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Antibody persistence after vaccination of adolescents with monovalent and combined acellular pertussis vaccines containing genetically inactivated pertussis toxin: a phase 2/3 randomised, controlled, non-inferiority trial

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Summary

Background The immunogenicity of acellular pertussis vaccines and persistence of immunity after vaccination might be improved by using genetically inactivated pertussis toxin (PTgen) instead of chemically inactivated pertussis toxin (PTchem) because of the preservation of conformational epitopes. We assessed the safety and immunogenicity of two vaccines containing PTgen 1 year after vaccination.

Methods We did a phase 2/3 non-inferiority, randomised, controlled trial involving 450 adolescents (age 12–17 years) enrolled between July 6, 2015, and Aug 20, 2015. Participants were randomised 1:1:1 to receive one dose of vaccine containing PTgen and filamentous haemagglutinin (FHA) either in a monovalent formulation ($aP_{[PTgen/FHA]}$) or in a combined formulation with tetanus and reduced-dose diphtheria toxoids ($TdaP_{[PTgen/FHA]}$) or to receive a commercial vaccine containing reduced-dose PTchem (Tdap) as a comparator. We report a secondary trial outcome, namely antibody persistence 1 year after vaccination, assessed per protocol in 150 randomly preselected participants (50 per group). Seroconversion was defined as antibody titres at least four times greater than at baseline. Safety was assessed in all trial participants. This study is registered in the Thai Clinical Trial Registry, number TCTR20150703002.

Findings Between June 5, 2016, and Aug 9, 2016, 442 (98%) of 450 enrolled participants attended a 1-year follow-up visit. After 1 year, persistent seroconversion for pertussis toxin neutralising antibodies was seen in 38 (76%, 95% CI 64–88) participants in the $aP_{(PTgen/FHA)}$ group and 41 (81%, 70–92) in the $TdaP_{(PTgen/FHA)}$ group, but in only four (8%, 1–16) in the Tdap comparator group. Seroconversion rates for IgG antibodies against pertussis toxin and FHA were also greater in the $aP_{(PTgen/FHA)}$ group (82%, 95% CI 71–93 and 64%, 51–77, respectively) and $TdaP_{(PTgen/FHA)}$ group (75%, 63–87 and 56%, 42–70, respectively) than in the Tdap group (4%, 0–9, p<0·0001, and 28%, 16–41, p=0·0007, respectively). 13 serious adverse events were reported in 12 participants and all were judged to be unrelated to the study vaccines. Five pregnancies were reported during follow-up, none of which had any maternal or neonatal complications.

Interpretation A monovalent and a combined recombinant acellular pertussis vaccine containing PTgen induced antibody responses that were greater and sustained for longer than those achieved with the Tdap comparator vaccine. New recombinant pertussis vaccines containing PTgen might offer new opportunities to limit pertussis resurgence and can be widely used, including in pregnant women.

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Introduction

Antibody responses and the duration of protection induced by acellular pertussis vaccines wane faster than those induced by whole-cell pertussis vaccines.[1–6] Exclusive use of these vaccines has contributed to a resurgence of pertussis disease and increased burden of disease in many countries, including in adolescents.[5,6] Reintroduction of whole-cell pertussis vaccine in these countries is unlikely for various reasons, including more systemic side-effects than acellular pertussis vaccines. Thus, acellular pertussis vaccines that can provide longer-lasting immune protection in children and adults are needed.[7]

Pertussis toxin is a major virulence factor of *Bordetella pertussis*, and antibody-mediated neutralisation of pertussis toxin is essential and sufficient to control pertussis disease.[8] Inactivated forms of pertussis toxin are important vaccine antigens and are the only *B pertussis* component that have been included in all acellular pertussis vaccines so far. Most vaccines use

Research in context

Evidence before this study

We used the term "pertussis, vaccine, immunogenicity AND (recombinant OR genetically detoxified OR genetically inactivated)" to search PubMed for human trials of acellular pertussis vaccines containing genetically inactivated pertussis toxin and reporting on persistence of immune responses that were published before Jan 1, 2018, and available online. We also used the same term and "acellular pertussis vaccine" to search ClinicalTrials.gov and the Thai Clinical Trial Registry for relevant clinical trials registered before Dec 1, 2017. Finally, we searched the reference lists of retrieved articles. Studies reporting on immunogenicity only up to 1 month after vaccination were excluded. We found four published studies that had used vaccines containing genetically inactivated pertussis toxin and no registered ongoing studies. Three studies were done in the 1990s. In the first study, 12 infants with perinatal HIV infections were vaccinated and antibody responses were measured 4 months after vaccination. The second study reported antibody responses in eight adults 6 months, 1 year, and 2 years after vaccination. The third study described antibody responses in 403 infants 15 months after completing primary immunisation with three doses of a genetically inactivated pertussis vaccine. A later phase 1 trial evaluated antibody responses in 420 adults 6 months, 1 year, and 3 years after vaccination.

Added value of this study

We present novel data on the persistence of immunogenicity in adolescents 1 year after vaccination with two acellular pertussis vaccines containing new genetically inactivated pertussis toxin (PTgen)—a monovalent vaccine and a combined vaccine also containing tetanus and reduced-dose diphtheria toxoids. Compared with a licensed combined vaccine containing tetanus and reduced-dose diphtheria toxoids and reduced-dose chemically inactivated pertussis toxin (PTchem), the two vaccines containing PTgen induced sustained higher antibody responses.

Implications of all the available evidence

Most of the acellular pertussis vaccines used for the past 20 years contain PTchem and induce short-term immune responses that decline within a few years of immunisation. In our phase 2/3 trial involving 450 adolescents, at 28 days after vaccination the two acellular pertussis vaccines containing PTgen showed non-inferior immunogenicity to a licensed combined vaccine containing acellular PTchem, and all had similar safety profiles. These findings led to the recombinant pertussis vaccines being licensed in Thailand for active immunisation in adults and children from age 11 years. At 1 year after vaccination, we saw persistent antibody responses, suggesting that the vaccines containing PTgen induce longer-lasting immunity than the comparator. Using recombinant DNA technologies to inactivate pertussis toxin improves the immunogenicity and persistence of acellular pertussis vaccines.

chemically inactivated pertussis toxin (PTchem), but the inactivation process can destroy up to 80% of epitopes, resulting in poor antibody binding and response to native pertussis toxin.[9,10] By contrast, genetic inactivation with recombinant DNA technologies maintains the toxin's native epitopic structure and immunogenic properties.[10] A paediatric acellular pertussis vaccine containing genetically inactivated pertussis toxin that was used in the early 1990s in several countries was more immunogenic than one containing PTchem.[11] However, the method of inactivation was not greatly considered until a resurgence of pertussis incidence was seen later and the limited duration of immune protection induced by vaccines using PTchem was recognised, leading to renewed interest in genetically inactivated pertussis vaccines.[7,12]

In a phase 2/3 trial we assessed the immunogenicity of two recombinant acellular pertussis vaccines containing genetically inactivated pertussis toxin (PTgen) and filamentous haemagglutinin (FHA) compared with a vaccine containing PTchem combined with tetanus and reduced-dose diphtheria toxoids (Tdap) in 450 adolescents.[13] One of the vaccines containing PTgen was a monovalent vaccine ($aP_{[PTgen/FHA]}$) and the other was the same vaccine in combination with tetanus and reduced-dose diphtheria toxoids ($TdaP_{[PTgen/FHA]}$). 28 days after vaccination, seroconversion rates for pertussis toxin neutralising antibodies and IgG antibodies against pertussis toxin and FHA were significantly higher in adolescents who received one dose of either of the two PTgen-containing vaccines than in those who received the comparator Tdap vaccine. The findings supported the non-inferior immunogenicity claim for these new-generation recombinant acellular pertussis vaccines.[13] These vaccines have since been licensed in Thailand for use in children older than 11 years and in adults.[13]

In a phase 1 study done in adults with another investigational recombinant acellular pertussis vaccine, genetically inactivated pertussis toxin was associated with vaccine antibody responses that were greater and sustained for longer by several years than those generated by a comparator vaccine containin PTchem.[14]

In this report we present our investigation of the persistence of antibody responses 1 year after vaccination of adolescents who received $aP_{(PTgen/FHA)}$ or $TdaP_{(PTgen/FHA)}$ in the phase 2/3 trial versus those in participants who received the Tdap comparator vaccine.[13] We also present safety data for this period.

Methods

Study design and participants

We did a phase 2/3, double-centre, single-blind, randomised, controlled non-inferiority vaccine trial in Thailand, involving 450 healthy adolescents. A detailed

description of the inclusion and exclusion criteria has been reported previously.[13] Briefly, study participants were recruited at two sites of Mahidol University, Bangkok, Thailand. Eligible participants were aged 12–17 years, had no obvious health problems, and had not received a diphtheria, tetanus, or pertussis vaccination within 1 year before recruitment. Girls who had menarche were tested to exclude pregnancy before study enrolment. No information was available on individual vaccination history and, therefore, in line with the Thai National Immunisation Program, we assumed that study participants had received the complete primary series of diphtheria, tetanus, and whole-cell pertussis vaccines in childhood (coverage for three doses by age 12 months has been 99% since 1996) and are likely to have received additional doses at age 18 months and 6 years.[15–17]

The trial was done according to International Conference on Harmonisation and Good Clinical Practice guidelines, the Declaration of Helsinki, and local ethics guidelines. Ethics approval was obtained from the Siriraj Hospital Institutional Review Board and the ethics committee of the Faculty of Tropical Medicine, Mahidol University. In line with Thai regulations, written informed assent and consent were obtained from, respectively, participants and their parents or legal guardians.

Randomisation and masking

The original cohort of 450 participants were randomly assigned 1:1:1 to receive one dose of $aP_{(PTgen/FHA)}$, $TdaP_{(PTgen/FHA)}$, or the Tdap comparator vaccine.[13] A randomisation list was generated by the Centre of Excellence for Biomedical and Public Health Informatics (Bangkok, Thailand) with the PROC PLAN procedure in SAS (version 9.4), applying random block sizes of three. The allocations were provided to investigators in sealed envelopes. At each site a pharmacist and a nurse who gave the vaccinations and were not masked to treatment allocation were responsible for vaccine preparation, administration, and adhering to the randomisation schedule. All other staff at the study sites, those involved in data management, statistics, and laboratory testing, and the study participants were unaware of vaccine group allocation for the duration of the 1-year follow-up period. 150 participants (50 per vaccine group made up of 25 per study site) in whom titres of pertussis toxin neutralising antibodies had been measured 28 days after vaccination[13] and who were randomly selected before the start of the study according to a list generated by the Centre of Excellence for Biomedical and Public Health Informatics were included in a 1-year immunogenicity analysis.

Study vaccines

The $aP_{(PTgen/FHA)}$ (Pertagen, BioNet-Asia, Ayutthaya, Thailand; batch number PE25002-2), and $TdaP_{(PTgen/FHA)}$ (Boostagen, BioNet-Asia; batch number TD25002-1) vaccines were produced with a recombinant *B pertussis* strain that was genetically inactivated by the introduction of mutations (Arg9Lys and Glu129Gly) in the *ptx* operon of the *S1* gene.[18,19] Each 0·5 mL dose of $aP_{(PTgen/FHA)}$ and $TdaP_{(PTgen/FHA)}$ contained 5 µg PTgen, 5 µg FHA, and 0·3 mg as aluminium cation (Al^{3+}); the $TdaP_{(PTgen/FHA)}$ dose additionally contained at least 7·5 Lf tetanus toxoid and at least 2·0 Lf diphtheria toxoid. Each 0·5 mL dose of the Tdap comparator vaccine (Adacel, Sanofi-Pasteur, North York, ON, Canada; lot number U4971AA) contained 2·5 µg PTchem, 5 µg FHA, 3 µg pertactin, 5 µg fimbriae types 2 and 3, 5·0 Lf tetanus toxoid, 2·0 Lf diphtheria toxoid, and 0·33 mg as Al^{3+}. All study vaccines were presented in single-dose prefilled syringes. Each participant received one intramuscular injection in the non-dominant deltoid region.

Safety assessment

Methods and results for safety up to 28 days after vaccination have been reported previously.[13] Information on serious adverse events, as defined per protocol, were collected between 28 days and 1 year after vaccination for all study participants, through telephone calls at 3–4 months and 7–8 months, and during individual visits a day 336 (or within 28 days before or after).

Immunogenicity assessment

To assess immunogenicity 1 year after vaccination, we took 5 mL venous blood samples from the 150 preselected participants. Pertussis toxin neutralising antibody titres were measured with a validated Chinese hamster ovary cell pertussis toxin neutralisation assay.[19,20] IgG antibodies against pertussis toxin and FHA were measured in serum with validated indirect ELISAs.[13] Samples with titres less than 5 IU/mL were arbitrarily attributed a titre of 5 IU/mL.

Titres of IgG specific to tetanus and diphtheria toxoids were measured in serum with commercially available ELISA kits (Serion ESR108G and ESR130G, respectively, Virion\Serion, Würzburg, Germany). Titres below the 0·1 IU/mL cutoff in this assay were attributed the cutoff value. We deemed titres greater than 0·1 IU/mL to be protective.

Seroconversion at 1 year for pertussis toxin neutralising antibodies and IgG against pertussis toxin or FHA was defined as titres at least four times more than those at baseline.[21] For IgG antibodies against tetanus and diphtheria toxoids, seroconversion at 1 year was classified as titres greater than 0·1 IU/mL.

Statistical analysis

Data management and statistical analyses were done by the Center of Excellence for Biomedical and Public Health Informatics, Bangkok, Thailand, with SAS version 9.4. Results were analysed per protocol. Proportions of participants with seroconversion and geometric mean titres of antibodies were calculated with exact 95% CIs. For categorical variables, we used the χ^2 or

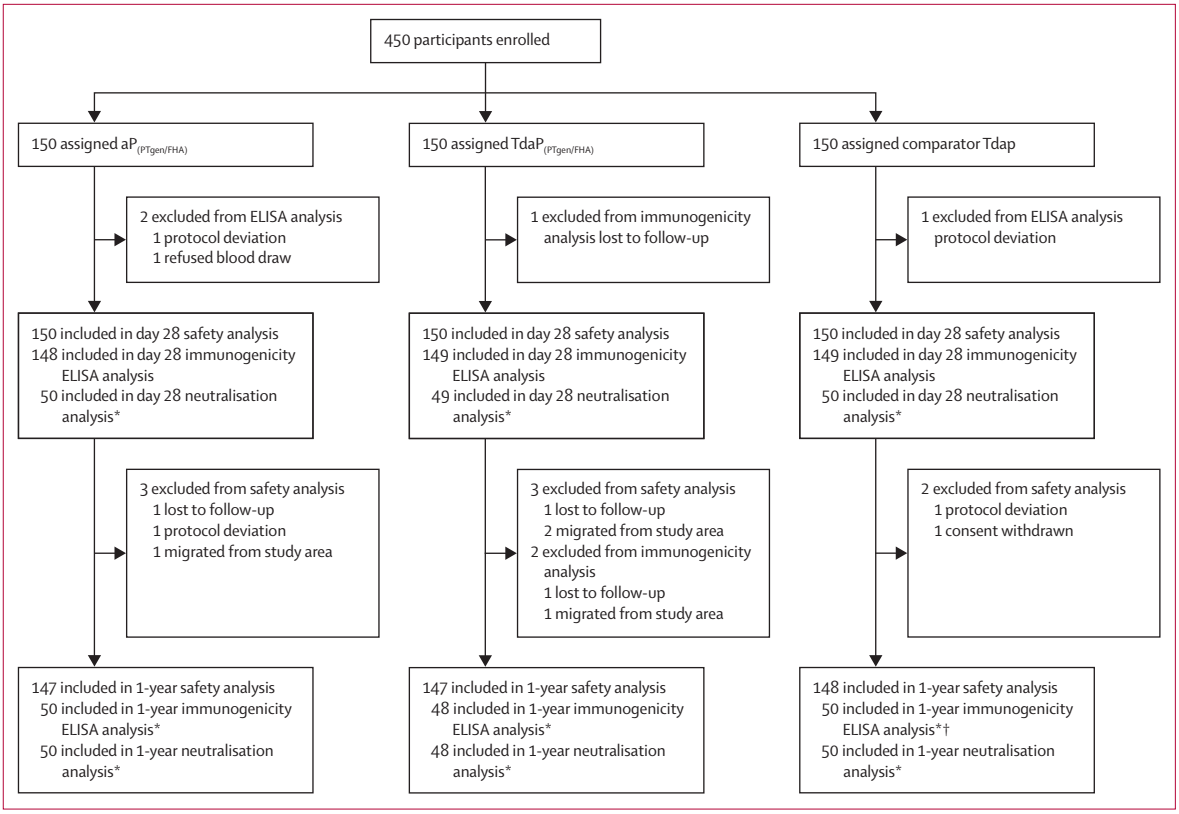

Figure 1: **Trial profile**

$aP_{(PTgen/FHA)}$=monovalent vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin. $TdaP_{(PTgen/FHA)}$=vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin combined with tetanus and reduced-dose diphtheria toxoids. Tdap=combined vaccine containing tetanus and reduced-dose diphtheria toxoids, and reduced-dose chemically inactivated acellular pertussis. *Randomly preselected subgroup. †One participant had pertussis toxin neutralising antibodies and IgG antibodies against pertussis toxin and FHA assessed at 1 year, but was excluded from analysis of antibody response to tetanus and diphtheria toxoids because of receiving a tetanus and diphtheria vaccination before blood draw.

Fisher's exact test to assess differences between vaccine groups, as appropriate. ANOVA was used to test for differences in normally distributed continuous variables. For differences in continuous variables that did not follow a normal distribution, we used the Kruskal-Wallis test or Mann-Whitney *U* test to compare three or two groups, respectively. A paired *t* test was used to compare geometric mean titres between baseline and postvaccination data. We plotted reverse cumulative distribution curves to describe the distribution of IgG antibody titres against pertussis toxin and FHA in the study population.

We took p values of 0·05 or less to be significant. This study is registered in the Thai Clinical Trial Registry, number TCTR20150703002.

Role of funding source

The funder of the study had a role in study design, data collection, data analysis, data interpretation, and the writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between June 5, 2016, and Aug 9, 2016, 442 (98%) of 450 study participants enrolled in the study attended the 1-year safety visit after vaccination (figure 1). Characteristics of the participants at baseline were similar across groups (table 1). The eight participants not followed up at 1 year included three participants vaccinated with $TdaP_{(PTgen/FHA)}$, three vaccinated with $aP_{(PTgen/FHA)}$, and two vaccinated with the Tdap comparator vaccine (figure 1). Of the 150 participants preselected for the immunogenicity assessment at 1 year, data were available for 149 (99%) at 28 days and 148 (99%) at 1 year after vaccination. The two participants not followed up for immunogenicity at 1 year were both in the $TdaP_{(PTgen/FHA)}$ group (figure 1).

Seroconversion rates for pertussis toxin neutralising antibodies and IgG antibodies against pertussis toxin and FHA remained significantly higher at 1 year after vaccination in the $aP_{(PTgen/FHA)}$ and $TdaP_{(PTgen/FHA)}$ groups than in the Tdap comparator vaccine group (table 2). Similarly, geometric mean titres for pertussis toxin neutralising antibodies and IgG antibodies against pertussis toxin and FHA remained higher at 1 year in the $aP_{(PTgen/FHA)}$ and

TdaP$_{(PTgen/FHA)}$ groups, but in the Tdap comparator group had fallen to close to prevaccination titres (figure 2, table 3). Geometric mean titres for pertussis toxin neutralising antibodies and IgG antibodies against pertussis toxin declined between 28 days and 1 year after vaccination at similar rates in the three vaccine groups. Pertussis toxin neutralising antibodies in the aP$_{(PTgen/FHA)}$ group decreased by 3·6 times (95% CI 3·0–4·2), those in the TdaP$_{(PTgen/FHA)}$ group by 3·2 times (2·7–3·8), and those in the Tdap comparator group by 2·9 times (2·6–3·4). For IgG antibodies against pertussis toxin, titres decreased by 3·9 times (95% CI 2·9–5·3), 3·9 times (3·2–4·3), and 2·9 times (2·3–3·6), respectively. Yet, geometric mean titres of pertussis toxin neutralising antibody at 1 year after vaccination were 7·9 times (95% CI 5·9–10·7) higher than at baseline in the aP$_{(PTgen/FHA)}$ group and 8·4 times (6·2–11·5) higher in the TdaP$_{(PTgen/FHA)}$ group, but were only 1·4 times (1·1–1·7) higher in the Tdap comparator vaccine group. For IgG antibodies against FHA, geometric mean titres at 1 year after vaccination remained 6·4 times (95% CI 4·6–8·8) and 5·4 times (3·9–7·5) higher than baseline in the aP$_{(PTgen/FHA)}$ and TdaP$_{(PTgen/FHA)}$ groups, respectively, compared with 2·0 times (1·5–2·8) higher in the Tdap comparator group, despite a relatively lower decrease in geometric mean titres between 28 days and 1 year after vaccination in the Tdap comparator group (decreased by 2·6 times, 95% CI 2·1–4·5) than in the aP$_{(PTgen/FHA)}$ group (3·4 times, 2·9–4·2) and the TdaP$_{(PTgen/FHA)}$ group (3·6 times, 95% CI 3·1–4·2).

Reverse cumulative distribution curves for pertussis toxin neutralising antibodies and IgG antibodies against pertussis toxin and FHA at baseline and 1 year after vaccination show persistently higher titres after vaccination with aP$_{(PTgen/FHA)}$ or TdaP$_{(PTgen/FHA)}$ than after vaccination with the Tdap comparator vaccine (figure 3). For pertussis toxin neutralising antibodies and IgG antibodies against pertussis toxin, titres were higher in larger proportions of participants 1 year after vaccination with aP$_{(PTgen/FHA)}$ or TdaP$_{(PTgen/FHA)}$ than 28 days after vaccination with the Tdap comparator vaccine (figure 3). For IgG antibodies against FHA, the 1-year titres for aP$_{(PTgen/FHA)}$ and TdaP$_{(PTgen/FHA)}$ had similar distributions to those seen at 28 days for the Tdap comparator vaccine (figure 3).

Geometric mean titres for IgG antibodies against tetanus and diphtheria toxoids were similar in the TdaP$_{(PTgen/FHA)}$ and Tdap comparator group 1 year after vaccination (appendix p 1). IgG antibody titres greater than the cutoff for protection (0·1 IU/mL) were seen for all participants against tetanus toxoid and in 144 (97%) of 148 participants against diphtheria toxoid. Of the four participants who did not have protective titres, two were in the TdaP$_{(PTgen/FHA)}$ and two were in the Tdap comparator group.

Adverse events up to 28 days after vaccination have been reported previously.[13] Between 28 days and 1 year after vaccination, 13 serious adverse events were reported in 12 study participants: five in the aP$_{(PTgen/FHA)}$ group, six (in five participants) in the TdaP$_{(PTgen/FHA)}$ group, and two in the Tdap comparator group. None of the serious adverse events was deemed to be vaccine related or led to participants discontinuing the study (appendix p 2). No participants had sustained cough or suspected or proven pertussis.

Five pregnancies were reported during 1-year follow-up. Two were in participants in the aP$_{(PTgen/FHA)}$ group and three in participants in the Tdap comparator group. No maternal complications were reported during delivery or the post-partum period. All neonates were born at full term with no congenital anomalies or birth defects, and a follow-up of infants at age 2 months showed good health and normal growth and development for age.

See Online for appendix

	aP$_{(PTgen/FHA)}$ group (n=150)	TdaP$_{(PTgen/FHA)}$ group (n=150)	Comparator Tdap group (n=150)	p value
Boys/girls	61 (41%)/89 (59%)	66 (44%)/84 (56%)	64 (43%)/86 (57%)	0·8412*
Age (years)	14·3 (1·6)	14·4 (1·7)	14·5 (1·7)	0·5267†
Height (cm)	158·7 (8·5)	159·4 (8·3)	159·1 (8·6)	0·7704‡
Weight (kg)	53·7 (15·7)	52·2 (13·6)	51·5 (13·5)	0·5494†

Data are n (%) or mean (SD). aP$_{(PTgen/FHA)}$=monovalent vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin. TdaP$_{(PTgen/FHA)}$=vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin combined with tetanus and reduced-dose diphtheria toxoids. Tdap=combined vaccine containing tetanus and reduced-dose diphtheria toxoids and reduced-dose chemically inactivated acellular pertussis. *Differences between groups for categorical variable measured with χ^2 test (two-sided). †Differences between groups for non-normally distributed continuous variables measured with Kruskal-Wallis test. ‡Differences between groups for normally distributed continuous variables measure with one-way ANOVA.

Table 1: **Participants' characteristics at baseline**

	Proportion (%) of participants (95% CI)			p value*
	aP$_{(PTgen/FHA)}$ group (n=50)	TdaP$_{(PTgen/FHA)}$ group (n=48)	Comparator Tdap group (n=50)	
Pertussis toxin neutralising antibodies				
Day 28	94% (87–100)	96% (90–100)	68% (55–81)	<0·0001
1 year	76% (64–88)	81% (70–92)	8% (1–16)	<0·0001
IgG antibodies against pertussis toxin				
Day 28	96% (91–100)	100% (100–100)	56% (42–70)	<0·0001
1 year	82% (71–93)	75% (63–87)	4% (0–9)	<0·0001
IgG antibodies against filamentous haemagglutinin				
Day 28	92% (85–100)	94% (87–100)	54% (40–68)	<0·0001
1 year	64% (51–77)	56% (42–70)	28% (16–41)	0·0007

Seroconversion was defined as antibody titres at least four times greater than at baseline. aP$_{(PTgen/FHA)}$=monovalent vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin. TdaP$_{(PTgen/FHA)}$=vaccine containing genetically inactivated pertussis toxin combined with tetanus and reduced-dose diphtheria toxoids. Tdap=combined vaccine containing tetanus and reduced-dose diphtheria toxoids and reduced-dose chemically inactivated acellular pertussis. *Difference between groups was measured with a two-sided χ^2 test.

Table 2: **Proportions of participants with persistent seroconversion after one dose of vaccine**

Discussion

The effectiveness of the most frequently used acellular pertussis vaccines is short lived.[1–6] Booster vaccinations are increasingly recommended, including for adolescents and adults in whom pertussis is less severe than in young

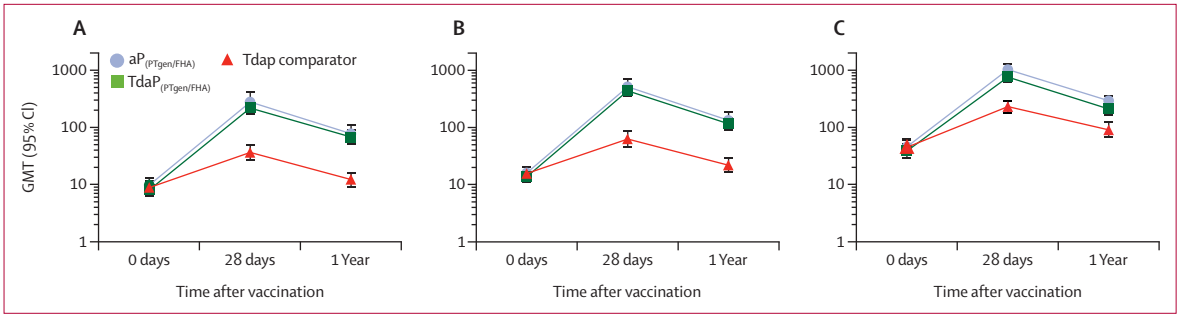

Figure 2: **GMTs for antibodies against vaccine components before and after one dose**
(A) Pertussis toxin neutralising antibodies. (B) IgG antibodies against pertussis toxin. (C) IgG antibodies against filamentous haemagglutinin. For all titres compared with baseline p<0·0001, except p=0·0005 for IgG against pertussis toxin at 1 year and p=0·0015 for pertussis neutralising antibodies at 1 year in the comparator Tdap group. For for all differences in responses between groups at 28 days and 1 year p<0·0001. GMT=geometric mean titre. $aP_{(PTgen/FHA)}$=monovalent vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin. $TdaP_{(PTgen/FHA)}$=vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin combined with tetanus and reduced-dose diphtheria toxoids. Tdap=combined vaccine containing tetanus and reduced-dose diphtheria toxoids, and reduced-dose chemically inactivated acellular pertussis.

	Geometric mean titre (95% CI)			p value
	$aP_{(PTgen/FHA)}$ group (n=50)	$TdaP_{(PTgen/FHA)}$ group (n=48)	Comparator Tdap group (n=50)	
Pertussis toxin neutralising antibodies (IU/mL)				
Baseline	9·8 (7·2–13·1)	8·0 (6·2–10·4)	8·8 (6·5–12·0)	0·8198*
28 days after vaccination	275·7 (171·6–418·6)	217·1 (164·1–287·1)	36·3 (25·7–51·1)	<0·0001*
1 year after vaccination	77·2 (53·3-111·7)†	67·5 (50·1–90·9)†	12·2 (8·9–16·7)†	<0·0001*
IgG antibodies against pertussis toxin (IU/mL)				
Baseline	16·0 (12·2–21·0)	13·7 (10·9–17·2)	15·6 (11·6–21·1)	0·8415*
28 days after vaccination	513·7 (366·4–720·3)	437·4 (342·7–558·3)	62·8 (44·1–89·5)	<0·0001‡
1 year after vaccination	132·8 (93·0–189·8)†	115·6 (88·1–151·8)†	21·9 (16·1–29·8)†	<0·0001‡
IgG antibodies against filamentous haemagglutinin (IU/mL)				
Baseline	45·6 (34·0–61·0)	38·5 (28·7–51·7)	44·6 (31·4–63·3)	0·7150‡
28 days after vaccination	1019·9 (804·0–1293·7)	756·4 (591·6–967·0)	229·9 (177·0–298·6)	<0·0001‡
1 year after vaccination	291·2 (230·9–367·1)†	208·6 (156·9–277·3)†	89·9 (64·5–125·4)†	<0·0001‡

$aP_{(PTgen/FHA)}$=monovalent vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin. $TdaP_{(PTgen/FHA)}$=vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin combined with tetanus and reduced-dose diphtheria toxoids. Tdap=combined vaccine containing tetanus and reduced-dose diphtheria toxoids and reduced-dose chemically inactivated acellular pertussis. *Kruskal-Wallis test for difference between groups. †Difference between baseline and after vaccination within groups was measured with the paired *t* test. ‡One-way ANOVA for difference between groups.

Table 3: **Geometric mean titres of antibodies after one dose of vaccine**

infants, but remains associated with substantial morbidity and potential spread of disease to more vulnerable and susceptible populations.[22] We have previously reported that two new acellular pertussis vaccines containing PTgen manufactured with recombinant DNA technologies induced greater immune responses in adolescents 28 days after vaccination than a widely used licensed Tdap comparator vaccine.[13] In this follow-up report of immune response at 1 year, we found that pertussis antibody responses were greater and sustained after vaccination with one dose of the monovalent vaccine $aP_{(PTgen/FHA)}$ and the combination vaccine $TdaP_{(PTgen/FHA)}$, whereas for the comparator Tdap vaccine responses had declined substantially 1 year after vaccination.

Although the cellular immune system probably plays a part in mediating protection against *B pertussis*, maternal pertussis immunisation programmes have shown that antibodies alone are sufficient to protect young infants against severe pertussis disease.[8,23,24] Antibody responses to pertussis toxin in acellular vaccines are fundamental for vaccine-induced protection against pertussis disease, and protection is likely to increase with increasing antibody titres.[25] We found that seroconversion rates for pertussis toxin neutralising antibodies remained substantially higher after 1 year in participants who received $aP_{(PTgen/FHA)}$ and $TdaP_{(PTgen/FHA)}$ than in those who received the Tdap comparator vaccine (76% and 81% *vs* 8%). Similar results were found for IgG antibodies against pertussis toxin and FHA. The responses we observed for the comparator Tdap vaccine 1 year after one dose correspond with those in earlier reports.[26,27] The higher seroconversion rates at 1 year in recipients of the

vaccines containing PTgen are due to initial higher immunogenicity, as seen at 28 days after vaccination.[13] Thus, acellular pertussis vaccines containing PTgen can induce longer-lasting protection than those containing PTchem.

The performance of the two vaccines containing PTgen is mostly attributable to the use of recombinant DNA technologies to inactivate the pertussis toxin.[18,28] Chemical inactivation processes alter the tertiary and quaternary structures of pertussis toxin, which affects conformational epitopes, including those involved in antibody binding and neutralisation of pertussis toxin. For instance the protective monoclonal antibody 1B7 cannot bind to formaldehyde-treated pertussis toxin.[10,29] Of note, titres and seroconversion rates of IgG antibodies against FHA also remained higher at 1 year in recipients of the vaccines containing PTgen than recipients of the Tdap comparator, despite the dose being the same in all three vaccines. The likely explanation for this difference is that in vaccines containing PTchem high concentrations of formaldehyde are needed to inactivate any residual pertussis toxin that has been coeluted with FHA. Consequently, the protein formation and immune recognition of FHA, and, therefore, its immunogenicity, are affected.[30] In recombinant acellular pertussis vaccines, FHA is coeluted with non-toxic genetically inactivated pertussis toxin and, therefore, needs no further chemical inactivation. Consequently, FHA in the vaccines containing PTgen retains its functional and immunological properties.

Follow-up of study participants over a period of 1 year since vaccination did not identify any safety concerns relating to the vaccines containing PTgen. Although these vaccines are yet to be studied in pregnant women, two girls vaccinated with $aP_{(PTgen/FHA)}$, and three vaccinated with the Tdap comparator vaccine became pregnant during the study after vaccination. All had healthy pregnancies and deliveries with no maternal or neonatal adverse events. The $aP_{(PTgen/FHA)}$ and $TdaP_{(PTgen/FHA)}$ vaccines are already in use in Thailand to vaccinate adolescents and adults, including pregnant women. So far, vaccination against pertussis, in general and in pregnant women, could only be delivered in combination vaccines including tetanus and diphtheria. Our findings for $aP_{(PTgen/FHA)}$, however, suggest that the monovalent formulation offers an alternative and more immunogenic option for vaccinating pregnant women against pertussis, which might be particularly useful, for example, if a woman received a tetanus booster or a tetanus and diphtheria booster dose a few years earlier and does not need a further dose. Maternal pertussis vaccination is currently recommended in the second or third trimester of pregnancy as a strategy additional to routine primary infant pertussis vaccination in countries or settings with high or increasing infant morbidity, mortality, or both, from pertussis.[31] However, owing to the high immunogenicity of the vaccines containing PTgen, perhaps vaccination could be given

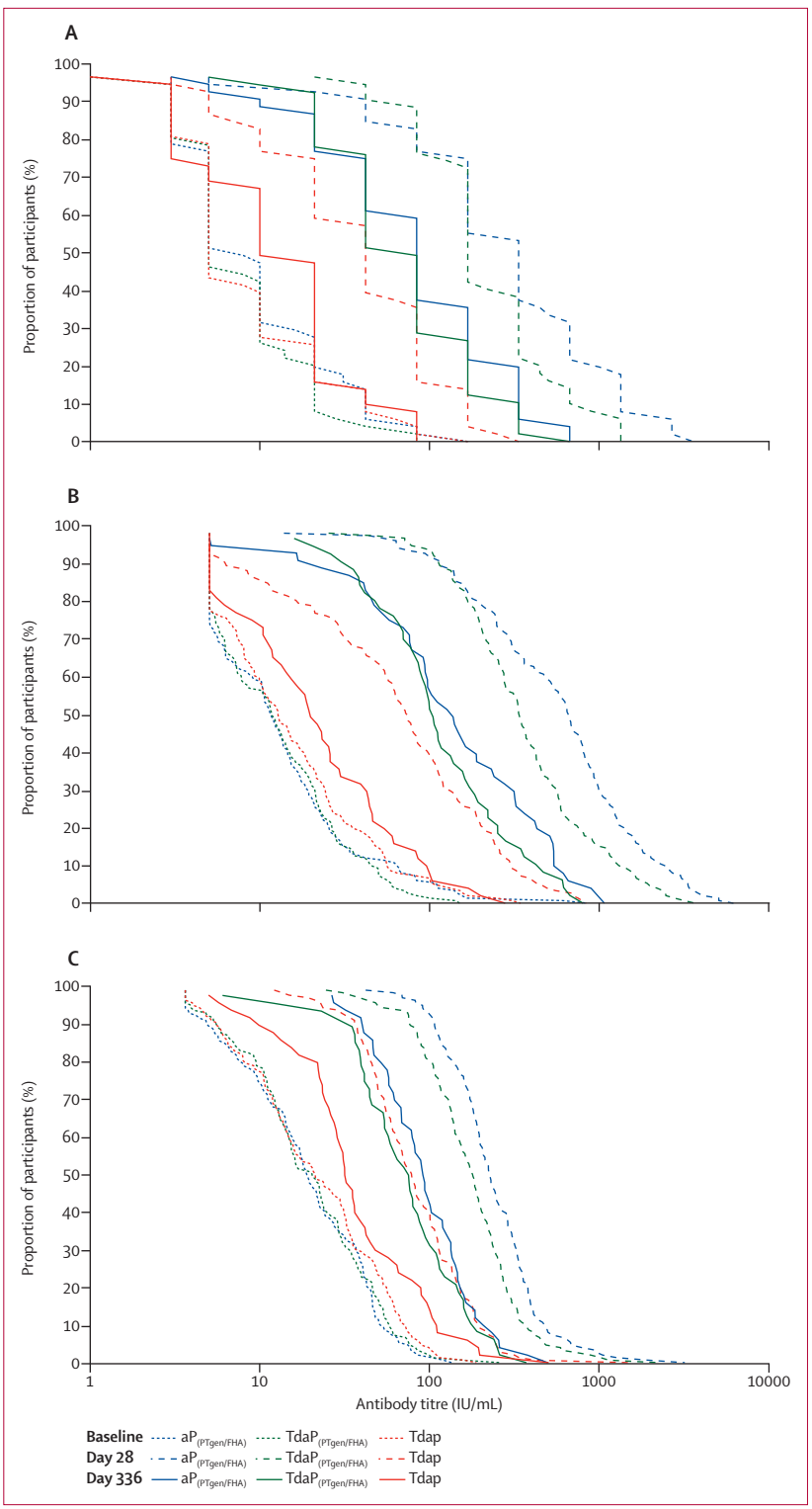

Figure 3: **Reverse cumulative distribution curves for antibody titres before and after one dose of vaccine**
(A) Pertussis toxin neutralising antibodies. (B) IgG antibodies against pertussis toxin. (C) IgG antibodies against filamentous haemagglutinin. $aP_{(PTgen/FHA)}$=monovalent vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin. $TdaP_{(PTgen/FHA)}$=vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin combined with tetanus and reduced-dose diphtheria toxoids. Tdap=combined vaccine containing tetanus and reduced-dose diphtheria toxoids and reduced-dose chemically inactivated acellular pertussis.

earlier in pregnancy. These vaccines might also improve mother-to-child transfer of protective antibodies when vaccination is preferred before planned pregnancies.

Our study has some limitations. First, longer follow-up of the study participants is needed to show the duration of immunogenicity beyond 1 year. Longer-term follow-up of participants vaccinated as part of this trial is planned. Second, the population included in this trial had been primed in infancy with whole-cell pertussis vaccines. Whether immunogenicity will persist following booster doses in adolescents who have been primed in childhood with acellular pertussis vaccines is being assessed in a separate trial (NCT02946190). Finally, because efficacy trials are no longer recommended for new pertussis vaccines,[21] we took the generally accepted approach of assessing antibody persistence as a marker of sustained efficacy.

Booster vaccination with monovalent and combined formulations pertussis vaccines containing PTgen led to greater and more sustained pertussis antibody responses and seroconversion rates than after vaccination with the comparator Tdap, with similar safety profiles. Genetically inactivated acellular pertussis vaccines licensed for immunisation of children aged 11 years and older and of adults could be advantageous in enabling maternal immunisation earlier in or before pregnancy and could address the fast-waning immunity of pertussis vaccines in adolescents.

Contributors

HTP and SV conceived and designed the study. PP, KC, CS, SS, JD, AP, WP, KB, KL, YS, and OW did the study and PP and SS were the principal investigators for the respective study sites. PC was responsible for study coordination and supervision. LF was responsible for pharmacovigilance management. GH and IKP designed and oversaw the immunological assays. MC made the investigational products. WW was responsible for regulatory submission. All authors contributed to interpretation of the data. AHvdB and SV prepared the first draft and final drafts of the manuscript. All authors reviewed and commented on drafts and approved the final version for submission.

Declaration of interests

MC, WW, GH, LF, PC, IKP, AHvdB, HTP, and SV are employed by BioNet-Asia. All other authors declare no competing interests.

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