The predicted persistence and kinetics of antibody decline 9 years after pre-school booster vaccination in UK children.

Authors:

Merryn Voysey^{a,c}, Rama Kandasamy^c, Ly-Mee Yu^a, Martine Baudin^b, Christine Sadorge^b, Stéphane Thomas^b, Tessa John^c, Andrew J Pollard^c

Institutional affiliations:

a. Nuffield Department of Primary Care Health Sciences, University of Oxford, Oxford, UK

b. Sanofi Pasteur MSD S.N.C, Lyon, France

c. Oxford Vaccine Group, Department of Paediatrics, University of Oxford, and NIHR Oxford Biomedical Research Centre, Oxford, United Kingdom.

Correspondence: Merryn Voysey

Email: Merryn.voysey@phc.ox.ac.uk

Nuffield Department of Primary Care Health Sciences,
University of Oxford.

Radcliffe Observatory Quarter,
Woodstock Road, Oxford, OX2 6GG, UK

[Tel: +44 \(0\)1865 289288](tel:+441865289288)

Keywords: diphtheria, tetanus, pertussis, polio, persistence, immunogenicity, children, United Kingdom

Word count: Abstract 197, Paper 3047.

Funding:

The parent study was sponsored by Sanofi Pasteur MSD in 2001.

Abstract

Background

Long term follow-up of vaccine trials is essential to establish the duration of protection. In the context of worldwide concern about rising pertussis incidence, estimates of antibody persistence after vaccination, which do not account for the rise in antibody due to natural boosting or infection, may overestimate the degree of protection afforded by pertussis vaccines.

Methods

This was a 5 year follow up study of a randomised controlled trial of diphtheria, tetanus, pertussis and polio booster vaccines in UK children aged 3.5 to 5 years. Antibody persistence was measured at 1 month, 1, 3, and 5 years after vaccination and the kinetics of antibody decline were modelled longitudinally. Estimates of predicted antibody persistence 9 years after the pre-school booster were derived from model parameters.

Results

Antibody levels 9 years after vaccination were predicted to be above accepted thresholds for protection for diphtheria, tetanus and polio. Antibody responses to pertussis toxoid were undetectable in 49% of children at the 5 year follow up visit, and responses were predicted to be undetectable in 69% (95% CI 45% to 88%) of children by the time of their teenage booster at 13 to 14 years of age.

Conclusions

There is no defined correlate of protection for pertussis. However, the large proportion of participants in this study with undetectable pertussis antibody levels at both measured and predicted timepoints suggests sub-optimal immunity in adolescence. Adding pertussis to the teenage booster for UK children as is done in other countries, would enhance immunity in adolescence.

Introduction

Pre-school aged children in the United Kingdom (UK) receive a combination booster vaccine, to enhance the immunity induced by priming against *Corynebacterium diphtheriae* toxin, *Clostridium tetani* toxin, *Bordetella pertussis*, and poliovirus during infancy. Unlike the situation in many countries, no booster vaccine is administered at 12 months of age, between the third dose of infant priming and the pre-school booster at age 4 years. To a smaller extent, some boosting of diphtheria and tetanus antibodies can occur after administration of tetanus or diphtheria protein conjugate vaccines. Circulating antibodies induced by the preschool booster vaccination gradually decline over time until a subsequent booster dose is administered during the early teen years [1]. In the UK, unlike some other countries, this booster does not contain pertussis antigens. This long period between booster doses creates a potential window of waning immunity where individuals may become susceptible to disease.

The incidence of diphtheria and tetanus in the UK is negligible and there have been no cases of natural polio infection in England or Wales since 1984 [2]. However, it is essential to maintain individual protection against tetanus, and to sustain herd immunity to prevent resurgence of diphtheria and to block the risk of outbreaks due to imported polio disease. Recent breakdown in immunisation due to civil war in Syria resulted in 35 children paralysed by polio [3]. This demonstrates the ease with which disease can return even 15 years after the last known case was observed. In contrast, an increasing number of pertussis diagnoses have been observed in the UK in recent years despite high levels of vaccination coverage. The reasons for increased pertussis notifications are multifactorial, due in part to enhanced diagnostic methods [4]. Other contributory factors include possible genetic modifications in *B. pertussis* and the change from a whole cell to an acellular vaccine in 2004 [5].

Between 2001 and 2002 a randomised clinical trial was conducted in the UK with 300 pre-school aged children (mean age 3.9 years) who had previously received 3 doses of whole cell pertussis-containing vaccine in infancy. Children received 3 different diphtheria, tetanus, acellular pertussis, and polio vaccine combinations and were followed up for 5-years. The results one month after vaccination [6] and after 5 years of follow up [7] are reported elsewhere.

We modelled the decline in vaccine antigen-specific antibody levels over the 5-year follow up period to predict the level of antibodies to components in the acellular pertussis vaccine 9 years after the booster vaccination, the age at which adolescent booster doses of vaccine are routinely administered in the UK, and to estimate the proportion of children still protected against diphtheria, tetanus, and poliovirus according to World Health Organization (WHO) recommended correlates of protection. This was an exploratory post-hoc analysis.

Methods

Study Participants

Participants were healthy male or female children aged from 3.5 years of age up to their fifth birthday [7].

Children were randomised to one of 3 groups; Combined adsorbed tetanus, low dose diphtheria, 5 component acellular pertussis and inactivated poliomyelitis vaccine (Td5ap-IPV: Repevax®), combined adsorbed tetanus, low dose diphtheria and 5 component acellular pertussis vaccine plus oral polio vaccine (Td5ap: Covaxis® + OPV), or combined adsorbed standard dose diphtheria, tetanus, two component acellular pertussis and inactivated poliomyelitis vaccine (DT2aP-IPV: Tetravac®). Live attenuated measles, mumps and rubella (M-M-R™ II) vaccine was given concomitantly (supplementary table 1).

Serological Analysis

Immune responses were measured as previously described by seroneutralisation (SN) for diphtheria and poliovirus antigens and by enzyme immunoassays (EIA) for tetanus (tetanus toxoid (TT)) and pertussis antigens (pertussis toxoid (PT), filamentous haemagglutinin (FHA), pertactin (PRN) and fimbrial agglutinogens (2+3) (FIM)) [6, 7]. Seroprotection was defined as diphtheria antibody titer (SN) ≥ 0.1 IU/mL; tetanus antibody titer (EIA) ≥ 0.1 IU/mL; and poliovirus types 1, 2 and 3 antibody titer (SN) ≥ 8 (1/dilution) respectively. Samples obtained at pre-vaccination, 1 month, and 1 year were analysed in Aventis Pasteur laboratories in Canada. For 3 and 5 year time points, assays were performed in Sanofi Pasteur laboratories in the United States. Concordance studies were conducted and conversion factors applied to obtain consistency of data between the two laboratories. Values less than the lower limit of assay detection were substituted with values half that level. The lower limit of detection (LLD) in the two laboratories was not the same for some antigens. For pertactin the LLD was < 3 and < 4 in the Canadian and US laboratories respectively, however few values

were below these limits. For FIM, the LLD was < 17 in Canada and < 4 in the US laboratory and thus predictions from the model of FIM antibodies may be affected by these differences.

Statistical Analysis

Data from all randomised participants were included in the analysis regardless of the number of visits attended. Mixed effects models for repeated measures [8] were fitted to antigen specific log₁₀-transformed antibody titers regressed on time and adjusted for baseline values. Anti-PT antibodies were analysed as a binary variable using a cutpoint of 5 IU/mL. Full details of statistical models are supplied in the supplementary material.

In order to model the waning of vaccine-induced antibody without the confounding influence of antibody increase due to exposure we attempted to identify those with antibody rises due to infection. There is no validated method for defining pertussis infection from cross-sectional or serial antibody measures. Other studies have used various criteria including anti-PT IgG > 50 EU/mL, or > 100 EU/mL, or anti-FHA > 12 EU/mL with a corresponding 100% rise [9-11]. We used a combination of such methods and classified children with pertussis antibody levels above 80 IU/mL at either the 3 or 5 year visit who also had increased antibody levels of at least 4-fold since their previous visit as likely to have had clinical or subclinical pertussis infection. This method is more likely to detect recent infection rather than infection occurring shortly after a blood draw. However, this was not clinically assessed during the study nor reported by the participants.

Results

Enrolment and demographics

One hundred children were randomised (1:1:1) into each of the 3 arms of the study. Blood samples were collected prior to vaccine administration, followed by 1 month, 1 year, 3 years and 5 years post vaccine administration [6]. Overall loss to follow up was 53% at the final visit 5 years post vaccination (Table 1). Baseline characteristics of participants who attended long term follow up and those who did not, were similar (Supplementary Table 1).

Measured and modelled persistence of immunogenicity to vaccine antigens

Diphtheria

Anti-diphtheria toxin immunoglobulin titers declined sharply in the first year post booster then stabilised (figure 1A). At 1 month post-booster, significant differences were observed between randomised groups with higher responses in the DT2aP-IPV group however at subsequent time-points thereafter the groups converged (figure 1a, supplementary table 2). Overall 78% of children had predicted anti-diphtheria toxin titers above 0.1 IU/mL at 9 years (table 2).

Tetanus

There were significant differences in average anti-tetanus toxin antibody levels between randomised groups. However, the observed rate of decline over time was the same. Although DT2aP-IPV vaccine contained more tetanus toxoid than the Td5ap vaccines, children in this group had lower tetanus antibody levels at 3 and 5 years after booster vaccination (figure 1B, table 1). Estimates from the statistical model predicted that 69% of children would remain seroprotected at 9 years post-booster in this group compared with 82% and 86% of children in the Td5ap-IPV and Td5ap + OPV groups respectively (table 2).

Poliomyelitis

Statistically significant interactions between treatment arm and time (rate of decline) were observed for immunoglobulins generated against serotypes 1 and 2 poliomyelitis (supplementary table 2). For serotype 1 poliomyelitis, the immediate post-booster antibody response at 1 month was significantly higher for IPV than OPV (interaction $p=0.0022$) and thereafter declined more rapidly. By 9 years, predicted protection was still high with more than 99% of children seroprotected. Serotype 2 poliomyelitis antibody titers also declined at a faster rate in the IPV groups ($p=0.0001$), but all antibody titers were estimated to be above the threshold of protection by 9 years.

Serotype 3 poliomyelitis antibody titers had similar patterns of decline for IPV and OPV however the IPV groups were significantly higher in the immediate post-booster period and thus remained higher throughout follow up ($p<0.0001$). In the OPV group only 2% of children were estimated to be seropositive to type 3 antibodies at 9 years compared with more than 94% of children in the IPV groups (table 2, figure 2).

Pertussis

Anti-pertussis toxin antibodies were below 5 IU/mL (the lower level of detection for the assays performed in Aventis Pasteur laboratories, Canada) for 49% of children after 5 years and 69% (95% CI 45% to 88%) of children were predicted to have antibody levels below this level at 9 years (figure 3). At 9 years post-booster the predicted rates of seropositivity were 25% (95% CI 9% to 46%) for those who received DT2aP-IPV, 37% (95% CI 18% to 60%) and 32% (95 CI 16% to 53%) in those who received Td5ap-IPV and Td5ap+OPV respectively (table 2).

In our study 16 children had antibody levels above 80 IU/mL at either 3 or 5 years post-boosting however not all these children had increases in antibody since their previous visit. Nine of the 16 children had pertussis antibody levels above 80 IU/mL as well as a 4-fold increase in antibody levels since their previous visit and were thus defined as likely to have had pertussis infection. A further 7 children had experienced a slower decline in antibody from a much higher starting value (figure 4). It is not possible to determine when within the preceding two year period prior to the study visit, pertussis exposure occurred so immediate pre-infection titers are unknown. In addition, this was not assessed clinically so it is unknown whether such infections represent clinical disease. Seven out of 9 infected children had PT antibody titers less than 5 IU/mL at their preceding visit and all children had FHA titers no higher than 25 IU/mL at the preceding visit. All 9 children with likely pertussis infection experienced correspondingly large increases in FHA. For the other pertussis components the responses were mixed. PRN titers increased from the previous visit in 4 infected children but decreased in 3 children. Antibodies to FIM increased in 5 children and decreased in two children. These antibodies were not measured in the 2-component pertussis vaccine group (DT2aP-IPV).

For the 9 children defined as having likely infection, all values post-infection for all 4 pertussis antigens (FHA, PRN, FIM, PT) were removed from the modelling of antibody decline. This accelerated the rate of decline observed for PRN, FIM and PT antibodies and changed the conclusions for the model for FHA. A significant increase in FHA antibodies over time was observed when children with likely pertussis infection were included, however after removal of the post-infection results the trajectory for FHA antibodies was flat (non-statistically significant) (supplementary table 3).

Discussion

This is the first study to model immunological persistence to 3 randomly allocated pre-school booster vaccines 9 years post vaccine administration in the United Kingdom. Regardless of treatment arm, children were predicted to maintain good protection against most antigens until the time of their adolescent booster (at 13 or 14 years of age). However, the majority (69%; 95% CI 45% to 88%) of children were predicted to have undetectable levels of anti-PT antibody after 9 years.

Estimates from statistical models have some limitations requiring caution in their interpretation. Furthermore, there is no defined serological correlate of protection against pertussis. However, anti-PT antibodies are considered the most important immunity defining pertussis antigen and are correlated with susceptibility to infections [11-13]. The low levels of immunity observed for pertussis antigens after 5 years of follow-up indicate that protection may be sub-optimal following the preschool booster vaccine in the UK and support the addition of an adolescent booster of pertussis, as is used in some countries, to improve protection in the second decade of life. Unlike other European countries, the UK does not administer a pertussis booster in the second year of life and the low levels of pertussis antibody observed at enrolment (prior to the booster) indicate that early schedules might also be further optimised.

Similar proportions of children in each group had protective antibody responses against tetanus toxoid immediately post boosting [6], however the geometric mean responses in each study arm for both tetanus toxoid and poliovirus antigens were significantly different. For tetanus, a lower response was noted directly post boosting in the higher tetanus toxoid antigen-content vaccine (DT2aP-IPV) group. This group also received a higher dose of diphtheria toxin but only generated equivocal responses to diphtheria. This may be due to vaccine interference, whereby higher doses of pertussis and diphtheria antigens may interfere with the immune response generated to tetanus toxoid or perhaps the context and formulation of the vaccines previously received in infancy generated an immune response that was more favourably boosted by one vaccine formulation than another [14, 15].

The observation that the OPV group had lower systemic antibody responses than IPV is not surprising, given that OPV replicates in the gut, generating local immune responses in the gastrointestinal lymphoid tissues, as opposed to IPV which is administered intramuscularly and hence leads to higher systemic responses. In addition, the co-administration of IPV with other potent antigens may create an adjuvant effect. The different routes of administration for OPV and IPV and hence their alternate mechanisms of providing protection make it difficult to compare the degree of protection between the two based on systemic antibody measures alone.

An initial rapid decline in antibody levels was observed for all antigens in the first year after vaccination and thereafter the rate of decline slowed considerably, consistent with the contribution to early responses by recently generated short lived plasma cells and persisting responses being dependent on long-lived plasma cells and/or turnover of memory B cells in a programmed manner or following nonspecific stimuli [16].

Antibody responses to serotypes 1 and 2 poliovirus in the study arms receiving IPV were higher after boosting followed by a rapid decline in the first year. The predicted rate of decline slowed after the first year but was still significantly different from the decline following OPV. A similar level of antibody in IPV and OPV groups was predicted at 9 years post vaccination. Antibody responses to serotype 3 poliovirus were less well maintained over the long-term, particularly in those receiving OPV, a finding also observed in cohort studies[17].

A combination of antibodies to the acellular components PT, PRN, and FIM are believed to be central to disease protection however the relative importance of each remains undefined [18, 19]. Prior studies have shown that persistence of antibody to PT in young children is short-lived [10, 20]. Findings from recent baboon studies show that although acellular pertussis vaccine reduces disease, unlike whole cell pertussis vaccine, it does not stop transmission [21], though the importance of this finding for human populations is unknown. Interestingly there has been resurgence of pertussis infections after some years of sole use of acellular pertussis vaccine [22]. The time to resurgence after change in vaccination policy is variable, and is likely to reflect the different policies used during transition from whole-cell to acellular vaccine, however many countries have experienced a rise in pertussis infections

approximately 6-8 years following the introduction of solely acellular schedules [14]. The resurgence observed may also be due to other factors such as; the natural disease cycling, and changes in the pathogen's virulence factors. In the context of the UK schedule, where no booster against pertussis is administered in the second year of life, as compared with most EU countries, our data suggest rapid waning of antibody and early susceptibility to pertussis disease after pre-school boosting.

We observed a rapid decline in anti-PT antibodies in all groups and no apparent development of a homeostatic antibody production level for anti-PT antibodies. Contrastingly anti-FIM and anti-PRN antibody levels appear to stabilise to a slower rate of decline, whilst FHA antibodies show no statistically significant long-term change. This has been hypothesised to be a result of environmental exposure to other FHA containing species such as *B. parapertussis* and microbes possessing epitopes that generate cross-reacting antibodies [23].

In the context of rising incidence of pertussis worldwide, long-term serological data on protection due to pertussis vaccination are confounded by results from children with natural exposure. After 3 doses of whole cell pertussis vaccine in infancy and a subsequent acellular booster, antigenic stimulation as a result of a significant natural exposure was still observed in some children, though we do not know if these were symptomatic infections. Estimates of persistence of antibody after vaccination which do not account for the rise in antibody due to infection may overestimate the protection afforded by pertussis vaccines. Conversely, cohort studies which classify those with recent pertussis infections according to a single serological cut point may overestimate the number with infection as these methods do not allow for a pattern of slow decline from a high starting titer.

The study has several strengths; the randomised trial design, the multiple time-points, long duration of follow-up and longitudinal analysis methods. Missing data are always an issue in a long term follow-up study, however use of appropriate modelling techniques which correctly deal with the missing values allow all data from all children to be retained in the analysis [24, 25].

This study has some limitations; this was an exploratory post-hoc analysis for which the study was not designed initially. The assumption was made that waning of antibody after the study period continued at the same rate as prior to the 5 year visit. In addition, the change in

laboratories between the first and third year visits may have impacted on the consistency of the data even though conversion factors were applied to achieve consistency. Validation of these predictive models in independent datasets from children who had received the same vaccines as those in this study, could provide further valuable information about model accuracy and calibration.

The current recommended pre-school booster in the UK is either Td5ap-IPV or DT2aP-IPV. This study suggests that apart from higher anti-tetanus toxoid GMT's following Td5ap-IPV, these vaccines provide similar levels of immunogenicity. The current strategy of the UK government for reducing pertussis in infants is via passive immunity generated by vaccinating pregnant women, however Public Health England estimate only approximately 60% of pregnant women in England are currently being vaccinated [26]. This study shows that school aged children in the UK are likely to be susceptible to pertussis infection and remain an avenue for transmission of pertussis into an infant's household [27]. Further studies exploring strategies to better sustain immunity after childhood pertussis vaccination, particularly a change in the booster schedule, are urgently needed as well as the development of new vaccines and new adjuvants that could better sustain pertussis protection.

Conflict of interest statement

The parent study was sponsored by Sanofi Pasteur MSD in 2001. With the exception of the authors employed by Sanofi Pasteur MSD (MB, ST, CS), no authors have received direct payment from Sanofi Pasteur MSD. The sponsor funded the study and developed the study protocol in collaboration with A.J.P and co-workers in 2001. Employees of the sponsor reviewed the manuscript before submission for publication but editorial control was maintained by the academic investigators. A.J.P. has previously conducted clinical trials on behalf of Oxford University funded by vaccine manufacturers but no longer does so.

Tables

Table 1 Estimates from linear mixed effects models of serotype-specific geometric mean titers at each visit and 9 year predictions.

<i>Antigen</i>	<i>Time since Booster vaccination</i>	<i>N</i>	<i>Td5ap-IPV GMT (95% CI)</i>	<i>N</i>	<i>Td5ap+ OPV GMT (95% CI)</i>	<i>N</i>	<i>DT2aP-IPV GMT (95% CI)</i>
Diphtheria	1 Month	98	22.84 (17.59, 29.67)	98	32.20 (24.79, 41.82)	89	48.45 (36.86, 63.68)
	1 Year	67	0.98 (0.77, 1.25)	72	1.22 (0.96, 1.54)	71	1.16 (0.91, 1.48)
	3 Years	47	0.25 (0.19, 0.32)	58	0.31 (0.24, 0.39)	46	0.29 (0.23, 0.38)
	5 Years	48	0.22 (0.17, 0.29)	53	0.28 (0.22, 0.35)	37	0.26 (0.20, 0.34)
	9 Years (predicted)		0.18 (0.10, 0.33)		0.22 (0.12, 0.40)		0.21 (0.12, 0.39)
Tetanus	1 Month	98	7.62 (6.76, 8.58)	98	10.36 (9.20, 11.68)	91	6.14 (5.43, 6.94)
	1 Year	67	2.85 (2.46, 3.30)	72	3.35 (2.90, 3.87)	72	1.71 (1.48, 1.97)
	3 Years	47	1.10 (0.93, 1.29)	58	1.29 (1.10, 1.51)	47	0.66 (0.56, 0.77)
	5 Years	48	0.69 (0.58, 0.83)	53	0.82 (0.68, 0.97)	37	0.42 (0.35, 0.50)
	9 Years (predicted)		0.28 (0.21, 0.37)		0.33 (0.24, 0.44)		0.17 (0.12, 0.22)
Type 1 polio	1 Month	99	5418.0 (4190.4, 7005.40)	98	711.41 (549.29, 921.38)	92	5091.0 (3903.4, 6639.95)
	1 Year	67	372.29 (281.82, 491.82)	72	105.62 (80.57, 138.45)	71	320.23 (243.37, 421.38)
	3 Years	47	206.57 (168.36, 253.45)	57	86.21 (70.66, 105.18)	47	216.58 (175.79, 266.82)
	5 Years	47	114.61 (88.76, 148.00)	52	70.37 (55.23, 89.65)	37	146.47 (110.97, 193.34)
	9 Years (predicted)		35.28 (20.59, 60.47)		46.88 (28.28, 77.71)		66.99 (37.39, 120.04)
Type 2 polio	1 Month	99	16542 (12564, 21780.9)	98	3408.4 (2584.4, 4495.07)	92	14879 (11213, 19742.5)
	1 Year	67	563.22 (438.74, 723.02)	72	140.07 (109.23, 179.63)	71	525.01 (408.20, 675.25)
	3 Years	47	340.61 (282.63, 410.48)	57	127.70 (106.56, 153.03)	47	343.24 (282.81, 416.58)

Antigen	Time since Booster vaccination	N	Td5ap-IPV GMT (95% CI)	N	Td5ap+ OPV GMT (95% CI)	N	DT2aP-IPV GMT (95% CI)
Type 3 polio	5 Years	47	205.98 (161.78, 262.26)	52	116.41 (92.69, 146.20)	37	224.40 (172.63, 291.70)
	9 Years (predicted)		75.33 (45.30, 125.27)		96.74 (59.57, 157.11)		95.91 (55.32, 166.30)
	1 Month	97	23454 (18643, 29505.9)	98	356.79 (283.71, 448.68)	91	26093 (20590, 33066.7)
	1 Year	67	992.87 (801.72, 1229.60)	72	94.77 (76.81, 116.91)	71	987.59 (798.35, 1221.68)
	3 Years	47	387.90 (319.80, 470.50)	57	37.02 (30.69, 44.66)	47	385.84 (316.98, 469.65)
FHA	5 Years	47	151.55 (122.34, 187.73)	52	14.46 (11.75, 17.81)	37	150.74 (120.79, 188.12)
	9 Years (predicted)		23.13 (16.50, 32.43)		2.21 (1.58, 3.08)		23.01 (16.25, 32.58)
	1 Month	100	125.43 (105.86, 148.62)	98	157.64 (132.94, 186.94)	92	188.50 (158.40, 224.32)
	1 Year	67	22.06 (18.39, 26.46)	73	27.72 (23.16, 33.19)	72	33.15 (27.65, 39.74)
	3 Years	46	22.98 (19.23, 27.45)	55	28.88 (24.23, 34.41)	47	34.53 (28.87, 41.30)
FIM (2+3)	5 Years	43	23.93 (19.17, 29.87)	51	30.08 (24.15, 37.45)	36	35.96 (28.74, 44.99)
	9 Years (predicted)		25.96 (17.83, 37.80)		32.63 (22.45, 47.41)		39.01 (26.72, 56.96)
	1 Month	100	955.61 (732.95, 1245.91)	98	875.07 (669.72, 1143.39)		
	1 Year	66	130.75 (100.10, 170.80)	72	119.73 (91.66, 156.41)		
	3 Years	47	86.64 (68.15, 110.14)	58	79.34 (62.58, 100.59)		
Pertactin	5 Years	44	57.41 (44.71, 73.72)	49	52.57 (41.15, 67.16)		
	9 Years (predicted)		25.21 (17.61, 36.08)		23.08 (16.24, 32.80)		
	1 Month	100	242.38 (197.20, 297.91)	98	315.47 (255.97, 388.81)		
	1 Year	67	36.14 (29.12, 44.84)	73	47.04 (37.87, 58.42)		
	3 Years	47	18.67 (14.84, 23.49)	58	25.88 (20.59, 32.54)		
	5 Years	44	15.20 (12.02, 19.22)	51	21.08 (16.67, 26.65)		
	9 Years (predicted)		10.09 (6.49, 15.68)		13.98 (8.99, 21.75)		

GMT: geometric mean titer, FIM (2+3): fimbriae types 2+3, FHA: filamentous haemagglutinin, Td5ap-IPV: Combined adsorbed tetanus, low dose diphtheria, 5 component acellular pertussis and inactivated poliomyelitis vaccine, Td5ap + OPV combined adsorbed tetanus, low dose diphtheria and 5 component acellular pertussis vaccine plus oral polio vaccine, DT2aP-IPV combined adsorbed standard dose diphtheria, tetanus, two component acellular pertussis and inactivated poliomyelitis vaccine.

Table 2. Percentage of children predicted to be above threshold at 9 years post-booster

Antigen	Threshold	Td5ap-IPV % (95% CI)	Td5ap + OPV % (95% CI)	DT2aP-IPV % (95% CI)
Diphtheria	% ≥ 0.1 IU/ml	73 (55, 90)	79 (63, 92)	81 (65, 91)
Tetanus	% ≥ 0.1 IU/ml	82 (74, 90)	86 (78, 92)	69 (58, 79)
Polio 1	% $\geq 1:8$	100 (98, 100)	100 (99, 100)	99 (98, 100)
Polio 2	% $\geq 1:8$	100 (98, 100)	100 (99, 100)	99 (98, 100)
Polio 3	% $\geq 1:8$	94 (84, 98)	2 (0, 7)	94 (85, 98)
PT	% ≥ 5 IU/mL	37 (18, 60)	32 (16, 53)	25 (9, 46)

Estimates and 95% confidence intervals from 1000 bootstrap samples. PT: pertussis toxoid, Td5ap-IPV: combined adsorbed tetanus, low dose diphtheria, 5 component acellular pertussis and inactivated poliomyelitis vaccine, Td5ap + OPV combined adsorbed tetanus, low dose diphtheria and 5 component acellular pertussis vaccine plus oral polio vaccine, DT2aP-IPV combined adsorbed standard dose diphtheria, tetanus, two component acellular pertussis and inactivated poliomyelitis vaccine.

Figure Legends

Figure 1. Serotype specific antibody concentrations, model based estimates and predicted 9 year antibody titers for diphtheria and tetanus. A: diphtheria, B: tetanus

Filled grey circles are antibody measurements. Open circles are model predicted values at 9 years.

Figure 2. Serotype specific antibody concentrations, model based estimates and predicted 9 year antibody titers for polio. A: type 1 polio, B: type 2 polio C: type 3 polio
 Filled grey circles are antibody measurements. Open circles are model predicted values at 9 years. Reference line indicates seroprotective titer 1:8

Figure 3. Serotype specific antibody concentrations, model based estimates and predicted 9 year antibody titers for pertussis antigens. A: pertussis toxoid, B: pertactin, C: fimbriae types 2+3 (FIM) D: filamentous haemagglutinin.

Filled grey circles are antibody measurements. Open circles are model predicted values at 9 years.

Reference lines on top left panel: 5 IU/mL (LLOQ) and 80 IU/mL (potential pertussis exposure). Pertussis toxoid modelled as a binary endpoint (<5 IU/mL) therefore no regression line is plotted.

Groups: — Td5aP-IPV; - - - Td5aP + OPV; - · - DT2aP-IPV

Figure 4. Antibody concentrations in individual children who had PT ≥ 80 IU/mL at year 3 or 5.

A: pertussis toxoid, B: filamentous haemagglutinin (FHA), C: fimbriae types 2+3 (FIM) D: pertactin. Each line represents an individual child's antibody concentration over time.

Solid lines: PT ≥ 80 IU/mL at year 3 or 5 with 4-fold rise in antibody from previous visit. Dotted lines: PT ≥ 80 IU/mL at year 3 or 5 without 4-fold rise in antibody from previous visit.

References

- [1] Siegrist C-A. Vaccine Immunology. In: Stanley Plotkin, Walter Orenstein, Offit P, editors. Vaccines. 5th ed: Elsevier Health Sciences; 2008.
- [2] Salisbury D, Ramsay M, Noakes K. Immunisation against infectious disease 2006-The Green Book. Updated. 2013;4:1-514.
- [3] World Health Organisation. Keeping Syrian children free from polio at home and across the border. <http://www.who.int/features/2015/polio-immunization-syria/en/>. 2015.
- [4] Public Health England. Laboratory confirmed cases of Pertussis infection by laboratory method, year and quarter, England: 2002-2013*. Enhanced Pertussis Surveillance. 2014.
- [5] Amirthalingam G, Gupta S, Campbell H. Pertussis immunisation and control in England and Wales, 1957 to 2012: a historical review. Euro Surveill Available online at <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20587>. 2012;18.
- [6] Collins C, Salt P, McCarthy N, Chantler T, Lane L, Hemme F, et al. Immunogenicity and safety of a low-dose diphtheria, tetanus and acellular pertussis combination vaccine with either inactivated or oral polio vaccine as a pre-school booster in UK children. Vaccine. 2004;22:4262-9.
- [7] John T, Voysey M, Yu L, McCarthy N, Baudin M, Richard P, et al. Immunogenicity of a low-dose diphtheria, tetanus and acellular pertussis combination vaccine with either inactivated or oral polio vaccine compared to standard-dose diphtheria, tetanus, acellular pertussis when used as a pre-school booster in UK children: A 5-year follow-up of a randomised controlled study. Vaccine. 2015;33:4579-85.
- [8] Brown H, Prescott R. Applied mixed models in medicine. 1999. New York: John Wiley & Sons.
- [9] Aase A, Herstad TK, Jørgensen SB, Leegaard TM, Berbers G, Steinbakk M, et al. Anti-pertussis antibody kinetics following DTaP-IPV booster vaccination in Norwegian children 7–8 years of age. Vaccine. 2014;32:5931-6.
- [10] Hallander HO, Andersson M, Gustafsson L, Ljungman M, Netterlid E. Seroprevalence of pertussis antitoxin (anti-PT) in Sweden before and 10 years after the introduction of a universal childhood pertussis vaccination program. APMIS. 2009;117:912-22.
- [11] Storsaeter J, Hallander HO, Gustafsson L, Olin P. Low levels of antipertussis antibodies plus lack of history of pertussis correlate with susceptibility after household exposure to Bordetella pertussis. Vaccine. 2003;21:3542-9.
- [12] Plotkin SA. Correlates of protection induced by vaccination. Clin Vaccin Immunol. 2010;17:1055-65.
- [13] Plotkin SA, Cadoz M. The acellular pertussis vaccine trials: an interpretation. Ped Infect Dis J. 1997;16:508-17.
- [14] Meriste S, Lutsar I, Tamm E, Willems P. Safety and immunogenicity of a primary course and booster dose of a combined diphtheria, tetanus, acellular pertussis, hepatitis B and inactivated poliovirus vaccine. Scand J Inf Dis. 2006;38:350-6.
- [15] Vidor E. The nature and consequences of intra-and inter-vaccine interference. J Comp Path. 2007;137:S62-S6.
- [16] Lanzavecchia A, Bernasconi N, Traggiai E, Ruprecht CR, Corti D, Sallusto F. Understanding and making use of human memory B cells. Immunol Rev. 2006;211:303-9.

- [17] Roebuck M, Chamberlain R. Prevalence of antibodies to poliovirus in 1978 among subjects aged 0-88 years. *BMJ*. 1982;284:697-700.
- [18] Cherry JD, Gornbein J, Heininger U, Stehr K. A search for serologic correlates of immunity to *Bordetella pertussis* cough illnesses. *Vaccine*. 1998;16:1901-6.
- [19] Deen JL, Mink CM, Cherry JD, Christenson PD, Pineda EF, Lewis K, et al. Household contact study of *Bordetella pertussis* infections. *Clin Inf Dis*. 1995;21:1211-9.
- [20] Mallet E, Matisse N, Mathieu N, Langue J, Boissard F, Soubeyrand Bt, et al. Antibody persistence against diphtheria, tetanus, pertussis, poliomyelitis and *Haemophilus influenzae* type b (Hib) in 5-6-year-old children after primary vaccination and first booster with a pentavalent combined acellular pertussis vaccine: immunogenicity and tolerance of a tetravalent combined acellular pertussis vaccine given as a second booster. *Vaccine*. 2004;22:1415-22.
- [21] Warfel JM, Zimmerman LI, Merkel TJ. Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. *Proceedings of the National Academy of Sciences*. 2014;111:787-92.
- [22] Strategic Advisory Group of Experts (SAGE) pertussis working group. WHO SAGE pertussis working group - background paper. <http://www.who.int/immunization/sage/meetings/2014/april/en/>. 2014.
- [23] Prince HE, Lieberman JM, Cherry JD. Age-related differences in patterns of increased *Bordetella pertussis* antibodies. *Clin Vaccin Immunol*. 2012;19:545-50.
- [24] Carpenter JR, Kenward MG. Missing data in randomised controlled trials — a practical guide. http://missingdata.lshtm.ac.uk/downloads/rm04_jh17_mk.pdf2007.
- [25] White IR, Horton NJ, Carpenter J, Pocock SJ. Strategy for intention to treat analysis in randomised trials with missing outcome data. *BMJ*. 2011;342.
- [26] Joint Committee on Vaccination and Immunisation. Minutes of the meeting on 4 June 2014. <https://www.gov.uk/government/groups/joint-committee-on-vaccination-and-immunisation>. 2014.
- [27] Wang K, Fry NK, Campbell H, Amirthalingam G, Harrison TG, Mant D, et al. Whooping cough in school age children presenting with persistent cough in UK primary care after introduction of the preschool pertussis booster vaccination: prospective cohort study. *BMJ*. 2014;348.

Supplementary Material

Statistical Methods

Data from all randomised participants were included in the analysis regardless of the number of visits attended. Mixed effects models for repeated measures ¹ were fitted to antigen specific log₁₀-transformed antibody titers regressed on time and adjusted for baseline values. Random intercepts and slopes were specified for each participant. A treatment group-time interaction variable was added to assess differential rates of antibody decline. Indicator variables for time at one month, and then for time at one year (fixed effects terms), were added to assess the linearity of response. The one year variable was retained in the model if statistically significant ($p < 0.05$). The one month variable was automatically retained because of strong prior belief of a different rate of decline over the first year. Models were compared for parsimony using AIC/AICc statistics ². For antigens with a defined correlate of protection, estimates of antibody levels were calculated for each participant from each model at 9 years post-vaccination as a linear combination of their individual random intercepts, random slope estimates along with the model fixed effect estimates. The proportion of participants above thresholds was determined from predicted antibody levels and the process repeated 1000 times using bootstrap resampling. Confidence intervals for proportions protected are the 2.5th to 97.5th percentile bootstrap proportions³.

Anti-PT antibodies were below the level of detection for many children by 5 years post-booster, resulting in a distribution which did not meet the assumptions of the model. These antibodies were instead modelled using a generalised linear mixed effects model for binary data (titer ≥ 5 IU/mL).

Formal imputation of missing data was not conducted as the mixed effects modelling framework inherently imputes missing data under a missing-at-random assumption ^{4,5}.

All analyses were conducted in SAS version 9.3.

References

1. Brown H, Prescott R. Applied mixed models in medicine. 1999. New York: John Wiley & Sons.
2. Burnham KP, Anderson DR. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach (2nd ed.): Springer-Verlag; 2002.
3. A.C. Davison, Hinkley DV. Bootstrap methods and their application. Cambridge: Cambridge University Press; 1997.
4. Carpenter JR, Kenward MG. Missing data in randomised controlled trials — a practical guide. http://missingdata.lshtm.ac.uk/downloads/rm04_jh17_mk.pdf; 2007.

5. White IR, Horton NJ, Carpenter J, Pocock SJ. Strategy for intention to treat analysis in randomised trials with missing outcome data; 2011.

Supplementary Table 1. Baseline characteristics of participants who participated in long term follow up compared with those who did not attend

	3 year follow up		5 year follow up	
	Attended	Did not attend	Attended	Did not attend
Sex (male)	90 (58%)	76 (52%)	80 (57%)	86 (53%)
Age at enrolment, years [min, max]	3.96 [3.5, 4.9]	3.92 [3.5, 5.0]	3.9 [3.5, 4.9]	3.9 [3.5, 5]
Antibody at baseline (GMC, 95%CI)				
Diphtheria	0.7 (0.5, 1.0)	1.2 (0.8, 1.7)	0.8 (0.5, 1.2)	1.0 (0.7, 1.4)
Tetanus	0.2 (0.2, 0.2)	0.2 (0.2, 0.3)	0.2 (0.2, 0.2)	0.2 (0.2, 0.3)
Pertussis	5.7 (4.6, 7.0)	4.8 (4.0, 5.7)	5.4 (4.4, 6.7)	5.1 (4.3, 6)
FHA	6.2 (4.8, 8)	5.0 (3.9, 6.6)	5.8 (4.5, 7.6)	5.4 (4.2, 7)
FIM	20 (16, 25.2)	18.9 (15, 23.8)	20 (15.8, 25.2)	19 (15.2, 23.8)
Pertactin	6.0 (4.7, 7.8)	5.1 (3.9, 6.8)	5.8 (4.5, 7.5)	5.4 (4.1, 7.2)
Polio 1	70.3 (56.1, 88.2)	84 (67.2, 105)	86.4 (68.8, 108.6)	69 (55.3, 86)
Polio 2	279.9 (228.3, 343.3)	279 (223.3, 348.5)	275.9 (220.1, 345.9)	282.6 (231, 345.7)
Polio 3	60.9 (47.9, 77.5)	63.1 (49.9, 79.7)	70 (54.8, 89.3)	55.7 (44.3, 70.1)

FIM: fimbriae types 2+3, FHA: filamentous haemagglutinin

Supplementary Table 2. Antigen concentrations of Td5ap-IPV, Td5ap+OPV and DT2aP-IPV

	<i>Td5ap-IPV</i>	<i>Td5ap + OPV</i>		<i>DT2aP-IPV</i>
		Td5ap	OPV	
Purified tetanus toxoid	≥20 IU	≥20 IU	-	≥40 IU
Purified diphtheria toxoid	≥2 IU	≥2 IU	-	≥30 IU
Purified pertussis toxoid (PT)	2.5 µg	2.5 µg	-	25 µg
Purified filamentous haemagglutinin (FHA)	5 µg	5 µg	-	25 µg
Purified fimbriae types 2+3 (FIM)	5 µg	5 µg	-	-
Purified pertactin (PRN)	3 µg	3 µg	-	-
Inactivated poliomyelitis virus type 1	40D antigen units	-	-	40D antigen units
Inactivated poliomyelitis virus type 2	8D antigen units	-	-	8D antigen units
Inactivated poliomyelitis virus type 3	32D antigen units	-	-	32D antigen units
Live attenuated type 1 poliovirus	-	-	≥10 ⁶ TCID50	-
Live attenuated type 2 poliovirus	-	-	≥10 ⁵ TCID50	-
Live attenuated type 3 poliovirus	-	-	≥10 ^{5.5} TCID50	-

Td5ap-IPV: Combined adsorbed tetanus, low dose diphtheria, 5 component acellular pertussis and inactivated poliomyelitis vaccine, Td5ap + OPV combined adsorbed tetanus, low dose diphtheria and 5 component acellular pertussis vaccine plus oral polio vaccine, DT2aP-IPV combined adsorbed standard dose diphtheria, tetanus, two component acellular pertussis and inactivated poliomyelitis vaccine.

Supplementary Table 3: Model estimates (standard error) for variables retained in each linear mixed effects model

	<i>Tetanus</i>	<i>Diphtheria</i>	<i>Serotype 1 Polio</i>	<i>Serotype 2 Polio</i>	<i>Serotype 3 Polio</i>	<i>FHA</i>	<i>FIM (2+3)</i>	<i>Pertactin</i>
Year	-0.09944 (0.0097)	-0.02331 (0.02434)	-0.0849 (0.0199)	0.2960 (0.0410)	0.4014 (0.0363)	0.00884 (0.0107)	-0.0894 (0.0107)	-0.0446 (0.0165)
log10 baseline titer	0.3959 (0.0480)	0.353 (0.02517)	0.2123 (0.0406)	-0.0923 (0.0186)	-0.2041 (0.0102)	0.2829 (0.0291)	0.5358 (0.0730)	0.4456 (0.0536)
Indicator 1 month	0.68 (0.0432)	2.148 (0.1103)	1.1235 (0.0717)	1.3678 (0.0422)	1.2348 (0.0535)	0.7629 (0.0344)	0.7819 (0.0377)	0.9834 (0.0685)
Age (Years)		-0.2821 (0.07967)						
Indicator 1 year	0.2155 (0.0304)	0.549 (0.07828)						0.1977 (0.0536)
Td5ap-IPV	0.5865 (0.0574)	0.6012 (0.3365)	2.2936 (0.1085)	2.1425 (0.1202)	2.4748 (0.0834)	1.1283 (0.0494)	1.5152 (0.1128)	1.0856 (0.0894)
Td5ap+OPV	0.6678 (0.0571)	0.7028 (0.3335)	1.6761 (0.105)	1.4219 (0.1238)	1.4739 (0.0823)	1.2089 (0.0501)	1.4756 (0.115)	1.2000 (0.0909)
DT2aP-IPV	0.3756 (0.0581)	0.6558 (0.3335)	2.1870 (0.1069)	2.1021 (0.1206)	2.4737 (0.0828)	1.3084 (0.0487)		
Year*Group			-0.0430 (0.0275)	-0.0169 (0.0230)	-0.04862 (0.0674)			
<i>Td5ap-IPV</i>								
<i>Td5ap+OPV</i>			0.0408 (0.0268)	0.07219 (0.0227)	-0.8462 (0.0663)			
<i>DT2aP-IPV</i>			ref	ref	ref			
Indicator 1 month*Group	-0.1285 (0.0325)	-0.2546 (0.08016)	-0.0778 (0.1016)					
<i>Td5ap-IPV</i>								
<i>Td5ap+OPV</i>	-0.06477 (0.0320)	-0.1983 (0.07913)	-0.3355 (0.0997)					
<i>DT2aP-IPV</i>	ref	ref	ref					

FIM (2+3): fimbriae types 2+3, FHA: filamentous haemagglutinin, Td5ap-IPV: Combined adsorbed tetanus, low dose diphtheria, 5 component acellular pertussis and inactivated poliomyelitis vaccine, Td5ap + OPV combined adsorbed tetanus, low dose diphtheria and 5 component acellular pertussis vaccine plus oral polio vaccine, DT2aP-IPV combined adsorbed standard dose diphtheria, tetanus, two component acellular pertussis and inactivated poliomyelitis vaccine.

Supplementary Table 4: Summary of all antibody data

<i>Antigen</i>	<i>Year</i>	<i>Td5ap-IPV</i>	<i>Td5ap+OPV</i>	<i>DT2aP-IPV</i>
		<i>N</i>	<i>N</i>	<i>N</i>
		<i>GMT (95% CI)</i>	<i>GMT (95% CI)</i>	<i>GMT (95% CI)</i>
Diphtheria	Pre-boost	100	100	99
		0.89 (0.58, 1.37)	0.82 (0.49, 1.36)	0.95 (0.63, 1.43)
	1 month	98	100	90
		22.39 (16.04, 31.25)	33.64 (23.95, 47.26)	48.06 (33.36, 69.24)
	1	67	74	72
		0.91 (0.67, 1.24)	1.23 (0.95, 1.59)	1.34 (0.98, 1.84)
	3	47	60	47
		0.21 (0.15, 0.31)	0.29 (0.21, 0.40)	0.24 (0.15, 0.37)
	5	48	54	38
Tetanus		0.23 (0.14, 0.35)	0.23 (0.16, 0.33)	0.34 (0.22, 0.52)
	Pre-boost	100	100	100
		0.24 (0.20, 0.27)	0.22 (0.19, 0.26)	0.22 (0.20, 0.25)
	1 month	98	100	92
		7.62 (6.82, 8.53)	10.55 (9.12, 12.20)	6.02 (5.21, 6.96)
	1	67	74	73
		2.73 (2.27, 3.29)	2.99 (2.47, 3.61)	1.78 (1.42, 2.24)
	3	47	60	48
		1.00 (0.79, 1.26)	1.25 (0.99, 1.59)	0.64 (0.47, 0.87)
Type 1 polio		0.74 (0.57, 0.97)	0.83 (0.62, 1.11)	0.44 (0.31, 0.62)
	Pre-boost	100	100	100
		81.00 (60.81, 107.89)	69.97 (53.40, 91.67)	79.45 (60.33, 104.64)
	1 month	99	100	93
		6008.6 (4692.2, 7694.41)	707.89 (490.18, 1022.31)	5116.9 (3984.6, 6570.79)
	1	67	74	72
		318.06 (260.14, 388.87)	119.89 (97.75, 147.05)	273.14 (218.34, 341.69)
	3	47	59	48
		257.88 (189.55, 350.85)	107.32 (81.29, 141.68)	322.54 (244.07, 426.23)
Type 2 polio		47	53	38
		101.85 (73.86, 140.43)	64.01 (46.87, 87.42)	124.55 (90.01, 172.34)
	Pre-boost	100	100	100
		265.21 (201.56, 348.96)	327.65 (256.02, 419.32)	251.20 (193.29, 326.47)
	1 month	99	100	93
		10284 (8529.6, 12398.1)	2716.0 (1997.5, 3693.04)	8849.1 (7331.6, 10680.6)
	1	67	74	72
		466.23 (378.49, 574.32)	178.09 (146.21, 216.91)	469.04 (374.06, 588.13)
	3	47	59	48
Type 3 polio		331.38 (256.56, 428.01)	154.47 (117.72, 202.68)	383.54 (281.24, 523.07)
		47	53	38
		202.19 (164.58, 248.39)	106.58 (84.37, 134.64)	211.37 (144.17, 309.90)
	Pre-boost	100	100	100
		64.45 (47.59, 87.27)	57.68 (43.62, 76.28)	64.00 (47.67, 85.92)

FHA	1 month	97 12137 (10205, 14434.0)	100 349.71 (262.84, 465.28)	92 12306 (10772, 14058.6)
	1	67 937.80 (724.24, 1214.32)	74 97.55 (74.72, 127.35)	72 890.58 (707.44, 1121.13)
	3	47 348.91 (218.42, 557.36)	59 36.62 (28.40, 47.23)	48 418.33 (287.80, 608.07)
	5	47 147.26 (101.81, 213.00)	53 16.75 (13.07, 21.47)	38 145.43 (101.79, 207.76)
	Pre-boost	100 5.37 (3.92, 7.36)	100 6.25 (4.45, 8.78)	100 5.23 (3.79, 7.22)
	1 month	100 122.98 (98.23, 153.96)	100 160.90 (130.11, 198.98)	93 187.14 (155.32, 225.48)
	1	67 25.66 (19.80, 33.26)	75 27.24 (21.61, 34.33)	73 30.14 (24.23, 37.50)
	3	46 18.35 (12.62, 26.69)	57 31.08 (21.69, 44.54)	48 39.54 (26.63, 58.71)
	5	46 26.86 (20.64, 34.95)	54 38.77 (28.53, 52.71)	38 41.29 (29.71, 57.38)
	3*	46 18.35 (12.62, 26.69)	55 28.09 (19.94, 39.57)	47 38.60 (25.85, 57.63)
Fimbriae types 2+3	5*	43 25.71 (19.49, 33.92)	51 34.71 (25.98, 46.37)	36 39.82 (28.24, 56.16)
	Pre-boost	100 19.45 (15.59, 24.27)	100 19.56 (15.43, 24.80)	
	1 month	100 826.38 (612.59, 1114.80)	100 996.52 (732.00, 1356.62)	
	1	66 118.72 (76.72, 183.71)	74 122.23 (82.61, 180.84)	
	3	47 62.59 (45.18, 86.70)	60 87.14 (63.34, 119.89)	
	5	47 63.62 (42.93, 94.28)	52 74.32 (52.39, 105.42)	
	3*	47 62.59 (45.18, 86.70)	58 83.42 (60.36, 115.28)	
	5*	44 65.32 (43.09, 99.02)	49 65.74 (48.04, 89.96)	
	Pre-boost	100 5.21 (3.98, 6.80)	100 6.00 (4.57, 7.87)	
	1 month	100 239.10 (190.21, 300.56)	100 338.03 (269.74, 423.61)	
Pertactin	1	67 35.60 (24.70, 51.31)	75 49.02 (34.54, 69.57)	
	3	47 18.55 (13.12, 26.24)	60 28.24 (20.60, 38.70)	
	5	47 18.13 (12.95, 25.39)	54 26.82 (18.14, 39.67)	

Pertussis toxoid	3*	47	58	
		18.55 (13.12, 26.24)	27.47 (19.97, 37.80)	
	5*	44	51	
		17.18 (12.07, 24.47)	23.52 (16.37, 33.80)	
	Pre-boost	100	100	100
		5.58 (4.38, 7.11)	6.03 (4.56, 7.96)	4.27 (3.57, 5.11)
	1 month	100	100	93
		158.69 (126.78, 198.64)	156.04 (117.86, 206.60)	97.10 (76.97, 122.48)
	1	65	74	72
		26.85 (19.45, 37.05)	27.80 (20.45, 37.79)	13.18 (9.87, 17.60)
	3	47	58	47
		6.05 (4.35, 8.41)	8.25 (5.33, 12.79)	7.09 (4.87, 10.31)
	5	38	41	33
		7.98 (4.88, 13.05)	8.06 (4.76, 13.66)	4.98 (3.23, 7.67)
	3*	47	56	46
		6.05 (4.35, 8.41)	6.87 (4.76, 9.92)	6.66 (4.64, 9.56)
	5*	35	38	31
		6.16 (4.02, 9.43)	6.06 (3.87, 9.49)	4.06 (2.89, 5.72)

3* 5*: 3 year and 5 year data after removal of those with pertussis infection

GMT: geometric mean titer, FHA: filamentous haemagglutinin, Td5ap-IPV: Combined adsorbed tetanus, low dose diphtheria, 5 component acellular pertussis and inactivated poliomyelitis vaccine, Td5ap + OPV combined adsorbed tetanus, low dose diphtheria and 5 component acellular pertussis vaccine plus oral polio vaccine, DT2aP-IPV combined adsorbed standard dose diphtheria, tetanus, two component acellular pertussis and inactivated poliomyelitis vaccine.