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Pertussis vaccination during pregnancy in Belgium: Follow-up of infants until 1 month after the fourth infant pertussis vaccination at 15 months of age

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Abstract

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

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

All infants were vaccinated with Infanrix Hexa® according to the standard Belgian vaccination schedule (8/12/16 weeks, 15 months). We report results from blood samples collected before and 1 month after the fourth vaccine dose. Immunoglobulin G (IgG) antibodies against Pertussis Toxin (PT), Filamentous Haemagglutinin (FHA), Pertactin (Prn), Tetanus Toxoid (TT) and Diphtheria Toxoid (DT) were measured using commercially available ELISA tests. Antibody levels were expressed in International Units per Milliliter.

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

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

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Keywords: pertussis, vaccination in pregnancy, maternal antibodies, blunting

Introduction

Pertussis, primarily caused by the gram negative bacteria *Bordetella pertussis*, is a worldwide endemic and epidemic respiratory disease. Despite the successful introduction of global vaccination programs with high immunization rates, pertussis remains an important public health issue [1]. Mainly young infants, too young to be protected by the currently available vaccination schedules, are prone to severe pertussis disease with the highest hospitalization and complication rates among the population [2].

In Belgium, pertussis vaccination with an acellular pertussis containing vaccine (aP) is recommended by the National Immunization Technical Advisory Group (NITAG) at 8, 12 and 16 weeks (primary vaccination). A fourth vaccine dose of an aP containing vaccine is recommended at 15 months of age. Additional booster doses for children and adolescents are equally put in place. Furthermore, maternal pertussis vaccination is recommended since August 2013 for pregnant women during every pregnancy between 24 and 32 weeks of gestation. Finally, adults in close contact with young infants are also advised to receive a booster aP vaccine [3]. Despite these national recommendations, the total number of confirmed pertussis cases increased significantly in Belgium from 243 cases in 2011 [4] to 1501 cases in 2014 [5]. The increase in pertussis cases was most prominent in adults between 40 and 60 years. However, the absolute (total) number of pertussis cases remained the highest in infants below one year of age [5].

As a consequence of the presence of high titers of maternal antibodies after maternal vaccination, a blunting effect of infant immune responses has been observed after the first three doses of an aP containing vaccine [6-9]. In a recent clinical study, this blunting effect disappeared after a fourth dose of a pertussis containing vaccine administered at the age of 12 months [6]. However, only limited data are available concerning the effect of a fourth

62 infant dose of an aP containing vaccine [6, 10] and data after the administration of a fourth
63 vaccine dose at the age of 15 months are, to our knowledge, lacking. Therefore, the
64 vaccination schedule in Belgium offers the unique opportunity to investigate the effect of
65 high titers of maternal antibodies on the humoral immune responses in infants after a fourth
66 dose of a pertussis containing vaccine at 15 months of age.

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

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Material and methods

A prospective controlled cohort study was conducted in accordance with the Declaration of Helsinki, ICH-GCP and the procedures established by Belgian law. The study was approved by the ethics committee of the University of Antwerp, Belgium (Clinicaltrials.gov identifier: NCT01698346). Informed consent was obtained from both parents of the participating infants. Extended information on material and methods can be found in a previous publication [7].

Children born from healthy women in 5 different hospitals in the province of Antwerp, Belgium, were included in the study and were followed until 1 month after their fourth pertussis containing vaccine dose, administered at 15 months of age. Participating children were included in either a vaccine group, i.e. children born from women vaccinated with an aP containing vaccine (Boostrix®) between 18 and 34 weeks of gestation or a control group, i.e. children born from women not vaccinated with a pertussis containing vaccine for at least 10 years. Women in both study groups did not differ in any underlying characteristics, but randomization was incomplete as explained in the previous publication [7].

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

Study vaccines

All infants were vaccinated with the licensed hexavalent vaccine (Infanrix hexa®, GSK Biologicals, Rixensart, Belgium). Infanrix hexa® contains 25 Lf of diphtheria toxoid (DT), 10 Lf

of tetanus toxoid (TT), 25µg pertussis toxoid (PT), 25 µg filamentous haemagglutinin (FHA) and 8 µg pertactin (Prn), inactivated poliovirus, hepatitis B surface antigens and *Haemophilus influenzae* type B polysaccharide.

Study procedures

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

Safety assessments

At each study visit, medical history of diseases in the household, mainly respiratory diseases, was assessed. All serious adverse events in the infants occurring during the study period were recorded. All infants were examined by a medical doctor at 15 or 16 months of age using the "Van Wiechen developmental test" [11]. This is a Dutch screening test for neurodevelopment used in the general practice to monitor the development of children from birth up to four years of age [12] in a few categories: fine motor activity, adaptive and personal social behavior, communication and gross motor activity (Annex 1).

Laboratory

All samples were tested with commercially available ELISA kits at the National Institute of Public Health in Brussels, Belgium. The Virion/Serion® kit (ANL, Copenhagen) was used to

detect anti-PT IgG antibodies and the Euroimmune® ELISA kit was used to detect anti-FHA and anti-Prn IgG antibodies. Anti-TT and anti-DT IgG antibodies were detected using the Virotech/Sekisui® ELISA kit. Serum samples were tested at a dilution of 1:100. ELISA results were expressed in International Units per milliliter (IU/mL), using respective WHO standards (NIBSC code 06/140 for pertussis, NIBSC code TE-3 for tetanus and NIBSC code 00/496 for diphtheria). For pertussis, these international units are equivalent to the CBER EU units of FDA [13]. The lower limit of detection of the assays was 0.7 IU/mL for PT, 1 IU/mL for FHA, 3 IU/mL for Prn, 0.01 IU/mL for TT and 0.03 IU/mL for DT.

An international independent validation was performed to guarantee the reliability of the results at the Canadian Center for Vaccinology in Halifax, Canada [7].

For pertussis, an actual protective antibody threshold (correlate of protection) is not known [14]. For tetanus and diphtheria, the protective antibody level is defined as 0.1 IU/mL for tetanus and 0.01 – 0.1 IU/mL for diphtheria.

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

Statistics

The initial sample size calculation was performed, based on previous results [15]: a population of 50 subjects in each study arm would be sufficient to detect significant

differences in antibody titers at several time points. However, during the conduct of the study, we were confronted with substantial drop-out rates resulting in a smaller sample size before and 1 month after the fourth vaccine dose, mainly in the control group.

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

Descriptive analyses were performed to identify possible differences between both study groups. Statistical tests included parametric tests: (paired) t-tests and chi-square tests and their non-parametric alternatives: (paired) Wilcoxon tests and Fisher exact tests whenever the underlying assumptions of the parametric tests were violated, i.e. normality and sparseness assumptions, respectively [16, 17]. Linear regression models were used to identify characteristics that could potentially impact infant antibody titers before and after the administration of a fourth vaccine dose.

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

Results

General characteristics of the study population

Characteristics of the mother-infant pairs until 5 months after delivery and exclusion criteria at baseline have been described previously [7]. 55 children (2 twins) were included in the vaccine group and 26 children were included in the control group. Children were born between April 2, 2012 and April 16, 2014. After the primary series of vaccines, 2 additional children from the control group were excluded due to loss to follow-up. In the vaccine group, 4 children were not vaccinated according to protocol for their fourth vaccine dose. As a consequence, these children were excluded for their blood sample 1 month after the fourth vaccine dose.

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

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

Safety results

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

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

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Laboratory results

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

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

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

Figure 1 shows the GMCs for antibodies to TT, DT, PT, FHA and Prn at all time points in both study groups, including the data that have been published before [7]. Significant differences are indicated with a star mark. The figure clearly shows the decay of all antibodies in both groups of infants between the post-primary vaccination and the pre-booster sampling time point. The decay was most pronounced for anti-PT IgG antibodies. For anti-PT IgG ($p<0.001$), anti-DT IgG ($p<0.001$) and anti-TT IgG (p=0.035), a significant correlation between the post-primary vaccination and the pre-booster antibody concentration was found.

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

Figure 1: Geometric Mean Concentrations for antibodies to TT, DT, PT, FHA and Prn in both groups of women and infants at all time points. 1A: Anti-TT antibodies. 1B: Anti-DT antibodies. 1C: Anti-PT antibodies. 1D: Anti-FHA antibodies. 1E: Anti-Prn antibodies. Significant differences are indicated with a star mark.

Results from the regression analysis

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

Discussion

This study is the first to investigate the effect of maternal vaccination with a combined tetanus, diphtheria and acellular pertussis vaccine (Tdap, Boostrix®) on the antibody titers in infants before and after a primary vaccination schedule at 8, 12 and 16 weeks of age and before and 1 month after their fourth aP containing vaccine on 15 months of age (Infanrix hexa®). We previously reported on the blunting of the infant immune response for anti-DT and anti-PT antibodies after the primary vaccination schedule [7]. Our new data still indicate a minor blunting effect on the anti-PT antibodies 1 month after the fourth vaccine dose at 15 months of age. However, a strong immune response with a significant rise in antibody titers for all measured antigens after the fourth vaccine dose was found in both the vaccine and the control group.

Before administration of the fourth infant pertussis vaccine dose at 15 months of age, lower IgG GMCs were found in the vaccine group compared to the control group, except for anti-TT IgG showing significantly higher antibody titers in the vaccine group. Although there is no known correlate of protection for pertussis, high IgG levels directed against PT and Prn are associated with protection against pertussis disease and mainly anti-PT antibodies are considered to be crucial for this protection [18, 19]. For diphtheria and tetanus, antibody concentrations remained above the protective threshold in both groups at all time points. After completing the primary infant vaccination schedule (8-12-16 weeks), we confirmed a rapid decay of vaccine-specific antibodies [20], resulting in relatively low antibody titers at 15 months of age. The differences in antibody titer before and 1 month after the administration of a fourth vaccine dose between the vaccine and control group can be explained by the blunting effect we already observed 1 month after completion of the

primary vaccination schedule with, for some antigens, (significantly) lower antibody concentrations in the vaccine group [7].

In a recent study performed by Muñoz et al [6], blunting of the antibody response after primary vaccination (2-4-6 months) was shown. This effect disappeared after the administration of a fourth vaccine dose at 12 months of age. In a study by Hardy-Fairbanks et al [10], a slight blunting of the immune response was also seen after primary vaccination. Yet, after administration of a fourth vaccine dose at 12-18 months of age, no notable differences in antibody concentrations were encountered any longer between children from vaccinated and unvaccinated mothers. In the present study, we report a persisting minor blunting effect on the humoral immune response in infants from the vaccine group for anti-PT antibodies (p=0.006) after the administration of a fourth vaccine dose at the age of 15 months. The differences observed between our study and the Hardy-Fairbanks and Muñoz study could be due to the use of different brands of vaccines, due to a different timing of the administration of the fourth vaccine dose, or due to other possible confounders between populations (e.g. different demographic composition of the study population, different disease-specific epidemiological background, different vaccination history, etc.).

In addition, the meaning of blunting of the infant immune response is not really understood. A decreased antibody production to vaccination in infants in the presence of maternal antibodies has been described for several pathogens, e.g. tetanus [21], poliovirus [22, 23], hepatitis B [24], pertussis [21, 25], and *Haemophilus influenzae* B [21, 26]. However, this blunting effect is not described when investigating cellular immune responses [27]. Moreover, blunting seemed to diminish [24] or disappear [28] when monitoring antibody production over longer time periods. In one study, infants who showed blunting on their first

two polio vaccine doses even tended to have higher antibody titers after the third vaccine dose [22]. Therefore, blunting might not necessarily be a sign of a less effective immunization.

In comparison with available literature on humoral responses to Infanrix hexa® at the age of 15 months [29, 30], the pertussis specific antibody titers were lower in our study, at both time points in both study groups. Gimenez-Sanchez et al [29] collected blood samples after a fourth dose of Infanrix hexa® at 11-15 months of age, concomitantly administered with PCV 7 or PCV 13. Tichmann et al [30] collected blood samples both before and after a fourth dose of Infanrix hexa® at 12-19 months of age. On the other hand, anti-TT and anti-DT IgG antibodies titers were higher in our study before and 1 month after the fourth vaccine dose in both study groups. Possible reasons for the difference in reported antibody titers are the use of different laboratory techniques, the use of other time points in the primary vaccination schedule, the different epidemiological background and the lower sample size in our study which is more sensitive to possible outliers.

We did not identify any clinical case of pertussis within our study population. However, the sample size of our study was too small to measure the potential clinical impact of maternal pertussis vaccination on infants up to one month after their fourth vaccine dose. In the UK however, this vaccination strategy was highly effective to protect newborn infants against pertussis [31]. The clinical impact of this vaccination strategy and the consecutive minor blunting effect later in life has not been investigated yet; e.g. possible higher susceptibility at older infant or childhood age because of the blunting effect.

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

Limitations of the study

Our study has some limitations. Firstly, we were not able to perform a strict randomization of the infants in either the vaccine or the control group, as explained in the previous publication on this trial [7]. A second limitation was the high drop-out rate experienced along the study, especially in the control group, resulting in a smaller sample size, larger confidence intervals of the results and lower statistical power. Conducting clinical trials in mother-infant pairs is not evident and retaining them into the study during the entire study period is challenging [32]. Since the study was conducted in one province in Belgium, the study should be repeated in other provinces and countries with a different epidemiological background, a different vaccination schedule and different vaccine compositions, before generalizations can be made. A last limitation of the study was that the “Van Wiechen developmental test” was not performed at the same age in every child, although ages did not differ significantly between both study groups.

Conclusion

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

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Conflict of interest statement

Authors do not have a commercial or other association that might pose a conflict of interest (e.g., pharmaceutical stock ownership, consultancy, pharmaceutical board membership, relevant patents, or research funding).

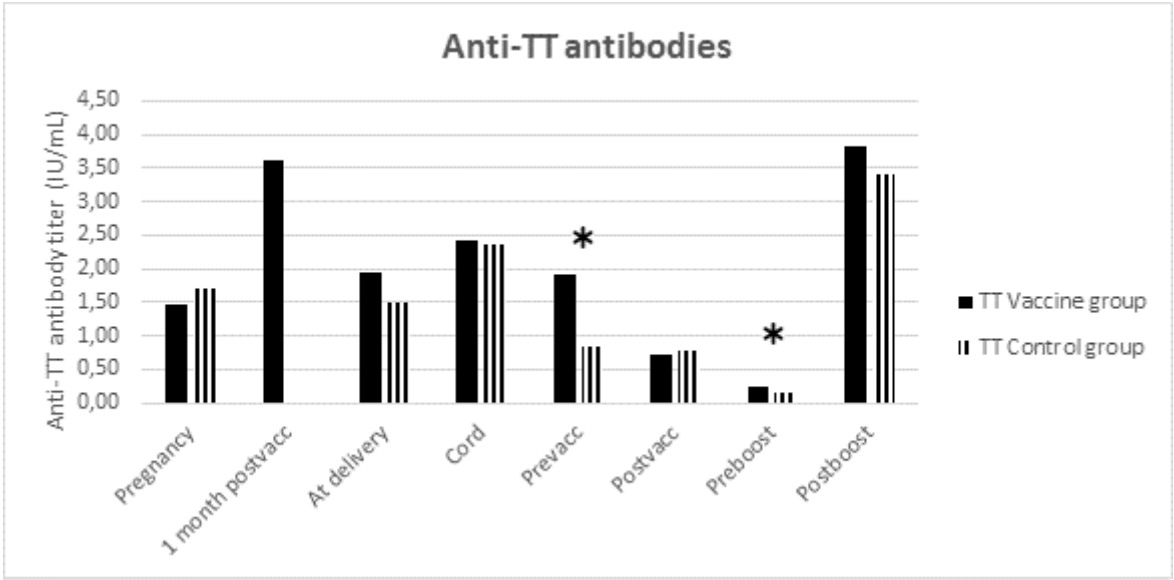
		Vaccine group	Control group	p-value
N (included infants)		55	24	
Infant gender, No. (%)	Male	27 (0.49)	12 (0.50)	0.910
	Female	28 (0.51)	12 (0.50)	
Mean weight month 15 in grams (SEM)		10316.30 (159.75)	10349.13 (172.00)	0.904
Mean length month 15 in centimeters (SEM)		77.82 (0.43)	79.40 (0.72)	0.067
Mean weight month 16 in grams (SEM)		10443.18 (157.72)	10406.30 (173.20)	0.891
Mean length month 16 in centimeters (SEM)		78.12 (0.44)	79.38 (0.66)	0.133
Mean age at blood sample before fourth vaccine dose in months (SEM)		14.93 (0.05)	15.00 (0.10)	0.475
Mean age at blood sample 1 month after fourth vaccine dose in months (SEM)		16.38 (0.07)	16.39 (0.11)	0.949
Mean age at vaccine dose 3 in months (SEM)		4.32 (0.07)	4.67 (0.14)	0.080
Mean age at fourth vaccine dose in months (SEM)		15.32 (0.06)	15.43 (0.14)	0.468
Mean interval between vaccine dose 3 – blood sample before fourth vaccine dose in months (SEM)		10.61 (0.09)	10.51 (0.14)	0.242
Mean interval between fourth vaccine dose– blood sample one month after fourth vaccine dose in months (SEM)		1.06 (0.02)	1.05 (0.02)	0.539
Mean interval between blood sample before fourth vaccine dose-fourth vaccine dose in months (SEM)		0.39 (0.06)	0.42 (0.09)	0.704

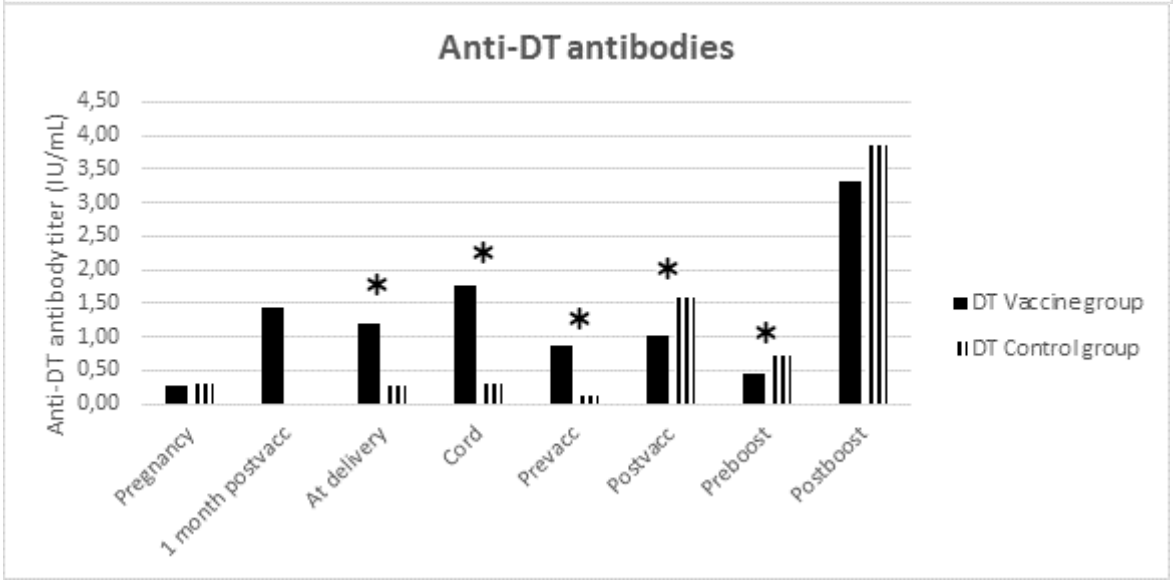
Table 1: Demographic and clinical characteristics of all study participants before and 1 month after the fourth vaccine dose.

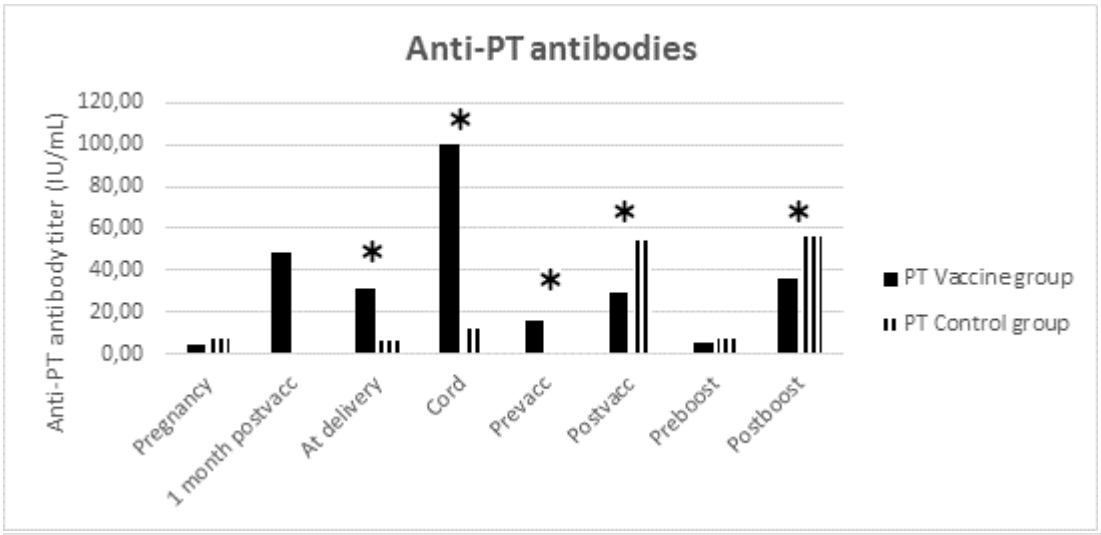
GMC (95% CI)	1 month after primary vaccination		Before fourth vaccine dose		1 m
	Vaccine group	Control group	Vaccine group	Control group	Vaccin
N	49	21	46	24	
Tetanus toxoid (IU/mL)	1.75 (1.69-1.82)	1.87 (1.68-2.07)	0.25 (0.21-0.30)	0.15 (0.11-0.21)	3 (3.39
p-value	0.560		0.007		
Diphtheria toxoid (IU/mL)	2.12 (1.95-2.21)	2.63 (2.48-2.97)	0.45 (0.35-0.58)	0.73 (0.56-0.94)	3 (2.94
p-value	0.002		0.023		
Pertussis toxin (IU/mL)	29.31 (24.60-34.93)	54.10 (42.36-69.09)	5.44 (4.49-6.58)	7.27 (5.80-9.12)	36 (30.93
p-value	<0.001		0.071		
Filamentous haemagglutinin (IU/mL)	64.86 (56.03-75.07)	53.73 (41.10-70.23)	14.83 (12.37-17.77)	15.98 (12.43-20.56)	10 (84.93
p-value	0.198		0.636		
Pertactin (IU/mL)	68.44 (55.85-83.89)	87.05 (62.17-121.89)	4.44 (3.66-5.39)	7.62 (5.67-10.25)	92 (67.04
p-value	0.220		0.003		

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

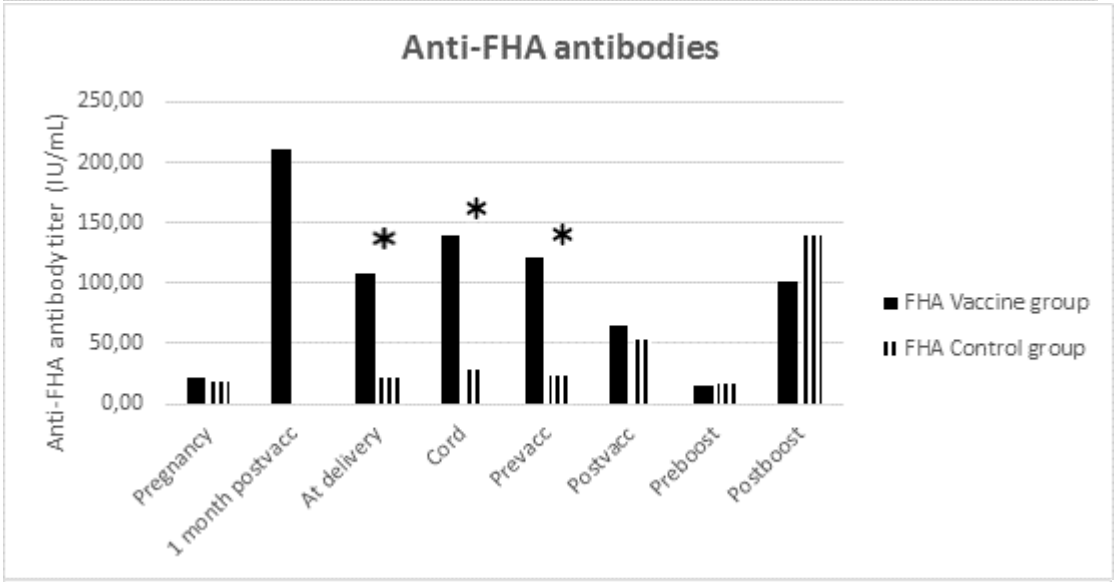
Figure 1: Geometric Mean Concentrations for antibodies to TT, DT, PT, FHA and Prn in both groups of women and infants at all time points. 1A: Anti-TT antibodies. 1B: Anti-DT antibodies. 1C: Anti-PT antibodies. 1D: Anti-FHA antibodies. 1E: Anti-Prn antibodies. Significant differences are indicated by a star mark.



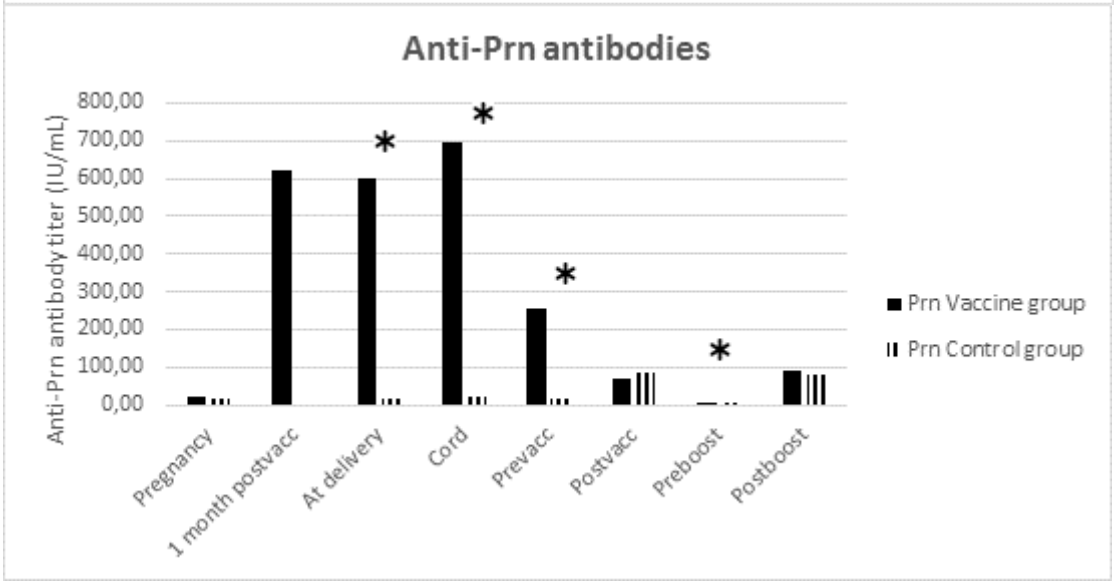



Anti-PT antibodies
Anti-PT antibody titer (IU/mL)
120,00
100,00
80,00
60,00
40,00
20,00
0,00
Pregnancy
1 month postvacc
At delivery
Cord
Prevacc
Postvacc
Preboost
Postboost
PT Vaccine group
PT Control group

511


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

512


Anti-Prn antibodies
Anti-Prn antibody titer (IU/mL)
800,00
700,00
600,00
500,00
400,00
300,00
200,00
100,00
0,00
Pregnancy
1 month postvacc
At delivery
Cord
Prevacc
Postvacc
Preboost
Postboost
Prn Vaccine group
Prn Control group

513