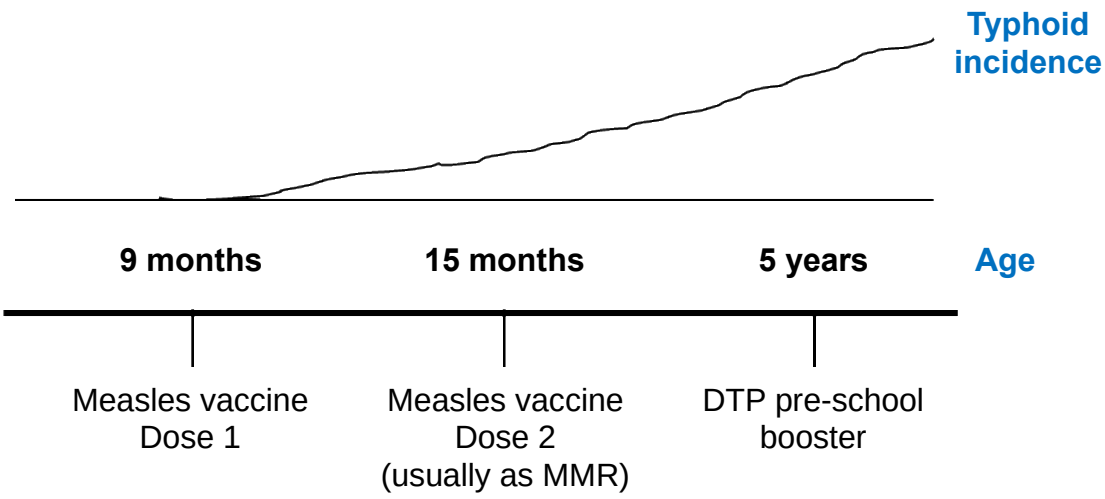
Supplementary Appendix

Vadrevu et al

Supplementary Figure 1: Typhoid burden is evident in ages 1 year and above[1,2].



Country	Typhoid Incidence rates[3] (2016 – 2018)				
	0–5 years	**5–9 years**	**10–14 years**	**15–29 years**	**30+**
Malawi	7.6	5.9	6.9	11.4	12.0
Nepal	10.7	19.7	19.6	15.8	15.0
Bangladesh	6.3	5.8	5.8	9.8	9.7

References:

1. World Health Organization. Typhoid vaccines: WHO position paper, March 2018 – Recommendations. Vaccine (2018), https://doi.org/10.1016/j.vaccine.2018.04.022.
2. Saha S, et al. Epidemiology of Typhoid and Paratyphoid: Implications for Vaccine Policy. Clin Infect Dis. 2019 Mar 7;68(Suppl 2):S117-S123.
3. Phillips MT, et al. A Bayesian approach for estimating typhoid fever incidence from large-scale facility-based passive surveillance data. Stat Med. 2021 Nov 20;40(26):5853-5870.

Supplementary Table 1: Study Sites, Principal Investigators and Ethical Committees

Site	PI Name	Hospital	Ethical Committee
1	Dr N S Mahantshetti	KLE Hospital, Belgaum	Ethics Committee, KLE University, Belgaum - 590010
2	Dr Vasant Khalatkar	Khalatkar Hospital, Nagpur	Jasleen Hospital's Ethics Committee, Nagpur - 440012
3	Dr Krishna Murthy	Cheluvambha Hospital, Mysore	Ethics Comittee, Mysore Medical College and Research Institute and associated Hospitals, Mysuru (Mysore) - 570021
4	Dr Sandeep Mogre	Mogre Childrens Hopsital, Nagpur	Jasleen Hospital's Ethics Committee, Nagpur - 440012
5	Dr Monjori Mitra	Institute of Child Health, Kolkata	Ethics Committee, Institute of Child health, Kolkata - 700017

Supplementary Table 2

Details of Serious Adverse Events

Group	Event Details	Event Intensity	Outcome of Event	Reported causality
Group 1A	Pneumonitis	Moderate	Resolved with treatment	Not related
Group 1B	Acute Gastroenteritis	Moderate	Resolved without sequelae	Not related
Group 1B	Focal Pneumonitis	Moderate	Resolved with treatment	Not related
Group 2	Acute gastroenteritis with moderate to severe dehydration	Moderate	Resolved with treatment	Not related
Group 3	Acute Gastroenteritis	Moderate	Resolved without sequelae	Not related
Group 3	Pneumonia	Moderate	Resolved without sequelae	Not related
Group 3	Acute gastroenteritis with moderate to severe dehydration with pneumonitis right with respiratory distress syndrome, with malarial fever	Moderate	Subject discharged from the hospital, but referred to another hospital, since condition persisted	Not related
Group 4	Acute gastroenteritis with bronchitis	Moderate	Resolved without sequelae	Not related
Group 4	Pneumonitis	Moderate	Resolved with treatment	Not related

Supplementary Table 3

Geometric mean titers (GMTs) with 95% CI of anti-Vi IgG antibodies from Day 0 (Baseline) to Day 720 measured by ELISA.

Days	Group 1A	Group 1B	Group 2	Group 3
0	10.74 (9.44, 12.19)	10.74 (9.44, 12.19)	--	8.68 (7.53, 10.00)
28	2272 (1624, 3185)	2272 (1624, 3185)	19.83 (12.68, 31.02)	1340 (898.0, 2002)
56	1841 (1423, 2383)	1657 (1281, 2142)	1218 (828.0,1792)	575.6 (241.0, 787.1)
90	--	--	768.6 (550.5, 1073)	475.3 (332.2, 680.0)
180	220.9 (167.1, 292.0)	135.1 (99.1, 184.3)	160.5 (117.5, 219.3)	183.7 (149.6, 225.6)
210	--	735.2 (554.0,975.7)	--	--
360	110.5 (87.9, 139.0)	161.2 (125.0, 208.0)	114.4 (90.91,143.9)	65.04 (50.49, 83.78)
720	26.94 (21.91, 33.13)	30.03 (24.10, 37.41)	22.13 (18.03, 27.16)	21.70 (17.33, 27.17)

SUPPLEMENTARY METHODS

Anti-Vi IgG ELISA

Anti-typhoid Vi antibodies were quantified using the VaccZyme Human anti-*Salmonella Typhi* Vi IgG kit. In brief, the contents of the kit were brought to room temperature. 100µL of each calibrator, control and appropriately diluted samples were added to wells of the ELISA plate and incubated at room temperature for 30 min. Well contents were then discarded and wells were washed three times with wash buffer. 100µL of the conjugate was then dispensed into each well and incubated at room temperature for 30 min before the wash procedure was repeated. 100µL of 3,3',5,5'-Tetramethylbenzidine (TMB) was added to each well and incubated at room temperature for 30 min before 100 µL of stop solution was added to each well. The optical density of each well was read at 450nm within 30 min on a microplate reader. Titer values were calculated using software (IBL-Spline Assays typhoid) provided by the kit manufacturer.

Reference: Human anti-salmonella Typhi Vi IgG Immunoassay ELISA kit (VaccZyme). Kit limits: negative: < 7.4. IU/ mL, positive: > 7.4, upper detection limit: > 600 IU/mL.

Correlate of protection: An anti-Vi IgG titer 2.0 µg/ml is a suggested estimate of protective titer [1].

1. Szu SC, Klugman KP, Hunt S. Re-examination of immune response and estimation of anti-Vi IgG protective threshold against typhoid fever-based on the efficacy trial of Vi conjugate in young children. Vaccine. 2014; 32:2359-63.

Anti-measles IgG ELISA

Measles IgG antibodies were quantified using a Serion-Virion ELISA kit. In brief, the contents of the kit were brought to room temperature and 100 µL of standard, controls (Positive and Negative) and appropriately diluted samples were added to the wells except for blank. Plates were covered with aluminum foil and incubated for 60 min at 37ºC in a moist chamber. Plate contents were discarded and the wells were washed four times with 300 µL wash buffer per well. The plate was inverted and wells were tapped to dry on gauze cloth or tissue after every wash. 100 µL of alkaline phosphatase conjugate (APC) solution was added to each well except for the blank and plates were incubated with aluminum foil for 30 min at 37ºC in a moist chamber. Well contents were discarded and wells were washed as before. 100 µL of substrate solution pNPP was added to each well including blank, plates were covered with aluminum foil and incubated for 30 min at 37ºC in a moist chamber before addition of 100 µL stop solution to each well including blank. Absorbance was read at 405 nm with a reference wavelength of 620-690 nm within one hour. IgG content was calculated as per the software (Infectious calculation) provided by the manufacturer (Serion ELISA kit).

Reference: Determination of anti-measles IgG antibodies using Serion ELISA kit (Cat #ESR102G). Kit Limits: negative: < 150 mIU/mL, equivocal: 150 and 200 mIU/mL, positive: > 200 mIU/mL, lower detection limit: 50 mIU/mL, upper detection limit: 5000 mIU/mL.

Correlate of protection: The correlate of protection against measles was lower than 0.345 IU/mL [1].

1. Woudenberg T, van Binnendijk R, Veldhuijzen I, Woonink F, Ruijs H, van der Klis F, Kerkhof J, de Melker H, de Swart R, Hahné S. Additional evidence on serological correlates of protection against measles: an observational cohort study among once vaccinated children exposed to measles. Vaccines (Basel). 2019 Oct 22;7(4):158.)

Anti-Mumps IgG ELISA

Mumps IgG antibodies were quantified using a Siemens ELISA kit. In brief, the contents of the kit were brought to room temperature and 200 µL non-dyed sample buffer was pre-dispensed into each well of the test plate. 20 µL of diluted Anti-Parotitis virus reference P/N (1+20) was dispensed into each well of the first pair (A1 Parotitis virus positive; A2 Parotitis virus-negative) and 20 µL of diluted samples (1+20) were dispensed into each well of the subsequent pair. 20 µL diluted Anti-Parotitis virus reference P/N (1+20) was dispensed into the last pair of wells. Well contents were mixed thoroughly by aspiring and expelling at least twice with a pipette tip within 15 min per test plate. The plate was sealed with aluminum foil and placed in the incubator at 37ºC for 60 min. Well contents were discarded and wells were washed with 250–300 µL diluted wash buffer and this process was repeated 3 times before 100 µL of diluted Anti-Human-IgG conjugate was added to each well; the plate was sealed again with foil and incubated for 60 min at 37ºC. Well contents were discarded and wells were washed as before. 100 µL of chromogen working solution was dispensed into each well and sealed with fresh foil and plates were incubated at temperature 18–25ºC for 30 min protecte from light before 100 µL stopping solution was dispensed into each well. The test plate was read at 450 nm in an ELISA reader with recommended reference wavelength at 650 nm within one hour. Calculations as per the software provided by the manufacture. (Siemens ELISA kit).

Reference: Determination of anti-Parotitis antibodies using Parotitis ELISA kit (Cat # OWLP-15) Siemens International Ltd. Kit Limit: negative: < 231 IU/mL, equivocal: $\Delta A \leq 0.100$ and $\Delta A \geq 0.200$, positive: $\Delta A \geq 0.200$: lower detection limit: $\Delta A \leq 0.100$, upper detection limit: $\Delta A > 2.5$ 5 (Samples need to be diluted further).

Correlate of Protection: There is no well-defined correlate of protection for mumps.

Anti-Rubella IgG ELISA

Rubella IgG antibodies were quantified using a Siemens ELISA kit. In brief, the contents of the kit were brought to room temperature, 200 µL non-dyed sample buffer was pre-dispensed into each well of the test plate. 20 µL of diluted Anti-Rubella virus reference P/N (1+20) was added to each well of the first pair (A1 Rubella virus positive; A2 Rubella virus-negative) and 20 µL of diluted samples (1+20) were dispensed into each well of the subsequent pair, and 20 µL diluted Anti-Rubella virus reference P/N (1+20) was added to the last pair of wells. Well contents were mixed thoroughly by aspiring and expelling at least twice with a pipette tip within 15 min per test plate. The plate was then sealed with aluminum foil and incubated at 37ºC for 60 min. Well contents were discarded and wells were washed with 250–300 µL diluted wash buffer and this process was repeated 3 times, before 100 µL of diluted Anti-Human-IgG conjugate was added to each well; the plate was again sealed with aluminum foil and incubated for 60 min at 37ºC. The contents were discarded and wells were washed and 100 µL chromogen working solution was added to each well and sealed with fresh foil. The wells were incubated at 18–25ºC for 30 min protected from light before 100 µL stopping solution was dispensed into each well. Calculations were done as per the software provided by the manufacture. (Siemens ELISA kit).

Reference: Determination of anti-rubella antibodies using Rubella ELISA kit (Cat # OWBF-15) Siemens International Ltd. Kit Limits: negative: <4 IU/ml, equivocal: $\Delta A \leq 0.100$ and $\Delta A \geq 0.200$, positive: $\Delta A \geq 0.200$, lower detection limit: $\Delta A \leq 0.100$, upper detection limit: $\Delta A > 2.5$.

Correlate of Protection: The correlate of protection for Rubella through immunoprecipitation assay was found to be 10–15 mIU/ml [1,2].

[1] Plotkin, S. A., and S. E. Reef. 2008. Rubella vaccines, p. 735–771. In S. A. Plotkin, W. A. Orenstein, and P. A. Offit (ed.), Vaccines, 5th ed. Elsevier Saunders, Philadelphia, PA.

[2] Plotkin SA. Correlates of protection induced by vaccination. Clin Vaccine Immunol. 2010;17(7):1055-1065.).