The safety and immunogenicity of a bivalent conjugate vaccine against *Salmonella enterica* Typhi and Paratyphi A in healthy Indian adults: a phase 1, randomised, active-controlled, double-blind trial

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Summary

Background Enteric fever caused by *Salmonella enterica* Typhi and *Salmonella* Paratyphi A is an important public health problem, especially in low-income and middle-income countries with limited access to safe water and sanitation. We present results from, to our knowledge, the first ever human study of a bivalent paratyphoid A-typhoid conjugate vaccine (Sii-PTCV).

Methods In this double-blind phase 1 study, 60 healthy Indian adults were randomly assigned (1:1) to receive a single intramuscular dose of either Sii-PTCV or typhoid conjugate vaccine (Typbar-TCV). Safety was assessed by observing solicited adverse events for 1 week, unsolicited events for 1 month, and serious adverse events (SAEs) over 6 months. Immunogenicity at 1 month and 6 months was assessed by measuring anti-capsular polysaccharide antigen Vi (anti-Vi) IgG and IgA against *Salmonella* Typhi and anti-lipopolysaccharide (LPS) IgG against *Salmonella* Paratyphi A by ELISA, and functional antibodies using serum bactericidal assay (SBA) against *Salmonella* Paratyphi A. This study is registered with Clinical Trial Registry–India (CTRI/2022/06/043608) and is completed.

Findings 60 participants were enrolled. Of these 60 participants, 57 (95%) participants were male and three (5%) participants were female. Solicited adverse events were observed in 27 (90%) of 30 participants who received Sii-PTCV and 26 (87%) of 30 participants who received Typbar-TCV. The most common local solicited event was pain in 27 (90%) participants who received Sii-PTCV and in 23 (77%) participants who received Typbar-TCV. The most common solicited systemic event was myalgia in five (17%) participants who received Sii-PTCV, whereas four (13%) participants who received Typbar-TCV had myalgia and four (13%) had headache. No vaccine-related unsolicited adverse events or SAEs were reported. The seroconversion rates on day 29 were 96·7% (95% CI 82·8–99·9) with Sii-PTCV and 100·0% (88·4–100·0) with Typbar-TCV for anti-Vi IgG; 93·3% (77·9–99·2) with Sii-PTCV and 100·0% (88·4–100·0) with Typbar-TCV for anti-Vi IgA; 100·0% (88·4–100·0) with Sii-PTCV and 3·3% (0·1–17·2) with Typbar-TCV for anti-LPS (paratyphoid); and 93·3% (77·9–99·2) with Sii-PTCV and 0% (0·0–11·6) with Typbar-TCV for SBA titres (paratyphoid). Paratyphoid anti-LPS immune responses were sustained at day 181.

Interpretation Sii-PTCV was safe and immunogenic for both typhoid and paratyphoid antigens indicating its potential for providing comprehensive protection against enteric fever.

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See Online for appendix

Introduction

Enteric fever, comprising typhoid and paratyphoid fever, is a disease of major public health importance, particularly in south Asia. Typhoid fever is a systemic infection caused by the Gram-negative bacilli *Salmonella enterica* subspecies serovar Typhi and paratyphoid fever is a systemic infection caused by *S enterica* subspecies serovars Paratyphi A, B, and C.[1] *S* Paratyphi A, like *S* Typhi, has adapted to human hosts; it causes a similar clinical picture to typhoid, including fevers, chills, and abdominal pain, and can be life-threatening in severe cases.[2] Antimicrobial resistance is one of the concerns in the management of enteric fever, with outbreaks in 2021 of extensively drug resistant strains making it even more challenging.[3] Currently available typhoid conjugate vaccines (TCVs) are safe, highly immunogenic, efficacious, and recommended for individuals who are aged 6 months or older.

An estimated 14·3 million (95% CI 12·5 million–16·3 million) cases of typhoid and paratyphoid fever occurred in 2017, resulting in about 135 000 (76 900–218 900) deaths.[4] Typhoid is endemic in Asia and sub-Saharan Africa, whereas paratyphoid fever is largely confined to South and

Research in context

Evidence before this study

We searched PubMed and WHO's website on May 17, 2021 using the search terms 'typhoid conjugate vaccine', 'paratyphoid vaccine', 'paratyphi A' with no date restrictions or language restrictions. We identified 25 articles relevant for a clinical trial of typhoid conjugate vaccines (TCVs) on PubMed. We identified one trial of a candidate paratyphoid conjugate vaccine. Previous phase 1 and phase 2 studies in Viet Nam showed that a *Salmonella enterica* Paratyphi A conjugate vaccine was safe and immunogenic. WHO recognises the need for development of an efficacious paratyphoid vaccine. WHO mentions seven paratyphoid vaccines that are in various stages of clinical development. CVD 1902, an oral paratyphoid vaccine, has completed a phase 1 study and is undergoing a human challenge study to assess the efficacy against paratyphoid A infection.

Added value of this study

This Article describes a phase 1 study of a bivalent paratyphoid A-typhoid conjugate vaccine (Sii-PTCV) to evaluate its safety and immunogenicity. Sii-PTCV was well tolerated by all recipients and no serious adverse events were reported over a 6-month period. Sii-PTCV showed robust immune responses to the paratyphoid A component at 4 weeks after vaccination, measured by anti-lipopolysaccharide IgG and serum bactericidal assay. Sii-PTCV also showed a comparable post-vaccination immune response for the typhoid component of an already licensed and WHO-prequalified TCV.

Implications of all the available evidence

Current TCVs provide protection against typhoid fever only and there is no vaccine against paratyphoid fever. Thus, comprehensive protection against enteric fever remains an unmet need. An effective bivalent vaccine would provide protection against enteric fever caused by *Salmonella* Typhi and *Salmonella* Paratyphi A, helping to mitigate increasing antimicrobial resistance. Sii-PTCV was safe and immunogenic in a phase 1 study. These findings warrant further evaluation in phase 2 and phase 3 studies.

southeast Asia.[2,5] South Asia has the highest mortality ratio, accounting for 69·6% (n=94700 [95% CI 54400–135200]) of global deaths from typhoid and paratyphoid fever in 2017.[4]

According to the Global Burden of Disease study 2019, 76·8% of enteric fever cases are typhoid infections and 23·2% are paratyphoid infections,[4] although epidemiological studies[6] show a gradual shift in enteric fever burden from *S* Typhi to *S* Paratyphi A. Furthermore, the emergence of antimicrobial resistance[7] emphasises the need for a vaccine against *S* Paratyphi A.

Among the serotypes of *S* Paratyphi, almost 90% of infections are caused by the A serotype.[8] A standalone vaccine against *S* Paratyphi A is unlikely to be used, and therefore a bivalent vaccine against *S* Typhi and *S* Paratyphi A could provide comprehensive protection against enteric fever. Currently, no vaccines against paratyphoid fever are available, although several candidate vaccines are in different stages of preclinical and clinical development. The conjugated polysaccharide (Vi)-containing typhoid conjugate and polysaccharide vaccines are also expected to protect against rare cases of *S* Paratyphi C, which also expresses the Vi capsule.[9]

TCVs have shown considerable advantage over conventional typhoid vaccines because the conjugates such as tetanus toxoid enhance immunogenicity by inducing T-cell dependent B-cell immune responses, which also generate immunological memory. For the paratyphoid component, a similar conjugate vaccine approach is being evaluated by several developers with monovalent and bivalent candidates at various stages of development.[2] Monovalent paratyphoid conjugate vaccine candidates were tested in Viet Nam in the 1990s with good results,[10] although no further updates on development of these vaccines are known.

A bivalent paratyphoid A-typhoid conjugate vaccine (Sii-PTCV) was developed in India. The *S* Typhi strain was isolated from the stool sample of a patient in Pune, India, and the O-specific polysaccharide was obtained from the ATCC 9150 *S* Paratyphi A strain. The typhoid antigen is Vi from *S* Typhi with tetanus toxoid as the carrier protein. The paratyphoid antigen is the O-specific polysaccharide from *S* Paratyphi A conjugated to diphtheria toxoid carrier protein. Sii-PTCV was safe and immunogenic against both antigens in pre-clinical studies (unpublished data). Subsequently, a first-in-human study of Sii-PTCV was conducted to assess its safety and immunogenicity.

Methods

Study design and participants

This was a phase 1, double-blind, randomised, active-controlled study to assess the safety and immunogenicity of Sii-PTCV compared with Typhoid Conjugate Vaccine (Typbar-TCV; an already licensed and WHO-prequalified typhoid conjugate vaccine) in healthy adults. The study was conducted from July 18, 2022, to March 6, 2023, after approval from the Indian regulatory authority and the Sri Venkateshwara Hospital Ethics Committee.

Participants were screened for eligibility after providing written informed consent.

Healthy adults (aged 18–45 years) with BMI 18·50 kg/m² to 24·99 kg/m² and with minimum 50 kg bodyweight were recruited at the Human Pharmacology Unit, Syngene International, Bangalore, India. At screening,

participants were asked if they had previously received a typhoid vaccine, had history of suspected or laboratory confirmed typhoid or paratyphoid infection, or had household exposure to these infections. Participants were excluded if they answered yes to any of these questions. No laboratory tests were performed at screening to rule out previous exposure to typhoid or paratyphoid A. Other exclusion criteria were history of serious events with previous receipt of tetanus or diphtheria toxoid vaccines, clinically significant systemic disease, and immunosuppression.

Procedures

Eligible participants visited the study site on day 1, day 8, day 29, and day 181. Study vaccines were administered on day 1. Sex was self-reported by study participants, with the options of male or female provided.

A single dose of 0·5 mL of Sii-PTCV (manufactured by Serum Institute of India, Pune, India) contains 25 µg of purified Vi polysaccharide from *S* Typhi conjugated to tetanus toxoid and 25 µg of purified O-specific polysaccharide from *S* Paratyphi A conjugated to diphtheria toxoid. A five-dose vial preparation (batch 474102, expiry May, 2023) was used.

A single dose of 0·5 mL of Typbar-TCV (manufactured by Bharat Biotech International, Hyderabad, India) contains 25 µg of purified Vi-capsular polysaccharide of *S* Typhi Ty2 conjugated to tetanus toxoid. A one-dose vial presentation (batch 76A21001A, expiry February, 2024) was used.

Both vaccines were stored at 2–8°C and were administered as a single dose by intramuscular route in the deltoid.

Randomisation and masking

A computer-generated randomisation list was generated using Advantage eClinical Interactive Web Response System (IWRS, Emmes, Rockville, MD, USA). Eligible participants were assigned a randomisation number using the IWRS on day 1 to receive either Sii-PTCV or Typbar-TCV in a randomisation ratio of 1:1. The participants, the study personnel responsible for the evaluation of study endpoints, and the laboratory researchers were unaware of the vaccine administered. The personnel involved in the handling of vaccines, their preparation, and their administration were unmasked to treatment assignments and not involved in clinical evaluations. The study vaccines were prepared out of view of the participants and the masked site staff. Because both vaccines had distinct appearances, even when drawn into syringes, the syringes were masked with an opaque wrapping before administration.

Outcomes

Primary outcomes were the occurrence of immediate adverse events within 60 min of vaccine administration, solicited (local and systemic) adverse events up to day 8, unsolicited adverse events up to day 29, and serious adverse events up to day 181. The secondary outcomes were immune responses to the typhoid (anti-Vi IgG and IgA) and paratyphoid A (anti-lipopolysaccharide [LPS] and serum bacterial assay [SBA] titres) by measuring geometric mean titres (GMTs) at baseline, day 29, and day 181; and seroconversion rates (defined as four-fold rise from baseline antibody titres to post-vaccination antibody titres) at day 29 and day 181. Exploratory outcomes were the effect of pre-existing anti-tetanus and anti-diphtheria antibodies on the immune responses to typhoid and paratyphoid A.

Participants were observed for 60 min after vaccination for the occurrence of any immediate adverse events. Active surveillance for solicited and unsolicited adverse events was conducted over a 7-day period by using a post-immunisation diary card and surveillance for unsolicited adverse events was continued up to day 29 by using another diary card. Active surveillance for serious adverse events continued up to and including day 181. Solicited events included: pain, redness, and swelling at the infection site, and fever, headache, malaise, anorexia, myalgia, and arthralgia.

The severity of events was graded by the site investigator according to Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (version 2.1; July, 2017).[11]

Physical examination, including the measurement of vital signs (ie, axillary temperature, blood pressure, pulse rate, and respiratory rate), was done at every visit. For safety laboratory tests (ie, haematology, biochemistry, and urinalysis), blood and urine samples were collected during screening and on day 8.

Blood samples were collected before vaccination (day 1) and on day 29 and day 181 after vaccination.

Immunogenicity assessments were conducted at the Oxford Vaccine Group Laboratory at the University of Oxford (Oxford, UK). Serum IgG antibody titres against typhoid Vi were measured using a commercial ELISA kit (VaccZyme, The Binding Site, Birmingham, UK) according to the manufacturer's instructions. Anti-Vi IgA titres were measured using Vi-coated plates and reagents supplied by The Binding Site and adapting protocol from the commercial VaccZyme assay.

Serum IgG titres against paratyphoid O-specific polysaccharide were measured using an in-house standardised indirect ELISA. 96-well Maxisorb ELISA plates were coated with 20µg/mL of O-specific polysaccharide (manufactured by Serum Institute of India) in carbonate–bicarbonate buffer (pH 9·7) and stored at 4°C overnight for 18 h. After coating, plates were washed five times with phosphate buffer solution containing 0·05% Tween and blocked with phosphate buffer solution containing 0·05% Tween and 5% non-fat milk powder for 2 h at room temperature. Thawed samples were diluted in phosphate buffer solution containing 0·05% Tween and 5% non-fat milk powder. Plates were washed five times

with phosphate buffer solution containing 0·05% Tween, plated in triplicate, and incubated for 1 h at room temperature. Internal positive controls to measure plate-to-plate variation were created from a pool of serum from individuals 90 days after they received oral *S* Paratyphi A challenge in an experimental challenge study. The standard curve was created from a pool of serum from individuals exposed to the live oral typhoid vaccine M01ZH09. The standard serum was used in a two-fold serial dilution to produce a ten point standard curve that was assigned arbitrary ELISA units. Goat anti-human IgG, conjugated to horseradish peroxidase, was added as a secondary antibody (1 in 10 000 diluted in assay buffer added and incubated at room temperature for 1 h) and the plates were washed five times using wash buffer. The plates were developed by adding 3,3□,5,5□-Tetramethylbenzidine followed by 2 M sulphuric acid. An ELx808 microplate reader (BioTek Instruments, Winooski, VT, USA) was used to provide optical density measurement of the plates at 450 and 630 nm. Standardised ELISA units for each sample were calculated from the standard curve (4-Parameter logistic model) using a single dilution of each sample, using BioTek Gen5 software version 3.09. (Agilent Technologies, Santa Clara, CA, USA).

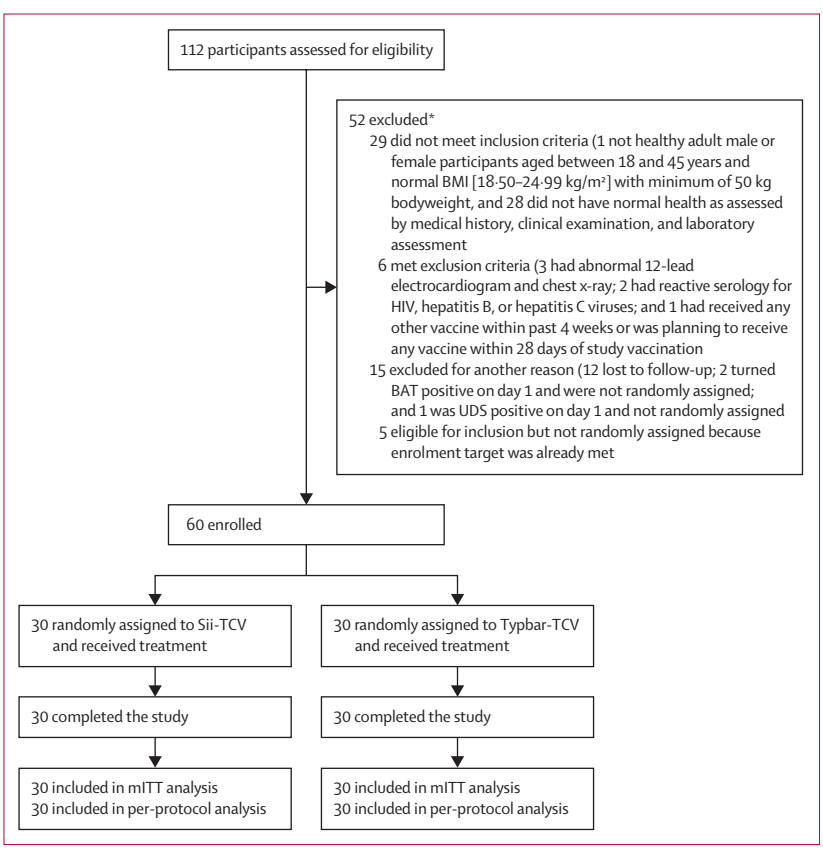

Figure: **Trial profile**
BAT=breath alcohol test. mITT=modified intention to treat. Sii-PTCV=bivalent paratyphoid A-typhoid conjugate vaccine. TCV=typhoid conjugate vaccine. UDS=urine drug screen for drugs that are abused. *Three participants both did not meet inclusion criteria and met the exclusion criteria.

SBA was assessed using an in-house assay developed for the purpose of this study.[12] Sera were heat inactivated for 30 min at 56°C and serially diluted (1:2) in Hanks' Balanced Salt Solution (Gibco, Paisley, Scotland, UK) containing 0·5% fetal bovine serum (heat inactivated fetal bovine serum, Sigma). Bacterial dilution yielding approximately 100 colony-forming units per 20 µL of the *S* Paratyphi A (ATCC9150 strain) were made and 20 µl of the bacterial suspension were added to each well, followed by 10 µL of baby rabbit complement (Pel-Freez Biologicals, Rogers, AR, USA) giving a final concentration of 12·5%. The reaction was incubated for 60 min at 37°C. The bacterial suspension was spotted as 10 µL spot per well onto a Luria–Bertani agar plate (Sigma-Aldrich, Bangalore, India) and allowed to dribble for spreading. The agar plates were incubated overnight (16–22 h) at 37°C and 5% CO_2, and the resultant colonies were quantified using a semi-automated colony counter. The SBA titre for each individual serum sample was calculated as the highest serum dilution giving at least 50% killing as compared with the colony-forming unit count obtained in the corresponding heat-inactivated complement-only condition wells. Internal positive controls created from a pool of serum from individuals 90 days after challenge with *S* Paratyphi A were ran on each plate.

The effect of pre-existing tetanus and diphtheria antibodies on the immune responses of typhoid and paratyphoid antigens was assessed. Baseline and day 29 serum samples were tested for quantitative estimation of anti-tetanus and anti-diphtheria IgG antibodies using SERION ELISA classic IgG tests (SERION Diagnostics, Würzburg, Germany). These tests were performed at Central Laboratory, Syngene International, Bangalore, India.

Statistical analysis

Because this was a phase 1 study, no formal sample size calculation was done. We judged a sample size of 30 per group as sufficient for assessing the initial safety of Sii-PTCV. Because this was a phase 1 study, statistics are descriptive only. Data analysis was performed using SAS (version 9.4M7). Demographics are represented as mean and SD for continuous variables and frequencies and percentages for categorical variables. Solicited and unsolicited events were reported as the number of participants with events, the percentage of participants with events, and the number of events. For immune response to typhoid and paratyphoid antigens, the GMTs and geometric mean fold rise were assessed. Seroconversion was defined as at least a four-fold rise in post-vaccination titres compared with pre-vaccination titres. For tetanus and diphtheria antibodies, a participant was considered positive if the values of titres were above the lower limit of quantitation (ie, 0·05 IU/mL). A participant was considered seroprotected for tetanus or diphtheria if titres were

above 0·1 IU/mL. Two-sided exact 95% CIs were assessed for the above variables. This study is registered with Clinical Trial Registry–India (CTRI/2022/06/043608) and is completed. The modified intention-to-treat (mITT) population included all participants who received the vaccines as per the random allocation. The mITT population was used to describe demographics, other baseline characteristics, and safety analyses. The mITT population served as a secondary population for immunogenicity analyses. The per-protocol population included all participants who received the vaccines as per the random allocation, gave blood samples at day 1 before vaccination and on day 29 after vaccination, and who did not have any major protocol deviations. The per-protocol population served as the primary population for immunogenicity analysis.

Role of the funding source

The funder was involved in conceptualisation, study design, data interpretation, writing of the study report, writing of the manuscript, and the decision to submit for publication.

Results

A total of 112 participants were screened for eligibility. 47 (42%) participants were not enrolled. Five (4%) were eligible for enrolment but were not randomly assigned because the enrolment target was completed, and 60 (54%) participants were randomly assigned (figure). All 60 randomly assigned participants received study vaccination as per random allocation and completed all three scheduled visits (figure). The mITT (n=60) and per-protocol (n=60) populations were the same, because all participants received the vaccines as per the randomisation allocation, provided samples for day 1 and day 29, and had no major protocol deviations.

The baseline demographics were similar between groups (table 1). 29 (97%) of 30 participants in the Sii-PTCV group and 28 (93%) of 30 participants in the Typbar-TCV group were male. The mean age at baseline was 30·7 years (SD 5·6) and 30·9 years (SD 5·8) in the Sii-PTCV and Typbar-TCV groups, respectively.

No immediate adverse events within 60 min of vaccination were observed. The most common local solicited event was pain with 27 events in 27 (90%) of 30 participants in the Sii-PTCV group and 23 events in 23 (77%) of 30 participants in the Typbar-TCV group. The most common solicited systemic event in the Sii-PTCV group was myalgia with six events in five (17%) participants, whereas the most common solicited systemic events in the Typbar-TCV group were myalgia and headache with four events in four (13%) participants each. All solicited events were mild in severity, except for one event each of fever, headache, and myalgia in the Sii-PTCV group that were of moderate severity. All participants with solicited events recovered without sequelae (table 2).

	Sii-PTCV (n=30)	Typbar-TCV (n=30)
Age, years	30·7 (5·6)	30·9 (5·8)
Sex		
Male	29 (97%)	28 (93%)
Female	1 (3%)	2 (7%)
Weight, kg	65·3 (5·3)	60·2 (6·3)
Height, cm	169·0 (5·8)	167·0 (6·7)
BMI, kg/m²	22·9 (1·5)	21·6 (1·9)

Data are mean (SD) or n (%). TCV=typhoid conjugate vaccine.

Table 1: **Baseline demographics**

	Sii-PTCV (n=30)	Typbar-TCV (n=30)
Solicited adverse events	27 (90%), 27	26 (87%), 40
Solicited local events	27 (90%), 27	23 (77%), 26
Pain	27 (90%), 27	23 (77%), 23
Redness	..	2 (7%), 2
Swelling	..	1 (3%), 1
Solicited systemic events	7 (23%), 20	9 (30%), 14
Fever	1 (3%), 1	1 (3%), 1
Headache	3 (10%), 4	4 (13%), 4
Malaise	3 (10%), 4	2 (7%), 2
Anorexia	2 (7%), 2	2 (7%), 2
Myalgia	5 (17%), 6	4 (13%), 4
Arthralgia	2 (7%), 3	1 (3%), 1
Unsolicited adverse events	1 (3%), 1	5 (17%), 6
Lymphadenitis	1 (3%), 1	..
Vomiting	..	1 (3%), 1
Limb injury	..	1 (3%), 1
γ-glutamyl transferase increased	..	1 (3%), 1
Glucose present in urine	..	1 (3%), 1
Aminotransferases increased	..	2 (7%), 2

Data are n (%), number of events. Sii-PTCV=bivalent paratyphoid A-typhoid conjugate vaccine. TCV=typhoid conjugate vaccine.

Table 2: **Reported solicited and unsolicited events**

One unsolicited adverse event occurred in one (3%) participant in the Sii-PTCV group and six events occurred in five (17%) of 30 participants in the Typbar-TCV group (table 2). All unsolicited events were mild, unrelated to vaccines, and the participants recovered without sequelae. No serious adverse events were reported.

At baseline, ten (33%) participants in the Sii-PTCV group and seven (23%) participants in the Typbar-TCV group had detectable anti-Vi IgG antibodies, whereas two (7%) participants in the Sii-PTCV group and one (3%) participant in the Typbar-TCV group had anti-Vi IgA antibodies. All participants had detectable paratyphoid anti-LPS and SBA antibodies. The baseline GMTs were similar between the groups (table 3).

On day 29 there was an increase in the post-vaccination GMTs for anti-Vi IgG and anti-Vi IgA in both groups compared with baseline (table 3). The seroconversion rates of anti-Vi IgG were 29 (97%, 95% CI 82·8–99·9) of

	Sii-PTCV (n=30)			Typbar-TCV (n=30)		
	GMT (95% CI)	GMFR (95% CI)	Seroconversion, n (%, 95% CI)	GMT (95% CI)	GMFR (95% CI)	Seroconversion, n (%, 95% CI)
Anti-Vi IgG (typhoid)						
Day 1	6·97 (4·75–10·22)	..	..	5·82 (4·13–8·20)	..	..
Day 29	1477·00 (867·80–2513·89)	211·96 (121·69–369·20)	29 (96·7%, 82·8–99·9)	996·38 (676·58–1467·35)	171·25 (103·13–284·38)	30 (100·0%, 88·4–100·0)
Day 181	480·46 (297·94–774·79)	68·95 (43·18–110·10)	29 (96·7%, 82·8–99·9)	482·54 (327·32–711·36)	82·93 (53·25–129·16)	30 (100·0%, 88·4–100·0)
Anti-Vi IgA (typhoid)						
Day 1	1·75 (1·48–2·07)	..	..	1·70 (1·43–2·01)	..	..
Day 29	75·66 (53·25–107·51)	43·27 (28·42–65·87)	28 (93·3%, 77·9–99·2)	85·19 (57·93–125·28)	50·15 (34·82–72·23)	30 (100·0%, 88·4–100·0)
Day 181	27·75 (18·90–40·74)	15·89 (10·51–23·95)	27 (90·0%, 73·5–97·9)	40·59 (27·2–60·59)	23·90 (16·74–34·12)	30 (100·0%, 88·4–100·0)
Anti-LPS (paratyphoid A)						
Day 1	360·46 (237·07–548·07)	..	..	181·04 (126·08–259·96)	..	..
Day 29	28 845·24 (19 679·44–42 280·06)	80·02 (54·93–116·58)	30 (100·0%, 88·4–100·0)	236·81 (169·24–331·37)	1·31 (1·09–1·58)	1 (3·3%, 0·1– 17·2)
Day 181	9535·52 (6281·40– 14 475·46)	26·45 (19·31–36·25)	30 (100·0%, 88·4–100·0)	222·86 (159·58– 311·22)	1·23 (1·05–1·44)	0
SBA (paratyphoid A)						
Day 1	8044·60 (5326·37– 12 150·05)	..	..	6765·70 (4672·43– 9796·85)	..	..
Day 29	155 737·80 (102 803·95–235 927·33)	19·40 (12·61–29·73)	28 (93·3%, 77·9–99·2)	5993·70 (4047·46–8875·91)	0·90 (0·66–1·19)	0 (NC)
Day 181	56 367·40 (33 580·12–94 617·93)	7·00 (3·98–12·32)	20 (66·7%, 47·2–82·7)	1782·30 (520·64–6101·18)	0·30 (0·09–0·81)	1 (3·3%, 0·1–17·2)

GMTs were calculated by taking the anti-log of the arithmetic mean of the log10-transformed titres. GMFR was calculated by taking the arithmetic mean of the difference in the log10-transformed titres, where difference was post-vaccination $\log_{10}$ titre minus baseline vaccination log10 titre. Seroconversion is defined as four-fold or higher rise in post-vaccination titres compared with pre-vaccination titres. GMFR=geometric mean fold rise from baseline. GMT=geometric mean titre. LPS=lipopolysaccharide. NC=not calculable. SBA=serum bactericidal assay. Sii-PTCV=bivalent paratyphoid A-typhoid conjugate vaccine. TCV=typhoid conjugate vaccine. Vi=capsular polysaccharide.

Table 3: **Immune response to typhoid and paratyphoid A antigen**

30 participants in the Sii-PTCV group versus 30 (100%, 88·4–100·0) of 30 participants in the Typbar-TCV group at both day 29 and day 181. For anti-Vi IgA, seroconversion was observed in 28 (93%, 77·9–99·2) of 30 participants on day 29 and in 27 (90%, 73·5–97·9) of 30 participants on day 181 in the Sii-PTCV group whereas seroconversion was observed in all 30 (100%, 88·4–100·0) participants at both timepoints in the Typbar-TCV group. The immune responses to the typhoid antigen in the Sii-PTCV and Typbar-TCV groups were similar, indicating there was no interference of the paratyphoid antigen (table 3).

For the paratyphoid A antigen, on day 29, there was an increase in the post-vaccination GMTs for anti-LPS and SBA titres compared with baseline in the Sii-PTCV group only (table 3). Seroconversion was observed in all 30 participants (100%, 95% CI 88·4–100·0) for anti-LPS in the Sii-PTCV group on day 29 and day 181, whereas only one (3%, 0·1–17·2) participant in the Typbar-TCV group had seroconversion for anti-LPS on day 29 and none had seroconversion on day 181. For SBA, seroconversion was observed in 28 (93%, 77·9–99·2) and 20 (67%, 47·2–82·7) participants in the Sii-PTCV group on day 29 and day 181, respectively, whereas in the Typbar-TCV group, no participants had seroconversion for SBA on day 29 and one (3%, 0·1–17·2) participant had seroconversion for SBA on day 181 (table 3).

The reverse cumulative distribution curves show the distribution of the immune responses between the two groups. Comparable immune responses were observed for anti-Vi IgG and IgA in both groups at day 29 and day 181 after vaccination. Higher antibody titres for anti-LPS and SBA were observed with Sii-PTCV at day 29 and day 181 after vaccination compared with Typbar-TCV (appendix pp 4–7).

At baseline, 29 (97%) participants in the Sii-PTCV group and 26 (87%) participants in the Typbar-TCV group had pre-existing antibodies against tetanus, and 15 (50%) participants in the Sii-PTCV group and 21 (70%) participants in the Typbar-TCV group had pre-existing antibodies against diphtheria (table 4). Seroprotection rates to tetanus toxoid were similar, increasing from 29 (97%, 95% CI 82·8–99·9) participants on day 1 to 30 (100%, 88·4–100·0) participants on day 29 in the Sii-PTCV group, whereas they increased from 24 (80%, 61·4–92·3) participants on day 1 to 29 (97%, 82·8–99·9) participants on day 29 in the Typbar-TCV group. Additionally, participants in the Sii-PTCV group showed increased seroprotection rates to diphtheria, increasing from eight (27%, 12·3–45·9) participants on day 1 to 24 (80%, 61·4–92·3) participants on day 29 (appendix p 3).

Discussion

In this first-in-human study of a typhoid–paratyphoid bivalent conjugate vaccine, Sii-PTCV showed an immune response to typhoid Vi-antigen similar to that of the

	Sii-PTCV (n=30)	Sii-PTCV (n=30)	Typbar-TCV (n=30)	Typbar-TCV (n=30)
Pre-existing anti-TT antibodies, n (%)	Yes, 29 (96·7%)	No, 1 (3·3%)	Yes, 26 (86·7%)	No, 4 (13·3%)
Geometric mean titres (95% CI)				
Anti-Vi IgG	1427·51 (826·42–2465·78)	3968·82 (NC)	875·23 (584·90–1309·69)	2314·13 (472·38–11 336·64)
Anti-Vi IgA	71·91 (50·79–101·82)	330·80 (NC)	74·44 (50·18–110·42)	204·71 (36·14–1159·49)
Seroconversion, n/N (%, 95% CI)				
Anti-Vi IgG	28/29 (96·7%, 82·2–99·9)	1/1 (100·0%, NC)	26/26 (100·0%, 86·8–100·0)	4/4 (100·0%, 39·8–100·0)
Anti-Vi IgA	27/29 (93·1%, 77·2–99·2)	1/1 (100·0%, NC)	26/26 (100·0%, 86·8–100·0)	4/4 (100·0%, 39·8–100·0)
Pre-existing anti-DT antibodies, n (%)	Yes, 15 (50%)	No, 15 (50%)	Yes, 21 (70%)	No, 9 (30%)
Geometric mean titres (95% CI)				
Anti-LPS	38 697·07 (23 013·65–65 068·49)	21 501·58 (12 008·45–38 499·37)	237·29 (158·56–355·10)	235·70 (110·38–503·31)
SBA	149 754·50 (84 354·52–265 858·88)	161 960·20 (82 468·68–31 8073·54)	6699·90 (4147·07–10824·27)	4622·00 (2046·84–10436·91)
Seroconversion, n/N (%, 95% CI)				
Anti-LPS	15/15 (100·0%, 78·2–100·0)	15/15 (100·0%, 78·2–100·0)	0/21 (NC)	1/9 (11·1%, NC)
SBA	14/15 (93·0%, 68·1–99·8)	14/15 (93·3%, 68·1–99·8)	0/21 (NC)	0/9 (NC)

DT=diphtheria toxoid. LPS=lipopolysaccharide. NC=not calculable. SBA=serum bactericidal assay. Sii-PTCV=bivalent paratyphoid A-typhoid conjugate vaccine. TCV=typhoid conjugate vaccine. TT=tetanus toxoid. Vi=capsular polysaccharide.

Table 4: **Effect of pre-existing tetanus and diphtheria antibodies on immune response on day 29 to typhoid and paratyphoid A antigens**

WHO-prequalified vaccine, Typbar-TCV. In addition, Sii-PTCV induced seroconversion in most of the participants for paratyphoid A antibodies. The vaccine was also safe and well tolerated, indicating the potential of a bivalent typhoid–paratyphoid conjugate vaccine that could comprehensively control enteric fever.

To our knowledge, this is the first study of a bivalent conjugate vaccine against *S enterica* serovars Typhi and Paratyphi A. Monovalent paratyphoid vaccines are less likely to be used. If this bivalent vaccine is found to be safe, immunogenic, and efficacious in late phase trials, it could replace the existing monovalent typhoid vaccines.

The first paratyphoid-antigen-containing vaccine was a whole-cell, heat-killed, and phenol-reserved combined typhoid plus paratyphoid A and B vaccine, which was developed in the UK during the First World War (in 1916) to replace a killed typhoid vaccine that was initially used at the end of the 19th century, also in the military.[13,14] However, the use of this vaccine declined after the 1960s[7] due to uncertain effectiveness and high reactogenicity. As a result, monovalent killed whole-bacterium typhoid vaccines were used, but still were considered to be too reactogenic.[15]

The development of live-attenuated (oral) and purified polysaccharide (intramuscular injection) typhoid vaccines provided an improved opportunity for protection against enteric fever but their use was largely restricted to travellers from high-income settings because of concerns over limitations in immunogenicity (ie, a T-cell independent immune response that results in low immunogenicity in children younger than 2 years, no immune memory, and no boosting, resulting only in short-term protection) and the duration of their effectiveness.[16] The first TCVs were tested in the 1990s[17] but a TCV product was not licensed until 2013.[18] TCVs are highly immunogenic and safe, and WHO now recommends they be used for children in the national programmes of all high-burden endemic countries.[19] However, none of these products can control *S* Paratyphi A, which has a considerable global burden. Therefore, a practical approach would be to use a bivalent vaccine targeting both bacteria and several are in development.[20]

Although not formally tested head to head, we note that the typhoid immune responses in this study are similar to those induced by other TCVs, which were also tested using the commercial assay from VaccZyme, including Typbar-TCV.[21–25] WHO recommends clinical evaluation of a new TCV by showing non-inferiority of anti-Vi IgG immunogenicity in comparison with an existing licensed typhoid polysaccharide vaccine or TCV.[26] Sii-PTCV showed robust immune responses for both anti-Vi IgG and anti-Vi IgA, which have been shown to correlate with protection in a typhoid human challenge model.[27,28]

Similarly, a high immune response against paratyphoid A, shown by both the anti-LPS ELISA and SBA, was seen in the Sii-PTCV vaccine group, and there was no response in the Typbar-TCV group to paratyphoid A. The immune responses to both components were sustained at day 181. Although no immune correlates of protection are known for paratyphoid A, the high immunogenicity shown by our vaccine in this first-in-human study is a positive finding that requires further evaluation.

When there are no immune correlates of protection known for an infection, an efficacy study is required for the candidate vaccine. Testing the efficacy against *S* Paratyphi A will be very challenging because the incidence of paratyphoid fever is relatively low (ie, 51·3 [95% CI 31·3–83·9] new cases per 100 000 in 2019).[29] In 2022, WHO recommended that the evaluation of paratyphoid-containing vaccines could include a test of

vaccine efficacy in a human challenge infection model,[30,31] with supporting immunogenicity and safety data from phase 3 trials in endemic countries.[20] This pathway will be followed for the Sii-PTCV bivalent vaccine. The effectiveness of the vaccine could further be tested in large post-licensure field studies after the vaccine is introduced in national programmes.

Because no paratyphoid vaccines are available currently, a WHO-prequalified typhoid polysaccharide vaccine or a TCV is the only option as a comparator. The typhoid vaccine will act as an active control to bridge the typhoid component and will act as a placebo for the paratyphoid component, because TCVs are not known to provide any cross-protection to paratyphoid fever.[32]

Most of the participants enrolled in this trial had pre-existing titres against diphtheria and tetanus at baseline. Participants who received Sii-PTCV showed increased seroprotection rates to tetanus and diphtheria on day 29 compared with day 1. Boosting of immunity to tetanus and diphtheria could be a further advantage of the vaccine.

This study has several limitations, including the inherent limitation of scale in a phase 1 study and the absence of statistical power. The majority of participants were male, although this is unlikely to have had a substantial effect on immune responses. Most studies reporting a sex difference in immunity show higher responses in females, indicating that our data could be even more robust in a balanced population of men and women.[33] Most of the participants had baseline antibody titres against typhoid and paratyphoid, reflecting expected immune responses in an endemic population. The strong responses observed in some individuals might reflect memory responses following natural infection. The need for a vaccine to cover both causes of enteric fever (ie, typhoid and paratyphoid) in highly endemic areas remains high because a single natural typhoid infection confers, at best, moderate or incomplete protection against subsequent infection.[31,34,35]

Sii-PTCV was safe and immunogenic for both the typhoid and paratyphoid A antigens after a single dose and will now be tested in phase 2 and phase 3 clinical studies. If found to be effective, it has the potential to address the problem of enteric fever in endemic countries.

Contributors

PSK, AVP, SB, AD, and CSP contributed to the study design, protocol development, manuscript preparation, and the decision to submit the manuscript. PSK, AVP, SB, and AD accessed and verified the data. VG, CDK, and AM contributed to the development and qualification of immunological assays. ADS and SSP contributed to the development and manufacturing of Sii-PTCV. SG contributed to quality control of the Sii-PTCV batches. AK, BN, and SY were involved in the conduct of the study including participant recruitment and data collection. RV contributed to statistical analysis. EJ, AF, YCK, and AJP contributed to clinical expertise, development, and qualification of immunological assays and testing of sera samples. All authors discussed the results and contributed to the final manuscript.

Declaration of interests

PSK, AVP, SB, AD, VG, CDK, ADS, SG, and SSP are full-time employees of the Serum Institute of India. CSP is the Chairman and Managing Director of the Serum Institute of India. AJP is Chair of the UK Department of Health and Social Care's Joint Committee on Vaccination and Immunisation and was a member of WHO's Strategic Advisory Group of Experts on Immunization until 2022. AJP and AF are contributors to intellectual property licensed by Oxford University Innovation to AstraZeneca on COVID-19 vaccines. AJP has received COVID-19 vaccine consulting fees from Shionogi. AJP has received grants on typhoid and paratyphoid vaccines from the Bill & Melinda Gates Foundation, the UK Medical Research Council, and the Wellcome Trust. RV, EJ, YCK, AK, BN, and SY declare no competing interests.

Data sharing

The anonymised study protocol is provided in the appendix (p 8). Individual de-identified participant data will be made available on direct request to the corresponding author with an appropriate research proposal. These data will be shared upon approval of analysis proposals and signed data-access agreements.

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