19. APPENDIX A: TRIAL FLOW CHART FOR PLANNED CONTACT



20. APPENDIX B: SCHEDULE OF PLANNED PROCEDURES

Table 1: Visit and sample schedule

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	X										
Randomisation	X										
Vaccination	X										
Medical history and exam	X										
Blood collection (b)	X		X						X		X
Height and weight (c)	X										X
Follow-up contact (d)		X**		X	X	X	X	X	X	X	X
Control group - vaccination with Vi-TCV(e)											X
Documentation of vaccine receipt(f)	X										X

a) Ideally, all of the visit 11 activities will occur simultaneously, but the follow-up contact may occur separately, if necessary

b) blood sampling for immunogenicity in a subset of 1000 Vi-TCV, 500 control participants. Planned blood draw = ~5 ml per visit. Total maximum volume ~20ml per participant
   - Blood draw on day 0 will occur before vaccination; blood draw at day 545 will aim to occur at the same time as follow-up; blood draw at day 730 will occur at some time as unblinding and vaccine documentation

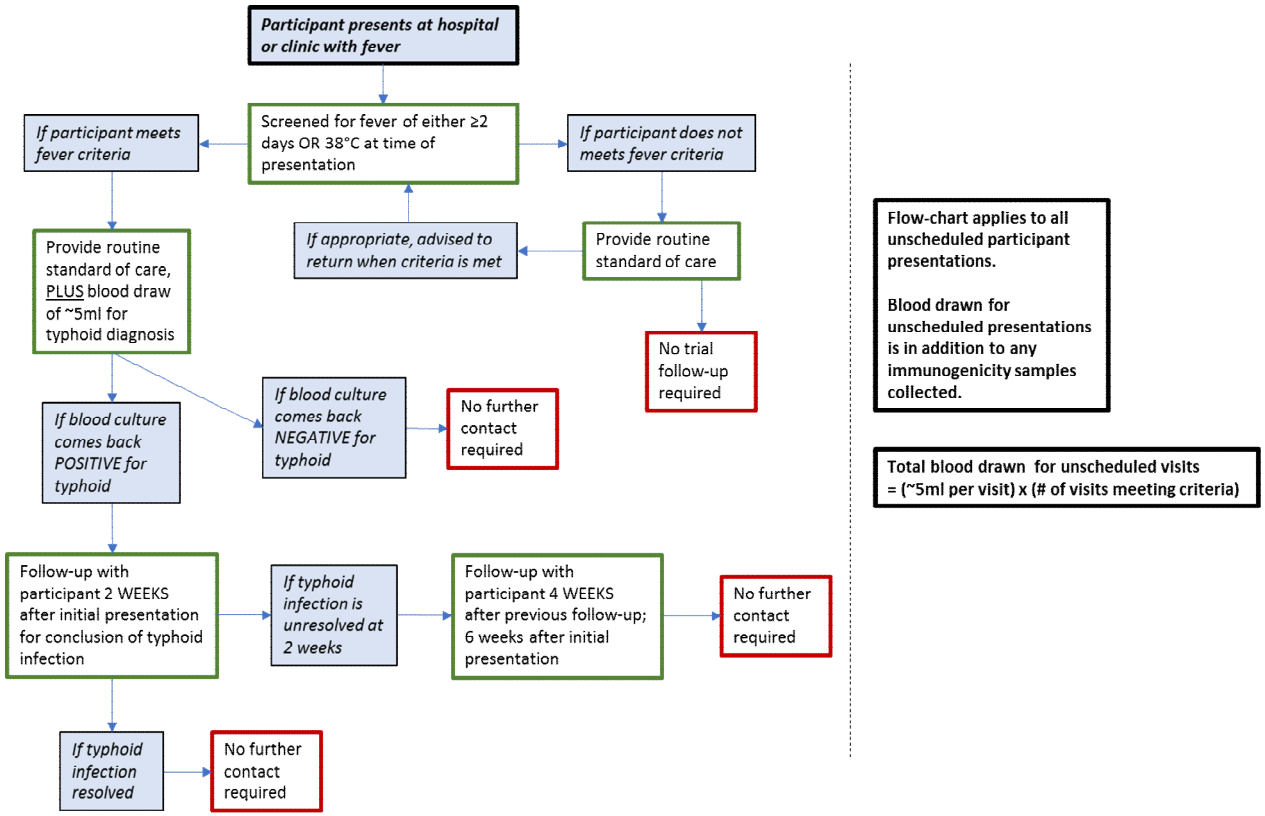
c) Only children under 5 years of age at the time of enrollment

d) Follow-up contact includes:
   - 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 trial health care facility if they develop fever of ≥2 days.
   - **: At 7 days full AEFI reporting will be collected.

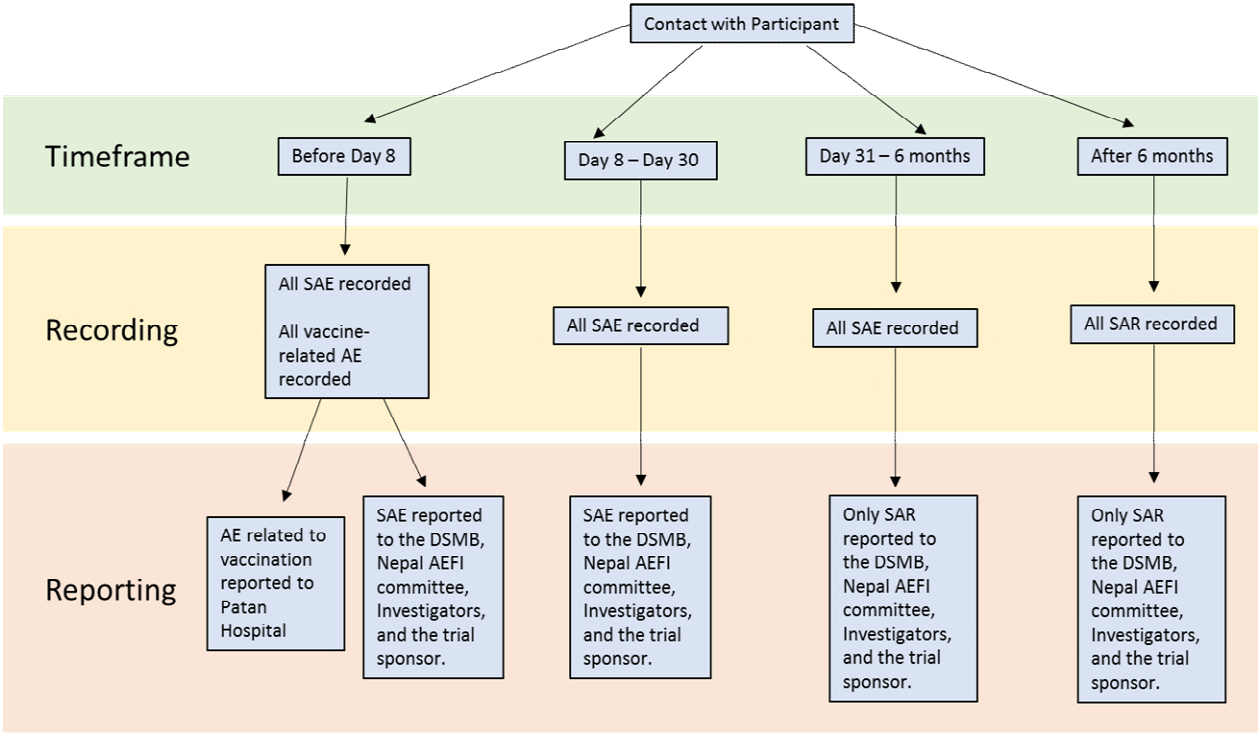
e) Control arm provided Vi-TCV at the end of the trial.

f) Both the intervention and control arms will be asked to return for un-blinding (at day 730 only) and documentation of vaccination

21. APPENDIX C: UNSCHEDULED PROCEDURES FLOWCHART



22. APPENDIX D: SAE REPORTING FLOW CHART



23. APPENDIX E: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made

Protocol amendments must be submitted to the Sponsor for approval prior to submission to the REC

Current Protocol

Trial Title: **Assessing the impact of a Vi-Polysaccharide Conjugate Vaccine in preventing typhoid infections among Nepali children – a Phase III trial**

Internal Reference Number / Short title: TyVAC Nepal: Typhoid Vaccine Study

Ethics Ref: OxTREC 15 - 17

OVG Ref: OVG2017/05

Date and Version No: 4th January 2019, Version 7.0

Chief Investigator:	Professor Andrew J Pollard, Oxford Vaccine Group, Department of Paediatrics, University of Oxford
Principal Investigator:	Professor Buddha Basnyat, Oxford University Clinical Research Unit Nepal – Patan Academy of Health Sciences, Kathmandu, Nepal
Investigators:	Dr Mila Shakya, Patan Hospital and the Oxford University Clinical Research Unit Nepal
	Dr Shrijana Shrestha, Department of Paediatrics Patan Hospital and the Patan Academy of Health Science, Kathmandu, Nepal
	Katherine Theiss-Nyland, Oxford Vaccine Group, Department of Paediatrics, University of Oxford
Sponsor:	The University of Oxford, University Offices, Wellington Square, Oxford, OX1 2JD
Funder:	Award from Bill and Melinda Gates Foundation (BMGF) to the University of Maryland, Baltimore, with the University of Oxford as a collaborating institution.
Chief Investigator Signature:	
Statistician Signature:	

There are no potential conflicts of interest.

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, host organisation, and members of the Research Ethics Committees, unless authorised to do so.

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24. KEY TRIAL CONTACTS

Chief Investigator	Professor Andrew J Pollard Professor of Paediatrics and Immunity Oxford Vaccine Group, University of Oxford, Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Churchill Hospital, Oxford, OX3 7LE, United Kingdom andrew.pollard@paediatrics.ox.ac.uk
Sponsor	The University of Oxford, University Offices, Wellington Square, Oxford, OX1 2JD Contact: Dr Rebecca Bryant
Statistician	Merryn Voysey Nuffield Department of Primary Care Health Sciences, University of Oxford T: +44 (0)1865 289288 E: merryn.voysey@phc.ox.ac.uk

25. SYNOPSIS

Trial Title	Assessing the impact of a Vi-Polysaccharide Conjugate Vaccine in preventing typhoid infection among Nepali children – a Phase III trial	
Internal ref. no. (or short title)	TyVAC Nepal: Typhoid Vaccine Study	
Clinical Phase	Phase III	
Trial Design	Participant- and observer-blind randomised-controlled trial	
Trial Participants	Children aged 9 months to <16 years in a defined catchment area of Kathmandu	
Planned Sample Size	20,000 children, allocated to two vaccination arms on a 1:1 ratio (10,000 each arm)	
Treatment duration	Single dose vaccination after enrolment.	
Follow up duration	Two years follow-up, post vaccination, for each participant	
Planned Trial Period	Nov 2017 – August 2020	
	Objective	**Outcome Measure**
Primary	To determine the efficacy and rate reduction of the Vi-TCV in preventing blood culture-confirmed symptomatic infection caused by S. Typhi	The Incidence of blood culture confirmed typhoid fever in each of the vaccination arms
Secondary	To determine the efficacy and rate reduction of the Vi-TCV in preventing blood culture-confirmed symptomatic infection caused by S. Typhi in participants who have had at least 3 days of fever	The Incidence of blood culture confirmed typhoid fever in participants who have had at least 3 days of fever
Secondary	To investigate safety outcomes associated with Vi-TCV vaccination, within the study population	The proportion of participants developing all adverse events within the first 7 days post-vaccination, and serious adverse events within 6 months of vaccination, as determined through self-reporting at follow-up contact
	To determine the impact of vaccination with Vi-TCV on the incidence of inpatient/outpatient admission rates for fever	Rates of participants with at least ≥2 days of subjective persistent fever, and/or a temperature of at least 38 degrees C at presentation, at Patan Hospital or trial clinics in each vaccination arm, stratified by duration and severity of fever

	To measure the difference in rates of hospital and clinic presentation for febrile illness in each vaccination arm	Rates of hospital or clinic presentation with febrile illness of any duration in each vaccination arm, measured by hospital presentation logs, hospital records, trial clinic records and self-reporting during three monthly follow-ups
	To determine Paratyphoid infection rates in each vaccination arm	Rates of blood culture confirmed Paratyphoid cases in each vaccination arm
	To measure days spent in hospital from febrile illness	Length of stay in hospital, collected from Patan hospital patient records, and parent/self-reported, in each vaccination arm
	To measure incidence of clinically-suspected enteric fever	Number of clinical diagnoses of typhoid fever, as determined by trial staff in Patan hospital outpatient clinics and trial clinic, in each vaccination arm.
Exploratory	To measure antibiotic/ antimicrobial use for inpatients/outpatients in each vaccination arm	Incidence of antibiotic/antimicrobial use in in/outpatient settings, from Patan hospital and trial clinic admission logs, and/or parent/self-reported in follow-up contact
	To measure the difference in the duration of febrile illness in each vaccination arm	Duration of fever recorded in Patan hospital and trial clinics, and parent/self-reported via follow-up contact
	To measure difference in rates of all-cause hospitalisation	Rates of hospitalization, identified through hospital admission logs and self-reporting in each vaccination arm
	To determine effect of vaccination on child growth and weight in children <5 years of age	Measurement of anthropomorphic parameters of children at baseline and at two years, in all children aged <5 years at the time of vaccination

	To determine the immunogenicity of Vi-TCV in a subset of participants, stratified by age groups	Assay of anti-Vi IgG antibodies in blood samples collected at baseline (Day 0) and at one month (Day 28) in a subset of participants (approximately 1000 in vaccine arm; 500 in control arm)
	To determine the persistence of antibodies induced by Vi-TCV in stratified age groups	Assay of anti-Vi IgG antibodies in blood samples collected at baseline (day 0), 18 months (day 545) and two years (day 730) in a subset of participants (approximately 1000 in vaccine arm; 500 in control arm)
	To measure the incidence of fever that does not result in medical treatment in each vaccination arm	Parent/self-reported fever at follow-up contact of participants.
	To determine rates of absenteeism from school/work in each vaccination arm	Rates of absenteeism from school or work, as applicable, as reported by parents at follow-up contact
	To measure all-cause mortality, and all-cause mortality with fever, in each vaccination arm	Rates and circumstances of mortality in each vaccination arm, recorded from hospital records and three-monthly follow-up
	To measure the rate of suspected and confirmed acute abdominal symptoms in each vaccination arm	Rates of presentation to Patan hospital or trial clinics with acute abdominal symptoms
	To measure the rate of surgical intervention for acute abdominal complaints in each vaccination arm	Rates of acute abdomen surgery in each vaccination arm, and gross surgical findings
	To analyse host genetic susceptibility to typhoid in individuals with and without Vi-TCV vaccination	DNA analysis of host genetics in individuals presenting with typhoid and healthy controls from each vaccination arm
Investigational Medicinal Product	Vi polysaccharide-tetanus toxoid conjugate vaccine (Vi-TCV) Licensed Trade name: Typbar-TCV®, Bharat-Biotech	
Formulation, Dose, Route of Administration	Each 0.5ml vaccine dose contains: Purified Vi-Capsular Polysaccharide of *S.* Typhi Ty2 conjugated to Tetanus Toxoid 25µg Sodium chloride 4.5 mg Water for Injection q.s. to 0.5ml Administration by intramuscular injection	

	2.5 ml 5-dose vials
Comparator (control)Treatment	Serogroup A meningococcal conjugate vaccine Licensed trade name: MenAfriVac™, Serum Institute of India PVT. Ltd. Dose 10µg for participants aged ≥1 year; 5µg for participants aged 9 to <12 months

26. ABBREVIATIONS

AE	Adverse event
AEFI	Adverse event following immunisation
AR	Adverse reaction
CI	Chief Investigator
CRF	Case Report Form
CT	Clinical Trials
DMC/DMSC	Data Monitoring Committee / Data Monitoring and Safety Committee
EPI	Expanded Programme on Immunizations
GCP	Good Clinical Practice
IB	Investigators Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
IRB	Independent Review Board
ISM	Independent Safety Monitor
NHRC	Nepal Health Research Council
OXTREC	Oxford Tropical Research Ethics Committee
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SMPC	Summary of Medicinal Product Characteristics
SOP	Standard Operating Procedure
STRATAA	Strategic Typhoid alliance across Africa & Asia
S. Typhi	*Salmonella enterica* serovar Typhi
SUSAR	Suspected Unexpected Serious Adverse Reactions
TMF	Trial Master File
Vi-PS	Vi antigen polysaccharide vaccine
Vi-TCV	Vi antigen typhoid conjugate vaccine

27. BACKGROUND AND RATIONALE

Enteric fever is a systemic illness caused by the human restricted pathogens *Salmonella enterica* serotypes

Typhi (*S*. Typhi) and Paratyphi A-C. It is estimated to affect >20 million people worldwide annually, with an estimated 200,000 fatalities per annum, primarily in lower income countries with poor sanitation(1). Areas with an incidence of >100/100,000 are considered endemic, including many countries in Africa, South Asia, South-East Asia and Central Asia(2). The burden of disease and mortality is increasingly recognised in the under 5 age group, as well as in older children and young adults(3–6). Enteric fever also remains a concern in high-income countries for travellers to endemic regions and laboratory workers (7,8).

Control of enteric fever, historically, has been established primarily through improved sanitation and infrastructure, leading to the elimination of disease as a public health problem from most developed countries. This remains the case, but there are substantial costs and difficulties implementing these measures in high typhoid incidence areas. As such, use of an effective vaccination programme targeting the highest risk populations will likely be a useful and cost-effective addition to control measures. Given the causative organisms are human-restricted, global eradication is possible and an effective vaccine will contribute to this.

27.1. Typhoid Vaccines

Currently, licensed vaccines exist only for the most prevalent serovar causing enteric fever, *S.* Typhi. The existing options are as follows:

Inactivated Whole Cell vaccine

This vaccine consists of heat-phenol-inactivated whole cell *S*. Typhi, which is injected subcutaneously in two doses four weeks apart. It had efficacy of 51-67% in controlled trials. It was associated with high degree of reactogenicity, causing fever and systemic symptoms in 9-34% of recipients, leading to school absence in 2-17% of cases (9). Due to these side-effects it has largely dropped out of mainstream use, however it is still used in several lower-income countries.

Vi Polysaccharide Vaccine (Vi-PS)

Developed in the 1980s this vaccine consists of purified Virulence factor (Vi antigen) capsular polysaccharide (Vi-PS) that forms the capsule of, and is specific to, *S.* Typhi. It elicits a T-cell independent antibody response, which means it has poor immunological memory and repeat doses do not result in an additional boosting response (10,11).

Similar to other polysaccharide vaccines, Vi-PS vaccine is poorly immunogenic and not licensed for use in children under 2 years old, presumably due to the absence of specific splenic marginal zone B-cells that are needed to produce an immunological response to polysaccharides. In clinical trials, clinical protection is non-comprehensive with protective efficacies of 64-72% (12–14). Additionally, protection is short lived, lasting only 2-3 years (15,16).

Live attenuated oral vaccine (Ty21a)

Also developed in the 1980s, this is an attenuated strain of *S*. Typhi (Ty21a) that has had many virulence genes mutated chemically, including the gene leading to failure to produce the Vi antigen. Ingestion of this strain induces local gut mucosal immunity as well as systemic antibody and cell mediated response(17,18). The strain is lyophilised and administered in either an oral enteric capsule or a liquid solution and requires 3-4 doses to induce effective protective immunity.

Clinical trials preformed in Chile and Indonesia demonstrated Ty21a vaccine had a protective efficacy of 67% and 53%, respectively (5,19). While the enteric-coated formulation is difficult to administer to young children, the alternative liquid formulation is better tolerated but may be less immunogenic in younger children (18,20). Ty21a vaccine is not licensed for children under the age of 6 years.

Vi-rEPA Vaccine

This vaccine was developed by the US National Institute for Health (US NIH) in 1994 utilising Vi-polysaccharide conjugated with a recombinant exoprotein A from *Pseudomonas aeruginosa* (rEPA) (21). A two-dose schedule six weeks apart was shown to be highly immunogenic with a protective efficacy of 91.1% in children aged 2 to 5 years in a trial in Vietnam (22). More recently, a study has demonstrated its immunogenicity in infants (23). However, the licensure of Vi-rEPA has been delayed due to lack of regulatory precedent for the use of rEPA carrier based vaccines.

Vi antigen typhoid conjugate vaccine (Vi-TCV)

Vi-TCV (Tybar-TCV™) is a newly available vaccine developed by Bharat Biotech consisting of 25 µg of Vi polysaccharide antigen conjugated to a nontoxigenic tetanus toxoid carrier protein. Similar to other vaccines, which are designed to protect against encapsulated bacterial pathogens and are conjugated to tetanus toxoid carrier proteins, Vi-TCV induces a T-cell dependent response. It can therefore produce an immunogenic response in infants under 2 years of age and has the potential to generate durable immune response via induction of immunological memory.

A Phase III randomised controlled trial comparing Vi-TCV with Vi-PS demonstrated seroconversion to anti-Vi IgG in the 6 month to 2 year age group (24). Additionally, a comparison of the sub-groups receiving boosters of either vaccine at two years demonstrated significantly higher anti-Vi IgG titres in the Vi-TCV group compared to the Vi-PS group (titres of 1685.3 EU/ml [95% CI: 1468-1797] in Vi-TCV vs 445.6 EU/ml [95% CI: 323-615] in Vi-PS). Safety data from the same study demonstrated that Vi-TCV was well tolerated by all age groups and that there were no differences in the number or variety of adverse events reported between the vaccine arms (25).

Efficacy data are available from a recent study performed using the typhoid challenge model at the University of Oxford (26). This study measured the efficacy of single-dose Vi-TCV, Vi-PS or a control vaccine in protecting against the development of typhoid infection after oral challenge. The study was conducted in healthy, UK adult volunteers and challenge was performed 28 days after vaccination. Using a composite diagnostic endpoint of clinical and/or microbiologically confirmed typhoid fever, the Vi-TCV and Vi-PS vaccines demonstrated comparable protective efficacy of 54.6% [95% CI: 26.8 – 71.8%] and 52.0% [95%CI: 23.2-70.0%], respectively, when compared to the control vaccine (26). This calculated Vi-TCV vaccine efficacy of 54.6% likely underestimates the protective effect of Vi-TCV in endemic settings. When applying a definition of typhoid fever which more closely approximates diagnosis in health care settings, i.e. fever followed by confirmatory bacteraemia, in a post-hoc analysis the protective efficacy of Vi-TCV vaccine increased to 87.1% [95%CI: 44.2-96.9%] compared to 52.3% [95%CI: -4.2%, 78.2%] for the unconjugated Vi-PS vaccine (26).

While Tybar-TCV is licensed for use in India and Nepal, and the data from seroconversion and efficacy studies are strong, field impact studies for Vi-TCV, demonstrating a reduction in the burden of disease attributable to typhoid infections, have not yet been conducted.

27.2. Description of TyVAC

This Vi-TCV trial falls within a larger multi-institution collaboration, called The Typhoid Vaccine Acceleration Consortium (TyVAC). TyVAC is a Bill and Melinda Gates Foundation funded project to generate evidence for Vi-TCV vaccine impact, and accelerate use of Typhoid Conjugate Vaccines in countries with significant typhoid burden. Managed by University of Maryland, in collaboration with University of Oxford, and PATH international, the TyVAC programme includes vaccination trials, health economics studies, country preparedness support for routine vaccine introduction, and the collation and synthesis of typhoid research and evidence.

Three sites have been identified for parallel field impact studies; Kathmandu, Nepal; Dhaka, Bangladesh; and Blantyre, Malawi. Each represents a geographical setting where enteric fever is endemic and has a substantial local burden of disease. In each site, independent studies with differing study designs will be implemented to identify a range of impact scenarios. Between the sites, there is a range of demographic and geographic variation to give confidence in the generalisability of the study results. The trial presented here, is one of the three planned studies, which will be conducted in Kathmandu, Nepal.

27.3. Aim of the Project

This study aims to assess the impact of Vi-TCV in a field setting in order to inform and support the use of the vaccine as a control measure for enteric fever in endemic settings, and to reduce global morbidity and mortality from enteric fever. Vi-TCV has shown promise from existing studies; it can produce seroconversion in infants; it potentially produces long lasting immunity; and it is efficacious in a controlled challenge setting. As such, it is an obvious candidate to test in a field impact study.

27.4. Rationale for Kathmandu as the study site

Kathmandu is the selected trial site for the following reasons:

- Enteric fever is endemic to Nepal, with a high incidence in Kathmandu.
- Enteric fever is recognised locally as a public health concern, both within the Nepal Ministry of Health and the local community.
- The National Immunisation Technical Advisory Group (NITAG) has prioritised control of the disease.
- The Ministry of Health is receptive to impact studies and subsequent vaccination introduction;
- Bharat-Biotech's Vi-TCV (Tybar-TCV) is already licensed in Nepal.
- The Strategic Typhoid Alliance Across Africa and Asia (STRATAA), funded by Wellcome Trust and the Bill and Melinda Gates Foundation, is a typhoid surveillance study, already running in 15 wards (community districts) of Lalitpur, Kathmandu, allowing for lower costs and potential synergy (27).
- STRATAA has identified that there is a sufficiently large population of children aged 9 months to <16 years within which to conduct this vaccination trial.

27.5. Rationale for Study Design

A randomised controlled trial will be performed with a two-year follow-up to assess the protective impact of the Vi-TCV vaccine.

The study will assess the impact of vaccination of children aged 9 months to <16 years of age living in a geographically defined catchment area in Lalitpur, Kathmandu. This age range has been selected because children bear a substantial burden of the disease in both mortality and morbidity, without an effective vaccine available in the routine vaccination schedule. Therefore, this demographic group has most to gain from vaccination with the Vi-TCV and would be the primary target for any subsequent vaccination campaign.

4.5.1 Rationale for Intervention vaccine

As discussed above, the Vi-TCV (Typbar-TCV®) is the most promising vaccine candidate for control of typhoid in an endemic area for the following reasons:

- One dose schedule,
- Immunogenic in children,
- Potentially prolonged immunogenicity,
- Shown to have minimal side effects.

Vi-TCV is licensed for use in Nepal and has been submitted for WHO prequalification. While the vaccine is currently licensed in Nepal, it is not routinely available for use. The results of this randomised trial will be used to inform country decision making for introduction of Vi-TCV into the routine expanded programme on immunisation (EPI).

4.5.2 Rationale for control vaccine

In this trial, the control vaccine should have the following features:

- Identical administration regime to Vi-TCV i.e. one dose,
- No provision of any direct protection against enteric fever,
- Potentially provides some additional health benefit to the trial participants.

The Men A vaccine (MenAfriVac) is a vaccine against *Neisseria meningitidis* serogroup A and is not currently given routinely in Nepal. It is a single dose vaccine, licensed for use from 9 months of age, and provides protection against group A *N. meningitidis*, the most common serotype in Nepal.

27.6. Summary of the known and potential risks and benefits, if any, to human participants

4.6.1 Potential benefits to participants

All participants enrolled will have the benefit of receiving Vi-TCV (the intervention arm at the beginning of the study, and the control arm at the end of the study), which is likely to provide protection against typhoid. Additionally, all participants will receive the control vaccine (the control arm at the beginning of the study and the intervention arm at the end of the study). The control vaccine can provide protection against group A meningitis, which, while not common in Nepal, can cause severe disease. There is no routine vaccination for typhoid or serogroup A meningitis in Nepal.

For the duration of the study, all participants will have access to free and accurate health assessments and diagnostics at the Patan hospital fever clinic, as well as at trial health clinics set up in the community, for all episodes of fever occurring during the study. Participants will also have access to information about general health issues, through trial staff.

Participants enrolled in the immunogenicity sub-study will be offered a free additional routine test, for either themselves or an adult family member, to be taken at the time of each study blood sample. Additional small non-monetary incentives, such as stationery items, small toys, or clothing items such as hats or gloves, will also be offered to participants.

Additionally, the trial will help improve the understanding of the impact of the Vi-TCV vaccine on typhoid infection rates, and help guide future implementation of Vi-TCV vaccination programmes. Whilst participants would not necessarily benefit directly from this, a positive result from this trial could lead to implementation of a Vi-TCV vaccine programme in typhoid endemic areas, including Nepal. This would provide direct and indirect protection to both those close to the participant and those in the wider population.

4.6.2 Potential risks to the participants

The Vi-TCV vaccine has demonstrated a favourable safety record in the approximately 400 people vaccinated in a Phase III study (24) and the Oxford challenge study (26), with vaccination being well tolerated with no side effects above that shown by comparator Vi-PS vaccines. However, this is the first study involving so many participants and there may be rare adverse events hereto unidentified that may become apparent in this study. While it is not anticipated that this will be the case, participants will be exposed to this potential risk. As a result, we will be conducting safety monitoring as a secondary outcome of this study.

28. OBJECTIVES AND OUTCOME MEASURES

	Objective	**Outcome Measure**
Primary	To determine the efficacy and rate reduction of the Vi-TCV in preventing blood culture-confirmed symptomatic infection caused by S. Typhi	The Incidence of blood culture confirmed typhoid fever in each of the vaccination arms
Secondary	To determine the efficacy and rate reduction of the Vi-TCV in preventing blood culture-confirmed symptomatic infection caused by S. Typhi in participants who have had at least 3 days of fever	The Incidence of blood culture confirmed typhoid fever in participants who have had at least 3 days of fever

	To investigate safety outcomes associated with Vi-TCV vaccination, within the study population	The proportion of participants developing all adverse events within the first 7 days post-vaccination, and serious adverse events within 6 months of vaccination, as determined through self-reporting at follow-up contact
	To determine the impact of vaccination with Vi-TCV on the incidence of inpatient/outpatient admission rates for fever	Rates of participants with at least ≥2 days of subjective persistent fever, and/or a temperature of 38 degrees C at presentation, at Patan Hospital or trial clinics in each vaccination arm, stratified by duration and severity of fever
	To measure the difference in rates of hospital and clinic presentation for febrile illness in each vaccination arm	Rates of hospital or clinic presentation with febrile illness of any duration in each vaccination arm, measured by hospital presentation logs, hospital records, trial clinic records and self-reporting during three monthly follow-ups
	To determine Paratyphoid infection rates in each vaccination arm	Rates of blood culture confirmed Paratyphoid cases in each vaccination arm
	To measure days spent in hospital from febrile illness	Length of stay in hospital, collected from Patan hospital patient records, and self-reported, in each vaccination arm
	To measure incidence of clinically-suspected enteric fever	Number of clinical diagnoses of typhoid fever, as determined by trial staff in Patan hospital outpatient clinics and trial clinic, in each vaccination arm
Exploratory	To measure antibiotic/ antimicrobial use for inpatients/outpatients in each vaccination arm	Incidence of antibiotic/antimicrobial use in in/outpatient settings, from Patan hospital and trial clinic admission logs, and/or self-reported in follow-up contact
	To measure the difference in the duration of febrile illness in each vaccination arm	Duration of fever recorded in Patan hospital and trial clinics, and self-reported via follow-up contact
	To measure difference in rates of all-cause hospitalization	Rates of hospitalization, identified through hospital admission logs and self-reporting in each vaccination arm

	To determine effect of vaccination on child growth and weight in children <5 years of age	Measurement of anthropomorphic parameters of children at baseline and at two years, in all children aged <5 years at the time of vaccination
	To determine the immunogenicity of Vi-TCV in a subset of participants, stratified by age groups	Assay of anti-Vi IgG antibodies in blood samples collected at baseline (Day 0) and at one month (Day 28) in a subset of participants (approximately 1000 in vaccine arm; 500 in control arm)
	To determine the persistence of antibodies induced by Vi-TCV in stratified age groups	Assay of anti-Vi IgG antibodies in blood samples collected at baseline (day 0), 18 months (day 545), and two years (day 730) in a subset of participants (approximately 1000 in vaccine arm; 500 in control arm)
	To measure the incidence of fever that does not result in medical treatment in each vaccination arm	Self-reported fever at follow-up contact of participants
	To determine rates of absenteeism from school/work in each vaccination arm	Rates of absenteeism from school or work, as applicable, as reported by parents at follow-up contact
	To measure all-cause mortality, and all-cause mortality with fever, in each vaccination arm	Rates and circumstances of mortality in each vaccination arm, recorded from hospital records and three-monthly follow-up
	To measure the rate of suspected and confirmed acute abdominal symptoms in each vaccination arm	Rates of presentation to Patan hospital or trial clinics with acute abdominal symptoms
	To measure the rate of surgical intervention for acute abdominal complaints in each vaccination arm	Rates of acute abdomen surgery in each vaccination arm, and gross surgical findings
	To analyse host genetic susceptibility to typhoid in individuals with and without Vi-TCV vaccination	DNA analysis of host genetics in individuals presenting with typhoid and healthy controls from each vaccination arm

29. TRIAL DESIGN SUMMARY

29.1. Details of study design and procedure

This is a participant- and observer-blind, individually randomised study of the typhoid conjugate vaccine (Vi-TCV), brand name: Tybar-TCV, in Nepali children. The population within a selected geographical catchment area of Lalitpur, Kathmandu, will be offered entry into the study. The aim is to enrol 20,000 children within the target age range (9 months to <16 years) residing in the target area. They will be randomised in a 1:1 ratio to receive Vi-TCV or control vaccine.

All participants will be contacted by telephone or in person seven (7) days after vaccination for follow-up and to record any adverse events following vaccination.

A subset of approximately 1500 participants (1000 Vi-TCV, 500 control vaccine) will be randomised to have blood samples collected at baseline (D0), at D28, at 18 months (D545), and at two years (D730) post-vaccination to study immunogenicity.

All Children 9 months to <5 years of age at the time of enrolment will have their height and weight measured at baseline and at 2 years post-vaccination.

The parent/guardians of participants will be contacted every three months at days 90, 180, 270, 365, 455 (1 year 3 months), 545 (1 year 6 months), 635 (1 year 9 months), and 730 (2 years). This contact will consist of either a telephone call, or an in-person visit. These follow-up contacts will:

- Confirm that the participant is still willing to continue with the study,
- Ensure participant and family still lives in area,
- Collect information about mortality and morbidity end points, including fever,
- Collect information about antibiotic use,
- Collect rates of school/work absenteeism (for both child and parent) in the last 3 months,
- Provide additional reminders to attend Patan Hospital or trial ward clinic sites if the participant develops fever of ≥2 days.

At two years after the initial vaccination campaign, the trial will end and all participants and trial staff will be unblinded. At this point, the control group will be offered vaccination with the Vi-TCV vaccine, the intervention group will be offered the Men A vaccine and both control and intervention groups will be informed of their vaccination status and have their vaccines documented on the patient record.

Duration of participation is two years from enrolment.

The number of planned participant contacts will be as follows:

- Study participants, not enrolled in the immunogenicity sub-study: up to 11 (2 face to face, 8 to 9 separate follow-up contact)
- Subset of participants enrolled in the immunogenicity sub-study: up to 13 (4 face to face, 7 to 9 separate follow-up contact).

Patients presenting at healthcare facilities with persistent fever will have a blood sample taken for confirmation of diagnosis. The samples will be processed by Patan Hospital Microbiology Laboratory and other microbiology laboratories with appropriate blood culture facilities over the two-year period of participant follow-up. Results of these blood samples will be obtained to acsertain the number of blood-

culture positive cases of *S.* Typhi in the trial cohort. Participants presenting at Patan hospital with fever will be directed to the Fever Clinic at Patan Hospital, and their data will be collected. Participants presenting at the trial ward clinics will be seen by a clinican and nurse and their data will be collected.

A trial flow chart and trial schedule can be seen in Appendix 1 and Appendix 2.

30. PARTICIPANT IDENTIFICATION

30.1. Trial participants

Children aged 9 months to <16 years (i.e., up to 15 years 364 days) who are in good health at the time of enrolment will be eligible to participate in this study. Participants will be identified as living within the defined catchment area of Lalitpur, Kathmandu, Nepal. Trial staff, including local community health volunteers, will identify households with children <16 years of age and approach them for participation.

30.2. Inclusion Criteria

The participant must satisfy all the following criteria to be eligible for enrolment:

- Parent/legal guardian is willing and competent to provide informed consent. If the participant is 7 years of age or older, assent will also be sought,
- Aged between 9 months (or eligible for measles vaccination according to local protocol) and <16 years (i.e. up to 15 years 364 days) at time of vaccination,
- In good health on the day of vaccination,
- Parent/legal guardian confirms that their child will be willing and be able to comply with study requirements including follow-up contact, according the schedule (Appendix B),
- Live within the study catchment area at the time of vaccination.

30.3. Exclusion Criteria

The participant will not be enrolled if any of the following criteria apply:

- They have knowingly received a typhoid vaccine in the last three years,
- They have a known allergy to any of the vaccine components,
- Any medical or social reasons that will prevent the participant from conforming to the study requirements as judged by a medical professional,
- They are planning to move away from the catchment area within the next six months.

30.4. Temporary exclusion criteria

Participants will be temporarily excluded from being vaccinated if, at point of vaccination, any of the following apply:

- Reported fever within the 24 hours prior to vaccination,
- Use of anti-pyretics within the 4 hours prior to vaccination.

If these apply, the participant will be temporarily excluded for vaccination until 48 hours has passed following cessation of fever. A re-assessment will be conducted to ensure these temporary exclusion criteria no longer exist.

30.5. Withdrawal of Patients/Subjects

Participants' parents/legal guardians can withdraw consent at any point. The Investigator may also discontinue a participant from the trial at any time if the Investigator considers it necessary for any reason including:

- Ineligibility (either arising during the trial or retrospectively having been overlooked at screening),
- Significant protocol deviation,
- Significant non-compliance with trial requirements,
- An adverse event or disease progression resulting in the inability to continue to comply with trial procedures and follow-up,
- Loss to follow up.

Withdrawal will result in cessation of any follow-up calls, visits, or blood tests (as applicable to the subset). Once vaccines have been administered no further treatment is required in the study, so no additional action will need to take place. Participants' parents/legal guardians will have the choice when withdrawing, to withdraw from active study procedures only (follow-up calls and visits) but remain in the passive surveillance for the primary outcome (allowing us to access their hospital records and blood test results), or withdraw from all study contact. In the case of a participant withdrawing from all study contact, we will not collect any further data of hospital presentation or blood culture results. Data and blood samples collected prior to the time of participant withdrawal will be kept and analysed as part of the study data. A participant who withdraws from the study has the option to re-engage at a future date if they choose to do so. All participants who withdraw from the study will be given information on how to re-engage with the study if they so choose. Reasons for withdrawal from the study, if known, will be recorded in the participant's CRF.

31. TRIAL PROCEDURES

31.1. Recruitment

Potential participants living in Lalitpur, Kathmandu will be identified. Trial staff, already embedded within each catchment area, including community health volunteers, will systematically approach each household in the area to identify households with children aged 9 months to <16 years (i.e. up to 15 years 364 days). Identified children will be screened as per the process below.

31.2. Screening and Eligibility Assessment

Once a household which includes a potential participant is identified, the parent/legal guardian of the child will be given basic information about the trial and invited to attend the nearest study clinic if they are interested. After arrival at the study clinic, screening according to inclusion/exclusion criteria will occur. Eligible participants will then have informed consent and if applicable assent obtained. After consent is taken, a medical examination, including temperature for temporary exclusion criteria, will be conducted.

Participants with a temporary exclusion criterion identified after the medical assessment will be informed of the reason and asked to return in ≥48 hours for repeat assessment and re-consent.

31.3. Informed Consent

The parent/legal guardian must personally sign and date the latest approved version of the Informed Consent form before any trial specific procedures are performed. Assent will also be sought from children 7 years of age or older, to participate in the trial.

Written and verbal versions of the Participant Information and Informed Consent/Assent will be presented to the participants and their parent/legal guardian in the local language detailing no less than: the exact nature of the trial; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant's parent/legal guardian is free to withdraw their child from the trial at any time for any reason without prejudice to future care, without affecting their legal rights and with no obligation to give the reason for withdrawal. Participants will also be informed that they can choose to have their remaining blood samples destroyed and not maintained for future analysis at the end of the trial.

The participant and their parents/legal guardians will be allowed as much time as they wish to consider the information, within the recruitment period until 20,000 children are enrolled, and the opportunity to question the Investigator or other independent parties to decide whether they will participate in the trial. Written Informed Consent/Assent will then be obtained, with additional opt-in consents to be randomised for blood sampling and to be contacted at the end of the trial, by means of participant's parent/legal guardians dated signature or thumbprint and dated signature of the person who presented and obtained the Informed Consent. The person obtaining consent will record assent on the same consent form, for children aged 7 and older. The person who obtained the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief/Principal Investigator. A copy of the signed Informed Consent will be given to the participant. The original signed form will be retained at the trial site.

If the parent/guardian is illiterate then a third party may act as a witness for the parent/guardian to attest that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the parent/guardian and that informed consent was freely given by the parent/guardian. In this event, the witness will also sign and date the consent form.

31.4. Randomisation and blinding

8.4.1. Randomisation

Computer generated randomisation lists will be prepared by the study statistician. Randomisation lists will allocate participants to receive either Vi-TCV or the control vaccine on a 1:1 basis using stratified block randomisation with randomly varying block sizes from 6-12. Stratification will be by age (9 months to <5 years old or >= 5 years old to <16 years). Participant randomisation will occur at the vaccination visit, using the pre-prepared randomisation lists. It will be performed by the staff member after collection of baseline data during the same visit.

In addition, a separate randomisation list will allocate 1500 individuals to blood sampling at D0, D28, 18 months, and at 2 years for the immunogenicity study. Blood sampling randomisation will be on a 2:1 basis (approximately 1000 Vi-TCV: 500 control) and be age stratified (< 5 years and >= 5 years).

The result of participant consent, and randomisation into both the vaccine trial and immunogenicity sub-study (the blinded trial randomisation ID number), will be directly entered onto the CRF.

Participants and parents will not be informed which vaccine was administered at this time.

8.4.2 Blinding

Vaccine allocation will be observer- and parent/guardian/participant-blind, with only the vaccinating trial staff aware of which vaccination has been given. To maintain observer blinding these staff members will not be involved in subsequent study procedures in wards in which they performed vaccinations until such time as study unblinding occurs. Efforts will be made to conceal vaccination preparation and vaccine supplies from participants and their families to maintain blinding.

Circumstances may arise where unblinding is needed prior to the end of the study e.g. occurrence of a SUSAR or requirement of a medical intervention that would be influenced by knowledge of which vaccine the individual has received. In such circumstances, the local Principal Investigator will be contacted to discuss whether urgent unblinding is warranted. If it is deemed necessary, unblinding will occur based on the recommendation of the DSMB. Any event of unblinding will be fully documented in the CRF.

31.5. Vaccination Visit

Potential participants will attend the study clinic situated within each ward. After potential participants have considered the PIL, and decided to participant, inclusion/exclusion will be assessed, and written informed consent will be formally obtained with the parent/legal guardian of the participant, and assent will be sought from the eligible subjects 7 years of age or older. Basic medical history will be taken and temporary exclusion will then be checked. If fever is identified, the participant will be asked to return in >48 hours following cessation of fever for re-affirmation of consent.

Once consented, a member of the study team will collect demographic information (including age and address) and participant contact details. Height/length and weight will be collected for all children <5 years of age. The participant will then be allocated via the randomisation lists, described above, to one of the study arms and, if they consented, to the immunogenicity study. If the participant is randomised to immunogenicity study, a blood sample will be taken by a suitably trained staff member at the clinic.

All details will be recorded in the CRF.

Based on the randomisation, the appropriate assigned vaccine will be administered by a trained member of the study team. The site of vaccination (right or left arm or thigh) will be recorded. The participant will be considered enrolled into the study at the point that any medical procedure takes place (i.e. blood sample taken or vaccination administered).

A study card containing the name of the study, and contact details for the study team will be given to parents/legal guardians to call if they have any concerns, or if their child is admitted to hospital at any time during the duration of the study. The card will also contain instructions to attend Patan Hospital fever clinic or the study clinics in the wards, if the participant develops prolonged fever (≥2 days) at any time over the next two years.

Parents/legal guardians of participants allocated to the immunogenicity study will be given details of the follow-up appointments at D28 and D545.

31.6. Subsequent Follow-up and Visits

See Appendix A and B for trial flow chart and visit timelines.

8.6.1 Adverse Events Follow-up (Day 7, -1/+7)

Follow-up contact at 7 days post-vaccination will collect parent/legal guardian-reported information on adverse events following immunisation (AEFIs).

Information collected will include:

- Verbal reconfirmation of consent for participation in the trial,
- Report from parent/legal guardian of adverse events related to vaccination, including: pain, swelling, fever, etc., (see section 10 of the protocol for action in the event of these occurring) and use of medications following vaccination,
- Reiteration of contact details and instructions to attend Patan Hospital / study clinic in the case of a prolonged fever (≥2 days).

8.6.2 Standard Follow-up

There will be routine participant follow-up via telephone or face-to-face contact every three months for the two-year duration of participation in the trial, for all participants enrolled in the trial. (See Appendix B for timelines.) A brief interview will be conducted by trial staff at each follow-up contact.

These interviews will collect parent/legal guardian-reported participant information, including:

- Confirmation that the parent/legal guardian of the participant is still willing to continue with the study,
- Ensure participant and family still live in area,
- A record of mortality and morbidity end points, including:
    - Mortality,
    - fever occurrence and duration with and without treatment seeking behavior,
    - use of antibiotics,
    - visits to clinics, hospitals, or pharmacies (and records requested if they attended a facility other than the study sites),
- Rates of school/work absenteeism (for both child and parent) in the last 3 months,
- A reminder to attend designated health care facilities if they develop fever of ≥2 days.

The results from this follow-up contact will be recorded in the participant's CRF. Appropriately qualified trial staff within the ward clinics will review the medical records of participants who report a febrile illness that results in seeking medical care, if parents/guardians agree to this and if this episode has not already been captured in the passive surveillance element of the trial. ,.

Text messages may be utilised to communicate with participants' parents/guardians. This may include items such as reminders about trial follow-up, encouragement of visits to Patan hospital or study clinics for fever of ≥2 days, and confirmation of test results.

8.6.3 Immunogenicity Study

The subset of participants enrolled in the immunogenicity sub-study will receive three additional face-to-face follow-up visits at their nearest trial clinic 28 days (D28+/-4 days), 18 months (D545 +/-56 days), and at two years (D730+/-90 days) after receiving vaccination. At these additional visits the following procedures will be performed:

- Confirmation of continued participation in the study,
- Draw of ~5ml blood for transport to, and processing at, the Patan Hospital Microbiology Laboratory,
- Offer of additional blood test (such as haemoglobin or blood group), free of charge, for either participant or adult family member
- A small token will be offered to participating children attending for immunogenicity follow-up tests - this will be non-monetary, but may include stationery items, small toys, or clothing items such as hats or gloves
- Confirmation of contact details and reiteration of instructions to attend Patan Hospital / trial ward clinic in the case of a prolonged fever ≥2 days (to be performed at the D28 and D545 visits only).

Effort will be made to perform the 18 month blood draw and the 18 month follow-up contact simultaneously, so as not to greatly increase the time burden of participants. The blood draw at two years post-vaccination will be performed at the same time as the study unblinding and vaccination recording.

The above information will be recorded in the participant's CRF.

8.6.4 Participant Presentation with Fever

Participants enrolled to this study who experience a fever lasting for ≥2 days and/or a fever of over 38ºC, as subjectively reported by a parent/guardian, will be asked to present to Patan Hospital or trial clinics. Regular community engagement activities will take place throughout the study period in order to remind and encourage participants to attend the clinics.

At the hospital or clinic, medically trained staff will assess the participant. Details of this assessment, including measurement of temperature, will be recorded and the participant will receive routine standard of care management and treatment, as deemed appropriate by the assessing member of staff. Trial staff will collect information about the participant illness episode and record it in the CRF. This will include information such as temperature and duration, hospital admission, surgeries, and antibiotics prescribed, etc. If the participant's parent/guardian confirms fever for ≥2 days, AND/OR if the participant is currently febrile at ≥38, this will result in a study sample being collected which constitutes a ~5ml blood sample. In order to compensate participants for their time and effort in attending clinic, a small token will be given to participating children attending trial clinics. This will be non-monetary, but may include stationery items, small toys, or clothing items such as hats or gloves.

Laboratory blood-culture confirmed enteric fever cases (i.e. blood culture positive for either *S.* Typhi or *S.* Paratyphi) will be followed-up approximately two weeks after presentation with fever, to record outcome of illness and resolution. If the illness is not resolved at that time of follow-up, an additional follow-up will occur two weeks later (four weeks after initial presentation with fever) to record outcome of illness and resolution, including any use of antibiotics.

See Appendix C for flow chart of unscheduled procedures.

31.7. Sample Handling

<u>Blood samples to confirm typhoid infections:</u>

Blood samples drawn in the Patan Hospital for diagnosis and confirmation of suspected typhoid fever will be handled, stored, processed, in accordance with standard operating procedures of Patan Hospital

Microbiology Laboratory. The results of these tests will be recorded in the participant CRF for use in this study. Plasma samples will be analysed for immune response.

Remaining samples will be stored at Patan Hospital for further analysis after the conclusion of the trial.

Immunogenicity study samples:

Blood samples taken for the immunogenicity study will be transported to Patan Hospital daily where they will be processed and stored by trained study staff, in accordance with standard operating procedures (SOP).

Plasma will be stored for investigation of novel diagnostic markers, for example, indications of an acute serological response indicating recent typhoid exposure, or identification of a metabolomic signature compatible with infection.

The primary laboratory technique performed will be anti-Vi IgG antibody ELISA performed on the extracted plasma sample, using a commercially available assay (Vacczyme Binding Site, or other comparable assay). This assay will be performed according to the manufacturer's instructions.

Any additional samples, taken for free routine testing, will be processed either in Patan Hospital or in other laboratories, with results later made available to participants.

Genetic analysis:

DNA from blood samples collected to confirm typhoid and from the immunogenicity study, will also be stored, with consent, for investigation of the genetic control of immunity to vaccines and susceptibility to infectious diseases like typhoid. DNA will be genotyped/sequenced and analysis will be performed (genome wide association study or GWAS analysis, and DNA sequence analysis).

As some laboratory processes, such as the ELISA and genetic analysis listed above, are currently not available in Nepal, the samples will be shipped to laboratories abroad (OUCRU-Vietnam; The University of Melbourne, Australia; the University of Oxford, UK; the Genome Institute of Singapore; and/or the Wellcome Trust Sanger Institute, UK), for performance of assays that are unavailable in Nepal. When an individual sample volume is sufficient enough so as not to compromise the ability to perform essential laboratory procedures, samples may be split. One set of samples will be retained in Nepal, and a second set of samples can be shipped abroad for the processes not available in Nepal.

At the end of the trial, once all study objectives have been achieved, all remaining samples abroad will either be sent back to Nepal or destroyed. Samples remaining in Nepal will be kept for future analysis.

31.8. Discontinuation/Withdrawal of Participants from Trial Treatment

Trial treatment consists of a single vaccination received at the point of enrolment into the study. It is not possible to withdraw from trial treatment after vaccination. For details of participant withdrawal from study procedures and follow-up, see section 7.5.

31.9. Catch-up vaccination

At the two-year follow-up visit, all participants will be informed which vaccination they received, and their vaccination records will be updated to reflect this. At this point, the study will become unblinded. Control

vaccine participants will be invited to receive the Vi-TCV vaccine and the intervention vaccine participants will be invited to receive the Men A vaccine Individuals accepting this offer will have the vaccines administered by a trained member of the study team.

All participants receiving the catch-up vaccines will be given information for reporting any adverse events occurring in the week following vaccination.

31.10. Definition of End of Trial

The end of trial is the date that the last sample is processed for the purposes of this study.

32. INVESTIGATIONAL MEDICINAL PRODUCT (IMP)

32.1. IMP Description

Trial treatment

Vi polysaccharide-tetanus toxoid conjugate vaccine (Vi-TCV). Trade name: Typbar-TCV®, Bharat-Biotech.

Each 0.5ml vaccine dose contains:

- Purified Vi-Capsular Polysaccharide of *S.* Typhi Ty2 conjugated to Tetanus Toxoid 25µg
- Sodium chloride 4.5 mg
- Water for Injection q.s. to 0.5ml

The vaccine is packaged as a 2.5ml 5-dose vial. It will be administered as an intramuscular injection in the antero-lateral thigh for younger children, or the upper arm for older children, according to local protocols.

Control vaccine

Meningococcal Group A conjugate vaccine Licensed trade name: MenAfriVac™, Serum Institute of India PVT Ltd. This vaccine is produced in two formulations; a standard 10µg/0.5 ml dose for individuals aged ≥1 year of age; and a 5µg/0.5ml single dose for individuals aged 9 – 24 months.

MenAfriVac™ is provided as a 10-dose presentation consisting of a vial and an ampoule. Each vial contains a lyophilised powder of meningococcal group A polysaccharide conjugated to tetanus toxoid protein and excipients. Each 5ml ampoule of diluent contains:

- Aluminium phosphate (≤1.25mg per single human dose) and
- Thimerosal (0.01%)

The lypohilised conjugate is reconstituted just before use with the contents of one ampoule of diluent to obtain 10 doses of the final vaccine in a white homogeneous suspension. A single dose of vaccine is equivalent to 0.5ml of the reconstituted suspension, with the doses as above depending on the formulation.

The vaccine will be administered as a deep intramuscular injection in the antero-lateral thigh for younger children, or the upper arm for older children, according to local protocols.

9.1.1 Labelling

The vaccines will be labelled by the manufacturer and will not be relabelled.

9.1.2 Supply

The Vi-TCV vaccine (Typbar-TCV®) will be provided by Bharat-Biotech. The Men A control vaccine (MenAfriVac ®) will be provided by the Serum Institute of India. The Study vaccines will be shipped to the Logistics Department at the Ministry of Health in Kathmandu before distribution to Patan Hospital.

32.2. Storage of IMP

Both the intervention vaccine (TypBar), and the control vaccine (MenAfriVac) will be stored according to manufacturer specifications, at 2° to 8° C (35 ° to 46 ° F), in a temperature monitored refrigerator at Patan Hospital, Nepal, when not in use for the daily activities. When in use for vaccination days, both vaccines will be stored in temperature monitored refrigerators or cold boxes. Fridge temperatures and cold chain transport will be audited during the vaccination campaign to ensure they are within range.

Both the intervention and control vaccine vials will be labelled with a "vaccine vial monitor"; a temperature-sensitive dot that provides an indication of the cumulative heat to which the vial has been exposed. It warns the end user when exposure to heat is likely to have degraded the vaccine beyond an acceptable level and should not be used.

Vi-TCV (TypBar)

The intervention vaccine is presented in 5 dose vials of active vaccine, ready for administration. The vaccine will be stored at 2° to 8° C, as described above. The vaccine should not be frozen, and if it has been, it should be discarded. Opened vials should be discarded 6 hours after opening.

MenA (MenAfriVac™)

The control vaccine is presented in 10 dose vials of active vaccine, and 10 dose ampoules of diluent. The vaccine will be stored at 2° to 8° C, as described above, and protected from light. The vaccine is stable and can be used when exposed up to 40°C for a period of 4 days immediately prior to reconstitution. Open vaccine vials will be discarded 6 hours after opening. The diluent will be stored at room temperature, according to manufacturer specification, in a clinical area. The reconstituted vaccine should be protected from direct sunlight.

32.3. Accountability of the Trial Treatment

The vaccines will be shipped to a central storage facility in Nepal and passed through customs. They will then be transported to Patan Hospital and distributed to local clinics whilst maintaining the cold-chain (aiming for temperature between 2-8°C), with the exception of the Meningococcal A vaccine diluent which will be kept at 25°C.

The number of doses of study vaccines that are received, used and wasted will be documented daily during the trial and checked weekly.

Unused vaccines at the end of the trial may be retained for laboratory use only (such as laboratory assay development). Any recall of study vaccines required for use in the study or reporting of defective vaccines will be performed according to trial SOPs.

33. SAFETY REPORTING

33.1. Definitions

Below are the various categories of Adverse Events Following Immunization (AEFIs).

Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect. Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out: • in the case of a product with a marketing authorisation, in the summary of product characteristics (SmPC) for that product

	• in the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the trial in question.

NB: to avoid confusion or misunderstanding of the difference between the terms "serious" and "severe", the following note of clarification is provided: "Severe" is often used to describe intensity of a specific event, which may be of relatively minor medical significance. "Seriousness" is the regulatory definition supplied above.

Brighton Collaboration case definitions for anticipated outcomes will be used to standardise the identification and reporting of all AEFIs.

Any pregnancy occurring during the clinical trial and the outcome of the pregnancy should be recorded and followed up for congenital abnormality or birth defect, at which point it would fall within the definition of "serious".

A flow chart for AEFI recording and reporting can be seen in Appendix D. Descriptions of these procedures are listed below (sections 10.3 and 10.4)

33.2. Causality

A medically qualified individual must determine the relationship of each adverse event to the trial medication according to the following definitions:

Related: The adverse event follows a reasonable temporal sequence from trial medication administration. It cannot reasonably be attributed to any other cause.

Not Related: The adverse event is probably produced by the participant's clinical state or by other modes of therapy administered to the participant.

33.3. Procedures for Recording Adverse Events

From vaccination through day 7

All adverse events related to vaccination (ARs), as judged by a medically qualified investigator or the Sponsor, occurring during the first 7 days post vaccination that are observed by the Investigator or reported by the participants parent/legal guardian, will be recorded on the CRF.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to trial medication, and action taken. Follow-up information should be provided as necessary.

The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

All Serious Adverse Events (SAEs) observed by the Investigator, members of the study team or reported by the parent/guardian will be recorded on the CRF.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to trial medication, other suspect drug or device and action taken. Follow-up information

should be provided as necessary.

Day 8 – 6 months

Serious Adverse Events (SAEs) observed by the Investigator, members of the study team or reported by the parent/guardian, will be recorded on the CRF.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to trial medication, and action taken. Follow-up information should be provided as necessary.

6 Months through end of trial
Serious Adverse Reactions (SARs), as judged by a medically qualified investigator, observed by the Investigator, members of the study team or reported by the parent/guardian, will be recorded on the CRF.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to trial medication, and action taken. Follow-up information should be provided as necessary.

All mortality occurring during the duration of the trial will be recorded in the CRF, and investigated by medically qualified trial staff.

Follow-up post Adverse Event

All adverse events recorded in the CRFs, as described above, for the duration of the study, from first vaccination until trial completion, will be followed by a medically qualified investigator either until resolution, or until the event is considered stable.

33.4. Reporting Procedures for Adverse Events

As per Nepal's immunisation reporting requirements all documented minor local and systemic adverse events following immunisation (AEFI), occurring within the first 7 days after vaccination, will be reported at the end of the trial to the Nepali AEFI committee.

All SAEs occurring within the first 30 days of vaccine administration, and SARs occurring until the end of the trial, must be reported to the Data Safety Monitoring Board (DSMB), the Principal Investigator (PI) in Nepal, the Chief Investigator in Oxford within 24 hours of the Site Study Team becoming aware of the event. A more detailed report form should be completed and sent, within 48 hours of the initial report to the Principal Investigator (PI) in Nepal, and the Chief Investigator in Oxford and the other study investigators. Additional and further requested information (follow-up or corrections to the original case) will be detailed on a CRF. All SAEs must be reported to the trial sponsor (University of Oxford) within 7 days.

SAEs will also be reported to the Nepal and Oxford RECs in the Annual Progress Reports. A final SAE/SAR report will be compiled and submitted to the Nepali AEFI committee at the end of the trial, including specific vaccine information, once the trial has been unblinded.

The DSMB will have the authority to halt the study, if any safety reports suggest it is needed. If any of the following halting criteria are met after study product is administered, then the study will be suspended and further doses of vaccine will not be administered pending review of data by the DSMB:

- Within 24 hours of receiving vaccine, any two subjects experience life-threatening hypersensitivity reaction.

- Within 7 days of vaccination, three or more subjects experience study-product-related SAEs within a single MedDRA category (e.g. gastrointestinal; respiratory; other infections and infestations; hypersensitivity/allergy).

33.5. Expectedness

Expectedness will be determined according to the Summary of Product Characteristics.

33.6. SUSAR Reporting

All SUSARs will be reported by the Nepal PI and Oxford CI to the relevant Competent Authority and to the REC and other parties as applicable. For fatal and life-threatening SUSARS, this will be done no later than 7 calendar days after the Sponsor or delegate is first aware of the reaction. Any additional relevant information will be reported within 8 calendar days of the initial report. All other SUSARs will be reported within 15 calendar days.

Principal Investigators will be informed of all SUSARs for the relevant IMP for all studies with the same Sponsor or funder, investigating the same IMP, whether or not the event occurred in the current trial.

33.7. Safety Monitoring Committee

An independent safety monitoring committee will be assembled to oversee data safety monitoring for the TyVAC typhoid vaccine trials conducted in Nepal, Bangladesh and Malawi. This committee will include physician representatives from each country, as well as an independent statistician.

In addition, a physician in Nepal with relevant study-related or therapeutic expertise will be identified as an Independent Safety Monitor(ISM). The ISM will not be an investigator for this study. As this is a single dose vaccine intervention, we anticipate that any circumstances warranting unblinding will be rare. However, circumstances may arise where unblinding is necessary prior to the end of the study e.g. occurrence of a Suspected Unexpected Serious Adverse Reactions (SUSAR) or requirement of a medical intervention that would be influenced by knowledge of which vaccine the individual has received. In such circumstances, the identified ISM would be requested to carry out an independent assessment of the child. The details of the assessment will be reported to the PI, CI, DSMB and discussed. If deemed necessary, the DSMB will recommend unblinding.

33.8. Development Safety Update Reports

A pharmacovigilance safety report will be submitted, annually in accordance with the Nepal regulations and guidelines.

In order to provide detailed safety information associated with Vi-TCV to global decision-makers at WHO, an unblinded report of safety data following vaccination of all vaccinated participants will be produced if requested by the WHO Global Advisory Committee on Vaccine Safety. The confidential report will be produced after completion of the routine day 180 follow up phone call. This report may also be shared with Bharat Biotech, the vaccine manufacturer, so that the data can be immediately made available to any regulatory agencies who require safety data during vaccine introduction. Trial participants and implementation staff will remain blinded. All vaccine efficacy data will stay blinded until the time of an official analysis as stated in section 11.

34. STATISTICS

34.1. Description of Statistical Methods

The primary endpoint will be blood culture positive typhoid fever obtained from enhanced passive surveillance in Patan hospital and study clinics.

The incidence of typhoid will be estimated as the number of cases divided by the total number of person-years of follow up. Incidence will be presented with 95% confidence intervals for each group and overall. Incidence rate ratio (IRR) will be computed as the ratio of the incidence in the Vi-TCV arm compared to the control arm.

Vaccine efficacy (VE) will be calculated as (1 – IRR) x 100%, where IRR is the incidence rate ratio (Vi-TCV: control).

The cumulative incidence of typhoid will be summarised using the Kaplan-Meier method. Participants will be censored in the analysis at the time of last known residence in or near the surveillance area, at the last known contact time or at the 2-year final visit. Statistical significance will be determined as a p value from a log-rank test of less than 0.05.

Subgroup analyses will include:

- age (< 5 years and >= 5 years)
- age (< 2 years and >= 2 years)
- male vs female

A fully detailed statistical analysis plan will be prepared and signed off by the Chief Investigator prior to conducting any data analyses.

34.2. The Number of Participants

Sample size calculations are based on the following assumptions:

5. An overall incidence of typhoid fever of 85 cases per year, per 100,000 persons in the entire population, with higher incidence rates in children under 16 years.
6. Age specific incidence rates were determined from the age distribution of typhoid cases, which is specific to Kathmandu, from published estimates and from site specific surveillance data.

7. A direct effect of vaccination of 75% and an indirect effect of 25% based on mathematical modelling.
8. 25% loss to follow up per year due to moving out of the area, based on current surveillance data from Patan.

Table 2. Sample Sizes under Varying Assumptions in Nepal (80% power, 5% alpha).

Overall incidence in the entire population without vaccination Per 100,000py	**Direct effect of vaccine**	**Total number to enroll**
100 cases py	80%	12,475
85 cases py	80%	14,677
100 cases py	75%	14,785
85 cases py	75%	17,395

The above assumptions are conservative; however, to allow for further variation in the assumptions, the total sample size has been increased to 20,000 children (10,000 in each vaccination arm).

Over the two-year follow-up of the trial it is expected to see approximately 36 cases of typhoid in the control arm and 9 cases in the Vi-TCV arm.

Investigation of safety outcomes:

If the background rate of a specific rare but serious adverse event (SAE) is ~30/100,000 individuals, (as seen with intussusception in the rotavirus vaccine trials), this study has 80% power to detect a five-fold increase in this SAE.

34.3. Procedure for Accounting for Missing, Unused, and Spurious Data.

All available data will be included in the analysis

34.4. Inclusion in Analysis

All vaccinated participants will be included in the analysis.

34.5. Interim analysis

Efficacy

An interim analysis may be undertaken if the incidence of blood culture positive typhoid fever is higher than anticipated in the sample size calculations. At least 1 full year of follow-up will be conducted before consideration will be given to conducting the interim analysis. The trigger for the interim analysis will be when 45 cases of typhoid have been detected as this is the number of cases expected in sample size calculations and represents a fully-powered analysis. Agreement from the international DSMB will be requested prior to the interim analysis being conducted. Individual participants will not be unblinded and follow-up will continue for the full two years in order to assess longer-term vaccine efficacy. Results of the interim analysis will be communicated to policy-makes, funders, and the wider scientific community through meetings and publications, and the local Lalitpur community through local public engagement activities.

Safety and immunogenicity

An unblinded interim analysis of safety and immunogenicity data will be undertaken following completion of the 6 month follow up contact and the Day 28 immunogenicity blood draw. Unblinded aggregated safety and immunogenicity results from the interim analysis will be communicated to policy-makes, funders, and the wider scientific community through presentations and publications. This analysis will provide detailed safety and immunogenicity date associated with Vi-TCV to global decision makers, which may be important as countries consider the introduction of the vaccine into routine schedules. Unblinded analysis will be completed by designated senior statisticians, who are not involved in trial delivery, participant follow-up, and data collection. Procedures will be put in place to ensure trial participants and blinded implementation staff remain blinded. Individual level safety and immunogenicity data and all vaccine efficacy data will remain blinded until the time that official unblinding occurs.

34.6. Procedures for Reporting any Deviation(s) from the Original Statistical Plan

A detailed statistical analysis plan will be prepared and signed off by the Chief Investigator prior to unblinding of any interim or final study data.

35. DATA MANAGEMENT

35.1. Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence.

CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All documents will be stored safely in confidential conditions. On all trial-specific documents, other than the signed consent, the participant will be referred to by the trial participant number/code, not by name.

35.2. Access to Data

Direct access will be granted to authorised representatives from the Sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

35.3. Data Recording and Record Keeping

Hand-held tablets or laptops will be used to collect and record all data in trial CRFs. All CRF and randomisation data will be collected off-line and uploaded to a secure server on a regular basis, when tablets/laptops are brought back to the central office, and reliable internet is available.

CRFs will be designed and maintained on REDCap, a secure web application for building and managing online surveys and databases. REDCap will be validated by data management and IT staff within Oxford University. The CRFs will be designed and maintained by a dedicated trial data manager, and quality control checks will be performed on a regular basis.

All participants will be identified by a unique trial specific number and/or code, this will not include any identifiable information. Individual names, addresses, and any other identifying detail will not be included in any trial data electronic file. CRFs will capture participant medical information from Patan hospital and trial clinic records, including but not limited to type of illness, severity, duration of illness, and treatment prescribed. Blood culture confirmed typhoid infections will be recorded in the CRFs. The results of three-monthly follow-up contact will be captured in the CRFs, including but not limited to previous illnesses occurring since last contact with or without medical treatment, and type of treatment sought, if any.

Trial staff will have access to REDCap via unique usernames and passwords. Each trial staff member will have an appropriate level of access to CRFs and collected data, according to their roles and responsibilities within the trial.

All participant data will be stored and maintained on servers within Nepal for the duration of the trial. Anonymised data will be uploaded to a central database in the UK. At the end of the trial, all individually identifiably data will be removed, and fully anonymised data will be retained for further analysis.

36. QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures.

Monitoring will be performed by representatives of the sponsor and according to the principles of ICH GCP. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following a risk based monitoring plan, the monitors will verify that the clinical study is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

37. SERIOUS BREACHES

A serious breach is defined as "A breach of GCP or the trial protocol which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the subjects of the trial; or

(b) the scientific value of the trial".

In the event that a serious breach is suspected the Sponsor must be contacted within one (1) working day. In collaboration with the CI, the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee and Regulatory authority within seven (7) calendar days.

38. ETHICAL AND REGULATORY CONSIDERATIONS

38.1. Declaration of Helsinki

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki.

38.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this trial is conducted in accordance with relevant regulations and with Good Clinical Practice.

38.3. Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to the appropriate Research Ethics Committees (REC) and regulatory authorities in Nepal and Oxford and Patan Hospital for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

38.4. Reporting

The CI shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the RECs (Nepal and Oxford), host organisation and Sponsor. In addition, an End of Trial notification and final report will be submitted to the RECs, host organisation and Sponsor.

38.5. Participant Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant ID number on all trial documents and any electronic database. All documents will be stored securely and will only be accessible by trial staff and authorised personnel. The trial will comply with the UK Data Protection Act, which requires data to be anonymised as soon as it is practical to do so and local regulations.

38.6. Expenses and Benefits

There will not be any payments or reimbursements made to participants, as incentive for participant recruitment. It is anticipated that provision of vaccination will be enough incentive to reach the necessary sample size. Local hospitals, clinics, and vaccination points are being used to deliver all trial components, which will not add additional travel or expense to participants and their families.

The trial will cover the costs of standard care treatment for participants presenting with fever (≥2 days) as part of the trial, including the cost of tests, antibiotics and/or other prescribed medications, and in-patient hospital stays and care, if medically necessary.

If participant presentation to hospital and ward trial clinics is less than expected, community health volunteers working in the trial area may be provided nominal incentives for referring participants with febrile illness to the trial health clinics and Patan hospital.

38.7. Other Ethical Considerations

All efforts will be made to conduct the research in a way that is sensitive to the Nepali culture and the social values. Nepali trial staff will be present at all times during the consent process, and the participant study related materials (information sheet, consent forms, etc) will be printed in Nepali.

Children aged 9 months – ≤16 years have been selected because children bear a substantial burden of the disease in both mortality and morbidity, without an effective vaccine available. Therefore, this demographic group has most to gain from vaccination with the Vi-TCV and would be the primary target for any subsequent vaccination campaign.

The meningococcal group A vaccine was selected as the control vaccine to ensure that the control group is receiving a beneficial intervention. The control vaccine will provide protection against group A meningitis, which is currently the most common serotype in Nepal, and can cause severe disease.

Samples and data collected may be shared with other researchers in Europe, Asia, and/or America, as some of the assays and analyses cannot be done in Nepal. Only anonymised samples and data will be sent outside of the research site. At the end of the study, all remaining samples in Nepal will be kept for storage in the Patan Hospital Microbiology Laboratory, as required by the Nepal Health Research Council (NHRC). All remaining samples overseas will be kept for storage under the oversight of Oxford University. All samples will be kept for a minimum of 10 years after the end of the trial. New and better tests may become available in the future. Storage of these samples may also allow important future research to be done without needing to take new samples from Nepali children.

Potential participants or their parents/legal guardians will be notified that they will be able to refuse to have the relevant biological samples stored, without this otherwise influencing participation in the study or the clinical care of their child. They will also be informed that should they no longer wish for their samples to be retained they may request their destruction.

39. FINANCE AND INSURANCE

39.1. Funding

This study is funded by the Bill and Melinda Gates Foundation to the University of Maryland, Baltimore, with the University of Oxford as a collaborating institution.

39.2. Insurance

The University of Oxford has a specialist insurance policy in place that would operate in the event of any participant suffering harm as a result of their involvement in the research.

40. PUBLICATION POLICY

The investigators will co-ordinate dissemination of data from this study. All publications, including manuscripts, abstracts, oral/slide presentations, and book chapters, etc., based on data from this study will be reviewed by each sub-investigator prior to submission. Authors will acknowledge that the study

was funded by BMGF. In accordance with BMGF, all publications related to this study will be open access. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

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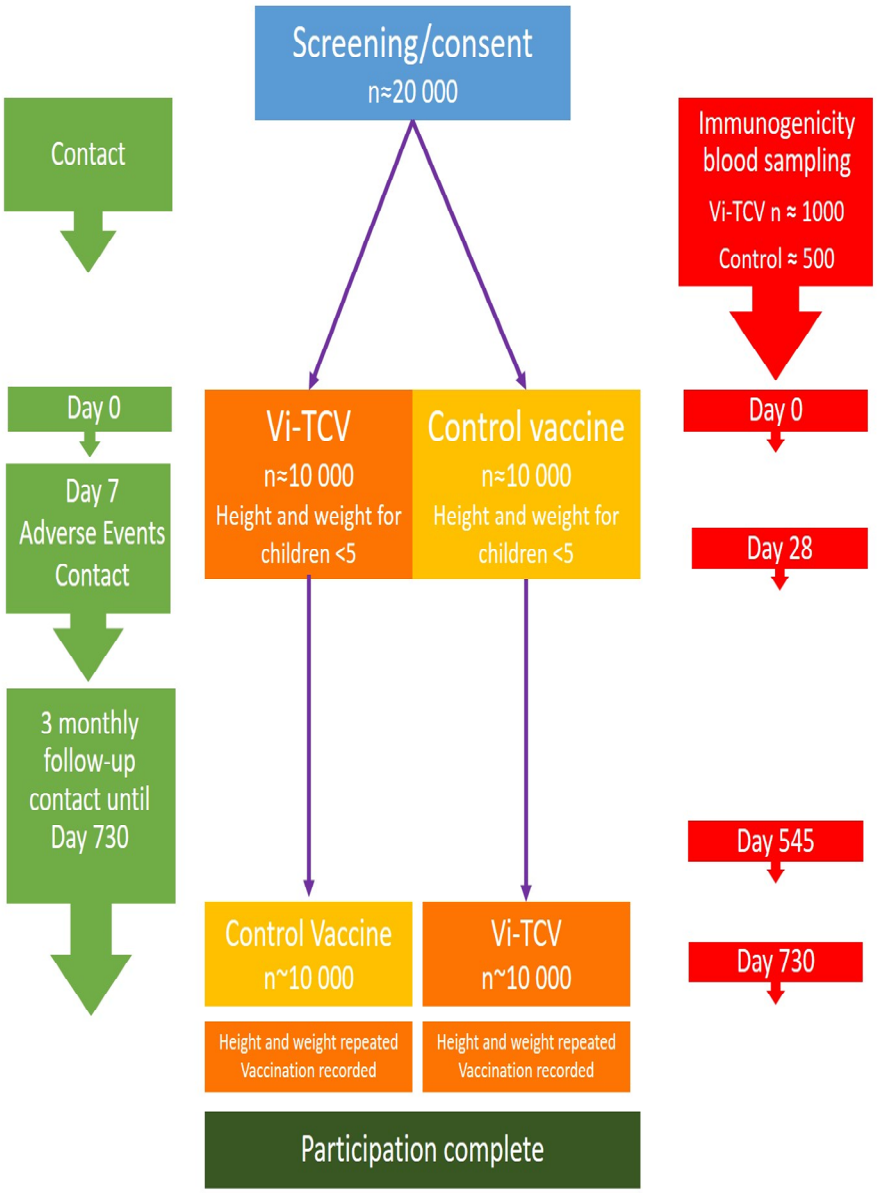
42. APPENDIX A: TRIAL FLOW CHART FOR PLANNED CONTACT


Screening/consent
n≈20 000
Contact
Immunogenicity blood sampling
Vi-TCV n ≈ 1000
Control ≈ 500
Day 0
Vi-TCV
n≈10 000
Height and weight for children <5
Control vaccine
n≈10 000
Height and weight for children <5
Day 0
Day 7
Adverse Events
Contact
Day 28
3 monthly follow-up contact until Day 730
Day 545
Control Vaccine
n~10 000
Vi-TCV
n~10 000
Day 730
Height and weight repeated
Vaccination recorded
Height and weight repeated
Vaccination recorded
Participation complete

43. APPENDIX B: SCHEDULE OF PLANNED PROCEDURES

Table 1: Visit and sample schedule

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	X										
Randomisation	X										
Vaccination	X										
Medical history and exam	X										
Blood collection (b)	X		X						X		X
Height and weight (c)	X										X
Follow-up contact (d)		X**		X	X	X	X	X	X	X	X
Vaccination with either Vi-TCV or Men A(e)											X
Documentation of vaccine receipt(f)	X										X

g) Ideally, all of the visit 11 activities will occur simultaneously, but the follow-up contact may occur separately, if necessary

h) Blood sampling for immunogenicity in a subset of approximately 1000 Vi-TCV, 500 control participants. Planned blood draw ~5 ml per visit. Total maximum volume ~20ml per participant
   - Blood draw on day 0 will occur before vaccination; blood draw at day 545 will aim to occur at the same time as follow-up; blood draw at day 730 will occur at some time as unblinding and vaccine documentation
   - Additional free blood tests will be offered only at the time of study blood draws

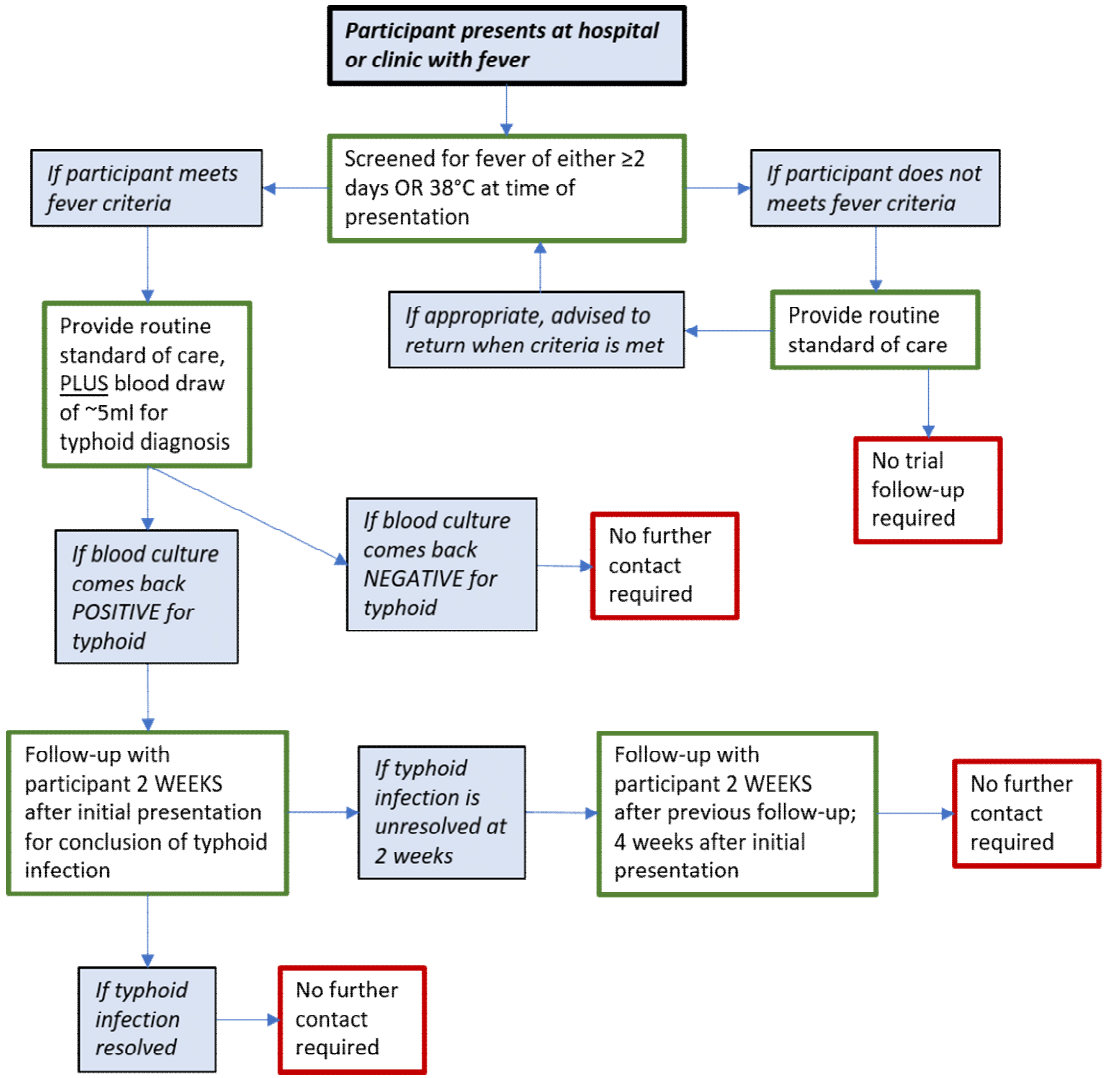
i) Only children under 5 years of age at the time of enrolment

j) Follow-up contact includes:
   - 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 trial health care facility if they develop fever of ≥2 days
   - **: At 7 days full AEFI reporting will be collected

k) Either the Vi-TCV or Men A vaccine will offered at the end of the trial depending on which vaccine the child initially received.

l) Both the intervention and control arms will be asked to return for un-blinding (at day 730 only) and documentation of vaccination

44. APPENDIX C: UNSCHEDULED PROCEDURES FLOWCHART

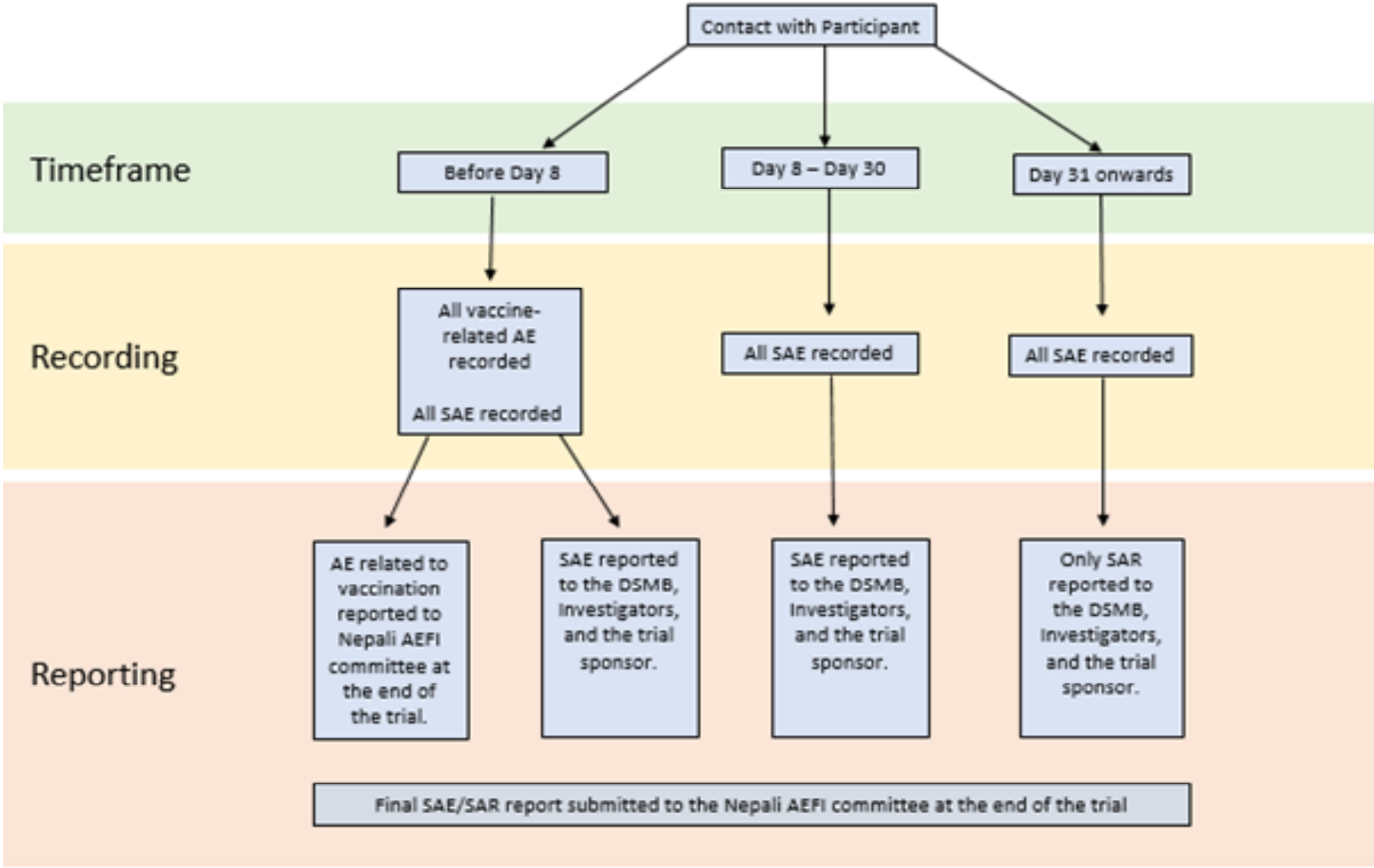


Flow-chart applies to all unscheduled participant presentations.

Blood drawn for unscheduled presentations is in addition to any immunogenicity samples collected.

Total blood drawn for unscheduled visits = (~5ml per visit) x (# of visits meeting criteria)

45. APPENDIX D: SAE REPORTING



46. APPENDIX E: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	V 3.0	12/10/2017	Rachel Colin-Jones	• Changes to AEFI reporting process • Wording of some sentences changed for clarity of meaning • Nepalese changed to Nepali as this is the correct term when referring to people
2	V 4.1	05/12/2017		• Addition of two Independent Safety Monitors • Inclusion of DSMB halting rules • Offering the intervention group the control (MenAfriVac) vaccine at the end of the study • Correction of OxTREC ethics number
3	V 4.2	03/01/2018		• NHRC number added
4	V 5.1	27/04/2018	Nicola Smith	• Change of follow-up in passive surveillance to correlate with STRATAA study • Offer of additional free blood tests for immunogenicity participants. • Addition of small incentives for passive surveillance and immunogenicity visits
5	V 6.0	17/09/2018	Rachel Colin-Jones	• Interim efficacy analysis proposed • Additional secondary objective to do an additional sensitivity analyses or secondary analyses using only blood cultures from participants with 3 days of fever. • Addition of a site for laboratory processing – University of Oxford • Proposal to produce Safety Update Reports • Clarifications throughout

6	V 7.0	04/01/2019	Rachel Colin-Jones	• Interim safety and immunogenicity analysis proposed

Summary of changes

Amendment No.	Protocol Version No.	Date issued	Details of Changes made
Changes made during the initial ethics approval process	V 2.0	07/08/2017	• The trial was changed from a phase IV trial to a phase III trial • An exploratory objective was added to look at host genetic susceptibility to typhoid in individuals with and without Vi-TCV vaccination • Following feedback from the Nepal Ethics Committee, the age of assent was changed from 12 years old to 7 years old to be in line with local regulations • Detail about the host genetic susceptibility laboratory analysis. • Further detail about the laboratory processing of plasma samples and locations where samples would be shipped to was added.
1	V 3.0	12/10/2017	• Changes to AEFI reporting process • Wording of some sentences changed for clarity of meaning • Nepalese changed to Nepali as this is the correct term when referring to people
2	V 4.1	05/12/2017	• Addition of two Independent Safety Monitors • Inclusion of DSMB halting rules • Offering the intervention group the control (MenAfriVac) vaccine at the end of the study • Correction of OxTREC ethics number
3	V 4.2	03/01/2018	• NHRC number added
4	V 5.1	27/04/2018	• Change of follow-up in passive surveillance to correlate with STRATAA study

			• Offer of additional free blood tests for immunogenicity participants. • Addition of small incentives for passive surveillance and immunogenicity visits
5	V 6.0	17/09/2018	• Interim efficacy analysis added • Additional secondary objective to do an additional sensitivity analyses or secondary analyses using only blood cultures from participants with 3 days of fever. • Addition of a site for laboratory processing – University of Oxford • Proposal to produce Safety Update Reports • Clarifications of wording throughout
6	V 7.0	04/01/2019	• Interim safety and immunogenicity analysis added

STATISTICAL ANALYSIS PLAN

TYVAC NEPAL

Short title: TyVAC Nepal: Typhoid Vaccine Study

OVG Ref: OVG2017/05

Ethics Ref: OxTREC 17-15

Sponsor: University of Oxford

	NAME	TITLE	SIGNATURE	DATE
Written by:	Merryn Voysey	Senior Statistician		15 Feb 2019
Reviewed by:	Xinxue Liu	Senior Statistician		21 Feb 2019
Approved by:	Andrew Pollard	Chief Investigator		21/2/2019

Version History

Version:	Version Date:	Changes:
1.0	15 February	First Draft

1 TABLE OF CONTENTS

2 INTRODUCTION

2.1 Preface

The TyVAC Nepal: Typhoid Vaccine Study falls within a larger multi-institution collaboration, called The Typhoid Vaccine Acceleration Consortium (TyVAC). TyVAC is a Bill and Melinda Gates Foundation funded project to generate evidence for Vi-TCV vaccine impact, and accelerate use of Typhoid Conjugate Vaccines in countries with significant typhoid burden. Managed by University of Maryland, in collaboration with University of Oxford, and PATH international, the TyVAC programme includes vaccination trials, health economics studies, country preparedness support for routine vaccine introduction, and the collation and synthesis of typhoid research and evidence.

Within the larger consortium, the TyVAC Nepal randomised controlled trial is an individually randomised, double-blind vaccine efficacy trial which will assess the impact of vaccination with Typbar TCV (Vi-TCV) compared with MenAfriVac in children aged 9 months to <16 years of age living in Lalitpur, Kathmandu.

2.2 Purpose and scope of the plan

This document details the proposed analysis of the main paper(s) reporting results from the TyVAC Nepal: Typhoid Vaccine Study. The results reported in these papers should follow the strategy set out here. Subsequent analyses of a more exploratory nature will not be bound by this strategy, though they are expected to follow the broad principles set out here. The principles are not intended to curtail exploratory analysis, nor to prohibit accepted practices, but they are intended to establish the principles that will be followed, as closely as possible, when analysing and reporting the trial.

2.3 Objectives

2.3.1 Primary Objective

To determine the efficacy and rate reduction of the Vi-TCV in preventing blood culture-confirmed symptomatic infection caused by S. Typhi

2.3.2 Secondary objectives

1. To investigate safety outcomes associated with Vi-TCV vaccination, within the study population
2. To determine the impact of vaccination with Vi-TCV on the incidence of inpatient/outpatient admission rates for fever
3. To measure the difference in rates of hospital and clinic presentation for febrile illness in each vaccination arm
4. To determine Paratyphoid infection rates in each vaccination arm
5. To measure days spent in hospital from febrile illness
6. To measure incidence of clinically-suspected enteric fever

2.3.3 Exploratory objectives

1. To measure antibiotic/ antimicrobial use for inpatients/outpatients in each vaccination arm
2. To measure the difference in the duration of febrile illness in each vaccination arm
3. To measure difference in rates of all-cause hospitalization
4. To determine effect of vaccination on child growth and weight in children <5 years of age
5. To determine the immunogenicity of Vi-TCV in a subset of participants, stratified by age groups.
6. To determine the persistence of antibodies induced by Vi-TCV in stratified age groups.
7. To measure the incidence of fever that does not result in medical treatment in each vaccination arm
8. To determine rates of absenteeism from school/work in each vaccination arm
9. To measure all-cause mortality, and all-cause mortality with fever, in each vaccination arm
10. To measure the rate of suspected and confirmed acute abdominal presentation in each vaccination arm
11. To measure the rate of surgical intervention for acute abdominal complaints in each vaccination arm
12. To analyse host genetic susceptibility to typhoid in individuals with and without Vi-TCV vaccination

2.4 Outcomes measures

2.4.1 Primary outcome measures

1. The incidence of blood culture confirmed typhoid fever in each of the vaccination arms.

Available fields for analysis

a. typhoid diagnosis confirmed [`fev_24_typhoid_con`, `fev_18_results`]
b. dates/days fever [`fev_8_start`]
c. typhoid diagnosis from notes review [`typh_rev_25_typh_con`]
d. dates/days from notes review [`typh_rev_8_fev_start`]
e. date of last follow-up [`fu_1_date`, `final_1_date`, `withdraw_6_date`, `sae_22_death_date`]

Analysis notes

1. Each participant will be classified as having blood culture confirmed typhoid fever if `fev_24_typhoid_con=1 or typh_rev_25_typh_con =1`. If there are participants with more than one blood culture confirmed typhoid fever, only the first event will be used in the Kaplan-Meier analysis but both will be included in incidence estimates.

2. The time to fever for Kaplan-Meier plots will be computed as the fever start date (`fev_8_start or typh_rev_8_fev_start`) minus the date of vaccination for that person.
3. For medical record review cases, if the start date of fever in unknown then the following dates will be substituted:
    a. the start date of fever as reported from the follow up phone call which led to the medical record review.
    b. the date of the follow up phone call
    c. the date of the medical record review
4. Length of follow up will be calculated as the date of last contact minus the date of vaccination.

2.4.2 Secondary outcome measures

1. The proportion of participants developing all adverse events within the first 7 days post-vaccination, and serious adverse events within 6 months of vaccination, as determined through self-reporting at follow-up contact.

    Adverse events within first 7 days:
    a. Fever [`d7_aefi_9_fever, d7_aefi_15`]
    b. pain [`d7_aefi_15_pain`]
    c. swelling [`d7_aefi_15_swell`]
    d. redness [`d7_aefi_15_red`]
    e. other local [`d7_aefi_16`]
    f. loss of appetite [`d7_aefi_21`]
    g. less active [`d7_aefi_23`]
    h. more irritable [`d7_aefi_25`]
    i. persistent crying [`d7_aefi_27`]
    j. sick child [`d7_aefi_29`]
    k. diarrhoea [`d7_aefi_31`]
    l. other serious illness [`d7_aefi_33_serious`]

    Serious adverse events within 6 months
    a. SAEs to be coded by MedDRA [`sae_4_desc`]

2. Rates of participants with at least ≥2 days of subjective persistent fever, or a temperature of 38 degrees C at presentation at Patan Hospital or trial clinics in each vaccination arm, stratified by duration and severity of fever
    a. Fever [`fev_6`]
    b. Fever dates/days [`fev_8_start, fev_10_end, fev_11_days`]

   c. temperature at presentation [fev_5_temp_c]
   d. dates/days fever that presented to Patan or clinic [fev_11_days]
3. Rates of hospital or clinic presentation with febrile illness of any duration in each vaccination arm, measured by hospital presentation logs, hospital records, trial clinic records and self-reporting during three monthly follow-up
   a. Follow up fever [fu*_12_fever, fu*_46a, fev_6]
   b. Follow up fever dates/days [fu*_13_fever_start, fu*_14_fever_end, fu*_15_fever_days, fev_8_start, fev_10_end, fev_11_days]
   c. temperature at presentation in Patan hospital [fev_5_temp_c]
   d. dates/days fever that presented to Patan or clinic [fev_11_days]
4. Rates of blood culture confirmed paratyphoid cases in each vaccination arm
   a. From fever passive surveillance data [fev_18_results]
   b. and medical records review [typh_rev_25_results]
5. Length of stay in hospital, collected from Patan hospital patient records, and self-reported, in each vaccination arm
   a. admission date [fev_1_date] and [fev_22_hospital]
   b. hospital discharge date [fev_29]
   c. duration of hospitalisation [fu_53, fu_5, fu_56]
6. Number of clinical diagnoses of typhoid fever, as determined by trial staff in Patan hospital outpatient clinics and trial clinic, in each vaccination arm. [fev_14, fu*l_23_typhoid_sus]

2.4.3 Exploratory outcome measures

1. Incidence of antibiotic/antimicrobial use in in/outpatient settings, from Patan hospital and trial clinic admission logs, and/or self-reported in follow-up contact
   a. antibiotic given [fev_19_antib]
   b. antibiotic prescribed [typh_rev_20_antib]
2. Duration of fever recorded in Patan hospital and trial clinics, and self-reported via follow-up contact
   a. typhoid diagnosis confirmed [fev_24_typhoid_con]
   b. dates/days fever [fev_8_start, fev_10_end, fev_11_days]
   c. typhoid diagnosis from notes review [typh_rev_25_typh_con]
   d. dates/days from notes review [typh_rev_8_fev_start, typh_rev_10_fev_end, typh_rev_11_fev_days]
3. Rates of hospitalization, identified through hospital admission logs and self-reporting in each vaccination arm
   a. hospitalisation [fu_48_hospital, typh_rev_12_hospital, fev_22_hospital]
4. Measurement of anthropomorphic parameters of children at baseline and at two years, in all children aged <5 years at the time of vaccination
   a. baseline [exam_4_height, exam_5_weight]
   b. at 2 years [final_8_weight, final_9_height]

5. Assay of anti-Vi IgG antibodies in blood samples collected at baseline (Day 0) and at one month (Day 28) in a subset of participants.
    a. Results from the lab in Vietnam
6. Assay of anti-Vi IgG antibodies in blood samples collected at baseline (day 0), 18 months (day 545), and two years (day 730) in a subset of participants.
    a. Results from the lab in Vietnam
7. Self-reported fever at follow-up contact of participants.
    a. Fever in first 7 days [`d7_aefi_9_fever, d7_aefi_15`]
    b. Number of days of fever [`d7_aefi_14`]
    c. Fever start date and end date [`d7_aefi_11, d7_aefi_13`]
    d. Follow up fever [`fu_12_fever, fev_6`]
    e. Follow up fever dates/days [`fu_13_fever_start, fu_14_fever_end, fu_15_fever_days, fev_8_start, fev_10_end, fev_11_days`]
8. Rates of absenteeism from school or work, as applicable, as reported by parents at follow-up contact
    a. missed days of work/school? [`fu2_41_parent_abs`]
    b. days missed [`fu2_43, fu2_45, fu2_46`]
9. Rates and circumstances of mortality in each vaccination arm, recorded from hospital records and three-monthly follow-up
    a. date of death [`sae_22_death_date`]
10. Rates of presentation to Patan hospital or trial clinics with acute abdomen
11. Rates of acute abdomen surgery in each vaccination arm, and gross surgical findings
    a. surgery [`fev_30_surgery, fev_33`]
    b. surgery type [`fev_32, fev1_32`]
    c. note review surgery and surgery type

3 ANALYSIS – GENERAL CONSIDERATIONS

Some of the data are likely to be skewed, and so skewed variables will be $\log_{10}$ transformed prior to analysis. If an appropriate transformation does not normalize the distribution, variables will be categorised.

Results of group comparisons on log scales will be presented as geometric means in each group, as well as the relative difference, 95% confidence interval (CI).

All statistical tests will be 2-sided and P values less than 0.05 will be considered significant unless specified otherwise in sections 4 & 5 below.

Normally distributed continuous variables will be summarised using mean, standard deviation and range values, and number of missing. Variables that are to be analysed non-parametrically (or variables far from normal distribution) will be summarised using median, interquartile range and range values. Categorical values will be presented as counts, percentages and/or centiles. 95% confidence intervals for means and differences will be

calculated, to demonstrate the plausible effects of random variation. Both numbers and proportions will be presented for binary data.

3.1 Characteristics of participants

Children aged 9 months to <16 years who are in good health at the time of enrolment. Baseline characteristics will be summarised for each group to describe the study population. No formal statistical comparisons of baseline characteristics between randomised groups will be conducted. Patient throughput from enrolment, through randomisation, follow up and analysis will be presented in a CONSORT flow diagram. This will contain the numbers of participants randomly assigned to each group, receiving vaccination, completing the study and analysed for the primary outcome. It will also include a breakdown of reasons for withdrawal.

3.2 Definition of population for analysis

All vaccinated participants will be included in the population for analysis.

3.3 Interim analysis

If 45 cases of typhoid are detected before the two year end of study visit the study will have full power and consideration will be given to limited unblinding of the data for analysis and publication of preliminary efficacy, safety and immunogenicity results. In the event of early unblinding and publication study follow up would continue for the full two years and individual participants and trial staff and clinicians would not be unblinded to the randomisation allocations for individual participants.

Interim analyses will follow the same analysis methods as detailed below for final analysis results.

4 PRIMARY ANALYSIS

4.1 Primary outcome

The primary is the incidence of blood culture confirmed typhoid fever.

The incidence of typhoid will be estimated as the number of cases divided by the total number of person-years of follow up. Follow up time will be calculated as the last date of contact (from follow up phone calls or other study contact) minus the date of randomisation. Incidence will be presented with 95% confidence intervals for each group and overall. Incidence rate ratio (IRR) will be computed as the ratio of the incidence in the Vi-TCV arm compared to the control arm.

Vaccine efficacy (VE) will be calculated as (1 – IRR) x 100%, where IRR is the incidence rate ratio (Vi-TCV: control).

Estimates of vaccine efficacy will be presented for typhoid cases occurring with one year of vaccination and for cases occurring in the second year post-vaccination separately as well as overall.

The cumulative incidence of typhoid will be summarised using the Kaplan-Meier method. For any participant with more than one blood culture confirmed typhoid fever, only the first event will be used in the Kaplan-Meier analysis but both will be included in incidence estimates. Participants without blood culture confirmed typhoid will be censored in the analysis at the time of last known residence in or near the surveillance area, at the last known contact time, at the 2 year final visit, or death.

The primary analysis will include all blood culture positive cases with the exception of those which occurred within two weeks of vaccination.

Statistical significance will be determined as a p value from a log-rank test of less than 0.05.

4.2 Secondary analysis of the primary outcome

Adjusted model

A secondary analysis of the primary outcome will be conducted using a Poisson regression model which will adjust for baseline characteristics (sex, age, previous typhoid diagnosis).

Sensitivity analysis

Secondary sensitivity analyses of the primary outcome will be conducted as follows:

1. exclusion of blood culture positive cases where confirmation of the culture results was not possible.
2. exclusion of blood culture positive cases that occurred in participants with fewer than 3 days of fever

4.3 Subgroup analyses

Subgroup analyses for the primary outcome will be conducted using adjusted Poisson models with interaction effects for the following subgroups:

- age (< 5 years and >= 5 years)
- age (< 2 years and >= 2 years)
- gender (male and female)

If any subgroup has less than 10 events per group then interactions will not be computed. p values from interaction effects will be adjusted for multiple comparisons using the Bonferroni method.

4.4 Missing data

All vaccinated participants will be included in the analysis up until the time of their last known follow up prior to withdrawal or unblinding. Missing data occurs when the participant attends for fever but does not consent to blood culture, or a blood sample is not able to be successfully taken, or does not attend passive surveillance for fever. In the primary analysis fever presentations that were eligible for blood culture but did not consent to blood culture will not be imputed. However, as an additional exploratory analysis, we will impute the blood culture results for these children based on the appropriate imputation methods and the effect of non-culturing of eligible fever cases and the predictors of missing data will be explored.

5 ANALYSIS OF SECONDARY OUTCOMES

1. The proportion of participants developing all adverse events within the first 7 days post-vaccination, and serious adverse events within 6 months of vaccination, as determined through self-reporting at follow-up contact.

Counts and percentages of each local and systemic solicited adverse reaction will be presented for each vaccine arm. Statistical comparisons will not be made.

Serious adverse events (SAE) will be coded using MedDRA by a blinded medical coder.

Counts and percentages of SAEs will be presented by MedDRA system organ class and preferred term for each group separately and overall. Data will also be presented by severity, expectedness, and relatedness to study medication.

No statistical comparisons of SAEs are planned.

2. Rates of participants with at least ≥2 days of subjective persistent fever, or a temperature of 38 degrees C at presentation at Patan Hospital or trial clinics in each vaccination arm, stratified by duration and severity of fever

3. Rates of hospital or clinic presentation with febrile illness <u>of any duration</u> in each vaccination arm, measured by hospital presentation logs, hospital records, trial clinic records and self-reporting during three monthly follow-up

Outcomes 2 & 3 will be analysed in a similar manner. The number of fevers will be divided by the total number of person-years of follow up in each group to obtain the incidence rate. Incidence rate ratio (IRR) will be computed as the ratio of the incidence in the Vi-TCV arm compared to the control arm and presented with 95% CI.

Analyses will be conducted for:

- fever of any duration (outcome 3)
- fever ≥2 days (outcome 2)
- fever ≥3 days (outcome 2)

- admitted to hospital vs not (severity) (outcome 3)

4. Rates of blood culture confirmed paratyphoid cases in each vaccination arm

This analysis will be the same as the analysis of blood culture confirmed typhoid (primary outcome)

5. Length of stay in hospital, collected from Patan hospital patient records, and self-reported, in each vaccination arm

The distribution of the length of stay variable is expected to be skewed but not log-normal. The distribution of the data will be examined and analysed using the non-parametric Mann-Whitney U test unless transformation to normalise the data is possible.

Analyses will be conducted on all hospital admissions. Further separate analyses will be conducted for hospital admissions for confirmed typhoid fever and for suspected typhoid fever to determine if the vaccine has an impact on the length and therefore severity of the illness.

6. Number of clinical diagnoses of suspected typhoid fever

This analysis will be the same as the analysis of blood culture confirmed typhoid (primary outcome)

6 ANALYSIS OF EXPLORATORY OUTCOMES

7. Incidence of antibiotic/antimicrobial use in in/outpatient settings, from Patan hospital and trial clinic admission logs, and/or self-reported in follow-up contact

Antibiotic use will be analysed for in-patient and outpatients separately, as well as for all patients overall. Chi-square tests will be used to compare the antibiotic use proportions between groups for; any antibiotic; and for each specific antibiotic separately.

8. Duration of fever recorded in Patan hospital and trial clinics, and self-reported via follow-up contact.

Self-reported duration of fever is a continuous variable but may not be normally distributed. If normally distributed then a t-test will be used to compare groups, otherwise a Mann Whitney U test will be used.

9. Rates of hospitalization, identified through hospital admission logs and self-reporting in each vaccination arm

Whilst many participants will have no hospitalisations during study follow-up, some participants may have multiple events. These will be analysed using a zero-inflated Poisson

or zero-inflated negative binomial model depending on the variability and distribution of the data.

10. Measurement of anthropomorphic parameters of children at baseline and at two years, in all children aged <5 years at the time of vaccination

Heights and weights will be converted into z-scores using the WHO growth score macros (http://www.who.int/childgrowth/software/en/).

The change in height and weight from baseline to 2 years for z-scores will be calculated for each child and analysed using linear regression model adjusted for baseline values.

11. Self-reported fever at follow-up contact of participants.
    This will be analysed in the same way as outcome 2.
12. Rates of absenteeism from school or work, as applicable, as reported by parents at follow-up contact

The proportion of typhoid fever cases that resulted in missed school or work for each arm will be summarised and compared using a chi-square test.

The number of days absent from school or work will be summarised for each group and overall. If sufficient typhoid cases are reported in both arms of the trial, and if sufficient numbers of absenteeisms are reported for these cases, and if the data are normally distributed, then a t-test will be used to compare groups, otherwise a Mann Whitney U test will be used or analyses will be descriptive in nature only.

13. Rates and circumstances of mortality in each vaccination arm, recorded from hospital records and three-monthly follow-up

The number and proportion of deaths in each arm of the trial will be summarised overall and by cause of death (major categories). No statistical comparisons will be conducted as it is not expected that there will be sufficient numbers of deaths for comparative analysis.

14. Rates of presentation to Patan hospital or trial clinics with acute abdomen
15. Rates of acute abdomen surgery in each vaccination arm, and gross surgical findings

The number of presentations for acute abdomen, the numbers of surgeries for acute abdomen will be divided by the total number of person-years of follow up in each group to obtain the incidence rate. Incidence rate ratio (IRR) will be computed as the ratio of the incidence in the Vi-TCV arm compared to the control arm and presented with 95% CI.

7 EXPLORATORY IMMUNOGENICITY OUTCOMES

16. Anti-Vi IgG antibodies in blood samples collected at baseline (Day 0) and at Day 28, 545, 730 in a subset of participants.

Data will be log-transformed prior to analysis. At each time point the number of samples below the lower limit of quantification will be summarised. The geometric mean concentration of anti-Vi IgG and associated 95% confidence interval will be summarised for each group at each visit, by computing the anti-log of the mean difference of the log-transformed data. Comparisons between groups will be made using a Mann Whitney U test.