The effect of pertussis vaccination in pregnancy on the immunogenicity of acellular or whole-cell pertussis vaccination in Gambian infants (GaPS): a single-centre, randomised, controlled, double-blind, phase 4 trial

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Summary

Background Vaccinating women against pertussis in pregnancy protects young infants from severe disease and death. Vaccination-induced maternally derived antibodies, however, might subsequently modulate (and specifically blunt) the infant's serological response to their primary series of pertussis vaccinations. We examined the effect of pertussis immunisation in pregnancy on the immunogenicity of primary acellular or whole-cell pertussis vaccines in a west African cohort.

Methods GaPs was a randomised, controlled, double-blind, phase 4 trial conducted in The Gambia. We used a predefined block randomisation scheme to randomly assign healthy, HIV-negative, pregnant participants (1:1) to receive a pertussis-containing (tetanus-diphtheria-acellular pertussis-inactivated polio virus [Tdap-IPV]) or tetanus-toxoid only vaccine at 28–34 weeks' gestation. At the same time, their infants were randomly assigned (1:1) to receive diphtheria-tetanus-acellular pertussis (DTaP) or diphtheria-tetanus-whole-cell pertussis (DTwP) primary vaccine at 8, 12, and 16 weeks postnatally. Participants and trial staff were masked to the allocation of the maternal vaccine. The field team and participants became unmasked to the allocation of the infant vaccine at 16 weeks; laboratory staff and all other investigators remained masked to infant vaccine allocation until the end of the trial. The primary outcome was geometric mean concentration (GMC) of infant pertussis toxin-specific antibodies at 20 weeks and 9 months postnatally and was assessed in infants who received all three doses of the primary vaccine. Secondary outcomes included memory B-cell responses, and exploratory outcomes were total pertussis-specific antibody binding concentrations and functional antibody titres (pertussis toxin-specific neutralising activity [PTNA] and serum bactericidal activity [SBA]). Vaccine reactogenicity was assessed in mothers and infants for 3 days after each vaccine dose. Pregnant women had an extra safety visit 7 days after vaccination. The study is registered with ClinicalTrials.gov, NCT03606096.

Findings Between Feb 13, 2019, and May 17, 2021, we enrolled 343 maternal–infant pairs. 239 (77%) infants were included in the per-protocol immunogenicity analysis. Among infants of mothers receiving Tdap-IPV in pregnancy, at 20 weeks postnatally, the GMCs of anti-pertussis toxin IgG were more than three-fold lower in infants vaccinated with three doses of DTwP (n=64) than in infants vaccinated with three doses of DTaP (n=53; adjusted geometric mean ratio 0·28, 98·75% CI 0·16–0·50). This difference persisted up to 9 months (0·31, 0·17–0·55). Conversely, among infants born to tetanus toxoid-immunised mothers, post-vaccination GMCs of anti-pertussis toxin IgG at 9 months were higher in those vaccinated with DTwP (n=58) than in those vaccinated with DTaP (n=64; 2·02, 1·15–3·55). Tdap-IPV immunisation in pregnancy blunted anti-pertussis toxin IgG following primary vaccination in all infants but particularly in those receiving DTwP, with GMCs of anti-pertussis toxin IgG more than eight-fold lower in DTwP-vaccinated infants born to Tdap-IPV-vaccinated mothers than in DTwP-vaccinated infants born to tetanus toxoid-immunised mothers (0·12, 98·75% CI 0·07–0·22 at 20 weeks; 0·07, 0·03–0·17 at 9 months). Similarly, DTwP-vaccinated infants born to Tdap-IPV-vaccinated mothers also showed significant blunting of PTNA, SBA, total pertussis-specific antibody binding, and memory B-cell responses after primary immunisation, whereas minimal blunting was observed among DTaP-vaccinated infants. However, the absolute levels of these responses generated by DTwP-vaccinated infants remained similar to or, in many cases, were higher than those generated by DTaP-vaccinated infants. There was no difference in reactogenicity between the two maternal vaccines, with most reactions graded 0 or 1. There were no serious adverse events related to vaccination or trial participation.

Interpretation Vaccinating women with Tdap-IPV in pregnancy was safe and well tolerated in a sub-Saharan African setting and boosted the quantity and quality of pertussis-specific antibodies in infants in early life. Although Tdap-IPV was associated with relative blunting of the immune response to the DTwP primary vaccination series, pertussis-specific

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antibody quality and memory B-cell responses were nevertheless preserved, regardless of the vaccine given during pregnancy.

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See Online for appendix

Introduction

Despite the availability of vaccines against pertussis (whooping cough) for over a century, effective control of this important respiratory disease remains an outstanding challenge worldwide because of the particularly high risk of morbidity and mortality it poses in children younger than 1 year.[1] Since late 2023, there has been a marked resurgence in pertussis across Europe, Asia, North America, and Australia, with a sharp increase in infant hospitalisation and deaths.[2] The reasons for this resurgence are multifactorial, but it has occurred against the background of a steady increase in pertussis cases over the last three decades, correlating in many high-income countries with the switch in infant primary immunisation from whole-cell to acellular pertussis vaccination schedules. This change was due to concerns over whole-cell pertussis-induced reactogenicity, although many of the alleged severe neurological complications have since been disproven. Acellular pertussis vaccines confer excellent protection against severe complications and death, but data from epidemiological studies and animal models have convincingly shown that these vaccines induce a shorter duration of immunity than do whole-cell pertussis vaccines and are less protective against upper airway *Bordetella pertussis* infection and transmission.[3] Contributing factors to shortened immunity and reduced protection against infection include differences in vaccine formulation, such as the number of antigens and adjuvants included. Whole-cell pertussis vaccines consist of inactivated whole *B pertussis* cells, whereas acellular pertussis subunit vaccines contain purified, detoxified *B pertussis* antigens including at least pertussis toxin, alone or combined with filamentous haemagglutinin and pertactin, with or without FIM2 and FIM3. Nevertheless, *B pertussis* is one of the most contagious human pathogens, and there is strong evidence that it continues to circulate even in whole-cell pertussis-primed populations with high vaccination rates.[4] These populations include those in many low-income and middle-income countries (LMICs), where whole-cell pertussis vaccination is recommended as part of the WHO's routine Expanded Programme of Immunization (EPI) schedule. Previously, we showed a waning of vaccine-induced immunity in The Gambia, with probable community circulation and reinfection;[5] other countries in sub-Saharan Africa have also shown much higher rates of *B pertussis* colonisation, household transmission, and ongoing circulation than previously appreciated among vaccinated populations.[6]

Due to increasing numbers of cases and infant deaths in the early 2010s, many countries introduced pertussis immunisation programmes for pregnant women to provide passive protection against disease to newborns immediately from birth, thereby bridging the period of vulnerability until primary vaccination.[7] Pertussis in infants has declined substantially following the introduction of antenatal immunisation, with estimates of the reduction in disease risk ranging from 70% to 95%.[8] Nonetheless, there is evidence that maternal antibodies might modulate—and specifically reduce or blunt—the infant's serological response to subsequent heterologous and homologous vaccine antigens.[9,10] This effect might differ according to the infant vaccine type used, with previous studies suggesting more marked blunting after vaccination with whole-cell pertussis than after vaccination with acellular pertussis.[11,12] To date, few studies have systematically assessed the effect of vaccination in pregnancy on infant pertussis-specific functional antibodies and memory B-cell responses, comparing infants vaccinated with either acellular or whole-cell pertussis. Importantly, without an internationally validated serological correlate of protection, the clinical significance of blunted antibody concentrations remains unclear.[13]

Here, we report the safety and reactogenicity of acellular pertussis vaccination in pregnancy in the sub-Saharan African setting and the immunogenicity of acellular versus whole-cell pertussis vaccination in infants in the presence or absence of pertussis immunisation in pregnancy.

Methods

Study design and participants

The Gambian Pertussis Study (GaPs) was a single-centre, randomised, controlled, double-blind, phase 4 trial in pregnant women combined with an open-label, randomised, controlled, phase 4 clinical trial in their infants, conducted by the Medical Research Council (MRC) Unit The Gambia at the London School of Hygiene & Tropical Medicine and part of the PERISCOPE (PERtussis Correlates Of Protection Europe) consortium.[14] The trial was conducted in two urban, governmental health-care facilities (5 km apart) in Sukuta and Faji Kunda

Research in context

Evidence before this study

We searched PubMed to identify articles in English published from database inception to Sept 1, 2024, using appropriate Boolean operators with the following search terms: "pertussis vaccine", "acellular", "whole-cell", "DTP", "infant", "maternal", "pregnancy", "randomised controlled trial", "clinical trial", "immunisation", "immunogenicity", and "antibody". There were no published randomised controlled trials in any low-income or middle-income country comparing the effect of pertussis vaccination in pregnancy versus the standard of care on infants' immune responses to the primary immunisation series with whole-cell or acellular pertussis vaccines. A randomised clinical trial conducted in 2020 with Thai maternal–infant pairs showed that infants who were born to tetanus-diphtheria-acellular pertussis (Tdap)-vaccinated mothers and received three doses of whole-cell pertussis vaccine at 2, 4, and 6 months showed a more marked reduction or blunting of post-vaccination IgG responses to *Bordetella pertussis* antigens (pertussis toxin and filamentous haemagglutinin) compared with infants who received the acellular pertussis primary schedule. However, the absence of a maternal control group in this study limited systematic comparison. Recently, Haidara and colleagues published the first trial to assess pertussis immunisation in pregnancy in a sub-Saharan setting. They reported Tdap-blunted responses for anti-pertussis toxin and anti-pertactin (but not anti-filamentous haemagglutinin) IgG concentrations in Malian infants 1 month after primary whole-cell pertussis immunisation at 6, 10, and 14 weeks. Responses following acellular pertussis primary immunisation have not been compared in this setting. The short-term and long-term implications of blunting remain unclear, partly because we do not yet have an internationally accepted immunological correlate of protection. Therefore, a comprehensive evaluation of the effect of pertussis vaccination in pregnancy on infant immunity that includes functional antibodies and memory-cell responses and compares acellular versus whole-cell pertussis priming series would be informative and help to optimise maternal immunisation guidelines and infant vaccination schedules.

Added value of this study

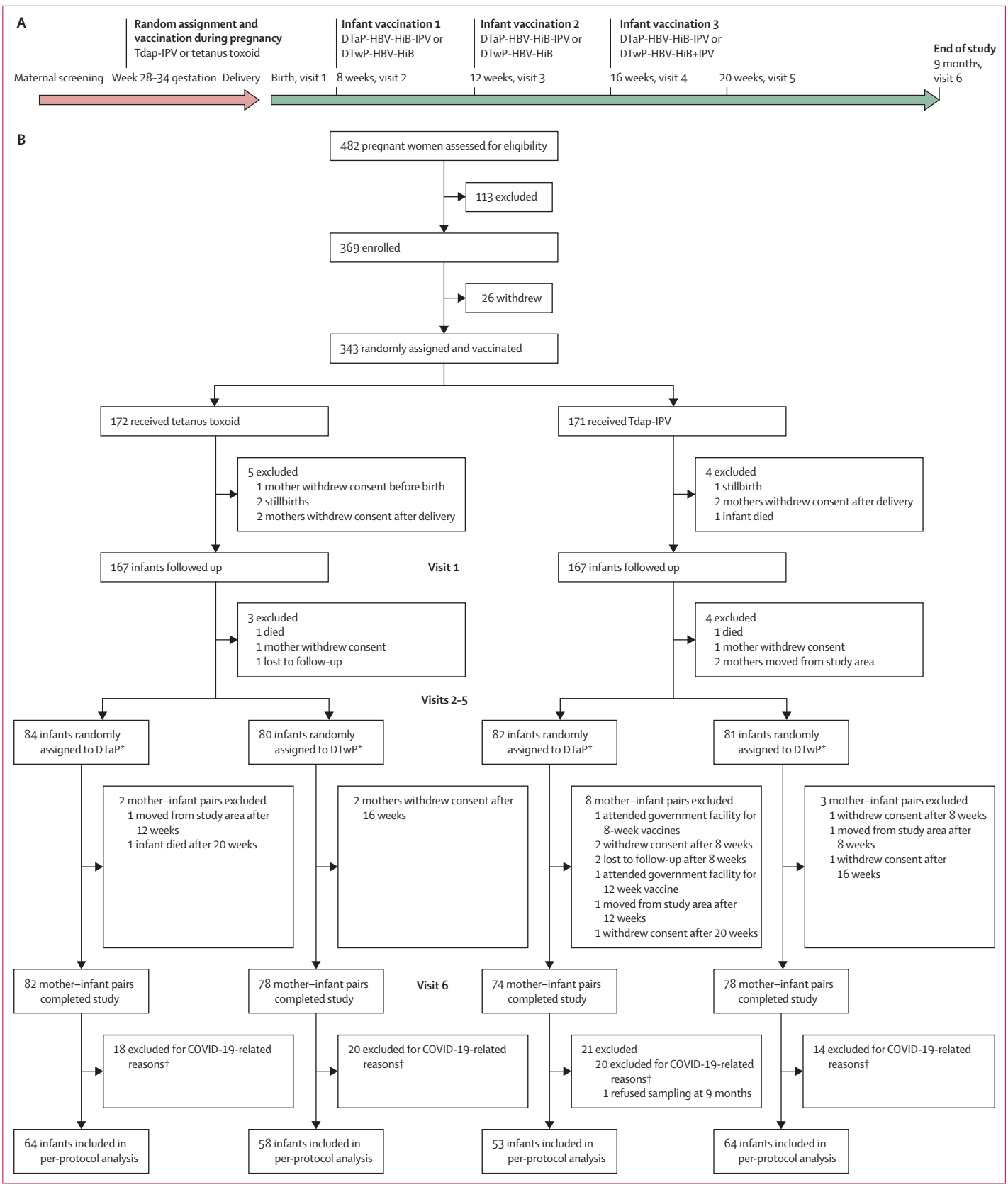
To our knowledge, this randomised phase 4 clinical trial is the first to have characterised the interaction between immunisation in pregnancy and primary vaccination with different pertussis vaccine types, particularly in a sub-Saharan African setting. Our study is also unique in comprehensively profiling pertussis-specific antibody responses in a large west African infant cohort, with the generation of robust quantitative data and evaluation of the antibodies' functional capacities, such as pertussis toxin neutralisation and bactericidal activity. Additionally, we evaluated longer-term vaccine-induced memory B-cell responses. Above all, we showed that vaccinating women with Tdap-inactivated polio virus (Tdap-IPV) in pregnancy in a sub-Saharan African setting is safe and well tolerated, and boosts both the quantity and quality of pertussis-specific antibodies in infants before primary vaccination. Following primary immunisation of the infants at 8, 12, and 16 weeks with diphtheria-tetanus-whole-cell pertussis (DTwP) vaccine or diphtheria-tetanus-acellular pertussis (DTaP) vaccine, we observed blunting of vaccine-specific responses dependent on the infant vaccine given, timepoint of sampling, and antigen-specific or functional response measured. In general, infants who received DTwP, particularly those born to mothers given a tetanus-toxoid only vaccine in pregnancy, had the strongest functional and memory B-cell responses. The findings are consistent with the current hypothesis that both DTaP and DTwP vaccines protect against severe disease, but DTwP vaccines induce a longer duration of protection and are more effective at preventing infection and transmission.

Implications of all the available evidence

The immunogenicity and safety data generated in the GaPs trial support existing encouraging clinical effectiveness data on pertussis immunisation in pregnancy as a successful strategy to prevent morbidity and death in young infants. Our finding that DTwP-vaccinated infants born to tetanus toxoid-vaccinated mothers consistently had the highest quantity, quality, and persistence of most vaccine-specific responses is reassuring in the sub-Saharan African context, where tetanus toxoid and DTwP vaccines are routinely used within national immunisation programmes. We also observed minimal or inconsistent blunting of DTaP-induced responses following pertussis vaccination in pregnancy, the combination currently used predominantly in high-income countries. However, infants who were born to Tdap-IPV-vaccinated mothers and subsequently received DTwP as their primary vaccine showed a marked reduction of almost all pertussis-specific and functional antibody responses compared with infants born to tetanus toxoid-vaccinated mothers. Nevertheless, even in the presence of blunting, antibody quality and memory-cell responses generated by DTwP-vaccinated infants were still comparable to or even higher than those in DTaP-vaccinated infants. The clinical implications remain unclear but need to be further explored when considering introducing antenatal pertussis immunisation programmes in sub-Saharan Africa.

in The Gambia, a low-income country in west Africa (2021 Human Development Index ranking 174). Full details of the trial protocol have been published previously.[15]

We included healthy, HIV-negative pregnant women, aged 18–40 years. Pregnant women with a high risk of pregnancy-related or other health-related complications were excluded (a full list of inclusion and exclusion criteria is in the protocol;[15] reasons for exclusion are also outlined in the appendix [p 8]). None of the infants born to enrolled mothers were excluded from the study unless the family withdrew or were no longer able to participate.


A
Random assignment and vaccination during pregnancy
Tdap-IPV or tetanus toxoid
Infant vaccination 1
DTaP-HBV-HiB-IPV or DTwP-HBV-HiB
Infant vaccination 2
DTaP-HBV-HiB-IPV or DTwP-HBV-HiB
Infant vaccination 3
DTaP-HBV-HiB-IPV or DTwP-HBV-HiB+IPV
End of study
9 months, visit 6
Maternal screening
Week 28–34 gestation
Delivery
Birth, visit 1
8 weeks, visit 2
12 weeks, visit 3
16 weeks, visit 4
20 weeks, visit 5
B
482 pregnant women assessed for eligibility
113 excluded
369 enrolled
26 withdrew
343 randomly assigned and vaccinated
172 received tetanus toxoid
171 received Tdap-IPV
5 excluded
1 mother withdrew consent before birth
2 stillbirths
2 mothers withdrew consent after delivery
4 excluded
1 stillbirth
2 mothers withdrew consent after delivery
1 infant died
167 infants followed up
Visit 1
167 infants followed up
3 excluded
1 died
1 mother withdrew consent
1 lost to follow-up
4 excluded
1 died
1 mother withdrew consent
2 mothers moved from study area
Visits 2–5
84 infants randomly assigned to DTaP*
80 infants randomly assigned to DTwP*
82 infants randomly assigned to DTaP*
81 infants randomly assigned to DTwP*
2 mother–infant pairs excluded
1 moved from study area after 12 weeks
1 infant died after 20 weeks
2 mothers withdrew consent after 16 weeks
8 mother–infant pairs excluded
1 attended government facility for 8-week vaccines
2 withdrew consent after 8 weeks
2 lost to follow-up after 8 weeks
1 attended government facility for 12 week vaccine
1 moved from study area after 12 weeks
1 withdrew consent after 20 weeks
3 mother–infant pairs excluded
1 withdrew consent after 8 weeks
1 moved from study area after 8 weeks
1 withdrew consent after 16 weeks
82 mother–infant pairs completed study
78 mother–infant pairs completed study
Visit 6
74 mother–infant pairs completed study
78 mother–infant pairs completed study
18 excluded for COVID-19-related reasons†
20 excluded for COVID-19-related reasons†
21 excluded
20 excluded for COVID-19-related reasons†
1 refused sampling at 9 months
14 excluded for COVID-19-related reasons†
64 infants included in per-protocol analysis
58 infants included in per-protocol analysis
53 infants included in per-protocol analysis
64 infants included in per-protocol analysis

The trial was registered with ClinicalTrials.gov (NCT03606096) and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Approval was given by the Gambian Government and MRC The Gambia Joint Ethics Committee, the London School of Hygiene & Tropical Medicine's Research Ethics Committee, and the Gambian Medicines Control Agency. It was sponsored by the London School of Hygiene & Tropical Medicine. Participants provided written or thumb-printed informed consent for themselves and their infants before any study-related procedures took place, with an impartial witness present for non-literate or non-English speaking women.

Randomisation and masking

Using a predefined block (n=4) randomisation scheme devised by an independent biostatistician, eligible pregnant women were randomly assigned (1:1) to receive either tetanus toxoid vaccine or tetanus-diphtheria-acellular pertussis-inactivated poliovirus (Tdap-IPV) vaccine. The same procedure was used to simultaneously randomly assign (1:1) their infants to receive either diphtheria-tetanus-acellular pertussis (DTaP) vaccine or diphtheria-tetanus-whole-cell pertussis (DTwP) vaccine. Thus, there were four infant groups: infants born to Tdap-IPV-vaccinated mothers and receiving DTaP (Tdap-IPV–DTaP group), infants born to Tdap-IPV-vaccinated mothers and receiving DTwP (Tdap-IPV–DTwP group), infants born to tetanus toxoid-vaccinated mothers and receiving DTaP (tetanus toxoid–DTaP group), and infants born to tetanus toxoid-vaccinated mothers and receiving DTwP (tetanus toxoid–DTwP group). Vaccine assignments were allocated to sequentially numbered, sealed, opaque, tamper-evident envelopes with a unique four-digit code and a validation letter (Damm algorithm). Unmasked nurses implemented randomisation by opening the next envelope in the sequence and subsequently administered the vaccines in the same way. Participants and all other trial staff were masked to the allocation of the maternal vaccine throughout the entire trial. The field team and participants became unmasked to the allocation of the infant vaccine at 16 weeks because an additional dose of inactivated polio virus (IPV) needed to be given to DTwP groups only as part of the Gambian EPI schedule (IPV is already included in the study DTaP vaccine). Laboratory staff and all other investigators remained masked to infant vaccine allocation until the end of the trial.

Figure 1: **Trial design and study profile**
(A) Figure created using Biorender.com. All infants on the pentavalent DTwP schedule also received IPV as a single vaccine dose at the 16 week visit (as per the Gambian national immunisation schedule; not required for DTaP-vaccinated infants because the combined hexavalent vaccine already contains IPV). Full details of vaccine content and schedule of trial visits are in the appendix (pp 3–4). (B) Study profile. DTaP=diphtheria-tetanus-acellular pertussis combined infant vaccine. DTwP=diphtheria-tetanus-whole cell pertussis combined infant vaccine. HBV=hepatitis B vaccine. HiB=*Haemophilus influenzae* type B. IPV=inactivated polio virus. Tdap-IPV=tetanus-diphtheria-acellular pertussis-inactivated polio virus combined maternal vaccine. *Random assignment of infant vaccine was done at the same time as random assignment of maternal vaccine. †COVID-19-related reasons for infant exclusion: receiving mixed DTaP and DTwP vaccines; primary immunisation completed but under the Expanded Programme of Immunisation schedule; not following randomised group allocation; follow-up at study visit timepoint but no sampling done; study visit or sampling outside of protocol window.

Procedures

At the first study visit, written informed consent was obtained from women who had been made aware of the study early in pregnancy and at least 24 h before the formal recruitment period (sensitised pregnant women), and screening was done (including a dating ultrasonography scan) to assess study eligibility. Eligible pregnant women were randomly assigned and vaccinated at their second visit, between 28 weeks' and 34 weeks' gestation. Participants received either tetanus toxoid vaccine (Serum Institute of India, Pune, India) or the combined Tdap-IPV vaccine, Boostrix-IPV (GSK, London, UK). Their infants received either pentavalent whole-cell-containing DTwP-HBV-HiB (Serum Institute of India, Pune, India) or hexavalent acellular pertussis-containing DTaP-HBV-HiB-IPV (Infanrix Hexa; GSK, London, UK) combination vaccines, as part of the EPI schedule at 8, 12, and 16 weeks (referred to as DTwP and DTaP, respectively, throughout; figure 1A). Antenatal tetanus toxoid and infant DTwP are part of the routine vaccination programmes in The Gambia; Boostrix-IPV (administered to pregnant women) and Infanrix Hexa (administered to infants) were considered investigational products (vaccine content is summarised in the appendix [p 2]). Routine antenatal care continued to be provided by the government-run antenatal services working alongside a delegated member of the clinical trial team. At each visit up to and including the delivery and then again at the final study visit (9 months postnatally), the mother had a symptom review and physical assessment, with or without an obstetric assessment. Each infant had a detailed examination at birth and then a clinical assessment, including anthropometry and vital signs, at each subsequent visit up to 9 months postnatally. The complete GaPs schedule is summarised in the appendix (p 3).

Solicited local and systemic adverse events were recorded for mothers and infants once per day for 3 days after each study vaccination during home visits conducted by trained field workers, with an additional visit for participants 7 days after vaccination during pregnancy. Solicited adverse events were graded for severity from mild (grade 1) to severe (grade 4). In line with standardised protocols, unsolicited adverse events were categorised by study clinicians using the Medical Dictionary for Regulatory Activities preferred terms,[16] graded for severity (1–5), assessed for expectedness and relatedness to the study vaccines, and subsequently managed.

Blood samples for immunological assays were collected longitudinally from each infant at birth (cord or peripheral blood sample), before the infants' first vaccine dose at age 8 weeks and after completion of the primary immunisation series at 20 weeks and 9 months postnatally. Full details of the immunological endpoint assays are described in the appendix (pp 4–6). Briefly, serum concentrations of IgGs against pertussis toxin, filamentous haemagglutinin, pertactin, FIM2, FIM3, diphtheria toxoid, and tetanus toxoid were quantified in cord samples and 8-week, 20-week, and 9-month infant samples using a fluorescent bead-based multiplex immunoassay.[17–19] At 8 weeks, 20 weeks, and 9 months, infant serum samples were also assessed for serum bactericidal activity (SBA) against *B pertussis* strain B1917, a clinical isolate that is representative of currently circulating strains,[20] and for pertussis toxin neutralising activity (PTNA) using Chinese hamster ovary cells.[21,22] In addition, total antibody binding by serum IgG deposition on *B pertussis* strain B1917 was assessed at birth (cord), 8 weeks, 20 weeks, and 9 months by flow cytometry.[23]

To measure vaccine-induced memory B-cell responses at 8 weeks, 20 weeks, and 9 months, peripheral blood mononuclear cells (PBMCs) were isolated from anticoagulated whole blood by density gradient centrifugation, as described previously.[24] We quantified pertussis-specific IgG-secreting memory B cells using fresh PBMCs with a protocol developed by the PERISCOPE consortium[25] that was adapted to this Gambian infant cohort.

Outcomes

There is no validated immunological correlate of protection against pertussis disease, but serum pertussis toxin-specific IgG is considered crucial in limiting disease severity.[26,27] In GaPs, the primary immunological outcome was geometric mean concentration (GMC) of anti-pertussis toxin IgG in infant serum at 20 weeks and 9 months, following three primary doses of DTaP versus DTwP, comparing the four infant vaccine groups. Secondary outcomes included comparisons of GMCs of serum IgGs specific to other vaccine antigens (ie, filamentous haemagglutinin, pertactin, FIM2 and FIM3, diphtheria toxoid, and tetanus toxoid) and geometric mean frequency of vaccine-specific memory B cells (other secondary outcomes not reported in this Article are outlined in the appendix [pp 6–7]). An exploratory outcome was assessment of the quality of pertussis-specific immune responses, specifically quantifying pertussis-specific functional antibodies (eg, antibodies with PTNA or SBA and total antibody binding).

Given that pertussis vaccination in pregnancy is not a routine policy in The Gambia, the following safety and reactogenicity endpoints were recorded in all vaccinated women: number and relatedness of serious adverse events in pregnant women from enrolment at 28–34 weeks' gestation up to 8 weeks from the end of pregnancy, and proportion of mothers between 28 weeks' and 34 weeks' gestation with local and systemic reactogenicity within the first 7 days of vaccination. A data safety monitoring board reviewed safety data and trial conduct throughout the duration of the study.

Statistical analysis

The primary study objective was to compare the GMCs of anti-pertussis toxin IgG in infants vaccinated with DTwP versus those in infants vaccinated with DTaP (ie, geometric mean ratio [GMR]) for both maternal groups at 20 weeks and 9 months (four tests). We calculated that a sample size of 127 infants per group had 80% power to detect a significant difference for a GMR of 1·3, equivalent to a Cohen's d-effect size of 0·4215. To allow for the test multiplicity, the power was calculated assuming a Bonferroni correction of 4. Assuming a participant attrition rate of 15%, we calculated a requirement for 150 infants per group and a total sample size of 600. This number was not reached at trial completion due to COVID-19 restrictions and national lockdown in The Gambia in 2020–21. The primary immunogenicity analysis was, therefore, carried out on the per-protocol population only—ie, infants who received all three doses of the primary vaccine and had samples collected for assessment of the primary immunological endpoint within the appropriate time windows, as outlined in the protocol. For safety and reactogenicity analysis, all mothers who received a study vaccine were included, as well as their infants.

Continuous variables were summarised descriptively with the mean and SD or median and range (or both). Categorical variables were presented as frequency counts and percentages. The observed GMC, geometric mean titre (GMT), geometric mean frequency, and two-sided 95% CI of the immune response parameters were reported for each infant group and timepoint.

Mixed-effect maximum likelihood regression models were fitted to the $\log_{10}$-transformed serological and memory B-cell data, with fixed factor effects for visit (baseline [8 weeks], 20 weeks, and 9 months), the four combinations of maternal and infant vaccinations, and the visit*vaccination interaction. Appropriate contrasts were used to compare infant responses between the 20 week and 9 month visits across vaccine groups. Infant identifier was fitted as a random effect and to allow for correlation between the three consecutive visits; the best fitting model was an unstructured correlation (compared with independent, exchangeable, and spherical power structures). The covariates, interval between vaccination in pregnancy to birth, birthweight, and sex of the infant were each tested as univariate fixed effects in the above model. At the 5% level, none of these terms were significant for any of the responses, and no multivariable covariate models were tested

(appendix pp 12, 20). Consequently, none of the models were adjusted for these covariates.

Model fits were manually assessed via diagnostic plots of residuals versus fitted values. 13% of anti-FIM IgG data, 33% of SBA data, 27% of anti-pertussis toxin memory B-cell measurements, and 22% of filamentous haemagglutinin memory B-cell measurements were below the sensitivity threshold. Therefore, a zero-inflated version of the above mixed model was used.[28] The algorithm created a two-part model whereby logistic regression was used to model the proportion of data below the limit of detection; data above the limit of detection were modelled with the mixed-effects model described previously. Two-sided p values were calculated for each post-vaccination group comparison, adjusted by Bonferroni correction for a multiplicity of 4; to be consistent, 98·75% CIs were reported for the adjusted GMRs. CIs were calculated using the SD estimate of the fixed effect from the mixed model of the $\log_{10}$-transformed data, assuming a t-distribution. The intervals were back-transformed to obtain intervals for the GMRs. Persistence of change was tested using the p value of the vaccine (maternal Tdap-IPV:tetanus toxoid or infant DTwP:DTaP)*(9 months:20 weeks) interaction (appendix pp 13–16, 21–22). PTNA-to-anti-pertussis toxin IgG ratios were compared between the four intervention groups using Kruskal-Wallis testing followed by Dunn's test for multiple comparisons. No adjustment for multiplicity was done for the group comparisons of memory B-cell responses or ratios or the longitudinal serological and memory B-cell responses analyses, given that these were not primary trial outcomes.

The association between antibody parameters was examined using an analysis of parallelism across vaccine groups. As the response and predictor in this analysis were antibody concentrations or titres, an errors-in-variable (Deming) regression was used. All statistical analyses were conducted using R (version 4.1.3).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Feb 13, 2019, and May 17, 2021, 482 pregnant women were assessed for eligibility for inclusion in this study. 343 (71%) women were enrolled and randomly assigned to receive tetanus toxoid (n=172) or Tdap-IPV (n=171) vaccines at 28–34 weeks' gestation (figure 1A); 113 (23%) did not meet the eligibility criteria (appendix p 8). The infants of the vaccinated participants subsequently commenced either DTwP (n=161) or DTaP (n=166) primary vaccination schedules at 8 weeks postnatally. There was a 9% attrition rate, with 312 mother–infant pairs completing the study. Outcomes were assessed in 239 (77%) infants for whom vaccination and sampling were completed as scheduled (per-protocol immunogenicity population; figure 1B), with a fairly even distribution across the four infant groups. Tables 1 and 2 show the baseline characteristics in the two maternal groups and four infant groups, respectively. The median age of pregnant women in the per-protocol population was 26·38 (18·11–40·06) years. 128 (54%) female and 111 (46%) male infants were born, with a mean gestation of 39·16 weeks (SD 1·5) and a mean birthweight of 2·95 kg (0·39). No infants in this population were born very or extremely premature (<34 weeks), and there were no babies of very low birthweight (<1·5 kg).

	Tdap-IPV (n=117)	Tetanus toxoid (n=122)
Maternal age, years		
Median (range)	26·47 (18·11–39·68)	26·3 (18·62–40·06)
Mean (SD)	27·13 5·07)	26·92 (4·64)
Maternal parity, n		
Median (range)	1 (0–4)	1 (0–4)
Mean (SD)	1·5 (1·34)	1·6 (1·31)
Maternal BMI at M01, kg/m²		
Median (range)	23·57 (16·5–38·69)	24·3 (14·7–37·62)
Mean (SD)	24·12 (4·14)	24·29 (3·92)
Maternal haemoglobin at M01, g/dL	10·55 (0·99)	10·72 (1·07)
Gestation at vaccination (M02), weeks	29·82 (1·63)	29·69 (1·5)
Interval between vaccination (M02) and delivery, days	65·74 (15·09)	65·86 (14·74)
Type of delivery, n (%)		
Vaginal delivery	110 (94%)	112 (92%)
Emergency caesarean section	5 (4%)	7 (6%)
Planned caesarean section	0	1 (<1%)
Breech delivery	2 (2%)	2 (2%)

Data are mean (SD) unless otherwise indicated. M01=screening visit. M02=random assignment and vaccination visit. Tdap-IPV=tetanus-diphtheria-acellular pertussis-inactivated polio virus combined maternal vaccine.

Table 1: **Baseline characteristics of mothers in the per-protocol immunogenicity population**

Immunological endpoints evaluated the effects of pertussis vaccination in pregnancy and infancy on two distinct antibody-dependent mechanisms of protection against pertussis in infants: binding to and neutralisation of pertussis toxin, which is considered essential for protection against (severe) disease (figure 2A); and binding to non-pertussis toxin vaccine antigens (figure 2B), including those involved in the recognition and killing of whole *B pertussis* bacteria (figure 2C). Observed GMCs and GMTs for all antibody endpoints in the four infant groups at the three timepoints are in the appendix (p 9).

At 8 weeks of age—ie, before their first primary vaccine dose—infants born to mothers vaccinated with Tdap-IPV had significantly higher anti-pertussis toxin IgG

	Tdap-IPV–DTaP group (n=53)	Tdap-IPV–DTwP group (n=64)	Tetanus toxoid–DTaP group (n=64)	Tetanus toxoid–DTwP group (n=58)
Gestational age at birth, weeks	39·15 (1·56)	39·31 (1·33)	39·12 (1·33)	39·03 (1·8)
Gestational age				
>37 weeks	51	63	62	51
34–36 + 6 weeks	2	1	2	7
<34 weeks	0	0	0	0
Infant sex, n (%)				
Female	29 (55%)	31 (48%)	37 (58%)	31 (53%)
Male	24 (45%)	33 (52%)	27 (42%)	27 (47%)
Infant birthweight, kg	2·94 (0·38)	2·96 (0·36)	2·99 (0·42)	2·90 (0·38)
Birthweight				
>2·5 kg	38	49	46	44
1·5–2·5 kg*	8	5	12	11
≤1·5 kg†	0	0	0	0
No data	7	10	6	3
Apgar score at 10 min	10	9·95 (0·28)	10·00 (0·18)	9·47 (1·99)

Data are n, n (%), or mean (SD). DTaP=diphtheria-tetanus-acellular pertussis combined infant vaccine. DTwP=diphtheria-tetanus-whole-cell pertussis combined infant vaccine. Tdap-IPV=tetanus-diphtheria-acellular pertussis-inactivated polio virus combined maternal vaccine. Tdap-IPV–DTaP group=infants vaccinated with DTaP born to mothers vaccinated with Tdap-IPV. Tdap-IPV–DTwP group=infants vaccinated with DTwP born to mothers vaccinated with Tdap-IPV. Tetanus toxoid–DTaP group=infants vaccinated with DTaP born to mothers vaccinated with tetanus toxoid. Tetanus toxoid–DTwP group=infants vaccinated with DTwP born to mothers vaccinated with tetanus toxoid. *Low birthweight. †Very low birthweight.

Table 2: **Baseline characteristics of infants in the per-protocol immunogenicity population**

concentrations (p<0·0001) and PTNA (p<0·0001) than did those born to mothers vaccinated with tetanus toxoid in pregnancy (figure 3A). Errors-in-variable regression showed a significant linear association between anti-pertussis toxin IgG and PTNA, which was strongest among infants whose mothers had been immunised with Tdap-IPV in pregnancy (p=0·043 for separate slopes; figure 3B). In parallel, infants born to Tdap-IPV-vaccinated mothers had significantly higher baseline IgG binding to whole *B pertussis* (p<0·0001) and significantly higher SBA (p<0·0001, figure 3C) than did infants born to tetanus toxoid-vaccinated mothers. IgG binding to *B pertussis* and SBA were significantly positively associated, with no difference between infant groups (p=0·17, figure 3D).

To evaluate the effect of primary infant vaccination on pertussis toxin antibody endpoints, we first compared anti-pertussis toxin IgG responses to the two different primary vaccination schedules in infants born to mothers who had received the same vaccine in pregnancy (figure 4A, appendix pp 15–16). At 20 weeks, in infants born to Tdap-IPV-immunised mothers, the GMCs of anti-pertussis toxin IgG were more than three-fold lower in the infants vaccinated with DTwP than in those vaccinated with DTaP (adjusted GMR 0·28, 98·75% CI 0·16–0·50), a difference that persisted to 9 months (0·31, 0·17–0·55). Conversely, in infants born to tetanus toxoid-immunised mothers, the GMCs of anti-pertussis toxin IgG at 9 months were higher in the infants vaccinated with DTwP than in those vaccinated with DTaP (2·02, 1·15–3·55). To investigate potential blunting effects on infant vaccine responses resulting from maternal vaccination-induced antibodies, we compared infants who had received the same primary vaccine but whose mothers had received different vaccines in pregnancy (figure 4B; appendix pp 13–14). For infants vaccinated with DTwP, at both post-vaccination timepoints, GMCs of anti-pertussis toxin IgG were more than eight-fold lower in those born to Tdap-IPV-vaccinated mothers than in those born to tetanus toxoid-immunised mothers, indicating severe blunting (0·12, 98·75% CI 0·07–0·22 at 20 weeks; and 0·07, 0·03–0·17 at 9 months). At 20 weeks, GMCs of anti-pertussis toxin IgG were similar to or significantly higher than pre-immunisation (8 weeks) concentrations in three of the infant groups (appendix pp 10–11); however, for DTwP-vaccinated infants born to Tdap-IPV-immunised mothers, post-vaccination anti-pertussis toxin IgG concentrations were almost three-fold lower than pre-vaccination concentrations (0·39, 95% CI 0·23–0·64). Tdap-IPV vaccination in pregnancy resulted in similar blunting after primary DTaP vaccination, albeit much less pronounced, with concentrations of anti-pertussis toxin IgG two-fold lower in infants born to Tdap-IPV-vaccinated mothers than in those born to tetanus toxoid-vaccinated mothers (0·56, 98·75% CI 0·31–0·99). By 9 months, however, there was no longer a significant difference (appendix pp 13–14).

Next, we assessed whether blunting of infant anti-pertussis toxin IgG concentrations translated into reduced neutralising capacity (figure 4B). Comparing the two groups of DTwP-vaccinated infants, post-vaccination PTNA titres were at least two-fold lower in those born to Tdap-IPV-vaccinated mothers than in those born to tetanus toxoid-vaccinated mothers, up to study completion (adjusted GMR 0·53, 98·75% CI 0·32–0·88 at 20 weeks; and 0·28, 0·14–0·56 at 9 months). This result was similar to the pattern of anti-pertussis toxin IgG responses, although the blunting was less pronounced, with the PTNA titres of DTwP-vaccinated infants born to Tdap-IPV-vaccinated mothers similar to those of both groups of DTaP-vaccinated infants (figure 4A). The neutralising capacity of antibodies generated by DTwP infants born to Tdap-IPV-vaccinated mothers was higher than expected, given the markedly lower level of anti-pertussis toxin IgG binding in this group compared with other groups (ie, the ratio of PTNA to pertussis toxin IgG was highest in this group; appendix p 36). Conversely, comparing groups of DTaP-vaccinated infants, PTNA was not significantly lower in those born to mothers who received Tdap-IPV than in those born to mothers who received tetanus toxoid in pregnancy (figure 4B). By 9 months, PTNA across all four groups had approximately halved, albeit remaining significantly higher than baseline in all infants born to tetanus toxoid-vaccinated mothers (appendix p 11).

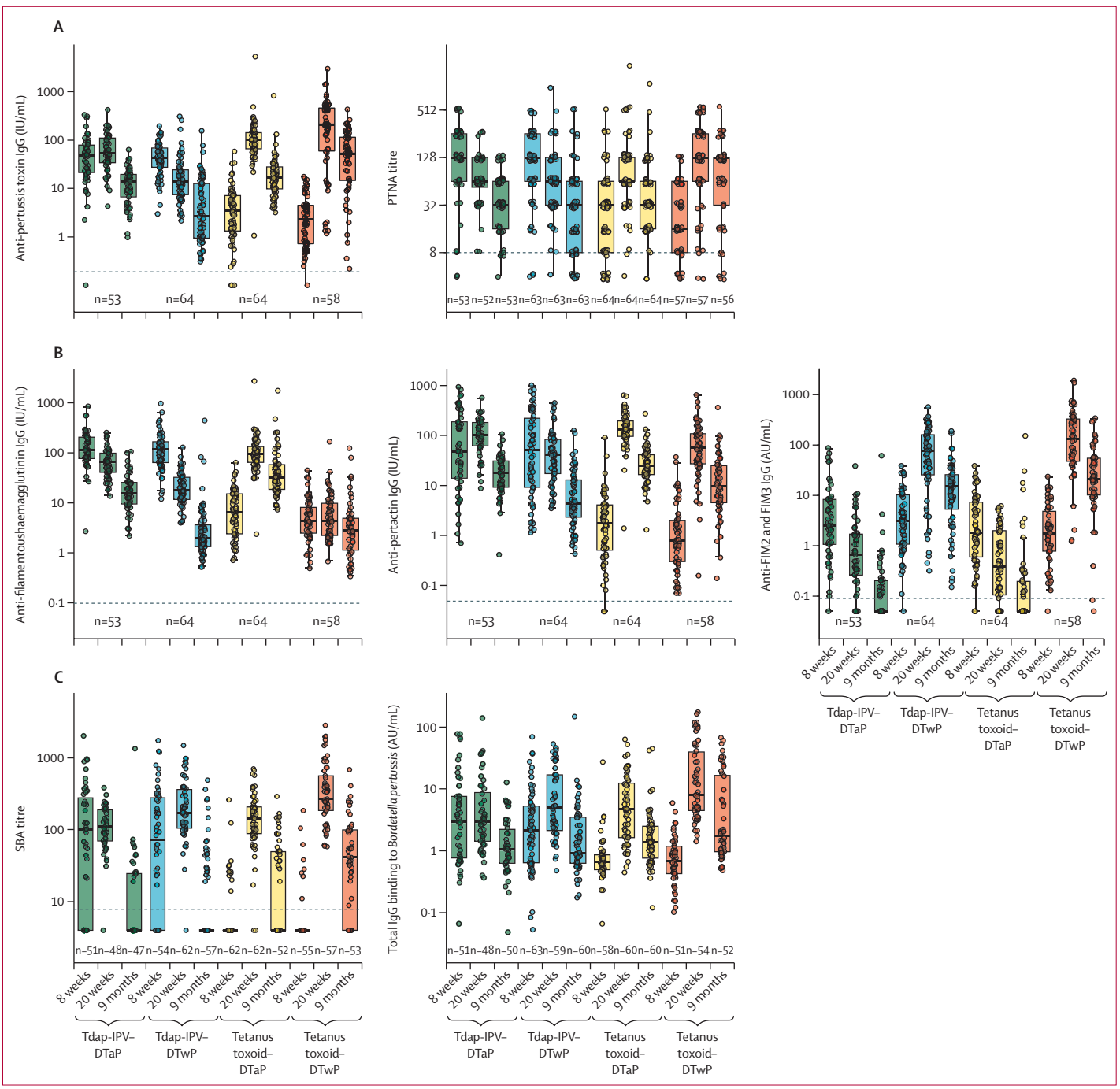


Figure 2: **Infant pertussis-specific IgG and functional antibody concentrations or titres related to pertussis-toxin-mediated or non-pertussis-toxin-mediated protection, according to study timepoint and infant group**

(A) Anti-pertussis toxin IgG concentrations (left) and neutralising antibody titres (right). (B) Concentrations of IgGs against filamentous haemagglutinin (left), pertactin (middle), and FIM 2 and FIM-3 (right). (C) SBA titres (left) and total IgG binding to *B pertussis* (right). Boxes show the IQRs; horizontal lines inside the boxes represent medians; whiskers represent the upper and lower adjacent values (a distance of 1·5 IQR beyond the first and third quartiles) as defined by Tukey; and dots represent all participant values (including outliers outside the whiskers). Dashed lines show the assays' lower limits of detection. Geometric mean concentrations are in the appendix (p 9). AU=arbitrary units. DTaP=diphtheria-tetanus-acellular pertussis combined infant vaccine. DTwP=diphtheria-tetanus-whole-cell pertussis combined infant vaccine. PTNA=pertussis toxin neutralising activity. SBA=serum bactericidal activity. Tdap-IPV=tetanus-diphtheria-acellular pertussis-inactivated polio virus combined maternal vaccine. Tdap-IPV–DTaP=infants vaccinated with DTaP born to mothers vaccinated with Tdap-IPV. Tdap-IPV–DTwP=infants vaccinated with DTwP born to mothers vaccinated with Tdap-IPV. Tetanus toxoid–DTaP=infants vaccinated with DTaP born to mothers vaccinated with tetanus toxoid. Tetanus toxoid–DTwP=infants vaccinated with DTwP born to mothers vaccinated with tetanus toxoid.

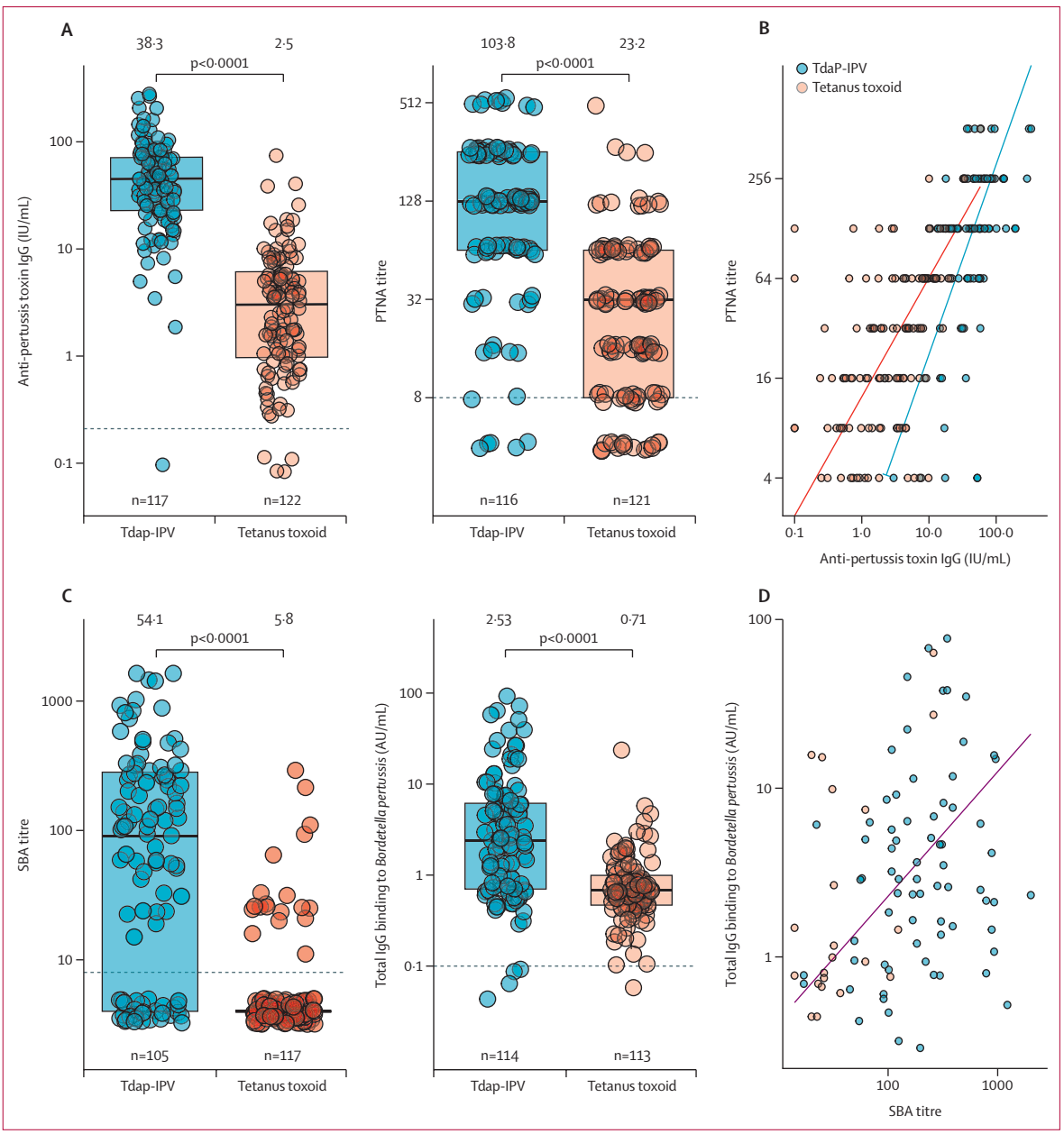


Figure 3: **Pre-immunisation pertussis-specific antibody levels in infants at 8 weeks and relationships between antibody parameters**
(A) Anti-pertussis toxin IgG concentrations (left) and neutralising antibody titres (right). (B) Relationship between anti-pertussis toxin IgG concentrations and pertussis-toxin neutralising antibodies before infant immunisation according to maternal vaccine given in pregnancy. (C) SBA titres (left) and total IgG binding to *B pertussis* (right). (D) Relationship between SBA and total IgG binding to *Bordetella pertussis* before infant immunisation according to maternal vaccine given in pregnancy. Boxes in (A) and (C) show the IQRs; horizontal lines inside the boxes represent medians; whiskers represent the upper and lower adjacent values (a distance of 1·5 IQR beyond the first and third quartiles) as defined by Tukey; and dots represent all participant values (including outliers outside the whiskers). The dashed line depicts the assays' lower limits of detection. The numbers above the box-and-whisker plots indicate the geometric mean concentrations. Solid lines in (B) and (D) indicate associations between antibody parameters estimated using errors-in-variable Deming regression; use of two coloured lines indicates a significant difference between vaccine groups, and a single line indicates no significant difference. Only values greater than the lower limits of detection for PTNA titres (B) and total antibody binding to *B pertussis* (D) were included in the regression analyses. PTNA=pertussis toxin neutralising activity. SBA=serum bactericidal activity. Tdap-IPV=tetanus-diphtheria-acellular pertussis-inactivated polio virus combined maternal vaccine.

To evaluate whether there was a longer-term effect on immune memory, we measured memory B cells in a representative subset of 81 infants who completed the trial per protocol and for whom cellular samples were available (figure 5). All four intervention groups showed a significant increase in pertussis toxin-specific memory B cells at 20 weeks, with higher responses in DTwP-vaccinated infants than in DTaP-vaccinated infants,

particularly at 9 months (appendix p 19, p 22). The blunting effect seen in the antibody compartment was also observed for pertussis toxin-specific memory B-cell responses, with reduced responses in those born to Tdap-IPV-vaccinated mothers compared with those born to tetanus toxoid-vaccinated mothers (adjusted GMR 0·21, 95% CI 0·07–0·59 at 9 months; appendix p 21). Consistent with the pattern of functional responses, however, for infants born to Tdap-IPV-vaccinated mothers, the frequency of memory B cells in DTwP-vaccinated infants was similar to or higher than that in DTaP-vaccinated infants at 20 weeks (2·09, 0·87–5·03 3·25) and 9 months (3·25, 1·42–7·44 ; appendix p 22) and remained significantly elevated above pre-vaccination baseline until study completion (13·07, 6·74–25·32; appendix p 19).

In addition to antibodies binding to pertussis toxin, which is secreted, we quantified antibodies binding to other pertussis vaccine antigens, which might be involved in direct recognition and clearance of *B pertussis*. Higher concentrations of IgG against both filamentous haemagglutinin and pertactin were observed in DTaP-vaccinated infants than in DTwP-vaccinated infants at both post-vaccination timepoints (figure 4A). Of note, although DTwP generated poor anti-filamentous haemagglutinin IgG responses, we observed higher numbers of filamentous haemagglutinin-specific memory B cells after vaccination than before vaccination (ie, compared with 8 weeks; appendix pp 19, 37). Responses to FIM2 and FIM3 antigens were measured predominantly as an immune readout for DTwP-vaccinated infants, as these antigens are not components of the hexavalent DTaP vaccine used in this study. Accordingly, only DTwP-vaccinated infants elicited significant FIM-specific post-vaccination responses (appendix p 10). Blunting by Tdap-IPV vaccination was dependent on the antigen-specific response measured, infant vaccine given, and sampling timepoint (figure 4B). Unexpectedly, in DTwP-vaccinated infants, the GMC of filamentous haemagglutinin-specific IgG was not blunted but was higher if the mothers of these infants had been given Tdap-IPV rather than tetanus toxoid in pregnancy (20 week adjusted GMR 3·86, 98·75% CI 2·50–5·97; figure 4B,

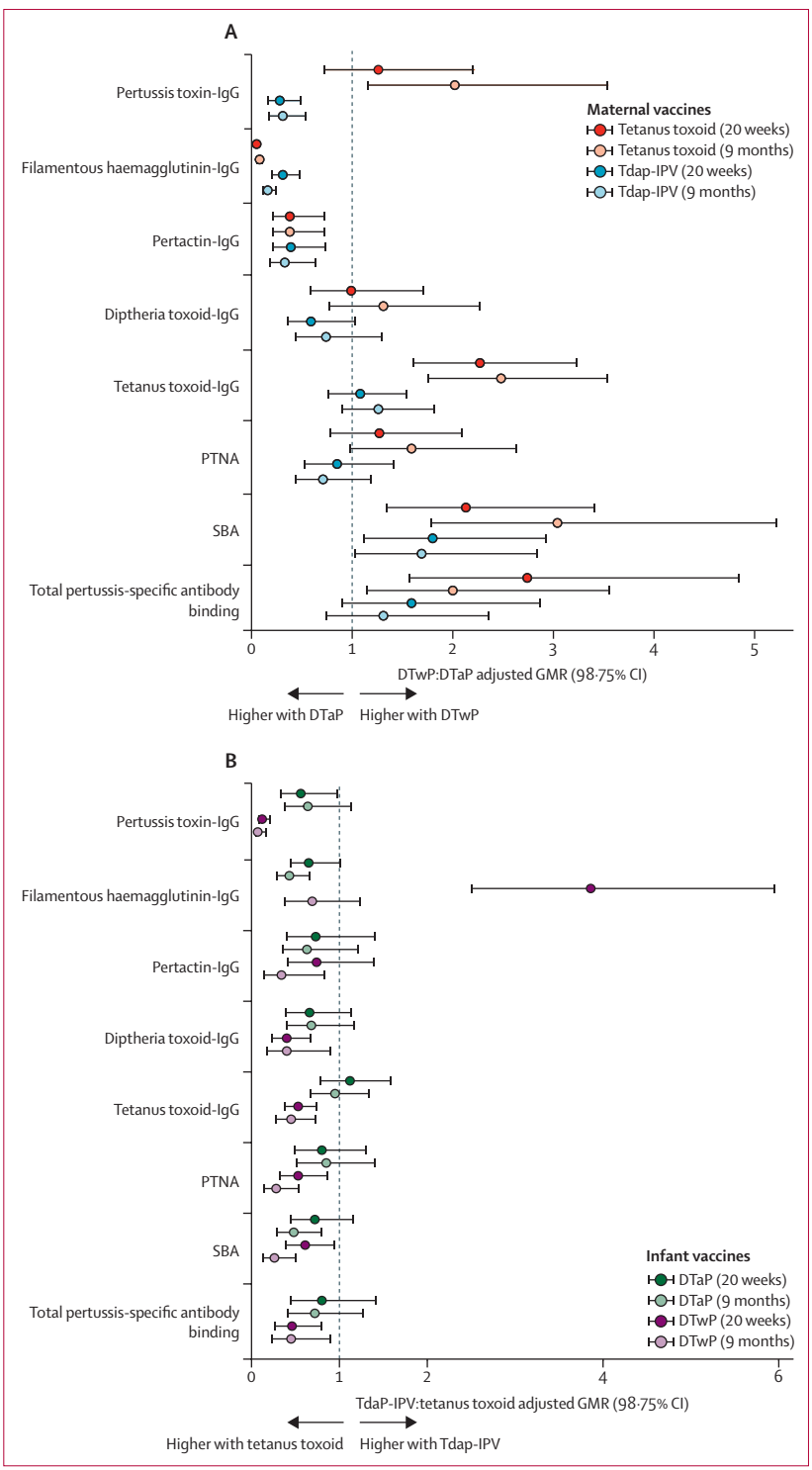


Figure 4: **Adjusted GMRs for immune parameters in vaccinated infants at 20 weeks and 9 months, comparing the two infant vaccines (A) and the two maternal vaccines (B)**

(A) Ratios of GMCs or GMTs in infants receiving DTwP versus DTaP primary immunisation for each vaccine antigen-specific and functional antibody response following primary immunisation (20 week and 9 month timepoints). Adjusted GMR values less than 1 suggest higher post-vaccination responses among DTaP-vaccinated infants; adjusted GMR values greater than 1 suggest higher post-vaccination responses among DTwP-vaccinated infants. Shading indicates maternal vaccine in pregnancy. (B) Ratios of GMCs or GMTs in infants born to mothers who received Tdap-IPV versus tetanus toxoid in pregnancy for each vaccine antigen-specific and functional antibody response following primary immunisation (20 week and 9 month timepoints). Adjusted GMR values less than 1 suggest blunting by maternal Tdap-IPV immunisation; adjusted GMR values greater than 1 suggest boosting by maternal Tdap-IPV immunisation. Shading indicates infant vaccine received. DTaP=diphtheria-tetanus-acellular pertussis combined infant vaccine. DTwP=diphtheria-tetanus-whole-cell pertussis combined infant vaccine. GMC=geometric mean concentration. GMR= geometric mean ratio. GMT=geometric mean titre. PTNA=pertussis toxin neutralising activity. SBA=serum bactericidal activity. Tdap-IPV=tetanus-diphtheria-acellular pertussis-inactivated polio virus combined maternal vaccine.

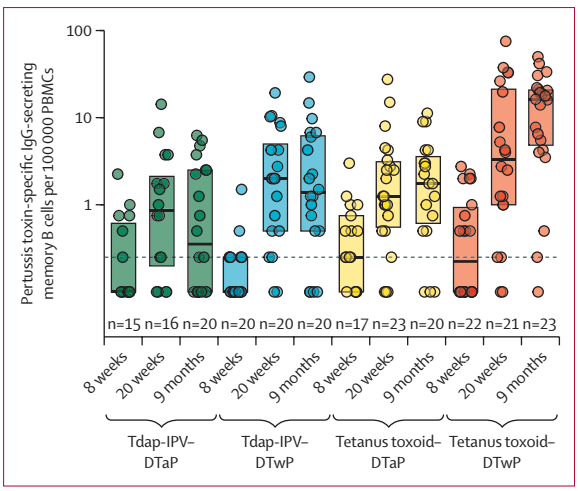


Figure 5: **Longitudinal changes in frequency of pertussis-specific IgG-secreting memory B-cell responses according to infant group and study timepoint, defined by spot-forming cells per 100 000 PBMCs**
Boxes show the IQRs; horizontal lines inside the boxes represent medians; whiskers represent the upper and lower adjacent values (a distance of 1·5 IQR beyond the first and third quartiles) as defined by Tukey, and dots represent all participant values (including outliers outside the whiskers). The dashed line depicts the assay lower limit of detection. DTaP=diphtheria-tetanus-acellular pertussis combined infant vaccine. DTwP=diphtheria-tetanus-whole-cell pertussis combined infant vaccine. PBMC=peripheral blood mononuclear cell. Tdap-IPV=tetanus-diphtheria-acellular pertussis-inactivated polio virus combined maternal vaccine. Tdap-IPV–DTaP=infants vaccinated with DTaP born to mothers vaccinated with Tdap-IPV. Tdap-IPV–DTwP=infants vaccinated with DTwP born to mothers vaccinated with Tdap-IPV. Tetanus toxoid–DTaP=infants vaccinated with DTaP born to mothers vaccinated with tetanus toxoid. Tetanus toxoid–DTwP=infants vaccinated with DTwP born to mothers vaccinated with tetanus toxoid.

appendix p 13). Of note, antibody responses to a non-pertussis antigen, diptheria toxoid, showed a similar pattern of blunting to that observed with pertussis toxin (figure 4B, appendix pp 13, 38). In DTwP-vaccinated infants, concentrations of tetanus toxoid-specific antibody were also two-fold lower in those born to Tdap-IPV-vaccinated mothers than in those born to tetanus toxoid-vaccinated mothers, even though all mothers had received a tetanus toxoid-containing vaccine in pregnancy (0·53, 0·37–0·76 at 20 weeks and 0·45, 0·27–0·75 at 9 months); this blunting was not seen following primary vaccination with DTaP (figure 4B, appendix pp 13, 38).

We also compared the effect of the two types of infant vaccines on antibody-mediated recognition and killing of whole *B pertussis* bacteria. Vaccination with DTwP resulted in higher concentrations of total antibody binding to *B pertussis* at both post-vaccination timepoints than did vaccination with DTaP (figure 4A), although this difference was significant only for infants born to tetanus toxoid-immunised mothers (adjusted GMR 2·74, 98·75% CI 1·55–4·85 at 20 weeks; and 2·0, 1·13–3·56 at 9 months). Moreover, DTwP-vaccinated infants had two-fold higher titres of SBA than did DTaP-vaccinated infants at 20 weeks (adjusted GMR 2·13, 98·75% CI 1·33–3·41 in infants born to tetanus toxoid-vaccinated mothers; 1·8, 1·10–2·93 in infants born to Tdap-IPV-vaccinated mothers). Both groups maintained this difference up to 9 months postnatally (appendix pp 15–16). Comparing the effect of maternal vaccines, infants born to mothers vaccinated with Tdap-IPV showed blunting of both SBA and total antibody binding compared with infants born to mothers vaccinated with tetanus toxoid, which was more pronounced in infants vaccinated with DTwP (figure 4B, appendix pp 13–14). Of note, despite blunting, absolute levels of SBA and total pertussis-specific antibody binding in DTwP-vaccinated infants remained similar to or even higher than those in DTaP-vaccinated infants (figure 4A, appendix pp 15–16). Finally, the decline in functional antibodies (as evaluated by PTNA, SBA, and total antibody binding) from 20 weeks to 9 months postnatally was largely similar between the four infant groups, suggesting that it was not dependent on the maternal or infant vaccine given.

To explore which of the individual pertussis vaccine antigens might be mediating these functional responses, we examined the association between antigen-specific antibodies and functional activity after vaccination, across the infant groups. At 20 weeks, there was a significant linear association between SBA and anti-pertactin IgG for DTaP-vaccinated infants but not for DTwP-vaccinated infants (appendix, p 39). Among DTaP-vaccinated infants, there were significantly different gradients (p=0.0059) for the Tdap-IPV versus tetanus-toxoid groups, with a stronger association for infants born to Tdap-IPV-vaccinated mothers. There was no significant association between SBA and anti-filamentous haemagglutinin, anti-pertussis toxin, or anti-FIM2 and anti-FIM3 IgG (data not shown).

Overall, the vaccines were well tolerated by both mothers and infants. There was no difference in reactogenicity between the two maternal vaccines, with few grade 2 events and no grade 3–4 events noted (appendix p 23–27). The most common solicited events following vaccination in pregnancy were local pain and tenderness (table 3). Only one infant (in the group of DTwP-vaccinated infants born to Tdap-IPV mothers) had a grade 3 temperature measured within the first 3 days after vaccination (appendix pp 28–33). In total, 92 serious adverse events were reported throughout the trial, 33 of which were maternal and 59 of which occurred in infants. These events included three stillbirths and four infant deaths (three within the neonatal period); there were no maternal deaths (appendix p 34). None of the serious adverse events were deemed to be related to vaccination or participation in the trial.

Discussion

This randomised, controlled phase 4 trial showed that vaccinating women with Tdap-IPV in pregnancy in a sub-Saharan African setting is safe, well tolerated, and boosts the quantity and quality of pertussis-specific

	Tetanus toxoid, n (%)	Tdap-IPV, n (%)
Systemic		
Acute allergic reaction		
Grade 0	172 (100%)	171 (100%)
Blood pressure		
Grade 0	167 (97%)	164 (96%)
Grade 1	3 (2%)	7 (4%)
Grade 2	2 (1%)	**0**
Diarrhoea		
Grade 0	168 (98%)	162 (95%)
Grade 1	4 (2%)	9 (5%)
Fatigue		
Grade 0	136 (79%)	142 (83%)
Grade 1	36 (21%)	28 (16%)
Grade 2	0	1 (<1%)
Heart rate		
Grade 0	133 (77%)	131 (77%)
Grade 1	39 (23%)	38 (22%)
Grade 2	0	2 (1%)
Headache		
Grade 0	150 (87%)	148 (87%)
Grade 1	22 (13%)	22 (13%)
Grade 2	0	1 (<1%)
Myalgia		
Grade 0	163 (95%)	156 (91%)
Grade 1	8 (5%)	15 (9%)
Grade 2	1 (<1%)	0 (0)
Respiratory rate		
Grade 0	170 (99%)	167 (98%)
Grade 1	1 (<1%)	3 (2%)
Grade 2	1 (<1%)	1 (<1%)
Fever		
Grade 0	171 (99%)	169 (99%)
Grade 1	1 (<1%)	1 (<1%)
Grade 2	0	1 (<1%)
Vomiting		
Grade 0	159 (92%)	162 (95%)
Grade 1	13 (8%)	8 (5%)
Grade 2	0	1 (<1%)
Local		
Erythema		
Grade 0	172 (100%)	171 (100%)
Induration		
Grade 0	170 (99%)	171 (100%)
Grade 1	2 (1%)	0
Pain at injection site		
Grade 0	76 (44%)	88 (51%)
Grade 1	96 (56%)	83 (49%)
Tenderness at injection site		
Grade 0	102 (59%)	105 (61%)
Grade 1	70 (41%)	66 (39%)

(Table 3 continues in next column)

(Continued from previous column)

No grade 3 reactions were observed. Tdap-IPV=tetanus-diphtheria-acellular pertussis-inactivated polio virus combined maternal vaccine.

Table 3: **Frequency of systemic and local adverse reactions in pregnant women in the first 7 days following immunisation according to grade and maternal vaccine given in pregnancy**

antibodies in infants before their primary vaccination schedule. To date, there has been little data available on the robustness of the immunogenicity conferred by pertussis vaccination in pregnancy, although previous studies have shown that infants born to mothers vaccinated in pregnancy are better protected against pertussis-related complications and death than those born to unvaccinated mothers.[8,29] To our knowledge, this study is also the first to systematically evaluate the effects of immunisation in pregnancy on the immune response to different infant pertussis vaccine types, focusing on functional antibodies and long-term memory B-cell responses in a west African cohort. In keeping with previously published data from our group and others,[9–11,30–32] we observed blunting of infant vaccine responses following vaccination in pregnancy. The relevance and persistence of this phenomenon, however, depend on the infant vaccine given, the timepoint of sampling, and the immunological readout examined. Although vaccination in pregnancy somewhat blunted the infants' immune response (particularly pertussis toxin-specific) to DTwP vaccination, overall functional antibody titres and memory-cell levels remained similar to or to even higher than those observed following vaccination with DTaP, irrespective of maternal immunisation status.

In the absence of a formal immunological correlate of protection, GaPs explored the effects of vaccination on two distinct potential mechanisms of antibody-mediated protection: antibody responses against pertussis toxin (which is secreted during infection), considered essential for protection against disease;[26,27] and antibodies that bind to whole *B pertussis* bacteria, which might drive other key effector functions, such as bacterial killing and clearance from the airways.[33] We first showed that before immunisation, Tdap-IPV-induced maternally derived antibodies had the capacity to both bind and neutralise pertussis toxin, as well as recognise and kill whole *B pertussis* bacteria. Next, we showed that Tdap-IPV in pregnancy significantly reduced the concentrations of particular antigen-specific and functional antibodies following primary infant immunisation. However, this effect is complex. At 1 month after completion of the primary immunisation series (ie, 20 weeks), antibody binding to pertussis toxin was significantly blunted following infant vaccination schedules that included either DTwP or DTaP, in line with previous studies.[9,11,12,30] The effect was much more pronounced in

DTwP-vaccinated infants than in DTaP-vaccinated infants, persisting to study completion at 9 months. The underlying mechanisms are unclear but might be related to differences in how antibody–antigen immune complexes are processed for soluble (ie, acellular pertussis) versus particulate (ie, whole-cell pertussis) antigens, pertussis toxin dosage, or the detoxification process for pertussis toxin. It will be important to evaluate whether novel maternal vaccine candidates containing genetically detoxified pertussis toxin will overcome the observed blunting effect. Crucially, in parallel with reduced IgG binding to pertussis toxin, both pertussis toxin-neutralising capacity and pertussis toxin-specific memory B-cell formation were also reduced in DTwP-vaccinated infants born to Tdap-IPV-vaccinated mothers. Considering the importance of immunity to pertussis toxin in decreasing disease severity, there might be clinical implications for infants who are exposed to *B pertussis* later in life. Conversely, the high PTNA-to-pertussis toxin IgG ratio suggested that partial functional capacity was retained, despite the lower anti-pertussis toxin antibody concentrations. This finding is consistent with data from a randomised trial in Thailand,[11] which showed preserved functional antibodies, as measured by a serum inhibition assay, despite similar blunting of pertussis toxin-specific IgG responses in DTwP-vaccinated infants born to mothers given Tdap in pregnancy. Unlike our study, there was no maternal control group in the Thai trial, which precluded a more comprehensive comparison between our studies. Interestingly, we found that immunisation with Tdap-IPV in pregnancy significantly blunted anti-tetanus toxoid IgG responses in DTwP-vaccinated infants, despite both maternal vaccines containing tetanus toxoid. These findings suggest that pertussis-specific maternal antibodies might have a bystander effect on infant immune cells in the germinal centre, further shaped by the primary vaccine used. The exact mechanisms warrant further investigation, which is currently ongoing.

Since the introduction of DTaP vaccines, immunogenicity endpoints have focused primarily on responses to pertussis toxin, filamentous haemagglutinin, and pertactin; such endpoints are inherently biased due to the higher doses of these purified antigens in DTaP than in DTwP. To achieve a more objective analysis of the overall effects of vaccination-induced antibodies on *B pertussis*, we measured total antibody binding to and antibody-dependent complement-mediated killing of whole *B pertussis*. Again, blunting was most pronounced following DTwP primary immunisation. Reassuringly, however, this blunting did not meaningfully affect the absolute SBA titres, which, in both DTwP-vaccinated groups, remained significantly above baseline and higher than in the DTaP-vaccinated groups. Because SBA is important in mediating bacterial clearance, our findings also support the current belief that, although both infant vaccines protect against severe disease, DTwP vaccines reduce bacterial colonisation and, therefore, diminish ongoing household or community transmission.[3] Interestingly, SBA titres and concentrations of IgGs against specific pertussis antigens were not significantly correlated following vaccination with DTwP, indicating that the high bactericidal activity generated is driven by antigens beyond the five acellular pertussis components. The strong positive association between anti-pertactin IgG and SBA afer vaccination with DTaP, however, might have clinical implications for protection against circulating pertactin-deficient *B pertussis* strains in populations vaccinated with DTaP rather than DTwP.[20]

Settings that use the combination of tetanus toxoid in pregnancy and DTwP primary vaccination in infants—ie, predominantly LMICs—should be encouraged by our finding that this schedule showed the highest quality pertussis-specific responses and most persistent memory B-cell responses compared with the other vaccine strategies. Moreover, the DTwP-containing primary vaccine used in our study was not significantly more reactogenic than DTaP, contrasting with the existing dogma. However, even in DTwP settings with high vaccine coverage, there is evidence of suboptimal control of pertussis and continued bacterial circulation,[5,6] largely because DTwP does not induce lifelong sterilising immunity. Because severe complication and mortality rates are highest among unvaccinated young infants, pertussis immunisation programmes in pregnancy might need to be considered in these settings to further reduce infant deaths. Our findings regarding Tdap-IPV-dependent blunting of DTwP-induced antibody responses to pertussis toxin suggest that caution and close monitoring are required if pertussis vaccination, particularly with monovalent pertussis toxin vaccines, is to be implemented during pregnancy in settings that currently have DTwP-based EPI schedules. By contrast, responses to pertussis toxin following vaccination with DTaP were minimally or transiently blunted by maternal Tdap-IPV vaccination in our cohort, which is reassuring for high-income countries in which this vaccination schedule is predominantly used. Nevertheless, our findings do not support immediately switching from DTwP to DTaP vaccines in EPI settings where antenatal pertussis immunisation programmes have been newly implemented. Although the GMC of anti-pertussis toxin IgG was lowest at both timepoints following a schedule of maternal vaccination with Tdap-IPV and infant vaccination with DTwP, other immunological endpoints, including total *B pertussis* antibody binding, SBA, and memory B-cell responses were similar to or, in many cases, even higher than in the DTaP-vaccinated groups. This finding raises an important question: should schedules aim to maximise pertussis toxin antibody responses, which are important for protection against disease, or should they maximise other immune responses that might provide protection against *B pertussis*

infection and potentially transmission? The possible introduction of the new live attenuated intranasal pertussis vaccine (BPZE1),[34] particularly alongside current parenteral vaccines, might help to reduce community circulation of *B pertussis* and, therefore, the severity of pertussis outbreaks every 3–5 years, which pose the biggest threat to unvaccinated infant cohorts, who are at high risk of infection and associated complications.

Importantly, antenatal acellular pertussis-based immunisation programmes have already been rolled out in South America, where the data so far have indicated that any blunting of anti-*B pertussis* antibodies following the DTwP primary series is not clinically significant in infants, at least up to 12 months of age; however, longer observation periods will be required.[35,36]

Our study had limitations. Due to the SARS-CoV-2-related lockdown and public health measures in The Gambia in 2020, we were unable to reach the planned sample size of 600 mother–infant pairs. There were no resources to extend recruitment, and we proceeded to analysis with a smaller sample size. Furthermore, due to the lockdown, some post-vaccination home visits were conducted by telephone; thus, reactogenicity signs, such as fever, could have been missed and their incidence underestimated. Nevertheless, given their close interactions with the trial team and the provision of a mobile telephone at study initiation, participants would have been likely to report severe adverse reactions by telephone or sought emergency care from the trial team, meaning that reactions more severe than grade 3 were unlikely to have been missed. Any missing data were also evenly distributed across intervention groups, minimising bias. The heterogeneity of DTwP vaccines used historically and worldwide, including batch-to-batch variability due to poor standardisation in manufacturing, might be another limitation; our study DTwP vaccine, however, has been distributed to more than 100 countries worldwide. We cannot comment on the effect of different vaccine strategies on clinical outcomes such as *B pertussis* colonisation, symptoms, hospitalisation, or other complications, because they were not trial objectives, given the anticipated sample size. Finally, given resource limitations, we only followed up infants up to 9 months of age and did not assess any immunological or clinical outcomes thereafter. It would be informative to evaluate the persistence of functional responses beyond this age, including up to and following the booster DTwP dose (given 1 year after the primary schedule in The Gambia).

In summary, GaPs comprehensively assessed vaccine-specific antibody and memory B-cell responses induced by different primary pertussis vaccines in the context of the presence or absence of pertussis immunisation in pregnancy, in a sub-Saharan African setting. The immunogenicity data generated support the existing data regarding the clinical effectiveness of pertussis immunisation programmes in pregnancy.

Contributors

DJ, QH, DK, DD, and BK developed the trial protocol. AS, EK, MO, MK, SR, PG, A-MBu, QH, SB, DK, DD, and BK contributed to and coordinated trial planning and implementation. AS, EK, MO, MK, ER, AF, HJ, PG, A-MBu, JF, A-MBa, QH, BC, EL, and DD contributed to data collection. AS, DJ, and NM analysed the data, with input from DD and BK. AS, DJ, NM, SR, PG, A-MBu, BC, EL, JF, A-MBa, QH, KT, SB, DK, DD, and BK contributed to data interpretation. All authors provided input into the manuscript and approved the final manuscript. AS, DJ, NM, MK, and BK accessed and verified the data underlying the study.

Declaration of interests

BK has received funding to Medical Research Council Unit The Gambia from multiple sources for the conduct of vaccine studies in pregnant women; none of the funding was related to this study beyond the original EU Innovative Medicines Initiative grant. DD reports funding to their institution from industry for conducting pertussis vaccine studies in adults; none of the objectives of the pertussis vaccine studies in adults are directly related to this study. All other authors declare no competing interests.

Data sharing

All laboratory data from GaPs are on the central PERtussIS Correlates Of Protection Europe (PERISCOPE) database, located on the DANS Data Station Life Sciences (https://doi.org/10·17026/dans-zcw-8x6j). Access to the dataset can be requested through the website or by contacting BK or DD (dimitri.diavatopoulos@radboudumc.nl) directly. These proposals will be reviewed and approved by the investigators and collaborators on the basis of scientific merit. To gain access, data requesters will need to sign a data access agreement. The study protocol (including statistical analysis plan) is available on Wellcomeopenresearch (https://wellcomeopenresearch.org/articles/9–487). Supporting documents, including clinical database dictionaries, informed consent form, and Independent Data and Safety Monitoring Committee (also known as DSMB) Charter, have been published previously on https://zenodo.org/doi/10.5281/zenodo.11567608.

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