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Quantity and quality of antibodies after acellular *versus* whole cell pertussis vaccines in infants born to mothers who received Tdap during pregnancy: a randomised trial

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Running title: Maternal Tdap and infant aP or wP vaccination

Summary: Infant wP vaccine responses are blunted after maternal Tdap vaccination. Pertussis antibody titers are higher in aP- than wP-vaccinated infants of immunized mothers, yet quality of antibodies, measured as serum-mediated bacterial growth inhibition, is better after wP than aP vaccination.

Abstract

Background:

The blunting effect of maternal pertussis immunization during pregnancy on infant antibody responses induced by whole cell pertussis (wP) vaccination is not well-defined.

Methods:

This randomized controlled trial (NCT02408926) followed term infants born to mothers vaccinated with tetanus-diphtheria-acellular pertussis (Tdap)-vaccine during pregnancy in Thailand. Infants received either acellular pertussis (aP)- or wP-containing vaccine at 2, 4, 6 and 18 months of age. A comparison group comprised wP-vaccinated children born to mothers not vaccinated during pregnancy. Antibodies against pertussis toxin (PT), filamentous haemagglutinin (FHA) and pertactin (PRN) were evaluated using commercial enzyme-linked immunosorbent assays (ELISA). Functionality of antibodies against *B. pertussis* was measured using *B. pertussis* growth inhibition assay (BGIA).

Results:

After maternal Tdap vaccination, 158 infants vaccinated with aP-containing vaccines possessed higher antibody levels ($p < 0.001$) against all tested *B. pertussis* antigens post-priming compared to 157 infants receiving wP-containing vaccines. At one-month post-booster, only anti-FHA and anti-PRN antibodies were still significantly higher ($p < 0.001$) in the aP group. Significantly higher anti-PT and anti-FHA ($p < 0.001$), but not anti-PRN IgG, were observed among 69 wP-vaccinated infants born to control mothers compared to wP-vaccinated infants of Tdap-vaccinated mothers after primary and booster vaccination. The antibody functionality was higher in all wP vaccinated infants at all times**.**

Conclusions:

Maternal Tdap vaccination inhibited more pertussis-specific responses in wP vaccinated infants compared to aP vaccinated infants, and the control group of unvaccinated women had highest pertussis-specific responses, persisting until after the booster dose. Antibody functionality was better in the wP groups.

1. Introduction

Pertussis remains difficult to control despite decades of worldwide vaccination. Infants are at highest risk for severe outcomes [1]. The most cost-effective method to protect infants is immunization during pregnancy [2-5]. During the last decade, maternal tetanus, diphtheria and acellular pertussis (aP) (Tdap) vaccination programs have been implemented, mainly in industrialized countries [6].

High titers of naturally-acquired maternal antibodies to pertussis toxin (PT) were previously reported to interfere with infant antibody responses to whole cell pertussis (wP) [7, 8], but not to aP vaccines [9]. In contrast, lowered antibody responses in infants born from Tdap-vaccinated mothers were observed following primary immunizations with aP-containing vaccines, with inconsistent results following a booster dose [10-13] In many countries, wP vaccines are used within the Expanded Programme on Immunization (EPI). Interference in infant immunity induced by aP vaccines cannot be extrapolated to wP vaccines without additional immunogenicity data [14].

Assessment on how immunization influences bactericidal immunity against *B. pertussis*, as means of measuring quality of antibodies, is of interest [15]. IgG-mediated binding of pathogen causes immobilization or agglutination. In the presence of complement, IgG may be bactericidal. Sera from subjects vaccinated with two-component (Filamentous Hemagglutinin (FHA), PT) aP vaccines did not activate complement-mediated killing [16]. Yet sera of individuals vaccinated with pertactin (PRN)-containing vaccines were able to generate bactericidal activity [17]. To our knowledge, little information exists on the difference in sera bactericidal activity induced by aP- or wP-containing vaccines and its correlations with serum IgG levels, after maternal immunization.

The wP-containing vaccine has been implemented in the Thai EPI program for more than 40 years, while the aP-containing vaccine was introduced ten years ago and is used in private hospitals [18]. Although there has been a resurgence of pertussis, especially among very young infants [19], maternal Tdap immunization has not been implemented. To evaluate the potential effects of implementing maternal Tdap on the responses to aP- *versus* wP-containing vaccines in children, we conducted a prospective randomized controlled clinical trial. The primary objective was to evaluate antibody levels in infants after priming and first booster vaccination with aP- or wP-containing vaccines, in comparison to the EPI schedule. Secondly, the functionality of these antibodies was evaluated.

2. Material and methods

2.1 Study design

This study (ClinicalTrials.gov NCT02408926) was approved by the Institutional Review Board at Chulalongkorn University and the ethical committee of the University of Antwerp. We enrolled healthy pregnant women at King Chulalongkorn Memorial Hospital, who consented to Tdap vaccination (Boostrix®). We assumed that all women received wP-containing vaccines during infancy. The inclusion and exclusion criteria, vaccine reactogenicity, and *B. pertussis*-specific antibody titers in maternal and cord blood were previously described [20]. Written informed consent was obtained from parents prior to infant enrollment. Healthy full-term and late preterm infants born at 36 weeks gestational age with birth weight higher than 2,500 grams, were randomized to receive either aP- (Infanrix hexa®) or wP-containing vaccine (Quinvaxem®). This study was not blinded since wP-vaccinated infants received oral polio vaccine (OPV) whereas aP-vaccinated infants received inactivated poliovirus (IPV) vaccine (hexavalent vaccine).

Simultaneously, a convenience sample of full-term infants born to non Tdap-vaccinated women was recruited in the same hospital, although not randomized, and this group received the wP-containing vaccine (Quinvaxem®) according to the current Thai EPI (EPI wP group).

2.2 Study vaccines

All women, except those from the EPI wP group, received Boostrix® (GSK Biologicals) during the third trimester of pregnancy, containing 8µg of PT, 8µg of FHA, 2.5µg of PRN, 2.5 Lf Diphtheria Toxoid (DT) and 5 Lf Tetanus Toxoid (TT).

All infants received aP- or wP-containing vaccines at 2, 4, 6 months of age (priming) and 18 months of age (booster).

Infanrix hexa® (GSK Biologicals) contains 25µg PT, 25µg FHA, 8µg PRN, 30 IU DT, 40 IU TT, 10µg Hepatitis B surface antigen (HBsAg), 10µg *Haemophilus influenzae* type b polysaccharide and 40, 8, and 32 D-antigen units of IPV type 1, 2, and 3. Quinvaxem® (Biogenetech) contains inactivated *B. pertussis* >4 IU/dose of potency, 30 IU DT, 60 IU TT, 10µg HBsAg and 10µg Hib oligosaccharide. Infants in the wP and EPI wP groups received bivalent OPV (Biofarma®) at 2, 4, 6 and 18 months. World Health Organization (WHO) recommended a switch from trivalent to bivalent OPV in April 2016, and all infants who reached the age of 4 months by 1 December 2015 also received trivalent IPV (IMOVAX polio, Sanofi Pasteur) vaccine containing 40, 8, and 32 D-antigen units of inactivated polioviruses type 1, 2, and 3.

According to the EPI, all infants received bacille Calmette-Guerin (BCG) and monovalent hepatitis B vaccine at birth, measles-mumps-rubella (MMR) vaccine (Priorix®, GSK Biologicals or M-M-R®II, Merck & Co.) at 9 months and Japanese Encephalitis (JE) (CD.JEVAX®, Chengdu Institute of Biological Products) vaccine at 12 and 19 months of age. They received trivalent influenza vaccine (Influvac®, Abbott Biologicals) at 7 and 9 months of age. Some infants received optional (decision by parents) rotavirus, pneumococcal, varicella zoster or rabies vaccines.

2.3 Sample collection

In the aP and wP groups, maternal and cord blood samples were collected at delivery (results published [20]). Cord antibody levels of the EPI wP infants were extrapolated from a Thai historical infant cohort born to mothers who did not receive Tdap during pregnancy [21]. Venous infant blood samples (2.5 mL) were collected at two months of age before the first pertussis-containing vaccine, 28-35 days after the last dose of priming (7 months of age), at 18

months of age before the first pertussis booster, and 28-35 days after the booster (19 months of age). In the EPI wP group, blood samples (2.5 mL) were taken at month 7 and 19.

2.4 ELISA for antibodies to *B. pertussis* antigens

Anti-PT, anti-FHA and anti-PRN IgG were analysed in a blinded manner using a commercial ELISA (EUROIMMUN, Lübeck, Germany) according to the manufacturer's instructions. Experiments were performed as previously described [20]. Samples with values below the lower limit of quantification (LLOQ), 5 IU/ml, were calculated as 50% of the LLOQ.

2.5 Bacterial Growth Inhibition Assay (BGIA)

Antibody-mediated *B. pertussis* growth inhibition was measured as described in Appendix. Bacterial growth inhibition activity was measured by the ratio of relative luminescence units (RLU) in the well of *B. pertussis* incubated with heat-inactivated sera (complement-independent activity) or untreated sera (complement-dependent activity) divided by RLU in the well of *B. pertussis* alone.

2.6 Statistical analysis

With significance level=0.05 and power =0.90, and if the geometric mean concentrations (GMC) of anti-PT IgG was expected to be 20% less in the wP group, with fixed variance, a population of 130 infants in both arms was sufficient. Baseline characteristics are reported as means and standard deviations (SD). Antibody titers are presented as GMC with 95% Confidence Interval (CI). The conventional *t*-test or ONE-WAY ANOVA was used to compare baseline characteristics, GMCs and functionality of antibodies. The paired *t*-test was

used to compare the antibody titers in order to make inference about the difference in GMC between month 2-7 and month 18-19 infant sera. The correlations between antibody titers at different time points and between antibody levels and their functionality were calculated using Pearson correlation. We analysed our results as per protocol with significance defined by a p-value of <0.01. Note that relaxing the significance level to 0.05 yields other insights. Blunting of vaccine-induced immune responses was defined as a significantly lower GMC of IgG at one time point in the wP *versus* the wP EPI group.

3. Results

3.1 Demographics

Overall, 370 pregnant women, recruited between April 2015 and September 2016, were vaccinated (Figure 1). From these women, 311 healthy infants were randomized to receive either Infanrix hexa® (aP group; N=156 term and 2 late preterm) or Quinvaxem® (wP group; N=155 term and 2 late preterm). Seventy-nine full-term infants born to non Tdap-vaccinated women received Quinvaxem® (EPI wP group). Baseline characteristics (Table 1) show no significant differences between the groups. Some infants were not vaccinated according to protocol (Table S1) as a result of illness or delayed visits.

3.2 Antibody responses to *B. pertussis* antigens

We discuss all available data (intention-to-treat analysis), since differences between all available data (Table S2) and data with full protocol adherence (Table S3) are not significant. The percentages of values below LLOQ ranged from 0.3% to 12% depending on antigen and time point.

Comparing wP group and EPI wP group, significantly lower anti-PT ($p < 0.001$), anti-FHA ($p < 0.001$), and somewhat lower anti-PRN ($p = 0.030$) titers were found one month after priming in the wP than in the EPI wP group, suggesting interference of maternal antibodies. At one month after the booster dose, interference still persisted for anti-PT ($p < 0.001$) and anti-FHA ($p < 0.001$) IgG.

The EPI wP group had significantly higher anti-PT ($p < 0.001$) IgG levels than the aP group at post-priming and post-booster, yet lower anti-FHA and anti-PRN levels.

Comparing the offspring of vaccinated women, GMCs of all *B. pertussis*-specific antibodies were significantly higher in the aP compared to the wP group following the primary

series ($p < 0.001$) (Figure 2). At 18 months of age, all antibody responses substantially waned and the remaining levels were lower than the levels at 2 months of age in both groups. Antibody titers increased significantly for all antigens one month after the booster vaccination. Anti-PT IgG was comparable between both groups, but the aP group possessed significantly higher anti-FHA ($p < 0.001$) and anti-PRN ($p < 0.001$) antibody titers.

Within the aP group, significantly higher anti-PT and anti-PRN IgG GMC ($p < 0.001$) were measured post-primary vaccination, compared to pre-priming levels, but their anti-FHA IgG remained at a comparable level (Figure 2). Although infants in the wP group also had significantly higher anti-PT IgG ($p < 0.001$) post-priming, their anti-FHA levels decreased significantly ($p < 0.001$) after priming, whereas the levels of anti-PRN IgG did not change significantly.

A comparison of *B. pertussis*-specific GMC's between infants who only received the vaccines foreseen in the study and infants who received optional vaccines showed no significant differences (data not shown).

3.3 Correlation between maternal antibodies and vaccine-induced antibody responses

Similar to Englund et al (9), we found negative correlations between anti-PT IgG levels at month 2 and month 7 in both the aP and wP group, with a higher coefficient in the wP group; Pearson's correlation coefficient (r) = -0.24, $p = 0.006$ (aP) vs. -0.32, $p < 0.001$ (wP) (Figure S1). In contrast, we found a statistically significant positive correlation between month 2 and month 7 for anti-FHA IgG levels in the wP group (r = 0.29, $p = 0.001$). The only positive and significant correlation was found for anti-PRN IgG between pre-priming (month 2) and post-booster (month 19) antibody levels, r = 0.23, $p = 0.007$ (Figure S2).

3.4 Functionality of antibodies

Sera from a subset (depending on the availability of samples at all time points) of samples (N=276) were tested for their ability to inhibit *B. pertussis* growth (Figure 1). Functional activity of all sera, was highly dependent on complement, as demonstrated by the decrease in activity in heat-treated compared to non-treated sera (compare panels A with B and C with D; Figure 3). However, even in the absence of complement, the serum samples expressed various levels of *Bordetella* growth inhibition (Fig. 3A and 3C), suggesting complement-independent *Bordetella* growth inhibition by anti-pertussis sera. This was stronger in maternal and cord blood than in infant sera, whereas the reverse was seen in the presence of complement.

In the absence of complement, functionality of antibodies in cord was not significant and maternal sera was not significantly different (Fig. 3A). In the presence of complement, maternal sera were significantly more inhibitory than cord sera (Fig. 3B). At one-month post primary infant vaccination, there was no difference between aP and wP groups for the heat-treated sera (Fig. 3C). However, at 18 months, heat-treated serum in the wP group was significantly more active than in the aP group (Fig. 3A), persisting for at least one month after the booster (Fig. 3C). Antibodies in infants born to Tdap-vaccinated mothers appeared to better inhibit bacterial growth than those of infants born to unvaccinated mothers after the primary series of wP vaccination, but this was reversed after booster vaccination (Figure 3C).

Analysis in the presence of complement, showed no difference between the aP and wP groups after the primary vaccination (Fig. 3D). However, after the booster vaccination, the wP group inhibited *B. pertussis* growth again significantly better than the aP group.

No correlations between bactericidal activity and anti-PT IgG and anti-FHA levels were found (Figure S3-S4). There were some positive correlations between functional activity and anti-PRN IgG levels in the wP group alone (Figure S5).

Discussion

Blunting of aP-vaccination in infants has been reported after maternal Tdap vaccination [10, 13], and we report for the first time in a large cohort equal blunting of the infant anti-PT and anti-FHA antibody responses to wP-containing vaccines. Our findings are consistent with data showing that naturally acquired maternal antibodies had a negative influence on PT antibody responses induced by DTwP vaccination in infants [9]. Ibrahim et al. [22] recently reported no attenuating effect on infant *B. pertussis*-specific post-primary immunization titers, yet, most infants did not receive the full three-dose wP regimen.

This blunting effect may be of clinical relevance. PT is a major virulence factor of *B. pertussis* [23], and humanised neutralizing anti-PT monoclonal antibodies have been shown to abolish disease manifestations in mice and non-human primates [24]. Furthermore, maternal vaccination with a monocomponent PT vaccine protected newborn baboons against disease following respiratory challenge with *B. pertussis* [25]. In humans low anti-PT IgG titers have been associated with high susceptibility to pertussis, although no correlate of protection is known [26]. From surveillance data in countries where maternal Tdap has been implemented, however, there are no signals of any clinical effect of the reported blunting of the aP infant responses [27]. In the UK e.g. , the maternal vaccine coverage has reached over 70% since May 2016. If blunting was clinically important, the rate of pertussis should have increased in children between 6 months – 1.5 years. However, there is no evidence of increased incidence of pertussis among English children. Since we report significantly lower antibody titers in wP- compared to aP- vaccinated children, the lack of clinical significance in aP-vaccinated children cannot be extrapolated to wP-vaccinated children.

Comparing aP and wP group immune responses, the aP group had significantly higher levels of all pertussis-specific IgG after a three-dose priming scheme and anti-FHA and anti-PRN antibody levels were still significantly higher after a booster dose. Previous comparative

studies, without maternal immunization, reported that aP-containing infant vaccines induce higher levels of antibodies, due to the higher amounts of antigens in aP compared to some of the wP-containing vaccines [28, 29]. In wP-containing vaccines, the levels of PT, FHA and PRN are not specified [30], resulting in wide ranges of immunogenicity between different manufacturers [31]. Quinvaxem® may contain reduced amount of FHA and PRN resulting in lower-than-expected immunogenicity following primary immunization.

Within the aP group, antibody levels to PT and PRN rose significantly after priming, but anti-FHA IgG did not. Ladhani et al. reported similar findings for anti-PT and anti-FHA IgG in a cohort of aP-vaccinated children [32].

Using a novel *Bordetella* growth inhibition assay **(**BGIA**),** complement-dependent growth inhibition was stronger in maternal than in cord blood, likely reflecting the different levels of complement in both tissues**.** Based on the growth inhibition results in infant sera, the blunting of antibodies induced by wP-containing vaccines in the presence of maternal antibodies after priming, did not imply a reduction of the bactericidal activity of the antibodies. Inhibition of growth was actually overall better in wP-vaccinated infant sera, and after maternal Tdap vaccination. This suggests that maternal antibodies may endorse this bactericidal activity or even promote the production of infant antibodies with specific biophysical features mediating efficient pathogen control. However, after boosting the bactericidal activity was stronger for wP-vaccinated infants born from unvaccinated mothers compared to infants born to vaccinated mothers, suggesting that the differences observed after priming are mainly due to the activity of maternal antibodies. Studies in a murine model of pertussis [33] indicated that maternal immunization may affect the functionality of antibodies induced by primary aP vaccination of the offspring. We report here a primary observation on the functionality of the induced antibodies during a human trial, although more research is certainly needed.

The effect of maternal Tdap vaccination on cell-mediated immunity (CMI) following wP- or aP-containing infant vaccines is also of importance [34]. CMI responses in the present cohort will be reported separately.

The study has a few shortcomings. Infants to the EPI wP group were not randomized and we lacked data on the baseline antibody levels at month 2 for these EPI wP infants, but it is expected that the antibody levels pre-priming were low, based on our previous study [21]. A fourth study arm, aP-vaccinated infants of non-vaccinated mothers, was not added, since many comparative data are already available. The largest relevant study was conducted by Halperin et al [13] reporting that infants born to Tdap-vaccinated mothers had significantly lower antibody titers following primary immunization, persisting until after the first booster.

Altogether we report that Tdap-induced maternal antibodies affect the immune responses to a primary series of vaccines, both quantitatively, especially for anti-PT and FHA IgG, persisting at least until after the booster dose, and qualitatively**.** No correlation between antibody levels against PT and levels of growth inhibition was observed, which is consistent with PT being mostly a secreted antigen **[35]** and therefore not an efficient target for antibodies that mediate growth inhibition or bacterial lysis**.**

In summary, if countries using wP-containing vaccines for priming of infants, would consider implementing maternal Tdap immunization, the blunting following wP vaccination should be considered. Vaccine-induced immune protection should then closely be monitored and pertussis surveillance should be strengthened.

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Contributor statement

EL is the principal investigator, she conceived the study, and followed up on the entire study conduct. YP and NW are the principal investigators on site. They included and sampled all the subjects, and are responsible for the laboratory analysis on site, and initiated the data analysis. YP, KM, PVD and CL are involved as co- investigators in the entire (Thrasher funded) study. NH and TMPT performed the statistics. TT and SV performed the ELISA laboratory tests.
All authors contributed to the writing of the manuscript.

Conflict of Interest

None of the authors have a conflict of interest to declare for this manuscript.

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Figure and Table legends

Table 1**:** Baseline characteristics of participants included in the study**.**

GA, Gestational age; SD, Standard Deviation; mo, month; N/D, Data not available.

Figure 1: The consort flow diagram. Tdap; Tetanus- diphtheria and acellular pertussis. GA; Gestational age. aP; acellular pertussis vaccine. wP; whole cell pertussis vaccine. mo; month, BGIA; *B. pertussis* growth inhibition assay. *One wP child received Quinvaxem® at month 7 which was not according to the protocol.

Figure 2: Geometric mean concentrations of **(**A**)** anti-PT, **(**B**)** anti-FHA and **(**C**)** anti-PRN IgG in the aP, wP and EPI wP groups at birth (cord), months 2, 7, 18 and 19. Cord antibody levels of the EPI wP infants were derived from the cord levels of Thai historical infant cohort born to mothers who did not receive Tdap during pregnancy [21]. Error bars indicate the upper bound of 95% confidence interval (CI). *statistically significant difference compared to pre-priming or pre-booster, ** statistically significant difference compared to other groups at month 7 and 19

Figure 3: Ratio of relative luminescence units (RLU) in different circumstances comparing the study groups aP, wP and EPI wP at different time points. Figure 3A & C compared the ratio of RLU in wells containing *B. pertussis* incubated with heat-treated antibody (ab) divided by RLU (ctr) in wells containing *B. pertussis* alone. Figure 3B & D compared the RLU in wells containing *B. pertussis* incubated with untreated antibody plus complement (ab+com) divided by RLU (ctr) in wells containing *B. pertussis* alone. Significance was evaluated using a two-tailed Student's t-test, fig. 3A: **p=0.0089, fig. 3B: **p=0.0017 and ***p<0.0001, fig. 3C: **p=0.005, ***p<0.0001 and ***p=0.0008 (aP vs wP group at month 19) and fig. 3D: **p=0.0043.

Supplementary Figures and Tables

Figure S1: Correlations of (A) anti-PT, (B) anti-FHA and (C) anti-PRN IgG between two and seven-month-old aP-vaccinated infant sera and (D) anti-PT, (E) anti-FHA and (F) anti-PRN IgG between two and seven-month-old wP-vaccinated infant sera. Pearson correlation coefficient for anti-PT (aP) = -0.165 (p = 0.005), anti-PT (wP) = -0.257 (p < 0.001), anti-FHA (aP) = 0.018 (p = 0.757), anti-FHA (wP) = 0.170 (p = 0.006), anti-PRN (aP) = -0.123 (p = 0.039) and anti-PRN (wP) = 0.114 (p = 0.065).

Figure S2: Correlations of (A) anti-PT, (B) anti-FHA and (C) anti-PRN IgG between two and nineteen-month-old aP-vaccinated infant sera and (D) anti-PT, (E) anti-FHA and (F) anti-PRN IgG between two and nineteen-month-old wP-vaccinated infant sera. Pearson correlation coefficient for anti-PT (aP) = -0.099 (p = 0.093), anti-PT (wP) = -0.113 (p = 0.07), anti-FHA (aP) = -0.048 (p = 0.418), anti-FHA (wP) = -0.087 (p = 0.158), anti-PRN (aP) = 0.145 (p = 0.016) and anti-PRN (wP) = 0.093 (p = 0.134).

Figure S3: Correlation between the growth inhibition activity of serum (ab+com) or decomplemented serum (ab) measured by the BGIA and the amount of anti-PT IgG measured by ELISA. The correlations were made for the aP, wP groups at month 7 (following vaccination) and before and after the boost (month 18 and 19, respectively). For the EPI wP group, correlations were made after vaccination and after the boost (month 7 and month 19, respectively). The BGIA was represented as a ratio of RLU in well containing serum and bacteria divided by RLU in well containing bacteria alone. The ELISA results were expressed in UI/ml on log10 scale. Correlation analysis was evaluated using a two-tailed Pearson's test.

Figure S4: Correlation between the growth inhibition activity of serum (ab+com) or decomplemented serum (ab) measured by the Bordetella growth inhibition assay (BGIA) and the amount of anti-FHA IgG measured by ELISA. The correlations were made for the aP, wP

groups at month 7 (following vaccination) and before and after the boost (month 18 and 19, respectively). For the EPI wP group, correlations were made after vaccination and after the boost (month 7 and month 19, respectively). The BGIA was represented as a ratio of RLU in well containing serum and bacteria divided by RLU in well containing bacteria alone. The ELISA results were expressed in UI/ml on log10 scale. Correlation analysis was evaluated using a two-tailed Pearson's test.

Figure S5: Correlation between the growth inhibition activity of serum (ab+com) or decomplemented serum (ab) measured by the Bordetella growth inhibition assay (BGIA) and the amount of anti-PRN IgG measured by ELISA. The correlations were made for the aP, wP groups at month 7 (following vaccination) and before and after the boost (month 18 and 19, respectively). For the EPI wP group, correlations were made after vaccination and after the boost (month 7 and month 19, respectively). The BGIA was represented as a ratio of RLU in well containing serum and bacteria divided by RLU in well containing bacteria alone. The ELISA results were expressed in UI/ml on log10 scale. Correlation analysis was evaluated using a two-tailed Pearson's test. Pearson's correlation coefficient for anti-PRN wP group at month 19 in decomplemented serum (ab) = 0.2714 (p=0.0045).

Table S1**:** Deviations in the study visits

Table S2: Geometric mean concentrations (GMC) with 95% CI of anti-PT, anti-FHA and anti-PRN IgG in cord and infant sera at different time points and *p* values indicating the difference in GMC between different groups or time points (all available data).

Table S3: Geometric mean concentrations (GMC) with 95% CI of anti-PT, anti-FHA and anti-PRN IgG in cord and infant sera at different time points and p values indicating the difference in GMC between different groups or time points (data from infants with full protocol adherence)

	aP group (n=158)	wP group (n=157)	EPI wP group (n=79)
Mean age of mothers at enrollment in years (SD)	29.0 (5.4)	28.4 (5.5)	28.0 (5.9)
Mean GA at delivery (SD)	38.7 (1.1)	38.6 (1.1)	38.6 (1.2)
Mean GA at vaccination (SD)	30.5 (2.4)	30.9 (2.2)	NA
Mode of delivery - vaginal, n (%) - cesarean, n (%)	 89 (56.3) 69 (43.7)	 87 (55.4) 70 (44.6)	 45 (56.3) 35 (43.8)
Gender -male, n (%) -female, n (%)	 77 (48.7) 81 (51.3)	 77 (49.0) 80 (51.0)	 44 (55.0) 36 (45.0)
Mean weight at birth in grams (SD)	3127.6 (389.7)	3122.0 (320.6)	3237.4 (417.5)
Mean length at birth in centimeters (SD)	49.6 (2.1)	49.7 (2.0)	N/D
Mean weight at mo 2 in kilograms (SD)	5.4 (0.6)	5.4 (0.6)	5.5 (0.6)
Mean length at mo 2 in centimeters (SD)	57.3 (2.3)	57.3 (2.6)	57.4 (2.3)
Mean weight at mo 4 in kilograms (SD)	6.7 (0.8)	6.8 (0.8)	6.9 (0.7)
Mean length at mo 4 in centimeters (SD)	63.0 (2.5)	63.3 (2.5)	63.5 (2.3)
Mean weight at mo 6 in kilograms (SD)	7.5 (1.0)	7.6 (0.9)	7.8 (0.8)
Mean length at mo 6 in centimeters (SD)	67.2 (3.0)	67.3 (2.5)	67.5 (2.1)
Mean weight at mo 7 in kilograms (SD)	7.9 (1.0)	7.9 (0.9)	8.1 (0.8)
Mean length at mo 7 in centimeters (SD)	69.0 (2.6)	69.3 (2.9)	69.4 (2.2)
Mean weight at mo 18 in kilograms (SD)	10.9 (1.5)	10.9 (1.5)	10.9 (1.2)
Mean length at mo 18 in centimeters (SD)	81.7 (3.4)	81.6 (3.4)	82.1 (2.9)
Mean weight at mo 19 in kilograms (SD)	11.2 (1.5)	11.2 (1.5)	11.2 (1.3)
Mean length at mo 19 in centimeters (SD)	83.2 (3.2)	83.0 (3.3)	83.0 (4.5)
Mean interval between birth and visit month 2 in days (SD)	63.0 (4.6)	62.6 (4.3)	61.6 (5.5)
Mean interval between visit month 2 and visit month 4 in days (SD)	59.9 (5.1)	60.0 (5.2)	61.8 (5.4)
Mean interval between visit month 4 and visit month 6 in days (SD)	60.5 (5.3)	61.6 (4.7)	61.5 (4.7)
Mean interval between visit month 6 and visit month 7 in days (SD)	30.8 (4.3)	31.1 (4.8)	31.7 (5.3)
Mean interval between visit month 18 and visit month 19 in days (SD)	31.8 (6.5)	32.1 (5.7)	31.6 (6.4)

Table 1**:** Baseline characteristics of participants included in the study**.**

GA, Gestational age; SD, Standard Deviation; mo, month; N/D, Data not available.

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Between birth and month 2					
No.	Group	Code	Interval (days)	Number of deviated days	Reasons
1	aP	C067	78	8	Illness
2	aP	C084	71	1	Delayed visit
3	aP	C100	73	2	Delayed visit
4	aP	C234	79	9	Delayed visit
5	aP	C289	73	3	Illness
6	aP	C333	80	10	Delayed visit
7	wP	C022	71	1	Illness
8	wP	C127	77	7	Delayed visit
9	wP	C153	79	9	Illness
10	wP	C173	71	1	Delayed visit
11	wP	C208	72	2	Delayed visit
12	wP	C215	73	3	Delayed visit
13	EPI wP	C501	88	18	Delayed visit
14	EPI wP	C504	74	4	Delayed visit
Between month 2 and month 4					
No.	Group	Code	Interval (days)	Number of deviated days	Reasons
1	aP	C062	80	10	Delayed visit
2	aP	C316	72	2	Delayed visit
3	aP	C333	77	7	Delayed visit
4	wP	C030	77	7	Illness
5	wP	C184	77	7	Illness
6	wP	C290	77	7	Delayed visit
7	EPI wP	C501	73	3	Delayed visit
8	EPI wP	C511	77	7	Illness
9	EPI wP	C558	73	3	Delayed visit
10	EPI wP	C577	84	14	Illness
Between month 4 and month 6					
No.	Group	Code	Interval (days)	Number of deviated days	Reasons
1	aP	C274	75	5	Illness
2	aP	C299	87	17	Delayed visit
3	aP	C316	84	14	Delayed visit
4	wP	C184	84	14	Delayed visit

5	wP	C230	73	3	Illness
6	wP	C264	77	7	Delayed visit
7	EPI wP	C518	73	3	Illness
8	EPI wP	C521	77	7	Illness
9	EPI wP	C529	77	7	Delayed visit
Between month 6 and month 7					
No.	Group	Code	Interval (days)	Number of deviated days	Reasons
1	aP	C032	42	7	Illness
2	aP	C034	36	1	Delayed visit
3	aP	C052	51	16	Delayed visit
4	aP	C062	27	-1	Limited availability
5	aP	C076	42	7	Illness
6	aP	C090	42	7	Illness
7	aP	C138	42	7	Illness
8	aP	C196	40	5	Illness
9	aP	C207	42	7	Delayed visit
10	aP	C326	27	-1	Limited availability
11	aP	C338	skip	-	Relocation
12	aP	C343	44	9	Delayed visit
13	wP	C041	42	7	Delayed visit
14	wP	C043	39	4	Illness
15	wP	C057	skip	-	Relocation
16	wP	C099	skip	-	Relocation
17	wP	C114	27	-1	Limited availability
18	wP	C119	42	7	Illness
19	wP	C132	49	14	Illness
20	wP	C157	42	7	Delayed visit
21	wP	C209	42	7	Illness
22	wP	C221	skip	-	Relocation
23	wP	C222	37	2	Delayed visit
24	wP	C229	skip	-	Relocation
25	wP	C260	37	2	Delayed visit
26	wP	C302	42	7	Delayed visit
27	wP	C337	42	7	Delayed visit
28	wP	C350	42	7	Illness
29	wP	C351	42	7	Delayed visit

30	wP	C364	47	12	Delayed visit
31	EPI wP	C518	53	18	Delayed visit
32	EPI wP	C519	42	7	Illness
33	EPI wP	C531	49	14	Delayed visit
34	EPI wP	C536	42	7	Delayed visit
35	EPI wP	C542	39	4	Delayed visit
36	EPI wP	C567	41	6	Delayed visit
Between month 18 and month 19					
No.	Group	Code	Interval (days)	Number of deviated days	Reasons
1	aP	C026	49	14	Delayed visit
2	aP	C033	42	7	Delayed visit
3	aP	C047	43	8	Illness
4	aP	C062	44	9	Illness
5	aP	C063	42	7	Delayed visit
6	aP	C088	25	-3	Limited availability
7	aP	C100	49	14	Delayed visit
8	aP	C122	42	7	Illness
9	aP	C150	21	-7	Limited availability
10	aP	C155	42	7	Delayed visit
11	aP	C196	42	7	Illness
12	aP	C206	56	21	Illness
13	aP	C207	42	7	Delayed visit
14	aP	C227	42	7	Illness
15	aP	C232	43	8	Delayed visit
16	aP	C243	36	1	Illness
17	aP	C268	36	1	Delayed visit
18	aP	C273	37	2	Delayed visit
19	aP	C291	42	7	Delayed visit
20	aP	C295	63	28	Delayed visit
21	aP	C320	42	7	Delayed visit
22	aP	C338	50	15	Illness
23	aP	C339	42	7	Delayed visit
24	aP	C342	42	7	Illness
25	wP	C036	59	24	Delayed visit
26	wP	C045	42	7	Delayed visit
27	wP	C115	37	2	Illness

28	wP	C119	42	7	Illness
29	wP	C127	42	7	Delayed visit
30	wP	C132	42	7	Delayed visit
31	wP	C160	49	14	Illness
32	wP	C161	42	7	Delayed visit
33	wP	C166	42	7	Illness
34	wP	C184	49	14	Illness
35	wP	C188	40	5	Illness
36	wP	C229	37	2	Delayed visit
37	wP	C260	42	7	Delayed visit
38	wP	C266	42	7	Delayed visit
39	wP	C359	42	7	Delayed visit
40	wP	C369	45	10	Illness
41	EPI wP	C547	61	26	Delayed visit
42	EPI wP	C550	49	14	Delayed visit
43	EPI wP	C565	25	-3	Limited availability
44	EPI wP	C568	40	5	Illness
45	EPI wP	C575	42	7	Illness

Table S1**:** Deviations in the study visits

Time point	GMC in IU/mL to B. pertussis antigens (95% CI)	aP	wP	wP EPI	p-value (aP vs wP)	p-value (aP vs EPI wP)	p-value (wP vs EPI wP)	p-value (aP before vs after vaccination)	p-value (wP before vs after vaccination)
Cord	N	129	121	NA		NA	NA	NA	NA
	Anti-PT	49.7 (42.0-58.8)	44.3 (37.7-52.1)		0.337				
	Anti-FHA	380.8 (311.0-466.2)	365.8 (302.0-443.0)		0.778				
	Anti-PRN	115.1 (81.3-162.9)	153.2 (106.6-220.0)		0.226				
Before primary vaccination (month 2)	N	137	132	NA		NA	NA	NA	NA
	Anti-PT	16.7 (14.3-19.5)	13.7 (11.7-16.1)		0.086				
	Anti-FHA	108.3 (91.1-128.7)	92.0 (77.3-109.5)		0.193				
	Anti-PRN	36.3 (26.8-49.1)	47.3 (34.8-64.3)		0.229				
One month after primary vaccination (month 7)	N	119	109	55					
	Anti-PT	48.9 (43.5-55.0)	28.3 (22.4-35.6)	93.7 (67.7-129.6)	<0.001	<0.001	<0.001	<0.001	<0.001
	Anti-FHA	111.8 (100.9-123.9)	29.6 (26.0-33.6)	55.6 (45.1-68.4)	<0.001	<0.001	<0.001	0.558	<0.001
	Anti-PRN	82.5 (70.9-96.1)	32.6(27.0-39.4)	44.7 (33.9-59.0)	<0.001	<0.001	0.063	<0.001	0.153
Before booster vaccination (month 18)	N	135	132	NA		NA	NA	NA	NA
	Anti-PT	9.2 (7.9-10.7)	11.6 (9.8-13.9)		0.049				
	Anti-FHA	18.6 (15.6-22.1)	10.2 (8.4-12.2)		<0.001				
	Anti-PRN	12.9 (10.7-15.5)	7.4 (6.3-8.7)		<0.001				
One month after booster vaccination (month 19)	N	111	111	60					
	Anti-PT	86.1 (75.2-98.6)	106.2 (92.4-121.9)	181.0 (139.7-234.5)	0.035	<0.001	<0.001	<0.001	<0.001
	Anti-FHA	231.8 (201.7-266.4)	66.1 (55.5-78.7)	109.3 (90.1-132.6)	<0.001	<0.001	<0.001	<0.001	<0.001
	Anti-PRN	321.7 (271.9-380.6)	76.3 (60.8-95.5)	75.7 (56.5-101.4)	<0.001	<0.001	0.965	<0.001	<0.001

542 Table S2: Geometric mean concentrations (GMC) with 95% CI of anti-PT, anti-FHA and anti-PRN IgG in cord and infant sera at different time points
543 and *p* values indicating the difference in GMC between different groups or time points (all available data).

GMC in IU/mL to B. pertussis antigens (95% CI)		aP	wP	wP EPI	p-value (aP vs wP)	p-value (aP vs EPI wP)	p-value (wP vs EPI wP)	p-value (aP before vs after vaccination)	p-value (wP before vs after vaccination)
Cord	N	137	127	NA		NA	NA	NA	NA
	Anti-PT	52.2 (44.2-61.6)	45.1 (44.2-52.7)		0.209				
	Anti-FHA	387.4 (318.9-442.9)	367.9 (305.6-442.9)		0.708				
	Anti-PRN	121.9 (87.1-170.6)	157.6 (111.0-223.9)		0.300				
Before primary vaccination (month 2)	N	143	138	NA		NA	NA	NA	NA
	Anti-PT	16.4 (14.1-19.1)	13.8 (11.8-16.1)		0.115				
	Anti-FHA	106.0 (89.6-125.6)	91.0 (76.9-107.7)		0.211				
	Anti-PRN	35.0 (26.0-47.2)	48.3 (35.7-65.4)		0.137				
One month after primary vaccination (month 7)	N	134	128	68					
	Anti-PT	48.4 (43.3-54.1)	27.5 (22.1-34.1)	94.1 (70.5-125.6)	<0.001	<0.001	<0.001	<0.001	<0.001
	Anti-FHA	109.8 (99.5-121.2)	30.0 (26.6-33.8)	57.1 (47.7-68.3)	<0.001	<0.001	<0.001	0.586	<0.001
	Anti-PRN	80.8 (70.1-93.1)	32.1 (26.9-38.4)	45.1 (35.1-57.9)	<0.001	<0.001	0.030	<0.001	0.027
Before booster vaccination (month 18)	N	135	132	NA		NA	NA	NA	NA
	Anti-PT	9.2 (7.9-10.7)	11.6 (9.8-13.9)		0.049				
	Anti-FHA	18.6 (15.6-22.1)	10.2 (8.4-12.2)		<0.001				
	Anti-PRN	12.9 (10.7-15.5)	7.4 (6.3-8.7)		<0.001				
One month after booster vaccination (month 19)	N	134	127	65					
	Anti-PT	87.7 (77.7-99.0)	105.4 (92.4-120.3)	176.4 (138.4-224.8)	0.044	<0.001	<0.001	<0.001	<0.001
	Anti-FHA	233.2 (205.3-264.9)	66.2 (56.3-77.8)	107.5 (88.9-130.1)	<0.001	<0.001	<0.001	<0.001	<0.001
	Anti-PRN	314.9 (269.4-368.0)	76.6 (62.1-94.6)	75.9 (56.8-101.6)	<0.001	<0.001	0.961	<0.001	<0.001

544 Table S3: Geometric mean concentrations (GMC) with 95% CI of anti-PT, anti-FHA and anti-PRN IgG in cord and infant sera at different time points
545 and *p* values indicating the difference in GMC between different groups or time points (data from infants with full protocol adherence).

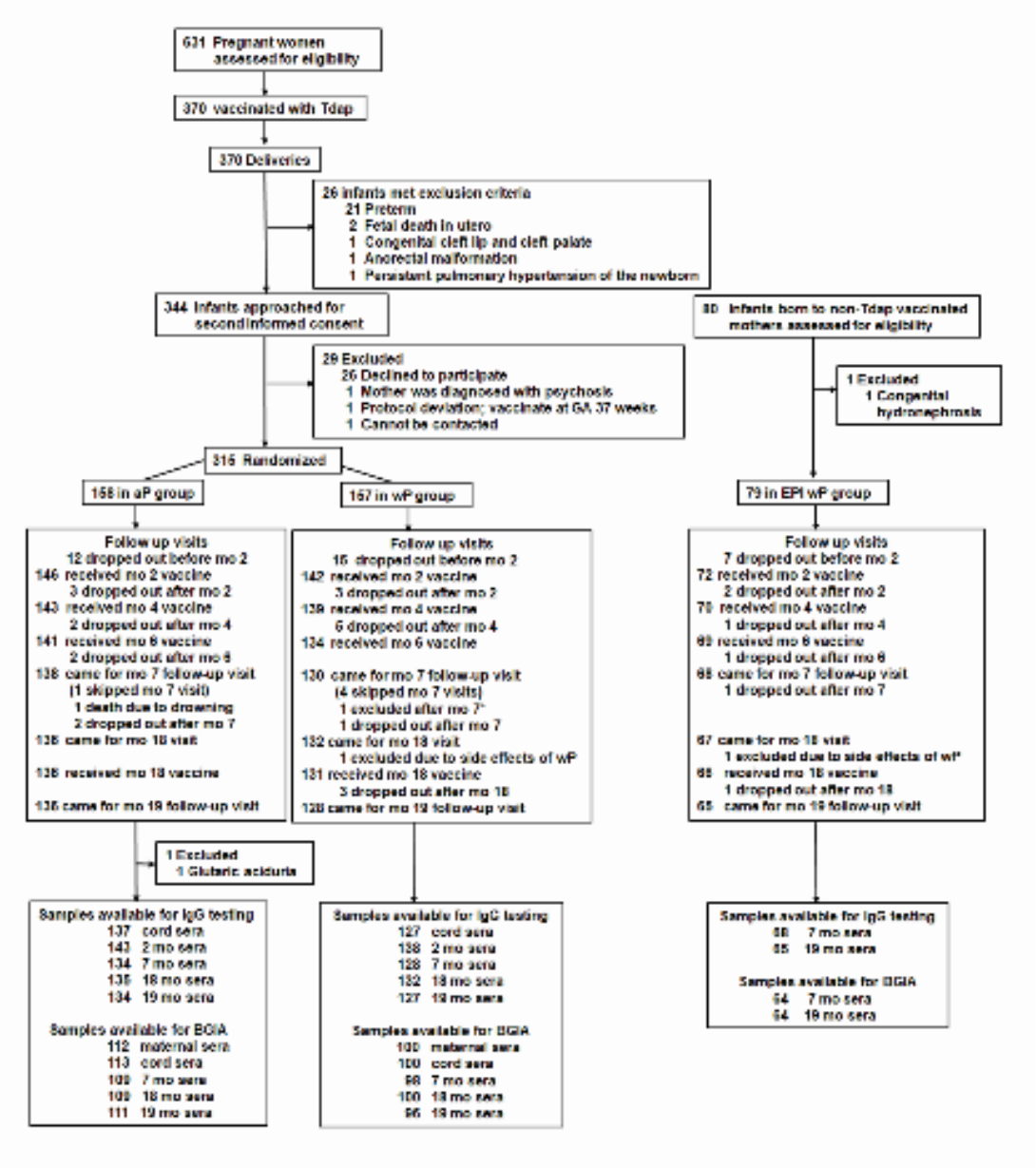
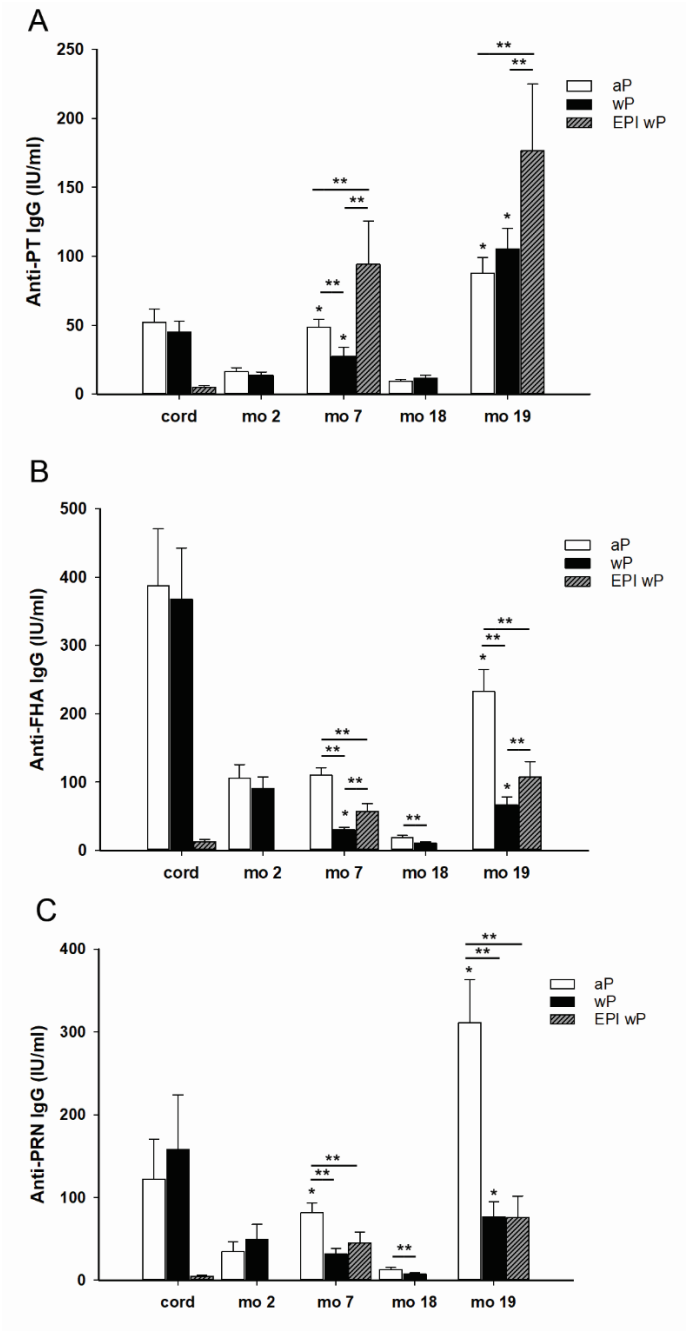


Figure 1: The consort flow diagram. Tdap; Tetanus- diphtheria and acellular pertussis. GA; Gestational age. aP; acellular pertussis vaccine. wP; whole cell pertussis vaccine. mo; month, BGIA; *B. pertussis* growth inhibition assay. *One wP child received Quinvaxem® at month 7 which was not according to the protocol.



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556 Figure 2: Geometric mean concentrations of **(**A**)** anti-PT, **(**B**)** anti-FHA and **(**C**)** anti-PRN IgG
557 in the aP, wP and EPI wP groups at birth (cord), months 2, 7, 18 and 19. Cord antibody levels
558 of the EPI wP infants were derived from the cord levels of Thai historical infant cohort born to
559 mothers who did not receive Tdap during pregnancy [21]. Error bars indicate the upper bound
560 of 95% confidence interval (CI). *statistically significant difference compared to pre-priming
561 or pre-booster, ** statistically significant difference compared to other groups at month 7 and
562 19

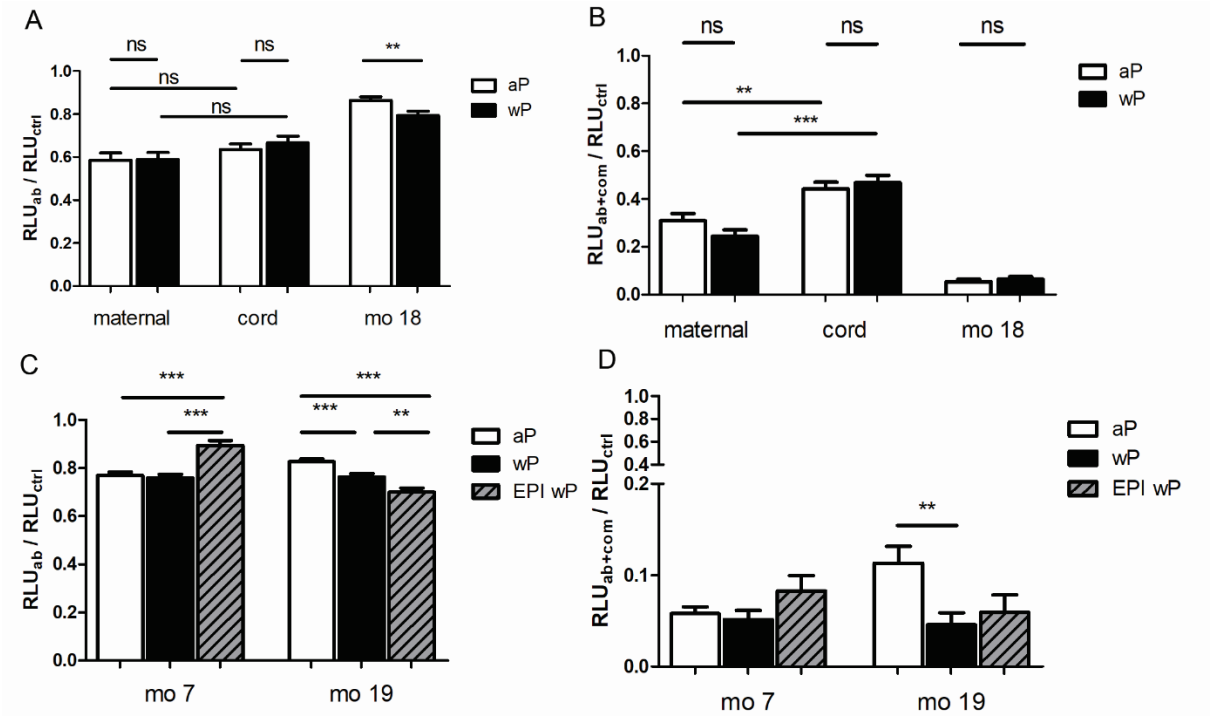
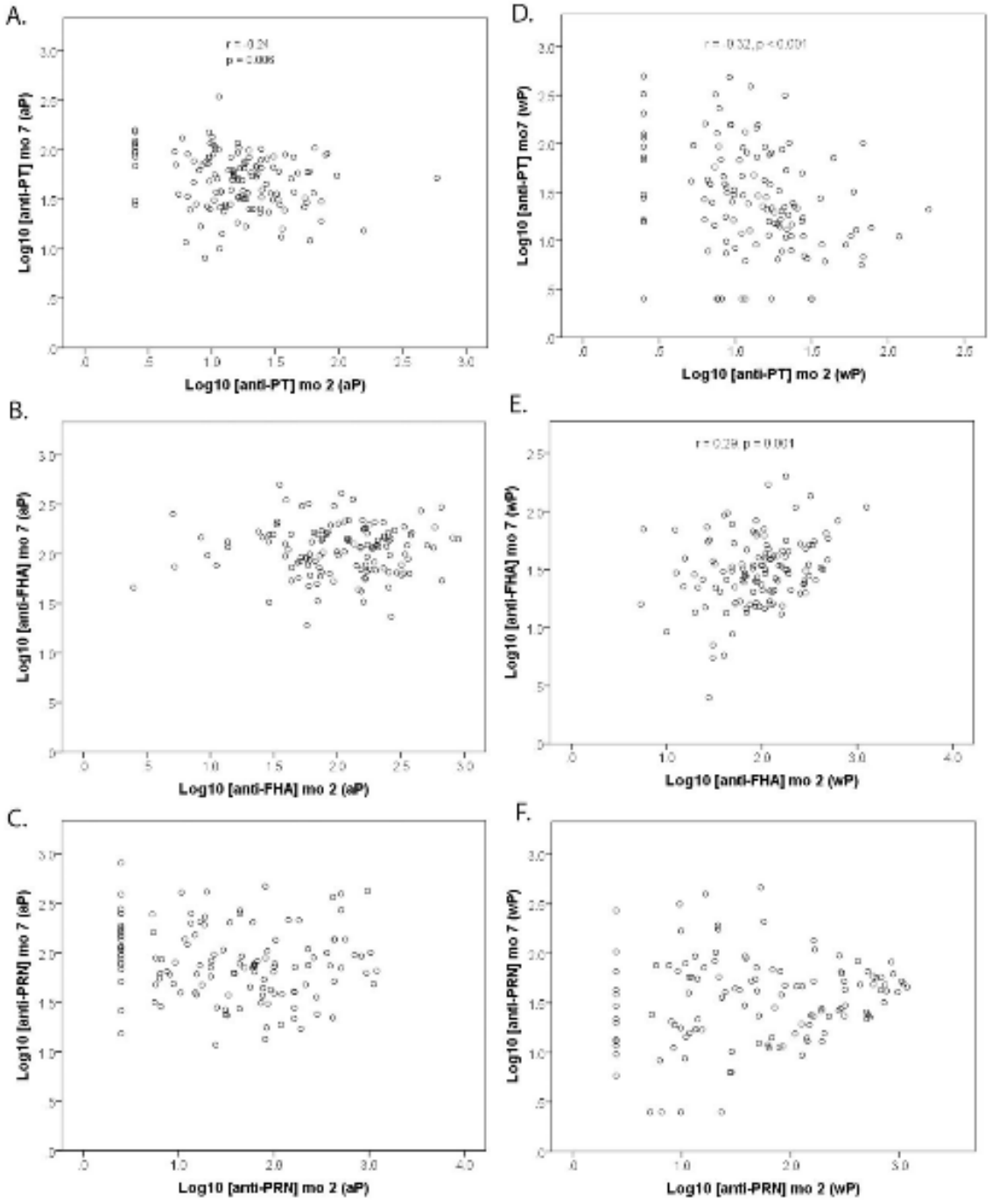
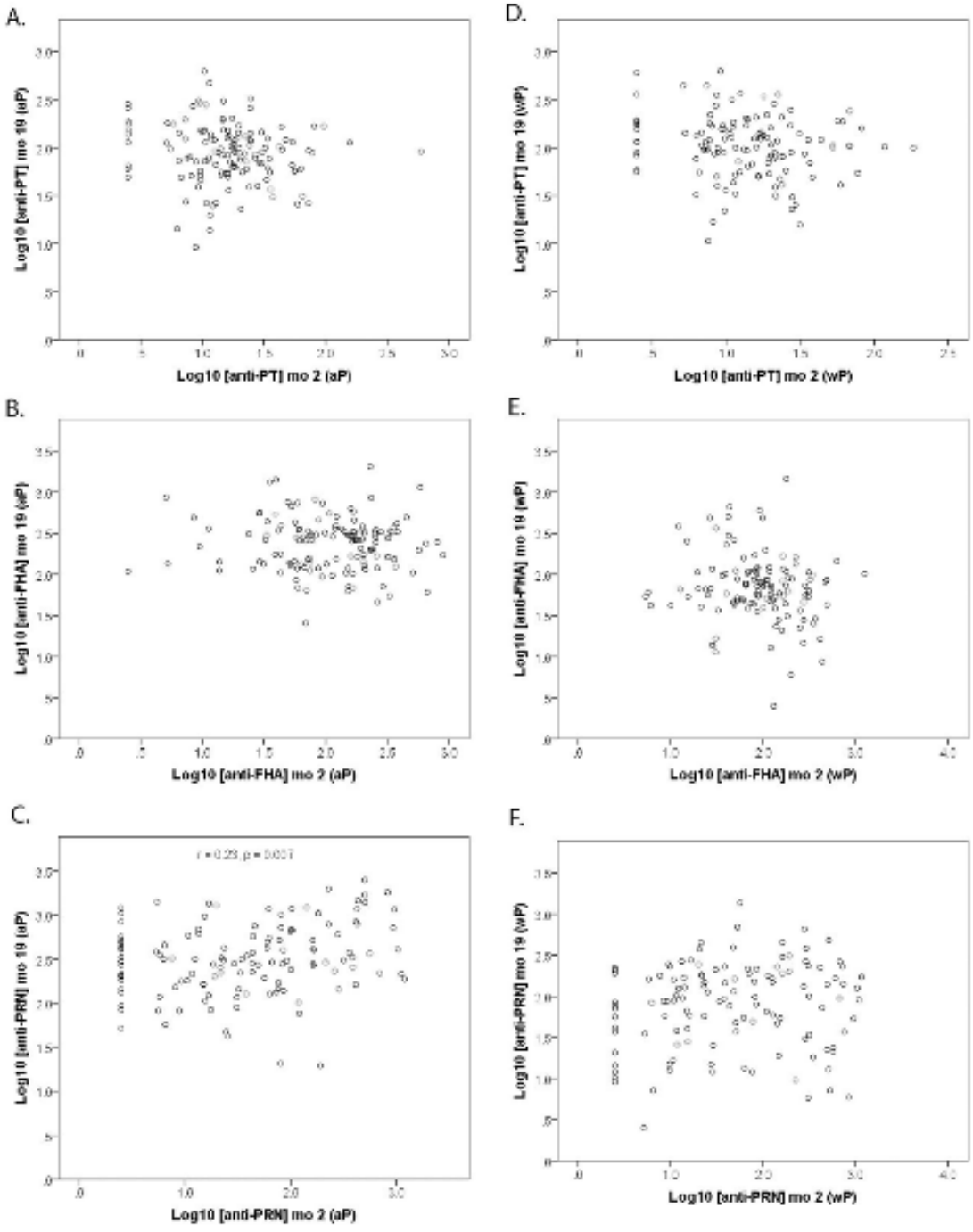


Figure 3: Ratio of relative luminescence units (RLU) in different circumstances comparing the study groups aP, wP and EPI wP at different time points. Figure 3A & C compared the ratio of RLU in wells containing *B. pertussis* incubated with heat-treated antibody (ab) divided by RLU (ctr) in wells containing *B. pertussis* alone. Figure 3B & D compared the RLU in wells containing *B. pertussis* incubated with untreated antibody plus complement (ab+com) divided by RLU (ctr) in wells containing *B. pertussis* alone. Significance was evaluated using a two-tailed Student's t-test, fig. 3A: **p=0.0089, fig. 3B: **p=0.0017 and ***p<0.0001, fig. 3C: **p=0.005, ***p<0.0001 and ***p=0.0008 (aP vs wP group at month 19) and fig. 3D: **p=0.0043.



578

579 Figure S1: Correlations of (A) anti-PT, (B) anti-FHA and (C) anti-PRN IgG between two and
580 seven-month-old aP-vaccinated infant sera and (D) anti-PT, (E) anti-FHA and (F) anti-PRN
581 IgG between two and seven-month-old wP-vaccinated infant sera. Pearson correlation
582 coefficient for anti-PT (aP) = -0.165 (p = 0.005), anti-PT (wP) = -0.257 (p < 0.001), anti-FHA
583 (aP) = 0.018 (p = 0.757), anti-FHA (wP) = 0.170 (p = 0.006), anti-PRN (aP) = -0.123 (p =
584 0.039) and anti-PRN (wP) = 0.114 (p = 0.065).



585

586 Figure S2: Correlations of (A) anti-PT, (B) anti-FHA and (C) anti-PRN IgG between two and
587 nineteen-month-old aP-vaccinated infant sera and (D) anti-PT, (E) anti-FHA and (F) anti-PRN
588 IgG between two and nineteen-month-old wP-vaccinated infant sera. Pearson correlation
589 coefficient for anti-PT (aP) = -0.099 (p = 0.093), anti-PT (wP) = -0.113 (p = 0.07), anti-FHA
590 (aP) = -0.048 (p = 0.418), anti-FHA (wP) = -0.087 (p = 0.158), anti-PRN (aP) = 0.145 (p =
591 0.016) and anti-PRN (wP) = 0.093 (p = 0.134).

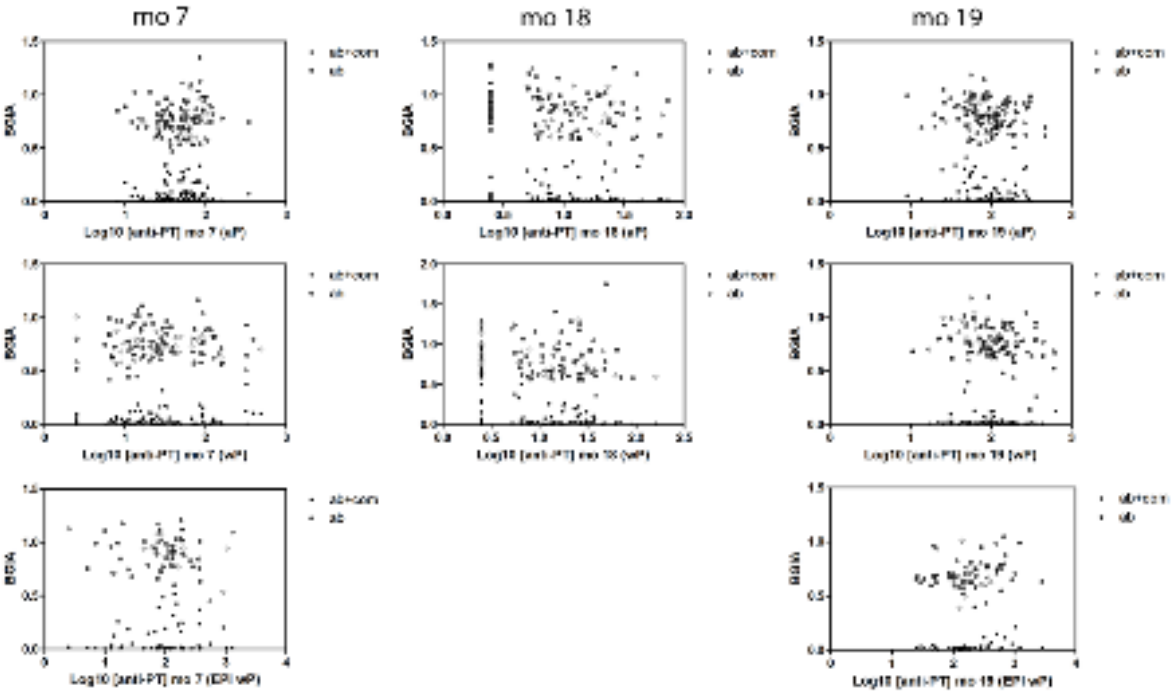


Figure S3: Correlation between the growth inhibition activity of serum (ab+com) or decomplemented serum (ab) measured by the BGIA and the amount of anti-PT IgG measured by ELISA. The correlations were made for the aP, wP groups at month 7 (following vaccination) and before and after the boost (month 18 and 19, respectively). For the EPI wP group, correlations were made after vaccination and after the boost (month 7 and month 19, respectively). The BGIA was represented as a ratio of RLU in well containing serum and bacteria divided by RLU in well containing bacteria alone. The ELISA results were expressed in UI/ml on log10 scale. Correlation analysis was evaluated using a two-tailed Pearson's test.

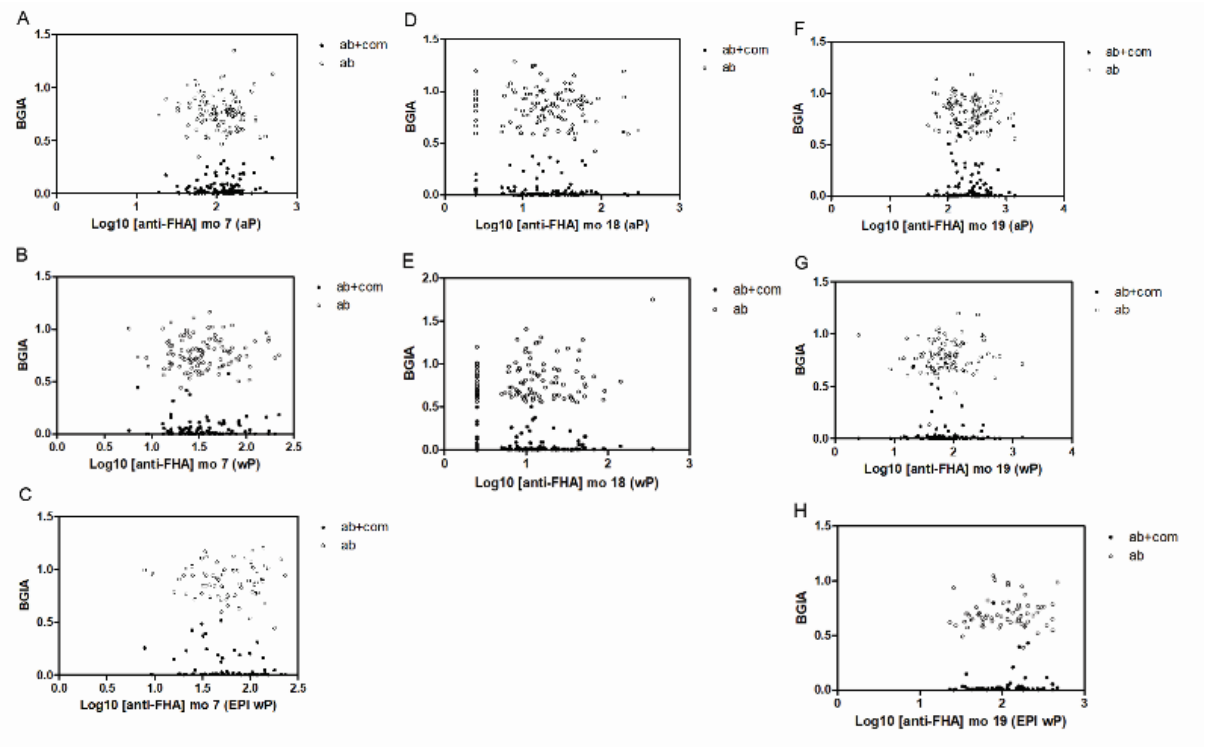
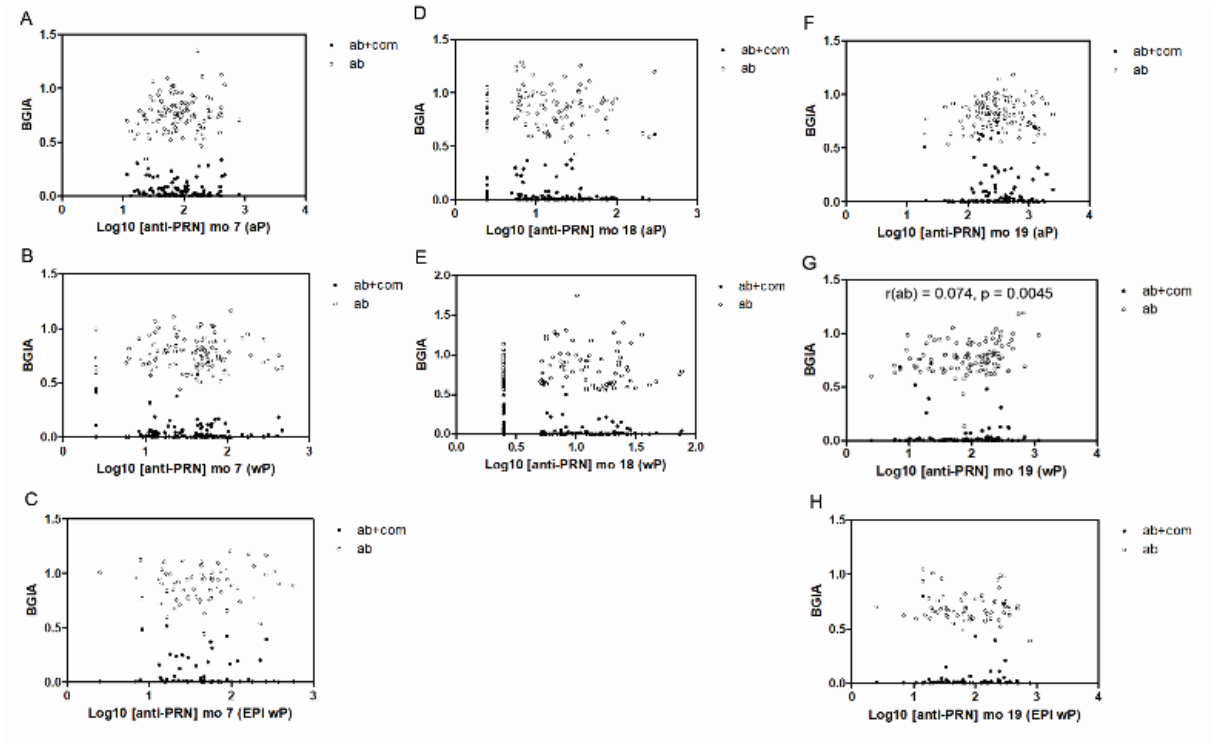


Figure S4: Correlation between the growth inhibition activity of serum (ab+com) or decomplemented serum (ab) measured by the Bordetella growth inhibition assay (BGIA) and the amount of anti-FHA IgG measured by ELISA. The correlations were made for the aP, wP groups at month 7 (following vaccination) and before and after the boost (month 18 and 19, respectively). For the EPI wP group, correlations were made after vaccination and after the boost (month 7 and month 19, respectively). The BGIA was represented as a ratio of RLU in well containing serum and bacteria divided by RLU in well containing bacteria alone. The ELISA results were expressed in UI/ml on log10 scale. Correlation analysis was evaluated using a two-tailed Pearson's test.



623

624 Figure S5: Correlation between the growth inhibition activity of serum (ab+com) or
625 decomplemented serum (ab) measured by the Bordetella growth inhibition assay (BGIA) and
626 the amount of anti-PRN IgG measured by ELISA. The correlations were made for the aP, wP
627 groups at month 7 (following vaccination) and before and after the boost (month 18 and 19,
628 respectively). For the EPI wP group, correlations were made after vaccination and after the
629 boost (month 7 and month 19, respectively). The BGIA was represented as a ratio of RLU in
630 well containing serum and bacteria divided by RLU in well containing bacteria alone. The
631 ELISA results were expressed in UI/ml on log10 scale. Correlation analysis was evaluated
632 using a two-tailed Pearson's test. Pearson's correlation coefficient for anti-PRN wP group at
633 month 19 in decomplemented serum (ab) = 0.2714 (p=0.0045).