A genetically inactivated two-component acellular pertussis vaccine, alone or combined with tetanus and reduced-dose diphtheria vaccines, in adolescents: a phase 2/3, randomised controlled non-inferiority trial

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Summary

Background Increasing evidence shows that protection induced by acellular pertussis vaccines is short-lived, requiring repeated booster vaccination to control pertussis disease. We aimed to assess the safety and immunogenicity of a recombinant acellular pertussis vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin, as either a monovalent vaccine ($aP_{[PTgen/FHA]}$) or in combination with tetanus and reduced-dose diphtheria vaccines ($TdaP_{[PTgen/FHA]}$), versus a licensed tetanus and reduced-dose diphtheria and acellular pertussis combination vaccine (Tdap).

Methods We did this phase 2/3, randomised controlled non-inferiority trial at two sites in Bangkok, Thailand. Healthy adolescents (aged 12–17 years) were randomly assigned (1:1:1), via a computer-generated randomisation list with block sizes of three, to receive one dose (0·5 mL) of $aP_{(PTgen/FHA)}$, $TdaP_{(PTgen/FHA)}$, or Tdap (comparator). Clinical research staff responsible for participant randomisation, vaccine preparation and administration, and accountability were aware of group allocation. However, allocation was concealed from all other site study staff, data management personnel, statisticians, laboratory staff, and study participants. The primary outcome was non-inferior immunogenicity of $TdaP_{(PTgen/FHA)}$ to Tdap based on seroconversion rates (a four-fold increase or more) for pertussis toxin and filamentous haemagglutinin IgG antibodies 28 days after vaccination, with a predefined 10% margin of equivalence. We did analysis by per protocol. This study is registered with the Thai Clinical Trial Registry, number TCTR20150703002.

Findings Between July 6 and Aug 20, 2015, we allocated 450 participants to receive one dose of $TdaP_{(PTgen/FHA)}$ (n=150), $aP_{(PTgen/FHA)}$ (n=150), or comparator Tdap (n=150). 28 days after vaccination, seroconversion rates for anti-pertussis toxin IgG were 96·6% (95% CI 93·8–99·5; n=144) in the $TdaP_{(PTgen/FHA)}$ group and 55·0% (47·1–63·0; n=82) in the comparator Tdap group (difference 41·6%, 95% CI 33·1–50·1; p<0·0001). Seroconversion rates for anti-filamentous haemagglutinin were 82·6% (95% CI 76·5–88·6; n=123) in the $TdaP_{(PTgen/FHA)}$ group and 54·4% (46·4–62·4; n=81) in the comparator group (difference 28·2%, 95% CI 18·1–38·2 p<0·0001). 28 days after vaccination, seroconversion rates in the $aP_{(PTgen/FHA)}$ group were 96·0% (95% CI 92·8–99·1; n=142) for anti-pertussis toxin IgG and 93·2% (89·2–97·3; n=138) for anti-filamentous haemagglutinin IgG. These findings support the non-inferior immunogenicity of $TdaP_{(PTgen/FHA)}$ over comparator Tdap. Reactogenicity and incidence of adverse events were similar between groups.

Interpretation The new $TdaP_{(PTgen/FHA)}$ vaccine is safe and induces higher pertussis responses 28 days after vaccination than does the available licensed Tdap booster vaccine. Results of our trial led to the licensure of new acellular pertussis vaccines containing genetically inactivated pertussis toxin in Thailand. The availability of recombinant monovalent pertussis vaccines that induce high antibody responses provides the medical community and consumers with the opportunity to vaccinate against pertussis when immunisation against diphtheria and tetanus is not required or not desired. Studies are underway to pave the way for licensure studies of this acellular pertussis vaccine in other countries.

Funding BioNet-Asia.

Introduction

Since the 1950s, pertussis vaccine in combination with tetanus and diphtheria vaccines has been at the core of infant immunisation programmes. In the 1990s, acellular pertussis vaccines replaced the more reactogenic whole-cell pertussis vaccines in most developed countries.[1] A resurgence of whooping cough in the past few years has been recorded in particular in countries using exclusively acellular pertussis vaccines.[2,3] This occurrence is partly explained by the fast-waning immunity of current acellular pertussis vaccines.[4,5] Repeated booster vaccination is now often recommended to maintain high levels of immune protection in the community,[6,7] as is vaccination during pregnancy to

Research in context

Evidence before this study

We searched PubMed for all papers published up to June 1, 2017, for trials in human volunteers of pertussis vaccines containing genetically detoxified pertussis toxin. Our search terms were "genetically engineered pertussis vaccine", "recombinant pertussis vaccine", "genetically detoxified pertussis vaccine", and "genetically inactivated pertussis vaccine". We identified ten publications reporting on results of a relevant pertussis vaccine studied in human participants. Nine studies were published in the 1990s, and were done in infants, young children, or adults. The tenth publication was a report on our phase 1/2 trial of genetically detoxified pertussis vaccine in adults. We also searched ClinicalTrials.gov and the Thai Clinical Trial Registry for relevant clinical trials registered before June 1, 2017, with the same terms as used in the previous search in addition to the more general search term "acellular pertussis vaccine". Besides the previous phase 1/2 trial, we identified one trial that was started after the trial in Thailand reported here and assessed the same pertussis vaccine in participants aged 11–15 years in Switzerland. We did not find any other studies in the registries that, according to the trial's descriptions, involved a relevant vaccine (ie, genetically detoxified, inactivated, engineered, or recombinant pertussis toxin).

Added value of this study

To our knowledge, this is the first study to assess the effect of a genetically inactivated two-component acellular pertussis vaccine in adolescents primed with whole-cell pertussis vaccine in infancy, and to investigate both a monovalent (pertussis only) formulation and the vaccine in combination with tetanus and reduced-dose diphtheria vaccines.

Implications of all the available evidence

The non-inferior immunogenicity and similar safety profile of the experimental vaccines compared with a licensed tetanus and reduced-dose diphtheria and acellular pertussis vaccine observed in this phase 2/3 trial has resulted in the licensure of these new-generation recombinant pertussis vaccines in Thailand for active immunisation in adolescents from 11 years of age and adults. This is the only recombinant acellular pertussis vaccine and the only monovalent acellular pertussis vaccine currently available. The availability of recombinant monovalent pertussis vaccines inducing high antibody responses provides the medical community and consumers with the opportunity to vaccinate against pertussis when immunisation against diphtheria and tetanus is not required or not desired, such as repeated maternal immunisation to protect newborn babies against pertussis. Studies are underway to pave the way for licensure studies of this acellular pertussis vaccine in countries other than Thailand, including trials in Europe and follow-up studies to prove the long-lasting protection induced by these new-generation recombinant pertussis vaccines.

provide protection to newborn babies during the first months of life.[8,9] Although these strategies might offer a short-term solution, improved pertussis vaccines are needed that induce more effective and long-lasting protection than available vaccines.

All acellular pertussis vaccines contain pertussis toxoid. Pertussis toxin is one of the major virulence factors produced by *Bordetella pertussis*, and immunity against pertussis toxin alone can be sufficient to mediate protection against pertussis disease.[10] Detoxification of the toxin is required to safely vaccinate human beings. In most vaccines, detoxification is achieved through chemical treatment. However, chemical inactivation can destroy up to 80% of epitopes and hence result in poor antibody responses to the toxin.[11,12] Genetic detoxification using recombinant technologies maintains the native epitope structure and immunogenic properties of the toxin.[12,13] In the early 1990s, an acellular pertussis vaccine containing genetically inactivated pertussis toxoid was developed and proven to be safe, efficacious, and more immunogenic in all age groups than vaccines containing chemically inactivated pertussis toxoid.[14,15] The vaccine was licensed for paediatric use as a diphtheria, tetanus, acellular pertussis combination vaccine (DTaP) in several countries in Europe, Asia, and Latin America in the 1990s, but withdrawn years later for commercial reasons.[16] Following the rise in pertussis disease in many countries, there is a renewed interest in new-generation pertussis vaccines containing genetically inactivated pertussis toxoid.[17]

Genetically engineered *B pertussis* strains producing genetically inactivated pertussis toxoid have been developed and patented by BioNet-Asia (Ayutthaya, Thailand),[18] and findings from a phase 1/2 vaccine trial[19] including 60 healthy adults showed that genetically inactivated pertussis toxoid containing pertussis vaccine was safe and induced significantly higher pertussis toxoid-specific antibody responses than a widely used tetanus and reduced-dose diphtheria and acellular pertussis (Tdap) vaccine.

We did this study to evaluate the safety and immunogenicity of a recombinant acellular pertussis vaccine containing two pertussis components—genetically inactivated pertussis toxin and filamentous haemagglutinin—as a monovalent pertussis vaccine ($aP_{[PTgen/FHAj]}$) or combined with tetanus and reduced-dose diphtheria vaccines ($TdaP_{[PTgen/FHAj]}$) by comparison with a licensed Tdap vaccine.

Methods

Study design and participants

We did this phase 2/3, randomised controlled non-inferiority trial at two sites in Mahidol University, Bangkok, Thailand: the Pediatric Infectious Diseases

Unit, Department of Pediatrics, Faculty of Medicine Siriraj Hospital; and the Vaccine Trial Centre, Faculty of Tropical Medicine.

We did the study according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice guideline, the Declaration of Helsinki, and local ethical guidelines. Ethics approval was obtained from the Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University, and ethics committee of the Faculty of Tropical Medicine, Mahidol University. We obtained written informed assent from participants and written informed consent from their parents or legal guardians.

We recruited healthy adolescents (aged 12–17 years) who had no obvious health problems as established by medical history and physical examination, were able to comply with the study protocol, and, for female participants who had reached menarche, were willing to take reliable birth control measures for the study duration. Main exclusion criteria were a history of clinically significant medical illness, including immune deficiency, uncontrolled diabetes or hypertension, and heart or renal or hepatic diseases; pregnancy or breastfeeding; a history of allergy to any vaccine component; a history of serious adverse event or neurological adverse event after diphtheria, tetanus, and pertussis vaccination; diphtheria, tetanus, or pertussis vaccination within 1 year before recruitment; progressive or severe neurological disorder, seizure disorder, or Guillain–Barré syndrome; history of alcoholism or intravenous drug abuse; presence of bleeding disorders, and abnormalities of splenic and thymic functions; and history of any illness that, in the opinion of the investigator, might interfere with the results of the study or pose additional risk to the participants because of participation in the study. Female participants who had menarche were tested to exclude pregnancy before enrolment.

Information about individual vaccination histories was not available for participants. The Thai national immunisation programme includes vaccination with whole-cell pertussis containing diphtheria, tetanus, and pertussis at 2, 4, 6, and 18 months of age, with an additional booster dose at 6 years of age. Because the national immunisation programme coverage for three doses of whole-cell pertussis containing diphtheria, tetanus, and pertussis has been 99% since 1996, participants were deemed likely to have completed the primary vaccination series in childhood.[20]

Randomisation and masking

Participants were randomly assigned (1:1:1), via a computer-generated (PROC PLAN, SAS version 9.4) randomisation list with block sizes of three, to receive one dose of $aP_{(PTgen/FHA)}$, $TdaP_{(PTgen/FHA)}$, or Tdap (comparator). The list was created by the biostatistics department and provided to the investigator in a sealed envelope. Clinical research staff responsible for participant randomisation, vaccine preparation and administration, and accountability were aware of group allocation. However, allocation was concealed from all other site study staff, data management personnel, statisticians, laboratory staff, and study participants.

Procedures

$aP_{(PTgen/FHA)}$ (batch number PE25002-2) and $TdaP_{(PTgen/FHA)}$ (batch number TD25002-1) were developed and produced by BioNet-Asia (Ayutthaya, Thailand) by use of a recombinant *B pertussis* strain containing a mutated (non-toxic) S1 gene (R9K and E129E) in the ptx operon.[18] One dose (0·5 mL) of $aP_{(PTgen/FHA)}$ contains 5 µg genetically inactivated pertussis toxin, 5 µg filamentous haemagglutinin, and 0·3 mg Al^{3+}; and $TdaP_{(PTgen/FHA)}$ additionally contains at least 7·5 flocculation units (Lf) tetanus toxoid and at least 2 Lf diphtheria toxoid. One dose (0·5 mL) of the comparator Tdap vaccine (Adacel; Sanofi-Pasteur, ON, Canada; batch number U4971AA) contains 2·5 µg chemically detoxified pertussis toxin, 5 µg filamentous hemagglutinin, 3 µg pertactin, 5 µg fimbriae types 2 and 3, 5 Lf tetanus toxoid, 2 Lf diphtheria toxoid, and 0·33 mg Al^{3+}. All study vaccines were presented as monodose prefilled syringes. Participants received one intramuscular injection in the non-dominant deltoid region with the allocated vaccine.

Of note, while lower case letters in the Tdap abbreviation denote reduced doses of diphtheria (d) toxoid and pertussis (p) vaccine used for booster vaccination in older children, adolescents, and adults, and upper case D, T, and P full-strength doses of diphtheria, tetanus, and pertussis vaccine used in the paediatric formulation, an upper case P is used for BioNet's pertussis vaccine formulation because it contains pertussis toxin and filamentous hemagglutinin antigens in the same amount as or higher than included in the paediatric recombinant pertussis vaccine licensed in the 1990s.

Venous blood samples (5 mL) were obtained on the same day just before vaccine was administered and 28 days after vaccination. Serum anti-pertussis toxin and anti-filamentous haemagglutinin IgG antibody titres were measured with validated indirect ELISA.[19] Titre results were calculated in IU/mL and calibrated to WHO International Standard Pertussis Antiserum (Human) 06/140. The WHO Reference Reagent Pertussis Antiserum (Human) 06/142 (National Institute for Biological Standards and Control [NIBSC], Hertfordshire, UK) was used as a positive control to determine the validity of test. The lower limit of quantitation for pertussis toxin and filamentous haemagglutinin IgG was below the assay cutoff of 5 IU/mL. Samples with titres below this cutoff were arbitrarily attributed a titre equal to 5 IU/mL. Functional anti-pertussis toxin antibody titres were assessed in a prespecified subset of 50 randomly selected study participants per group using

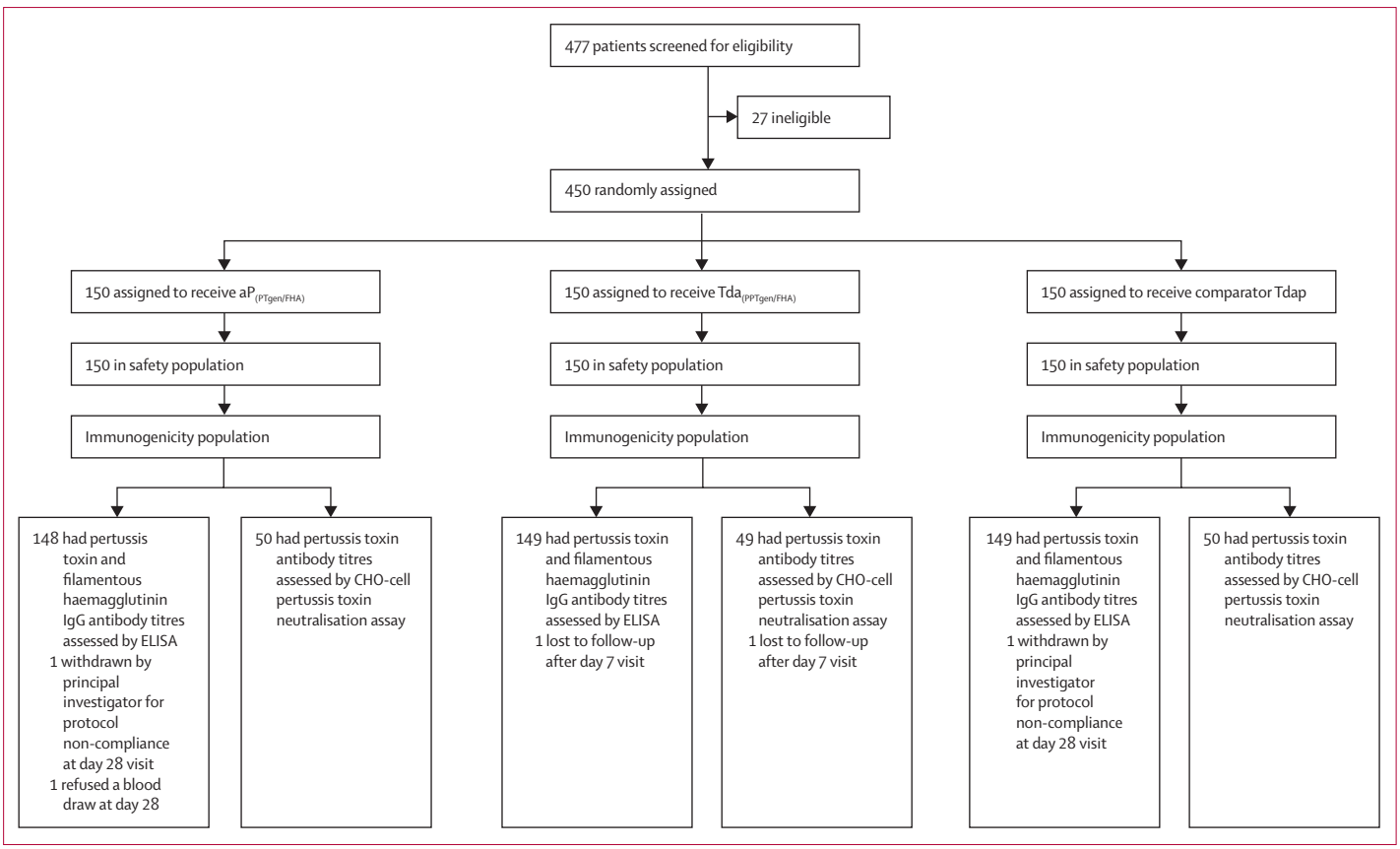

***Figure 1*: Trial profile**
Functional anti-pertussis toxin antibody titres were assessed in a prespecified subset of 50 randomly selected study participants per group. $aP_{(PTgen/FHA)}$=acellular pertussis vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin. $TdaP_{(PTgen/FHA)}$=tetanus with reduced-dose diphtheria and acellular pertussis vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin. Tdap=tetanus with reduced-dose diphtheria and acellular pertussis combination vaccine. CHO=Chinese hamster ovary.

a validated Chinese hamster ovary-cell pertussis toxin neutralisation assay and JNIH-5 (NIBSC) as the source of pertussis toxin, as reported previously.[19,21] The pertussis toxin neutralising titre was reported as IU/mL on the basis of the relative activity of the WHO International Standard Pertussis Antiserum (Human) 06/140. Serum tetanus toxoid and diphtheria toxoid IgG titres were measured with commercially available ELISA kits (Serion ELISA classic Tetanus [ESR108G] and Diphtheria [ESR130G] IgG kits, respectively; Virion\Serion, Würzburg Germany). Samples with titres below the assay cutoff of 0·1 IU/mL were attributed the value of the cutoff. Diphtheria and tetanus toxoid IgG titres greater than 0·1 IU/ml were deemed protective. All ELISA tests were done by an independent certified laboratory in Europe (VisMederi, Siena, Italy) and the toxin neutralisation assay at BioNet-Asia.

Participants were monitored for 30 min after vaccination for immediate adverse events and reactogenicity. Diary cards were distributed on the day of vaccination to record solicited local (pain, redness, and induration) and systemic (fever, headache, fatigue, arthralgia, chills, malaise, myalgia, and vomiting) reactions for 7 days after vaccination. Adverse events were recorded for 1 month after vaccination, and serious adverse events for the entire study duration (1 year). Here we report data collected up to day 28. The relation of adverse and serious adverse events to study vaccines was determined by the investigator according to ICH guidelines and protocol-specified safety considerations.

Outcomes

The primary outcome was non-inferior immunogenicity of $TdaP_{(PTgen/FHA)}$ to the comparator Tdap based on the rate of seroconversion for pertussis toxin and filamentous haemagglutinin IgG antibodies 28 days after vaccination. We defined seroconversion as the proportion of participants who achieved a titre increase of four fold or more from baseline to day 28. Secondary outcomes were non-inferior immunogenicity of $aP_{(PTgen/FHA)}$ to Tdap based on pertussis toxin and filamentous haemagglutinin IgG seroconversion rates, geometric mean titres (GMTs) for pertussis toxin and filamentous haemagglutinin IgG titres and pertussis toxin neutralising antibody titres 28 days after vaccination, reactogenicity during 7 days after vaccination, adverse and serious adverse events

	TdaP$_{(PTgen/FHA)}$ (n=149)	aP$_{(PTgen/FHA)}$ (n=148)	Comparator Tdap (n=149)	Difference for TdaP$_{(PTgen/FHA)}$ *vs* comparator Tdap*	Difference for aP$_{(PTgen/FHA)}$ *vs* comparator Tdap*
Pertussis toxin	96·6% (93·8–99·5); n=144	96·0% (92·8–99·1); n=142	55·0% (47·1–63·0); n=82	41·6% (33·1–50·1)†	40·9% (32·3–49·5)†
Filamentous haemagglutinin	82·6% (76·5–88·6); n=123	93·2% (89·2–97·3); n=138	54·4% (46·4–62·4); n=81	28·2% (18·1–38·2)†	38·9% (29·9–47·8)†

Data are seroconversion rate (95% CI), unless otherwise specified. Seroconversion rates were defined as the proportion of participants with an increase of four fold or more in antibody titres compared with baseline values. TdaP$_{(PTgen/FHA)}$=tetanus with reduced-dose diphtheria and acellular pertussis vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin. aP$_{(PTgen/FHA)}$=acellular pertussis vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin. Tdap=tetanus with reduced-dose diphtheria and acellular pertussis combination vaccine. *Based on non-inferiority test with 10% margin of equivalence. †Non-inferiority criteria satisfied.

***Table 1*: Anti-pertussis toxin and anti-filamentous haemagglutinin ELISA IgG seroconversion rates 28 days after vaccination**

during 28 days after vaccination, and comparison of seroconversion rates and GMTs for diphtheria and tetanus toxoid 28 days after vaccination between TdaP$_{(PTgen/FHA)}$ and the comparator vaccine. As part of the protocol participants will be followed up after 1 year for safety and persistence of immunogenicity; these findings will be reported elsewhere.

See Online for appendix

Statistical analysis

The sample size was calculated based on a non-inferiority test, an α level of 0·05, and 80% power, and in the assumption of an 85% seroconversion rate in the control group. Calculations of the sample size required for the non-inferiority assessment were based on formulas derived by Farrington and Manning.[22]

Seroconversion rates and GMTs were calculated with exact 95% CIs. For categorical variables, we assessed differences between vaccine groups with either χ^2 or Fisher's exact tests. We used ANOVA to test for differences in normally distributed continuous variables; Kruskal–Wallis or Mann–Whitney *U* tests were used to test for differences in continuous variables that did not follow a normal distribution, comparing three or two groups, respectively. We used paired *t* tests to compare GMTs between baseline and after vaccination. Non-inferiority testing for vaccine-induced seroconversion rates was done with the Wald test for non-inferiority, with a predefined 10% margin of equivalence and calculation of confidence limits based on a one-sided α of 0·025. We did analysis in the per-protocol population.

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. This study is registered with the Thai Clinical Trial Registry, number TCTR20150703002.

Role of funding source

The sponsor of the study had a role in study design, data collection, data analysis, data interpretation, or 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 July 6 and Aug 20, 2015, we allocated 450 participants to receive one dose of TdaP$_{(PTgen/FHA)}$ (n=150). aP$_{(PTgen/FHA)}$ (n=150), or comparator Tdap (n=150; figure 1). The safety population comprised 450 (100%) participants and the immunogenicity population comprised 446 (99%) participants (figure 1). The study population comprised more female than male participants, but baseline characteristics were otherwise similar between groups (appendix p 1).

Non-inferiority based on a predefined 10% margin of equivalence was confirmed for TdaP$_{(PTgen/FHA)}$ over the comparator vaccine for both pertussis toxin and filamentous haemagglutinin IgG, with differences in seroconversion rates of 41·6% (95% CI 33·1–50·1) for pertussis toxin and 28·2% (18·1–38·2) for filamentous haemagglutinin (p<0·0001 for both; table 1). The aP$_{(PTgen/FHA)}$ vaccine met non-inferiority criteria for pertussis toxin and filamentous haemagglutinin IgG seroconversion rates over the comparator vaccine, with differences in seroconversion rates of 40·9% (95% CI 32·3–49·5) for pertussis toxin and 38·9% (29·9–47·8) for filamentous haemagglutinin (p<0·0001 for both; table 1).

28 days after vaccination, anti-pertussis toxin GMTs were significantly higher in participants vaccinated with aP$_{(PTgen/FHA)}$ (562 IU/mL, 95% CI 468–675) or TdaP$_{(PTgen/FHA)}$ (365 IU/mL, 315–423) than in those vaccinated with Tdap (63 IU/mL, 51–78; p<0·0001; figure 2). Anti-filamentous haemagglutinin GMTs were likewise higher in the candidate vaccine groups (aP$_{(PTgen/FHA)}$ 924 IU/mL, 95% CI 809–1054; TdaP$_{(PTgen/FHA)}$ 632 IU/mL, 550–727) than in the comparator group (242 IU/mL, 209–280; p<0·0001; figure 2). Vaccination with TdaP$_{(PTgen/FHA)}$ or aP$_{(PTgen/FHA)}$ resulted in higher anti-pertussis toxin and anti-filamentous haemagglutinin IgG antibody titres in a larger proportion of the vaccinated population than did vaccination with Tdap (figure 3).

Neutralising (Chinese hamster ovary-cell assay) anti-pertussis toxin GMTs were similar at baseline across the three vaccine groups. 28 days after vaccination, neutralising anti-pertussis toxin GMTs were significantly higher in the aP$_{(PTgen/FHA)}$ (276 IU/mL, 95% CI 182–419) and TdaP$_{(PTgen/FHA)}$ (216 IU/mL, 164–284) groups than in the comparator group (36 IU/mL, 26–51; p<0·0001; figure 2). In a post-hoc analysis, seroconversion rates for neutralising anti-pertussis toxin antibody titres 28 days after vaccination were significantly higher in the aP$_{(PTgen/FHA)}$ group (94%, 95% CI 87–100; n=47 of 50) and TdaP$_{(PTgen/FHA)}$ group (96%, 90–100; n=47 of 49) than in the

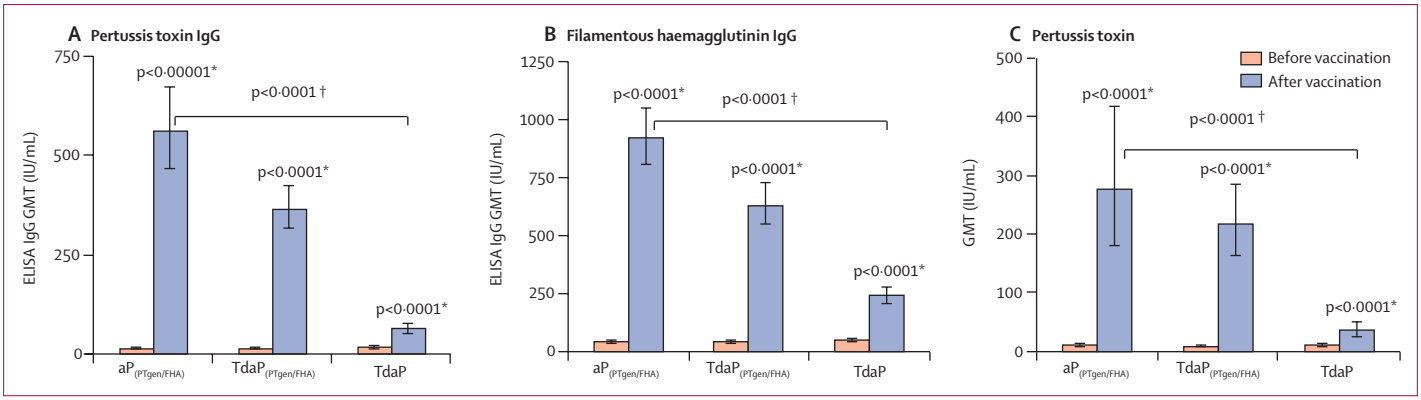

***Figure 2*: Pertussis toxin and filamentous haemagglutinin ELISA IgG GMTs and pertussis toxin neutralising antibody GMTs before and 28 days after vaccination**
Error bars show 95% CIs. Pertussis toxin and filamentous haemagglutinin antibody titres were assessed by ELISA and pertussis toxin neutralising antibody titres by the Chinese hamster ovary-cell neutralisation assay. GMT=geometric mean titre. $aP_{(PTgen/FHA)}$=acellular pertussis vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin. $TdaP_{(PTgen/FHA)}$=tetanus with reduced-dose diphtheria and acellular pertussis vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin. Tdap=tetanus with reduced-dose diphtheria and acellular pertussis combination vaccine. *We used paired *t* tests to compare GMTs between baseline and after vaccination. †To compare post-vaccination titrer, we used the Kruskal–Wallis test for pertussis toxin ELISA and neutralising GMTs, and one-way ANOVA for filamentous haemagglutinin ELISA GMTs. Differences in baseline titres did not differ significantly between the vaccination groups for any of the outcomes (Kruskal–Wallis p>0·05; Kruskal–Wallis).

comparator Tdap group (68%, 55–81; n=34 of 50; p<0·0001 for both; figure 2).

28 days after vaccination, the proportion of participants who reached cutoff values of 80 IU/mL or more of ELISA pertussis toxin IgG and neutralising pertussis toxin antibody titrers with $aP_{(PTgen/FHA)}$ or $TdaP_{(PTgen/FHA)}$ was more than double that in the Tdap group (figure 4).

Anti-tetanus toxoid and anti-diphtheria toxoid IgG GMTs were similar in the $TdaP_{(PTgen/FHA)}$ and comparator Tdap groups before vaccination, and significantly increased (p<0·0001) to titres that did not differ significantly between both groups at day 28 after vaccination (appendix p 2). Before vaccination, 148 (99%) participants in the $TdaP_{(PTgen/FHA)}$ group and 147 (99%) in the Tdap group had protective antibody titres against tetanus, and 127 (85%) and 132 (89%), respectively, had protective antibody titres against diphtheria (>0·1 IU/mL; appendix p 2). 28 days after vaccination, all participants had protective anti-tetanus toxoid antibody titres greater than 0·1 IU/mL, and 146 (98%) in the $TdaP_{(PTgen/FHA)}$ group and 142 (95%) in the Tdap group had reached protective titres against diphtheria toxoid (appendix p 2).

No immediate adverse events occurred in the first 30 min after vaccination. Significantly more study participants in the comparator Tdap group than in the $TdaP_{(PTgen/FHA)}$ and $aP_{(PTgen/FHA)}$ groups reported local pain and redness during the first 30 minutes after vaccination (table 2). Neither local nor systemic side-effects in the 7 days after vaccination differed significantly between the candidate vaccine and the comparator Tdap groups (table 2). Overall, pain at the injection site was the most reported local side-effect during this period (table 2). Myalgia was the most common systemic reaction after vaccination in all groups, followed by fatigue, headache, malaise, and arthralgia (table 2).

The incidence of both unrelated and related adverse events was similar in the three vaccine groups (appendix p 3). The most commonly reported related adverse events were pain in the limbs (n=2 vaccinated with $TdaP_{(PTgen/FHA)}$ and n=3 vaccinated with comparator Tdap), and myalgia (n=3 vaccinated with $aP_{(PTgen/FHA)}$, n=1 vaccinated with $TdaP_{(PTgen/FHA)}$, and n=1 vaccinated with Tdap). Related

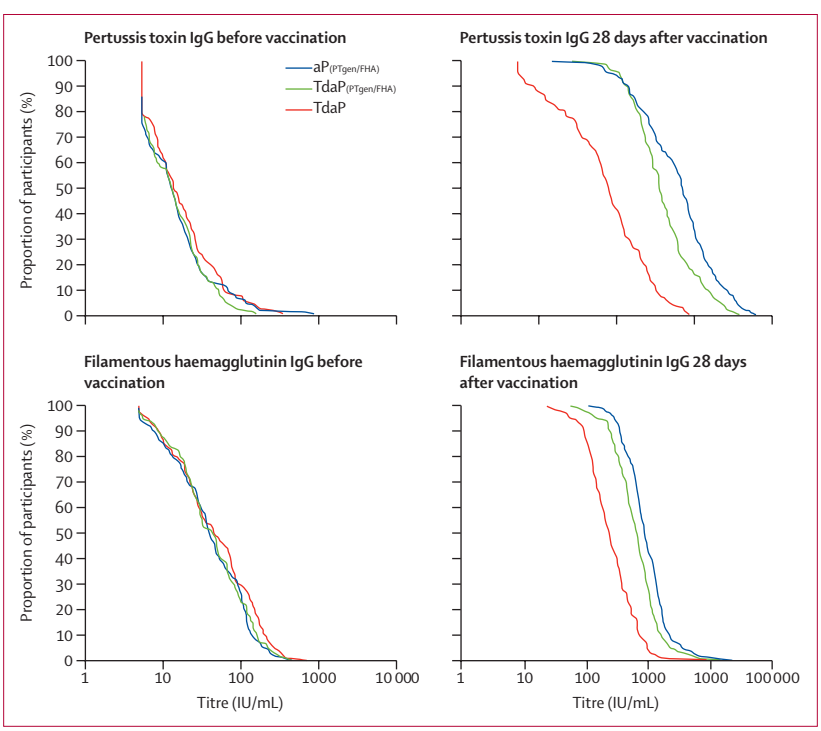

***Figure 3*: Reverse cumulative distribution curves for anti-pertussis toxin and anti-filamentous haemagglutinin IgG titres before and 28 days after vaccination**
$aP_{(PTgen/FHA)}$=acellular pertussis vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin. $TdaP_{(PTgen/FHA)}$=tetanus with reduced-dose diphtheria and acellular pertussis vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin. Tdap=tetanus with reduced-dose diphtheria and acellular pertussis combination vaccine.

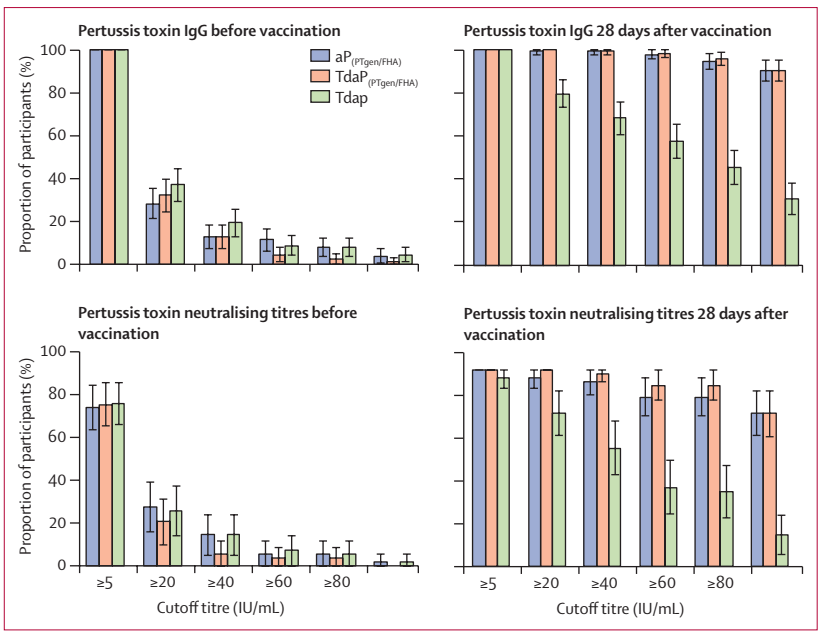

***Figure 4*: Proportion of participants with increasing cutoff titres of pertussis toxin ELISA IgG and pertussis toxin neutralising antibodies before and 28 days after vaccination**
Error bars show 95% CIs. $aP_{(PTgen/FHA)}$=acellular pertussis vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin. $TdaP_{(PTgen/FHA)}$=tetanus with reduced-dose diphtheria and acellular pertussis vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin. Tdap=tetanus with reduced-dose diphtheria and acellular pertussis combination vaccine.

adverse events were mostly mild in severity, except in the case of three (1%) participants: one participant vaccinated with $TdaP_{(PTgen/FHA)}$ reported severe nausea 5 days after vaccination that lasted for 1 day and resolved without sequelae; one participant vaccinated with $aP_{(PTgen/FHA)}$ had eye pain with onset on the day of vaccination, which was moderate in severity, lasted for 2 days, and resolved without sequelae; and one participant vaccinated with comparator Tdap reported headache of moderate severity that started 1 day after vaccination, lasted longer than 7 days, and resolved without sequelae.

During the 28 day observation period after vaccination, one serious adverse was reported in one (1%) participant in the $aP_{(PTgen/FHA)}$ group. The participant was involved in a motorbike accident 18 days after vaccination. This serious adverse event was reported as unrelated to the study vaccine administration by the investigator.

Discussion

Placebo-controlled efficacy trials for licensing of new paediatric acellular pertussis vaccines are no longer ethical. Therefore, WHO recommends that trials are designed to show the non-inferior safety and immunogenicity of a new vaccine to a licensed pertussis vaccine by comparing, as the primary outcome, vaccine-induced antibody responses and the proportion of responders (ie, the proportion of vaccinated participants with a significant increase in antibody concentration to greater than pre-immunisation titres) and, as the secondary outcome, functional antibody responses.[23] What the magnitude of this significant increase should be is not predefined. Most studies apply a four-fold versus a two-fold increase dependent on whether pre-vaccination titres are less or more than a predefined cutoff value. The criterion of a lower fold-increase is in particular applied in studies of booster responses in adolescents and adults who might have higher baseline titres due to previous pertussis vaccination and natural exposure than younger populations. Indeed, in our study all participants had baseline anti-pertussis toxin IgG titres of 5 IU/mL or more and roughly 30% had titres of 20 IU/mL or more. Nevertheless, we applied a stringent predefined criterion of a four-fold increase in antibody titres irrespective of baseline levels, rather than a two-fold increase as used in other studies. With this criterion, the primary objective of non-inferiority of pertussis toxin IgG and filamentous haemagglutinin IgG seroconversion rates for $TdaP_{(PTgen/FHA)}$ versus the comparator Tdap vaccine was met on the basis of a predefined 10% margin of equivalence. Our study also met its secondary objective to show non-inferior immunogenicity of $aP_{(PTgen/FHA)}$ versus Tdap with the same margin of equivalence. Results from this phase 2/3 trial led to the licensure of these two new-generation recombinant pertussis vaccines in Thailand as Pertagen ($aP_{(PTgen/FHA)}$) and Boostagen ($TdaP_{(PTgen/FHA)}$) for active booster immunisation in adolescents (aged ≥11 years) and adults.

A post-hoc analysis showed significantly higher seroconversion rates for $TdaP_{(PTgen/FHA)}$ and $aP_{(PTgen/FHA)}$ over the comparator. This finding confirms that a criterion of a four-fold increase discriminates well between the immunogenicity of the tested vaccines. The seroconversion rate we observed in the group vaccinated with the licensed Tdap vaccine was of the same magnitude as seroconversion rates previously observed for this vaccine in other trials (NCT01311557, NCT01689324). We additionally found that $TdaP_{(PTgen/FHA)}$ and $aP_{(PTgen/FHA)}$ induced significantly higher pertussis toxin and filamentous haemagglutinin IgG GMTs and pertussis toxin neutralising antibody titres than the comparator vaccine.

Correlates of protection are not established for pertussis vaccines; however, antibody responses to pertussis toxin are fundamental for acellular pertussis vaccine-induced protection against pertussis disease, and higher pertussis toxin antibody titres are likely to mean better protection.[24] This notion might also explain why pertussis toxin is the only pertussis component always included in any formulation of acellular pertussis vaccine so far commercialised.[1,17,25] In fact, a paediatric acellular pertussis vaccine containing only pertussis toxin and no other pertussis component has been used in Denmark since 1997, with no evidence of pertussis resurgence as yet. However, this vaccine contains a very high dose of pertussis toxin.[10] In all vaccines, pertussis toxin needs to be inactivated before it can be safely administered to human beings. Chemical treatment is mostly used,

	30 min after vaccination			p value	0–7 days after vaccination			p value
	$aP_{(PTgen/FHA)}$ (n=150)	$TdaP_{(PTgen/FHA)}$ (n=150)	Comparator Tdap (n=150)		$aP_{(PTgen/FHA)}$ (n=150)	$TdaP_{(PTgen/FHA)}$ (n=150)	Comparator Tdap (n=150)	
Local reactogenicity								
Pain	9 (6·0%, 2·2–9·8)	9 (6·0%, 2·2–9·8)	22 (14·7%, 9·0–20·3)	0·010*†	110 (73·3%, 66·3–80·4)	113 (75·3%, 68·4–82·2)	118 (78·7%, 72·1–85·2)	0·553†
Redness	1 (0·7%, 0·0–2·0)	0	6 (4·0%, 0·9–7·1)	0·019‡§	10 (6·7%, 2·7–10·7)	21 (14·0%, 8·5–19·6)	19 (12·7%, 7·3–18·0)	0·099†
Induration	0	0	0	..	6 (4·0%, 0·9–7·1)	19 (12·7%, 7·3–18·0)	12 (8·0%, 3·7–12·3)	0·024†¶
Systemic reactogenicity								
Fever (≥37·5°C)	1 (0·7%, 0·0–2·0)	0	0	1·000§	7 (4·7%, 1·3–8·0)	5 (3·3%, 0·5–6·2)	9 (6·0%, 2·2–9·8)	0·549†
Headache	2 (1·3%, 0·0–3·2)	1 (0·7%, 0·0–2·0)	4 (2·7%, 0·1–5·2)	0·515§	47 (31·3%, 23·9–38·8)	52 (34·7%, 27·1–42·3)	45 (30·0%, 22·7–37·3)	0·671†
Fatigue	0	0	2 (1·3%,0·0–3·2)	0·332§	57 (38·0%, (30·2–45·8)	52 (34·7%, 27·1–42·3)	71 (47·3%, 39·3–55·3)	0·068†
Arthralgia	0	0	0	..	36 (24·0%, 17·2–30·8)	32 (21·3%, 14·8–27·9)	36 (24·0%, 17·2–30·8)	0·819†
Chills	0	0	0	..	14 (9·3%, 4·7–14·0)	12 (8·0%, 3·7–12·3)	16 (10·7%, 5·7–15·6)	0·730†
Malaise	0	1 (0·7%, 0–2·0)	0	1·000§	34 (22·7%, 16·0–29·4)	38 (25·3%, 18·4–32·3)	44 (29·3%, 22·1–36·6)	0·414†
Myalgia	2 (1·3%, 0·0–3·2)	1 (0·7%, 0·0–2·0)	0	0·776§	68 (45·3%, 37·4–53·3)	76 (50·7%, 42·7–58·7)	88 (58·7%, 50·8–66·6)	0·067†
Vomiting	0	0	0	..	8 (5·3%, 1·7–8·9)	3 (2·0%, 0·0–4·2)	2 (1·3%, 0·0–3·2)	0·163§

$TdaP_{(PTgen/FHA)}$=tetanus with reduced-dose diphtheria and acellular pertussis vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin. $aP_{(PTgen/FHA)}$=acellular pertussis vaccine containing genetically inactivated pertussis toxin and filamentous haemagglutinin. Tdap=tetanus with reduced-dose diphtheria and acellular pertussis combination vaccine. *p=0·014 for $aP_{(PTgen/FHA)}$ versus Tdap and $TdaP_{(PTgen/FHA)}$ versus Tdap. †Overall p value (two sided) based on χ^2 test. ‡p=0·030 for $TdaP_{(PTgen/FHA)}$ versus Tdap. §Overall p value (two sided) based on Fisher's exact test. ¶p=0·007 for $aP_{(PTgen/FHA)}$ versus Tdap.

***Table 2*: Local and systemic reactions after vaccination**

but results in denaturation of the binding epitopes, whereas detoxification through recombinant technology conserves most or all epitopes. As a consequence, vaccination with chemically detoxified pertussis toxin induces antibodies that are impaired in their ability to bind and neutralise native pertussis toxin.[12,15] Indeed, in this trial we show that $TdaP_{(PTgen/FHA)}$ and $aP_{(PTgen/FHA)}$ induced significantly higher concentrations of pertussis toxin IgG and significantly higher anti-pertussis toxin neutralising antibody titres than the licensed comparator Tdap vaccine, thereby confirming that immunisation with genetically detoxified pertussis toxin results in higher quantitative and qualitative immune responses.[15]

Recombinant acellular pertussis vaccines are not new: a paediatric DTaP vaccine containing 5 µg of genetically detoxified pertussis toxin was evaluated in previous DTaP efficacy trials and shown to elicit high persisting antibody responses in infants and sustained efficacy over a 6 year period similar to another DTaP vaccine containing a higher dose (25 µg) of chemically detoxified pertussis toxin.[26] This paediatric DTaP vaccine was commercialised in many countries until 2001. Although acellular pertussis vaccines used in adolescents and adults contain a lower dose of pertussis antigens than paediatric formulations to limit reactogenicity, the 5 µg of genetically inactivated pertussis toxin contained in BioNet's recombinant acellular pertussis-based vaccines were shown to elicit high levels of antibodies against pertussis antigens (pertussis toxin and filamentous haemagglutinin) while having little reactogenicity, similar to Tdap vaccine.

Pertussis antigens other than pertussis toxin might have a role in vaccine protection. Filamentous haemagglutinin is present in nearly all acellular pertussis vaccines, while some acellular pertussis vaccines also include pertactin and fimbriae types 2 and 3. Although, according to some systematic reviews,[27,28] high-component acellular pertussis vaccines seem to have slightly higher short-term efficacy than one-component or two-component acellular vaccines, the experience of large-scale and long-term use of low-component acellular pertussis vaccines shows that these vaccines are highly effective in preventing pertussis disease. In a position paper on pertussis vaccines,[7] WHO concluded that evidence was not sufficient to establish any substantial difference in vaccine effectiveness of acellular pertussis vaccines on the basis of differing numbers of components. Furthermore, increases in pertactin-negative *B pertussis* isolates in several countries[29,30] have led to debate about the inclusion of antigens, such as pertactin, in new acellular pertussis formulations. Therefore, the quality and amount of pertussis antigens included in a vaccine can be as important or even more important for effectiveness and long-lasting protection against pertussis disease as the number of pertussis antigens included in a vaccine.[15]

Our study has some limitations. First, the population included had been primed in infancy with whole-cell pertussis vaccines and data cannot be extrapolated to other settings where adolescents have been primed with acellular pertussis vaccines. Although children worldwide are still vaccinated with whole-cell pertussis vaccines, in countries that have shifted to acellular pertussis vaccines in the late 1990s or early 2000s, first cohorts of exclusively acellular pertussis-vaccinated individuals are reaching adulthood. It will be important to show that this new-generation pertussis vaccine also induces high immune responses in these populations.

Second, we included Adacel as a comparator, meaning that it is not known how the BioNet vaccines perform compared with Boostrix—a licensed Tdap vaccine with a higher pertussis toxin content. A trial comparing $aP_{(PTgen/FHA)}$ with Boostrix in an adolescent population that had been previously immunised with five doses of conventional acellular pertussis vaccines is underway in Switzerland (NCT02946190). Finally, although antibody responses 1 month after vaccination were significantly higher with $TdaP_{(PTgen/FHA)}$ or $aP_{(PTgen/FHA)}$ than with the licensed comparator Tdap vaccine, no conclusions can be drawn about the persistence of vaccine-induced immunogenicity on the basis of these results. As part of the protocol, 1 year follow-up studies are underway to show the persistence of immune responses in participants vaccinated with $aP_{(PTgen/FHA)}$ or $TdaP_{(PTgen/FHA)}$.

No available monovalent pertussis vaccines can be used when vaccination against diphtheria and tetanus is not required. In fact, all current acellular pertussis vaccines available for use in adolescents or adults are combined with diphtheria and tetanus vaccines. This could be a particular concern in immunisation of pregnant women, in view of the growing recommendation to repeat immunisation with acellular pertussis vaccine during each pregnancy.[8,9] This recommendation means that with no monovalent pertussis vaccine available, women might receive repeated Tdap vaccination with fewer than 2 years between each vaccination. In high-income countries, tetanus–diptheria vaccination is generally recommended to be repeated only every 10 years, whereas in low-income countries, tetanus vaccination might be recommended during each pregnancy to prevent neonatal tetanus. Therefore, the availability of recombinant monovalent pertussis vaccines that induce high antibody responses can provide the medical community with a new way to approach pertussis immunisation when only boosting pertussis immunity is sought, and to address an unmet medical need for repeated maternal immunisation to protect newborn babies against pertussis only.

Contributors

SV, PHT, and JP were responsible for the conceptualisation and design of the study. SS, CS, KC, PP, JD, AP, WP, KB, KL, YS, and OW conducted the study. SS and PP were the principal investigators at the study sites in Mahidol University: Department of Pediatrics, Faculty of Medicine Siriraj Hospital (SS) and the Vaccine Trial Centre, Faculty of Tropical Medicine (PP). PC was responsible for study coordination and supervision. IKP was responsible for designing and overseeing the immunological assays. All authors contributed to data interpretation. SS and SV prepared the first draft of the manuscript. All authors reviewed, commented on, and approved the final manuscript for submission.

Declaration of interests

PC, IKP, JP, PHT, and SV are employed by BioNet-Asia. All other authors declare no competing interests.

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