**Effectiveness of the TYPHIBEV® typhoid conjugate vaccine in Nepal:**

**a test-negative, case control study protocol**

Version 2

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## I. Background

Typhoid fever, a bacterial infection caused by *Salmonella* Typhi, causes global morbidity and mortality. The World Health Organization (WHO) estimates an annual global burden of 11-20 million typhoid cases a year and 128,000-161,000 deaths per year.[1] In many countries, autochthonous typhoid infection has been largely eliminated with improvements to water and sanitation. However, typhoid remains a prominent cause of disease in many low- and middle-income countries.

In recent years, advances in typhoid vaccine technology have offered new prevention strategies. The WHO prequalified a typhoid conjugate vaccine (TCV), Typbar TCV® by Bharat, in 2017.[2] In 2020, the WHO prequalified another typhoid conjugate vaccine, TYPHIBEV® from Biological-E, based on immunogenicity, safety, and non-inferiority data.[3] To date, there are no real-world effectiveness data for TYPHIBEV®.[4]

In Nepal, typhoid fever presents an ongoing public health problem.[5,6] Typhoid estimates at surveillance sites in Nepal exceeded the “high burden” threshold for disease. Typhoid remains a problem in both rural and urban communities in Nepal.[7] In April 2022, Nepal introduced TCV and conducted a national vaccination campaign using the TYPHIBEV® vaccine.[8] The campaign aimed to reach 7.5 million children and reported 95% achievement; a post-campaign coverage survey is planned.[9] Nepal introduce

To measure vaccine effectiveness in Nepal and of the TYPHIBEV® vaccine, we propose to conduct a test-negative design case-control study.[10] We will leverage an extant typhoid clinical surveillance program, the Surveillance for Enteric Fever in Asia Project (SEAP), to identify blood culture-positive typhoid case-patients and culture-negative controls.

This study is sponsored by the Bill & Melinda Gates Foundation. It is expected to run from 1 October 2022 through 30 September 2024.

## II. Objectives

### Primary objective

The primary objective of this study is to estimate the effectiveness of TYPHIBEV®, a typhoid conjugate vaccine manufactured by Biological E, against clinically presenting, symptomatic, blood culture-positive typhoid infection among children eligible for the typhoid conjugate vaccination campaign in April 2022.

### Secondary objectives

- Estimate vaccine effectiveness of TYPHIBEV® among age-stratified subgroups.
- Estimate vaccine effectiveness in the first two year after vaccination vs. more than two year after vaccination.

## III. Methods

### Design

We will conduct a retrospective 3:1 matched case-control study among vaccine-eligible case-patients who present for care, comparing those who are positive for *Salmonella* Typhi by blood culture with controls who test negative with blood culture for *Salmonella* Typhi. The study will evaluate vaccine effectiveness on the primary outcome of symptomatic, clinically presenting typhoid infection. We will identify cases and matched controls using surveillance data from the SEAP study.

### Ethics

This protocol has been submitted to and approved by the Nepal Health Research Council (protocol registration number 395/2022 P) and the institutional review boards at all study facilities.

### Study sites

Through the surveillance in the SEAP project, we will recruit participants from seven hospitals: Bir Hospital, Dhulikhel Hospital, Helping Hands Foundation, Siddhi Memorial Hospital, Civil Service Hospital, Ishan Children and Women's Hospital, Kanti Children's Hospital, Kathmandu Model Hospital (KMH), and Nepal Medical College Hospital (NMC).

Five of the facilities (Bir, Ishan, Kanti, Civil Service, KMH, and Helping Hands) are located in Kathmandu city. Kathmandu is the largest and capital city of Nepal. While recent, unpublished SEAP data indicate a drastic drop in typhoid cases, Kathmandu has previously been called "the typhoid capital of the world" due to widespread, endemic typhoid in the city.[11] These three

facilities all have a large number of pediatric consultations and have historically reported a large number of typhoid patients. NMC is in Kathmandu district, but outside the city. Siddhi is in Bhaktapur, a city next to Kathmandu proper.

Dhulikhel Hospital is located in Kavrepalanchok District, approximately 30 km east of Kathmandu. Kavrepalanchok is a rural district, and patients visit Dhulikhel, a large reference hospital, from throughout the district.

**Data sources**

We will identify potentially eligible participants through the SEAP project through routine clinical surveillance in the five health facilities described above: Dhulikhel Hospital, Helping Hands Foundation, Kanti Children's Hospital, Kathmandu Model Hospital, and Nepal Medical College Hospital.

Individuals are recruited into the SEAP project after seeking health care services (outpatient, inpatient, or laboratory services) at sentinel facilities. At inpatient and outpatient settings at Dhulikhel and KMH, patients are prospectively recruited into SEAP if they present to outpatient services with three or more days of fever within the past seven days or are inpatients with suspected enteric fever. Prospectively recruited participants are then tested for typhoid and paratyphoid (among other pathogens) by blood culture. At laboratory sites (Kanti, NMC, Helping Hands) and through the laboratory departments at Dhulikhel at KMH, SEAP participants are enrolled retrospectively following a blood culture positive for typhoid or paratyphoid.

From the list of children prospectively enrolled in SEAP, we will identify eligible case-patients and controls who tested positive or negative for typhoid on blood culture, respectively. We will additionally review laboratory logs at all sites to identify children who tested negative on blood culture for typhoid as an additional source of controls.

SEAP data are compiled and reviewed on a biweekly basis. Potential case-patients and controls will be identified through routine data monitoring procedures and assessed for eligibility (see below).

**Study population**

*Inclusion criteria*

- Resided in Nepal in April 2022.
- who were eligible for typhoid vaccine catch up campaign: born between 1 May 2006 and 1 January 2021 (between 15 years 11 months old and 15 months old on 1 April 2022) and who are eligible for typhoid vaccine in routine immunization after 1 July 2022 (15 month or older at the time of blood culture)
- Valid blood culture results at a participating study site.
- Valid phone number in study site records.

*Exclusion criteria*

- Did not live in Nepal in April 2022.

- Was not eligible to receive the typhoid vaccine during April 2022 campaigns or routine vaccination after July 2022
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**Case definition and eligibility**

We will identify eligible case-patients among SEAP participants who are eligible members of the study population (see above) with a blood sample that was culture-positive for *S.* Typhi after May 2022.

**Control definition and eligibility**

We will identify eligible controls among children who are eligible members of the study population (see above) and who:

- Presented to care at a study site.
- Had a sample that was blood culture-negative for *S.* Typhi.

**Matching**

Test-negative controls will be matched 3:1 to the case-patients. Matching factors include variables that are anticipated to be related to the likelihood of vaccine receipt, risk of infection, and care-seeking behaviors:

- Age, categorized as:
    - 15 months to under 5 years
    - 5 years to 9 years
    - 10+ years
- Geography, categorized as:
    - Kathmandu
    - Bhaktapur
    - Lalitpur
    - Kavrepalanchok District (urban)
    - Kavrepalanchok District (rural)
- Window of +/- 28 days between collection of culture-positive specimen among case-patients and culture-negative specimen for controls.

Where possible, we will preferentially recruit controls who have laboratory-confirmed alternate illness etiologies. Blood culture is very specific but has only moderate sensitivity, and some culture-negative controls are true positives.[12] In reviewing available controls, we will first focus on matching criteria. If there are more than three identified controls who meet all matching criteria, patients with a confirmed alternate etiology will be screened first.

**Exposure definition**

Typhoid conjugate vaccine:

- Received the TYPHIBEV® typhoid conjugate vaccine manufactured by Biological E as reported by either:
    - Recall.
    - Vaccination card.

**Primary outcome**

The primary outcome of this study is blood culture-confirmed typhoid infection.

**Implementation**

SEAP study staff will regularly review case data at least biweekly to identify potential case-patients based on age and diagnosis. Upon identification, study staff will call the parents or guardians of potential case-patients or their guardians to assess interest in study participation and screen for eligibility.

When an eligible case-patient is identified, study staff will review laboratory logs and SEAP data (among typhoid-negative participants) to identify control-patients who have the appropriate matching characteristics. If necessary, matched controls will be prioritized by closest specimen collection time to the case-patient. Study staff will call the parent or guardian of the potential control to assess interest in the study and screen for eligibility.

If a case- or control-patient is eligible and the parent or guardian assents, study staff will visit the residence of the patient. During the home visit, a research assistant (RA) will conduct informed consent procedures. The RA will ask to see the vaccination card, photograph the card if available, and fill a short, electronic questionnaire (Appendix A).

Study data will be collected and managed using REDCap electronic data capture tools hosted at Stanford University.[13,14] REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies. Study staff will use Android tablets to collect all data; tablets will be synced daily. Tablets will be password-protected and kept in a locked cabinet when not in use.

**Statistical analyses**

We will evaluate vaccine effectiveness of TYPHIBEV® against a primary outcome of symptomatic, clinically presenting, culture-positive typhoid. We will use conditional logistic regression to estimate the odds ratio of vaccination among cases and controls, accounting for the matched design. We additional adjust for age (as a continuous variable) and sex. We will estimate vaccine effectiveness as 1 minus the odds ratio under the standard assumptions of a test-negative design.

In addition to estimating vaccine effectiveness in the overall study population, we will evaluate vaccine effectiveness in each age strata. Additionally, we will evaluate effectiveness among individuals who received the vaccine >24 months prior to blood culture and among those who receiving the vaccine <24 months prior to blood culture.

Children who have received any typhoid vaccination other than TYPHIBEV® will not be analyzed as part of either group, as we aim to describe the effectiveness of the specific vaccine only. As the vaccine type is not identified until after study enrollment, we will exclude participants in our analysis.

### Sample size

Power for a matched case-control study design depends on the assumed odds ratio and the number of discordant pairs, which relates to the expected prevalence of vaccination among controls in this study. Here, we present sample size calculations for the number of cases needed required to achieve 90% power, according to vaccine coverage and effectiveness (Table 1).

**Table 1. Sample size calculation by effectiveness and estimated coverage level.**

| Number of cases required to achieve sample size according to vaccination coverage and effectiveness | | | |
|---|---|---|---|
| Vaccination coverage in controls | Effectiveness | | |
| | 70% | 80% | 90% |
| 80% | 44 | 23 | 11 |
| 90% | 64 | **31** | 13 |
| 95% | 109 | 50 | 18 |

As our base case, we assume 80% vaccine effectiveness and 90% vaccination coverage among controls. If there is correlation of exposures upon matching (e.g. demographic characteristics, location) of $\phi$=0.3, would require **44** cases (and 132 controls). We plan to recruit 10% more participants than needed, as some children may be excluded in analysis based on vaccine type.

**Appendix A.** Electronic questionnaire for data collection.

SEAP Typhoid
Vaccine Effectiveness

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