

Pertussis vaccination during pregnancy in Belgium: Results of a prospective controlled cohort study

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1 **Pertussis vaccination during pregnancy in Belgium: results of a prospective controlled cohort**  
2 **study**

3 Running title: Pertussis vaccination during pregnancy

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19

20 **Abstract**

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

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

37 Infants born to vaccinated women had significantly higher GMC at birth and during the first 2  
38 months of life for all vaccine antigens compared to the offspring of unvaccinated women,  
39 thereby closing the susceptibility gap for pertussis in infants. However, blunting was noticed for

40 infant diphtheria and pertussis toxin vaccine responses ( $p < 0.001$ ) in the infants from vaccinated  
41 women after the primary vaccination schedule (week 8-12 and 16).

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

45 Clinicaltrials.gov identifier: NCT01698346

46 Key words: pertussis, vaccination in pregnancy, maternal antibodies, blunting

47

## 48 **Introduction**

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

53 However, a decade after the switch from the whole-cell (wP) vaccine to the acellular pertussis  
54 (aP) vaccine, a cyclic resurgence has been reported in several industrialized countries. The  
55 reason is presumed to be multifactorial, with waning immunity after the primary or booster  
56 vaccination as the primary cause. A resurgence has been observed in all age categories;  
57 however, severe morbidity and mortality occurs primarily in young infants who are not fully  
58 vaccinated [2, 3]. The majority of cases are found in adolescents and adults, due to waning  
59 immunity [4], and these populations represent sources of infection for young infants.

60 In Belgium, pertussis vaccination with a hexavalent aP-containing vaccine is offered at 8, 12, and  
61 16 weeks and 15 months of age. Booster doses for children 4-6 years of age (since 2004) and for  
62 adolescents 14-16 years of age have been recommended since 2009. Additionally, receiving a  
63 booster dose once during adulthood has been recommended since 2013 [5]. Nevertheless, the  
64 total number of confirmed cases increased in Belgium from 93 in 2005 to 843 cases in 2013 [6],  
65 of which many (25.4% in 2013) were found in infants under the age of 1 year.

66 Partial primary protection against infectious diseases is offered at birth by maternal  
67 immunoglobulin G (IgG) antibodies [7, 8], with an estimated half- life of 6 weeks for pertussis  
68 [8]. The amount of transmitted antibodies depends on the placental function and the

69 concentration of maternal antibodies in the pregnant woman [9]. The latter depends on the  
70 time lapse since the last vaccination or infection [10] and the titer of passively transmitted  
71 pertussis maternal antibodies is often low [11]. Thus, increasing the load of maternal antibodies  
72 by vaccination during pregnancy is, with the currently available vaccines, the only way to offer  
73 passive protection to the newborn at birth [12]. During the first weeks of life, these maternal  
74 antibodies disappear in the newborn due to natural clearance [9, 13].

75 Vaccination during pregnancy is recommended in an increasing number of countries (e.g. UK,  
76 USA, Belgium, New Zealand, etc). Research has been performed on the immunological and  
77 safety aspects of the strategy [14-18]; nevertheless, many aspects are still unknown, and the  
78 possible interference of maternal antibodies with the infant's immune responses is one of the  
79 concerns.

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

84

85 **Material and methods**

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

91 Healthy pregnant women and their healthy offspring from 5 different hospitals in the province  
92 of Antwerp, Belgium, were included in the study, and follow-up remains ongoing. Pregnant  
93 women were included in either a vaccine group, receiving an acellular pertussis vaccine, or a  
94 control group, if they had not received any pertussis-containing vaccine for at least 10 years.  
95 Strict randomization was not possible because some women were advised positively or  
96 negatively by their treating physician on the pertussis vaccination in pregnancy and were  
97 included accordingly. The recommendation for receiving the pertussis vaccination during  
98 pregnancy by the Belgian National Immunization Technical Advisory Group (NITAG, since August  
99 2013) was not yet in place during the recruitment phase of this study, only a recommendation  
100 for cocoon vaccination. However, by 2012, the VVOG (Association of Flemish Obstetricians and  
101 Gynecologists) had recommended the ACIP as a valuable alternative for cocoon vaccination on  
102 its website. This recommendation was followed by some Belgian clinicians.

103 Strict inclusion and exclusion criteria were used (Annex 1).

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

#### 108 Study vaccines

109 Licensed Tdap vaccine (Boostrix<sup>®</sup>, GSK Biologicals, Rixensart, Belgium) was used to immunize  
110 pregnant women. Boostrix<sup>®</sup> contains 5 Lf of tetanus toxoid (TT), 2.5 Lf of diphtheria toxoid (DT),  
111 8 mcg of inactivated pertussis toxoid (PT), 8 mcg of filamentous haemagglutinin (FHA), and 2.5  
112 mcg of pertactin (Prn). Infants were vaccinated with a hexavalent vaccine (Infanrix hexa<sup>®</sup>, GSK  
113 Biologicals, Rixensart, Belgium). Infanrix hexa<sup>®</sup> contains 25 Lf of DT, 10 Lf of TT, 25 mcg PT, 25  
114 mcg FHA and 8 mcg Prn, inactivated poliovirus, hepatitis B surface antigens and *Haemophilus*  
115 *influenzae* type B polysaccharide.

#### 116 Study procedures

117 Venous blood (10 cc) was collected from all participating women immediately preceding the  
118 vaccination, at 1 month (28-31 days) after vaccination, and at delivery. The maternal vaccination  
119 was performed by the study physician or study nurse under supervision. Cord blood was  
120 collected at delivery (10 cc). Blood samples (2 cc) were collected from the infants before starting  
121 the primary schedule (week 8  $\pm$  4days) and at month 5 (28-35 days after the third vaccine dose).  
122 Infant vaccines were administered in the regular health care system at the well-baby clinics or a  
123 pediatrician. Further follow-up is ongoing, with blood samples being collected before and after



124 an Infanrix hexa<sup>®</sup> booster dose is given at month 15 (data not shown). The samples were  
125 centrifuged at 2000 rpm within 24 hours and stored at -20°C.

#### 126 Safety assessments

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

#### 134 Laboratory

135 All samples were tested with commercially available ELISA kits at the National Reference Centre  
136 for Bordetella. The Virion/Serion<sup>®</sup> kit (ANL, Copenhagen) was used to detect anti-PT IgG  
137 antibodies, and the EuroImmune<sup>®</sup> ELISA kit was used to detect anti-FHA and anti-Prn IgG  
138 antibodies. Anti-TT and anti-DT IgG antibodies were detected using the Virotech/Sekisui<sup>®</sup> ELISA.  
139 Serum samples were tested in duplicate at a dilution of 1:100 (PT, TT and DT), 1:400 (FHA) and  
140 1:800 (Prn). All OD results were converted into international units per milliliter (IU/mL). For  
141 tetanus and diphtheria, the limits of detection were 0.01 IU/mL and 0.03 IU/mL, respectively. All  
142 titers are expressed in International Units IU/ml , using respective WHO standards (NIBSC 06/140 for  
143 pertussis, NIBSC code TE-3 for tetanus and NIBSC 00/496 for diphtheria). For Pertussis these

144 international units are equivalent to the CBER EU units of FDA (A. van der Zee et al, Clin Microbiol Rev  
145 28: 1005-1026, 2015)).

146 An international independent validation was performed to guarantee the reliability of the  
147 results. A random selection of samples (N=177) was reanalyzed at the Canadian Center for  
148 Vaccinology in Halifax, where CBER equivalent sera based on the WHO standard lot number 3 were  
149 used. A positive correlation was found in the results from both laboratories. The protective  
150 threshold of antibodies (a correlate of protection) is not known for pertussis [19]. For tetanus  
151 and diphtheria, a correlate of protection is defined as 0.1 IU/mL for tetanus and 0.01-0.1 IU/mL  
152 for diphtheria.

### 153 Statistics

154 A sample size calculation was performed, based on previous results [20]. Accordingly, a  
155 population of 50 subjects in both study arms would be sufficient to detect significant differences  
156 in antibody titers at several time points.

157 Statistical tests included parametric tests: (paired) t-tests and chi-square tests and their non-  
158 parametric alternatives: (paired) Wilcoxon tests and Fisher Exact tests whenever the underlying  
159 assumptions of the parameteric tests were violated, ie normality and sparseness, respectively  
160 [21, 22].

161 The presence of twins in the data resulted in using different sample sizes for outcomes related  
162 to women and children. Data were assumed to be missing completely at random and complete  
163 case analyses were conducted rendering unbiased estimates though at the cost of a loss of

164 efficiency [23]. The loss of efficiency resulting from excluding observations was limited due to  
165 the limited amount of missing data (12.4%).

166 Assessing the impact of the different mother and child characteristics on GMC for each of the  
167 different time points was done using a regression approach with outcome the log titer values,  
168 symmetrizing the response, and consisting of three consecutive steps: (1) variable selection  
169 using random forests [24] (Annex 2); (2) backward model selection using multiple linear  
170 regression based on AIC; and (3) further model reduction using likelihood ratio tests [22]. This  
171 model building procedure was used before [25].

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

175

176 **Results**

177 General characteristics of the study population (Table 1)

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

190 Safety results (Table 2)

191 Of the 57 women in the vaccine group, 50 adverse events (AE) were reported in 46 women.  
192 Most symptoms were mild and self-limited and were resolved within 72 hours after vaccination.  
193 Stiffness of the arm at the injection site was the most commonly reported AE (N=42), followed  
194 by minor swelling at the injection spot. Five AE (vaginal thrush, reflux, fever <38.5°C, extensive  
195 limb swelling, and rashes on the abdomen and arms) required the use of concomitant

196 medication. Fever was described in only 1 vaccinated women (1.75%). The mean duration of all  
197 AE was 2.30 days (1-10 days).

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

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

#### 209 Laboratory results

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

212 At baseline, no significant differences were found between both groups for any measured  
213 antibody. Protective antibody concentrations for tetanus and diphtheria were measured at all  
214 other time points in mothers and infants. Women in the vaccine group had significantly higher  
215 GMCs to all antigens at delivery compared with women from the control group, except for

216 tetanus ( $p=0.064$ ). Significantly higher antibody concentrations were found in the cord blood of  
217 the vaccine group compared with the control group for all antigens, except again for tetanus  
218 ( $p=0.888$ ).

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

223 At 1 month after the third hexavalent vaccine dose, GMCs to PT ( $p<0.001$ ) (*Figure 2*) and DT  
224 ( $p=0.002$ ) were significantly lower in the vaccine group compared with the control group.

225 However, antibody GMCs for both antigens had risen from week 8 to month 5 (*Figure 1*). For  
226 Prn ( $p=0.220$ ), TT ( $p=0.560$ ) and FHA ( $p=0.198$ ), non-significant differences in GMCs were found  
227 in the vaccine group compared to the control group. For these three antigens a decay in  
228 antibody titer from week 8 to month 5 is notified in the vaccine group (*Figure 1*).

229 *Figure 1* demonstrates the log distribution of the prevaccination and postvaccination (after 3  
230 doses) IgG titers for all vaccine antigens in both the vaccine and control group. There is a  
231 significant difference in the distribution of antibodies for all prevaccination titers in the vaccine group  
232 versus the control group, in favor of the vaccine group. The postvaccination titers differ significantly  
233 between both groups for PT and DT. *Figure 2* shows the individual data for PT antibodies only, but  
234 expressed as the individual correlation of pre-vaccination and post-vaccination IgG titers for  
235 each infant in both groups.

236

237 Transplacental transport rate

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

#### 242 Results from the regression analysis

243 We report only the significant influences of all variables included in the random forest analysis.

244 At baseline, a higher parity had a positive effect on the anti-FHA antibody concentration in the  
245 control group ( $p=0.03$ ), and older women had higher anti-Prn GMCs in the control group  
246 ( $p=0.017$ ). A negative influence of the receipt of a tetanus-only vaccine within the past 10 years  
247 was observed on the anti-FHA antibody concentration ( $p=0.01$ ) and the anti-Prn antibody  
248 concentration ( $p=0.04$ ) at baseline in the control group.

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

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

256

257 **Discussion**

258 This study is the first to investigate the effect of the administration of Boostrix® in pregnant  
259 women on transmitted maternal antibodies and to assess the immune responses of infants  
260 administered acellular pertussis-containing vaccines (Infanrix hexa®) according to a schedule of  
261 8, 12 and 16 weeks of age. The presented data show that vaccinating during pregnancy closes  
262 the susceptibility gap for pertussis infection.

263 Safety data have been reported from far larger studies [16, 26], showing that pertussis  
264 vaccination during pregnancy is safe and well tolerated. The AE reported within this study  
265 (73.7% showed mild to moderate injection site pain and swelling) do not differ from the  
266 expected side effects described in the Boostrix® summary of product characteristics (SmPC:  
267 23.7% - 80.6%) [27]. Because we did not use a placebo in the control group, it is not possible to  
268 make a comparison of the rate of adverse events between both groups. SAE were encountered  
269 in this study. However, the number of reported serious adverse events was small and did not  
270 differ from what is expected within the general population [28]. The safety data in the offspring  
271 did not demonstrate an unexpected risk pattern; no congenital disorders were detected.

272 Transplacental transport was effective in both groups for all vaccine antigens. Inter-pathogen  
273 specific IgG differences in the effectiveness of this transport have been described before [29],  
274 despite a common mechanism for transplacental IgG transport involving the FcRn receptor.  
275 Healy et al. reported a lower transplacental transport rate for PT compared with the rate  
276 reported in this study [30]. Conversely, for FHA and Prn, similar transport rates were found in  
277 both studies. In a randomized controlled trial, Muñoz et al [16] also reported an effective



278 transplacental transport for PT antibodies, similar to what we report in this study, but less for  
279 FHA, Prn, DT and TT. However, a comparison of serological results from various studies should  
280 be interpreted with caution because different laboratory techniques are utilized.

281 At baseline, all IgG GMCs were comparable in both groups. There was an adequate maternal  
282 immune response to all vaccine antigens except for tetanus, yet all women were already  
283 protected for tetanus at baseline. We showed in another paper [31] that humoral responses to  
284 pertussis vaccines in pregnancy are as robust as in non-pregnant women. The RCT performed by  
285 Muñoz et al [16] describes equally high antibody titers post-vaccination as during pregnancy.  
286 Again, immune responses in the latter study were measured with other laboratory techniques  
287 and the women were vaccinated with another vaccine brand (Adacel®); therefore, titers cannot  
288 easily be compared.

289 At delivery and in cord samples, IgG GMC were still significantly higher in the vaccine group  
290 compared with their baseline values for all vaccine antigens. These increased titers persisted  
291 until the age of 8 weeks, before the start of the primary vaccination schedule, suggesting a  
292 closure of the susceptibility gap of in newborns. A decay of maternal antibodies during the first  
293 weeks of life has been described before [16, 20], and although there is no known correlate of  
294 protection for pertussis, high concentrations of PT, FHA and Prn IgG are associated with  
295 protection against (severe) disease [32, 33]. A decay of maternal antibodies during the first  
296 weeks of life has been described before [16, 20]. Yet, at week 8, immediately preceding the  
297 vaccination, infants of vaccinated women still had significantly higher antibody titers compared  
298 with the control group.

299 Naturally acquired maternal pertussis antibodies have been shown to interfere with humoral  
300 responses to wP, yet not to aP vaccines [7, 34-39]. Nevertheless, the recent study by Muñoz et  
301 al showed a trend of blunting by maternal antibodies for FHA ( $p < 0.01$ ) after a 3-dose priming  
302 schedule [16]; however, this blunting effect disappeared with the booster dose offered during  
303 the second year of life, whereas the clinical significance of the interference has not been  
304 demonstrated. This result confirmed the finding by Hardy- Fairbanks et al [15]. In the study  
305 presented here, the blunting of the infant immune response is also suggested in infants from  
306 mothers in the vaccine group for anti-PT antibodies ( $p < 0.001$ ). The differences between our  
307 study and the Muñoz study could be due to different brands of vaccine used during pregnancy  
308 and to other confounders in both populations (e.g., different epidemiological background for  
309 pertussis, different vaccination histories, etc.). Jones et al measured the effect of high levels of  
310 maternal antibodies on the humoral immune responses to vaccines in infancy in general and  
311 found the inhibition of immune responses to tetanus and pneumococcus [40]. The present  
312 study does not confirm this blunting effect for tetanus, and pneumococcal antibody titers have  
313 not (yet) been analyzed. Mouse models seem to indicate however, that maternal antibodies,  
314 and blunting, do not solely have a negative effect on infant immune responses but might  
315 enhance the B cell maturation in infants [41]. In a study conducted in parallel in Vietnam, we  
316 describe less blunting effect by maternal antibodies. A possible explanation could be that we  
317 used different brands of acellular pertussis vaccines in mothers and infants in Vietnam (cross  
318 reference to manuscript JVAC-D-15-01293), resulting in different antibodies. This difference in  
319 interference was already shown in a mouse model experiment (personal communication  
320 Camille Locht, Institut Pasteur de Lille).

321 Abu Raya et al [18] also used Boostrix® vaccine during pregnancy, as in the present study,  
322 whereas Muñoz et al [16] and Hardy Fairbanks et al [15] used Adacel. A comparison of antibody  
323 titers induced by vaccines of distinct manufacturers has never been analyzed in a single  
324 pregnant population.

325 A random forest regression analysis revealed no consistent influence of any factor on the entire  
326 study population, except for vaccination during pregnancy. Only isolated significant influences  
327 of some variables on one specific time point in one specific group were described, never  
328 indicating any plausible relationship. Unlike Abu Raya et al [18], we could not confirm the  
329 influence of gestational age at vaccination on the titer of antibodies encountered in the cord.  
330 Our study was not powered for the analysis of this specific influence; considerably larger  
331 cohorts are needed to show this effect.

### 332 Limitations of the study

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

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

339 Conducting clinical trials in pregnant women and their offspring is difficult. Recruitment is time  
340 consuming and labor intensive. Moreover, it is a challenge to retain both mothers and infants

341 throughout the entire study period [42]. In our study, there were limited amounts of missing  
342 data (12.4%). Therefore, we performed a complete case analysis which assumes that the  
343 missingness process was unrelated to the observed and unobserved titer values. We were  
344 confronted with a large drop-out rate, especially in the control group, which resulted in wider  
345 confidence intervals of the results.

346 And lastly, despite validation of the laboratory results, comparison with other studies and  
347 laboratories remains a major challenge, as in many other trials.

348

## 349 **Conclusion**

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

358

359

360

361 **Tables**

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

362

363 **Table 1:** Demographic and clinical characteristics of all study participants

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

364

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

GMC (95%CI)	Women						Infants					
	Baseline		1 Month after vaccination		At delivery		Cord		Before primary vaccination		1 Month after primary vaccination	
	Vaccine	Control	Vaccine	Control	Vaccine	Control	Vaccine	Control	Vaccine	Control	Vaccine	Control
	N		N		N		N		N		N	
	57 (54 for anti-PT)	31	57	0	57 (56 for anti-PT and anti-FHA)	41	58 (57 for anti-PRN)	41	51	26	49	21
Tetanus toxoid, IU/ml	1.5 (1.3-1.7)	1.7 (1.4-2.1)	3.6 (3.5-3.8)	NA	1.9 (1.6-2.3)	1.5 (1.2-1.7)	2.4 (2.3-2.5)	2.4 (1.9-2.9)	1.9 (1.8-2)	0.8 (0.7-1)	1.7 (1.7-1.8)	1.9 (1.7-2.1)
p-Value	0.212		NA		0.064		0.888		<0.001		0.560	
Diphtheria toxoid, IU/ml	0.3 (0.2-0.4)	0.3 (0.2-0.5)	1.4 (1.3-1.7)	NA	1.2 (1-1.5)	0.3 (0.2-0.4)	1.7 (1.5-2.1)	0.3 (0.2-0.5)	0.9 (0.7-1)	0.12 (0.1-0.17)	2.1 (1.9-2.2)	2.6 (2.4-2.9)
p-Value	0.749		NA		<0.001		<0.001		<0.001		0.002	
Pertussis toxin, IU/ml	4.5 (3.2-6.4)	7.5 (5-11)	48 (39-59)	NA	31.4 (26-38)	6.4 (4.3-9.6)	100.7 (82-123)	12.4 (8-19)	15.5 (12.1-20)	1.1 (0.7-1.6)	29 (25-35)	54 (42-69)
p-Value	0.078		NA		<0.001		<0.001		<0.001		<0.001	
Filamentous Haemagglutinin, IU/ml	21 (17-26)	17.6 (13-24)	211 (170-263)	NA	107 (91-126)	21.4 (16.6-27.5)	140 (109-180)	27.5 (21.5-35)	121 (100-145)	23 (19-27)	65 (56-75)	54 (41-70)
p-Value	0.409		NA		<0.001		<0.001		<0.001		0.198	
Pertactin, IU/ml	24 (18-31)	16.9 (11.6-24.6)	622 (511-756)	NA	602 (485.5-747)	18 (13-24)	697 (573-848)	21 (15.5-28)	253 (183-351)	17 (14.5-21)	68 (56-84)	87 (62-121)
p-Value	0.147		NA		<0.001		<0.001		<0.001		0.220	

366

367 Table 3: Geometric Mean Concentrations with 95% confidence interval (CI) for antibodies to  
368 tetanus, diphtheria, pertussis toxin, filamentous haemagglutinin, pertactin in both groups of  
369 women and infants

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

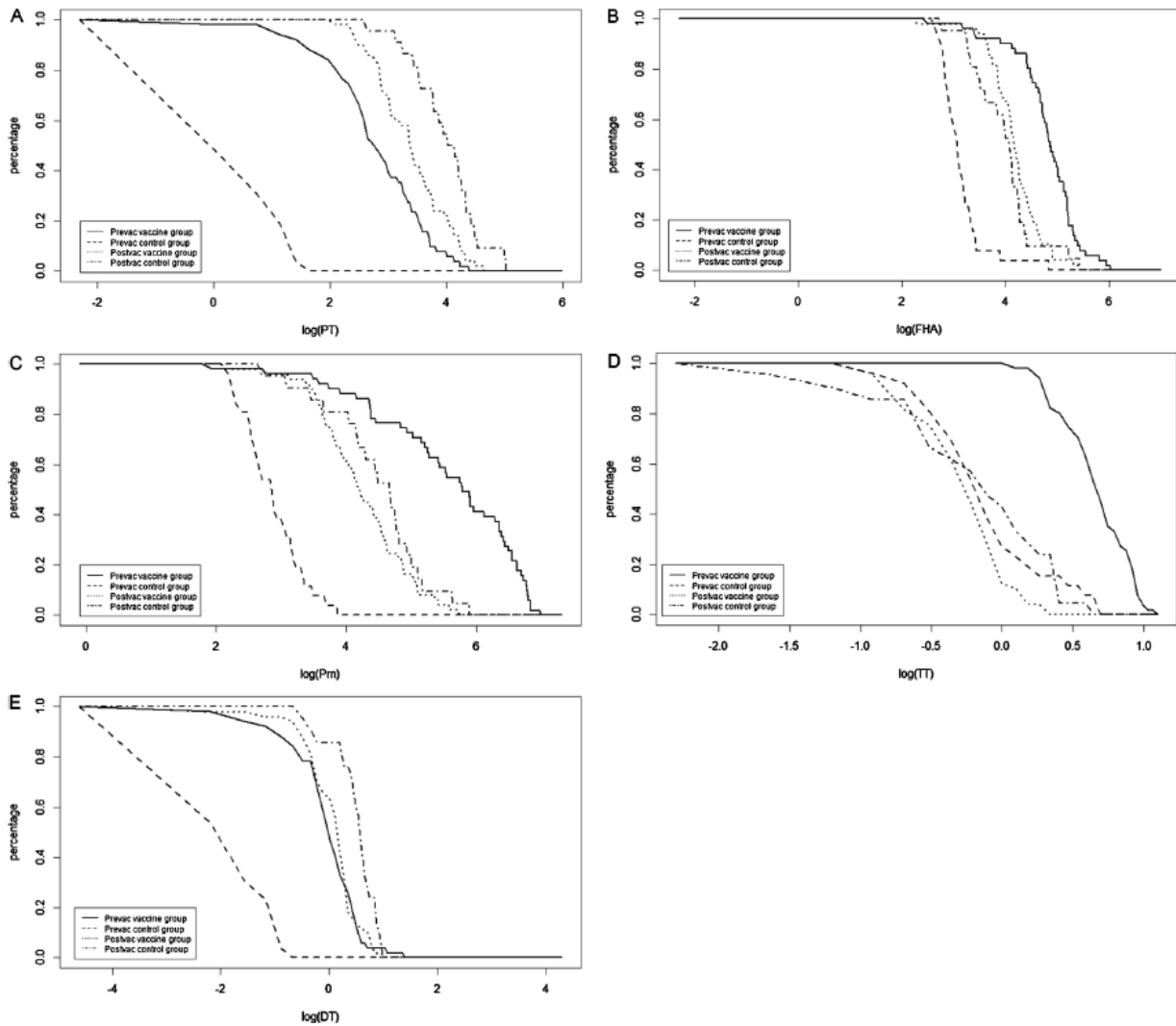
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371 Table 4: The rate of the cord titer/ maternal titer with standard deviation (SD) for tetanus,  
372 diphtheria, pertussis toxin, filamentous haemagglutinin and pertactin in both study groups

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375 **Figures**

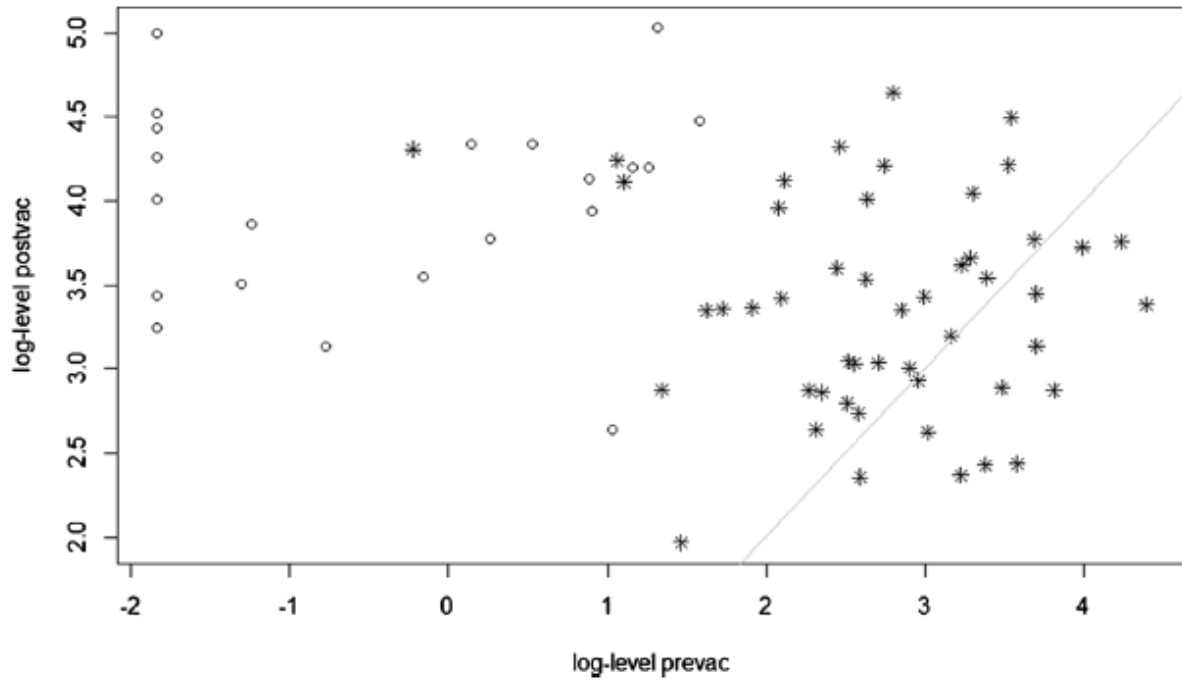


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377 Figure 1: Comparison of both groups of infants before and after the priming vaccination

378 regarding the antigen specific (log) antibody levels. 1A: Anti-PT antibodies. 1B: Anti-FHA

379 antibodies. 1C: Anti-Prn antibodies. 1D: Anti-TT antibodies. 1E: Anti-DT antibodies

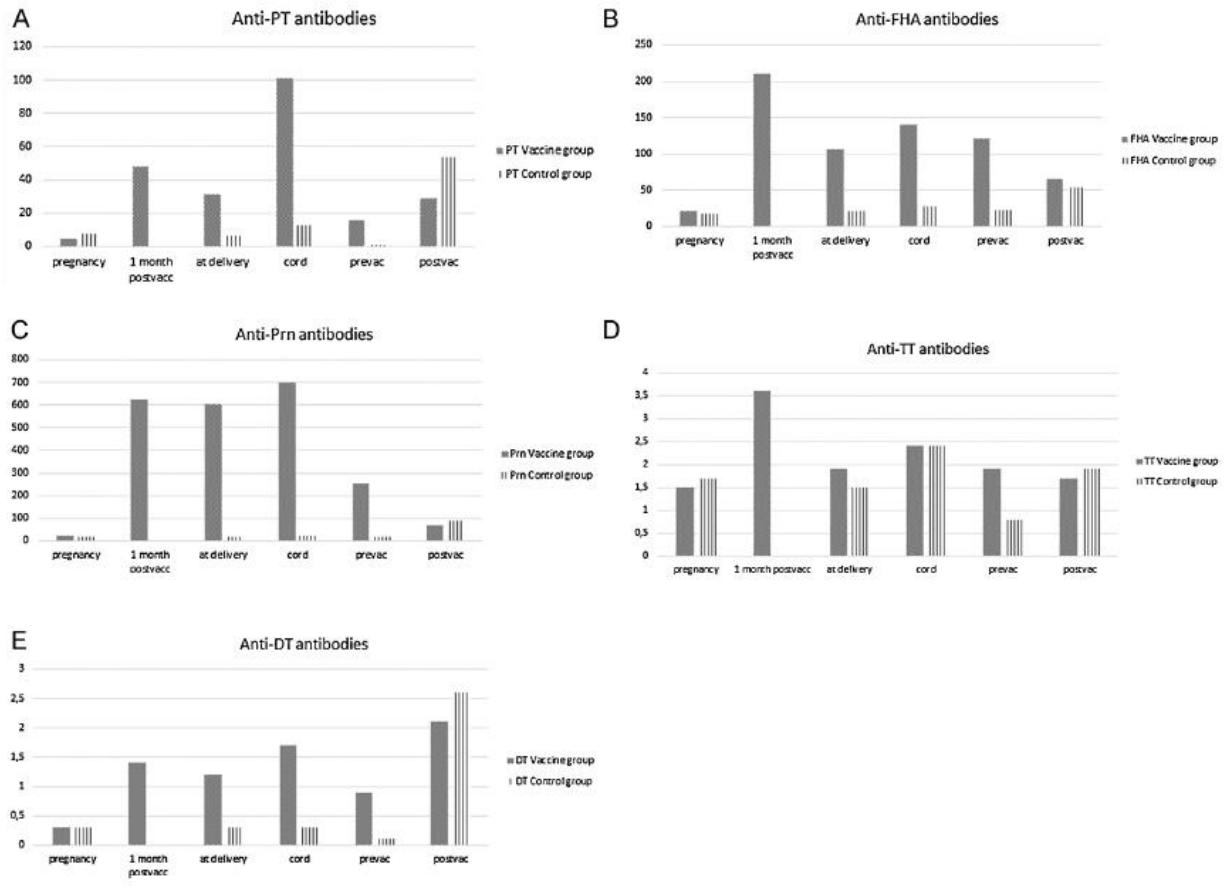


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381 Figure 2: The individual correlation of the anti-PT antibody titers pre- and post-primary

382 vaccination in infants in the control group (dots) and the vaccine group (stars).





383

384 Figure 3: Geometric Mean Concentrations for antibodies to tetanus (TT), diphtheria (DT),  
 385 pertussis toxin (PT), Filamentous Haemmaglutinin (FHA) and Pertactin (Prn) in both groups of  
 386 women and infants at all time points.

387 **Legends**

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546 **Conflict of interest statement**

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