



Maternal pertussis vaccination and its effects on the immune response of infants aged up to 12 months in the Netherlands: an open-label, parallel, randomised controlled trial

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Summary

Background Maternal tetanus, diphtheria, and acellular pertussis (Tdap) vaccination offers protection for neonates against clinical pertussis until primary vaccinations, but maternal antibodies also interfere with infants' immune responses to primary vaccinations. We investigated the effect of maternal Tdap vaccination on the pertussis antibody responses of infants starting primary vaccinations at age 3 months.

Methods In an open-label, parallel, randomised, controlled trial, pregnant women aged 18–40 years with a low risk of pregnancy complications were recruited through independent midwives at 36 midwife clinics in the Netherlands and received Tdap vaccination either at 30–32 weeks of pregnancy (maternal Tdap group) or within 48 h after delivery (control group). All term-born infants were vaccinated with the diphtheria, tetanus, and pertussis-inactivated poliomyelitis-*Haemophilus influenzae* type B-hepatitis B six-in-one vaccine and a ten-valent pneumococcal vaccine at 3 months, 5 months, and 11 months. Randomisation was done using a number generator in a 1:1 ratio and with sealed envelopes. Participants and clinical trial staff were not masked, but laboratory technicians were unaware of study group assignments. The primary endpoint was serum IgG pertussis toxin antibody concentrations at age 3 months. Cord blood and infant blood samples were collected at age 2 months, 3 months, 6 months, 11 months, and 12 months. Analysis was done by modified intention to treat with all randomly assigned participants in case a laboratory result was available. This trial is registered with ClinicalTrialsRegister.eu (EudraCT 2012-004006-9) and trialregister.nl (NTR number NTR4314). The trial is now closed to new participants.

Findings Between Jan 16, 2014, and March 4, 2016, 118 pregnant women were enrolled into our study, with 58 in the maternal Tdap group and 60 in the control group. The geometric mean concentration (GMC) of pertussis toxin antibodies were higher in infants in the maternal Tdap group than in the control group infants at age 3 months (GMC ratio 16·6, 95% CI 10·9–25·2) and also significantly higher compared with control infants at age 2 months. After primary vaccinations, antibody concentrations for pertussis toxin, filamentous haemagglutinin, and pertactin were significantly lower at all timepoints in infants of the maternal Tdap group than in infants in the control group. No safety issues after maternal Tdap vaccination were encountered.

Interpretation In view of the high pertussis toxin antibody concentrations at age 3 months, maternal vaccination supports a delay of the first pertussis vaccination in infants until at least age 3 months. Maternal antibody interference affects antibody concentrations after primary and booster vaccinations. The clinical consequences of this interference remain to be established.

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Introduction

Pertussis, also known as whooping cough, is caused by the *Bordetella pertussis* bacterium and is a highly contagious and potentially life-threatening respiratory illness, particularly in infants during their first weeks of life.¹ Despite high pertussis vaccination coverage in infants and young children, pertussis is still endemic in many countries.² Since the late 1990s, pertussis has re-emerged in the Netherlands with a disease pattern showing peaks every 2–3 years, with the largest epidemics occurring in 2012 and 2014. In 2016, three children died of pertussis, all of whom were too young to have received a first pertussis

vaccination.³ The Dutch schedule starts with pertussis vaccinations around age 6–8 weeks, with repeat vaccinations at age 3 months and 4 months and a booster vaccination at age 11 months (3 + 1 schedule).

Newborn babies depend on maternal antibodies for protection against pertussis until primary vaccinations. Maternal IgG antibodies are actively transferred across the placenta from mother to child starting at 12–17 weeks of pregnancy, but antibodies rapidly decline in infants during the first months of life.⁴ Maternal tetanus, diphtheria, and acellular pertussis (Tdap) vaccination in the second or third trimester of pregnancy induces high

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Research in context

Evidence before this study

Maternal tetanus, diphtheria, and acellular pertussis (Tdap) vaccination provides protection against clinical pertussis morbidity and high disease severity and mortality during the first months of life, the period during which most hospital admissions and deaths from pertussis occur. However, maternal antibodies are known to interfere with infants' primary immune response after vaccination, resulting in lower circulating pertussis antibody concentrations even after booster vaccinations at a later age than in infants whose mothers were not vaccinated. This interference might have consequences for the optimal timing of the start of infant primary vaccination schedules. To search for available data on the relation between maternal pertussis vaccination, the infants' pertussis antibody response, and age of first pertussis vaccination, we searched PubMed for publications between July 19, 2013 and Sept 6, 2018, with no language restrictions, using the search terms "pregnant women", "vaccination", "maternal Tdap vaccination", "*Bordetella pertussis*", "maternal antibodies", "blunting", "infant immune response", "interference", and "infant vaccination schedules". Given that the search yielded no relevant trials and no studies were found on these topics, we did a randomised clinical trial to explore the effect of maternal Tdap vaccination on infant antibody responses after a reduced 2 + 1 schedule starting at age 3 months instead of the current age of first vaccination at 2 months. Data on infants from the maternal Tdap group were compared with control neonates without Tdap maternal vaccination.

Added value of this study

To our knowledge, this study is the first open-label randomised, parallel, controlled trial that compares the effect of maternal

pertussis vaccination on infant immune response after a delayed and reduced primary 2 + 1 dose-vaccination schedule. Our study shows that maternal pertussis vaccination induces high pertussis antibody concentrations that are well above those in the control group for at least the first 3 months of life. Despite rapid waning of maternal antibodies in the first 3 months of life, antibody concentrations at age 3 months in the maternal Tdap group are also still well above the geometric mean concentrations (GMCs) of infants aged 2 months without maternal vaccination. Maternal vaccination reduced GMCs after primary and booster vaccinations. The clinical relevance of maternal antibody interference remains unclear, since no correlate of protection is known, but 75% of the infants still had high antibody levels above 50 IU/mL after the booster vaccination.

Implications of all the available evidence

The timing of infant vaccination schedules after maternal pertussis vaccination should be reconsidered to optimise the efficacy of infant vaccinations and reduce the interference of maternal antibodies with infant immune responses. Delay of first vaccination until at least 3 months instead of the current first pertussis vaccination at 6–8 weeks of age could reduce interference of maternal antibodies. Implementation of a reduced 2 + 1 schedule requires a high coverage of the maternal Tdap vaccination; therefore, without timely maternal vaccination or in case of premature delivery, the first pertussis vaccination is still advised at 6–8 weeks of age.

pertussis IgG concentrations in vaccinated women, with accompanying high pertussis IgG antibody concentrations in cord blood and in the infants of these women during the first weeks of life.^{5–9} In the UK, maternal Tdap vaccination was implemented in 2012 during an epidemic of infant pertussis. An overall 91% effectiveness in protection against pertussis in infants during the first 3 months of life was observed after the introduction of maternal Tdap when administered at least 1 week before delivery,¹⁰ and maternal Tdap vaccination was also shown to reduce the number of pertussis-associated hospital admissions.¹¹

Along with protection against clinical pertussis, maternal antibodies also reduce antibody generation after primary vaccinations, resulting in lower concentrations of pertussis antibodies after pertussis vaccinations in infancy, which is known as interference or blunting.^{6–8,12–15} The interference of maternal antibodies in the response of infants to primary immunisations continues to affect post-booster antibody concentrations, with antibody concentrations that remain lower than in infants whose mothers were not vaccinated. Mathematical modelling

has shown that starting primary vaccinations later in the life of the infant leads to less maternal antibody interference, given that concentrations of maternal antibodies decline rapidly over the first months of life.¹⁶ We aimed to establish whether it would be better to delay the first primary immunisation in children.

We studied the effect of maternal Tdap vaccination on the infants' vaccination response during the first year of life by measuring pertussis antibodies before and after a delayed and reduced vaccination schedule (2 + 1), in which primary vaccinations were given at age 3 months and repeat vaccinations at age 5 months and 11 months. First, antibody concentrations in infants aged 3 months after maternal Tdap were compared with those of control infants without maternal immunisation. Second, pertussis-specific IgG antibody concentrations after primary vaccination (at age 3 months and 5 months) and before and after booster vaccinations (at age 11 months) were compared between the maternal Tdap group and the control group. To establish whether postponing primary infant vaccinations was sound, we compared antibody concentrations after maternal Tdap vaccination in infants

aged 3 months with antibody concentrations in infants in the control group aged 2 months (the time at which they receive the first pertussis vaccination in the national immunisation programme in the Netherlands). Lastly, antibody concentrations after the reduced 2+1 schedule in infants with and without maternal Tdap vaccination aged 3 months, 5 months, and 11 months were compared with historical vaccination data for the 3+1 schedule in infants without maternal Tdap vaccination aged 2 months, 3 months, 4 months, and 11 months, to establish serological response on both schedules.

Methods

Study design and participants

Through the National Institute for Public Health and the Environment in the Netherlands, we carried out an open-label, parallel, randomised, controlled trial. Pregnant women were recruited through 36 independent midwife clinics. Women received a leaflet with information about the trial and a reply card around 20 weeks of pregnancy. The exclusion criteria for pregnant women were abnormalities revealed by the 20 week ultrasound, pertussis vaccination in the past 5 years or a tetanus or diphtheria vaccine in the past 2 years, known or suspected immunocompromising condition (eg, malignancy, autoimmune disease, or immunodeficiency), immune-modulating medication or the receipt of blood products within 3 months before study entry, known severe adverse reactions to any vaccine component, and immunisation within 2 weeks before study entry (except for influenza). Exclusion criteria for infants included premature birth before 37 weeks of gestational age or an underlying medical condition that could interfere with the results of the study. Fathers of the newborn babies in both the maternal immunisation group and control group were excluded from pertussis vaccination after birth of the infant if they had had a previous severe adverse reaction to any vaccine. A full list of the inclusion and exclusion criteria is available in the appendix (p 1).

Ethical approval was given by the Central Committee on Research Involving Human Subjects, The Hague, the Netherlands. All parents provided written informed consent.

Randomisation and masking

Pregnant women were randomly assigned at a 1:1 ratio to receive a Tdap vaccine during pregnancy between 30 weeks and 32 weeks of gestation (maternal Tdap group) or within 48 h after birth (control group). Randomisation envelopes were generated with a computer containing a letter indicating allocation of participants to the maternal Tdap group or the control group. The envelopes were generated and sealed by a person who was not involved in the trial. On the day of the first study visit around 30–32 weeks of pregnancy, the investigator opened the envelope with the lowest number. Participants were assigned to a group on the

basis of the randomisation envelope. A new set of sealed randomisation envelopes was created during the study after recruitment of 100 participants because of the early withdrawal of some participants who needed replacements, as per the protocol. Only the laboratory personnel were masked to the study group assignments (investigators and participants were aware of group assignments).

Procedures

Maternal data were collected through a questionnaire at recruitment, including age, number of household members and their age, smoking habits, use of antibiotics in the past 3 months, pertussis infection in the past 5 years, and presence of chronic diseases. During follow-up, data on the use of non-steroidal anti-inflammatory drugs around the time of vaccination (mother and newborn baby), infant birthweight, duration of breastfeeding, day-care attendance, and number of episodes and diagnosis and treatment of respiratory tract infections of household members were collected at every home visit (nine to ten visits).

The maternal Tdap group received a Tdap vaccine (Boostrix; GlaxoSmithKline Biologicals, Rixensart, Belgium; containing 2.5 limit of flocculation [Lf] diphtheria toxoid, 5 Lf tetanus toxoid and *B pertussis* antigens; 8 µg pertussis toxin, 8 µg filamentous haemagglutinin, and 2.5 µg pertactin) during weeks 30–32 of pregnancy. Mothers in the control group received a Tdap vaccine (Boostrix) within 48 h after delivery. Fathers in both groups received the Tdap vaccine (Boostrix) within 48 h after birth if they had not had any previous adverse reactions to vaccines. Infants in both groups received routine vaccines according to the national immunisation programme, including Infanrix Hexa (GlaxoSmithKline Biologicals), a conjugate vaccine for diphtheria, tetanus, pertussis (acellular component), hepatitis b (rDNA), poliomyelitis (inactivated), and *Haemophilus influenzae* type B; and Synflorix (ten-valent; GlaxoSmithKline Biologicals), a pneumococcal polysaccharide conjugate vaccine (adsorbed), at age 3 months, 5 months, and 11 months (within 5 days before and after these timepoints).

Finger-prick blood samples of 300 µL were collected from mothers immediately before maternal Tdap vaccination, and within 48 h of delivery, 6 months after delivery, and 12 months after delivery in both groups. Cord blood and 300 µL blood samples from infants were collected before primary vaccinations at age 2 months and 3 months, after primary series at 6 months, and before and after booster vaccination at 11 months and 12 months. We collected local and systemic injection site reactions after the Boostrix vaccination in pregnant women in a diary during the first 7 days after vaccination. Antibody concentrations of IgG against pertussis antigens (pertussis toxin, filamentous haemagglutinin, and pertactin) were measured with a bead-based fluorescent

See Online for appendix

multiplex immunoassay as previously described.¹⁷ This assay showed high correlation with US Food and Drug Administration-based ELISA.¹⁸ The lower limits of assay quantification were 0.85 IU/mL for pertussis toxin, 0.82 IU/mL for filamentous haemagglutinin, and 1 IU/mL for pertactin.

Outcomes

The primary endpoint was serum IgG pertussis toxin antibody concentrations at age 3 months. Secondary outcomes were pertussis-specific IgG and IgA antibody concentrations and serum IgG antibody concentrations against the concomitantly given vaccines after primary vaccination (at 3 months and 5 months) and before and after booster vaccinations at age 11 months, 2 years, and 4 years. Pertussis-specific IgG antibody concentrations in the serum of the mothers, IgA antibody concentrations in breast milk, cellular immune responses before and after booster doses at age 11 months and 4 years, infant pain response, and maternal attitude to blood collection were monitored. In a post-hoc analysis, the 3-5-11 schedule used in this study was compared with a historical cohort from 2011 from a pneumococcal intervention study that included the group that was vaccinated according to the Dutch national immunisation programme. In the study, 66 infants were vaccinated with the same vaccines although in a 3 + 1 schedule at age 2 months, 3 months, 4 months, and 11 months.¹⁹ In this historical cohort, blood was collected at age 5 months (1 month after the third dose of the primary schedule), 11 months (before booster vaccination), and 12 months (1 month after booster vaccination). Antibody concentrations after the primary vaccination series at age 5 months were compared with blood collected at age 6 months in our study and before and after booster vaccination at age 11 months and 12 months. Antibody analysis in the historical cohort was done in the same laboratory with the same methodology as the present study (bead-based fluorescent multiplex immunoassay).¹⁷

Adverse events and serious adverse events were monitored for mothers and infants during pregnancy and within a week after blood collections. All serious adverse events were reviewed by an independent physician and reported to the Central Committee on Research Involving Human Subjects.

Statistical analysis

On the basis of a historical group of 50 infants from a large Dutch serosurvey from 2006–07,²⁰ we expected a geometric mean concentration (GMC) of 5 IU/mL for IgG antibodies against pertussis toxin at age 2 months in the control group, with an SD of log-10-transformed pertussis toxin of 0.5. We assumed slightly more variation and an SD of 0.6 in infants of mothers vaccinated during pregnancy. To detect a 2.5 fold difference in GMC (GMC of 12.5 IU/mL in infants of maternally vaccinated mothers) with a significance level of 5% and power of

	Maternal Tdap group (n=58)	Control group (n=60)
Mothers		
Age, years	32.2 (3.3)	32.3 (3.9)
Gestational age at immunisation, weeks	31.2 (0.8)	..
Interval between vaccination and delivery, days	61 (6)	..
Infants		
Sex		
Male	24 (41%)	22 (37%)
Female	34 (59%)	38 (63%)
Gestational age, weeks	39.7 (1.5)	39.7 (1.2)
Birthweight, g	3425 (480)	3439 (456)
Age at first infant blood sample, days	61 (2.1)	61 (1.8)
Age at second blood sample and first Infanrix Hexa dose, days	91 (3.4)	91 (2.7)
Age at second Infanrix Hexa dose, days	153 (3.3)	153 (2.7)
Age at third blood sample, after primary vaccinations, days	183 (3.5)	184 (3.9)
Age at fourth blood sample, before booster vaccination, days	335 (4.2)	334 (4.5)
Age at fifth blood sample, after booster vaccination, days	365 (4.5)	364 (4.7)
Data are mean (SD) or n (%). Tdap=maternal tetanus, diphtheria, and acellular pertussis.		
Table 1: Baseline characteristics of 118 mother–infant pairs		

90%, we needed 48 infants per group. To account for a dropout of 10% during the study and exclusion of premature infants with a gestational age of less than 37 weeks (6%), we needed 58 pregnant women per group.

All analyses were done in the modified intention-to-treat population (for primary outcomes, given that dropouts were randomly distributed between the two groups, we did not perform imputation, which would have been required for an intention-to-treat analysis; secondary outcomes did not differ between intention-to-treat and per-protocol analysis), including all samples available from the randomly assigned mother–infant pairs and excluding babies born before 37 weeks of gestational age (post-randomisation exclusion criterion). IgG antibody concentrations below the detection limit were set at 0.5 times the limit of detection. For each group, GMCs and 95% CIs were calculated for IgG antibodies against pertussis toxin, filamentous haemagglutinin, and pertactin at different time points. Differences in GMCs between the two groups were analysed with a t-test and GMC ratios with 95% CIs were calculated. All reported p values are two sided and p values of less than 0.05 are significant. Log-transformed data followed a normal distribution. Statistical analyses were done using SPSS version 24. No data monitoring committee was involved. This trial is registered with ClinicalTrialsRegister.eu (EudraCT 2012-004006-9) and trialregister.nl (number NTR4314).

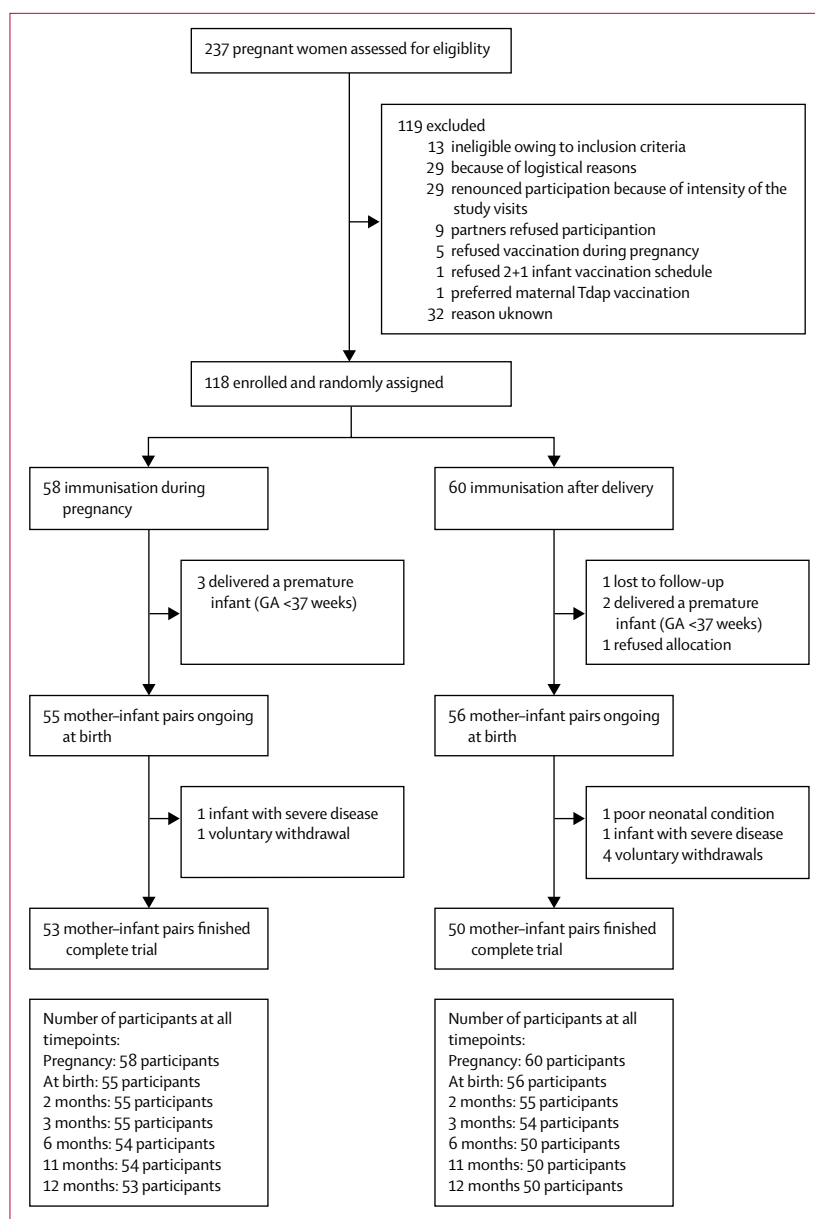


Figure 1: Trial profile
GA=gestational age. Tdap=maternal tetanus, diphtheria, and acellular pertussis.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of the 237 pregnant women who returned the reply card, 118 women were enrolled in the study between Jan 16, 2014, and March 4, 2016. Of these 118 women, 58 women were vaccinated during pregnancy with a mean gestational age

of 31.2 weeks (range 29.9–33.0; maternal Tdap group) and 60 women were randomly assigned for vaccination within 48 h after birth (control group). Baseline characteristics are shown in table 1 and baseline characteristics of the historical cohort in the appendix (p 2). No substantial differences in baseline characteristics existed between the groups of the present study or between the present study groups and the historical cohort. In the maternal Tdap group, 53 mother–infant pairs completed the trial and in the control group 50 pairs completed the trial (figure 1).

Infants' IgG antibody concentrations to pertussis toxin, filamentous haemagglutinin, and pertactin in cord blood and at age 2 months, 3 months, 6 months, 11 months, and 12 months are presented (table 2; figure 2).

At age 3 months, before primary vaccinations, anti-pertussis IgG GMCs in infants of the maternal Tdap group were at least 16.6 times higher than those in infants from the control group (table 2). GMCs for all pertussis antigens in infants aged 3 months in the maternal Tdap group were also at least 9.5 times higher than in infants aged 2 months in the control group, as established by the modified intention-to-treat analysis. In cord blood and at age 2 months anti-pertussis IgG GMCs in the maternal Tdap group were also significantly higher than those in infants from the control group. The control group showed an increase in GMCs for all pertussis antigens after primary vaccinations, but different kinetics were observed for pertussis toxin, filamentous haemagglutinin, and pertactin in the maternal Tdap group. For pertussis toxin, the GMC increased in both groups, but the pertussis toxin GMC after primary vaccination in infants in the maternal Tdap group was significantly lower than the GMC in infants in the control group (table 2). GMCs for filamentous haemagglutinin and pertactin even decreased after primary vaccination (6 months) in the maternal Tdap group, which also resulted in significantly lower GMCs in the maternal Tdap group than in the control group (table 2).

Between primary and booster vaccination, IgG antibody concentrations against all pertussis antigens declined in all infants, and GMCs in the maternal Tdap group remained significantly lower than in the control group (table 2).

Booster vaccination at age 11 months induced a nine times higher increase in pertussis toxin IgG antibody concentrations in infants of both groups, regardless of maternal Tdap vaccination as observed at 12 months. However, despite this increase, the GMC of pertussis toxin antibodies in the maternal Tdap group remained half that of the GMC in the control group (table 2). For the additional filamentous haemagglutinin and pertactin, we observed a higher increase in the maternal Tdap group after booster vaccination than in the control group, but GMC after booster vaccination remained significantly lower than in the control group.

In cord blood, nine (17%) of 53 infants in the control group had antibody concentrations lower than the lower limit of detection (LLOD; table 2). By contrast, all infants

	Maternal Tdap group	Infants with antibody concentrations $\leq$ LLOD*	Control group	Infants with antibody concentrations $\leq$ LLOD*	GMC ratio of the maternal Tdap group vs the control group	GMC ratio of the maternal Tdap group at 3 months of age vs the control group at 2 months of age
Cord blood	n=54	0	n=53	9 (17%)	..	..
Pertussis toxin	125.1 (94.0–166.3)	0	5.6 (3.7–8.6)	7 (13%)	22.2 (13.5–36.7)	..
Filamentous haemagglutinin	330.9 (261.2–419.3)	0	15.6 (11.1–22.0)	1 (2%)	21.2 (14.1–31.8)	..
Pertactin	500.5 (322.5–776.7)	0	11.4 (7.7–16.9)	3 (6%)	44.0 (24.7–78.2)	..
2 months	n=55	1 (2%)	n=53	24 (45%)	..	..
Pertussis toxin	27.3 (20.1–37.1)	1 (2%)	1.8 (1.2–2.5)	16 (30%)	15.7 (9.9–24.8)	..
Filamentous haemagglutinin	83.7 (67.4–103.9)	0	5.0 (3.5–7.0)	4 (8%)	16.9 (11.4–25.1)	..
Pertactin	110.3 (71.6–170.0)	0	3.6 (2.3–5.4)	12 (23%)	31.1 (17.3–56.1)	..
3 months	n=54	1 (2%)	n=50	34 (68%)	..	..
Pertussis toxin	16.6 (12.6–21.9)	0	1.0 (.7–1.4)	28 (56%)	16.6 (10.9–25.2)	9.5 (6.1–14.9)
Filamentous haemagglutinin	48.5 (38.5–61.1)	0	2.8 (2.0–3.8)	4 (8%)	17.5 (12.1–25.4)	9.8 (6.5–14.7)
Pertactin	65.5 (41.9–102.43)	1 (2%)	2.0 (1.4–2.8)	17 (34%)	33.1 (18.9–58.1)	18.5 (10.1–33.9)
6 months	n=53	0	n=50	0	..	..
Pertussis toxin	35.6 (28.1–45.0)	0	83.0 (65.6–105.2)	0	0.4 (0.3–0.6)	..
Filamentous haemagglutinin	31.2 (25.8–37.8)	0	82.6 (69.8–97.7)	0	0.4 (0.3–0.5)	..
Pertactin	28.9 (21.9–38.0)	0	61.9 (47.7–80.4)	0	0.5 (0.3–0.7)	..
11 months	n=51	6 (12%)	n=48	1 (2%)	..	..
Pertussis toxin	8.4 (6.1–11.5)	1 (2%)	16.6 (13.2–20.8)	0	0.5 (0.4–0.7)	..
Filamentous haemagglutinin	7.3 (5.3–10.1)	1 (2%)	19.9 (15.7–25.2)	0	0.4 (0.3–0.5)	..
Pertactin	2.9 (2.2–3.8)	5 (10%)	8.4 (6.3–11.3)	1 (2%)	0.3 (0.2–0.5)	..
12 months	n=53	0	n=50	0	..	..
Pertussis toxin	75.7 (62.0–92.4)	0	157.7 (124.6–199.5)	0	0.5 (0.4–0.7)	..
Filamentous haemagglutinin	67.3 (54.9–82.6)	0	125.5 (102.68–153.25)	0	0.5 (0.4–0.7)	..
Pertactin	91.7 (69.2–121.4)	0	142.0 (109.7–183.7)	0	0.7 (0.5–0.9)	..

Data are number in IU/mL (95% CI) unless otherwise stated. GMC=geometric mean concentration. Tdap=maternal tetanus, diphtheria, and acellular pertussis vaccination. LLOD=lower limits of assay quantification.*The LLOD were 0.85 IU/mL for pertussis toxin, 0.82 IU/mL for filamentous haemagglutinin, and 1 IU/mL for pertactin. Antibody concentrations were less than or equal to the LLOD for a single antigen or to more than one antigen.

Table 2: GMCs for IgG antibodies against pertussis toxin, filamentous haemagglutinin, and pertactin in infants during their first year of life

in the maternal Tdap group had detectable antibody concentrations. At age 2 months, 24 (45%) of 53 infants in the control group had antibody concentrations that were lower than or equal to the LLOD: 17 infants for one vaccine antigen, including nine infants for pertussis toxin, one for filamentous haemagglutinin, and seven for pertactin; four infants for both pertussis toxin and pertactin; two infants for both pertussis toxin and filamentous haemagglutinin; and one infant for all three antigens. In the maternal Tdap group, only one (2%) of 54 infants had pertussis toxin concentrations that were less than or equal to the LLOD. At age 3 months, only one (2%) of 54 infants in the maternal Tdap group showed pertactin antibody concentrations that were lower than or equal to the LLOD, but in the control group, more than half of the control infants (34 [68%] of 50) showed

antibody concentrations that were lower than or equal to the LLOD. Antibody concentrations lower than or equal to the LLOD for a single antigen were observed in 21 infants (16 infants for pertussis toxin and five infants for pertactin), and 11 infants had antibody concentrations lower than or equal to the LLOD for two antigens (two infants for pertussis toxin and filamentous haemagglutinin and nine infants for pertussis toxin and pertactin). Undetectable concentrations for all vaccine antigens were observed in two infants in the control group. After the primary vaccinations, all infants of both groups showed antibody concentrations that were higher than the LLOD. Before booster vaccination, six (12%) of 51 infants in the maternal Tdap group had undetectable antibody concentrations compared with one (2%) of 48 infants in the control group. Following booster

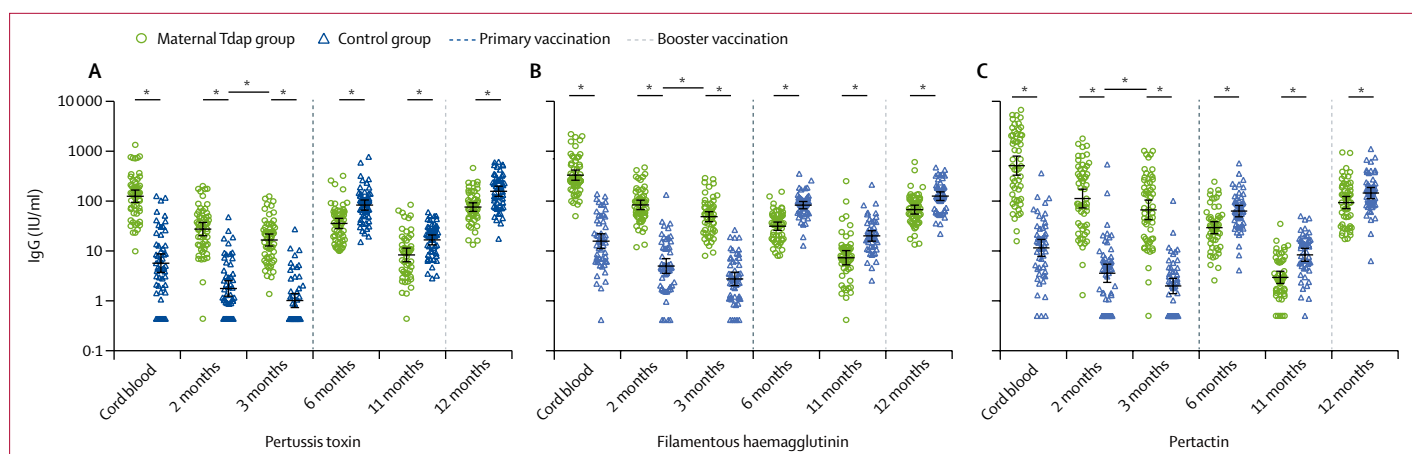


Figure 2: Pertussis-specific antibody concentrations in infants at all study time points

Anti-pertussis toxin (A), anti-filamentous haemagglutinin (B), and anti-pertactin (C) IgG antibody concentrations in infants from mothers vaccinated during pregnancy (green) and infants from mothers vaccinated directly after delivery (blue) are presented. Results represent individual antibody concentrations, expressed in IU/mL. Horizontal bars represent the geometric mean concentrations and their 95% CIs of each group. * $p < 0.05$.

vaccination, all infants of both groups showed antibody concentrations that were higher than the LLOD.

1 month after the primary vaccination series at age 3 months and 5 months, GMCs for pertussis toxin and filamentous haemagglutinin antibodies in the control group were similar to antibody GMCs of the historical cohort with three primary vaccinations at 2 months, 3 months, and 4 months according to the national immunisation schedule (appendix p 3). After the primary vaccination series, pertactin antibody concentrations were significantly lower in the control group in the present study than in the historical cohort. Before the booster vaccination at age 11 months, GMCs for pertussis toxin and filamentous haemagglutinin were comparable between the control group after the 2+1 schedule and the historical cohort, but pertactin antibody GMC remained significantly lower in the control group than in the historical cohort (appendix p 3). After the booster vaccination, the GMC for pertussis toxin was significantly higher after the reduced 2+1 schedule in the control group than in the historical cohort, and antibody concentrations were the same between the groups for filamentous haemagglutinin, but significantly lower for pertactin. In the maternal Tdap group, GMCs of all pertussis antigens at all timepoints were significantly lower than those of the control group and the historical cohort group, although large overlapping CIs between antibodies between groups was observed.

The antibody GMCs of the mothers are provided in the appendix (pp 4, 7). At birth, GMCs for all pertussis antigens were significantly higher in the mothers vaccinated with Tdap than those in the control group. All pertussis antibody concentrations in the cord blood were higher than those from the maternal finger-prick blood around birth, with a ratio of 2.0 (95% CI 1.8–2.1) for pertussis toxin, 2.0 (1.9–2.2) for filamentous haemagglutinin, and 1.8 (1.7–1.9) for pertactin in the maternal

Tdap group. A significantly lower ratio was observed for pertussis toxin in the control group (1.7, 95% CI 1.6–1.9) compared with the maternal Tdap group. Ratios did not differ significantly between the maternal Tdap group and the control group for filamentous haemagglutinin and pertactin (data not shown).

After delivery, antibody concentrations in mothers of both groups gradually declined over time. In a post-hoc analysis, 6 months after giving birth, the GMCs of pertussis toxin, filamentous haemagglutinin, and pertactin antibodies in mothers of the maternal Tdap group showed no significant difference compared with those of mothers vaccinated within 2 days of giving birth (appendix pp 3, 7). 1 year after giving birth, the GMCs for pertussis toxin were slightly higher after maternal Tdap vaccination than vaccination after delivery (appendix pp 3, 7). In both groups, pertussis antibody GMCs remained significantly higher than GMCs within the first 2 days after birth in unvaccinated mothers. Because of the age of the mothers, we considered that they had previously been primed with a whole-cell pertussis vaccine.

The main adverse events in women of the maternal Tdap group after vaccination were redness in 14 (25%) of 55 women and pain in 49 (86%) of 55 women. Systemic reactions were predominantly fatigue in 25 (44%) of 57 women and myalgia in 38 (67%) of 57 women. The number of local site reactions and systemic reactions within 7 days after the Boostrix vaccination in pregnant women were in accordance with the European Public Assessment Report for Boostrix, although more accounts of fatigue and pain were recorded.²¹ Fatigue was also reported before Tdap vaccination and did not increase afterwards (appendix p 5). Serious adverse events in mothers and infants are shown in the appendix (p 6). In the maternal Tdap group, serious adverse events were reported by 27 (47%) of 58 women and in five (9%) of

58 infants. In the control group they were reported by 16 (27%) of 60 women and in 11 (18%) of 60 infants. In the maternal Tdap group, three premature infants were born compared with two infants in the control group. Caesarean sections were done in seven pregnant women of the maternal Tdap group versus four women in the control group. All serious adverse events were considered unrelated to maternal Tdap vaccination.

Data on IgG and IgA antibodies to other vaccine components, IgA antibody concentrations in breast milk, cellular immune responses, infant pain response, and maternal attitude to blood collection are not yet available.

Discussion

In this open-label, parallel, randomised, controlled study, we show that maternal Tdap vaccination around 30–32 weeks of gestational age results in term-born children with at least 22 times higher serum cord blood pertussis IgG antibody GMCs and 9·5 times higher serum pertussis IgG antibody GMCs at age 3 months, compared with infants in the control group without maternal immunisation at age 2 months (the current age of the first pertussis vaccination in the national vaccination programme). All infants of the maternal Tdap group had detectable pertussis toxin antibody concentrations at age 3 months, whereas more than half of the children in the control group had pertussis toxin antibody concentrations that were below the LLOD. After the primary series of vaccinations, infants of mothers who received a maternal Tdap vaccination had significantly reduced antibody concentrations (pertussis toxin GMC ratio of 0·4) and the effect of maternal antibody interference on the achieved antibody concentrations for pertussis toxin, filamentous haemagglutinin, and pertactin persisted until after the booster vaccination at age 11 months (pertussis toxin GMC ratio of 0·5). These changes were apparent despite the fact that the increase after booster vaccination was similar in both groups for pertussis toxin and higher in the maternal Tdap group than in the control group for filamentous haemagglutinin and pertactin. Although antibody GMCs achieved after infant primary and booster vaccination were significantly different in children with and without maternal Tdap vaccination, both groups included infants with similar antibody concentrations. Despite the fact that there is no established correlate of protection for pertussis, pertussis toxin antibody GMCs were substantial after booster vaccination.

Many more children in the control group than in the Tdap group had antibody concentrations below the LLOD. A Swedish trial²² reported that undetectable antibodies to more than one type of antigen, including pertussis toxin, pertactin, and *B pertussis* fimbriae (but not filamentous haemagglutinin) and especially undetectable concentrations of antibody to multiple antibody classes, were associated with low or absent vaccine effectiveness against pertussis in cases in which a household contact had contracted pertussis.²² These findings suggest that

protection against pertussis can be considerably improved after timely maternal Tdap vaccination until at least age 3 months.

In the case of maternal Tdap vaccination and primary vaccinations starting at age 2 months, more blunting is likely to be expected compared with starting primary vaccinations at 3 months.¹⁶ We found that despite the rapid decrease of maternal antibodies in the first months of life of the infant, delaying primary vaccinations until age 3 months does not prevent blunting of primary vaccinations, with antibody concentrations being up to 40% lower than without maternal vaccination. In studies done in the UK and Belgium, maternal Tdap vaccination, with primary vaccinations at age 2 months, 3 months, and 4 months also resulted in the same extent of interference, with lower pertussis antibodies being recorded after primary vaccination, results that were similar to those of our study with a first vaccination at age 3 months.^{8–13} In the cohort study in the UK, despite the interference and thus low antibody concentration, after primary vaccination the number of pertussis cases did not increase between primary and booster pertussis vaccinations in the 3 years following maternal Tdap vaccinations, nor was this the case later in infancy.¹⁰ Generally, later infant primary immunisations induce higher antibody concentrations than earlier primary immunisations.²³ Infants who are older at the time of primary immunisations benefit from less maternal antibody interference than those who are younger, but also from a more experienced immune system with higher protective B-cell responses later in life.²⁴ A meta-analysis by Voysey and colleagues,¹⁶ who studied maternal antibody interference without maternal vaccination and did mathematical modelling of delays of vaccination and blunting, suggested that pertussis toxin antibody concentrations would increase by 15%, filamentous haemagglutinin by 22%, and pertactin antibody by 22% per additional month of age at which primary vaccinations were started. We showed that maternal Tdap vaccination significantly increases maternal antibody concentrations and likewise significantly enhances interference with primary vaccinations. This finding further supports considering a delay of primary immunisations after maternal Tdap vaccination against pertussis.

Together, our data suggest that timely maternal Tdap vaccination around 30–32 weeks of pregnancy leads to term-born children having high pertussis antibody concentrations until at least age 3 months, which might allow for a delay in primary vaccinations up to the age of at least 3 months. Our data also support introduction of a reduced 2+1 schedule starting at age 3 months, given that antibody concentrations are similar between infants on this reduced schedule without maternal Tdap vaccination and historical data of a 3+1 schedule starting at age 2 months without maternal immunisation. However, the value of this delayed and reduced schedule will depend on the coverage of Tdap vaccination in

pregnancy and the effectiveness of this schedule should be closely monitored.

Interference of maternal antibodies following maternal Tdap vaccination both after primary vaccinations and after booster vaccinations has also been reported in other studies.^{6–8,12–15,25} However, these clinical studies reported miscellaneous effects of maternal Tdap vaccination on infants' immune response after (primary and booster) vaccination. Results vary because of small sample sizes, wide range of timing of maternal Tdap vaccination, different brands of maternal and infant vaccines, different infant schedules, and observational study designs that usually have low statistical power.

Use of a historical comparator has its limitations. The cohort was not randomised, and a different timepoint of blood sampling might have affected pertussis antibody responses. However, incidence of pertussis in young infants during recruitment of the historical cohort (2011–12) was similar to 2014–16 when the present cohort was recruited, and recruitment and results were obtained by the same research institute and laboratory, according to the same methods.³

Another limitation of our study is that we studied maternal Tdap vaccination in only a small window of 30–32 weeks' gestational age as advised in other countries, and only term babies were studied.^{26,27} This time window might not reflect real life, with maternal vaccination occurring in the second or third trimester, premature delivery, and infants with enhanced risk for severe pertussis. The optimal timing of maternal vaccination is still under debate.^{28,29} When less transfer of maternal antibodies is expected, as in the case of prematurely born babies, neonates without maternal vaccination, infants whose mothers received maternal Tdap within 2 weeks before delivery, or infants with enhanced risk for another reason, primary vaccination at age 6 weeks and a 3+1 schedule might provide the best protection against clinical pertussis. We need more research to investigate maternal vaccination in the second trimester, after 32 weeks, and in premature infants.

In conclusion, our data support a start of pertussis vaccination at age 3 months instead of 2 months in the case of timely administration of maternal Tdap vaccination and when infants are born after term, and in developed countries with vaccine-preventable disease epidemiology that is comparable to the Netherlands. Infants of immunised mothers have high antibody concentrations during the first months of life, which correlate with protection against clinical pertussis.²² Infants will benefit from a later start of primary vaccination because of less interference because of lower concentration of maternal antibodies. The infants' vaccination schedule after maternal pertussis vaccination should be reconsidered to optimise the efficacy of infants' vaccinations and reduce the interference of maternal antibodies with the infants' immune response, not only in countries that still want to implement maternal Tdap, but also in those that have

already implemented this vaccination. A later age at the start of vaccination might also allow reduction of the number of doses in the primary series.

Contributors

NYR, IP, MAVH, FGAV, MJK, and EAMS designed the trial. NYR, IP, MAVH, and DB coordinated and did the clinical trial, clinical data collection, and clinical data management. DB and GAMB generated the Luminex data. DB, MJK, and JvdK did the statistical analysis. DB, EAMS, and NYR wrote the first draft of the manuscript and all authors contributed to subsequent drafts. All authors read and approved the final manuscript.

Declaration of interests

We declare no competing interests.

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References

- de Greeff SC, Mooi FR, Westerhof A, et al. Pertussis disease burden in the household: how to protect young infants. *Clin Infect Dis* 2010; **50**: 1339–45.
- World Health Organization meeting of the strategic advisory group of experts on immunization, April 2014—conclusions and recommendations. *Wkly Epidemiol Rec* 2014; **89**: 221–36.
- van Alebeek R, Benschoop K, van Benthem B, et al. The National Immunisation Programme in the Netherlands: surveillance and developments in 2016–2017. Bilthoven: National Institute for Public Health and the Environment, 2017.
- Malek A, Sager R, Kuhn P, Nicolaides KH, Schneider H. Evolution of maternofetal transport of immunoglobulins during human pregnancy. *Am J Reprod Immunol* 1996; **36**: 248–55.
- Abu Raya B, Sruogo I, Kessel A, et al. The effect of timing of maternal tetanus, diphtheria, and acellular pertussis (Tdap) immunization during pregnancy on newborn pertussis antibody levels - a prospective study. *Vaccine* 2014; **32**: 5787–93.
- Munoz FM, Bond NH, Maccato M, et al. Safety and immunogenicity of tetanus diphtheria and acellular pertussis (Tdap) immunization during pregnancy in mothers and infants: a randomized clinical trial. *JAMA* 2014; **311**: 1760–69.
- Hoang HT, Leuridan E, Maertens K, et al. Pertussis vaccination during pregnancy in Vietnam: results of a randomized controlled trial pertussis vaccination during pregnancy. *Vaccine* 2016; **34**: 151–59.
- Maertens K, Cabore RN, Huygen K, Hens N, Van Damme P, Leuridan E. Pertussis vaccination during pregnancy in Belgium: results of a prospective controlled cohort study. *Vaccine* 2016; **34**: 142–50.
- Gall SA, Myers J, Pichichero M. Maternal immunization with tetanus-diphtheria-pertussis vaccine: effect on maternal and neonatal serum antibody levels. *Am J Obstet Gynecol* 2011; **204**: 334.e1–e5.
- Amirthalingam G, Campbell H, Ribeiro S, et al. Sustained effectiveness of the maternal pertussis immunization program in England 3 years following introduction. *Clin Infect Dis* 2016; **63** (suppl 4): S236–43.
- Amirthalingam G, Andrews N, Campbell H, et al. Effectiveness of maternal pertussis vaccination in England: an observational study. *Lancet* 2014; **384**: 1521–28.
- Jones C, Pollock L, Barnett SM, Battersby A, Kampmann B. Specific antibodies against vaccine-preventable infections: a mother-infant cohort study. *BMJ Open* 2013; **3**: e002473.
- Ladhani SN, Andrews NJ, Southern J, et al. Antibody responses after primary immunization in infants born to women receiving a pertussis-containing vaccine during pregnancy: single arm observational study with a historical comparator. *Clin Infect Dis* 2015; **61**: 1637–44.

- 14 Hardy-Fairbanks AJ, Pan SJ, Decker MD, et al. Immune responses in infants whose mothers received Tdap vaccine during pregnancy. *Pediatr Infect Dis J* 2013; **32**: 1257–60.
- 15 Abu Raya B, Edwards KM, Scheifele DW, Halperin SA. Pertussis and influenza immunisation during pregnancy: a landscape review. *Lancet Infect Dis* 2017; **17**: e209–22.
- 16 Voysey M, Kelly DF, Fanshawe TR, et al. The influence of maternally derived antibody and infant age at vaccination on infant vaccine responses: an individual participant meta-analysis. *JAMA Pediatr* 2017; **171**: 637–46.
- 17 van Gageldonk PG, van Schaijk FG, van der Klis FR, Berbers GA. Development and validation of a multiplex immunoassay for the simultaneous determination of serum antibodies to *Bordetella pertussis*, diphtheria and tetanus. *J Immunol Methods* 2008; **335**: 79–89.
- 18 Markey K, Douglas-Bardsley A, Fry N, Barkoff A-M, He Q. External quality assessment scheme for *Bordetella pertussis* serology 2016. Stockholm: European Centre for Disease Prevention and Control, 2018.
- 19 Wijmenga-Monsuur AJ, van Westen E, Knol MJ, et al. Direct comparison of immunogenicity induced by 10- or 13-valent pneumococcal conjugate vaccine around the 11-month booster in dutch infants. *PLoS One* 2015; **10**: e0144739.
- 20 van der Klis FR, Mollema L, Berbers GA, de Melker HE, Coutinho RA. Second national serum bank for population-based seroprevalence studies in the Netherlands. *Neth J Med* 2009; **67**: 301–08.
- 21 US Federal Drug Administration. Highlights of prescribing information. <https://www.fda.gov/downloads/BiologicsBloodVaccines/UCM152842.pdf> (accessed Oct 10, 2018).
- 22 Storsaeter JI, Hallander HO, Gustafsson L, Olin P. Low levels of antipertussis antibodies plus lack of history of pertussis correlate with susceptibility after household exposure to *Bordetella pertussis*. *Vaccine* 2003; **21**: 3542–49.
- 23 Spijkerman J, Veenhoven RH, Wijmenga-Monsuur AJ, et al. Immunogenicity of 13-valent pneumococcal conjugate vaccine administered according to 4 different primary immunization schedules in infants: a randomized clinical trial. *JAMA* 2013; **310**: 930–37.
- 24 Siegrist CA, Aspinall R. B-cell responses to vaccination at the extremes of age. *Nat Rev Immunol* 2009; **9**: 185–94.
- 25 Halperin SA, Langley JM, Ye L, et al. A randomized controlled trial of the safety and immunogenicity of tetanus, diphtheria, and acellular pertussis vaccine immunization during pregnancy and subsequent infant immune response. *Clin Infect Dis* 2018; **67**: 1063–71.
- 26 Updated recommendations for use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine (Tdap) in pregnant women and persons who have or anticipate having close contact with an infant aged <12 months. Advisory Committee on Immunization Practices (ACIP), 2011. *MMWR Morb Mortal Wkly Rep* 2011; **60**: 1424–26.
- 27 Updated recommendations for use of tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine (Tdap) in pregnant women. Advisory Committee on Immunization Practices (ACIP), 2012. *MMWR Morb Mortal Wkly Rep* 2013; **62**: 131–35.
- 28 Healy CM, Rench MA, Baker CJ. Importance of timing of maternal combined tetanus, diphtheria, and acellular pertussis (Tdap) immunization and protection of young infants. *Clin Infect Dis* 2013; **56**: 539–44.
- 29 Eberhardt CS, Blanchard-Rohner G, Lemaitre B, et al. Maternal immunization earlier in pregnancy maximizes antibody transfer and expected infant seropositivity against pertussis. *Clin Infect Dis* 2016; **62**: 829–36.