**Title:** Pertussis antibody concentrations in infants born prematurely to mothers vaccinated in pregnancy

**Running title:** Maternal pertussis vaccine in preterm infants

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**Financial Disclosure Statement:**

PTH and SNL have conducted studies on behalf of St George’s, University of London funded by vaccine manufacturers but do not receive any personal payments or travel support. All other authors have no financial relationships relevant to this article to disclose.

**Source of Funding:**

This was an investigator-led study funded by Pfizer Ltd. The funder had no input into the study design, data analysis or manuscript preparation. This study was supported by the NIHR CRN.

**Conflicts of Interest Statement:**

PTH and SNL have conducted studies on behalf of St George’s, University of London funded by vaccine manufacturers but do not receive any personal payments or travel support. All other authors have no conflicts of interest relevant to this article to disclose.

**Clinical trial registration:** For the parent trial: EudraCT number 2007-007535-23

**Abbreviations:**

FHA Filamentous haemagglutinin

Fim 2 & 3 Fimbriae types 2 and 3

IgG Immunoglobulin G

IPV Inactivated polio vaccination

mTdap Maternal Tetanus, diphtheria and acellular pertussis (Tdap-IPV) vaccination

PT Pertussis toxin

**Key words:**

*Bordetella pertussis*

Infant, premature

Vaccination

Antibody

Maternal vaccination

**Word count:** 1356

**What’s known on this subject:**

Antenatal pertussis vaccination is highly effective at preventing neonatal pertussis due to the transfer of maternal antibody. This transfer is limited by premature birth and preterm infants, already at higher risk of disease, may not benefit from maternal vaccination.

**What this study adds:**

Premature infants born to mothers vaccinated in pregnancy have higher antibody concentrations than infants of unvaccinated mothers at the time of their first vaccination but lower filamentous haemaggglutinin antibodies after primary immunisations. These differences resolved by 12 months of age.

**Contributors’ Statement Page:**

Dr Kent coordinated and supervised the study, carried out the statistical analysis and prepared the manuscript

Dr Ladhani assisted with the study and reviewed and revised the manuscript.

Dr Andrews carried out the statistical analysis and reviewed and revised the manuscript

Dr Matheson and Dr England performed the sample analysis and reviewed and revised the manuscript

Prof Miller and Prof Heath developed and supervised the study and analysis and reviewed and revised the manuscript

All authors approved the final manuscript as submitted.

**Abstract**

**Background and objectives**

Maternal antenatal pertussis-containing vaccination is recommended for the prevention of neonatal pertussis but the ability of maternal vaccination to protect premature infants is unknown.

We hypothesised that that infants born prematurely to antenatally vaccinated women would have higher pertussis antibody concentrations than those born to unvaccinated women.

**Methods**

Mothers had been offered a combined tetanus, diphtheria, 5-component acellular pertussis, inactivated polio vaccine (Tdap/IPV; Repevax®; Sanofi Pasteur) from 28 weeks gestation as part of their routine antenatal care. Premature infants of vaccinated and unvaccinated mothers enrolled in a randomised controlled trial of pneumococcal conjugate vaccine schedules had antibody concentrations (pertussis toxin - PT, filamentous haemoagglutinin – FHA, and fimbriae 2 and 3 - Fim) measured at 2 months (prior to primary vaccination), 5 months (1 month after primary vaccination) and 12 months of age.

**Results**

Mothers of 31/160 (19%) premature infants had received Tdap/IPV in pregnancy. Compared with infants of unvaccinated mothers, those born to vaccinated mothers had significantly higher antibody concentrations at 2 months for all measured vaccine antigens (p<0.001). The number of days between maternal vaccination and delivery and IgG concentration at 2 months of age was positively correlated for PT (p=0.011) and FHA (p=0.001). After primary immunisation, infants of vaccinated mothers had significantly lower antibody concentrations for FHA (p=0.003) compared with infants of unvaccinated mothers, these differences had resolved by 12 months of age.

**Conclusions**

Maternal vaccination administered early in the third trimester may provide protection for infants born prematurely.

**Introduction**

The United Kingdom (UK) introduced a temporary immunisation programmme against pertussis for pregnant women in September 2012, following a significant increase in pertussis-related hospitalisations and deaths in young infants.1 A combined tetanus, diphtheria, 5-component acellular pertussis, inactivated polio vaccine (Tdap/IPV; Repevax®; Sanofi Pasteur) was offered to all pregnant women from 28 weeks gestation. The programme achieved 64% vaccination coverage in the first year after its introduction, with an estimated 91% effectiveness in preventing confirmed pertussis infections in infants younger than three months of age.2 Earlier administration within the recommended 28-34 week gestation window appears to result in higher infant antibody concentrations and affinity at birth and at 2 months of age.3–5

Premature infants have an increased risk of pertussis infection and are more likely to develop severe illness, resulting in prolonged hospitalisation, intensive care admission and death.6,7 At the same time, because transplacental transfer of maternal antibodies to the foetus occurs predominantly in the last trimester of pregnancy8, premature infants may not benefit from maternal vaccination to the same extent as their term-born peers.

This observational sub-study of a larger multi-centre, randomised controlled vaccination trial in premature infants (Prems Under New Schedule – PUNS) aimed to compare pertussis antibody concentrations before and after primary immunisation in premature infants whose mothers received Tdap/IPV in pregnancy with those born to unvaccinated mothers.

**Materials and Methods**

The PUNS trial was conducted at 8 neonatal units in England between May 2012 and May 2014. The maternal immunisation programme was introduced on 01 September 2012. Since infants were recruited into the clinical trial after birth, the study investigators had no influence on whether mothers were offered Tdap/IPV as part of their routine antenatal care, whether they accepted or declined vaccination or on the timing of vaccination in pregnancy. This information was collected only after informed parental consent was obtained for the infant to be included in the PUNS study. Whilst any infant with a gestational age less than 35 weeks was potentially eligible for the RCT, only those whose mothers would have been eligible for pertussis vaccination in pregnancy (>28 weeks gestation) are included in this sub-study. In addition, infants had to be medically fit for vaccination, between 7 and 12 weeks of age and with written parental consent obtained.

All infants received a combined DTaP-IPV-*Haemophilus influenzae* type b vaccine (Pediacel; Sanofi Pasteur MSD) at 2, 3 and 4 months old and meningococcal C-CRM197 vaccine (Menjugate; Novartis Vaccines) at 3 and 4 months of age. In addition they were randomly assigned (1:1:1) to receive pneumococcal conjugate vaccine (Prevenar13; Pfizer Ltd) at 2 and 4 or, 2, 3 and 4 or 2, 4 and 6 months of age.

Mothers who were vaccinated received Repevax containing pertussis toxoid (2.5mcg), filamentous haemagglutinin (5mcg), pertactin (3mcg), fimbriae types 2 & 3 (5mcg), diphtheria toxoid (≥2IU), tetanus toxoid (≥20 IU) and inactivated poliovirus from 28 weeks of pregnancy. These pertussis antigen concentrations are equivalent to those in Adcel (Sanofi Pasteur).

Infant IgG concentrations against pertussis toxin (PT), filamentous haemagglutinin (FHA), Fimbriae types 2 & 3 (Fim), diphtheria and tetanus were measured by ELISA at the Public Health England Immunoassay Laboratory at Porton Down, UK, before (~2 months of age) and 1 month after their primary immunisations (~5 months of age) and at 12 months of age. An antibody concentration of 0.1IU/mL was accepted as a serological correlate of protection for diphtheria and tetanus but there is no established correlate for pertussis9.

For statistical analysis, IgG concentrations were log transformed to normality. Statistical significance was testing using Kruskal-Wallis test or Fisher’s exact test (proportions) as appropriate and a p value<0.05 was classed as statistically significant. The main clinical trial was registered (EudraCT number 2007-007535-23) and East of England – Essex research ethics committee (REC reference 07/HO301.11).

**Results**

In this sub-study, mothers of 31/160 (19%) premature infants born at 28-35 weeks gestation had received Tdap/IPV in pregnancy (mTdap). The median gestation at mTdap administration was 28.5 weeks (IQR 28.0-29.6) and the median interval between vaccination and delivery was 24 days (IQR 9-35). The median birth gestation was slightly older in infants of vaccinated mothers compared with unvaccinated mothers (32.6 weeks vs. 31.0 weeks), although this difference was not statistically significant (p=0.057).

Compared with infants of unvaccinated mothers, those born to vaccinated mothers had significantly higher antibody concentrations at 2 months for all measured vaccine antigens (p<0.001) (Table 1) resulting in increased seroprotection rates for diphtheria (mTdap: 66.7% [20/30] vs no mTdap: 15.7% [19/121], p<0.001) and tetanus (mTdap: 90.0% [27/30] vs no mTdap: 66.1% [80/121], p=0.012). The number of days between maternal vaccination and delivery and IgG concentration at 2 months of age was positively correlated for PT (4% increase in PT concentration per day [95% CI 1-6]; p=0.011), FHA (7% [95% CI 3-10]; p=0.001), tetanus (10% [95% CI 5-14]; p<0.001) and diphtheria (6% [95% CI 1-11]; p=0.008) but not significantly so for Fim 2&3 (5% [95% CI -1-11]; p=0.061).

After primary immunisation, both groups had significantly higher antibody concentrations for all measured vaccine antigens compared with pre-immunisation concentrations. However, infants of vaccinated mothers had significantly lower antibody concentrations for FHA (p=0.003) and diphtheria (p=0.003), compared with infants of unvaccinated mothers (Table 1). All infants had seroprotective antibody concentrations for tetanus and only one infant (in the ‘no mTdap’ group) did not have protective antibody concentrations against diphtheria.

Table 2 shows the antibody concentrations and seroprotection rates at 12 months of age; there were significantly higher tetanus antibody concentrations in the mTdap group (p=0.015) but no other significant differences between groups were found.

**Discussion**

The emergency introduction of a maternal immunisation programme to control a national pertussis outbreak serendipitously provided an opportunity to assess antibody concentrations to maternal vaccine antigens in premature infants. We found significantly higher antibody concentrations at 2 months of age for all measured antigens in premature infants of vaccinated mothers compared with those born to unvaccinated mothers. Moreover, antibody concentrations at two months of age increased with the interval between maternal vaccination and birth for all but one measured vaccine antigen.

However the pre-immunisation geometric mean concentrations were substantially lower than those of term infants born to vaccinated mothers in a separate evaluation using the same laboratories: PT (3.5 vs. 11.2), FHA (17.5 vs. 46.0) and FIM (33.6 vs. 123.0)1. This is likely due to a shortened period for maternal immunological response and transfer of antibody to the foetus.8 After primary immunisation, antibodies to PT (37.2 vs. 28.8), FHA (23.0 vs. 25.5) and FIM (119 vs. 114) were generally comparable between premature and term infants born to vaccinated mothers, but lower than in the sera of the infants of unvaccinated mothers.1 Given the UK schedule does not include any further pertussis immunisation until children are 3 years old, it is reassuring that the observed differences between the groups had resolved by 12 months of age, a finding also previously reported in term infants. 10

Although post hoc analysis of clinical trial data should be interpreted with caution, these results are biologically plausible and the observed trends are consistent with the recent studies in term infants. 1,3,5,10,11 Our findings, therefore, suggest that maternal vaccination early in the third trimester (i.e. closer to 28 weeks gestation – or even early, as administered in some settings12) may also protect infants born prematurely against pertussis in early life. There are some limitations with these data; as an observational sub-study of a larger clinical trial, the trial design did not permit measurement of antibody concentrations (either maternal or infant) at birth. By assessing antibody at 2 months we have therefore estimated the nadir of maternal antibody concentrations prior to immunisation of the infant. In addition, we have limited information on maternal vaccination history outside of pregnancy. It is however, unlikely that women (in either group) would have been recently vaccinated as there is no routine pertussis vaccination programme after childhood in the UK and the maternal vaccination programme which was introduced during the study.

**Conclusion**

Maternal vaccination delivered early in the third trimester may provide protection for infants born prematurely and any potential negative impact on the infant’s immunological response to primary immunisation appears to resolve by 12 months of age.

**Acknowledgements:**

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Scorrer, Tim (1)

Snape, Matthew D (2)

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Clarke, Paul (3)

Hughes, Stephen (4)

Satodia, Prakash (5)

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9. Neonatal Unit, Royal Berkshire Hospital, Reading, UK
10. Neonatal Unit, Royal Cornwall Hospital, Truro, UK
11. NIHR Welcome Trust Clinical Research Facility, University of Southampton and University Hospital Southampton NHS Foundation Trust, UK

We would like to thank all children who took part in the study as well as their parents/guardians, the study staff at all the research centres, Pauline Kaye, Deborah Cohen, Teresa Gibbs and all other members of the team at Public Health England for their invaluable help and support and Pfizer for financially supporting this investigator-led study.

**Previous presentation**

This work has been previously presented at the European Sociaty of Paediatric Infectious Disease Annual Meeting, Leipzig, Germany, 15th May 2015 (abstract number 456)

**References**

1. Ladhani SN, Andrews NJ, Southern J, et al. Antibody responses after primary immunization in infants born to women receiving a pertussis-containing vaccine during pregnancy: single arm observational study with a historical comparator. *Clin Infect Dis*. 2015:pii: civ695 [Epub ahead of print].

2. Amirthalingam G, Andrews N, Campbell H, et al. Effectiveness of maternal pertussis vaccination in England: an observational study. *Lancet*. 2014;6736(14):1-8. doi:10.1016/S0140-6736(14)60686-3.

3. Abu Raya B, Srugo I, Kessel A, et al. The effect of timing of maternal tetanus, diphtheria, and acellular pertussis (Tdap) immunization during pregnancy on newborn pertussis antibody levels - a prospective study. *Vaccine*. 2014;32(44):5787-5793. doi:10.1016/j.vaccine.2014.08.038.

4. Abu Raya B, Bamberger E, Almog M, Peri R, Srugo I, Kessel A. Immunisation of pregnant women against pertussis: The effect of timing on antibody avidity. In: *European Society of Paediatric Infectious Diseases Annual Conference*. Leipzig, Germany; 2015.

5. Eberhardt CS, Blanchard-Rohner G, Lemaître B, et al. Maternal Immunization Earlier in Pregnancy Maximizes Antibody Transfer and Expected Infant Seropositivity Against Pertussis. *Clin Infect Dis*. 2016:ciw027. doi:10.1093/cid/ciw027.

6. Berger JT, Carcillo J a, Shanley TP, et al. Critical pertussis illness in children: a multicenter prospective cohort study. *Pediatr Crit Care Med*. 2013;14(4):356-365. doi:10.1097/PCC.0b013e31828a70fe.

7. Marshall H, Clarke M, Richmond P, Buttery J, Reynolds G, Nissen M. Predictors of Disease Severity in Children Hospitalised for Pertussis during an Epidemic. *Pediatr Infect Dis J*. 2015;34(4):339-345.

8. Van den Berg JP, Westerbeek E a M, van der Klis FRM, Berbers G a M, van Elburg RM. Transplacental transport of IgG antibodies to preterm infants: a review of the literature. *Early Hum Dev*. 2011;87(2):67-72. doi:10.1016/j.earlhumdev.2010.11.003.

9. Plotkin SA. Correlates of protection induced by vaccination. *Clin Vaccine Immunol*. 2010;17(7):1055-1065. doi:10.1128/CVI.00131-10.

10. Hardy-Fairbanks AJ, Pan SJ, Decker MD, et al. Immune responses in infants whose mothers received Tdap vaccine during pregnancy. *Pediatr Infect Dis J*. 2013;32(11):1257-1260. doi:10.1097/INF.0b013e3182a09b6a.

11. Knuf M, Schmitt H-J, Jacquet J-M, et al. Booster vaccination after neonatal priming with acellular pertussis vaccine. *J Pediatr*. 2010;156(4):675-678. doi:10.1016/j.jpeds.2009.12.019.

12. Vizzotti C, Neyro S, Katz N, et al. Maternal immunization in Argentina: A storyline from the prospective of a middle income country. *Vaccine*. 2015:[Epub ahead of print]. doi:10.1016/j.vaccine.2015.07.109.

**Tables**

Table 1: IgG Geometric mean concentrations (GMC) (µg/mL) at 2 and 5 months of age. Due to the design of the vaccination study, fewer infants were sampled at 5 months of age.

Table 2: IgG Geometric mean concentrations (GMC) (µg/mL) and percentage of infants with seroprotective antibody concentrations at 12 months of age.