Sero-efficacy of Vi-polysaccharide tetanus-toxoid typhoid conjugate vaccine (Typbar-TCV)

Merryn Voysey[1], Andrew J Pollard[2]

1. Merryn Voysey, M.Biostat,

Corresponding author

Affiliations: Nuffield Department of Primary Care Health Sciences, University of Oxford, Oxford, UK and Oxford Vaccine Group, Department of Paediatrics, University of Oxford, Oxford, UK.

Address: Nuffield Department of Primary Care Health Sciences, University of Oxford, Radcliffe Observatory Quarter, Woodstock Road, Oxford, OX2 6GG, UK.

Merryn.voysey@phc.ox.ac.uk, Tel: +44 (0)1865 289288

2. Andrew J Pollard, PhD

Affiliations: Oxford Vaccine Group, Department of Paediatrics, University of Oxford, and NIHR Oxford Biomedical Research Centre, Oxford, UK.

Room 02-46-07, Level 2, Children's Hospital, Oxford OX3 9DU

Andrew.pollard@paediatrics.ox.ac.uk, Tel +44 (0)1865 234226/611313

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Summary:

Vi-tetanus toxoid conjugate typhoid vaccine (Typbar TCV) substantially reduced the number of serologically defined (sub)clinical infections compared with Vi-polysaccharide vaccine (RR=0.37, p<0.0001). Vaccine seroefficacy was 85% (95%CI 80–88%).

1 Abstract

Background

Salmonella Typhi is the major cause of enteric fever in lower income countries. New conjugate vaccines show promise as public health interventions, however there are no efficacy data available from endemic areas.

Methods

Data were obtained from a previously published phase 3 randomised controlled trial comparing Vi-polysaccharide tetanus-toxoid conjugate vaccine (**Typbar-TCV;** Bharat Biotech Intl Ltd, India**):** (Vi-TT) with Vi-polysaccharide (**Typbar;** Bharat Biotech Intl Ltd, India**):** (Vi-PS) in participants aged 2- 45 years. An additional open-label arm administered Vi-TT to children aged 6 months to 23 months. The proportion of participants with presumed clinical or subclinical infection ('seroincidence'), was determined using mixture models and compared using relative risks.

Results

81/387 (21%) participants were classified as having presumed typhoid infection during the 2 year period post-vaccination.

Seroincidence was lower in those randomised to Vi-TT than Vi-PS in those aged 2-45 years; 21/155 (13.5%) vs 47/129 (36.4%); RR 0.372 (95%CI 0.235–0.588), p<0.0001 and in those aged 2-15 years RR 0.424 (95%CI 0.231–0.778), p=0.0039. There was no difference in seroincidence in those receiving Vi-TT aged 2-45 years and those aged 6-23 months; 21/155 (13.5%) vs 13/103 (12.6%); RR 1.073 (0.563, 2.046), p=0.8293. Vaccine seroefficacy was 85% (95%CI 80–88%).

Conclusion

This is the first field estimate of the seroefficacy of a Vi-TT vaccine and shows that Typbar TCV substantially reduces the number of serologically defined (sub)clinical infections in infants, children and adults. These results support the recent World Health Organisation recommendations for deployment of typhoid conjugate vaccines in high burden areas.

2 Introduction

Salmonella enterica subspecies *enterica* serovar Typhi (*S.* Typhi) is a major cause of invasive bacterial infection, particularly in children in low and middle income countries. Vaccines available for prevention of typhoid fever include: Vi-polysaccharide (Vi-PS) vaccines, and live attenuated oral vaccines. Both types of vaccine induce protection of limited duration, are not licensed for administration in infants and young children (aged less than 2 years for Vi-PS; less than 6 years for live oral vaccines), and have not been widely implemented as routine public health interventions.[1,2]

More recently, new Vi-polysaccharide protein-conjugate vaccines have been developed for widespread use and show greater promise as routine public health interventions.[3,4] Two conjugate vaccines with a tetanus toxoid carrier protein are licensed in India,[3,4] but only one of these is currently being considered by WHO for prequalification (Tybar TCV, Bharat Biotech Intl Ltd).[4] Unlike plain polysaccharide vaccines, conjugate vaccines induce robust immune responses in young children,[3-5] and the technology has been used effectively to produce vaccines that prevent other bacterial infections such as *Haemophilus influenzae* type b, meningococcal, and pneumococcal disease.[6-10] However, there are no field efficacy estimates for the Vi polysaccharide -tetanus toxoid (Vi-TT) conjugate vaccine, and limited data on immunogenicity and the duration of protection.[4,11,12]

We provide an independent report of the seroefficacy, and protective antibody levels from a phase III study of Vi-TT conjugate vaccine. Antibody concentrations generally peak at 4-6 weeks post-vaccination or post-infection, decay sharply thereafter, then plateau.[13] However, if a subsequent infection occurs, antibody will increase. Increases in antibody when decline is expected, or sharp declines when antibody is expected to have plateaued, provide serological

evidence of infection which we used to assess the comparative protection afforded by these vaccines.

Typhoid conjugate vaccines were recently recommended by the World Health Organisation's Strategic Advisory Group of Experts, and subsequent decisions on financing will be made by the Global Alliance for Vaccines and Immunisation. In the absence of any field efficacy studies with clinical endpoints for Typbar TCV, these results provide the only available evidence of the efficacy of this vaccine in an endemic field setting, and are therefore important for the comprehensive review of the benefits of typhoid conjugate vaccines by global policy makers.

3 Methods

Immunogenicity and demographic data were provided by Bharat Biotech International Ltd from their phase III, randomised, multicentre, controlled study that evaluated the immunogenicity and safety of Vi-TT vs Vi-PS vaccine in healthy subjects. Data from the primary phase of the study have been previously published.[4] Briefly, participants aged 2-45 years were randomised to receive Vi-TT or Vi-PS vaccine. In an open-label arm participants aged 6 months to 23 months received Vi-TT with no comparator vaccine group (Vi-TT OLT group).

3.1 Vaccines

The vaccines used in the study were as follows:

Vi-TT (**Typbar-TCV;** Bharat Biotech Intl Ltd, Hyderabad, India**):** Typhoid Vi capsular polysaccharide-tetanus toxoid conjugate vaccine. Dose: 25µg/0.5mL administered intramuscularly.

Vi-PS (Typbar; Bharat Biotech Intl Ltd Hyderabad, India**):** A Typhoid Vi capsular polysaccharide vaccine. Dose 25µg/0.5 ml administered intramuscularly.

3.2 Laboratory Assays

Serum anti-Vi IgG antibody levels were tested using the commercially available VaccZyme™ Enzyme Linked Immunosorbent Assay (ELISA) kit (The Binding Site, UK), as per the manufacturer's instructions. The lower limit of assay quantification was 7.4 EU/mL.

3.3 Statistical analyses

Antibody results were $\log_{10}$-transformed and values below the lower limit of quantification of the assays were replaced with values of half the lower limit.

To estimate comparative protection in the two year period after a single dose of vaccine, we used antibody levels at days 42, 540 and 720 to classify participants as having a presumed typhoid infection (symptomatic or asymptomatic) or not using Gaussian finite mixture models. Mixture models are probabilistic models which assume data are generated from a fixed number of underlying distributions and can be used to detect subpopulations within the data. The rate of decay of log-transformed antibody levels was calculated for each time period (day 42 to day 540, and day 540 to day 720) and Gaussian mixture models were fitted to the antibody decay rates at each time period separately. Antibody titres at time points some distance removed from the original vaccination, are a combination of residual circulating vaccine-induced antibody, and antibody which is generated in response to exposure to antigens in the intervening period. In the absence of other exposures, vaccine-induced antibody from conjugate vaccines decays rapidly in the first year post-vaccination and then less rapidly in the second year.[13,14] We classified participants as having presumed typhoid infection if their antibody kinetics did not follow this pattern and they were thus classified by mixture models as coming from a different subpopulation.

The proportion of infected persons in each group (seroincidence) was compared using relative risks and vaccine seroefficacy (VSE) computed as:

$$VSE = 1 - \left[RR_{C/P} \times \left(1 - VE_{P/0}\right)\right];$$

where $RR_{C/P}$ is the relative risk of infection from the data (Vi-TT versus Vi-PS); and $VE_{P/0}$ is the estimate of vaccine efficacy (Vi-PS versus no Vi vaccine) from the published literature.

Only participants with data at all three post-vaccination time points were included in the analysis. Those with missing data at follow up time points were compared with those with no missing data to determine if this sub-set of participants was representative of the total randomised cohort in terms of their age, sex, and initial antibody response to vaccination.

The relationship between log-Vi-IgG at day 42 post-vaccination and seroincidence of typhoid infection was examined using logistic regression models.

All analyses were conducted using R version 3.3.2. Mixture models were fit using the mixtools package.[15]

4 Results

4.1 Baseline characteristics of participants included in the analysis

Antibody data at day 540 and day 720 were available for 387 (41%) of 944 participants. 47% of participants in the Vi-TT (RCT) group had full data as did 42% of participants in the Vi-PS group. In comparison, the children in the younger open label cohort had less data with only 34% with full data included in the analysis (Table 1).

In those receiving Vi-TT in the randomised trial, participants with full data available were older than those with missing data (median 10 years vs 8 years, p=0.013) and had lower antibody titres at day 42 (GM 1093, 95%CI 931–1282 vs 1497, 95%CI 1274–1760, p=0.007) (Table 1). For the other two groups there were no significant differences in age, sex or antibody responses in those with missing data and those with full data (Table 1).

4.2 Relative risk of infection using a serological definition of typhoid

The results of fitting mixture models to classify participants are shown in Figure 1. Mixture models fit to antibody decay rates during the first time period (day 42 to day 540) classified 13 participants as infected. All 13 participants had antibody levels which increased during this period (Figure 1B), consistent with exposure or infection, and in contrast to those classified as presumed uninfected whose antibody levels decayed (Figure 1A).

Mixture models fitted to antibody differences in the second time period (day 540 to day 720) denoted two sets of participants with different antibody kinetics. The first group had antibody levels which increased during this 6-month period, consistent with exposure or infection during the intervening gap (Figure 1C), and the second group had antibody levels which decayed steeply during this 6 month period (Figure 1D), in contrast to the general population for whom antibody decay rates had flattened out by this time (Figure 1A). As it is also

possible that some people do have antibody which decays steeply during the entire 2 year period following an initial high antibody concentration after vaccination, we only included participants in this group if the rate of decay in the second time period was greater than the rate of decay in the first time period, as this is the opposite to what is seen in the uninfected group. These participants are therefore indicative of people who have had exposure or infection not long before the day 540 time point, and as such their antibody is relatively high at day 540 and decaying rapidly. Some people were classified as infected using more than one method as these definitions are not mutually exclusive.

Overall, 81/387 (21%) participants were classified using mixture models as having presumed typhoid infection at some point during the 2 year period post-vaccination (Table 2), and 34 of these received Vi-TT, resulting in an estimated seroincidence of infection of 13.2% after Vi-TT vaccination. Seroincidence in the Vi-PS group was 36%.

The risk of serologically defined typhoid infection was lower in those randomised to Vi-TT than those receiving the Vi-PS vaccine 21/155 (13.5%) vs 47/129 (36.4%), RR 0.372 (95%CI 0.235–0.588), p<0.0001 (Table 2). Similar relative risks were seen when restricting analyses to those aged 2–15 years, although with slightly wider confidence intervals (RR 0.424, 95%CI 0.231–0.778, p=0.039). There was no significant difference in serologically defined typhoid infection rates between those receiving Vi-TT age 2-45 years in the randomised trial, and those receiving Vi-TT aged 6 months to 23 months in the open-label study (RR: 0.932 95%CI 0.489–1.776), p=0.829 (Table 2). In their systematic review Anwar *et al* report the 2-year efficacy of Vi polysaccharide vaccines as RR 0.41, (95%CI 0.31–0.55), VE 59% (95%CI 45–69%).[12] Using these estimates, the vaccine seroefficacy of Vi-TT was 85% (95%CI 80–88%).

There was a strong relationship between seroincidence and anti-Vi IgG levels in logistic regression (OR 0.327, 95%CI 0.206–0.518). The probability of serologically defined infection calculated from the logistic model when anti-Vi IgG at day 42 was 1000 EU/mL was 19% (15–23%) (Figure 2).

5 Discussion

In the absence of large field studies with suitable clinical endpoints we estimated vaccine efficacy using a serological definition of typhoid infection, and for the first time show that instances of presumed typhoid infection in an endemic setting occur significantly less often with Vi-TT than Vi-PS vaccine. The relative protection afforded by Vi-TT was the same when estimated in the full RCT cohort aged 2-45 years, and when restricted to those aged 2-15 years. In the open-label trial enrolling children aged 6 months to 23 months, seroincidence after Vi-TT vaccination was the same as in those aged 2-45 years receiving this vaccine in the RCT. This may suggest a similar impact of vaccination in infants and toddlers as in older age groups. However, there was no control group for the younger aged children therefore differential exposure rates in different age groups may also have influenced these comparisons. These findings are especially important since the World Health Organisation has recently recommended the use of Vi-conjugate vaccine programmes as a public health policy to control enteric fever.

A serological definition of infection is a simple method which can be used to compare groups in randomised trials. Infections defined using these methods are likely to include both clinical and subclinical infections thus the seroincidence of 13.2% estimated for those receiving the conjugate vaccine may be higher than the prevalence of clinical disease endpoints detected in field studies. However, subclinical infections associated with gastrointestinal shedding likely

contribute to disease transmission, so vaccine efficacy against this endpoint is important in developing herd immunity.[16]

Estimates of seroincidence derived using probabilistic methods are affected by misclassification which can result in either over- or under-estimation of the true rate of infection. A rise in antibody levels is likely due to exposure to *S.* Typhi bacteria however could also be due to cross-reactive epitopes expressed on other organisms or Vi-polysaccharide capsules on the surface of unrelated bacteria such as some strains of Citrobacter.[17] Alternatively, persistence of vaccine antigen might occur in some individuals and cause a rise in antibody that continues beyond the 6 week post-vaccination timepoint in some individuals, but would not account for rises after the later timepoints in this study. In contrast, an infected individual may generate a limited antibody response which decays by the time of the next blood sample and they will thus be misclassified as non-infected. Caution needs to be applied when interpreting incidence of infection from serological data alone. One of the benefits of randomisation in clinical trials is that the effect of misclassification will be balanced between groups and therefore only add random 'noise' to comparisons, rather than inducing bias.

Using our serological definition of infection and the estimated vaccine efficacy for Vi-PS from a Cochrane review, we estimate the two-year vaccine efficacy of Vi-TT to be 85% (95%CI 80–88%) after a single dose. This is similar to the only other robust field estimate of vaccine efficacy for a Vi-conjugate vaccine which comes from a randomised trial in Vietnam.[5] The Vi-conjugate vaccine in that study contained recombinant *Pseudomonas aeruginosa* exotoxin A (rEPA) as the carrier protein. The study administered two doses to children aged 2–5 years and observed vaccine efficacy of 91% (79–97%).[5] Vaccine efficacy for Vi-TT has also been estimated in a challenge study in typhoid-naïve adults in Oxford,

where vaccine efficacy was 55% (27–72%) against symptomatic or asymptomatic infection during 14 days of follow up.[18]

This finding is important as this is the first attempt to estimate the efficacy of a Vi-TT vaccine from field data, and potentially provides strong support for the use of this vaccine as part of programmatic control of enteric fever. However, large field efficacy trials such as those being conducted by the TyVAC consortium are needed to confirm or refine this estimate.[19]

In logistic regression models of seroincidence there was a strongly significant relationship between higher antibody levels measured 6 weeks after vaccination and protection against infection during the 2 year period post-vaccination, however no threshold antibody level provided complete protection. Even at antibody levels of 1000 EU/mL at day 42 post-vaccination, there was still a 19% chance of serologically defined typhoid infection, similar to findings seen in Oxford typhoid challenge studies in which a 25% chance of infection corresponded with an anti-Vi IgG concentration of 1000 EU/mL at the time of challenge.[18] Defining a serological correlate of protection is desirable for vaccine development and can simplify the testing of new vaccines by comparing the number of vaccinees in immunogenicity studies with an immune response higher than a threshold level rather than conduct large field studies with rare clinical endpoints.[20] Correlates of protection for typhoid vaccines have been suggested previously based on different assays,[21] however the methods used to calculate such thresholds necessarily assume that such a threshold does exist, i.e. that there is a level of Vi IgG antibody at which the probability of infection drops in a step-function from a high to a very low probability of infection. Our findings confirm those seen in typhoid challenge studies that whilst higher anti-Vi IgG is associated with greater protection against typhoid infection, there is no threshold level where the probability of infection becomes negligible within the range of antibody levels induced by vaccination in

this study. Even at the highest antibody level of 1500 EU/mL the 2-year probability of infection is still 10%.

Conjugate vaccines against *Haemophilus influenzae* type b, pneumococcus and meningococcus are thought to confer direct protection by both preventing acquisition of the organism on the upper airway mucosa and facilitating killing of organisms invading through the mucosa to the blood through bactericidal activity or opsonophagocytosis. In the context of invasive Salmonella infection it is not clear how antibody-mediated protection operates and whether similar processes are involved, since the pathogens of enteric fever occupy an intracellular niche, at least during part of the infection process, and are therefore presumed to be inaccessible to antibody. It may be that anti-Vi antibody induced by the vaccine reduces or even blocks organisms invading through the mucosa or limits multiplication and spread between cells after invasion.

5.1 Limitations

Not all participants were followed up at all time points in this study and thus many participants were excluded from the analysis. More data were available for the randomised trial than the open-label arm. Those receiving Vi-TT in the randomised trial who were included in the analysis were a slightly older cohort with lower antibody responses than those with missing data. A cohort with lower antibody responses would presumably be more susceptible to infection and thus our analysis may over-estimate seroincidence in this arm of the trial. This would have the effect of reducing any differences seen between study groups and seroefficacy may be underestimated in our analysis.

6 Conclusion

Administration of Typbar TCV induces a robust Vi antibody response in children and adults that substantially reduces the seroincidence of infection by an estimated 85%, similar to the

efficacy reported in a field trial of a different Vi conjugate vaccine.[5] In the context of typhoid which occurs most often in children, a vaccine which can induce robust antibody responses in young infants and children, and is effective in the prevention of infection is key to overcoming the current limitations of licensed typhoid vaccines.

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Contributions

MV analysed the data. MV and AJP wrote the paper.

Role of the Funding Source

No specific funding was obtained for this work.

Declarations of interest statement

AJP has previously conducted studies on behalf of Oxford University funded by vaccine manufacturers, but currently does not undertake industry funded clinical trials. AJP chairs the UK Department of Health's (DH) Joint Committee on Vaccination and Immunisation (JCVI) and is a member of the World Health Organization's (WHO) Strategic Advisory Group of Experts.

The views expressed in this manuscript are those of the authors and do not necessarily reflect the views of the JCVI, the DH, or the WHO.

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Table 1 Baseline characteristics of participants included and excluded from the analysis according randomised booster groups

			N	Missing data (excluded from analysis)	N	No Missing Data (included in analysis)	P value*
Age (years)	Vi-TT (RCT)	Median (IQR) [min, max]	177	8.0 (4.5, 13) [2.0, 45.0]	155	10.0 (5.0, 18.0) [3.0, 40.0]	0.013
	Vi-TT (OLT)	Median (IQR) [min, max]	204	1.2 (0.9, 1.5) [0.5, 2.0]	103	1.1 (0.8, 1.6) [0.6, 2.0]	0.845
	Vi-PS	Median (IQR) [min, max]	176	8.5 (5.0, 18) [2.0, 41.0]	129	9.0 (5.0, 22.0) [2.0, 45.0]	0.230
Sex (male)	Vi-TT (RCT)	N (%)	176	83 (47%)	155	70 (45%)	0.716
	Vi-TT (OLT)	N (%)	205	109 (53%)	103	59 (57%)	0.471
	Vi-PS	N (%)	176	77 (44%)	129	67 (52%)	0.157
Vi-IgG at Day 42	Vi-TT (RCT)	Geometric mean (95%CI)	177	1497.4 (1273.9, 1760.2)	155	1092.5 (931.2, 1281.7)	0.007
	Vi-TT (OLT)	Geometric mean (95%CI)	204	1970.2 (1794.5, 2163.1)	103	1874.1 (1592.0, 2206.3)	0.573
	Vi-PS	Geometric mean (95%CI)	176	386.9 (324.9, 460.8)	129	446.6 (358.8, 555.8)	0.301
All	Vi-TT (RCT)	N=332		177 (53%)		155 (47%)	
	Vi-TT (OLT)	N=307		204 (66%)		103 (34%)	
	Vi-PS	N=305		176 (58%)		129 (42%)	

Vi-PS: Vi polysaccharide vaccine; Vi-TT: Vi polysaccharide tetanus toxoid conjugate vaccine; RCT: Randomised trial of participants aged 2 – 45 years; OLT: Open label trial of participants aged 6 months – 23 months. *p values computed: for comparisons of age, Wilcoxon Rank-Sum test; for comparisons of sex, chi-square tests; for comparisons of antibody titres at day 42, independent samples t-tests.

Table 2 Seroincidence and relative risk of presumed typhoid infection

Kinetics of antibody in those classified as presumed infected by Gaussian mixture models	**Vi-TT (RCT) (age 2-45 years) N=155**	**Vi-TT (OLT) (age 6-23 months) N=103**	**Vi-PS (RCT) (age 2-45 years) N=129**	**RR (95% CI) Vi-TT vs Vi-PS *(randomised comparison)***	**RR (95% CI) Vi-TT age 2-45 years vs age 6-23 months *(non-randomised comparison of Vi-TT groups)***
Increase in antibody between day 42 to day 540 (Fig 2B)	3 (1.9%)	2 (1.9%)	8 (6.2%)		
Increase in antibody between day 540 to day 720 (Fig 2C)	13 (8.4%)	5 (4.8%)	28 (21.7%)		
Decrease in antibody between day 540 to day 720 (Fig 2D)	6 (3.9%)	6 (5.8%)	19 (14.7%)		
Total seroincidence	**21 (13.5%)**	**13 (12.6%)**	**47 (36.4%)**	**0.372 (0.235, 0.588), p<0.0001**	**1.073 (0.563, 2.046), p=0.8293**
Total seroincidence in those aged 2-15 years	**13/108 (12.0%)**		**25/88 (28.4%)**	**0.424 (0.231, 0.778), p=0.0039**	

Only participants with data at all three time points (day 42, day 540 and day 720) were included

RR: Relative risk; RCT: Randomised controlled trial in participants aged 2-45 years; OLT: Open label trial in children aged 6 months to 23 months

Figure 1 Individual participant anti-Vi IgG antibody kinetics according to serological classification of typhoid infection

A: Presumed uninfected individuals

B: Participants classified as having evidence of infection during first time period (day 42 to 540)

C: Participants with antibody levels that increased during second time period (day 540 to 720)

D: Participants with antibody levels that decreased during second time period (day 540 to 720)

Classification of infection done using 2-component Gaussian mixture model.

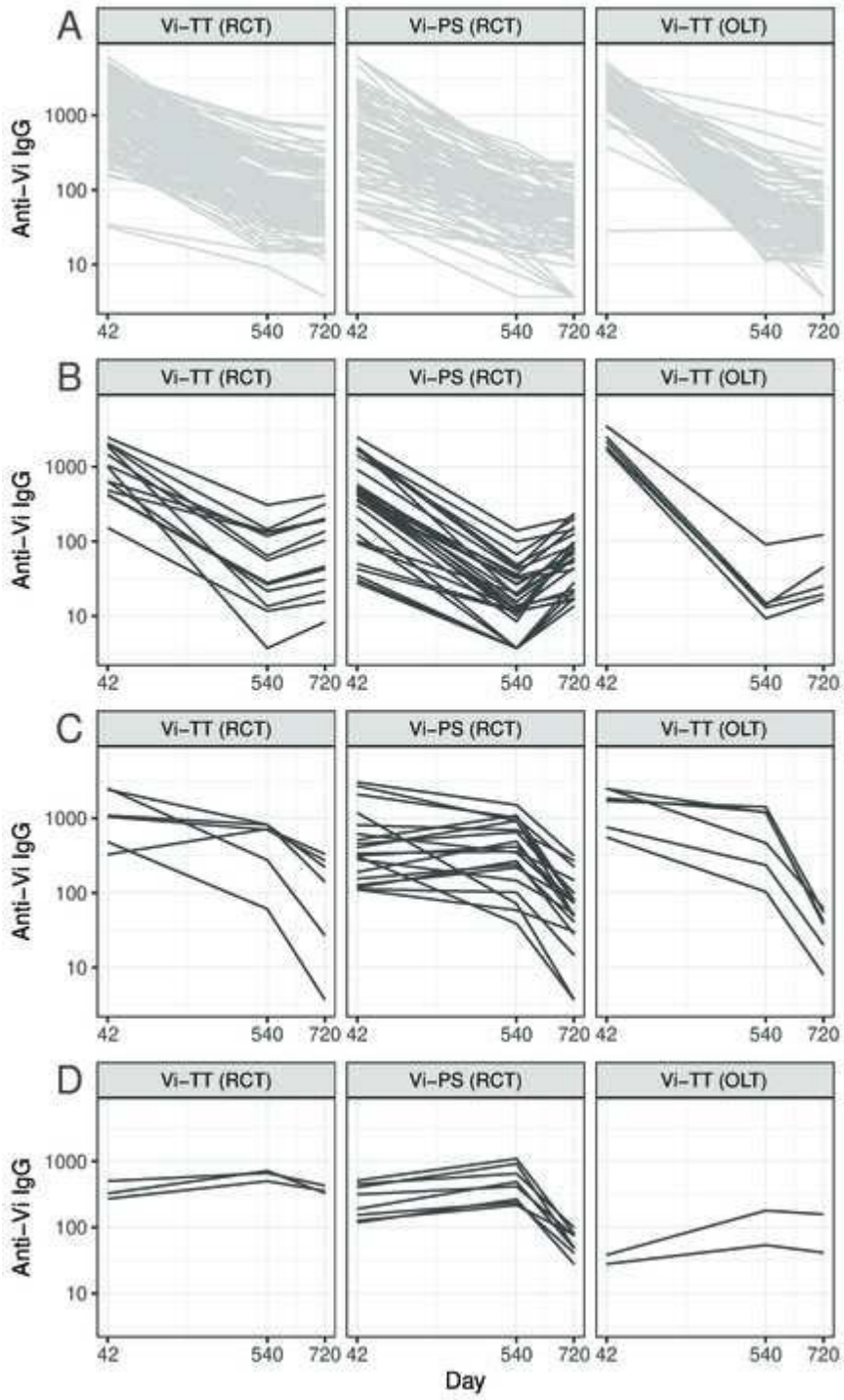
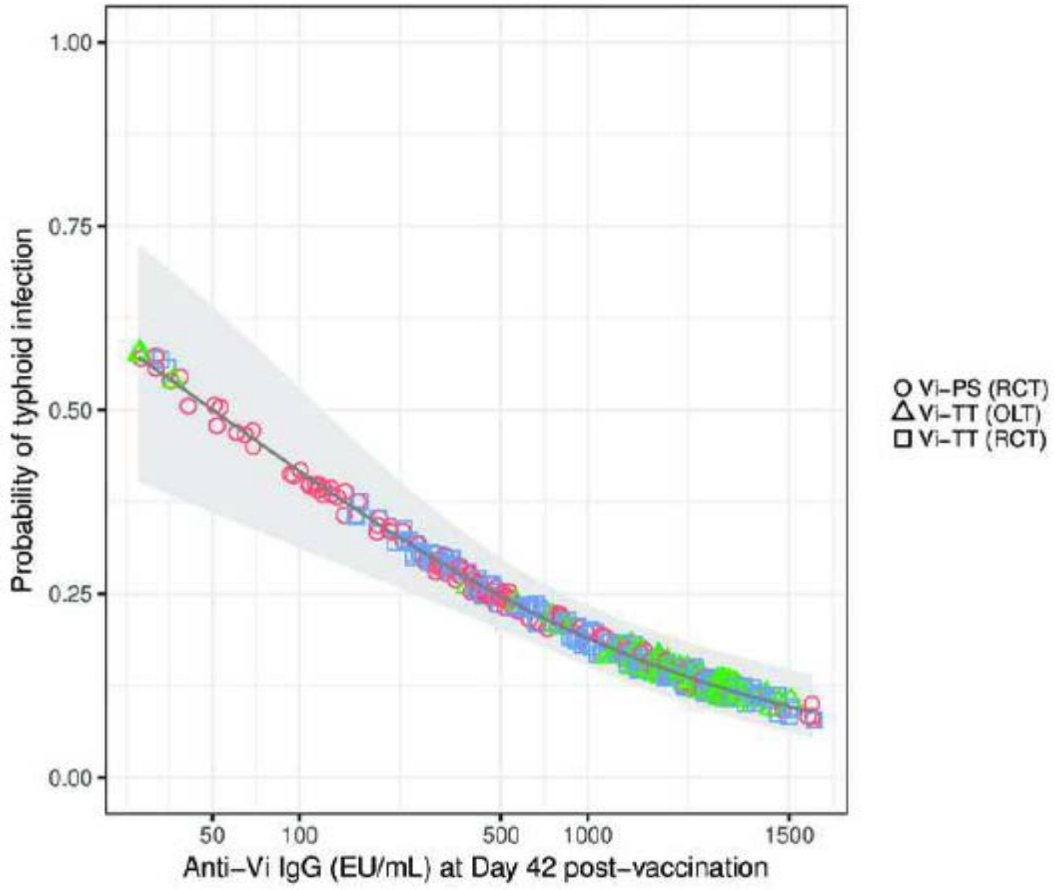

A
B
C
D
Vi–TT (RCT)
Vi–PS (RCT)
Vi–TT (OLT)
Anti–Vi IgG
1000
100
10
42
540
720
Day

Figure 2 The two-year probability of serologically defined typhoid infection according to anti-Vi IgG concentration 42 days after vaccination with Vi-TT or Vi-PS vaccine.



RCT: Randomised controlled trial in participants aged 2-45 years; OLT: Open label trial in children aged 6 months to 23 months.