Pertussis vaccination during pregnancy: Scientific evidence behind the recommendation

Kinkhoestvaccinatie tijdens de zwangerschap: Wetenschappelijke achtergrond om de aanbeveling te ondersteunen

Dissertation for the degree of doctor in the Medical Sciences at the University of Antwerp

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“It always seems impossible until it’s done” (N. Mandela)

“There is no greater warrior than a mother protecting her child” (N.K. Jemisin)

Content

Summary

Young infants are prone to severe pertussis disease and death. Globally, according to the World Health Organization (WHO), 142,512 cases of pertussis and 56,694 pertussis related deaths occurred in 2015 in children under 5 years of age. Universal pertussis vaccination programs have been introduced with success. Nevertheless, the current programs fail to protect infants younger than 6 months of age, too young to be completely protected by the currently available vaccines and vaccination schedules.

In order to protect these young infants, the National Immunization Technical Advisory Groups (NITAG) from several countries, including Belgium, decided to implement the cocoon strategy, i.e. vaccination of all close contacts of the infant, including postpartum women.

In the **first chapter** of this PhD thesis, we looked at the effect of administering an acellular pertussis (aP) containing vaccine between two successive pregnancies, on the maternal protection still offered at the time of the second pregnancy. This is in fact the effect of cocoon vaccination on maternal antibodies offered at a next pregnancy. We reported significantly lower antibody titers for all measured pertussis specific antigens (Pertussis Toxin (PT), Filamentous Hemagglutinin (FHA), Pertactin (Prn)) in children born after the Tdap booster dose compared to children born before the Tetanus, Diphtheria and aP (Tdap) booster dose at 12 months of age. The median interval between Tdap vaccination and the second delivery was 16.80 months. Mathematical modelling of the data was performed in order to estimate the potential interval needed between two aP containing booster doses in women of childbearing age still inducing a possible beneficial effect for the consecutive offspring: this interval should be no longer than 30 months, which is approximately 2.5 years, in women of reproductive age to have sufficient antibody levels to PT at a next delivery.

However, research showed that cocoon vaccination was not cost-effective and that within this strategy, it was difficult to reach sufficient coverage to adequately protect the infants, leaving them with a susceptibility gap for infection. Therefore, there was a need to look for another vaccination strategy to optimally protect vulnerable infants.

Since protection against infectious diseases in infants immediately after birth is provided by maternal antibodies transferred from mother to infant via transplacental transport and lactation, the amount of transmitted antibodies depends on the placental function and on the concentration of maternal antibodies in the pregnant women. This concentration depends on the vaccination status of the women and the time since last vaccination or disease. After vaccination against pertussis in childhood, antibodies start to decline. Therefore, the amount of pertussis-specific antibodies transferred from mother to child during pregnancy, is low. Thus, increasing the load of maternal antibodies by maternal vaccination is the only way, with the currently available vaccines, to offer passive protection to the newborn from immediately after birth.

Therefore, NITAGs from several countries, including Belgium, recommend the administration of an aP containing vaccine during pregnancy. However, at the time of implementation, the decision to recommend this strategy was mainly based on epidemiological need and many immunological and safety aspects of this promising public health strategy were lacking. Therefore, within this PhD thesis, the main aim was to generate scientific evidence to further support the maternal pertussis immunization strategy.

In a **second chapter**, humoral and cellular immune responses in pregnant and non-pregnant women of childbearing age after a booster vaccination with an aP containing vaccine (Boostrix®) were compared. While no differences in humoral immune responses for all antigens included in the Tdap vaccine were found between pregnant and non-pregnant women, significantly weaker cellular immune responses were found in pregnant women compared to non-pregnant women. We further demonstrated that already one year after Tdap vaccination, significantly waning of pertussis-specific antibodies was seen, supporting the current recommendations for repeat boosters in consecutive pregnancies.

In a **third chapter**, the safety and immunogenicity of pertussis vaccination during pregnancy was studied in parallel in Belgium and Vietnam. In Belgium, pregnant women were vaccinated with the aP containing vaccine Boostrix® while in Vietnam pregnant women were vaccinated with the aP containing vaccine Adacel®. All children in Belgium and Vietnam received the hexavalent aP containing vaccine Infanrix Hexa® and were followed until one month after their booster vaccination in the second year of life to see whether high titers of maternal antibodies induced by maternal vaccination interfere with infant immune responses in the first years of life. Safety outcomes were monitored during the entire study period.

Within both the Belgian and Vietnamese study, no unexpected side effect in the women other than the side effects described on the summary of product characteristics were encountered. In the infants, no unexpected side effects and no congenital disorders were detected. Therefore, our studies add to the evidence that vaccination during pregnancy is safe. Blunting, defined as a significantly lower antibody titer at a certain time point in the offspring of women vaccinated with an aP containing vaccine during pregnancy compared to the offspring of women not vaccinated with an aP containing vaccine during pregnancy, of the anti-PT and anti-Diphtheria Toxin (DT) immune responses was observed after primary vaccination and of the anti-PT immune responses after booster vaccination in the Belgian study. In the Vietnamese study, blunting of the anti-Prn and anti-DT immune responses is seen after primary immunization yet not anymore after booster immunization.

In the past, blunting of the infant immune responses was not only seen to the same vaccine antigens than the ones included in the Tdap vaccine, but also to other vaccine antigens included in the infant vaccination schedule. Therefore, in a **fourth chapter**, we checked whether maternal Tdap vaccination interferes with the infant's immune responses to 13-valent pneumococcal vaccines. We were able to confirm the blunting effect that was already observed in the UK with blunting for 9 out of 13 vaccine-included pneumococcal serotypes after primary immunization and 2 out of 13 serotypes after booster immunization. We also showed that while pneumococcal immune responses were blunted, the proportion of infants achieving protective concentrations was similar after primary and booster vaccination irrespective of the maternal vaccination status.

In a **fifth chapter**, the effect of maternal immunization on breast milk composition was studied. A review of the available literature on the effect of maternal vaccination on breast milk composition and possible clinical protection was carried out and showed that evidence was limited or even lacking. Therefore, in a second section of this chapter, an observational study looking at the effect of different maternal pertussis vaccination strategies on breast milk composition was performed. This study demonstrated that women receiving an aP containing vaccine during pregnancy or shortly after delivery (cocoon vaccination) show significantly higher levels of anti-PT sIgA antibodies in breast milk 8 weeks postpartum compared to women who did not receive a pertussis containing vaccine for at least 5 years before delivery.

Finally, in a **sixth chapter**, the coverage of the recently implemented recommendation for pertussis vaccination during pregnancy in Flanders along with the coverage of the cocoon strategy in partners, was determined in two subsequent studies performed after the free of charge availability of the aP containing vaccine in Flanders. Additionally, predictors for non-vaccination were assessed. In the first study, conducted between October 2014 and May 2015, a coverage of 64.0% for pertussis vaccination during pregnancy is reported. In the second study conducted in the first half of 2016, a coverage of 69.3% for pertussis vaccination during pregnancy is reported. Yet, there is still room for improvement by targeting the underserved populations of pregnant women like multiparous women and women with lower socio-demographic background.

To conclude, we demonstrated in this PhD thesis that maternal pertussis vaccination is a safe and immunogenic strategy for both mother and infants. We also showed that maternal pertussis vaccination has a positive influence on breast milk composition. By using a mathematical model, we provided scientific evidence for the recommendation to repeat the vaccine every pregnancy. We were also able to show that with a good approach, it is possible to reach a high coverage of the strategy within a few years after implementation.

Samenvatting

Zuigelingen zijn erg vatbaar voor kinkhoest en kinkhoest gerelateerde sterfte. Wereldwijd werden er in 2015 volgens de cijfers van de Wereldgezondheidsorganisatie 142.512 kinkhoestgevallen en 56.694 kinkhoest gerelateerde sterfgevallen in kinderen onder de leeftijd van 5 jaar gerapporteerd. Ondanks de introductie van succesvolle universele kinkhoestvaccinatieprogramma's, slagen deze programma's er echter niet in om zuigelingen onder de leeftijd van 6 maanden, die te jong zijn om reeds volledig beschermd te zijn door de huidig beschikbare kinkhoestvaccins en kinkhoestvaccinatieprogramma's, te beschermen.

In een poging om deze zuigelingen te beschermen introduceerde de Nationale Vaccinatieraad van verschillende landen, waaronder België, de "cocoon strategie". Dit is een strategie waarin alle nauwe contacten van zuigelingen, inclusief pas bevallen moeders, worden gevaccineerd. In het **eerste hoofdstuk** van deze doctoraatsthesis hebben we gekeken naar het effect van het toedienen van een acellulair kinkhoest bevattend vaccin (Boostrix®) tussen twee opeenvolgende zwangerschappen. Hier meten we eigenlijk het effect van cocoon vaccinatie op maternale antistoffen tijdens een volgende zwangerschap. We rapporteerden significant lagere antistoftiters voor alle kinkhoest specifieke antigenen (Pertussis Toxine (PT), Filamenteus Hemagglutine (FHA) en Pertactine (Prn)) die werden gemeten op de leeftijd van 12 maanden in kinderen geboren na het toedienen van het kinkhoest bevattend vaccin vergeleken met kinderen geboren voor het toedienen van het kinkhoest bevattend vaccin. Het mediane interval tussen het toedienen van het kinkhoest bevattend vaccin en de volgende bevalling was 16,80 maanden. Mathematische modellering van de data werd uitgevoerd om een schatting te bekomen van het interval nodig tussen twee opeenvolgende boosterdosissen van het acellulair kinkhoest bevattend vaccin in vrouwen van vruchtbare leeftijd dat nog steeds een voordelig effect heeft op een volgend geboren kind: dit interval mag niet langer dan 30 maanden zijn, wat ongeveer 2,5 jaar is, om nog voldoende hoge antistoftiters voor PT te hebben in de vrouw bij een volgende bevalling.

Onderzoek toonde echter aan dat cocoon vaccinatie niet kosteneffectief is en dat het moeilijk is om een voldoende hoge vaccinatiegraad te bekomen om zuigelingen adequaat te beschermen, waardoor zuigelingen binnen deze vaccinatiestrategie met een vatbaarheidsvenster voor infectie achterblijven. Daarom was het noodzakelijk om naar een andere vaccinatiestrategie te zoeken die wel in staat was om zuigelingen optimaal te beschermen.

Pasgeborenen en zuigelingen worden beschermd tegen kinkhoest door maternale antistoffen die ze ofwel krijgen via de placenta tijdens de zwangerschap en bij de bevalling of via de borstvoeding na de geboorte. De hoeveelheid antistoffen die wordt doorgegeven van moeder op kind is afhankelijk van de placentaire functie en van de concentratie antistoffen bij de zwangere vrouw. Deze concentratie hangt af van de vaccinatiestatus van de vrouw en de tijd die is verstreken sinds de laatste kinkhoestvaccinatie of de laatste infectie met kinkhoest. Na kinkhoestvaccinatie in de kindertijd neemt de hoeveelheid antistoffen snel af waardoor vrouwen op vruchtbare leeftijd slechts een beperkte bescherming hebben tegen kinkhoest. Hierdoor zal de hoeveelheid antistoffen overgedragen van moeder op kind tijdens de zwangerschap relatief laag zijn. Het verhogen van de hoeveelheid antistoffen door vaccinatie tijdens de zwangerschap is, met de huidig beschikbare vaccins, de enige mogelijkheid om vanaf de geboorte passieve bescherming te bieden aan de zuigeling.

Daarom adviseren Nationale vaccinatieraden van verschillende landen, waaronder België, momenteel de toediening van een acellulair kinkhoest bevattend vaccin tijdens de zwangerschap. Echter, op het moment dat deze aanbevelingen werden geïntroduceerd waren ze grotendeels gebaseerd op epidemiologische data en verschillende veiligheids- en immunologische aspecten van deze strategie ontbraken. Het doel van deze doctoraatsthesis was dus om wetenschappelijke achtergrond te vergaren om de aanbevelingen voor maternale kinkhoestvaccinatie te ondersteunen.

In het **tweede hoofdstuk** werden humorale en cellulaire immuunresponsen in zwangere en niet-zwangere vrouwen van vruchtbare leeftijd vergeleken na vaccinatie met een acellulair kinkhoest bevattend vaccin (Boostrix®). Terwijl er geen verschillen in humorale immuunresponsen werden gezien voor alle antigenen in het vaccin, zagen we wel een verminderde cellulaire immuunrespons in zwangere vrouwen vergeleken met niet-zwangere vrouwen. We toonden ook aan dat er al een significante daling van de kinkhoest specifieke antistoffen aanwezig was 1 jaar na vaccinatie. Dit ondersteunt de aanbeveling voor herhaalde boostervaccinaties in opeenvolgende zwangerschappen.

In het **derde hoofdstuk** werden de veiligheid en immunogeniciteit van kinkhoestvaccinatie tijdens de zwangerschap in parallel in België en Vietnam bestudeerd. In België werden zwangere vrouwen gevaccineerd met het acellulair kinkhoest bevattend vaccin Boostrix®. In Vietnam werden zwangere vrouwen gevaccineerd met het acelluair kinkhoest bevattend vaccin Adacel®. Alle kinderen in België en Vietnam werden gevaccineerd met het hexavalent acellulair kinkhoest bevattend vaccin Infanrix Hexa® en werden opgevolgd tot 1 maand na de

boostervaccinatie in het tweede levensjaar. Er werd nagegaan of hoge titers maternale antistoffen geïnduceerd door maternale vaccinatie interfereren met de immuunrespons in zuigelingen tijdens de eerste levensjaren. Veiligheidsgegevens werden tijdens de hele studieperiode verzameld.

In zowel de Belgische als Vietnamese studie werden geen onverwachte nevenwerkingen in zwangere vrouwen gezien buiten diegene die beschreven staan op de bijsluiter. Ook in de kinderen werden geen onverwachte nevenwerkingen of congenitale afwijkingen gezien. Onze studies dragen dus bij tot de evidentie dat vaccinatie met een kinkhoest bevattend vaccin tijdens de zwangerschap veilig is. Interferentie, gedefinieerd als een significant lagere antistoftiter in kinderen van moeders gevaccineerd met een kinkhoest bevattend vaccin tijdens de zwangerschap vergeleken met kinderen van moeders niet gevaccineerd met een kinkhoest bevattend vaccin tijdens de zwangerschap, werd gezien in de Belgische studie voor PT en Difterie immuunresponsen na primaire vaccinatie en voor PT immuunresponsen na boostervaccinatie. In Vietnam werd interferentie vastgesteld voor Prn en Difterie immuunresponsen na primaire vaccinatie. Na boostervaccinatie werd geen interferentie meer gezien.

In het verleden werd interferentie niet enkel gezien bij de antigenen die in het kinkhoest bevattend vaccin vervat zijn, maar ook tegen andere vaccin antigenen die opgenomen zijn in het zuigelingenvaccinatieschema. In het **vierde hoofdstuk** werd gekeken of maternale vaccinatie met een acellulair kinkhoest bevattend vaccin interfereert met de immunrespons van zuigelingen tegen het 13-valent pneumokokkenvaccin. We slaagden erin om het interferentie-effect dat reeds geobserveerd werd in het Verenigd Koninkrijk te bevestigen. Interferentie voor 9 van de 13 serotypes werd gezien na primaire vaccinatie en voor 2 van de 13 serotypes na boostervaccinatie. We toonden ook aan dat ondanks de interferentie er in beide groepen toch gelijke proporties van kinderen waren met beschermende antistoftiters na primaire en boostervaccinatie ongeacht de maternale vaccinatiestatus.

In het **vijfde hoofdstuk** werd gekeken naar het effect van maternale vaccinatie op de samenstelling van moedermelk. Een review van de beschikbare literatuur over het effect van maternale vaccinatie op de samenstelling van moedermelk en de mogelijke bescherming door moedermelk werd uitgevoerd en leerde ons dat wetenschappelijke evidentie rond dit thema zeer beperkt en zelfs afwezig is. Daarom werd in een tweede luik van dit hoofdstuk een observationele studie uitgevoerd die keek naar het effect van verschillende kinkhoestvaccinatiestrategieën bij de moeder op de samenstelling van moedermelk. Deze studie leerde ons dat vrouwen die gevaccineerd waren met een acellulair kinkhoest bevattend vaccin tijdens de zwangerschap of vrouwen die kort na de geboorte gevaccineerd waren met een acellulair kinkhoest bevattend

vaccin (cocoon vaccinatie) significant hogere PT antistoftiters hadden in hun moedermelk vergeleken met vrouwen die al meer dan 5 jaar niet gevaccineerd waren met een acellulair kinkhoest bevattend vaccin.

Tot slot werd in het **zesde hoofdstuk** de vaccinatiegraad van de recent geïmplementeerde aanbeveling voor kinkhoestvaccinatie tijdens de zwangerschap en de vaccinatiegraad voor cocoonvaccinatie van de partners in 2 opeenvolgende studies in Vlaanderen gemeten. Deze metingen werden uitgevoerd na het gratis op de markt brengen van het kinkhoest bevattend vaccin in Vlaanderen. Ook werden de risicofactoren voor niet-vaccinatie geïdentificeerd. In de eerste studie, uitgevoerd tussen oktober 2014 en mei 2015, werd een vaccinatiegraad voor kinkhoestvaccinatie tijdens de zwangerschap van 64,0% gerapporteerd. In de tweede studie, uitgevoerd in de eerste helft van 2016, werd een vaccinatiegraad van 69,3% voor kinkhoestvaccinatie tijdens de zwangerschap gemeten. Ondanks de relatief hoge vaccinatiegraad is er nog steeds ruimte voor verbetering in Vlaanderen door het bereiken van de moeilijk bereikbare populaties zoals multipare vrouwen en vrouwen met een lagere socio-demografische achtergrond.

In deze doctoraatsthesis waren we in staat om aan te tonen dat kinkhoestvaccinatie tijdens de zwangerschap en veilige en immunogene strategie is voor zowel moeders als zuigelingen. We toonden ook aan dat kinkhoestvaccinatie tijdens de zwangerschap een positief effect heeft op de samenstelling van moedermelk. Aan de hand van een mathematisch model werd wetenschappelijke evidentie bekomen voor de aanbeveling om het kinkhoestvaccin elke zwangerschap te herhalen. We toonden ook aan, dat met een goede aanpak, het mogelijk is om een hoge vaccinatiegraad van de strategie te bereiken enkele jaren na de implementatie van de aanbeveling.

List of abbreviations

ACIP	Advisory Committee on Immunization Practices
ACT	Adenylaat Cyclase Toxin
AE	Adverse Event
AFP	Alpha-Fetoprotein
aP	Acellular Pertussis
ARI	Acute Respiratory Infection
CDC	Center for Disease Control and Prevention
CHC	Commune Health Center
CI	Confidence Interval
CMI	Cell-Mediated Immunity
CPM	Counts Per Minute
DT	Diphtheria Toxoid
DTaP	Diphtheria Tetanus Acellular Pertussis
DTPw	Diphtheria Tetanus Whole Cell Pertussis
ELISA	Enzyme-Linked Immunosorbent Assay
EMA	European Medicine Agency
EPI	Expanded Program on Immunization
EU	Elisa Units
FCS	Fetal Calf Serum
FDA	Food and Drug Administration
FHA	Filamentous Haemagglutinin
FIM	Fimbriae

GMC	Geometric Mean Concentration
GP	General Practitioner
GSK	GlaxoSmithKline
GZA	Gasthuiszusters Antwerpen
HCG	Human Chorion Gonadotrophin
HCP	Health Care Provider
HCW	Health Care Worker
Hib	Haemophilus Influenzae Type b
HIC	High Income country
HIV	Human Immunodeficiency Virus
ICH-GCP	International Conference on Harmonization – Good Clinical Practice
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
INF	Interferon
IPD	Invasive Pneumococcal Disease
IPV	Inactivated Polio Vaccine
IQR	Interquartile Range
IRB	Institutional Review Board
IU	International Units
LIC	Low Income Country

LLOQ	Lower Limit of Quantification
LMIC	Low and Middle-Income Country
LPS	Lipooligosaccharide
MD	Medical Doctor
MOH	Ministry of Health
mL	Milliliter
NIHE	National Institute of Hygiene and Epidemiology
NITAG	National Immunization Technical Advisory Group
NLMM	Non-Linear Mixed Model
OPA	Opsonophagocytosic assay
PAPP-A	Pregnancy Associated Serum Protein – A
PBMC	Peripheral Blood Mononuclear Cell
PCV	Pneumococcal Vaccine
Prn	Pertactin
PT	Pertussis Toxin
PWM	Pokeweed Mitogen
QC	Quality Control
RIF	Respiratory Illness with Fever
RCT	Randomized Controlled Trial
RPM	Rotations per Minute
SAE	Serious Adverse Event
SAEM	Stochastic Approximation Expectation Maximization
SAGE	Strategic Advisory Group of Experts

SD	Standard Deviation
SEM	Standard Error of the Mean
sIgA	Secretory Immunoglobulin A
SmPC	Summary of Product Characteristics
SPSS	Statistical Package for Social Sciences
TCT	Tracheal Cytotoxin
TD	Tetanus Diphtheria
Th	T-Helper
TT	Tetanus Toxoid
TTR	Transplacental Transport Ratio
UK	United Kingdom
USA	United States of America
VVOG	Vlaamse Vereniging voor Obstetrie en Gynaecologie
WHO	World Health Organization
WIV-ISP	Wetenschappelijk Instituut Volksgezondheid – Institut Scientifique de Santé Publique
wP	Whole Cell Pertussis
ZNA	Ziekenhuis Netwerk Antwerpen

Introduction

Pathogenesis and clinical appearance of pertussis

Pertussis, better known as whooping cough, is a highly contagious respiratory infection caused by the gram-negative bacteria *Bordetella pertussis*. The disease process is multifactorial and dependent on numerous toxins and virulence factors, including Pertussis Toxin (PT), Filamentous Hemagglutinin (FHA), Pertactin (Prn), Fimbriae type 2 and type 3 (FIM2/3), Adenylate Cyclase Toxin (ACT), Tracheal Cytotoxin (TCT) and Lipooligosaccharide (LPS), of which PT is the most pathogenic [1]. Pertussis is mainly a toxin-mediated disease. The *Bordetella pertussis* bacteria attach to the cilia of the respiratory epithelial cells and produce toxins that paralyze the cilia and cause inflammation of the respiratory tract. This interferes with the clearing of the pulmonary secretions. The pertussis antigens also allow the organism to evade host defense mechanisms resulting in promoted lymphocytosis and impaired chemotaxis [2].

The incubation period varies between 7-10 days after exposure with a range of 4-21 days. There are typically three clinical phases of pertussis disease: the catarrhal, the paroxysmal and the convalescent phase. In the catarrhal phase, the symptoms are comparable with those of a common cold with a runny nose, a low-grade fever and a mild cough. This cough gradually becomes more severe and after 1-2 weeks the paroxysmal phase starts. Within this phase, patients experience long lasting episodes of coughing followed by an inspiratory “whoop” and post-tussive vomiting. Infants younger than 6 months of age do not have the characteristic whoop but may have apneic episodes resulting in cyanosis and exhaustion. The paroxysmal phase can persist for up to 10 weeks before turning into the convalescent phase characterized by a gradual recovery and less persistent cough [3]. The clinical presentation of the disease in adolescents and adults is often atypical leading to delays in diagnosis and thus eliminating the change for timely treatment [4].

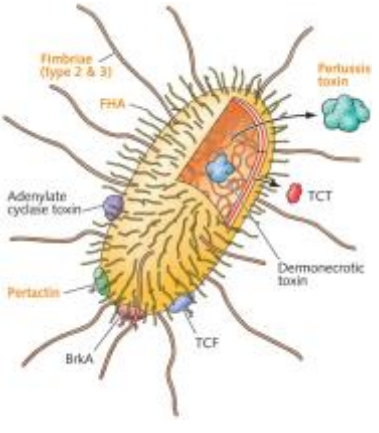

Figure 1: *Bordetella pertussis* bacteria. Adapted from [5].

Epidemiology

Despite the availability of successful universal pertussis vaccination programs (pertussis is one of the diseases targeted by the Expanded Program on Immunization (EPI) [6]; 86% global DTP3 coverage in 2015 [7]), the disease remains an important public health problem and is nowadays one of the most common vaccine-preventable diseases in the world [4]. During recent years, some countries have experienced an increase in the incidence of pertussis [8].

The World Health Organization (WHO) Strategic Advisory Group of Experts (SAGE) pertussis working group recently collected data from 19 countries, both high-income and upper middle-income countries, on pertussis incidence, vaccination coverage, surveillance methods, case definitions and type of vaccines used. Looking at these data, they found no evidence for the resurgence of pertussis at the global level. Only data from 5 out of 19 countries, including Australia, USA, UK, Portugal and Chile, supported the presence of a true resurgence in recent years [9]. The article by Domenech de Cellés et al. [10] confirms this stating that although there is a resurgence of pertussis in some high-income countries (HIC) maintaining high vaccination coverage, this resurgence is not global. However, global data need to be interpreted with caution. Assuming that most of the burden of pertussis occurs in low-middle income countries (LMIC) and no data from these countries are included in most articles. Since pertussis surveillance data from LMIC are largely missing or incomplete due to poor surveillance systems and limited laboratory capacities [11].

Regarding the resurgence of pertussis in HIC, recent outbreaks of pertussis were described in the USA [12], Australia [13] and some parts of Europe [14, 15] including Belgium [16]. The main reasons for the resurgence of pertussis in the vaccine era are multifactorial [17] including waning immunity both after natural infection and vaccination [18, 19], pathogen adaptation with changes in the antigenic and genotypic characteristics of circulating *Bordetella pertussis* strains resulting in the emergence of Prn deficient strains [20, 21], the switch from whole cell (wP) to acellular pertussis (aP) vaccines resulting in a shorter duration of protection after vaccination [19], increased awareness due to the strengthening of surveillance systems and laboratory confirmation capacities [22] and other factors such as variable vaccine uptake and inadequate adult booster dose coverage.

Disease burden

Globally, approximately 142,512 cases of pertussis [7] and 56,694 pertussis related deaths were reported in 2015 in children under the age of 5 year [23], accounting for approximately 1% of all under-5-deaths. More than half of these pertussis related deaths occur in Africa. However, according to the estimates of the WHO (1999), 20 to 40 million pertussis cases take place annually and 300,000 deaths occur due to the disease [24]. This number corresponds with the estimated 24.1 million pertussis cases and 160,700 pertussis related deaths in 2014 reported in the paper by Yeung et al [25]. This is confirmed by seroprevalence studies performed in The Netherlands, Belgium, Czech Republic and The Gambia, where a discrepancy between the number of individuals with signs of a recent pertussis infection in their blood and the actual reported pertussis incidence is observed [26-30].

During the last years, a shift in the age distribution of pertussis towards older age groups, mainly adolescents and young adults, has been seen [31]. However, the highest incidence and disease burden of pertussis disease can still be found in infants below one year of age, too young to be completely protected by the currently available vaccines and vaccination schedules [32]. The case fatality rate (CFR) from pertussis is also the highest in infants below one year of age [24]. A geographical variability in the rate of death shows a CFR of 0.2% in HIC and a CFR as high as 4.0% in LMIC in children under one year of age [33].

Since most pertussis vaccination programs do not start before the age of six weeks, infants are left with a susceptibility gap for pertussis infection. Therefore, alternative vaccination strategies are needed to protect these vulnerable infants during the currently ongoing outbreaks.

In Belgium, the total number of reported pertussis cases increased during the last decade from <200 cases in the period 2004-2010 to 1127 cases in 2015 [34]. The incidence of clinical pertussis cases was the highest in infants below one year of age (87.7/100,000 in 2015), an age group of children extremely vulnerable to pertussis related morbidity and mortality.

Pertussis vaccines

Two types of pertussis vaccines are currently available: the wP and the aP vaccines. The wP vaccines were first licensed in the United States in 1914 and became available combined with diphtheria and tetanus in 1948. The wP vaccines are suspensions of the inactivated entire *Bordetella pertussis* organism. The aP vaccines were first brought on the market in Japan in 1981 and are based on one or more highly purified pertussis antigens [35]. The currently available aP vaccines contain one or more of the following purified antigens: PT, FHA, Prn and FIM types 2 and 3. For the moment, there is no commercially available monovalent pertussis vaccine on the market so all pertussis vaccines are produced as combination vaccines with other antigens [36].

Despite the fact that immune responses to aP vaccines wane more rapidly than immune responses to wP vaccines [19, 37], many HIC and some LMIC have replaced the wP vaccine by the less reactogenic aP vaccine. At the moment, aP vaccines have entirely replaced wP products in the USA, Canada, Australia, many European countries and some countries in Asia. Despite this replacement, the wP vaccine is still the most widely used pertussis vaccine worldwide and the WHO currently recommends that countries using wP vaccines for their primary immunization schedules should continue to do so [1].

For pertussis, no correlate of protection (an immune response that is responsible for and statistically interrelated with protection) is known [38]. However, high IgG levels directed against PT and Prn, rather than FHA and FIM are associated with protection against disease and mainly anti-PT antibodies are considered to be crucial for this protection [39, 40]. Besides protection provided by pertussis-specific antibodies, effector mechanisms of the cellular mediated immune response like IFN-γ are thought to play an important role in the protection against pertussis [41].

Globally, more than 80 different pertussis vaccination schedules are used [9, 42]. In Belgium, pertussis vaccination with a hexavalent aP containing vaccine is offered at 8, 12 and 16 weeks and at 15 months of age. Booster doses are recommended for children at 4-6 years of age (since 2004) and for adolescents at 14-16 years of age (since 2009). Additionally, receiving a booster dose of a pertussis containing vaccine once during adult life is recommended since 2013 [43].

Immune responses to natural infection and vaccination

Despite the fact that both aP and wP vaccines and natural infection are able to induce a significant increase of antibodies against pertussis-specific antigens, humoral immune responses wane quite rapidly and do not provide durable protection against future (re)infection [19].

Studies on T-cell immune responses on the other hand show that these T-cell responses last significantly longer than the presence of serum antibodies and that whereas humoral immunity is waning after a few years, cellular immune responses are still elevated [44-46]. Cellular immune responses to wP vaccines more closely mirror the immune response after natural pertussis infection, with both natural infection and wP vaccination inducing a Th1-polarized immune response. In contrast, responses to aP vaccines are predominantly Th2-polarized [47, 48]. Therefore, we can say that humoral immunity alone is expected not to be sufficient to confer protection against Bordetella pertussis infection and that additionally cellular immunity is required.

Animal models

Human challenge studies can provide the opportunity to study the natural course and immune responses to infection. However, due to a variety of logistical but mainly ethical problems associated with the human challenge model, like the potential for developing severe disease after pertussis challenge, the lack of an effective therapeutic strategy and the highly contagious nature of the pathogen, these human challenge studies were never performed until now [49].

A variety of animal models including mice, rabbits, guinea pigs and newborn piglets have already been proposed and even used in the past to study pertussis disease. While some models were useful to study certain aspects of pertussis, none of these models were able to adequately reproduce the full spectrum of pertussis disease as it is observed in humans [50, 51]. As an example, within the mouse model, researchers were able to study the innate and adaptive immune responses to both pertussis infection and vaccination. However, infected mice do not cough or transmit the disease to naïve mice. Therefore, mouse models were not useful to study the mechanisms of pertussis pathogenesis, circulation and transmission [52].

In addition, when thinking about animal maternal immunization studies, some other problems show up like differences between humans and animals in length of gestation, placental physiology and the relative contribution of placental antibody transfer versus postnatal transfer of antibodies via breast milk. Some animals like ruminants, horses and pigs have no or little placental transfer of maternal antibodies and are therefore not useful for testing maternal

vaccination strategies. Other animals like mice show a more comparable placental and postnatal antibody transfer with humans. However, differences remain with more and longer lasting antibodies transferred through breastfeeding and therefore, mice do not completely replicate the human physiology in terms of pregnancy [53].

To overcome these problems, a nonhuman primate model >96% genetically identical to humans, the baboon model, for pertussis was developed. Within this model, researchers were not only able to investigate host immune responses to pertussis infection and vaccination, but also to look at pertussis pathogenesis and transmission. The baboon is the only model that is able to reproduce the cough illness and to develop disease very similar to severe clinical pertussis in humans. Also, the route of transmission in baboons, which is airborne, is the route of transmission postulated to occur between humans. Further, the baboon proved to be also a relevant model for maternal immunization studies since baboons form a single discoid placentation very similar to humans, they possess the same 4 immunoglobulin G (IgG) subclasses and they have a similar transplacental transport of IgG from mother to fetus as in humans [54]. Despite all advantages, this model has also some limitations. The use of it is quite expensive with fewer animals available for research compared to smaller animal models resulting in relatively low sample sizes in trials where baboons are involved [55, 56]. Also, when using baboons, some ethical considerations should be taken into account since baboons are intelligent animals and the harm caused to these animals should be reduced as much as possible [57]. Additionally, there is a paucity of immunological reagents validated for baboons compared to humans and mice. Finally, it is not possible to link an immune response in baboons to clinical protection. However, for pertussis, this is also not possible in humans since there is no real correlate of protection [50].

Vaccination strategies to protect newborn infants from pertussis

NEONATAL VACCINATION

Immunization of newborns at birth or shortly after birth is a first possible strategy to provide protection to infants in the first months of life when they are most vulnerable to pertussis disease related morbidity and mortality. Some old and recent studies have investigated whether this vaccination strategy is capable to close the neonatal susceptibility gap for infection.

Studies on vaccination with Diphtheria, Tetanus, wP (DTPw) at birth have been conducted decades ago. These studies reported immune tolerance resulting in reduced infant immune responses to both primary and booster vaccination [58].

Recently, four clinical trials investigating neonatal aP immunization have been published [59-64]. Within these trials, different vaccines (Diphtheria, Tetanus, Acellular pertussis (DTaP) and aP) were used and follow-up of the children (bleeding time points, vaccination schedule) was not completely comparable. In the three studies in which newborns were vaccinated with an experimental monovalent aP vaccine (GSK Biologicals), comparable or enhanced immune responses to pertussis antigens were seen following primary vaccination. However, a decreased responsiveness to booster vaccination later in life is seen following neonatal pertussis vaccination. In contrast, lower antibody levels were seen for almost all pertussis-specific antigens after primary and booster vaccination when the child was vaccinated with DTaP vaccine at birth. In all studies, earlier protection was seen after vaccination at birth compared to infants who start their vaccination at 2 months of age.

However, despite the accelerated immune response to vaccination at birth, neonatal vaccination is not capable to completely close the susceptibility gap for infection and leave the infants with a window of vulnerability during the first weeks of life [65]. Also, neonatal aP vaccination showed to interfere with antibody responses to other recommended infant vaccines such as Hepatitis B, *Heamophilus Influenzae type B* and diphtheria [60]. On top of that, regarding the cost-effectiveness of this vaccination strategy, some contradictory results were found. An Australian study found neonatal vaccination as the most cost-effective strategy compared to vaccination at the age of one month and cocoon vaccination [66]. Studies in The Netherlands on the other hand found neonatal vaccination as the least cost-effective strategy compared to maternal vaccination and cocoon vaccination [67, 68].

COCOON VACCINATION

Another strategy intending to protect newborns from pertussis is the cocoon strategy. Within this strategy, close contacts of the newborn, including postpartum women, are vaccinated in order to provide indirect protection to infants who are too young to be immunized or to be protected by immunization [69]. The aim of this strategy is to create a protective barrier towards the infant during the period in which they are at highest risk for infection and complications.

Household contacts are described as the most common source of pertussis infection in young infants. Siblings and parents, especially the mother, have been identified as the primary source of transmission [70-72]. However, when vaccinating postpartum women, we have to keep in mind that a maximum immune response to Tdap is reached only 14 days after vaccination leaving both mothers and infants with a possible gap for infection [73].

The effectiveness of cocoon vaccination on the incidence of pertussis disease in young infants has been studied. In a USA study, postpartum Tdap vaccination of mothers was not associated with a decrease in pertussis cases in infants below 6 months of age [74]. In an Australian study, immunizing both parents with a pertussis containing vaccine was associated with a decreased risk of pertussis in infants below 4 months of age of 51%. The effectiveness of cocooning on pertussis related outcomes was also investigated using statistical models. Most of these models concluded that the cocoon strategy is not an effective strategy to prevent pertussis infection in young infants under the conditions of low pertussis incidence. These models estimated that a large number of individuals need to be vaccinated to prevent one disease-related outcome [75-77].

On top of the not reassuring data on the effectiveness of cocoon vaccination, widespread implementation of the strategy is difficult due to logistic and financial barriers resulting in rather low coverage [78, 79]. Also, the costs related to cocoon vaccination are high and the benefit-to-cost ratio is rather low compared to maternal vaccination [80].

Notwithstanding these hurdles, the cocoon vaccination strategy is still recommended in a number of countries in order to provide protection to newborn infants [81]. In Belgium, cocoon vaccination is recommended to all close contacts of young infants, with special attention for personnel at pediatric departments, maternities and nurseries. Also for mothers, cocoon vaccination is recommended in the immediate postpartum if they were not vaccinated during pregnancy [43].

UNIVERSAL INFANT VACCINATION AND ADOLESCENT AND ADULT BOOSTER VACCINATION

A following strategy possibly contributing to the protection of newborn infants is universal infant vaccination and adolescent and adult booster vaccination. The main aim of this vaccination strategy is to prevent transmission of the pathogen across all age groups and thus to eradicate *Bordetella pertussis* infection in the population [82].

According to the WHO, all children should be immunized against pertussis during infancy. Every country should seek to achieve early and timely vaccination of all their infants initiated at 6 weeks and not later than 8 weeks of age and should maintain a high coverage (≥90%) with at least 3 doses of quality assured pertussis vaccines with an interval of at least 4 weeks between the different doses. Any reduction in global coverage can lead to an increase of pertussis cases [1].

Universal adolescent and adult booster vaccination does not only have an effect in the directly targeted population. Several articles describe a decrease in pertussis incidence among all age groups following the introduction of a national vaccination schedule for adolescents and adults [83, 84]. When immunizing adolescents and adults previously vaccinated against pertussis during childhood with an aP containing vaccine, a seropositivity rate for pertussis antigens of more than 90% was seen a few weeks after vaccination [85, 86] and still a significantly higher antibody concentration was measured approximately 5 years after Tdap vaccination compared to the concentration before Tdap vaccination despite the fact that there was already a remarkable waning of the antibodies at that time point [87, 88]. This shows that the duration of protection following vaccination is relatively short and that with our present vaccines, we are not able to induce lifelong immunity. Therefore, frequent pertussis booster doses might be necessary. However, the optimal time interval between the booster vaccinations is uncertain and may differ according to the initial vaccines received in childhood, with those infants receiving only aP vaccines possibly requiring more frequent boosting to maintain protection [89].

In 2002, the Global Pertussis Initiative including 37 experts from 17 countries already recommended to include Tdap boosters for adolescents and adults within the immunization programs of developed countries [82]. Unfortunately, the suggestions of this expert group had only partial effect because currently only 18 European countries have an adolescent pertussis booster dose in their national immunization schedules. For adults, there are only 7 European countries including a single Tdap dose and only 3 countries recommending multiple Tdap doses in their national adult immunization schedule [90]. In Flanders, the Tetanus-Diphtheria (Td) vaccine, which is recommended every ten year for adults, is recently replaced by Tdap in the national immunization schedule [91].

MATERNAL VACCINATION

Immunizing pregnant women to protect both the mother, fetus and infant from infection has been used increasingly over the last decade [92]. During recent influenza epidemics and pertussis outbreaks, pregnant women have been identified as a target group for vaccination. However, the strategy of vaccinating pregnant women is not a new strategy. Already at the beginning of the previous century, several clinical trials were conducted where they vaccinated pregnant women with multiple doses of wP vaccine during pregnancy and looked at the safety, immunogenicity and effectiveness of this vaccination strategy. These studies showed only local side effects. Also, the strategy turned out to be immunogenic and effective in preventing infant pertussis (Table 1).

Currently, maternal vaccination with aP containing vaccines, is considered as the most successful, effective and cost-effective strategy to prevent infant pertussis disease and has emerged as an important public health approach against both maternal and childhood infections [80].

Study	Year	Number of vaccinated mothers	Vaccine/Doses administered during pregnancy	Timing of vaccination	Safety outcomes	Immunogenicity of the strategy	Effectiveness of the strategy
Lichty et al. [93]	1938	28	wP; 3 doses	Third trimester of pregnancy	Local adverse reactions, one systemic reaction.	Neonatal antibody concentration influenced by maternal antibody level, history of pertussis and active immunization	Not reported
Mishulow et al. [94]	1942	29	wP; 3 doses	Third trimester of pregnancy	No adverse pregnancy outcomes	Increase in protective, agglutinating and complement-fixing antibodies in mothers	Not reported
Cohen et al. [95, 96]	1943, 1946	±170	wP; 6 doses	Second or third trimester of pregnancy	Arm pain, swelling, fever in 2% of cases; no adverse pregnancy outcomes	Transfer of protective antibodies to neonate; rapid decline of protective antibodies in infants	0/8 immunized and 3/6 unimmunized exposed infants developed pertussis
Kendrick et al. [97]	1945	57	wP; 3 doses	Not reported	Not reported	Increase in maternal antibody concentration; transfer of antibodies to infants	Not reported
Adams et al. [98]	1946	16	wP; 3 doses	Last trimester of pregnancy	Not reported	Good immune response to vaccination in mothers; rapid decline in antibodies in infants	Not reported

Table 1: Summary of historical maternal pertussis vaccination studies

a) Antibody titer in pregnant women

Pertussis-specific antibody titers in pregnant women, both in serum and breast milk, are influenced by several factors. First of all, immunological adaptations managed by the increased estrogen and progesterone levels occur during pregnancy. These increased hormone levels are essential for the survival of the fetus resulting in a Th1-to-Th2 shift in pregnancy and thus an alternation of the Th1-Th2 balance [99, 100]. However, despite these immunological adaptations, no difference in humoral and cell-mediated immune (CMI) responses following both natural infection and vaccination between pregnant and non-pregnant women were seen up to the start of this thesis [101, 102]. By contrast, pregnancy is associated with a relative decrease in the concentration of IgG antibodies to approximately 75% of its initial value. This decrease occurs mainly during the third trimester of pregnancy due to the growing circulatory volume creating haemodilution [103]. After delivery, maternal titers increase back to almost their pre-delivery levels.

Secondly, a difference in vaccine immunogenicity is often reported between LMIC and HIC, mostly due to health conditions of the mother. Some infectious diseases like malaria and Human Immunodeficiency Virus (HIV) are thought to have an effect on maternal antibody responses. A recently conducted South African study reported impaired immune responses to influenza vaccination in pregnant women infected with HIV resulting in lower antibody titers and seroconversion rates, but comparable efficacy rates with uninfected women [104, 105]. Another study reported no effect of malaria infection on antibody responses to tetanus toxoid vaccination during pregnancy [106]. However, studies on the effect of infectious diseases on antibody responses in pregnancy are limited and additional research is certainly needed. Also, some studies are suggesting an effect of nutrition on vaccine responses during pregnancy [107, 108].

Finally, the disease history or vaccination status of the women and the time interval since last vaccination or disease has an influence on the antibody titers. After pertussis vaccination or disease during childhood, antibodies start to decline during adolescence leading to low concentrations of circulating pertussis specific antibodies in women of childbearing age [109, 110], making these women again susceptible to pertussis infection or disease.

Pertussis vaccination during pregnancy induces a relatively prompt antibody response. Both Immunoglobulin G (IgG) and Immunoglobulin A (IgA) serum antibody levels start to increase by day 5-7 post-vaccination reaching a peak concentration at day 14 [73]. After this peak, pertussis specific antibody levels start to decline rapidly with already significant lower IgG antibody levels 9-15 months postpartum compared to the antibody levels at delivery. However, despite this

significant decrease, the antibody concentration in Tdap vaccinated women is still higher after a 9-15-month follow-up period compared to the antibody concentration in a control group of not recently vaccinated women [111]. This raises the question whether high pertussis specific antibody levels induced by vaccination during pregnancy might not be sustained through subsequent pregnancies.

In breast milk, an increase of pertussis-specific IgA and IgG antibodies has been seen following postpartum Tdap vaccination starting at day 7, peaking at day 10-14 post-vaccination and slowly decreasing from day 28 onwards [73]. In an Israeli trial where women were vaccinated with Tdap during pregnancy, significantly higher pertussis specific IgA and IgG antibody levels were seen in colostrum samples and breast milk samples taken at 2 weeks postpartum. At 8 weeks postpartum, pertussis specific antibody levels in breast milk were still detectable. However, by this time, already a significant decline compared to the antibody levels in colostrum was seen [112].

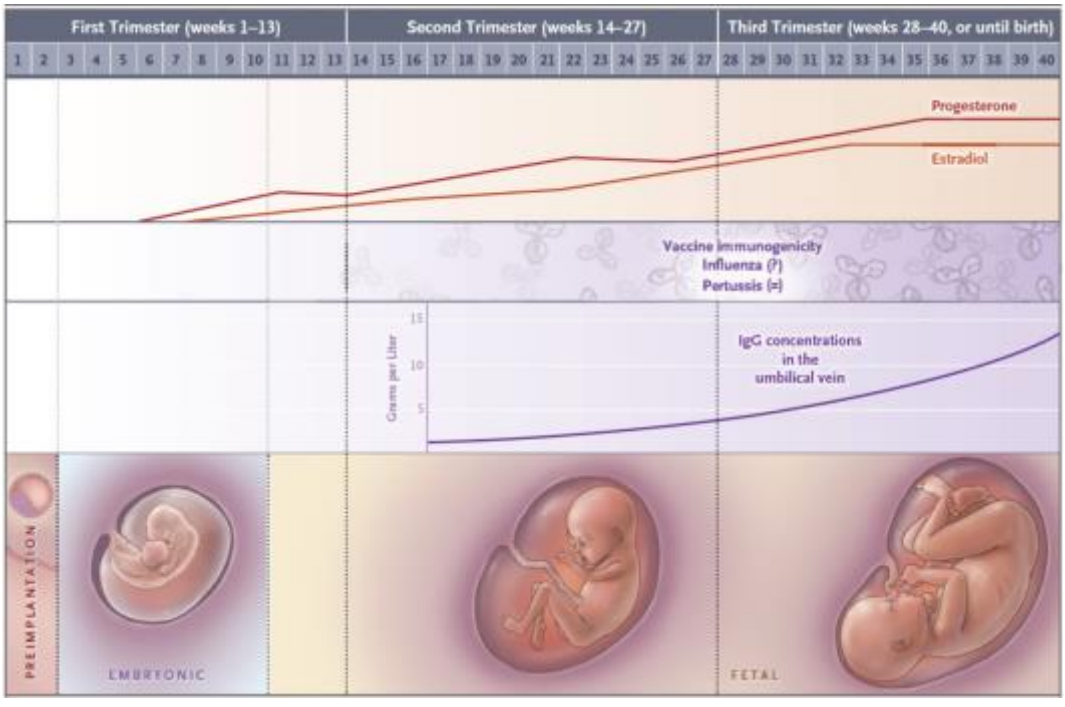

Figure 1: Vaccine and immune responses during the course of pregnancy. Adapted from [100]. Reproduced with permission from [100], Copyright Massachusetts Medical Society.

b) Transport of antibodies from mother to infant

Maternal vaccination elicits both systemic and mucosal antibodies which can be transported from mother to fetus during pregnancy, at delivery or after delivery through transplacental transport or breastfeeding.

Systemic IgG antibodies are actively transported from mother to infant through the placenta. Maternal IgG antibodies are endocytosed into the villous syncytiotrophoblast at the maternal surface and bind to the FcRn receptor in the acidic environment of the endosomes. The FcRn-IgG complexes are then transported to the fetal surface of the syncytiotrophoblast where the IgG antibodies dissociate from the FcRn receptor due to the neutral pH at the fetal side. The IgG antibodies then pass through the villous stroma and fetal capillary endothelium before entering into the fetal circulation [53, 113].

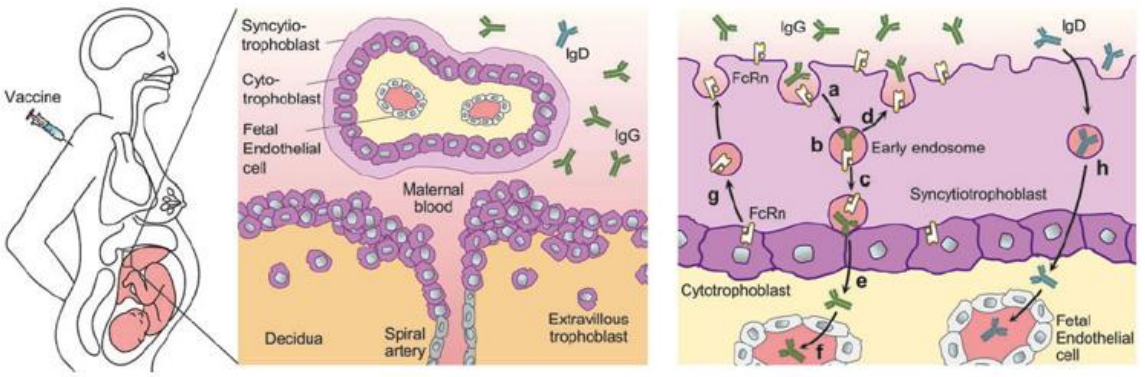

Figure 2: Transplacental transport of antibodies from mother to infant (Adapted from [53]; Permission obtained from The Journal Human Reproduction Update).

The placental transport system is highly selective for IgG antibodies and essentially excludes the transport of other major immunoglobulin classes including Immunoglobulin M (IgM), Immunoglobulin E (IgE) and IgA [114]. Within the IgG antibodies, preferential transport of the IgG_1 and IgG_4 is seen over the IgG_2 and IgG_3 subclasses [115]. Vaccines containing protein antigens, like Tdap, elicit predominantly IgG_1 and IgG_3 antibodies, transferred more efficiently than IgG_2 antibodies elicited by vaccines containing polysaccharide antigens [116]. Also, a preferential transport of high avidity maternal antibodies across the placenta has been described [117, 118].

The amount of antibodies transported from mother to fetus during the pregnancy gradually increases as the pregnancy proceeds [119]. In the first trimester, only a limited amount of IgG antibodies is transported to the fetus [113]. In the second trimester, the fetal IgG gradually increases from 10-20% of the maternal concentration at 17-22 weeks of gestation to approximately 50% of the maternal concentration at 28-32 weeks of gestation [120-122]. In the third trimester of pregnancy, the fetal IgG concentration further increases until it equals or even exceeds the maternal concentration at full term with a sharp increase of maternal IgG in cord blood after 36 weeks of gestation [53, 123].

Some chronic maternal infections like malaria [124, 125] and HIV [126, 127] can cause damage to the placental integrity leading to an impairment of the FcRn receptor function and thus a lower transplacental transport of antibodies from mother to child.

Mucosal non-specific and specific antibodies, including IgA, IgM and IgG are secreted into colostrum and breast milk. After ingestion of these antibodies by the neonate during breastfeeding, they provide mucosal immune protection in the gastrointestinal tract by binding and opsonizing pathogenic organisms, thus inhibiting pathogen adhesion and invasion and by promoting immune exclusion and neutralization. In this way, the ingested antibodies, mainly IgA, function as a first line barrier protecting the epithelium from pathogens and toxins [128]. In the gut of the neonate, the ingested IgA can undergo retrograde transport across the M cells of the gut via an unknown receptor or across the duodenal epithelial cells via the transferrin receptor. Ingested IgG can also undergo retrograde transport via the FcRn receptor expressed on the apical surface of the intestinal epithelial cells [53]. However, since pertussis is a respiratory infection, the mechanisms in which mucosal antibodies transferred through breast milk can potentially add to the clinical protection of the newborn is largely unknown.

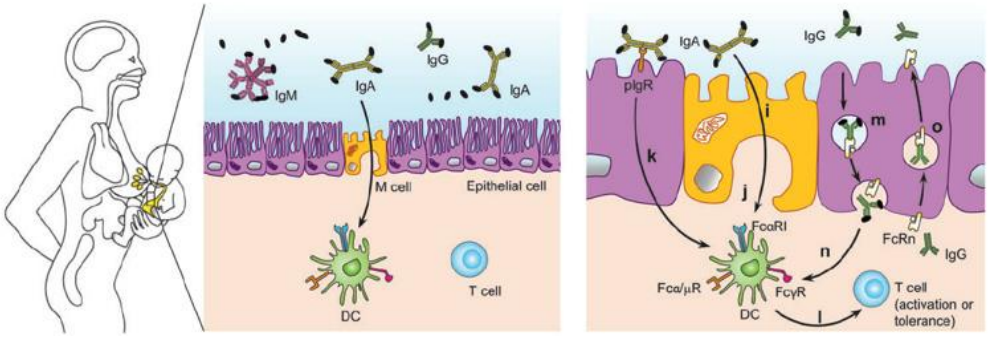

Figure 3: Transport of antibodies from mother to infant through breastfeeding (Adapted from [53]; Permission obtained from the Journal Human Reproduction Update).

Maternal conditions that are known to negatively affect the transplacental transfer of antibodies do generally not affect the transfer of antibodies through breast milk. In contrast to the transplacental transport, prematurity increases the transfer of immune factors, particularly IgA, in colostrum and breast milk [129]. Also, the concentration of total and pathogen-specific antibodies in breast milk are not negatively affected by maternal HIV infection and malnutrition [130, 131].

c) The role of maternal antibody titers in infants

Maternal antibodies, either transported to the fetus via the placenta or breast milk, function as the primary source of protection against infectious diseases in neonates during the first weeks of life. The maternal antibodies compensate for the biological immaturity of the infant immune system at birth [132]. To provide clinical protection to the infant, a critical concentration of antibodies, known as the correlate of protection, has to be reached. This concentration needs to be maintained until the infants are no longer at risk and are protected by their own immunizations.

How long maternal antibodies persist in the newborn is dependent on the half-life. The mean half-life of IgG antibodies, which is approximately 23 days, is the longest of any plasma proteins [133]. For several infectious diseases with pertussis as an example, different half-life values can be found in literature [134, 135]. This can be due to the fact that the half-life is not a fixed value but varies inversely with the concentration of IgG. The same FcRn receptor responsible for the transport of IgG antibodies through the placenta is also found in the endothelial cells of the neonate. IgG antibodies bound to this receptor are described to be protected from degeneration. So, when IgG levels are high, the FcRn receptor becomes overwhelmed resulting in an increase of the catabolic rate. In contrast, when IgG levels are low, there is a diminished competition for the FcRn receptor resulting in a decrease of the catabolic rate. This process is called the concentration-catabolic effect [136]. Despite this effect, infants starting with a higher level of maternal antibodies at birth, for example due to maternal vaccination, will still have a longer persistence of these antibodies until the start of the infant's own primary immunization schedule [135].

High concentrations of maternal antibodies in the infant are known to interfere with the infant's humoral immune response with an inhibition of the antibody generation after their own vaccination and lower antibody titers as a consequence [132, 137]. The maternal antibodies not only affect antibody concentrations, they can also influence their quality [138].

In most cases, interference is described as a temporary effect which mainly influences immune responses after primary vaccination and to a lesser extent after booster vaccination [53]. The interference effect is highly variable for different vaccines and even in different studies on the same vaccine. As an example, for Tetanus and Hepatitis B, interference is not described.

Many mechanisms on how these maternal antibodies inhibit the humoral immune response were postulated in the past with epitope masking as one of the most common. But, these studies were conducted in the absence of a maternal immunization program [139]. Therefore, studies on how the maternal antibodies induced by maternal immunization actually interfere with vaccine-induced immunity in infants are needed since the cellular and molecular mechanisms of this interference effect remain incompletely understood at the moment.

d) Currently existing recommendations for maternal vaccination

Several countries, including Belgium, have already put in place a recommendation for pertussis vaccination during pregnancy.

In the USA, Tdap vaccination during pregnancy is recommended for every pregnant woman in the late second or third trimester of pregnancy by the Advisory Committee on Immunization practices (ACIP) (Centers for Disease Control and Prevention (CDC)) since August 2011. If the vaccination is not given during pregnancy, it should be administered in the immediate postpartum as part of the cocoon strategy [140]. In October 2012, this recommendation was updated stating that every woman should be vaccinated with a Tdap vaccine during every pregnancy [141].

In the UK, Tdap-Inactivated Polio Vaccine (IPV) vaccination during pregnancy is also recommended by the Department of Health since October 2012 for every pregnant woman during every pregnancy. Originally, the recommendation was to vaccinate the pregnant women preferably between 28 and 32 weeks of pregnancy. In April 2016, this time window was extended to 16 to 32 weeks of pregnancy. Although the vaccination can be offered up till 38 weeks of pregnancy [142].

Also in Belgium, maternal pertussis vaccination is recommended by the Superior Health Council since August 2013 for every pregnant woman during every pregnancy between 24 and 32 weeks of gestation. If women are not vaccinated during pregnancy, they should receive the Tdap vaccine in the immediate postpartum as part of the cocoon strategy [43]. In Flanders, the Tdap vaccine (Boostrix®) is available free of charge for all adults [143], whereas in the Walloon region, the Tdap vaccine (Boostrix®) is only free of charge for all pregnant women [144].

In addition to the recommendation in the USA, UK and Belgium, maternal vaccination is also recommended in a variety of countries worldwide including The Netherlands [145], Israel [146], Australia [147] and Argentina [148], Brazil, Colombia, Mexico, El Salvador, New Zealand, Panama, Switzerland, Ireland [149], Czech Republic [149], Spain [150], Italy [149] and Greece [137].

Figure 4: Countries with existing maternal immunization programs (Inspired by Amirthalingham G. Immunizing pregnant women – Success and challenges. ESPID 2017

Research questions and aim of the thesis

Research questions

The main aim of this PhD thesis was to fill some of the most important knowledge gaps regarding maternal pertussis vaccination. Therefore, we tried to find an answer to the following main research questions within this PhD thesis:

- Does cocoon vaccination after a first pregnancy still have an influence on a subsequent born child (**CHAPTER 1**)?
- What is the potential interval needed between two aP containing booster doses in women of childbearing age which still has a protective effect for the consecutive offspring (**CHAPTER 1**)?
- Is there a difference in humoral and cellular immune responses between pregnant and non-pregnant women (**CHAPTER 2**)?
- Is it safe for both mother and child to administer an aP containing vaccine during pregnancy (**CHAPTER 3)**?
- Do high titers of maternal antibodies induced by maternal vaccination interfere with infant's humoral immune response to its own immunizations (**CHAPTER 3** and **CHAPTER 4**)?
- What is the optimal gestational age to administer the aP containing vaccine during pregnancy (**CHAPTER 3**)?
- Is there a difference in safety and immunogenicity of maternal pertussis vaccination in both mother and child between different countries with different background epidemiology and vaccination history (**CHAPTER 3**)?
- Is there an effect of different pertussis vaccination strategies in adult women on breast milk composition (**CHAPTER 5**)?
- What is the coverage of maternal pertussis vaccination and the cocoon strategy in partners in Flanders? How can we do better to increase the coverage (**CHAPTER 6**)?
- What is the attitude towards the existing recommendations for maternal and cocoon vaccination in health care workers in Flanders (**CHAPTER 6**)?

Aim of the thesis

Outbreaks of pertussis occur in HIC with high burden of disease particularly in young, not completely vaccinated infants. In order to protect these infants, the National Immunization Technical Advisory Group (NITAG) in Belgium decided in 2009 to implement the cocoon strategy.

In the **first chapter** of this PhD thesis, we looked at the effect of administering an aP containing vaccine between two successive pregnancies on the second pregnancy, which is in fact the effect of cocoon vaccination on maternal antibodies offered at a next pregnancy. We checked whether vaccination with an aP containing vaccine after a first pregnancy still has an influence on a subsequent born child in terms of high maternal antibody titers at birth and possible blunting on infant immune responses. Mathematical modelling of the data was performed in order to estimate the potential interval needed between two aP containing booster doses in women of childbearing age which has still a beneficial protective effect for the consecutive offspring.

➔ *Maertens K. & Tran T.P.H., Hens N., Van Damme P, Leuridan E. Effect of pre-pregnancy pertussis vaccination in young infants. JID 2017; 215(12):1855-1861 [151].*

However, research showed that cocoon vaccination was not cost-effective and it was difficult to reach sufficient vaccination coverage to adequately protect the infants. Bearing in mind the outbreaks of pertussis disease with the highest incidence in infants below one year of age, another strategy was sought to close the susceptibility gap for infection in this age category. In response to these outbreaks, the NITAGs of several countries, including Belgium, decided to implement pertussis vaccination during pregnancy. In Belgium, pertussis vaccination during pregnancy is recommended by the Superior Health Council since August 2013. However, at the time of implementation the decision to recommend this strategy was mainly based on epidemiological data and many immunological and safety aspects of this strategy, both for mother and child, of this promising public health strategy were lacking. Within this PhD thesis, we tried to generate the scientific evidence to support the implementation of the maternal (pertussis) vaccination strategies.

In the **second chapter** of this PhD thesis, the difference in humoral and cellular immune response after booster vaccination with an aP containing vaccine (Boostrix®) between pregnant and non-pregnant women of childbearing age was studied.

➔ *Huygen K., Caboré R.N., Maertens K., Van Damme P., Leuridan E. Humoral and cell mediated immune responses to a pertussis containing vaccine in pregnant and non-pregnant women. Vaccine 2015 Aug 7; 33(33): 4117-23 [152].*

In a **third chapter**, the safety and immunogenicity of pertussis vaccination during pregnancy in both women and children was studied. A clinical study was performed in parallel in Belgium and Vietnam. In Belgium, pregnant women were vaccinated with the aP containing vaccine Boostrix®. In Vietnam, pregnant women were vaccinated with the aP containing vaccine Adacel®. All children in Belgium and Vietnam were vaccinated with the hexavalent aP containing vaccine Infanrix Hexa® and were followed until one month after their booster vaccination in the second year of life to see whether the high titers of maternal antibodies induced by maternal vaccination interfere with the infant immune responses in the first years of life. (Serious) adverse events ((S)AE) and possible congenital abnormalities were monitored during the entire study period.

- ➔ *Maertens K., Caboré R.N., Huygen K, Hens N., Van Damme P. Leuridan E. Pertussis vaccination during pregnancy in Belgium: results from a prospective controlled cohort study. Vaccine 2016 Jan 2; 34(1):142-50 [153].*
- ➔ *Hoang T.T.H., Leuridan E., Maertens K., Nguyen T.D., Hens N., Vu N.H., Caboré R.N., Duong H.T., Huygen K., Van Damme P., Anh D.D. Pertussis vaccination during pregnancy in Vietnam: results from a randomized controlled trial. Vaccine 2016 Jan 2; 34(1):151-9 [154].*
- ➔ *Maertens K., Caboré R.N., Huygen K., Vermeiren S., Hens N., Van Damme P., Leuridan E. Pertussis vaccination during pregnancy in Belgium: follow-up of infants until 1 month after the fourth pertussis containing vaccine at 15 months of age. Vaccine 2016 Jun 30; 34(31):3613-19 [155].*
- ➔ *Maertens K., Hoang T.T.H., Nguyen D.T., Caboré R.N., Duong T.H., Huygen K., Hens N., Van Damme P., Anh D.D., Leuridan E. The effect of maternal pertussis immunization on infant vaccine responses to a booster pertussis-containing vaccine in Vietnam. CID 2016 Dec 1; 63 (suppl 4):S197-S204 [156].*

Next, in a **fourth chapter**, we looked whether high infant antibody titers of tetanus, diphtheria and pertussis antigens induced by maternal Tdap vaccination (Boostrix®) could also interfere with other components of the infant vaccination schedule. In this chapter, we specifically looked whether maternal Tdap vaccination interferes with the infant's immune responses to 13-valent pneumococcal vaccines, as was also observed in the UK [157].

- ➔ *Maertens K., Burbidge P., Van Damme P., Goldblatt D., Leuridan E. Pneumococcal immune response in infants whose mothers received Tdap vaccination during pregnancy. PIDJ 2017; 36(12):1186-92 [158].*

In a **fifth chapter**, the effect of maternal immunization on breast milk composition was studied. In a first section, a review of the available literature on the effect of maternal vaccination on breast milk was carried out. In this review, we both looked at the effect of maternal vaccination on breast milk composition and possible clinical protection against disease. In a second section, we performed an observational study and looked at the effect of different pertussis vaccination strategies in adult women on breast milk composition.

- ➔ *Maertens K., De Schutter S., Braeckman T., Baerts L., Van Damme P., De Meester I., Leuridan E. Breastfeeding after maternal immunisation during pregnancy: providing immunological protection to the newborn: a review. Vaccine 2014 Apr 1; 32(16):1786-92 [159].*
- ➔ *De Schutter S., Maertens K., Baerts L., De Meester I., Van Damme P., Leuridan E. Quantifiction of vaccine-induced anti-pertussis toxin sIgA antibodies in breast milk: comparison of different vaccination strategies in women. PIDJ 2015 Feb 21; 34:e149-e152 [160].*

Finally, in a **sixth chapter**, we determined the coverage of the recently implemented recommendation of pertussis vaccination during pregnancy in Flanders together with the coverage of the cocoon strategy in partners and predictors for non-vaccination with a pertussis containing vaccine during pregnancy were assessed. We also evaluated the attitude of health care workers towards the existing recommendations for maternal and cocoon vaccination in Flanders and determined their pertussis vaccination coverage.

- ➔ *Maertens K., Braeckman T., Top G., Van Damme P., Leuridan E. Maternal pertussis and influenza immunization coverage and attitude of health care workers towards these recommendations in Flanders. Vaccine 2016 Nov 11;34(47):5785-5791 [161].*
- ➔ *Maertens K., Braeckman T., Blaizot S., Theeten H., Roelants M., Hoppenbrouwers K., Leuridan E., Van Damme P., Vandermeulen C. Coverage of recommended vaccines during pregnancy in Flanders, fairly good but can we do better? Submitted to Vaccine [162].*

CHAPTER 1

The effect of pre-pregnancy pertussis booster vaccination on young infants

This chapter is published: "Maertens K. & Tran T.M.P., Hens N., Van Damme P., Leuridan E. Effect of a pre-pregnancy pertussis vaccination in young infants. JID 2017;215(12):1855-1861."

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Abstract

Maternal pertussis antibodies can hamper infant immune responses to pertussis vaccines. The effect of offering a maternal tetanus, diphtheria, acellular pertussis (Tdap) booster between 2 consecutive pregnancies is investigated.

A prospective study was conducted (Belgium, 2008-2014) on kinetics of maternal pertussis antibodies in unvaccinated women and their infants (group A, N=86) and in siblings (group B, N=58), born after the woman received a Tdap vaccination. Anti-Pertussis Toxin (PT), anti-Filamentous Hemagglutinin (FHA) and anti-Pertactin (Prn) antibodies were measured in maternal blood before and after vaccination and at both deliveries, in cord blood from both siblings and before and after a priming series of infant pertussis vaccines.

All pertussis antibodies were significantly higher in group B siblings at birth, even with growing time interval since maternal vaccination. Blunting of the infant pertussis vaccines response was detected in group B siblings. We estimated a maximum time interval between repeat Tdap doses in adult women in order to have a beneficial effect for the consecutive infant.

Pre-pregnancy Tdap vaccination increases significantly maternal antibody concentrations at consecutive delivery. However, similarly to the effect of Tdap vaccination during pregnancy, blunting of the infant immune responses occurs after a pre-pregnancy immunization.

Background

Despite universal infant vaccination programs against *Bordetella pertussis*, there is an increase in reported whooping cough cases, particularly in industrialized countries. Most outbreaks occur in adolescents and young adults [19] and they are representing a source of infection for unvaccinated infants and newborns. Highest incidence of pertussis disease is recorded in very young children who have not yet been (fully) vaccinated and/or did not receive sufficient maternal antibodies [163].

At first, cocoon vaccination was recommended to protect young infants from disease, but cost effectiveness studies, as well as difficulties to reach high coverage among all persons in contact with young infants, discouraged the strategy [164]. Since 2012, maternal vaccination during pregnancy is a recommended strategy in an increasing number of (industrialized) countries to protect young infants from disease. Many countries advise to immunize at every pregnancy, since antibodies are rapidly waning after an adult booster [73], and a high concentration of maternal antibodies is needed for transfer of antibodies from mother to fetus during pregnancy [165]. For pertussis, a correlate of protection is not well defined, yet high concentrations of anti-Pertactin (Prn) and mainly anti-Pertussis Toxin (PT) antibodies, are related to protection [166]. After birth, maternal antibodies decrease rapidly in the offspring, mostly within two months [167].

The present paper reports results of a prospective study on the transfer and persistence of maternal antibodies in newborns, whenever a tetanus, diphtheria and pertussis (Tdap) booster vaccination is given between two consecutive pregnancies. Previously, we reported an interim analysis on a subsample of this cohort study [168]. The primary aim of the study is to evaluate whether a pre-pregnancy booster helps to increase antibody concentrations to pertussis in neonates, thereby closing the susceptibility gap. A secondary aim is to evaluate possible blunting of the infant immune responses to pertussis containing vaccines after a pre-pregnancy maternal booster vaccination, similar to blunting of the infant immune responses after Tdap administration in pregnancy [153]. In addition, modelling the anti-PT IgG antibody concentrations enables drafting possible recommendations regarding the timing of consecutive maternal booster doses.

Methods

STUDY DESIGN

A prospective multicenter study was conducted in Antwerp, Belgium, between 2008 and 2014, in accordance with the Helsinki declaration, ICH-GCP and procedures established by Belgian law. Ethical approval was obtained (University Hospital of Antwerp). Women participating in a study on maternal antibody kinetics were recruited [169-171]. Informed consent was obtained from the women and from both parents of the participating children. For the women, the exclusion criteria were pertussis vaccination within ten years prior to study participation, immunological disorders and recent administration of immunoglobulins prior to vaccination. Exclusion criteria for the children were low birth weight (<2400g), prematurity (<36 weeks gestation) and immunological disorders. Serum samples were taken from all women at delivery (10mL), from the cord (10mL), and from the infant at 1 month of age, 6 or 9 months of age (randomly) and 12 months of age (2mL) (Group A). Time points were chosen based on waning of maternal measles antibodies [169].

Women were offered a Tdap vaccine (Boostrix®, GSK Biologicals, Rixensart, Belgium) after the first delivery. One month after vaccination, a blood sample (10mL) was taken. At consecutive delivery, blood was again taken from the woman and the cord and later on from the sibling at 1 month of age, 6 or 9 months of age (randomly and equal to the older sibling) and 12 months of age (Group B).

All infants were vaccinated within the standard Belgian vaccination schedule with the hexavalent aP containing vaccine Infanrix Hexa® (GSK Biologicals, Rixensart, Belgium) at 8, 12 and 16 weeks.

An extended questionnaire collected information on obstetrical risk factors, demographics, vaccination history, and general medical history. Growth parameters, breastfeeding data, immunization data, and medical history were collected at each visit.

STUDY VACCINES

Licensed Tdap vaccine (Boostrix®, GSK Biologicals, Rixensart, Belgium) was used to immunize women in the deltoid muscle. Boostrix® contains 5 Lf of Tetanus Toxoid (TT), 2.5 Lf of Diphtheria Toxoid (DT), 8 µg of inactivated PT, 8 µg of Filamentous Hemagglutinin (FHA) and 2.5 µg of Prn. Infants were vaccinated with the hexavalent vaccine Infanrix Hexa® (GSK Biologicals, Rixensart, Belgium), containing 25 Lf of DT, 10 Lf of TT, 25 µg PT, 25 µg FHA and 8 µg Prn, inactivated poliovirus, hepatitis B surface antigens and *Haemophilus influenzae* type B polysaccharide.

LABORATORY

All serum samples were centrifuged at 2000 rpm within 24 hours after withdrawal, and stored between -20°C and -40°C. Serum leftovers from women and infants in group A were selected for analysis in view of the inclusion of their siblings (group B siblings). An in-house ELISA was used to test all samples for anti-PT, anti-FHA and anti-Prn IgG antibodies at GSK Biologicals, Belgium. The limit of detection of the assay was 5 EU/mL for all three antibodies. For women, a booster response was defined as a post-vaccination antibody concentration ≥20 EU/mL with a pre-vaccination antibody concentration <5 EU/mL, a post-vaccination rise of at least 4 times the pre-vaccination antibody concentration in subjects with a pre-vaccination antibody concentration ≥ 5 EU/mL and < 20 EU/mL; or at least twice the pre-vaccination antibody concentration in subjects with a pre-vaccination antibody concentration ≥ 20 EU/mL. The pre-vaccination value was the maternal sample taken at the first delivery.

STATISTICS

Antibody geometric mean concentrations (GMCs) with 95% confidence intervals (CI) were calculated. Statistical tests included parametric tests: (paired) t-tests and chi-square tests and their non-parametric alternatives: (paired) Wilcoxon tests and Fisher exact tests. The analysis was performed using SPSS statistical software version 23.0.

Non-linear mixed effect models (NLMM) were employed to model the dynamics of anti-PT antibodies in both mothers and infants. Anti-PT antibodies were chosen because they, as well as anti-Prn antibodies, correlate with protection [38]. The model building procedure is motivated in the Appendix. The models were fitted using Monolix software [172]. The anti-PT antibody values < 5EU/mL are treated as left-censored data. Results of the NLMM are expressed as medians and interquartile range (IQR), because of possible asymmetry for the quantiles under study as well as for ease of interpretation. Figure 1 in the Appendix shows a presumed visualization of the dynamics of the anti-PT antibody levels in the infants, indicating the different slopes and rates, used in the NLMM model.

Finally, a robust simple linear regressions model was fitted to investigate the association between the antibody levels in women at delivery and in the cord.

All rates mentioned in the manuscript are exponential decay or growth rates expressed in months, unless indicated differently. A significance level of 5% was used for all analysis. Blunting of vaccine responses was defined as a significantly lower GMC of pertussis specific IgG antibodies in group B siblings.

Results

GENERAL CHARACTERISTICS OF THE STUDY POPULATION

In total, 86 women received a Tdap vaccine after a first pregnancy. All women had been vaccinated against pertussis during childhood with whole cell pertussis (wP) containing vaccines and received no documented pertussis booster for at least 10 years prior to the study booster. Of the 86 vaccinated women, 58 women became pregnant again and delivered within the study period.

The median interval between the first delivery and the Tdap vaccination was 16.07 months (min-max: 8.46-43.31). The median interval between the Tdap booster and the consecutive delivery was 16.80 months (min-max: 6.20-56.49). Three women had a negative pregnancy test at the time of vaccination but were pregnant 1 month later, despite contraceptive advice. The mean age of the women was 29.97 years at first, and 32.04 years at consecutive delivery. Mean duration of both pregnancies was comparable. No significant difference in birth weight and length of both siblings was seen, nor gender ratio differences (Table 1).

Group, Characteristic	Value		P[a]
Women			
Mean age at vaccination, y	30.53 ± 0.36		
Median interval between delivery of first child and vaccination, mo	16.07 (11.90)		
Mean interval between vaccination and blood sample collection	1.00 ± 0.01		
Median interval between vaccination and delivery of child 2, mo	16.80 (15.48)		
At delivery of infants	Infant A	Sibling B	
Mean age of the mother at delivery, y	29.97 ± 0.37	32.04 ± 0.41	NS
Gestational age at birth, wk	39.43 ± 0.13	39.25 ± 0.17	.395
Mean duration of breastfeeding, mo	4.43 ± 0.31	4.72 ± 0.40	.555
Infants	Group A	Group B	
Sex, no. (%)			
Male	39 (45.88)	30 (51.72)	.383
Female	46 (54.12)	28 (48.28)	
Mean age at blood sample collection, mo, by collection time			
Mo 1	1.01 ± 0.01	1.02 ± 0.01	.758
Mo 6	6.01 ± 0.03	6.56 ± 0.11	.256
Mo 9	9.00 ± 0.02	9.19 ± 0.11	.120
Mo 12	12.04 ± 0.02	12.46 ± 0.10	<.001
Mean age at receipt of hexavalent vaccine, mo, by dose			
Dose 1	2.25 ± 0.04	2.13 ± 0.10	.532
Dose 2	3.43 ± 0.05	3.49 ± 0.10	.572
Dose 3	4.62 ± 0.07	4.73 ± 0.12	.450
Mean interval between receipt of hexavalent vaccine dose 3 and blood sample collection, mo, by collection time			
Mo 6	1.26 ± 0.11	2.16 ± 0.10	.918
Mo 9	4.42 ± 0.10	4.39 ± 0.30	.984
Mo 12	7.42 ± 0.07	7.82 ± 0.13	.007

Data are mean values ± standard errors of the mean or median values (interquartile ranges), unless otherwise indicated.

Abbreviation: NS, not significant.

[a]By the unpaired *t* test or χ^2 test.

Table 1: Demographic characteristics of the participating women and infants.

LABORATORY RESULTS

a) Maternal results

Table 2 summarizes the GMC of IgG antibody concentrations to PT, FHA, and Prn in all women at delivery of the first born infants (Group A) (=pre-vaccination sample of the mother), 1 month post-booster vaccination, and at the moment of delivery of the Group B siblings.

At baseline, 93% of the women had detectable anti-FHA IgG antibodies and 79% had detectable anti-Prn IgG antibodies, while 52% had detectable anti-PT IgG antibody concentrations > 5 EU/mL. 16% of participating women were seronegative for both anti-PT and anti-Prn antibodies at baseline. One month after vaccination, 97.7% of the women showed a booster response to FHA and Prn while 90.7% showed a booster response to PT. All but one woman responded with a rise in all pertussis-specific antibodies, and there was no difference in magnitude of the response for anti-PT responses, nor for anti-Prn responses, between seronegative women and seropositive women pre-vaccination. At the next delivery, the mean maternal antibody levels for all 3 antigens had declined significantly ($p<0.001$) compared to one month after vaccination, but were still significantly higher compared to baseline concentrations for all antibodies ($p<0.001$). In 8 women, the anti-PT antibody concentration dropped below the threshold of 5 EU/mL at the consecutive delivery.

Group, Specimen Collection Time or Type	Anti-FHA, EU/mL			Anti-Prn, EU/mL			Anti-PT, EU/mL		
Women									
At delivery of infant A									
GMC (95% CI)	21.50 (16.96–27.22)			15.84 (11.97–20.96)			6.47 (5.16–8.11)		
Samples, no.[a]	84			85			85		
1 mo after vaccination									
GMC (95% CI)	770.75 (638.43–930.49)			658.88 (503.92–861.49)			69.97 (55.87–87.62)		
Samples, no.	86			86			86		
At delivery of child B									
GMC (95% CI)	149.59 (118.22–189.28)			147.91 (100.37–217.96)			13.43 (10.10–17.85)		
Samples, no.	55			55			55		
Infants	Group A	Group B	P[b]	Group A	Group B	P[b]	Group A	Group B	P[b]
Cord									
GMC (95% CI)	32.16 (22.49–45.99)	239.85 (187.93–306.12)	<.001	22.60 (15.66–32.63)	253.74 (175.35–367.17)	<.001	9.68 (6.86–13.65)	22.61 (17.18–29.74)	<.001
Samples, no.	46	55		48	55		48	55	
Age 1 mo									
GMC (95% CI)	16.70 (10.84–25.73)	133.63 (105.38–169.44)	<.001	10.89 (7.04–16.86)	143.49 (101.31–203.25)	<.001	5.46 (3.91–7.63)	11.84 (8.81–15.92)	.001
Samples, no.	34	53		35	53		35	53	
Age 6 mo									
GMC (95% CI)	206.42 (116.36–366.17)	92.40 (67.79–125.96)	.039	70.34 (41.63–118.85)	25.27 (14.45–44.18)	.016	59.63 (34.25–103.83)	36.31 (27.17–48.53)	.175
Samples, no.	15	11		15	11		15	11	
Age 9 mo									
GMC (95% CI)	84.28 (64.82–109.57)	32.58 (22.19–47.85)	<.001	36.89 (25.72–52.90)	12.19 (6.97–21.33)	.002	23.76 (17.33–32.58)	16.23 (11.29–23.31)	.154
Samples, no.	20	10		19	10		19	10	
Age 12 mo									
GMC (95% CI)	57.75 (45.28–73.65)	27.99 (21.02–37.28)	.001	12.31 (8.79–17.24)	7.05 (5.52–9.01)	.009	10.87 (8.50–13.90)	6.39 (5.07–8.04)	.003
Samples, no.	33	47		32	47		32	47	

Abbreviations: CI, confidence interval; EU, enzyme-linked immunosorbent assay units.

[a]Sufficient levels of sample were not available for all women.

[b]By the unpaired *t* test.

Table 2: Geometric Mean Concentrations (GMCs) of antibodies against Filamentous Hemagglutinin (FHA), Pertactin (Prn), and Pertussis Toxin (PT) among women and infants, by specimen collection time or type

b) Cord results

Table 3 shows the transplacental transport ratio in both cohorts of infants. In general, GMC in cord blood exceeds GMC in the mother at delivery for both siblings. Ratios did not significantly differ at consecutive pregnancies.

	TTR, Mean ± SEM		
IgG	Group A	Group B	P^a
PT	1.96 (0.18)	1.76 (0.08)	.300
FHA	1.78 (0.09)	1.74 (0.07)	.899
Pertactin	1.84 (0.13)	1.62 (0.08)	.147

Abbreviations: IgG, immunoglobulin G; SEM, standard error of the mean.
[a]By the unpaired *t* test.

Table 3: Transplacental Transport Ratio (TTR) for Pertussis Toxin (PT), Filamentous Hemagglutinin (FHA) and Pertactin antibodies in Group A and Group B infants.

c) Infant results

Group B siblings have significantly higher antibody levels to all pertussis specific antigens at birth compared to group A infants, lasting up to the age of 1 month, before the start of the infant vaccination program. After three doses of primary vaccination, all antibody concentrations are consistently lower in group B siblings (month 6). For anti-FHA and anti-Prn, the IgG concentrations are significantly lower at the ages of 9 and 12 months. For anti-PT on the other hand, the antibody concentrations are only significantly lower at the age of 12 months (Table 2).

MODELLING RESULTS

a) Maternal results

Based on the NLMM, the median time for anti-PT antibodies in women to decrease by 50% (i.e. half-life) is 15.87 months (IQR: 14.86-16.74). The age of the mother at vaccination had no significant effect on this half-life.

Since there is no correlate of protection for pertussis, it is unclear how high the maternal antibody concentration at delivery should be in order to protect the offspring. Figure 1 shows the median time point (with IQR) whenever antibody concentrations in women fall below a pre-specified level (from 5-25 EU/mL) after a booster vaccination. After a median time of 55.56 months (IQR: 51.76-59.33) post-vaccination, the anti-PT IgG antibody levels in women decline below the

threshold value of 5 EU/mL, and after a median time of 30.24 months (IQR: 27.77-33.25), the anti-PT IgG antibody levels reached the threshold of 15 EU/mL. For comparison, the GMC of maternal antibodies at delivery of the group B siblings was 13.5 EU/mL, and this titer corresponded with possibly enough maternal antibodies in the cord to protect young infants.

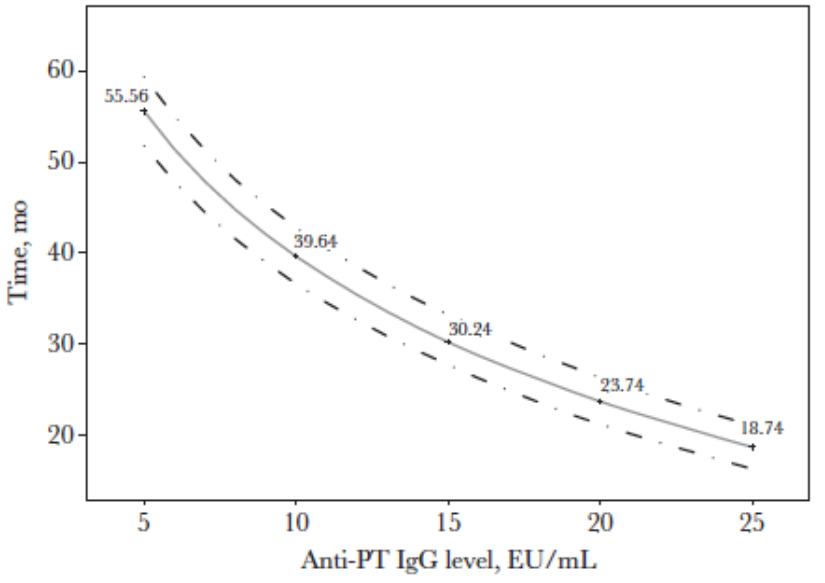

Figure 1: Median time points (interquartile ranges) after booster vaccination at which anti-pertussis toxin (PT) Immunoglobulin G (IgG) antibodies in women declined below a prespecified threshold of 5-25 enzyme-linked immunosorbent assay units (EU)/mL.

b) Infant results

The results of the NLMM confirm that siblings in group B have a significantly higher antibody concentration at birth (i.e. A_0 in Figure 1 in Appendix) compared to group A infants. After birth and before the start of the infant vaccination program, maternal antibody levels decrease very fast in both groups (Figure 2): the median time for anti-PT antibody levels in group A infants to fall below 5 EU/mL, is 1.21 months (IQR: 1.08-1.36), while it takes about 2.21 months (IQR: 2.09-2.39) to drop below 5 EU/mL in group B siblings. The half-life of maternal anti-PT antibody levels in infants is approximately 5 weeks (33 days and 30 days for children in group A and B, respectively).

Infants in group B have a significantly lower increase rate (denoted by γ in Figure 1 in Appendix) of anti-PT IgG antibody levels after priming with 3 vaccine doses. The time point h, at which we observe the highest antibody concentration before waning of antibodies, is estimated to be at 6.82 months (IQR: 6.63-7.01). The last scheduled vaccination was administered at 16

weeks of age, but the effect of 3 subsequent primary doses made the antibody levels still increase approximately 2.82 months after the last dose.

We included gender and birth weight of the infants, centered around its mean, in the NLMM: boys were born with significantly higher anti-PT antibody concentrations compared to girls in both groups. Time point h in boys is significantly lower (5.55 months (IQR: 5.33-5.77)), compared to the estimated value of h (7.06 months (IQR: 6.84-7.28)) for girls. Newborns with higher birth weight tend to have higher estimated decay rates $\hat{\beta}$ albeit this small difference is not clinically relevant.

c) Correlation between maternal and infant antibody levels

The robust simple linear regression model shows a significant positive association between the anti-PT IgG antibody levels in women at delivery and in the cord. The predicted anti-PT IgG antibody levels in the cord of the infant whose mothers had anti-PT IgG antibody levels at delivery of 5, 10, 15, 20 and 25 EU/mL, are respectively: 10.43 (IQR: 9.31-11.55), 18.80 (IQR: 17.04-20.56), 26.53 (IQR: 23.87-29.19), 33.88 (IQR: 30.12-37.64) and 40.96 (IQR: 35.97-45.95) EU/mL.

In order to evaluate the possible interval between several booster doses of Tdap in 1 woman, the correlation of maternal antibodies at delivery, with titers in cord and the loss of these maternal antibodies in the young infant over time (as a measure for protection from disease) could offer insights in the need for repeat booster, also considering possible interference of infant immune responses on vaccines. If, at delivery, the anti-PT antibody titer in the mother is 15 EU/mL, it takes roughly 2.5 months in infants for anti-PT antibody levels to decline below 5EU/mL and 1 month to fall below 10 EU/mL (Figure 2).

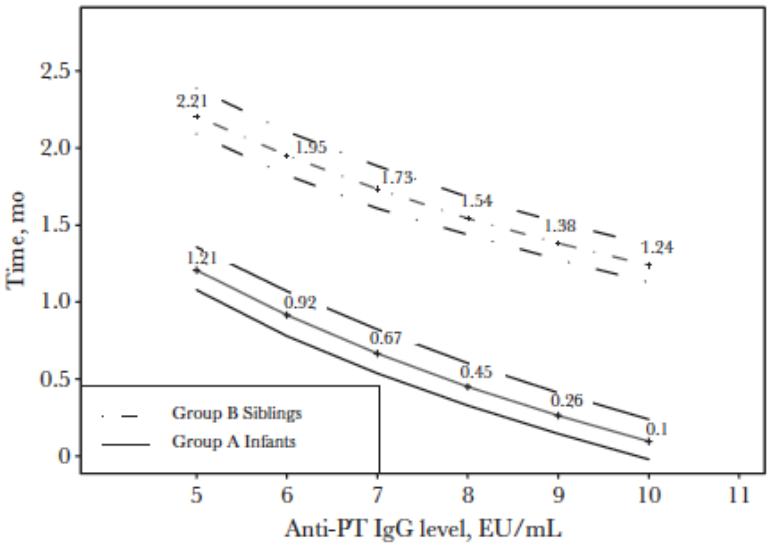

Figure 2: Decline in maternal antibody levels during the first months of life in group A infants and group B siblings. Data are median time points (interquartile ranges) at which maternal anti-pertussis toxin (PT) immunoglobulin G (IgG) antibodies in infants start to decline below a prespecified threshold of 5-10 enzyme-linked immunosorbent assay units (EU)/mL (when no infant vaccination is performed).

Discussion

At baseline, 48% of the women in the present long-term follow-up study, had undetectable anti-PT antibody levels, indicating the lack of maternal antibodies to be transferred and hence the lack of protection offered at birth. After a pre-pregnancy Tdap booster vaccination, good humoral responses were measured. Yet, similarly to other vaccination studies with Tdap in adults [173-175], a rapid drop in antibody concentration is measured after vaccination: the half-life of anti-pertussis antibodies is estimated to be 15.87 months. However, median antibody concentrations remained significantly higher in women at a next delivery, even if the booster dose was offered more than 2 years before the consecutive delivery. And significantly increased anti-pertussis antigen specific maternal antibody concentrations were still encountered in that offspring, born after the booster dose. In 13% (8/58) of the women, the anti-PT IgG had declined below the lower limit of 5 EU/mL by the time of the next delivery, indicating possible susceptibility to disease for the young infant, and underlining the importance of repetitive boosting with each new pregnancy.

Transplacental transport in children born before and after the pre-pregnancy booster vaccination was equally effective. Previously, adequate transport has been described in prospective cohorts [102, 153, 154]. Yet, this is the first study describing transplacental transport in siblings, and no effect of vaccination or parity was found on the adequacy of the transport. We would like to stress that our cohort consisted of healthy pregnant women with mostly healthy pregnancies, while transplacental transport can be influenced by placental dysfunction or disease. We confirm in the present study the recently published Swiss data that the presence of high titers of maternal antibodies during the entire pregnancy, or a long period thereof, results in adequate transport and high concentrations at birth [176].

Using a NLMM, we were able to calculate the impact of time lapse between repeat boosters in healthy adult women on transferred antibody levels towards the fetus. Based on these results, the time frame to be considered between consecutive pertussis booster vaccines should be no longer than 30 months (roughly 2.5 years), in order to have median anti-pertussis antibody concentrations in pregnant women as high as 15 EU/mL at the moment of delivery. This level corresponds to anti-PT IgG antibody levels in cord blood of 26.53 EU/mL in the present study (IQR: 23.87-29.19). These results are based on the assumption that the estimated decay rate derived from our model is the same as the one we would obtain with a larger number of samples (See Appendix), and on the assumption that the positive effect that we found in group B siblings, is protective against disease. This is not a recommendation, yet a finding that has to be confirmed based on additional persistence studies, and possibly including information on antibody levels at

multiple time points to inform antibody dynamics. In addition, similar calculations when vaccination is offered during pregnancy are needed and planned.

Infants born after a pre-pregnancy booster had significantly higher anti-PT GMC at birth that endured up to one month of age, thus closing the susceptibility gap for infection. These results are in line with the effect of Tdap vaccination during pregnancy [102, 153, 154]. The median half-life of anti-PT IgG antibodies in infants is relatively short (5 weeks) which is again in line with literature [134, 177, 178]. Since no correlate of protection is known for pertussis, it is not sure whether the elevated antibody concentrations in the second born cohort are sufficiently protective against disease, or whether the blunting later in infant life, after a primary series of three vaccines, is meaningful in terms of protection. Indeed, the effect of these maternal antibodies on the infant immune responses to the primary infant vaccination, is still detectable in group B siblings born after a pre-pregnancy booster dose. This so-called blunting effect has been described after maternal Tdap vaccination during pregnancy [102, 153, 154, 157], however this is the first study to describe the same effect after a pre-pregnancy (or postpartum) booster dose. What we measure is in fact the effect of cocoon vaccination on offspring that is born later on. Effectiveness data are lacking whether the elevated titer of maternal antibodies is protecting young infants from disease.

At month 6 and 9, after primary vaccination, differences between both siblings are not always significant for all 3 tested antibody types, perhaps due to the small sample size. GMC in infants at 12 months of age, measured in previous studies [179], are comparable with GMC in children at 1 month of age after a pre-pregnancy booster, suggesting that GMC in group B siblings at birth would be compatible with clinical protection.

The present study shows that also countries with a recommendation for cocoon vaccination, or with repeated booster recommendations, should be aware that significantly higher antibody concentrations are transferred to the offspring, thereby leading to possible early life protection but also potential interference of the maternal antibodies with the infant's immune response. Data could be used for cost-benefit calculations for subsequent doses in women of childbearing age.

There are a few shortcomings in this study. There is a rather low number of infants born after the booster dose as a result of the difficulty to plan this upfront. Besides, the study was not powered to detect small differences in antibody levels between both siblings, with growing time interval between vaccination and delivery of a second born child. In addition, the time points of blood sampling were chosen according to the original study on measles antibody kinetics, and

those were likely not the most optimal time points for pertussis responses. Nevertheless, pre- and post-vaccination samples were available and are always taken at the same time points. In order to understand better the relevance of the findings, measurement of the functionality of the antibodies, would be valuable, ideally comparing functionality of antibodies elicited during pregnancy, and as in this project, in a non-pregnant status.

To conclude, the present study is the first study describing the effects of a pre-pregnancy Tdap booster dose. The maternal antibodies at birth are significantly higher in the second born siblings, even with a growing time interval between booster and subsequent delivery.

Acknowledgments

Authors express their gratitude to Mrs Aline Bontenakel, senior study nurse, for performing all blood samplings. The obstetricians and pediatricians of the Antwerp University Hospital (UZA), the hospitals of the Gasthuiszusters of Antwerp and the ZNA hospitals of Antwerp are gratefully acknowledged for their participation.

Appendix

MODEL BUILDING FOR DATA IN INFANTS

Dynamics of antibodies in infants

There are maximum four data points of anti-PT IgG antibody in each infant: at delivery (cord blood sample), 1 month after birth, 6 or 9 months after birth and 12 months after birth. Based on the recommended vaccination schedule in Belgium, infants were vaccinated at the ages of 8, 12 and 16 weeks. We propose to use the model with three ODEs (see Figure 1).

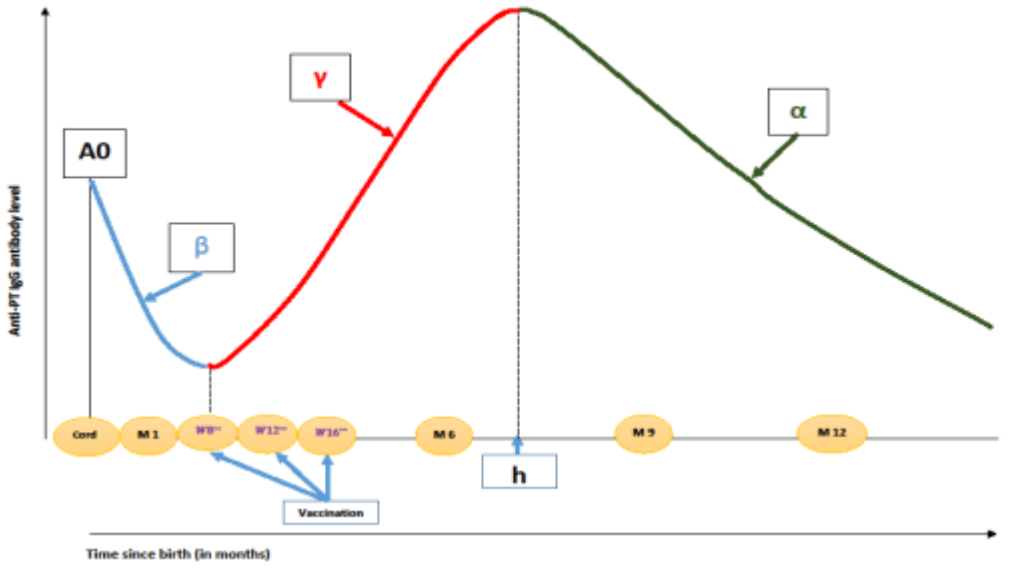

Figure 1: Illustrated presumed dynamics of anti-pertussis antibody levels in infants: A_0 is the antibody level at birth. After birth, the antibody levels decrease with decay rate β. After the administration of the first aP containing vaccine at week 8, the antibodies start to increase up to time point h at an overall rate ɣ. It is hypothesized that h>4 since the infants are vaccinated at week 8, 12 and 16. After time point h, antibodies decrease again at rate α. Cord, M1, M6, M9 and M12 indicate the sampling time points. W8**, W12** and W16** indicate the vaccination time points.

The dynamic change in anti-pertussis antibodies is written down as follows:

$$t \leq 2: \frac{dA}{dt} = -\beta A$$

$$2 < t \leq h: \frac{dA}{dt} = \gamma A$$

$$t > h: \frac{dA}{dt} = -\alpha A$$

This ODE system assumes that anti-PT antibodies firstly decrease with a decay β right after birth till the first vaccination at month 2. After the first vaccination, antibodies increase with an overall rate of ɣ which is accounted for the general increase due to three consecutive vaccine doses. Since there are no enough data points to model the increasing rate after each vaccination dose, only the overall rate ɣ is expressed and modelled. After the vaccination at month four, antibodies remain increasing, till point h, and start decrease again with decay α.

Model building and selection

Model selection is performed based on AIC obtained from models without any covariate fit in Monolix software. The anti-PT IgG antibody level at T0 (corresponding to cord sample) is A_0 . Since there are maximum four measurements per subject, we allow maximum four random effects. We let h fixed, and let $A_{0,i}$, β_i, γ_i and α_i be random. The individual parameters are assumed to have log-normal distribution.

To perform model selection, we fit different models with several scenarios: models with only one random effect, models with two random effects, models with three random effects and models with four random effects. Some models did not achieve convergence in Monolix software. Among all models whose convergence was achieved, the model with four random effects had the lowest AIC. As a result, we used this model to continue our analysis, where covariates were taken into account.

First of all, we consider the order of the infants (covariate *child2: child2*=0 if the child from group A infants and *child2*=1 if the child from group B siblings) as a categorical variable with group A infants as reference group. The model where *child2* is assumed to affect the estimate of all parameters did not converge in Monolix. The results presented in the manuscript, hence, were obtained from the model where *child2* was assumed to affect the estimate of four parameters ($A_{0,i}$, $\beta_i, \gamma_i, \alpha_i$). Directly after, the effect of gender (covariate *gender: gender*=1 if male and *gender*=0 if female where baby girl is the reference group), birth weight (covariate *bweight*

centered around its mean *bweight_mean*) of infants (in gram) were additionally taken into account.

Specification for final models

- Model with only one covariate (*child2*):

Let denote A_0, β, γ and α the four population parameters. Individual parameters are assumed to have log-normal distribution, it follows:

$$log(A_{0,i}) = log(A_0) + \rho_{A_0} child2_i + \eta_{A_{0,i}}$$

$$log(\beta_i) = log(\beta) + \rho_{\beta} child2_i + \eta_{\beta,i}$$

$$log(\gamma_i) = log(\gamma) + \rho_{\gamma} child2_i + \eta_{\gamma,i}$$

$$log(\alpha_i) = log(\alpha) + \rho_{\alpha} child2_i + \eta_{\alpha,i}$$

Where:

$$\eta_{A_{0,i}} \sim N(0, {\delta_{A_0}}^2),$$

$$\eta_{\beta,i} \sim N(0, {\delta_{\beta}}^2),$$

$$\eta_{\gamma,i} \sim N(0, {\delta_{\gamma}}^2)$$

$$\eta_{\alpha_i} \sim N(0, {\delta_{\alpha}}^2),$$

We use anti-PT IgG antibody levels on the log 10 scale and assume an additive residual error model, that is:

$$log_{10}(A_{obs,ij}) = log_{10}(A_{pred,ij}) + \varepsilon_{ij}$$

where $A_{obs,ij}$ and $A_{pred,ij}$ are the observed and predicted anti-PT IgG antibodies for the child i at time point j. The residual error is assumed to have a normal distribution with mean 0, that is:

$$\varepsilon_{ij} \sim N(0, \delta^2),$$

- Model with three covariates (*child2, gender, bweight*):

Initially, we fit the models where only *gender* or *bweight* were considered as covariates separately. In the next step, only statistically significant relationships of *gender* and *bweight* were retained in the final model with three covariates since the central interference lies on the effect of *child2*. The individual parameters in the final model were assumed to have a log-normal distribution:

$$log(A_{0,i}) = log(A_0) + \rho_{child2,A_0}\, child2_i + \rho_{gender,A_0}\, gender_i + \eta_{A_{0,i}}$$

$$log(\beta_i) = log(\beta) + \rho_{child2,\beta} child2_i + \rho_{bweight,\beta}(bweight_i - bweight\_mean) + \eta_{\beta,i}$$

$$log(\gamma_i) = log(\gamma) + \rho_{child2,\gamma} child2_i + \eta_{\gamma,i}$$

$$log(\alpha_i) = log(\alpha) + \rho_{child2,\alpha} child2_i + \rho_{gender,\alpha} gender_i + \eta_{\alpha,i}$$

Moreover, gender is assumed to affect the estimate of h.

- Left-censored observations:

There are anti-PT IgG antibody values less than the limit of detection (5EU/mL). these data will be treated as left censored during the estimation procedure in Monolix Software.

Model diagnosis:

We perform model diagnosis for the final model by the means of the SAEM (Stochastic Approximation Expectation Maximization) convergence plot and residual plots (residuals vs time and residuals vs prediction).

Furthermore, the scatter plot of the observed antibody levels and predicted values are presented to partially evaluate the prediction ability of the model (See Figure 2).

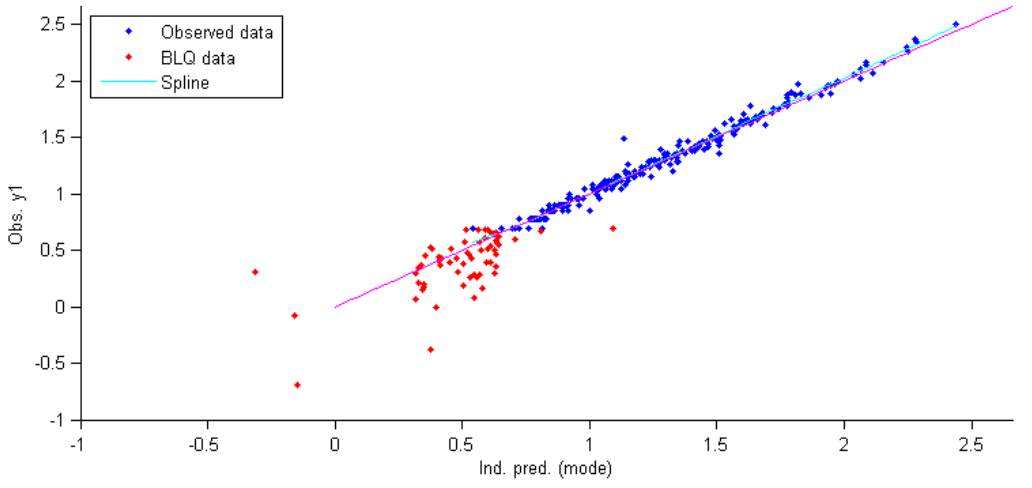

Figure 2: Scatter plot of the observed anti-PT IgG antibodies with respect to the predicted anti-PT IgG antibodies using the individual parameters (model in infants with only one covariate *child2*, output from Monolix Software).

Important results:

In the model with three covariates, it is shown that boys were born with significantly higher anti-PT IgG concentrations compared to girls in both groups of infants. The median anti-PT IgG antibody levels at birth in group A infants is respectively 5.51 EU/mL (IQR: 5.96-11.06) for girls and 13.3 EU/mL (IQR: 9.18-17.42) for boys; and in group B siblings 18.20 EU/mL (IQR: 17.63-18.77) for girls and 28.40 EU/mL (IQR: 27.55-29.25) for boys, respectively.

MODEL BUILDING FOR DATA IN PREGNANT WOMEN

Dynamics of antibodies in pregnant women:

There were three blood sampling occasions in each pregnant woman: at the first delivery, one month after booster vaccination and at the delivery of the second child.

We denoted the antibody level at T_0 (first delivery), antibody levels decrease over time until the women received a booster vaccination. From that moment, antibodies are assumed to immediately increase. Since we do not have enough data to observe the whole process, what we can model was the process illustrated in Figure 3. In these presumed dynamics, the solid line (in black) illustrates the theoretical change of antibodies. The dashed-dotted line (in red) is the curve based on the collected data. It is assumed that the decay rates after the peak (time point h) were

the same in solid line and dashed line. However, it is a strong assumption and the conclusion based on the result of α should be interpreted with caution.

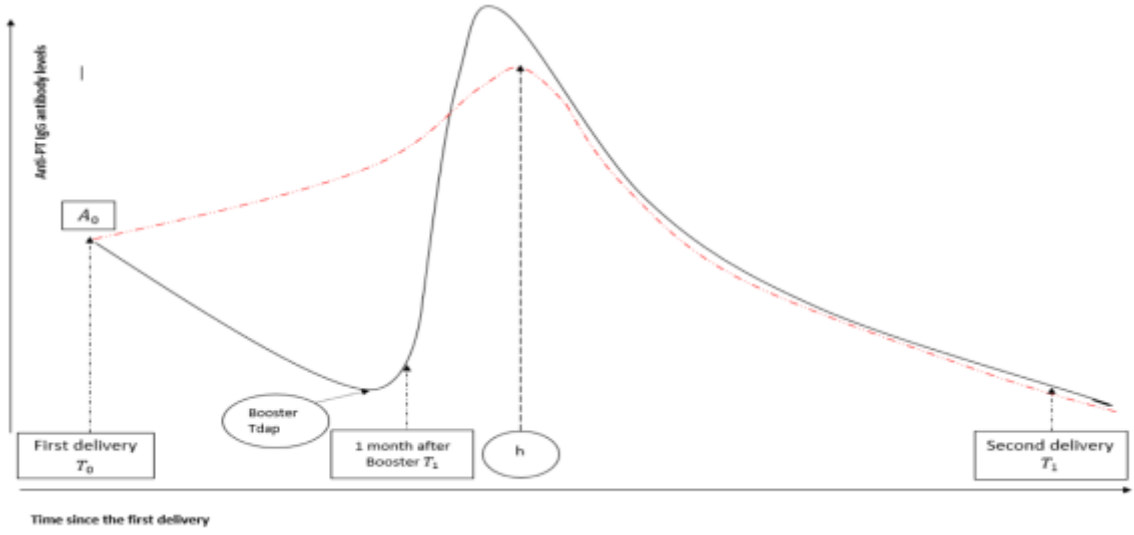

Figure 3: Illustrated presumed dynamics of antibody levels in pregnant women. Solid line (in black): the theoretical dynamics of antibodies, where it is assumed to decrease over time and then immediately increase after pregnant women received the booster vaccination. Dashed-dotted line (in red): the hypothesized dynamics of antibodies based on the collected data where it is assumed that antibodies increase between T_0 and T_1 with the rate ω then decrease between T_1 and T_2 with the decay α.

The ODEs for the evolution of antibodies in pregnant women are written as follows:

$$t \leq h: \frac{dA}{dt} = \omega A,$$

$$t > h: \frac{dA}{dt} = -\alpha A.$$

It is noticed that some notations used in this section, though might be the same as those in infants' model, in fact carry their own meaning and values.

Model building and selection:

We fit non-linear mixed effects model using Monolix Software. Model selection was performed based on AIC (smaller is better). Since there were maximum three measurements per subject, we allowed maximum three random effects. We let h fixed and let A_0, ω and α be random (Model A). This model, however, did not converge in Monolix. Based on the SAEM convergence plot, there seemed to be a negative correlation between h and ω. To further explore this notice, we extracted the sampled values of h and ω during SAEM in Monolix. The scatter plot (in Figure 4) shows a curvature relationship between two variables.

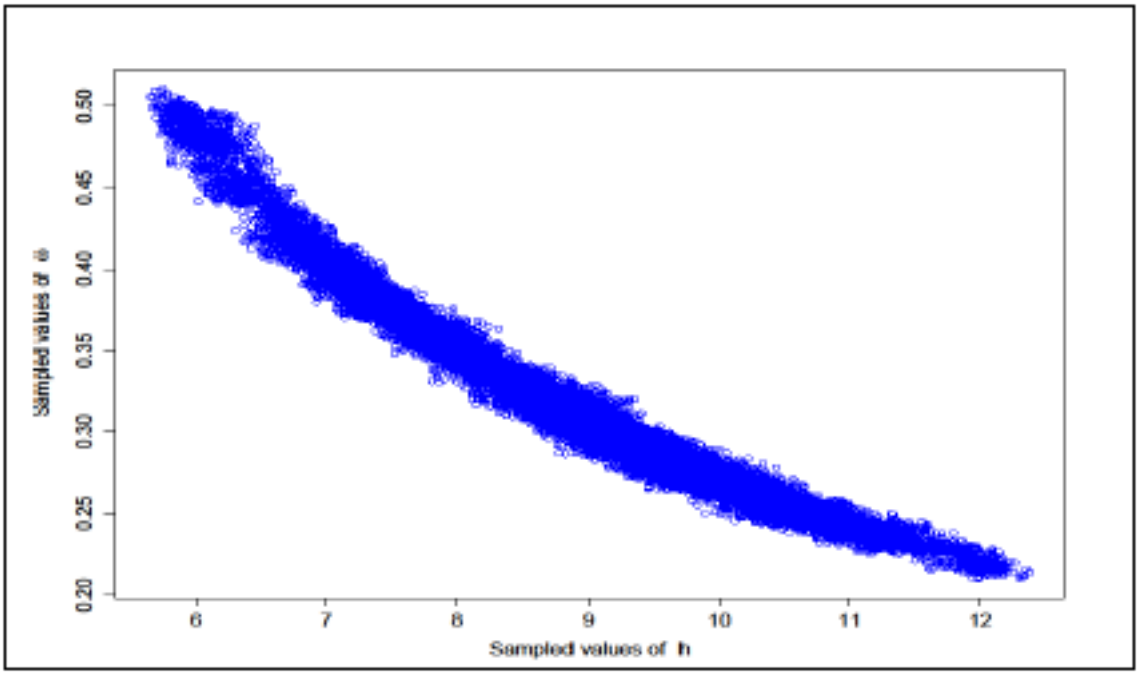

Figure 4: The scatter dot between sampled values of h and sampled values of ω from running model A in Monolix software.

We then applied the transformation to stabilize procedure in Monolix: $\omega = \frac{h}{e^{\gamma}}$. As a result, instead of estimating ω directly, we estimate γ.

The four-parameter model (A_0, γ, α and h) with three random effects $(A_{0,i}, \gamma_i, \alpha_i)$ were fit. Based in the SAEM convergence plot produced from Monolix software, the convergence of γ seems to be very unstable. Hence, a two-random-effect model $(A_{0,i}, \alpha_i)$ was fit. The convergence of this model was well achieved.

To perform model selection, we run two models with only 1 random effect: $A_{0,i}$ or α_i separately. The AIC obtained from these two models are higher compared with two random effects ($A_{0,i}$, α_i). As a result, the final model is the model with two random effects ($A_{0,i}$, α_i).

Specification for final model:

Let denote A_0 and α the two population parameters. The age at Boostrix (centered around its mean: *age_mean*) is assumed to affect the estimate of all four parameters. We assume $A_{0,i}$ and α_i have log-normal distribution:

$$log(A_{0,i}) = \log(A_0) + \beta_{A_0}(age_i - age\_mean) + \eta_{A_{0,i}}$$

$$log(\alpha_i) = \log(\alpha) + \beta_{\alpha}(age_i - age\_mean) + \eta_{\alpha,i}$$

Where

$$\eta_{A_{0,i}} \sim N(0, {\delta_{A_0}}^2),$$

$$\eta_{\alpha_i} \sim N(0, {\delta_{\alpha}}^2),$$

We use anti-PT IgG antibody levels on the log10 scale and assume an additive residual error model, that is:

$$log_{10}(A_{obs,ij}) = log_{10}(A_{pred,ij}) + \varepsilon_{ij}$$

where where $A_{obs,ij}$ and $A_{pred,ij}$ are the observed and predicted anti-PT IgG antibodies for women *i* at time *j*. The residual error is assumed to have a normal distribution with mean 0, that is:

$$\varepsilon_{ij} \sim N(0, \delta^2),$$

There are antibody values less than the limit of detection (5 EU/mL). These data will be treated as left censored during the estimation procedure in Monolix software.

Model diagnosis:

We perform model diagnosis for the final model by the means of the SAEM convergence plot and residual plots (residuals vs time and residuals vs prediction).

Moreover, the scatter dot of the observed values and the predicted values are assessed to partially evaluate the prediction ability of the model (see Figure 5).

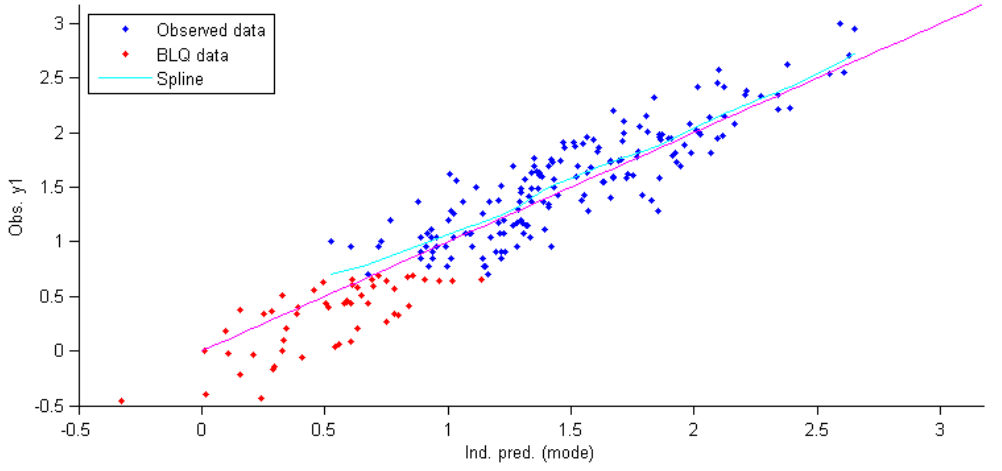

Figure 5: Scatter dot of the observed anti-PT IgG antibody levels with respect to the predicted anti-PT IgG antibody levels using the individual parameters (model in pregnant women, output from Monolix Software).

ROBUST LINEAR REGRESSION MODEL TO INVESTIGATE THE ASSOCIATION BETWEEN THE ANTI-PT IgG ANTIBODIES IN PREGNANT WOMEN AT DELIVERY AND THAT IN CORDS OF THEIR INFANTS

Figure 6 shows the scatter plot between anti-PT IgG antibodies in cords of infants and that in pregnant women at delivery. It is expected to see a linear trend between the two measurements.

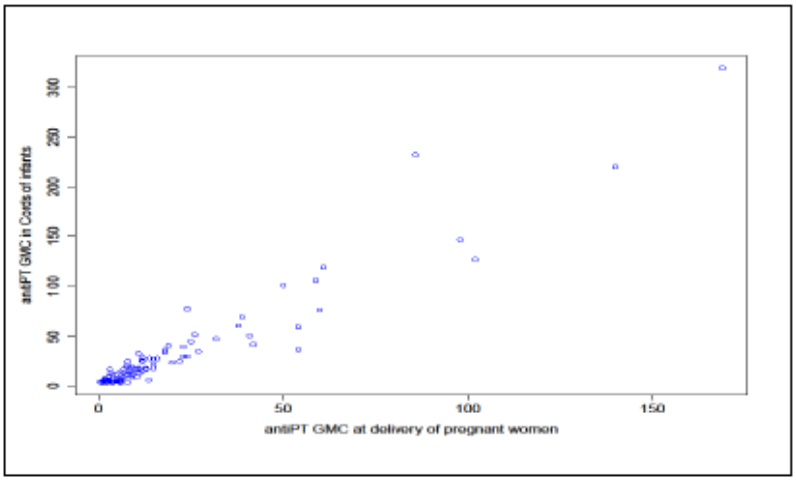

Figure 6: Scatter plot between anti-PT IgG antibodies in cords of infants and that in pregnant women at delivery. Left censored observations were replaced by the individual predicted mode values obtained from the final NLMM models for data in infants and data in pregnant women.

Model building and selection:

The response variable is *antiCord* (anti-PT IgG antibodies in Cords of infants). Initially, a full model with four covariates: anti-PT IgG antibodies of pregnant women at delivery (*antiPTMum* covariate), the order of the child (*child2*), the gender (*gender*) and birthweight of the child (*bweight* covariate) along with the interaction between *child2* and *antiPTMum* was considered. However, this model did not satisify two important assumptions, namely, the normality and the homocesdasticity of the residuals. Hence, it is decided to transform the response variable and the *antiPTMum* covariate into natural log scale. The motivation to choose this transformation is that it helps to satisfy the assumptions of linear model, and among other potential transformations, it serves the purpose of easier interpretation. The results from model on transformed data shown that the effects of *gender*, *bweight*, *child2* and the interaction between *antiPTMum* and *child2* were not significant. Since the central interest lies in the association between the antibody levles in pregnant women at delivery and that in Cords of their infants, the final model is the simple linear regression model where only *antiPTMum* is considered as covariate.

Specification for final model:

The final model could be written down as follows: $\log(antiCord_i) = \log(antiPTMum_i) + \varepsilon_i$,

where $i = 1, 2, \ldots, 97$.

The assumption of homocesdasticity of the residuals is not satisfied. Hence, a robust linear regression model was fit using *rlm* function in the MASS package. The result from this robust linear regression was used for making inference.

CHAPTER 2

Differences in humoral and cellular immune responses between pregnant and non-pregnant women

This chapter is published: "Huygen K., Caboré R.N., Maertens K., Van Damme P., Leuridan E. Humoral and cell mediated immune responses to a pertussis containing vaccine in pregnant and non-pregnant women. Vaccine. 2015 Aug 7; 33(33):4117-23."

doi: 10.1016/j.vaccine.2015.06.108.

Abstract

Vaccination of pregnant women is recommended for some infectious diseases in order to protect both women and offspring through high titers of maternal IgG antibodies. Less is known on the triggering of cellular immune responses by vaccines administered during pregnancy. In an ongoing study on maternal pertussis vaccination (2012-2014), 18 pregnant women were vaccinated with a tetanus-diphtheria-acellular pertussis (Tdap) containing vaccine (Boostrix®) during the third pregnancy trimester. Sixteen age-matched non-pregnant women received the same vaccine in the same time period. A blood sample was taken at the moment of, but before vaccination and one month and one year after vaccination. Anti-pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (Prn), tetanus toxin (TT) and diphtheria toxin (DT) antibodies were measured by ELISA. Cellular immune responses were analyzed using a diluted whole blood assay, measuring proliferation, and cytokine release in response to vaccine antigens PT, FHA, TT, and to pokeweed mitogen (PWM) as polyclonal stimulus.

Antibody levels to all five vaccine components increased significantly and to the same extent after vaccination in pregnant and non-pregnant women. One year after vaccination, antibody titers had decreased particularly to PT, but they were still significantly higher to all antigens than before vaccination. In contrast, proliferative and IFN-ɣ responses were increased to TT, PT and FHA in non-pregnant women one month after vaccination, whereas in pregnant women only TT specific T-cell responses were increased and to a lesser extent than in the control group. One year after vaccination, cellular responses equaled the baseline levels detected prior to vaccination in both groups. In conclusion, a Tdap vaccination can increase vaccine specific IgG antibodies to the same extent in pregnant and in non-pregnant women, whereas the stimulation of vaccine specific Th1 type cellular immune responses with this acellular vaccine is transient and impaired during pregnancy.

Introduction

Pertussis, caused by *Bordetella pertussis*, is a contagious, potentially life-threatening respiratory illness and a major cause of childhood morbidity and mortality. Although disease incidence declined steadily since the introduction of whole-cell-based vaccines in the fifties, the number of reported cases across all age groups has increased again during the past two decades in many industrialized countries with high vaccination coverage [22]. The underlying mechanism of this evolution is probably multifactorial: waning immunity in adults, switch from whole cell pertussis (wP) to less reactogenic acellular pertussis (aP) vaccines, increased awareness of physicians coupled to an easier and earlier diagnosis by PCR and possibly changes in virulence of the circulating pertussis strains. In adults, *Bordetella pertussis* infection can often cause only mild disease, as we confirmed in a recent seroprevalence study of 'healthy' adults aged 20-39 years old [27].

In order to protect young infants, who are most vulnerable to severe disease and even death, various strategies such as cocoon vaccination and pre-pregnancy boosting have been proposed [168, 180-182]. Pertussis vaccination in the second or third trimester of pregnancy has been implemented in 2011 in the USA [141], in 2012 in the United Kingdom [183], and in 2013 in Ireland [184] and Belgium [43].

Babies born from mothers who received Tdap during pregnancy have significantly higher tetanus, diphtheria, and pertussis antibody concentrations at birth when compared to newborns from mothers who did not receive Tdap [185, 186]. A Tdap booster vaccination at 30-32 weeks' gestation is safe [187] and elevated pertussis antibodies can be measured at delivery, and in the infants at birth and at age 2 months [102]. A vaccine effectiveness of 91% for infants younger than 3 months was demonstrated in an observational study, conducted between January 2008 and September 2013 in England [188].

Some immunological questions related to this maternal vaccination strategy, have not been answered yet. For one, protection against pertussis is dependent both on antibody- and cell-mediated immune (CMI) responses [189]. Vaccination induces these pertussis-specific CMI responses in infants [190], adolescents [191-193], and adults [44, 194] but CMI responses to pertussis vaccination during pregnancy have so far not been reported. In a number of papers reported on maternal vaccination (for a more recent review [195]), focus has been so far on the induction of antibodies. The only published data on CMI responses stimulated by maternal vaccination are to our knowledge by Yamaguchi et al. who described that the efficacy of a flu vaccination was similar in all stages of pregnancy [196] and by Kay et al. who reported on

enhanced natural killer cell and T-cell responses to inactivated influenza vaccination during pregnancy [197].

In this paper, we report on Tdap (Boostrix®) induced specific antibody and CMI responses of women enrolled during the third trimester of pregnancy as compared to the responses of age-matched non-pregnant women who received the same vaccine in the same time period.

Methods

STUDY DESIGN

A total of 18 pregnant women who participated in an ongoing study (Clinicaltrials.gov: NCT01698346) on maternal pertussis vaccination [198], received a combined pertussis vaccine (Boostrix®, GSK Biologicals, Rixensart) during the third trimester of pregnancy (25-32 weeks). Inclusion criteria of the study were: pregnant women aged 18-40 years with a normal pregnancy, consenting to be immunized during pregnancy. The pregnancy was considered to be 'normal' by the responsible physician, i.e., all standard prenatal care tests had to be normal, including tests for gestational diabetes and congenital malformations detectable on ultrasound checkup at 20 weeks of gestation. The chromosomal test was not obligatory to enter the study. But when a woman had an abnormal antenatal test, she was not allowed to participate. Chromosomal testing is performed before the gestational age of 20 weeks and women were only included after the testing was performed (if they chose antenatal testing). A detailed ultrasound is performed in all Belgian women at week 20 and the result was obtained for all participating women. Antenatal steroids were not an exclusion criterium for this study.

A control group was identified during the same time period and consisted of 16 age-matched non-pregnant women receiving the same vaccination.

Five cc of heparinized blood was collected at the moment of but before vaccination (month 0), at 28-31 days (month 1) and at year 1 (month 12) after vaccination. Ethical approval has been obtained by the Ethical Committee of the University Hospital of Antwerp on April 4, 2011.

PROLIFERATION ASSAYS

Proliferation assays were performed as described before [199]. Briefly, heparinized whole blood, collected by venipuncture, was diluted 1/10 in RPMI-1640 medium, supplemented with Hepes, L-glutamine, penicillin/streptomycin, and 5 x 10^{-5} M 2-mercapto-ethanol. Cells were cultured in round bottom microwell plates (Greiner) in a humidified CO_2 incubator at 37°C for 7 days. A volume of 180 µl of diluted blood (in 10% autologous plasma) was added to 20 µl of tetanus toxoid (TT) (provided by Dr. Y. Fikri, WIV-ISP), heat inactivated pertussis toxin (PT), filamentous hemagglutinin (FHA) (both kindly provided by Dr. M. De Ridder) (GlaxoSmithKline) to Dr. R. Vanhoof [200]) or Pokeweed Mitogen (PWM) (Sigma, L'Isle D'Abeau, France). Antigens were used at final concentration of 5 µg/mL and PWM at 4 µg/ml. Tritiated thymidine (Perkin Elmer) was added to the cells during the last 20 hours of culture (0.4 µCi/well). Cells were harvested on a Skatron Cell Harvester and filters were counted in a Beckman LS Betaplate scintillation counter.

Mean counts per minute (cpm) were calculated from quintuplicate cultures. Mean cpm values of cultures unstimulated cells (negative control) ranged between 50 and 100 cpm. Because of these very low negative control values, the analysis of antigen/mitogen stimulated responses was based on total cpm values without subtraction of the negative control values. Responses were considered positive when the stimulation index was >5.

CYTOKINE PRODUCTION

To avoid interference with circulating cytokine levels, heparinized blood was centrifuged for 10 min at 1500 rpm and plasma was recovered and stored at -20°C for antibody assays. Plasma was replaced by a same volume of RPMI-1640 medium, supplemented with Hepes, L-glutamine, penicillin/streptomycin, 5 x 10^{-5} M 2-mercapto-ethanol, and 10% Fetal Calf Serum (FCS). Blood was diluted to a final concentration of 10^6 leucocytes/ml in the same complete medium with 10% FCS and cells were stimulated as described for the proliferation assay. Culture supernatans from three wells were pooled after 7 days of culture and stored at -20°C until assay.

CYTOKINE ASSAY

IFN-ɣ was detected using Human IFN gamma ELISA Ready-SET-Go! (eBioscience, Cat.nr. 88-7316). Sensitivity 4 pg/ml, Standard curve range 4-500 pg/ml.

IL-10 and IL-13 levels were quantified using Human IL-10 ELISA Ready-SET-Go! (Affymetrix Ref. 887106-88) and Human IL-13 ELISA Reday-SET-Go! (Affymetrix 88-7439-88) respectively. Sensitivity of the assays is 2 pg/ml and 4 pg/ml respectively.

DETECTION OF ANTI-PERTUSSIS, ANTI-TETANUS AND ANTI-DIPHTHERIA ANTIBODIES

Anti-PT antibodies were detected in plasma by ELISA, using the Virion/Serion kit (ANL Copenhagen). Anti-FHA and anti-Prn IgG antibodies were detected using Euroimmune ELISA. Anti-TT and anti-DT IgG antibodies were detected using the Virotech/Sekisui ELISA.

Plasma was tested in duplicate at a dilution of 1:100 (PT, TT and DT), 1:400 (FHA) and 1:800 (Prn). OD results were converted into international units IU/ml. In order to calculate the Geometric Mean Concentration (GMC), all values >1100 IU/mL were used as 1200 IU/ml.

Vaccine responsiveness for pertussis antigens was defined as a post-vaccination antibody concentration ≥20 IU/ml if the pre-vaccination antibody concentration was <5 IU/ml, a post-vaccination rise of at least four times when the pre-vaccination antibody concentration ranged between 5 and 20 IU/ml and at least a doubling of pre-vaccination antibody concentration of ≥20 IU/ml. For diphtheria, vaccine responsiveness was only calculated for women with initial anti-DT titers <1IU/ml (requiring a booster vaccination) and a twofold increase in antibody titer was

considered as a positive vaccine response. For tetanus, all women had initial antibody titers >1 IU/ml, requiring a booster vaccination at the earliest after 5 year, and vaccine responsiveness was not calculated.

STATISTICAL ANALYSIS

Geometric Mean Concentrations (GMCs) of antibodies, proliferative responses and cytokine levels and the lower and upper 95% confidence intervals of GMCs were calculated using colums statistics of GraphPad Prism. Statistical analyses were performed using two-tailed Mann-Whitney for intergroup comparisons and two-tailed Wilcoxon matched-pairs signed rank test for intragroup comparisons. P values <0.05 were considered as statistically significant.

Results

A total of 18 pregnant women and 16 age-matched controls were identified and vaccinated. All subjects were vaccinated between October 2012 and April 2013. The mean age of both groups was 28.5 years. Mean gestational age at the moment of vaccination of the included pregnant women was 29 (25-32) weeks. Adverse events were monitored after vaccination in both groups. Ten of 18 pregnant women and five of 16 non-pregnant women had a history of tetanus (but no pertussis) booster vaccination within the last 10 years. Prior vaccination information was obtained through anamnesis and if available, vaccination booklet of the women. If no booklet was available, both the participant and her parents were questioned on vaccination history.

For the 1 year follow-up, blood samples of 17/18 and 11/16 women were collected among the previously pregnant and control group, respectively. Exclusions were due to a new pregnancy or lost to follow-up.

PERTUSSIS SPECIFIC ANTIBODY TITERS IN PREGNANT AND NON-PREGNANT WOMEN

As shown in Figure 1 and Table 1, IgG antibody titers to PT, FHA and Prn increased significantly in both groups one month after the vaccination. Highest antibody titers were found in response to Prn and even at a serum dilution of 1:800, anti-Prn titers were above the detection range (>1100 IU/ml) of the kit for 7/18 pregnant and 6/16 control women.

According to the definition of vaccine responsiveness, all participants responded to FHA, all but two women (in the control group) to Prn and 15/18 of pregnant and 13/16 of control women to PT (Table 1). The three non-responders to PT among the pregnant women increased from 14 to 31 IU/ml, from 18.5 to 32 IU/ml and from 7.8 to 28 IU/ml/ For the control group, the three non-responder women had an anti-PT antibody increase from 4 to 17 IU/ml, from 31.5 to 39 IU/ml, and from 19 to 50 IU/ml.

There was no statistical difference in GMC either before or after the immunization, between control and pregnant women for any of the antigens (using Mann-Whitney and considering $p<0.05$ as statistically significant). For pertussis, anti-PT IgG antibodies higher than 100 IU/ml are considered in serodiagnosis as indicative of an acute infection (in the absence of recent vaccination) [201]. In our study, none of the subjects except one woman in the control group, had an antibody level above 100 IU/ml prior to vaccination, but at 1 month after vaccination, antibody titers above this threshold value were measured in 5/18 pregnant and 5/16 of control women.

One year after vaccination (month 12), mean GMC levels were significantly lower in both groups as compared to GMC levels at month 1 for PT and FHA but not for Prn. (Figure 1, Table 1).

For PT only 2/17 and 2/11 of the women still had antibody titers indicative of a vaccine response (according to our criteria) and the woman in the control group who had an anti-PT titer above 100 IU/ml prior to vaccination (and had a doubling of the titer at month 1) still had an anti-PT level of 126 IU/ml at month 12. At month 12, vaccine responses for FHA were still detected in 16/17 pregnant and 10/11 control women and for Prn in 15/17 pregnant and 7/11 control women. GMC values at 12 months as compared to GMC values prior to vaccination at month 0 were significantly higher for all antigens in the women who had received the vaccine during pregnancy, whereas in the control group mean response to PT was not significantly different from GMC prior to vaccination. However, when values for the control woman with the high anti-PT value prior to vaccination were not taken into account, the mean anti-PT GMC in the control group at month 12 was also significantly ($p<0.05$) higher than at month 0.

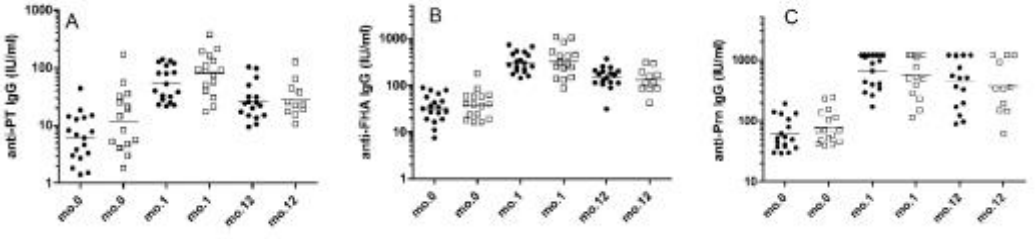

Figure 1: Pertussis specific antibody titers in pregnant and non-pregnant women before, 1 month and 1 year after a Tdap vaccination. Anti-PT (A), anti-FHA (B), and anti-Prn (C) IgG antibody levels of pregnant (black circles) and control (open squares) women, as detected by ELISA before booster, 1 month after booster, and 12 months after booster vaccination. Results represent individual antibody titers, expressed in IU/ml. Horizontal bars represent the geometric mean concentrations (GMC) of each group.

TETANUS AND DIPHTHERIA SPECIFIC ANTIBODY TITERS IN PREGNANT AND NON-PREGNANT WOMEN

Likewise, tetanus and diphtheria toxin specific antibody levels increased significantly after vaccination (Figure 2, Table 1). For tetanus, 10/18 pregnant and 5/16 control women had written evidence of a tetanus booster vaccine during the last 10 years prior to the present vaccination. However, GMC of TT specific antibodies in these 15 women was not statistically different from GMC of not recently vaccinated women and not different within the groups. Prior to vaccination, protective levels for tetanus (>1 IU/ml) were present in all women from both groups and vaccine responsiveness was therefore not calculated (Table 1). For diphtheria, high antibody levels (>1

IU/ml) were detected in 4/18 pregnant and 6/16 control women prior to vaccination (Figure 2, Table 1). Among the other women, 13/14 of the pregnant and 10/10 of the control women showed at least a doubling of their anti-DT antibodies at 1 month after the vaccination. At month 12, 10/13 women (with initial anti-DT titers < 1IU/ml) vaccinated during pregnancy and 4/7 of the vaccinated control women (with initial anti-DT titers < 1IU/ml) still had at least twofold higher titers as compared to month 0.

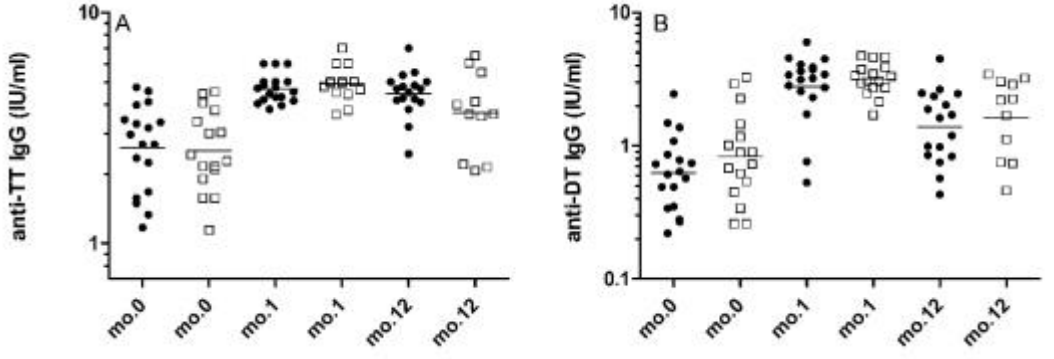

Figure 2: Tetanus and diphtheria specific antibody titers in pregnant and non-pregnant women before, 1 month and 1 year after a Tdap vaccination. Anti-TT (A) and anti-DT (B) IgG antibody levels of pregnant (black circles) and control (open squares) women, as detected by ELISA before booster, 1 month after booster and 12 months after booster vaccination. Results represent individual antibody titers, expressed in IU/ml. Horizontal bars represent the geometric mean concentrations of each group.

IgG (IU/mL)		PT	FHA	Prn	TT	DT
PREGNANT (*N* = 18)	**GMC mo.0** (95% CI)	**6.1** (3.9–9.5)	**32.1** (22.8–45.2)	**59.2** (43.9–80.0)	**2.59** (2.01–3.22)	**0.62** (0.45–0.86)
	GMC mo.1 (95% CI)	**52.7** (37.3–74.5)	**305** (238–390)	**667** (479–927)	**4.67** (4.36–5.01)	**2.78** (2.05–3.76)
	Response mo.1	15/18	18/18	18/18	–	13/14
p cf. mo 0		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
N = 17	**GMC mo.12** (95% CI)	**26.0** (18.0–37.6)	**148** (111–195)	**449** (275–733)	**4.45** (3.96–5.01)	**1.39** (1.00–1.92)
	Response mo.12	2/17	16/17	15/17	–	10/13
p cf. mo 1		$p < 0.01$	$p < 0.001$	$p < 0.01$	ns	$p < 0.01$
p cf. mo 0		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
CONTROL (*N* = 16)	**GMC mo.0** (95% CI)	**11.9** (6.2–22.9)	**38.1** (26.7–54.4)	**78.4** (56.6–108)	**2.52** (2.03–3.13)	**0.83** (0.5–1.26)
	GMC mo.1 (95% CI)	**79.5** (50.4–125.3)	**319** (214–476)	**574** (374–882)	**4.91** (4.51–5.36)	**3.17** (2.73–3.68)
	Response mo.1	13/16	16/16	14/16	–	10/10
p cf. mo. 0		$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
N = 11	**GMC mo.12** (95% CI)	**28.3** (17.6–45.6)	**129** (87–191)	**368** (186–730)	**3.66** (2.79–4.82)	**1.64** (1.02–2.62)
	Response mo.12	2/11	10/11	7/11	–	4/7
p cf. mo.1		$p < 0.05$	$p < 0.05$	ns	$p < 0.05$	$p < 0.05$
p cf. mo.0		ns	$\boldsymbol{p} < 0.01$	$p < 0.001$	ns	$P < 0.05$

N = number of women ns: statistically not significant CI: lower and upper confidence interval

Table 1: Geometric mean concentrations of anti-PT, anti- FHA, anti-Prn, anti-TT and anti-DT antibodies in pregnant and control women before and one month and 12 months after a Tdap vaccination.

PROLIFERATIVE RESPONSES OF PREGNANT AND NON-PREGNANT WOMEN

As shown in Figure 3 and Table 2, prior to vaccination positive antigen-specific proliferative responses in diluted whole blood cultures could be detected in response to TT, but not to PT nor to FHA, and TT specific responses were comparable in both groups. One month after vaccination, T-cells from non-pregnant women showed about five- to tenfold higher proliferative responses that were now directed against TT and also against PT and FHA. In contrast, immunization of pregnant women resulted only in a threefold stronger proliferation to TT and only a slightly increased response to the two pertussis antigens. One year after vaccination, proliferative responses were back to baseline levels measured at month 0 for all antigens, indicating that the Tdap vaccination had only transiently stimulated the existing memory T-cell population.

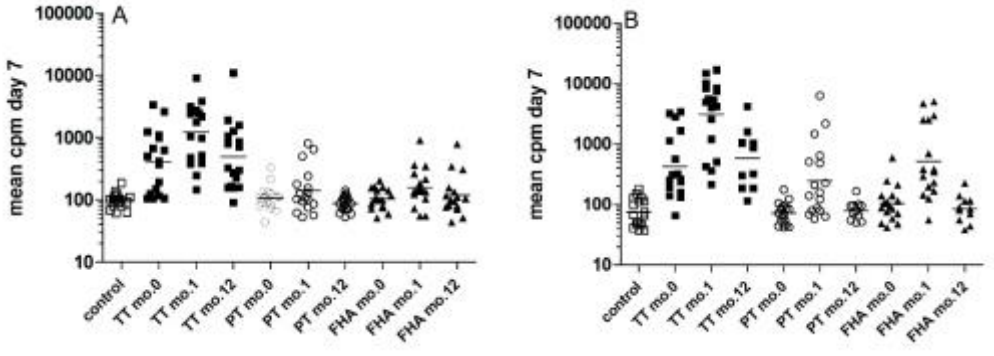

Figure 3: Proliferative responses of pregnant and non-pregnant women before, 1 month and 1 year after a Tdap booster vaccination. Proliferative responses (expressed in cpm) measured in 7 day cultures of diluted whole blood of pregnant women (A) and control women (B); stimulated with TT (black squares), PT (white circles) or FHA (black triangles) before booster, 1 month after booster, and 12 months after booster vaccination. Responses of non-stimulated cells are shown as white squares. Horizontal line represents mean cpm values of each group.

IFN-ɣ RESPONSES OF PREGNANT AND NON-PREGNANT WOMEN

Vaccine specific IFN-ɣ levels in 7 day culture supernatans could be detected only in response to TT stimulation and responses increased more after boosting in control than in pregnant women (Figure 4, Table 2). IFN-ɣ levels in PT or FHA stimulated cultures were overall very low and not different from levels in non-stimulated cells in this whole blood cell assay. As for the proliferative responses, TT specific IFN-ɣ levels at one year after vaccination were back to month 0 baseline levels.

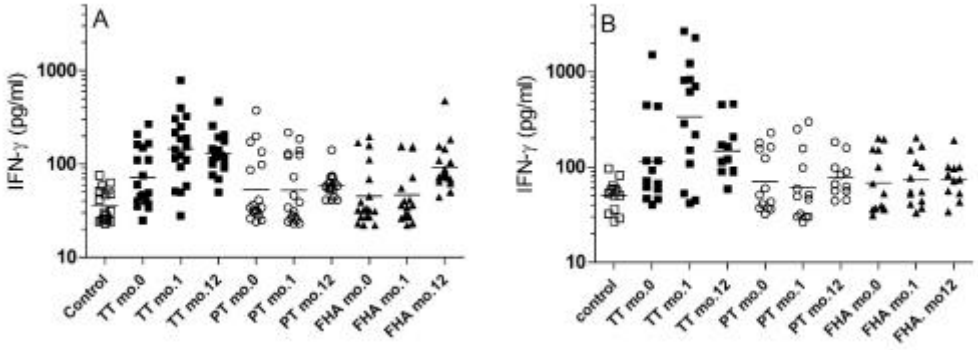

Figure 4: IFN-ɣ responses of pregnant and non-pregnant women before, 1 month and 1 year after a Tdap booster vaccination. IFN-ɣ responses (expressed in pg/ml) measured in 7 day cultures of diluted whole blood of pregnant women (A) and control women (B), stimulated with TT (black squares), PT (white circles) or FHA (black triangles) before booster, 1 month after booster, and 12 months after booster vaccination. Responses of non-stimulated cells are shown as white squares. Horizontal lines represent mean IFN-ɣ titers of each group.

PROLIFERATIVE AND CYTOKINE RESPONSES IN RESPONSE TO POLYCLONAL POKEWEED MITOGEN

Overall CMI potential was assessed by stimulating the cells with Pokeweed mitogen, which is a polyclonal T- and B-cell mitogen that served as a positive control. Proliferative response was comparable in both groups prior to vaccination, and increased after vaccination in control but not in pregnant women. Responses were statistically and almost twofold higher in the control group ($p=0.008$) (Table 3). PWM also induced strong IFN-ɣ responses in both groups (about 100 ng/ml at month 0). After vaccination, PWM-induced IFN-ɣ responses were significantly higher in control than in pregnant women as well ($p=0.027$) (Table 3). At month 12, mean proliferative and IFN-ɣ responses were again comparable between the two groups of women.

Production of two other cytokines, i.e. IL-13 and IL-10, was also measured in these PWM stimulated cultures for all pregnant and control women prior to vaccination and 1 month and 1 year after vaccination. Vaccination did not influence PWM-induced IL-13 or IL-10 production. However, GMC of PWM-induced IL-13 was significantly higher in control than in pregnant women prior to vaccination ($p=0.028$), whereas IL-10 responses showed the same tendency as the proliferative and IFN-ɣ responses, control women having significantly higher levels than the pregnant women at month 1 ($p=0.0204$).

	Pregnant women Proliferation (95% CI)	Control women Proliferation (95% CI)	Proliferation Ratio Co/P	Pregnant women Interferon-γ (95% CI)	Control women Interferon-γ (95% CI)	IFN-γ Ratio Co/P
Control	97 (84–113)	74 (57–97)	ns	36 (29–44)	50 (39–63)	ns
TT mo.0	404 (223–732)	426 (227–799)	ns	71 (50–102)	114 (59–222)	ns
TT mo.1	1213 (678–2168)**	3077 (1514–6253)***	*	145 ((96–219)**	336 (148–763)**	ns
TT mo.12	501 (266–944)ns	585 (279–1224)ns	ns	129 (98–170)**	147 (95–230)ns	ns
PT mo.0	107 (85–135)	72 (59–89)	*	53 (35–80)	71 (45–110)	ns
PT mo.1	142 (93–217)ns	250 (123–509)***	ns	52 (35–79)ns	61 (37–102)ns	ns
PT mo.12	87 (75–101)ns	79 (62–101)ns	ns	59 (50–68)ns	77 (56–105)ns	ns
FHA mo.0	105 (86–128)	103 (73–145)	ns	45 (31–65)	68 (44–105)	ns
FHA mo.1	158 (110–228)*	518 (255–1052)***	**	47 (33–67)ns	72 (50–105)ns	ns
FHA mo.12	119 (82–172)ns	85 (60–121)ns	ns	92 (69–124)*	74 (54–102)ns	ns

IFN-γ responses expressed in pg/ml. The lower and upper 95% confidence intervals of mean are given in parentheses. Statistical significance calculated as compared to Month 0 values by Wilcoxon rank test. Statistical significance of ratios of GMC of control women to GMC of pregnant women were calculated using Mann–Whitney *U* test * $p < 0.05$, ** $p < 0.01$.

Table 2: Geometric mean proliferative and interferon-ɣ responses of diluted whole blood cultures of pregnant and control women prior (mo.0) and 1 month (mo.1) after vaccination (pregnant n=18, control n=16) and 12 months (mo.12) after vaccination (pregnant n=17, control n=11) in response to tetanus toxoid (TT), pertussis toxin (PT), or filamentous hemagglutinin (FHA). Proliferative responses expressed as mean counts per minute (cpm).

Pregnant women GMC (95% CI)	Control women GMC (95% CI)	Ratio C/P (p)	Pregnant women GMC (95% CI)	Control women GMC (95% CI)	Ratio C/P (p)
Proliferation			**Interferon-γ**		
13,476 (9768–17,184)	15,063 (9099–21,027)	1.12 (ns)	99,388 (31,266–167,550)	121,747 (52,393–191,102)	1.22 (ns)
12,764 (8606–16,922) ns	23,116 (16,441–29,791)*	1.81**	69,177 (36,553–101,800) ns	143,063 (82,819–203,307) ns	2.07*
6604 (3875–9338) ns	8950 (6003–11,897) ns	1.35 (ns)	65,631 (46,434–84,827) ns	80,536 (32,203–128,869) ns	1.23 (ns)
Interleukin-13			**Interleukin-10**		
138 (102–186)	241 (161–360)	1.75*	687 (515,915)	836 (667–1050)	1.22 (ns)
217 (148–317) ns	281 (164–482) ns	1.29 (ns)	594 (473–745) ns	909 (747–1105) ns	1.53*
206 (131–325) ns	357 (173–739) ns	1.73 (ns)	647 (437–958) ns	1113 (879–1408) ns	1.72 (ns)

Proliferative responses in counts per minute cpm. IFN-γ, IL-13, and IL-10 responses expressed in pg/ml. The lower and upper 95% confidence intervals of mean are given in parentheses. Statistical significance calculated as compared to Month 0 values by Wilcoxon rank test. Statistical significance of ratios of GMC of control women to GMC of pregnant women were uncited references calculated using Mann–Whitney U test. ns: not significant * $p < 0.05$, ** $p < 0.01$.

Table 3: Geometric mean proliferative, interferon-ɣ, IL-13, and IL-10 responses of diluted whole blood cultures of pregnant and control women prior to and 1 month after vaccination (pregnant n=18, control n=16) and 12 months (n=17 respectively n=11) after vaccination in response to polyclonal T-and B-cell mitogen Pokeweed Mitogen (PWM).

Discussion

In view of the resurgence of whooping cough in a number of industrialized countries with high aP vaccination coverage, maternal pertussis vaccination may be the most effective strategy to close the susceptibility gap of young infants to pertussis and thus reduce the burden of disease in the youngest age group [198]. The precise immunological mechanisms by which pertussis vaccines confer protection against infection and disease are not fully defined, but it has become clear that besides strong antibody-mediated also Th1/Th17 mediated immune responses are important [189]. Here we have compared the effect of Tdap vaccination on humoral and cell-mediated immune responses at 1 month and 1 year after vaccination in pregnant women vaccinated with a pertussis containing vaccine during the third trimester of pregnancy and in age-matched non-pregnant women.

Antibody titers to the three pertussis antigens (PT, FHA and Prn), to TT and to DT increased significantly one month after maternal vaccination. Increases were comparable to those observed after the Tdap boost in the age-matched group of non-pregnant control women. At 1 year after vaccination, antibody levels to PT, FHA and DT had decreased to about half the values observed at 1 month after vaccination, but with the exception of anti-PT titers, they were still significantly higher than before vaccination at month 0. Antibodies to Prn and TT were very high after the boost and not significantly lower after 1 year. The 18 pregnant women enrolled in this pilot study, were part of a larger study (Clinicaltrials.gov: NCT01698346) analyzing antibody responses in 57 women vaccinated during pregnancy and 42 control pregnant women who did not receive a booster vaccine. Results of this study will be published soon (E. Leuridan et al, manuscript in preparation).

T-cells are also important for protection against whooping cough, particularly IFN-ɣ producing Th1-cells [202]. To our knowledge, the Th1 cell-mediated immune response in response to a Tdap vaccination in adult pregnant women was not studied before. In our study, we found that the Tdap boost stimulated stronger vaccine specific proliferative and IFN-ɣ responses in control than in pregnant women. Proliferative and IFN-ɣ responses of control women were also higher in response to the polyclonal powekeed mitogen. During pregnancy, a progressive shift in the Th1/Th2 balance has been described, particularly when tested against non-specific stimuli [197, 203-206] or assessed at the systemic level [207]. However, using the diluted whole-blood assay, we could not really demonstrate vaccine specific Th2 responses neither in cultures of pregnant nor of control women. Pregnancy-induced suppression of the cellular immune response

to promote foetal tolerance is well known and it is more likely that the reduced Th1 response that we observed in our group of pregnant women, was a reflection of these tolerogenic mechanisms and an overall anti-inflammatory state, which is most pronounced during the second pregnancy trimester [208-210].

Although the Tdap boost stimulated CMI responses, this stimulation was transient at one year after vaccination; cellular responses equaled the baseline levels detected prior to vaccination in both groups. In 2007, Meyer et al. analyzed antibody and cell-mediated immunity (proliferation and IFN-ɣ release by Ficoll-purified PBMCs) in 49 healthy adolescents and adults at 1 month and 1 year postimmunization with a trivalent acellular pertussis vaccine composed of three antigens PT, FHA and Prn [44]. Meyer's study reported that the decay in cell-mediated immune responses to the acellular vaccine was less than the decay in antibody levels. However, this conclusion has to be considered with some caution, as antibody levels had decayed by about 50% after 1 year (similar to our findings), whereas IFN-ɣ levels had diminished by 60 to 70% relative to 1 month postvaccination [44]. Moreover, a comparison of the two studies is difficult, as the initial number of precursor T-cells in purified PBMC cultures was likely higher than in diluted whole blood assay and proliferative responses were expressed as stimulation indices as compared to actual cpm values in our study. Also, the booster vaccine was somewhat different as the trivalent acellular pertussis vaccine lacked diphtheria and tetanus toxoid. Finally, vaccination history of the two populations may have been different, as no vaccine specific proliferation was measured in the German study prior to boosting, whereas in our study initial CMI response could be detected, particularly to TT. Tetanus rather than pertussis-specific T-cell responses were augmented - in both groups of women - in contrast to antibody responses which were increased in the two groups to the same extent and to both tetanus and pertussis antigens. Past tetanus booster immunizations are a likely explanation, as quite strong anti-tetanus antibodies were found in both control and pregnant women prior to boosting. Gall et al. also reported on high anti-TT antibodies in 96% of pregnant women prior to Tdap boosting [185]. It is well known that tetanus toxoid contains an immunodominant, promiscuously recognized T-cell epitope [211] and it could be hypothesized that boosting with a hexavalent vaccine could lead to antigenic competition at the T-cell level between TT on the one hand and the pertussis and diphtheria antigens on the other, at least in populations such as ours, in which all women had particularly strong anti-tetanus immunity.

In conclusion, Tdap vaccination during the third trimester of pregnancy resulted in significantly increased antibody response to all five antigens after the vaccination and the increase was comparable to the one observed in a group of non-pregnant control women. Antibodies against pertussis antigens, particularly pertussis toxin, decreased after one year, supporting the recommendations for repeat vaccination with repeat pregnancies in order to transport high titers of maternal antibodies to subsequently born offspring. In contrast, vaccine specific CMI responses were boosted to a lesser extent in pregnant than in control women and moreover this stimulation was transient and only observed at 1 month but not at 1 year after the boost.

Acknowledgments

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CHAPTER 3

Pertussis vaccination during pregnancy in different epidemiological settings

CHAPTER 3/A

Vaccine responses in Belgian children after primary vaccination

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Abstract

Vaccination during pregnancy has been recommended in some countries as a means to protect young infants from severe infection. Nevertheless, many aspects are still unknown and possible blunting of the infant's immune responses by maternal antibodies, is one of the concerns with maternal vaccination. We report the first prospective controlled cohort study in women and infants on the effects of using Boostrix®, a combined tetanus, diphtheria and acellular pertussis vaccine, during pregnancy. The primary aim was to measure the influence of this booster dose on the titer and duration of the presence of maternal antibodies in the infants and assess possible interference with infant immune responses.

In a controlled cohort study, 57 pregnant women were vaccinated with Tdap vaccine (Tetanus Diphtheria acellular Pertussis, Boostrix®, GSK Biologicals), at a mean gestational age of 28.6 weeks. A control group of pregnant women (N=42) received no vaccine. Antibody geometric mean concentrations (GMCs) against tetanus (TT), diphtheria (DT), pertussis toxin (PT), filamentous hemagglutinin (FHA) and pertactin (Prn) were measured with commercial ELISA tests in samples taken preceding maternal vaccination and one month afterwards, at delivery and from the cord blood, and in infants before and 1 month after the primary series of 3 pertussis containing hexavalent vaccines.

Infants born to vaccinated women had significantly higher GMC at birth and during the first 2 months of life for all vaccine antigens compared to the offspring of unvaccinated women, thereby closing the susceptibility gap for pertussis in infants. However, blunting was noticed for infant diphtheria and pertussis toxin vaccine responses ($p<0.001$) in the infants from vaccinated women after the primary vaccination schedule (weeks 8, 12 and 16).

Since pertussis vaccination has been recommended during pregnancy already, the results of this study support that recommendation and provide additional scientific evidence to document possible interference by maternal antibodies.

Introduction

Pertussis, caused by *Bordetella pertussis*, is a highly contagious respiratory illness and a major cause of infant morbidity and mortality. Global pertussis vaccination programs have been introduced with success and approximately 86% of infants worldwide have received 3 doses of the diphtheria-tetanus-pertussis (DTP3) vaccine [1].

However, a decade after the switch from the whole-cell (wP) vaccine to the acellular pertussis (aP) vaccine, a cyclic resurgence has been reported in several industrialized countries. The reason is presumed to be multifactorial, with waning immunity after the primary or booster vaccination as the primary cause. A resurgence has been observed in all age categories; however, severe morbidity and mortality occurs primarily in young infants who are not fully vaccinated [30, 212]. The majority of cases are found in adolescents and adults, due to waning immunity [24], and these populations represent sources of infection for young infants.

In Belgium, pertussis vaccination with a hexavalent aP-containing vaccine is offered at 8, 12 and 16 weeks and 15 months of age. Booster doses for children at 4-6 years of age (since 2004) and for adolescents at 14-16 years of age have been recommended (since 2009). Additionally, receiving a booster dose once during adulthood has been recommended since 2013 [43]. Nevertheless, the total number of confirmed cases increased in Belgium from 93 in 2005 to 843 cases in 2013 [213], of which many (25.4% in 2013) were found in infants under the age of 1 year.

Partial primary protection against infectious diseases is offered at birth by maternal immunoglobulin G (IgG) antibodies [134, 214], with an estimated half-life of 6 weeks for pertussis [214]. The amount of transmitted antibodies depends on the placental function and the concentration of maternal antibodies in the pregnant woman [120]. The latter depends on the time lapse since the last vaccination or infection [215] and the titer of passively transmitted pertussis maternal antibodies is often low [160]. Thus, increasing the load of maternal antibodies by vaccination during pregnancy is, with the currently available vaccines, the only way to offer passive protection to the newborn at birth [216]. During the first weeks of life, these maternal antibodies disappear in the newborn due to natural clearance [120, 171].

Vaccination during pregnancy is recommended in an increasing number of countries (e.g. UK, USA, Belgium, New Zealand, etc.). Research has been performed on the immunological and safety aspects of the strategy [102, 185, 186, 217, 218]; nevertheless, many aspects are still unknown, and the possible interference of maternal antibodies with the infant's immune responses is one of the concerns.

To the best of our knowledge, no other data have been published on the effects of using the combined tetanus, diphtheria and acellular pertussis vaccine Boostrix® (GSK, Rixensart, Belgium) during pregnancy. The primary aim was to measure the influence of this booster vaccination on the titer and the duration of maternal antibodies in infants and to assess possible interference.

Methods

A prospective controlled cohort study was conducted in accordance with the Declaration of Helsinki, ICH-GCP, and the procedures established by Belgian law and was approved by the ethics committee of the University of Antwerp, Belgium (Clinicaltrials.gov identifier: NCT01698346). Written informed consent was obtained from all participants and from both parents of the participating infants (in accordance with the Belgian law and IRB regulations).

Healthy pregnant women and their healthy offspring from 5 different hospitals in the province of Antwerp, Belgium, were included in the study, and follow-up remains ongoing. Pregnant women were included in either a vaccine group, receiving an acellular pertussis vaccine, or a control group, if they had not received any pertussis-containing vaccine for at least 10 years. Strict randomization was not possible because some women were advised positively or negatively by their treating physician on the pertussis vaccination in pregnancy and were included accordingly. The recommendation for receiving the pertussis vaccination during pregnancy by the Belgian National Immunization Technical Advisory Group (NITAG, since August 2013) was not yet in place during the recruitment phase of this study, only a recommendation for cocoon vaccination. However, by 2012, the VVOG (Association of Flemish Obstetricians and Gynecologists) had recommended the ACIP as a valuable alternative for cocoon vaccination on its website. This recommendation was followed by some Belgian clinicians. Strict inclusion and exclusion criteria were used (Annex 1).

An extended questionnaire collected information on obstetrical risk factors, demographics, a general vaccination and pertussis-specific history, and a general medical history. Growth parameters, breastfeeding data, day-care attendance, immunization data and medical histories for all household members were collected at each visit.

STUDY VACCINES

Licensed Tdap vaccine (Boostrix®, GSK Biologicals, Rixensart, Belgium) was used to immunize pregnant women. Boostrix® contains 5 Lf of tetanus toxoid (TT), 2.5 Lf of diphtheria toxoid (DT), 8 mcg of inactivated pertussis toxoid (PT), 8 mcg of filamentous hemagglutinin (FHA), and 2.5 mcg of pertactin (Prn). Infants were vaccinated with a hexavalent vaccine (Infanrix Hexa®, GSK Biologicals, Rixensart, Belgium). Infanrix Hexa® contains 25 Lf of DT, 10 Lf of TT, 25 mcg PT, 25 mcg FHA and 8 mcg Prn, inactivated poliovirus, hepatitis B surface antigens and *Haemophilus influenza* type B polysaccharide.

STUDY PROCEDURES

Venous blood (10cc) was collected from all participating women immediately preceding the vaccination, at 1 month (28-31 days) after vaccination, and at delivery. The maternal vaccination was performed by the study physician or study nurse under supervision. Cord blood was collected at delivery (10cc). Blood samples (2cc) were collected from the infants before starting the primary schedule (week 8 ± 4 days) and at month 5 (28-35 days after third vaccine dose). Infant vaccines were administered in the regular health care system at the well-baby clinics or a pediatrician. Further follow-up is ongoing, with blood samples being collected before and after an Infanrix Hexa® booster dose given at month 15 (data not shown). The samples were centrifuged at 2000 rpm within 24 h and stored at -20°C.

SAFETY ASSESSMENTS

Systemic reactions were monitored by a medical doctor in all women for 30 min post-vaccination. Adverse events were monitored for 30 days post-vaccination and included: injection site pain, swelling, erythema, and general symptoms such as myalgia and fever. Serious adverse events during the pregnancy and follow-up period were documented. Whether an adverse event reaction was caused by the immunization was judged by the investigators who considered temporality, biologic plausibility, as well as the identification of alternative etiologies for each event. Possible congenital abnormalities were also monitored in the offspring.

LABORATORY

All samples were tested with commercially available ELISA kits at the National Reference Centre for Bordetella. The Virion/Serion® kit (ANL, Copenhagen) was used to detect anti-PT IgG antibodies, and the Euroimmune® ELISA kit was used to detect anti-FHA and anti-Prn IgG antibodies. Anti-TT and anti-DT IgG antibodies were detected using the Virotech/Sekisui® ELISA. Serum samples were tested in duplicate at a dilution of 1:100 (PT, TT and DT), 1:400 (FHA) and 1:800 (Prn). All OD results were converted into international units per milliliter (IU/ml). For tetanus and diphtheria, the limits of detection were 0.01 IU/ml and 0.03 IU/ml, respectively. All titers are expressed in IU/ml, using respective WHO standards (NIBSC 06/140 for pertussis, NIBSC code TE-3 for tetanus and NIBSC 00/496 for diphtheria). For pertussis, these international units are equivalent to the CBER EU units of FDA [219].

An international independent validation was performed to guarantee the reliability of the results. A random selection of samples (N=177) was reanalyzed at the Canadian Center for Vaccinology in Halifax, where CBER equivalent sera based on the WHO standard lot number 3 were used. A positive correlation was found in the results of both laboratories. The protective threshold of antibodies (a correlate of protection) is not known for pertussis [38]. For tetanus and diphtheria, a correlate of protection is defined as 0.1 IU/ml for tetanus and 0.01-0.1 IU/ml for diphtheria.

STATISTICS

A sample size calculation was performed, based on previous results [168]. Accordingly, a population of 50 subjects in both study arms would be sufficient to detect significant differences in antibody titers at several time points.

Statistical tests included parametric tests: (paired) t-test and chi-square tests and their non-parametric alternatives: (paired) Wilcoxon tests and Fisher exact tests whenever the underlying assumptions of the parametric tests were violated, i.e. normality and sparseness, respectively [220, 221].

The presence of twins in the data resulted in using different sample sizes for outcomes related to women and children. Data were assumed to be missing completely at random and complete case analyses were conducted rendering unbiased estimates though at the cost of a loss of efficiency [222]. The loss of efficiency resulting from excluding observations was limited due to the limited amount of missing data (12.4%).

Assessing the impact of the different mother and child characteristics on GMC for each of the different time points was done using a regression approach with outcome the log titer values, symmetrizing the response, and consisting of three consecutive steps: (1) variable selection using random forests [223] (Annex 2); (2) backward model selection using multiple linear regression based on AIC; and (3) further model reduction using likelihood ratio tests [221]. This model building procedure was used before [224].

Blunting of vaccine immune responses among infants was defined as a lesser GMC of specific IgG antibodies at a certain point in time in the offspring of the vaccinated women compared to the titer in the offspring of the control group.

Results

GENERAL CHARACTERISTICS OF THE STUDY POPULATION

In total, 57 healthy pregnant women were vaccinated (the vaccine group), and 42 women were identified as controls (the control group). The children were born between April 2, 2012 and April 16, 2014. Blood samples were taken between February 6, 2012 and September 18, 2014. The mean interval between the Tdap immunization and delivery was 77.1 days (39-117 days). The mean gestational age at vaccination was 28.6 weeks (22-33 weeks). After delivery, 55 children were included in the vaccine group (including 2 sets of twins) and 26 children in the control group. Reasons for exclusion included: premature delivery (N=1), children not vaccinated according to protocol (N=2), and the failure to obtain informed consent signed by both parents (N=17). Additionally, 2 children from the control group were excluded after the blood sample on week 8 due to delayed primary vaccination. No significant differences in demographics were found between both groups. The clinical history performed at every visit did not identify any clinical case of pertussis (Table 1).

		Vaccine group	Control group
N (women)		57	42
Mean age at delivery in years (SD)		30.7 (4.0)	32.3 (3.8)
Level of education, no. (%)			
	Unknown	1 (1.8)	0
	Secondary school	13 (22.8)	1 (2.4)
	Bachelor	18 (31.6)	23 (54.8)
	Master	25 (43.9)	18 (42.9)
Race mother, no. (%)			
	Caucasian	55 (96.5)	41 (97.6)
	Other	2 (3.5)	1 (2.4)
Mean gestational age at delivery in weeks (SD)		39.7 (1.4)	39.7 (1.0)
Primiparity, no. (%)		43 (75.4)	28 (66.7)
Mean gestational age at vaccination in weeks (SD)		28.6 (2.8)	NA
Mean interval between vaccination and delivery in days (SD)		77.1 (17.5)	NA
Tetanus dose <10 years ago, no. (%)		26 (45.6)	19 (45.2)
Exposure pertussis disease <10 years ago, no. (%)		0	1 (2.4)
Twin pregnancies, no. (%)		2 (3.5)	0
Mode of delivery, no. (%)			
	Vaginal	46 (80.7)	35 (83.3)
	Cesarean	11 (19.3)	7 (16.7)
Induction of labor, no. (%)		19 (33.3)	8 (19.0)
Epidural anesthesia, no. (%)		40 (70.2)	24 (57.1)
N (included infants)		55	26
Infant gender, no. (%)	Male	30 (50.8)	17 (40.5)
	Female	29 (49.2)	25 (59.5)
Mean weight at birth in gram (SD)		3351.7 (485.2)	3404.8 (479.4)
Mean length at birth in centimeters (SD)		50.3 (2.6)	49.5 (2.7)
Mean weight week 8 in gram (SD)		5165.6 (584.2)	5058.2 (492.3)
Mean length week 8 in centimeters (SD)		57.3 (2.2)	57.2 (1.9)
Mean weight month 5 in gram (SD)		7376.5 (940.9)	7345.9 (707.3)
Mean length month 5 in centimeters (SD)		66.2 (2.5)	66.6 (1.7)
Mean age at vaccine dose 1 in days (SD)		63.0 (7.4)	67.5 (7.4)
Mean age at vaccine dose 2 in days (SD)		95.9 (11.2)	101.5 (12.6)
Mean age at vaccine dose 3 in days (SD)		131.2 (15.1)	135.6 (16.6)
Mean age at blood sample before primary vaccination in days (SD)		55.9 (3.8)	55.2 (6.9)
Mean age at blood sample 1 month after primary vaccination in days (SD)		162.3 (15.1)	166.0 (16.5)
Mean interval between vaccine dose 3—blood sample month 5 in days (SD)		31.7 (3.0)	30.9 (2.8)

Table 1: Demographic and clinical characteristics of all study participants.

SAFETY RESULTS

Of the 57 women in the vaccine group, 50 adverse events (AE) were reported in 46 women. Most symptoms were mild and self-limited and were resolved within 72 h after vaccination. Stiffness of the arm at the injection site was the most commonly reported AE (N=42), followed by minor swelling at the injection spot. Five AE (vaginal thrush, reflux, fever <38.5°C, extensive limb swelling and rashes on the abdomen and arms) required the use of concomitant medication. Fever was described in only 1 vaccinated woman (1.75%). The mean duration of all AE was 2.30 days (1-10 days).

A total of 11 serious adverse events (SAE) were reported in the vaccine group; none of these SAE were related to the vaccination (according to the investigator's opinion). In the control group, 3 SAE were reported. The reported SAE included 1 case of preterm preeclampsia, 4 of term preeclampsia, 4 of premature contractions, 3 of hypertension, 1 of oligohydramnios and 1 of placenta previa.

In total, 8 SAE requiring hospitalizations for at least 1 h, were reported in the infants: 7 in the vaccine group and 1 in the control group. The mean duration of the SAE was 7.75 days (1-31 days). The reported SAE included: 1 premature delivery, 1 fever at birth, 1 hypoglycemia at birth, 1 pneumonia at birth, 2 infections that required hospitalizations at the age of 1 and 5 months, 1 episode of febrile seizures at the age of 2 months and 1 episode of extreme vomiting at the of 5 months. No congenital disorders were detected among the infants in the study (Table 2).

	Vaccine group (*N*= 57)	Control group (*N*= 41)
Preterm preeclampsia (number/proportion)	1 (1.75%)	0 (0%)
Term preeclampsia (number/proportion)	3 (5.26%)	1 (2.44%)
Premature contractions	4 (7.02%)	0 (0%)
Hypertension	2 (3.50%)	1 (2.44%)
Oligohydramnion	1 (1.75%)	0 (0%)
Placenta praevia	0 (0%)	1 (2.44%)
Total number of serious adverse events	11	3

Table 2: Overview of the reported serious adverse events within the study.

LABORATORY RESULTS

Table 3 provides an overview of the Geometric Mean Concentrations (GMCs) of IgG antibodies to Tdap vaccine antigens in the sera from all mothers and infants.

At baseline, no significant differences were found between both groups for any measured antibody. Protective antibody concentrations for tetanus and diphtheria were measured at all other time points in mothers and infants. Women in the vaccine group had significantly higher GMCs to all antigens at delivery compared with women from the control group, except for tetanus ($p=0.064$). Significantly higher antibody concentrations were found in the cord blood of the vaccine group compared with the control group for all antigens, except again for tetanus ($p=0.888$).

Despite a significant decrease in antibody titers between birth and the age of 8 weeks, right before the administration of the first infant vaccine dose, the GMCs to all antigens were still significantly higher in infants from vaccinated mothers compared with infants from unvaccinated mothers (Table 3) at the age of 8 weeks.

At 1 month after the third hexavalent vaccine dose, GMCs to PT ($p<0.001$) (Figure 2) and DT ($p=0.002$) were significantly lower in the vaccine group compared with the control group. However, antibody GMCs for both antigens had risen from week 8 to month 5 (Figure 1). For Prn ($p=0.220$), TT ($p=0.560$) and FHA ($p=0.198$), non-significant differences in GMCs were found in the vaccine group compared to the control group. For these three antigens, a decay in antibody titer from week 8 to month 5 is notified in the vaccine group (Figure 1).

Figure 1 demonstrates the log distribution of the pre-vaccination and post-vaccination (after 3 doses) IgG titers for all vaccine antigens in both the vaccine and control group. There is a significant difference in the distribution of antibodies for all pre-vaccination titers in the vaccine group versus the control group, in favor of the vaccine group. The post-vaccination titers differ significantly between both groups for PT and DT. Figure 2 shows the individual data for PT antibodies only, but expressed as the individual correlation of pre-vaccination and post-vaccination IgG titers for each infant in both groups. Figure 3 shows the GMCs for antibodies to TT, DT, PT, FHA and Prn at all time points in both groups of women and infants.

GMC (95%CI)	Women						Infants					
	Baseline		1 Month after vaccination		At delivery		Cord		Before primary vaccination		1 Month after primary vaccination	
	Vaccine	Control	Vaccine	Control	Vaccine	Control	Vaccine	Control	Vaccine	Control	Vaccine	Control
N	57 (54 for anti-PT)	31	57	0	57 (56 for anti-PT and anti-FHA)	41	58 (57 for anti-PRN)	41	51	26	49	21
Tetanus toxoid, IU/ml												
	1.5 (1.3–1.7)	1 .7 (1.4–2.1)	3 .6 (3.5–3.8)	NA	1.9 (1.6–2.3)	1.5 (1.2–1.7)	2.4 (2.3–2.5)	2.4 (1.9–2.9)	1.9 (1.8–2)	0.8 (0.7–1)	1.7 (1.7–1.8)	1.9 (1.7–2.1)
p-Value	0.212		NA		0.064		0.888		<0.001		0.560	
Diphtheria toxoid, IU/ml												
	0.3 (0.2–0.4)	0.3 (0.2–0.5)	1.4 (1.3–1.7)	NA	1.2 (1–1.5)	0.3 (0.2–0.4)	1.7 (1.5–2.1)	0.3 (0.2–0.5)	0.9 (0.7–1)	0.12 (0.1–0.17)	2.1 (1.9–2.2)	2.6 (2.4–2.9)
p-Value	0.749		NA		<0.001		<0.001		<0.001		0.002	
Pertussis toxin, IU/ml												
	4.5 (3.2–6.4)	7.5 (5–11)	48 (39–59)	NA	31.4 (26–38)	6.4 (4.3–9.6)	100.7 (82–123)	12.4 (8–19)	15.5 (12.1–20)	1.1 (0.7–1.6)	29 (25–35)	54 (42–69)
p-Value	0.078		NA		<0.001		<0.001		<0.001		<0.001	
Filamentous Heammaglutinin, IU/ml												
	21 (17–26)	17.6 (13–24)	211 (170–263)	NA	107 (91–126)	21.4 (16.6–27.5)	140 (109–180)	27.5 (21.5–35)	121 (100–145)	23 (19–27)	65 (56–75)	54 (41–70)
p-Value	0.409		NA		<0.001		<0.001		<0.001		0.198	
Pertactin, IU/ml												
	24 (18–31)	16.9 (11.6–24.6)	622 (511–756)	NA	602 (485.5–747)	18 (13–24)	697 (573–848)	21 (15.5–28)	253 (183–351)	17 (14.5–21)	68 (56–84)	87 (62–121)
p-Value	0.147		NA		<0.001		<0.001		<0.001		0.220	

Table 3: Geometric mean concentrations with 95% confidence interval (CI) for antibodies to tetanus, diphtheria, pertussis toxin, filamentous hemagglutinin, pertactin in both groups of women and infants.

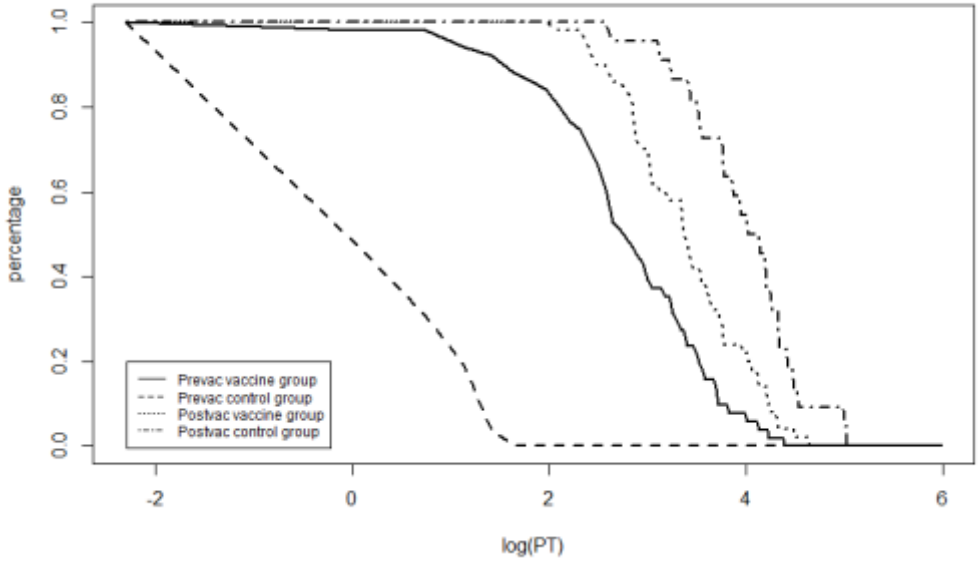


Figure 1A: Comparison of both groups of infants before and after the priming vaccination regarding the antigen specific (log) antibody levels. Anti-PT antibodies.

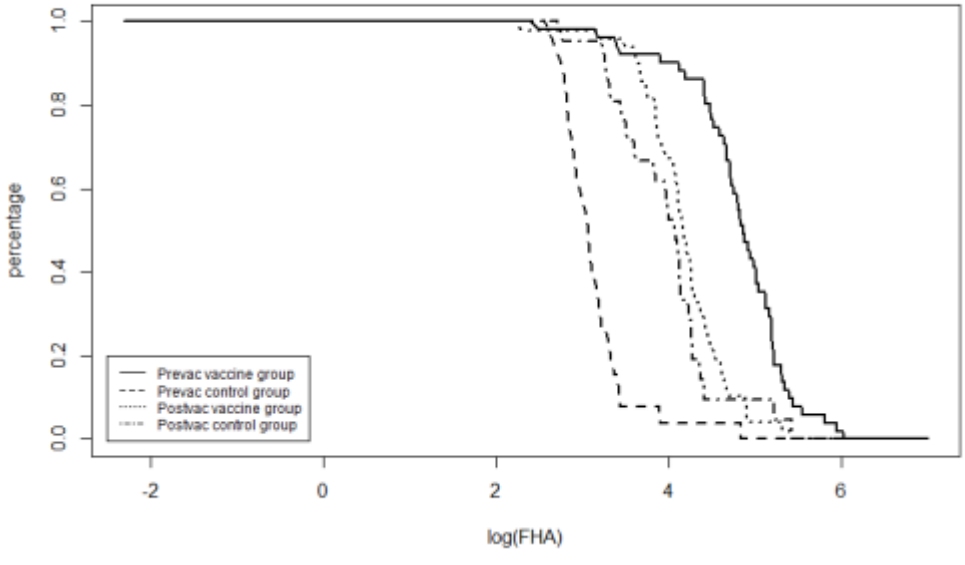


Figure 1B: Comparison of both groups of infants before and after the priming vaccination regarding the antigen specific (log) antibody levels. Anti-FHA antibodies.

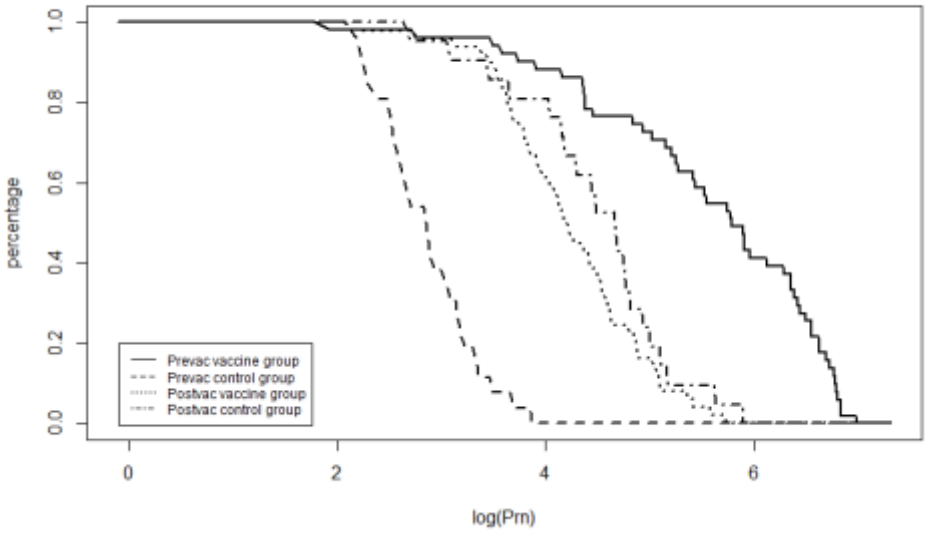


Figure 1C: Comparison of both groups of infants before and after the priming vaccination regarding the antigen specific (log) antibody levels. Anti-Prn antibodies.

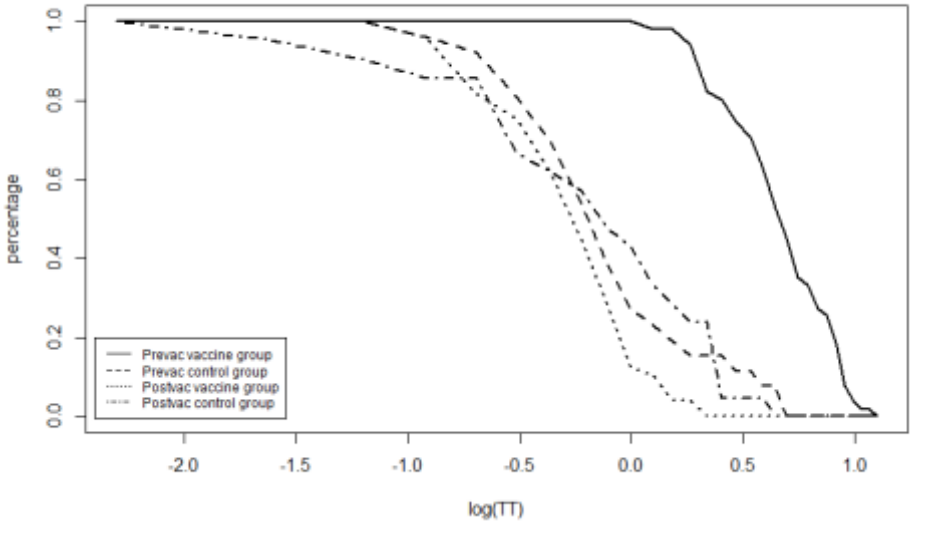


Figure 1D: Comparison of both groups of infants before and after the priming vaccination regarding the antigen specific (log) antibody levels. Anti-TT antibodies.

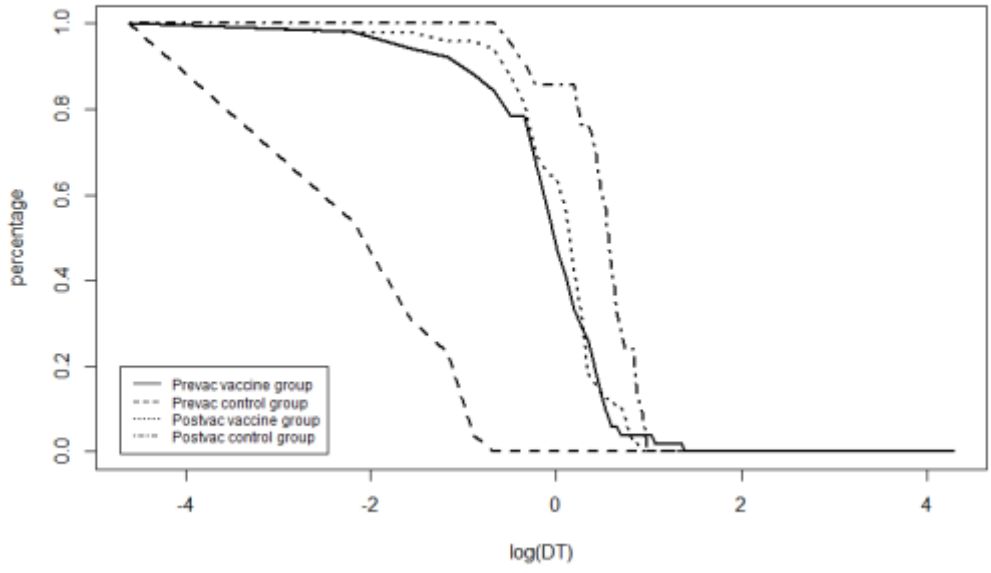


Figure 1E: Comparison of both groups of infants before and after the priming vaccination regarding the antigen specific (log) antibody levels. Anti-DT antibodies.

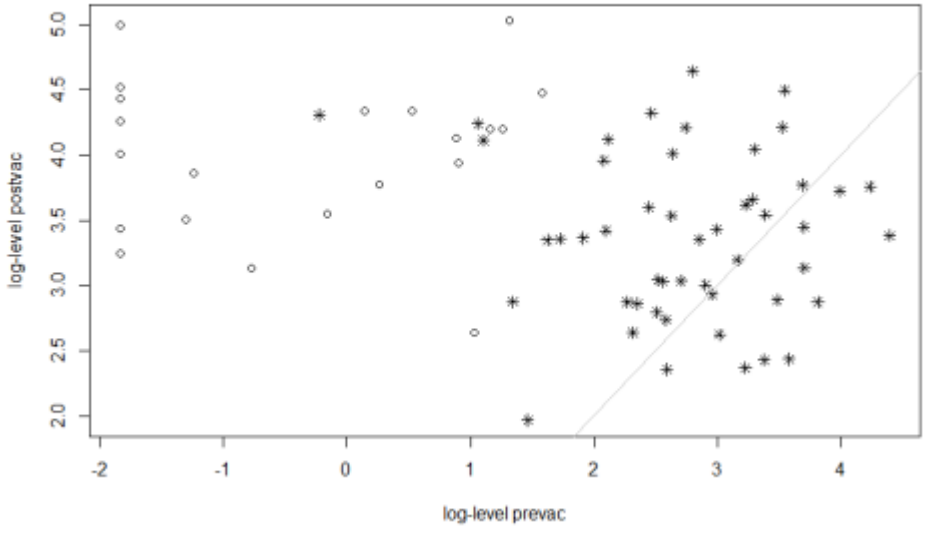
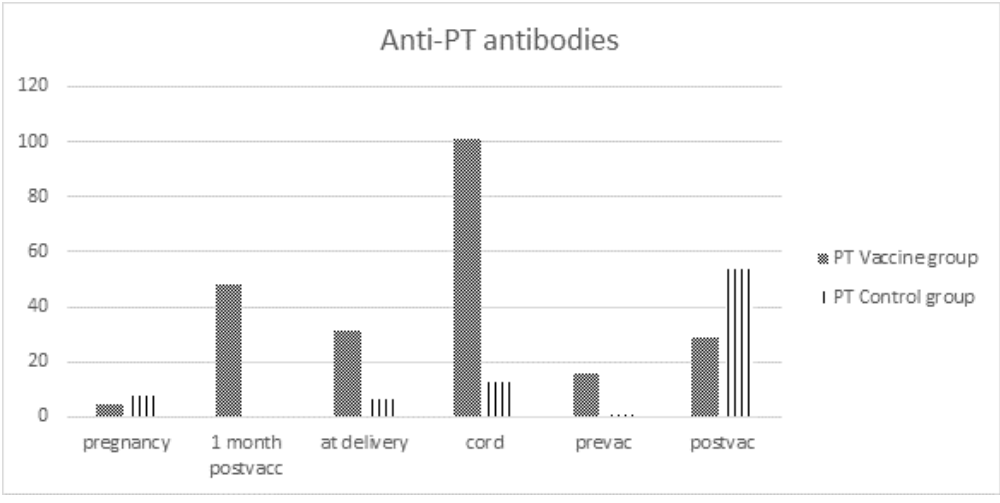
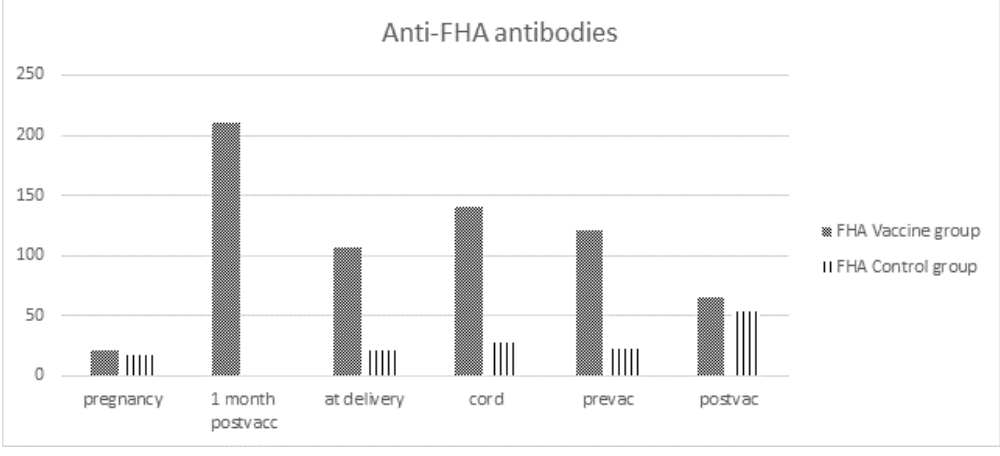
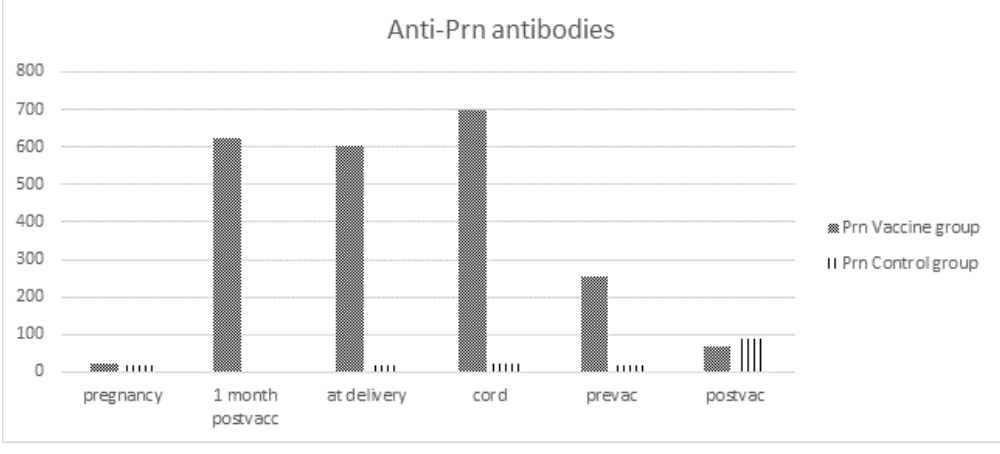


Figure 2: The individual correlation of the anti-PT antibody titers pre- and post-primary vaccination in infants in the control group (dots) and the vaccine group (stars)


Anti-PT antibodies
120
100
80
60
40
20
0
pregnancy
1 month postvacc
at delivery
cord
prevac
postvac
PT Vaccine group
PT Control group


Anti-FHA antibodies
250
200
150
100
50
0
pregnancy
1 month postvacc
at delivery
cord
prevac
postvac
FHA Vaccine group
FHA Control group


Anti-Prn antibodies
800
700
600
500
400
300
200
100
0
pregnancy
1 month postvacc
at delivery
cord
prevac
postvac
Prn Vaccine group
Prn Control group

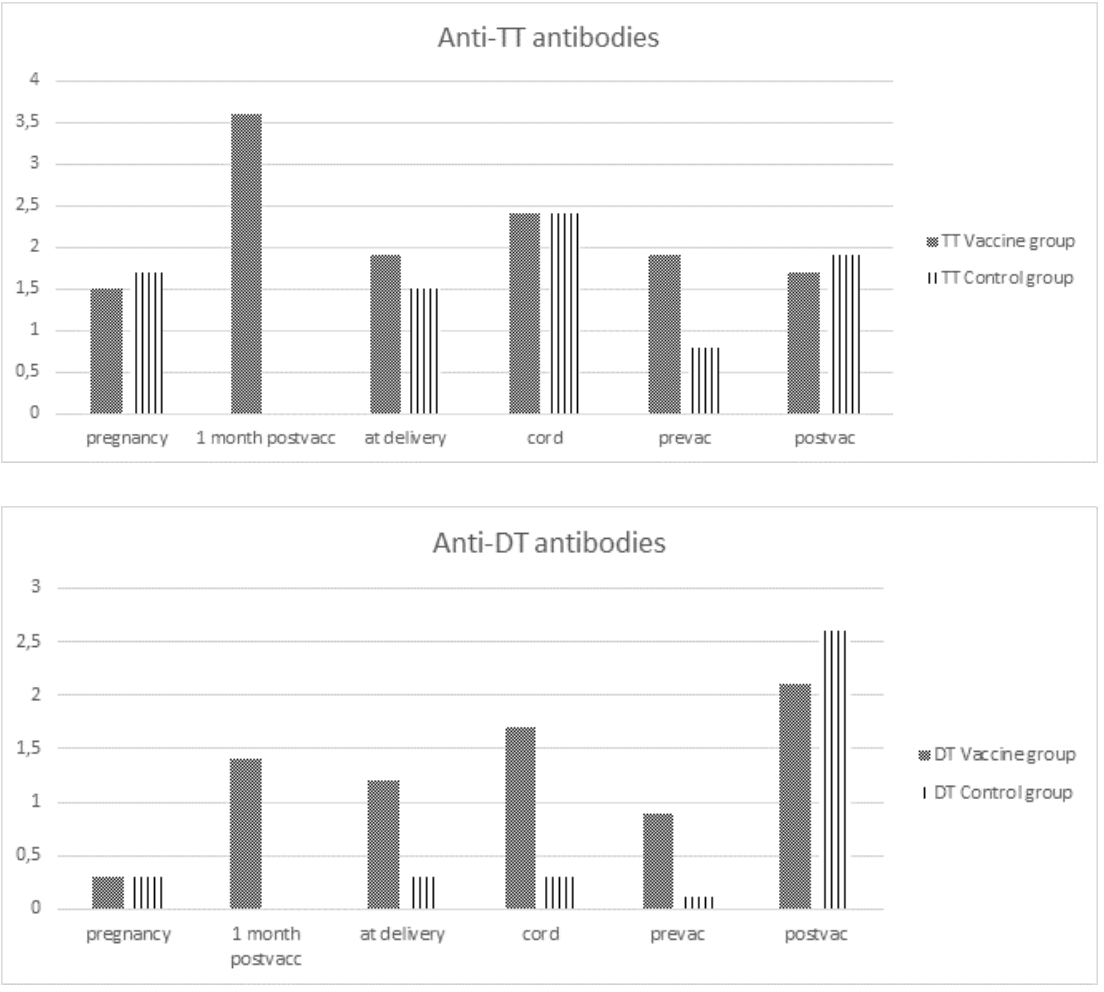


Figure 3: Geometric mean concentrations for antibodies to tetanus (TT), diphtheria (DT), pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (Prn) in both groups of women and infants at all time points.

TRANSPLACENTAL TRANSPORT RATE

No significant difference was observed for the transplacental transport rate (Fetal/Maternal titer) for all three pertussis antibodies between both groups. For TT, a significant lower transplacental transport rate was found in the vaccine group, whereas for DT, a significantly higher transplacental transport rate was found in the vaccine group (Table 4).

	Cord/maternal titer (SD)		
	Vaccine	Control	*p*-Value
Tetanus toxoid	1.17 (0.35)	1.65 (0.42)	<0.001
Diphtheria toxoid	1.42 (0.37)	1.20 (0.40)	0.007
Pertussis toxin	3.47 (1.40)	2.90 (3.52)	0.269
Filamentous haemagglutinin	1.81 (1.99)	1.35 (0.40)	0.096
Pertactin	1.24 (0.38)	1.33 (0.58)	0.322

Table 4: The rate of the cord titer/maternal titer with standard deviation (SD) for tetanus, diphtheria, pertussis toxin, filamentous hemagglutinin and pertactin in both study groups.

RESULTS FROM THE REGRESSION ANALYSIS

We report only the significant influences of all variables included in the random forest analysis. At baseline, a higher parity had a positive effect on the anti-FHA antibody concentration in the control group (p=0.03), and older women had higher anti-Prn GMCs in the control group (p=0.017). A negative influence of the receipt of a tetanus vaccine (TD) within the past 10 years was observed on the anti-FHA antibody concentration (p=0.01) and the anti-Prn antibody concentration (p=0.04) at baseline in the control group.

At delivery, a higher parity had a positive effect on the anti-PT antibody concentration in the women in the vaccine group (p=0.01). A higher gestational age at delivery negatively influenced the anti-PT antibody concentration at delivery in the control group (p=0.01) and the anti-FHA antibody concentration in the vaccine group (p=0.004).

The gestational age at vaccination did not demonstrate an influence on the titer of antibodies in the cord. At both time points in the infants (week 8 and month 5), no significant influence on the antibody concentrations was encountered by any of the variables studied.

Discussion

This study is the first to investigate the effect of the administration of Boostrix® in pregnant women on transmitted maternal antibodies and to assess the immune responses of infants administered acellular pertussis-containing vaccines (Infanrix Hexa®) according to a schedule of 8, 12 and 16 weeks of age. The presented data show that vaccinating during pregnancy closes the susceptibility gap for pertussis infection.

Safety data have been reported from far larger studies [102, 187], showing that pertussis vaccination during pregnancy is safe and well tolerated. The AE reported within this study (73.7% showed mild to moderate injection site pain and swelling) do not differ from the expected side effects described in the Boostrix® summary of product characteristics (SmPC: 23.7-80.6%) [225]. Because we did not use a placebo in the control group, it is not possible to make a comparison of the rate of adverse events between both groups. SAE were encountered in this study. However, the number of reported serious adverse events was small and did not differ from what is expected within the general population [226]. The safety data in the offspring did not demonstrate an unexpected risk pattern; no congenital disorders were detected.

Transplacental transport was effective in both groups for all vaccine antigens. Inter-pathogen specific IgG differences in the effectiveness of this transport have been described before [227], despite a common mechanism for transplacental IgG transport involving the FcRn receptor. Healy et al. reported a lower transplacental transport rate for PT compared with the rate reported in this study [167]. Conversely, for FHA and Prn, similar transport rates were found in both studies. In a randomized controlled trial, Muñoz et al. [102] also reported an effective transplacental transport for PT antibodies, similar to what we report in this study, but less for FHA, Prn, DT and TT. However, a comparison of serological results from various studies should be interpreted with caution because different laboratory techniques are utilized.

At baseline, all IgG GMCs were comparable in both groups. There was an adequate maternal immune response to all vaccine antigens except for tetanus, yet all women were already protected for tetanus at baseline. We showed in another paper [152] that humoral responses to pertussis vaccines in pregnancy are as robust as in non-pregnant women. The RCT performed by Muñoz et al. [102] describes equally high antibody titers post-vaccination as during pregnancy. Again, immune responses in the latter study were measured with other laboratory techniques and the women were vaccinated with another vaccine brand (Adacel®); therefore, titers cannot easily be compared.

At delivery and in cord samples, IgG GMC were still significantly higher in the vaccine group compared with their baseline values for all vaccine antigens. These increased titers persisted until the age of 8 weeks, before the start of the primary vaccination schedule, suggesting a closure of the susceptibility gap in newborns. A decay of maternal antibodies during the first weeks of life has been described before [102, 168], and although there is no known correlate of protection for pertussis, high concentrations of PT, FHA and Prn IgG are associated with protection against (severe) disease [40, 228]. Yet, at week 8, immediately preceding the vaccination, infants of vaccinated women still had significantly higher antibody titers compared with the control group.

Naturally acquired maternal pertussis antibodies have been shown to interfere with humoral responses to wP, yet not to aP vaccines [58, 134, 178, 229-232]. Nevertheless, the recent study by Muñoz et al. showed a trend of blunting by maternal antibodies for FHA ($p<0.01$) after a 3-dose priming schedule [102]; however, this blunting effect disappeared with the booster dose offered during the second year of life, whereas the clinical significance of the interference has not been demonstrated. This result confirmed the finding by Hardy-Fairbanks et al [186]. In the study presented here, the blunting of the infant immune response is also suggested in infants from mothers in the vaccine group for anti-PT antibodies ($p<0.001$). The differences between our study and the Muñoz study could be due to different brands of vaccine used during pregnancy and to other confounders in both populations (e.g. different epidemiological background for pertussis, different vaccination histories etc.). Jones et al. measured the effect of high levels of maternal antibodies on the humoral immune responses to vaccines in infancy in general and found the inhibition of immune responses to tetanus and pneumococcus [233]. The present study does not confirm this blunting effect for tetanus, and pneumococcal antibody titers have not (yet) been analyzed. Mouse models seem to indicate however, that maternal antibodies, and blunting, do not solely have a negative effect on infant immune responses but might enhance the B cell maturation in infants [132]. In a study conducted in parallel in Vietnam, we describe less blunting effect by maternal antibodies. A possible explanation could be that we used different brands of acellular pertussis vaccines in mothers and infants in Vietnam [154], resulting in different antibodies. This difference in interference was already shown in a mouse model experiment (personal communication Camille Locht, Institut Pasteur de Lille).

Abu Raya et al. [218] also used Boostrix® vaccine during pregnancy, as in the present study, whereas Muñoz et al. [102] and Hardy-Fairbanks et al. [186] used Adacel®. A comparison of antibody titers induced by vaccines of distinct manufacturers has never been analyzed in a single pregnant population.

A random forest regression analysis revealed no consistent influence of any factor of the entire study population, except for vaccination during pregnancy. Only isolated significant influences of some variables on one specific time point in one specific group were described, never indicating any plausible relationship. Unlike Abu Raya et al., we could not confirm the influence of gestational age at vaccination on the titer of antibodies encountered in the cord. Our study was not powered for the analysis of this specific influence; considerably larger cohorts are needed to show this effect.

LIMITATIONS OF THE STUDY

Strict randomization was not possible, as explained in the methods section. No significant differences in demographic characteristics were observed between the vaccine and control group, which suggested that the groups of pregnant women were comparable.

Another potential limitation of the study is that the results are unlikely generalizable to countries with different epidemiological profiles as well as other vaccine compositions and vaccination schedules, as the study was only performed in 1 province (Antwerp) in Belgium.

Conducting clinical trials in pregnant women and their offspring is difficult. Recruitment is time consuming and labor intensive. Moreover, it is a challenge to retain both mothers and infants throughout the study period [234]. In our study, there were limited amounts of missing data (12.4%). Therefore, we performed a complete case analysis which assumes that the missingness process was unrelated to the observed and unobserved titer values. We were confronted with a large drop-out rate, especially in the control group, which resulted in wider confidence intervals of the results. And lastly, despite validation of the laboratory results, comparison with other studies and laboratories remains major challenge, as in many other trials.

Conclusion

The pertussis vaccination has been recommended for every pregnant woman during each pregnancy by the Superior Health Council in Belgium since August 2013 and many other countries in Western Europe and North America; the results of this study support these recommendations and provide additional scientific evidence to continue this vaccination strategy. The susceptibility gap for pertussis in the youngest age group, before immunization starts, was closed. Blunting was found for the anti-PT antibody immune response in infants of vaccinated women; however, follow-up of the children until after the booster dose at 15 months of age will shed further light on whether this blunting will persist.

Acknowledgments

The authors would like to thank all participating women and their children. We would also like to thank all participating hospitals for their recruitment assistance and Mrs. Aline Bontenakel for performing the blood sampling in the infants. The excellent technical assistance for the laboratory testing of Caroline Rodeghiero and Christophe Van den Poel is gratefully acknowledged.

Annex 1

INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria for pregnant women were: aged 18-40 years, vaccinated with a pertussis containing vaccine during pregnancy between 18 and 34 weeks of gestation (vaccine group), available for follow-up visits and phone calls through 16 months following delivery and willing to have their infant immunized with hexavalent vaccine according to the Belgian vaccination schedule (at 8, 12 and 16 weeks and at 15 months of age).

In the 18th-32nd week of pregnancy, the women should be considered at low risk for complications as determined by the treating obstetrician and by the following criteria: a/ second trimester ultrasound with no significant abnormalities; b/ pregnancy estimated to be at low risk (<1/300) for Down's syndrome (trisomy 21), trisomy 13 and trisomy 18 tested at 11-13 weeks of gestation. Appropriate screening tests included (any one of the following): a/second trimester screening based on nuchal translucency measurement or pregnancy-associated serum protein A (PAPP-A) and free beta-human chorionic gonadotropin (β-HCG) taking into account maternal age and pregnancy duration; b/ first trimester ultrasound screening and second trimester maternal serum screening test that looks for four specific substances: AFP, total HCG, Estriol and Inhibin-A, with risk estimated using an integrated, sequential, or contingency approach, and taking into account maternal age; or c/ second trimester maternal serum screening test that looks for four specific substances: AFP, total hCG, Estriol and Inhibin-A.

Pregnant subjects who met any of the following exclusion criteria were excluded at baseline: serious underlying medical condition (e.g., immunosuppressive disease or therapy, human immunodeficiency virus (HIV) infection, collagen vascular disease, diabetes mellitus, chronic uncontrolled hypertension, moderate to severe asthma, lung/heart disease, liver/kidney disease, chronic or recurrent infections), significant mental illness (e.g. schizophrenia, psychosis, major depression); history of a febrile illness (temperature higher than or equal to 38° Celsius) within the past 72 hours before vaccination; previous severe reaction to any vaccine; receipt of tetanus-diphtheria toxoid immunization within the past month; receipt of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine (Tdap) in the last 10 years (for the control group); receipt of a vaccine (excluding influenza), blood product (excluding Rhogam) or experimental medicine within the 4 weeks prior to vaccination through 4 weeks following vaccination; deemed high risk for serious obstetrical complications; anything in the opinion of the investigator that would prevent volunteers from completing the study or put the volunteer at risk.

Exclusion criteria for infants were: serious underlying medical condition (e.g., immunosuppressive disease or therapy, human immunodeficiency virus (HIV) infection, collagen vascular disease, diabetes mellitus, chronic hypertension, moderate to severe asthma, lung/heart disease, liver/kidney disease, chronic or recurrent infections); no signed informed consent by both parents; severe reactions to any vaccine; anything in the opinion of the investigator that would prevent volunteers from completing the study or put the volunteer at risk.

Annex 2

RANDOM FOREST ANALYSIS VARIABLES

The following variables were tested for their influence on antibody titers for PT, FHA and Prn in the regression analysis, on several time points:

1. At baseline: age mother at delivery, race mother, tetanus booster previous 10 years, recent contact with pertussis disease, parity and gestational age at vaccination.
2. At delivery: age mother at delivery, race mother, tetanus booster previous 10 years, recent contact with pertussis disease, parity, gestational age at vaccination, gestational age at delivery and titer on previous time points.
3. Cord: age mother at delivery, race mother, tetanus booster previous 10 years, recent contact with pertussis disease, parity, gestational age at vaccination, gestational age at delivery, labor induction, delivery method, epidural anesthesia and titer on previous time points.
4. Infant aged 8 weeks: age mother, race mother, tetanus booster previous 10 years, recent contact with pertussis disease, parity, gestational age at delivery, labor induction, delivery method, epidural anesthesia, nutrition and titer on previous time points.
5. Infant aged 5 months: age mother, race mother, tetanus booster previous 10 years, recent contact with pertussis disease, parity, gestational age at delivery, labor induction delivery method, epidural anesthesia, nutrition, duration of breastfeeding, childcare and titer on previous time points.

CHAPTER 3/B

Vaccine responses in Belgian children after booster vaccination at 15 months of age

This chapter is published: "Maertens K., Caboré R.N., Huygen K., Vermeiren S., Hens N., Van Damme P. Leuridan E. Pertussis vaccination during pregnancy in Belgium: Follow-up of infants until 1 month after the fourth infant pertussis vaccination at 15 months of age. Vaccine. 2016 Jun 30;34(31):3613-3619."

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Abstract

Vaccination of pregnant women with a pertussis containing vaccine is a recommended strategy in some industrialized countries, to protect young infants from severe disease. One of the effects of the presence of high titers of passively acquired maternal antibodies in young infants is blunting of immune responses to infant vaccination. We present infant immune responses to a fourth pertussis containing vaccine dose at 15 months of age, as a follow-up of previously presented data.

In a prospective cohort study, women were either vaccinated with an acellular pertussis vaccine (Boostrix®) during pregnancy (vaccine group) or received no vaccine (control group).

All infants were vaccinated with Infanrix Hexa® according to the standard Belgian vaccination schedule (8/12/16 weeks, 15 months). We report results from blood samples collected before and 1 month after the fourth vaccine dose. Immunoglobulin G (IgG) antibodies against pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (Prn), tetanus toxoid (TT) and diphtheria toxoid (DT) were measured using commercially available ELISA tests. Antibody levels were expressed in International Units per milliliter.

Demographic characteristics were similar in the vaccine and control group. Before the fourth vaccine dose, significantly lower antibody titers were measured in the vaccine group compared to the control group for anti-Prn IgG (p=0.003) and anti-DT IgG (p=0.023), with a steep decay of antibody titers since post-primary vaccination. One month after the fourth dose, antibody titers were only significantly lower in the vaccine group for anti-PT IgG (p=0.006). For all antigens, there was a rise in antibody titer after the fourth vaccine dose.

The present results indicate still a minor blunting effect 1 month after a fourth vaccine dose for anti-PT antibodies. However, a good humoral immune response on all measured antigens was elicited in both groups of children. The clinical significance of such blunting effect is yet unknown.

Introduction

Pertussis, primarily caused by the gram-negative bacteria *Bordetella pertussis*, is a worldwide endemic and epidemic respiratory disease. Despite the successful introduction of global vaccination programs with high immunization rates, pertussis remains an important public health issue [1]. Mainly young infants, too young to be protected by the currently available vaccination schedules, are prone to severe pertussis disease with the highest hospitalization and complication rates among the population [235].

In Belgium, pertussis vaccination with an acellular pertussis containing vaccine (aP) is recommended by the National Immunization Technical Advisory Group (NITAG) at 8, 12 and 16 weeks (primary vaccination). A fourth vaccine dose of an aP containing vaccine is recommended at 15 months of age. Additional booster doses for children and adolescents are equally put in place. Furthermore, maternal pertussis vaccination is recommended since August 2013 for pregnant women during every pregnancy between 24 and 32 weeks of gestation. Finally, adults in close contact with young infants are also advised to receive a booster aP vaccine [43]. Despite these national recommendations, the total number of confirmed pertussis cases increased significantly in Belgium from 243 cases in 2011 [236] to 1501 cases in 2014 [237]. The increase in pertussis cases was most prominent in adults between 40 and 60 years. However, the absolute (total) number of pertussis cases remained the highest in infants below one year of age [237].

As a consequence of the presence of high titers of maternal antibodies after maternal vaccination, a blunting effect of infant immune responses has been observed after the first three doses of an aP containing vaccine [102, 153, 187, 188]. In a recent clinical study, this blunting effect disappeared after a fourth dose of a pertussis containing vaccine administered at the age of 12 months [102]. However, only limited data are available concerning the effect of a fourth infant dose of an aP containing vaccine [102, 186] and data after the administration of a fourth vaccine dose at the age of 15 months are, to our knowledge, lacking. Therefore, the vaccination schedule in Belgium offers the unique opportunity to investigate the effect of high titers of maternal antibodies on the humoral immune responses in infants after a fourth dose of a pertussis containing vaccine at 15 months of age.

We have previously reported on the effect of high titers of maternal antibodies on infant immune responses on the primary infant vaccination schedule at 8, 12 and 16 weeks, after maternal vaccination during pregnancy with the combined tetanus, diphtheria and acellular pertussis (Tdap) vaccine Boostrix® (GSK Biologicals, Rixensart, Belgium). Here we have analyzed possible remaining interference of maternal antibodies with the infant humoral responses after a fourth aP containing vaccine dose administered at 15 months of age.

Methods

A prospective controlled cohort study was conducted in accordance with the Declaration of Helsinki, ICH-GCP and the procedures established by Belgian law. The study was approved by the ethics committee of the University of Antwerp, Belgium (Clinicaltrials.gov identifier: NCT01698346). Informed consent was obtained from both parents of the participating infants. Extended information on material and methods can be found in a previous publication [153].

Children born from healthy women in 5 different hospitals in the province of Antwerp, Belgium, were included in the study and were followed until 1 month after their fourth pertussis containing vaccine dose, administered at 15 months of age. Participating children were included in either a vaccine group, i.e. children born from women vaccinated with an aP containing vaccine (Boostrix®) between 18 and 34 weeks of gestation or a control group, i.e. children born from women not vaccinated with a pertussis containing vaccine for at least 10 years. Women in both study groups did not differ in any underlying characteristics, but randomization was incomplete as explained in the previous publication [153].

For all children, an extended questionnaire on demographics, growth parameters, breastfeeding and immunization data and day-care attendance was completed at every visit.

STUDY VACCINES

All infants were vaccinated with the licensed hexavalent vaccine Infanrix Hexa® (GSK Biologicals, Rixensart, Belgium). Infanrix Hexa® contains 25 Lf of diphtheria toxoid (DT), 10 Lf of tetanus toxoid (TT), 25 mcg pertussis toxoid (PT), 25 mcg filamentous hemagglutinin (FHA) and 8 mcg pertactin (Prn), inactivated poliovirus, hepatitis B surface antigens and *Haemophilus influenzae* type B polysaccharide.

STUDY PROCEDURES

Blood samples were collected from the infants before (1-14 days) and 1 month after the fourth vaccine dose (28-49 days). Infant vaccines were administered in the regular health system at the well-baby clinics, by a general practitioner or by a pediatrician at the age of 15 months. The samples were centrifuged at 2000 rpm within 24 h after blood collection and stored at -20°C.

SAFETY ASSESSMENTS

At each study visit, medical history of diseases in the household, mainly respiratory diseases, was assessed. All serious adverse events in the infants occurring during the study period were recorded. All infants were examined by a medical doctor at 15 or 16 months of age using the "Van Wiechen Developmental test" [238]. This is a Dutch screening test for neurodevelopment used in the general practice to monitor the development of children from birth up to four years of age [239] in a few categories: fine motor activity, adaptive and personal social behavior, communication and gross motor activity (Annex 1).

LABORATORY

All samples were tested with commercially available ELISA kits at the National Institute of Public Health in Brussels, Belgium. The Virion/Serion® kit (ANL, Copenhagen) was used to detect anti-PT IgG antibodies and the Euroimmune® ELISA kit was used to detect anti-FHA and anti-Prn IgG antibodies. Anti-TT and anti-DT IgG antibodies were detected using the Virotech/Sekisui® ELISA kit. Serum samples were tested at a dilution of 1:100. ELISA results were expressed in International Units per milliliter (IU/ml), using respective WHO standards (NIBSC code 06/140 for pertussis, NIBSC code TE-3 for tetanus and NIBSC code 00/496 for diphtheria). For pertussis, these international units are equivalent to the CBER EU units of FDA [219]. The lower limit of detection of the assays was 0.7 IU/ml for PT, 1IU/ml for FHA, 3 IU/ml for Prn, 0.01 IU/ml for TT and 0.03 IU/ml for DT.

An international independent validation was performed to guarantee the reliability of the results at the Canadian Center for Vaccinology in Halifax, Canada [153].

For pertussis, an actual protective antibody threshold (correlate of protection) is not known [38]. For tetanus and diphtheria, the protective antibody level is defined as 0.1 IU/ml for tetanus and 0.01-0.1 IU/ml for diphtheria.

Blunting of the immune response on the fourth vaccine dose among infants was defined by the authors as a lower geometric mean concentration (GMC) of antigen specific IgG antibodies 1 month after the fourth vaccine dose in the vaccine group compared to the control group.

STATISTICS

The initial sample size calculation was performed, based on previous results [168]: a population of 50 subjects in each study arm would be sufficient to detect significant differences in antibody titers at several time points. However, during the conduct of the study, we were confronted with substantial drop-out rates resulting in smaller samples size before and 1 month after the fourth vaccine dose, mainly in the control group.

Antigen specific antibody GMCs and 95% confidence interval (CI) were calculated at each time point in both study groups.

Descriptive analyses were performed to identify possible differences between both study groups. Statistical tests included parametric tests: (paired) t-tests and chi-square tests and their non-parametric alternatives: (paired) Wilcoxon tests and Fisher exact tests whenever the underlying assumptions of the parametric tests were violated, i.e. normality and sparseness assumptions, respectively [220, 221]. Linear regression models were used to identify characteristics that could potentially impact infant antibody titers before and after the administration of a fourth vaccine dose.

Data were assumed to be missing completely at random. The analysis was performed using SPSS statistical software version 23.0 and R.3.1.2. Two-sided p-value <0.05 was considered statistical significant.

Results

GENERAL CHARACTERISTICS OF THE STUDY POPULATION

Characteristics of the mother-infant pairs until 5 months after delivery and exclusion criteria at baseline have been described previously [153]. 55 children (2 twins) were included in the vaccine group and 26 children were included in the control group. Children were born between April 2, 2012 and April 16, 2014. After the primary series of vaccines, 2 additional children from the control group were excluded due to loss to follow-up. In the vaccine group, 4 children were not vaccinated according to protocol for their fourth vaccine dose. As a consequence, these children were excluded for their blood sample 1 month after the fourth vaccine dose.

Blood samples before and 1 month after the fourth vaccine dose were taken between June 24, 2013 and September 29, 2015. No significant differences in demographics were present between the vaccine and the control group (Table 1).

	Vaccine group	Control group	*p*-value
N (included infants)	55	24	
Infant gender, No. (%)			
Male	27 (0.49)	12 (0.50)	0.910
Female	28 (0.51)	12 (0.50)	
Mean weight month 15 in grams (SEM)	10,316.30 (159.75)	10,349.13 (172.00)	0.904
Mean length month 15 in centimeters (SEM)	77.82 (0.43)	79.40 (0.72)	0.067
Mean weight month 16 in grams (SEM)	10,443.18 (157.72)	10,406.30 (173.20)	0.891
Mean length month 16 in centimeters (SEM)	78.12 (0.44)	79.38 (0.66)	0.133
Mean age at blood sample before fourth vaccine dose in months (SEM)	14.93 (0.05)	15.00 (0.10)	0.475
Mean age at blood sample 1 month after fourth vaccine dose in months (SEM)	16.38 (0.07)	16.39 (0.11)	0.949
Mean age at vaccine dose 3 in months (SEM)	4.32 (0.07)	4.67 (0.14)	0.080
Mean age at fourth vaccine dose in months (SEM)	15.32 (0.06)	15.43 (0.14)	0.468
Mean interval between vaccine dose 3–blood sample before fourth vaccine dose in months (SEM)	10.61 (0.09)	10.51 (0.14)	0.242
Mean interval between fourth vaccine dose–blood sample one month after fourth vaccine dose in months (SEM)	1.06 (0.02)	1.05 (0.02)	0.539
Mean interval between blood sample before fourth vaccine dose-fourth vaccine dose in months (SEM)	0.39 (0.06)	0.42 (0.09)	0.704

Table 1: Demographic and clinical characteristics of all study participants before and 1 month after the fourth vaccine dose

SAFETY RESULTS

The clinical history performed at every visit did not identify a pertussis disease case in the infants nor in the households during the entire study period. The proportion of infants hospitalized during the study period did not differ between both study groups: vaccine group 10.9% versus control group 12.5% (p=0.838). The reported reasons for hospitalization were the following: pneumonia at birth (N=1), child suspected of meningitis infection (N=1), rotavirus infection (N=1), removal of birthmark by esthetics surgery (N=1), dehydratation (N=1) and febrile seizures (N=4).

In total, 54 children in the vaccine group and 24 children in the control group were examined using the "Van Wiechen developmental test", as an indication of normal neurological development in three clusters: fine motor development and adaptation and social behavior; communication; gross motor development. There was no significant difference in the age of the examined children between the vaccine and the control group (p=0.629). According to the age category of the infants (15-16 months of age), 11 developmental items in all 3 subcategories were identified for examination. Some significant differences in the infants' development between the vaccine and the control group were identified. Infants in the vaccine group were significantly better developed for 2 items in comparison with infants from the control group, yet these skills were not expected to be present among all infants of that age (Annex 2). In addition, the test has no overall score and is mostly used for referral of infants. Therefore, these results are considered as a very rough interpretation of possible neurodevelopment level of the participating infants. We decided, since there is no cutoff or end score to judge the development of the infants as normal or slow, not to report the results of the test in detail in the paper.

LABORATORY RESULTS

Table 2 provides an overview of the GMCs of IgG antibodies to tetanus, diphtheria and pertussis antigens in the sera of all infants 1 month after the primary vaccination schedule and before and 1 month after the administration of the fourth pertussis containing vaccine dose. The antibody titers for tetanus and diphtheria were above the protective threshold at all time points. After a primary series of 3 doses of a hexavalent aP vaccine administered at 8, 12 and 16 weeks of age, significant lower antibody titers for anti-DT IgG (p=0.002) and anti-PT IgG (p<0.001) were observed in infants from the vaccine group. For anti-TT IgG and anti-FHA IgG, non-significant lower antibody titers were observed in infants from the vaccine group compared to infants from the control group. For anti-Prn IgG however, non-significant higher antibody titers were observed in infants from the vaccine group compared to infants from the control group.

Before the administration of the fourth vaccine dose, GMCs to anti-DT IgG (p=0.023) and anti-Prn IgG (p=0.003) were significantly lower in infants from the vaccine group compared to infants from the control group. For anti-PT IgG and anti-FHA IgG, non-significantly lower antibody concentrations were found in infants from the vaccine group compared to infants from the control group. For anti-TT however, significantly higher antibody concentrations were found in infants from the vaccine group compared to infants from the control group (p=0.007).

One month after the administration of the fourth vaccine dose, GMC to anti-PT IgG (p=0.006) was significantly lower in infants from the vaccine group compared to infants from the control group. For anti-DT and anti-FHA IgG, non-significantly lower antibody concentrations were found in infants from the vaccine group compared to infants from the control group. For anti-TT IgG and anti-Prn IgG, non-significantly higher antibody concentrations were found in infants from the vaccine group compared to infants from the control group. However, for all antigens, there was a rise in antibody concentration after the administration of the fourth vaccine dose at month 15 in both the vaccine group and the control group without significant differences in increase rate between both study groups. Only for anti-Prn IgG, this rate was significantly higher (p=0.001) in the vaccine group compared to the control group.

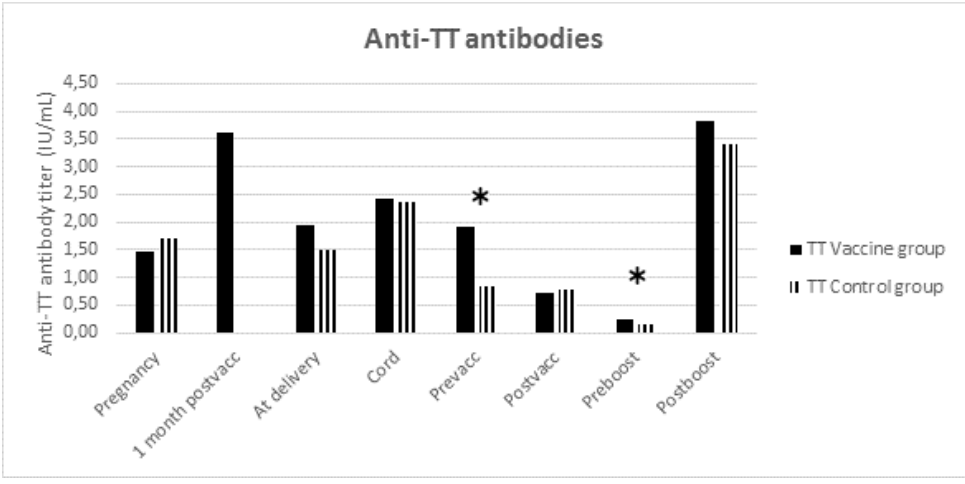
Figure 1 shows the GMCs for antibodies to TT, DT, PT, FHA and Prn at all time points in both study groups, including the data that have been published before [153]. Significant differences are indicated with a star mark. The figure clearly shows the decay of all antibodies in both groups of infants between the post-primary vaccination and the pre-booster sampling time point. The decay was most pronounced for anti-PT IgG antibodies. For anti-PT IgG (p<0.001), anti-DT IgG (p<0.001) and anti-TT IgG (p=0.035), a significant correlation between the post-primary and the pre-booster antibody concentration was found.

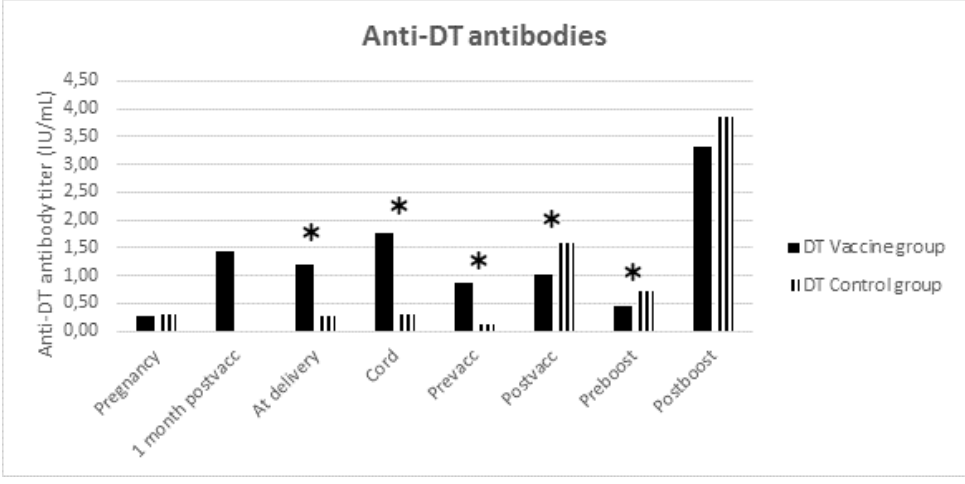
RESULTS FROM THE REGRESSION ANALYSIS

We only report the significant influences of variables on the antibody titers found before and 1 month after the fourth vaccine dose. A significant influence of weight (p=0.01) and length (p=0.001) of the child on the anti-PT antibody titer one month after the fourth vaccine dose was found. Children with a lower weight had lower anti-PT antibody titers one month after the fourth vaccine dose, whereas children with a lower length had higher anti-PT antibody titers one month after the fourth vaccine dose. No other significant influences of variables on antibody titers at the distinct time points were found.

GMC (95% CI)	1 month after primary vaccination		Before fourth vaccine dose		1 month after fourth vaccine dose	
	Vaccine group	Control group	Vaccine group	Control group	Vaccine group	Control group
N	49	21	46	24	45	23 (22 for FHA and Prn)
Tetanus toxoid (IU/mL)	1.75 (1.69–1.82)	1.87 (1.68–2.07)	0.25 (0.21–0.30)	0.15 (0.11–0.21)	3.83 (3.39–4.32)	3.40 (2.67–4.33)
p-value	0.560		0.007		0.394	
Diphtheria toxoid (IU/mL)	2.12 (1.95–2.21)	2.63 (2.48–2.97)	0.45 (0.35–0.58)	0.73 (0.56–0.94)	3.32 (2.94–3.74)	3.85 (3.44–4.31)
p-value	0.002		0.023		0.221	
Pertussis toxin (IU/mL)	29.31 (24.60–34.93)	54.10 (42.36–69.09)	5.44 (4.49–6.58)	7.27 (5.80–9.12)	36.29 (30.93–42.57)	56.60 (42.36–75.65)
p-value	<0.001		0.071		0.006	
Filamentous hemagglutinin (IU/mL)	64.86 (56.03–75.07)	53.73 (41.10–70.23)	14.83 (12.37–17.77)	15.98 (12.43–20.56)	100.86 (84.93–119.77)	139.42 (112.68–172.51)
p-value	0.198		0.636		0.651	
Pertactin (IU/mL)	68.44 (55.85–83.89)	87.05 (62.17–121.89)	4.44 (3.66–5.39)	7.62 (5.67–10.25)	92.73 (67.04–128.25)	81.20 (58.40–112.90)
p-value	0.220		0.003		0.272	

Table 2: Geometric mean concentration (GMC) with 95% confidence interval (CI) for antibodies to TT, DT, PT, FHA, and Prn 1 month after primary vaccination and before and 1 month after the fourth vaccine dose in both groups of infants.


Anti-TT antibodies
Anti-TT antibody titer (IU/mL)
4,50
4,00
3,50
3,00
2,50
2,00
1,50
1,00
0,50
0,00
Pregnancy
1 month postvacc
At delivery
Cord
Prevacc
Postvacc
Preboost
Postboost
TT Vaccine group
TT Control group


Anti-DT antibodies
Anti-DT antibody titer (IU/mL)
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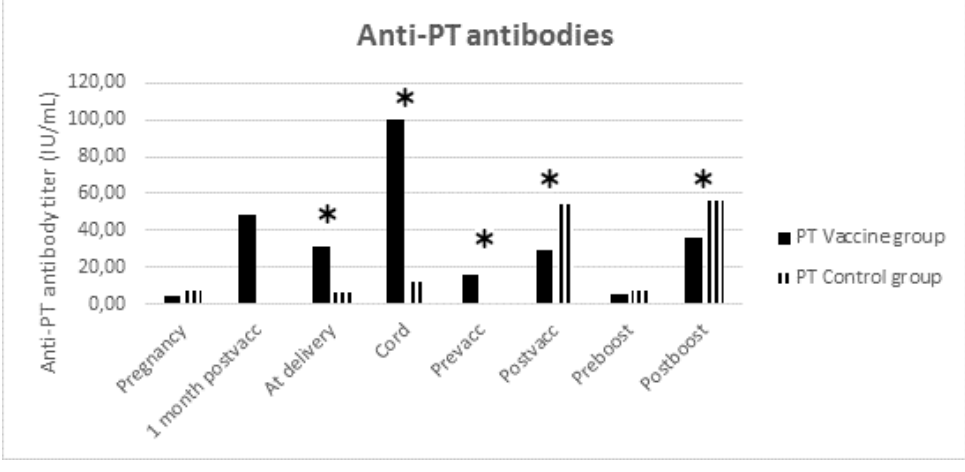
3,50
3,00
2,50
2,00
1,50
1,00
0,50
0,00
Pregnancy
1 month postvacc
At delivery
Cord
Prevacc
Postvacc
Preboost
Postboost
DT Vaccine group
DT Control group


Anti-PT antibodies
Anti-PT antibody titer (IU/mL)
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100,00
80,00
60,00
40,00
20,00
0,00
Pregnancy
1 month postvacc
At delivery
Cord
Prevacc
Postvacc
Preboost
Postboost
PT Vaccine group
PT Control group

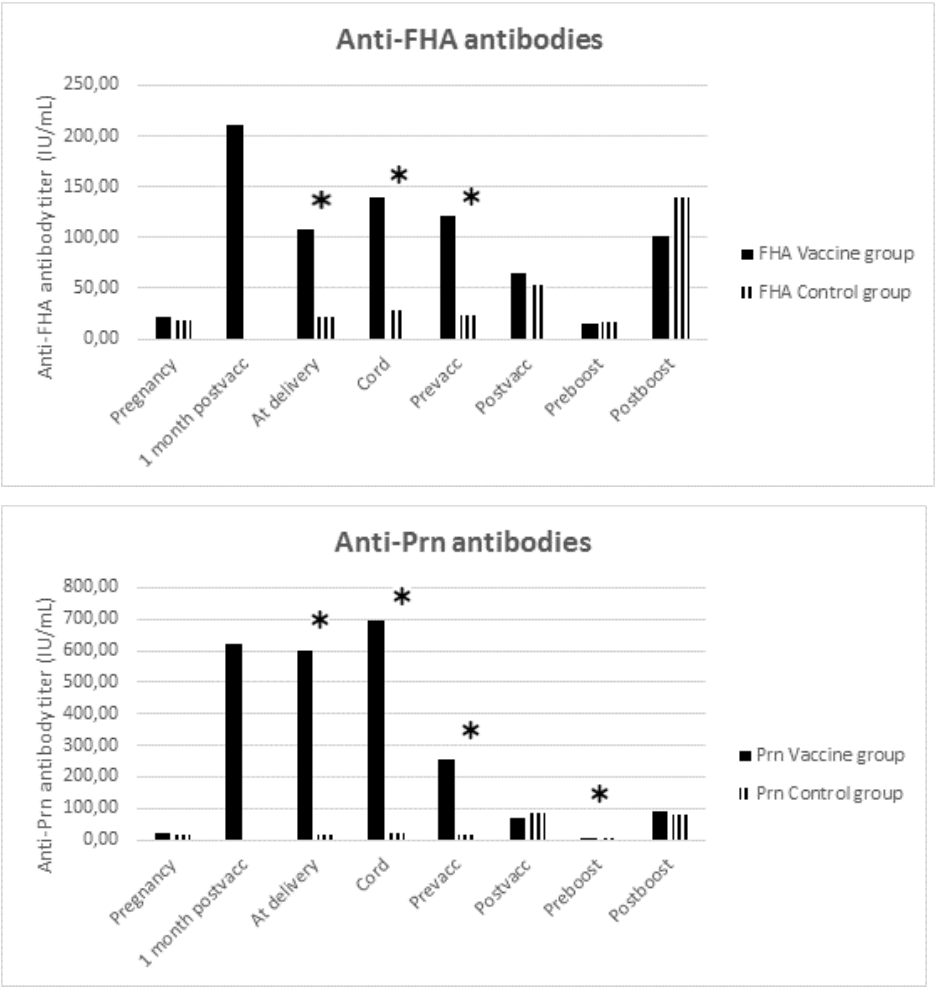

Figure 1: Geometric mean concentration for antibodies to TT, DT, PT, FHA and Prn in both groups of women and infants at all time points. Significant differences are indicated by a star mark.

Discussion

This study is the first to investigate the effect of maternal vaccination with a combined tetanus, diphtheria and acellular pertussis vaccine (Tdap, Boostrix®) on the antibody titers in infants before and after a primary vaccination schedule at 8, 12 and 16 weeks of age and before and after their fourth aP containing vaccine on 15 months of age (Infanrix Hexa®). We previously reported on the significant blunting of the infant immune response for anti-DT and anti-PT antibodies after the primary vaccination schedule [153]. Our new data indicate still a significant blunting effect on the anti-PT antibodies and a minor non-significant blunting effect on the anti-DT and anti-FHA antibodies 1 month after the fourth vaccine dose at 15 months of age. However, a strong immune response with a significant rise in antibody titers for all measured antigens after the fourth vaccine dose was found in both the vaccine and the control group.

Before administration of the fourth infant pertussis vaccine dose at 15 months of age, lower IgG GMCs were found in the vaccine group compared to the control group, except for anti-TT IgG showing significantly higher antibody titers in the vaccine group. Although there is no known correlate of protection for pertussis, high IgG levels directed against PT and Prn are associated with protection against pertussis disease and mainly anti-PT antibodies are considered to be crucial for this protection [39, 40]. For diphtheria and tetanus, antibody concentrations remained above the protective threshold in both groups at all time points. After completing the primary infant vaccination schedule (8-12-16 weeks), we confirmed a rapid decay of vaccine-specific antibodies [240], resulting in relatively low antibody titers at 15 months of age. The differences in antibody titer before and 1 month after the administration of a fourth vaccine dose between the vaccine and control group can be explained by the blunting effect we already observed 1 month after completion of the primary vaccination schedule with, for some antigens, (significantly) lower antibody concentrations in the vaccine group [153].

In a recent study performed by Muñoz et al. [102], blunting of the antibody response after primary vaccination (2-4-6 months) was shown. This effect disappeared after the administration of a fourth vaccine dose at 12 months of age. In a study by Hardy-Fairbanks et al. [186], a slight blunting of the immune response was also seen after primary vaccination. Yet, after administration of a fourth vaccine dose at 12-18 months of age, no notable differences in antibody concentrations were encountered any longer between children from vaccinated and unvaccinated mothers. In the present study, we report a persisting blunting effect on the humoral immune response in infants from the vaccine group for anti-PT antibodies after the administration of a fourth vaccine dose at the age of 15 months. The differences observed between our study and the

Hardy-Fairbanks and Muñoz study could be due to the use of difference brands of vaccines, due to a different timing of the administration of the fourth vaccine dose, or due to other possible confounders between populations (e.g. different demographic composition of the study population, different disease-specific epidemiological background, different vaccination history, etc.).

In addition, the meaning of blunting of the infant immune response is not really understood. A decreased antibody production to vaccination in infants in the presence of maternal antibodies has been described for several pathogens, e.g. tetanus [233], poliovirus [241, 242], hepatitis B [243], pertussis [229, 233], and *H. Influenzae B [233, 244]*. However, this blunting effect is not described when investigating cellular immune responses [245]. Moreover, blunting seemed to diminish [243] or disappear [246] when monitoring antibody production over longer time periods. In one study, infants who showed blunting on their first two polio vaccine doses even tended to have higher antibody titers after the third vaccine dose [241]. Therefore, blunting might not necessarily be a sign of a less effective immunization.

In comparison with available literature on humoral responses to Infanrix Hexa® at the age of 15 months [179, 247], the pertussis specific antibody titers were lower in our study, at both time points in both study groups. Gimenez-Sanchez et al. [247] collected blood samples after a fourth dose of Infanrix Hexa® at 11-15 months of age, concomitantly administered with PCV7 or PCV13. Tichmann et al. [179] collected blood samples both before and after a fourth dose of Infanrix Hexa® at 12-19 months of age. On the other hand, anti-TT and anti-DT IgG antibody titers were higher in our study before and 1 month after the fourth vaccine dose in both study groups compared to refs [247] and [179]. Possible reasons for the difference in reported antibody titers are the use of different laboratory techniques, the use of other time points in the primary vaccination schedule, the different epidemiological background and the lower sample size in our study which is more sensitive to possible outliers.

We did not identify any clinical case of pertussis within our study population. However, the sample size of our study was too small to measure the potential clinical impact of maternal pertussis vaccination on infants up to one month after their fourth vaccine dose. In the UK however, this vaccination strategy was highly effective to protect newborn infants against pertussis [248]. The clinical impact of this vaccination strategy and the consecutive minor blunting effect later in life has not been investigated yet; e.g. possible higher susceptibility at older infant or childhood age because of the blunting effect.

The linear regression identified no persistent influencing factors on the antibody titers in our study population. Only single significant influences of some variables on one specific antigen at one specific time point were found (e.g. weight and length).

LIMITATIONS OF THE STUDY

Our study has some limitations. Firstly, we were not able to perform a strict randomization of the infants in either the vaccine or the control group, as explained in the previous publication on this trial [153]. A second limitation was the high drop-out rate experienced along the study, especially in the control group, resulting in a smaller sample size, larger confidence intervals of the results and lower statistical power. Conducting clinical trials in mother-infant pairs is not evident and retaining them into the study during the entire study period is challenging [234]. Since the study was conducted in one province in Belgium, the study should be repeated in other provinces and countries with a different epidemiological background, a different vaccination schedule and different vaccine compositions, before generalizations can be made. A last limitation of the study was that the "Van Wiechen developmental test" was not performed at the same age in every child, although ages did not differ significantly between both study groups.

Conclusion

Maternal pertussis vaccination has been recommended for every pregnant woman during every pregnancy by the NITAG in Belgium, as is recommended in many other industrialized countries. The results of this study are supportive for these recommendations and provide additional scientific data to continue this already implemented maternal vaccination strategy. Pertussis vaccination during pregnancy closes the susceptibility gap for infection in young unvaccinated infants. Previously, significant blunting of the infant immune response after 3 doses of a pertussis containing vaccine, when vaccination is performed in the presence of high titers of maternal antibodies at a schedule of 8, 12 and 16 weeks of age, has been reported for the anti-PT and anti-DT antibody immune response in infants. After the fourth dose of a pertussis containing vaccine at 15 months of age, we report still a significant blunting effect for anti-PT IgG antibodies. However, a strong humoral immune response was noted in both groups of infants from the vaccine and the control group, with an increase in antibody titer for all vaccine antigens 1 month after the fourth vaccine dose. The clinical significance of the minor blunting effect at 16 months of age is yet unknown.

Acknowledgments

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Annex 1

VAN WIECHEN DEVELOPMENTAL TEST 15-54 months of age

Behavioural state	Social responsiveness	Name:
0 = child is awake	0 = child is co-operative	
1 = child seems tired	1 = child is reserved and needs encouragement	Date of birth:
2 = child is fussy	2 = child is reserved or shy without active resistance	
3 = child cries continuously	3 = child resists and struggles	Gestational age in weeks:
4 = otherwise: describe under 'remarks'	4 = otherwise: describe under 'remarks'	

System of motivation:

1. Always indicate the calender age in the appropriate column, also in case of prematurity
2. Use a new column for each test. The columns after 1 1/2 year can be used for an extra test.
3. Record the result as + or -; if doubt use -.
4. Separately note right and lefted when asked
5. Observe as much as possible; if needed, information on features marked with (M) (Mentioned) may gained from parents; if positive, note M.
6. * Repeat features

	15 mth	1 1/2 yrs		2 yrs	2 1/2 yrs	3yrs	3 1/2 yrs	4 yrs	4 1/2 yrs	Remarks
Age										
Behavioural state										
Social responsiveness										

Fine motor activity, adaptive and personal / social behaviour

	R L	R L	R L	R L	R L	R L	R L	R L	R L	
11. Puts cube in and out a box										
12. Plays "give and take" (M)										

13. Builds tower of 2 cubes		R L	R L	R L	R L	R L	R L	R L	R L	
14. Explores environment (M)										
15. Builds tower of 3 cubes				R L	R L	R L	R L	R L	R L	
16. Imitates others (M)										
17. Builds tower of 6 cubes										
18. Places round form in form-box										
19. Undresses himself (M)										
20. Imitates building a truck										
21. Places 3 forms in form-box										
22. Imitates drawing vertical line										
23. Imitates building a bridge										
24. Places 4 forms in form-box										
25. Puts on own garment (M)										
26. Copies a circle										
27. Holds pencil with fingers										(with R/L hand)
28. Copies a cross										

Communication	15 mth	1 1/2 yrs		2 yrs	2 1/2 yrs	3 yrs	3 1/2 yrs	4 yrs	4 1/2 yrs	
37. Says 2 "sound-words" with comprehension (M)										
38. Understands a few daily-used sentences (M)										
39. Says 3 "words" (M)										
40. Understands 'play' orders (M)										
41. Says "sentences" of 2 words (M)										
42. Points at 6 parts of body of a doll (M)										
43. Refers to self-using "me" or "I" (M)										

44. Points at 5 pictures in the book										
45. Says "sentences" of 3 or more words (M)										
46. Speech is understood by acquaintances (M)										
47. Talks spontaneously about events at home/playgroup (M)										
48. Asks questions about "who", "what", "where" and "how" (M)										
49. Speech is easily understood by examiner										
50. Asks questions about "how much", "when", "why"? (M)										
51. Understands analogies and opposites										

Grove motor activity

66. Crawls, abdomen off the floor (M)										
67. Walks along (M)										
68. Walks alone/walks well alone/walks smoothly										(first time: _____ mth)
69. Throws ball without falling down										
70. Spuats or bends to pick up things										
71. Kicks ball					R L	R L	R L	R L	R L	
72. Can rotate fluently in sitting position										
73. Rides (tri)cycle (M)										
74. Jumps with both feet simultaneously										
75. Can stand on one foot at least 5 seconds									R L	

Annex 1: The Van Wiechen developmental test.

Annex 2

		Vaccine group	Control group	p-value
Number of examined children		54	24	
Age child, No. (%)	15 months	40 (74.1)	19 (79.2)	0.629
	16 months	14 (25.9)	5 (20.8)	
Item 1: Puts cube in and out of a box, No. (%)	One-sided	4 (7.4)	1 (4.2)	0.563
	Two-sided	50 (92.6)	23 (95.8)	
Item 2: Plays "give and take", No. (%)		54 (100.0)	24 (100.0)	NA
Item 3: Builds tower of 2 cubes, No. (%)		33 (68.8)	18 (75.0)	0.582
Item 4: Explores environment, No. (%)		27 (87.1)	15 (68.2)	0.094
Item 5: Says minimal 2 "sound-words" with comprehension, No. (%)		46 (85.2)	23 (95.8)	0.159
Item 6: Understands a few daily-used sentences, No. (%)		54 (100.0)	24 (100.0)	NA
Item 7: Says 3 "words", No. (%)		32 (65.3)	14 (58.3)	0.562
Item 8: Crawls, abdomen off the floor, No. (%)		54 (100.0)	24 (100.0)	NA
Item 9: Walks along (a table…)		51 (98.1)	20 (83.3)	0.019
Item 10: Walks alone/walks well alone/walks smoothly, No. (%)		43 (84.3)	17 (73.9)	0.205
Item 11: Throws ball without falling down, No. (%)		27 (73.0)	9 (42.9)	0.023

Annex 2: Results from the "Van Wiechen developmental test": comparison between vaccine and control group.

CHAPTER 3/C

Vaccine responses in Vietnamese children after primary vaccination

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Abstract

A pertussis vaccination during pregnancy has recently been adopted in several countries to indirectly protect young infants. This study assessed the effect of adding a pertussis component to the tetanus vaccination, in the pregnancy immunization program in Vietnam.

A randomized controlled trial was performed. Pregnant women received either a Tdap (tetanus, diphtheria, acellular pertussis) vaccine or a tetanus only vaccine between 19 and 35 weeks' gestational age. Immunoglobulin G (IgG) against tetanus (TT), diphtheria (DT), pertussis toxin (PT), filamentous hemagglutinin (FHA) and pertactin (Prn) were measured using commercial ELISA tests, at baseline, 1 month after maternal vaccination, at delivery, and in infants from cord blood and before and after the primary series (EPI: month 2-3-4) of a pertussis containing vaccine.

Significantly higher geometric mean concentrations (GMC) were observed for all 3 measured pertussis antigens in the offspring of the Tdap group, up to 2 months of age. One month after completion of the primary infant vaccination schedule, anti-Prn GMC, but not anti-PT and anti-FHA GMCs, was significantly (p=0.006) higher in the control group.

Maternal antibodies induced by vaccination during pregnancy close the susceptibility gap for pertussis in young infants. Limited interference with the infant vaccine responses was observed. Whether this interference effect disappears with the administration of a fourth vaccine dose is further studied.

Introduction

In 2008, the World Health Organization (WHO) estimated that there were 16 million pertussis cases worldwide [35]. Most cases occur in low- and middle- income countries (LMICs) [11], and case-fatality rates (CFR) for infants in developing countries are as high as 4%. Global coverage of the 3 vaccine doses for DTP (Diphtheria, Tetanus, Pertussis) for infants is as high as 86% [249]. Despite universal infant vaccination, the disease has re-emerged in some industrialized countries, resulting in morbidity and mortality in young infants who are not fully vaccinated [30]. Adolescents and young adults are susceptible to pertussis due to waning antibodies after vaccination (for both aP (acellular pertussis) and wP (whole cell pertussis) vaccines) and declining naturally acquired immunity [19, 212]. They represent a source of infection for newborns. It is likely that the shift in ages of those diagnosed with pertussis that has been observed in industrialized countries will eventually be observed in developing countries [250], depending on the vaccine that is used in infancy. The WHO recommends the use of wP vaccines in the Expanded Programme on Immunization (EPI) [251] whenever a 3+1 infant-only schedule is used, but aP vaccines can be chosen when coverage decreases due to wP side effects [35].

Protection against infectious diseases at birth is provided in part by maternal antibodies transferred though the placenta and lactation [160, 170]. IgG pertussis antibodies have a half-life of 6 weeks [165]. The amount of antibodies transmitted depends on the placental function and on the maternal antibody concentration [252]. After pertussis vaccination/infection during childhood, antibody levels decline by childbearing age [253]. Therefore, the amount of anti-pertussis maternal antibodies transmitted is often low. Thus, increasing the load of maternal antibodies by maternal vaccination is, with the currently available vaccines, the way to offer protection to newborns [185, 186, 216, 217]. Some countries recommend pertussis vaccination during pregnancy (including the USA, UK, Belgium, and others). However, no studies were performed in LMICs, where the background epidemiology and vaccination statuses are different.

In Vietnam, pertussis vaccination began in 1985. Prior to that, the incidence of pertussis was up to 84.4/100,000 (1984) [254]. Overall, the reported incidence is now low (2012-2013: 0.1/100,000 to 0.06/100,000 [254]). Nevertheless, between 95 and 108 pertussis cases were recorded in 2011-2013, and over 50% of those cases occurred in infants under one year of age. In 2014, 92 out of 102 pertussis cases were reported in infants aged less than 6 months [255]. Cases are identified and confirmed based on a clinical diagnosis; laboratory confirmation is not obtained because standard laboratory diagnostic equipment is not available at the community level, and hospital diagnosis are not routinely reported. Therefore, underreporting and underdiagnosis is highly probable.

The aim of the present study was to assess the effect of vaccinating pregnant women in Vietnam with an aP vaccine, on the amount of transferred maternal antibodies and the possible interference of the vaccine with humoral immune responses in the infants.

Methods

A randomized controlled study was conducted in accordance with the Helsinki Declaration, Good Clinical Practice (ICH-GCP) and the procedures established by Vietnamese law. Ethical approval was obtained (National Institute of Hygiene and Epidemiology (NIHE), Vietnam No. 05IRB-120412; Ministry of Health (MOH): No. 978/CN-BYT-131112). Written informed consent was obtained from all participants and from both parents of the infants.

A sample size calculation was performed based on previous results [185]. The goal was to vaccinate 50 pregnant women with a combined Tdap (tetanus, diphtheria, acellular pertussis) booster vaccine (Tdap group) between 18 and 36 weeks of pregnancy and 50 pregnant control women with a tetanus only vaccine, as recommended within the EPI (TT group). The study was conducted in three villages of 1 region, Ha Nam province, in Northern Vietnam. Both study groups were present in each village and the same medical doctor (MD) and nurse performed the study visits for both groups. Pregnant women were randomly recruited for either the Tdap group or the TT group during routine preventive visits. Infants were offered pertussis immunization at 2, 3 and 4 months of age using a hexavalent vaccine on fixed days by the same study personnel.

A questionnaire was completed on each woman's general medical history, obstetrical factors, demographics, and general and pertussis-specific vaccination histories. Growth parameters, breastfeeding statuses, day care attendance, immunization data, and medical histories were recorded at each visit. Inclusion and exclusion criteria can be viewed in Annex 1.

STUDY VACCINES

Women in the Tdap group received Adacel® (Sanofi Pasteur, Canada) containing 5 Lf tetanus toxoid (TT), 2 Lf diphtheria toxoid (DT), 2.5 mcg pertussis toxin (PT), 5 mcg filamentous hemagglutinin (FHA), 3 mcg pertactin (Prn) and 5 mcg fimbriae types 2 and 3 (FIM2 and FIM3). The TT group received the monovalent tetanus vaccine TT-VAC (IVAC®, Vietnam) including at least 10 Lf of TT.

Infants received the hexavalent vaccine Infanrix Hexa® (GSK Biologicals, Belgium), containing 10 Lf TT, 25 Lf DT, 25 mcg PT, 25 mcg FHA and 8 mcg Prn plus inactivated poliovirus, hepatitis B surface antigens and *Haemophilus influenzae* type B polysaccharide.

STUDY PROCEDURES

Venous blood was taken from the women immediately preceding vaccination (5 cc) and at 1 month after vaccination (in the Tdap group) (5 cc). At delivery, a maternal blood sample (5 cc) and a cord blood sample (5 cc) were taken. Blood samples from infants were taken at week 8 before the first vaccine dose was administered (2.5 cc) and at 1 month after the third vaccine dose (2.5 cc). All samples were collected at the Commune Health Center and transported to the Ha Nam Preventive Medicine Center on the same day. Samples were centrifuged and stored at -80°C. All samples were monthly sent to the Department of Bacteriology at NIHE.

SAFETY ASSESSMENTS

Systemic reactions were monitored in both groups by a medical doctor for 30 min post-vaccination. Other adverse events were monitored for 30 days post-vaccination through a diary and visits to the local health center. Solicited adverse events included pain at the injection site, swelling, erythema and general symptoms, e.g., myalgia and fever. Serious adverse events (SAE) were recorded throughout each pregnancy. The causality of an adverse event was judged by the investigators based on its temporality, biologic plausibility and the identification of alternative etiologies. Possible congenital abnormalities were monitored in the offspring.

LABORATORY

Anti-PT IgG antibodies were detected using the Virion/Serion® kit (ANL Copenhagen). Anti-FHA and anti-Prn IgG antibodies were detected with the Euroimmune® ELISA kit. Anti-TT and anti-DT IgG antibodies were detected using the Virotech/Sekisui® ELISA. Serum samples were tested in duplicate at a dilution of 1:100 (PT, TT and DT), 1:400 (FHA) and 1:800 (Prn). All titers are expressed in International Units IU/ml, using respective WHO standards (NIBSC 06/140 for pertussis, NIBSC code TE-3 for tetanus and NIBSC 00/496 for diphtheria). The limit of detection was 0.01 IU/ml and 0.03 IU/ml for tetanus and diphtheria, respectively. All analyses were performed at the laboratory of the Scientific Public Health Institute in Brussels (Belgium), except for TT ELISA in the infant samples. An international independent validation of the pertussis toxin results was performed to guarantee the reliability of the results at the Canadian Center for Vaccinology, Halifax. The correlate of protection is 0.1 IU/ml for tetanus and 0.01-0.1 IU/ml for diphtheria. No correlate of protection is known for pertussis [38].

STATISTICS

All statistical analyses were performed with R statistical software. IgG antibodies were expressed as geometric mean concentration (GMC) with their 95% confidence intervals. p-values <0.05 were considered significant.

Statistical tests included parametric tests: (paired) t-tests and chi-square tests and their non-parametric alternatives: (paired) Wilcoxon tests and Fisher Exact tests whenever the underlying assumptions of the parametric tests were violated, i.e. normality and sparseness, respectively [221].

Data were assumed to be missing completely at random and complete case analyses were conducted rendering unbiased estimates though at the cost of a loss of efficiency [222]. The loss of efficiency resulting from excluding observations was limited due to the limited amount of missing data (16.1%).

Assessing the impact of the different mother and child characteristics (Annex 2) on titer values for each of the different time points was done using a regression approach with outcome the log titer values, symmetrizing the response, and consisting of three consecutive steps: (1) variable selection using random forests [223]; (2) backward model selection using multiple linear regression based on AIC; and (3) further model reduction using likelihood ratio tests [221]. This model procedure was used before [224].

Results

GENERAL CHARACTERISTICS

Fifty-two women were vaccinated with Tdap (Adacel®) (Tdap group), of whom 51 received follow-up care; 51 women were vaccinated with a TT vaccine, of which 48 received follow-up care (TT group). Early dropouts were caused by the participants moving.

The mean gestational age at vaccination was 25.8 weeks for the Tdap group and 24.9 weeks for the TT group (p=0.155). The children in the study were born between February 22, 2013 and October 7, 2013. The mean gestational age at delivery was 39.5 weeks in both study groups. The demographic and clinical characteristics of both study groups were comparable (Table 1).

The blood collection at the age of 2 months, before the start of the priming pertussis vaccination schedule, was performed according to protocol. However, the first vaccine dose was administered at a mean age of 3 months, being later than foreseen according to protocol. All 3 priming vaccines were administered with the scheduled 1 month interval and the blood sample post-priming was obtained according to protocol at a mean age of 26-29 days after the third vaccine dose.

			Tdap group	TT group
Women	*N* (women)		52	51
	Mean age at delivery in years (SD)		26.7 (5.3)	26.5 (5.8)
	Level of education, no. (%)	Unknown	1 (1.9)	1 (2.0)
		Secondary school	1 (1.9)	22 (43.1)
		Bachelor	48 (92.3)	23 (45.1)
		Master	2 (3.8)	5 (9.8)
	Race mother, no. (%)	Vietnamese	52 (100)	51 (100)
	Mean gestational age at delivery in weeks (SD)		39.5 (1.6)	39.5 (2.1)
	Primiparity, no. (%)		17 (32.7)	22 (43.1)
	Mean gestational age at vaccination in weeks (SD)		25.8 (2.2)	24.9 (3.0)
	Mean interval between vaccination and delivery in days (SD)		96.3 (16.8)	104.1 (18.9)
	Previous tetanus vaccination, No. (%)	<5 years ago	25 (48.1)	14 (27.5)
		>5 years ago	11 (21.2)	9 (17.6)
		No previous tetanus vaccine	16 (30.8)	28 (54.9)
	Exposure to pertussis disease <10 years ago, no. (%)		0	0
	Mode of delivery, no. (%)	Unknown	1 (1.9)	3 (5.9)
		Vaginal	51 (92.7)	47 (92.2)
		Cesarean	0	1 (2.0)
	Induction of labor, no. (%)		2 (3.8)	1 (2.0)
Infants	*N* (included infants)		51	48
	Infant gender, no. (%)	Male	28 (54.9)	21 (43.8)
		Female	23 (45.1)	27 (56.3)
	Mean weight at birth in gram (SD)		3108.8 (401.7)	3103.1 (350.1)
	Mean length at birth in centimeters (SD)		49.1 (1.7)	49.3 (2.2)
	Mean weight week 8 in gram (SD)		5423.5 (792.6)	5405.4 (764.0)
	Mean length week 8 in centimeters (SD)		57.8 (3.0)	55.7 (7.9)
	Mean weight month 5 in gram (SD)		7084.3 (755.6)	6795.4 (845.1)
	Mean length month 5 in centimeters (SD)		64.9 (3.7)	62.3 (3.2)
	Mean age at vaccine dose 1 in days (SD)		91.4 (31.7)	84.2 (28.1)
	Mean age at vaccine dose 2 in days (SD)		121 (31.8)	116.8 (31.4)
	Mean age at vaccine dose 3 in days (SD)		150.4 (33.1)	148.7 (30.6)
	Mean age at blood sample before primary vaccination in days (SD)		64.6(17.0)	69.9 (23.5)
	Mean age at blood sample 1 month after primary vaccination in days (SD)		180.3 (33.4)	174.6 (28.6)
	Mean interval between vaccine dose 3—blood sample month 5 in days (SD)		29.9 (14.7)	25.9 (12.9)

Table 1: Demographic and clinical characteristics of all study participants.

SAFETY RESULTS

Of the 52 women in the Tdap group, 23 women experienced at least 1 solicited adverse event (AE), with a mean duration of 1.3 days. Of the 51 women who received TT, 22 women presented with at least 1 adverse event with a mean duration of 1.2 days. The most common adverse events were stiffness, swelling and itching at the injection site.

In total, 7 serious adverse events (SAEs) were reported in 6 women. After Tdap vaccination, fever was reported 1 day after vaccination (N=1), and another woman complained of fatigue. Both women were hospitalized and monitored for 3 days in the medical center of Ha Nam. Three episodes of premature contractions were reported: 2 in the Tdap group and 1 in the TT group, all more than 1 month after vaccination. There was one preterm delivery with stillbirth at seven months' gestational age in the TT group (5 weeks following vaccination); no causal information was available. Common symptoms of respiratory and gastrointestinal diseases were recorded in the infants, but these episodes were never serious, never related to vaccination and required no hospitalization.

LABORATORY RESULTS

Table 2 provides an overview of the GMC values per vaccine antigen and per time point.

At baseline, lower GMC values were measured for tetanus in the TT group (p=0.039). After vaccination, all women responded to all antigens included in the vaccines. Women in the Tdap group had significantly higher concentrations for all pertussis and tetanus IgGs (p=0.001). Significantly higher concentrations were observed for all antigens in the cord blood samples in the Tdap group.

At month 2, GMCs to all antigens, except for tetanus, were still significantly higher in the Tdap group. At month 5, the antibody concentration for tetanus (p=0.001) was significantly higher and the antibody concentration for Prn (p=0.006) and DT (p<0.001) were significantly lower in the Tdap group, yet the titers of anti-PT (p=0.198) and anti-FHA (p=0.753) antibodies did not differ significantly between both groups.

Figure 1 displays the log titer distributions for vaccine antigens in infants in both intervention groups before (prevac) and one month after the three infant hexavalent vaccine doses (postvac). The responses to Prn, DT and TT are better and higher in the infants from the TT group. Figure 2 displays the same data for PT antibodies only, but expressed as the individual correlations of pre- and post-vaccination IgG titers for each infant in both groups. Figure 3 shows the antibody titers in women and children comparing both groups at several time points and per antigen.

GMC (95%CI)	Women						Infants					
	Before vaccination		1 month after vaccination		At delivery		Cord		Before primary vaccination		1 month after primary vaccination	
	Tdap group*	TT group**	Tdap group*	TT group**	Tdap group*	TT group**	Tdap group*	TT group**	Tdap group*	TT group**	Tdap group*	TT group**
N	53	51	51	0	49 (51 for anti-PT)	47 (48 for anti-PRN)	49 (50 for anti-PT)	47 (46 for anti-FHA)	45 (51 for anti-TT and anti-dipht)	48 (35 for anti-PRN and anti-FHA and anti-PT)	35 (51 for anti-TT)	35 (49 for anti-TT)
Tetanus toxoid (IU/mL)												
	0.9 (0.7–1.3)	0.54 (0.4–0.8)	3.9 (3.3–4.6)	NA	3.3 (2.9–3.7)	1.6 (1.2–2.6)	2.2 (1.5–3.2)	1.1 (0.6–1.9)	0.36 (0.2–0.6)	0.25 (0.2–0.4)	1.5 (1.3–1.8)	1.0 (0.8–1.2)
p-Value	0.039		NA		0.001		0.046		0.329		0.001	
Diphtheria toxoid (IU/mL)												
	0.03 (0.02–0.05)	0.03 (0.02–0.04)	0.4 (0.2–0.7)	NA	0.2 (0.1–0.4)	0.03 (0.02–0.04)	0.24 (0.1–0.4)	0.05 (0.04–0.07)	0.14 (0.1–0.2)	0.05 (0.04–0.06)	1.96 (1.62–2.3)	2.80 (2.48–3.12)
p-Value	0.545		NA		<0.001		<0.001		<0.001		<0.001	
Pertussis toxin (IU/mL)												
	8.2 (6.4–10.6)	7.9 (4.9–10.4)	33.1 (26–41.8)	NA	17.3 (13–22)	5.7 (4.3–7.6)	21 (16–28)	7.2 (5.6–9.4)	4.2 (2.9–5.9)	0.8 (0.5–1.3)	70 (58–84)	67 (53–84)
p-Value	0.839		NA		<0.001		<0.001		<0.001		0.753	
Filamentous Heammaglutinin (IU/mL)												
	16.7 (15.9–24.6)	19.1 (15.1–24.1)	270 (211–343)	NA	139 (109–176)	17.3 (14–21.4)	93 (65–133)	27.6 (20.9–36.7)	59 (48–73)	23.1 (19.7–27)	77 (66–90)	66.6 (56–78)
p-Value	0.677		NA		<0.001		<0.001		<0.001		0.198	
Pertactin (IU/mL)												
	6.3 (4.6–8.6)	8.9 (6.6–12.1)	229 (166–317)	NA	111 (76–163)	9.4 (6.9–12.5)	124 (86–179)	13.9 (10.5–18.2)	46 (32–66)	7.8 (6.6–9.4)	83 (65–104)	132.6 (104–168)
p-Value	0.114		NA		<0.001		<0.001		<0.001		0.006	

Table 2: Geometric Mean Concentration (GMC) with 95% confidence interval (CI) of IgG antibodies against tetanus, diphtheria, PT, FHA and Prn at all time points. *Tdap group: Women vaccinated with tetanus, diphtheria and pertussis containing vaccine (Adacel®). ** TT group: Women vaccinated with tetanus only vaccine (IVAC®) during pregnancy.

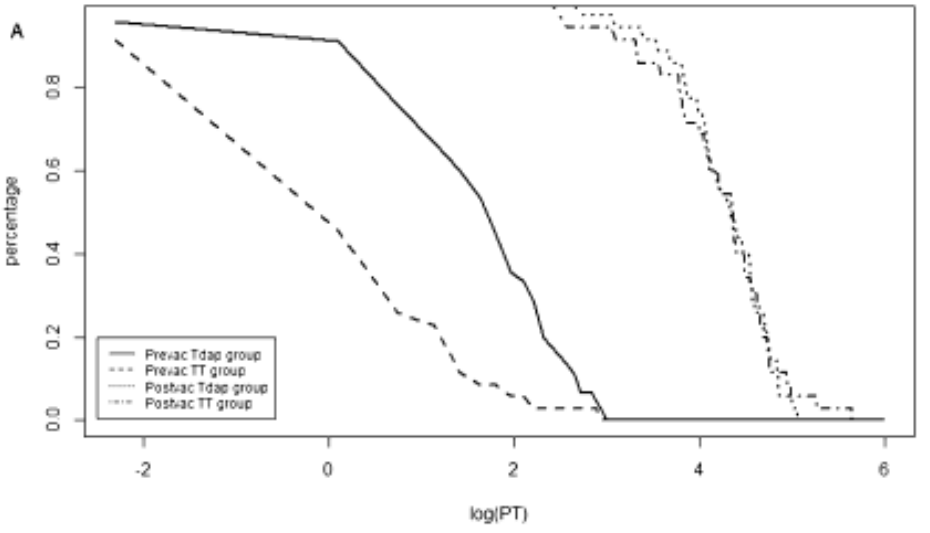


Figure 1A: The comparison of both groups of infants before and after the primary vaccination regarding the antigen specific (log) antibody levels. Anti-PT antibodies.

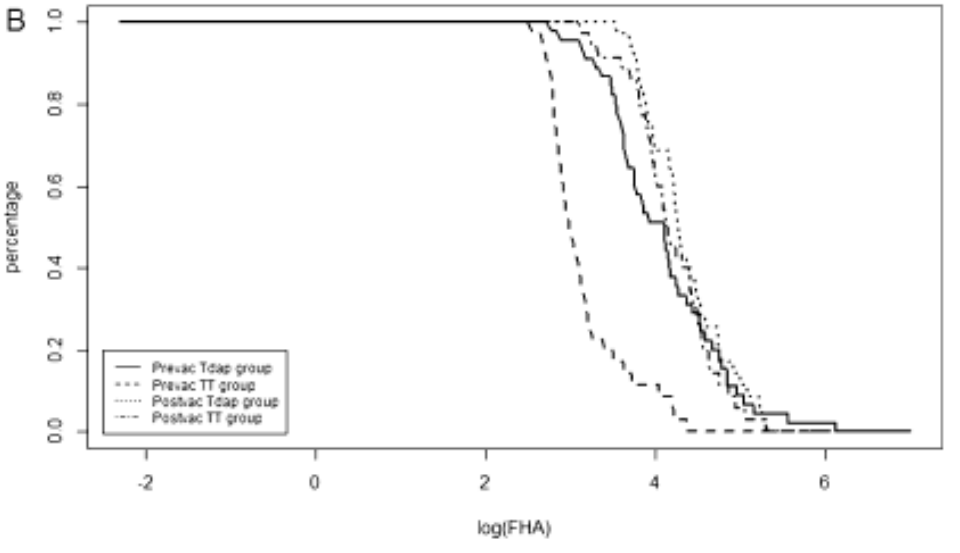


Figure 1B: The comparison of both groups of infants before and after the primary vaccination regarding the antigen specific (log) antibody levels. Anti-FHA antibodies.

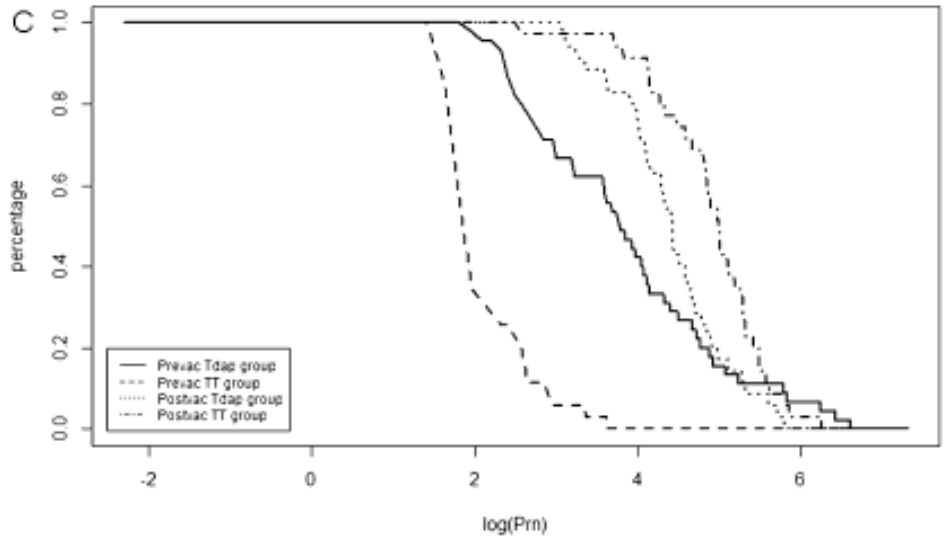


Figure 1C: The comparison of both groups of infants before and after the primary vaccination regarding the antigen specific (log) antibody levels. Anti-Prn antibodies.

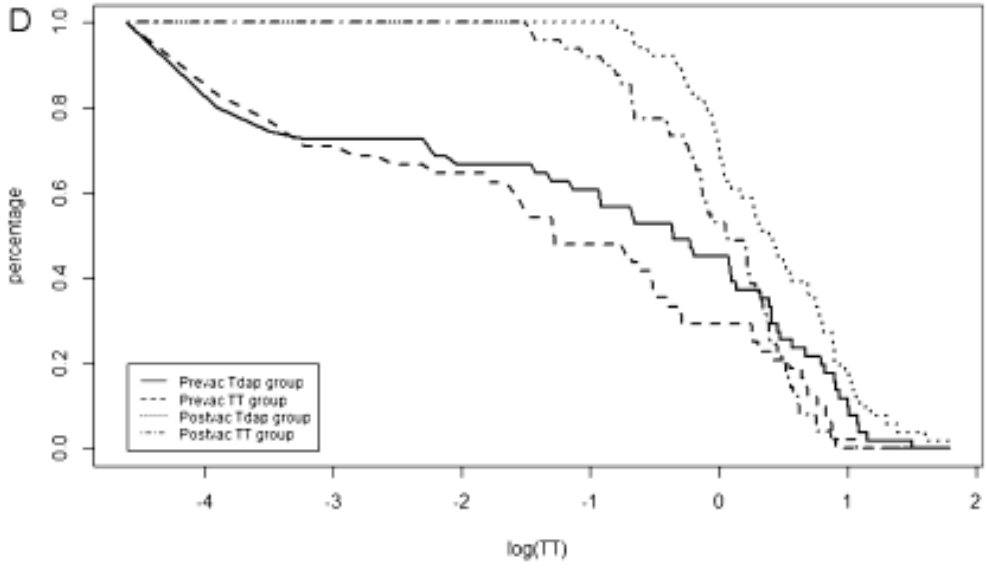


Figure 1D: The comparison of both groups of infants before and after the primary vaccination regarding the antigen specific (log) antibody levels. Anti-TT antibodies.

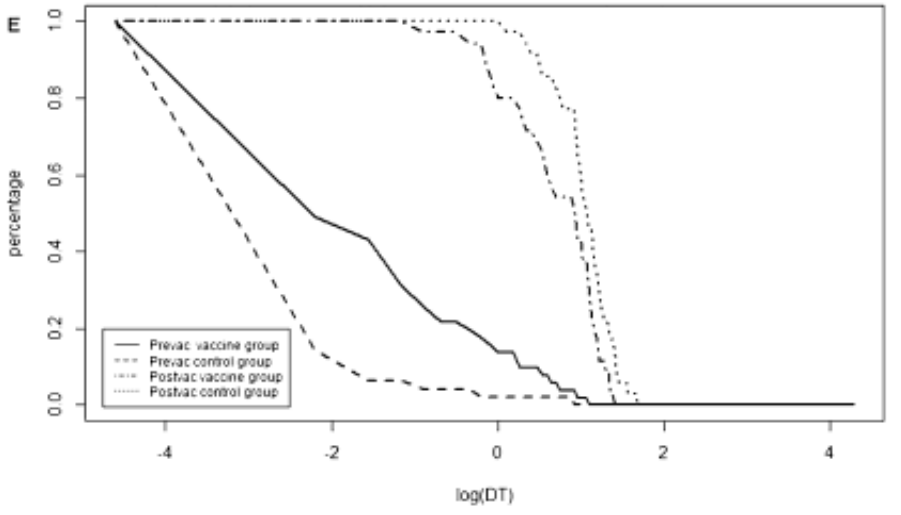


Figure 1E: The comparison of both groups of infants before and after the primary vaccination regarding the antigen specific (log) antibody levels. Anti-DT antibodies.

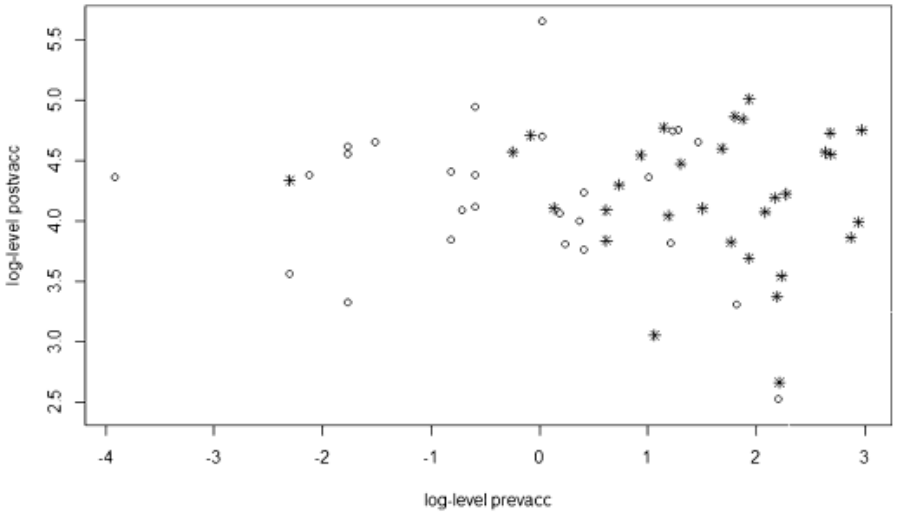
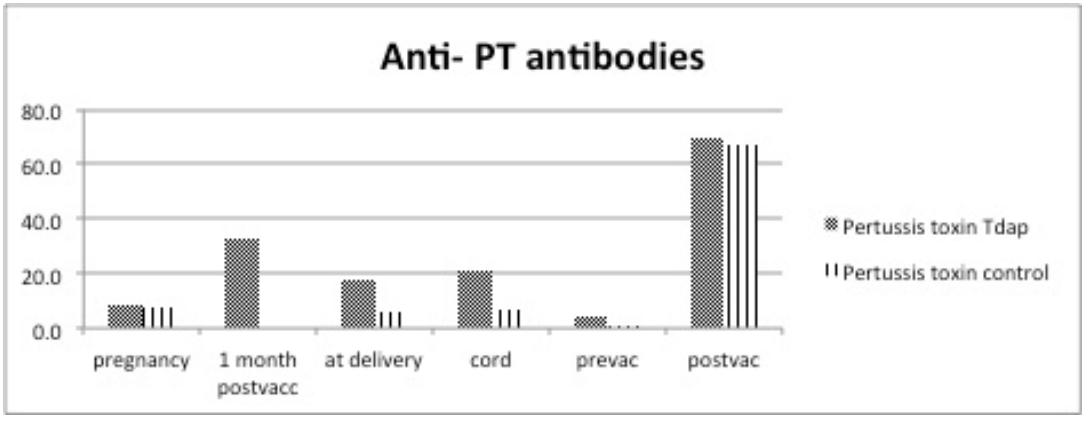
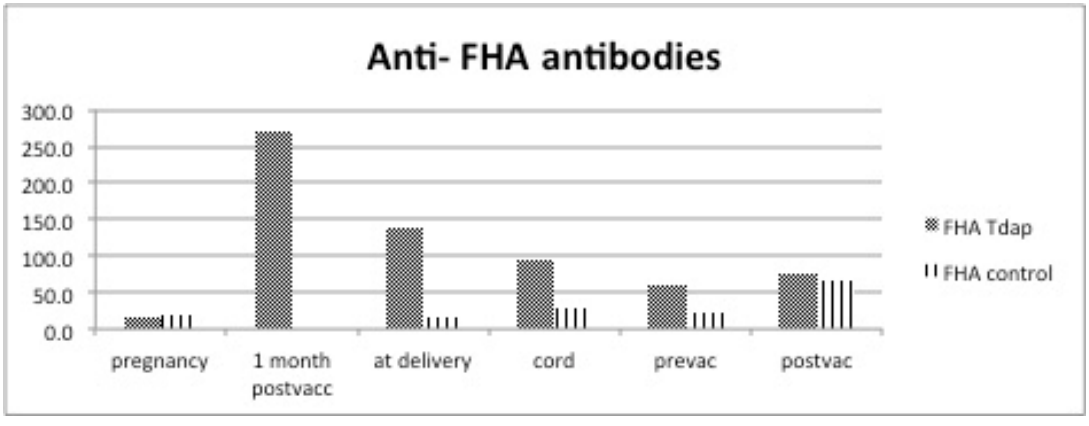
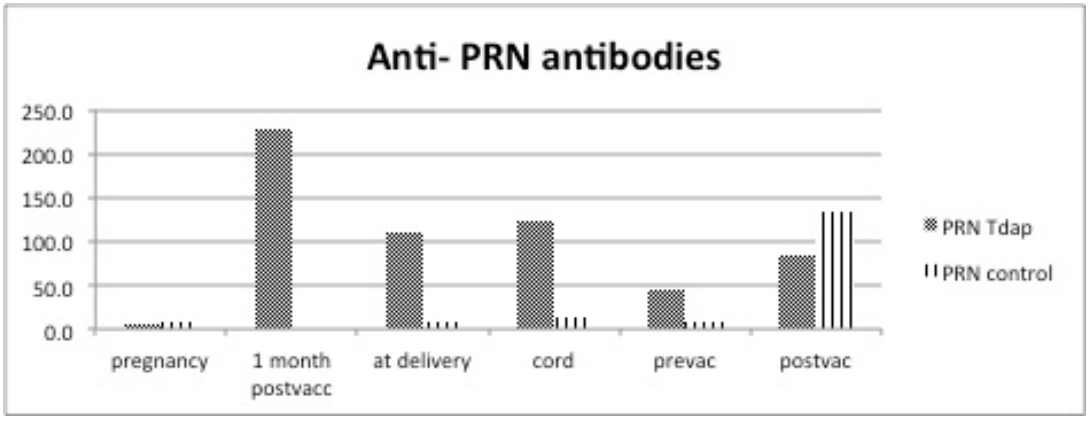


Figure 2: The individual correlation of the anti-PT antibody titers and pre- and post-primary vaccination in infants in the TT group (dots) and the Tdap group (stars).


Anti- PT antibodies
80.0
60.0
40.0
20.0
0.0
pregnancy
1 month postvacc
at delivery
cord
prevac
postvac
Pertussis toxin Tdap
Pertussis toxin control


Anti- FHA antibodies
300.0
250.0
200.0
150.0
100.0
50.0
0.0
pregnancy
1 month postvacc
at delivery
cord
prevac
postvac
FHA Tdap
FHA control


Anti- PRN antibodies
250.0
200.0
150.0
100.0
50.0
0.0
pregnancy
1 month postvacc
at delivery
cord
prevac
postvac
PRN Tdap
PRN control

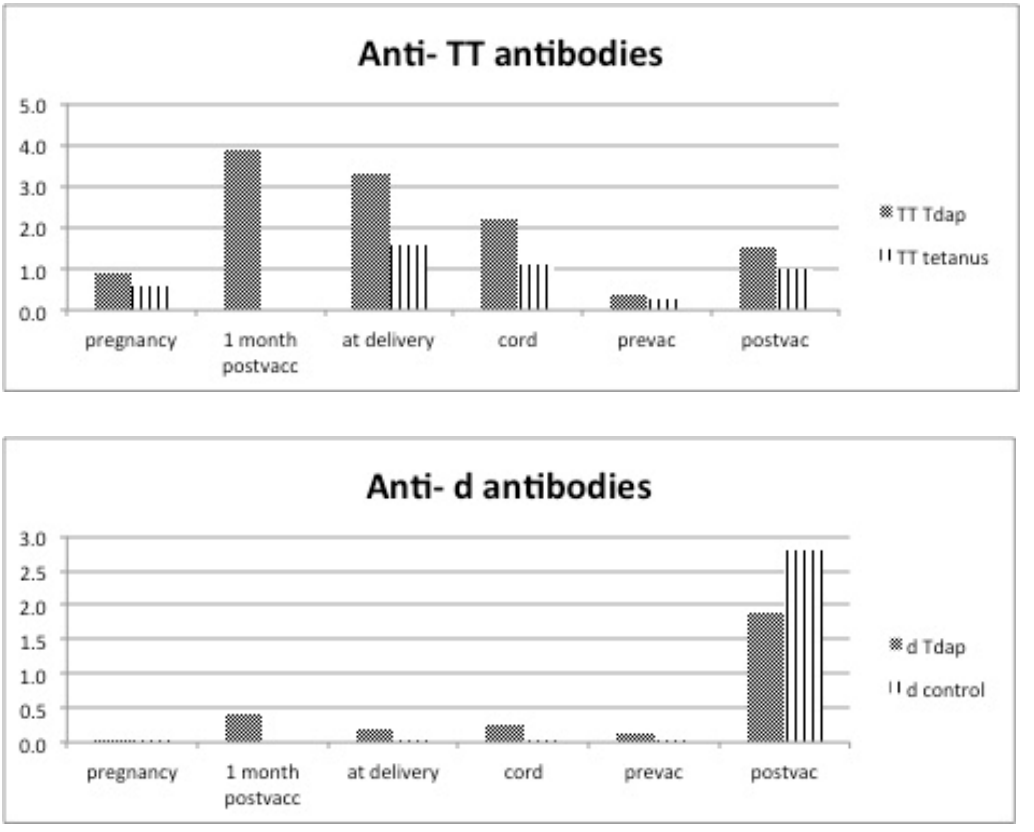


Figure 3: Geometric mean antibody titer per antigen and per time point, comparing both group of women and offspring.

TRANSPLACENTAL TRANSPORT RATE

Table 3 displays the transplacental transport rates for all of the antigens. A significant difference ($p<0.001$) was observed between both groups for FHA antibodies. Different rates were measured depending on the antigen, from 1 (tetanus) to 8 (diphtheria).

	Tdap group	TT group	*p*-Value
Tetanus toxoid IgG	0.95 (0.47)	1.50 (2.31)	0.106
Diphtheria toxoid IgG	8.51 (34.69)	5.05 (14.17)	0.527
Pertussis toxin IgG	1.38 (0.58)	1.73 (1.79)	0.204
Filamentous haemaglutinin IgG	1.04 (0.96)	1.83 (0.89)	<0.001
Pertactin IgG	1.40 (0.96)	1.73 (1.04)	0.110

Table 3: Transplacental transport rate (cord/maternal titer at delivery) for all vaccine antigens in vaccine and control group.

RESULTS FROM THE RANDOM FOREST REGRESSION

At baseline, there was a negative influence of older age of the mother (p=0.020) on the anti-Prn IgG concentrations in the Tdap group. There was also a negative influence of higher parity (p=0.020) on the anti-Prn IgG concentrations in the TT group.

At delivery, there was a negative influence of older age of the mother (p=0.040) on both anti-PT and anti-Prn antibody concentrations in the TT group.

In the infants, no significant influences of any of the included variables on GMCs were observed.

Discussion

This is a controlled, prospective, randomized clinical trial in a LMIC that provides new and important data to the international community. In addition, we report on the use of vaccines from different brands for maternal (Sanofi Pasteur) and infant (GSK Biologicals) vaccination for the first time.

No unexpected adverse events were observed following immunization in the women other than the expected side effects based on the product characteristics (SmPC) of both vaccines [256]. There were no significant differences in safety issues between the Tdap and TT group. Mild to moderate injection site pain and swelling was observed in 45% of all pregnant participants in the Tdap group after vaccination. This proportion was higher than described in the study conducted by Muñoz et al., in which 36.4% experienced local adverse events [102] but it lies within the expected rate of reactions based on the Adacel® SmPC (24.7-65.7%). Safety data in the infants did not reveal unexpected patterns of risk, and no congenital disorders were detected. One woman in the TT group had a stillbirth, but this has no causal obvious relationship with the vaccine that was administered 5 weeks before. Our results add to the evidence that the vaccination of pregnant women with the Tdap vaccine can be considered safe for both the mother and the infant, despite the small study size [187].

At baseline, the GMCs for DT and all of the pertussis antibodies were comparable in both groups. It is unclear why the TT group had lower tetanus antibody titers at baseline. Within the Vietnamese EPI, pregnant women receive a TT vaccine during every pregnancy, at a high coverage rate (89%) [257]. As a consequence, all of the multiparous women (N=62) within the study were supposed to have received a TT vaccine previously. However, there was no statistical influence of parity on the baseline anti-TT GMC; an influence was only observed on the anti-Prn titer, which is hard to explain. All participating women had low antibody titers for pertussis antibodies at baseline. More troublesome was the finding that all women were susceptible to diphtheria at baseline. Within the EPI, 6-16 diphtheria cases are confirmed yearly during the last 5 years, indicating an existing susceptibility for the disease in the Vietnamese population.

Immunization during pregnancy offers early protection to infants through transplacental transport of maternal antibodies [168]. Adequate humoral responses to vaccines in pregnant women versus non-pregnant women have been discussed before [102, 152] and are confirmed by the present study. Post-vaccination titers can hardly be compared between all of the available studies due to the use of different laboratory techniques and vaccines being administered at different gestational ages (20-30 weeks in our study compared to 30-32 weeks in [102]).

At delivery, antibodies against all of the antigens were still significantly higher in the Tdap group. The tetanus antibody titers were higher than the baseline values for both groups, yet these values were significantly lower in the TT group (p=0.001) despite there being a higher tetanus toxoid dosage in the TT-VAC (10Lf) vaccine compared to the Adacel® (5Lf) vaccine and a significantly (p=0.039) higher antibody titer at baseline in the Tdap group. Differences in recent tetanus boosters could not explain this difference. The specific combinations of antigens in the Tdap and TT vaccines might be an advantage, as has been observed before with an improved anti-HBs response after Twinrix® vaccination compared to monovalent hepatitis B vaccine [258].

Relatively high amounts of vaccine-specific antigens were found in the umbilical cords in both study groups, except for the TT IgG in the Tdap group. Rates of individual and group cord/maternal titers did differ among the various disease-specific IgGs, as has been described before [171, 259]. The responsible FcRn receptor is commonly used for all IgG antibodies and transfers preferentially IgG1 subtype antibodies. The differences in the types of IgG antibodies induced by vaccination are known to be influenced by the type of vaccine: IgG2 antibodies are produced after polysaccharide vaccines [227]. However, antibodies to TT, DT and pertussis antigens mostly induce IgG1 antibodies [227].

As expected [102, 169], waning of all maternal antibodies occurred during the first 2 months of life, but the positive effect of Tdap vaccination in pregnancy remained until the starting age of infant vaccination, resulting in a closure of the susceptibility gap for the youngest infants.

The presence of high titers of all maternally acquired pertussis antibodies could possibly interfere with humoral infant immune responses, as was described for wP vaccines [229, 230], but not for aP vaccines [134, 178, 229, 232]. Muñoz et al. [102] observed a trend of blunting for PT and FHA after administration of the third dose of the priming aP schedule in the presence of high titers of maternal antibodies. Fortunately, this blunting effect disappeared with a fourth vaccine dose; the clinical significance of this blunting has not been explained [102]. In the present study, we did not confirm blunting of the anti-PT and anti-FHA antibody response in the presence of high titers of maternal antibodies. A plausible explanation could be that maternal antibodies had declined substantially between the measuring of the titer at 8 weeks postpartum and the administration of the first vaccine, which was delayed to a mean age of 3 months due to field circumstances. Planned mathematical modelling of the antibody kinetics will shed more light on this issue. Another explanation could be that different brands of vaccines are used in mothers and infants, inducing different immune responses to the distinctive composition of antigens in both vaccines, resulting in non-blunting effect in infants, as has been described in a mouse model [260].

An alternative explanation could be that the maternal antibodies induced in Vietnamese women might be of lower affinity, since the Tdap booster during pregnancy could have been a first in a lifetime vaccine dose, if these women never received infant vaccination. Low affinity maternal antibodies [261] would be transferred to the fetus, possibly resulting in less blunting effect.

However, we observed a significantly higher GMC for TT in the Tdap group's offspring at 5 months of age, although both groups had a significant GMC increase compared to their pre-vaccination titers. The infants in the TT group were found to have a statistically significant higher anti-Prn GMC ($p=0.006$) and anti-DT GMC ($p<0.001$) than the infants in the Tdap group, yet there was, again, a significant increase in titers in both groups when compared with the results from week 8. We can conclude that there is a minimal blunting effect for anti-Prn and anti-DT antibodies.

A few significant influences of general variables on GMCs were not consistent with physiologically plausible possibilities (e.g., a negative influence of older age of the mother on the anti-Prn IgG concentration in the Tdap group). At delivery, negative influences of older age of the mother on both anti-PT and anti-Prn antibody concentrations were encountered in the TT group. We have no plausible explanation for this finding.

The present study is the first to describe antibody responses in woman-infant pairs in which the women and infants received aP vaccines produced by different manufacturers. The influence of the use of different antigens in the vaccine formulas on the titers and affinities of the antibodies, as well as the effects on the possible blunting of the infants' immune responses, will be further investigated in a comparative study.

LIMITATIONS

The original aim of the study was to measure the effects of Tdap vaccination during pregnancy on immune responses to wP vaccines in young infants in a LMIC. However, after ethical approval, there were some severe adverse events within the national vaccination program, though not within the present study, occurring after the administration of the pentavalent Quinvaxem® vaccine (Berna Biotech, Korea). The Vietnamese MOH decided to suspend the use of Quinvaxem® for an undetermined period of time. Therefore, Infanrix Hexa® was used in the study based on its availability in the Vietnamese market and the advice of the ethical committee. Despite this unexpected change to the protocol, the population is exemplary for a country in transition, and the strategy proved to be beneficial.

The study is a randomized controlled trial conducted in field conditions. Drop-out rates due to people moving were unforeseen and could not be addressed. Recruiting and retaining both mothers and infants throughout the entire study protocol is not simple, but subject retention and follow-up was reasonable [234]. Another consequence of field conditions, is the delayed start of the first vaccine dose. Pre-priming blood collections were performed according to protocol, but due to the above-mentioned change in the infant vaccine supplies, the first dose was administered at a mean age of 3 months instead of 2 months. Intervals between the three were however respected and the post-priming blood sample was obtained within the obligatory time frame.

Laboratory analyses of the children's samples were performed following training in laboratories in Belgium and Vietnam. All of the samples from the women and the umbilical cords were tested in Belgium; the intention was to analyze the children's samples in Vietnam to avoid transport. However, cross-validation of a subset of the children's samples revealed major differences in the results. Therefore, all samples were transported to and tested in Belgium. We used the leftover samples, which constituted 77% and 72% of the samples from the pre- and post-vaccination time points, respectively, resulting in a limited amount of missing data (16.1%). A complete case analysis was performed with the assumption that the missingness process is unrelated to the observed and unobserved titer values. Cross-validation in a Canadian laboratory indicated good correlation of the data. Despite this validation comparison of laboratory results with other studies and laboratories remains a major challenge, as in many other pertussis vaccination trials.

Conclusion

This study adds to the scientific evidence that pertussis vaccination during pregnancy is safe and can be used as a means to close the susceptibility gap for pertussis among young infants. Further research is needed to assess the effects of high maternal antibody titers on the immune responses of infants to wP vaccines used in LMICs. A comparative study on different brands of pertussis vaccines in pregnancy could shed light on the induction of qualitative and quantitative differences between the induced maternal antibodies.

Acknowledgments

The authors would like to thank all participating women and their children. We thank all collaborators in the health centers for assistance in the recruitment and performing blood sampling. We would like to thank Msc Nguyen Thuy Tram, Msc Pham Thanh Hai, Msc Minh Hoa at the Department of Bacteriology, National Institute of Hygiene and Epidemiology, Hanoi, Vietnam, for their assistance and dedication. The excellent technical assistance for the laboratory testing of Caroline Rodeghiero and Christophe Van den Poel is gratefully acknowledged.

Annex 1

INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria for women were: aged 18-41 years, willing to be vaccinated during pregnancy between 18 and 32 weeks of gestation, intended to be available for follow-up visits and phone call access through 16 months following delivery and willing to have their infant immunized with the hexavalent study vaccine at 8, 12 and 16 weeks at the Commune Health Center.

In the 18th-32nd week of pregnancy, they should be considered at low risk for complications with no significant abnormalities as determined by the obstetrician by second trimester ultrasound. A gynecologist was present at the health care center on all inclusion days. Pregnant women who met any of the following criteria at baseline were excluded from the study: serious underlying medical condition (e.g., immunosuppressive disease or therapy, human immunodeficiency virus (HIV) infection, collagen vascular disease, diabetes mellitus, chronic hypertension, moderate to severe asthma, lung/heart disease, liver/kidney disease, chronic or recurrent infections); significant mental illness (e.g. schizophrenia, psychosis, major depression); history of a febrile illness (temperature greater than or equal to 38° Celsius) within the past 72 hours before injection; previous severe reaction to any vaccine; receipt of tetanus (+ diphtheria) toxoid immunization within the past month; receipt of Tdap immunization in the past 10 years; receipt of a vaccine (excluding influenza), blood product (excluding Rho(D) immunoglobulin) or experimental medicine within the 4 weeks prior to injection through 4 weeks following injection; deemed high risk for serious obstetrical complication; anything in the opinion of the investigator that would prevent volunteers from completing the study or put the volunteer at risk.

Children with following criteria were excluded: serious underlying medical condition (e.g., immunosuppressive disease or therapy, human immunodeficiency virus (HIV) infection, collagen vascular disease, diabetes mellitus, chronic hypertension, moderate to severe asthma, lung/heart disease, liver/kidney disease, chronic or recurrent infections); no signed informed consent by both parents available; severe reactions to any vaccine; anything in the opinion of the investigator that would prevent volunteers from completing the study or put the volunteer at risk.

The date of delivery was estimated based on the first day of the last menstruation. There is no recommendation for a first trimester ultrasound in routine practice in Vietnam.

Annex 2

The following variables were tested for their influence on antibody titers for PT, FHA and Prn in the regression analysis, on several time points:

1) At baseline: age mother at delivery, race mother, parity and gestational age at vaccination
2) At delivery: age mother at delivery, race mother, parity, gestational age at vaccination, gestational age at delivery and titer on previous time points
3) Cord: age mother at delivery, race mother, parity, gestational age at vaccination, gestational age at delivery, tetanus booster previous 10 years, recent contact with pertussis disease, labour induction, epidural anesthesia, delivery method and titer on previous time points
4) Infant aged 8 weeks: age mother at delivery, race mother, parity, gestational age at vaccination, gestational age at delivery, tetanus booster previous 10 years, recent contact with pertussis disease, labour induction, epidural anesthesia, delivery method, nutrition and titer on previous time points
5) Infant aged 5 months: age mother at delivery, race mother, parity, gestational age at vaccination, gestational age at delivery, tetanus booster previous 10 years, recent contact with pertussis disease, labour induction, epidural anesthesia, delivery method, nutrition, duration of breastfeeding, childcare and titer on previous time points

CHAPTER 3/D

Vaccine responses in Vietnamese children after booster vaccination in the second year of life

This chapter is published: "Maertens K., Hoang T.T.H., Nguyen D.T., Caboré R.N., Duong T.H., Huygen K., Hens N., Van Damme P., Duc Anh D., Leuridan E. The effect of maternal pertussis immunization on infant vaccine responses to a booster pertussis containing vaccine in Vietnam. CID 2016 Dec 1; 63(suppl 4) : S197-S204"

doi: 10.1093/cid/ciw551

Abstract

Maternal vaccination with an acellular pertussis (aP) containing vaccine, is a recommended strategy in a growing number of industrialized countries, to protect young infants from disease. Little is known on the effect of this strategy in low- and middle-income countries. Following a previous report on the effect of adding a pertussis and diphtheria component to the tetanus vaccination program in pregnant women in Vietnam, we report on infant immune responses to a booster aP vaccine dose in this randomized, controlled clinical trial.

Thirty infants of Tdap (Tetanus, Diphtheria, aP) vaccinated pregnant women and 37 infants of women vaccinated with a tetanus only vaccine, received a fourth aP containing vaccine dose in the second year of life. Blood was taken 1 month after the fourth vaccine infant dose. Immunoglobulin G (IgG) antibodies against Pertussis Toxin (PT), Filamentous Hemagglutinin (FHA), Pertactin (Prn), Tetanus Toxoid (TT) and Diphtheria Toxoid (DT) were measured using commercially available ELISA tests.

One month after the booster dose, significantly lower antibody titers were measured in the Tdap group for anti-TT IgG ($p<0.001$) only. Anti-DT IgG, anti-PT IgG, anti-Prn IgG and anti-FHA IgG antibody titers were comparable for both groups. A rise in antibody concentrations was elicited for all (except DT) antigens after boosting.

The present results indicate that the blunting by maternal immunization, measured after a primary series of aP vaccines, was resolved with the booster aP vaccine dose. These results add to the evidence for national and international decision makers on maternal immunization as a vaccination strategy for protection of young infants against infectious diseases.

Introduction

In 2014, global coverage of the 3 primary infant DTP (Diphtheria, Tetanus, Pertussis) vaccine doses was as high as 86%. Despite these successful global pertussis vaccination programs, the disease remains an important public health issue, causing an estimated 63,000 deaths in children under five years of age (2013) [1]. Mainly young infants, too young to be protected by the currently available vaccines and vaccination schedules, are prone to severe pertussis disease with the highest hospitalization and complication rates among the population [235].

Vaccination during pregnancy has been implemented in national vaccination programs to elicit high titers of maternal antibodies, as a means to protect young infants from disease [102, 153, 154]. High titers of maternal antibodies induced by maternal vaccination have already been shown to interfere with the infant humoral immune response on primary acellular pertussis (aP) vaccination [102, 153, 154, 157]. This blunting effect ceased after a fourth aP vaccine dose at the age of 12 months in a randomized controlled trial conducted in the United States [102]. Yet few data are available on infant immune responses to a fourth pertussis vaccine dose using different intervals in infant immunization schedules.

In Vietnam, infant pertussis vaccination with whole cell pertussis (wP) vaccines started in 1985. Prior to that, the incidence of pertussis was up to 84.4/100,000 (1984) [254]. Overall, the reported incidence is now relatively low. In 2015, based on clinical criteria, 309 pertussis cases were reported resulting in an incidence of 0.3/100,000 (personal communication National Institute of Hygiene and Epidemiology (NIHE), Vietnam). In the period 2011-2013, over 50% of the cases occurred in infants below one year of age. In 2014, 92 out of 102 pertussis cases were reported in infants aged less than 6 months [255].

The World Health Organization (WHO) recommends the use of wP vaccines within the Expanded Program on Immunization (EPI) [251] whenever a 3+1 infant-only schedule is used. National programs currently administering wP vaccination should continue to use wP vaccines for the primary vaccination schedule. A switch from wP to aP vaccines for primary infant immunization should only be considered when additional boosters or maternal immunization are included in the national immunization schedule [1].

We have previously reported on the effect of high titers of maternal antibodies on the primary infant immune responses to aP infant vaccines in Vietnam, after maternal vaccination during pregnancy with a combined tetanus, diphtheria and aP (Tdap) vaccine Adacel® (Sanofi Pasteur, Canada) [154]. The present paper assesses the possible remaining blunting effect of maternal immunization with the infant humoral immune responses after a fourth aP containing vaccine dose, administered in the second year of life.

Methods

A randomized controlled study was conducted in accordance with the Helsinki Declaration, Good Clinical Practice (ICH-GCP) and the procedures established by Vietnamese law. Ethical approval was obtained (NIHE, Vietnam No. 05IRB-120412; No. IRB-VN1059-02; and Ministry of Health: No. 978/CN-BYT-131112). Written informed consent was signed by all participants and both parents of the infants. Extended information on material and methods has been reported previously.

Participating children were included in either a Tdap group, i.e. children born from women vaccinated with an aP containing vaccine (Adacel®, Sanofi Pasteur, Canada) between 18 and 36 weeks of pregnancy or a TT group, i.e. children born from women vaccinated with a tetanus only vaccine (TT-IVAC, Hanoi, Vietnam) during pregnancy, as recommended within the EPI.

Within the present study, all infants received Infanrix Hexa® (GSK Biologicals, Rixensart, Belgium) for primary vaccination at the age of 2-3-4 months [154]. A fourth Infanrix Hexa® dose was planned to be administered at the age of 18 months. Due to a delay in the approval of the ethics committee, resulting in a suspension of approximately 4 months, some of the infants within this study were already vaccinated with a wP containing vaccine (DTP) within the EPI, whereas most children received Infanrix Hexa® as a booster dose in the second year of life.

From all children in the study, data on health status and growth parameters were collected at the moment of the fourth vaccine dose.

STUDY VACCINES

Infants received either the hexavalent vaccine Infanrix Hexa® (GSK Biologicals, Belgium) or a DTwP (Diphtheria, Tetanus, wP) vaccine (Institute of Vaccine and Biological products (IVAC), Hanoi, Vietnam). Infanrix Hexa® contains 10 Lf Tetanus Toxoid (TT), 25 Lf Diphtheria Toxoid (DT), 25 µg Pertussis Toxin (PT), 25 µg Filamentous Hemagglutinin (FHA) and 8 µg Pertactin (Prn) plus inactivated poliovirus, hepatitis B surface antigens and *Haemophilus influenza* type B polysaccharide. The DTwP vaccine used in the study contains purified diphtheria anatoxin (30 IU), purified tetanus anatoxin (60 IU) and inactivated whole cell pertussis (4 IU) adsorbed by aluminium phosphate.

STUDY PROCEDURES

All infant vaccines were administered at the Commune Health Center (CHC) during the second year of life. Blood samples were collected from the infants 1 month after the fourth vaccine dose. All blood samples were collected at the CHC and transported to the Ha Nam Preventive Medicine Center on the same day. Samples were centrifuged and stored at -80°C. All samples were monthly sent to the department of Bacteriology at NIHE.

LABORATORY

All frozen samples were transported to the Scientific Institute of Public Health in Brussels, Belgium and tested with commercially available ELISA kits. The Virion/Serion® kit (ANL; Copenhagen) was used to detect anti-PT IgG antibodies and the Euroimmune® ELISA kit was used to detect anti-FHA and anti-Prn IgG antibodies. Anti-TT and anti-DT antibodies were detected using the Virotech/Sekisui® ELISA kit. Serum samples were tested at a dilution of 1:100. ELISA results were expressed in International Units per Milliliter (IU/ml), using respective WHO standards (NIBSC code 06/140 for pertussis, NIBSC code TE-3 for tetanus and NIBSC code 00/496 for diphtheria). For pertussis, these international units are equivalent to the CBER EU units of FDA [219]. The lower limit of detection of the assays was 0.7 IU/ml for PT, 1 IU/ml for FHA, 3 IU/ml for Prn, 0.01 IU/ml for TT and 0.03 IU/ml for DT.

To guarantee the reliability of the results, an international independent validation was performed at the Canadian Center for Vaccinology in Halifax, Canada [153-155].

For pertussis, a protective threshold of antibodies (correlate of protection) is not known [38]. However, low antibody concentrations are correlated with susceptibility to pertussis infection [39, 262]. For tetanus and diphtheria, the correlate of protection is defined as 0.1 IU/ml for tetanus and 0.01-0.1 IU/ml for diphtheria.

In this paper, blunting of the immune response after the fourth vaccine dose among infants was defined by the authors similarly to a previous publication [154] as a significantly lower geometric mean concentration (GMC) of specific IgG antibodies, measured 1 month after the fourth vaccine dose in the Tdap group compared to the TT (control) group.

STATISTICS

The initial sample size calculation was based on previous results [168]: a population of 50 subjects in each study arm would be sufficient to detect significant differences in antibody titers if IgG in cord and newborns. No additional sample size calculation has been performed, due to a lack of data for the post-booster time point at the conception of the study. The original aim was to vaccinate all infants with an aP containing vaccine for their fourth vaccine dose. Due to unforeseen circumstances, some children were vaccinated with a wP containing vaccine resulting in a smaller number of aP vaccinated infants in both study groups, mainly in the Tdap group. Therefore, the study might be underpowered because of these unforeseen circumstances.

Disease specific antibody geometric mean concentrations (GMCs) and 95% confidence intervals (CI) were calculated at each time point in both study groups. Descriptive analysis were performed to identify possible differences between both study groups. Statistical tests included parametric tests: (paired) t-tests and chi-square tests and their non-parametric alternatives: (paired) Wilcoxon tests and Fisher exact tests whenever the underlying assumptions of the parametric tests were violated, i.e. normality and sparseness assumptions, respectively [220, 221]. Linear regression models were used to identify characteristics that could potentially impact infant antibody titers 1 month after the administration of a fourth vaccine dose.

The analysis was performed using SPSS statistical software version 23.0. A two-sided p-value <0.05 was considered statistical significant.

Results

GENERAL CHARACTERISTICS OF THE STUDY POPULATION

Characteristics of the mother-infant pairs until 5 months after delivery as well as exclusion criteria at baseline have been described in a previous publication. Children were born between February 22th, 2013 and October 7th, 2013. After birth, 51 children were included in the Tdap group and 48 children in the TT group. After the primary series of 3 aP containing vaccines, 15 children from the Tdap group and 4 children from the TT group were vaccinated 'not according to protocol' with a wP vaccine as a fourth vaccine dose. Those results are reported in a separate paragraph. Due to loss to follow-up, 6 additional children from the Tdap group and 7 additional children from the TT group were excluded from the study. In the end, 30 infants were included in the Tdap group and 37 infants in the TT group.

Infants were vaccinated with a fourth aP containing vaccine (Infanrix Hexa®) between April 4th, 2015 and May 10th, 2015 at a mean age of 22.18 months (range: 18.5-24.7 months). All children were in good health at the moment of vaccination. Blood samples were taken on average 30.2 days (range: 30-33 days) after the fourth vaccine dose between May 7th, 2015 and June 10th, 2015.

Comparing demographics between children from the Tdap group and children from the TT group, a significantly smaller interval between vaccine dose 3 and vaccine dose 4 was found in the TT group (p=0.010) (Table 1).

Characteristic	Tdap Group	TT Group	*P* Value
No. (included infants)	30	37	
Infant sex, No. (%)			
Male	20 (66.7)	21 (56.8)	.458
Female	10 (33.3)	16 (43.2)	
Mean weight, kg (SEM)	10.75 (0.20)	10.59 (0.18)	.562
Mean length, cm (SEM)	83.20 (0.59)	81.71 (0.58)	.078
Mean age at vaccine dose 4, mo (SEM)	22.18 (0.27)	21.44 (0.29)	.071
Mean age at blood sample 1 mo after fourth vaccine dose, mo (SEM)	23.18 (0.27)	22.43 (0.29)	.069
Mean interval between vaccine dose 4 and blood sample 1 mo after fourth vaccine dose, mo (SEM)	0.99 (0.01)	0.98 (0.00)	.175
Mean interval between vaccine dose 3 and vaccine dose 4, mo (SEM)	17.44 (0.17)	16.58 (0.26)	.010

Abbreviations: SEM, standard error of the mean; Tdap, tetanus, diphtheria, and acellular pertussis vaccine; TT, tetanus toxoid.

Table 1: Demographic characteristics of infants at booster vaccination.

The clinical history of the participants did not identify any pertussis disease case in the infants nor in the households during the entire study period.

LABORATORY RESULTS

Table 2 provides an overview of the GMCs of IgG antibodies to TT, DT and three pertussis specific antigens in the sera of all infants at delivery, before the start of the primary pertussis vaccination, 1 month after the primary pertussis vaccination and 1 month after administration of the fourth aP containing vaccine dose during the second year of life.

One month after a primary series of 3 doses of the hexavalent aP vaccine, significantly lower antibody titers were observed in infants from the Tdap group compared to infants from the TT group for anti-Prn IgG (GMC 83 (CI 65-104) versus 132 (CI 104-168); p=0.006) and anti-DT IgG (GMC 1.96 (CI 1.62-2.30) versus 2.80 (CI 2.48-3.12); p<0.001) antibodies. For anti-TT IgG, anti-PT IgG and anti-FHA IgG, higher antibody titers were reported in infants from the Tdap group compared to infants from the TT group [154].

One month after the administration of the fourth aP containing vaccine, GMCs to anti-TT IgG (GMC 2.7 (CI 2.4-3.1) versus 4.2 (CI 3.7-4.7); p<0.001) were significantly lower in infants from the Tdap group compared to infants from the TT group. For anti-DT IgG, anti-PT IgG, anti-FHA IgG and anti-Prn IgG, comparable but lower antibody titers were found in infants from the Tdap group compared to infants from the TT group.

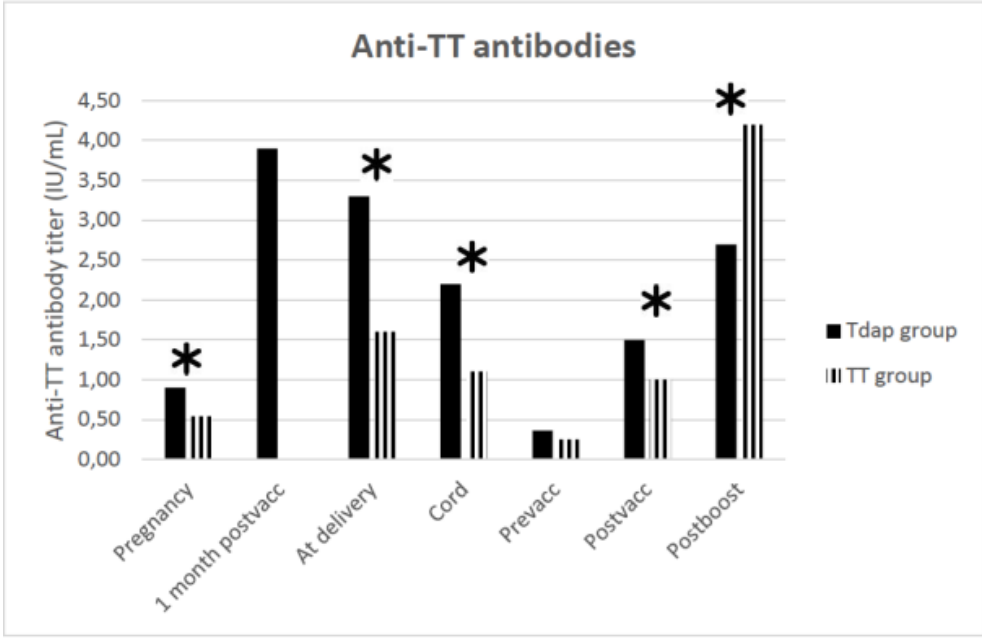
Figure 1 shows the GMCs for antibodies to TT, DT, PT, FHA and Prn at all time points in both study groups, including the data that have been published before [154]. For all antigens, except for anti-TT IgG, a significant correlation was found between the antibody titers one month after the primary vaccination and 1 month after the fourth vaccine dose.

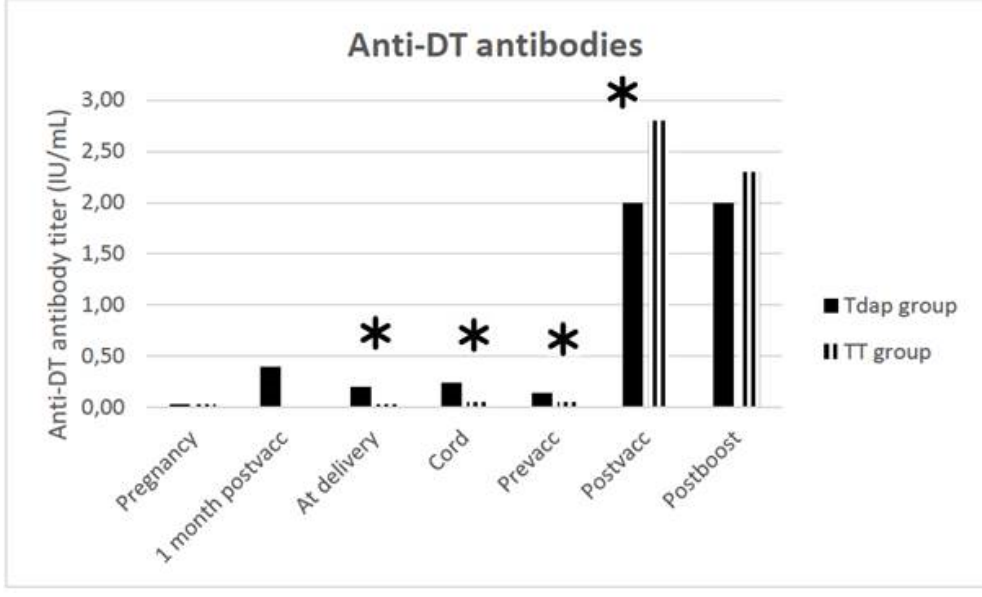
Antigen Included in the Infant Vaccine	Cord		Before Primary Vaccination		1 mo After Primary Vaccination		1 mo After Fourth Vaccine Dose	
	Tdap Group	TT Group	Tdap Group	TT Group	Tdap Group	TT Group	Tdap Group	TT Group
No. of samples	49 (50 for anti-PT)	47 (46 for anti-FHA)	45 (51 for anti-TT and anti-DT)	48 (35 for anti-Prn and anti-FHA and anti-PT)	35 (51 for anti-TT)	35 (49 for anti-TT)	30	37
Tetanus toxoid, IU/mL	2.2 (1.5–3.2)	1.1 (.6–1.9)	0.36 (.2–.6)	0.25 (.2–.4)	1.5 (1.3–1.8)	1.0 (.8–1.2)	2.7 (2.4–3.1)	4.2 (3.7–4.7)
P value	.046		.329		.001		<.001	
Diphtheria toxoid, IU/mL	0.24 (.1–.4)	0.05 (.04–.07)	0.14 (.1–.2)	0.05 (.04–.06)	1.96 (1.62–2.3)	2.80 (2.48–3.12)	2.0 (1.6–2.4)	2.3 (2.1–2.6)
P value	<.001		<.001		<.001		.187	
Pertussis toxin, IU/mL	21 (16–28)	7.2 (5.6–9.4)	4.2 (2.9–5.9)	0.8 (.5–1.3)	70 (58–84)	67 (53–84)	129.0 (97.5–170.7)	133.7 (106.6–167.6)
P value	<.001		<.001		.753		.845	
Filamentous hemagglutinin, IU/mL	93 (65–133)	27.6 (20.9–36.7)	59 (48–73)	23.1 (19.7–27)	77 (66–90)	66.6 (56–78)	161.3 (134.1–193.9)	181.7 (160.3–206.0)
P value	<.001		<.001		.198		.285	
Pertactin, IU/mL	124 (86–179)	13.9 (10.5–18.2)	46 (32–66)	7.8 (6.6–9.4)	83 (65–104)	132.6 (104–168)	159.0 (141.2–179.0)	187.1 (163.8–213.6)
P value	<.001		<.001		.006		.085	

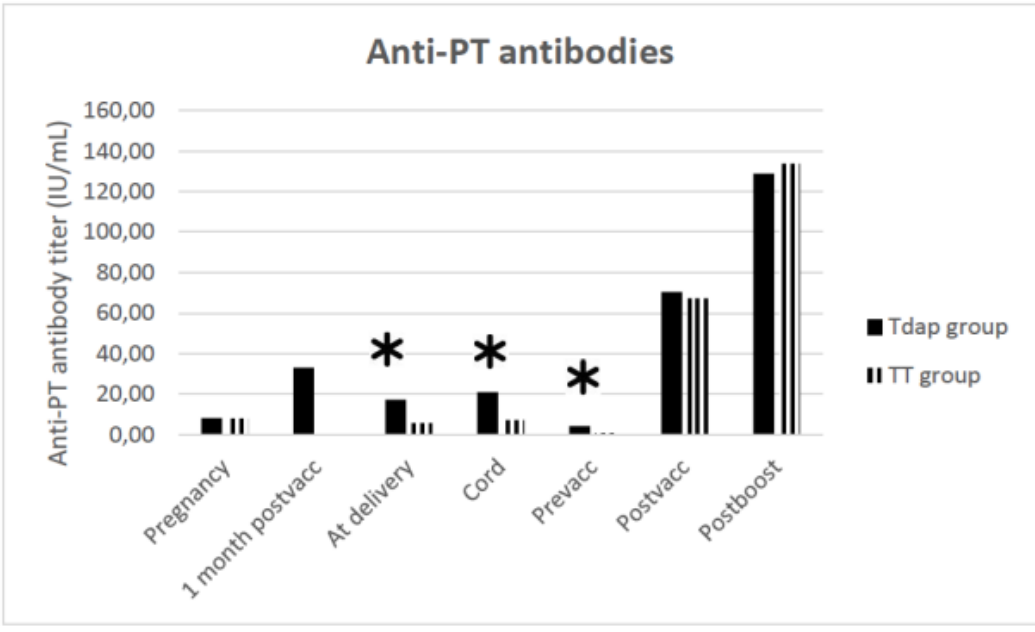
Data are presented as geometric mean concentration (95% confidence interval) unless otherwise indicated.

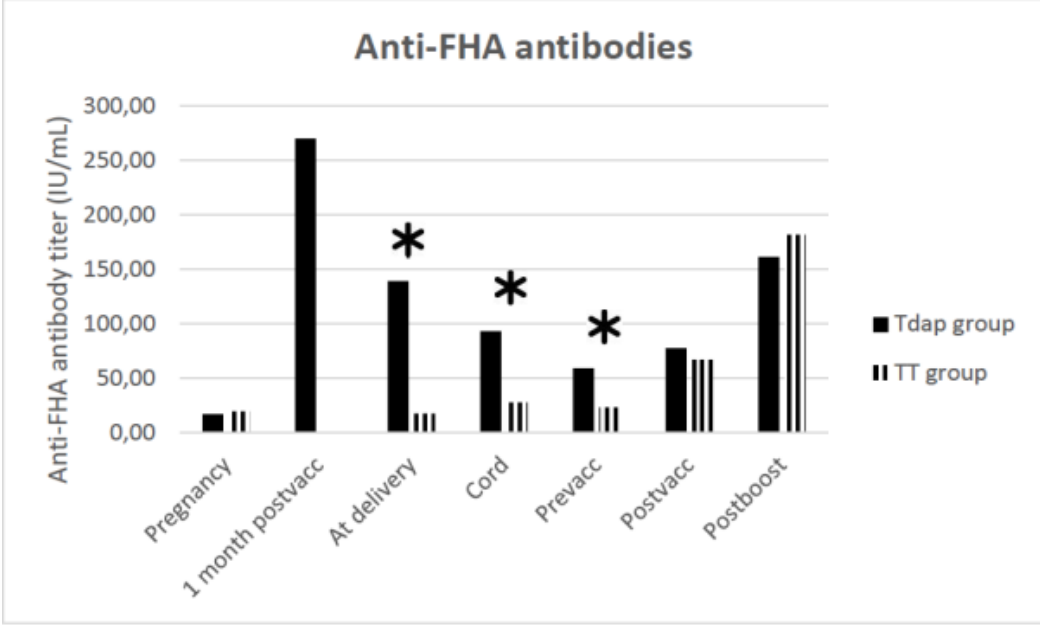
Abbreviations: DT, diphtheria toxoid; FHA, filamentous hemagglutinin; Prn, pertactin; PT, pertussis toxin; Tdap, tetanus, diphtheria, and acellular pertussis vaccine; TT, tetanus toxoid.

Table 2: Antibodies to Tetanus Toxoid, Diphtheria Toxoid, Pertussis Toxin, Filamentous Hemagglutinin, and Pertactin at delivery, before primary vaccination, 1 month after primary vaccination and 1 month after the fourth Acellular Pertussis-containing vaccine dose in both groups of infants.


Anti-TT antibodies
Anti-TT antibody titer (IU/mL)
4,50
4,00
3,50
3,00
2,50
2,00
1,50
1,00
0,50
0,00
Pregnancy
1 month postvacc
At delivery
Cord
Prevacc
Postvacc
Postboost
Tdap group
TT group


Anti-DT antibodies
Anti-DT antibody titer (IU/mL)
3,00
2,50
2,00
1,50
1,00
0,50
0,00
Pregnancy
1 month postvacc
At delivery
Cord
Prevacc
Postvacc
Postboost
Tdap group
TT group


Anti-PT antibodies
Anti-PT antibody titer (IU/mL)
160,00
140,00
120,00
100,00
80,00
60,00
40,00
20,00
0,00
Pregnancy
1 month postvacc
At delivery
Cord
Prevacc
Postvacc
Postboost
Tdap group
TT group


Anti-FHA antibodies
Anti-FHA antibody titer (IU/mL)
300,00
250,00
200,00
150,00
100,00
50,00
0,00
Pregnancy
1 month postvacc
At delivery
Cord
Prevacc
Postvacc
Postboost
Tdap group
TT group

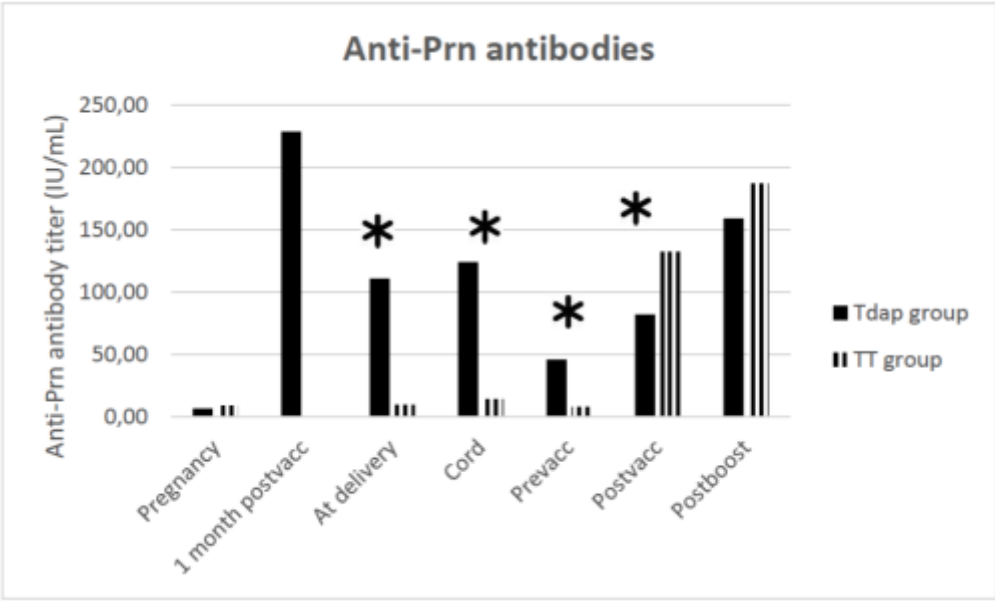


Figure 1: Geometric mean concentration (GMC) for antibodies to TT, DT, PT, FHA and Prn in both groups of women and infants at all time points. Significant differences between the Tdap and the TT group are indicated with a star.

RESULTS FROM THE REGRESSION ANALYSIS

We only report the significant influences of variables on the antibody titers 1 month after the booster infant dose. The variables used in the linear regression analysis are described in Table 1.

In the TT group, a significant influence of the interval between vaccine dose 3 and 4 (16.58 months (SEM 0.26)) on the anti-FHA IgG (p=0.028) was found: the antibody titer decreases with an increasing interval between vaccine dose 3 and 4. Also a significant influence of gender (p=0.013) on the anti-DT IgG was found. Male infants have a significantly higher antibody titer compared to female infants.

In the Tdap group, no significant influences of any variable on the antibody titer was found.

ADDITIONAL RESULTS ON THE WP BOOSTED INFANTS

Since only 4 children in the TT group were boosted with a wP containing vaccine, the sample size of this group was insufficient to analyze the results separately.

Within the Tdap group, 15 children received a wP containing vaccine as a fourth vaccine dose. A significantly higher mean age at vaccine dose 4 (22.18 months (SEM 0.27) versus 20.81 months (SEM 0.43); p=0.007) and a significantly higher mean interval between vaccine dose 3 and 4 (17.44 months (SEM 0.17) versus 15.24 months (SEM 0.29); p<0.001) were calculated in aP vaccinated infants compared to wP vaccinated infants, due to the delay in the IRB approval for the use of aP contanining vaccines for booster. As a consequence, a significantly lower interval between vaccine dose 4 and blood sampling (0.99 months (SEM 0.01) versus 3.16 months (SEM 0.20); p<0.001) was calculated in aP vaccinated infants compared to wP vaccinated infants within the Tdap group (Annex 1).

Overall, higher antibody titers were found in fully aP (4 doses) vaccinated infants, yet only for anti-PT IgG, these antibody titers were significantly higher in the aP boosted infants (GMC 129 (CI 97.5-170.7) versus GMC 19.4 (CI 11.9-31.8); p<0.001). Figure 2 shows the GMCS for antibodies to TT, DT, PT, FHA and Prn after the fourth aP or wP containing vaccine in the Tdap group.

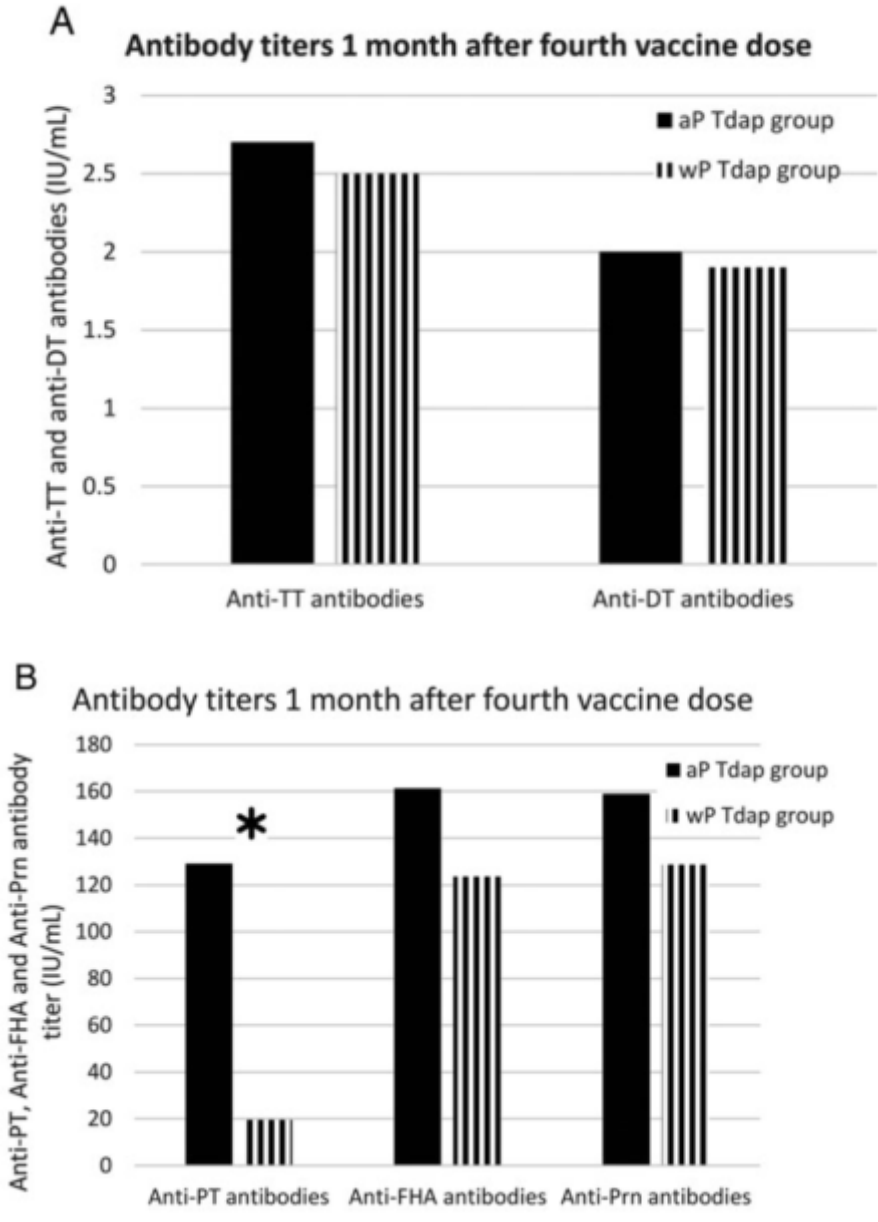


Figure 2: Geometric mean concentration (GMC) for antibodies to TT, DT, PT, FHA and Prn in infants from the Tdap group after the administration of the fourth aP or wP containing vaccine dose. Significant differences are indicated with a star. A: Anti-TT and Anti-DT antibodies. B: Anti-PT, Anti-FHA and Anti-Prn antibodies.

Discussion

The present study describes the effect of maternal vaccination with a Tdap vaccine (Adacel®) on the antibody titers in infants after a booster vaccination with an aP containing vaccine during the second year of life. Previously, blunting of the infant immune response by maternal vaccination during pregnancy, in comparison with a control group receiving a tetanus only vaccine during pregnancy, has been described for anti-DT and anti-Prn antibodies after a primary series of 3 aP infant vaccines [154].

The present data indicate that a blunting effect by maternal immunization only persists on the anti-TT IgG titers in the Tdap group, 1 month after a fourth vaccine dose is offered in the second year of life, compared to the TT group. For anti-PT IgG, anti-FHA IgG, anti-Prn IgG and anti-DT IgG, comparable but lower titers are measured in the Tdap group compared to the TT group. Nevertheless, a good humoral immune response is reported in both study groups, with a significant rise of antibody titers for all measured antigens, except DT oriented antibodies, upon the fourth vaccine dose. The interval between vaccine dose 3 and 4 was significantly smaller in the TT group (16.58 months (SEM 0.26) versus 17.44 months (SEM 0.17) compared to the Tdap group. But this was only affecting the anti-FHA antibody titers in the regression analysis.

In comparison with the available literature on general infant humoral immune responses to a booster dose of Infanrix Hexa® in the second year of life [179, 247], some slight differences were found. Tichmann et al. [179] collected blood samples after a fourth dose of Infanrix Hexa® administered at 12-19 months of age. Gimenez-Sanchez et al. [247] collected blood samples after a fourth dose of Infanrix Hexa® at 11-15 months of age, administered concomitantly with PCV7 or PCV 13. Different laboratory tests were used in both studies compared to this study. Antibody titers to anti-PT IgG, measured at 1 month after the fourth vaccine dose are comparable [179] or higher [247] in the here presented study in both groups. Antibody titers of anti-FHA IgG, anti-Prn IgG and anti-DT IgG are lower in our study compared to both other publications [179, 247]. On the other hand, we report lower anti-TT antibody titers after the booster dose compared to the Tichmann et al. study [179], but higher compared to the Gimenez-Sanchez et al. study [247].

The clinical relevance of the lower antibody titers in children from vaccinated mothers after a fourth vaccine dose, yet rising titers compared to the post-primary time point within one study group, is point of discussion, since no correlate of protection is known for pertussis. But high concentrations of anti-PT IgG and anti-Prn IgG are associated with protection against pertussis disease and mainly anti-PT antibodies are considered to be crucial for this protection [39, 40]. No clinical cases of pertussis were identified within our study population. However, in Vietnam,

pertussis disease is only diagnosed based on a clinical definition. Laboratory diagnosis is not obtained because diagnostic equipment is not available at the community level. Therefore, underdiagnosis is highly probable. Antibody titers for tetanus and diphtheria remained above the correlate of protection both after primary and booster vaccination.

In the study performed by Muñoz et al [102]., blunting of the antibody response after primary vaccination (2-4-6 months) was also reported. This effect disappeared with the administration of a fourth vaccine dose at 12 months of age. Similarly, Hardy-Fairbanks et al. [186], reported a slight blunting of the immune response after primary vaccination. Yet, after administration of a fourth vaccine dose at 12-18 months of age, no notable differences in antibody concentrations were encountered anymore between infant groups. In a Belgian study, a similar blunting effect on the immune response after primary vaccination (2-3-4 months) was described [153]. After the administration of a fourth vaccine dose at 15 months of age, only a significant blunting effect remained for the anti-PT antibodies [155].

The differences observed between the present data and the studies described above [102, 153, 155, 186] could be due to the use of other vaccine brands in pregnant women or during infancy, to distinct primary vaccination schedules, to another timing of the administration of the fourth vaccine dose, different laboratory tests used [102, 186], or possible confounders between populations (e.g. different demographic composition of the study population, different disease-specific epidemiological background, different vaccination history, etc.).

The blunting effect described, is in contradiction with the observations in mice by Feunou et al. where less blunting effect is described whenever different brands of vaccines are used in mothers and infants [260] compared to the same brand in mother and offspring. However, taking into consideration the small sample size of our study, the possible effects of the use of vaccines from several manufacturers certainly needs to be further investigated in future studies.

The linear regression model identified no persistent influencing factor on the antibody titers measured at one month after the fourth vaccine dose in our study population. Only single significant influences of some variables on one specific antigen at one specific time point were found (e.g. gender on anti-DT IgG and interval between vaccine dose 3 and 4 on anti-FHA IgG).

The original design of this study was to vaccinate all participating infants with the wP vaccine used within the EPI. Due to previously described fatalities among young infants in Vietnam, and subsequent disruption of the national program, Infanrix Hexa® was approved to be administered to all participating infants [154]. Then again, due to an unforeseen delay in the ethical approval of the booster dose administration within the study, nineteen children overall

(Tdap and TT group) received a fourth (booster) wP vaccine dose within the regular Vietnamese EPI services. This situation created the unique opportunity to report on different infant vaccination schedules after maternal immunization. Within the Tdap group, all measured antibody titers in wP boosted infants were lower compared to the antibody titers in aP boosted infants. For anti-PT IgG, these antibody titers were even significantly lower. These lower antibody titers could potentially be influenced by the longer interval between the fourth vaccine dose and blood sampling in the wP boosted infants (Annex 1 for details). Yet the difference in the anti-PT antibody titers in unlikely to be solely the consequence of the longer time lapse between booster vaccine and blood sampling. It is well known that higher antibody responses to aP vaccination are elicited compared to wP vaccination in infants after both primary and booster vaccination [194, 263].

LIMITATIONS OF THE STUDY

Our study has a number of limitations. Firstly, no blood samples were taken before the administration of the fourth vaccine dose. Consequently, we could not describe the antibody decay between the third and fourth vaccine dose.

Second, due to a delay in ethical approval, not all children were vaccinated with the same vaccine as a fourth vaccine dose. Some children were already vaccinated within the standard EPI health care system before ethical approval was obtained. However, these unforeseen circumstances offered the opportunity to investigate different schedules of boosting.

During the follow-up of the study, we experienced a drop-out rate due to moving of participants to other provinces. The lower sample size of the study resulted in larger confidence intervals and lower statistical power, but we were still able to detect significant differences one month after the fourth vaccine dose.

Conclusion

Pertussis vaccination during pregnancy closes adequately the susceptibility gap for infection in young unvaccinated infants. Previously, blunting of the infant immune response after 3 doses of an aP containing vaccine, has been reported for the anti-DT and anti-Prn antibody immune response in infants in Vietnam, when vaccination is performed in the presence of high titers of maternal antibodies after a three dose priming schedule. After the fourth dose with a pertussis containing vaccine in the second year of life, significant blunting is reported for anti-TT antibody immune responses. However, a strong humoral immune response on the fourth vaccine dose is elicited for all antigens, except DT, in both groups of infants from either Tdap vaccinated or TT vaccinated women.

The data reported in this manuscript can add evidence for national and international decision makers on maternal immunization as a vaccination strategy for protection of young infants against infectious diseases. Further research on pertussis vaccination during pregnancy in LMIC is certainly needed to assess the impact of high maternal antibody levels on the immune responses of infants both primed with aP or wP containing vaccines. An additional comparative study on different brands of pertussis vaccines could shed further light on the induction of qualitative and quantitative differences between the induced immune responses.

Acknowledgments

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Annex 1

		Tdap group		**p-value**
		aP vaccinated infants	**wP vaccinated infants**	
N (included infants)		30	15	
Infant gender, No. (%)	Male	20 (66.7)	8 (53.3)	0.517
	Female	10 (33.3)	7 (46.7)	
Mean weight in kilograms (SEM)		10.75 (0.20)	10.77 (0.32)	0.963
Mean length in centimeters (SEM)		83.20 (0.59)	82.71 (0.87)	0.639
Mean age at vaccine dose 4 in months (SEM)		22.18 (0.27)	20.81 (0.43)	0.007
Mean age at blood sample 1 month after fourth vaccine dose in months (SEM)		23.18 (0.27)	23.98 (0.32)	0.065
Mean interval between vaccine dose 4 – blood sample 1 month after fourth vaccine dose in months (SEM)		0.99 (0.01)	3.16 (0.20)	<0.001
Mean interval between vaccine dose 3 and vaccine dose 4 in months (SEM)		17.44 (0.17)	15.24 (0.29)	<0.001

Annex 1: Demographic characteristics of infants in the Tdap group receiving a fourth aP or wP containing vaccine dose.

CHAPTER 4

Effect of pertussis vaccination during pregnancy on pneumococcal immune responses

This chapter is published: "Maertens K., Burbidge P., Van Damme P., Goldblatt D., Leuridan E. Pneumococcal immune responses in infants whose mothers received Tdap vaccination during pregnancy. Pediatric Infectious Disease Journal. 2017 Apr 10; 36(12):1186-1192"

doi: 10.1097/INF.0000000000001601.

Abstract

Maternal immunization with a tetanus, diphtheria and acellular pertussis (Tdap) vaccine may blunt infant pneumococcal immune response after a primary series of vaccines.

As part of a prospective controlled cohort trial of Tdap (Boostrix®, GSK Biologicals) vaccination in pregnancy, infants born to vaccinated mothers and controls were immunized at 8 and 16 weeks and 12 months of age with 13-valent pneumococcal conjugate vaccine (Prevenar13®, Pfizer). Sera were tested for pneumococcal antibody concentrations against vaccine serotypes following primary and booster immunization.

Geometric Mean Concentration (GMC) of antibodies to serotype 1, 3, 4, 5, 6A, 7F, 9V, 14 and 19A were significantly lower after 2 doses of Prevenar13® vaccine in the offspring of mothers vaccinated in pregnancy. This blunting effect disappeared after a booster dose at the age of 12 months, except for serotype 1 and 4. Despite this blunting, the percentage of children achieving the threshold of protection of 0.35μg/mL was comparable in the vaccine and the control group both after primary and booster vaccination with only a significant lower rate of seroprotection in the vaccine group for serotype 3 after primary vaccination. After booster vaccination, seroprotection rates increased further for serotype 3, 5, 6B, 9V and 23F.

The present results indicate a blunting effect post-primary vaccination for some serotypes resolving after booster vaccination. Seroprotection rates were comparable both after primary and booster vaccination, except for serotype 3 with a significant lower seroprotection rate in the vaccine group after primary vaccination.

Introduction

Pertussis, caused by the gram-negative bacteria *Bordetella pertussis*, is a global endemic respiratory disease and an important cause of morbidity and mortality among infants [1]. Worldwide, pertussis is estimated to cause 63,000 deaths in <5-year-old children (2013), although there is considerable uncertainty around these estimates due to the paucity of reliable surveillance data, particularly from developing countries [1]. In industrialized countries, outbreaks of pertussis have been reported during the last decade and the overall incidence is increasing, with most severe cases and fatalities among young, not fully vaccinated infants [235].

An important way to offer protection against pertussis disease from birth is, with the currently available vaccines and vaccination schedules, to include immunization during pregnancy. High concentrations of maternal antibodies, elicited by maternal vaccination, are transported transplacentally to the fetus, offering protection until the start of the primary infant vaccination schedule and thereby closing the susceptibility gap for infection [102, 153, 154, 157, 186]. Several industrialized countries have already put in place a recommendation for this strategy although some safety and immunological aspects were unknown at implementation. While blunting of infant immune responses to pertussis antigens included in the infant vaccination, has been described after primary series of infant vaccination in the presence of maternal antibodies [153, 157], the impact of maternal tetanus, diphtheria, acellular pertussis (aP) (Tdap) vaccination on other non-Tdap antigens, included in the regular infant vaccination schedule, is a potential concern.

A UK study [157] showed a blunting effect on some serotypes of the primary pneumococcal infant humoral immune response, in infants born to mothers immunized with a Tdap vaccine (Repevax®, Sanofi Pasteur, France) during pregnancy. The proposed explanation is that the diphtheria vaccination during pregnancy interferes with the immune response of the infants to the CRM-197 carrier protein included in the infant pneumococcal vaccine. Any additional data from controlled clinical trials on the beneficial and possible unknown side effect of maternal Tdap vaccination is therefore welcomed to assist decision-making with regard to maternal immunization as a global strategy to protect young infants.

We report the effect of maternal Tdap vaccination (Boostrix®, GSK Biologicals, Rixensart, Belgium) on infant responses to pneumococcal conjugate vaccine one month after 2-dose pneumococcal priming (at 8 and 16 weeks of age) and 2.5 months after the pneumococcal booster dose (at 12 months of age). Importantly, this last dose is not foreseen in the global Expanded Program on Immunization (EPI) schedule. This output adds to the body of knowledge on potential blunting of infant immune responses to childhood vaccination following maternal Tdap vaccination. The study will ultimately contribute to inform decision-making bodies on implementing maternal immunization, both in industrialized and low-and middle-income (LMIC) countries.

Material and Methods

A prospective controlled cohort study was conducted in Belgium in 2011-2015, in accordance with the Declaration of Helsinki, ICH-GCP, and procedures established by Belgian law (Clinicaltrials.gov identifier: NCT01698346). The present analysis was a spin-off study on leftover blood samples from a trial on maternal Tdap vaccination [153, 155] and was approved by the ethics committee of the University of Antwerp, Belgium. Written informed consent was obtained from both parents of the participating infants, and details on the study procedures can be consulted in previous publications [153, 155].

Participating women were included in either a vaccine group: women vaccinated with Tdap (Boostrix®, GSK Biologicals, Rixensart, Belgium) between 18 and 34 weeks of gestation (as per protocol), or a control group of pregnant women not vaccinated with a pertussis containing vaccine for at least 10 years. The offspring was included in 2 groups according to the vaccination status of their mother. Within the regular health care system, infant pneumococcal vaccines were administered at well-baby clinics, by a pediatrician or general practitioner at the age of 8 and 16 weeks and 12 months. All infants were vaccinated according to the Belgian vaccination schedule with hexavalent vaccine, MMR (Measles, Mumps, Rubella) vaccine and rotavirus vaccine.

STUDY VACCINES

Licensed Tdap vaccine (Boostrix®, GSK Biologicals, Rixensart, Belgium) was used to vaccinate pregnant women. Boostrix® contains 5 Lf of Tetanus Toxoid (TT), 2.5 Lf of Diphtheria Toxoid (DT), 8 μg inactivated Pertussis Toxin (PT), 8 μg Filamentous Hemagglutinin (FHA) and 2.5 μg Pertactin (Prn).

Infants were vaccinated with the 13-valent pneumococcal vaccine Prevenar13® (Pfizer, United Kingdom) containing 2.2 μg of serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F and 23F and 4.4 μg of serotype 6B.

STUDY PROCEDURES

Blood samples were collected from the infants at month 5 (28-35 days after the second pneumococcal vaccine dose) and at month 15 (2.5 months after the pneumococcal booster dose). Blood samples were centrifuged at 2000 rpm within 24 hours after blood collection and stored at -20°C. Since this study is a spin-off study from a maternal Tdap vaccination trial measuring infant pertussis immune responses, bleeding time points were originally not chosen to measure pneumococcal immune responses resulting in late bleeding after the pneumococcal booster dose.

LABORATORY

Sera were tested for antibodies to the 13 vaccine-type capsular polysaccharides (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F) at the University College London, Institute of Child Health. Immunoglobulin G (IgG) antibody levels were measured by the World Health Organization (WHO) reference enzyme-linked immunosorbent assay (ELISA) after adsorption with cell wall and 22F polysaccharide [264]. The lower limit of quantification (LLOQ) has been set at 0.15 μg/mL. The protective threshold for all 13 pneumococcal serotypes is set at 0.35 μg/mL.

Laboratory procedures used to test the samples were exactly the same as in the Ladhani et al. [157] study which was also analyzed in the WHO Reference laboratory. Results of both studies are therefore comparable.

STATISTICS

Statistical tests included parametric tests: (paired) t-tests and chi-square tests, and non-parametric alternatives: (paired) Wilcoxon tests and Fisher exact tests whenever the underlying normality and sparseness assumptions of parametric tests were violated. Linear regression models were used to identify characteristics that could potentially impact infant antibody concentrations. The analysis was performed using SPSS statistical software 23.0. Two-sided p-value <0.05 was considered as statistical significant. Pneumococcal antibody concentrations below the LLOQ were replaced by a value of 0.075 μg/mL, which is half of the limit of detection, to perform the analysis.

Blunting of the infant immune response is defined as a statistically significant lower GMC of serotype-specific antibodies in infants from one group compared to the other group.

Results

GENERAL CHARACTERISTICS OF THE STUDY POPULATION

All infants were vaccinated with Prevenar13® at the respective immunization visits. Fifty-two mother-infant pairs were included in the vaccine group and 25 pairs in the control group. Women in the vaccine group were vaccinated with a Tdap vaccine (Boostrix®, GSK Biologicals, Rixensart, Belgium) between 22 and 32 weeks of gestation. Women in our study were not vaccinated with a pneumococcal vaccine during childhood or later in life. However, they could have pre-existing antibodies due to natural exposure. Children were born between April 2nd, 2012 and April 16th, 2014. Blood samples were taken between August 27th, 2012 and July 27th, 2015. The mean interval between Tdap vaccination and delivery was 77.7 days. The mean gestational age at vaccination was 28.8 weeks. Table 1 summarizes the characteristics of all mother-infant pairs, vaccination data and intervals between vaccination and blood sampling in the infants. All women in the control group had additional education after secondary school resulting in a significant difference in education (p=0.008) between both groups. Significantly lower mean age at hexavalent vaccine dose 1 (p=0.026) and dose (p=0.015) were calculated in the vaccine group compared to the control group.

Characteristic	Vaccine Group	Control Group	*P*
N (women)	52	25	
Mean age at delivery (yr) (SEM)	30.78 (0.55)	31.86 (0.74)	0.257
Race mother, N (%)			
Caucasian	51 (98.08)	25 (100.00)	1.000
Other	1 (1.92)	0 (0.00)	
Level of education, N (%)			
Secondary school	11 (21.15)	0 (0.00)	0.008*
Bachelor degree	17 (32.69)	16 (64.00)	
Master degree	24 (46.15)	9 (36.00)	
Mean gestational age at delivery (wk) (SEM)	39.91 (0.16)	39.89 (0.21)	0.972
Mean gestational age at vaccination (wk) (SEM)	28.80 (0.39)	NA	NA
Mean interval between Tdap vaccination and delivery (d) (SEM)	77.65 (2.40)	NA	NA
Tetanus booster < 10 yr ago, N (%)			
Yes	25 (48.08)	11 (44.00)	0.808
No	27 (51.92)	14 (56.00)	
Delivery method, N (%)			
Vaginal	45 (86.54)	21 (84.00)	0.741
Cesarean	7 (13.46)	4 (16.00)	
N (children)	54 (2 twins)	25	
Gender, N (%)			
Male	26 (48.15)	12 (48.00)	1.000
Female	28 (51.85)	13 (52.00)	
Mean birth weight (g) (SEM)	3383.67 (58.71)	3476.56 (90.16)	0.383
Mean birth length (cm) (SEM)	50.37 (0.33)	49.43 (0.60)	0.146
Mean age at visit month 5 (mo) (SEM)	5.32 (0.07)	5.44 (0.11)	0.337
Mean weight at visit month 5 (g) (SEM)	7360.28 (128.19)	7346.91 (150.81)	0.949
Mean length at visit month 5 (cm) (SEM)	66.30 (0.40)	66.74 (0.37)	0.445
Mean age at visit month 15 (mo) (SEM)	14.93 (0.05)	15.00 (0.10)	0.482
Mean weight at visit month 15 (g) (SEM)	10,316.30 (159.75)	10,349.13 (173.00)	0.904
Mean length at visit month 15 (cm) (SEM)	77.82 (0.43)	79.40 (0.72)	0.067
Mean duration breastfeeding (mo) (SEM)	5.04 (0.54)	5.69 (1.14)	0.560
Mean age at hexavalent vaccine dose 1 (mo) (SEM)	2.07 (0.03)	2.27 (0.11)	0.026*
Mean age at hexavalent vaccine dose 2 (mo) (SEM)	3.14 (0.05)	3.42 (0.13)	0.015*
Mean age at hexavalent vaccine dose 3 (mo) (SEM)	4.31 (0.07)	4.55 (0.14)	0.105
Mean age at pneumococcal vaccine dose 1 (mo) (SEM)	2.11 (0.05)	2.27 (0.11)	0.142
Mean age at pneumococcal vaccine dose 2 (mo) (SEM)	4.33 (0.07)	4.54 (0.17)	0.196
Mean age at pneumococcal vaccine dose 3 (mo) (SEM)	12.41 (0.07)	12.73 (0.20)	0.151
Mean interval between pneumococcal vaccine dose 2 and blood sample month 5 (mo) (SEM)	1.07 (0.26)	1.06 (0.06)	0.817
Mean interval between blood sample month 5 and pneumococcal vaccine dose 3 (mo) (SEM)	9.61 (0.09)	9.53 (0.13)	0.632
Mean interval between pneumococcal vaccine dose 3 and blood sample month 15 (mo) (SEM)	2.52 (0.06)	2.58 (0.12)	0.633

*Significant difference.
NA indicates not tested in the analysis.

Table 1: Demographic characteristics of mother-infant pairs and vaccination and blood sampling data from infants in both study groups. * Significant difference.

SEROPROTECTION RESULTS

Table 2 provides an overview of the percentage of infants with an antibody concentration above the correlate of protection (0.35 μg/mL) 1 month after the second and 2.5 months after the third Prevenar13® dose. After priming, a significantly lower seroprotection rate was seen in the vaccine group compared to the control group for serotype 3 (p=0.045). Low levels of seroprotection are described in both study groups for serotype 6B and 23F after priming, but there is a significant rise after the third vaccine dose for both serotypes. After the booster dose, comparable seroprotection rates are found in both study groups for all serotypes.

Overall, following primary vaccination, the vaccine was immunogenic in both groups with similar proportions achieving protective concentrations. For most infants in the vaccine group, immune responses following the booster vaccination were higher than immune responses after primary immunization despite the fact that post-primary immune responses were measured one month, but booster responses 2.5 months after vaccination.

Serotype	Month 5		Month 15	
	Vaccine Group	Control Group	Vaccine Group	Control Group
Serotype 1	95.56 (89.44–100.00)	91.67 (80.51–100)	95.92 (90.28–100.00)	95.46 (86.66–100.00)
P	0.606		1.00	
Serotype 3	73.81 (60.41–87.21)	95.55 (86.83–100.00)	94.74 (87.75–100.00)	83.33 (66.01–100.00)
P	0.045*		0.314	
Serotype 4	97.78 (93.30–100.00)	91.67 (80.51–100.00)	87.78 (78.51–97.05)	100
P	0.276		0.167	
Serotype 5	65.91 (51.80–80.02)	87.50 (74.17–100.00)	95.92 (90.28–100.00)	90.91 (78.80–100.00)
P	0.083		0.583	
Serotype 6A	84.09 (73.18–95.00)	91.67 (80.51–100.00)	100	100
P	0.476		NA	
Serotype 6B	37.78 (23.51–52.05)	41.67 (21.85–61.49)	95.92 (90.28–100.00)	100
P	0.475		0.566	
Serotype 7F	97.73 (93.23–100.00)	95.83 (87.731–100.00)	100	100
P	1.00		NA	
Serotype 9V	77.78 (65.53–90.03)	87.50 (74.17–100.00)	91.84 (84.07–99.61)	95.46 (86.66–100.00)
P	0.519		0.673	
Serotype 14	95.56 (89.44–100.00)	95.65 (87.21–100.00)	100	100
P	1.00		NA	
Serotype 18C	88.89 (79.61–98.17)	91.67 (80.51–100.00)	81.63 (70.69–92.57)	77.27 (59.66–94.88)
P	1.00		0.750	
Serotype 19A	88.64 (79.16–98.12)	100	100	100
P	0.153		NA	
Serotype 19F	100	100	100	100
P	NA		NA	
Serotype 23F	57.78 (43.25–72.31)	62.50 (43.03–81.97)	87.78 (78.51–97.05)	100
P	0.799		0.167	

*Significant difference.
CI indicates confidence interval; NA, not tested in the analysis.

Table 2: Percentage of children with pneumococcal antibody concentration above the correlate of protection (0.35 μg/mL) in both study groups 1 month after the second pneumococcal vaccine dose and 2.5 months after the third pneumococcal vaccine dose. *Significant difference.

SEROTYPE SPECIFIC IgG CONCENTRATIONS

Table 3 provides an overview of the GMC's per serotype in both groups at all time points. One month after the priming, significantly lower GMC's are seen in the vaccine group for serotype 1, 3, 4, 5, 6A, 7F, 9V, 14 and 19A. For serotypes 6B, 18C, 19F and 23F, comparable but lower antibody concentrations were found in the vaccine group. After the administration of the third pneumococcal vaccine dose, significantly lower GMC's were only seen in the vaccine group for serotypes 1 and 4, with a slight increase in antibody concentration after the booster for serotype 1. In general, the increase in antibody concentration between both time points is higher in the vaccine group, except for serotype 4, 6B, 18C and 23F. Figure 1 shows the reverse distribution curve (RDC) of the data for serotype 1, as an example. RDC's for other serotypes can be found in Annex 1.

	1 mo After the Second Pneumococcal Vaccine			2.5 mo After the Third Pneumococcal Vaccine		
Serotype	Vaccine Group	Control Group	Ratio: Vaccine/ Control Group	Vaccine Group	Control Group	Ratio: Vaccine/ Control Group
N samples	49 (44 for type 3, 45 for type 19F, 46 for type 5/6A/7F/19A)	24 (22 for type 3, 23 for type 14)		49 (38 for type 3)	24 (19 for type 3)	
Type 1, μg/mL	1.00 (0.79–1.28)	1.99 (1.31–3.02)	0.51	1.15 (0.92–1.44)	1.88 (1.29–2.75)	0.61
P	0.004*			0.025*		
Type 3, μg/mL	0.53 (0.39–0.71)	0.86 (0.64–1.14)	0.62	0.71 (0.60–0.85)	0.70 (0.51–0.95)	1.02
P	0.044*			0.902		
Type 4, μg/mL	0.97 (0.80–1.17)	1.53 (1.03–2.25)	0.63	0.84 (0.69–1.02)	1.40 (1.02–1.91)	0.60
P	0.024*			0.008*		
Type 5, μg/mL	0.48 (0.37–0.61)	0.93 (0.66–1.31)	0.51	0.93 (0.77–1.13)	1.01 (0.78–1.30)	0.93
P	0.003*			0.659		
Type 6A, μg/mL	0.80 (0.59–1.10)	1.49 (0.98–2.25)	0.54	5.32 (4.26–6.65)	7.68 (5.38–10.96)	0.69
P	0.025*			0.084		
Type 6B, μg/mL	0.24 (0.17–0.34)	0.29 (0.19–0.45)	0.84	2.57 (1.95–3.39)	3.87 (2.70–5.63)	0.66
P	0.528			0.095		
Type 7F, μg/mL	1.67 (1.37–2.04)	2.71 (1.75–4.20)	0.62	1.85 (1.54–2.22)	2.55 (1.93–3.38)	0.73
P	0.027*			0.062		
Type 9V, μg/mL	0.54 (0.42–0.70)	1.18 (0.79–1.77)	0.46	0.78 (0.65–0.93)	0.99 (0.69–1.41)	0.79
P	0.001*			0.192		
Type 14, μg/mL	3.41 (2.47–4.70)	7.41 (4.44–12.35)	0.46	5.11 (3.96–6.59)	7.65 (4.87–12.01)	0.67
P	0.011*			0.108		
Type 18C, μg/mL	0.86 (0.68–1.08)	1.10 (0.72–1.68)	0.78	0.62 (0.52–0.75)	0.81 (0.53–1.24)	0.77
P	0.270			0.289		
Type 19A, μg/mL	1.17 (0.92–1.50)	2.21 (1.55–3.14)	0.53	3.61 (2.85–4.58)	3.60 (2.39–5.41)	1.00
P	0.005*			0.985		
Type 19F, μg/mL	3.31 (2.51–4.36)	4.88 (3.08–7.73)	0.68	3.80 (3.09–4.67)	3.77 (2.68–5.31)	1.00
P	0.136			0.966		
Type 23F, μg/mL	0.41 (0.30–0.56)	0.59 (0.35–0.99)	0.70	1.09 (0.84–1.41)	1.65 (1.16–2.35)	0.66
P	0.214			0.073		

*Significant difference.
CI indicates confidence interval.

Table 3: Geometric Mean Concentrations (GMCs) with 95% confidence intervals (CI) for antibodies to serotype 1, 3, 4, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F post-primary and post-booster vaccination in both groups of infants. *Significant difference.

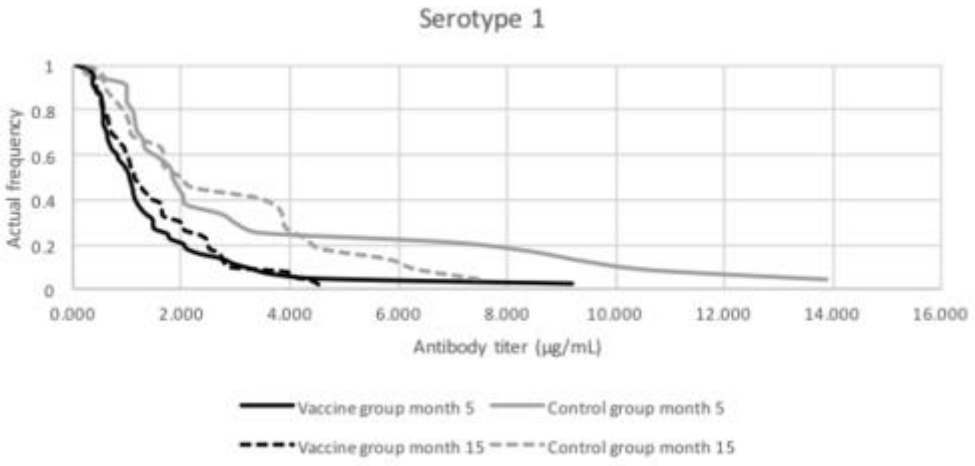


Figure 1: Reverse Cumulative Distribution curve of serotype 1, as an example.

RESULTS FROM THE REGRESSION ANALYSIS

We only report significant influences of variables on the serotype-specific pneumococcal antibody titers after primary and booster vaccination. Some variables like gender, lactation, gestational age at delivery and weight randomly influence serotype-specific antibody concentrations at one time point. There is no consistency in these effects, and they are hard to explain (Table 4). Other factors do seem to influence more consistently serotype-specific antibody responses. For example, a higher age at blood sampling correlates with a higher antibody concentration in the vaccine group. In contrary, a higher age at blood sampling correlates with a lower antibody concentration in the control group. A higher age at pneumococcal booster vaccination correlates with higher antibody concentrations in both study groups and a higher interval between pneumococcal vaccine dose 3 and blood sampling correlates with a lower antibody concentration in both study groups (Table 4).

Characteristic	Vaccine Group Month 5	Control Group Month 5	Vaccine Group Month 15	Control Group Month 15
Gender	7F (higher in females)	/	/	/
Nutrition (formula-lactation)	/	/	5 (higher with lactation)	/
Age at blood sample month 5	18C (higher with higher age)	14 (higher with lower age)	NA	NA
Age at blood sample month 15	NA	NA	-6A (higher with higher age) -14 (higher with higher age)	-5 (higher with lower age) -6B (higher with lower age) -18C (higher with lower age)
Age at pneumococcal vaccine dose 2	18C (higher with higher rage)	14 (higher with lower age)	NA	NA
Interval pneumococcal vaccine dose 2 and blood sample month 5	18C (higher with shorter interval)	14 (higher with longer interval)	NA	NA/
Weight	6B (higher with higher weight)	6B (higher with higher weight)	/	/
Gestational age at delivery	/	4 (higher with higher Gestational Age)	NA	NA/
Gestational age at vaccination	/	/	/	/
Age hexavalent vaccine dose 3	/	/	/	/
Interval between pneumococcal vaccine doses 1 and 2	14 (higher with longer interval)	/	/	/
Age at pneumococcal vaccine dose 3	NA	NA	-6A (higher with higher age) -14 (higher with higher age)	-5 (higher with higher age) -6B (higher with higher age) -18C (higher with higher age)
Interval between pneumococcal vaccine dose 3 and blood sample month 15	NA	NA	-6A (higher with shorter interval) -14 (higher with shorter interval)	-5 (higher with shorter interval) -6B (higher with shorter interval) -18C (higher with shorter interval)
Interval between pneumococcal vaccine doses 2 and 3	NA	NA	/	/

NA indicates not tested in the analysis; "/,"no effect found.

Table 4: Influencing factors on distinct serotypes in the multiple regression analysis. NA means not tested in the analysis. '/' means no effect found.

Discussion

This study of pneumococcal vaccine responses in infants born to mothers who received Tdap vaccine (Boostrix®, GSK Biologicals, Rixensart, Belgium) during pregnancy, shows that while infant response to PCV are blunted, proportions of infants achieving protective concentrations of serotype specific IgG are similar irrespective of maternal vaccination status. Our study is the first study that has addressed the impact of Tdap in pregnancy on the responses to a pneumococcal booster dose. All serotype specific concentrations reached a high percentage of seroprotection after the booster dose, with lowest rate for serotype 4 and 23F (87.78%), but no significant differences in seroprotection rates between both study groups. This finding is reassuring that the blunting effect is temporary and time limited.

These results support those previously published for British mothers and their infants by Ladhani et al [157]. Our findings are important as it confirms that the impact of maternal Tdap vaccination on pneumococcal humoral responses is not an isolated result in the UK and needs to be considered whenever recommending the maternal pertussis vaccination strategy with the available combination vaccines.

In comparison with the UK data, we confirm low levels of seroprotection for serotype 3 (significantly lower in the vaccine group), 6B and 23F but otherwise good levels of seroprotection (>65%) in both study groups for most other serotypes post-primary vaccination. In contrast to the UK data, we did not confirm lower protection for serotypes 5 and 9V. Reaching the seroprotection levels of antibodies ensures closing the susceptibility gap of infection for invasive pneumococcal disease (IPD).

Belgian and British population are expected to be quite similar in characteristics and both countries have a vaccination program with high coverages. Circulation of vaccine serotypes is therefore limited and the protective relevance of blunting needs to be interpreted carefully. This is certainly different in countries where other schedules and coverage of pneumococcal vaccination are reported, e.g. LMIC, where no booster doses are foreseen or coverage of pneumococcal vaccination might be lower resulting in ongoing circulation of vaccine included serotypes.

When comparing serotype specific IgG concentrations, we found a significant blunting effect for serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14 and 19A after 2-dose primary immunization. In the UK, blunting of the immune responses was described for the same serotypes apart from serotypes 14 and 19A. While our vaccinees and controls were recruited in the same study period, the UK

used historical control data collected in a period where maternal Tdap immunization was not yet in place. The use of different vaccines for maternal Tdap vaccination (Repevax® versus Boostrix®) and possible boosting by nasopharyngeal carriage of pneumococci in the historical controls may explain why responses to 14 and 19A were comparable between both study groups in the UK study, but blunted in the vaccine group of our study after 2-dose primary immunization.

In our study, the second blood sample was taken relatively late after the third pneumococcal vaccination (2.5 months), hence the concentrations measured at that point might already be waning. However, as the study was randomized, we assume that waning of concentrations is similar in both groups of infants, thus allowing comparison of the results between both study groups. Post-booster GMC results were comparable for both groups and blunting only persisted for serotype 1 and 4. Booster responses to serotypes 6A, 6B, 7F, 14, 19A and 19F were excellent. The high response to these specific serotypes has already been reported previously [265, 266].

Interestingly, booster responses in the control group were lower for seven serotypes 2.5 months after boosting compared to primary responses. This may be due to rapid decay following boosting and the delayed time point of blood sampling in the infants which may have missed the peak of the response. This phenomenon was only seen for two serotypes (4 and 18C) in the infants born to vaccinated mothers.

Comparison of GMC to post-vaccination data after vaccination with distinct vaccination schedules is useful to interpret the present results. Spijkerman et al [266] compared several PCV13 vaccination schedules. Children receiving a 3+1 schedule, reached higher levels of antibodies after the primary vaccination compared to our study, yet all schedules had similar high seroprotection levels after completion. Nevertheless, we report similar antibody concentrations after a short primary schedule as do Rodgers et al [267] in their comparative overview on different priming schedules with PCV13. Higher response rates are reported for serotype 4 but similar lower rates for serotype 23F.

Gestational age at maternal vaccination did not influence the antibody concentrations. Our cohort was not powered to detect differences in GMC when vaccinating at different gestational ages, as has been recently suggested [176, 218]. Nevertheless, some variables like age at blood sampling, age at pneumococcal booster vaccination and interval between pneumococcal vaccine dose 3 and blood sampling at month 15 do seem to have influence on several serotype antibody responses. However, we have to be careful drawing conclusions on influencing factors taking into account the relatively small sample size of the study.

In Belgium, the circulating strains causing invasive pneumococcal disease in children <2 years of age, the period where children are most vulnerable to IPD, were 3, 7F, 19A and non PCV-13 types in 2014 [268]. In the same year, 186 IPD's were diagnosed in children below 16 years of age (N=283 in 2013 and N=334 in 2012). The overall incidence of IPD <2 years of age was 53.2/10,000 (compared to 156/10,000 before the start of the vaccination recommendation). Coverage for the third dose of pneumococcal vaccine among infants in Flanders is >96.5%. The present results show interference, resolving after the booster dose. Since there is low circulation of the vaccine serotypes, the blunting effect will probably not have clinical significance unless long term blunting in the population might affect the circulation of vaccine strains again.

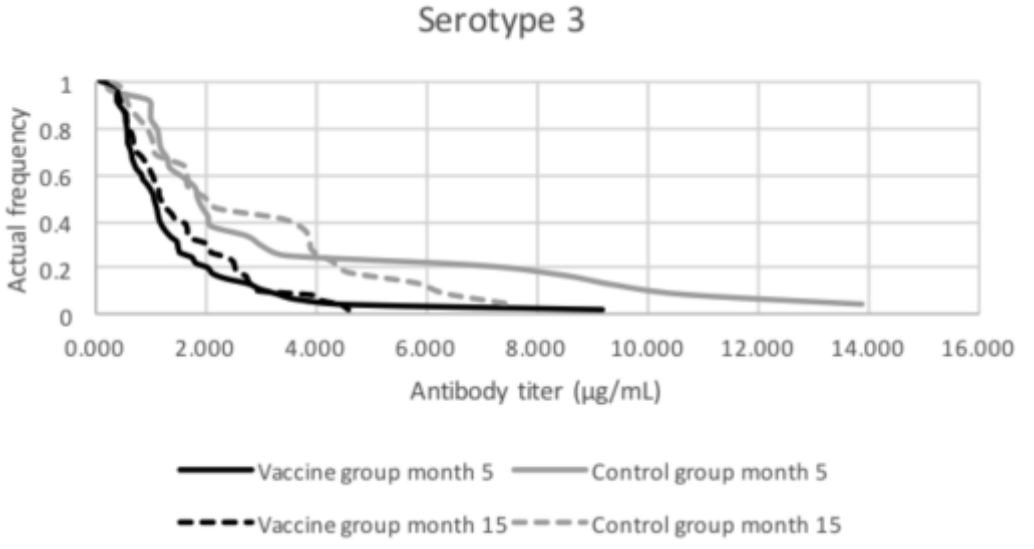
In view of global recommendations for maternal immunization to protect infants from disease, the effect of maternal Tdap vaccination on infant immune responses regarding both seroprotection rate and clinical efficacy is important. In particular, when considering implementation of recommendations for maternal vaccination in LMIC with varied regional epidemiology and infant immunization schedules (three dose priming without booster dose) as used in the EPI since no deduction from the Belgian study can be drawn and no data from LMIC are available to confirm these results.

The study has a few limitations. The present project builds on an existing serumbank of leftovers and is therefore a convenience sample, without having a power calculation on beforehand to answer this research question. In addition, the main research aim was initially not to identify blunting of pneumococcal immune responses, and the moments for blood sampling are therefore not adapted to the pneumococcal vaccination schedule. Maternal samples were not tested for pre-existing anti-pneumococcal antibody titers; hence, maternal antibody interference could not be analyzed, but as the mothers were randomized this should not impact on the analysis. The interval between vaccination and blood sampling after the primary series (1 month) differs from the interval between the booster dose and blood sampling (2.5 months). Also, no functional data were reported in this paper since the sample volumes of the infants following multiple testing were limited and we were therefore not able to undertake additional analysis of functional anti-pneumococcal antibody. However, the correlation between serotype specific IgG measured by ELISA compared to opsonophagocytosis assay (OPA) is good following PCV in infancy especially after the booster dose [269]. Despite this limitation, the samples were all taken at the same time point, allowing us to compare the results post-booster vaccination between both groups, taking into consideration the possible waning of antibodies.

Conclusions

The blunting effect of maternal Tdap vaccination on pneumococcal immune responses in young infants, is confirmed in the present study. The clinical effect on protection from pneumococcal disease will likely be low in the Belgian setting, since protective levels of antibody are achieved for almost all serotypes and circulation of vaccine serotypes is almost non-existent.

Annex 1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Actual frequency
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0.6
0.4
0.2
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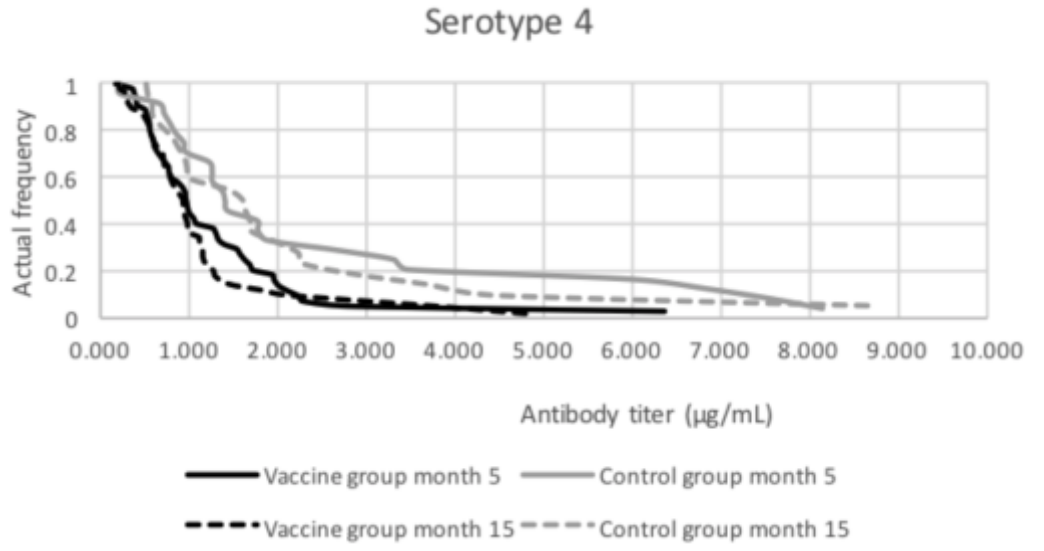
4.000
6.000
8.000
10.000
12.000
14.000
16.000
Antibody titer (µg/mL)
Vaccine group month 5
Control group month 5
Vaccine group month 15
Control group month 15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Actual frequency
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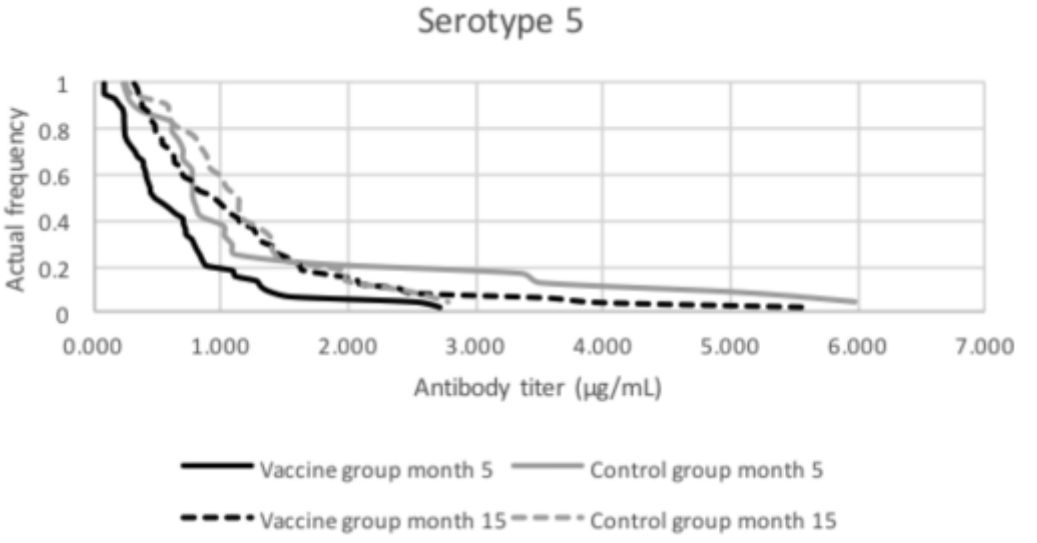
2.000
3.000
4.000
5.000
6.000
7.000
8.000
9.000
10.000
Antibody titer (µg/mL)
Vaccine group month 5
Control group month 5
Vaccine group month 15
Control group month 15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Actual frequency
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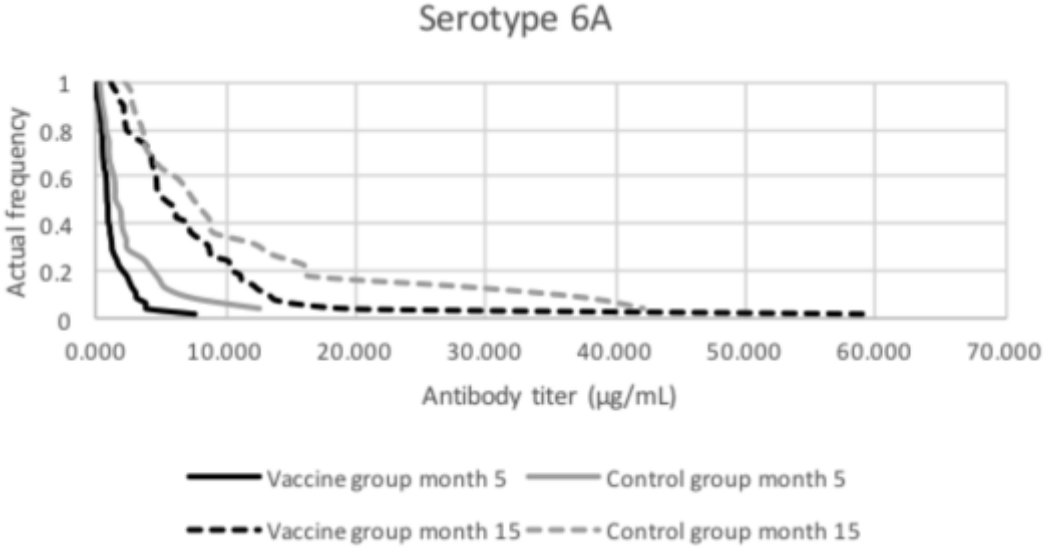
0.6
0.4
0.2
0
0.000
1.000
2.000
3.000
4.000
5.000
6.000
7.000
Antibody titer (µg/mL)
Vaccine group month 5
Control group month 5
Vaccine group month 15
Control group month 15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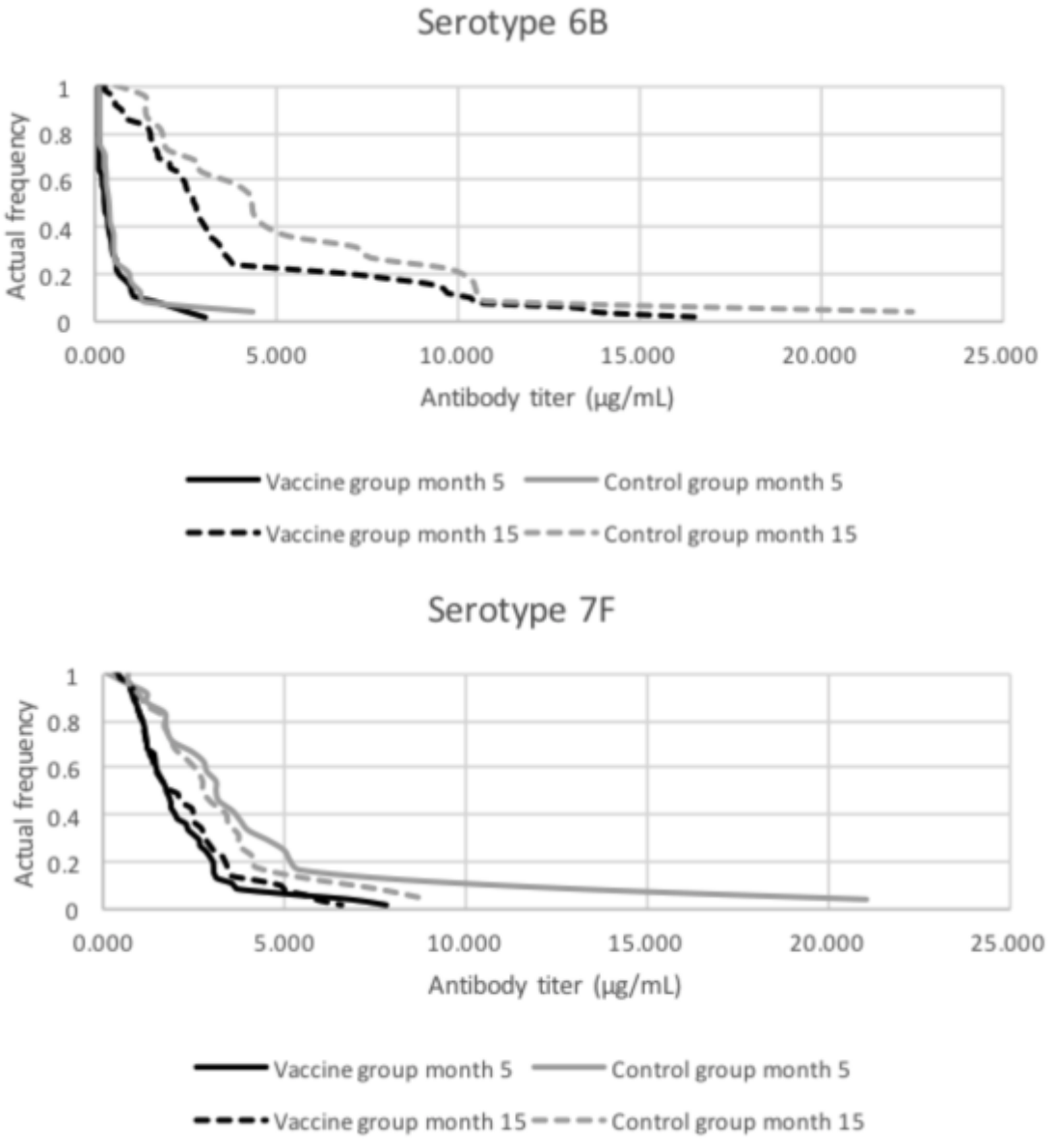
0.4
0.2
0
0.000
10.000
20.000
30.000
40.000
50.000
60.000
70.000
Antibody titer (µg/mL)
Vaccine group month 5
Control group month 5
Vaccine group month 15
Control group month 15


Serotype 6B
Actual frequency
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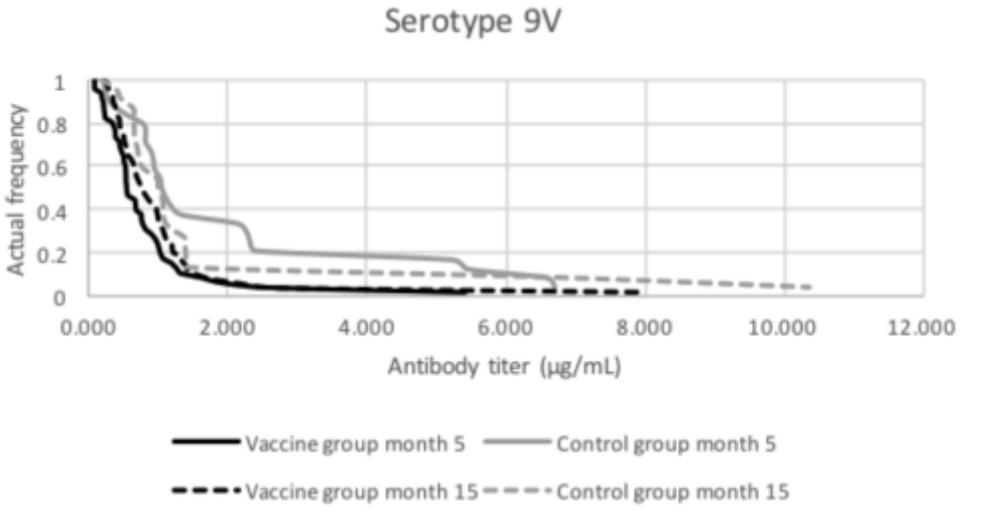
0.6
0.4
0.2
0
0.000
5.000
10.000
15.000
20.000
25.000
Antibody titer (µg/mL)
Vaccine group month 5
Control group month 5
Vaccine group month 15
Control group month 15
Serotype 7F
Actual frequency
1
0.8
0.6
0.4
0.2
0
0.000
5.000
10.000
15.000
20.000
25.000
Antibody titer (µg/mL)
Vaccine group month 5
Control group month 5
Vaccine group month 15
Control group month 15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Actual frequency
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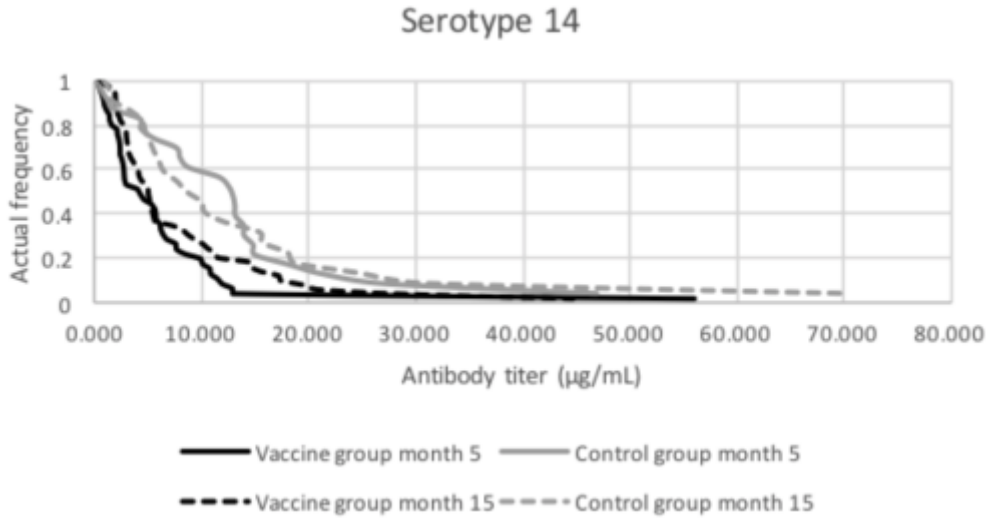
0.6
0.4
0.2
0
0.000
2.000
4.000
6.000
8.000
10.000
12.000
Antibody titer (µg/mL)
Vaccine group month 5
Control group month 5
Vaccine group month 15
Control group month 15


Serotype 14
Actual frequency
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0.4
0.2
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0.000
10.000
20.000
30.000
40.000
50.000
60.000
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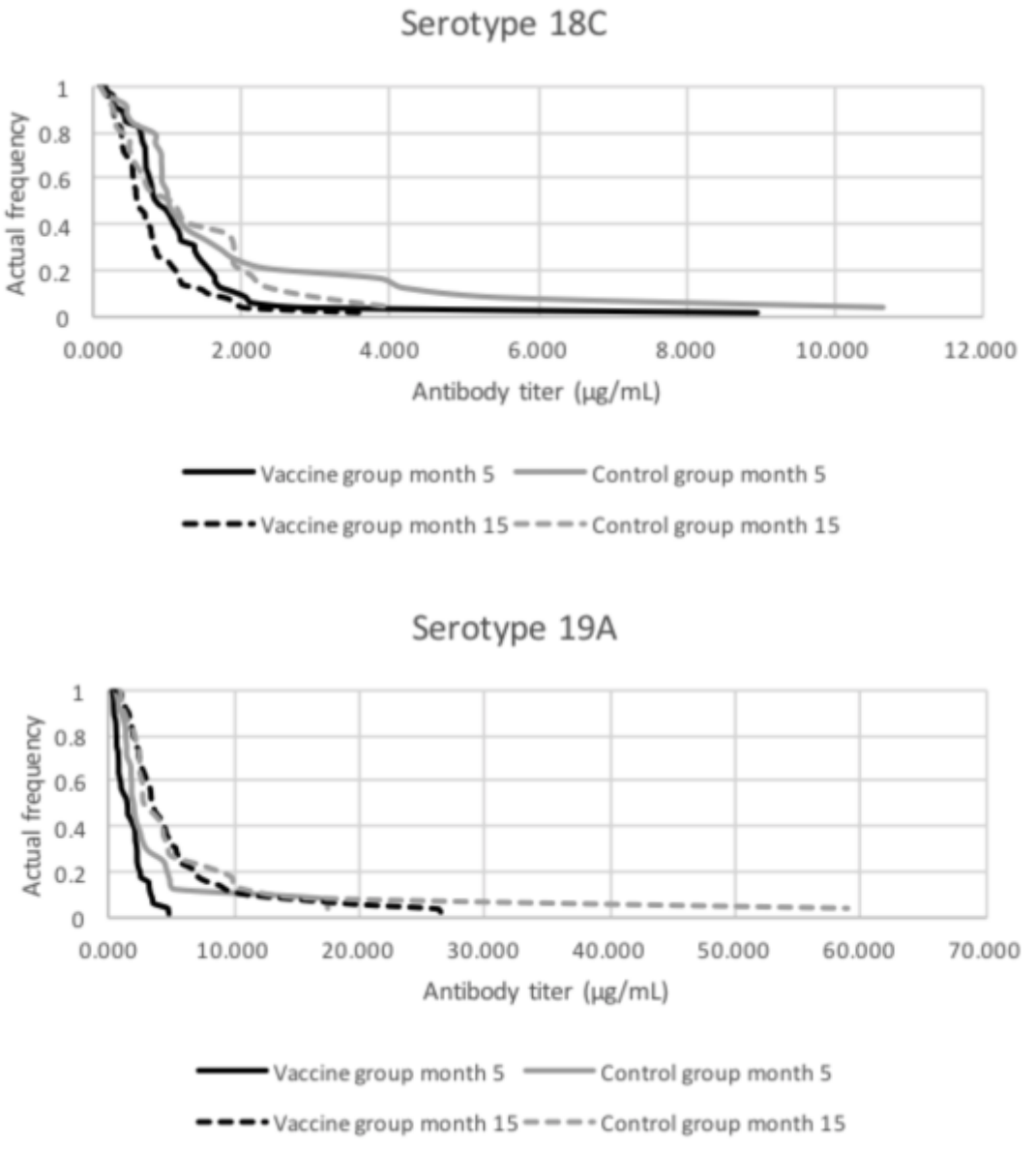
80.000
Antibody titer (µg/mL)
Vaccine group month 5
Control group month 5
Vaccine group month 15
Control group month 15


Serotype 18C
Actual frequency
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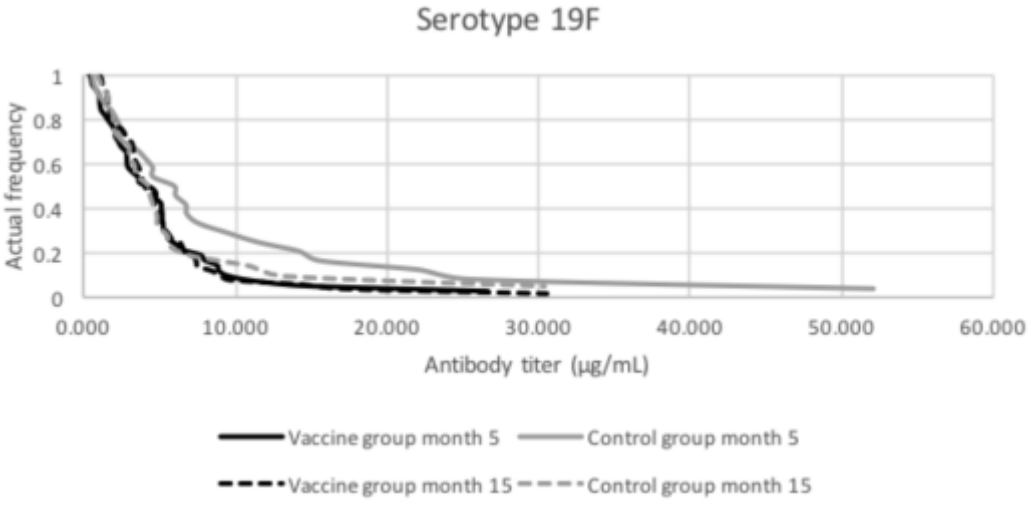
0.6
0.4
0.2
0
0.000
2.000
4.000
6.000
8.000
10.000
12.000
Antibody titer (µg/mL)
Vaccine group month 5
Control group month 5
Vaccine group month 15
Control group month 15

Serotype 19A
Actual frequency
1
0.8
0.6
0.4
0.2
0
0.000
10.000
20.000
30.000
40.000
50.000
60.000
70.000
Antibody titer (µg/mL)
Vaccine group month 5
Control group month 5
Vaccine group month 15
Control group month 15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0
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10.000
20.000
30.000
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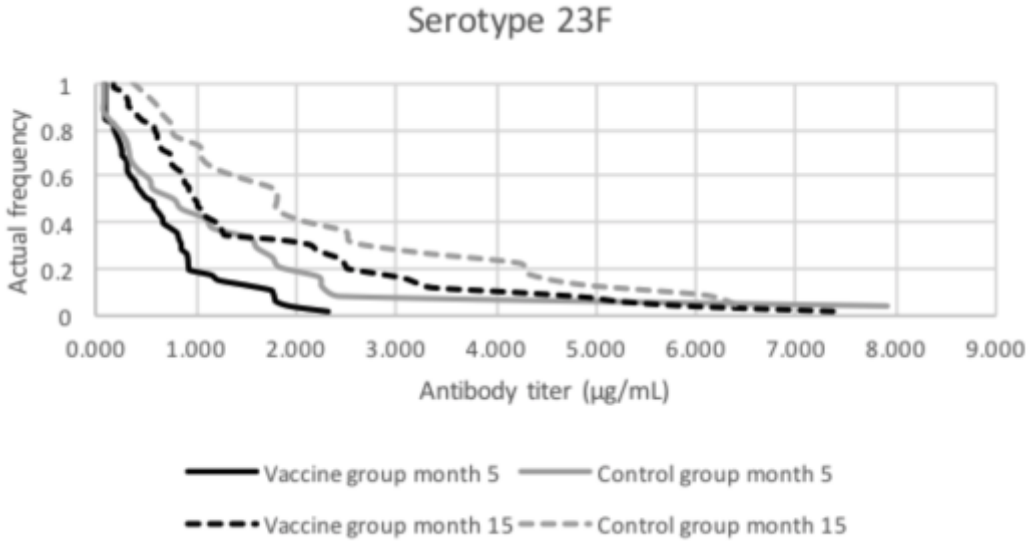
50.000
60.000
Antibody titer (µg/mL)
Vaccine group month 5
Control group month 5
Vaccine group month 15
Control group month 15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3.000
4.000
5.000
6.000
7.000
8.000
9.000
Antibody titer (µg/mL)
Vaccine group month 5
Control group month 5
Vaccine group month 15
Control group month 15

CHAPTER 5

Effect of vaccination during pregnancy on breastfeeding

CHAPTER 5/A

Effect of vaccination during pregnancy on immunological protection provided by breast milk

This chapter is published: "Maertens K., De Schutter S., Braeckman T., Baerts L., Van Damme P., De Meester I., Leuridan E. Breastfeeding after maternal immunisation during pregnancy: Providing immunological protection to the newborn: A Review. Vaccine. 2014 Apr 1;32(16):1786-1792."

doi: 10.1016/j.vaccine.2014.01.083.

Abstract

Vaccination during pregnancy results in an augmentation of disease specific maternal antibodies. Immunoglobulin G (IgG) is mainly transferred through the placenta during the third trimester of pregnancy, while secretory Immunoglobulin A (sIgA) is passed through breast milk. At birth, newborns are partially protected against infectious diseases by these antibodies.

This review aims to provide an overview of the effect of vaccination during pregnancy on the immunological protection of the newborn by the presence of disease specific sIgA antibodies in breast milk and their possible protective function against disease.

Our search produced 11 relevant papers; 1 on pertussis, 7 on pneumococcus, 2 on influenza and 1 on meningococcus.

All of the studies in this review that measured disease specific antibodies in breast milk (n=8 papers), stressed the beneficial effect of maternal vaccination during pregnancy on the amount of disease specific sIgA in breast milk. Only a few studies demonstrated a potential protective effect, particularly with influenza vaccines. In an era where maternal vaccination is increasingly considered as a valuable strategy to protect both the mother and infant, further research is needed to assess the effect on breast milk sIgA and to understand the potentially beneficial effects to the infant.

Introduction

In industrialized countries, the use of vaccines in pregnant women has been controversial. Live attenuated vaccines are contraindicated in pregnant women because of the possible transplacental transmission of the attenuated virus to the foetus. However, this recommendation is based on a theoretical risk rather than on evidence [270]. In contrast, vaccination with killed or inactivated vaccines has not been shown to cause any risk to the foetus when administered during pregnancy [271-274]. Some of these vaccines are even recommended during pregnancy [274]. This strategy has the potential to protect the pregnant woman and foetus from serious illness during pregnancy (e.g., from influenza). In addition, it provides better protection to the infants after birth and during the first months of life when they are too young to be vaccinated for certain diseases (e.g., from tetanus, influenza and pertussis) [274]. The Centers for Disease Control and Prevention (CDC) have published a summary of vaccines that may be given during pregnancy, i.e., vaccines against typhoid fever infection, Japanese encephalitis, tick-borne encephalitis, pneumococci (both conjugate and polysaccharide), hepatitis A, hepatitis B, meningococci (both conjugate and polysaccharide), cholera, inactivated polio, rabies, inactivated influenza, pertussis, tetanus and diphtheria [275].

As a rule, breastfeeding is not a contraindication to maternal vaccination nor is vaccination a contraindication to breastfeeding. The only exception is the yellow fever vaccination, as reports have demonstrated that the live attenuated virus may be transmitted through breast milk [276]. Vaccination with the yellow fever vaccine should therefore be avoided during breastfeeding.

According to the World Health Organization (WHO), colostrum is the perfect food for the newborn and should be given within the first hours of life. Furthermore, breast milk provides essential nourishment to the newborn and assures healthy growth and development. Breast milk is also known to have a protective effect against sudden infant death syndrome, infant mortality, allergic disease, necrotizing enterocolitis, gastrointestinal tract infections and respiratory tract infections [277]. The WHO recommends exclusive breastfeeding up to the age of 6 months, followed by a combination of breast milk and supplementary food up to 2 years of age or older [278].

In general, the transfer of maternal antibodies from mother to child via placental transport [120, 279] has been well documented. The placental transport of Immunoglobulin G (IgG) depends on the placental function and on the concentration of maternal antibodies in the pregnant woman [120, 252]. The concentration of IgG in women at childbearing age is defined by the previous exposure to the antigen through either disease and/or vaccination. Increased levels of maternal IgG antibodies have been described after vaccination during pregnancy [185, 280, 281]. In contrast, little is known about the effect of vaccination during pregnancy on the transfer of vaccine-induced secretory Immunoglobulin A (sIgA) maternal antibodies via breastfeeding, which is the principal immunoglobulin in breast milk [282]. SIgA protects infants by binding and opsonizing pathogenic microorganisms, thus inhibiting the colonization and invasion of the mucosal membranes of the child [128]. Via this mechanism, sIgA functions as a first-line barrier protecting the epithelium from pathogens and toxins. Other factors that are responsible for the protective effect of breast milk are lactoferrin, oligosaccharides, interleukin-10, epidermal growth factor and other anti-inflammatory factors [128, 283].

In this paper, we review the literature on the possible immunological protection provided through breastfeeding in women who were vaccinated during pregnancy. Animal studies were not selected because we cannot extrapolate the results to humans. The immune system and the role of breastfeeding in animals are entirely different from humans. In mice, for example, maternal antibodies are mainly transmitted to the offspring through breastfeeding and less often through placental transfer, as confirmed by foster feeding studies. These breast milk derived maternal antibodies of these animals provide a longer lasting protection in comparison to the antibodies transferred via the placenta [284].

The current recommendations for vaccination during pregnancy are based on evidence that vaccination during pregnancy or shortly before pregnancy has a positive effect on the amount of IgG antibodies transported through the placenta [280]. The question remains whether this vaccination also has a positive effect on the amount of maternal sIgA antibodies found in breast milk and if these breast milk antibodies will provide actual protection against infectious diseases.

Methods

SYSTEMATIC LITERATURE REVIEW

A review of the literature on transfer of maternal antibodies through breastfeeding from mothers vaccinated during pregnancy was performed according to the MOOSE (Meta-analysis of Observational Studies in Epidemiology) [285]. A Medline search was conducted using the National Library of Medicine's PubMed online search engine with a combination of the following Medical Subject Headings (MESH) terms: Vacc*, Lact*, Breast milk, Breastfeeding, Colostrum, Preg*, Pertussis, Pneumo*, Influenza, Meningo*, Tetanus, Diphtheria, Hepatitis A, Hepatitis B and Polio. We only included studies on inactivated licensed vaccines for adults that are not contraindicated for administration during pregnancy. Supplemental information was consulted based on the available references of the selected papers. No language priority was chosen. No time limitations were set.

One study was obtained through personal communication and was presented during a meeting (Annecy, Fondation Mérieux) [286].

INCLUSION AND EXCLUSION CRITERIA

The abstracts and full articles were reviewed on October 3, 2013 and were selected based on the title and the available abstract. If an abstract mentioned vaccination in combination with pregnancy and lactation, breast milk or breastfeeding, the full text was reviewed. Only human studies were included.

Results

RESULTS OF THE SEARCH

The overall search produced a total of 208 papers, of which 70 papers were repeatedly selected. A searching of the bibliographies revealed 5 further publications. On the basis of the inclusion/exclusion criteria, 22 abstracts were selected and the full papers were obtained and read. A second selection was made using the same inclusion/exclusion criteria on the full text of the papers. Only 10 publications and 1 presentation were selected of the present review (Table 1).

A summary of the selected articles and test procedures used is presented in Table 2.

	PUBMED search	**Duplicates**	**Articles selected from reference list of other publications**	**Selected on abstract**	**Final selection on full paper**
Pertussis	21 papers	1 paper	0 papers	3 papers	1 paper [93]
Pneumococcus	29 papers	5 papers	3 papers	11 papers	6 papers and 1 presentation [286-292]
Influenza	37 papers	10 papers	3 papers	7 papers	2 papers [293, 294]
Meningococcus	4 papers	3 papers	0 papers	1 paper	1 paper [295]
Tetanus	37 papers	17 papers	0 papers	0 papers	0 papers
Diphtheria	20 papers	17 papers	0 papers	0 papers	0 papers
Hepatitis A	9 papers	2 papers	0 papers	0 papers	0 papers
Hepatitis B	40 papers	10 papers	0 papers	0 papers	0 papers
Polio	11 papers	5 papers	0 papers	1 paper	0 papers
Total	**208 papers**	**70 papers**	**6 papers**	**23 papers**	**10 papers and 1 presentation**

Table 1: Overview of the number of papers selected by the inclusion/exclusion criteria for this review.

TETANUS, DIPHTHERIA, HEPATITIS A, HEPATITIS B, POLIO

The PUBMED search on tetanus, diphtheria, hepatitis A, hepatitis B and polio produced a total of 117 papers; however, none of these papers met the criteria for inclusion in the review.

PERTUSSIS

One publication was found for pertussis. This was a US (United States) study (1938) with pregnant women (N=28) who were vaccinated with 3 doses of a vaccine containing *Heamophilus pertussis* (old nomenclature for *Bordetella pertussis*). To test whether colostrum had a positive influence on the phagocytic capacity in the blood of young infants, blood was taken before and after ingestion of colostrum in a limited subsample of infants (N=5). No influence was found on opsonophagocytic activity in the blood after colostrum intake [93].

INFLUENZA

For influenza, two relevant articles from the same vaccine trial in Bangladesh were found. This trial assessed the effect of influenza vaccination during pregnancy on the transfer of maternal antibodies through breastfeeding.

In a first epidemiological paper from Bangladesh (2012), 340 pregnant women were vaccinated against influenza (N=166) or pneumococcus (N=165). A follow-up on the frequency of occurrence of respiratory illness with fever (RIF)-episodes in infants was performed from birth up to 6 months of age. All children received breastfeeding, but the duration of the breastfeeding period was variable. From the epidemiological results, there was a significant, independent protective effect of exclusive breastfeeding on the occurrence of RIF-episodes. In addition, infants of mothers who received the influenza vaccine had a reduced risk on the occurrence of RIF-episodes compared to infants from mothers who received the pneumococcal vaccine [293].

The second paper reports a subanalysis of the same Bangladesh study [294]. Of the 340 pregnant women who were vaccinated during pregnancy, breast milk samples from 57 women were analyzed, 30 of which had received trivalent inactivated influenza vaccine, and 27 received a 23-valent pneumococcal polysaccharide vaccine. At different time points in the study, breast milk samples were collected and analyzed for the presence of anti-influenza sIgA with a neutralization assay and the total amount of sIgA using an ELISA technique. In addition to these laboratory parameters, the occurrence of RIF-episodes was also monitored. The mean specific anti-influenza sIgA antibody titer was higher in the breast milk of women who received the influenza vaccine during pregnancy, and the effect lasted up to 6 months after birth. The highest titer was detected immediately after birth. The neutralization antibodies were also higher and a decreased number of RIF-episodes was detected in children of women vaccinated with influenza vaccine during pregnancy [294].

MENINGOCOCCUS

One relevant paper was selected concerning polysaccharide quadrivalent meningococcal vaccination (serotypes A, C, Y and W-135) during pregnancy and the effect on antibody concentration in breast milk. The article describes a study from Bangladesh, in which 55 pregnant women were vaccinated with either a pneumococcal or a meningococcal polysaccharide vaccine during pregnancy. In addition, all of the participants received a tetanus toxoid vaccine in the contralateral arm. At fixed time points in the study, up to 5 months after delivery, the mothers donated a breast milk sample that was analyzed for the presence of anti-meningococcal group A sIgA. The results of this study clearly showed that vaccination of the mother during pregnancy had a positive effect on the amount of meningococcal antibodies detected in breast milk in comparison to the control group up to 6 months after delivery [295].

PNEUMOCOCCUS

Most of the published manuscripts (N=6) on the transfer of maternal sIgA antibodies through breastfeeding have assessed the impact of pneumococcal polysaccharide vaccination in pregnant women. To our knowledge, no studies have been published on the use of conjugate pneumococcal vaccines during pregnancy.

The same group that reported on meningococcal vaccination during pregnancy [295], also described the impact of the pneumococcal vaccine (1995). Of the 55 pregnant women included in the study, 29 were vaccinated with the pneumococcal vaccine. Breast milk samples were collected at the same time point (Table 2), and the analysis revealed a significantly higher titer of specific sIgA in the breast milk samples of the study group compared to the control group up to 5 months after delivery [287].

Another paper describes a study in the US where 60 pregnant women were enrolled and vaccinated with either a pneumococcal (serotypes 6B, 14, 19F and 23F) vaccine (study group) or a *Haemophilus influenzae* type b (Hib) conjugate vaccine (control group) (2001). Breast milk samples were analyzed for pneumococcal and Hib specific sIgA and IgG at 2 and 7 months after delivery. The investigators found a significantly higher antibody level for both anti-pneumococcus sIgA and IgG in the study group when compared to the control group. The levels of sIgA remained higher in the study group until 7 months postpartum, while the differences in the IgG levels had disappeared at the same time point [288].

In The Gambia [289], 113 pregnant women were randomized and vaccinated with a pneumococcal (N=56) or meningococcal (N=57) polysaccharide vaccine between 24 and 32 weeks of pregnancy. Breast milk samples were collected at 0, 2, 4 and 6 months after birth. The samples

were assessed for sIgA subclass distribution and avidity of pneumococcal antibodies. The ratio of the sIgA concentration to pneumococcal polysaccharide antigens in colostrum was at least three times higher in subjects vaccinated against pneumococcus when compared to subjects vaccinated against meningococcus. The antigen-specific sIgA concentration remained higher for at least 6 months after birth [289].

A sub-study [290] of the previous study in The Gambia [289] describes an analysis of the colostrum samples from 16 women who were vaccinated during pregnancy with either a pneumococcal vaccine (study group) or a meningococcal vaccine (control group). Both the concentration and the avidity of pneumococcal sIgA antibodies were higher in the study group when compared to the control group [290].

Other research was conducted in Papua New Guinea where a total of 258 pregnant women were recruited [291]. In this study, 177 women were immunized with the pneumococcal vaccine, and the control group contained 81 non-immunized women. In addition to colostrum, the concentration of anti-pneumococcal sIgA antibodies in breast milk samples from 1-3 and 4-6 months postpartum were analyzed. Vaccination induced a humoral immune response that was measurable in both the colostrum and breast milk. The antibody concentrations were generally higher in the vaccinated group than the control group [291].

Another article describes a study in Brazil [292]. A total of 139 pregnant women were randomized and divided into 3 study groups as follows: (1) received 23-valent polysaccharide vaccination during pregnancy (group 1), (2) received the same vaccination after pregnancy (group 2), (3) received no vaccination at all (group 3). From those 139 women, 65 women (22 in group 1, 19 in group 2 and 24 in group 3) were exclusively breastfeeding until 6 months after birth. In the infant who were exclusively breastfed, the occurrence of acute respiratory infection (ARI)-episodes was monitored during the first 6 months of life. No effect was found of exclusively breastfeeding in the occurrence of ARI-episodes in either group or on pneumococcal nasopharyngeal colonization [292].

In Australia (2012), a vaccine trial was conducted on 227 pregnant women who were divided into the following three study groups: (1) received a pneumococcal vaccination during pregnancy, (2) received a vaccination at delivery and (3) received a vaccination 7 months after delivery. Breast milk specimens were taken at delivery and at month 1, 2 and 7. The amount of pneumococcal specific sIgA was measured in the breast milk samples and the highest titer was found in the group that had been vaccinated during pregnancy [286].

Disease	Number of participants	Administered vaccine	Time of administration	Country of execution	Methodology and parameter analyzed	Time of sampling and storage conditions	Outcome
PERTUSSIS							
Lichty J.R., 1938 [93]	5	Hemophilus *pertussis* containing vaccine	Third trimester of pregnancy	USA	Measurement of the opsono-cytophagic reaction for H. pertussis bacilli in blood of the infant	Blood from newborn in first period of nursing and end of first week of life Storage conditions not specified	No effect of colostrum on phagocytic reaction in the blood of the newborn
MENINGOCOCCUS							
Shahid N.S., 2002 [295]	55	Meningococcal polysaccharide vaccine (n=26; study) or 23-valent pneumococcal polysaccharide vaccine (n=29; control) and tetanus toxoid vaccine (all)	30-34 weeks of pregnancy	Bangladesh	*In-house* ELISA anti-meningococcal group A sIgA [1] (coated Ag[2]: meningococcus group A)	Colostrum at 0-3 days after delivery. Breast milk at 1 and 2 weeks and 1,3 and 5 months after delivery Storage of defatted milk at -20 °C until analysis (transported on dry ice)	Higher sIgA[1] levels in vaccinated group (both in colostrum (2.6 times higher) and breast milk at 3-6 months of age (4 times higher))
INFLUENZA							
Schlaudecker E.P., 2013 [294]	57	Trivalent inactivated influenza vaccine (n=30; study) or 23-valent pneumococcal polysaccharide vaccine (n=27; control)	Third trimester of pregnancy	Bangladesh	① *In-house* ELISA anti-influenza sIgA[1] (coated Ag[2]: recombinant hemagglutinin derived from H1N1 strain) ②*In-house* ELISA total sIgA[1] (coated Ag[2]: Rabbit anti-human IgA) ③Neutralization assay for influenza (H1N1) ④Epidemiological study to detect RIF-episodes[3]	Breast milk at birth, 6 weeks, 6 and 12 months Storage of defatted milk at -70 °C until analysis	① Anti-influenza sIgA[1] was significantly higher in influenza vaccinees up to 6 months postpartum ② Total sIgA[1] in breast milk was similar between vaccine groups ③ Neutralization titers significantly higher in influenza vaccinees at birth ④ Reduction of RIF-episodes[3] in exclusively breastfed children of influenza vaccinees up to 6 months
Henkle E., 2012 [293]	331	Trivalent inactivated influenza vaccine (n=166; study) or 23-valent pneumococcal polysaccharide vaccine (n=165; control)	Third trimester of pregnancy	Bangladesh	Epidemiological study to detect RIF-episodes[3]	Anamnestic follow-up of RIF-episodes[3] from birth up to 6 months at weekly intervals	Exclusively breastfeeding and maternal influenza or pneumococcal vaccination results in significant reduction of RIF-episodes[3] in infants

PNEUMOCOCCUS							
Munoz F.M., 2001 [288]	60	23-valent pneumococcal polysaccharide vaccine (n= 20; study) or Hib[6] conjugate vaccine (n= 40; control)	30-36 weeks of pregnancy	USA	*In-house* ELISA pneumococcal IgG and sIgA[1] (coated Ag[2]: Types 6B, 14, 19F or 23F pneumococcal capsular polysaccharide)	Breast milk at 2 and 7 months Storage of defatted milk at -70 °C until analysis	At 2 months postpartum PV[4] vaccinees had higher SIgA[1] and IgG levels in breast milk while sIgA[1] remained higher up to 7 months postpartum
Shahid N.S., 1995 [287]	55	23-valent pneumococcal polysaccharide vaccine (n=29; study) or meningococcal polysaccharide vaccine (n=26; control) and tetanus toxoid vaccine (all)	30-34 weeks of pregnancy	Bangladesh	*In-house* ELISA pneumococcal IgG and sIgA[1] (coated Ag[2]: Types 6B or 19F pneumococcal capsular polysaccharide)	Colostrum 0-3 days after delivery. Breast milk samples at 1 and 2 weeks and 1,3 and 5 months after delivery Storage of breast milk at -20°C until analysis (transported on dry ice)	Colostrum of PV[4] vaccinees contained higher anti-type 6B sIgA[1] (3x) and anti-type 19F (7x) SIgA[1] compared to controls sIgA[1] remained higher (3x) in breast milk until 5 months postpartum
Deubzer H.E., 2004 [290]	16	Polyvalent (2 types) pneumococcal polysaccharide vaccine (n=8; study) or meningococcal polysaccharide vaccine A and C (n=8; control)	Late second to early third trimester of pregnancy	The Gambia	① Adherence of *S. pneumonia* serotype 6B and 14 polysaccharides to human pharyngeal epithelial-cell line (Detroit 562) ②*In-house* ELISA pneumococcal sIgA[1] and avidity (coated Ag[2]: Types 6B or 14 pneumococcal capsular polysaccharide)	Colostrum first week after delivery Storage of defatted milk at -20 °C until analysis	Colostrum of PV[4] vaccinees induced a higher inhibition of adherence and contained significantly higher concentration and avidity of sIgA[1]
Lehmann D., 2003 [291]	258	14-valent pneumococcal polysaccharide vaccine (n=177; study) and no vaccine (n=81; control)	Between 28 and 38 weeks of pregnancy	Papua New Guinea	*In-house* ELISA pneumococcal sIgA[1] (coated Ag[2]: Types 5, 7F, 14 or 23F pneumococcal capsular polysaccharide)	Colostrum immediately postpartum. Breast milk at 1-3 months and 4-6 months postpartum Whole milk transported at -20 °C, storage of defatted milk at -70 °C until analysis	Breast milk of vaccinated women contained higher (1.1 – 1.8x) sIgA[1] levels against four serotypes (5, 7F, 14 and 23 F) up to 90 months postpartum No differences in sIgA[1] levels from 90 days until 6 months postpartum

Obaro S.K., 2004 [289]	113	23-valent pneumococcal polysaccharide vaccine (n=56; study) or meningococcal polysaccharide vaccine (n=57; control)	Between 24 and 32 weeks of pregnancy	The Gambia	*In-house* ELISA pneumococcal sIgA[1] and avidity (coated Ag[2]: Types 4, 6B, 14, 19F or 23F pneumococcal capsular polysaccharide)	Breast milk at 0, 2, 4 and 6 months postpartum Storage of defatted milk at -20 °C until analysis	Colostral sIgA[1] concentrations specific to all pneumococcal polysaccharide Ag were significantly higher among PV[4] vaccinees Titers for serotypes for 4, 6B and 14 remained significantly higher during 6 months, and those for 19F were higher up to 4 months
Lopes C.R.C., 2009 [292]	65	23-valent pneumococcal polysaccharide vaccine (n=41); and no vaccine (n=24)	After pregnancy (n=19) or between 30 and 34 weeks of pregnancy (n=22)	Brasil	Epidemiological study to detect ARI-episodes[5]	Anamnestic follow-up of ARI-episodes[5] from birth up to 6 months	No effect of exclusively breastfeeding on the occurrence of ARI-episodes[5]
Andrews R., 2012 [286]	227	Pneumococcal polysaccharide vaccine during pregnancy (n=75), pneumococcal vaccine at birth (n=75), pneumococcal vaccine at 7 months (n=77)	Between 30 and 36 weeks of pregnancy	Australia	Method not specified, sIgA[1] levels to pneumococcal polysaccharide serotype 6B, 10A, 19A and 33 F were measured.	Colostrum at delivery. Breast milk at 1,2 and 7 months postpartum Storage conditions not specified	Higher amount of sIgA[1] against all serotypes in breast milk from PV[4] vaccinees

[a]SIgA is the secretory Immunoglobulin A, [b] Ag is the antigen, [c] RIF-episode is the respiratory illness with fever episode, [d] Hib is the *Heamophilus Influenzae* type B, [e] PV is the pneumococcal polysaccharide vaccine, [f] ARI-episode is the acute respiratory infection-episode.

Table 2: Summary of selected articles and used test procedures

Discussion

Based on this review, we conclude that very little data are available on the effect of vaccination during pregnancy on the composition of breast milk and, in particular, on the presence of disease specific sIgA antibodies.

The concept of providing protection to children through breast milk is not new. Considering the US article on pertussis [93], it is obvious that there is a long history of research on the possible protective effects of breast milk. However, most research does not focus on the transfer of antibodies through breast milk that is induced by vaccination during pregnancy.

In general, the evidence retrieved from the literature suggests that vaccination during pregnancy results in better protection of the offspring during the most vulnerable months of life. This is reflected by a higher concentration of sIgA to different diseases in the breast milk samples at different time points during the first months of life (Table 2) or by a lower incidence of RIF-episodes in the young infants of influenza-vaccinated mothers [294]. There is particularly strong evidence to suggest a beneficial effect on breast milk composition when pregnant women are vaccinated against influenza [293, 294].

For some diseases there is a “correlate of protection” defined by an immune response that is responsible for and/or related to protection against this specific disease [38]. For pertussis, there is no generally accepted correlate of protection defined as a specific threshold of anti-pertussis IgG antibodies in human serum [44]. As a consequence, we cannot be sure whether the amount of antibodies transferred to the child is sufficient to provide an accurate protection during the first months of life. For influenza, pneumococcus and meningococcus on the other hand, there is a known correlate of protection [38]. However, it is unclear whether we can extrapolate these correlates of protection in serological IgG assessments to sIgA titers in breast milk. Therefore, no conclusion can be drawn on protection through breastfeeding for vaccine preventable diseases unless other outcome measures are used in the study design. In the articles by Henkle et al. [293] and Schlaudecker et al. [294], the protective effect of breast milk is determined by the occurrence of RIF- or ARI-episodes and in Lopes et al. [292] by the effect on ARI and pneumococcal nasopharyngeal colonization. Schlaudecker et al. and Henkle et al. proved that maternal influenza vaccination reduces significantly the number of RIF, although no effect was reported by Lopes et al. on ARI or nasopharyngeal carriage after maternal pneumococcal immunization.

In some animal studies the protective effect of breast milk is proven by performing foster feeding studies. In these studies, the offspring of immunized mothers are nursed by non-immunized mothers and vice versa [296]. However, this study design is not feasible in humans due to ethical constraints.

This review has some limitations. Due to the limited number of articles on one specific disease and the limitations of the study designs (small number of participants), it is difficult to draw conclusions regarding the potential protective effect of breast milk after vaccination during pregnancy. Even for pneumococcal disease, which was the most studied vaccine according to the searching results, vaccination with polysaccharide pneumococcal vaccines results in a rise in the amount of maternal antibodies in breast milk that may have a protective effect on the child; however, no effect was found on ARI-episodes [292].

The studies analyzed were conducted in different countries with different infectious disease epidemiology, which can result in a bias. Immunity is more likely to be naturally boosted in higher endemicity regions. Consequently, these boosted women have a much higher titer of naturally acquired antibodies compared to women in other countries where the pathogen is no longer circulating. These women will therefore transfer a higher amount of antibodies to their children.

A caveat in sIgA research is the fact that, at this time, no validated commercial assay for the detection of sIgA exists. Most of the commercial assays used for the analysis of breast milk are only validated for the detection of IgA. In the various studies, breast milk samples were analyzed with an in-house ELISA technique following site-specific procedures. For example, the pneumococcal antibodies in breast milk were determined with five different in-house ELISA methods. The major difference between these methods is the antigen that was coated on the microtiter plate. Furthermore, various secondary antibodies and substrates are used. The last two modifications can result in varying specificities and sensitivities. These variations can also be found in all other in-house methods. In addition, there is a lack of standardization concerning the time of collection and storage of the breast milk samples. It is likely that there is a difference in the concentrations of antibodies in the different types of milk samples at different ages and at different points during feeding (start-middle-end of feeding). The storage temperature and handling of the sample before analysis has an influence on the amount of antibodies found in the samples [297]. For example, most of the breast milk samples were centrifuged to remove the fat layer, but the exact procedures are different for the various studies.

The potential roles of T cells and cellular immunity should be investigated in view of maternal vaccination during pregnancy. In particular, in colostrum, higher amount of activated CD4+ T cells are encountered in comparison to the peripheral blood, suggesting selective migration to breast milk [298]. However, we are not aware of information in the literature regarding the role of the cellular immune response and its effect on breast milk after maternal vaccination.

In view of the current recommendations on vaccination during pregnancy for influenza, pertussis and tetanus [275], additional research is necessary to determine the influence of vaccination on specific antibodies (and, if possible, other immunologically active substances) in breast milk and possible effect of vaccination on the protection of the breastfed infant.

CHAPTER 5/B

Effect of different maternal pertussis vaccination strategies on breast milk composition

This chapter is published: "De Schutter S., Maertens K. Baerts L. De Meester I., Van Damme P., Leuridan E. Quantification of vaccine-induced Antipertussis Toxin Secretory IgA antibodies in breast milk. The Pediatric Infectious Disease Journal. 2015 Jun;34(6):e149-152."

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Abstract

Pertussis vaccination during pregnancy or immediately after delivery is a strategy that is increasingly being recommended to protect young infants from disease. Breast milk contains disease-specific antibodies that can contribute to the protection of young infants. The composition of breast milk could be altered by vaccination during pregnancy or near delivery. However, the quantification of these antibodies in breast milk lacks standardization.

In this paper, sample preparation procedures and detection methods for total and anti-pertussis toxin (anti-PT) secretory immunoglobulin A (sIgA) are proposed that can be accurately repeated and are in accordance with European Medicines Agency and Food and Drug Administration requirements. Both antibody analytes were measured in breast milk samples of lactating women obtained 8-9 weeks postpartum to compare different maternal pertussis vaccination strategies: vaccination during pregnancy, shortly after or at delivery (cocoon), less than 5 years before delivery or more than 5 years before delivery.

The validated immunoassays could quantitatively detect total and anti-PT sIgA in the processed breast milk samples. Significantly higher levels of anti-PT sIgA were measured in breast milk after pertussis vaccination during pregnancy or at delivery [Geometric mean concentration (GMC): 2.56 and 2.15 IU/mg] in contrast to mothers with no recent (>5 years) pertussis vaccination (GMC: 0.96 IU/mg; P=0.014 and P=0.028).

Vaccination against pertussis in the second/third trimester of pregnancy or immediately postpartum significantly increased the levels of anti-PT sIgA in breast milk.

Introduction

In several industrialized countries with long-standing and successful infant vaccination programs, pertussis has become an increasing health problem, particularly in young vulnerable infants, who are too young to be protected by the current infant vaccination programs. As a result of epidemiological changes, some countries have decided to implement additional recommendations for pertussis vaccination, such as additional booster doses in adolescents and adults [43, 299], the cocoon strategy [300] (i.e., vaccinating adults that are in regular contact with newborns and young infants) and vaccination during pregnancy (gestational vaccination), to reduce the number of (severe) pertussis cases in young infants (e.g., in United States, United Kingdom and Belgium) [43, 275, 301]. Since August 2013, pregnant women in Belgium have been recommended to receive a booster vaccination against pertussis during each pregnancy between 24 and 32 weeks of gestation; if this vaccination was missed, a pertussis booster vaccination is offered immediately at or soon after delivery (as part of the cocoon vaccination strategy) [43].

Human breast milk contains a variety of components that positively influence the nutritional status, general development and overall health of newborns and young infants. The World Health Organization recommends colostrum within the first hours of life and exclusive breastfeeding up to the age of 6 months [278]. Breast milk contains several classes of immunoglobulins (Ig): IgG, secretory Ig (sIg) M and sIgA, the latter being the predominant Ig in breast milk [302]. These sIgA antibodies are secreted into the lumens of mucosal organs [303] and can provide protection to the infant by binding pathogenic microorganisms, thereby inhibiting the invasion and colonization of the mucosal membranes [304]. Disease-specific sIgA antibodies are able to bind microorganisms directly, although the Fc part of the sIgA molecule can bind bacterial carbohydrates [305]; sIgA functions as a first-line barrier to protect the epithelium from pathogens and toxins. Maternal vaccination (during pregnancy as well as immediately after delivery) can increase the amount of specific sIgA transmitted through breast milk [159], which was recently shown for pertussis vaccination during pregnancy by Abu Raya et al. [112].

A thorough literature survey demonstrated that no detection methods for the measurement of sIgA against vaccine-preventable diseases in breast milk have been thoroughly validated [159]. In general, data on pre-analytical procedures for breast milk are rather scarce [306]. Well-validated assays are needed to generate accurate data that can be easily compared among laboratories. An ongoing study on pertussis vaccination in pregnant women in Belgium (Clinicaltrial.gov NCT01698346) offered the opportunity to validate commercial immunoassays and to analyze the influence of different maternal vaccination strategies on total sIgA and anti-pertussis toxin sIgA in breast milk.

Methods

STUDY DESIGN

A prospective study on pertussis vaccination in pregnant women is ongoing in Antwerp, Belgium (clinicaltrials.gov NCT01698346). Breast milk samples were collected from some of the participants in this study (N=35). Additional breast milk samples (N=39) were collected randomly from other women who had delivered 8-9 weeks before. Ethical approval was obtained from the ethical committee of the University Hospital Antwerp/University of Antwerp. Informed consent was obtained from all participants. This study was conducted in accordance with the International Conference on Harmonization – Good Clinical Practice and procedures established by Belgian law. All the participating women were divided into 4 groups according to their pertussis vaccination status: vaccinated during pregnancy (group 1), vaccinated shortly after or at birth (group 2), vaccinated less than 5 years before delivery (group 3) and no vaccination for at least 5 years before delivery (group 4). The combined tetanus, diphtheria, acellular pertussis (Tdap) vaccine Boostrix® (GlaxoSmithKline Biologicals, Rixensart, Belgium) was used in groups 1-3. Vaccination during pregnancy was performed during the second or third trimester. Women who delivered prematurely or who had received another vaccine or any blood product in the previous month were excluded.

Human breast milk samples were collected between March 11, 2013 and June 2, 2014. The samples were obtained at a median of 58 days after delivery (min-max: 44-91 days). A questionnaire on vaccination status was completed, and the patient's medical history, side of sampling (right or left breast), method of expression (machine vs. manual sampling), exclusive breastfeeding or breastfeeding in combination with formula feeding and time of sampling in relation to last breastfeeding session were recorded. The women were asked to keep the samples in the refrigerator until transport. A cold-chain transport to the laboratory was organized. Upon arrival, the samples were immediately aliquoted and processed.

SAMPLE PREPARATION

Three different centrifugation conditions that are described in the literature were applied to clarify the breast milk [129, 131, 297, 307-313]. In the first procedure, fat and cellular elements were removed by 2 consecutive centrifugations. The first centrifugation was performed at 1000g for 10 minutes at 4°C. After the intermediate aqueous phase was collected, this fraction was centrifuged again at 10,000g for 30 minutes at 4°C. The other 2 procedures consisted of a single centrifugation: one at 1000 g for 10 minutes at 4°C and the other one at 10,000g for 30 minutes at 4°C. Subsequently, the aqueous phase was aliquoted to avoid repeated freeze-thaw cycles and stored at -20°C. The breast milk samples were evaluated using commercial enzyme-linked immunosorbent assays (ELISAs) for total sIgA ("ELISA for human IgA", Mabtech AB, Nacka Strand, Sweden) and anti-pertussis toxin (anti-PT) sIgA ("anti-*Bordetella pertussis* toxin IgA ELISA", Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany). Both of the immunoassays were performed according to the manufacturer's recommendations except sample dilution in this series. Breast milk samples were diluted 1:30,000 for the analysis of total sIgA and 1:10 for analysis of anti-PT sIgA. Only readouts within the standard curve were included in the analysis.

VALIDATION OF THE "TOTAL sIgA" AND ANTI-PT sIgA" ASSAY FOR ANALYSIS OF BREAST MILK

The following validation criteria were examined: sensitivity, linearity, precision and accuracy based on the guidelines from European Medicines Agency and Food and Drug Administration [314, 315]. Supplemental content presenting results of the validation of total sIgA and anti-PT sIgA assays for analysis of breast milk and the effect of different centrifugation methods on the amount of antibodies detected is available upon request.

STATISTICAL ANALYSIS

All of the statistical analyses were performed using Statistical Package for Social Science Version 20. Total anti-PT and adjusted sIgA antibody levels in breast milk are expressed as the geometric mean concentration (GMC) with confidence intervals (CI). Because of the fluctuations in the concentration of total sIgA in breast milk, the anti-PT sIgA levels were adjusted by dividing anti-PT sIgA levels by the total sIgA levels. The 4 vaccination strategies were compared using a one-way analysis of variance statistical test. A post-hoc analysis was performed using the Fisher's least significant difference test. The influence of several variables on the GMC was studied using an independent samples t-test, a linear regression and Pearson's and Spearman correlations. P-values less than 0.05 were considered significant for all of the statistical tests used.

Results

All of the validation criteria for the total sIgA and anti-PT sIgA measurements with the proposed commercial immunoassays are in accordance with the guidelines by the European Medicines Agency and Food and Drug Administration.

The best procedure to ensure no interference from lipids while retaining maximal antibody concentrations was to centrifuge the breast milk samples twice (at 1000g for 10 minutes and then at 10,000g for 30 minutes).

Of the 74 participating women, 19 were vaccinated during pregnancy (group 1), 34 were vaccinated shortly after or at delivery (group 2), 9 were vaccinated less than 5 years before delivery (group 3) and 12 had received no vaccination for at least 5 years before delivery (group 4). The median number of postpartum days was significantly higher for group 3 compared with group 1 ($p=0.001$), group 2 ($p=0.001$) and group 4 ($p=0.005$). However, no significant influence of time since delivery on antibody levels was found. Additionally, no significant difference in time interval since last breastfeeding (in minutes) was found between the different groups (Table 1).

In addition to the timing of maternal vaccination, several other variables were analyzed as possibly influencing the antibody levels: the time interval between the last breastfeeding session and sampling, exclusive breastfeeding or combined nutrition (breast milk and formula milk) as well as the method of milk expression (manual vs. mechanical) and the side of sampling (left or right breast).

No significant difference was found for the total sIgA levels in the 4 groups (Table 2). The total sIgA titer was influenced by the time interval since the last breastfeeding: there was a significant linear relation ($p=0.003$ and R^2 value=0.121) between the time since previous breastfeeding and the total sIgA titer. The longer the time interval since breastfeeding was, the higher the titer of total sIgA. In addition, a significant difference in total sIgA level was detected between women giving exclusively breast milk and women also feeding their child with formula milk ($p=0.001$), with higher titers in women giving combined nutrition. No significant difference was observed with regard to the other variables: method of milk expression or the breast side of sampling.

Regarding anti-PT sIgA, a significant difference was found between the groups (Table 2). Women who were vaccinated during pregnancy ($p=0.012$) or shortly after or at birth ($p=0.001$) showed significantly higher levels of anti-PT sIgA compared with women with no vaccination for at least 5 years before delivery. In addition, significantly higher anti-PT sIgA titers ($p=0.013$) were encountered in the breast milk of women who breastfeed in combination with formula feeding

compared with women who exclusively breastfeed. No significant difference was observed for time since previous breastfeeding, method of milk expression or breast side of sampling.

Maternal vaccination during pregnancy or at birth with a PT-containing vaccine significantly increased the adjusted GMC of anti-PT sIgA antibody compared with women without recent (>5 years) pertussis vaccination (Table 2). A statistically significant difference was found between groups 1 and 4 ($p=0.014$) and between groups 2 and 4 ($p=0.028$). No significant difference was observed for time interval between last breastfeeding session and sampling, combined nutrition, method of milk expression or the side of sampling.

TABLE 1. Characteristics of the Breast Milk Sampling in Each Study Group

Characteristics	Vaccination During Pregnancy (Group 1)	Vaccination Shortly After or at Birth (Group 2)	Vaccination Less Than 5 Years Before Delivery (Group 3)	No Vaccination 5 Years Before Delivery (Group 4)
Number of samples	19	34	9	12
Median number of postpartum days at sampling (Min–Max)	58 (50–64)	57 (44–79)	62 (56–91)	58 (53–68)
Median number of minutes since last breastfeeding (Min–Max)	129 (30–765)	112.5 (10–570)	150 (5–645)	110 (30–285)
Median number of days from vaccination to sampling (Min–Max)	132 (111–169)	55 (35–76)	997(417–1782)	Not known

Table 1: Characteristics of the breast milk sampling in each study group.

TABLE 2. Geometric Mean Concentration with the 95% CI Between Brackets for Anti-PT sIgA, Total sIgA and Adjusted anti-PT sIgA in Breast Milk Samples in the 4 Study Groups

	Vaccination During Pregnancy (Group 1)	Vaccination Shortly After or at Delivery (Group 2)	Vaccination Less Than 5 Years Before Delivery (Group 3)	No Vaccination 5 Years Before Delivery (Group 4)
Anti-PT sIgA in IU/mL (95% CI)	0.55 (0.31–0.98)	0.66 (0.44–0.97)	0.51 (0.29–3.22)	0.19 (0.16–0.23)
Total sIgA in mg/mL (95%CI)	0.22 (0.17–0.28)	0.31 (0.25–0.38)	0.29 (0.18–0.48)	0.20* (0.15–0.28)
Adjusted anti-PT sIgA in IU/mg (95% CI)	2.56 (1.42– .00)	2.15 (1.53–3.02)	1.73 (1.07–2.80)	0.96* (0.67–1.38)

*The concentration of total sIgA was not determined for one sample in study group 4.

Table 2: Geometric mean concentration with the 95% CI between brackets for anti-PT sIgA, total sIgA and adjusted anti-PT sIgA in breast milk samples in the 4 study groups.

Discussion

Human breast milk is a valuable source of bioactive components necessary for the health and development of the neonate. Comparison of laboratory techniques to measure these bioactive components is difficult because many different in-house methods are used, and papers often do not provide adequate details regarding method validation and applied procedures. Therefore, for the first time, we validated commercial immunoassays to detect anti-PT sIgA in breast milk. The validation results of the total sIgA and the anti-PT sIgA immunoassay support the proposed sample preparation procedure as well as the use of the tested commercial assays.

For the first time, this paper describes the effect of several pertussis vaccination strategies in adult women (vaccination during pregnancy, vaccination shortly after/at delivery, vaccination less than 5 years before delivery and no vaccination for 5 years prior to delivery), on anti-PT sIgA levels in breast milk. Breast milk from mothers vaccinated during pregnancy or shortly after/at delivery shows significantly higher levels of anti-PT sIgA and adjusted anti-PT sIgA levels at 8-9 weeks postpartum compared with mothers without recent (>5 years) pertussis vaccination. Total sIgA levels are affected by the interval since the last breastfeeding session. Both total sIgA levels and anti-PT sIgA levels are influenced by giving combined nutrition to infants in the form of breast milk and formula milk. The lower frequency of breastfeeding might be compensated by a higher load of sIgA in the breast milk.

There are some limitations to this study. First, the women were free to choose the moment of sampling with regard to previous breastfeeding session (time interval), method of expression, the side of sampling and the moment within the breastfeeding session (start-middle-end). Despite the fact that no significant difference was observed between groups with regard to method of expression and the side of sampling, the use of more standardized collection methods is advisable for future studies, whenever feasible. Second, the breast milk samples were obtained at one time point, that is, 8-9 weeks postpartum, which makes it impossible to describe the kinetics of anti-PT sIgA levels in breast milk from mothers vaccinated shortly after/at delivery and mothers vaccinated during pregnancy. Finally, a limited number of breast milk samples (N=73) were collected.

Notwithstanding these limitations, our study shows that even at 8-9 weeks postpartum, breast milk from mothers who have recently been vaccinated, contains significantly higher anti-PT sIgA and adjusted anti-PT sIgA levels. Although children born at term transplacentally acquire disease-specific IgG, which offers protection to the neonates [169], passively acquired maternal IgG antibodies are lower or lacking in premature born infants because of immature transplacental

transport [116, 123]. Women who deliver prematurely have higher total sIgA levels in their colostrum compared with women who deliver at term [129]. This increased sIgA could perhaps compensate for the lack of transplacentally acquired IgG in preterm born infants. Halperin et al. [73] report that the anti-pertussis sIgA levels in breast milk from women vaccinated postpartum (within 24 hours of delivery) reach a maximum at 10 days after vaccination and then slowly decrease. The potentially higher titers in colostrum after vaccination during pregnancy, in contrast to vaccination shortly after/at delivery, may be a clear benefit for preterm born children, who lack transplacental IgG maternal protection. Nevertheless, if vaccination during pregnancy is not performed, then preterm infants can still benefit if their mothers are vaccinated shortly after/at delivery.

Until very recently, no data on specific anti-PT sIgA levels in breast milk were available. Using an analytical procedure based on the method we presented at the Tenth International Symposium on Bordetella (Dublin, September 8-11, 2013) [316], Abu Raya et al. reported that colostral pertussis IgA antibody levels were significantly higher among women vaccinated with Tdap during pregnancy compared with unvaccinated women. They used a 1 of 101 dilutions of the breast milk samples, as recommended by the ELISA insert, and detected adequately the sIgA in colostrum. A significant decline in the anti-PT sIgA level over time was observed in breast milk at 2, 4 and 8 weeks postpartum [112]. This decrease in anti-PT sIgA could reflect a parallel decrease in total sIgA from colostrum to mature milk [317]. In contrast to their findings, we observed significantly higher anti-PT sIgA levels in breast milk at 8-9 weeks postpartum after vaccination during pregnancy. These observed differences could be the result of different epidemiological settings with more or fewer boosting opportunities for adults, or differences in sample handling and detection methods. We show in this paper that samples taken at 8 weeks postpartum need an adapted dilution protocol compared with the ELISA insert of the company. In addition, we included women vaccinated shortly after/at delivery and found that their breast milk anti-PT sIgA levels at 8-9 weeks postpartum were comparable with women vaccinated during pregnancy. Both vaccination during pregnancy and shortly after delivery were associated with increased levels of anti-PT sIgA in breast milk at 8 weeks postpartum.

New cohort studies are planned with several collection time points for breast milk samples both in term and preterm delivering women offering the opportunity to assess the influence of different vaccination strategies on the kinetics of specific antibody concentrations in colostrum and breast milk.

Acknowledgments

The authors thank all participating subjects, women and children, as well as Mrs. Aline Bontenakel, study nurse, who performed the sampling.

Annex 1

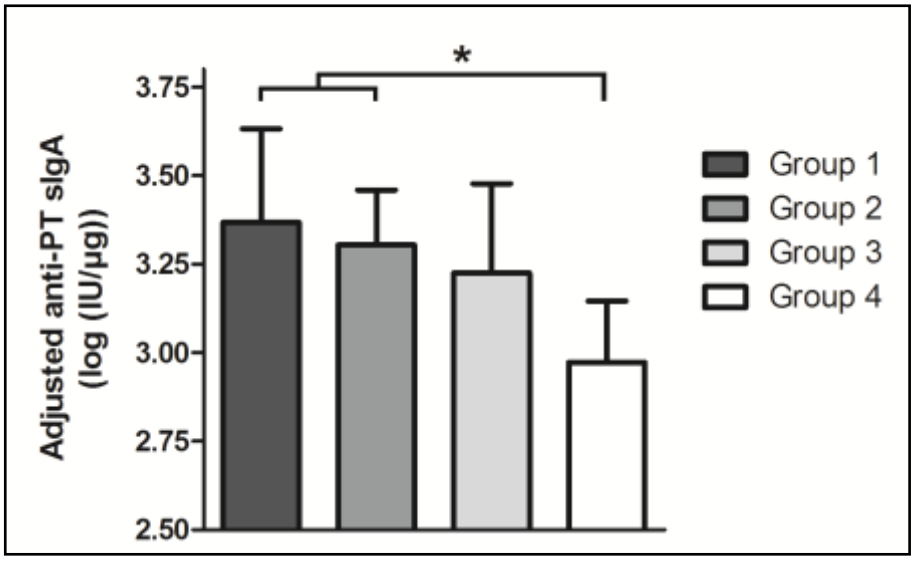

Figure 1: Geometric mean concentration (with 95% CI) of log transformed adjusted anti-PT sIgA in breast milk after different maternal vaccination strategies. Vaccinated during pregnancy (group 1), vaccinated shortly after/at delivery (group 2), vaccinated less than 5 years before delivery (group 3) and no vaccination 5 years before delivery (group 4).

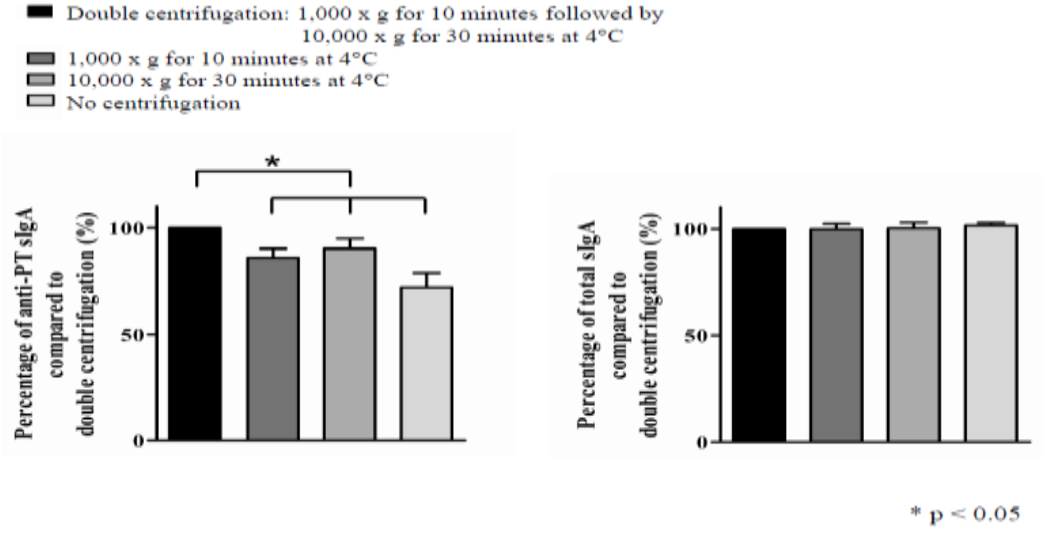

Figure 2: Concentration of anti-PT sIgA and total sIgA after different centrifugation procedures described in the literature.

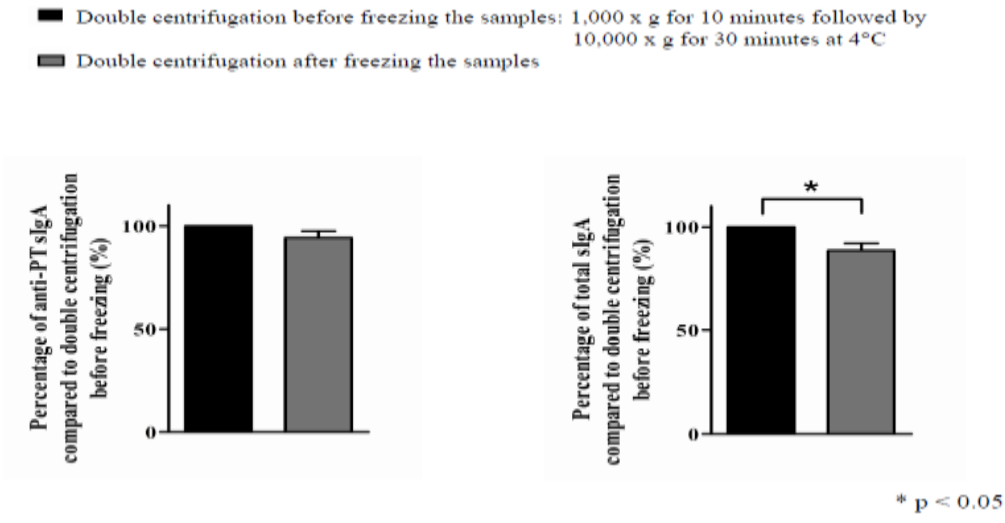

Figure 3: Concentration of anti-PT sIgA and total sIgA after the double centrifugation procedure before and after breast milk was frozen at -20°C.

Annex 2

VALIDATION OF THE 'TOTAL sIgA' AND 'ANTI-PERTUSSIS TOXIN sIgA' ASSAY FOR ANALYSIS OF BREAST MILK: METHODS AND CRITERIA

Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were established by analyzing the sample buffer in quadruplicate. The mean of this measurement was count up with the standard deviation which was multiplied by three or ten to receive the LOD and LOQ respectively.

Linearity

The linearity was verified by measuring the concentration in the aqueous fraction of a breast milk sample in several different dilutions (total sIgA: n=5; anti-PT sIgA: n=6) that were measured in quadruplicate for total sIgA and in duplicate for anti-PT sIgA. The precision of the final concentration after correction for dilution should not exceed 20%, according to the requirements from European Medicines Agency (EMA) [314].

Precision

Precision may be considered at different levels: within-run and between-run. Breast milk samples with diverse concentrations of the analyte were used for determining the precision. The maximum variation of precision (expressed as coefficient of variation (CV)) may not exceed 15% (20% at LOQ) or 20% (25% at LOQ) according to the guidelines from Food and Drug Administration (FDA and EMA respectively [314, 315]. The within-run precision was obtained by analyzing 6 or 7 breast milk samples in quadruplicate for total and anti-PT sIgA respectively. The between-run precision was defined by measuring 6 or 10 samples in three separated runs for total and anti-PT sIgA respectively.

Accuracy

This validation criterion was determined by adding a known amount of the analyte to a breast milk sample (quality control sample (QC)). We preferred to add a concentration of the analyte reflecting the amounts of the analyte in breast milk samples. Afterwards, the total recovery of the analyte was determined. According to the guidelines from Food and Drug Administration (FDA), a minimum of 67% of the QC samples should be within 15% of the nominal and a maximum of 33% of the QC samples may exceed 15% of the nominal concentration [315]. Following the guidelines from EMA for ligand binding assay, the mean concentration should be within 20% at the nominal concentration at each concentration level and 25% at the LOQ [314].

When defining the accuracy for total sIgA, purified sIgA (sigma-aldrich) was added to a breast milk sample. In addition, the nominal concentration was analyzed by adding the same amount of purified sIgA to the sample buffer.

CHAPTER 6

Coverage of pertussis vaccination during pregnancy in Flanders, Belgium

CHAPTER 6/A

Coverage of pertussis and influenza vaccination during pregnancy in Flanders, Belgium: October 2014 – May 2015

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Abstract

In Belgium, pertussis vaccination is recommended for all pregnant women in every pregnancy. Adults in close contact with young infants are equally advised to receive a pertussis containing booster dose. Maternal influenza vaccination is likewise recommended in Belgium in the second or third trimester of pregnancy, within the influenza season.

A quantitative multicenter survey study has been performed between October 2014 and May 2015 in both postpartum women (N=823, response rate= 89.2%) and health care workers (HCWs) (N=261) to assess the coverage of both vaccines during pregnancy along with the coverage of the pertussis cocoon strategy, and to evaluate the knowledge and recommending attitude of HCWs towards maternal vaccination strategies and the cocoon strategy among surveyed women and HCWs.

Overall coverage of pertussis vaccination during pregnancy was 64.0%. Most women were vaccinated by their general practitioner (GP) (82.4%), and most often in the third trimester (74.0%) of pregnancy. Overall coverage of influenza vaccination during pregnancy was 45.0%. Again, the GP administered most vaccines (67.6%); vaccines were equally administered in the second or third trimester of pregnancy. Educational level had a significant influence on both the pertussis and influenza vaccination coverage during pregnancy while working situation and parity had only an influence on the maternal pertussis vaccination coverage and country of birth only on the maternal influenza vaccination coverage.

Overall, 78.4% of gynecologists and GPs recommends both maternal pertussis and influenza vaccination and 67.0% recommends both maternal vaccination strategies and the cocoon strategy. Within the group of the midwives, only 23.7% recommends both maternal pertussis and influenza vaccination and 10.5% recommends both maternal vaccination strategies and the cocoon strategy.

High coverage is reached among pregnant women for pertussis and influenza vaccination. Several underserved populations of pregnant women regarding maternal immunization, are identified.

Introduction

Pregnant women and neonates are at increased risk for vaccine-preventable disease-related morbidity and mortality [318]. Pertussis is a global cause of morbidity and mortality in infants too young to be protected by the currently available vaccines and vaccination schedules. In 2013, whooping cough caused an estimated 63,000 deaths in children below 4 years of age worldwide [1]. In Belgium, the number of confirmed pertussis cases also increased during the last decade with the highest incidence in the youngest infants. Some of these cases ended fatal, with 1-5 cases yearly before 2012. After 2012, no infant fatalities due to pertussis have been notified (Oral communication Scientific Institute of Public Health Belgium). Globally, yearly influenza epidemics are estimated to result in 3-5 million influenza cases and 250,000-500,000 deaths [319]. Pregnant women as well as children under 6 months of age who are too young to be vaccinated with the currently available vaccines, are vulnerable to severe disease resulting in a high rate of influenza related hospitalizations and deaths [320].

Maternal pertussis and influenza vaccination programs have already proven to be effective in preventing illness and hospitalization in both pregnant women and newborn infants [188, 321]. According to the recommendations of the World Health Organization (WHO), vaccination of pregnant women with a tetanus, diphtheria, acellular pertussis (Tdap) vaccines in the second or third trimester of pregnancy (at least one week prior to delivery) should be introduced as a routine complementary strategy in countries with increasing infant morbidity and mortality from pertussis [1]. For influenza, WHO recommends vaccination with inactivated influenza vaccines at any stage of pregnancy. However, the Strategic Advisory Group of Experts (SAGE) of WHO emphasized in April 2015, that maternal influenza vaccination is not a universal recommendation but a recommendation to maximize beneficial effects of influenza vaccines in countries with existing, or initiating new, influenza vaccination programs [322, 323].

In Belgium, national recommendations are made by the National Immunization Technical Advisory Group (NITAG). Implementation of the vaccination policy is managed at the subnational level of the 3 regions: the Flemish, Brussels Capital and Walloon region. Pertussis vaccination during pregnancy has been recommended since August 2013 for pregnant women during every pregnancy between 24 and 32 weeks of gestation. If the vaccine is not given during pregnancy, it should be administered in the immediate postpartum within the cocoon strategy. Additionally, all adults in close contact with young infants have been advised to receive a pertussis booster dose once during adult life as part of the cocoon strategy since 2009 [43]. Maternal influenza vaccination has been recommended in Belgium for pregnant women in the second or third

trimester of pregnancy coinciding with the influenza season, for more than 10 years [324]. In Flanders, adult pertussis booster has been free of charge since July 2014 and the influenza vaccine is available for pregnant women at a reduced fee.

Health care workers (HCWs) are frequently involved in nosocomial outbreaks of pertussis and influenza infection [325, 326]. Therefore, all HCWs, especially those in contact with risk groups such as pregnant women and newborn infants, should be immunized with an acellular pertussis (aP) containing vaccine and influenza vaccine to minimize potential exposure to patients [43, 324].

Achieving vaccine acceptance among both pregnant women and their health care providers is an important healthcare challenge. To identify potential barriers that could be addressed in order to improve the maternal vaccination coverage, a quantitative cross-sectional survey study has been performed. The main aim of the study was to determine the coverage of pertussis and influenza vaccination during pregnancy, along with the assessment of the pertussis cocoon strategy in Flanders. In addition, we aimed to assess the women's awareness and attitude towards the existing maternal immunization recommendations. In a second part of the study, HCWs were interviewed to evaluate their attitude towards the existing maternal vaccination strategies and the cocoon strategy and to determine the pertussis vaccination coverage among this occupational group.

Methods

STUDY DESIGN

A quantitative cross-sectional multicenter study was performed in all five provinces in Flanders, Belgium, between October 2014 and May 2015. Within a group of postpartum women, questions regarding awareness, coverage and attitude towards the existing maternal vaccination recommendations and the cocoon strategy were asked (Annex A). Within a group of HCWs, questions regarding awareness, informing and recommending attitude on maternal vaccination and cocoon vaccination were asked (Annex B). The study was approved by the ethical committee of the University hospital of Antwerp, Belgium (leading ethical committee) and by the regional ethical committees of all collaborating hospitals.

STUDY POPULATION

Postpartum women

In Flanders, 35 hospitals with more than 800 deliveries per year were identified. From these hospitals, 10 hospitals were selected for participation in the study through random sampling; all selected hospitals agreed to participate. The number of participating hospitals per province was proportional to the number of hospitals per province. Surveys were taken by trained investigators from hospitalized postpartum women. All potential participants were informed on the background, objectives and privacy rules related to the survey. Written informed consent was obtained from all participating women. Exclusion criteria were: aged below 18 years; languages other the Dutch, English, French and Arabic or absence of signed informed consent. The participants did not receive any payment.

Health care workers

Gynecologists and midwives in each participating hospital (in-hospital HCWs) and general practitioners (GPs) in Flanders were invited to complete an encoded questionnaire. In-hospital HCWs received a cover letter together with the questionnaire to explain the purpose of the survey. Several reminder e-mails were sent and the study was also promoted during scheduled staff meetings. GPs were surveyed during symposia or training courses. HCWs did not receive any incentive for participation.

DATA COLLECTED

Both questionnaires used a combination of check boxes and free text answers. A pilot survey in both target groups was performed to ensure comprehensiveness.

From postpartum women, data were collected on socio-demographic background and obstetrical conditions including gestational age at delivery and complications during pregnancy. Knowledge, attitudes and behavior towards recommendations for vaccination during pregnancy and the cocoon strategy were addressed as well as their vaccination status.

From in-hospital HCWs and GPs, data were collected on demographical background, knowledge and attitude towards current recommendations for maternal pertussis and influenza vaccination and the cocoon strategy and their current pertussis vaccination status (Questionnaires in annex, can be provided upon request).

STATISTICAL ANALYSIS

Questionnaires from both postpartum women and HCWs were collected and encoded data were entered into two separate Microsoft Access 2013 databases.

Statistical analysis was performed using SPSS version 23.0. Statistical tests included parametric tests: t-tests and chi-square tests and their non-parametric alternatives: (paired) Wilcoxon tests and Fisher exact tests whenever the underlying assumptions of the parametric tests were violated i.e. normality and sparseness, respectively [220, 221]. Multiple logistic regression models were used to identify determinants that could potentially influence maternal pertussis and influenza vaccination coverage. Only significant influences of variables on the vaccination coverage were reported. A p-value <0.05 was considered as statistically significant.

Results

DEMOGRAPHICS

Questionnaires from postpartum women were collected between October 20th 2014 and May 6th 2015. Of the 923 women approached, 823 agreed to participate (overall response rate: 89.2%). Main reasons for non-participation were: failure to obtain informed consent, minor aged participant, language, etc. All participants were pregnant during the period in time whereas the recommendations were in place and influenza vaccines were available. Therefore, the opportunity to receive the influenza vaccine during pregnancy was completely dependent on the recommending behavior of the HCWs. Surveys were collected on average 2.4 days postpartum. Demographic details of the participating women are summarized in Table 1. The surveyed women were demographically representative for the total population of young mothers in Flanders [327].

Questionnaires of HCWs were collected between October 11th 2014 and May 20th 2015. In total, 261 HCWs were surveyed: 103 in-hospital HCWs and 158 GPs. Demographic details of the participating HCWs can be found in Table 1. Mean age of the in-hospital HCWs was significantly lower ($p<0.001$) compared to the mean age of the GPs.

Demographic characteristics of the study participants.

Women		
Mean age in years (SD)		29.8 (4.8)
Number of days postpartum at moment survey (SD)		2.4 (1.7)
Mean gestational age at birth in weeks (SD)		39.3 (1.6)
Gestational age at birth, No. (%)	<37 weeks	39 (4.7)
	⩾37 weeks	784 (95.3)
Country of birth, No. (%)	Belgium	618 (75.1)
	EU-country	59 (7.2)
	Non EU-country	146 (17.7)
Country of birth of parents, No. (%)	Belgium	523 (63.5)
	EU-country	77 (9.4)
	Non EU-country	223 (27.1)
Highest level of education, No. (%)	No education	13 (1.6)
	Primary school	65 (7.9)
	Secondary school	334 (40.6)
	Bachelor degree	256 (31.1)
	Master degree	150 (18.2)
	Doctoral degree	4 (0.5)
	Unknown	1 (0.1)
Employment situation, No. (%)	Full-time	410 (49.8)
	Part-time	141 (17.1)
	Independent	47 (5.7)
	Unemployed with alimony	87 (10.6)
	Unemployed without alimony	118 (14.3)
	Student	18 (2.2)
	Other	2 (0.2)
Parity, No. (%)	Primiparous	372 (45.2)
	Multiparous	451 (54.8)
In-hospital health care workers		
Mean age in years (SD)		35.6 (11.1)
Gender, No. (%)	Male	10 (9.7)
	Female	93 (90.3)
Occupation, No. (%)	Gynecologist	27 (26.2)
	Midwife	76 (73.8)
General practitioners		
Mean age in years (SD)		46.9 (12.2)
Gender, No. (%)	Male	78 (49.4)
	Female	80 (50.6)

Table 1: Demographic characteristics of the study participants.

UPTAKE OF PERTUSSIS AND INFLUENZA VACCINE DURING PREGNANCY

Of the 823 respondents, 527 women (64.0%) were vaccinated with an aP containing vaccine during their current pregnancy. Most women were vaccinated in the second (24.3%) or third (74.0%) trimester of pregnancy. For women who recalled their vaccination date (N=225), the moment of vaccination was on average at 30.8 weeks of pregnancy (SD 3.8). No seasonality in pertussis vaccination coverage was seen (Fig. 2A). Maternal pertussis vaccination was in most cases recommended by the gynecologist (87.9%). In contrast, vaccination was mostly performed by the GP (82.0%) (Fig. 1). Main reasons for not being vaccinated with Tdap during pregnancy were: vaccine not offered during pregnancy by HCW (49.7%), Tdap vaccination during previous pregnancy (19.9%), vaccination discouraged by HCW (7.8%), forgetfulness (6.4%), safety concerns (4.7%) and others (8.1%).

From the 296 women who did not receive the pertussis vaccine, only ten women (3.4%) were vaccinated postpartum at the moment of the survey. Main reasons for not yet receiving the cocoon vaccination were: vaccine not yet offered by HCW (50.3%), vaccinated during previous pregnancy (21.3%), recently delivered and no opportunity yet to get vaccinated (17.5%) and others (10.6%). Among the 286 women who did not yet receive the cocoon vaccination at the moment of the survey, 117 women (40.9%) were planning to get vaccinated in the upcoming days/weeks.

370 women (45.0%) were vaccinated against influenza during this pregnancy. Again, most women were vaccinated during the second (47.3%) or third (50.3%) trimester of pregnancy. A variation in vaccination coverage and trimester of vaccination was clear throughout the study period due to programmatic reasons as the vaccine is available in October during a limited period in time, and women receive in the fall the vaccine, regardless of second or third trimester (Fig. 2B). Main recommenders for maternal influenza vaccination were the gynecologist (64.1%) and the occupational physician (14.7%). Main vaccinators were the GP (68.1%) and the occupational physician (21.9%) (Fig. 1). Main reasons for not being vaccinated with an influenza vaccine during pregnancy were: vaccine not offered during pregnancy by HCW (40.2%), not convinced of the necessity to be vaccinated against influenza (19.9%), vaccination discouraged by HCW (6.8%), reasons of belief (6.4%), safety concerns (5.7%), forgetfulness (5.1%), vaccine not available (6.2%) and others (9.7%).

To summarize, 213 women (25.9%) were vaccinated with a Tdap vaccine only and 56 women (6.8%) were vaccinated with an influenza vaccine only; 314 women (38.2%) received both vaccines and 240 women (29.2%) received not vaccine at all (Fig. 3).

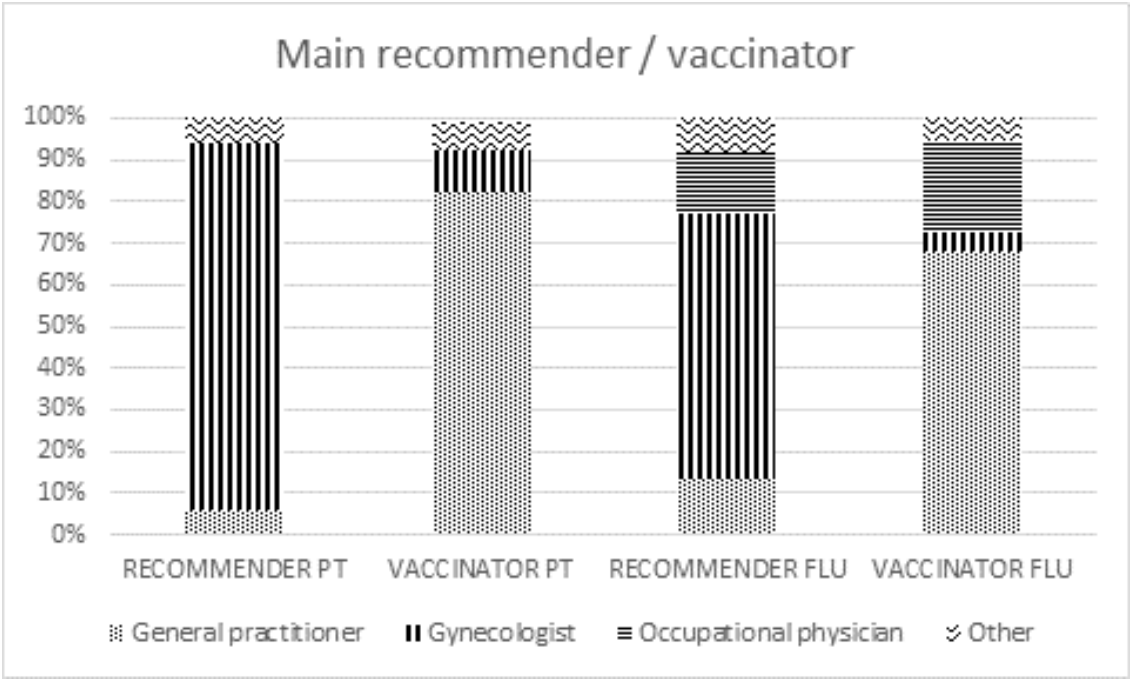

Figure 1: Main recommenders and vaccinators of the maternal vaccination strategies.

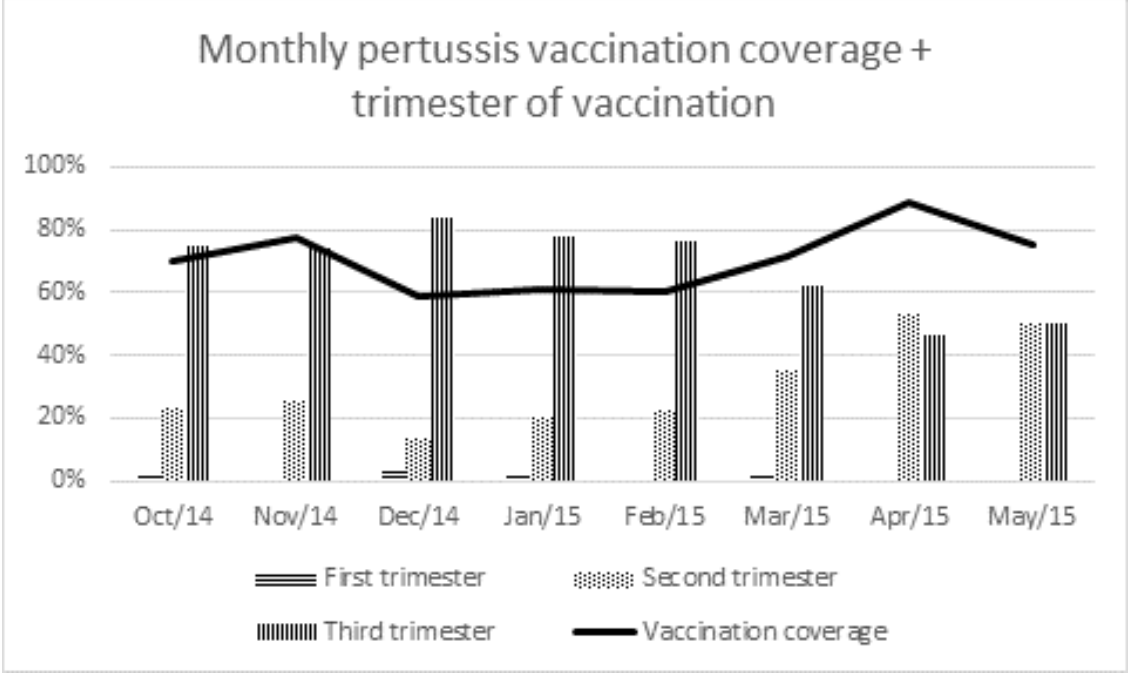

Figure 2A: Pertussis vaccination coverage and trimester of vaccination by month of delivery.

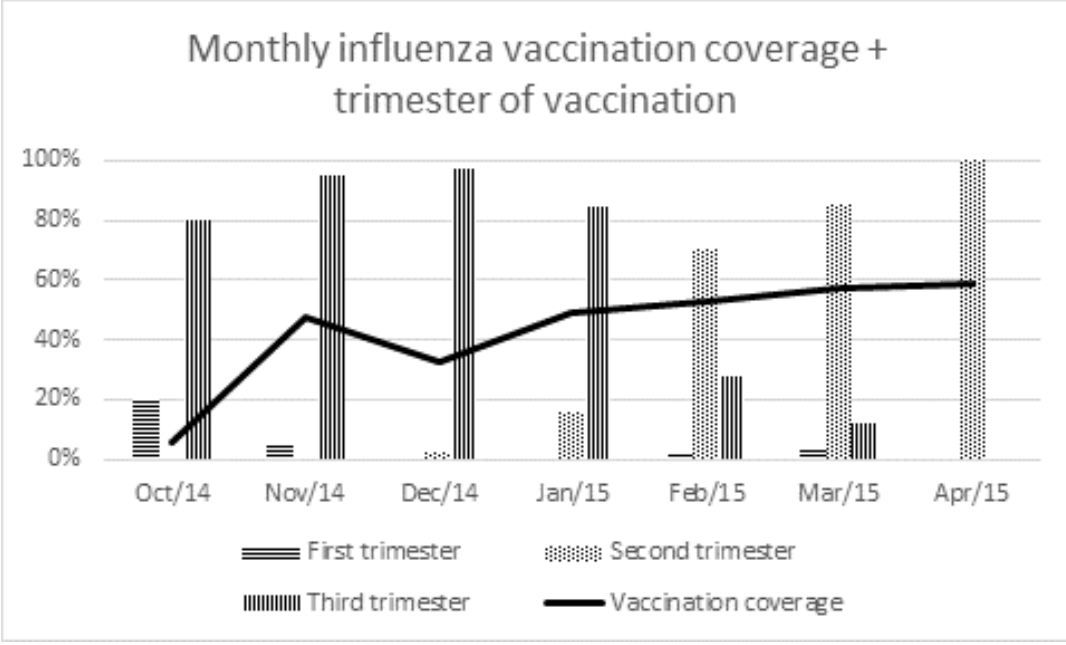

Figure 2B: Influenza vaccination coverage and trimester of vaccination by month of delivery.

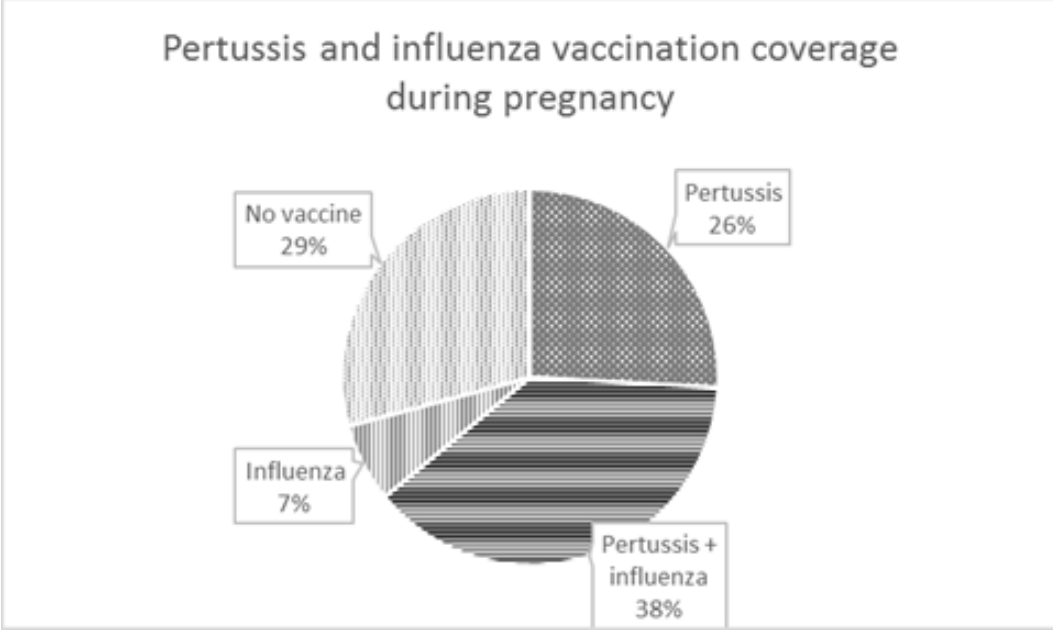

Figure 3: Coverage pertussis and influenza vaccination during pregnancy.

INFLUENCING FACTORS ON UPTAKE OF MATERNAL PERTUSSIS AND INFLUENZA VACCINATION

The multiple logistic regression model indicated a significant influence of maternal level of education ($p<0.001$), maternal work situation ($p<0.001$) and parity ($p=0.005$) on the maternal pertussis vaccination status. A higher level of education and full-time employment was associated with a higher pertussis vaccination coverage. Primiparous women were significantly better vaccinated than multiparous women.

In this multiple logistic regression model for influenza vaccination during pregnancy, a significant influence of country of birth of the grandparents of the newborn ($p=0.003$) and maternal level of education ($p<0.001$) was found. A significantly higher influenza vaccination coverage was obtained if the grandparents of the newborn were born in Belgium or an EU-country. Again, a higher maternal level of education was associated with a higher influenza vaccination coverage.

UPTAKE OF THE COCOON STRATEGY IN PARTNERS

815 women reported to have a partner and 510 partners (62.6%) were vaccinated with a pertussis containing vaccine during the last 10 years. From these 510 vaccinated partners, 372 partners (72.9%) were vaccinated during the current pregnancy.

AWARENESS AND RECOMMENDING ATTITUDE OF HCWs TOWARDS THE MATERNAL VACCINATION STRATEGIES AND THE COCOON STRATEGY

In total, 86.2% of all surveyed HCWs were aware of the current NITAG recommendation on pertussis vaccination during pregnancy. 70.1% of all HCWs inform pregnant women on pertussis vaccination during pregnancy; 75.1% recommends maternal pertussis vaccination, whereas 72.0% recommends maternal influenza vaccination. The cocoon strategy is recommended by 64.4% of the HCWs.

In general, gynecologists and GPs are better aware of the current recommendation for pertussis vaccination compared to midwives. Both groups of HCWs are more likely to inform and recommend the maternal vaccination strategies and the cocoon strategy compared to midwives (Table 2).

		In-hospital HCWs (N = 103)		GPs (N = 158)	Total (N = 261)
		Gynecologists (N = 27)	Midwives (N = 76)		
Aware of recommendation pertussis vaccination during pregnancy, No. (%)	Yes	27 (100)	53 (69.7)	145 (91.8)	225 (86.2)
	No	0 (0)	23 (30.3)	13 (8.2)	36 (13.8)
Inform pregnant women on pertussis vaccination during pregnancy, No. (%)	Yes	27 (100)	30 (39.5)	126 (79.7)	183 (70.1)
	Sometimes	0 (0)	17 (22.4)	13 (8.2)	30 (11.5)
	No	0 (0)	29 (38.2)	19 (12.0)	48 (18.4)
Recommend pertussis vaccination during pregnancy, No. (%)	Yes	27 (100)	32 (42.1)	137 (86.7)	196 (75.1)
	Sometimes	0 (0)	9 (11.8)	7 (4.4)	16 (6.1)
	No	0 (0)	35 (46.1)	14 (8.9)	49 (18.8)
Recommend cocoon strategy, No. (%)	Yes	24 (88.9)	21 (27.6)	123 (77.8)	168 (64.4)
	Sometimes	1 (3.7)	4 (5.3)	8 (5.1)	13 (5.0)
	No	2 (7.4)	51 (67.1)	27 (17.1)	80 (30.7)
Recommend influenza vaccination during pregnancy, No. (%)	Yes	20 (74.1)	35 (46.1)	133 (84.2)	188 (72.0)
	Sometimes	6 (22.2)	10 (13.2)	13 (8.2)	29 (11.1)
	No	1 (3.7)	31 (40.8)	12 (7.6)	44 (16.9)

Table 2: Attitude of HCWs towards maternal vaccination and cocoon vaccination.

INFLUENCING FACTORS ON THE ATTITUDE OF HCWs TOWARDS MATERNAL IMMUNIZATION AND COCOON VACCINATION

Older age of in-hospital HCWs relates positively to recommending pertussis ($p<0.001$) and influenza ($p=0.034$) vaccination during pregnancy, yet this is not true for the cocoon strategy ($p=0.089$). For the GPs on the other hand, younger age relates to increased likelihood of recommending influenza vaccination ($p=0.024$). In general, HCWs who recommend pertussis vaccination during pregnancy are more likely to also recommend influenza vaccination during pregnancy and vice versa.

PERTUSSIS VACCINATION STATUS OF HCWs

Overall, 70.1% of all HCWs were vaccinated with a pertussis containing vaccine during the previous 10 years. For the in-hospital HCWs, 63.0% of the gynecologists and 80.3% of the midwives were vaccinated with a Tdap vaccine during the last 10 years. 65.8% of the GPs were up to date with their pertussis vaccination status. No association between the pertussis vaccination status of HCWs and their recommending attitude was found.

Discussion

The present study reveals a relatively high coverage of pertussis (64.0%) and influenza (45.0%) vaccination during pregnancy and of the cocoon strategy (62.6% of partners of pregnant women) in Flanders, Belgium. The main recommenders and administrators of maternal vaccination as well as the main reasons for women not to get vaccinated, were identified. Underserved populations of pregnant women for these recommendations are women with a migration background (non-EU country), lower maternal educational level and employment status. The attitude of gynecologists and GPs towards maternal pertussis and influenza vaccination and the cocoon strategy, is overall positive; 78.4% of gynecologists and GPs recommends both maternal pertussis and influenza vaccination and 67.0% recommends both maternal vaccination strategies and the cocoon strategy. In contrast, only 23.7% of midwives recommends both maternal pertussis and influenza vaccination and 10.5% recommends both maternal vaccination strategies and the cocoon strategy. This in line with lower trust towards vaccination in the particular group of HCWs, as has been described before [328]. The overall measured pertussis immunization status of HCWs during the last 10 years in Flanders is 70.1%

Compared to the coverage described in a recently conducted monocentric Belgian study by Laenen et al. [329], we report higher coverage for both vaccines. However, in contrast to our study results, higher coverage rates for influenza (42.8%) were described compared to pertussis (39.2%). Most plausible explanations for the discrepancy are that pregnant women were surveyed before delivery and thus, vaccines administered after 32 weeks of pregnancy were not considered in this assessment of the coverage. Moreover, the study was performed before the free availability of Tdap in the Flemish health care facilities. As for influenza vaccines, several countries have observed that co-payment could be a barrier for the uptake, although this has been assessed in populations at risk other than pregnant women [330, 331]. This observation might also be relevant in Flanders; offering influenza vaccines free of charge might enhance the uptake. However, no literature data are available on comparison of both strategies in pregnant women, and we observe comparable coverage in the UK, where the vaccine is offered free of charge for pregnant women, and Belgium, despite the difference in payment system.

During the last decade, maternal vaccination strategies are becoming more important with an increasing number of countries issuing recommendations on maternal influenza and pertussis vaccination. A study in the United States [332] reported a maternal influenza vaccination coverage of 35.0% during the 2014-2015 influenza season, in the same period as the here presented study. In the United Kingdom, an influenza vaccination uptake during pregnancy of

30.0% was found in the 2014-2015 influenza season [333], which is lower compared to the coverage measured here in Flanders.

Since the recommendation for maternal pertussis vaccination is more recently adopted in a number of countries, only limited studies on the coverage of this strategy are available. In a study in the United States [334], conducted between April 2012 and March 2014, a coverage of 35.0% was found, with increasing coverages from 13.8% in January 2013 to 51.0% in March 2014. A possible explanation for the higher coverage we report, could be the free availability of Tdap in Flemish health care facilities and the support of professional organizations to stimulate maternal immunization. In the United Kingdom, where maternal pertussis vaccination is also free of charge and highly stimulated through information campaigns, a lower maternal pertussis vaccination coverage of 56.4% was reported between April 2014 and March 2015 [335].

The majority of women in our study were vaccinated for pertussis and influenza during the second of third trimester of gestation (according to the recommendations), a time point that is considered to be biologically optimal to maximize the placental transport of maternal antibodies from mother to infant [176]. In Flanders, main recommenders for maternal vaccination were the gynecologist whereas the GPs were the main vaccinators. Antenatal care is mainly performed by gynecologists in Belgium, although shared care with GPs is gaining more interest. In contrast to prenatal care, vaccination is historically not one of the task for gynecologists, but fits neatly within the GP consultation, explaining the interaction between both groups of HCWs in informing on vaccination and performing the vaccination.

One of the main reasons not to get vaccinated, was that vaccination was not offered or was even discouraged by a HCW. This finding highlights the concept that provider attitude is the key to achieve high maternal vaccination coverages, as previously demonstrated [336, 337]. In contradiction to the article by Wilson et al. where safety is identified as one of the main concerns (41.0%), not many safety issues were reported in the present study for both vaccines (4.8% for pertussis and 5.7% for influenza) [338].

Several underserved populations have been identified. Ethnicity, level of education, work situation and parity have a significant influence on either the pertussis or influenza vaccination coverage. The influence of ethnicity on vaccination coverage in general [339] and among pregnant women [329, 340, 341], has already been shown in previous studies with lower vaccination coverage among ethnic minorities. Identification of reasons for lower coverage, whether to deal with an underserved population or an under informed population and additional campaigns and education of both HCWs and the target group, could enhance the coverage of the vaccination

[342]. Other influencing factors as described by Wilson et al. as the social context factors, are confirmed in the present analysis: the maternal educational level and being a second generation immigrant [338]. Also, lower maternal education has been previously associated in Belgium with a lower maternal influenza vaccination coverage [329]. Our present multicenter retrieved results, confirm the findings in the single center evaluation in Belgium, yet Wiley et al. did not identify maternal education as an influencing factor for pertussis vaccine uptake in Australia [343].

For women who were not immunized during pregnancy, low vaccination coverages for maternal cocoon vaccination were counted. This could be a consequence of collecting surveys very soon after delivery (average 2.4 days postpartum), resulting in women not yet having the opportunity to receive the vaccine. Most of these women showed the intention to comply with the cocoon strategy at a later time point.

We report a higher coverage rate (62.2% vaccinated during the last decade) of the partners compared to a Swiss study, whereas only 17.0% of the partners were accurately protected against pertussis [78]. At the moment of conduct, cocooning was the only strategy in place in Switzerland to protect young infants from pertussis. In Australia, however, as a public health response to an ongoing pertussis epidemic, high coverage rates of cocoon strategy were reached: up to 70.0% of the fathers reported vaccination in relation to the birth of their most recent child. Another 10% of the fathers received a pertussis containing vaccine during the last 10 years [344]. HCWs, especially gynecologists, seem to be the most important stakeholders in the recommendations of the maternal vaccination programs and the cocoon strategy. On the other hand, GPs are the main vaccinators within these strategies in Flanders. According to the survey results from HCW, most rather gynecologists and GPs recommend maternal pertussis and influenza vaccination during pregnancy, confirming the high recommendation percentage found in the survey of the target group. Nonetheless, special attention needs to go to the group of midwives, whose awareness on the current recommendations could be improved. In Flanders, among this occupational group, there is a tendency of vaccine hesitancy when vaccines are administered during pregnancy, mainly due to safety reasons. In the future, further research on this hesitancy is absolutely needed. This survey highlights the need for proper education for all vaccine providers, regardless of their role, because consistent messages from all HCWs are necessary to further improve immunization rates.

This study has several potential limitations. First of all, we only included hospitals with a relatively high number of deliveries. Therefore, the results of this study may not be completely generalizable to the entire population in Flanders. Consequently, the Flemish maternal pertussis and influenza vaccination coverage in women delivering at home (only 0.8% of total number of deliveries in Flanders in 2013) or in smaller maternity wards remains unknown. A planned vaccination coverage study among a sample of infants in Flanders in 2016, will include a survey of the parents and could shed light on this gap in the data. Secondly, the maternal pertussis and influenza vaccination status is self-reported and can therefore be subject to erroneous recall resulting in an over- or underestimation of the vaccination coverage in pregnant women. However, since recall is limited to the last 9 months and no other vaccines (e.g. tetanus) are administered during pregnancy in Belgium, the bias is less presumable. A third potential limitation is that most surveys of GPs were collected at congresses or training sessions. It could be that the public attending these sessions is not representable for the general population of GPs in Flanders, resulting in a possible selection bias because of over-representation of vaccinators. However, the high percentage of GPs vaccinating pregnant women, as was reported by the target group itself is an argument that GPs in general are aware of the recommendation and do vaccinate during pregnancy.

Surveys were collected in many different languages (Dutch, French, English, Arabic) to maximize the response rate. Nevertheless, we were still not able to survey all women, mainly due to language problems. The migration background might be a relevant influencing factor on vaccine uptake.

A few strengths of this study include the study design, which is a randomized, multicenter study, in comparison with the Laenen et al. study [329]. Also, the surveys from HCWs were collected anonymously thereby allowing HCWs to freely express their opinions.

Conclusion

The overall positive attitude of HCW towards maternal immunization is reflected by a relatively high vaccination coverage. However, there is still room of improvement by targeting underserved populations of pregnant women and improve both the awareness and attitude of midwives on the current recommendations.

Acknowledgments

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Annex A

Survey of post-partum women: Pertussis vaccination during pregnancy

Code: ……
Date of testing: ……./……./……..

☐ Not completed due to language problem

1. Age: ……	7. Did your pregnancy proceed normally?
2. Country of birth:	☐ Yes
☐ Belgium	☐ No: …… …… ……
☐ EU country	
☐ non-EU country	
3. Parents' country of birth:	
☐ Belgium	
☐ EU country	8. Weight of child at birth:……..g
☐ non-EU country	9. Length of child at birth:……..cm
4. Highest degree completed:	10. Length of pregnancy at birth:…….weeks
☐ Primary school	11. Delivery date: ……../……../…….
☐ Secondary school	12. Location of delivery:…….. …… ……
☐ Bachelor/University college	
☐ Master/University	
☐ Other: …….	

5. What is your current employment situation? ☐ Full-time ☐ Part-time ☐ Self-employed ☐ Unemployed, with social welfare benefits ☐ Unemployed, with no social welfare benefits ☐ Other: …….. …… 6. Postal code + place of residence: ……..	
13. Were you aware of the recommendation for pertussis vaccination during your pregnancy? ☐ Yes ☐ No	13a. **If yes**, from whom had you received this information? ☐ Gynaecologist ☐ General Practitioner ☐ Midwife ☐ Occupational Physician ☐ Family/friends ☐ Pharmacist ☐ Other:…… …… …..

14. Were you vaccinated for pertussis during your pregnancy? ☐ Yes ☐ No	14a. **If yes,** who recommended this vaccination? ☐ Gynaecologist ☐ General Practitioner ☐ Midwife ☐ Occupational Physician ☐ Other:…… …… …. 14b. **If yes,** who administered this vaccination? ☐ Gynaecologist ☐ General Practitioner ☐ Midwife ☐ Occupational Physician ☐ Other:…… …… ….. 14c. **If no**, why were you not vaccinated? ☐ Afraid of the injection ☐ Practitioner advised against it ☐ Family/friends advised against it ☐ Afraid of side-effects ☐ Vaccination was not offered to me ☐ Forgot ☐ Spiritual convictions

	☐ Other: ……. …… ……
15. If you were vaccinated for pertussis during your pregnancy: **when** during your pregnancy were you vaccinated for pertussis? ☐ During the first three months of the pregnancy ☐ Between the third and sixth months of the pregnancy ☐ During the last three months of the pregnancy ☐ Date: ……/……/……	
16. Were you vaccinated during a previous pregnancy? ☐ Yes ☐ No ☐ Not applicable	16a. **If yes**, for what were you vaccinated? ☐ Pertussis ☐ Flu ☐ Both ☐ Other:…….. …….
17. If you were not vaccinated during the pregnancy, were you vaccinated for pertussis after delivery? ☐ Yes ☐ No	17a. **If yes,** who recommended this vaccination? ☐ Gynaecologist ☐ General Practitioner ☐ Midwife ☐ Occupational Physician ☐ Other:…… ……

	17b. **If yes,** who administered this vaccination? ☐ Gynaecologist ☐ General Practitioner ☐ Midwife ☐ Occupational Physician ☐ Other:……. ……. 17c. **If no**, why were you not vaccinated? ☐ Afraid of the injection ☐ Practitioner advised against it ☐ Family/friends advised against it ☐ Afraid of side-effects ☐ Vaccination was not offered to me ☐ Forgot ☐ Spiritual convictions ☐ Other: …… …….. …….
18. If you were not vaccinated for pertussis after delivery, are you planning to be vaccinated? ☐ Yes ☐ No	18a. **If yes**, when are you planning to be vaccinated? ☐ This week ☐ This month ☐ Don't know

	18b. **If no**, why not? ☐ Afraid of the injection ☐ Practitioner advised against it ☐ Family/friends advised against it ☐ Afraid of side-effects ☐ Vaccination was not offered to me ☐ Forgot ☐ Spiritual convictions ☐ Other: …… …… …. …… ….
19. Was your partner vaccinated for pertussis during the pregnancy or after the delivery? ☐ Yes ☐ No	19a. Who recommended this? ☐ Gynaecologist ☐ General Practitioner ☐ Midwife ☐ Occupational Physician ☐ Other:…….. …… …. 19b. **If yes,** who administered this vaccination? ☐ Gynaecologist ☐ General Practitioner ☐ Midwife ☐ Occupational Physician

	☐ Other:…….. ……. 19c. **If no**, is your partner planning to be vaccinated? ☐ Yes ☐ No 19d. **If no**, why was he not vaccinated? ☐ Afraid of the injection ☐ Practitioner advised against it ☐ Family/friends advised against it ☐ Afraid of side-effects ☐ Vaccination was not offered to him ☐ Forgot ☐ Spiritual convictions ☐ Other: …… …… ….. …… …..
20. Were you vaccinated for flu during this pregnancy? ☐ Yes ☐ No	20a. **If yes,** who recommended this vaccination? ☐ Gynaecologist ☐ General Practitioner ☐ Midwife ☐ Occupational Physician ☐ Other:…… ……

	20b. **If yes,** who administered this vaccination? ☐ Gynaecologist ☐ General Practitioner ☐ Midwife ☐ Occupational Physician ☐ Other:…… …… … 20c. **If no**, why were you not vaccinated? ☐ Afraid of the injection ☐ Practitioner advised against it ☐ Family/friends advised against it ☐ Afraid of side-effects ☐ Vaccination was not offered to me ☐ Forgot ☐ Spiritual convictions ☐ Other: …… …… …. ……..

16. If you were vaccinated for influenza during your pregnancy: when during the pregnancy were you vaccinated for influenza?

☐ During the first three months of the pregnancy

☐ Between the third and sixth months of the pregnancy

☐ During the last three months of the pregnancy

☐ Date: ……/……./……

Do you have any comments about vaccination during pregnancy?

……
……
……
……..
……
……
…….

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Annex B

Survey of health-care providers: Pertussis vaccination during pregnancy

Code: ……
Date of testing: ……./……./……..

☐ Not completed due to language problem

Dear participant,

The Superior Health Council of Belgium has issued a new recommendation regarding pertussis vaccination during pregnancy. We at the Centre for the Evaluation of Vaccinations at the University of Antwerp are conducting a survey of health-care providers in order to study the follow-up to this recommendation.

We would like to ask you to take a moment of your time to answer the following brief questions.

1.Sex: ☐ Male ☐ Female 2. How old are you? …….. 3. In which province do you work primarily? ……	4. What is your training? ☐ Gynaecologist ☐ General Practitioner ☐ Midwife ☐ Other: ……..
5. Are you aware of the new recommendation of the Superior Health Council of Belgium regarding pertussis vaccination during pregnancy? ☐ Yes ☐ No	5a. **If yes**, from whom/in what way did you receive information about it? ……

6. Are you aware of the new recommendation of the Flemish Association for Obstetrics and Gynaecology (VVOG) regarding pertussis vaccination during pregnancy? ☐ Yes ☐ No	6a. **If yes**, from whom/in what way did you receive information about it? ……
7. Do you systematically inform pregnant women about the existence of the recommendation for pertussis vaccination during pregnancy? ☐ Yes ☐ No ☐ Sometimes	7a. **If no**, why not? ☐ Not informed ☐ No time during the consultation ☐ Disagree with recommendation: why?..... ☐ Other: …….. 7b. **If sometimes**, why sometimes? ☐ No time during the consultation ☐ Disagree with recommendation: why?..... ☐ Other: ……..
8. Do you systematically advise pregnant women to be vaccinated for pertussis during pregnancy? ☐ Yes ☐ No ☐ Sometimes	8a. **If no**, why not? ☐ Not informed ☐ No time during the consultation ☐ Disagree with recommendation: why?..... ☐ Other: ……..

	8b. **If sometimes**, why sometimes? ☐ No time during the consultation ☐ Disagree with recommendation: why?..... ☐ Other: ……..
9. Do you systematically recommend the cocoon strategy? ☐ Yes ☐ No ☐ Sometimes	9a. **If no**, why not? ☐ Not informed ☐ No time during the consultation ☐ Disagree with recommendation: why?..... ☐ I systematically vaccinate women during pregnancy. ☐ Other: …….. 9b. **If sometimes**, why sometimes? ☐ No time during the consultation ☐ Disagree with recommendation: why?..... ☐ Other: ……..
10. Do you systematically advise pregnant women to be vaccinated for influenza during pregnancy? ☐ Yes ☐ No ☐ Sometimes	10a. **If no**, why not? ☐ Not informed ☐ No time during the consultation ☐ Disagree with recommendation: why?..... ☐ Other: ……..

	10b. **If sometimes**, why sometimes? ☐ No time during the consultation ☐ I vaccinate only high-risk groups ☐ Disagree with recommendation: why?..... ☐ Other: ……..
11. Have you been vaccinated for pertussis in the last 10 years? ☐ Yes ☐ No	11b. **If yes**, date of last pertussis vaccination:……..

Do you have any comments about vaccination during pregnancy?
……
……
……
……
……..

Thank you for you cooperation. The results of this survey will be available in an anonymised form on the CEV website: www.uantwerpen.be/cev .

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CHAPTER 6/B

Coverage of pertussis and influenza vaccination during pregnancy in Flanders, Belgium: anno 2016

This chapter is submitted as an article to Vaccine": "Maertens K., Braeckman T., Blaizot S., Theeten H., Roelants M., Hoppenbrouwers K., Leuridan E., Van Damme P., Vandermeulen C. Coverage of recommended vaccines during pregnancy in Flanders, Belgium. Fairly good but can we do better?"

Abstract

In Flanders, Belgium, pertussis vaccination is recommended since 2013 and free-of-charge for all pregnant women in every pregnancy between 24 and 32 weeks of gestation. Influenza vaccination is recommended for more than 10 years with a co-payment system in the second or third trimester of pregnancy, when pregnancy coincides with the influenza season.

A vaccination coverage study using the Expanded Program on Immunization (EPI)-based two-stage cluster sampling design has been performed in postpartum women (N=481) in 2016 to estimate the coverage of pertussis and influenza vaccination during pregnancy. Additionally, predictors for non-vaccination using a multivariate logistic regression model were assessed.

Vaccination coverage of pregnant women was 69.3% for pertussis and 47.2% for influenza. For pertussis, 65.3% of women were vaccinated inside the recommended gestational window while for influenza this was the case for 96.9% of women.

Parity and size of the hospital were associated with both maternal pertussis and influenza vaccination coverage while income was only associated with pertussis vaccination coverage. Ethnicity, educational level and health care provider responsible for pregnancy follow-up were associated with influenza vaccination coverage.

Among women who were informed about the risks, recommendations and financial framework of maternal vaccination, still 12.4% were not vaccinated for pertussis and 23.9% for influenza during pregnancy.

In Flanders, the overall coverage for pertussis vaccination during pregnancy is fairly good. However, there is room for improvement for both maternal vaccination programs by targeting the underserved populations and diminishing the hurdles to get vaccinated.

Introduction

Despite a good universal compliance to infant vaccination [249, 345, 346], the *Bordetella pertussis* pathogen remains responsible for a substantial disease burden, especially among neonates, who are most susceptible to develop severe or even fatal disease. [347]. Several vaccination strategies have been set forward to prevent infection of the vulnerable group of infants too young to be immunized, including cocoon vaccination as parents have been identified to be among the main source of infection [348], development of new-generation pertussis vaccines and the immunization of pregnant women to generate transplacental transport of IgG antibodies from mother to infant [349, 350]. Given the need for an effective approach to prevent pertussis cases and considering the demonstrated effectiveness and safety of pertussis-containing vaccines among pregnant women [188, 351], various National Immunization Technical Advisory Groups (NITAGs) have adopted this strategy. The Belgian NITAG recommends maternal pertussis immunization during every pregnancy as of August 2013, preferably between 24 and 32 weeks of gestation. Postpartum vaccination is indicated if the vaccine was not administered during pregnancy. Following the cocoon strategy, all adults, especially those in close contact with infants, are recommended to receive a single booster dose in adulthood [352]. In Flanders, pertussis containing vaccines for adults are available free-of-charge.

For over a decade, influenza vaccination during the second or third trimester of pregnancy is recommended in case this period coincides with the influenza season, with a co-payment modality [353].

To evaluate compliance to the national recommendations, the Flemish government commissioned the conduct of a survey to estimate the uptake of recommended vaccines in pregnant women, the adherence to timeliness of administration and to identify the underserved in order to develop specific strategies to continuously augment and maintain maternal vaccination coverage. Additionally, knowledge and trust regarding maternal vaccination were assessed.

Methods

The methodology of the Expanded Program on Immunization (EPI)-based two-stage cluster sampling design for vaccination coverage studies in Flanders was extensively described elsewhere [224, 354]. A sample size of 660 women was calculated based on a minimal anticipated coverage of 64% [161], a design effect of 1.5, a margin error of the confidence interval of 2.5% and a drop-out rate of 10%. The clusters of the mothers were proportionally distributed over the 5 different Flemish provinces. Per cluster, five mothers were randomly selected, based on the date of birth of their latest child (January or February 2016) as registered in the Flemish register of natural persons. Selected families were surveyed at home by a trained interviewer. Families were replaced only if (i) the family could not be contacted after three home visits, (ii) the interviewee was not able to understand Dutch, or (iii) the family no longer lived on the designated address. Mothers who refused to participate were not replaced.

Surveys were performed between April 4th and August 16th, 2016. After obtaining informed consent from the mother, the following information was collected through a standardized electronic questionnaire (SNAP software): socio-demographic characteristics, vaccination history (documented and by recall) and medical information related to the pregnancy. The vaccination data obtained at home were first checked against the Flemish immunization registry (Vaccinnet) [354] and missing or doubtful vaccination data were in a next step sent to the general physician (GP) or gynecologist (if contact information was available) with a request to verify, correct and/or complete these data.

To assess knowledge of pregnant women concerning pertussis and influenza, they were asked if they were aware of (i) the risks of the pathogen for either the neonate or fetus, (ii) the recommendations regarding vaccination during pregnancy, (iii) the financial framework of pertussis and influenza vaccination. In case they were aware, their information source was asked for.

Trust towards immunization during pregnancy was assessed using an adapted version of the vaccine hesitancy survey tool developed by WHO [355].

This study was authorized by the National Privacy Commission and received approval on March 8th 2016 from the ethics committee of the University of Leuven.

IBM SPSS Statistics 23 was used for descriptive analysis. Survey-based vaccination coverage with its 95% confidence interval (CI) as well as odds ratios predicting missed vaccination from univariate and multiple logistic regression were calculated using R version 3.3.2. Final models were selected using a backward selection, p-values <0.05 were considered significant.

Results

A total of 627 mothers were approached of whom 486 mothers agreed to be interviewed at home (refusal rate=22.4%), but five were excluded afterwards due to a lost informed consent form. Participating mothers were unequally distributed among the clusters which was accounted for by weighing. Demographic characteristics of participants were highly comparable with the census-data in Flanders, except that higher educated mothers and fathers were slightly overrepresented.

The weighted pertussis vaccination coverage during pregnancy was 69.3% (95%CI: 63.9-74.3). Less than 1/5 of vaccinated women (18.0%) had documented proof of pertussis vaccination, the majority was based on recall, of which a substantial proportion (62.1%) was confirmed using Vaccinnet. Pertussis-containing vaccine was in most cases administered by the GP (71.9%), and in 18.9% of cases by the gynecologist. Remarkably, 34.7% of the vaccinated women received the vaccine outside the recommended gestational window of whom the majority (55.2%) after 32 weeks of gestation.

Mothers who were not vaccinated during pregnancy mentioned as main reason that the vaccine was not recommended or offered by the health care provider (27.0%) or they had even been advised against vaccination (17.5%), mainly because of a recent pertussis vaccination in multiparous women (90.5%). However, less than 1/4 of women not vaccinated during pregnancy had received a pertussis vaccine during the last 10 years, of whom 30.0% in the postpartum period. Only a minority of the unvaccinated women (5.1%) reported to have made a deliberate choice not to vaccinate due to fear concerning possible adverse effects of the vaccine.

The weighted influenza vaccination coverage during pregnancy was 47.2% (95%CI: 42.1-52.3). Approximately 1/3 of vaccinated women (30.3%) had documented proof of influenza vaccination during pregnancy, the remainder was based on recall, of which only 11.7% was confirmed using Vaccinnet and 14.9% through contact with a health care provider. Influenza vaccination was performed by the GP in 68.0%, followed by the occupational physician (15.3%) and the gynecologist (10.8%). Few vaccinated women (3.1%) reported being vaccinated during the first trimester of pregnancy. Main reason mentioned for not being vaccinated was that the vaccine was not recommended, offered or even discouraged by the health care provider (39.5%). Over 1/4 women (28.9%) reported to have made a deliberate choice not to vaccinate against influenza, either due to fear concerning possible adverse effects or because they had never been vaccinated against influenza previously.

Assessing the combined vaccination status of pertussis and influenza vaccination showed that in case the pregnant woman was immunized against influenza, a pertussis vaccination was generally administered too. More than ¼ women received neither vaccine (Figure 1).

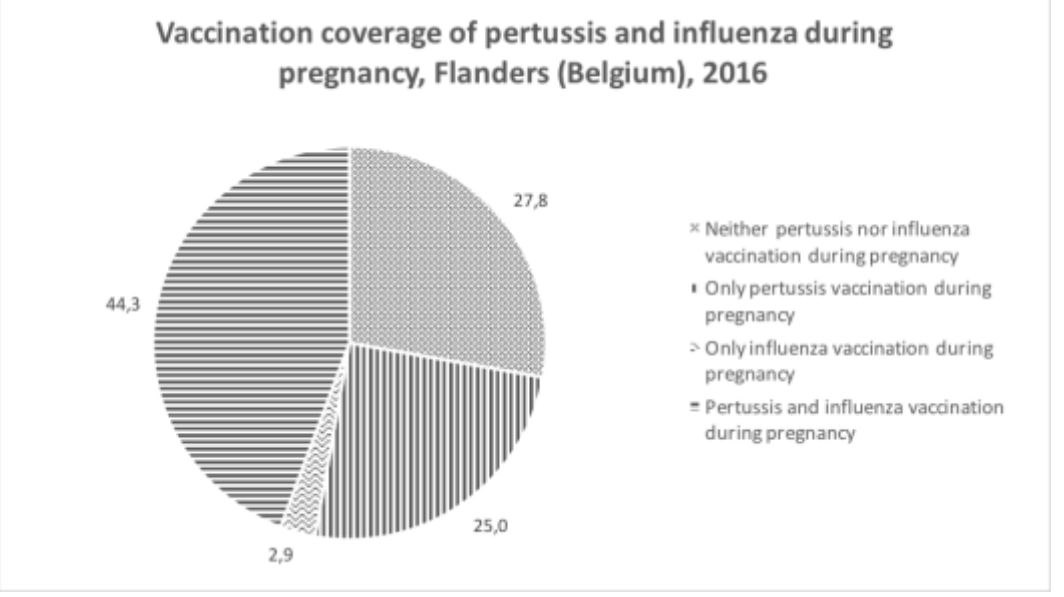

Figure 1: Weighted vaccination coverage of pertussis and influenza vaccination during pregnancy, Flanders (Belgium), 2016.

Several predictors of missed vaccination in pregnancy were identified by multiple logistic regression. For pertussis, multiparas, and mothers whose monthly family income was below 3000 euro or was unknown were more often unvaccinated, as well as women who delivered in a hospital with yearly rate of more than 800 deliveries (Table 1). For influenza, a significantly lower vaccination coverage was observed in multiparas, women with an ethnic background outside the European Union and women who delivered in a hospital outside Flanders or a Flemish hospital of which the number of deliveries per year could not be retrieved. A significantly higher influenza vaccination coverage was seen when women had a high educational level (master degree) compared to a secondary school educational level and when medical follow-up of the pregnancy was performed by the gynecologist in combination with another health care provider compared to when medical follow-up of the pregnancy was not performed by the gynecologist (Table 2).

	Odds Ratio
Multipara *(Reference: unipara)*	**2.09 (1.29-3.39)****
Family income *(Reference: 3001-4000€)*	
<1200-2000€	**2.37 (1.04-5.37)***
2001-3000€	**2.69 (1.35-5.39)****
>4000€	1.38 (0.64-2.97)
Don't know	**2.78 (1.30-5.97)***
Does not wish to disclose	0.99 (0.30-3.24)
Place of residence (Reference: Cities)	
Towns and suburbs	1.47 (0.74-2.94)
Rural area	0.72 (0.37-1.41)
Location of delivery (Reference: hospital with <800 annual deliveries)	
hospital with >800 annual deliveries	**2.87 (1.64-5.01)*****
home birth	4.19 (0.77-22.94)
hospital located outside Flanders or unknown annual number of deliveries	**17.64 (5.54-56.15)*****

Table 1: Odds ratios (95% CI) of determinants for non-vaccination against pertussis during pregnancy.

	Odds Ratio
Multipara *(Reference: unipara)*	**1.77 (1.18-2.65)****
Educational level of the mother *(Reference: secondary school, second cycle)*	
Secondary school, first cycle or lower	1.88 (0.92-3.82)
Bachelor	0.62 (0.37-1.05)
Master	**0.34 (0.18-0.65)****
Ethnicity of the mother *(Reference: Belgium)*	
European	1.83 (0.85-3.92)
Non-European	**2.32 (1.30-4.12)****
Place of residence (Reference: Cities)	
Towns and suburbs	1.53 (0.80-2.94)
Rural area	0.91 (0.46-1.81)
Location of delivery (Reference: hospital with <800 annual deliveries)	
hospital with >800 annual deliveries	1.46 (0.95-2.24)
home birth	§
hospital located outside Flanders or unknown annual number of deliveries	**6.15 (2.04-18.51)****
Medical monitoring of pregnancy *(Reference: no gynecologist)*	
Gynecologist	0.67 (0.24-1.88)
Gynecologist and other health care worker	**0.26 (0.08-0.86)***

Table 2: Odds ratios (95% CI) of determinants for non-vaccination against influenza vaccination. during pregnancy

The trust assessing questions showed that 78.8% of the women were convinced of the importance of maternal vaccination and 80.1% was convinced that these vaccinations are safe for the unborn child. A clear association was found between attitude towards both importance and safety, and vaccination status for pertussis and influenza (p<0.0001).

For all of the assessed knowledge topics (risks, recommendations and financial framework), more than half of the women mentioned the gynecologist as being the main information source. All assessed knowledge turned out to be significantly related with vaccination coverage during pregnancy (Table 3). Interestingly, the vaccination coverage against both pertussis and influenza was much lower than the proportion of women aware of either the risk of the pathogen for the neonate/fetus and the recommendation to vaccinate, but is comparable with the proportion of women knowledgeable of the financial framework.

	Pertussis			**Influenza**		
Vaccination coverage	69.3% (95%CI: 63.9-74.3)			47.2% (95%CI: 42.1-52.3)		
Main vaccinator	GP			GP		
	Risks disease	Recommendation	Free-of-charge	Risks disease	Recommendation	Co-Payment
Awareness (%)	83.2	86.1	73.6	68.6	69.0	52.2
Main information source	Gynecologist	Gynecologist	Gynecologist	Gynecologist	Gynecologist	Gynecologist (+GP)
OR for vaccination (OR; 95% CI)	8.14*** (4.53-14.62)	36.63*** (14.55-92.20)	5.22*** (3.21-8.50)	6.50*** (3.98-10.60)	12.61*** (7.21-22.06)	5.87*** (3.76-9.17)

Table 3: Awareness regarding maternal vaccination and influence on vaccination coverage, Flanders, Belgium, 2016.

The subgroup of women who quoted positive on all 3 knowledge topics was assessed separately, and a significantly lower pertussis and influenza vaccination coverage was observed in multiparas and in women who visited no gynecologist compared to women who consulted both a gynecologist and another health care provider for their pregnancy follow-up. Additionally, being born outside the European Union and partus in a hospital with more than 800 deliveries per year was associated with missed pertussis vaccination in this subgroup, whereas educational level lower than secondary school was associated with missed influenza vaccination (data not shown).

Discussion

This study is the first that assessed the coverage of maternal pertussis and influenza vaccination among the Flemish population using a 2-stage cluster design survey. Previous studies were restricted to either one large university hospital [329] or multiple hospitals with a large maternity unit (i.e. more than 800 deliveries per year) [161]. The current 2016 estimate of 69.3% pertussis coverage during pregnancy is higher than the formerly reported 64.0% between October 2014 and May 2015 [161] suggesting that the maternal coverage against pertussis is increasing over time in Flanders. However, among women who delivered in a hospital with a large maternity unit the coverage was lower at 66.7%, so the higher coverage in 2016 is mainly attributable to the vaccination status of women who delivered in smaller hospitals which had not been surveyed previously. The trend in time thus has to be interpreted with caution.

Coverage data for maternal pertussis vaccination in other recommending countries are limited and often sub-optimal. In the US, an observational study from the Vaccine Safety Datalink in 2013 found that 41.7% of insured women with live births across seven health systems received Tdap during pregnancy [356]. Argentina started a maternal Tdap vaccination program in 2012 and national coverage increased from 51% to 67% in the following two years [148]. In England, a coverage estimate of 73.8% for maternal pertussis immunization was reported between February and March 2017 by the sentinel system, the highest level recorded since the introduction of the program in 2012 [357].

Despite the fact that all surveyed women in the current study were pregnant during the influenza season and the recommendation is already in place for over a decade, merely 47.2% received an influenza vaccine during pregnancy. Several countries have highlighted the low coverage within this at-risk population. In Spain, a similar 40.5% of the pregnant women in the Valencia health department were vaccinated. The vaccination coverage during the 2014-2015 influenza season was estimated at 44.1% in England and at 35.0% in the United States [332, 358].

For pertussis, 34.7% of women were vaccinated outside the recommended gestational window while for influenza this was only the case in 3.1%. Main reason is the relatively small recommended window for maternal Tdap vaccination in Belgium (24-32 weeks of gestation) compared to that for influenza (second and third trimester) [352, 353]. Some countries, including the UK, recently broadened their time window for maternal Tdap vaccination following a Swiss study showing that both second and third trimester vaccination are effective in transferring antibodies from mother to infant [176]. Nevertheless, a UK study showed that vaccination of

pregnant women at least 7 days before delivery had a vaccine effectiveness of approximately 90%. After that time point, a significant drop in vaccine effectiveness was measured [188].

In our study, the main predictors for non-vaccination against pertussis were multiparity, unknown income or monthly family income below 3000 € and delivery in a hospital with more than 800 deliveries per year. The observation that multiparous women are less vaccinated confirms previously published results [161, 359] and could be related with a lack of knowledge on repeat boosters in each pregnancy. Suggested reasons for the significant difference between smaller and larger hospitals include a higher turn-over of medical staff and subsequent lack of appropriate training, a less personal/individual approach in the medical follow-up of the pregnant women, and less focus on preventive measures as larger centers more often provide tertiary care. The effect of income on the pertussis vaccination status is surprising, since this vaccine is offered free-of charge in Flanders and most women are aware of this free availability.

Parity and location of birth were also identified as predictors for non-vaccination against influenza. Additionally, educational level and ethnicity of the mother as well as health care provider responsible for pregnancy follow-up predicted non-vaccination against influenza. Women with an ethnic background outside the European Union, often referred to as ethnic minorities, have also been reported at risk in other studies [339, 341]. The higher influenza vaccination coverage in women with higher educational level in this study is in line with previous findings [161, 329]. Education and origin are often referred to as social context factors and are an indication of the equity of the current campaigns.

Remarkably, combined clinical monitoring of the pregnancy by a gynecologist together with another health care provider results in a fourfold higher chance to be vaccinated against influenza. In Flanders, Belgium, pregnancy monitoring is mainly performed by gynecologists, but there is growing interest for the concept of shared care with GPs. From a historical point of view, vaccination does not belong to the main tasks of gynecologist but is daily practice for general practitioners, hence, cross-disciplinary interactions are of utmost importance. The fact that higher influenza vaccination coverage is seen if a gynecologist is involved in the follow-up of the pregnancy might reflect the impact of this interaction with GPs and possibly also with occupational physicians.

Gynecologists and other health care providers play a major role in correctly informing pregnant women on preventive interventions [360]. In general, Flemish women have high confidence in the health gains and few cautions regarding safety of vaccination in pregnancy, and are well aware of recommendations. Still, a substantial proportion of pregnant women does not

benefit from protection offered from maternal vaccination despite being fully aware of the existing recommendations. This indicates that there are still hurdles in the health care system to actually vaccinate the pregnant women and that pregnant women can remain hesitant even if fully informed.

This study has several potential limitations. More than half of the vaccination data are self-reported and can therefore be subject to erroneous recall. However, since recall is limited to a short time period and no other vaccines than pertussis and influenza are administered during pregnancy in Belgium, such bias is not likely. Another limitation is that that families not able to understand Dutch were not surveyed in this study, and those families might easier be missed by the program. An important strength of this study is that surveys were taken a few weeks postpartum allowing to capture cocoon vaccination in a broader time period after birth than in previously conducted studies in Flanders [161, 329]. Also, within this study, we were able to double check vaccination data against Vaccinnet.

Conclusion

In Flanders, the overall coverage for pertussis and influenza vaccination during pregnancy is fairly good. However, there is still room for improvement by targeting underserved populations. Additional strategies are needed to reach the underserved, such as increasing knowledge and awareness and diminishing hurdles in target groups, with lower socio-economic background, women of non-European origin and multipara.

Acknowledgments

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General discussion and conclusion

As NITAG's from several countries, including Belgium, have currently adopted the cocoon and the maternal pertussis vaccination strategy, the main aim of this thesis was to provide sufficient scientific evidence behind these recommendations. At the time of implementation, the decision to recommend maternal immunization was mainly based on epidemiological data while many immunological and safety aspects of these strategies, both for mother and child, were lacking.

Main findings of this PhD thesis

SAFETY

At the time of introduction of the maternal vaccination strategy in several industrialized countries, the safety of this strategy was only based on studies in small cohorts of pregnant women indicating no safety signals [102].

The first large prospective safety study of Tdap vaccination in pregnancy was actually performed after the implementation of the recommendation in the UK. Within this UK study, 20,074 pregnant women who were vaccinated with a Tdap vaccine during pregnancy were compared with a historical control group of unvaccinated women. A number of safety outcomes were monitored, showing no increased risk of stillbirth within 14 days after vaccination, no maternal and neonatal death, no (pre-) eclampsia and no other predefined adverse events related to pregnancy [187].

In the US, 123,494 singleton pregnancies were monitored for adverse reactions. Within this cohort of pregnant women, 26,229 women received a Tdap vaccine during pregnancy. This study showed that maternal Tdap vaccination was not associated with an increased risk for preterm delivery, being small for gestational age at birth or hypertensive disorders during pregnancy. Only a small increased relative risk for chorioamnionitis during pregnancy was seen. However, these data should be interpreted carefully since there was no increased risk for preterm birth, a direct consequence of chorioamnionitis, within the study cohort [361]. To further assess this possible relation between maternal Tdap vaccination and chorioamnionitis, a review of the Vaccine Adverse Event Reporting System (VAERS) was carried out. Within this review, all cases of chorioamnionitis after any vaccine in pregnancy between 1990 and 2014 were considered. Only few cases of chorioamnionitis were found accounting for 1% of all pregnancy reports to the VAERS [362]. Also, two other small observational studies could not replicate this association [363, 364]. In contrast, a recent study carried out in the US supports the results found by Kharbanda et al with a small but positive association between maternal Tdap and chorioamnionitis, but no increased risk for associated indicators of infant morbidity [365]. To further clarify the possible relation

between maternal Tdap and chorioamnionitis, future studies should use strict case definitions for chorioamnionitis.

In our own studies, having a relative small sample size compared to the two previously mentioned studies from the UK and US, we looked at the safety of maternal pertussis vaccination both in Belgium and Vietnam [153, 154]. We observed no unexpected side effects in the women other than the side effects described on the summary of product characteristic (Boostrix® and Adacel®). Also, safety data in the infants did not reveal unexpected patterns of risk and no congenital disorders were detected. Within the study, some SAE's were reported but the frequency of these serious adverse events did not differ from what is expected within the general Belgian and Vietnamese population. So, the safety data in our study did not demonstrate an unexpected risk pattern and add to the evidence that vaccination of pregnant women in the second or third trimester of pregnancy is safe for both mother, fetus and infant, apart from the possible association with chorioamnionitis. Meanwhile, several additional studies monitoring the safety of maternal Tdap vaccination across the globe have been published [351, 366] and do not indicate additional risks other than the ones mentioned above.

When looking at the safety of concomitant administration of influenza and Tdap vaccination during pregnancy, a US study showed that there was no increased risk for adverse outcomes following concomitant administration of influenza and Tdap vaccination during pregnancy compared to sequential administration of both vaccines [367]. In the light of implementing maternal Tdap vaccination in countries with existing recommendations for tetanus vaccination during pregnancy, a US study examined the safety of Tdap vaccination in relation with the prior receipt of tetanus-containing vaccines in the last 5 years. The study concluded that relatively recent receipt of prior-tetanus containing vaccines (less than two years ago; 2-5 years ago; more than 5 years ago) does not increase the risk for adverse outcomes after Tdap vaccination in pregnancy [368].

Recently, the Global Alignment of Immunisation Safety Assessment in Pregnancy (GAIA) consortium, supported by the Brighton collaboration, offered a very detailed framework for the collection and analysis of safety data gathered during pregnancy vaccine trials [369, 370]. These unified guidelines for the surveillance of pregnancy associated (serious) adverse events are absolutely necessary for the harmonization and standardization of case definitions regarding (serious) adverse events occurring during pregnancy, the fetal period and the neonatal period. Especially when thinking about introducing newly developed vaccines which can possibly be used during pregnancy.

IMMUNOGENICITY OF THE VACCINE DURING PREGNANCY

a) Pregnant women

Protection against pertussis is dependent on both humoral and CMI responses. Despite the fact that vaccination with an aP containing vaccine induces adequate pertussis-specific humoral and CMI responses in adolescents and women of childbearing age [73, 192, 193], little is known about the immune responses in pregnant women.

A US study performed in a small group of pregnant and non-pregnant women showed comparable humoral immune responses after Tdap vaccination between both groups [102]. Within this PhD thesis, we were able to confirm the comparable humoral immune response between pregnant and non-pregnant women, with no statistical differences in GMC before, one month and one year after vaccination for all antigens included in the Tdap vaccine [152].

Additionally, a good humoral immune response to Tdap vaccination during pregnancy was observed in our study with significantly higher antibody levels one month after Tdap vaccination compared to baseline levels [152]. This is in line with previous observations in non-pregnant women of childbearing age and postpartum women [73]. One year after Tdap vaccination, we already detected a significant decline of these antibody levels like it was also the case in the study by Abu Raya et al [111].

This finding of rapidly waning antibodies after maternal Tdap vaccination supports the recommendation for repeat booster vaccinations with consecutive pregnancies in order to transport sufficiently high titers of maternal antibodies to protect subsequently born infants. However, in one of our studies we modelled anti-PT IgG antibody concentrations in mothers and two siblings born before and after a pre-pregnancy Tdap booster vaccination. We were able to demonstrate that the interval between consecutive booster doses should be no longer than 30 months, which is approximately 2.5 years, in women of reproductive age to have sufficient antibody levels to PT at a next delivery [151]. But, we need to carefully interpret these results since the vaccine in this study was administered before pregnancy and the estimated interval between consecutive booster doses is an assumption based on mathematical modelling. The interval is not a strict recommendation but a finding that needs to be confirmed in additional studies on antibody persistence and studies where similar calculations are made when vaccination is offered during pregnancy. Therefore, we are currently developing a NLMM to model the dynamics of antibodies induced by vaccination during pregnancy. From this model, we hope to shed further light on the need for repeat boosters in repeat pregnancies and the interval needed

between two consecutive booster doses administered during pregnancy to optimally protect the offspring.

Evidence regarding CMI responses in pregnant women after maternal Tdap vaccination was so far lacking. Until recently, only data on CMI responses to influenza vaccines are available indicating enhanced natural killer cell and T-cell responses to inactivated influenza vaccination in pregnant women compared to non-pregnant women [197] and similar efficacy of influenza vaccination in all stages of pregnancy [196]. Yet, since pregnancy is a complex immunologic state where responses to some stimuli are suppressed while others are enhanced [99], we cannot extrapolate the immunological findings for influenza vaccination to vaccination with aP containing vaccines. In our study, we looked at the CMI responses in pregnant and non-pregnant women one month after Tdap vaccination and found that the Tdap vaccine stimulated significantly weaker proliferative and IFN-γ responses in pregnant compared to non-pregnant women. Proliferative and IFN-γ responses in responses to the polyclonal pokeweed mitogen were also significantly weaker in pregnant compared to non-pregnant women. Although the Tdap vaccine was able to stimulate CMI responses in both pregnant and non-pregnant women, the responses equaled baseline levels again one year after vaccination indicating that there is only a transient stimulation of CMI responses. A limitation of these results is the low sensitivity of the technique used. Preliminary results presented at the INMIS congress in September 2017 indicated however a similar rise in TNF-α and IFN-γ responses in pregnant women in response to aP vaccination upon stimulation of either whole blood (UK) or PBMC (Brazil) (confidential results). What this finding means in terms of the offered protection for the woman by the vaccine when administered during pregnancy, is not clear.

Overall, we were able to show that vaccination of pregnant women with aP containing vaccines is immunogenic and induces a sufficient amount of maternal antibodies in the women that is transported to the fetus to protect the offspring in the first weeks of life. We demonstrated that while CMI responses were boosted to a lesser extent in pregnant women, humoral immune responses were comparable between pregnant and non-pregnant women.

b) <u>Transplacental transport</u>

In all studies, we conducted within this PhD, we observed an active transplacental transport of maternal antibodies from mother to infant for all antigens with a good correlation between the maternal and infant antibody levels at birth [151, 153, 154]. As was already described in literature, we found a variation in transport ratio for the different antigens possibly due to the

production of variable proportions of IgG subclasses after exposure to different antigens [119]. This was also observed in a study by Jones et al with a more efficient transport of pertussis and tetanus antibodies compared to Hib antibodies across the placenta [371].

The possible influence of several factors on the transplacental transport ratio was also studied. The FcRn receptor, responsible for the transport of maternal antibodies from mother to infant, is more expressed with advancing gestation resulting in more efficient transplacental transport later in pregnancy [120]. However, we were not able to observe an influence of gestational age on the transplacental transport since all children were born term (>36 weeks of gestation; according to the WHO definition at the time the study was initiated). As already described in previous papers [123, 125] and confirmed in our papers, no influence of maternal age, parity or mode of delivery on the transplacental transport ratio was found. Also, since all women in our study were healthy, we could not assess the influence of diseases during pregnancy like HIV, malaria and immunodeficiency's on the transplacental transport [126].

A difference in transplacental transport ratio between the study we conducted in Belgium and Vietnam was found with in general lower transplacental transport ratio's in Vietnam, except for DT. This finding might potentially be explained by the fact that avidity is described to play a role on the efficiency of antibody transfer across the placenta with more efficient placental transfer for high avidity maternal antibodies [118] since higher avidity of anti-PT antibodies is seen in both mother and cord samples from Belgian mother-infant pairs compared to Vietnamese mother-infant pairs [261].

c) Infants

Interference or blunting of immune responses by maternal antibodies of the infant immune response is one of the major concerns of the maternal immunization strategy. High levels of circulating maternally derived antibodies are known to interfere with the infant immune responses to primary and even booster immunization [132]. In the nineties, interference of naturally acquired maternal pertussis antibodies with the infant's humoral immune response to wP, yet not to aP vaccines was already described [229].

In this PhD thesis, blunting is defined as a significantly lower antibody titer at a certain time point in the offspring of women vaccinated with an aP containing vaccine during pregnancy compared to the offspring of women not vaccinated with an aP containing vaccine during pregnancy. Blunting of the anti-PT and anti-DT immune response was observed after primary immunization and of the anti-PT immune response after booster immunization in the second year

of life in the Belgian study [153, 155]. In the Vietnamese study, blunting of the anti-Prn and anti-DT immune responses is seen after primary immunization yet not anymore after booster immunization [154, 156]. Several other recently conducted studies [102, 157, 186, 372] confirm this blunting effect on the infant immune responses after maternal Tdap vaccination (Table 1). However, this interference effect is highly variable for different aP containing vaccines and even in different studies on the same aP containing vaccine (Table 1). Possible reasons for these differences besides the use of different brands of aP containing vaccines during pregnancy and infancy could be the different demographic composition of the populations, the variable epidemiological background for pertussis between different populations, differences in vaccination history, the use of different infant vaccination schedules, a variable gestational age at maternal vaccination, the use of different laboratory techniques… Even when comparing our Belgian and Vietnamese study with results obtained in the same laboratory, we see less blunting by maternal antibodies in Vietnam. One of the possible explanations for this could be the use of different brands of vaccines in the mother versus infant in Vietnam, resulting in different antibodies and less blunting as was already shown in a mouse model by Feunou-Feunou et al [260].

At the moment, the clinical significance of blunting after maternal Tdap immunization is not clear, mainly because of a lack of an established correlate of protection for pertussis [38]. The impact of blunting on the possible reemergence of pertussis at a later age is likely to be dependent on country-specific immunization schedules. For example, in the UK, no booster dose is administered to children until the age of 3 year. Therefore, blunting is more a concern in countries with similar schedules as the UK compared to countries like Belgium that foresee a booster dose in their infant immunization schedule in the second year of life. Ongoing surveillance to detect pertussis cases in older infants from Tdap vaccinated women will be critical to understand the possible long-term impact and the clinical significance of this blunting effect. However, we have to keep in mind that the main objective of maternal immunization is to protect young infants from severe pertussis, which is in my opinion and in the opinion of pediatricians more important than the potential risk of contracting pertussis at an older age.

A recently conducted study (UK) showed that there is not only interference of the infant immune responses to the same vaccine antigens than the ones included in the Tdap vaccine, but also to vaccine antigens conjugated to CRM or TT, resulting in blunting of the pneumococcal immune responses after primary infant immunization [157]. In Belgium, we were able to confirm these results with blunting for 9 out of 13 vaccine-included pneumococcal serotypes after primary immunization and 2 out of 13 serotypes after booster immunization [158]. We also showed that

while pneumococcal immune responses were blunted, the proportion of infants achieving protective concentrations was similar after primary and booster vaccination irrespective of the maternal vaccination status which reassures that the blunting effect will likely have no impact in our Belgian setting. Worthwhile to mention is that the study we conducted in Belgium was the first study looking at the impact of maternal Tdap vaccination on pneumococcal booster vaccination. Unfortunately, we did not have the opportunity to further confirm these results in our Vietnamese study since the pneumococcal vaccine is not included in the EPI infant immunization schedule in Vietnam [254].

As we describe in the third chapter of this PhD thesis, blunting is not only observed after maternal immunization, but also after a pre-pregnancy (or postpartum) booster dose depending on the interval between vaccination and a subsequent delivery. In our study, after a median interval of 16.80 months between Tdap vaccination and a subsequent delivery, children born after a booster dose of an aP containing vaccine showed significantly lower antibody titers for all measured pertussis specific antigens at month 12 compared to children born before the booster dose of an aP containing vaccine.

This thesis shows that not only countries who recommend maternal vaccination, but also countries who recommend cocoon vaccination or repeated boosters in adults should be aware that high concentrations of maternal antibodies are transported to the fetus during pregnancy not only leading to protection of the infant in the first weeks of life, but also potentially blunting the infant immune responses to their own immunizations.

	Country	*Period*	*Study type*	*Number of participants*	*Maternal vaccine; Gestational age at vaccination*	*Infant vaccine; Infant vaccination schedule*	*Blood sampling time points*	*Infant's outcome measure*	*Interference*
STUDIES CONDUCTED BY OTHER RESEARCH GROUPS									
Hardy-Fairbanks et al., 2013 [186].	USA	2006-2009	Retrospective cohort study	Tdap group: 54 mother-infant pairs Control group: 16 mother-infant pairs	Adacel® (Sanofi Pasteur); any timester	Different vaccine products from different brands; 2, 4, 6 months and 12-18 months	Before and 1 month after primary vaccination; before and 1 month after booster vaccination	Antibody levels to PT, FHA, Prn, FIM2/3, TT, DT, HBV, polio 1/2/3	*No interference reported after primary and booster vaccination.
Muñoz et al., 2014 [102].	USA	2008-2012	Randomized, double-blind, placebo controlled trial with cross-over design	Tdap group: 33 mother-infant pairs Control group: 15 mother-infant pairs	Adacel® (Sanofi Pasteur); 30-32 weeks of gestation	Pentacel® (Sanofi Pasteur); 2, 4, 6 months and 12 months	Before and 1 month after primary vaccination; 1 month after booster vaccination.	Antibody levels to PT, FHA, Prn, FIM2/3, TT and DT	*After primary vaccination, interference reported for FHA. * No interference reported after booster vaccination.
Ladhani et al., 2015 [157].	UK	2012-2014	Case-control study with historical control group	Tdap group: 141 mother-infant pairs Historical control group: 246 mother-infant pairs	Repevax® (Sanofi Pasteur); not mentioned	Pediacel® (Sanofi Pasteur); 2, 3, 4 months	Before and 1 month after primary vaccination (For historical control group, only 1 month after primary vaccination).	Antibody levels to PT, FHA, FIM2/3, DT, TT, Hib, MenC, 13 pneumococcal serotypes	* After primary vaccination, interference reported for PT, FHA, FIM2/3, DT and some pneumococcal serotypes.
Villarreal Perez et al, 2017 [372].	Mexico	2011-2015	Randomized, double-blind, parallel group controlled	Tdap group: 102 mother-infant pairs Control group: 102 mother-infant pairs	Not mentioned; 30-32 weeks of gestation	Pentavalent vaccine with aP component; 2, 4, 6 months	Before each dose of the primary vaccination schedule	Antibody levels to PT and Prn	*Blunting reported for PT before the second and third dose of the primary vaccination.

STUDIES CONDUCTED BY OUR RESEARCH GROUP									
Maertens et al., 2015 [153]. Maertens et al., 2016 [155]. Maertens et al., 2017 [158].	Belgium	2012-2015	Prosepctive controlled cohort study	Tdap group: 55 mother-infant pairs Control group: 26 mother-infant pairs	Boostrix® (GlaxoSmithKline Biologicals); 22-33 weeks of gestation	Infanrix Hexa® (GlaxoSmithKline Biologicals); 8, 12, 16 weeks and 15 months	Before and 1 month after primary vaccination and before and 1 month after booster vaccination.	Antibody levels to PT, FHA, Prn, TT and DT.	* After primary vaccination, interference reported for PT, DT and 9 pneumococcal serotypes. * After booster vaccination, interference reported for PT and 2 pneumococcal serotypes.
Hoang et al., 2016 [154]. Maertens et al., 2016 [156].	Vietnam	2013-2015	Randomized controlled trial.	Tdap group: 51 mother-infant pairs. Control group: 48 mother infant-pairs.	Adacel® (Sanofi Pasteur); 18-36 weeks of gestation	Infanrix Hexa® (GlaxoSmithKline Biologicals); 2, 3, 4 months and second year of life	Before and 1 month after primary vaccination and 1 month after booster vaccination.	Antibody levels to PT, FHA, Prn, TT and DT.	* After primary vaccination, interference reported for Prn and DT. * No interference reported after booster vaccination.

Table 1: Summary of clinical trials on maternal immunization describing blunting.

INFLUENCE OF TIMING OF MATERNAL VACCINATION

The timing of maternal vaccination during pregnancy plays possibly an important role on both the amount and quality (avidity) of antibodies measured in maternal and cord samples at delivery and eventually on the incidence of pertussis disease in infants below 12 weeks of age.

In a small prospective cohort study conducted in Israel, higher anti-PT and anti-FHA antibody levels in cord were seen in children from women immunized between 27 and 31+6 weeks of gestation compared to children from women immunized at a later time point in pregnancy [218]. These results were confirmed in the study from Naidu et al., where higher antibody titers were found in maternal samples at delivery in women vaccinated between 28 and 32+6 weeks of gestation compared to women vaccinated between 33 and 36+6 weeks of gestation [373]. A recently conducted Swiss study found similarly significantly higher antibody titers in cord from term born infants for women vaccinated in the second trimester between 13 and 25 weeks of gestation compared to women vaccinated in the third trimester after 26 weeks of gestation [176]. The same effect was also seen in the cord of preterm born infants, even when they were born before 33 weeks of gestation [374], a time point where the transplacental transport is considered to be suboptimal [123]. Unfortunately, we were not able to confirm the effect of gestational age at vaccination on antibody titers in cord samples in the studies included in this PhD thesis. Presumably due to the fact that our study was not powered to perform this analysis and larger cohorts are needed to show this effect. However, in a randomized controlled trial that we are currently conducting in Bangkok, Thailand, preliminary analysis of maternal and cord samples showed an influence of the time interval between Tdap vaccination and delivery on anti-PT antibody titers in cord with higher antibody titers when the interval between Tdap vaccination and delivery was higher (Wanlapakorn et al. Abstract accepted for oral presentation at INMIS-2017, Belgium) [375].

Besides the effect of the timing of maternal vaccination on antibody titers, also an effect on antibody quality has been described. In an Israeli study, a higher PT-specific Relative Avidity Index (RAI) was noted in women vaccinated between 27 and 30+6 weeks of gestation compared to women vaccinated later in pregnancy. Also, a positive correlation between the RAI and time interval between Tdap vaccination and delivery was seen [376]. However, we were not able to confirm these results in our Belgian and Vietnamese study where RAI against anti-PT antibodies in both maternal and cord samples was determined. In general, higher avidity was found in Belgian compared to Vietnamese samples possibly due to a difference in vaccination history, previous exposure to infection and other factors within these two cohorts. In Belgium, no correlation

between RAI and gestational age at vaccination in cord samples was observed. In the Vietnamese study, even an inverse trend was seen, with lower RAI in maternal samples when the interval between Tdap vaccination and delivery was higher [377].

Despite the effect of timing of maternal vaccination on antibody quantity and quality, also a clinical effect on preventing pertussis in infants below 12 weeks of age has been described. Vaccination between 27 and 36 weeks of gestation turned out to be more effective in preventing pertussis in newborns compared to vaccination in the second trimester [359]. However, this result is contradictory to the previously reported effects of timing on antibody quantity and quality and should be interpreted with caution since the number of pertussis cases described in this article is rather low (N=1562) resulting in an overfitting of the model and a probable overestimation of the association between trimester of vaccination and prevention of disease [378].

In summary, the results from all above-mentioned studies need to be interpreted carefully since the number of participants in all studies were rather low. Therefore, further data regarding the influence of timing of maternal vaccination on antibody levels, quality of antibodies and effectiveness against disease are absolutely necessary since other factors (such as comorbidities, parity, vaccination status of the mother, vaccination history of the mother…) apart from gestational age at vaccination could possibly confound this relation.

EFFECTIVENESS OF THE STRATEGY

Until recently, limited or no evidence regarding the effectiveness of maternal pertussis vaccination was available except from two UK studies conducted in the first months following the introduction of their national program (2012) [379]. The first study, a case-control study with a relative small sample size of 58 cases and 55 controls showed a vaccine effectiveness (VE) against laboratory confirmed pertussis for maternal Tdap vaccination of 93% [248]. A comparable VE was found in the study by Amirthalingham et al using the screening method with an overall VE against laboratory confirmed pertussis of 91% in infants below 3 months of age if mothers were vaccinated at least 7 days before delivery. A significant drop in VE was observed if the vaccine was given too late in pregnancy [188].

In the last year, more data on the effectiveness of the maternal pertussis vaccination strategy became gradually available. A case-control study conducted in Spain showed similar results to the UK results with a VE against laboratory confirmed pertussis of 90.9% [380]. A US study performed on a large cohort of infants demonstrated a VE against laboratory confirmed pertussis of 91.4% in the first two months of life and of 69.0% during the first year of life. The effectiveness in between the different doses of the primary infant vaccination schedule was also

checked and no negative VE estimate of maternal Tdap vaccination after infant DTaP vaccination or lower than expected VE estimates after infant DTaP vaccination was found indicating that neither type of evidence for interference was present. Also receipt of a Tdap vaccine within two years before pregnancy, which is in fact pre-pregnancy vaccination as we described in the third chapter of this PhD thesis, appeared to provide some protection against disease with a VE against laboratory conformed pertussis of 55.6% in the first year of life. Postpartum vaccination on the other hand does not result in a significant reduction of the risk for contracting pertussis in the first year of life [381]. This was also shown in the study by Winter et al where prenatal Tdap vaccination was 85.0% more effective than postpartum vaccination in preventing pertussis in infants below 8 weeks of age [359]. If infants, despite maternal immunization, contract pertussis disease, maternal vaccination is 58.0% effective in preventing hospitalization and ICU admission [382]. In Argentina, where they introduced maternal pertussis vaccination in February 2012, a relative reduction of 51.0% in pertussis cases was measured in high coverage states (maternal Tdap coverage >50.0%) compared to low coverage states shortly after the start of the recommendation. They also observed a reduction of 44.0% in pertussis illness in infants between 2 and 6 months. This reduction was not significant in infants between 6 months and 2 years [383].

Unfortunately, we were not able to look at VE within our studies due to the small sample size and the fact that no clinical cases of pertussis were identified in our study cohorts.

EFFECT ON BREASTFEEDING

As we highlighted in our review, data on the effect of vaccination during pregnancy on the composition of breast milk, in particular on the presence of disease specific sIgA antibodies, and on possible protection provided by breastfeeding, are very limited. At the time we conducted the literature review, no data on the effect of vaccination with an aP containing vaccine during pregnancy on breast milk composition were available [159]. This knowledge gap was also recognized in the article by Beigi et al stating that investigations into the effect of breast milk derived antibodies elicited by maternal immunization is absolutely necessary [384].

When starting this PhD, no commercially available ELISA tests were validated for the measurement of anti-PT sIgA and total sIgA antibodies in breast milk and also data on the pre-analytical procedures for breast milk handling were rather scarce. Therefore, we tested several pre-analytical procedures for breast milk handling and we validated commercial immunoassays to detect anti-PT sIgA and total sIgA in breast milk [316]. After this validation, we compared the effect of several pertussis vaccination strategies in adult women on breast milk composition and demonstrated that women receiving a pertussis containing vaccine during pregnancy or at/shortly

after delivery show significantly higher levels of anti-PT sIgA antibodies in breast milk 8 weeks postpartum compared to women who did not receive a pertussis containing vaccine for at least 5 years before delivery [160].

A limitation of our study was that samples were only taken at one time point postpartum, i.e. 8 weeks postpartum, which made it impossible to describe breast milk antibody kinetics. However, in an Israeli study, breast milk samples were taken at birth (colostrum), 2, 4 and 8 weeks postpartum. In that study, a significant decline in anti-PT and anti-FHA sIgA levels over time is described [112]. This was also seen in the study by Halperin et al., where women were vaccinated postpartum, with already a decrease in breast milk antibody levels at day 28 post-vaccination [73]. Our study, the Israeli study and the one by Halperin et al demonstrated that both vaccination in pregnancy and postpartum vaccination increases disease specific sIgA in breast milk. However, when vaccinating women postpartum, no influence on antibody concentrations in colostrum is present since a peak in antibody concentrations is only achieved between day 10-14 post-vaccination [73].

Currently, studies regarding the protective effect of breastfeeding against pertussis are rare. In our studies, we did not have the opportunity to look at the possible protective effect of breast milk with high amounts of sIgA against pertussis. A study conducted in 1994 stated that although breastfeeding seems to protect infants aged 0-6 months against acute lower respiratory infections requiring hospitalization, no protection against pertussis-like illness is conferred [385]. This finding was countered in a recently conducted study where a possible protective effect of breastfeeding against pertussis was suggested in infants from mothers who did not receive a Tdap vaccine during pregnancy [380]. Worthwhile to mention is that in the 1994 study, none of the women received a pertussis containing vaccine during pregnancy while in the recent study half of the women received a pertussis containing vaccine during pregnancy suggesting that the possible protective effect of breastfeeding found in that study may originate from specific anti-PT sIgA produced by the mother as a result of maternal vaccination rather than by natural components of breast milk.

VACCINATION COVERAGE AMONG THE TARGET GROUP AND ACCEPTANCE OF THE STRATEGY

Coverage data for maternal pertussis vaccination in other countries where the strategy is currently implemented are very limited and if available, coverage often remains sub-optimal. In the US, a coverage of Tdap during pregnancy of 45.9% was reported between March 2013 and December 2014 [386]. Argentina started a maternal Tdap vaccination program in 2012 and national coverage increased from 51% to 67% in the following two years [148]. In Catalonia, Spain, a maternal Tdap vaccination coverage of only 25.6% in the first-year post-implementation was reported [387]. In England, a coverage estimate of 73.8% for maternal pertussis immunization was reported between February and March 2017 by the sentinel system. This coverage level is the highest level recorded since the introduction of the program in 2012 [388] and might be related to the recent broadening of the optimal gestational window of vaccination in the UK from 28-32 weeks of gestation to 16-32 weeks of gestation [142]. The longer period available for vaccination, including a greater opportunity for reminders, could contribute to the high coverage.

In this PhD thesis, two studies that determined the maternal pertussis vaccination coverage in Flanders, Belgium, are included. Both studies were conducted after the start of the free of charge availability of the Tdap vaccination program in Flanders. In the first study conducted between October 2014 and May 2015, a coverage of 64.0% is reported [161]. In the second study conducted in the first half of 2016, a slightly higher coverage of 69.3% is reported. Looking at both studies, it seems that the coverage is increasing over time in Flanders. However, some caution in interpreting the data must be taken as the first study was only conducted in hospitals with more than 800 deliveries per year and coverage in the second study was slightly lower in the subgroup of women delivering in a hospital with a larger maternity unit. Therefore, it can't be excluded that the maternal pertussis vaccination coverage was also higher in hospitals with a smaller maternity unit in the 2014-2015 study.

If we compare the pertussis vaccination coverage found in our studies [161, 162] with the coverage of 39.2% found in the study by Laenen et al in Flanders (Belgium), much higher coverages were found in our studies. This can be explained by the fact that the study by Laenen et al. was conducted shortly after the implementation of the recommendation in Flanders and before the free availability of the vaccine on the Flemish market. Also, women in the study by Laenen et al. were interviewed in the third trimester of their pregnancy. Therefore, it can't be excluded that some additional women received the vaccine after the survey resulting in a higher vaccination coverage at birth [329].

As already described in literature and confirmed in this PhD thesis, several social context factors like degree of education, work situation, parity, family income… negatively influence the maternal pertussis vaccination coverage resulting in underserved populations of pregnant women [329, 338, 340, 341, 389]. While safety concerns are mentioned in literature as one of the main reasons not to get vaccinated [338], no issues around safety were found in all Belgian studies [161, 162, 329]. One of the main reasons not to get vaccinated in Flanders was the fact that maternal Tdap vaccination was not offered or even discouraged by a health care worker. This was also pointed out in the article by Agricola et al where the absence of a recommendation by a physician was one of the main reasons for the refusal to get vaccinated against Tdap in pregnancy [390]. This highlights the fact that provider attitude is one of the key factors to achieve high maternal vaccination coverage.

We can conclude that the coverage of maternal pertussis vaccination in Flanders is fairly good. Yet, there is still room for improvement by targeting underserved populations of pregnant women and diminishing the hurdles to get vaccinated. In general, barriers to vaccination in pregnancy are complex and vary depending on the context and the population. Therefore, more research into the most effective strategies to optimize the uptake is needed so that the full potential of maternal immunization can be realized.

Strengths and limitations of this thesis

Like every research that is conducted, also this thesis has his strengths and limitations. One of the main strengths is that we were the first study group worldwide that was able to conduct a clinical trial on maternal immunization in field conditions in parallel in two different countries, i.e. Belgium and Vietnam. In fact, the original aim of the study in Vietnam was to compare infants vaccinated with aP containing vaccines with infants vaccinated with wP containing vaccines after maternal immunization. However, right before we started the study in Vietnam, some adverse events following immunization within the National Immunization Program happened after the administration of the pentavalent wP vaccine Quinvaxem® resulting in a suspension of the vaccine from the Vietnamese market. As a result, we were forced to change our study design and the hexavalent vaccine Infanrix Hexa® was used in all infants. Due to this unexpected change resulting in a comparable study design in Belgium and Vietnam, a unique opportunity was offered to compare infant immune responses to aP containing vaccines after maternal immunization in a HIC and LMIC, which is unique in the world. At the moment of publication, our Belgian and Vietnamese

studies reported on the largest cohort of mother-infant pairs worldwide up till then in a trial on maternal pertussis immunization.

A possible limitation of the trial conducted in Belgium is that it was difficult to strictly randomize pregnant women in a vaccine and control group due to the fact that the VVOG already recommended maternal vaccination at the time our study started. Despite this limitation, no differences in demographics between the vaccine and control group were seen.

Another strength of this thesis is that samples from both the Belgian and Vietnamese study were tested in the same laboratory in Belgium (National Institute of Public Health, Brussels) using the same ELISA kits. In addition, an international independent validation of the samples showed a good correlation of the data was performed (Canadian Center for Vaccinology, unpublished data).

The combination of comprehensible study designs and up to date statistical methods is certainly an asset for this thesis. Conducting clinical trials in pregnant women and their offspring is a challenge. Recruitment is time consuming and labor intensive. Also, retaining mother-infant pairs throughout the entire study period is not easy [234]. When conducting our studies, we were confronted with drop out of participants, resulting in a smaller sample size and larger confidence intervals. Luckily, only a limited amount of missing data was reported in the study giving us the opportunity to perform a complete case analysis which assumes that the 'missing data' process was unrelated to the observed and unobserved titer values. Additionally, in chapter 1, we had the opportunity to work with an innovative NLMM to model the dynamics of anti-PT antibodies after pre-pregnancy vaccination in both mothers and infants which enabled us to draft a possible recommendation regarding the time needed between two consecutive maternal booster doses. In the future, the NLMM will be further adapted and used to model dynamics of maternal antibodies after maternal pertussis vaccination.

One of the limitations of this PhD thesis is that the time points for blood sampling were not always ideal. In Chapter 1, time points for blood sampling were mainly chosen based on the kinetics of maternal measles antibodies [169] and were therefore not the most optimal time point for pertussis antibodies. Also, in Chapter 4, leftover samples from the trial on maternal pertussis immunization were used and thus time points were not ideal chosen for pneumococcal antibodies. Another limitation of this thesis is that there is no established correlate of protection for pertussis [38]. Therefore, we cannot speak of protection, but rather of the presence and titers of maternal antibodies, possibly correlating with protection.

Another limitation is that vaccination data of mothers in the survey studies were often self-reported and can therefore be subject to erroneous recall resulting in an under- or overestimation of the vaccination coverage. However, since recall is limited to the last year and no other vaccines except from Tdap and influenza are recommended to be administered during pregnancy in Belgium, the bias is less plausible.

Future challenges within the field of maternal (pertussis) immunization

Despite all the progress that has been made in the field of maternal immunization during the last years, there are still some remaining knowledge gaps. The question whether preterm infants can also benefit from maternal immunization and if so, whether we have to adapt the timing window of vaccination to optimally protect these preterm infants is currently unanswered.

When thinking about the broader implementation of maternal pertussis immunization programs, studies on infant immune responses to a wP containing vaccine after maternal Tdap vaccination are absolutely necessary. Also, epidemiological studies to estimate the burden of diseases preventable by maternal immunization in LMIC and research on the implementation of these vaccination programs in different countries including HIC, with a variety of local sensitivities need to be done. Currently, Bill & Melinda Gates Foundation has launched an initiative to evaluate the potential impact of maternal immunization in LMIC with a specific focus on 5 eligible diseases (tetanus, pertussis, influenza, Respiratory Syncytial Virus (RSV) and Group B Streptococcus (GBS)). For three of these, vaccines are already available [391].

Up till now, various brands of aP containing vaccines (with a different antigenic composition) have been used in different clinical studies on maternal immunization, both in pregnant women and infants [102, 177, 186, 218]. However, in the future, it would be beneficial to use several brands of aP containing vaccines in mothers and infants in one clinical study within one single population with the same epidemiological background to compare immune responses and possible blunting between those brands.

Yet, we only looked at the influence of maternal pertussis immunization on infant humoral immune responses. The influence of maternal immunization on pertussis specific cellular immune responses is not yet known and should be further investigated.

The success of maternal pertussis immunization has opened minds and doors to go further with this strategy. At the moment, a number of new vaccines are in various stages of development. There is a great potential for maternal immunization for diseases that affect mother, fetus and infant like Cytomegalovirus (CMV), RSV, GBS and Zika Disease… If these vaccines become available, the development of combination vaccines would certainly benefit the implementation of these vaccines into the existing health care programs.

Ongoing projects

This thesis is not an end point. In contrary, from this thesis, new research questions emerged to fill remaining knowledge gaps.

At the moment, we are conducting a clinical study in Belgium, the MAMA study (FWO funded), where we are looking at the differences in pertussis specific humoral and cellular immune responses between term and preterm born infants after maternal Tdap immunization. Additionally, we are also looking at the effect of Tdap administration on breast milk composition of women who delivered term and preterm at different time points postpartum. This study started in January 2015. Recruitment of this study has finished and we are now further following up the infants. The final results of this study are expected by the end of 2019.

Currently we are also conducting a clinical trial in Bangkok, Thailand. In this trial, we look at the differences in infant primary and booster immune responses to both aP or wP containing vaccines in infancy after maternal Tdap vaccination. Besides, also the quality of infant antibodies is monitored. Like in the MAMA study, the recruitment phase has already ended and we are currently following the infants until after the booster vaccination. The final results of this study are expected by the end of 2018.

Due to the high number of clinical trials on maternal antibody kinetics and maternal immunization conducted by our research team, we have a huge amount of leftover samples stored in our freezers. In the near future, the plan is to perform systems serology on these samples to further characterize missing information on both humoral and cellular immune responses in mothers and infants after maternal Tdap vaccination.

Conclusion

The strategy of vaccination during pregnancy turned out to be safe and immunogenic in both mothers and infants and also has a positive influence on breast milk composition and possible protection provided by breastfeeding. By using a mathematical model, we provided scientific evidence for the recommendation to repeat the vaccine every pregnancy. We were also able to show that with a good approach, it is possible to reach a high coverage of the strategy within a few years after implementation.

The results of this thesis are therefore supportive for the currently existing recommendations on maternal pertussis vaccination and provide additional scientific evidence to continue this vaccination strategy. On the basis of the results found in this work, we believe that this thesis could play a pivotal role in decision-making related to implementing of maternal immunization as a broader strategy to protect mothers, fetuses and young infants. Also, the results found on maternal pertussis vaccination in this thesis will be useful to future vaccination programs against CMV, RSV, GBS…

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List of publications

Peer reviewed national and international journal articles

- Maertens K, De Schutter S, Braeckman T, Baerts L, Van Damme P, De Meester I, Leuridan E. Breastfeeding after maternal immunisation during pregnancy. Providing immunological protection to the newborn: A review. Vaccine 2014 Apr 1; 32(16):1786-1792.
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Other publications (proceedings, non-peer reviewed articles, scientific reports)

- Hoang TTH, Leuridan E, Maertens K, Duong TH, Dang DT, Vu NH, Nguyen CK, Van Damme P, Dang DA. Report on the adverse events following immunization (AEFI) with Tdap in pregnant women in Vietnam and Belgium. Vietnam Journal of Preventive Medicine 2014.
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(presenting author marked with *)

- Huygen K*, Jurion F, Maertens K, Rodegiero C, Caboré RN, Van den Poel C, Van Damme P, Leuridan E. *Vaccination during pregnancy: humoral and cell-mediated immune responses to a pertussis containing vaccine in pregnant and non-pregnant women*. EUpert Strain & Genomics, Copenhagen.
- Maertens K*, Van Dyck W, Hens N, Van Damme P, Leuridan E. *Pertussis vaccination during pregnancy in Belgium*. Research Day Faculty of Medicine, 24 April 2015, Antwerp. **Award for best oral presentation.**
- Maertens K*, Caboré RN, Huygen K, Hens N, Van Damme P, Leuridan E. *Pertussis vaccination during pregnancy in Belgium*. EUpert Strain & Genomics Meeting, 8-9 May 2015, Brussels.
- Maertens K*, Leuridan E. *Pertussis vaccination during pregnancy in Belgium*. Vaccine Symposium "Maternal Vaccination", 17 September 2015, Utrecht. (Invited speaker).
- Maertens K*, Van Damme P. *Mother and child health: prevention of vaccine-preventable diseases.* The Belgo-Vietnamese Rector's Mission, 19-23 October 2015, Antwerp. (Invited speaker).
- Maertens K*, Van Damme P, Hens N, Leuridan E. *Effect of pre-pregnancy pertussis booster vaccine on the kinetics of pertussis antibodies in the infants up to 12 months of age.* 3rd International Neonatal & Maternal Immunization Symposium (INMIS-2015), 4-6 November 2015, The Gambia.
- Maertens K*, Leuridan E. *Pertussis vaccination during pregnancy: results from a Belgian and Vietnamese Clinical study.* Hoge Gezondheidsraad België, 10 December 2015, Brussels. (Invited speaker).
- Leuridan E, Maertens K*. *Vaccinatie tijdens de zwangerschap.* Bijscholing Intensieve Neonatale Zorg. 23 February 2016, Antwerp. (Invited Speaker).

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- Huygen K*, Caboré RN, Maertens K, Hoang TTH, Van Damme P, Dang DA, Leuridan E. Prospective cohort studies on pregnancy vaccination against pertussis, diphtheria and tetanus in Belgium and Vietnam. EUpert Strain & Genomics, 12-13 October 2016, Utrecht.
- Maertens K*. *Vaccinatiegraad in Vlaanderen: Bereik bij zwangeren*. Vijftiende Valentijn Vaccinatiesymposium, 10 February 2017, Antwerp. (Invited speaker).
- Wanlapakorn N*, Maertens K, Srimuan D, Chaithongwongwattana S, Thongmee T, Vongpunsawad S, Van Damme P, Locht C, Pooworavan Y, Leuridan E. *Assessment of IgG antibody to pertussis toxin after Tdap vaccination in Thai pregnant women.* 4th International Neonatal & Maternal Immunization Symposium (INMIS-2017), 10-12 September 2017, Brussels.
- Tran TMP*, Hoang TTH, Caboré RN, Maertens K, Van Damme P, Leuridan E, Hens N. *Mathematical modelling of maternal antibodies: the quest for the kinetics.* 4th International Neonatal & Maternal Immunization Symposium (INMIS-2017), 10-12 September 2017, Brussels.

Poster presentations

- Leuridan E, Hoang TTH, Maertens K, Duong TH, Anh DA, Van Damme P. *Adverse events following immunization (AEFI) with Tdap in pregnant women in Vietnam and Belgium.* 10th International symposium on Bordetella, 8-11 September 2013, Dublin.
- De Schutter S, Leuridan E, Maertens K, Baerts L, Van Damme P, De Meester I. *Quantification of Bordetella pertussis toxin antibodies in breast milk: optimazation of sample preparation and evaluation of commercial ELISAs.* 10th International symposium on Bordetella, 8-11 September 2013, Dublin.

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- Maertens K*, Burbide P, Orije M, Van Damme P, Goldblatt D, Leuridan E. *Pneumococcal immune response in infants whose mothers recieved Tdap vaccination during pregnancy.* 35th Annual Meeting of the Society of Pediatric Infectious Diseases, 23-26 May 2017, Madrid. **Poster selected for oral presentation.**
- Maertens K & Braeckman T, Theeten H, Roelants M, Hoppenbrouwers K, Blaizot S, Top G, Van Damme P, Leuridan E, Vandermeulen C. *Increasing coverage of pertussis vaccination during pregnancy in Flanders, but is there more than meets the eye?* 35th Annual Meeting of the Society of Pediatric Infectious Diseases, 23-26 May 2017, Madrid.

- Braeckman T, Theeten H, Roelants M, Blaizot S, Hens N, Maertens K, Hoppenbrouwers K, Van Damme P, Top G, Vandermeulen C. *Childhood vaccination achievements in Flanders from 2005 to 2016.* 35th Annual Meeting of the Society of Pediatric Infectious Diseases, 23-26 May 2017, Madrid.
- Vandermeulen C, Roelants M, Braeckman T, Maertens K, Top G, Van Damme P, Hoppenbrouwers K, Theeten H. *Vaccine confidence measured in 3 different parent populations in Flanders.* 35th Annual Meeting of the Society of Pediatric Infectious Diseases, 23-26 May 2017, Madrid.
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