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Quantity and Quality of Antibodies After Acellular Versus Whole-cell Pertussis Vaccines in Infants Born to Mothers Who Received Tetanus, Diphtheria, and Acellular Pertussis Vaccine During Pregnancy: A Randomized Trial

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Background. The blunting effect of pertussis immunization during pregnancy on infant antibody responses induced by whole-cell pertussis (wP) vaccination is not well-defined.

Methods. This randomized controlled trial (NCT02408926) followed term infants born to mothers vaccinated with tetanus, diphtheria, and acellular pertussis (Tdap) vaccine during pregnancy in Thailand. Infants received either acellular pertussis (aP)- or wP-containing vaccine at 2, 4, 6, and 18 months of age. A comparison group comprised wP-vaccinated children born to mothers not vaccinated during pregnancy. Antibodies against pertussis toxin (PT), filamentous hemagglutinin (FHA), and pertactin (PRN) were evaluated using commercial enzyme-linked immunosorbent assays. Functionality of antibodies against *Bordetella pertussis* was measured using *Bordetella pertussis* growth inhibition assay.

Results. After maternal Tdap vaccination, 158 infants vaccinated with aP-containing vaccines possessed higher antibody levels ($P < .001$) against all tested *B. pertussis* antigens postpriming compared to 157 infants receiving wP-containing vaccines. At 1 month postbooster, only anti-FHA and anti-PRN antibodies were still significantly higher ($P < .001$) in the aP group. Significantly higher anti-PT and anti-FHA ($P < .001$), but not anti-PRN immunoglobulin G, were observed among 69 wP-vaccinated infants born to control mothers compared with wP-vaccinated infants of Tdap-vaccinated mothers after primary and booster vaccination. The antibody functionality was higher in all wP-vaccinated infants at all times.

Conclusions. Maternal Tdap vaccination inhibited more pertussis-specific responses in wP-vaccinated infants compared to aP-vaccinated infants, and the control group of unvaccinated women had highest PT-specific responses, persisting until after the booster dose. Antibody functionality was better in the wP groups.

Clinical Trials Registration. NCT02408926.

Keywords. pertussis; pregnancy; maternal immunization; humoral immune response; functionality.

Pertussis remains difficult to control despite decades of worldwide vaccination. Infants are at highest risk for severe outcomes [1]. The most cost-effective method to protect infants is immunization during pregnancy [2–5]. During the last decade, maternal tetanus, diphtheria, and acellular pertussis (Tdap) vaccination programs have been implemented, mainly in industrialized countries [6].

High titers of naturally acquired maternal antibodies to pertussis toxin (PT) were previously reported to interfere with infant antibody responses to whole-cell pertussis (wP) [7, 8], but not to acellular pertussis (aP) vaccines [9]. In contrast, lowered antibody responses in infants born from Tdap-vaccinated mothers were observed following primary immunizations with aP-containing vaccines, with inconsistent results following a booster dose [10–13]. In many countries, wP vaccines are used within the Expanded Programme on Immunization (EPI). Interference in infant immunity induced by aP vaccines cannot be extrapolated to wP vaccines without additional immunogenicity data [14].

Assessment on how immunization influences bactericidal immunity against *Bordetella pertussis*, as means of measuring quality of antibodies, is of interest [15]. Immunoglobulin G

(IgG)–mediated binding of pathogen causes immobilization or agglutination. In the presence of complement, IgG may be bactericidal. Sera from subjects vaccinated with 2-component (filamentous hemagglutinin [FHA], PT) aP vaccines did not activate complement-mediated killing [16]. Yet sera of individuals vaccinated with pertactin (PRN)–containing vaccines were able to generate bactericidal activity [17]. To our knowledge, little information exists on the difference in sera bactericidal activity induced by aP- or wP-containing vaccines and its correlations with serum IgG levels, after maternal immunization.

The wP-containing vaccine has been implemented in the Thai EPI program for >40 years, while the aP-containing vaccine was introduced 10 years ago and is used in private hospitals [18]. Although there has been a resurgence of pertussis, especially among very young infants [19], maternal Tdap immunization has not been implemented. To evaluate the potential effects of implementing maternal Tdap on the responses to aP- vs wP-containing vaccines in children, we conducted a prospective randomized controlled clinical trial. The primary objective was to evaluate antibody levels in infants after priming and first booster vaccination with aP- or wP-containing vaccines, in comparison to the EPI schedule. Second, the functionality of these antibodies was evaluated.

MATERIALS AND METHODS

Study Design
This study (ClinicalTrials.gov NCT02408926) was approved by the Institutional Review Board at Chulalongkorn University and the Ethical Committee of the University of Antwerp. We enrolled healthy pregnant women at King Chulalongkorn Memorial Hospital who consented to Tdap vaccination (Boostrix). We assumed that all women received wP-containing vaccines during infancy. The inclusion and exclusion criteria, vaccine reactogenicity, and *B. pertussis*–specific antibody titers in maternal and cord blood were previously described [20]. Written informed consent was obtained from parents prior to infant enrollment. Healthy full-term and late preterm infants born at 36 weeks' gestational age with birth weight >2500 g were randomized to receive either aP-containing (Infanrix hexa) or wP-containing (Quinvaxem) vaccine. This study was not blinded as wP-vaccinated infants received oral polio vaccine (OPV) whereas aP-vaccinated infants received inactivated polio (IPV) vaccine (hexavalent vaccine).

Simultaneously, a convenience sample of full-term infants born to non-Tdap-vaccinated women was recruited in the same hospital, although not randomized, and this group received the wP-containing vaccine (Quinvaxem) according to the current Thai EPI (EPI wP group).

Study Vaccines
All women, except those from the EPI wP group, received Boostrix (GSK Biologicals) during the third trimester of pregnancy, containing 8 µg of PT, 8 µg of FHA, 2.5 µg of PRN, 2.5 Lf diphtheria toxoid (DT), and 5 Lf tetanus toxoid (TT).

All infants received aP- or wP-containing vaccines at 2, 4, and 6 months of age (priming) and 18 months of age (booster).

Infanrix hexa (GSK Biologicals) contains 25 µg PT, 25 µg FHA, 8 µg PRN, 30 IU DT, 40 IU TT, 10 µg hepatitis B surface antigen (HBsAg), 10 µg *Haemophilus influenzae* type b (Hib) polysaccharide, and 40, 8, and 32 D-antigen units of IPV types 1, 2, and 3, respectively. Quinvaxem (Biogenetech) contains inactivated *B. pertussis* >4 IU/dose of potency, 30 IU DT, 60 IU TT, 10 µg HBsAg, and 10 µg Hib oligosaccharide. Infants in the wP and EPI wP groups received bivalent OPV (Biofarma) at 2, 4, 6, and 18 months. In April 2016, The World Health Organization (WHO) recommended a switch from trivalent to bivalent OPV in all countries. The Department of Disease Control, Ministry of Public Health, Thailand, announced that one dose of IPV (IMOVAX polio, Sanofi Pasteur) containing 40, 8, and 32D-antigen units of inactivated polio types 1, 2 and 3 should be given to Thai infants receiving OPV at the age of four months starting from December 2015. The schedule for polio vaccine in the Thai EPI program from December 1st 2015 to April 29th, 2016 was trivalent OPV at months 2, 4, 6 and 18 with an additional one dose of IPV at month 4. After April 29th, 2016, Thailand replaced trivalent OPV with bivalent OPV.

According to the EPI, all infants received BCG and monovalent hepatitis B vaccine at birth, measles-mumps-rubella vaccine (Priorix, GSK Biologicals or M-M-R II, Merck & Co) at 9 months, and Japanese encephalitis (CD.JEVAX, Chengdu Institute of Biological Products) vaccine at 12 and 19 months of age. They received trivalent influenza vaccine (Influvac, Abbott Biologicals) at 7 and 9 months of age. Some infants received optional (decision by parents) rotavirus, pneumococcal, varicella zoster, or rabies vaccines.

Sample Collection
In the aP and wP groups, maternal and cord blood samples were collected at delivery (results published in [20]). Cord antibody levels of the EPI wP infants were extrapolated from a Thai historical infant cohort born to mothers who did not receive Tdap during pregnancy [21]. Venous infant blood samples (2.5 mL) were collected at 2 months of age before the first pertussis-containing vaccine, 28–35 days after the last dose of priming (7 months of age), at 18 months of age before the first pertussis booster, and 28–35 days after the booster (19 months of age). In the EPI wP group, blood samples (2.5 mL) were taken at months 7 and 19.

Enzyme-linked Immunosorbent Assay for Antibodies to *B. pertussis* Antigens
Anti-PT, anti-FHA, and anti-PRN IgG were analyzed in a blinded manner using a commercial enzyme-linked immunosorbent assay (EUROIMMUN, Lübeck, Germany) according to

the manufacturer's instructions. Experiments were performed as previously described [20]. Samples with values below the lower limit of quantification (LLOQ), 5 IU/mL, were calculated as 50% of the LLOQ.

Bacterial Growth Inhibition Assay

Antibody-mediated *B. pertussis* growth inhibition was measured as described in the Appendix. Bacterial growth inhibition activity was measured by the ratio of relative luminescence units (RLU) in the well of *B. pertussis* incubated with heat-inactivated sera (complement-independent activity) or untreated sera (complement-dependent activity) divided by RLU in the well of *B. pertussis* alone.

Statistical Analysis

With a significance level of 0.05 and power of 0.90, and if the geometric mean concentration (GMC) of anti-PT IgG was expected to be 20% less in the wP group, with fixed variance, a population of 130 infants in both arms was sufficient. Baseline characteristics are reported as mean and standard deviation. Antibody titers are presented as GMC with 95% confidence interval. The conventional *t* test or 1-way ANOVA was used to compare baseline characteristics, GMCs and functionality of antibodies. The paired *t* test was used to compare the antibody titers to make inference about the difference in GMC between month 2–7 and month 18–19 infant sera. The correlations between antibody titers at different time points and between antibody levels and their functionality were calculated using Pearson correlation. We analyzed our results as per protocol with significance defined by a *P* value of <.01. Note that relaxing the significance level to .05 yields other insights. Blunting of vaccine-induced immune responses was defined as a significantly lower GMC of IgG at 1 time point in the wP vs the wP EPI group.

RESULTS

Demographics

Overall, 370 pregnant women, recruited between April 2015 and September 2016, were vaccinated (Figure 1). From these women, 315 healthy infants were randomized to receive either Infanrix hexa (aP group; n = 156 term and 2 late preterm) or Quinvaxem (wP group; n = 155 term and 2 late preterm). Seventy-nine full-term infants born to non-Tdap-vaccinated women received Quinvaxem (EPI wP group). Baseline characteristics (Table 1) show no significant differences between the groups. Some infants were not vaccinated according to protocol (Supplementary Table 1) as a result of illness or delayed visits.

Antibody Responses to *B. pertussis* Antigens

We discuss all available data (intention-to-treat analysis), since differences between all available data (Supplementary Table 2) and data with full protocol adherence (Supplementary Table 3)

are not significant. The percentages of values below the LLOQ ranged from 0.3% to 12% depending on antigen and time point.

Comparing the wP and EPI wP groups, significantly lower anti-PT (*P* < .001), anti-FHA (*P* < .001), and somewhat lower anti-PRN (*P* = .030) titers were found 1 month after priming in the wP than in the EPI wP group, suggesting interference of maternal antibodies. At 1 month after the booster dose, interference still persisted for anti-PT (*P* < .001) and anti-FHA (*P* < .001) IgG.

The EPI wP group had significantly higher anti-PT (*P* < .001) IgG levels than the aP group at postpriming and postbooster, yet lower anti-FHA and anti-PRN levels.

Comparing the offspring of vaccinated women, GMCs of all *B. pertussis*–specific antibodies were significantly higher in the aP compared with the wP group following the primary series (*P* < .001) (Figure 2). At 18 months of age, all antibody responses substantially waned and the remaining levels were lower than the levels at 2 months of age in both groups. Antibody titers increased significantly for all antigens 1 month after the booster vaccination. Anti-PT IgG was comparable between both groups, but the aP group possessed significantly higher anti-FHA (*P* < .001) and anti-PRN (*P* < .001) antibody titers.

Within the aP group, significantly higher anti-PT and anti-PRN IgG GMC (*P* < .001) were measured post–primary vaccination, compared to prepriming levels, but their anti-FHA IgG remained at a comparable level (Figure 2). Although infants in the wP group also had significantly higher anti-PT IgG (*P* < .001) postpriming, their anti-FHA levels decreased significantly (*P* < .001) after priming, whereas the levels of anti-PRN IgG did not change significantly.

A comparison of *B. pertussis*–specific GMC between infants who only received the vaccines foreseen in the study and infants who received optional vaccines showed no significant differences (data not shown).

Correlation Between Maternal Antibodies and Vaccine-induced Antibody Responses

Similar to Englund et al [9], we found negative correlations between anti-PT IgG levels at month 2 and month 7 in both the aP and wP group, with a higher coefficient in the wP group: Pearson correlation coefficient (*r*) = −0.24, *P* = .006 (aP) vs −0.32, *P* < .001 (wP) (Supplementary Figure 1). In contrast, we found a statistically significant positive correlation between month 2 and month 7 for anti-FHA IgG levels in the wP group (*r* = 0.29, *P* = .001). The only positive and significant correlation was found for anti-PRN IgG between prepriming (month 2) and postbooster (month 19) antibody levels (*r* = 0.23, *P* = .007; Supplementary Figure 2).

Functionality of Antibodies

A subset (depending on the availability of samples at all time points) of sera (n = 276) was tested for their ability to inhibit
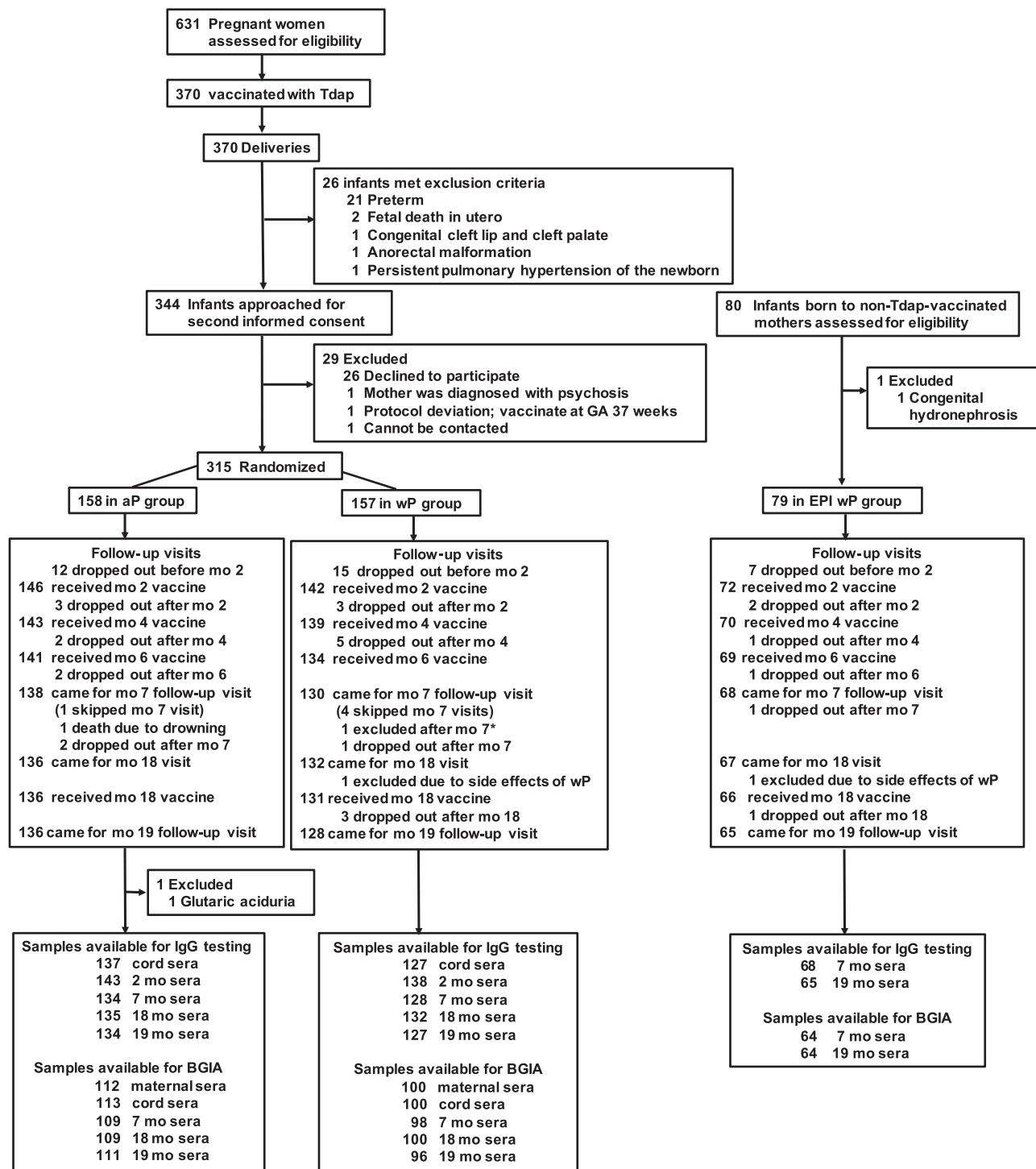
Figure 1. The Consolidated Standards for Reporting Trials flow diagram. *One child in the wP group received Quinvaxem at month 7, which was not according to the protocol. Abbreviations: aP, acellular pertussis vaccine; BGIA, *Bordetella pertussis* growth inhibition assay; EPI, Expanded Programme on Immunization; GA, gestational age; IgG, immunoglobulin G; Tdap, tetanus, diphtheria, and acellular pertussis vaccine; wP, whole-cell pertussis vaccine.

B. pertussis growth (Figure 1). Functional activity of all sera was highly dependent on complement, as demonstrated by the decrease in activity in heat-treated compared with nontreated sera (Figure 3). However, even in the absence of complement, the serum samples expressed various levels of

Bordetella growth inhibition (Figure 3A and 3C), suggesting complement-independent *Bordetella* growth inhibition by antipertussis sera. This was stronger in maternal and cord blood than in infant sera, whereas the reverse was seen in the presence of complement.

Table 1. Baseline Characteristics of Participants Included in the Study

Characteristic	aP Group (n = 158)	wP Group (n = 157)	EPI wP Group (n = 79)
Mean age of mothers at enrollment, y (SD)	29.0 (5.4)	28.4 (5.5)	28.0 (5.9)
Mean GA at delivery, wk (SD)	38.7 (1.1)	38.6 (1.1)	38.6 (1.2)
Mean GA at vaccination, wk (SD)	30.5 (2.4)	30.9 (2.2)	NA
Mode of delivery, No. (%)			
Vaginal	89 (56.3)	87 (55.4)	45 (56.3)
Cesarean	69 (43.7)	70 (44.6)	35 (43.8)
Sex, No. (%)			
Male	77 (48.7)	77 (49.0)	44 (55.0)
Female	81 (51.3)	80 (51.0)	36 (45.0)
Mean weight at birth, g (SD)	3127.6 (389.7)	3122.0 (320.6)	3237.4 (417.5)
Mean length at birth, cm (SD)	49.6 (2.1)	49.7 (2.0)	NA
Mean weight at month 2, kg (SD)	5.4 (0.6)	5.4 (0.6)	5.5 (0.6)
Mean length at month 2, cm (SD)	57.3 (2.3)	57.3 (2.6)	57.4 (2.3)
Mean weight at month 4, kg (SD)	6.7 (0.8)	6.8 (0.8)	6.9 (0.7)
Mean length at month 4, cm (SD)	63.0 (2.5)	63.3 (2.5)	63.5 (2.3)
Mean weight at month 6, kg (SD)	7.5 (1.0)	7.6 (0.9)	7.8 (0.8)
Mean length at month 6, cm (SD)	67.2 (3.0)	67.3 (2.5)	67.5 (2.1)
Mean weight at month 7, kg (SD)	7.9 (1.0)	7.9 (0.9)	8.1 (0.8)
Mean length at month 7, cm (SD)	69.0 (2.6)	69.3 (2.9)	69.4 (2.2)
Mean weight at month 18, kg (SD)	10.9 (1.5)	10.9 (1.5)	10.9 (1.2)
Mean length at month 18, cm (SD)	81.7 (3.4)	81.6 (3.4)	82.1 (2.9)
Mean weight at month 19, kg (SD)	11.2 (1.5)	11.2 (1.5)	11.2 (1.3)
Mean length at month 19, cm (SD)	83.2 (3.2)	83.0 (3.3)	83.0 (4.5)
Mean interval between birth and visit month 2, d (SD)	63.0 (4.6)	62.6 (4.3)	61.6 (5.5)
Mean interval between visit month 2 and visit month 4, d (SD)	59.9 (5.1)	60.0 (5.2)	61.8 (5.4)
Mean interval between visit month 4 and visit month 6, d (SD)	60.5 (5.3)	61.6 (4.7)	61.5 (4.7)
Mean interval between visit month 6 and visit month 7, d (SD)	30.8 (4.3)	31.1 (4.8)	31.7 (5.3)
Mean interval between visit month 18 and visit month 19, d (SD)	31.8 (6.5)	32.1 (5.7)	31.6 (6.4)

Abbreviations: d, days; GA, gestational age; NA, data not available; SD, standard deviation; wk, weeks; y, years.

In the absence of complement, functionality of antibodies in cord and maternal sera was not significantly different (Figure 3A). In the presence of complement, maternal sera were significantly more inhibitory than cord sera (Figure 3B). One month after primary infant vaccination, there was no difference between aP and wP groups for the heat-treated sera (Figure 3C). However, at 18 months, heat-treated serum in the wP group was significantly more active than in the aP group (Figure 3A), persisting for at least 1 month after the booster (Figure 3C). Antibodies in infants born to Tdap-vaccinated mothers appeared to better inhibit bacterial growth than those of infants born to unvaccinated mothers after the primary series of wP vaccination, but this was reversed after booster vaccination (Figure 3C).

Analysis in the presence of complement showed no difference between the aP and wP groups after the primary vaccination (Figure 3D). However, after the booster vaccination, the wP group inhibited *B. pertussis* growth again significantly better than the aP group.

No correlations between bactericidal activity and anti-PT IgG and anti-FHA levels were found (Supplementary Figures 3 and 4). There were some positive correlations between functional activity and anti-PRN IgG levels in the wP group alone (Supplementary Figure 5).

DISCUSSION

Blunting of aP vaccination in infants has been reported after maternal Tdap vaccination [10, 13], and we report for the first time in a large cohort equal blunting of the infant anti-PT and anti-FHA antibody responses to wP-containing vaccines. Our findings are consistent with data showing that naturally acquired maternal antibodies had a negative influence on PT antibody responses induced by DTwP vaccination in infants [9]. Ibrahim et al [22] recently reported no attenuating effect on infant *B. pertussis*–specific post–primary immunization titers, yet most infants did not receive the full 3-dose wP regimen.

This blunting effect may be of clinical relevance. PT is a major virulence factor of *B. pertussis* [23], and humanized neutralizing anti-PT monoclonal antibodies have been shown to abolish disease manifestations in mice and nonhuman primates [24]. Furthermore, maternal vaccination with a monocomponent PT vaccine protected newborn baboons against disease following respiratory challenge with *B. pertussis* [25]. In humans, low anti-PT IgG titers have been associated with high susceptibility
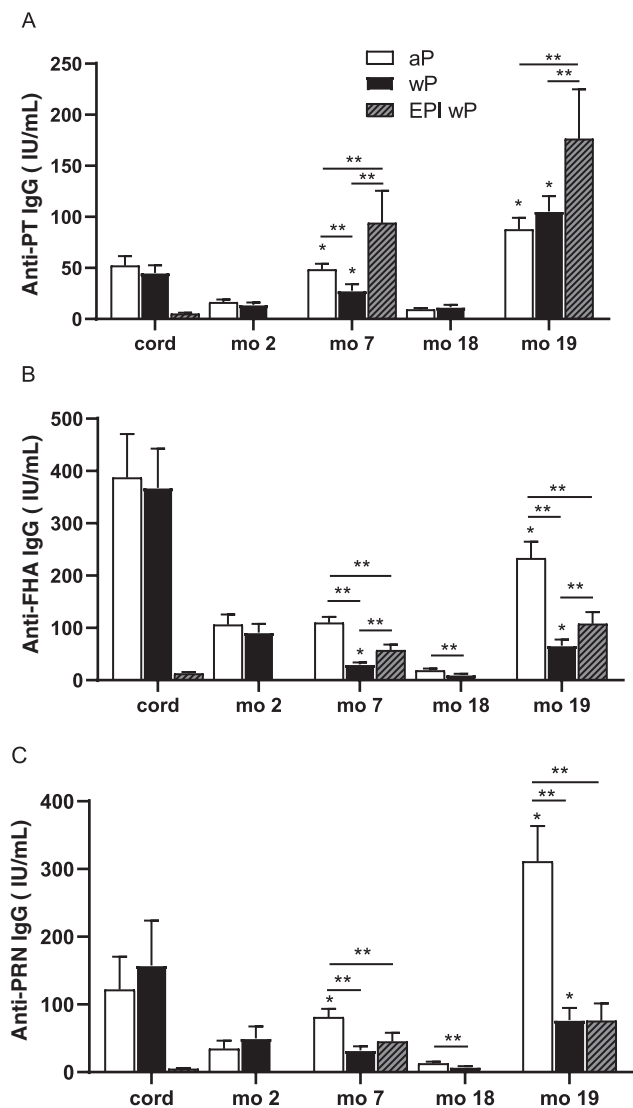
Figure 2. Geometric mean concentrations of anti–pertussis toxin (PT; *B*), anti–filamentous hemagglutinin (FHA; *A*), and anti-pertactin (PRN; *C*) immunoglobulin G (IgG) in the acellular pertussis (aP), whole-cell pertussis (wP), and Expanded Programme on Immunization (EPI) wP groups at birth (cord) and months 2, 7, 18, and 19. Cord antibody levels of the EPI wP infants were derived from the cord levels of Thai historical infant cohort born to mothers who did not receive tetanus, diphtheria, and acellular pertussis vaccine during pregnancy [21]. Error bars indicate the upper bound of the 95% confidence interval. *Statistically significant difference compared to prepriming or prebooster. **Statistically significant difference compared to other groups at month 7 and 19. Abbreviations: aP, acellular pertussis; EPI, Expanded Programme on Immunization; wP, whole-cell pertussis.

to pertussis, although no correlate of protection is known [26]. From surveillance data in countries where maternal Tdap has been implemented, however, there are no signals of any clinical effect of the reported blunting of the aP infant responses [27]. In the United Kingdom, for example, the maternal vaccine coverage has reached >70% since May 2016. If blunting was clinically important, the rate of pertussis should have increased in children between 6 months and 1.5 years. However, there is

no evidence of increased incidence of pertussis among English children. Since we report significantly lower antibody titers in wP- compared to aP-vaccinated children, the lack of clinical significance in aP-vaccinated children cannot be extrapolated to wP-vaccinated children.

Comparing aP and wP group immune responses, the aP group had significantly higher levels of all pertussis-specific IgG after a 3-dose priming scheme and anti-FHA and anti-PRN antibody levels were still significantly higher after a booster dose. Previous comparative studies, without maternal immunization, reported that aP-containing infant vaccines induce higher levels of antibodies, due to the higher amounts of antigens in aP compared to some of the wP-containing vaccines [28, 29]. In wP-containing vaccines, the levels of PT, FHA, and PRN are not specified [30], resulting in wide ranges of immunogenicity between different manufacturers [31]. Quinvaxem may contain reduced amounts of FHA and PRN, resulting in lower than expected immunogenicity following primary immunization.

Within the aP group, antibody levels to PT and PRN rose significantly after priming, but anti-FHA IgG did not. Ladhani et al reported similar findings for anti-PT and anti-FHA IgG in a cohort of aP-vaccinated children [32].

Using a novel *Bordetella* growth inhibition assay, complement-dependent growth inhibition was stronger in maternal than in cord blood, likely reflecting the different levels of complement in both tissues. Based on the growth inhibition results in infant sera, the blunting of antibodies induced by wP-containing vaccines in the presence of maternal antibodies after priming did not imply a reduction of the bactericidal activity of the antibodies. Inhibition of growth was actually overall better in wP-vaccinated infant sera, and after maternal Tdap vaccination. This suggests that maternal antibodies may endorse this bactericidal activity or even promote the production of infant antibodies with specific biophysical features mediating efficient pathogen control. However, after boosting the bactericidal activity was stronger for wP-vaccinated infants born from unvaccinated mothers compared to infants born to vaccinated mothers, suggesting that the differences observed after priming are mainly due to the activity of maternal antibodies. Studies in a murine model of pertussis [33] indicated that maternal immunization may affect the functionality of antibodies induced by primary aP vaccination of the offspring. We report here a primary observation on the functionality of the induced antibodies during a human trial, although more research is certainly needed.

The effect of maternal Tdap vaccination on cell-mediated immunity (CMI) following wP- or aP-containing infant vaccines is also of importance [34]. CMI responses in the present cohort will be reported separately.

The study has a few shortcomings. Infants to the EPI wP group were not randomized and we lacked data on the baseline
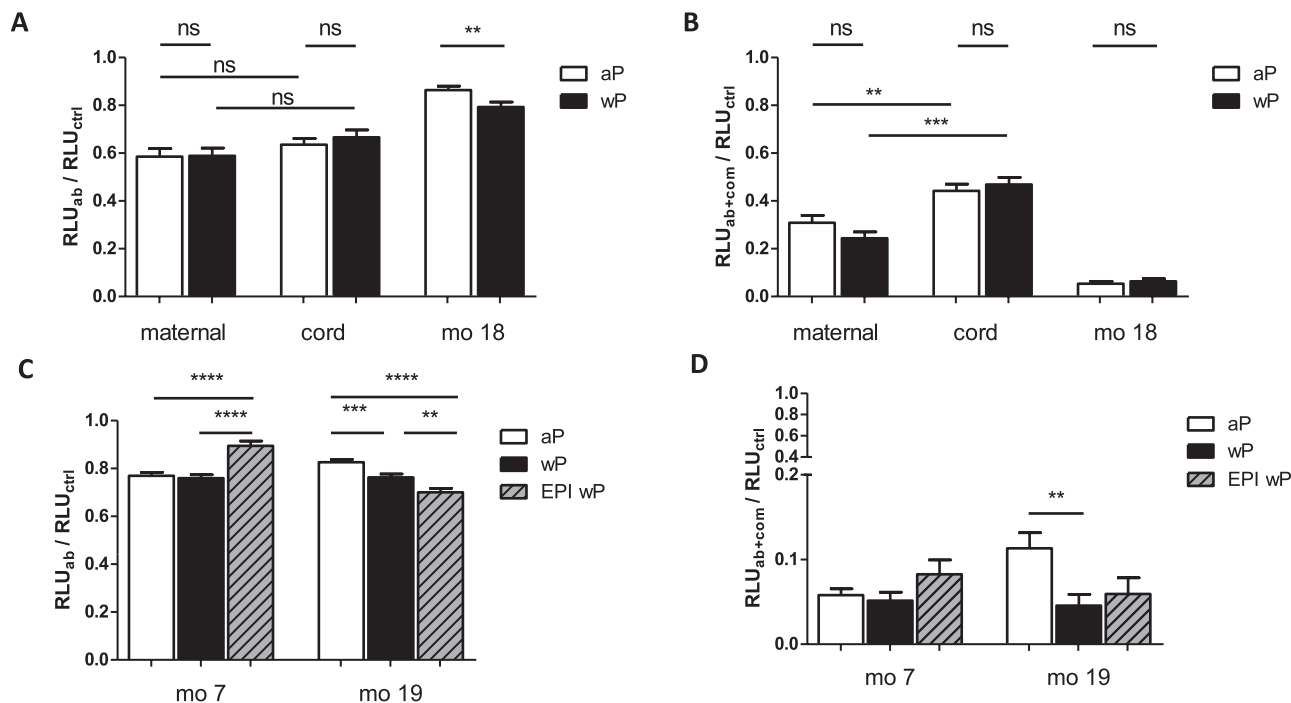
Figure 3. Ratio of relative luminescence units in different circumstances comparing the study groups (acellular pertussis [aP], whole-cell pertussis [wP], and Expanded Programme on Immunization wP [EPI wP]) at different time points. *A* and *C*, Comparing the ratio of RLU in wells containing *Bordetella pertussis* incubated with heat-treated antibody divided by RLU in wells containing *B. pertussis* alone. *B* and *D*, Comparing the RLU in wells containing *B. pertussis* incubated with untreated antibody plus complement divided by RLU in wells containing *B. pertussis* alone. Significance was evaluated using a 2-tailed Student *t* test. *A*, **$P = .0089$. *B*, **$P = .0017$ and ****$P < .0001$. *C*, **$P = .005$, ***$P < .0001$, and ***$P = .0008$ (aP vs wP group at month 19). *D*, **$P = .0043$. Abbreviations: ab, antibody; aP, acellular pertussis; com, complement; ctrl, control; EPI, Expanded Programme on Immunization; ns, not significant; RLU, relative luminescence units; wP, whole-cell pertussis.

antibody levels at month 2 for these EPI wP infants, but it is expected that the antibody levels before priming were low, based on our previous study [21]. A fourth study arm, aP-vaccinated infants of nonvaccinated mothers, was not added, as many comparative data are already available. The largest relevant study was conducted by Halperin et al [13], who reported that infants born to Tdap-vaccinated mothers had significantly lower antibody titers following primary immunization, persisting until after the first booster.

Altogether we report that Tdap-induced maternal antibodies affect the immune responses to a primary series of vaccines, both quantitatively, especially for anti-PT and FHA IgG, persisting at least until after the booster dose, and qualitatively. No correlation between antibody levels against PT and levels of growth inhibition was observed, which is consistent with PT being mostly a secreted antigen [35] and therefore not an efficient target for antibodies that mediate growth inhibition or bacterial lysis.

In summary, if countries using wP-containing vaccines for priming of infants are considering implementing maternal Tdap immunization, the blunting following wP vaccination should be taken into account. Vaccine-induced immune protection should then closely be monitored and pertussis surveillance should be strengthened.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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APPENDIX: Bacterial Growth Inhibition Assay

Antibody-mediated *B. pertussis* growth inhibition was measured as follows. The streptomycin-resistant *B. pertussis* Tohoma I derivative BPSM, a gift from the National University of Singapore, was grown in a modified Stainer-Scholte medium supplemented with 100 μg/mL of streptomycin at 37°C overnight [36]. Absorbance reading of the bacterial culture was performed at optical density 600 nm and the bacterial density was adjusted to approximately 0.07 by diluting with the growth medium to yield approximately 9×10^7 colony-forming units/mL. In each well of a 96-well microplate (Greiner Bio-One), 9 μL of bacteria and 9 μL of serum sample (diluted 1:3) were incubated at 37°C overnight. Parallel experiments included the use of decomplemented serum samples obtained by heating the serum samples at 56°C for 30 minutes. Positive control wells comprised bacteria incubated in the absence of serum, while negative control wells included the addition of 1% Triton

X-100. After 18-hour incubation, 18 μL of the BacTiter-Glo substrate (Promega) was added. Microplate was mixed for 2 minutes and subsequent reading of luminescence (in RLU) was performed after 5 minutes of incubation. The averaged luminescence readouts from triplicate wells represent the amount of ATP released by viable bacteria. Bacterial growth inhibition activity of the antisera was measured by the ratio of RLU in the well of *B. pertussis* incubated with heat-inactivated sera divided by RLU in the well of *B. pertussis* alone. The role of complement in mediating bacterial growth inhibition via antibody-dependent complement-mediated killing was measured by the ratio of RLU in the well of *B. pertussis* incubated with non-heat-inactivated sera divided by RLU in the well of *B. pertussis* alone.