EVALUATION OF PERTUSSIS IMMUNIZATION DURING PREGNANCY

by

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Abstract

Pertussis disease is most severe among young infants, leading to high morbidity and mortality. To reduce the burden of pertussis disease among young infants, immunization against pertussis during pregnancy has been implemented in an increasing number of countries over the past decade. My research goals have focused on addressing important knowledge gaps in the field of pertussis immunization during pregnancy to inform an evidence-based immunization program.

Using data on hospitalized pertussis cases admitted to pediatric tertiary care centers in Canada, I report that the highest morbidity and mortality from pertussis is among infants <2 months of age with an incidence rate of 116.4/100,000/year, 38% intensive-care unit admission rate, and 2.3% case fatality rate. Age <16 weeks, encephalopathy and prematurity were independently associated with a 5-fold, 21-fold and 6-fold increased risk for intensive-care unit admission, respectively. I also developed a novel approach that enables comprehensive characterization of anti-pertussis immunoglobulin G (IgG) avidity using a range of bond-breaking agent concentrations combined with high-dimensional biology statistical tools. I applied this approach on cord blood samples, and found that vaccination against pertussis during pregnancy was associated with high levels of high-avidity antibodies. I also found that maternal pertussis vaccination at 28–32 weeks gestation was associated with higher cord blood anti-pertussis IgG avidity that vaccination at 33–36 weeks gestation. Furthermore, I compared antibody responses after primary and/or booster immunization in infants born to women with and without pertussis immunization during pregnancy. I found lower vaccine-induced antibody

responses to pertussis, diphtheria and some *Streptococcus pneumoniae* serotypes in infants born to women vaccinated against pertussis during pregnancy compared with infants of unvaccinated women.

The body of work presented here assists public health policy makers to reach evidence-based recommendations across countries. Supporting earlier immunization in the 3rd trimester will be of particular clinical relevance for preterm infants who would completely miss out on protection via maternal antibodies if immunization only occurred in late pregnancy. These data from the meta-analysis supports enhanced surveillance of pertussis, diphtheria and invasive pneumococcal disease in infants to determine the clinical significance of this effect.

Lay Summary

Young babies are at high risk of severe whooping cough disease. Whooping cough vaccine has been increasingly recommended for pregnant women, to prevent disease in their babies. I have shown that infants under 2 months are at highest risk of ending up in an intensive care unit in the hospital or die from whooping cough. We don't know when is best to give the whooping cough vaccine in pregnancy. I also found that vaccination early in the third trimester is associated with higher levels of more potent antibodies as compared to vaccination later in the third trimester. I found that antibody levels to pertussis are lower in infants born to women vaccinated against pertussis during pregnancy compared to unvaccinated women after primary and booster immunization. Altogether, my PhD work helps understand how whooping cough vaccine works in pregnancy, and how to best use it to prevent whooping cough in babies.

Preface

Portions of **Chapter 1** are modified versions based on one publication:

Sections 1.1 are based on a published review

Abu-Raya B, Michalski C, Sadarangani M, Lavoie P. Maternal Immunological Adaptation During Normal Pregnancy. Front. Immunol. 07 October 2020. doi.10.3389/fimmu.2020.575197. Review. PubMed PMID: 33133091.

Co-author Michalski C and I were responsible for writing the manuscript under the supervision of Dr. Lavoie. The content in the sections described in this thesis chapter was originally written by me, with the exceptions of the sections describing the innate immune system changes (which are modified versions of sections initially written by Michalski C). The tables were originally written by me. Sadarangani M reviewed the manuscript and provided comments and edits.

Version of **Chapter 2** has been published:

Abu-Raya B, Bettinger JA, Vanderkooi OG, Vaudry W, Halperin SA, Sadarangani M; Members of the Canadian Immunization Monitoring Program, Active (IMPACT). Burden of Children Hospitalized With Pertussis in Canada in the Acellular Pertussis Vaccine Era, 1999-2015. J Pediatric Infect Dis Soc. 2020 Apr 30;9(2):118-127. doi: 10.1093/jpids/piy128.PubMed PMID: 30535079.

For this publication, I developed the idea and was responsible for conception and design of the study, data analysis, drafting the manuscript, preparing the figures and tables and addressing comments from reviewers. The analysis, manuscript write up and addressing comments from reviewers were supervised by Dr. Manish Sadarangani. Bettinger JA, Vanderkooi OG, Vaudry W and Halperin SA provided feedback on the study design, reviewed the manuscript, and provided comments and edits.

Versions of **Chapter 3** have been published:

Abu-Raya B, Giles ML, Kollmann TR, Sadarangani M.
Profiling avidity of antibodies elicited by vaccination using enzyme-linked immunosorbent assay-based elution - Insights into a novel experimental and analytical approach. Vaccine. 2020 Jul 22;38(34):5389-5392. doi: 10.1016/j.vaccine.2020.06.060. Epub 2020 Jun 30.PubMed PMID: 32620372

Abu-Raya B, Giles ML, Kollmann TR, Sadarangani M. The Effect of Timing of Tetanus-Diphtheria-Acellular Pertussis Vaccine Administration in Pregnancy on the Avidity of Pertussis Antibodies. Front Immunol. 2019 Oct 11;10:2423. doi: 10.3389/fimmu.2019.02423. eCollection 2019.PubMed PMID: 31681310

For the two publications, I developed the idea and was responsible for conception and design of the studies, obtaining the funding (principal investigator), doing the laboratory work, data analysis, drafting the manuscript, preparing the figures and tables and addressing comments from reviewers. Specifically, I optimized the avidity assay, proposed the analysis plan and statistical approach and performed the statistical analyses. I performed the laboratory work and measured the avidity of antibodies. The experimental design, manuscripts write up and addressing comments from reviewers were supervised by Dr. Manish Sadarangani. Giles ML and Kollmann TR provided feedback on the study design, reviewed the manuscript, and provided comments and edits.

Chapter 4 is an unpublished material and part of a manuscript in preparation: individual-participant data meta-analysis of antibody response after vaccination in infancy of infants born to women vaccinated against pertussis during pregnancy. I conceptualized the project and proposed the analytical approach. The systematic review and screening of identified articles was done in duplicate with Dr. Kirsten Maertens (University of Antwerp, Belgium). I analyzed the data. Early results were selected for an oral presentation at the international neonatal and maternal immunization symposium held in Vancouver on September 2019.

Chapter 5 is unpublished material written by myself.

All human research studies performed in the thesis were conducted under the human ethics approval of the UBC Research Ethics Board: Certificate number: H17-00050 (Avidity of pertussis antibodies) and H17-02635 (Active immune response in infants born to mothers immunized with Tdap: a systematic review and meta-analysis). Appropriate approvals for the study presented in Chapter 2 were obtained in all participating hospitals.

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List of Abbreviations

AAU: Absolute Avidity Units

AB: Alberta

abs: absolute

AIC: Akaike information criterion

aP: acellular pertussis

AU: Avidity Units

BC: British Columbia

Bregs: B regulatory cells

CENTRAL: Cochrane Central Register of Controlled Trials

CFR: case fatality rate

CH50: Complement hemolytic activity

CI: confidence interval

COP: Correlates of protection

CS: caesarean section

DFA: direct fluorescent assay

DT: Diphtheria toxoid

DTaP: diphtheria-tetanus-acellular pertussis

DTaP-HB-IPV-Hib: Diphtheria, Tetanus, acellular Pertussis, Hepatitis B, Inactivated Polio Virus, Haemophilus Influenzae type B vaccine

DTaP-IPV-Hib: Diphtheria, Tetanus, acellular Pertussis, Inactivated Polio Virus, Haemophilus Influenzae type B vaccine

ELISA: Enzyme-linked immunosorbent assay

EU: ELISA units

F: fractional

Fc: fragment crystallization

FcRn: neonatal Fc receptors

FHA: filamentous hemagglutinin

FIM2/3: fimbriae 2/3

Foxp3: Forkhead box p3

GMR: geometric mean ratios

GSK: GlaxoSmithKline

HBV: Hepatitis b virus

hCG: Human chorionic gonadotropin

Hib: *Haemophilus influenzae* type b

HIV: human immunodeficiency virus

HLA-DR: Human Leukocyte Antigen –DR;

ICU: intensive-care unit

IFN-γ: Interferon- γ

Ig: immunoglobulin

IgA: immunoglobulin A

IgE: immunoglobulin E

IgG: immunoglobulin G

IgM: immunoglobulin M

IL: interleukin

ILCs: Innate lymphoid cells

IMPACT: Immunization Monitoring Program Active

iNKT: invariant NK T cells

IPD: invasive pneumococcal disease

IQR: Interquartile range

IU/mL: international unit/ml

LAMP-1: lysosome-associated membrane protein-1

LLOQ: lower levels of quantification

LOS: length of stay

LPS: Lipopolysaccharides

M: Molar

Mat: maternal

MB: Manitoba

MDSC: myeloid-derived suppressor cell

Menc: meningococcal C

MHC: major histocompatibility complex

NA: Not applicable

N/Av: not available

NB: New Brunswick

NH_4SCN: ammonium thiocyanate

NK cells: Natural killer cells

NKT: natural killer T

NL: Newfoundland and Labrador

NS: Nova Scotia

NT: Northwest Territories

NU: Nunavut

NVD: normal vaginal delivery

ON: Ontario

PCR: polymerase chain reaction

PCP: pneumococcal capsular polysaccharide

PCV: pneumococcal conjugate vaccine

PE: Prince Edward Island

PHA: Phytohemagglutinin

Post-boost: post-booster

Post-prim: post-primary

Post-vac: post-vaccination

Pre-prim: pre-primary

Pre-vac: pre-vaccination

PRP: polyribosylribitol phosphate

PT: pertussis toxin

PMA: phorbol-12-myristate-13-acetate

Pre-boost: pre-booster

PRN: pertactin

QC: Quebec

RAI: relative avidity index

ROS: Reactive oxygen species

SD: standard deviation

SK: Saskatchewan

SPN: *Streptococcus pneumoniae*

T: total

Tdap: tetanus-diphtheria-acellular pertussis

Tdap-IPV: Tetanus, diphtheria, acellular pertussis, Inactivated Polio Virus vaccine

Th: T helper

TIM-3: T-cell immunoglobulin- and mucin domain-containing-3

TLR: Toll-like receptors

TNFα: tumor necrosis factor α

Tr1: type 1 regulatory T cells

Tregs: T regulatory cells

TT: tetanus toxoid

UK: United Kingdom

US: United States

VE: vaccine effectiveness

WBC: white-blood cell

WG: Weeks gestation

WK: weeks

wP: whole-cell pertussis

YT: Yukon

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To Vanier Canada, thank you so much for your support and trust in providing me with the highly prestigious graduate scholarship of the Canadian Health Research Institute. I hope that the research performed during my PhD will benefit pregnant women and their infants in Canada and worldwide. Merci beaucoup.

Dedication

For my loving wife Dr. Karama Asleh, who's without her support, this journey could not have been made possible.

1. Introduction

1.1 Susceptibility of pregnant women and newborns to infection and underlying immunology

1.1.1 Susceptibility of pregnant women, fetus and newborn to infections

Increased risks for certain infections during pregnancy suggest significant changes during gestation[1]. Some infections are more common (e.g. urinary tract infections) or more severe (e.g. pneumonia) due to physiological and physical changes occurring during pregnancy[2,3]. Pregnant women are at increased susceptibility for particular infections and some infections are more severe in pregnant women, probably due to immunological adaptations associated with pregnancy. Furthermore, certain infections might have minimal impact on pregnant women themselves but can severely affect the fetus and lead to long-term health consequences for the neonate and infant (Table 1.1). Altogether, these data point towards clinically important and unique interactions between physiological, hormonal and immunological elements occurring during pregnancy.

Table 1. 1: Infections associated with increased maternal susceptibility or severity during pregnancy, or severe adverse fetal outcomes.

Infection	Reference
Increased maternal susceptibility	
Listeriosis	[4-10]
Tuberculosis (during the puerperium)	[11, 12]
Malaria	[13-16]
Increased maternal severity	
Influenza	[17-22]
Varicella Zoster Virus infection	[23-27]
Hepatitis E virus infection	[28-31]
Malaria	[14, 32-35]
Invasive *Haemophilus influenzae* infection	[36-38]
Invasive pneumococcal disease	[39]
Invasive group A streptococcal disease	[39]
Dengue fever	[40]
Lassa Fever	[41, 42]
Ebola virus	[41]
Primary Herpes Simplex Virus infection	[43-45]
Coccidiomycosis†	[46-50]
Measles	[51, 52]
Severe adverse fetal outcomes	
Toxoplasmosis	[53, 54]
Influenza	[17, 19, 21, 55-58]
Primary varicella zoster virus infection	[24, 59]
Malaria	[33]
Rubella	[60-62]
Parvovirus B19	[63]
Listeriosis	[4, 9, 64, 65]
Tuberculosis	[66, 67]
Zika virus	[68, 69]
Measles	[52, 61, 70, 71]
Mumps	[70]
Cytomegalovirus	[72]

†: Some data suggest increased maternal severity while other data do not suggest this association.

1.1.2 Immunological changes during pregnancy

During pregnancy, major adaptations and fine balance occur in the maternal systemic immune system to protect the mother and her future baby from pathogens while avoiding

detrimental immune responses against the allogeneic fetus. A better understanding of immunological changes during pregnancy may also be important in considering optimal strategies for use of pertussis vaccines, to protect both the pregnant woman and infant[73]. The dynamic changes occurring in the peripheral maternal immune system during normal pregnancy are described below.

INNATE IMMUNITY

Complement system

Studies suggest increased complement activity during pregnancy (Table 1.2). Plasma levels of C3a, C4a, C5a, C4d, C3a, C3, C9, and the Serum Complement Membrane Attack Complex SC5b9 are elevated during pregnancy[74.75,76]. The balance in complement system is maintained through high levels of regulatory proteins such as factor H which blocks the alternative C3 convertase[77]. Complement hemolytic activity (CH50) reflects activity of the classical complement pathway and increases as pregnancy progresses[78,79].

Granulocytes

Eosinophil and basophil counts are not affected by pregnancy (Table 1.2)[80, 81]. However, urinary eosinophil-derived neurotoxin secretion is elevated during the second and third trimester, suggesting increased eosinophil degranulation. In contrast, urinary N-methylhistamine concentrations are lower in the third trimester, suggesting reduced mast cell degranulation[82]. There is a gradual, marked increase in neutrophils from the first trimester onwards[80, 83].

Granulocyte colony-stimulating factor and Granulocyte monocyte colony-stimulating factor, two cytokines mediating bone marrow neutrophil production, are also elevated during pregnancy[80, 84]. The function of neutrophils may be altered during pregnancy, as suggested by reduced phagocytosis of zymosan molecule[85]. Elastase and lactoferrin are secreted from primary and secondary neutrophil granules, respectively, and are elevated in the first trimester [80].

Monocytes

Three main subsets of monocytes have been characterized in humans. Classical monocytes ($CD14^{high}CD16^{-}$) are the main subset in the peripheral blood of healthy adults (~80% of all monocytes) and have phagocytic functions. Non-classical monocytes ($CD14^{low}CD16^{high}$) are inflammatory[86]. Intermediate monocytes ($CD14^{high}CD16^{intermediate}$) may represent a transitional state, displaying both inflammatory and phagocytic capacity[86]. Monocytes also present antigens to T cells, hence modulating adaptive immune responses. Monocytes increase during pregnancy, beginning in the first trimester[87, 88]. The impact of pregnancy on maternal monocyte function has been reviewed elsewhere and is summarized (Table 1.2)[89, 90].

Table 1. 2: Changes in complement, granulocytes and monocytes during normal pregnancy.

Component	Main findings	References
Complement		
Levels	Elevated C3a, C4a and C5a in the second and third trimester in comparison to non-pregnant women.	[74]
	Elevated C4d, C3a, C3, C9, the Serum Complement Membrane Attack Complex SC5b9 during pregnancy.	[75]
Regulatory proteins	High levels of regulatory proteins (e.g. Factor H).	[77]
	Increased levels of the C3 inhibitor pregnancy-associated plasma protein A during the second and third trimesters.	[91, 92]
CH50	Increase in CH50 levels in healthy pregnancy as compared to non-pregnant women and increases as pregnancy progressed.	[79]
Granulocytes		
	Eosinophil and basophil counts were not affected by pregnancy.	[80, 81]
	Increased eosinophil degranulation during the second and third trimester compared to non-pregnant women.	[82]
	Increase in neutrophil counts from the first trimester onwards.	[80, 83]
	In vitro activated neutrophils from pregnant women show reduced respiratory burst activity and are refractory to priming with IFN-γ.	[93-95]
	Reduced phagocytosis of neutrophils during pregnancy.	[85]
	Increased levels of TLR4 co-receptor CD14 and the Fc receptor CD64 on granulocytes in the second and third trimesters compared to non-pregnant women. Reduced expression of the neutrophil maturity marker CD16 and the MHC II molecule HLA-DR on granulocytes in pregnant women.	[96]
Monocytes		
	Granulocytic but not monocytic MDSCs are elevated in pregnancy.	[97]
	Increases in monocyte numbers during pregnancy, mainly due to a higher number of "intermediate" monocytes, where classical monocytes decrease, with no change in the proportion of non-classical monocytes.	[87, 88 98, 99]
	Elevated stimulation-induced IL-12 and TNFα production by monocytes from pregnant women throughout all three trimesters.	[100, 101]
	Increased levels of activation markers CD11a, CD11b, CD14 and CD64, and ROS production by monocytes from pregnant women.	[83, 96]
	Reduced LPS-induced IL-12 and TNFα production by monocytes of third trimester pregnant women as compared to non-pregnant women.	[88]
	Reduction in non-classical monocytes and an increase in classical monocytes in the third trimester compared to healthy controls.	[102]

Abbreviations: CH50: 50% haemolytic complement; IFN-γ: Interferon- γ; ROS: Reactive oxygen species; TLR: Toll-like receptors; MHC: *major histocompatibility complex*; HLA-DR: Human Leukocyte Antigen –DR; MDSC: myeloid-derived suppressor cell; TNFα: tumor necrosis factor α; LPS: Lipopolysaccharides.

Innate Lymphoid Cells

Innate lymphoid cells (ILCs) lack CD3 and antigen-specific receptors [103]. Natural killer (NK) cells are the best characterized ILCs[104]. In blood, most NK cells express low levels of the adhesion molecule CD56 and the Ig receptor CD16. Most studies report no change in NK subsets ($CD56^{dim}$, $CD56^{bright}$), invariant NK T cells (iNKT) and type II non-classical NK T cells in peripheral blood between pregnant and non-pregnant women[105-107] despite a reduction in NK cell numbers[108, 109] (Table 1.3). Maternal NK cells and monocytes have increased expression of the immune checkpoint protein TIM-3 in pregnancy[107, 110], potentially induced by high IL-4 and low IFN-γ levels[111]. TIM-3 is important for NK cell-mediated IFN-γ production and may contribute to increased phagocytosis in pregnancy[111]. High surface levels of TIM-3, a characteristic of lymphocyte exhaustion[112], potentially indicate that pregnancy NK cells are anergic. The augmented inflammatory NK cell capacity during pregnancy is further supported by studies showing increased expression of the activation marker CD69 on $CD4^{neg}$ iNKT cells as pregnancy progresses[106]. Together, this indicates elevated baseline activity and heightened potential to upregulate pro-inflammatory responses, underlining increased innate immunity during pregnancy. This might serve as an important defense mechanism against infections in pregnancy. In contrast, IFN-γ production is reduced and IL-10 production upon *ex vivo* stimulation with PMA-ionomycin is increased by NK cells from the first trimester, compared to non-pregnant women[113]. This anti-inflammatory capacity could contribute to the dampening of the adaptive immune system which could help in

protection of fetus from rejection by the mother and thus optimize immunity without collateral damage to the fetus.

Table 1. 3: Changes in systemic innate lymphoid cells during normal pregnancy.

Component	Main findings	References
NK cells		
	No change in total numbers or frequency of NK subsets ($CD56^{dim}$, $CD56^{bright}$), iNKT and NKT cells in peripheral blood between non-pregnant and pregnant women, regardless of the trimester of pregnancy.	[105-107]
	Reduction in NK cell numbers in pregnant vs. non-pregnant women	[108, 109]
	Decreased ratio of type 1 NK cells (defined as expressing IL18R1) to type 2 NK cells (defined as expressing IL1RL1) in the third trimester compared to healthy controls suggesting reduced inflammatory responses.	[114]
	Increased expression of surface-marker immune checkpoint protein TIM-3 on NK cells and monocytes in pregnancy.	[107, 110]
	Increase in expression of the activation marker CD69 on $CD4^{neg}$ iNKT cells from the first to the third trimester, although the levels are not significantly different to age-matched non-pregnant controls.	[106]
	Increased expression of the degranulation marker LAMP-1 (CD107a) on $CD56^{dim}$ cells after PMA-ionomycin stimulation (reflects NK cell activity) and baseline levels of the natural cytotoxicity receptor NKp46 CD335 (regulator of NK cells function) in the third trimester as compared to non-pregnant women. These changes highlight the increased activity of NK cells which could contribute to protection from infections.	[99, 107]
	Reduced IFN-γ production and increased IL-10 production upon *ex vivo* stimulation with PMA-ionomycin by NK cells from the first trimester compared to non-pregnant women.	[113]

Abbreviations: NK: Natural killer; iNKT: Invariant natural killer T; NKT: natural killer T; TIM-3: T-cell immunoglobulin- and mucin domain-containing-3; LAMP-1: lysosome-associated membrane protein-1; PMA: phorbol-12-myristate-13-acetate; IFN-γ: Interferon – γ.

ADAPTIVE IMMUNITY

T cells

The absolute lymphocyte count and the percentage of total T cells does not differ significantly during the first, second, and third trimesters of pregnancy[115, 116], while the

numbers of T cells during pregnancy are lower than before pregnancy (Table 1.4)[117]. Pregnancy has also been associated with changes in T cell subsets, although the data are conflicting and the significance is unclear[117,118,115, 116]. The percentage of $CD4^+$ and $CD8^+$ T cells of women at various stages of gestation does not differ significantly[115, 116]. In another study, no significant changes were found in the percentage of $CD4^+$ cells, $CD8^+$ cells, nor of $CD4^+/CD8^+$ ratio at any stage of pregnancy[118]. However, compared to pre-pregnancy, the number of T helper cells and cytotoxic T cells was lower in third and first trimesters of pregnancy, respectively, while the number of suppressor T cells was higher in the first trimester of pregnancy[117].

Studies have investigated the ratio of Th2 to Th1 cells as measured by the circulatory levels of secreted Th1 or Th2 serum cytokines, or levels of $CD4^+$ cells producing Th1 or Th2 cytokines, or expression of chemokine receptors CXCR3 (associated with Th1 cells) and CCR4 (associated with Th2 cells) on $CD4^+$ T cells. The view of pregnancy as a Th2 state is supported by numerous studies[119-123], but also rejected by others[124]. Viewing pregnancy as a Th2 state is supported by a rise in anti-inflammatory cytokines, and by studies showing that Th1 and Th17 -type autoimmune disorders are improved[125-127] while Th2-type autoimmune disorders worsen in pregnancy[128]. A progressive shift from cell-mediated, pro-inflammatory, Th1 cell responses to humoral, anti-inflammatory, Th2 cell responses is initiated early in pregnancy[1, 129]. The ratio of Th17 cells (important against extracellular bacteria or fungal pathogens) to $CD4^+$ T cells is similar to healthy non-pregnant women during all stages of pregnancy[130,131, 132] (Table 1.4).

B cells

Maternal antibodies are the main maternal immune component that protect the neonate immediately after birth[133]. Peripheral blood B cell counts vary during normal pregnancy and the post-partum period, also compared to healthy non-pregnant women (Table 1.4)[117, 118, 134-142,143]. A reduction in circulating B cells is particularly prominent during the third trimester, revealing a "physiological" B cell lymphopenia[144] due to the effect of elevated estrogens on lymphopoiesis[145,146]. This B cell lymphopenia has also been attributed to cellular migration into tissues, including the placental decidua, and suggests that B cells play a particularly important role maintaining tolerance at the maternal-fetal interface[147]. In a mouse model, fetal trophoblasts converted B cells into IL-10-producing B cells which are thought to be important in regulating feto-maternal tolerance [148].

In a mouse model, treatment of mice with estrogen upregulated expression of CD22 receptor and the intracellular tyrosine phosphatase SHP-1 genes in B cells. Overexpression of these genes led to diminished calcium response in B cells after activation of BCR, thus supporting a role of these molecules in reduction in B cell receptor signaling[149]. B cells can also induce tolerance of immune system. In a mice model, a population of B cells promoted the proliferation of T-regulatory cells (Tregs). However, whether this happens in humans and in pregnancy has not been investigated[150]. Pregnancy is also associated with lower frequency or total levels of $CD5^+$ B cells during pregnancy, at delivery or early in the postpartum period[117, 134, 138, 142]. These are innate B-1 cells that produce natural IgM antibodies that are important in early protection following infection.

The function of B cells decreases as pregnancy advances. Loss of responsiveness to mitogens and infectious agents, which may increase the risk of infection, has been reported (Table 1.4)[151].

Immunoglobulins

Studies from the 1960s-70s reported conflicting results regarding immunoglobulin (Ig) levels during pregnancy (Table 1.4). Some studies suggest that total IgG levels remain stable during pregnancy[152-154], while other studies show a decrease in late pregnancy[155-161]. IgG1 levels were higher in pregnancy compared to non-pregnant women, while IgG3 levels were higher in pregnant women in their second trimester, compared to non-pregnant women[162]. IgG1 and IgG3 are important contributors to different functions of IgG including neutralization and opsonization of pathogens or antigens, activation of complement system, and sensitization of killing by NK cells (through antibody-dependent-cellular toxicity). IgG2 and IgG4 levels remain stable during pregnancy and levels are comparable to non-pregnant women[162].

IgGs are glycoproteins and contain N-glycans at both the Fc and Fab portion of IgGs. These N-glycans consist of a constant heptasaccharide core, fucose, N acetylglucosamine (GlcNAc), galactose(s), and sialic acid(s)[163, 164]. Pregnancy has been shown to be associated with changes in IgG Fc domain glycosylation, with an increase of galactosylation and sialylation of the Fc portion of IgG[163, 165, 166], whereas Fc fucosylation was shown to remain at high and very similar levels during pregnancy[163, 166]. IgG Fc domain glycosylation can have immune regulatory functions and modulate IgG effector functions as Fc-linked glycans alter the three-dimensional structure of the protein, thus

influencing the binding to Fc-receptors[167, 168]. Glycan–glycan interactions occur between IgG and Fc Receptor IIIa[169], with core fucose decreasing the affinity of this interaction[170]. Thus, high fucosylation of the Fc portion of the IgG, that is reported to occur during pregnancy, has the potential to inhibit the binding with Fc Receptor IIIa expressed on NK cells, and thus decreasing antibody-dependent cellular cytotoxicity, suggesting that this post-translational modification might be associated with an increased risk for infections in pregnancy.

Table 1. 4: Changes in T cells, B cells and immunoglobulins during normal pregnancy.

Component	Main findings	References
T cells		
Total levels	Lower levels of T cells during pregnancy than before pregnancy.	[117]
	No differences in the total lymphocyte count and the percentage of total T cells during the first, second, and third trimesters.	[115, 116]
Subsets	No difference in the percentages of T helper [$CD4^+$] and T suppressor [$CD8^+$] cells during the first, second, and third trimesters.	[115, 116].
	No significant changes in the percentage of $CD4^+$ cells, CD8+ cells, nor $CD4^+/CD8^+$ ratio at any stage of pregnancy.	[118]
	Pregnancy is associated with a Th-2 or anti-inflammatory state.	[119-123]
	A progressive shift from Th1 cell responses to Th2 cell responses initiated early in pregnancy.	[1, 129]
	Lower plasma IL-2 levels (indicative of $CD4^+$ Th1 cells) in the post-partum period when compared to all trimesters.	[171].
	Lower percentage of Th1 cells ($CD4^+$ cells producing IFN-γ) in the third trimester compared to the first trimester and no changes in the percentage of Th2 ($CD4^+$ cells producing IL-4) was observed.	[172]
	No change in the proportion of Th1 or Th2 cells during pregnancy. No differences in the percentage of $CD3^+CD8^-IFN\text{-}\gamma^+$ cells (Th1) across gestation. No change in the percentage of resting $CD4^+$ T-cells expressing CXCR3 (associated with Th1 cells) and CCR4 (associated with Th2 cells) during different stages of pregnancy.	[173]
	Increase in the numbers of IFN-γ and IL-4 secreting cells as pregnancy progressed compared with postpartum	[174]
	No change in the Th17/$CD4^+$ T cells ratio during all stages of pregnancy compared to that of healthy non-pregnant women.	[131]
Function	Reduced PHA-Stimulated T lymphocytes proliferation in pregnant women at various times throughout gestation compared with those from non-pregnant controls.	[175].
	Decreased lymphocyte proliferation to mitogenic stimulation in the first, second and third trimesters as compared to non-pregnancy.	[115]
	Decreased in IL-2 and IFN-γ production and increased in production of IL-4 and IL-10, during normal pregnancy in response to antigen- and mitogen stimulation.	[176]
	The ability of T cells to form colonies varied during pregnancy.	[177]
B cells		
Total B cells	Lower numbers and/or frequency of total B cells in pregnant women compared to post-partum levels or to healthy non-pregnant women.	[117, 118, 134-142 143,144]
	No changes in absolute levels of total B cells during the entire course of pregnancy.	[134, 178, 179]
	Decrease in the absolute levels of total B cells during the entire course of pregnancy.	[117, 137]

Component	Main findings	References
Subsets of B cells	Lower frequency or total levels of $CD5^+$ B cells during pregnancy, at delivery or early in the postpartum period.	117, 134, 138, 142
	Lower absolute counts of transitional B cells, unswitched memory B cells, resting memory B cells, and plasmablasts during the third trimester than in non-pregnancy.	143, 162
Markers of B cell activation and function	No difference in the percentage of activated B cells during the three trimesters compared to non-pregnant women.	162
	Higher B cell activating factor levels during their third trimester.	161
	Loss of responsiveness of B cells to mitogens and infectious agents.	151
Immunoglobulins		
Total IgG levels	No significant changes in total IgG levels during pregnancy.	152-154
	Decreased total IgG levels during pregnancy, especially in late pregnancy.	155-161
Subclass levels	Higher IgG1 levels in the three trimesters when compared to non-pregnant women. Higher IgG3 levels in the second trimester, when compared to non-pregnant women. No differences in IgG2 and IgG4 levels in any trimester as compared to non-pregnant women.	162
Glycosylation	Increase in galactosylation and sialylation of the Fc portion of IgG.	163, 165, 166
	High and similar levels of fucosylation of Fc portion of IgG during pregnancy.	163, 166
	No changes in glycosylation in the Fab of IgG during pregnancy.	163
IgA		
Total levels	No significant change in IgA levels during pregnancy.	135, 153, 154, 157, 160
	Higher IgA levels in the first compared to second or third trimester.	158
	Higher IgA levels in the first trimester compared to non-pregnancy.	162
	Lower IgA levels in the third trimester compared to non-pregnancy.	161
IgM		
Total levels	No changes total IgM levels during the course of pregnancy.	135, 153, 155, 157
	Decrease in IgM levels in the second and third trimester compared to first trimester.	152, 156, 158
	Increase in total IgM levels during late-third compared with early-third trimester.	156, 180
	Increase in total IgM levels in the first trimester	162
	No differences in IgM levels in the third trimester	161
IgE		
Total levels	No change in IgE levels during the course of pregnancy.	162

Abbreviations: IFN-γ: Interferon- γ; Th: T helper; PHA: Phytohemagglutinin; IgG: immunoglobulin G; Fc: fragment crystallization; IgA: immunoglobulin A; IgM: immunoglobulin M; IgE: immunoglobulin E.

T regulatory cells

T regulatory cells (Tregs) induce peripheral tolerance by suppressing the proliferation and cytokine production of CD4 and CD8 T cells, Ig production by B cells, cytotoxic activity of NK cells, and maturation of dendritic cells, resulting in tolerance induction[181, 182]. Tregs express low levels of IL7R and high levels of the alpha chain of IL-2 receptor (CD25) [183] and the transcription factor Forkhead box p3 (Foxp3)[184]. Other suppressive T cell subsets have been described [185] including, $CD4^+CD25^+$Foxp3- type 1 regulatory T cells (Tr1), and $CD4^+CD25^{low}$ Th3 cells[186, 187] that are induced by, and exert their suppressive activity through IL-10[188] and TGF-β[189]. The dynamics of Tregs during pregnancy are controversial, which might be in part due to difference in how Tregs are defined between studies (Table 1.5). Estrogen augmented Foxp3 expression *in vitro* and *in vivo*, and treatment with estrogen increased $CD4^+CD25^+$ "Tregs" in animal model, potentially promoting maternal fetal tolerance[190].

While CD25 and Foxp3 are often used as Treg markers, activated conventional T cells can also express Foxp3 in addition to dim levels of CD25[191, 192]. In one study, a higher percentage of $CD4^+CD25^{dim}$ T cells was observed at term as compared to 17-24 weeks into gestation, however, no significant changes were observed in $CD4^+CD25^{bright}$ T cells[193]. In another study, the number of $CD4^+CD25^+FoxP3^+$ T cells decreased during the first trimester then increased at 24-30 weeks of gestation then again declined after 31 weeks until term[194]. Some studies showed that the proportion of Tregs in circulation increases during early pregnancy[183,195] and peaks in the second trimester[183, 196], with one study showing that these cells express Foxp3[183] to further support that they are Tregs

(Table 1.5). However, in the latter studies[183, 196], no distinction between $CD4^+CD25^{dim}$ and $CD4^+CD25^{bright}$ T cells was made, thus limiting the definite conclusion about the true dynamics of Tregs during human pregnancy. Both $CD4^+CD25^{bright}$ and $CD4^+CD127^{low}CD25^+$ T cells subsets were significantly elevated at the time of delivery compared to non-pregnant women[197].

B regulatory cells

B regulatory cells (Bregs) express high levels of CD24, CD27 and/or CD38, and have the capacity to suppress T cell responses in part through production of the anti-inflammatory cytokine IL-10[198-200]. There is phenotypic heterogeneity of Bregs indicating that Bregs may not represent a distinct lineage[201]. $CD19^+CD24^{hi}CD27^+$ Breg levels increase in the first trimester of pregnancy[202] (Table 1.5). Human chorionic gonadotropin (hCG) enhances the function of Bregs as hCG induces IL-10 production in B cells and ~95% $CD19^+CD24^{hi}CD27^+$ cells expressed the hCG receptor[202]. Bregs' role may be to suppress maternal Th1 responses, thus preventing allogeneic responses against the fetus[202].

Table 1. 5: Changes in systemic T- and B- regulatory cells during pregnancy.

Component	Main Findings	References
T regulatory cells		
	Increased proportion of T regulatory cells in early pregnancy, peaking in the second and declining in the third trimester.	[183, 196]
	Higher percentage of $CD4^{+}CD25^{dim}$ T cells in samples obtained at term (>37 weeks) as compared to 17-24 weeks, while no significant changes in $CD4^{+}CD25^{bright}$ T cells.	[193]
	Increased $CD4^{+}CD25^{bright}$ T cells during early pregnancy compared to non-pregnant women, from 6% to 8%.	[195]
	Decreased number of $CD4^{+}CD25^{+}FoxP3^{+}$ T cells from 5 to 23 weeks gestation, then increased during 24-30 weeks gestation, then declined after 31 weeks until term.	[194]
B regulatory cells		
	Lower IL-10-producing B cells and $CD24^{hi}CD38^{hi}$ B cells during third trimester and at delivery than in post-partum.	[143]
	Increased $CD19^{+}CD24^{hi}CD27^{+}$ B cells in the first trimester as compared to non-pregnant women.	[202]

1.1.3 Summary of immune system changes during pregnancy and reasons for discrepant results

Based on review of the scientific literature, it is evident that there are dynamic changes in maternal immune system during normal pregnancy (Figure 1.1).

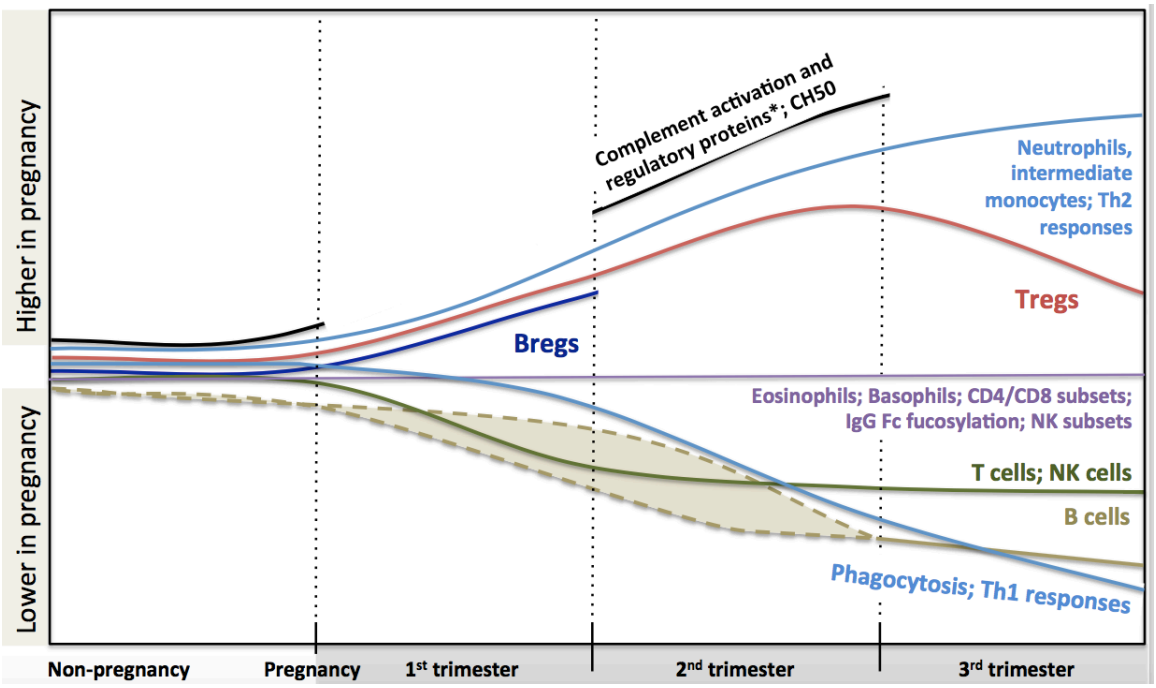


Figure 1. 1: Immune system in pregnancy

Changes that are not depicted in the scientific literature are not shown (represented as gaps and stops in lines). Dashed lines indicate that reduction in B cell might happen during first or second trimester. Controversies in the literature regarding the dynamics of total and subclasses of IgG combined enabled drawing a definite pattern (not described in the figure, see full text). Fucosylation of Fc portion of IgG is similar to non-pregnancy but at very high levels. *Complement activation proteins: C3a, C4a, C5a, Serum Complement Membrane Attack Complex SC5b9; Complement regulatory proteins: Decay-accelerating factor (CD55), C3 inhibitor pregnancy-associated plasma protein A.

There are different reasons for discrepancies in the literature regarding some changes in the immune system during pregnancy. First, inclusion of pregnant women from different populations and different ethnic backgrounds with different levels of exposure to different pathogens might lead to different levels of pre-existing immunity and dynamics during pregnancy. Second, timing of sampling during pregnancy is also an important determinant of the level of function of immune system. Third, the comparative group to

which pregnant women are compared is important. In some studies pregnant women were compared to women of childbearing age pre-pregnancy or post-partum, while others compared pregnant women during different times in pregnancy. Forth, the use of different laboratory assays could have led to different results. Finally, variability of the definitions of specific immune cells might also contribute to variations in results and conclusions.

1.2 Pertussis disease

1.2.1 Resurgence of pertussis

Pertussis (or "whooping cough" disease) is caused in humans mainly by *Bordetella pertussis*, a gram-negative, aerobic coccobacillus[203]. Pertussis was a common disease in the early 20th century and before the availability of a pertussis vaccine. In the US, the yearly rate of reported pertussis was 157/100,000 population in the early 20th century[204]. It should be noted however that this number should be interpreted with caution, as the true incidence at that time was underreported due to the limitation in sensitivity of relying on culture for diagnosing pertussis[205].

There are 2 types of pertussis vaccines. The whole-cell pertussis (wP) vaccine is composed of the whole inactivated organism, with all the virulence factors and antigens that are in the bacteria. The wP vaccine was associated in some reports with local reactions and serious neurological diseases (e.g. convulsions and hypotonic hyporesponsive episodes)[206, 207]. Thus, there was an interest in development of a less

reactogenic vaccine. This was achieved with acellular pertussis (aP) vaccines, which are composed of purified bacterial antigens (pertussis toxin [PT], filamentous hemagglutinin [FHA], pertactin [PRN], fimbriae [FIM2/3]) (for role of vaccine antigens in pathogenesis of disease please see 1.2.2, and for vaccine-induced immunity please see 1.2.3). The latter vaccine is associated with fewer side effects, when compared with the former[153,208]. Thus, the aP vaccine has replaced the wP vaccine in most high-income countries, and is currently used in these countries for infants' and adults' immunization schedules.

Immunization with the wP vaccine started in the 1940s in the US. By 1960s the average annual incidence rate declined to 10/100,000 population, and by 1970s the yearly incidence rate was low at 1/100,000 population in the US[204]. In Canada, the same pattern was observed. The incidence of reported pertussis disease was ~ 150/100,000 population before the introduction of wP vaccines (introduced in Canada in 1943). Following wP vaccine introduction, the incidence declined to ~ 10/100,000 population during 1980s[209].

In the past 2 decades, there has been an increase in pertussis disease rates, despite continued high aP vaccination uptake and coverage[210, 211]. Globally, it has been estimated that in 2014 there were 24.1 million pertussis cases and 160,700 deaths caused by pertussis in < 5 years children[212]. Several reasons have been suggested to explain the increase in incidence rates of pertussis. Specifically, increased awareness of pertussis disease by health care providers, improvements in diagnostics and surveillance methods (e.g. the use of polymerase chain reaction). In Canada, the increase use of a more

sensitive polymerase chain reaction assays was associated with 5-fold increase in pertussis incidence[213].

Adaption of *B. pertussis* to the vaccine is another potential reason for the increase of pertussis disease. Specifically, the circulation of pertussis vaccines not containing pertactin[214, 215], which may represent escape from immunity to *B. pertussis*. In addition, polymorphism of PT subunit 1 has also be observed in circulating bacteria following widespread pertussis vaccination , and this is important because it is implicated in binding to the T-cell receptor[216, 217]. Waning immunity reflected by faster decline of antibodies in individuals given aP booster vaccines has been suggested to contribute also to the resurgence of pertussis. A decrease in effectiveness of aP vaccine as time since last dose increased was noted in several studies[218-221], suggesting that waning immunity is an important contributor to the resurgence of pertussis disease. However, detailed data were lacking on the burden of pertussis disease in hospitalized infants in Canada over the past two decades.

1.2.2 Microbiology and clinical manifestations

Several virulence factors of *B. pertussis* contribute to the development of pertussis disease in humans, including PT, FHA, FIM and PRN. The disease is initiated by the adherence of the bacteria to the respiratory epithelium, mainly via FHA and FIM [222].

Attachment via FHA and FIM is later followed by local damage to the mucosa of the respiratory tract[223, 224]. This might lead to pathological findings such as necrotizing bronchitis and diffuse alveolar damage[222, 223, 225]. However, some evidence suggests that FHA and FIM suppress inflammation in the airways. Human monocyte-derived dendritic cells stimulated with full-length FHA secreted IL-10, a suppressive cytokine[226]. In animal models, mice inoculated with *B. pertussis* strains deficient with FIM have a higher inflammatory response when compared with those inoculated with wild-type *B. pertussis* strains[227]. This findings provide clues that FIM might suppress the inflammatory immune response[227].

PRN is a surface-associated protein[228], and it is suggested to contribute to adherence of *B. pertussis* to ciliated respiratory epithelium[229]. In addition, PRN resists neutrophil-mediated clearance[230]. PT an adenosine diphosphate-ribosylating protein toxin and is an important virulence factor. PT enters the host cell by receptor-mediated endocytosis[231]. In addition to the local reaction, PT can induce lymphocytosis (thus termed previously as lymphocytosis-promoting factor), which can lead to pulmonary hypertension, leading to respiratory failure and death [222, 232-234]. In the cytoplasm, PT inhibits signaling of inhibitory G proteins which inhibit adenylate cyclase activity[235]. In addition, it is proposed that PT reduced leukocytes retention in bone marrow and spleen and inhibits extravasation of leukocytes[236-239].

The incubation period of *B. pertussis* ranges between 7-10 days[240]. Clinical manifestations of the disease vary according to the age of the patient but most often

present as “whooping cough”, which consists of paroxysms of many violent and rapid coughs followed by a high-pitch “whoop” voice[203]. There are three classical stages of pertussis disease in infants, young children, adolescents and adults: the catarrhal, paroxysmal and convalescent stages. The catarrhal stage is manifested as a flu-like disease presenting as low-grade fever, malaise, nasal congestion, rhinorrhea, sneezing and mild cough. The paroxysmal stage is characterized by the classical “whooping cough”, which might be associated with vomiting (post-tussive vomiting). The convalescent stage is characterized by a decrease in paroxysmal cough frequency. Each of the stages lasts ~1-3 weeks[241, 242]. Pertussis is most severe in youngest infants leading to substantial morbidity and mortality[243, 244]. Infants with pertussis can have severe complications such as apnea, seizures (reported in 3% of infants <30 days with pertussis[245]), and can also die as a complication of the disease[246, 247]. Complications of pertussis in adults are rare but can include syncope[248]. Death is a rare event in adults with pertussis but was reported [249].

1.2.3 Natural and vaccine-induced immunity to pertussis

Protection following natural infection with *B. pertussis* or vaccination against pertussis disease is not lifelong[250, 251]. Among adults with pertussis, anti-PT IgG levels reached a peak 2 months after illness onset and then rapidly declined to reach a level that is 2-fold higher than 1 week after onset of symptoms by 28 months[252]. The decline was more rapid for anti-PT IgG compared with anti-PRN IgG and anti-FHA IgG. For IgA, anti-PT IgA levels increased 3-fold between 1 week and 2 months after symptoms onset, and returned to baseline at 1 year, while anti-FHA and anti-PRN IgA levels measured 28 months after onset of illness were still higher than levels measured 1 week after onset. In another

study, it was also demonstrated that 3 years after a symptomatic pertussis infection, anti-*B. pertussis* IgG levels declined. Anti-PT IgG levels waned the fastest when compared to other anti-*B. pertussis* IgG antibodies and anti-PT IgA levels waned faster than anti-PT IgG. IgG1 was the main IgG subclass detected after infection[253]. Among Danish infants and adults with bacteriologically confirmed *B. pertussis* infection, the median half-life for anti-PT IgG after infection was 221 days, to further confirm that immunity after natural infection is not durable[254]. Avidity of anti-*B. pertussis* antibodies was also assessed after infection. Among adolescents and adults with microbiology-confirmed pertussis disease, the avidity of anti-PT IgG increased within one month after infection[255], supporting that also functional anti-*B. pertussis* antibodies increase after infection. Peripheral blood mononuclear cells from children infected with *B. pertussis* and recovered produced IFN-γ but low or undetectable interleukin (IL)-5, suggesting that Th1 cells may mediate protection from pertussis[256], a finding that is supported by an animal study[257]. IL-12, which also polarizes Th cell to Th-1 lineage, might also mediate protection against *B. pertussis* [258].

Both cellular and humoral immunity have been detected after immunization with pertussis-containing vaccines (wP and aP). In mice, Th1 and IL-17-producing Th cells (Th-17) are induced after wP vaccination[259]. In children, immunization with aP was associated with an increase in IFN-producing cells (Th-1) up to 2 years after immunization[260]. Another study in children supports that aP vaccines induce a Th-2 CD4-positive immune response [261].

Children primed with aP vaccine had higher pertussis-specific IgG1, and IgG4 levels after booster with aP as compared to infants primed with wP and boosted with aP, suggesting that subclass distribution following pertussis immunization is affected by priming[260]. In another study, IgG1 was the predominant pertussis subclass measured after pertussis immunization, and elevated IgG4 levels were present in children who had been primed with aP, when compared with wP vaccine[262], a finding that supports polarization of the immune response to Th2 after aP vaccination.

It is important to note that currently there are no well-established correlates of protection (COP) against pertussis disease, which complicates interpretation of immunogenicity data. However, higher anti-PT, anti-FHA, and PRN IgG levels are associated with clinical protection from pertussis disease[263-265].

1.2.4 Susceptibility of newborns to pertussis disease

Different factors could put young infants at high risk for pertussis disease. The notion that anti-*B. pertussis* antibodies wane after infection and vaccination results in lower anti-*B. pertussis* antibodies in pregnant women that are transferred to newborns. Recently, it has been shown that newborn mice are susceptible to *B. pertussis* and enriched with erythroid suppressor $CD71^+$ cells. These cells express and CD71 and TER119 in mice, which might suppress the immune response through production of suppressive molecules (e.g. TGF-B)[266, 267]. Upon depletion of these cells, which are also enriched in human cord blood, reduced susceptibility to *B. pertussis* infection was noted as examined by reduced

pathological changes in mice lungs [266]. Preterm infants are at particular risk for severe respiratory infection as they have immature respiratory control, smaller airways and immature lungs.

1.3 Immunization against pertussis during pregnancy

1.3.1 How does immunization mediate protection?

Immunization with a vaccine induces immune responses that result in the production of different immune effectors that are capable of controlling the replication of a specific pathogen, that is the target of immunization, and/or inhibiting its toxins[268]. These immune system effectors that are induced via immunization are effector and memory immune cells or molecules. Namely, the most common measure of immune responses are antibodies that are capable of binding to a toxin of a pathogen or a pathogen itself[269]. $CD4^+$ Th cells produce and secrete cytokines, provide support to the generation of B-cells and $CD8^+$ T-cells and are also be induced by immunization. Cytotoxic $CD8^+$ T cells kill infected cells or secrete cytokines that are specific to the vaccine antigen. A subset of $CD4^+$ Th cells are follicular Th cells that are located in the lymph nodes and support B-cell differentiation into antibody-secreting-cells[270-273] . Tregs are involved in maintaining immune tolerance, and thus control immune responses[274].

There are several functions of antibodies. Antibodies have the capacity to neutralize toxins, reduce adhesion to host cells (thus reduce pathogen colonization on mucosal surfaces) and limit viral replication[275], functions that are mediated through recognition of

the antigen via the antigen-binding fragment of the antibody. Effector functions that are mediated via the antibodies' Fc domain include phagocytosis of the pathogen by macrophages and neutrophils, antibody-dependent cellular cytotoxicity, and complement-dependent cytotoxicity[276]. Antibodies are important in host defense against infection[277]. This could be indirectly concluded from studies showing that subjects with deficits in IgG are vulnerable to pneumococcal infection[278] and that antibody concentration above a threshold cut-off is associated with protection from some diseases (e.g. *Haemophilus influenzae* type b [Hib][279]).

The type of vaccine influences the type of immune responses that are generated. For example, capsular polysaccharides vaccines induce B cell responses in a T-cell independent manner, leading to production of IgG2 and IgG4 subclasses[280]. Conjugation of capsular polysaccharides to a protein carrier, or immunization with toxoid or protein vaccines leads to recruitment of antigen-specific $CD4^+$ Th follicular cells and production of antibodies in a T-cell dependent manner[281, 282], thus leading to production of IgG1 and IgG3 as well high affinity antibodies and immune memory[268]. IgG1 is the subclass that is most efficiently trans-placentally transferred to the newborn and is a stronger inducer of Fc-mediated effector mechanisms (e.g. antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity, and antibody-dependent cellular phagocytosis[283]).

Vaccine-induced antigen-specific T cells may contribute to the protection conferred after vaccination. For example, T cells may confer protection against pertussis disease in

vaccinated children after vaccine-induced antigen-specific antibodies have waned[284, 285]. For many of these reasons, vaccination represents a window of opportunity to protect young infants in the first few, most vulnerable, months of life.

1.3.2 Immune response to vaccines in pregnant compared with non-pregnant women

The above-mentioned systemic immunological changes and adaptations in the maternal immune system that occur during normal pregnancy might indicate that immune responses to vaccines administered during pregnancy might be different and lower than immune responses to vaccines administered to non-pregnant women. Specifically, the decrease in B cells, increase in Bregs and Tregs. However, the current evidence does not fully support this.

In one study, antibody levels to components of tetanus-diphtheria-acellular pertussis (Tdap) vaccine (pertussis antigens: PT, FHA, PRN; tetanus-toxoid [TT]; diphtheria-toxoid [DT]) increased significantly after vaccination and to the same level between pregnant and non-pregnant women[286]. Studies for influenza vaccines have also addressed this question, but were less consistent. Some studies that compared the immune response to influenza vaccines showed similar antibody titers in pregnant and non-pregnant women after influenza vaccination. This has been true for pandemic H1N1/2009 monovalent inactivated vaccine and seasonal trivalent inactivated vaccine[287-289]. However, other studies showed lower sero-conversion rates and lower antibody titers after vaccination of pregnant women when compared to non-pregnant women[290-292]. The effect of pregnancy status on the cellular immune responses to vaccines has been less

well studied. INF-γ levels to the *B. pertussis* antigens (PT, FHA) increased one month after immunization with Tdap but were not significantly different in pregnant and non-pregnant women[286].

1.2.3 Factors affecting transfer of maternal antibodies via the placenta to the newborn

Among the five Ig isotypes (IgG, IgM, IgA, IgE, IgD), IgG is the only isotype that crosses the placenta[293]. The efficiency of transfer of IgG (defined as the antibody levels in the newborn/cord divided by antibody levels in the mother) across the placenta increases as pregnancy progresses. Cord levels of IgG are ~ 50% of maternal levels at weeks 28–32 of gestation, and 120%–130% maternal levels at term delivery[294-297]. The transfer of IgG from the mother to the fetus across the placenta is mediated by the neonatal Fc receptors (FcRn), which is in the syncytiotrophoblasts[293]. FcRn-mediated transport has been suggested by examining the transport of a recombinant, humanized IgG1 antibody with that of a mutated variant that does not bind to FcRn in *ex vivo* perfused placenta[298]. FcRn actively transports IgG into the fetal circulation via binding to its constant domain (Fc fragment)[299, 300]. This is suggested as whole IgG molecules or Fc fragments of IgG pass into the fetal circulation more readily than antigen-binding fragment[301]. Recently, it has been shown that there is no association between the levels of placental FcRn and efficiency of transfer total IgG across the placenta[302], suggesting that factors other than the levels of FcRn are responsible for the time-dependent efficiency of trans-placental transfer of antibodies as pregnancy advances.

Several factors affect the transfer of IgG from the mother to the newborn via the placenta. IgG subclasses have differences in efficiency of transfer across the placenta. IgG1 is the subclass transferred with the highest efficiency, achieving higher levels in cord blood compared with the maternal blood [303]. IgG2 is transferred with the least efficiency,[293, 304-307,303, 308, 309]. Transfer of IgG across the placenta to the newborn can also be influenced by several maternal clinical conditions. For example, infants of women infected with human immunodeficiency virus (HIV)[310], malaria[311], and women with high IgG levels[312], had lower cord IgG levels when compared with infants of women without these maternal conditions.

1.2.4 Immunogenicity of vaccines administered in pregnancy-tetanus, influenza and pertussis

1.2.4.1 Tetanus and influenza

Immunization with TT-containing vaccines induces anti-TT IgG antibodies, particularly of the IgG1[303, 313], which are transferred across the placenta to the newborn, leading to protective antibody levels (anti-TT IgG $\geq$ 0.1 IU/mL) in the infant[314-317]. At the age of one month, ~80% of maternally-derived antibodies that are transferred to the infant remain in the infant's circulation[318].

Immunization with influenza vaccine (a protein) induces IgG1 antibodies[319]. Influenza-specific hemagglutinin antibodies are higher in neonates born to women vaccinated against influenza during pregnancy[320-322]. The rate of decline of influenza antibodies might provide clues for the duration of protection mediated via influenza vaccination

during pregnancy. Maternally-derived antibodies against seasonal influenza viruses have a half-life of ~ 45 days in infants after maternal vaccination and by 4 months of age antibody levels in infants born to vaccinated mothers are similar to those born to unvaccinated mothers[323, 324]. In another study, infants born to mothers vaccinated against influenza in pregnancy had seroprotective influenza-specific antibody levels up to 5 months after birth[325].

1.2.4.2 Pertussis vaccines

In Canada, there are two brands in Canada of aP vaccines that are administered during pregnancy. These vaccines are licensed primarily for immunization of adolescents and adults. Adacel (Sanofi Pasteur) contains PT, FHA, PRN, FIM2/3 vaccine antigens, and Boostrix (GlaxoSmithKline) contains PT, FHA, PRN vaccine antigens. Studies showed significant increase in anti-*B. pertussis* antibody levels one month after vaccination against pertussis during pregnancy[326-328]. Vaccination with aP vaccines (a protein) induces mainly IgG1 subclass antibodies[262, 329], and this subclass is actively transferred across the placenta to the newborn, resulting in higher levels of antibodies (e.g. anti-PT IgG levels) in the newborn than in the mother[316, 330, 331].

1.4. Strategies to protect infants from severe pertussis other than immunization during pregnancy

1.4.1 Cocooning strategy

The cocooning strategy aims to vaccinate those in close contact with the newborn (e.g. parents), and thus potentially indirectly protect infants from pertussis by reducing risk of transmission of the bacteria. The effectiveness of this strategy has been evaluated. In one study from the US, vaccination of mothers after delivery was not associated with a decrease in the number of infants <6 months of age with pertussis[332]. In another study that indirectly assessed the cocooning strategy in Australia, immunization of mothers and fathers was associated with a decrease the risk of pertussis by 51% in their infants[333]. In Canada, the number needed to vaccinate for parental immunization was at least 1 million to prevent 1 infant death from pertussis, and >10,000 to prevent 1 hospitalization[334]. These data suggest that cocooning strategy is not highly effective in reducing the burden of pertussis in young infants, and would require vaccination of a large number of close contacts to prevent severe disease. Thus this strategy might not be ideal and cost-effective.

1.4.2 Neonatal pertussis vaccination

Infants' vaccination against pertussis usually begins during age 6 to 8 weeks. As the most severe disease of pertussis occurs in the first months of life, then adding a dose of vaccine at the time of birth might theoretically add protection and this strategy has been investigated and yielded inconsistent results. At birth dose of aP vaccine in Italian infants, followed by aP vaccination at 3, 5, and 11 months, resulted in lower anti-PT IgG

levels, at 7 to 8 months compared with infant who did not receive at birth dose of aP of age[335]. At birth immunization with aP was associated with significantly reduced anti-*B. pertussis* antibody levels between 6-18 months of age (after primary and booster immunization), when compared with infants not immunized at birth[336]. A recent Australian study showed that at birth immunization with aP vaccine with subsequent immunization at 2, 4, and 6 months of age was associated with a similar levels at 6 months of age, when compared to controls who did not receive at birth vaccination[337].

1.4 Rationale for thesis and research objectives

Pertussis is a major global public health concern, despite high vaccination coverage, and there has been a resurgence of the disease, both globally and in Canada since 2010[338-340]. To protect infants too young to be vaccinated, immunization against pertussis in pregnancy is recommended by public health policy makers in a number of countries (e.g. the United States [US][341, 342], United Kingdom [UK][343], Australia[344]). At the start of my PhD in 2017, immunization during pregnancy had not been routinely recommended in Canada. The Canadian National Advisory Committee on Immunization updated its guidelines in February 2018, and since then has recommended pertussis immunization during pregnancy of all Canadian pregnant women[345]. While the mechanism of protection following immunization in pregnancy is unknown, it is thought to be, at least partially, mediated by maternal antibodies transferred to the newborn via the placenta[73]. As immunization during pregnancy is a new strategy and there are important knowledge gaps, these must be addressed to inform an evidence-based immunization program. This is what I aimed to do during my PhD.

The aims of my PhD were to address critical knowledge gaps related to immunization against pertussis in pregnancy. Aim 1 was to determine the burden of age–specific epidemiology, morbidity and mortality of hospitalized pertussis disease in Canada in the aP vaccine era over a 17-year period and to establish risk factors associated with morbidity and mortality. Aim 2 was to enhance our understanding of the immune response to immunization in pregnancy by assessing the avidity of anti-pertussis antibodies elicited after immunization in pregnancy and how this is affected by timing of immunization during pregnancy. Aim 3 was to determine whether immunization against pertussis in pregnancy modifies the infants' active immune response to vaccination. The results generated in the first aim are presented in Chapter 2, the results generated in the second aim are presented in Chapter 3, and the results generated in the third aim are presented in Chapter 4. Chapter 5 summarizes the body of work presented in the thesis and provides perspectives for the future in pertussis immunization during pregnancy.

2. Burden of pediatric pertussis disease in Canada

2.1 Synopsis

Increases in pertussis morbidity and mortality among young infants over the past decade have led to recommendation of vaccination against pertussis during pregnancy in increasing number of countries. Data on cases of hospitalized pediatric pertussis from a large population over a long period of time in Canada are important to establish the true burden of disease in the aP vaccine era in Canadian settings. In this chapter, I describe the age-specific epidemiology, morbidity and mortality of children hospitalized with pertussis over 17 years across Canada in the aP vaccine era. Patients ≤16 years admitted with pertussis to 12 pediatric tertiary-care hospitals across Canada during 1999–2015 with confirmed (laboratory–confirmed or epidemiologically–linked) or probable (clinically diagnosed) pertussis were included.

Overall, 1402 pediatric patients with pertussis were included. The overall mean annual pertussis hospitalization and intensive care unit (ICU) admission incidence in all age groups was, 2.61 (95%CI: 2.03–3.18) and 0.50 (95% CI: 0.40–0.60) per 100,000 population, respectively. Infants aged <2 months had the highest mean annual pertussis hospitalization and ICU admission incidence, 116.40 (95% CI: 85.32–147.49) and 33.48 (95% CI: 26.35-40.62) per 100,000 population, respectively. The overall proportion of children requiring ICU admissions among all age groups was 25.46% and this was highest in infants <2 months of age at 37.90%. There were 21 deaths. Age <16 weeks, prematurity, encephalopathy and confirmed pertussis diagnosis were found to be

independent risk factors for ICU admission. Age <4 weeks, prematurity and female sex were found to be independent risk factors for death. These data support that in the aP vaccine era, endemic pertussis still contributes considerably to childhood morbidity and mortality, particularly in infants aged <2 months. Vaccination against pertussis during pregnancy has the potential to reduce this disease burden.

2.2 Introduction

B. pertussis is a gram-negative coccobacillus that causes a respiratory disease, "whooping cough" disease. Pertussis remains a major global public health concern with a recent global estimate of 24.1 million cases and 160,700 deaths from pertussis in children <5 years of age in 2014. Globally, nearly 21% of pertussis cases and an estimated 53% deaths were in infants younger than 1 year[212]. Most industrialized countries use diphtheria-tetanus-acellular pertussis (DTaP) vaccines for primary and/or booster immunization against pertussis[346, 347]. In spite of high vaccination coverage with aP vaccine, pertussis outbreaks with substantial morbidity and mortality continue to occur[210, 339]. Previous reports on the burden of pertussis disease in the aP vaccine era described incidence rates and clinical outcomes in small populations, a single center, a single region, a specific epidemic period or other limited time period or years during which both wP and aP vaccines were used[210, 348,349, 350,245, 246, 351]. Moreover, the estimates of burden of severe pertussis disease requiring ICU admission included studies that spanned a limited time period[246, 352] Data on the burden of pediatric pertussis from a large population over time are important to establish the true burden of disease in the aP vaccine era, not only in an epidemic or outbreak setting, and to inform cost-effectiveness analyses of different immunization strategies. This is especially important, as several countries have recommended universal vaccination against pertussis during pregnancy in response to a recent rise in pertussis morbidity and mortality among young infants[342, 343, 353].

In Canada, vaccination against pertussis commenced in 1943 with whole-cell pertussis

(wP) formulations. Between 1997-1998, all Canadian provinces and territories changed from wP to aP vaccine, given at 2, 4, 6, and 18 months and 4 to 6 years of age (Table 2.1) [354].

Table 2. 1: Vaccination schedule against pertussis in Canada (as of January 2020).

Vaccine	British Columbia, Alberta, Prince Edward Island, Yukon	Saskatchewan, Manitoba, Ontario, New Brunswick, Nova Scotia, Newfoundland and Labrador, Northwest Territories, Nunavut	Quebec
DTaP-IPV-Hib	18 months	2,4,6,18 months	12 months
DTaP-HB-IPV-Hib	2,4,6 months	N/A	2, 4, months
Tdap-IPV	4-6 years (4 years in Alberta, 4-5 years in Prince Edward Island)	4-6 years (4 years in New Brunswick)	4-6 years
TdaP	Grade 9	Grade 8 in Saskatchewan and Manitoba;14-16 years in Ontario; Grade 7 in New Brunswick; Nova Scotia and Northwest Territories; Grade 9 in Newfoundland and Labrador; Grade 6 in Nunavut	Not publically funded

Abbreviations: DTaP-IPV-Hib: Diphtheria, Tetanus, acellular Pertussis, Inactivated Polio Virus, *Haemophilus Influenzae* type B vaccine; DTaP-HB-IPV-Hib: Diphtheria, Tetanus, acellular Pertussis, Hepatitis B, Inactivated Polio Virus, *Haemophilus Influenzae* type B vaccine; Tdap-IPV: Tetanus, diphtheria, acellular pertussis, Inactivated Polio Virus vaccine. Source: Government of Canada website[355].

The burden of pertussis disease among children in Canada during the wP and the wP-aP vaccine transition period have been described[354, 356]. Despite the ongoing impact on the health system of this severe yet preventable infection, there are limited data on long-term studies assessing the burden of pertussis disease in pediatric hospitalized pertussis

disease. In this chapter, I reported the age–specific epidemiology, morbidity and mortality of children hospitalized with pertussis disease in Canada in the aP vaccine era. I also identified risk factors associated with poor outcome (morbidity and mortality) among pediatric patients hospitalized with pertussis.

2.3 Methods

2.3.1 Study Locations

Pertussis cases admitted to hospitals which are part of the Immunization Monitoring Program Active (IMPACT) were included. IMPACT is an active surveillance network collecting data from 12 pediatric tertiary-care hospitals across Canada since 1991.[354] The 12 IMPACT centers account for approximately 90% of the pediatric tertiary-care beds in Canada, with referrals from all provinces and territories. All IMPACT centers actively report all hospitalized pertussis cases to IMPACT data center, located in the Vaccine Evaluation Center at British Columbia Children's Hospital Research Institute, Vancouver, British Columbia.

2.3.2 Study Subjects

Inclusion Criteria: Patients ≤16 years of age admitted to an IMPACT hospital with pertussis between 1st January 1999 and 31st December 2015 were included.

Clinical case definition of pertussis: A clinical case of pertussis was defined as a cough illness lasting for ≥2 weeks, with paroxysmal coughing. Post–tussive vomiting, whoop, cyanosis during coughing, or apnea episodes were supportive evidence of a case.

Confirmed case of pertussis: Consistent with the Canadian national pertussis case definitions[357], confirmed cases were laboratory–confirmed (at least one positive microbiological test for *B. pertussis* (culture, polymerase chain reaction [PCR], direct fluorescent assay [DFA], or serology)) or epidemiologically–linked (meeting the clinical case definition above and contact with a laboratory-confirmed case). Co-infections with *B. pertussis* and another *Bordetella* species were included.

Probable case of pertussis: A probable case was defined as a case meeting the clinical case definition but none of the confirmed case criteria (i.e. absence of positive laboratory tests and not epidemiologically linked to a laboratory-confirmed case).

Exclusion criteria: Compatible illnesses demonstrated to be due to another cause were excluded. Cases confirmed to be caused only by *Bordetella* species other than *B. pertussis* were excluded.

2.3.3 Data Collection and Management

Standardized case report forms were used at all IMPACT hospitals. Pertussis cases were identified via microbiology laboratories, ward and ICU admission lists, infection control practitioners and/or search of hospital records for ICD9 and ICD10 discharge codes that included terms for pertussis. Clinical data were collected from patient health records. This included the patient's date of birth, sex, pre-existing medical conditions, the diagnostic method, evidence for diagnosis, complications (seizures, encephalopathy, death), level of care required (duration of hospital stay (length of stay [LOS], need for ICU admission, ICU LOS). Prematurity was defined as birth before 37 weeks gestation.

Encephalopathy was defined as a decreased level of consciousness not associated with post-ictal period. For each patient admitted with pertussis, vaccination records were initially reviewed per the hospital record. Data in the hospital records was confirmed from the relevant vaccine provider (public health or family physician), which varies by region. If there was discrepancy the vaccine provider record was considered accurate. All data were reviewed at the IMPACT data center in Vancouver before being entered into an electronic database by means of a dual–entry system with preprogrammed consistency checks. Errors were corrected by a data manager before files were added to the database.

2.3.4 Pertussis vaccination status

A valid vaccine dose was as any dose administered ≥28 days before hospital admission with pertussis, and this information was used to classify children ≥3 months with laboratory–confirmed pertussis disease as unimmunized, under-immunized, or as having received an age-appropriate number of pertussis vaccinations (Table 2.2). This is because there is a minimum of 2 weeks from vaccination to induce antibody response and the clinical cases definition of all cases includes cough of 2 or more weeks. Vaccinations received < 4 weeks prior to admission were not counted for determination of age-appropriate vaccination status, and patients were considered unvaccinated if they had only received pertussis vaccinations within 4 weeks prior to admission to the hospital.

Table 2. 2: Classification of age-specific vaccination status according to number of received pertussis-containing vaccine doses.

	Unvaccinated	Under-vaccinated	Age-appropriately vaccinated
3-4 months	0	Not applicable	≥ 1
5-6 months	0	1	≥ 2
7-18 months	0	1 or 2	≥ 3
19 months-6 years	0	1 or 2 or 3	≥ 4
7-16 years	0	1 or 2 or 3 or 4	≥ 5

2.3.5 Statistical analysis

Pearson's Chi–squared test was used to compare categorical variables (Fisher's exact test was used for comparisons with cell number <5), Student's t–test was used for normally distributed continuous variables and Mann Whitney U test for non–normally distributed continuous variables. Annual age–specific pertussis hospitalization incidence was calculated using population estimates of each study hospital's catchment area obtained from the 2006 Canadian census[358]. Each IMPACT hospital defined its estimated local population catchment area. Pertussis cases from the IMPACT hospital were matched to this catchment area using the first 3 characters of the postal code. The first 3 characters of the postal code form the forward sortation area, which represents a specific area within a major geographic region or province. Cases from outside the hospital catchment areas and one hospital with a large referral population and an undefined catchment area (The Hospital for Sick Children, Toronto) were not included in hospitalization incidence calculations (Table 2.3). Annual age–specific pertussis ICU admission incidence was calculated using relevant provincial population estimates obtained from the 2006 Canadian census[358]. Outside the province of Ontario, IMPACT hospitals are the only

pediatric centers admitting to the ICU. Age–specific population estimates of the study population areas included the respective province. Pertussis cases admitted to IMPACT hospitals' ICUs were matched to the respective province. Cases admitted to IMPACT hospitals' ICUs but residing in the Canadian territories were excluded from the ICU incidence analysis. Ontario was also excluded from the ICU incidence analysis because cases may also have been admitted to other ICUs, not included in the IMPACT network (Table 2.3). The overall mean annual pertussis hospitalization and ICU admission incidence rates and the 95% confidence interval (CI) were calculated for the 17-year period using the annual hospitalization and ICU admission incidence rates, respectively. The variability of the median hospital length of stay (LOS), the proportion of children requiring ICU admissions during the study period and the proportion of children requiring ICU admissions by their gestation age at birth were analyzed using the Kruskal-Wallis test and the Chi-squared Test for trend, for continuous and categorical variables, respectively.

Age-specific ICU admission rate of patients hospitalized with pertussis was reported as the proportion of patients admitted to the ICU among patients admitted with pertussis to IMPACT centers. Age-specific case-fatality rates were reported as the proportion of deaths among pertussis hospitalized cases.

Univariate logistic regression analysis was used to identify risk factors for ICU admission and death. To identify the most appropriate age cut-off as a risk factor for ICU admission and death, regression models were generated using all ages between 0 up to 16 years, by

1-week intervals. The Akaike information criterion (AIC) of each model, which is a function of its maximized log-likelihood (ℓ) and the number of estimable parameters (*K*) (AIC=-2l +2K), was computed[359]. The model with the lowest AIC was used as the final model. Forward stepwise multivariable logistic regression models were developed to identify independent risk factors for ICU admission and death. These included all variables identified in the univariate regression model with p–value ≤0.25 and the age-cut off with the lowest AIC. Variables with a p-value <0.05 were retained in the final model. P values of <0.05 were considered statistically significant for all tests. R version 3.4.0 was used for all analyses.

Table 2. 3: Numerator and denominator used for pertussis hospitalization incidence rate and intensive care unit incidence rate calculations, Canada, 1999-2015.

Rate	Numerator	Denominator*
Hospitalization incidence rate	**Included:** Pertussis cases admitted to 11/12 IMPACT hospitals and matched to the hospital's catchment area: 1. Alberta Children's Hospital, Calgary, AB. 2. BC Children's Hospital, Vancouver, BC. 3. Le Centre Mère-Enfant de Québec City, QC. 4. Children's Hospital of Eastern Ontario, Ottawa, ON. 5. CHU-Sainte-Justine, Montreal, QC. 6. IWK Health Centre, Halifax, NS. 7. Eastern Health Janeway Child Health and Rehabilitation Centre, St. John's, NL. 8. The Montreal Children's Hospital, Montreal, QC. 9. Royal University Hospital, Saskatoon, Sk. 10. Stollery Children's Hospital, Edmonton, AB. 11. Children's Hospital, Winnipeg, MB. **Excluded:** Pertussis cases admitted to The Hospital for Sick Children, Toronto, ON.**	**Included:** Population estimates of the catchment areas corresponding to the 11/12 IMPACT hospitals included. **Excluded:** The catchment area of The Hospital for Sick Children, Toronto.
Intensive-care unit admission incidence rate	**Included:** Pertussis cases admitted to the intensive care unit of 10/12 IMPACT hospitals: 1. Alberta Children's Hospital, Calgary, AB. 2. B.C. Children's Hospital, Vancouver, BC. 3. Le Centre Mère-Enfant de Québec City, QC. 4. CHU-Sainte-Justine, Montreal, QC. 5. IWK Health Centre, Halifax, NS. 6. Eastern Health Janeway Child Health and Rehabilitation Centre, St. John's, NL. 7. The Montreal Children's Hospital, Montreal, QC. 8. Royal University Hospital, Saskatoon, SK. 9. Stollery Children's Hospital, Edmonton, AB. 10. Children's Hospital, Winnipeg, MB. **Excluded:** Pertussis cases admitted to the intensive care unit of 2 IMPACT hospitals: 1. The Hospital for Sick Children, Toronto 2. Children's Hospital of Eastern Ontario, Ottawa	**Included:** Population estimates of the provinces of 10/12 IMPACT hospitals:1. BC; 2. AB; 3. SK; 4. MB; 5. QC; 6. NS; 7. NL. **Excluded:** 1. Population estimates of the province of ON, NB, PE; 2. NT, YT, NU.

* Based on 2006 Canadian census data.

** This hospital was excluded because it has a large referral population and an undefined catchment area.

2.4 Results

2.4.1 Study population

Overall, 1402 children were included, of which 1157 (82.5%) were confirmed cases (1145 [81.7%] laboratory–confirmed, 12 [0.8%] epidemiologically-linked) and 245 (17.5%) were probable cases. The majority (810/1145 [70.7%]) of laboratory-confirmed cases were diagnosed by PCR alone (Figure 2.1).

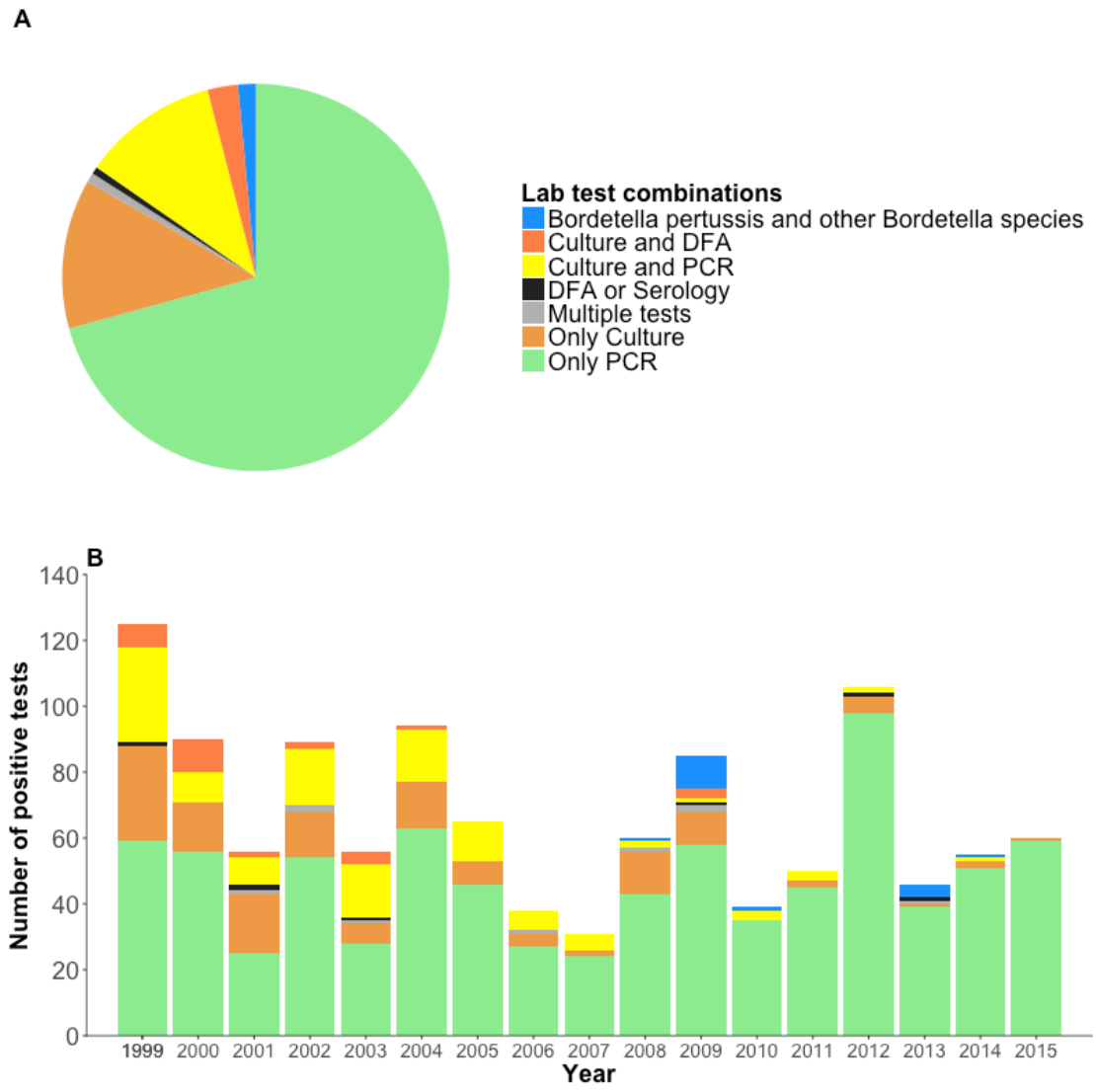


Figure 2. 1: Laboratory–confirmed *Bordetella pertussis* cases.

Graph shows (A) laboratory–confirmed *Bordetella pertussis* cases stratified by type of laboratory test and (B) the number of the positive laboratory tests per year, MPACT hospitals, 1999–2015. * During 2010-2015, the laboratory diagnosis of pertussis was made by PCR in 8 out of 11 IMPACT centers. One center switched from culture to PCR testing in 2014. One center used PCR only for severe cases until 2012 and for all samples after 2012. In one center, the laboratory diagnosis of pertussis was made by either culture or PCR.

The baseline characteristics of pertussis hospitalized cases and stratified by evidence of diagnosis are summarized (Table 2.3). The majority of pertussis cases were among infants younger than 1 year old, specifically, younger than 4 months old. Of the total cases, nearly one quarter required admission to ICU. Prematurity (birth at gestational age <37 weeks gestation) was the most common risk factor (Table 2.4). Confirmed pertussis cases were significantly younger, had longer hospital and ICU LOS, and were more likely to be admitted to the ICU than probable cases (Table 2.4). The median hospital LOS was 8 days among infants <6 months of age (Table 2.4). There was significant year-to-year variation in the median hospital LOS over the study period ranging from 4 to 10 days (p=0.002).

Table 2. 4: Characteristics of pertussis cases admitted to IMPACT hospitals in Canada, 1999–2015.

Characteristic	All Pertussis cases [a] (n=1402)	Confirmed pertussis cases [b] (n=1157)	Probable pertussis cases [c] (n=245)	P-value [d]
Demographics				
Male sex n (% of total)	655 (46.7)	525(45.4)	130 (53.1)	0.034
Age				
Median ([overall range], [Interquartile range [IQR]) (weeks)	10 ([1-886][6-17])	9 ([1-884] [6-16])	11 ([1-886] [7-22])	0.003
Age groups n (% of total)				
< 1 year	1265 (90.2)	1056 (91.3)	209 (85.3)	
0-1 months	612 (43.6)	523 (45.2)	89 (36.3)	
2-3 months	460 (32.8)	381 (32.9)	79 (32.2)	
4-5 months	119 (8.5)	91 (7.9)	28 (11.4)	
6-11 months	74 (5.3)	61 (5.3)	13 (5.3)	
1-4 years	72 (5.1)	53 (4.6)	19 (7.7)	
5-9 years	24 (1.7)	15 (1.3)	9 (3.7)	
10-16 years	41 (2.9)	33 (2.8)	8 (3.3)	
Clinical features				
Comorbidity [e] n (% of total)				
Underlying condition(s)	178 (12.7)	152 (13.1)	26 (10.6)	0.500
Prematurity [f]	50 (3.6)	44 (3.8)	6 (2.4)	0.735
Pulmonary	31 (2.2)	23 (2.0)	8 (3.3)	
Neurologic	27 (1.9)	24 (2.1)	3 (1.2)	
Congenital Cardiac	23 (1.6)	20 (1.7)	3 (1.2)	
Gastrointestinal	20 (1.4)	16 (1.4)	4 (1.6)	
Genetic-Metabolic	13 (0.9)	10 (0.9)	3 (1.2)	
Failure to thrive	12 (0.8)	11 (0.9)	1 (0.4)	
Immuno-compromised	10 (0.7)	10 (0.9)	0	
Other	34 (2.4)	30 (2.6)	4 (1.6)	
Seizures n (% of total)				
New seizures	30 (2.1)	28 (2.4)	2 (0.8)	0·120
Exacerbation of an existing seizure disorder	8 (0.6)	8 (0.7)	0	
Encephalopathy n (% of total)				0.344
Present	8 [g] (0.6)	8 (0.7)	0	

Characteristic	All Pertussis cases [a] (n=1402)	Confirmed pertussis cases [b] (n=1157)	Probable pertussis cases [c] (n=245)	P-value [d]
Outcome				
Hospitalization				
Median length of stay ([overall range], [IQR]) (days)	7 ([1-185], [3-13])	8 ([1-185], [4-14])	4 ([1-45], [2-7])	< 0.0001
< 1 year	8 ([1-185], [3-14])	9 ([1-185], [4-15])	4 ([1-45], [2-8])	< 0.0001
0-1 months	10 ([1-87], [5-16])	11 ([1-87], [6-17])	5 ([1-45], [2-9])	< 0.0001
2-3 months	6 ([1-185], [3-12])	7 ([1-185], [3-12])	4 ([1-37], [2-9])	< 0.001
4-5 months	4 ([1-59], [2-8])	5 ([1-59], [3-9.50])	3 ([1-15], [2-4])	< 0.001
6-11 months	4 ([1-38], [2-9.75])	4 ([1-38], [2-10])	3 ([1-8], [2-5])	0.051
1-4 years	3 ([1-60], [1-5])	3 ([1-60], [2-5])	2 ([1-13], [1-4.50])	0.231
5-9 years	2 ([1-8], [1-5])	2 ([1-68], [1-4.5])	3 ([1-5], [2-5])	0.502
10-16 years	3 ([1-14], [1-5])	1 ([1-14], [2-5])	1 ([1-5], [1-5])	0.124
ICU admission				
ICU admission n (% of total)	357 (25.5)	316 (27.3)	41 (16.7)	< 0.001
Median ICU length of stay ([overall range], [IQR]) (days)	4 ([1-79], [2-9])	5 ([1-79], [2-10])	3 ([1-23], [2-7])	0.027
< 1 year	5 ([1-79], [2-10])	5 ([1-79], [2.5-10])	3 ([1-23], [2-7])	0.033
0-1 months	5 ([1-79], [3-9])	5 ([1-79], [3-9.75])	3 ([1-23], [2-6])	0.031
2-3 months	4 ([1-62], [2-10])	4 ([1-62], [2- 9])	3 ([1-13], [3-11])	0.888
4-5 months	7 ([1-42], [2-12])	7 ([1-42], [2.50-12.25])	7 ([1-8], [4-7.50])	0.559
6-11 months	2.5 ([1-16], [1.75-9])	5 ([1-16], [2.25-13])	1.5 ([1-2], [1.25-1.75])	0.238
1-4 years	2 ([1-29], [1-4])	2.5 ([1-29], [1.75-5.25])	1 ([1-1], [1- 1])	0.323
Death n (% of total)	21 (1.5)	21 (1.8)	0	0.037

[a] Includes confirmed and probable pertussis cases; [b] Laboratory-confirmed (n=1145) or epidemiologically linked (n=12) pertussis cases; [c] Clinical pertussis cases ; [d] For the comparison of confirmed versus probable pertussis cases; [e] As clinically denoted in the records. A patient can have more than one comorbidity; [f] Prematurity is defined as birth at gestational age <37 weeks gestation; [g] 5 cases had both new seizures and encephalopathy comorbidity.

2.4.2 Incidence of pertussis hospitalization

In total, 747/1270 (58.81%) of pertussis cases occurred in the defined catchment areas for the study hospitals and were included in the hospitalization incidence calculations. The cases used for the hospitalization incidence analysis had similar baseline characteristics (age, sex and comorbidities) as the cases not included in this analysis (data not shown). The overall mean annual pertussis hospitalization incidence was highest among infants <2 months of age (Table 2.5).

Table 2. 5: Mean annual pertussis hospitalization and Intensive-care unit admission incidence (per 100,000 population) at IMPACT hospitals by age groups, 1999-2015.

Age	Hospitalization incidence [a] (95% CI)	Intensive-care unit admission incidence [b] (95% CI)
< 1 year	42.3 (32.5–52.1)	8.6 (6.7–10.3)
< 2 months	116.4 (85.3–147.5)	33.5 (26.3-40.6)
2-3 months	95.9 (71.6–120.2)	14.6 (10.5-18.6)
4-5 months	28.3 (19.5–37.2)	2·5 (1.1–3.9)
6-11 months	5.1 (3.4–6.8)	0.4 (0.1–0.7)
1-4 years	0.8 (0.6–1.1)	0.1 (0.0-0.1)
5-9 years	0.2 (0.1–0.2)	0.0 (0-0.02)
10-16 years	0.2 (0.1–0.3)	0.0 (0-0.01)
All age groups	2.6 (2.0–3.2)	0.5 (0.4–0.6)

[a] This analysis included 11/12 IMPACT hospitals (excluding The Hospital for Sick Children, Toronto); [b] This analysis included 10/12 IMPACT hospitals and their 7 respective provinces (excluding 2 hospitals from the province of Ontario [The Hospital for Sick Children, Toronto; Children's Hospital of Eastern Ontario, Ottawa]).

Pertussis hospitalization incidence rates in young infants fluctuated over time with peaks every 2–5 years (Figure 2.2).

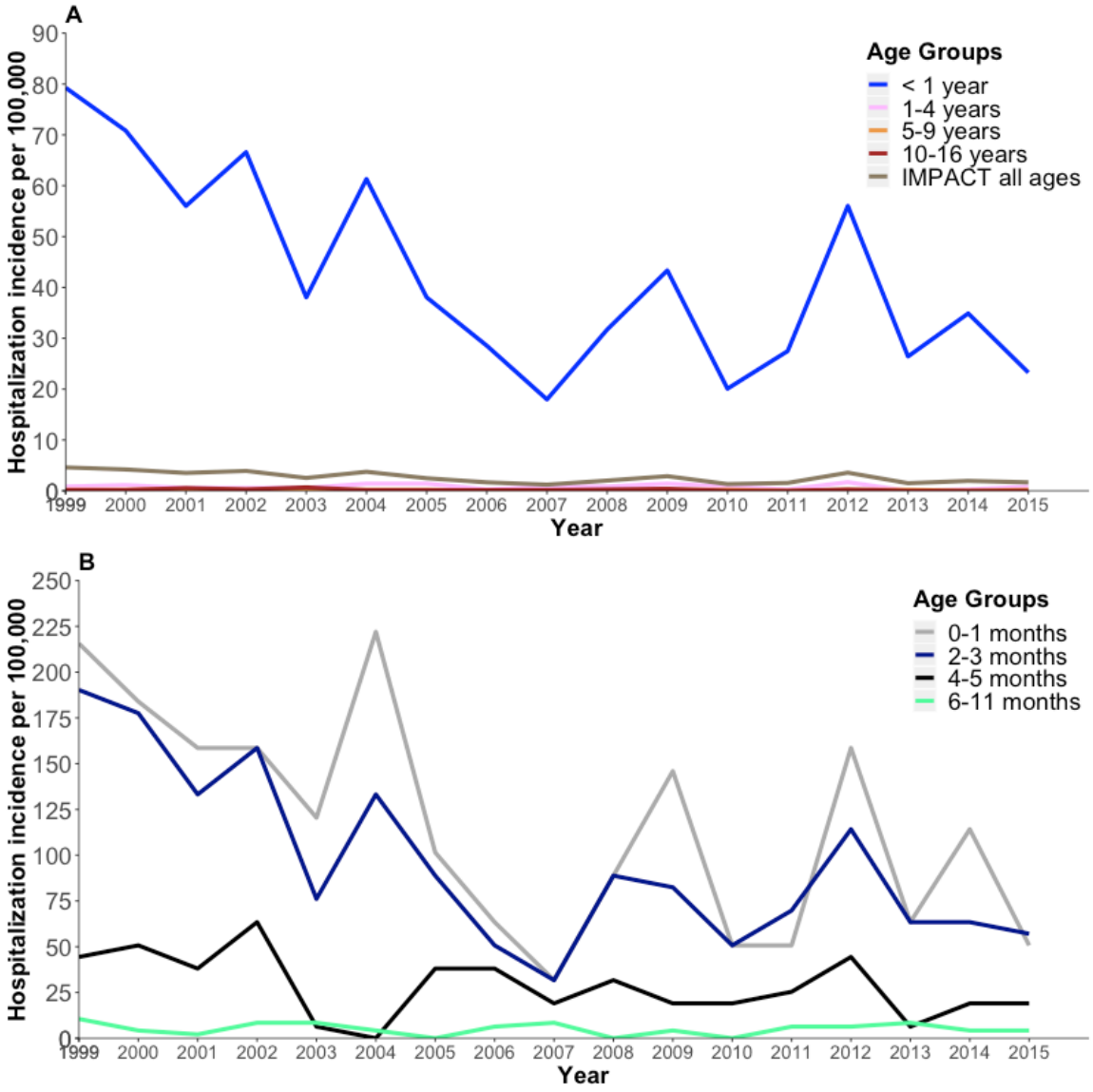


Figure 2. 2: Pertussis population hospitalization incidence at IMPACT hospitals, 1999-2015.

Graphs show age-specific population-based pertussis hospitalization incidence in (A) children ≤ 16 years and (B) younger than 1 year of age, IMPACT hospitals, 1999–2015.

2.4.3 Intensive care admission

In total, 357/1402 (25.5%) of children were admitted to ICU. There was significant year-to-year variation between years in the proportion of children admitted to the ICU with a low of 14.0% and high of 41.7% ($p<0·0001$) over the 17 years. Of the cases admitted to the ICU, 316/357 (88.5%) were confirmed pertussis cases. Pertussis cases requiring admission to the ICU were younger, had higher rates of neurological complications, longer hospital LOS and were more likely to have comorbidities compared with cases not admitted to the ICU (Table 2.6). Of note, prematurity was the most common comorbidity, reported in 8.4% of ICU cases. The proportion of cases admitted to ICU was 37.9% (232/612) for infants <2 months of age, 19.8% (91/460) for infants 2–3 months of age, 12.6% (15/119) for infants 4–5 months of age, 10.8% (8/74) for those 6–11 months of age and 27.3% (346/1265) for infants <1 year of age.

Table 2. 6: Characteristics of pertussis cases admitted to IMPACT hospitals ICU in Canada, 1999–2015.

Characteristic	Admitted to the ICU (n=357)	Not admitted to the ICU (n=1045)	P-value [a]
Demographics			
Male sex n (% of total)	171 (47.9)	484 (46.3)	0.648
Age			
Median ([overall range], [IQR]) (weeks)	7 ([2-872], [4-10])	11 ([1-886], [7-19])	<0.0001
Age groups n (% of total)			
< 1 year	346 (96.9)	919 (87.9)	
0-1 months	232 (64.9)	380 (36.4)	
2-3 months	91 (25.5)	369(35.3)	
4-5 months	15 (4.2)	104(9.9)	
6-11 months	8 (2.2)	66(6.3)	
1-4 years	9 (2.5)	63(6.0)	
5-9 years	1 (0.3)	23(2.2)	
10-16 years	1 (0.3)	40(3.8)	
Clinical features			
Comorbidity [b] n (% of total)			
Underlying conditions n (% of total)	61 (17.1)	117 (11.2)	0.013
Prematurity [c]	30 [d] (8.4)	20 [e] (1.9)	<0.0001
Congenital Cardiac	8 (2.2)	10 (0.9)	
Pulmonary	3 (0.8)	24 (2.3)	
Genetic-Metabolic	3 (0.8)	5 (0.5)	
Gastrointestinal	3 (0.8)	8 (0.8)	
Failure to thrive	2 (0.6)	5 (0.5)	
Neurologic	1 (0.3)	14 (1.3)	
Immuno-compromised	0 (0)	9 (0.9)	
Other	11 (3.1)	22 (2.1)	
Evidence of diagnosis n (% of total)			<0.001
Confirmed [f]	316 (88.5)	841 (80.5)	
Probable [g]	41 (11.5)	204 (19.5)	
Seizures n (% of total)			<0.0001
New seizures	17 (4.8)	13 (1.2)	
Exacerbation of an existing seizure disorder	4 (1.1)	4 (0.4)	
Encephalopathy n (% of total)			0.005
Present	6 (1.7)	2 (0.2)	
Outcome			
Hospitalization			
Median length of stay ([overall range], [IQR]) (days)	13 ([1-185], [7-22])	5 ([1-60], [3-10])	<0.0001
Mortality n (% of total)			
Death	21 (5.9)	0 (0)	<0.0001

[a] For the comparison of pertussis cases admitted to the ICU versus not admitted to the ICU; [b] As clinically denoted in the records. A patient can have more than one comorbidity; [C] Prematurity is defined as birth at gestational age <37 weeks gestation; [d] Data on gestational age at birth was available for 28/30 premature cases admitted to the intensive care unit; 5 were born at 24-26 weeks gestation, 7 were born at 27-31 weeks gestation, 16 were born at 32-36 weeks gestation; [e] Data on gestational age at birth was available for 19/20 premature cases not admitted to the intensive care unit; 1 was born at 25 weeks gestation, 4 were born at 27-31 weeks gestation, 14 were born at 32-36 weeks gestation; [f] Laboratory-confirmed or epidemiology-linked pertussis cases; [g] Clinical pertussis cases.

The proportion of cases admitted to ICU decreased as gestational age at birth increased (p<0·0001) (Figure 2.3).

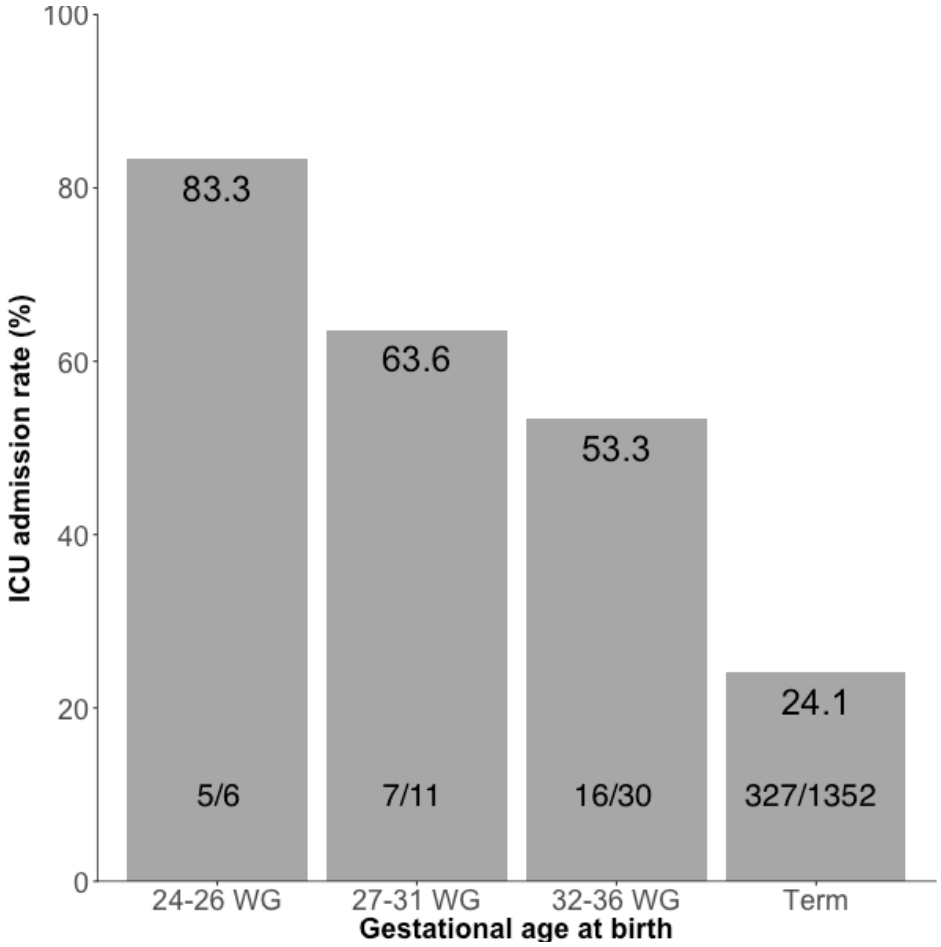


Figure 2. 3: Proportion of children admitted to the intensive care unit due to pertussis at IMPACT hospitals by their gestation age at birth, 1999-2015.

The absolute number of patients admitted to the intensive care unit out of total children for each gestation age group is shown in the bar.

With the exception of 2007, the proportion of children admitted to the ICU was higher for infants <2 months of age compared with infants aged 2–3 months (Figure 2.4).

In total, 298/357 (83.5%) of pertussis cases admitted to the ICU were from provinces where almost all ICU admissions are captured by IMPACT hospitals (excluding Ontario and the Canadian territories) and were included in the ICU incidence calculations. The

overall mean annual incidence of ICU admission over the 17-year period was highest among infants <2 months of age (Table 2). With the exception of 2006, the incidence of ICU admission was higher for cases <2 months compared with cases 2–3 months with three peaks in 2004, 2009, and 2012 (Figure 2.4).

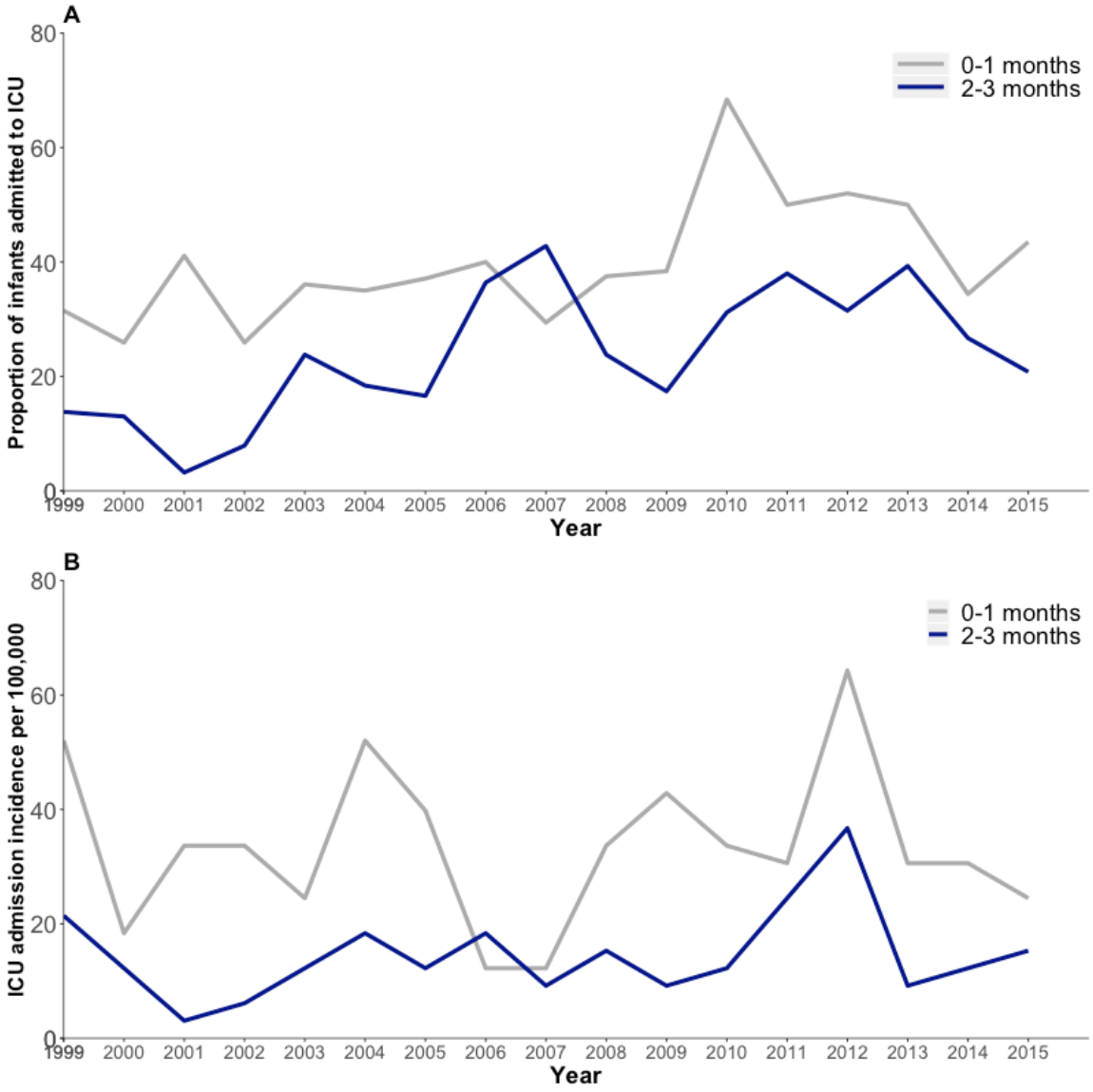


Figure 2. 4: Intensive care unit admission rate and admission incidence at IMPACT hospitals, 1999-2015.

Abbreviation: ICU: intensive care unit. Graphs show age-specific (A) intensive care unit admission rate (in percentage) and (B) population–based intensive-care unit admission incidence (per 100,000 population) of patients hospitalized with pertussis among infants < 4 months, IMPACT hospitals, 1999–2015. This figures describes the Intensive-care unit admission rate and admission incidence in infants < 4 months of age. The small numbers of patients older than 4 months and admitted to the intensive care precluded the description of the incidence rates in this figure.

2.4.4 Mortality

There were 21 deaths in children aged 2-14 weeks at admission reported to IMPACT centers during 17 years period (Table 2.7). None of the infants who died had received a valid dose of vaccine although 17/21 infants were aged <3 months and therefore too young to have received 1 valid pertussis vaccine dose.

Table 2. 7: Characteristics of IMPACT hospitals pertussis fatalities, 1999–2015.

Characteristic	Deaths (n=21)	No death (n=1381)	P-value
Male sex n (% of total)	4 (19.0)	651 (47.1)	0.019
Age			
Median ([overall range], [IQR]) (weeks)	5 ([2-14], [3-9])	10 ([1-886], [6 -17])	<0.0001
Age groups			
< 1 year, n (% of total)	21 (100)	1244 (90.1)	
0-1 months	14 (66.7)	598 (43.3)	
2-3 months	7 (33.3)	453 (32.8)	
4-5 months	0 (0)	119 (8.6)	
6-11 months	0 (0)	74 (5.4)	
1-4 years	0 (0)	72 (5.2)	
5-9 years	0 (0)	24 (1.7)	
10-16 years	0 (0)	41 (2.9)	
Evidence of diagnosis			0.057
Confirmed	21 (100)	1124 (81.4)	
Probable	0 (0)	257 (18.6)	
Morbidity			
Seizures n (% of total)			0.665
No seizure	20 (95.2)	1344 (97.3)	
New seizures	1 (4.8)	29 (2.1)	
Exacerbation of an existing seizure disorder	0 (0)	8 (0.6)	
Encephalopathy [a] n (% of total)			< 0.0001
Not present	19 (90.5)	1373 (99.4)	
Present	0 (0)	8 (0.6)	
Hospitalization			
Median length of stay ([overall range], [IQR]) (days)	3 ([1-79], [2-14])	7 ([1 185], [2-14])	0.050
Comorbidity [b] n (% of total)			
Underlying conditions	5 (23.8)	173 (12.5)	0.303
Prematurity [c]	4 (19.0)	46 (3.3)	0.001

[a] 2 fatal cases with no data on encephalopathy; [b] As clinically denoted in the records. A patient can have more than one comorbidity; [c] Prematurity is defined as birth at gestational age <37 weeks gestation. One case was born at 29 weeks gestation, two cases were born at 34 weeks gestation, and one case was born at 35 weeks gestation.

Death occurred 1-47 days after admission with 61.9% (13/21) occurring in the first three days after admission (Figure 2.5). The overall case fatality rate (CFR) was 1.5% (21/1402). The CFR was 2.3% (14/612), 1.5% (7/460) and 1.7% (21/1265) for infants <2 months of age, 2–3 months of age, and <1 year of age, respectively.

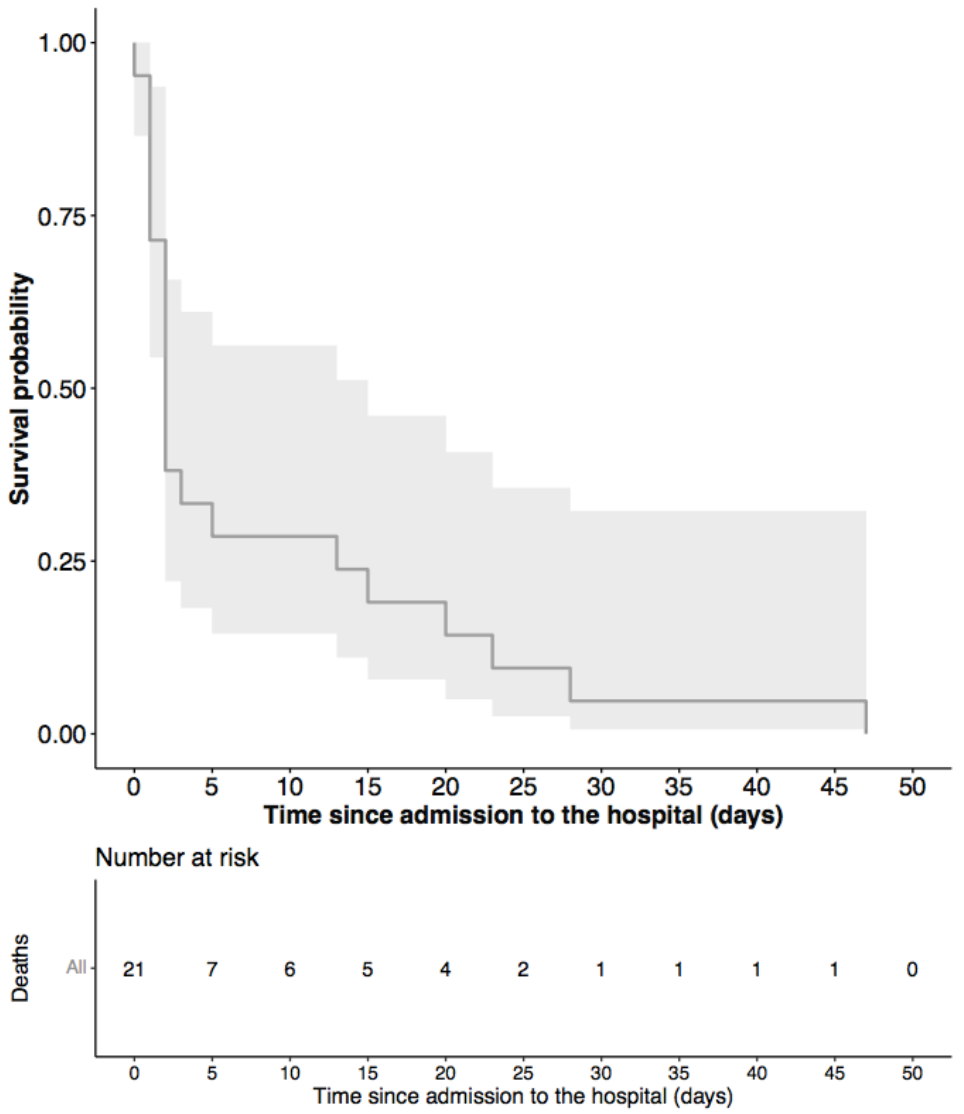


Figure 2. 5: Kaplan-Meier survival plot for pertussis fatal cases at IMPACT hospitals, 1999-2015.

Graph shows time from admission with pertussis to death (n=21), IMPACT hospitals, 1999–2015.

2.4.5 Risk factors for admission to intensive care unit and death

In univariate analyses, different variables were associated with increased risk to ICU admission and death (Table 2.8). In multivariate analyses, independent risk factors for ICU admission were age <16 weeks, prematurity, encephalopathy, confirmed pertussis diagnosis and later year of admission (Table 2.8). Independent risk factors for death were age <4 weeks, prematurity, female sex and later year of admission (Table 2.8).

Table 2. 8: Risk factors for admission to intensive care unit and death in patients with pertussis (n=1392).

Variable	P value	Odds ratio (95% CI)	P value	Adjusted odds ratio (95% CI)
	Univariate analysis		Multivariable analysis [a]	
Risk factors for admission to intensive care unit				
Age: ≥ 16 weeks (380) < 16 weeks (1012)	< 0.0001	Ref 4.47 (3.12-6.59)	< 0.0001	Ref 4.83 (3.30-7.27)
Male (648) Female (744)	0.569	Ref 0.93 (0.3-1.18)	Not included	
No new seizures (1363) New seizures (29)	< 0.001	Ref 3.77 (1.80-8.06)	Not included*	
Encephalopathy: No (1385) Yes (7)	0.007	Ref 18.08 (3.07-342.22)	0.007	Ref 21.13 (3.18-425.21)
Prematurity [b]: No (1343) Yes (49)	<0.0001	Ref 4.59 (2.58-8.35)	<0.0001	Ref 5.81 (3.04-11.37)
Comorbidity No (1252) Yes (140)	0.580	Ref 1.11 (0.75-1.64)	Not included	
Evidence for diagnosis: Probable (245) Confirmed (1147)	< 0.001	Ref 1.84 (1.29-2.67)	0.040	Ref 1.51 (1.03- 2.27)
Admission date (year)	< 0.0001	1.05 (1.03- 1.08)	<0.001	1.05 (1.02-1.07)
Vaccination status [c] (3-4 months) (n=184): Unvaccinated (102) AAV (n=82)	0.665	Ref 0.83 (0.35- 1.89)	Not included	
Risk factors for death				
Age: ≥ 4 weeks (1280) < 4 weeks (112)	< 0.0001	Ref 7.04 (2.57-17.89)	0.0002	Ref 6.73 (2.39- 17.88)
Male (648) Female (744)	0.034	Ref 3.31 (1.19- 11.66)	0.032	Ref 3·46 (1.21- 12.47)
Prematurity [b]: No (1343) Yes (49)	0.009	Ref 5.40 (1.23-16.95)	0.015	Ref 5.36 (1.15-18.61)
Comorbidity No (1252) Yes (140)	0.493	Ref 0.49 (0.03-2.41)	Not included	
Admission date (year)	0.022	1.11 (1.01-1.22)	0.014	1.13 (1.03-1.25)

[a] The multivariable intensive care unit analysis was adjusted to patient's age, occurrence of encephalopathy, existence of prematurity, admission year, IMPACT hospital and evidence for diagnosis. The multivariable death analysis was adjusted to patient's age, admission year, IMPACT hospital, and sex.
[b] Prematurity is defined as birth at gestational age <37 weeks gestation.
[c] This sub-analysis included laboratory-confirmed case age 3-4 months who received more than one vaccine dose or were unvaccinated.
* Not included because some patients had both seizures and encephalopathy, so encephalopathy outcome was chosen. Abbreviation: AAV: Age-appropriately vaccinated

2.4.6 Vaccination status of laboratory-confirmed pertussis cases

Among hospitalized patients with laboratory–confirmed pertussis aged 3 months–16 years (n=355), 31.8% (113/355) had received an age-appropriate number of pertussis vaccine doses and 51.5% (183/355) were unvaccinated (0 doses) (Figure 2.6). The percentage of unimmunized patients hospitalized with pertussis was 55.4% (102/184), 42.6% (23/54) and 59.6% (34/57), 58.3% (14/24) and 27.8% (10/36), among infants 3-4 months old, 5-6 months old, 7-18 months old, 19 months- 6 years and 7-16 years, respectively (Figure 2.6). Among patients aged ≥7 months, 28.2% (33/117) had received an age-appropriate number of pertussis vaccine doses, 22.2% (26/117) were under-vaccinated and 49.6% (58/117) were unvaccinated. Among infants aged 7 months–18 months, 22.8% (13/57) patients had received 3 valid pertussis vaccine doses. Of those cases, 84.6% (11/13) occurred ≥7 months after the third dose (Figure 2.6).

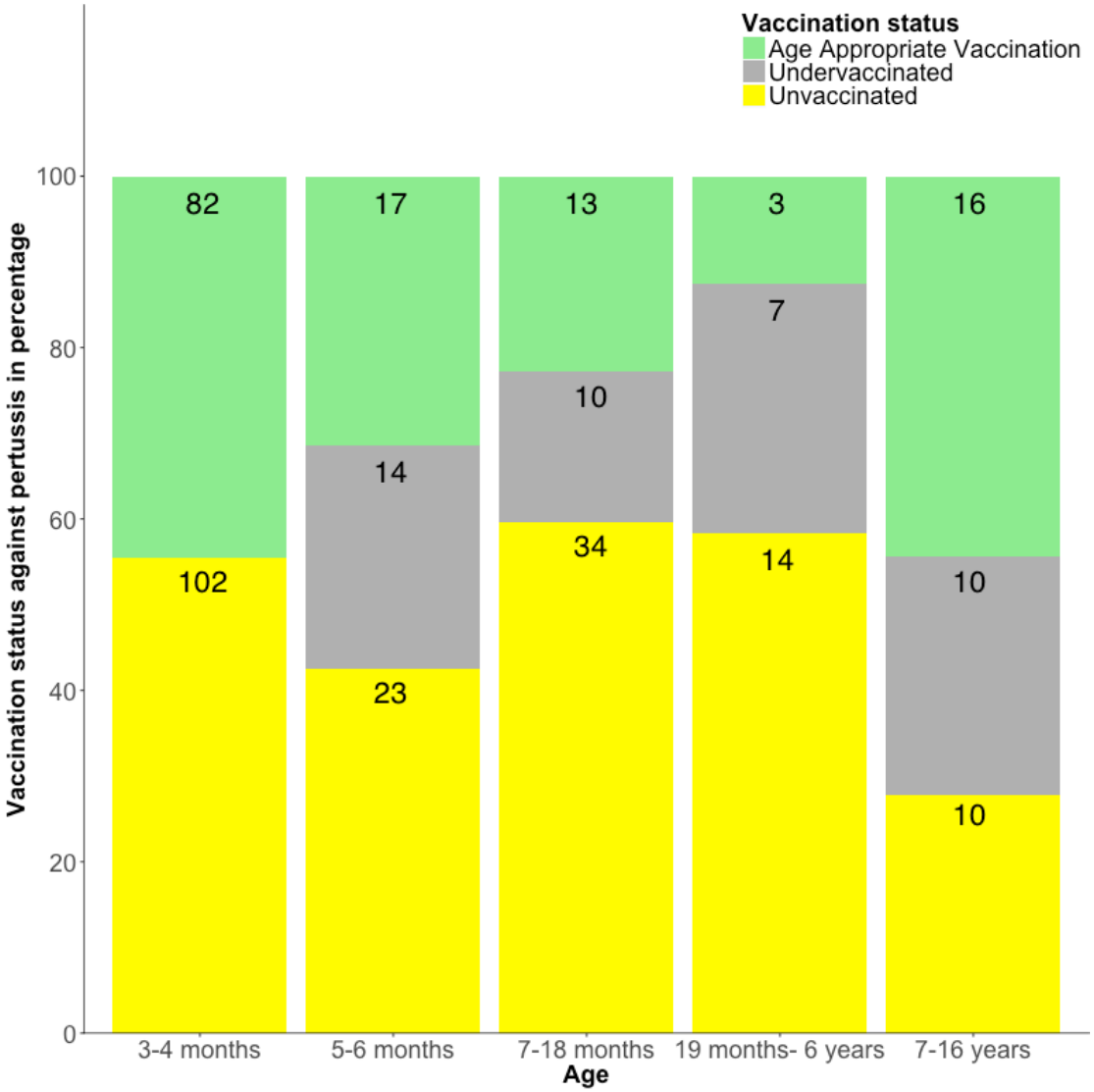


Figure 2. 6: Vaccination status of patients hospitalized with pertussis at IMPACT hospitals, 1999-2015.

Graph shows vaccination status of patients hospitalized with laboratory-confirmed pertussis 3 months-16 years, IMPACT hospitals, 1999–2015. The absolute number of patients for each vaccination status is shown in the bar.

The median interval between the third vaccine dose and admission was 254 days (overall range=71–392, IQR= 218–317) (Figure 2.7).

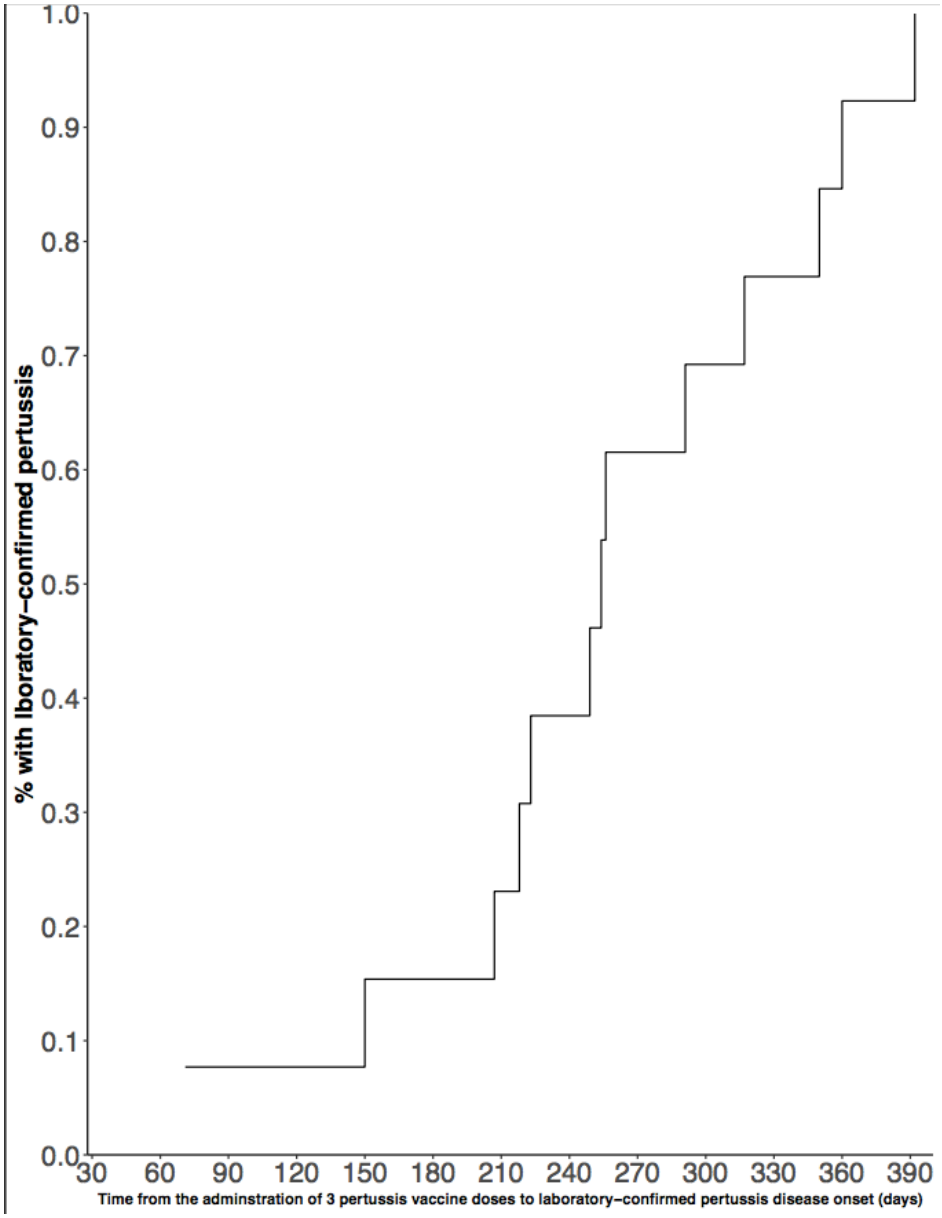


Figure 2. 7: Cumulative incidence plot of hospital admission due to pertussis at IMPACT hospitals, 1999-2015.

Graph shows time from vaccination with 3 received pertussis vaccine doses to disease onset among infants 7–18 months with laboratory-confirmed pertussis (n=13), IMPACT hospitals, 1999–2015.

2.5 Discussion

In this chapter, I report on the longitudinal burden of hospitalized pertussis among pediatric population admitted to pediatric tertiary care centers in the aP vaccine era in Canada over a 17-year period. I show that that infants too young to be vaccinated were the major contributor to the burden of hospitalized pertussis disease. Specifically, infants <2 months of age displayed the highest hospitalization incidence rate. The rate of pertussis hospitalization fluctuated during this period but was still significant and the highest among infants younger than 2 months. Almost 25% of all cases were admitted to ICU, with the majority in infants aged <2 months. Age <16 weeks was independently associated with a 5-fold increase in odds for ICU admission compared with older children. Prematurity and confirmed pertussis diagnosis were independently associated 6-fold and 1.5-fold increased odds for ICU admission, respectively. Age <4 weeks was the most significant independent risk factor for mortality, associated with 7-fold increased odds of death. Prematurity and female sex was significantly associated with 5-fold and 3.5-fold increased odds of death, respectively. These data have important implications, establishing the true burden of endemic pertussis disease, and thus assisting public health policy makers to reach evidence-based conclusions regarding the optimal cost-effective preventative approach. Identification of risk factors for poor outcomes aids appropriate prioritization in management of young infants with pertussis, and counseling for families during hospitalization.

In this chapter, I report that the majority of children hospitalized with pertussis were <4

months of age, consistent with previous data from Canada, USA and Australia in the aP vaccine era[210, 339, 349, 354]. While almost 21% of global pertussis cases are in infants <1 year of age[212], 90% of hospitalized pertussis cases in the aP era and 92% of hospitalized pertussis cases in the wP vaccine era in Canada were in this age group[356]. In addition, 85% of hospitalized pertussis cases in this study and 79.1% of hospitalized pertussis cases in the wP vaccine era in Canada were in infants <6 months of age. This emphasizes that young infants are at disproportionate high risk for severe pertussis. Pertussis hospitalization incidence among infants <2 months of age (116.40/100,000) was lower than reported in the wP vaccine era in England during 1995–1997 (164/100,000 among infants <3 months of age)[360], and in Australia four years after the introduction of aP vaccine (~200/100,000 among infants <2 months of age)[349]. Thus, although the incidence of hospitalized pertussis among infants aged <2 months in the aP era is lower than in the wP era, this age group accounts for a high proportion of pertussis–related hospital admissions. In addition, pertussis hospitalization incidence among infants <1 year of age (42.3/100,000) is lower than reported in the wP vaccine era in Canada (136/100,000)[354].

Children with pertussis had long hospitalization with median LOS of 7 days, notably higher than the median LOS of 4 days reported among patients hospitalized with pertussis (median age 2.6 months) during the 2010 California pertussis epidemic[339]. This difference might stem from the fact that IMPACT hospitals are pediatric tertiary-care centers admitting the more severe cases of pertussis. Moreover, the median LOS of 8 days among infants <6 months of age in this study is comparable to the median LOS of 9.3 days among infants aged <6 months admitted to IMPACT centers with pertussis

during the wP era, and both studies used the same clinical case definition of pertussis[356]. In this study, 77.2% of patients aged 7-18 months had received fewer than 3 vaccine doses while 44.9% of patients 6-24 months of age admitted to IMPACT centers with pertussis during the wP era received fewer than 3 vaccine doses. This emphasizes that in the aP era, under-vaccination is an important contributor to pertussis hospitalization. However, infection despite of completion of three vaccination doses among this age group was observed in 23% (13/57) of hospitalized pertussis cases, which could be due to waning immunity. In contrast to many other infectious diseases, immunity following pertussis vaccination is not long lasting. Waning immunity is expected 4-12 years after vaccination[221, 251, 361], and within 5 years after five doses of aP vaccine[221]. A recent study from New Zealand reported that the vaccine effectiveness (VE) of 3 doses of aP in preventing hospitalization due pertussis among infants 5-11 months old was 93%. This VE remained high, in a range of 91-98% during the first 2-3 years of life[362]. In this study, I found that among infants 7-18 months old, 13 cases out of 57 had infection although completed a series of primary immunization with 3 doses. This emphasized that 3 pertussis vaccine doses were not sufficient to prevent hospitalized pertussis and reaffirm that pertussis short-lived vaccine-induced immunity. Although VE was not calculated in this analysis as the data included were collected prior to recommendation of pertussis vaccination in pregnancy in Canada, these data challenge the recent observational study in Australia showing that VE against hospitalized pertussis among infants 6-11 months old increased for dose 1 to 2 (from 55% to 83%) and remained high (85%) after the third dose[361] and question the approach to delay the third dose of pertussis vaccine during infancy, a practice in Scandinavian countries[363] and France[364].

Neurological manifestations (new onset seizures and/or encephalopathy) were observed in 2.6% of patients admitted with pertussis, consistent with data from IMPACT centers in the wP era (2.4% among patients <2 years of age). Thus, these data emphasize that neurological manifestations still occur in the aP era in Canada. Moreover, the CFR in this study was 1.5% for all age groups (2.3% for infants <2 months of age), higher than the CFR reported in the wP era in Canada (0.9% among patients <2 years of age). In this study, two thirds of deaths were amongst <2 months infants, comparable with the data from IMPACT centers during the wP era where 80% of fatalities due to pertussis were among infants <2 months of age.

Patients hospitalized with pertussis had frequent ICU admissions (25%). The range of ICU admission rate of patient hospitalized with pertussis is in the range of 4.6%-33% in studies reported from both high and middle income countries[365,348,366]. The highest ICU admission rate was among infants <2 months of age (37.9%). In a single center in California, a third of infants < 3 months admitted during 2009-2010 were admitted to the ICU[367]. The proportion of ICU admission among infants <1 year, is comparable to the rate reported in an epidemic in California (33% among hospitalized infants aged <1 year)[348], and higher than the rate reported during the wP era in Canada (16% among children aged <2 years and 19.2% among infants aged <6 months)[356]. In addition, median ICU LOS reported in this study (4 days) is comparable to that reported in Australia and New Zealand of 3.6 and 3.9 days[366, 368]. Similar to previous literature, nearly two third of infants admitted to the ICU were too young to be vaccinated[365, 366] and 17% of infants

admitted to the ICU had at least one comorbidity, the most common was prematurity[366, 369].

The mean CFR in this study was 1.5% for all age groups (2.3% for infants <2 months). Notably, this is slightly higher than the CFR reported in the wP (among patients < 2 years of age) and aP (< 16 years old) era by Canadian IMPACT centers, 0.9% and 0.7%, respectively[356,354] but similar to the CFR reported during the California 2010 outbreak (CFR of 1.3% among infants < 3 months old) [339]. In another study, a CFR of 2% was reported in a multinational study of infants < 1 year admitted to the ICU[365]. All deaths were among infants younger than 14 weeks old, and two thirds of deaths were amongst patients too young to be vaccinated with any vaccine dose, consistent with previous literature[339,222, 246,350] . Two-third of patients died by the fifth day of admission while one patient died at day 47 of admission. This is consistent with 4 reported cases died in a range of 21-41 days in Australia in 2001[351].

There are no data on risk factors for admission to the ICU and scarce data on the risk factors for death due to pertussis. Identifying higher–risk infants can help physicians in their clinical management decisions and thus close monitoring and early consideration of need for ICU admission is required in infants displaying these risk factors. Infants with confirmed pertussis had longer hospital and ICU LOS than those with probable pertussis demonstrating that the clinical severity of confirmed pertussis disease is higher than clinically diagnosed pertussis disease, which may be because some probable cases did not have pertussis or had a lower bacteria load that led to less severe disease and was not also

detected by diagnostic methods. A case-control study during a period in which both wP and aP vaccine were used in Canada found that white-blood cell (WBC) count was risk factor for death from pertussis[370]. This is consistent with recent data from California showing that fatal pertussis cases had higher WBC counts compared to nonfatal cases[371]. Lower birth weight, higher peak WBC count, pulmonary hypertension, seizures[244] and female sex[372] were independent risk factors for death from pertussis among infants. Encephalopathy was independently associated with 21-fold increased odds for ICU admission, however, this is based on a small number of patients (8) and thus the precision of this estimate is unclear as the 95 CI was large. In the US in the aP vaccine era, prematurity was a risk factor for death from pertussis in univariate analyses only[244,372]. Premature infants were overrepresented (12/20) in a cohort of fatal pertussis cases in the US in the wP vaccine era[373]. This study sets prematurity as independent risk factor for death from pertussis in the aP era. The finding that female sex is risk factor for death is unexpected and surprising as female usually mount stronger innate and adaptive immune responses than males[374].

The data presented in this chapter has a number of strengths. This is a detailed characterization of pediatric hospitalized pertussis cases in the aP vaccine era. The study is unique for its inclusion of a national population, long duration and extensive active case finding. IMPACT reporting is active, prospective, standardized and performed by trained nurse monitors, and as such the accuracy and completeness of the data is high. The 17-year time period enabled evaluation of the burden and characteristics of pertussis disease that spanned over a prolonged period. IMPACT hospitals constitute 90% of

Canada's tertiary-care pediatric beds, thus providing good estimates of severe pediatric pertussis cases that require hospitalization in tertiary care centers. Moreover, IMPACT hospitals' catchment area covers 57% of the Canadian population <16 years of age.

This study has some limitations. Less severe pertussis cases would have been admitted at smaller hospitals not part of the IMPACT network or diagnosed and treated in the community. Thus, the true burden of all pertussis cases is underestimated in this study. This limitation is less concerning when assessing severe pertussis requiring ICU admission as IMPACT centers comprise most of the pediatric tertiary-care beds in Canada. Misclassification of probable cases is possible, although this was a minority of cases. These data did not capture readmissions. However, this is expected to be uncommon as pertussis is a monophasic disease. This study is based on information collected from medical databases and as such there might be misclassification bias leading to underestimation of burden of pertussis disease during study period. The limited variables available for inclusion in risk factors analyses is another limitation as other important factors (e.g. maternal age, ethnicity) could be an important confounders of morbidity and mortality. There is a possibility that some of the patients included in this study were primed with wP vaccine, however the proportion of those cases is expected to be low as 90% of the patients were <1 year of age and admitted after 1999 (aP was introduced in Canada in 1997-1998). These data did not capture the onset of cough, an important variable in defining a vaccine dose as valid. However, the 4 week interval between recent vaccine dose and admission, used in this study, minimizes this misclassification. The diagnostic tests used during the study period might have affected

detection of cases with the increased use of PCR over culture. Literature supports that PCR is more sensitive than culture in diagnosing pertussis, mainly due to lower sensitivity of culture [205, 375].

Two main potential strategies for reducing the burden of pertussis in infants include immunization of close contacts ("cocooning") and antenatal maternal immunization. The cocooning strategy of all close contacts of infants too young for vaccination was shown to be difficult to implement[376], unsuccessful in preventing pertussis illness in infants ≤6 months of age[377], inefficient and resource intensive for the prevention of serious outcomes in early infancy[334]. Several countries (e.g. USA, UK) have responded to the increase in pertussis morbidity and mortality among young infants by universal recommendation of immunization of pregnant women against pertussis[342, 343]. Protection of infants against pertussis following maternal immunization is assumed to result from direct protection provided by maternally transferred pertussis-specific antibodies and indirect protection resulting from reduced risk of *B. pertussis* infection in the mother and thus lower risk for infant exposure. Vaccination against pertussis in pregnancy has proved to be effective in preventing pertussis disease among infants aged <3 months[343, 378, 379],and to decrease the risk of hospitalization, risk of ICU admission and hospital LOS[380].Given the severe morbidity of endemic pertussis disease among infants during the first months of life, as shown in this study, vaccination against pertussis in pregnancy has the potential to control the burden of pertussis among young infants in countries with long standing use of aP vaccine. Vaccination against pertussis in pregnancy has proved to be highly (nearly 90%) effective in preventing pertussis disease and hospitalization

among infants aged <3 months in the UK and USA[343, 379]. Assuming that vaccination against pertussis in pregnancy is 90% effective in the prevention of pertussis hospitalization among infants aged <3 months, approximately 825 cases of hospitalized pertussis could have been prevented via maternal immunization in the study hospitals during this 17-year period. Universal vaccination against pertussis in pregnancy is now recommended in increasing number of countries and most recently in Canada[353]. While the optimal timing of vaccination against pertussis in pregnancy is under research, my finding that prematurity is independent risk factor for ICU admission and death from pertussis may support vaccination earlier in pregnancy.

3. Avidity of anti-*Bordetella pertussis* antibodies induced after vaccination during pregnancy

3.1 Synopsis

Vaccination of all pregnant women with Tdap vaccine is recommended in an increasing number of countries (e.g. the UK, US, Australia, Canada) in order to protect infants too young to be vaccinated themselves. The optimal timing of vaccination in pregnancy to provide maximal protection to young infants remains an important knowledge gap leading to variable national recommendations, for example 16-32 weeks gestation (WG) in the UK and 27-32 WG in Canada. These recommendations are based on studies showing higher levels of anti-*B. pertussis* antibodies in infants born to women vaccinated during early third trimester compared with vaccination in late third trimester or showing higher anti-*B. pertussis* antibody levels in infants born to women vaccinated in second compared to third trimester.

Evaluation of vaccine-induced antibody immune response includes assessment of the quantity and function of antibodies. No well-established specific anti-pertussis antibody level correlates with protection, suggesting the importance of antibody function such as avidity. Avidity (or functional affinity) of antibodies is a measure of the binding strength of bi- or poly-valent antibody with bi- or poly-valent antigens and is a functional measure of affinity maturation of antibodies following exposure to an antigen (e.g. vaccine components). Enzyme-linked immunosorbent assay (ELISA)-based elution assay is a common method for the measurement of antibody avidity and consists of the assessment

of the stability of antigen–antibody complexes in the presence of a chaotropic agent. The application of single chaotropic agent concentration to the ELISA-based elution assay, a frequently used approach in research and diagnostics, leads to an arbitrary separation of high and low avidity antibodies, presented in relative measures (e.g. relative avidity index [RAI]). However, the true avidity profile, induced following exposure to an antigen, includes continuous range of quantity of antibodies heterogeneous in their avidities. There are scare data on the laboratory and analytical approaches needed in order to profile the avidity of antibodies following antigen exposure (e.g. vaccination).

In this chapter, I propose a step-by-step laboratory analysis and novel analytical approaches in order to allow in deep analysis of the profile of antibody avidity using ELISA-based elution. I also aimed to determine the effect of timing of vaccination with Tdap in pregnancy on the avidity of cord anti-PT IgG. Included were newborns born in a tertiary hospital (Melbourne, Australia) born to women vaccinated with Tdap in pregnancy. Ammonium thiocyanate was used as a bond-breaking agent to measure the avidity of anti-PT IgG using concentrations between 0.25M (to measure low avidity antibodies) and 3M (to measure very high avidity antibodies).

I also showed that using a range of concentrations of chaotropic agent, the fractional relative avidity index and the absolute levels of antibodies with different avidities can be accurately quantified. In addition, a single weighted value of total absolute avidity levels, that incorporates both quantitative and qualitative avidity characteristics can also be calculated. Using this approach, I found that cord specimens of women vaccinated with

Tdap in pregnancy had higher total absolute avidity levels and fractional absolute levels of low–medium, medium, medium–high, high, and very-high avidity levels compared with those of cord specimens of unvaccinated women. Vaccination was associated with avidity profile consisting of high levels of high avidity antibodies.

Anti-PT IgG levels achieved at each ammonium thiocyanate concentration in cord samples of women vaccinated during 28–32 WG *vs.* 33–36 WG, and women vaccinated 5–12 *vs.* 1–4 weeks prior to delivery were compared using *t*-tests. I found that newborns of women vaccinated with Tdap during 28–32 WG (n=43) had statistically significant higher concentrations of medium and high avidity anti-PT IgG compared with newborns of women vaccinated during 33–36 WG (n=47), 11.6 IU/ml (95% CI, 8.8–15.2) IU/ml *vs.* 6.7 IU/ml (95% CI, 5.2–8.6) and 10.1 IU/ml (95% CI, 7.4–13.8) *vs.* 5.7 (95% CI, 3.6–8.9) IU/ml, (p=0.007 and p=0.035), respectively. Newborns of women vaccinated 5–12 weeks before delivery (n=64) had statistically significant higher concentrations of high and very high avidity anti-PT IgG compared with newborns of women vaccinated within 4 weeks before delivery (n=25), 10.3 IU/mL (95% CI, 7.9–13.4) *vs.* 3.3 IU/mL (95% CI, 1.7–6.4), 12.6 IU/mL (95% CI, 9.4–16.9) *vs.* 4.3 IU/mL (95% CI, 2.2–8.5), (all $p<0.03$), respectively.

In conclusion, quantification of levels of anti-PT IgG with different avidities demonstrated that pertussis vaccination 5–12 weeks before delivery was associated with higher anti-PT IgG avidity compared with vaccination within 4 weeks before delivery. Pertussis vaccination during 28–32 WG was associated with higher anti-PT IgG avidity

compared with vaccination during 33–36 WG, supporting vaccination at 28–32 over 33–36 WG for optimal protection against pertussis in infancy.

3.2 Introduction

Vaccination of all pregnant women with Tdap vaccine is recommended in an increasing number of countries, including the UK[381], US[382], Australia[383], Canada[384], Brazil[385] and Argentina[386] to protect infants too young to be vaccinated themselves. The optimal timing of vaccination in pregnancy to provide maximal protection to young infants remains an important knowledge gap[73] leading to variable national recommendations, for example 16-32 WG in the UK and 27-32 WG in Canada[381-384]. In addition, although vaccination against pertussis in pregnancy has been shown to be effective in preventing pertussis disease in infancy, breakthrough cases do occur in infants born to women vaccinated according to their national recommendations[378, 387-389,343, 390], suggesting the need for data to further understand the variables affecting vaccine effectiveness. The impact of timing of Tdap vaccination during pregnancy on the infants' immunity to pertussis is one of the important variables.

Data on the effect of timing of antenatal pertussis vaccination on the avidity of anti–pertussis antibodies are scarce, conflicting and derived from two small studies which assessed avidity with limited approach of using a single concentration of bond-breaking agent[391,392]. Acellular pertussis vaccines contain components of *B. pertussis* such as PT, FHA, FIM2/3 and PRN. PT is an important virulence factor of *B. pertussis*[393, 394]. PT is thought to be the cause of leukocytosis[223, 225, 395, 396], which is associated with poor outcome among infants with pertussis[222, 370]. Anti-PT antibodies are thus important in protecting from pertussis disease.

Higher anti-pertussis antibody levels are associated with clinical protection from pertussis disease[397], but there is no specific anti-pertussis antibody level that correlates with protection. This emphasizes the importance of evaluating anti-pertussis antibody function and not only antibody concentration. Antibody avidity is one important measure of function, which examines the overall binding strength between a specific antibody and a target antigen[398,399]. Antibody functions have been established as a correlate of post-vaccination protection from other bacterial invasive diseases (e.g. bactericidal antibody in meningococcal disease)[400] and are likely also be important for protection against pertussis disease.

Affinity (or intrinsic affinity) of antibodies is a measure of the binding strength of monovalent antigen with monovalent antibody while avidity (or functional affinity) of antibodies is a measure of the binding strength of bi- or poly-valent antigens with bi- or poly-valent antibodies. Using interaction measures to describe the binding strength between antigens and antibodies, antibody affinity is described as the sum of attractive and repulsive physical and chemical forces between antigenic determinant and immunoglobulin combining site. Avidity is a measure of the stability of the antigen-antibody complex and is affected, in addition to the sum of attractive and repulsive forces, also by other factors (e.g. antigen valency [i.e number of binding sites], antibody valency, structural arrangement of the antigen-antibody complex, the density of epitopes, and the antibody polyreactivity[401].

There are different laboratory techniques to assess the avidity of antibodies. ELISA-based elution assay is a common method for the estimation of antibody avidity and consists of the assessment of the stability of antigen–antibody complexes in the presence of chaotropic agent[402]. In this technique, the preformed antigen-antibody complex is transiently exposed to a chaotropic agent and antigen-antibody complexes resisting dissociation, at a specific chaotropic agent concentration, are quantified and presented as a RAI. The RAI is calculated as antibody levels in samples treated with the chaotropic agent divided by antibody levels in samples not treated with chaotropic agent and is expressed as a percentage. Thus the RAI measurement after the application of single concentration of chaotropic agent leads to an arbitrary separate antibodies into high and low avidity antibodies.

Previous studies evaluating the avidity of vaccine-induced antibodies are based on a comparison of antibody levels with *vs.* without the addition of a single concentration of bond-breaking agent, leading to an arbitrary and artificial separation of antibodies into 'low' and 'high' avidity antibodies[398, 399, 403]. However, immune response to vaccination is polyclonal and results in antibodies with different avidities. True avidity spectrum (or profile) should reflect the range of antibodies with heterogeneity of avidities that are produced in a polyclonal response to an antigen. Vaccination is expected to produce a spectrum of antibodies with different avidities, ranging from very low to very high avidity antibodies and measuring this requires the use of a gradient of increasing concentrations of chaotropic agent to dissociate antigen–antibody complexes. No published studies have assessed the full avidity profile of anti–pertussis antibodies after

vaccination in general and in pregnancy in particular[255, 404, 405]. Assessing the full spectrum of antibody avidity after vaccination will provide comprehensive insight on avidity maturation after vaccination.

In this chapter, I aimed to develop a laboratory analysis and novel analytical approach in order to allow in deep analysis of the profile of antibody avidity using ELISA-based elution. I used this methodology aimed in order to determine the effect of timing of vaccination with Tdap in pregnancy on anti–PT antibodies conveyed to the newborn at the time of delivery in cord blood of a cohort of pregnant women, evaluating and contrasting both antibody concentration as well as avidity.

3.3 Methods

3.3.1 Study design

Pregnant women at a tertiary obstetric hospital (Monash Health, Melbourne, Australia) were prospectively recruited (April–September 2014) as previously reported.[406] Inclusion criteria were healthy pregnant women with a singleton pregnancy between 28–36^{+6} WG[406]. Women were excluded if they had one or more of the following: receipt of Tdap vaccine during their current pregnancy, having an immunosuppressive disorder (including human immune deficiency virus infection), or were considered at high risk for preterm delivery. Women were invited to participate in the study during the third trimester of their pregnancy. Women who agreed to vaccination were vaccinated with Tdap ([Boostrix] containing ≥2 IU diphtheria toxoid, ≥20 IU tetanus toxoid, 8 μg PT, 8 μg FHA and 2.5 μg PRN and were allocated to either early third (28-32^{+6} [thereafter 28-32] WG) or late third (33-36^{+6} [thereafter 33-36] WG) trimester vaccination group according to time of vaccination in pregnancy. Women who declined to receive Tdap but were willing to participate in the study were the unvaccinated control group. Data were prospectively collected from women's medical records and participant questionnaire upon enrollment.

3.3.2 Laboratory analysis

Cord serum was separated from cord blood by centrifugation at the time of collection and stored at – 80 ^{0}C. Samples were shipped in temperature-controlled conditions to the Vaccine Evaluation Center (Vancouver, Canada) for avidity analysis.

Determination of anti- PT IgG antibody avidity

Avidity analysis of anti-PT IgG was performed as previously described using anti-PT IgG ELISA (EUROIMMUN) with ammonium thiocyanate (NH_4SCN) (SIGMA–ALDRICH, St. Louis, MO) as a bond-breaking agent[391, 404]. Briefly, 100 microliters (μL) of serum (1/101 dilution achieved by dilution 10 μL of serum with 1000 μL of sample buffer), 100 μL of standards provided by the manufacturer and 100 μL of positive and negative control sera (1/101 dilution) were added per well and incubated at 37 °C for 60 minutes. After incubation, the plates were washed three times with 200 μL of washing buffer. After washing, 100 μL of phosphate buffered saline (PBS) or different NH_4SCN concentrations (see below) were added for 20 minutes at 37 °C. One hundred μL of enzyme conjugate (peroxidase-labeled anti-human IgG) were added per well and incubated for 30 minutes at room temperature, after which the plates were washed three times with 200 μL of washing buffer. Positive reactions were developed by adding 100 μL chromogen/substrate solution for 15 minutes at room temperature. The process was terminated by adding 100 μL of 0.5 M sulfuric acid, and the plates are developed. Optical density was measured immediately at 450 nm. All standards, controls and study samples were analyzed in duplicate with the average of the two samples taken as the final value.

Determination of the optimal range of bond-breaking agent (chaotrope)

In order to characterize the spectrum of antibody avidity in a sample, I used a range of concentrations of the chaotrope. I calculated the RAI achieved at each concentration (as above). Next, I determined the lowest and the highest chaotrope concentrations of this

range which provide helpful discrimination of antibody avidity. The lowest concentration was the chaotrope concentration that achieved the highest RAIs that was still different from the RAIs achieved at the next lower concentration. Chaotrope concentrations below this lowest concentration are thus less discriminatory and were not used. The highest concentration of the chaotrope was the highest concentration which still yielded antibody levels above the lower levels of quantification (LLOQ) of the ELISA. In my initial experiments, the range of ammonium thiocyanate concentrations was 0.25 molar (M), 0.5M, 1M, 1.5M, 2M and 3M, while concentrations of 0.125M and 4M were rejected.

Sample selection

Samples not treated with the chaotrope or treated with the lowest concentration and yielded values lower than the ELISA's LLOQ were excluded from further analysis. Avidity cannot be reliably measured in sera with very low total antibody levels[404], or undetectable antibody levels following the addition of the lowest chaotrope concentration. Including such samples has the potential to introduce an error to the results, as these samples have undetectable antibody levels rather than low avidity antibodies.

3.3.3 Calculation of Relative Avidity Index (RAI), fractional and total RAI of anti-PT IgG

The RAI for every sample at each ammonium thiocyanate concentration was calculated and expressed as a percentage (Table 3.1 and Table 3.2). Samples not treated with ammonium thiocyanate or treated with the lowest ammonium thiocyanate concentration

(0.25 M) with optical density values lower than the ELISA's lower levels of quantification were excluded from further avidity analysis.

The fractional RAI of anti-PT IgG achieved at a specific ammonium thiocyanate concentration was calculated (Table 3.1 and Table 3.2). Within the range of concentrations of ammonium thiocyanate used (0.25 M–3 M), the data demonstrated high linear correlation between increasing ammonium thiocyanate concentration and decreasing RAI ($r=-0.88$, $p<0.001$). Thus, a total RAI value of anti-PT IgG that reflected the weighted contribution of the fractional RAIs of anti-PT IgGs achieved at the specific ammonium thiocyanate concentrations was calculated (Table 3.1 and Table 3.2) and expressed in Avidity Units (AU).

3.3.4 Quantification of fractional and total absolute avidity levels of anti-PT IgG

The fractional absolute avidity levels of anti-PT IgG achieved at a specific ammonium thiocyanate concentration was quantified and expressed in IU/mL (Table 3.1 and Table 3.2). The quantified fractional absolute avidity levels of anti-PT IgG at 0.25 M, 0.5 M, 1 M, 1.5 M, 2 M, and 3 M of ammonium thiocyanate were classified as low, low-medium, medium, medium-high, high and very high avidity anti-PT IgG antibodies, respectively. The levels of anti-PT IgG eluted by the lowest ammonium thiocyanate concentration (0.25 M) were classified as very low avidity anti-PT IgG antibodies. The total absolute avidity levels of anti-PT IgG reflecting the weighted contribution of the fractional absolute avidity levels of anti-PT IgG were calculated and expressed in Absolute Avidity Units (AAU)/mL (Table 3.1 and Table 3.2). Anti-PT IgG levels

measured without the addition of ammonium thiocyanate (T_0 in Table 3.1 and 3.2), were referred to as total anti-PT IgG.

Table 3. 1: Calculation of relative avidity index, fractional relative avidity index, total relative avidity index and quantification of fractional and absolute avidity levels of anti-PT IgG.

NH_4SCN Concentration (molar [M])	**3 M**	**2 M**	**1.5 M**	**1 M**	**0.5 M**	**0.25 M**	**0 M**	**NA*** **
Anti-PT IgG levels (IU/mL)	T_3	T_2	$T_{1.5}$	T_1	$T_{0.5}$	$T_{0.25}$	T_0	NA
RAI* (%)	$RAI_3 = T_3/T_o * 100$	$RAI_2 = T_2/T_o * 100$	$RAI_{1.5} = T_{1.5}/T_o * 100$	$RAI_1 = T_1/T_o * 100$	$RAI_{0.5} = T_{0.5}/T_o * 100$	$RAI_{0.25} = T_{0.25}/T_o * 100$	NA	NA
Fractional (F) RAI (%)	$F\ RAI_3 = RAI_3$	$F\ RAI_2 = RAI_2 - RAI_3$	$F\ RAI_{1.5} = RAI_{1.5} - RAI_2$	$F\ RAI_1 = RAI_1 - RAI_{1.5}$	$F\ RAI_{0.5} = RAI_{0.5} - RAI_1$	$F\ RAI_{0.25} = RAI_{0.25} - RAI_{0.5}$	NA	$F\ RAI_{<0.25} = 100\% - RAI_{0.25}$
Total RAI (AU)	$F\ RAI_3*3 + F\ RAI_2*2 + F\ RAI_{1.5}*1.5 + F\ RAI_1*1 + F\ RAI_{0.5}*0.5 + F\ RAI_{0.25}*0.25 + F\ RAI_{<0.25}*0.125$							
Fractional (F) absolute (abs) avidity levels** (IU/mL)	$F\ abs_3 = F\ RAI_3 * T_0$	$F\ abs_2 = F\ RAI_2 * T_0$	$F\ abs_{1.5} = F\ RAI_{1.5} * T_0$	$F\ abs_1 = F\ RAI_1 * T_0$	$F\ abs_{0.5} = F\ RAI_{0.5} * T_0$	$F\ abs_{0.25} = FRAI_{0.25} * T_0$	NA	$F\ abs_{<0.25} = FRAI_{0<0.25} * T_0$
Total absolute avidity levels (AAU/mL)	$F\ abs_3*3 + F\ abs_2*2 + F\ abs_{1.5}*1.5 + F\ abs_1*1 + F\ abs_{0.5}*0.5 + F\ abs_{0.25}*0.25 + F\ abs_{<0.25}*0.125$							

Abbreviations: NA: not applicable; IU:mL: international unit/ml; T: total; F: fractional; abs: absolute. * Samples treated with PBS or the lowest NH_4SCN concentration (0.25M NH_4SCN) with optic density values lower than the ELISA's lower levels of quantification (LLOQ) were excluded from further avidity analysis. Samples treated with 0.5M, 1M, 1.5M, 2M, 3M concentrations of NH_4SCN and with optic density values lower than the ELISA's LLOQ were assigned an arbitrary RAI value of 2.5%, 5%, 7.5%, 10% and 12.5%, respectively, for the respective NH_4SCN concentrations.

** Fractional absolute avidity levels of anti-PT IgG at a specific NH_4SCN concentration quantified as 0 were assigned an arbitrary value of 0.04 IU/mL

*** This column includes the Fractional (F) RAI and Fractional (F) absolute (abs) avidity levels of anti-PT IgG antibodies eluted at the lowest NH_4SCN concentration.

Table 3. 2: Example of calculation of relative avidity index, fractional relative avidity index, total relative avidity index and quantification of fractional and absolute avidity levels of anti-PT IgG.

NH_4SCN Concentration (molar [M])	**3 M**	**2 M**	**1.5 M**	**1 M**	**0.5 M**	**0.25 M**	**0 M**	**NA*** **
Anti-PT IgG levels (IU/mL)	18 IU/mL	42 IU/mL	60 IU/mL	84 IU/mL	96 IU/mL	108 IU/mL	120 IU/mL	NA
RAI* (%)	18/120 *100= 15	42/120 *100= 35	60/120* 100=50	84/120* 100=70	96/120* 100=80	108/120 *100= 90	NA	NA
Fractional (F) RAI (%)	15	35-15=20	50-35=15	70-50=20	80-70=10	90-80=10	NA	100-90=10
Total RAI (AU)	15*3+ 20*2 + 15*1.5 + 20*1 + 10*0.5 + 10*0.25 + 10*0.125= 136.25 AU							
Fractional (F) absolute (abs) avidity levels** (IU/mL)	15%* 120= 18	20%* 120= 24	15%* 120=18	20%* 120=24	10%* 120=12	10%* 120=12	NA	10%* 120=12
Total absolute avidity levels (AAU/mL)	18*3 + 24*2 + 18*1.5 + 24*1 + 12*0.5 + 12*0.25 + 12*0.125= 163.5							

* Samples treated with PBS or the lowest NH_4SCN concentration (0.25M NH_4SCN) with optic density values lower than the ELISA's lower levels of quantification (LLOQ) were excluded from further avidity analysis. Samples treated with 0.5M, 1M, 1.5M, 2M, 3M concentrations of NH_4SCN and with optic density values lower than the ELISA's LLOQ were assigned an arbitrary RAI value of 2.5%, 5%, 7.5%, 10% and 12.5%, respectively, for the respective NH_4SCN concentrations.

** Fractional absolute avidity levels of anti-PT IgG at a specific NH_4SCN concentration quantified as 0 were assigned an arbitrary value of 0.04 IU/mL

*** This column includes the Fractional (F) RAI and Fractional (F) absolute (abs) avidity levels of anti-PT IgG antibodies eluted at the lowest NH_4SCN concentration

3.4.5 Statistical Analyses

The demographic and baseline characteristics of the entire cohort have been published[406]. In this chapter, I report only data for participants included in the avidity analysis. The demographic and baseline characteristics of pregnant women vaccinated during 28–32 WG, 33–36 WG (per the original study classification)[406] and unvaccinated women and their newborns were compared using Pearson's chi-squared test for categorical variables, and one-way analysis of variance for normally distributed continuous variables. The demographic and baseline characteristics of pregnant women vaccinated during 28–32 WG and 33–36 WG and their newborns were compared using Pearson's chi-squared test for categorical variables, and independent sample t-test for normally distributed continuous variables. The natural log of total anti-PT IgG levels, fractional absolute avidity levels of anti-PT IgG and total absolute avidity levels of anti-PT IgG were used for further analysis.

Anti-PT IgG levels and the total RAI of anti-PT IgG were compared between newborns of women vaccinated with Tdap *vs.* unvaccinated women, and newborns of women vaccinated during 28–32 WG *vs.* 33–36 WG using independent sample *t*–tests. In addition, newborns were classified according to time elapsed between maternal Tdap vaccination in pregnancy and delivery initially into three groups: 1-4, 5-8 and 9-12 weeks prior to delivery, and later into two groups: 1-4 and 5-12 weeks prior to delivery. Anti-PT IgG levels and the total RAI of anti-PT IgG were compared between newborns of women vaccinated 1–4 *vs.* 5–12 weeks prior to delivery using independent sample *t*–tests. Univariate linear regression analysis was used to identify baseline characteristics

variables that could potentially impact anti-PT IgG levels and total RAI of anti-PT IgG. This was followed by multivariable linear regression models in which the response variable was anti-PT IgG levels or total RAI of anti-PT IgG levels, and all variables identified in the univariate regression model with p-value ≤ 0.25 were included. In all models, the variable of interest (Tdap vaccination status in pregnancy [Tdap vaccinated or Tdap unvaccinated] or timing of vaccination in pregnancy) was also included. Pearson correlation assessed the relationship between the timing of Tdap vaccination in pregnancy (in WG) or the time interval between vaccination and delivery (in weeks) and anti-PT IgG levels as well the total RAI of anti-PT IgG. Density estimates of total absolute avidity levels of anti-PT IgG according to vaccination status and timing of vaccination in pregnancy were performed using Gaussian kernels.

In order to study the correlation between fractional absolute avidity levels of anti-PT IgG, a correlation matrix of correlation coefficients of pairs of logged anti-PT IgG levels achieved at two different NH_4SCN concentrations among vaccinated and unvaccinated groups based on Pearson's correlation coefficient test was performed. Statistical significance of each correlation coefficient of each paired comparison was set using a Bonferroni correction.

Principal component analysis of the 7 logged fractional absolute anti-PT IgG levels was performed in order to display the potential clustering of absolute avidity of newborns born to women by their vaccination status.

Unsupervised clustering of the fractional absolute avidity levels of anti-PT IgG for all newborns were performed. The timing of Tdap administration in pregnancy and time interval between vaccination and delivery were independently displayed to enable visualization of the relationship between clinical variables and clusters of anti-PT IgG avidity profiles. R version 3.4.0 was used for all analyses.

3.4.6 Ethical aspects

The original study was approved by Monash Health Human Research Ethics Committee (HREC Ref:13426B) and all participants provided informed and signed consent [406]. The current study was approved by University of British Columbia Children's and Women Research Ethics Board (Certificate number: H17–00050).

3.4 Results

In the original study, where only samples with paired maternal–cord sera were analyzed for total anti-PT IgG levels, analysis was performed on 29 samples for unvaccinated women, 42 samples for women vaccinated during 28–32 WG and 45 for women vaccinated during 33–36 WG. In this study, a total of 125 cord serum samples were available for avidity analysis (33 for unvaccinated women, 44 vaccinated with Tdap between 28–32 WG and 48 vaccinated with Tdap between 33–36 WG) and 112 were included in the final analysis (Figure 3.1).

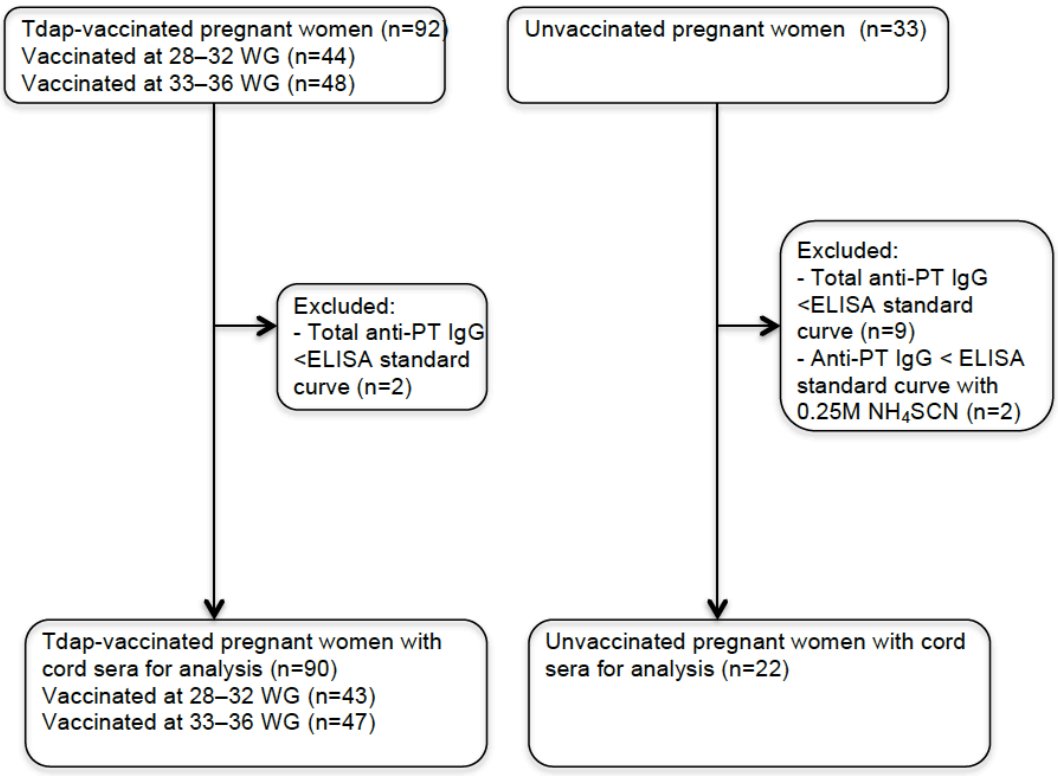


Figure 3. 1: Flow chart of study participants.

3.4.1 Correlation between the different fractional absolute avidity levels of antibodies

In order to address this, the correlation between the different fractional absolute avidity levels of antibodies was tested. Specifically, a correlation matrix of Pearson's correlation coefficients of pairs of fractional absolute avidity levels achieved at two different chaotrope concentrations was performed. Statistical significance of each correlation coefficient of each paired comparison was set using Bonferroni correction to adjust for multiple comparisons. Overall, I did not find a high correlation between pairs of fractional absolute avidity levels achieved at two different chaotrope concentrations, to further confirm the need to use a range of chaotrope concentrations and to highlight the limitations of previous studies[391,392] (Figure 3.2).

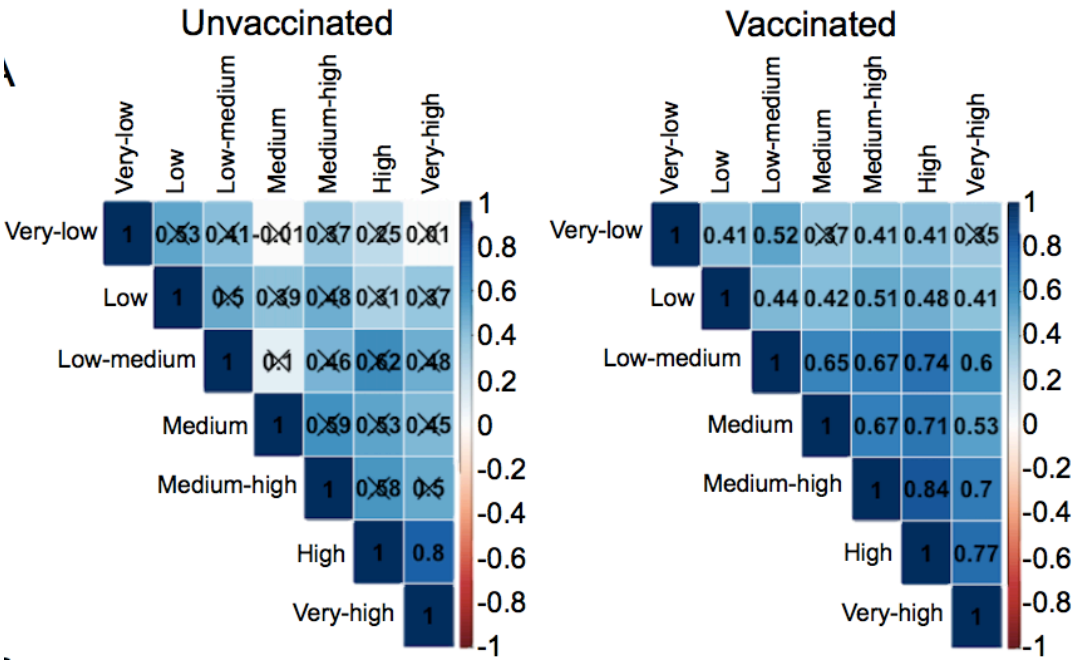


Figure 3. 2: Correlation matrix of fractional absolute avidity levels of anti-PT IgG achieved at different pairs of chaotrope concentrations based on Pearson's correlation coefficient test.

Each box represents the correlation coefficient of a pair of logged fractional absolute avidity levels of antibodies achieved at a pair of bond-breaking concentrations between all subjects by vaccination status (vaccinated [right], unvaccinated [left]). The boxes are colored according to the correlation coefficient from red (-1) to blue (+1) and the number represent the correlation coefficient rho value. Statistical significance of each correlation coefficient of each paired comparison was set using bonferroni correction. The X sign represents correlation coefficients that did not reach statistical significance, while absence of X sign represents statistical significance.

3.4.2 Correlation between the total antibody levels and the total RAI

To explore whether antibody levels influence the avidity, the correlation between the total anti-PT IgG levels and the total RAI was tested. I did not find high correlation (Pearson's r=0.38) between total anti-PT IgG levels and total RAI, suggesting that avidity is not determined by total antibody levels (Figure 3.3). This confirms that the avidity is

not mainly driven by total antibody levels.

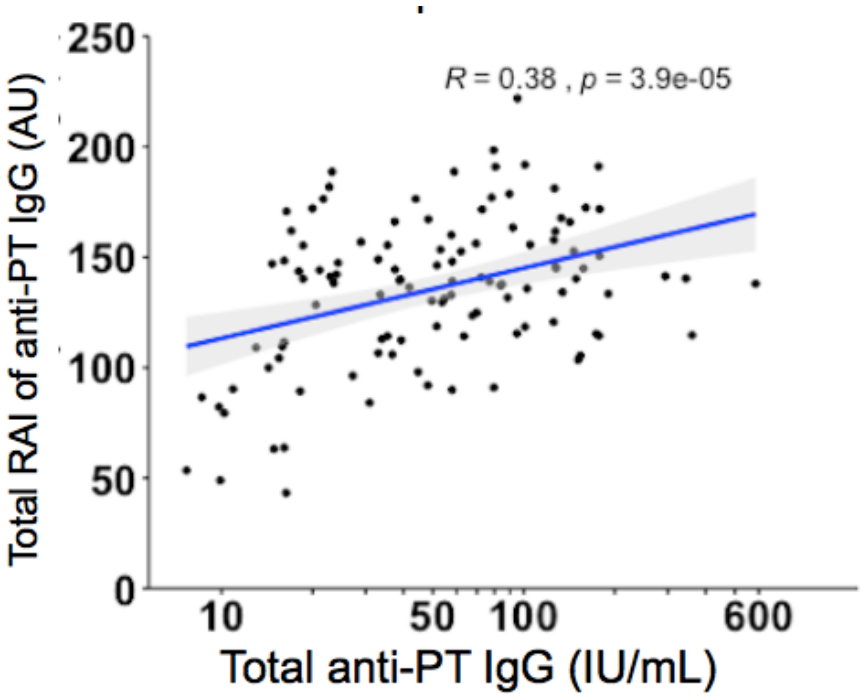


Figure 3. 3: Scatter plot of total anti-PT IgG levels and total relative avidity index of anti-PT IgG.

3.4.3 Anti-PT IgG avidity by vaccination status in pregnancy

Newborns of women vaccinated with Tdap in pregnancy had higher total anti-PT IgG levels, total RAI of anti-PT IgG, total absolute avidity levels of anti-PT IgG, and fractional absolute levels of low, low-medium, medium, medium-high, high, and very avidity anti-PT IgG compared with newborns of unvaccinated women, after adjustment for multiple variables (Table 3.3). To investigate the potential effect of pre-pregnancy pertussis vaccination status on anti-PT IgG levels at delivery, I compared total anti-PT IgG levels in women vaccinated against pertussis during 5 years before pregnancy to levels of anti-PT IgG in women not vaccinated against pertussis in the past, vaccinated

more than 5 years before pregnancy or their vaccination status was not determined. The GMC of total anti-PT IgG was 25.4 IU/mL (95% CI: 17.3-37.2, n=15) *vs.* 18.6 IU/mL (95% CI: 13.4-25.9, n=85), in the former *vs.* the latter group, respectively, P=0.243.

Table 3. 3: Cord anti-PT IgG levels with different avidities of women vaccinated with Tdap during pregnancy and unvaccinated women.

	Vaccinated (n=90)	**Unvaccinated (n=22)**	**P**	**Adjusted P***
Total anti-PT IgG levels (IU/mL), GMC (95% CI)	62.5 (52.3-74.8)	21.4 (16.6-27.6)	<0.001	<0.001[1]
Total RAI of anti-PT IgG (AU), mean (SD)	140.1 (31.9)	117.8 (35.7)	0.012	0.024[2]
Total absolute avidity levels of anti-PT IgG (AAU/mL), GMC (95% CI)	84.9 (69.2-104.1)	24.0 (17.0-33.8)	<0.001	<0.001[1]
Fractional absolute anti-PT IgG levels (IU/mL) with different avidities, GMC (95% CI)				
Very low	1.0 (0.6-1.7)	1.4 (0.7-2.5)	0.495	0.629[3]
Low	2.0 (1.3-3.1)	1.3 (0.8-2.2)	0.209	0.672[4]
Low–medium	8.7 (6.8-11.1)	3.0 (1.8-5.1)	0.001	0.005[1]
Medium	8.7 (7.2-10.6)	3.4 (2.2-5.4)	<0.001	<0.001[4]
Medium–high	9.1 (7.2-11.7)	2.8 (1.6-4.8)	<0.001	<0.001[5]
High	7.5 (5.6-9.9)	1.6 (0.9-2.9)	<0.001	<0.001[6]
Very high	9.2 (6.8-12.3)	1.4 (0.8-2.6)	<0.001	<0.001[7]

Abbreviations: SD: standard deviation. * For each specific anti-PT IgG levels, Univariate linear regression analysis was used to identify baseline characteristics variables that could potentially impact the anti-PT IgG levels. This was followed by multi-variate regression model, for each anti-PT IgG levels, that adjusted for potential confounders detected in univariate regression analysis as well vaccination status in pregnancy. The adjusted P value is presented.[1] Adjusted for gestational age at birth (weeks), parity (yes, no) and

delivery mode (Caesarian Section or vaginal delivery); [2] Adjusted for gestation at delivery (≤37 weeks, >37 weeks) and delivery mode (Caesarian Section or vaginal delivery); [3] Adjusted for ethnicity and delivery mode (Caesarian Section or vaginal delivery); [4] Adjusted for gestational age at birth (weeks) and gestation at delivery (≤37 weeks, >37 weeks); [5] Adjusted for gestational age at birth (weeks), gestation at delivery (≤37 weeks, >37 weeks), parity (yes, no) and ethnicity (Australian born, not-Australian born); [6] Adjusted for gestational age at birth (weeks), parity (yes, no), ethnicity and delivery mode (Caesarian Section or vaginal delivery); [7] Adjusted for maternal age (years), gestational age at birth (weeks), gestation at delivery (≤37 weeks, >37 weeks), parity (yes, no), ethnicity (Australian born, not-Australian born), and delivery mode (Caesarian Section or vaginal delivery).

3.4.4 Distribution of avidity by vaccination status

In order to further explore whether vaccinated and unvaccinated subjects differed by the quality and quantity of antibodies combined, I performed analysis of density estimates of total absolute avidity levels. I found that vaccination resulted in a shift of the distribution of total absolute avidity levels, demonstrating that there are more vaccinated subjects with higher avidity than unvaccinated subjects (Figure 3.4).

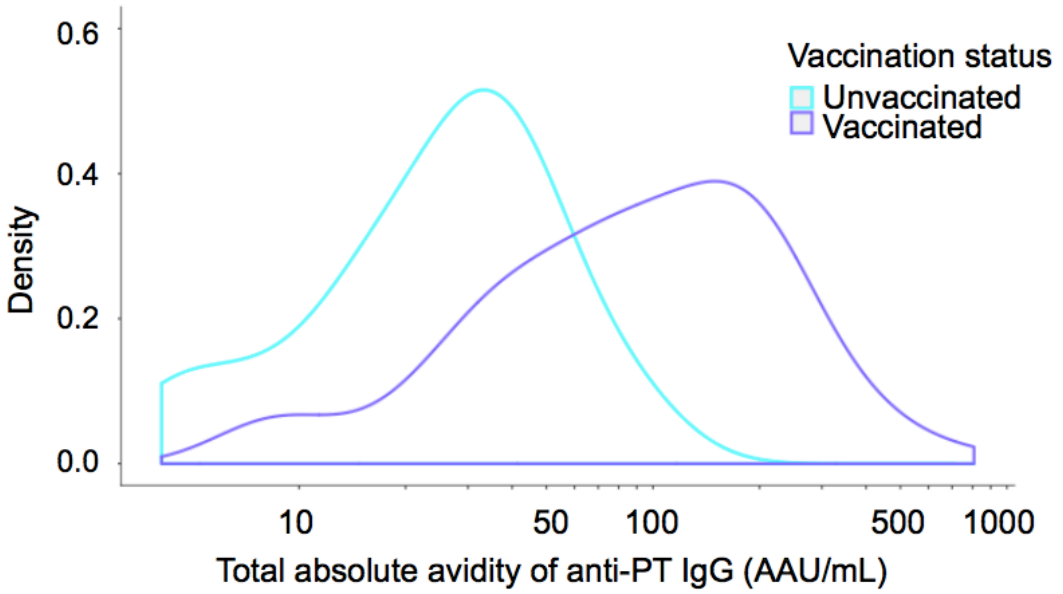


Figure 3. 4: Distribution of total absolute avidity of anti-PT IgG by vaccination status.

Kernel Density plot shows the total absolute avidity of antibodies in vaccinated versus unvaccinated subjects.

3.4.5 Clustering and separation of vaccinated and unvaccinated groups by avidity

In order to gain further enhanced resolution on the results and to investigate whether it is possible to separate vaccinated and unvaccinated subjects based on their avidity, a dimensional reduction method (principal component analysis) was applied. I found that the vaccinated and unvaccinated groups could be separated based on their fractional absolute antibody levels (Figure 3.5).

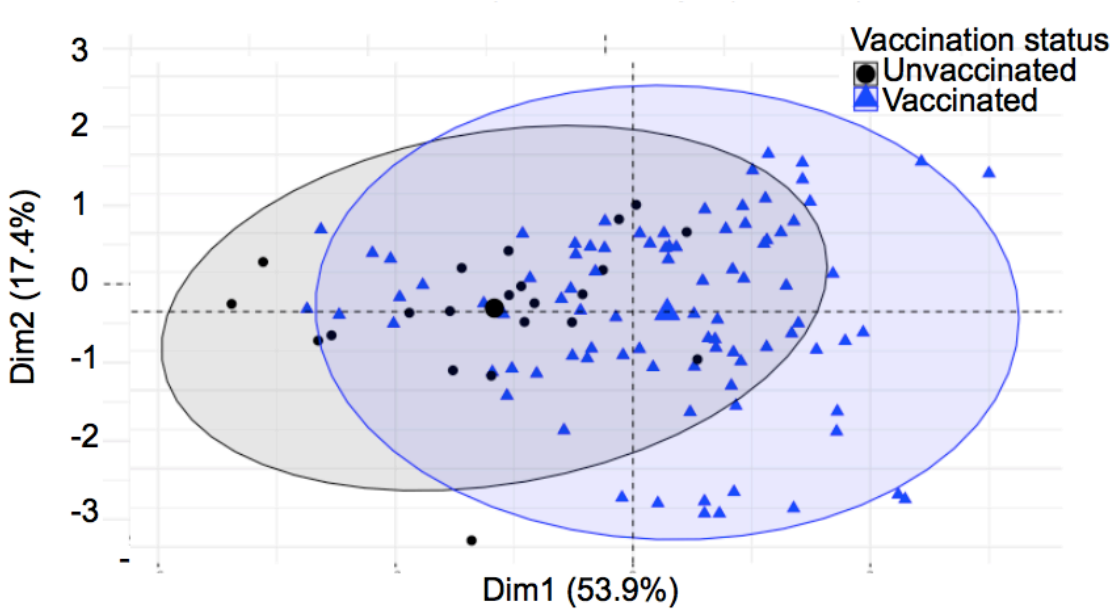


Figure 3. 5: Principal component analysis of the 7 fractional absolute anti-PT IgG levels by vaccination status.

This principal component analysis shows each vaccination status as indicated by distinct colors. Each colored circle/triangle in space represents and individual avidity profile and similar avidity profiles are grouped more closely together in two-dimensional space. The principal components are ordered according to the amount of variance in the data they explain. The plot is based on principal component 1 and 2, which explains 53.9% and 17.4% of the total variance of the data, respectively.

3.4.6 Results by timing of vaccination during pregnancy

3.4.6.1 Baseline characteristics

There were no significant differences in the baseline characteristics of pregnant women vaccinated during 28–32 WG compared with vaccination during 33–36 WG (Table 3.4).

Table 3. 4: Demographic and baseline characteristics of study participants.

Characteristic	Tdap-vaccinated (n=90)	Unvaccinated (n=22)	P[1]	Vaccinated during 28–32 WG (n=43)	Vaccinated during 33–36 WG (n=47)	P[2]	P[3]
Ethnicity							
Australian born, n (%)	44 (48.9)	11 (50)	1	23 (53.5)	21 (44.7)	0.533	0.703
Maternal age, years							
Mean (SD)	30.0 (4.4)	32.1 (4.8)	0.072	30.3 (4.2)	29.8 (4.6)	0.531	0.126
Parity							
Nulliparous, n(%)	46 (51.1)	5 (22.7)	0.031	21 (48.8)	25 (53.2)	0.840	0.052
Mode of delivery, n (%)							
Elective CS	6 (6.7)	15 (68.2)		3 (6.9)	3 (6.4)		<0.001
Emergency CS	15 (16. 7)	0 (0)		8 (18.6)	7 (14.9)		
NVD	50 (55.5)	5 (22.7)		22 (51.1)	28 (59.6)		
Instrumental	19 (21.1)	2 (9.1)	<0.001	10 (23.2)	9 (19.1)	0.882	
Gestational age at delivery, weeks							
Mean (SD)	39.3 (1.3)	38.6 (1.1)	0.015	39.2 (1.4)	39.4 (1.2)	0.523	0.054
Gestational age at delivery, n (%)							
<37 weeks	1[4] (1.1)	1[4] (4.5)		1 (2.3)	0 (0)		0.529
37–42 weeks	86 (95.6)	21 (95.5)		40 (93.0)	46 (97.9)		
>42 weeks	3 (3.3)	0 (0)	0.386	2 (4.6)	1 (2.1)	0.454	
Pertussis vaccination history before pregnancy							
No vaccination	8 (8. 9)	2 (9. 1)		5 (11.6)	3 (6.4)		0.001
Not sure	49 (54.4)	5 (22.7)		23 (53.5)	26 (55.3)		
<5 years before pregnancy	7 (7. 8)	10 (45.4)		2 (4.6)	5 (10.6)		
>5 years before pregnancy	26 (28. 9)	5 (22.7)	<0.001	13 (30.2)	13 (27.6)	0.616	
Gestational age at vaccination (weeks gestation)							
Mean (SD)	32.6 (2.7)	NA		30.2 (1.4)	34.9 (1.1)	<0.001	NA
Time between vaccination and delivery							
Time interval in weeks (SD)	6.7 (2.9)	NA		9.0 (2.0)	4.5 (1.5)	< 0.001	NA

Abbreviations: CS: caesarean section; NVD: normal vaginal delivery; [1] P-value for comparison between women vaccinated with Tdap vs. unvaccinated; [2] P-value for comparison between women vaccinated with Tdap during 28-32 WG vs. 33-36 WG; [3] P-value for comparison between women vaccinated with Tdap during 28-32 WG vs 33-36 WG vs unvaccinated; [4] Born at 36 WG

3.4.6.2 Anti-PT IgG avidity by timing of vaccination in pregnancy

There was a significant negative association between later timing of Tdap administration and total anti-PT IgG levels and fractional absolute levels of low–medium, medium, medium–high and high avidity anti-PT IgG (Figure 3.6 and Figure 3.7).

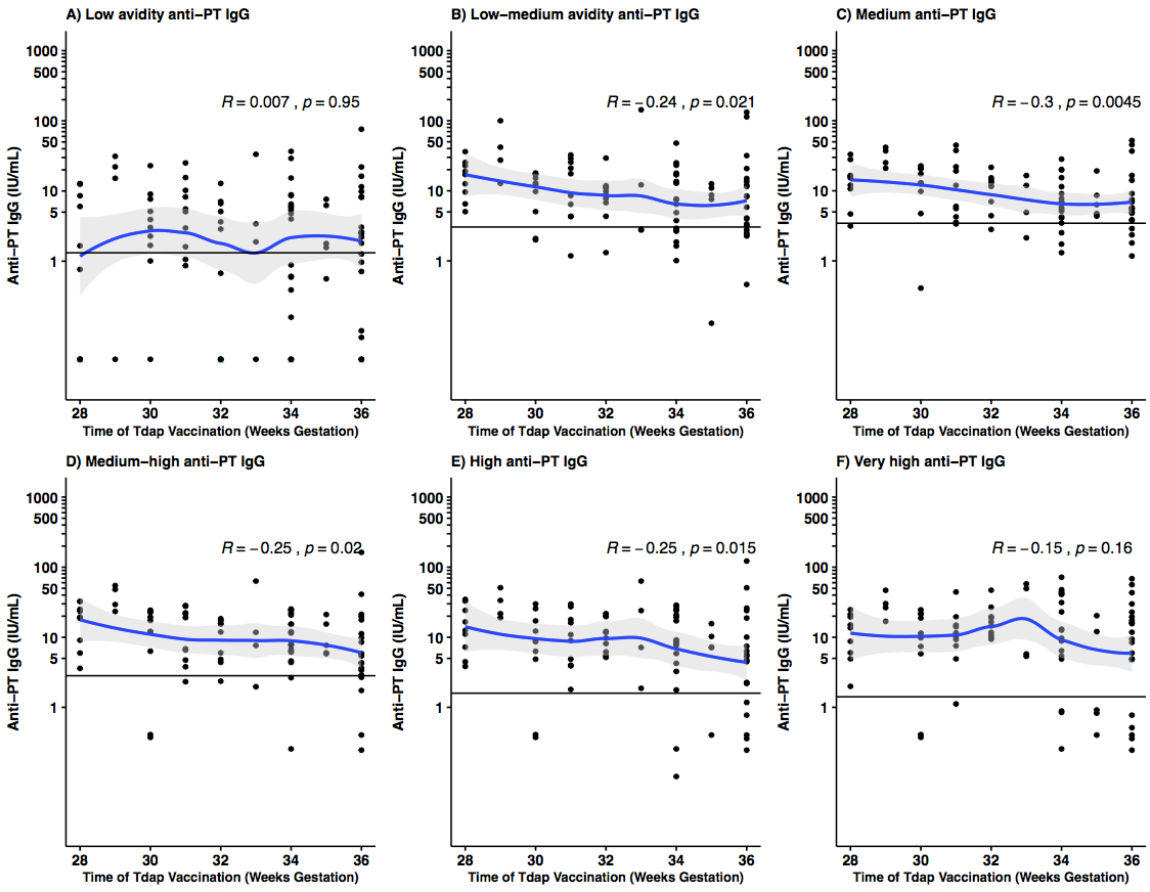


Figure 3. 6: Fractional absolute anti-PT IgG levels by time of vaccination against pertussis in pregnancy achieved at the different ammonium thiocyanate concentrations.

The quantified fractional absolute avidity levels of anti-PT IgG at 0.25 M, 0.5 M, 1 M, 1.5 M, 2 M, and 3 M of ammonium thiocyanate are classified as low, low–medium, medium, medium–high, high and very high avidity anti-PT IgG antibodies, respectively. The horizontal line denotes the cord mean levels in newborns born to unvaccinated women.

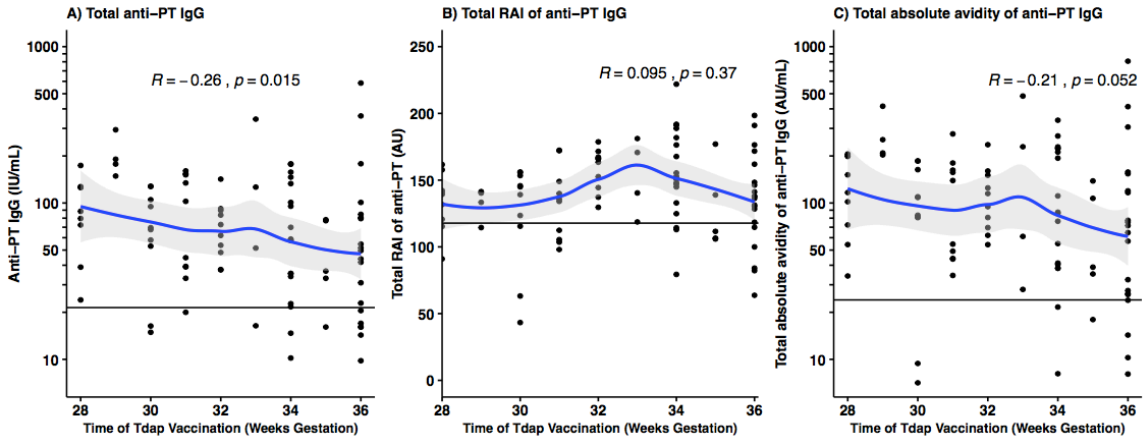


Figure 3. 7: Cord (A) total anti-PT IgG, (B) total relative avidity index, (C) total absolute avidity levels anti-PT IgG levels by time of vaccination against pertussis in pregnancy.

The horizontal line denotes the cord mean levels in newborns born to unvaccinated women. This figure shows that the earlier Tdap is given in pregnancy the higher total anti–PT levels are achieved at birth.

Newborns of women immunized with Tdap during 28–32 WG had higher total anti-PT IgG levels and fractional absolute levels of medium and high avidity anti-PT IgG compared with newborns of women immunized during 33–36 WG (Table 3.5). In multivariate analysis, early vaccination remained significantly associated with higher total anti-PT IgG levels and fractional absolute levels of medium and high avidity anti-PT IgG after adjustment for gestational age at birth, ethnicity and delivery mode (Table 3.5).

Table 3. 5: Cord anti-PT IgG levels with different avidities of women vaccinated with Tdap during early and late third trimester.

	Vaccinated during 28-32 WG (n=43)	**Vaccinated during 33-36 WG (n=47)**	**P**	**Adjusted P***
Total anti-PT IgG levels (IU/mL), GMC (95% CI)	75.3 (61.2–92.9)	52.66 (39.9–69.6)	0.046	0.038 [1]
Total RAI of anti-PT IgG (AU), mean (SD)	136.5 (28.4)	143.3 (34.7)	0.313	0.317**
Total absolute avidity levels of anti-PT IgG (AAU/mL), GMC (95% CI)	100.0 (78.3–127.8)	73.1 (53.3–100.3)	0.128	0.119**
Fractional absolute anti-PT IgG levels (IU/mL) with different avidities, GMC (95% CI)				
Very low	1.2(0.5–2.6)	0.9 (0.5–1.7)	0.667	0.675 [2]
Low	2.0 (1.1–3.9)	2.0 (1.1–3.6)	0.986	0.759 [3]
Low–medium	11.2 (8.5–14.7)	6.9 (4.7–10.2)	0.051	0.054**
Medium	11.6 (8.8–15.2)	6.7 (5.2–8.6)	0.005	0.007 [4]
Medium–high	11.4 (8.2–15.8)	7.5 (5.2–10.7)	0.088	0.090**
High	10.1 (7.4–13.8)	5.7 (3.6–8.9)	0.042	0.035[1]
Very high	11.2 (8.1–15.3)	7.7 (4.7–12.5)	0.210	0.268 [5]

* For each specific anti-PT IgG levels, Univariate linear regression analysis was used to identify baseline characteristics variables that could potentially impact the specific anti-PT IgG levels. This was followed by multi-variate regression model, for each anti-PT IgG levels, that adjusted for potential confounders detected in univariate regression analysis as well timing of vaccination in pregnancy. The adjusted P value is presented.

** No other variable (other than timing of vaccination) with $p \leq 0.25$; [1] Adjusted for gestational age at birth (weeks); [2] Adjusted for ethnicity (Australian born, not-Australian born) and delivery mode (Caesarian Section or vaginal delivery); [3] Adjusted for gestational age at birth (weeks), gestation at delivery (≤37 weeks, >37 weeks); [4] Adjusted for gestation at delivery (≤37 weeks, >37 weeks); [5] Adjusted for ethnicity (Australian born, not-Australian born).

3.4.6.3 Anti-PT IgG avidity by interval between vaccination to delivery

There was a significant positive association between increasing time between Tdap administration during the third trimester and delivery and total anti-PT IgG levels, total absolute avidity levels of anti-PT IgG and fractional absolute levels of low–medium, medium, medium–high and high avidity anti-PT IgG (Figure 3.8 and Figure 3.9).

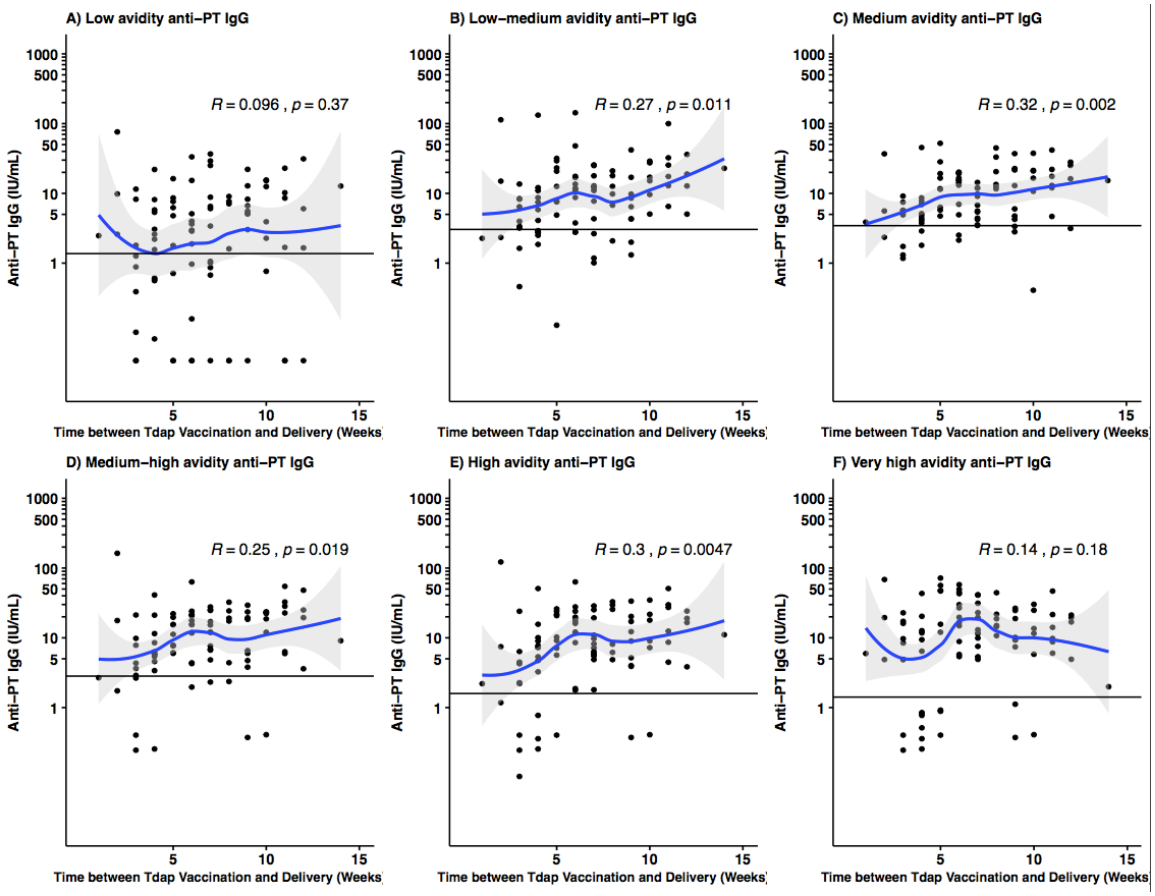


Figure 3. 8: Fractional absolute anti-PT IgG levels by time elapsed from vaccination against pertussis in pregnancy to delivery achieved at the different ammonium thiocyanate concentrations.

The quantified fractional absolute avidity levels of anti-PT IgG at 0.25 molar (M,) 0.5 M, 1 M, 1.5 M, 2M, and 3 M of ammonium thiocyanate are classified as low, low-medium, medium, medium-high, high and very high avidity anti-PT IgG antibodies, respectively. The horizontal line denotes the cord mean levels in newborns born to unvaccinated women. This figure shows that the longer the interval between Tdap administration

during the third trimester and delivery, the higher the fractional absolute levels of low–medium, medium, medium-high and high avidity anti-PT IgG achieved at birth.

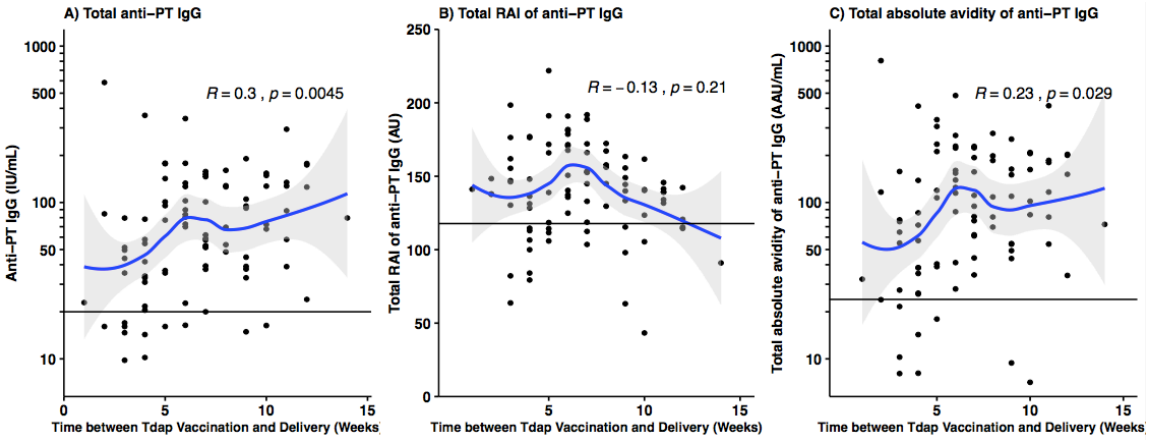


Figure 3. 9: Cord (A) Total anti-PT IgG, (B) total relative avidity index, (C) total absolute avidity levels anti-PT IgG levels by time elapsed from vaccination against pertussis in pregnancy to delivery.

The horizontal line denotes the cord mean levels in newborns born to unvaccinated women. This figure shows that the longer the interval between Tdap administration during the third trimester and delivery, the higher the total anti–PT IgG levels and total absolute avidity levels of anti–PT IgG achieved at birth.

No significant differences were observed in anti-PT IgG of newborns born to women vaccinated 5–8 vs. 9–12 weeks prior to delivery, thus the two groups were combined (Table 3.6).

Table 3. 6: Cord anti-PT IgG levels with different avidities of women vaccinated at 1-4 vs. 5-8 vs. 9-12 weeks prior to delivery.

Time interval between vaccination and delivery	Vaccinated 1-4 weeks before delivery* (n=25)	Vaccinated 5-8 weeks before delivery (n=39)	Vaccinated 9-12 weeks before delivery (n=25)	P1	P2	P3
Total anti-PT IgG levels (IU/mL), GMC (95% CI)	37.2 (25.3–54.8)	76.1 (60.9-90.1)	76.5 (55.9-104.4)	0.003	0.006	0.979
Fractional absolute anti-PT IgG levels (IU/mL) with different avidities, GMC (95% CI)						
Very low	1.5 (0.7–3.3)	0.7 (0.3-1.7)	1.0 (0.4-3.0)	0.234	0.593	0.641
Low	1.6 (0.8–3.4)	1.8 (0.9-3.5)	2.9 (1.3-6.6)	0.825	0.305	0.385
Low–medium	5.6 (3.5–9.0)	9.2 (6.3-13.4)	12.0 (8.1-17.7)	0.118	0.019	0.337
Medium	5.0 (3.6–7.0)	10.5 (8.2-13.2)	11.1 (7.3-16.9)	0.001	0.006	0.806
Medium–high	5.1 (2.9–9.1)	11.5 (9.0-14.7)	11.4 (6.9-18.8)	0.015	0.044	0.966
High	3.3 (1.7–6.4)	10.5 (7.7-14.3)	9.9 (6.1-16.0)	0.004	0.011	0.841
Very high	4.3 (2.2–8.5)	15.1 (10.5-21.9)	9.4 (5.8-15.2)	0.002	0.070	0.129

* One newborn born to woman vaccinated 1 week before delivery, 3 newborns born to women vaccinated 2 weeks before delivery, 9 newborns born to women vaccinated 3 weeks before delivery and 12 newborns born to women vaccinated 4 weeks before delivery.
P1 comparison between women vaccinated 1-4 vs. 5-8 weeks prior to delivery
P2 comparison between women vaccinated 1-4 vs. 9-12 weeks prior to delivery
P3 comparison between women vaccinated 5-8 vs. 9-12 weeks prior to delivery

Newborns of women vaccinated with Tdap 5–12 weeks prior to delivery had higher total anti-PT IgG levels, total absolute avidity levels of anti-PT IgG and fractional absolute levels of low–medium, medium, medium–high and high avidity anti-PT IgG compared

with newborns of women vaccinated four weeks or less prior to delivery (Table 3.7). In multivariate analysis, vaccination with Tdap 5–12 weeks prior to delivery remained significantly associated with higher total anti-PT IgG levels, total absolute avidity levels of anti–PT IgG and fractional absolute anti-PT IgG levels of low–medium, medium, medium–high and high avidity anti-PT IgG compared to newborns of women vaccinated four weeks or less prior to delivery (Table 3.7).

Table 3. 7: Cord anti-PT IgG levels with different avidities of women vaccinated at different time intervals prior to delivery.

Time interval between vaccination and delivery	**Vaccinated 1-4 weeks prior to delivery* (n=25)**	**Vaccinated 5-12 weeks prior to delivery (n=64)**	**P**	**Adjusted P****
Total anti-PT IgG levels (IU/mL), GMC (95% CI)	37.2 (25.3–54.8)	76.2 (63.6–91.3)	0.002	<0.001[1]
Total RAI of anti-PT IgG (AU), mean (SD)	132.9 (33.8)	143.6 (30.5)	0.174	0.151***
Total absolute avidity levels of anti-PT IgG (AAU/mL), GMC (95% CI)	47.8 (30.9–73.9)	105.9 (86.3–130.0)	0.002	<0.001***
Fractional absolute anti-PT IgG levels (IU/mL) with different avidities, GMC (95% CI)				
Very low	1.5 (0.7–3.3)	0.9 (0.5–1.6)	0.282	0.378[2]
Low	1.6 (0.8–3.4)	2.2 (1.3–3.6)	0.522	0.950[3]
Low–medium	5.6 (3.5–9.0)	10.2 (7.7–13.5)	0.039	0.030***
Medium	5.0 (3.6–7.0)	10.7 (8.6–13.3)	<0.001	<0.001[1]
Medium–high	5.1 (2.9–9.1)	11.5 (8.9–14.6)	0.016	0.005***
High	3.3 (1.7–6.4)	10.3(7.9–13.4)	0.004	<0.001[1]
Very high	4.3 (2.2–8.5)	12.6 (9.4–16.9)	0.007	0.002[4]

* One newborn born to woman vaccinated 1 week before delivery, 3 newborns born to women vaccinated 2 weeks before delivery, 9 newborns born to women vaccinated 3 weeks before delivery and 12 newborns born to women vaccinated 4 weeks before delivery; ** For each specific anti-PT IgG levels, Univariate linear regression analysis was used to identify baseline characteristics variables that could potentially impact the specific anti-PT IgG levels. This was followed by multi-variate regression model, for each anti-PT IgG levels, that adjusted for potential confounders detected in univariate regression analysis as well timing between vaccination in pregnancy and delivery. The adjusted P value is presented; *** No other variable (other than timing of vaccination) with $p \leq 0.25$; [1] Adjusted for gestational age at birth (weeks); [2] Adjusted for ethnicity and delivery mode; [3] Adjusted for maternal age (years); [4] Adjusted for ethnicity

3.4.6.5 Distribution of anti-PT IgG avidity by timing of vaccination

Vaccination with Tdap during 28–32 WG resulted in a shift in the overall distribution of total absolute avidity levels of anti-PT IgG, with higher levels total absolute avidity levels of anti-PT IgG in newborns of women vaccinated during 28–32 WG compared with newborns born to women vaccinated during 33–36 WG. Vaccination with Tdap 5–12 weeks prior to delivery resulted in a shift in the overall distribution of total absolute avidity levels of anti-PT IgG, with higher levels in newborns of women vaccinated 5–12 weeks pre delivery compared with vaccination within 4 weeks pre delivery (Figure 3.10).

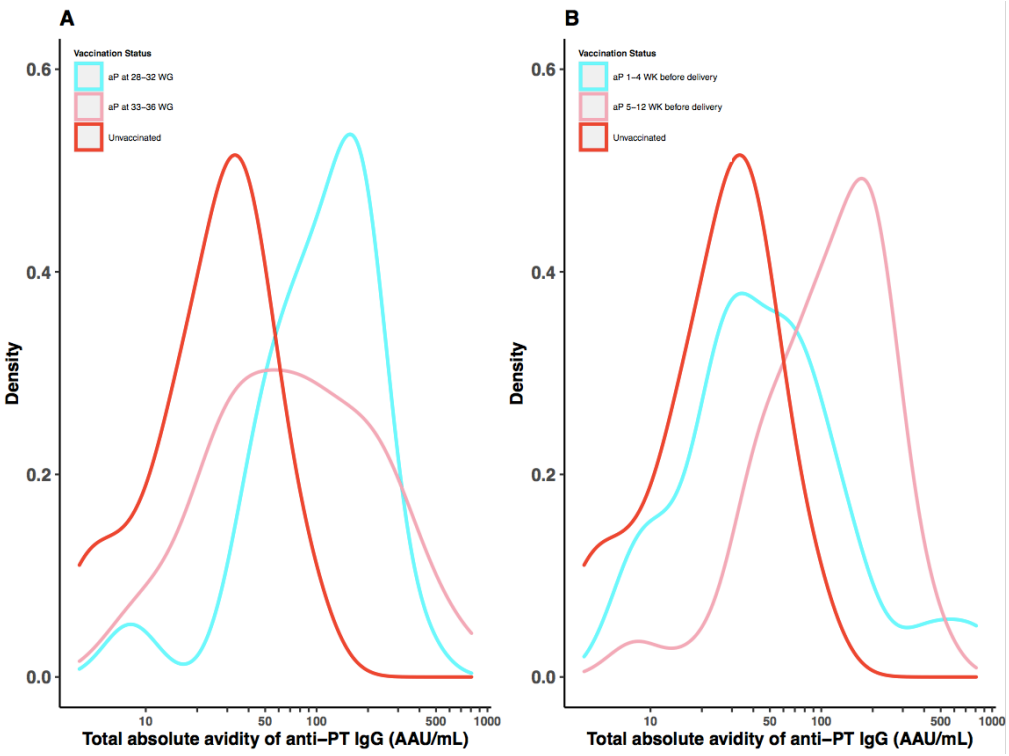


Figure 3. 10: Distribution of total absolute avidity of anti–PT IgG by timing of vaccination in pregnancy (A) and time elapsed between vaccination and delivery (B).

Kernel Density plot shows the total absolute avidity of anti-PT IgG in cord sera of newborns of women vaccinated against pertussis in pregnancy at different times. The density curves were obtained using a Gaussian kernel. Abbreviations: WK: weeks.

3.4.6.6 Clustering of newborns by anti-PT IgG avidity

Among newborns of women vaccinated during 28–32 WG, 36/43 (83.7%) had an avidity profile consisting of high levels of high fractional absolute anti-PT IgG levels. Among newborns of women vaccinated more than 4 weeks prior to delivery, 52/64 (81.3%) had an avidity profile consisting of high fractional absolute anti–PT IgG levels (Figure 3.11).

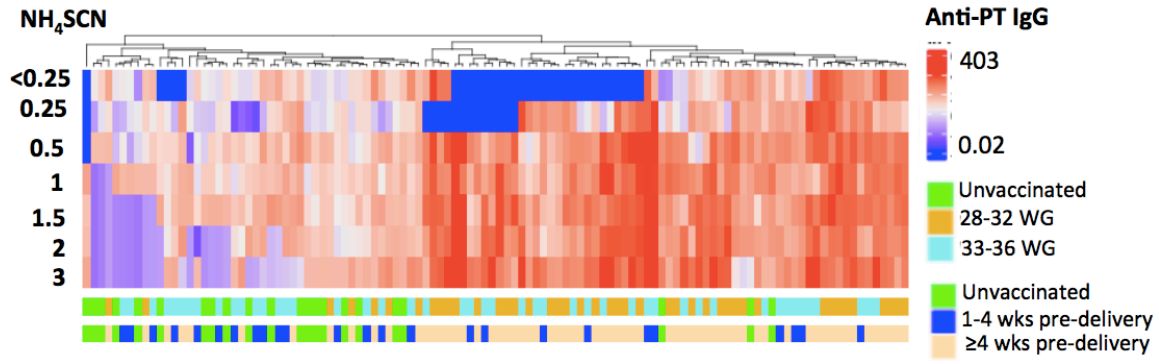


Figure 3. 11: Heat-map analysis based on hierarchical unsupervised clustering.

Fractional absolute levels of anti-PT IgG with different avidities for 112 cord samples are illustrated. In the heat-map, natural log fractional absolute anti-PT IgG levels are shown by column. The natural log fractional absolute anti-PT IgG levels were color-coded as indicated by the scale in the right, in which levels range from blue to red indicating high (red) and low (blue) levels. Timing of tetanus diphtheria and acellular pertussis (Tdap) administration is displayed by the different rows. This figure shows that most newborns of women vaccinated during 28–32 WG or more than 4 weeks prior to delivery had an avidity profile consisting of high levels of high fractional absolute anti-PT IgG levels.

3.5 Discussion:

In this chapter, I described a novel experimental and analytical approach, which enabled comprehensive characterization of the full avidity profile of anti-pertussis antibodies induced by vaccination in pregnancy. This analytical approach can be adopted in studies assessing the avidity of antibodies after vaccination or infection with a range of vaccines and pathogens and settings, beyond pertussis immunization in pregnancy. I found that newborns of women vaccinated with Tdap in pregnancy had higher total absolute avidity levels of anti-PT IgG, and higher levels of medium to very high avidity anti-PT IgG compared with newborns of unvaccinated women. Furthermore, I found that newborns born to women vaccinated with Tdap during 28–32 WG had higher levels of medium and high avidity anti-PT IgG antibodies compared with newborns born to women vaccinated during 33–36 WG. In addition, newborns of women vaccinated 5–12 weeks prior to delivery achieved higher total absolute avidity levels of anti-PT IgG antibodies, and higher levels of medium to very high avidity anti-PT IgG antibodies compared with vaccination within 4 weeks prior to delivery. This is the first study that characterizes the full avidity profile of anti-PT IgG elicited by pertussis vaccination and highlights important changes in antibody avidity related to timing of vaccination in pregnancy, supporting vaccination in early *vs.* late third trimester of pregnancy.

Data on the effect of timing of pertussis vaccination in pregnancy on the avidity of anti-pertussis antibodies are scarce. In a small study, Tdap vaccination in pregnancy between 27–31 WG resulted in higher RAI of cord anti-PT IgG compared with vaccination beyond 31 WG[391]. Conversely, in a small cohort of Belgium and Vietnamese women,

there was no significant correlation between cord anti-PT IgG RAI and gestational age at vaccination (categorized as <27, 27-30 and 31–36 WG)[392]. These two studies used different, fixed, ammonium thiocyanate concentrations, with the former using 0.25 M and the latter using 1.5 M. In these studies, avidity measurement was based on comparison of antibody levels with *vs.* without addition of a single, fixed concentration of a bond-breaking agent that disrupts binding between antibodies and the target antigen. This arbitrarily separated antibodies into 'low' or 'high' avidity. Both studies therefore suffer the limitation of reporting results as a single relative measure and thus are only able to provide a limited perspective on antibody avidity. The approach taken in this chapter with a range of ammonium thiocyanate concentrations enables complete profiling of avidity of antibodies generated following vaccination. The data presented in this chapter of pregnant women using in depth profiling of antibody avidity confirms that concentration as well as avidity increase with increasing time elapsed between vaccination and delivery.

Antigen–antibody bond is a result of reversible non-covalent intermolecular forces. Four types of non-covalent forces that can be identified in the antigen–antibody bond exist. Firstly, *hydrogen bonding, which* is established when the positive charge surrounding a hydrogen atom belonging to a residue in one molecule shares the negative charge of a chemical group in a residue in another molecule. There is prominent role of bound water molecules in forming hydrogen bond networks between the antigen and the antibody. Secondly*, Van der Waals forces, which* result when polarities oscillate in the outer electron clouds of two neighboring atoms, creates either attractive or repulsive

forces between the two atoms. The high frequency of aromatic amino acids in the pockets of antibody proteins increases the charge in this region, promoting both hydrogen and Van der Waals bonding. Thirdly*, hydrophobic bonds, which* are formed in aqueous solution when polar water molecules force hydrophobic, non-polar chemical groups (e.g. amino acids Leucin, isoleucine or valine) together in an effort to generate the minimum non-polar surface area possible. The larger the hydrophobic regions involved, the stronger the hydrophobic association between them. Finally, *electrostatic* or *ionic bonds,* which are the result of attraction between charged residues with opposite polarities[407]. Different factors can affect the relative contribution of each force to the overall antigen-antibody binding complex, namely, the identity and location of the amino acids or other chemical groups in both the antibody and antigen molecules. The more closely the relevant chemical groups can approach one another, the more efficient is the antigen-antibody binding. Similarly, the more complementary the shapes of the antigenic epitope and the antigen-binding site on the antibody, the more contact sites will simultaneously be brought into close proximity, increasing the number of non-covalent bonds of all types and resulting in a stronger overall binding[408, 409].

Different molecular mechanism of inhibition of binding of antibody to an antigen by chaotropic agents has been proposed. Chaotropic agents in general can disturb hydrophobic interactions, hydrogen bonding, and Van der Waals forces between antigen and antibodies. Thus, variation in chaotrope resistance according to the relative contributions to the antigen-antibody binding of van der Waals or hydrophobic versus polar interactions is expected[410]. NH_4SCN is a stronger ionic chaotrope, and as such can

also dissociate electrostatic interactions[411]. NH_4SCN can also interfere with conformational changes that occur as a consequence of antigen-antibody interactions[412]. Chaotropic agents has also been shown to disrupt the ordered shell of water that juxtaposes non-polar patches on the protein surfaces, so the tendency of water molecules to form shells around macromolecules is diminished, thus leading to reduction of protein-protein interactions[410]. Ionic chaotropes, such as NH_4SCN, also interact directly with the protein backbone resulting in a shift in the equilibrium towards the unfolded state[413]. This direct interaction between the chaotropic agent and proteins partly stem from ionic interactions with the peptidic backbone and the side chains, the latter in particular making the susceptibility of each antibody-antigen complex unique, like the amino-acid composition of the parts contributing to the antigen and the antibody[410].

Different factors have the potential to affect the determination of the avidity of antibodies as measured by ELISA-based elution assays, raising concerns regarding the standardization of this laboratory technique for the specific antigen-antibody complex in question. Elution of antigen–antibody complexes can be performed using different chaotropic agents (e.g. urea, NH_4SCN, diethylamine). Different chaotropic anions have differential ability to alter the hydrogen bonding network of water, leading to changes in protein stability[414] and protein–protein interactions[415, 416]. Thiocyanate is the anionic chaotropic agent with the highest ability to decrease protein stability, to increase protein denaturation and solubility of proteins, followed by ClO4, I, NO3, Br, Cl, respectively, in decreasing order[417]. Incubation with different chaotropeic agents has been performed for different lengths of incubation[402, 418-421], which also has the potential to affect the

resultant avidity of specific antibodies and thus the standardization of the technique. Pertinent to the avidity of anti-PT IgG using a commercial assay (EUROIMMUN), it has been shown that NH_4SCN dissociates PT- anti-PT IgG complexes[404]. Specifically, accurate measurement of anti-PT IgG avidity was obtained using NH_4SCN in concentrations lower than 3.0 M for 20 min time of incubation at 37 °C. Thus, these optimized laboratory conditions were used in my study.

The difference in avidity development after antigen exposure is the result of affinity maturation and increased production of antigen-specific antibody-producing plasma cells during maturation of immune response in the germinal center of lymphoid follicles[422,423-426]. Maturation of plasma cells to produce high-affinity antibodies, after an exposure to an antigen, is due to somatic hypermutation in the antibody variable region genes that encode the regions of antibodies that form the interface with the antigens[422]. One study showed that affinity maturation of antibodies occurs through simple changes in the complementarity-determining regions of the immunoglobulin heavy-chain variable region gens region[427]. However, other study showed that in order to acquire its full affinity activity, influenza-specific antibody needed to accumulate a minimum of seven specific mutations in two regions of its immunoglobulin heavy-chain variable region genes[424]. Somatic hypermutation is followed by selection of B cells based on the affinity of their B cell receptors for the specific antigen, with positive selection of B cells with improved affinity for a specific antigen[426]. It is also important to note that follicular dendritic cells are important in the immune response to antigen. Antigens (in the form of antigen:antibody:complement) bind to follicular dendritic cells in the lymph node and can

bind to B cells in the germinal center. B cells later take up this complex based on their affinity to the antigen[428]. Altogether, avidity maturation in response to an antigen occurs through a process of clonal proliferation, somatic hypermutation, and selection[425]. It has been shown that the affinity of antibodies progressively increases over time after antigen exposure[426, 429-432]. In addition, the increase in antibody avidity as time after antigen exposure increases has also been shown after vaccination with pertussis vaccine[433,255, 405], Hib conjugate vaccine[398, 434], and different pneumococcal vaccines[399]. However, those studies used a single concentration of bond-breaking agent in order to assess avidity. I hypothesize that the longer the interval between vaccination and delivery, the more maturation of immune response with somatic hypermutation and selection of B cells with higher affinity/avidity to the vaccine antigens. The mutations accumulate in a stepwise and time-dependent manner.

Defining the optimal timing for vaccination against pertussis in pregnancy that provides maximal clinical protection to the infant is important and represents a critical gap in current knowledge[73]. Furthermore, data to define the preferred timing of vaccination against pertussis within the third trimester are limited and inconclusive[389,390]. Early third trimester vaccination was associated with infants' lower odds ratio (0.43) to have pertussis infection at age <8 weeks compared with vaccination in late third trimester. However, the findings were limited by the wide CI of the odds ratio (0.02–7.58)[389]. In a small number of infants whose mothers received vaccine up to 1 week before delivery, vaccine effectiveness was 43%, with negative lower limit of the CI, limiting a firm conclusion from this study[390]. Vaccination earlier in the third trimester also has the added

advantage to increase immunization opportunities for pregnant women subsequently giving birth to premature infants. In the UK, among infants <3 months of age with laboratory–confirmed pertussis, 10/66 were premature (born at 32–36 WG)[343]. The latter findings, combined with these data that vaccination more than 4 weeks before delivery provides higher levels of high and very high avidity of anti-pertussis antibodies, suggests that vaccination earlier in the third trimester of pregnancy should be preferred. Although the clinical significance of high levels of medium and high avidity anti-PT IgG needs further study, function of antibodies may correlate with protection from infections. Function of anti-pertussis antibodies is important, as there is no anti–pertussis antibody level that correlates with protection. Function of meningococcal vaccine-induced antibodies (bactericidal titers) has been found to correlate with protection from the meningococcal disease[400]. Antibody avidity was reported to be an important surrogate for determining protective immunity for Hib conjugate vaccine[398]. The amount of human anti-pneumococcal capsular polysaccharide (PCP) 6B-seroytpe specific antibodies required for the prevention of lethal pneumococcal serotype 6B bacteremia in mice was lower for high avidity antibodies demonstrating potential clinical implication in the protection from invasive pneumococcal disease[435]. In addition, higher avidity antibodies to PPS 6B and 23F were more effective than lower avidity antibodies in mediating opsonophagocytosis[435].

In addition to the highest vaccine-induced immune responses, there are different variables that affect the uptake of a vaccine in pregnancy that need to be considered when determining and recommending the ideal timing of pertussis vaccination in pregnancy.

The uptake of a specific vaccine in pregnancy is influenced by timing and number of antenatal care visits, timing of administration of other vaccines (e.g. influenza) and access to vaccination services[436]. Achieving the highest vaccine uptake is ideal for optimal protection against pertussis disease in infancy and should be balanced against vaccination in a narrow window that is associated with the highest vaccine-induced immune response.

This analysis has a number of strengths. This is the first detailed characterization of full antibody avidity spectrum of anti-pertussis antibodies in a large cohort of well characterized subjects. The use of a concentration gradient of bond-breaking agent enabled accurate quantification of fractional absolute anti–PT IgG levels according to their binding characteristic to PT antigen. The high linear correlation between anti-PT IgG RAI and ammonium thiocyanate, within the range of ammonium thiocyanate concentration used in the study, enabled the calculation of a weighted measure of total RAI and the quantification of total absolute avidity levels of anti-PT IgG. Altogether, this analytical approach enabled us to perform novel and in-depth analyses of the immune response following vaccination in pregnancy and to link it to clinical variables. The calculated single value of total absolute avidity levels of anti-PT IgG, incorporating both antibody quantity and function (avidity) can be used in future research aimed at establishing correlates of protection against pertussis. In addition, given the ability to characterize the composition of antibodies with different avidity characteristics, I was also able to show that newborns can be clustered according to the timing of vaccination in the third trimester.

This analysis also has some limitations. The effect of timing during the third trimester was explored; however, optimal timing of vaccination during the entire pregnancy should be explored, as vaccination against pertussis in the second trimester has been shown to be associated with higher anti-pertussis antibody levels as compared to vaccination in the third trimester[437]. This study was not a randomized controlled trial and there were imbalances in some baseline characteristics (e.g. mode of delivery) between the different cohorts; however, adjustments were made for co-variates in multivariable analysis. There is a possibility of selection bias as healthier pregnant women could have chosen to receive the vaccine. In addition, some other variables that could have affected immune responses (e.g. maternal body mass index, smoking) could have affected the outcome. Including women vaccinated against pertussis within 5 years before pregnancy, and their high percentage in unvaccinated women, is another limitation as it could have affected the results of the comparison of anti-PT IgG levels of newborns born to vaccinated and unvaccinated women. However, the GMC of anti-PT IgG levels of this subgroup was 18.6 IU/mL and thus it is expected to have has minimal effect on anti-PT IgG. A study by Abu-Raya et al. followed women vaccinated with Tdap during the third trimester of pregnancy and reported that anti-PT IgG levels declined significantly from 21.5 IU/mL to 11.7 IU/mL, 9-15 months after delivery[327]. A study by Maertens et al. found that after pre-pregnancy Tdap vaccination, anti-PT IgG levels decreased significantly from 69.9 ELISA Units (EU)/mL 1-month post-vaccination to 13.43 EU/mL at delivery (within a mean interval of 16 months after pre-pregnancy Tdap vaccination)[328]. The small number of the study participants might have limited the ability to detect significant differences in

the anti-PT IgG levels achieved at 0.5 M and 1.5 M of ammonium thiocyanate. In addition, this study included only cord sera for analysis and did not include premature infants. Thus, additional studies in preterm infants are needed to investigate the avidity profile of preterm infant born within short time after maternal pertussis vaccination in pregnancy. In this study, only one full-term infant was born 1 week after maternal Tdap vaccination in pregnancy, a time period not sufficient for induction of immune response. Thus, the inclusion of this newborn is unlikely to affect the results. Lastly, these data did not have full details of previous pertussis immunization of the participants.

In conclusion, in this study I characterized in-depth the profile of the avidity of anti-pertussis antibodies elicited by vaccination in pregnancy and it's relation to timing of vaccination in pregnancy. Neonates born to women vaccinated against pertussis in during 28–32 WG had higher levels of medium and high avidity anti-pertussis antibodies compared with newborns born to women vaccinated during 33–36 WG. Furthermore, most newborns of women vaccinated during 28–32 WG have avidity profile consisting of high levels of high avidity anti-pertussis antibodies. Future studies need to determine the profile of avidity of anti-pertussis antibodies that is generated after vaccination even earlier in pregnancy and to determine the correlation of these findings with clinical protection from pertussis disease.

4. Modification of immune response following vaccination during pregnancy- a systematic review and individual participant data meta-analysis

4.1 Synopsis

Immunization against pertussis in pregnancy might affect the antibody response of infants to their own vaccinations. In this chapter, I aimed mainly to determine the impact of Tdap immunization in pregnancy on antibody levels to routine immunizations in infants of women who did or did not receive pertussis-containing vaccine in pregnancy and factors affecting these. Systematic literature search was performed for randomized and non-randomized studies published between January 1st, 1990 and January 6th, 2020 and investigating antibody responses to vaccinations in infants. After reviewing the studies, I performed an individual participant data meta-analysis with the geometric mean ratios (GMRs) of pertussis-specific (pertussis toxin [PT], filamentous haemagglutinin [FHA], pertactin [PRN], fimbriae 2/3 [FIM2/3]), tetanus-toxoid [TT]; diphtheria-toxoid [DT], pneumococcal polysaccharide; and *Haemophilus influenzae* type b polyribosyl ribitol phosphate [PRP]) antibody levels/titres after primary and booster immunization, as the primary outcome. Mixed-effects models were used. Seroprotection rates against TT, DT (anti-TT and anti-DT IgG ≥0.1 IU/mL) and *Streptococcus pneumoniae* (SPN) (anti-SPN IgG ≥0.35 μg/mL) were calculated. As secondary objectives, factors influencing antibody responses to Tdap vaccination in pregnant women and primary and booster immunizations with DTaP in infants of immunized women were also determined using mixed-effects models. From 8391 citations identified, 16 articles met the inclusion criteria, resulting in 14 articles included in the individual participant data meta-analysis

(data from 2 studies was not shared). After primary immunization, infants of Tdap-immunized women had significantly lower PT (GMR, 0.65; 95%CI, 0.57-0.74), FHA (0.68;0.53-0.87), PRN (0.65;0.58-0.72) and FIM2/3 (0.41; 0.32-0.52) antibody levels compared with infants of unimmunized women. These low antibody levels persisted at time of booster for PT, FHA, PRN and after booster immunization for FHA and FIM2/3. Anti-TT IgG levels were higher in infants born to Tdap-immunized women after booster immunization with DTaP, compared with infants of women who did not receive TT-containing vaccines during pregnancy (1.59; 1.04-2.42). Anti-DT IgG levels were lower in infants born to Tdap-immunized women after primary immunization, at time of booster immunization and after booster immunization with DTaP compared to infants of women who did receive DT-containing vaccines (0.63; 0.5-0.79), (0.68; 0.54-0.87), and (0.81; 0.71-0.91), respectively. Anti-SPN IgG levels were lower in infants born to Tdap-immunized women after primary immunization with pneumococcal conjugate vaccine 13 (PCV-13) for serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 18C, 19A, 19F, 23F with a reduction ranging from 15%-35%.

After primary immunization, infants of Tdap-immunized women had lower seroprotection rates against diphtheria (90% [843 /973] *vs* 98% [566/579]; $p<0.001$) and invasive pneumococcal disease (IPD) caused by 5 *Streptococcus pneumoniae* (SPN) serotypes (SPN5, SPN6B, SPN9V, SPN19A, SPN23F), and higher seroprotection rates against *Haemophilus influenzae* type b (short-term and long-term seroprotection rates, 86%[471/547] *vs* 76%[188/247] and 62%[337/547] *vs* 49%(121/247), respectively, all $p=0.001$).

Among infants born to women immunized against pertussis in pregnancy, doubling of pre-existing antibody levels at primary immunization resulted in 9% (GMR 0.92, 95% CI: 0.88-0.95) lower post-primary immunization levels and doubling of anti-PT and anti-FHA IgG levels at primary immunization resulted in 10% (GMR 0.90. 95% CI: 0.85-0.97) and 8% (GMR 0.92, 95% CI: 0.86-0.99) lower post-booster immunization levels. Timing of vaccination in pregnancy did not affect post-primary immunization anti-*B. pertussis*, anti-TT and anti-DT antibody levels. Vaccination schedule did not affect anti-PT, anti-FHA, anti-PRN, anti-FIM and anti-DT antibody levels, while a 2,3,4 months schedule was associated with lower post-primary immunization anti-TT antibody levels, compared with a 2,4,6 months vaccination schedule.

This large, longitudinal analysis demonstrates lower infants' antibody levels to pertussis, diphtheria and some SPN serotypes, after maternal pertussis immunization compared with unvaccinated women. This supports enhanced surveillance of pertussis, diphtheria and invasive pneumococcal disease in infants to determine the clinical significance of this effect.

4.2 Introduction

Immunization against pertussis in pregnancy is thought to reduce pertussis disease morbidity and mortality in the offspring by reducing the risk of transmission of the bacteria[438] and enhancing the trans-placental transfer of vaccine-specific antibodies to the newborn[316, 439]. However, several early studies suggested that high pre-existing maternally derived antibody levels, not induced by vaccination in pregnancy, can have a suppressive effect on infants' active immune responses to their own vaccination[440-443] leading to lower post-vaccination antibody levels in infants. There is an increasing evidence to support that immunization against pertussis in pregnancy can modify infants' active immune responses to immunization in infancy, leading to lower anti-*B. pertussis* antibody levels in infants born to vaccinated compared with unvaccinated women[316, 330, 444, 445]. Current formulations of Tdap vaccines used in pregnancy include tetanus toxoids and diphtheria toxoids. Thus, infants' immune responses to TT and diphtheria toxoid DT components of vaccines and vaccines conjugated these toxoids as carrier proteins (e.g. Hib vaccine, *Neisseria meningitidis*, and PCVs) might also be modified. However, data are conflicting regarding the antigen-specific antibodies affected, the degree and the duration of such modification in immune responses. In addition, it is unknown yet whether this translates into lower seroprotection rates for some diseases in which COP exist (e.g. tetanus disease, diphtheria disease, and invasive pneumococcal disease [IPD]). Primary vaccination against pertussis is given to infants as part of a three (2, 4, 6 months; 2, 3, 4 months) or 2 doses (3, 5 months) schedules in different countries. Factors affecting immune response to vaccines in infants born to vaccinated women are not yet known,

including the ideal vaccination schedule. Thus, the aim of work presented in this chapter is to determine the association between immunization of mothers against pertussis in pregnancy and infants' active immune responses to their own vaccinations. In addition, to explore factors that are associated with immune responses to Tdap immunization during pregnancy and routine vaccines of infants born to women vaccinated against pertussis in pregnancy and unvaccinated women.

4.3 Methods

4.3.1 Search strategy and selection criteria

PubMed, MEDLINE, Embase, CINAHL, and the CENTRAL databases were searched for English language literature reporting on antibody levels/titers following primary and booster immunization in infants born to mothers immunized against pertussis during pregnancy versus unimmunized women, published between January 1st, 1990 and January 6th, 2020 following PRISMA guidelines (original search performed on February 21st, 2017 and updated searches performed on June 4th, 2018 and January 6th, 2020)[446]. The following search terms were used for all databases: "pertussis immunization" or "pertussis vaccination" or "Tdap vaccination" or "Tdap vaccine" or "Tdap immunization" or "Tdap pregnancy" and "interference" or "antibody response" or "immunogenicity" or "immune responses" and "pregnancy" (PROSPERO: CRD42017079171). Additional studies were identified by contacting experts in the field of immunization in pregnancy. Reference lists of identified publications and trial registries (clinical trials.gov) were searched for completed studies that have not been

identified through any of the above databases. Included were randomized and non-randomized studies. Letters, editorials and review articles containing no primary data were excluded.

A systematic literature search was performed by two independent researchers and references were de-duplicated automatically by EndNote™ Web/Basic and manually according to the last name of the first author[447, 448]. De-duplicated references were screened by title and abstract by two researchers, with a third researcher consulted when necessary. Articles screened and found to be possibly eligible were fully assessed for eligibility (full-text fully assessed against inclusion and exclusion criteria) by two researchers with a third researcher consulted when necessary.

Inclusion criteria were all of the following: The study included infants born to healthy women immunized at any time during pregnancy with a single dose of vaccine against pertussis; The study included a control group: infants born to healthy women unimmunized against pertussis during pregnancy; The study included infants after primary and/or booster (at age 9-24 months) immunization against pertussis-containing vaccines and vaccines containing TT or DT as a carrier protein; The study reported on antibody levels and/or titres of at least one of the following antigens (PT, FHA, PRN, FIM2/3, TT, DT, Hib, *Neisseria meningitidis* and/or SPN) following infants' primary and/or booster immunization.

Exclusion criteria were any of the following: The study included infants born to mothers

having an immunologic disorder, received immunoglobulins in the previous year, received immunosuppressive drugs during the current pregnancy (including high-dose steroids), or received blood products 3 months prior to delivery; The study included infants having immunologic disorder, received immunoglobulins within the previous year, received immunosuppressive drugs (including high-dose steroids), received blood products 3 months prior to antibody response assessment.

Studies meeting the inclusion criteria were included in the systematic review and the authors of the included studies were contacted to share individual participant data for the meta-analysis.

4.3.1 Data analysis

I performed an individual-participant data meta-analysis of antibody levels to primary and booster immunizations in infants of women who did or did not receive pertussis vaccine in pregnancy. Antibody levels were $\log_2$-transformed and meta-analyzed using mixed-effects models for each antigen-specific antibody and time point. Time points included were: at time of immunization in pregnancy, 4 weeks after immunization in pregnancy, at delivery (maternal sera and cord), at time of primary immunization, after primary immunization, at time of booster immunization, after booster immunization.

For the time points (4 weeks after immunization and at delivery [maternal sera and cord sera] and at primary immunization), the mixed-effects models included co-variates that were shown to affect immune responses to immunization and that were available within the datasets of the included studies [449] (maternal age at vaccination, pre-existing homologous antibody levels). For the infants' post-primary, at booster and post-booster immunization time points, the mixed-effects included co-variates that were shown to affect immune responses to immunization to account for their influence[449] (infants' sex, infants' age at primary immunization).

The antilog (2^x) of the coefficients from models and their 95% CI were presented as GMRs and their 95% CIs. For maternal time points, the GMR was interpreted as the ratio of antigen-specific antibody levels in women immunized against pertussis in pregnancy versus unimmunized women. For infants' time points, the GMR was interpreted as the ratio of antigen-specific antibody levels in infants born to women immunized in pregnancy versus unimmunized women (a GMR of ≥ 1 indicates higher antibody levels in infants of mothers immunized in pregnancy versus unimmunized, while a GMR ≤ 1 indicates lower antibody levels in infants of mothers immunized in pregnancy versus unimmunized).

In order to determine whether immunization in pregnancy affects protection against vaccine-preventable diseases with known COP, seroprotection rates against tetanus disease (anti-TT IgG ≥0.1 IU/mL), diphtheria disease (anti-DT IgG ≥0.1 IU/mL), IPD

(anti-SPN ≥0.35 μg/mL), short and long term Hib (anti-polyribosylribitol phosphate [PRP] IgG ≥ 0.15 mcg/ml and anti-PRPR IgG ≥ 1 mcg/ml, respectively) were calculated[450]. A chi-square test was used to determine whether the percentage of protection is different among immunized versus unimmunized pregnant women, or in the groups of infants born to pertussis-immunized versus pertussis-unimmunized women at the different time points.

To explore maternal factors that affect the maternal immune response to pertussis vaccination in pregnancy, co-variates influencing antibody responses to pertussis immunizations in pregnancy were determined using mixed-effects models. This analysis was restricted to studies that included antibody levels at time of vaccination during pregnancy and at delivery. Co-variates included were maternal age, timing of vaccination in pregnancy in weeks gestation, and pre-existing homologous antibody levels at time of vaccination.

To explore factors that can affect the immune response of infants' to their own vaccination, co-variates influencing antibody responses to primary and booster immunizations in infants born to women immunized and unimmunized against pertussis in pregnancy were determined using mixed-effects models. This analysis was restricted to studies that used a 2,3,4 or 2,4,6 months primary vaccination schedule and that included antibody levels at time primary vaccination and post-primary vaccination (for the post-primary vaccination model), or at time primary vaccination and post-booster vaccination (for the post-booster vaccination model).

For the primary immunization model, co-variates included were infants' sex, gestational age at delivery, timing of vaccination in pregnancy (for the cohort of infants born to vaccinated mothers), pre-existing homologous antibody levels at time of primary immunization, age at initiation of primary vaccination and primary immunization schedule (2,3,4 months versus 2,4,6 months). For the booster immunization model, co-variates included were infants' sex, time elapsed between vaccination in pregnancy and delivery in weeks (for the cohort of infants born to vaccinated mothers), pre-existing homologous antibody levels at time of primary immunization, age at primary vaccination, primary immunization schedule (2,3,4 months versus 2,4,6 months), age at booster immunization (except for anti-TT and anti-DT antibodies of unvaccinated women, in order to enable fitting the model with more than one level of vaccination schedule).

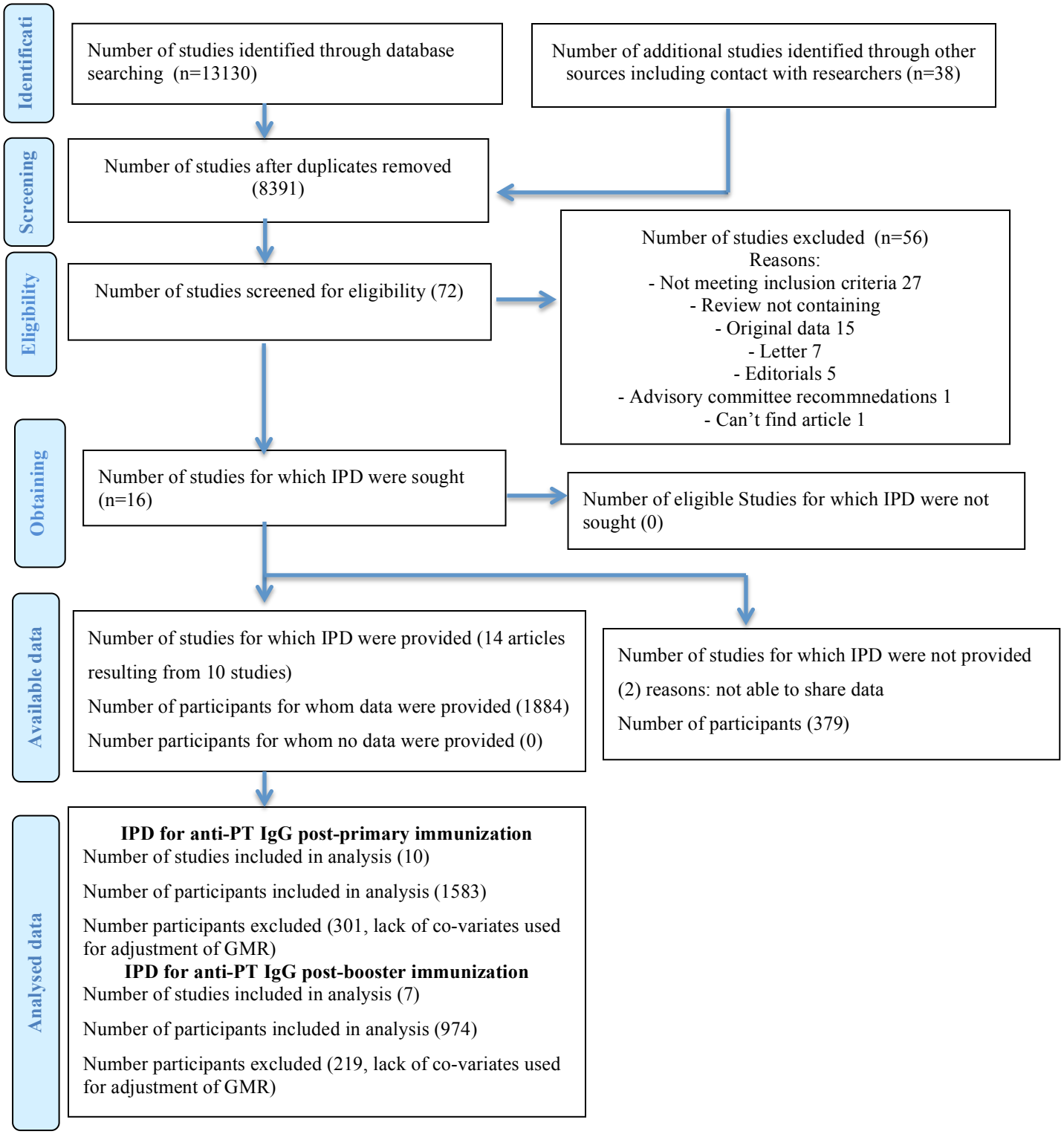
The antilog (2^x) of the coefficients from models and their 95% CIs are presented as GMRs and their 95% CIs. For this analysis, the GMR indicates the relative increase (fold rise) in antibody levels in response to vaccination associated with 1 unit change in a co-variate (e.g. the relative increase in antibody response for a 1-week older infant, or the relative increase in antibody response associated with a doubling in antibody levels).

R version 3.4.0 was used for all analysis (meta package, version 4.9-1). Study was registered at PROSPERO International prospective register of systematic reviews (CRD42017079171).

4.4 Results

4.4.1 Studies characteristics

A total of 8391 studies were screened for eligibility and 8319 were excluded. Seventy two full-text articles were assessed for eligibility and 56 articles were excluded. Sixteen articles met the inclusion criteria and were included in the systematic-review (Figure 4.1).



Abbreviations: IPD: individual-participant data; GMR: geometric mean ratio.

The PRISMA IPD flow diagram. © Reproduced with permission of the PRISMA IPD Group, which encourages sharing and reuse for non commercial purposes

Figure 4. 1: PRISMA 2009 flow chart

The studies included in the systematic review originated from 7 randomized-controlled trials and 5 non-randomized trials (Table 4.1). The vast majority (11/12) of the trials were performed in high-income countries (The United States, United Kingdom, Belgium, Canada, Spain, Italy, Australia, Finland, Netherlands, Czech Republic) with 1 trial conducted in a middle-income country (Vietnam). Different Tdap formulations were administered during the second and third trimester of pregnancy (Adacel [Sanofi Pasteur] in 4/11 trials, Boostrix [GlaxoSmithKline] in 5/11 trials, and Repevax [Sanofi Pasteur] in 2/11 trials). In one trial, Tdap formulation was not specified[451]. Infants were immunized with different DTaP formulations administered per a 2-3-4 months, 2-4-6 months, 3-5 months and 6 weeks-4-6 months schedules in 5/12, 4/12, 1/12 and 1/12 trials, respectively. In one international trial infants were vaccinated per the country's vaccination schedule[452]. Antibody responses one month after both primary and booster immunization were assessed in 8/12 trials, and after primary immunization only in 4/12 trials.

Authors of the articles included in the systematic review were contacted to obtain individual level data; 14/16 articles were included in the meta-analysis, which originated from 10 trials (Table 4.1).

Table 4. 1: Characteristics of studies identified through the systematic review.

Author (study location, time period)	Study design	Vaccine in pregnancy (timing of vaccination in pregnancy)	Pertussis vaccine administered to infants for primary immunization and schedule	Pertussis vaccine administered to infants for booster immunization and schedule	Infants' outcomes measure (antibody levels/titers to vaccine specific antigens)
Hardy-Fairbanks (US, 2006, 2008-2009)[453]	Retrospective cohort study	Adacel, Sanofi Pasteur (any trimester [Trimester 1: 4 women Trimester 2: 8 women Trimester 3: 4 women])	Tdap group: Pediarix, GSK; 2, 4, 6 months Control group: Pediarix, GSK or Pentacel, Sanofi Pasteur or Infanrix, GSK or a combination of these vaccines; 2, 4, 6 months	Tdap group: Infanrix, GSK or TriHIBit, Sanofi Pasteur or Pediarix®, GSK; 12-18 months. Control group: Infanrix, GSK or Pediarix®, GSK or Daptacel® Sanofi Pasteur, or Pentacel®, Sanofi Pasteur; 12-18 months	PT, FHA, PRN, FIM2/3, TT, DT, HBV, Polio1/2/3
Ladhani (UK, 2012-2014)[454]	Case-control study with historical cohort	Repevax®, Sanofi Pasteur (median interval between vaccination and delivery: 9.9 WG)	Pediacel, Sanofi Pasteur; 2, 3, 4 months Prevenar-13®, Pfizer; 2, 4 months Neivac-C, Pfizer or Menjugate, Sanofi Pasteur or Meningitec, Pfizer; 3, 4 months	N/A	PT, FHA, FIM2/3, TT, DT, Hib, MenC, SPN 1, 3, 4, 6A, 6B, 7B, 9V, 14, 18C, 19A, 19F, 23F.
Hoang (Vietnam, 2013-2013)[455] *	Randomized Controlled Trial	Adacel, Sanofi Pasteur (18-36 WG)	Infanrix Hexa, GSK Biologicals; 2, 3, 4 months of age	N/A	TT, DT, PT, FHA, PRN
Maertens (Belgium, 2012-2014)[330] *	Prospective controlled cohort study	Boostrix, GSK (22-33 WG)	Infanrix Hexa®, GSK; 8, 12 and 16 weeks of age	N/A	TT, DT, PT, FHA, PRN
Maertens (Belgium, 2012-2014)[456] *	Prospective controlled cohort study	Boostrix, GSK (22-33 WG)	N/A	Infanrix Hexa®, GSK Biologicals; 15 months of age (booster vaccination)	TT, DT, PT, FHA, PRN

Author (study location, time)	Study design	Vaccine in pregnancy (timing of vaccination in pregnancy)	Pertussis vaccine administered to infants for primary immunization and schedule	Pertussis vaccine administered to infants for booster immunization and schedule	Infants' outcomes measure (antibody levels/titers to antigens)
Maertens (Vietnam, 2013)[457]*	Randomized Controlled Trial	Adacel, Sanofi Pasteur (18-36 WG)	N/A	Infanrix Hexa®, GSK; Second year of life (mean age Tdap group: 22.18 months; mean age control group: 21.44 months)	TT, DT, PT, FHA, PRN
Munoz (US, 2008-2012)[316]*	Randomized controlled clinical trial	Adacel, Sanofi Pasteur (30-32 WG)	Pentacel, Sanofi Pasteur; 2, 4, 6 months	Pentacel®, Sanofi Pasteur; 12 months	PT, FHA, PRN, FIM2/3, TT, DT
Maertens (Belgium, 2011-2015)[458]*	Prospective controlled cohort study	Boostrix, GSK (22-33 WG)	Prevenar-13, Pfizer; 2, 4 months Infanrix Hexa, GSK; 2, 3, 4 months	Prevenar-13, Pfizer at 12 months	SPN 1, 3, 4, 6A, 6B, 7B, 9V, 14, 18C, 19A, 19F, 23F.
Halperin (Canada, 2007-2011 and 2012-2014)[331]*	Randomized Controlled Trial	Adacel, Sanofi Pasteur (33–35 WG)	DTaP-IPV-Hib; Pediacel, Sanofi Pasteur; 2, 4, and 6, months	DTaP-IPV-Hib; Pediacel, Sanofi Pasteur; 12 months	PT, FHA,PRN , FIM2/3, TT, DT, Hib
Barug (Netherlands, N/Av)[459]*	Randomized controlled trial	Boostrix, GSK (30-32 WG).	Infanrix Hexa, GSK; Synflorix, GSK; at 3, 5 months of age	Infanrix Hexa, GSK; Synflorix, GSK; at 11 months of age	PT, FHA, PRN
Zimmermann (Australia, 2013-2016)[460]*	Randomized controlled trial	Boostrix, GSK (N/Av)	Infanrix Hexa, GSK; Prevenar 13, Wyeth; At 6 weeks, 4 months and 6 months of age	Menitorix, GSK; at 12 months of age	PT, FHA, PRN, TT, DT,,Hib, SPN 1, 3, 4, 6A, 6B, 7B, 9V, 14, 18C, 19A, 19F, 23F. Polio (types 1, 2, 3), MenC,measls ,mumps, rubella

Author (study location, time)	Study design	Vaccine in pregnancy (timing of vaccination in pregnancy	Pertussis vaccine administered to infants for primary immunization and schedule	Pertussis vaccine administered to infants for booster immunization and schedule	Infants' outcomes measure (antibody levels/titers to antigens)
Rice (UK, 2014-2016)[461]*	Prospective controlled cohort study	Repevax, Sanofi Pasteur (prior to July 2014) and Boostrix-IPV GSK (after July 2014) (N/Av).	DtaP5-IPV-Hib Pediacel, Sanofi Pasteur or DtaP3-IPV-Hib (Infanrix-IPV-Hib;GSK) at 2, 3 and 4 months. Prevenar 13 (Pfizer) at 2 and 4 months .	NA	PT, FHA, PRN, TT, DT, Hib, SPN 1, 3, 4, 6A, 6B, 7B, 9V, 14, 18C, 19A, 19F, 23F.
Perret (Australia, Canada, Czech Republic, Finland, Italy and Spain, 2016-2018)[452]*	Phase IV, multi-center, observer-blind, randomized, placebo controlled	Boostrix, GSK (27–36 WG)	2 or 3 doses of DTaP-HepB-IPV/Hib (Infanrix Hexa, GSK) co-administered with PCV13 (Prevnar 13, Pfizer Inc.) at 2 and 4 months; or 3 and 5 months; or 2, 4 and 6 months; or 2, 3 and 4 months	NA	PT, FHA, PRN, TT, DT, Hib, HBV, SPN 1, 3, 4, 6A, 6B, 7B, 9V, 14, 18C, 19A, 19F, 23F; Polio (types 1, 2, 3)
Barug (Netherlands, N/Av)[462]*	Randomized controlled trial	Boostrix, GSK (30-32 WG).	Infanrix Hexa, GSK; Synflorix, GSK; at 3, 5 months of age	Infanrix Hexa, GSK; Synflorix, GSK; at 11 months of age	DT, TT, Hib, SPN 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F, 6A, 19A
Klein (US, 2-0014-2015)[451]*	Randomized controlled trial	Pertussis vaccine (trade name N/Av), (timing, N/Av)	Infanrix Hexa, GSK Pentacel, Sanofi Pediarix, GSK co-administered with with PCV13 (Prevnar 13, Pfizer Inc.) at 2, 4, 6 months	Hiberix, GSK ActHIB, Sanofi Pentacel, Sanofi at 11 months	PT, FHA, PRN, TT, DT, Hib, HBV, Polio (types 1, 2, 3)
(Belgium, 2015-2019)[463]	Prospective controlled cohort study	Boostrix, GSK (Mean GA at vaccination: 29.3 weeks (13.4-36.9 weeks).	Hexyon, Sanofi Pasteur at 8-12-16 weeks. Synflorix, GSK at 8-16 weeks and 12 months.	Hexyon, Sanofi Pasteur at 15 months. Synflorix, GSK at 12 months. Neivac-C, Pfizer at 15 months.	PT, FHA, PRN, TT, DT, Hib, HBV, Polio (types 1, 2, 3)

* Study included in the individual-participant-data meta-analysis. Abbreviations: GSK: GlaxoSmithKline; HBV: Hepatitis b virus; Menc: meningococcal C; SPN: N/Av: not available.

4.4.2 Effect of maternal pertussis immunization during pregnancy on antigen-specific antibody response in mothers and their infants

4.4.2.1 Anti-*B. pertussis* antibodies

Anti-*B. pertussis* IgG levels were comparable at time of immunization during pregnancy in pregnant women who later received Tdap compared to women who later did not receive Tdap. Anti-*B. pertussis* IgG levels were higher in women who received Tdap during pregnancy 4 weeks after immunization and at delivery compared to women who did receive Tdap. Anti-*B. pertussis* IgG levels were higher in infants born to Tdap-immunized women at birth and at time of primary immunization. After primary immunization, infants of Tdap-immunized women had significantly lower PT (GMR, 0.65; 95%CI, 0.57-0.74), FHA (0.68;0.53-0.87), PRN (0.65;0.58-0.72) and FIM2/3 (0.41; 0.32-0.52) antibody levels compared with infants of unimmunized women. These low antibody levels persisted at time of booster for PT, FHA, PRN and after booster immunization for FHA and FIM2/3 (Figure 4.2, Figure 4.3, Figure 4.4, Figure 4.5). The range of reduction in anti *B. pertussis*-specific antibody levels in infant born to pertussis vaccinated women when compared with unvaccinated women was 32-59% after primary immunization for PT, FHA, PRN and FIM2/3; 33-43% before booster immunization for PT, FHA and PRN, and 28-47% after booster immunization for FHA and FIM2/3.

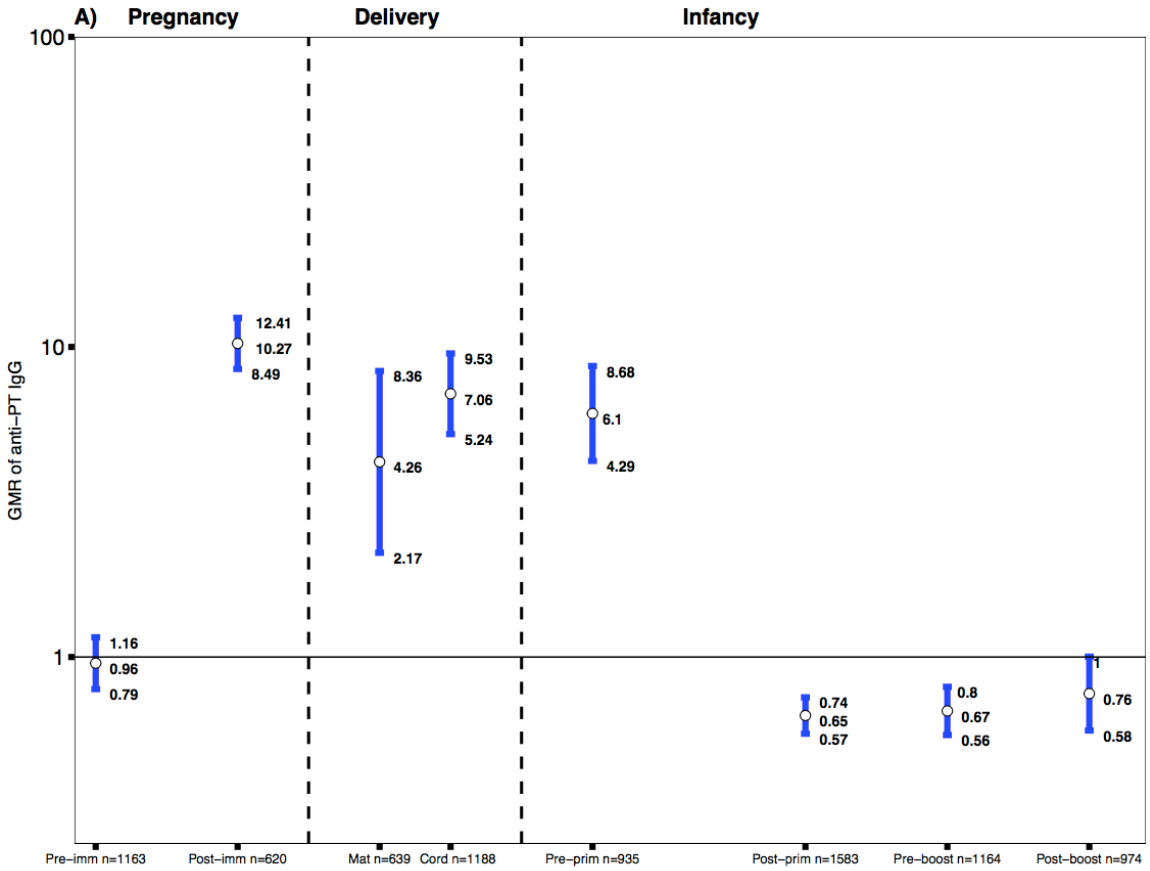


Figure 4. 2: Antibody responses to PT in women after vaccination in pregnancy, at delivery, cord sera, in infants before and after primary immunization, before and after booster immunization with tetanus- diphtheria -acellular pertussis vaccine.

Vertical blue lines indicate the GMR at the different time points with the 95% CI. Horizontal line indicates a GMR of 1. Numbers (n) indicate the number of infants whom serotype-specific antibody levels were available for meta-analysis. This figure shows higher anti-PT IgG levels in pregnant women Tdap -vaccinated vs. unvaccinated and their infants until primary immunization. Anti-PT IgG levels are lower in infants born to Tdap-vaccinated vs –unvaccinated after primary immunization, at booster immunization and after booster immunization against pertussis. Abbreviation: Pre-vac: pre-vaccination; Post-vac: post-vaccination; Mat: maternal; Pre-prim: pre-primary; Post-prim: post-primary; Pre-boost: pre-booster; Post-boost: post-booster.

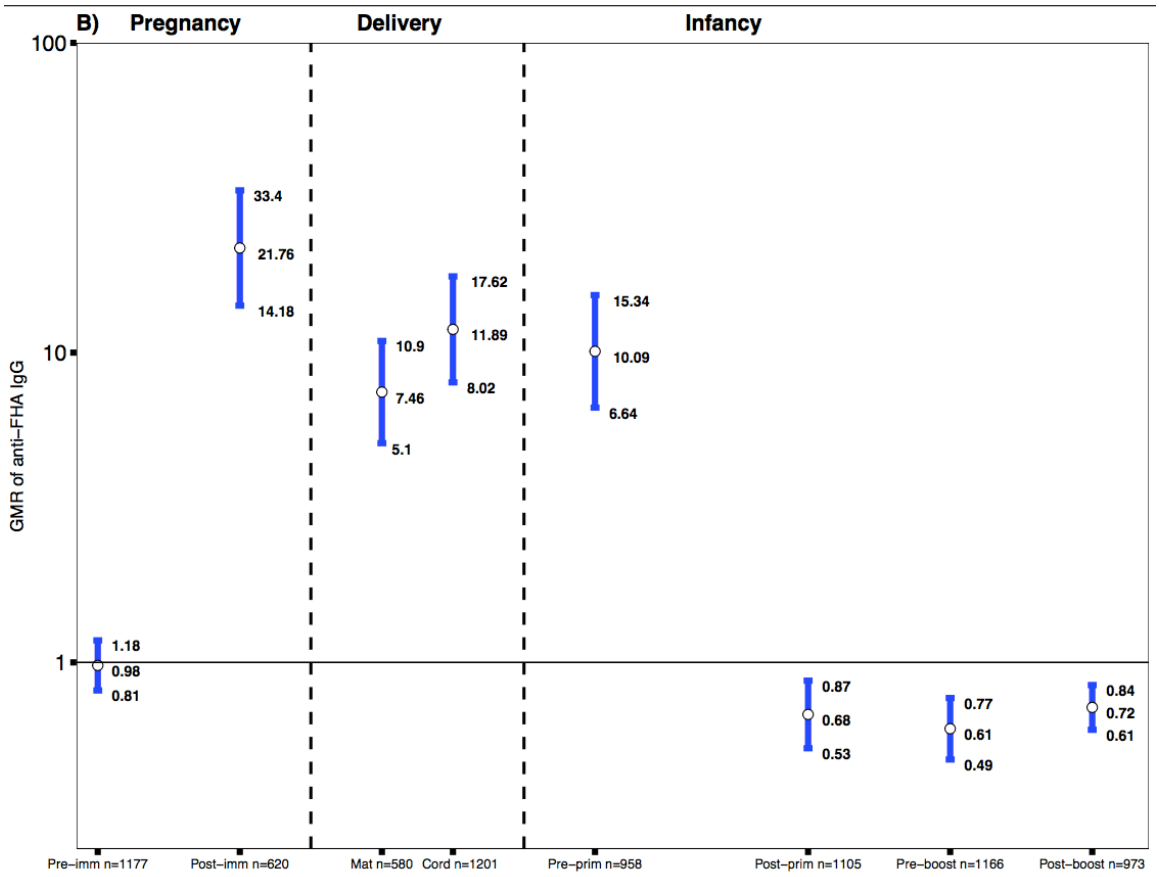


Figure 4. 3: Antibody responses to FHA in women after vaccination in pregnancy, at delivery, cord sera, in infants before and after primary immunization, before and after booster immunization with tetanus- diphtheria -acellular pertussis vaccine.

Vertical blue lines indicate the GMR at the different time points with the 95% CI. Horizontal line indicates a GMR of 1. Numbers (n) indicate the number of infants whom serotype-specific antibody levels were available for meta-analysis. This figure shows higher anti-FHA IgG levels in pregnant women Tdap -vaccinated vs. unvaccinated and their infants until primary immunization. Anti-FHA IgG levels are lower in infants born to Tdap-vaccinated vs –unvaccinated after primary immunization, at booster immunization and after booster immunization against pertussis.

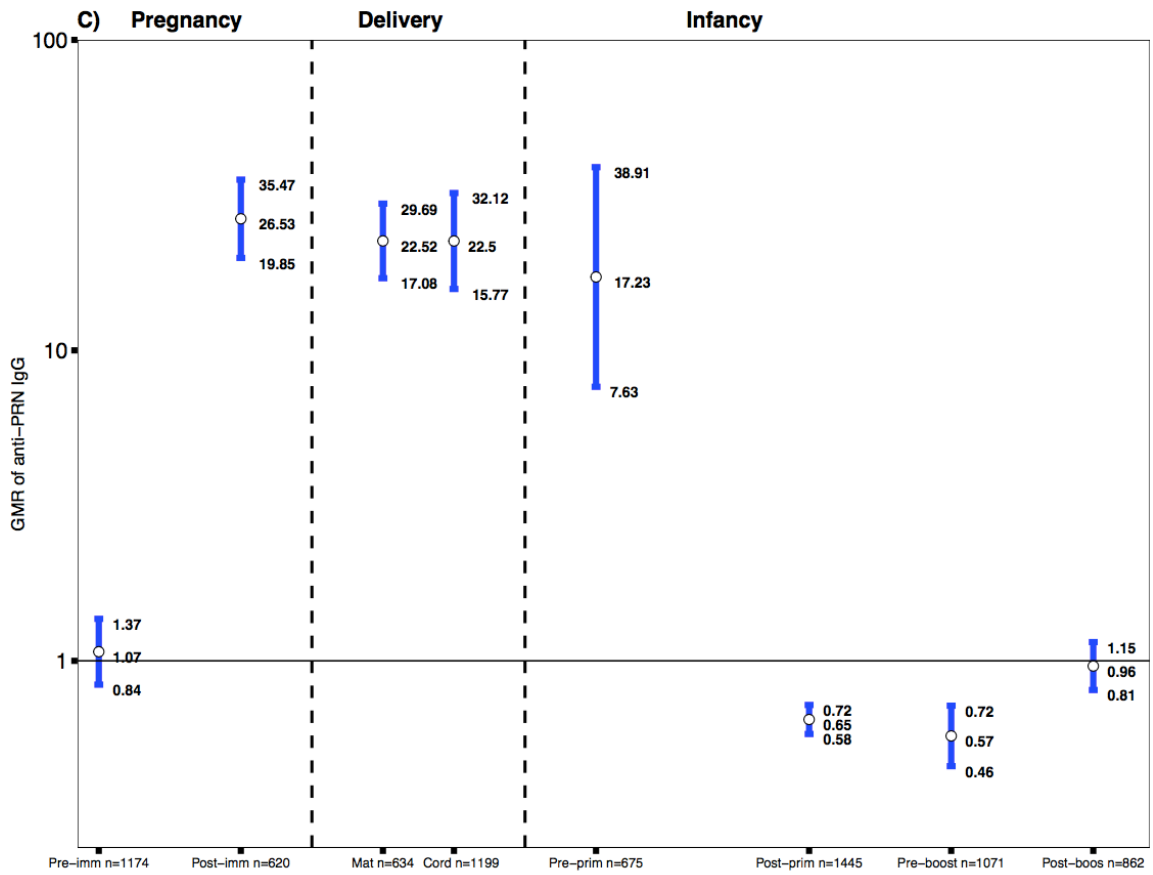


Figure 4. 4: Antibody responses to PRN in women after vaccination in pregnancy, at delivery, cord sera, in infants before and after primary immunization, before and after booster immunization with tetanus- diphtheria -acellular pertussis vaccine.

Vertical blue lines indicate the GMR at the different time points with the 95% CI. Horizontal line indicates a GMR of 1. Numbers (n) indicate the number of infants whom serotype-specific antibody levels were available for meta-analysis. This figure shows higher anti-PRN IgG levels in pregnant women Tdap -vaccinated vs. unvaccinated and their infants until primary immunization. Anti-PRN IgG levels are lower in infants born to Tdap-vaccinated vs –unvaccinated after primary immunization and at booster immunization.

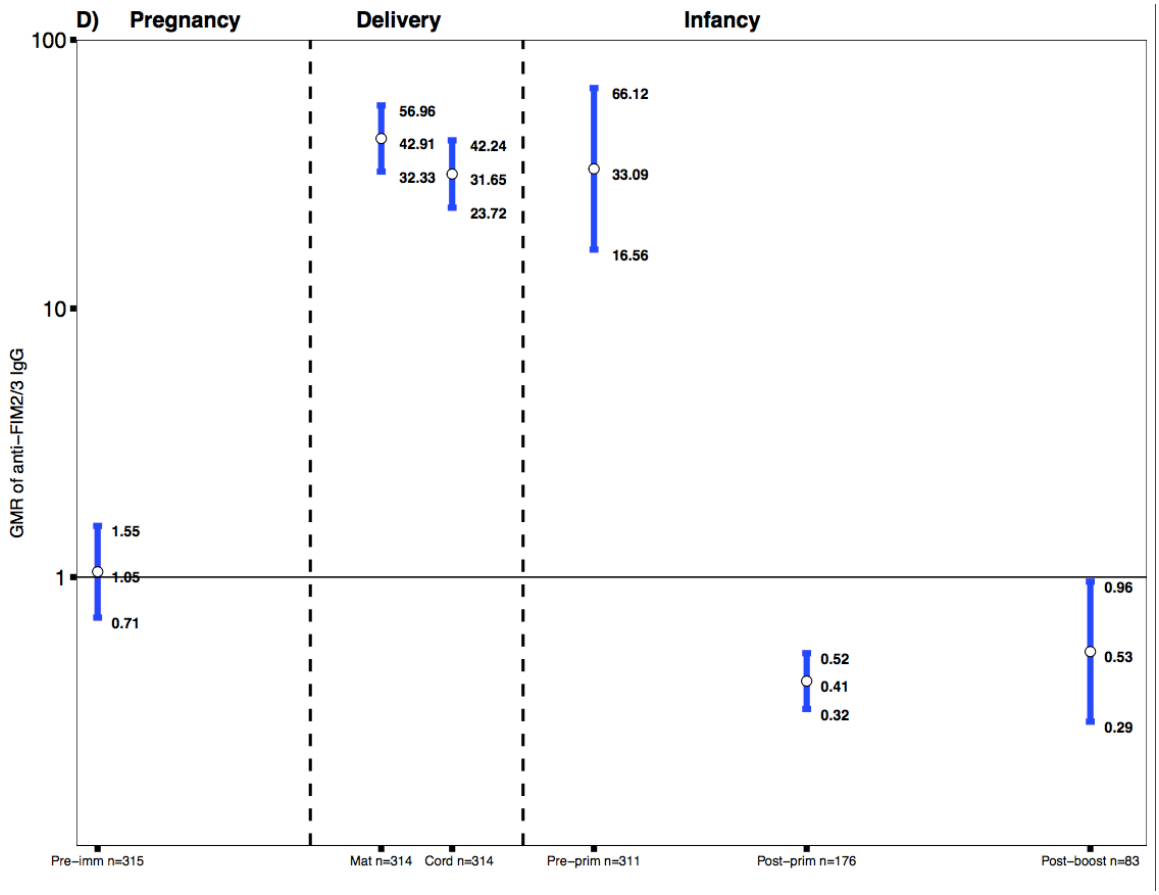


Figure 4. 5: Antibody responses to FIM2/3 in women at delivery, cord sera, in infants before and after primary immunization, and after booster immunization with tetanus- diphtheria -acellular pertussis vaccine.

Vertical blue lines indicate the GMR at the different time points with the 95% CI. Horizontal line indicates a GMR of 1. Numbers (n) indicate the number of infants whom serotype-specific antibody levels were available for meta-analysis. This figure shows higher anti-FIM2/3 IgG levels in pregnant women Tdap -vaccinated vs. unvaccinated and their infants until primary immunization. Anti-FIM2/3 IgG levels are lower in infants born to Tdap-vaccinated vs –unvaccinated after primary immunization and at booster immunization. GMR was not computed post-vaccination in pregnancy and pre-booster in infancy as data were available for one study on these time points precluding meta-analysis.

4.4.2.2 Anti-TT and anti-DT antibodies

Anti-TT IgG levels were higher in infants born to Tdap-immunized women at birth, before primary immunization and after booster immunization with DTaP, compared with infants of women who did not receive TT-containing vaccines during pregnancy, (4.53; 1.55-13.25), (5.46; 3.98-7.49) and (1.59; 1.04-2.42), respectively (Figure 4.6).

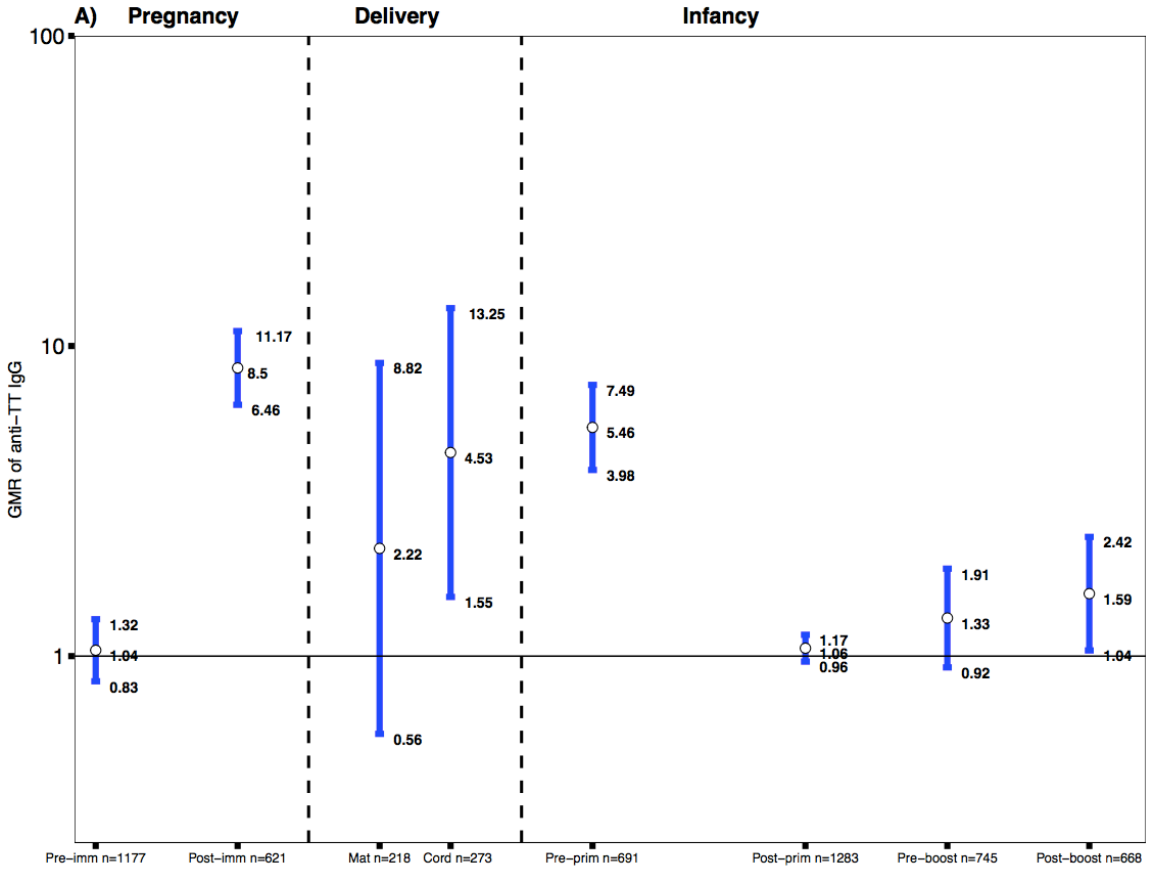


Figure 4. 6: Antibody responses to TT in women at delivery, cord sera, in infants before and after primary immunization, and after booster immunization with tetanus- diphtheria -acellular pertussis vaccine.

Vertical blue lines indicate the GMR at the different time points with the 95% CI. Horizontal line indicates a GMR of 1. Numbers (n) indicate the number of infants whom serotype-specific antibody levels were available for meta-analysis. This figure shows higher anti-TT IgG levels in pregnant women after Tdap vaccination compared with unvaccinated women, and their infants until primary immunization. Anti-FIM2/3 IgG levels are higher in infants born to Tdap-vaccinated vs –unvaccinated after booster

immunization.

Anti-DT IgG levels were comparable at time of immunization during pregnancy in pregnant women who later received Tdap compared to women who later did not receive DT-containing vaccines. Anti-DT IgG levels were higher in women who received Tdap during pregnancy 4 weeks after immunization and at delivery compared to women who did receive DT-containing vaccines. Anti-DT IgG levels were higher in infants born to Tdap-immunized women at birth and at time of primary immunization with DTaP compared to infants of women who did receive DT-containing vaccines. Anti-DT IgG levels were lower in infants born to Tdap-immunized women after primary immunization, at time of booster immunization and after booster immunization with DTaP compared to infants of women who did receive DT-containing vaccines (0.63; 0.5-0.79), (0.68; 0.54-0.87), and (0.81; 0.71-0.91), respectively (Figure 4.7).

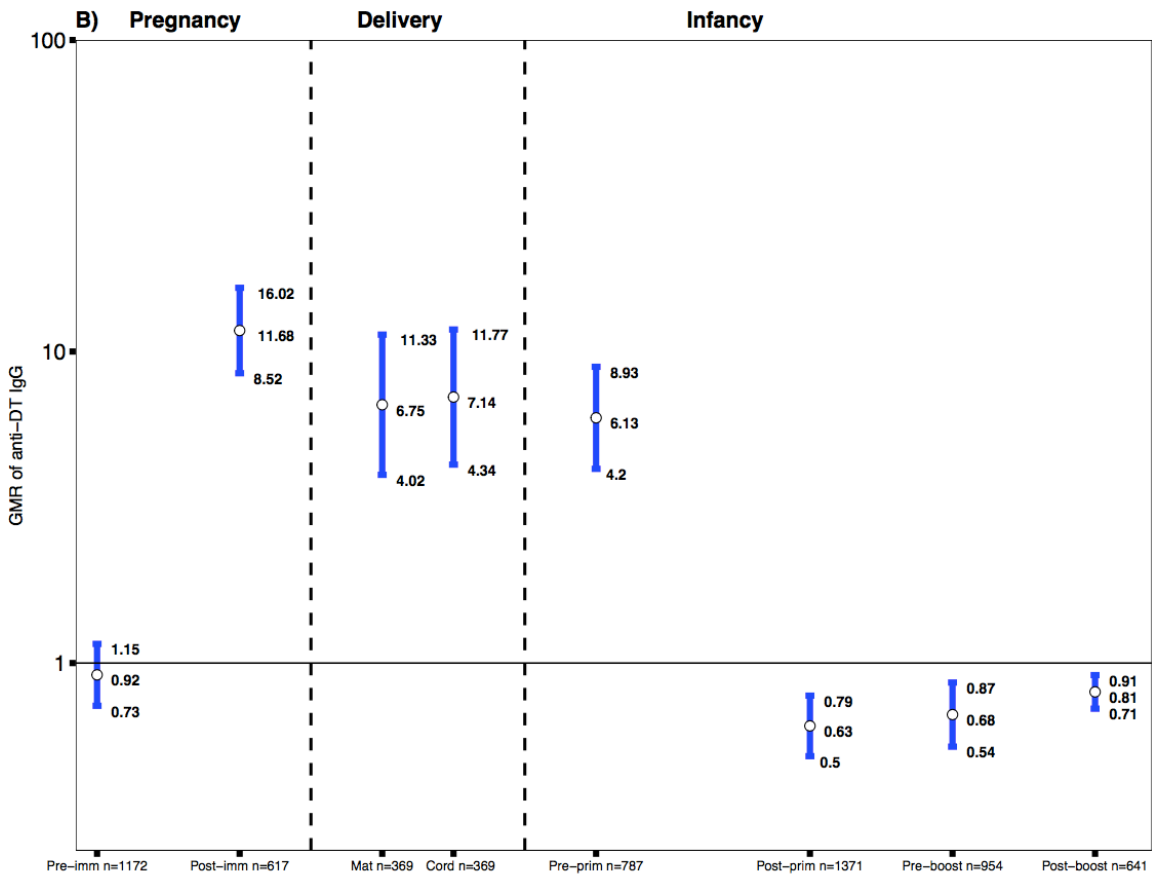


Figure 4. 7: Antibody responses to DT in women at delivery, cord sera, in infants before and after primary immunization, and after booster immunization with tetanus- diphtheria -acellular pertussis vaccine.

Vertical blue lines indicate the GMR at the different time points with the 95% CI. Horizontal line indicates a GMR of 1. Numbers (n) indicate the number of infants whom serotype-specific antibody levels were available for meta-analysis. This figure shows higher anti-DT IgG levels in pregnant women Tdap -vaccinated vs. unvaccinated and their infants until primary immunization. Anti-DT IgG levels are lower in infants born to Tdap-vaccinated vs –unvaccinated after primary immunization, at booster immunization and after booster immunization against pertussis.

4.4.2.3 Anti-SPN antibodies

Anti-SPN IgG levels were lower in infants born to Tdap-immunized women after primary immunization with PCV-13 for serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 18C, 19A, 19F, 23F

with a reduction ranging from 15%-35% (Figure 4.8).

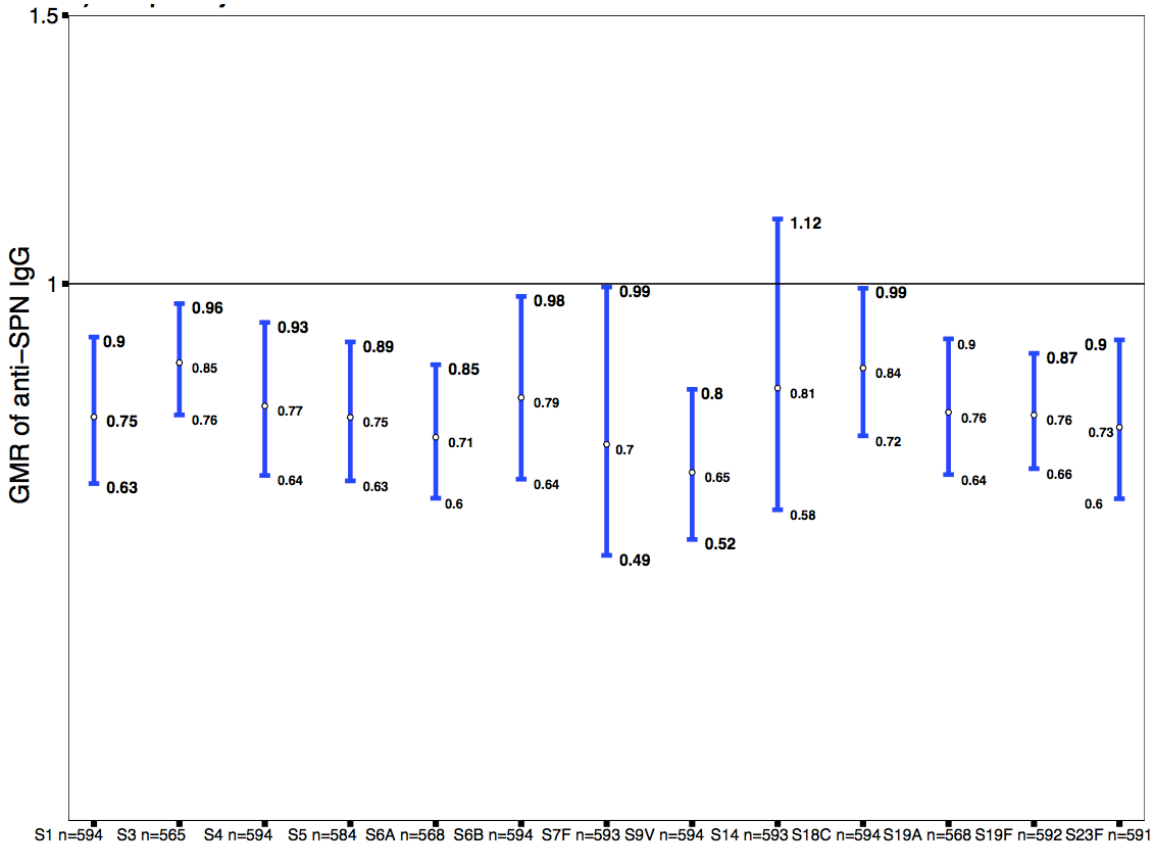


Figure 4. 8: Antibody responses to SPN. GMR of antibody levels to SPN serotypes in infants after primary immunization pneumococcal conjugate vaccine-13.

Horizontal line indicates a GMR of 1. Numbers (n) in brackets indicate the number of infants whom serotype-specific antibody levels were available for meta-analysis. This figure shows lower anti-SPN IgG levels in infants born to Tdap-vaccinated vs – unvaccinated after primary immunization with pneumococcal conjugate vaccine 13 for 12/13 serotypes

4.4.2.4 Anti-PRP antibodies

Anti-PRP IgG levels were not significantly different in infants born to Tdap-immunized women after primary immunization, at time of booster immunization and after booster immunization with Hib-including vaccines, compared with infants of women who did not receive TT-containing vaccines during pregnancy (Figure 4.9).

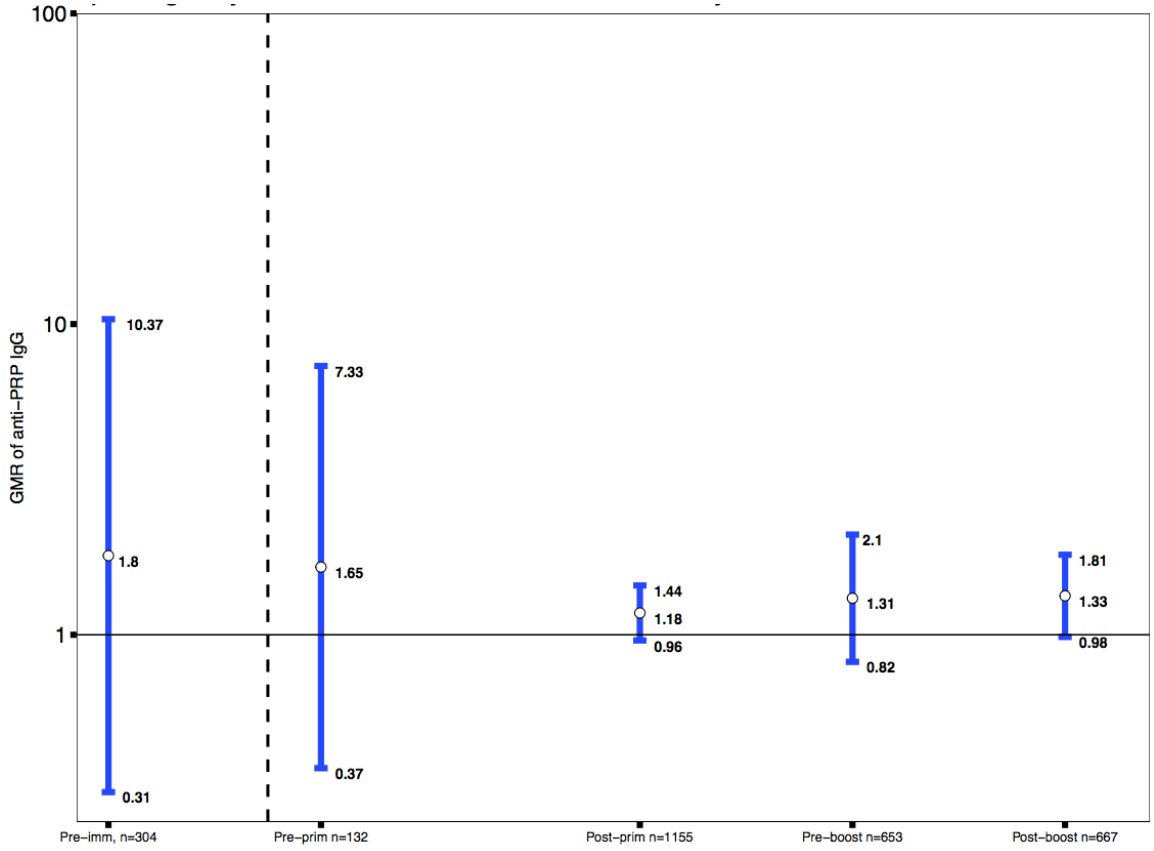


Figure 4. 9: Antibody responses to PRP in infants before and after primary immunization, and after booster immunization with tetanus- diphtheria -acellular pertussis vaccine.

Vertical blue lines indicate the GMR at the different time points with the 95% CI. Horizontal line indicates a GMR of 1. Numbers (n) indicate the number of infants whom serotype-specific antibody levels were available for meta-analysis. This figure shows no significant change in anti-PRP levels in infants born to Tdap-vaccinated vs –unvaccinated before and after primary and booster immunization. GMR was not computed post-

vaccination in pregnancy (no data), in maternal sera (one study), cord sera (one study) precluding meta-analysis.

4.4.3 Effect of maternal pertussis immunization during pregnancy on seroprotection rates against tetanus, diphtheria, *Haemophilus influenzae* type b and invasive pneumococcal disease

4.4.3.1 Seroprotection rates against tetanus disease

At time of vaccination with Tdap, ~93% of women had seroprotective antibody levels against tetanus disease. This rate increased to nearly 100% at birth in women vaccinated with Tdap during pregnancy and in cord sera both from pregnant women vaccinated and unvaccinated with TT-containing vaccines during pregnancy. Infants of women vaccinated with Tdap during pregnancy had significantly higher seroprotection rate against tetanus at time of primary and booster vaccination compared with infants of women unvaccinated with TT-containing vaccines during pregnancy, and comparable high seroprotection rate after primary and booster immunization (Figure 4.10).

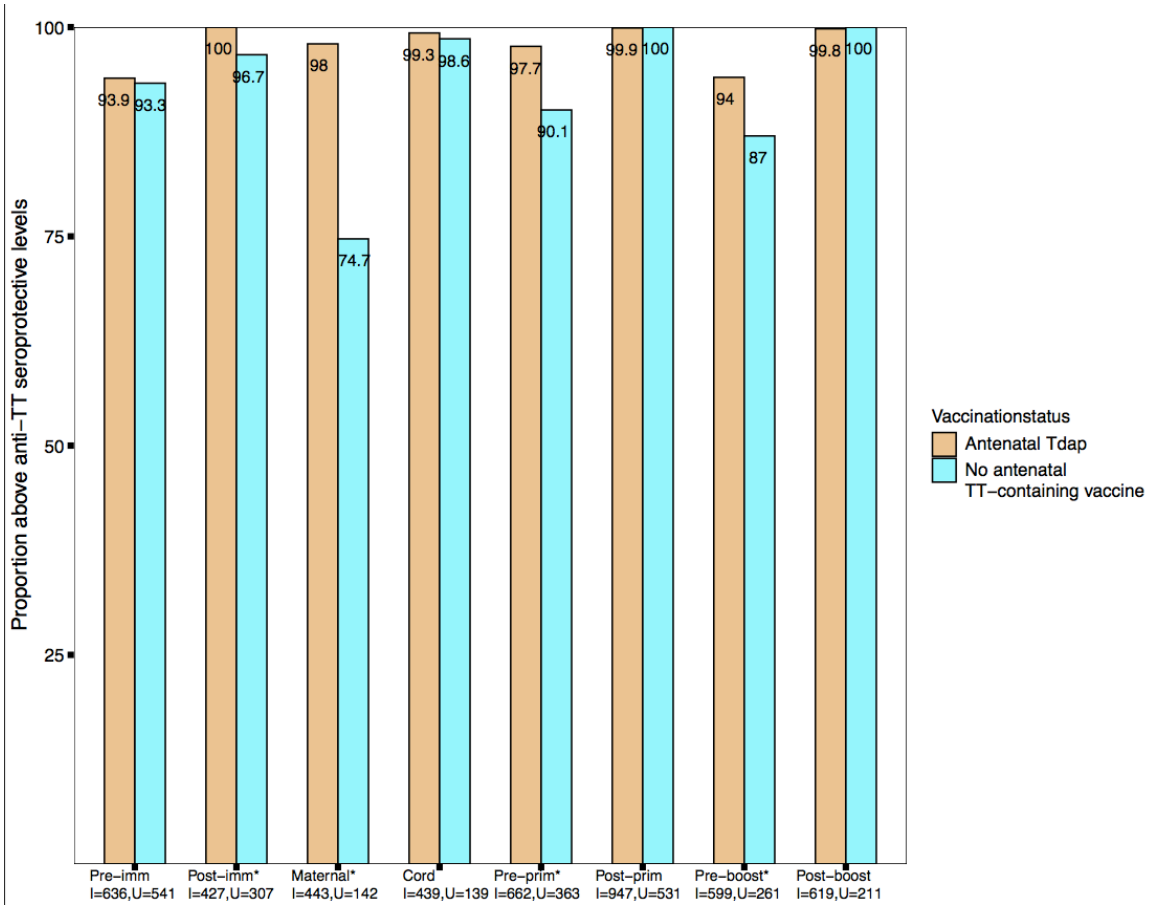


Figure 4. 10: Seroprotection rates against tetanus.

Rates of participants with anti–tetanus toxoid (TT) (anti-TT IgG ≥0.1 IU/mL) levels in women immunized with tetanus-diphtheria-acellular pertussis (Tdap) versus women who did not receive Tdap or diphtheria and tetanus toxoids (dT) vaccine or TT in pregnancy at time of immunization and after immunization, at delivery (women and cord blood); in infants born to women immunized Tdap versus infants of women not immunized with Tdap or dT or TT in pregnancy before and after primary immunization, before and after booster immunization with diphtheria-tetanus-acellular pertussis vaccine (*comparisons with p-values <0.05). This figure shows significantly higher seroprotection rates against tetanus disease in Tdap-vaccinated pregnant women after vaccination and at delivery, in their infants before primary immunization. This figure also shows significant lower seroprotection rates against tetanus in infants born to Tdap-vaccinated women at time of booster immunization. P-values for the time points: Pre-vac:P=0.806; Post-Vac:P= 0.001; Maternal:P<0.001; Cord:P= 0.754; Pre-prim:P<0.001;Post-prim:P=1; Pre-boost:P=0.001;Post-boost:P=1.

4.4.3.2 Seroprotection rates against diphtheria disease

At time of vaccination in pregnancy, nearly 60% of pregnant women had seroprotection antibody levels against diphtheria disease. Seroprotection rates against diphtheria disease were significantly higher in women vaccinated with Tdap during pregnancy after vaccination, at birth and in their infants at time of primary vaccination, compared with women who did not receive DT-containing vaccines during pregnancy. Infants of Tdap-vaccinated pregnant women had statistically significant lower seroprotection rates after primary immunization compared with infants of women who did not receive DT-containing vaccines during pregnancy. Nearly 60% of infants born to women vaccinated with Tdap during pregnancy had seroprotective anti-DT levels at time of booster immunization, increasing to nearly 100% after booster vaccination (Figure 4.11).

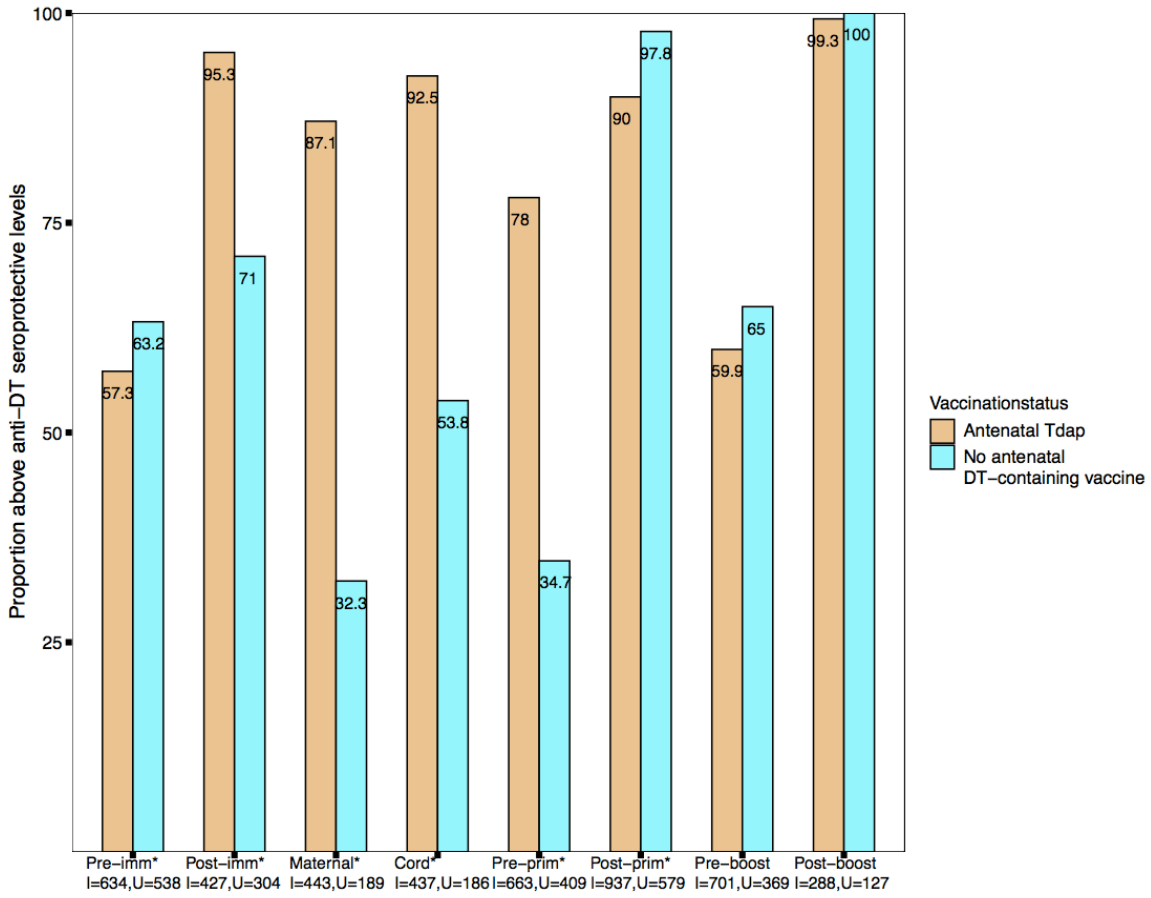


Figure 4. 11: Seroprotection rates against diphtheria.

Rates of participants with anti-diphtheria toxoid (DT) (anti-DT IgG ≥0.1 IU/mL) levels in women immunized with tetanus-diphtheria-acellular pertussis (Tdap) versus women who did not receive Tdap or diphtheria and tetanus toxoids (dT) vaccine in pregnancy at time of immunization and after immunization, at delivery (women and cord blood); in infants born to women immunized Tdap versus infants of women not immunized with Tdap or dT vaccine in pregnancy before and after primary immunization, before and after booster immunization with diphtheria-tetanus-acellular pertussis vaccine (*comparisons with p-values <0.05). This figure shows significantly higher seroprotection rates against diphtheria disease in Tdap-vaccinated pregnant women after vaccination and at delivery, in their infants at delivery and before primary immunization. This figure also shows significant lower seroprotection rates against diphtheria in infants born to Tdap-vaccinated women after primary immunization. P-values for the time points: Pre-vac:P= 0.045; Post-Vac:P<0.001; Maternal:P<0.001; Cord:P<0.001; Pre-prim:P<0.001;Post-prim: <0.001; Pre-boost:P=0.116;Post-boost:P=0.863.

4.4.3.3 Seroprotection against invasive pneumococcal disease

After primary immunization with PCV-13, seroprotection rates for serotypes 5, 6B, 9V, 19A, 23F were significantly lower in infants of Tdap-immunized women compared with unimmunized women (Figure 4.12).

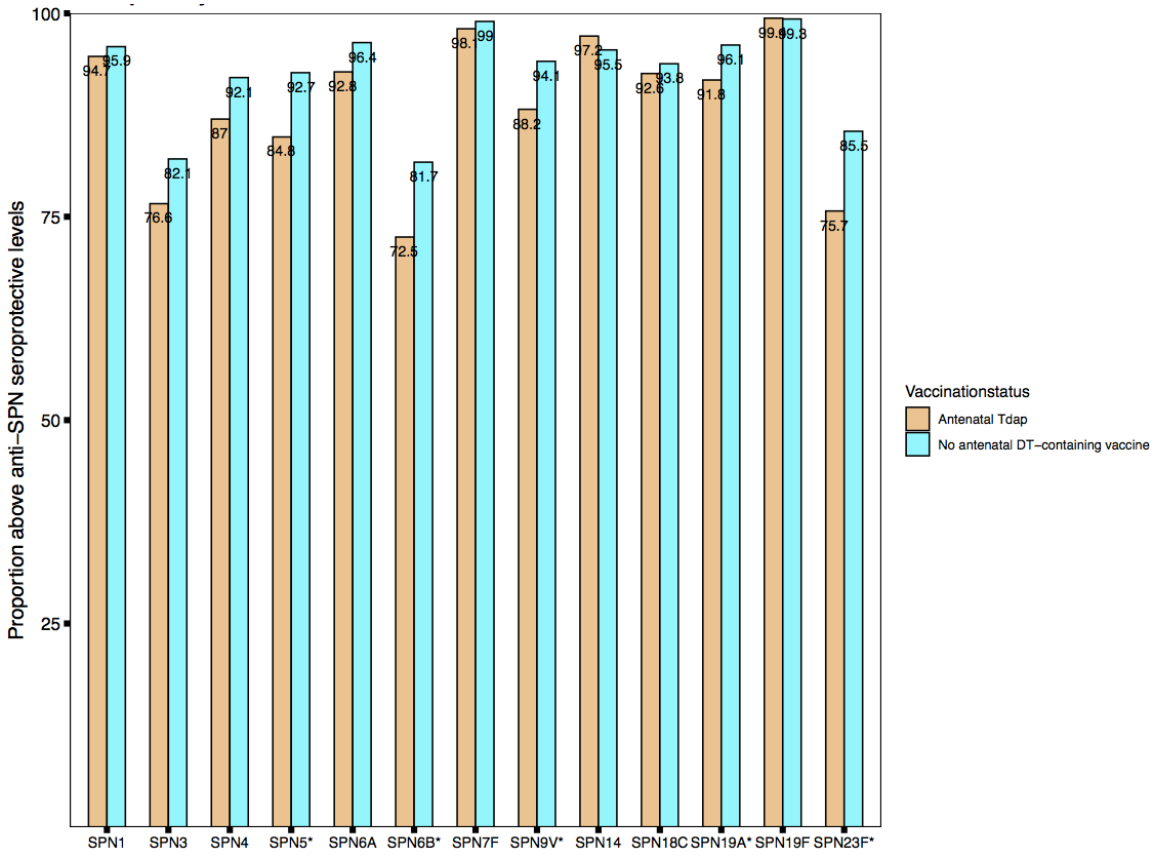


Figure 4. 12: Seroprotection rates against invasive pneumococcal disease.

Rates of subjects with anti–*streptococcus pneumonia* (SPN) IgG levels ≥0.35 mg/mL in infants in infants born to women immunized tetanus-diphtheria-acellular-pertussis (Tdap) versus infants of women not immunized with Tdap or diphtheria and tetanus toxoids vaccine in pregnancy after primary immunization with pneumococcal conjugate vaccine 13 (PCV13) (*denotes comparisons where p-values <0.05 [Serotypes 5, 6B, 9V, 19A, 23F]). Number of infants born to women vaccinated and unvaccinated during pregnancy was in the range of 304-323 and 279-290, respectively, for the different serotypes. This figure shows significantly lower seroprotection rates against Serotypes 5, 6B, 9V, 19A, 23F in infants of Tdap-vaccinated pregnant women after primary vaccination with PCV-13. P-values for the specific serotypes: 1:P= 0.642;3:P= 0.13;4:P= 0.057; 5:P= 0.004;

6A:P= 0.079 ; 6B:P= 0.009 ; 7F:P= 0.607; 9V:P= 0.016 ; 14:P= 0.358 ; 18C: 0.661 ; 19A:P= 0.048; 19F:P=1; 23F:P=0.003.

4.4.3.4 Seroprotection against Hib

At time of immunization in pregnancy, nearly 85% of pregnant women had protective antibody levels against Hib using the short term cut off for protection. Infants of women vaccinated with Tdap during pregnancy had significantly higher seroprotection rates against Hib after primary immunization, comparable rates at booster immunization, increasing to nearly 95% after booster immunization (Figure 4.13). Infants of women vaccinated with Tdap during pregnancy had significantly higher seroprotection rates against Hib after primary immunization, at booster immunization, and after booster immunization, when using the cut-off of long term protection against Hib (Figure 4.14).

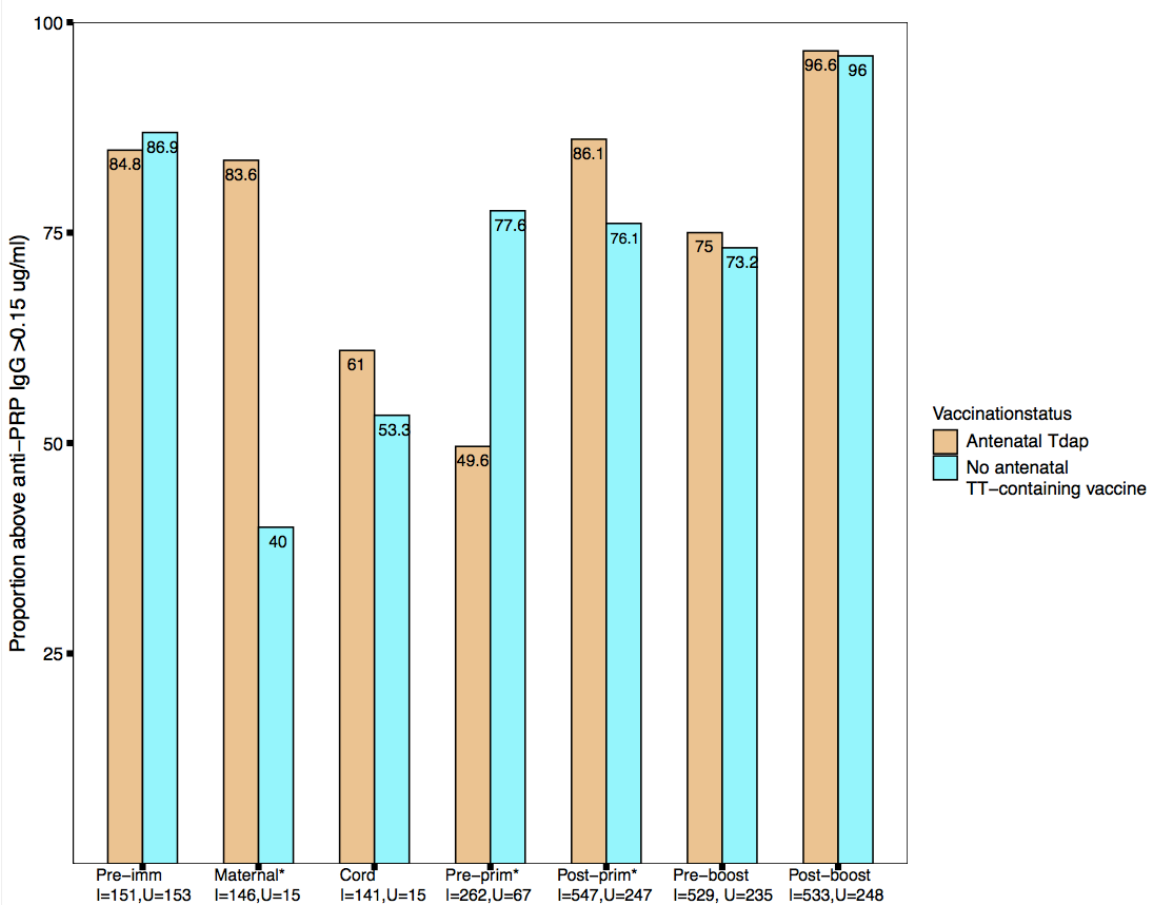


Figure 4. 13: Short term seroprotection rates against Hib disease.

Rates of subjects with seroprotective anti–PRP IgG levels (anti-PRP IgG ≥0.15 mcg/ml) Rates of subjects with anti–polyribosylribitol phosphate (PRP) IgG ≥0.15 mcg/ml in women immunized with tetanus-diphtheria-acellular pertussis (Tdap) versus women who did not receive Tdap vaccine or tetanus-toxoid (TT) vaccine in pregnancy at time of immunization, at delivery (women and cord blood); in infants born to women immunized Tdap versus infants of women not immunized with Tdap vaccine or TT vaccine in pregnancy before and after primary immunization with diphtheria-tetanus-acellular-pertussis-Hib (DTaP-Hib), before and after booster immunization with DTaP-Hib vaccine (*comparisons where p-values <0.05). This figure shows significantly lower seroprotection rates against Hib disease in infants of Tdap-vaccinated women before primary immunization and higher rates after primary vaccination. This figure also shows that most infants of Tdap-vaccinated and unvaccinated have achive protective levels after booster immunization. P-values for the time points: Pre-vac:P= 0.707; Maternal:P<0.001; Cord:P=0.765; Pre-prim:P<0.001;Post-prim: P= 0.001; Pre-boost:P= 0.651;Post-boost:P=0.801.

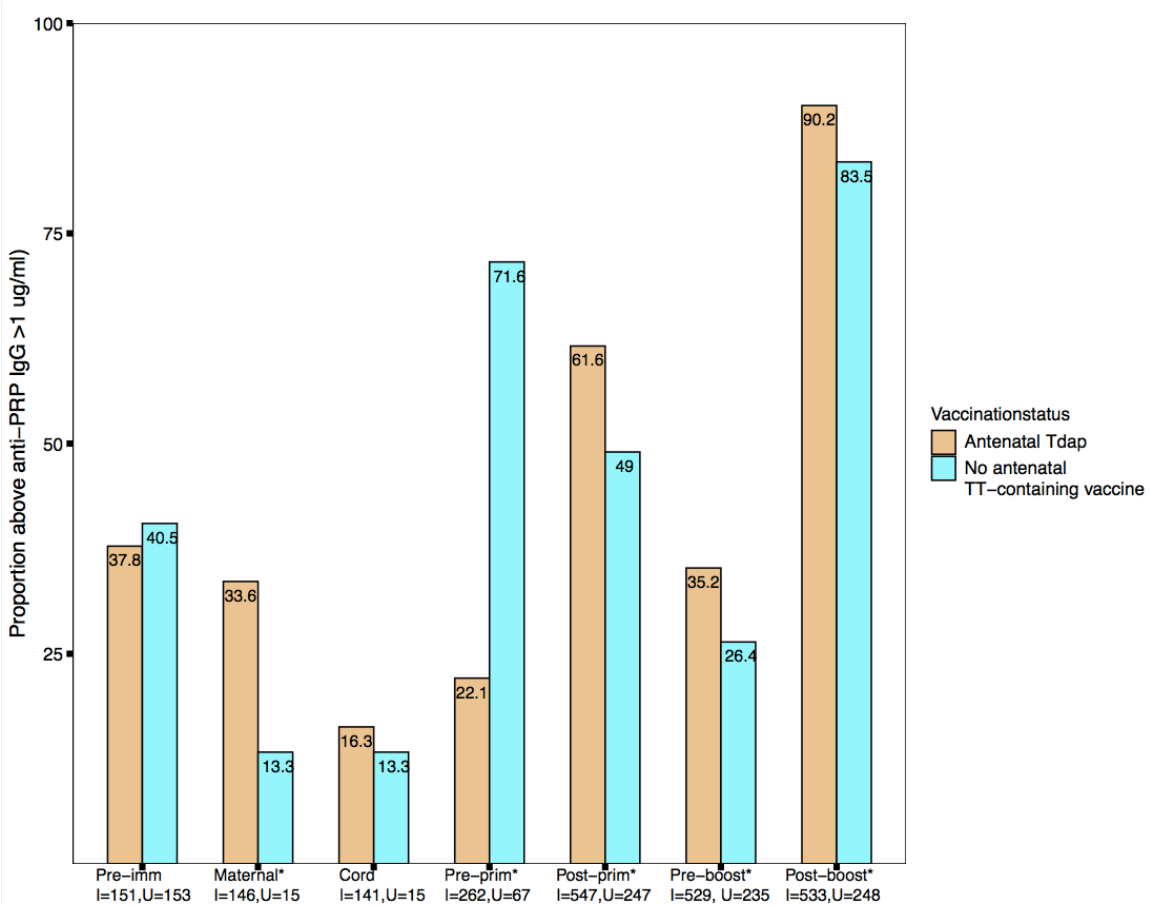


Figure 4. 14: Long term seroprotection rates against Hib disease.

Long-term seroprotection rates against *haemophilus influenzae* type b (Hib) disease. Rates of subjects with anti–polyribosylribitol phosphate (PRP) IgG ≥1 mcg/ml in women immunized with tetanus-diphtheria-acellular pertussis (Tdap) versus women who did not receive Tdap vaccine or tetanus-toxoid (TT) vaccine in pregnancy at time of immunization, at delivery (women and cord blood); in infants born to women immunized Tdap versus infants of women not immunized with Tdap vaccine or TT vaccine in pregnancy before and after primary immunization with diphtheria-tetanus-acellular-pertussis-Hib (DTaP-Hib), before and after booster immunization with DTaP-Hib vaccine (*comparisons where p-values <0.05). This figure shows significantly lower seroprotection rates against Hib disease in infants of Tdap-vaccinated women before primary immunization and higher rates after primary vaccination, at booster immunization and after booster immunization. P-values for the time points: Pre-vac:P= 0.705; Maternal:P= 0.189; Cord:P=1; Pre-prim:P<0.001;Post-prim: P= 0.001; Pre-boost:P= 0.021;Post-boost:P=0.009.

4.4.4 Factors affecting antibody response to Tdap vaccination during pregnancy

Among pregnant women immunized with Tdap in pregnancy, higher anti-*B. pertussis*-specific antibody levels at of immunization were associated with increase in vaccine-induced immune responses to Tdap vaccines, with doubling homologous antigen-specific antibody levels resulting in 14%-28% higher maternal post-immunization antibody levels measured at delivery. Doubling of anti-DT antibody levels at time of vaccination resulted in 24% higher post-immunization antibody levels measured at delivery, while pre-existing anti-TT IgG level at time of vaccination did not affect maternal anti-TT IgG levels measured at delivery. Maternal age, and timing of vaccination in pregnancy, did not affect post-immunization antibody levels measured at delivery (Figure 4.15).

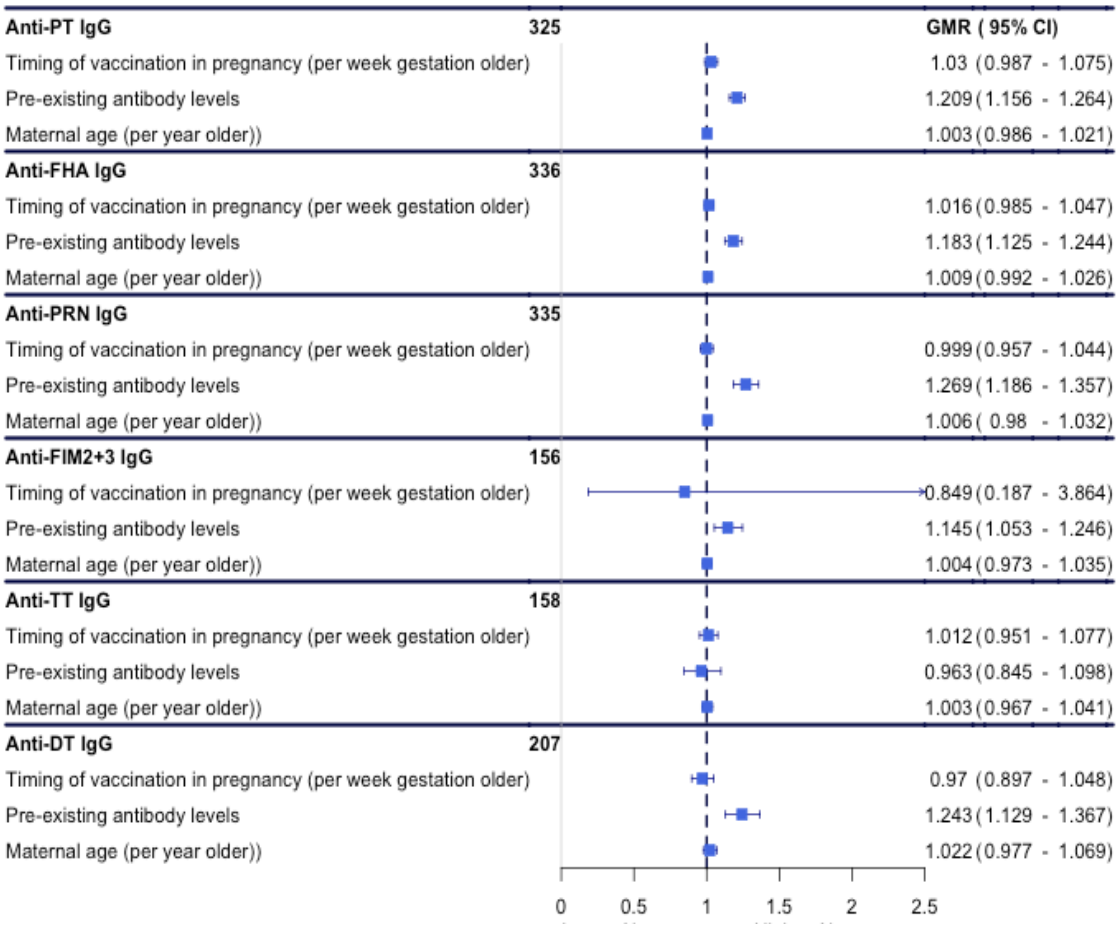


Figure 4. 15: Variables affecting antibody immune response to Tdap vaccine during pregnancy.

Effect of different variables on antibody levels against PT, FHA, PRN, FIM2/3, TT, DT, after vaccination with Tdap vaccine during pregnancy. The GMR indicates the relative increase (fold rise) in antibody levels after primary vaccination that is associated with 1 unit change in a co-variate. GMRs less than 1.0 indicate that a 1 unit increase in the variable that is associated with lower antibody levels after primary vaccination, while GMR >1 indicates that a 1 unit increase in the variable is associated with higher antibody levels after the third dose. This figure shows that doubling of homologous antigen-specific anti-*B. pertussis* antibody levels results in 14%-28% higher maternal post-immunization antibody levels measured at delivery. Doubling of anti-DT antibody levels at time of vaccination resulted in 24% higher post-immunization antibody levels measured at delivery.

4.4.5 Factors affecting infants' antibody immune response to primary immunization

4.4.5.1 Infants of Tdap-vaccinated women

Among infants born to women immunized against pertussis in pregnancy, anti-PT and anti-DT antibody levels at primary immunization were associated with reduction of antibody immune responses to TDaP vaccines, with doubling antibody levels resulting in 8% and 10% lower post-primary immunization levels, respectively (Figure 4.16a).
Timing of vaccination in pregnancy did not affect post-primary immunization anti-PT, anti-FHA, anti-PRN, anti-FIM, anti-TT and anti-DT antibody levels (Figure 4.16a).
Age at initiation did not affect post-primary immunization anti-PT, anti-FHA, anti-FIM, anti-TT and anti-DT antibody levels. Older age at first vaccination was associated with higher post-vaccination antibody levels against PRN (Figure 4.16a).
Vaccination schedule did not affect anti-PT, anti-FHA, anti-PRN, anti-FIM and anti-DT antibody levels, while 2,3,4 months schedule that was associated with lower post-immunization anti-TT antibody levels, compared with 2,4,6 months vaccination schedule.

4.4.5.2 Infants of unvaccinated women

Among infants born to women unimmunized with Tdap during pregnancy, doubling of anti-*B. pertussis* antibodies resulting in 8-15% lower post-primary immunization levels (Figure 4.16b). Anti-TT and anti-DT antibody levels at primary immunization were associated with reduction of antibody immune responses to TDaP vaccines, with doubling of antibody levels resulting in 12% and 17% lower post-primary immunization levels, respectively. Older age at initiation of primary vaccination did not affect post-

immunization anti-PT, anti-FHA, anti-FIM2/3 and anti-TT antibody levels, but was associated with higher post-primary immunization antibody levels against PRN and DT (Figure 4.16b). Vaccination schedule did not anti-PT, anti-FHA, anti-PRN, anti-FIM antibody levels, while vaccination schedule 2,3,4 months was associated with lower post-immunization anti-TT and anti-DT antibody levels, compared with 2,4,6 months vaccination schedule (Figure 4.16b).

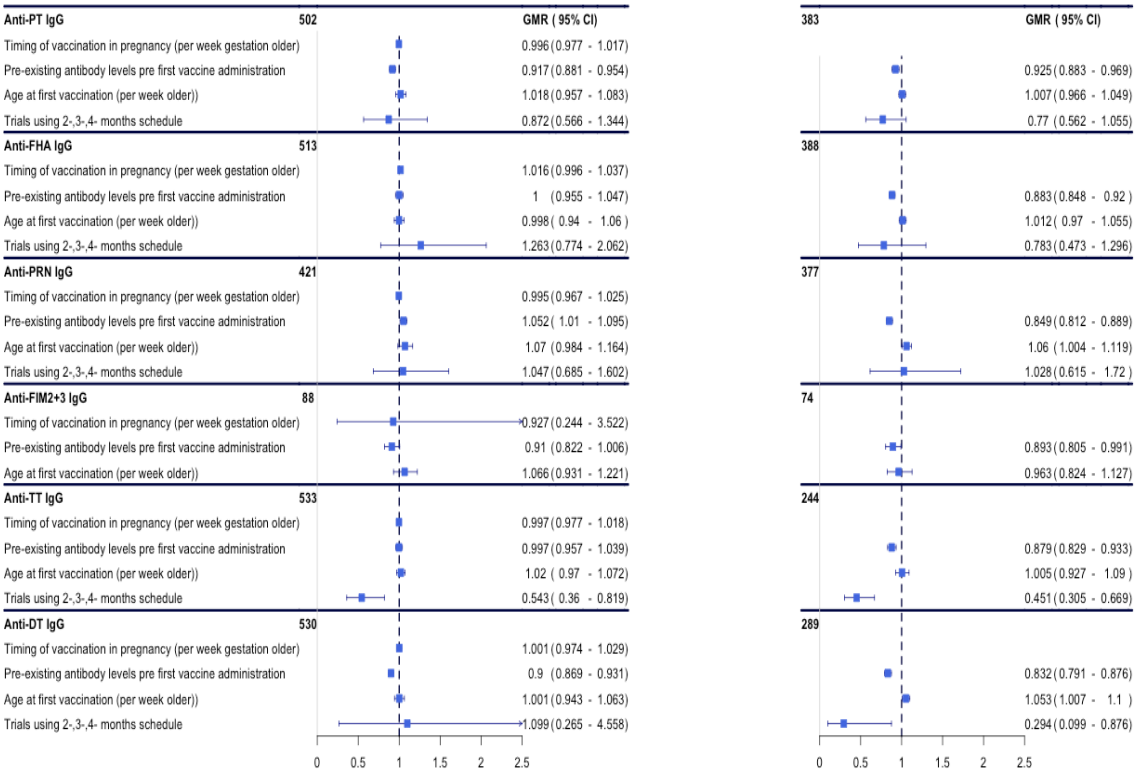


Figure 4. 16: Variables affecting antibody immune response to primary immunization with DTaP vaccine during infancy.

Effect of different variables on antibody levels against PT, FHA, PRN, FIM2/3, TT, DT, after the third dose of vaccination with Diphtheria-Tetanus-acellular pertussis vaccine (2-, 3-, and 4-month schedules and 2-, 4- and, 6-month schedules) in infants born to women vaccinated against pertussis during pregnancy (left). The GMR indicates the relative increase (fold rise) in antibody levels after primary vaccination that is associated with 1 unit change in a co-variate. GMRs less than 1.0 indicate that a 1 unit increase in the variable (for continuous variables, or a 2-3-4 month schedule) is associated with lower antibody levels after primary vaccination, while GMR >1 indicates that a 1 unit increase in the variable is associated with higher antibody levels after the third dose; Results of infants born to women unvaccinated against pertussis during pregnancy (right).

4.4.6 Factors affecting infants' antibody immune response to booster immunization

4.4.6.1 Infants of Tdap-vaccinated mothers

Among infants born to women immunized with Tdap during pregnancy, doubling of anti-PT and anti-FHA IgG levels at primary immunization resulted in 10% (95% CI: 10-15%) and 8% (95% CI:1-15%) lower post-booster immunization levels. Doubling of anti-PRN IgG levels at primary immunization resulted in 12% (95% CI: 2-23%) higher post-booster immunization levels.

Time difference between Tdap vaccination and delivery did not affect post-primary immunization anti-PT, anti-FHA, anti-PRN, anti-TT and anti-DT antibody levels (Figure 4.17 a). Age at initiation of primary vaccination and at booster immunization did not affect anti-PT, anti-FHA and anti-PRN antibody levels. Spacing of vaccinations (2,3,4 vs. 2,4,6 months) did not affect anti-PT, anti-PRN, anti-TT and anti-DT post-booster immunization antibody levels, while vaccination schedule 2,3,4 months was associated with higher post-immunization anti-FHA antibody levels, compared with 2,4,6 months vaccination schedule (Figure 4.17 a).

4.4.6.2 Infants of unvaccinated mothers

Among infants born to women immunized against pertussis in pregnancy, doubling of anti-FHA antibody levels at primary immunization resulted in 8% lower post-booster immunization levels (Figure 4.17 b). Age at initiation of primary vaccination did not affect post-immunization anti-PT, anti-FHA and anti-PRN, anti-TT and anti-DT antibody

levels. Older age at booster vaccination did not affect post-immunization anti-FHA, and anti-TT antibody levels, but was associated with higher post-primary immunization antibody levels against PT. Spacing of vaccinations (2,3,4 vs. 2,4,6 months) did not affect post-booster immunization antibody levels except for anti-PT where 2,3,4 months schedule was associated with lower post-immunization anti- antibody levels, compared with 2,4,6 months vaccination schedule (Figure 4.17 b).

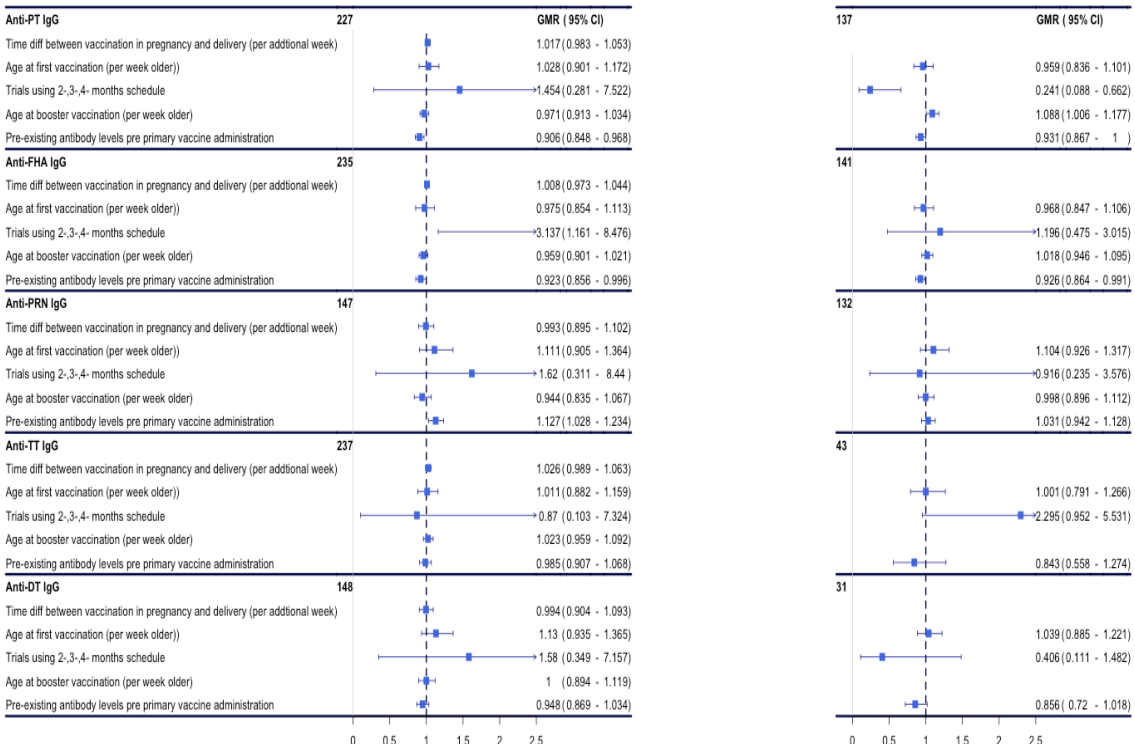


Figure 4. 17: Variables affecting antibody immune response to booster immunization with DTap vaccine during infancy.

Effect of different variables on antibody levels against PT, FHA, PRN, TT, DT, after booster vaccination with Diphtheria-Tetanus-acellular pertussis vaccine (2-, 3-, and 4-month schedules and 2-, 4- and, 6-month schedules) in infants born to women vaccinated against pertussis during pregnancy (left). The GMR indicates the relative increase (fold rise) in antibody levels after primary vaccination that is associated with 1 unit change in a co-variate. GMRs less than 1.0 indicate that a 1 unit increase in the variable (for continuous variables, or a 2-3-4 month schedule) is associated with lower antibody levels after primary vaccination, while GMR >1 indicates that a 1 unit increase in the variable is associated with higher antibody levels after the third dose; Results of infants born to women unvaccinated against pertussis during pregnancy (right).

4.5 Discussion

This large, international, longitudinal meta-analysis demonstrates reduction in infants' immune responses to pertussis, diphtheria and some SPN serotypes, after Tdap immunization in pregnancy. This effect was observed after primary and booster immunization. This also resulted in lower sero-protection rates for some SPN serotypes and diphtheria after primary immunization in infants born to women immunized with Tdap in pregnancy when compared to unimmunized women. Enhanced immune response to tetanus and Hib vaccine components was observed in infants born to women vaccinated with Tdap during pregnancy compared with infants of unimmunized women. High maternally derived antibody levels have inhibitory effect to antibody immune response to PT and DT among infants of vaccinated mothers and this effect persisted at booster immunization for PT. Primary vaccination schedule at 2,3,4 months, compared with 2,4,6 months schedule was associated with lower post-primary anti-TT antibody levels and higher post-booster anti-FHA levels, in infants born to women vaccinated and unvaccinated with Tdap during pregnancy.

These data have important implications in establishing the effect of pertussis immunization in pregnancy on the antibody immune response to various vaccine antigens in infancy and can assist public health policy makers in countries where maternal pertussis immunization programs have been recommended.

In this chapter, I report on the different statistical approaches which were undertaken to address the question of modification of infants' immune responses after vaccination with Tdap during pregnancy. The GMR of antigen-specific antibodies of infants born to vaccinated compared with unvaccinated women at different time points. A GMR and an upper bound of the GMR CI below 1 implies significantly lower antibody levels (or titers) in infants born to vaccinated compared with unvaccinated women, and thus supports interference. This approach has the advantage of adjustment for co-variables that could affect the infants' immune responses, such as gestational age, sex or age at vaccination. However, this approach does not take into account whether a reduction in antibody levels is associated with a reduction in protection against specific diseases. Investigating whether a reduction in antibody levels is associated with a potentially higher risk of infection is feasible for diseases for which a correlate of protection [COP] exists is thus another approach to investigate interference and can be used for diseases like tetanus, diphtheria, invasive pneumococcal disease, and Hib. A statistically significant reduction in seroprotection rate in infants born to vaccinated compared with unvaccinated women might also be used to define interference. However, this approach is not possible for vaccination against diseases for which a well-defined COP does not exist (e.g. pertussis).

This meta-analysis shows reduced immune responses to pertussis in infants born to women vaccinated against pertussis during pregnancy after primary and booster immunization. Several studies from the pre-maternal pertussis immunization era suggested that high pre-existing maternally derived antibody titers can have a suppressive

effect on infants' immune responses to primary immunization against pertussis[440-443]. This meta-analysis provides further support to these findings in the era of maternal pertussis immunization and also extends these findings to pertussis booster immunization. This reduction might potentially put infants born to women immunized against pertussis in pregnancy at increased risk for pertussis disease later in their infancy. However, the clinical significance of these findings is not clear, as current surveillance data from the UK do not indicate that this reduction in anti-pertussis antibody levels is clinically significant [390]. This is because incidence of pertussis disease in infants after primary and booster immunization did not increase years after the introduction of maternal pertussis immunization program in the UK[390]. However, more data are needed to definitely assess the true clinical significance of such reduction as the cohort of infants born to pertussis-vaccinated women is increasing[464].

As current formulations of Tdap vaccines used in pregnancy also include tetanus toxoids and diphtheria toxoids, infants' immune responses to TT and DT components of vaccines and vaccines conjugated these toxoids as carrier proteins (e.g. Hib vaccine, and PCVs) might also be modified. I found reduced immune responses to diphtheria and some SPN serotypes in infants born to women vaccinated with Tdap during pregnancy after primary and booster immunization, and a lower sero-protection rates for some SPN serotypes and diphtheria after primary immunization. This might increase the risk of infection with these pathogens in infants born to immunized women. Although diphtheria disease incidence has decreased since the implementation of 3 diphtheria-containing vaccine

doses, outbreaks do still occur, especially in low-middle income countries and among unvaccinated subjects[465] . In high-income countries diphtheria is a rare disease[466]. In Canada, between 1993-2012, 19 cases were reported with a range of 0-4 cases annually[467]. This supports the need to enhance surveillance for diphtheria disease among infants born to women immunized against pertussis in infancy. The clinical significance of reduction of seroprotection rates against SPN is not clear and should be investigated by future research. While anti-SPN >0.35 ug/ml cut-off is used for licensure of pneumococcal vaccines, some studies showed that the COP are serotype-specific, and is different for protection against disease compared with colonization[468, 469]. In addition, there are changes in the pneumococcal vaccination programs in different countries and these should be considered in the setting of immunization in pregnancy. In the UK, PCV primary vaccination has been reduced from 2 primary doses followed by a booster dose to a one primary dose followed by a booster dose[470]. This meta-analysis showed also enhanced immune response to tetanus and Hib vaccine components was observed in infants born to women vaccinated with Tdap during pregnancy compared with infants of unimmunized women.

The mechanism of interference has not been fully investigated. Inhibition of B cell activation through the FcγRIIB on B cells has been proposed as a possible mechanism of interference. Specifically, vaccine antigen–antibody complexes cross-link the B-cell receptor with the FcγRIIB, thus inhibiting antigen specific B-cell activation[471]. It was recently shown in a mouse model of influenza vaccination, that interference of influenza vaccination with maternal antibodies was antigen-specific and in a dose-dependent

manner. In addition, maternal immunization was associated with reduction in the number of germinal center B cells that differentiate into plasma cells and memory B cells, potentially explaining the durable effect of maternal immunization during pregnancy on booster immunization in infancy[472]. Other proposed mechanisms of interference include removal of vaccine antigen by macrophages through binding to FcR, although this has never been shown[473]. Inhibition of B cell via epitope masking is another suggested mechanism. In this mechanism, the B cell epitopes on a vaccine antigen(s) are covered by antibodies and thus are not recognized by B cells[473]. However, this does not explain the inhibitory effect observed following booster immunization.

The association between Tdap administration in pregnancy and infants immunization PCV13 is probably mediated via anti-DT antibodies that are transferred to infants, because each of the 13 polysaccharides included in PCV13 is conjugated to CRM197 (a non-toxic mutant of DT). This is supported by finding from a recent meta-analysis that reported that maternal pre-existing anti-DT levels were associated with lower immune response to serotypes 4, 6B and 9V after vaccination with PCV-7, and lower response to 19F (the only serotype conjugated to diphtheria protein) after vaccination with PCV-10[474]. The mechanism of enhancement of immune response to TT and vaccines conjugated to TT is yet to be explored. Studies not in the setting of maternal immunization showed that vaccination with Hib vaccine conjugated to TT was associated with higher anti-PRP levels when given concomitantly with meningococcal serogroup C polysaccharide vaccine conjugated to TT, supporting the enhancement of immunogenicity of Hib vaccines conjugated to TT[475, 476].

This meta-analysis also identified factors that have the potential to affect vaccine-induced immune response in the setting of maternal immunization. In pregnant women, high antigen-specific antibody levels are associated with higher anti-*B. pertussis* and anti-DT antibody levels at delivery. There are scarce data assessing the effect of pre-existing immunity on immune response to vaccination during pregnancy. Data from non-pregnant population showed a positive relationship between pre-vaccination antibody levels and post-immunization antibody response. Among young children (4 years of age), higher pre-vaccination influenza titres were associated with higher post-vaccination odds ratios for seroprotection[477]. Among subjects older than 61 years of age, those who were seronegative at time of vaccination, might not seroconvert after one dose of influenza vaccine[478].

Maternal age (range 16-44 years) was not associated with post-immunization antibody response. Age is an important factor affecting immune response to vaccination especially in the extremes of age (newborns and elderly) (reviewed in[449]). It is thus possible that within the range of age of pregnant women, this factor is not affecting immune response. Timing of vaccination during pregnancy was also not associated with immune response in this analysis. However, this should be interpreted with caution, as the included studies were not primarily designed to answer this question. In addition, antibody response to Previous studies showed that vaccination in the second trimester is associated with higher anti-*B. pertussis* antibody levels than vaccination in the third trimester[479], and vaccination

in the early third trimester is associated with higher anti-*B. pertussis* antibody levels at delivery when compared with vaccination in the late third trimester[439].

I also found that high maternally-derived anti-PT and anti-DT antibody levels at primary immunization were independent factors associated with lower post-primary antibody levels to the same vaccine antigen. This inhibitory effect of maternal antibodies extended to booster immunization for PT. This finding is consistent with previous literature showing that high maternal antibody concentrations reduces infants' vaccine immune responses to primary and booster immunization[474]. A recent meta-analysis found that preexisting maternally-derived antibody levels, not in the context of maternal immunization, were associated with inhibition of immune response to primary vaccination of 20/21 antigens including pertussis antigens (PT, FHA, PRN), diphtheria and tetanus[474]. The latter meta-analysis also showed that the inhibitory effect of maternal antibodies extended to booster immunization for PT, FHA and PRN. Thus, my findings in this chapter further confirm these findings and extend them to infants born to women vaccinated against pertussis during pregnancy. These findings should stimulate the search for factors affecting antibody levels at primary immunization, for example timing of vaccination in pregnancy and delaying primary immunization. This is because vaccination early in pregnancy (the second trimester or early third trimester) were associated with higher *B. pertussis*-specific antibody levels compared to later times in pregnancy[406, 437, 439]. The effect of timing of vaccination against pertussis in pregnancy on post-immunization anti-*B. pertussis* antibody levels is being investigated (NCT03908164). Delaying initiation of primary immunization beyond 2 months of age is

another approach and has been recommended in some HICs (the Netherlands)[480]. While delaying pertussis primary immunization in the context of maternal immunization might minimize the inhibitory effect of maternal immunization, additional aspects need to be considered. This approach might not be ideal for optimal protection against other vaccine-preventable diseases included in aP vaccine formulations (e.g. hepatitis B, Hib), for which delaying primary immunization might increase the risk for these infections.

In addition to pre-existing antibody levels, I found that primary vaccination schedule at 2,3,4 months, compared with 2,4,6 months schedule was associated with lower post-primary anti-TT antibody levels and lower post-booster anti-PT levels, in infants born to women vaccinated and unvaccinated with Tdap during pregnancy. Spacing of vaccine doses has been shown to affect post-immunization immune response with findings supporting that vaccine schedules that allow longer intervals between the vaccine doses are associated with higher immune responses. Infants immunized with a 2, 4, 6-months primary vaccination schedule had a significantly higher immune response to pertussis (measured by neutralization assay) than infants immunized with a 2-, 3, 4-months schedule[481]. In another study, immunization with 3, 5, 9-months schedule was associated with significantly higher antibody levels against diphtheria and tetanus, than immunization with 2, 3, 4-months schedule[442].

Age at initiation of primary vaccination was not found to affect post-primary and post-booster antibody levels. This could be explained by the narrow range of initiation of primary vaccination in this subgroup (restricted to 2,3,4 or 2,4,6 primary schedules).

This meta-analysis highlights the need to enhance pertussis, diphtheria and invasive pneumococcal disease surveillance in countries implementing maternal pertussis programs in order to investigate the clinical significance of interference on vaccine-preventable disease in infancy. In addition, this meta-analysis also highlights the need to determine the serological correlate(s) of protection for pertussis disease against which clinical trails results can be tested. This is of particular importance for countries where surveillance systems are not well established.

This project has a number of strengths. This is the first detailed and longitudinal analysis of the largest number of samples combined to establish the effect of maternal pertussis immunization on immune response to different vaccine antigens, routinely given to infants worldwide. Given the individual-participant nature of this meta-analysis, I was able to determine seroprotection rates for some vaccine-preventable disease and investigate modifiable factors that could affect vaccine-induced immune responses in the setting of maternal pertussis immunization.

This project has also some limitations. The lack of serological correlate of protection for pertussis disease, against which data can be tested, necessitates that these results should be backed up by epidemiological surveillance data. This meta-analysis included randomized and non-randomized studies. While adjustments for different co-variates were made in different analyses, there is still a potential of residual confounding. Residual confounding happens when there is error in measurement of a co-variate or when a confounding co-variate is not measured. An important limitation is that most

studies were conducted in HICs were aP is used in infants' immunizations programs. As a significant proportion of pertussis cases are in low-middle income countries using whole cell pertussis vaccines, these data are less relevant to countries where whole-cell pertussis is being used for primary and booster vaccination.

5. Overall conclusions and future directions

5.1 Conclusions

During my PhD, I report that the highest morbidity and mortality from pertussis is among infants <2 months of age. Age of <16 weeks, encephalopathy and prematurity were independently associated with an increased risk for intensive-care unit admission. Age of <4 weeks and prematurity were independently associated with increased risk for death. These data determined the burden of pertussis disease among young infants in Canada and supported the recommendation for pertussis immunization during pregnancy because these young and highly vulnerable infants cannot be protected by the infant program which commences at 2 months of age.

I also developed a novel analytical approach that allowed me to comprehensively characterize the full avidity profile of anti-pertussis antibodies using a range of bond-breaking agent. Applying this methodology on cord samples collected from women vaccinated against pertussis during pregnancy I found that vaccination against pertussis during pregnancy was associated with high levels of high avidity antibodies. Pertussis vaccination during 28–32 weeks gestation was associated with higher anti-pertussis IgG avidity compared with vaccination during 33–36 weeks gestation. These findings support vaccination against pertussis during the early third trimester of pregnancy.

Using a meta-analysis of individual participant level data of antibody response after primary and/or booster immunization in infants born to mothers immunized against pertussis in pregnancy *vs.* unimmunized women, I found lower antibody levels to pertussis, diphtheria and some *Streptococcus pneumoniae* serotypes in infants born to women vaccinated against pertussis during pregnancy compared with infants of unvaccinated women. Furthermore, I found that antibody levels at time of primary immunization are the main determinant of this lower immune response in infants after their own vaccination. These data provide evidence that immunization against pertussis in pregnancy modifies the infants' active immune response to their own vaccination. This also supports enhanced surveillance of pertussis, diphtheria and invasive pneumococcal disease in infants to determine the clinical significance of this effect. This also supports delaying primary pertussis immunization in infants born to women vaccinated against pertussis during pregnancy.

5.2 Future directions

5.2.1 Further investigation of function of anti-*B. pertussis* antibodies

Functions of anti-*B. pertussis* antibodies, other than avidity, should be explored by future research. This will be important and could be deployed to investigate correlates of protection against pertussis disease.

5.2.1.1. Anti-adhesion of anti- *B. pertussis* antibodies

FHA and FIM are presumed to mediate adherence of *B. pertussis* to host tissues[482]. aP vaccines efficiently protect against the symptomatic disease of pertussis disease but fail to prevent colonization[483]. Thus, it is important to explore the effect of anti-*B. pertussis* vaccine-induced antibodies on colonization by testing the functionality of vaccine-induced antibodies in inhibiting the bacterial adhesion in a cell culture. Adhesion assay offers the possibility to test the functionality of vaccine-induced antibodies in inhibiting the *B. pertussis* adhesion to the host cells. Indeed, during my PhD, I developed an assay that measures inhibition of anti-*B. pertussis* antibodies of the adhesion of *B. pertussis* to epithelial cells based on previous publication[484] using *B. pertussis* Tohama 1 strain (Kindly provided by Dr. Rachel Fernandez, Department of Microbiology and Immunology, University of British Columbia, Canada). This assay can be used in future studies.

5.2.1.2. Antibody-dependent complement deposition

B. pertussis binds C4b-binding protein via its surface protein FHA. The host complement regulator C4b-binding protein inhibits complement activation (reviewed in[485])**.** Vaccines should contain antigen preparations that, in addition to inducing long-lasting immunity, can prevent suppression of the innate immune response by *B. pertussis*. To generate the membrane attack complex, antigen on the surface of the bacteria must complex with complement-fixing antibody. It is important to explore whether aP vaccines elicit deposition of complement on target cells. For example, studies with serum from

vaccinated subjects depleted and not depleted for complement can be used to test bactericidal activity of antibodies against pertussis.

5.2.1.3. Antibody-dependent phagocytosis

Neutrophils are an important part of the innate immune response, and opsonizing antibodies enable neutrophils to contribute to microbial clearance in the presence of an acquired immune response. Neutrophil infiltration was observed in the lungs of mice following aerosol challenge with *B. pertussis*[486]. Neutrophils contribute to clearance of *B. pertussis*, in a naive mouse model[487]. Two virulence factors (FHA and adenylate cyclase toxin) influence phagocytosis of *B. pertussis* by neutrophils[488-490]. Previous studies have shown that opsonization with a human immune serum could inhibit both attachment and phagocytosis of wild-type *B. pertussis* by neutrophils[488-491].

The role of antibody-dependent cellular phagocytosis in protection from pertussis has not also been established. During my PhD, I developed (Technologist, Tony Harn, Vaccine Evaluation Center, Vancouver, Canada) an assay to test the phagocytosis of *B. pertussis* antigen-antibody complexes based on previous literature[492, 493]. This assay can be used in future studies.

5.2.1.4. Antibody-dependent NK cell activation

Following *B. pertussis* infection of mice, NK cells provide the initial source of IFN–γ, which is essential for containing the bacteria within the respiratory tract[494], and

promoting Th1 responses that mediate bacterial clearance[257, 495]. Depletion of NK cells resulted in dissemination of *B. pertussis* to the liver[494]. NK cells crosslink CD16 by antigen–antibody immune complexes and thus might contribute to immune responses after vaccination[496]. Antibodies produced after vaccination against pertussis could be tested for their NK cell activation.

5.2.1.5. Antibody-dependent respiratory burst

B. pertussis survives intra-cellularly within human polymorphonuclear leukocytes. Internalization of these bacteria is IgG dependent, and survival depends, at least in part, on inhibition of phagosome-lysosome fusion. Two purified exoproducts of *B. pertussis*, PT and adenylate cyclase toxin, have also been reported to inhibit phagocyte respiratory burst activity[497-503]. Antibodies produced after vaccination against pertussis should be tested for their inhibition of respiratory burst in human polymorphonuclear leukocytes.

5.2.1.6. Neutralization of *B. pertussis* by anti-*B. pertussis* antibodies

Toxins from *B. pertussis* induces clustering of Chinese hamster ovary cells[504], and anti-*B. pertussis* antibodies with neutralizing activity against PT have been observed to inhibit this effect[505]. A significant rise in anti-PT antibodies titer by neutralization test was found in 70% of patients with culture-confirmed pertussis infection[506]. Thus, neutralization of *B. pertussis* by anti-*B. pertussis* antibodies following vaccination should be explored.

5.2.2. Gaps in knowledge in immunization against pertussis during pregnancy

Although during my PhD I have addressed critical gaps in knowledge related to immunization against pertussis in pregnancy, there are still important gaps in knowledge that should be addressed by future research

5.2.2.1 The clinical significance of interference to pertussis immunization in pregnancy

In Chapter 4, I found that infants of women immunized against pertussis in pregnancy had lower anti-*B. pertussis*, anti-diphtheria and anti-pneumococcal antibodies levels. Currently, there are data to support that there is no increase in pertussis incidence later in infancy in infants born to women immunized in pregnancy. However, enhanced surveillance should continue to ensure that this is not a clinical problem in the years to follow. This is also true for diphtheria and invasive pneumococcal disease, where surveillance needs to be enhanced and data provided by future research.

5.2.2.2 Establishing the immune correlates for protection against pertussis disease

This is important for further evaluation of maternal vaccination strategies as it might provide clues for clinical significance of lower anti-*B. pertussis* antibody levels in settings where surveillance is problematic (e.g. Low-middle income countries[464]). This could be studied from cohort studies in which pre-existing immunity (anti-*B. pertussis*

antibodies) is known and later subsequent infection with *B. pertussis* or not is captured. The association between pre-existing immunity and later infection could provide clues for potential correlate of protection.

5.2.2.3 Understanding the cellular basis of the transplacental transfer of maternal anti- *B. pertussis* antibody

\While it is now well established that FcRn mediates transfer of IgG across the placenta, involvement of other receptors should be explored and will aid in better understanding the cellular basis of the transplacental transfer of maternal anti- *B. pertussis* antibody and it's relation with timing of delivery. This could be done using immunohistochemistry studies on placental tissues.

5.2.2.4 Investigating the induction of anti-*B. pertussis* IgG subclasses

Investigating the induction of *B. pertussis* IgG subclasses after vaccination could help evaluate new pertussis vaccines. Vaccines capable of inducing IgG1 and IgG3 will be preferable to be used in pregnant population as it will be expected that these vaccines will lead to higher anti-*B. pertussis* antibodies in newborns and infants.

5.2.2.5 The effect of timing of immunization during pregnancy on the function of anti- *B. pertussis* antibodies

During my PhD, I found that vaccination early in the third trimester is associated with higher anti-*B. pertussis* antibody levels in newborns compared to vaccination in late third

trimester, confirming my previous findings in a more detailed approach. However, it will be important also to explore the effect of timing of immunization during pregnancy on other functions of anti- *B. pertussis* antibodies. Such data will provide further support to immunization at a specific time window.

5.2.2.6 The mechanism(s) of interference and modifiable factors that can mitigate it

The exact mechanism of interference needs to be explored. One of the mechanisms that has not been explored is removal of vaccine antigens by macrophages. This could be done using assays that measure the phagocytic activity of anti-*B. pertussis* antibodies generated after immunization in pregnancy compared to antibodies from unimmunized host. If interference is found to have clinical significance in the future, modifiable factors that can mitigate this effect need to be explored. An important modifiable factor is timing of vaccination during pregnancy.

5.2.2.7 Maternal health conditions and placental conditions that affect transfer of B. pertussis IgG across the placenta

It will be important to investigate maternal health conditions that could affect the transfer of anti-*B. pertussis* after vaccination in pregnancy. For example, the effect of maternal HIV infection on transfer of anti-*B. pertussis* antibodies have not been explored following maternal immunization. In addition, placental conditions (insufficiency, malaria) that affect transfer of *B. pertussis* IgG across the placenta also needs to be explored.

5.2.2.8 The additional protective effect of breastfeeding in the protection against pertussis in young infants and the duration of persistence of antibodies in breast milk

My previous work before my PhD, and consistent with others, have shown that immunization against pertussis in pregnancy results in induction of anti-*B. pertussis* antibodies measured up to 8 weeks after delivery[507, 508]. However, the duration of persistence of antibodies in breast milk of pregnant women immunized against pertussis in pregnancy beyond 8 weeks has not been explored. It will also be important to investigate whether this has clinical significance. However, this might be challenging due to the large number of infants needed to answer this question where clinical outcome (infection with pertussis or not) needs to be linked to breastfeeding status.

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