# Acellular Pertussis Vaccine at Birth and One Month Induces Antibody Responses By Two Months of Age

*Nicholas Wood, MB BS, FRACP,*†‡ Peter McIntyre, MB BS, FRACP, FAFPHM, PhD,*†‡ Helen Marshall, MB BS, DCH, MPH,§ and Don Roberton, MD, FRACP, FRCPA¶*

**Background:** Infants less than 3 months of age are at highest risk of hospitalization and death from pertussis. Several studies have examined antibody responses to pertussis vaccines at birth but no previous study has evaluated 2 doses of monovalent acellular pertussis vaccine (aPV) before 2 months of age.

**Methods:** Seventy-six newborns were randomized at birth to 3 groups–aPV at birth and 1 month, aPV at birth, and control. All infants received hepatitis B vaccine (HBV) at birth followed at 2, 4, and 6 months by a combination vaccine including aPV, diphtheria, tetanus, *Haemophilus influenzae* type b (Hib), hepatitis B, polio antigens and 7 valent conjugate pneumococcal vaccine. IgG antibody responses to pertussis toxoid (PT), filamentous hemagglutinin (FHA), and pertactin (PRN) were measured in maternal serum and in infants at 2, 4, 6, and 8 months of age. Antibody responses to hepatitis B, diphtheria, tetanus, and Hib were measured at 8 months only. A parental diary and active telephone follow-up occurred for 7 days after each vaccination.

**Results:** The aPV birth dose was well tolerated. By 2 months of age, 22 of 25 (88%) of 2 dose recipients had detectable IgG antibody to PT (IgG PT) compared with 9 of 21 (43%) who received a birth dose only and 3 of 20 (15%) of controls. Infants in the 2 dose group had a geometric mean concentration (GMC) of IgG PT of 16 ELISA units per mL (EU/mL), 95% CI: 11 to 25, significantly higher than birth dose only (5 EU/mL, 95% CI: 3–8) and controls (3 EU/mL, 95% CI: 2–5). At 8 months of age, following 5, 4, and 3 doses of aP-containing vaccine, respectively, IgG PT had plateaued but IgG to FHA and PRN increased with successive doses. There was a trend to lower antibody responses for hepatitis B and Hib with higher numbers of Pa doses.

**Conclusion:** These data suggest that aPV at birth and 1 month induces significantly higher IgG antibody against pertussis antigens by 2 months of age without reducing subsequent pertussis antibody responses. Larger and more detailed studies of aPV from birth are needed to evaluate other antibody responses and the potential of this approach to reduce death and morbidity from *Bordetella pertussis* infection in the first 3 months of life.

Accepted for publication August 17, 2009.

From the *National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases, The Children's Hospital at Westmead and the University of Sydney, New South Wales, Australia; †Kids Research Institute, The Children's Hospital, Westmead, Sydney, South Wales, Australia; ‡Faculty of Medicine, The University of Sydney, New South Wales, Australia; §Vaccine and Immunology Research trials Unit, The University of Adelaide and Women's and Children's Hospital, Adelaide, Australia; and ¶Faculty of Medicine, The University of Otago, New Zealand.

Supported by a grant from the Financial Markets Foundation for Children, Australia (S112 – 2004). GlaxoSmithKline provided the investigational aPV and performed all serologic assays. Engerix and Infanrix hexa are trademarks of the GlaxoSmithKline group of companies; Prevnar is a trademark of Wyeth Pharmaceuticals Inc.

Address for correspondence: Nicholas Wood, National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases (NCIRS), Locked Bag 4001, Westmead, New South Wales 2145, Australia. E-mail: nicholw3@chw.edu.au.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pidj.com).



ISSN: 0891-3668/10/2903-0209
DOI: 10.1097/INF.0b013e3181bc98d5

**Key Words:** acellular pertussis vaccine, birth, immunogenicity

(*Pediatr Infect Dis J* 2010;29: 209–215)

Pertussis is a significant cause of mortality in early infancy worldwide. Nearly 300,000 deaths occur each year, most in developing countries, but deaths are probably underestimated in both rich and poor countries.[1,2] Death and hospitalization from pertussis occur predominantly in infants too young to receive more than 1 dose under current schedules, with over 80% of 145 reported deaths in the United States, between 2000 and 2006, occurring under 3 months of age.[3] Two doses of a pertussis-containing vaccine provide significant protection against severe disease, and even 1 dose may provide some protection against death.[4,5] The earliest age at which the first pertussis vaccine dose is currently recommended is 6 weeks under the Expanded Programme of Immunization (EPI) schedule of the World Health Organization (WHO) and can be given from 6 weeks in Europe, North America, and Australia. The second dose is given at 10 weeks under the EPI, 12 weeks in some European countries and 16 weeks elsewhere.[6] This means that, even if optimally delivered, current pertussis immunization schedules cannot provide direct protection to infants less than 8 weeks of age. When delays in immunization are taken into account, protection is often delayed even more.[7,8]

High infant morbidity and mortality from pertussis in infants was recognized more than 60 years ago,[3,9] leading to trials of maternal[10–13] and neonatal vaccination[14–17] with whole cell vaccine preparations. Following the suggestion that pertussis immunization in the neonatal period could induce immune tolerance,[16] the emphasis shifted to commencing whole cell vaccines later in the first year, even though the validity of these concerns was later questioned.[18] Currently, only BCG and hepatitis B vaccines are routinely administered at birth and their inclusion in the WHO's EPI schedule and many national vaccination programs is well-established as safe, feasible, and effective.[6,19]

Strategies to prevent early infant pertussis include universal adult and adolescent vaccination, "cocoon" vaccination of those in close contact with infants, maternal vaccination, and neonatal vaccination. No studies of maternal acellular pertussis vaccination have been published and the results of 3 recent small studies examining administration of acellular pertussis-containing vaccines in the first week of life are conflicting.[20–22] Two studies using different monovalent acellular pertussis vaccines at birth suggested that earlier antibody responses could be achieved,[20,22] but the study which used a combined diphtheria-tetanus-acellular pertussis (DTPa) vaccine at birth[21] showed inferior later antibody responses. We report the immunologic and clinical outcomes comparing 2 doses of a monovalent acellular pertussis vaccine (Glaxo Smith Kline, Belgium) at birth and 4 weeks of age with monovalent acellular pertussis vaccine at birth only and standard practice.

## METHODS

### Design

This pilot study was a randomized, nonblinded trial of administration of monovalent acellular pertussis vaccine (aPV) to newborn infants. This study was conducted according to Good Clinical Practice guidelines, the Declaration of Helsinki 1999 and had the approval of 3 ethics committees (The Children's Hospital at Westmead, Westmead Hospital, and the Children, Youth and Women's Health Service, Adelaide). Written informed consent was obtained from parents/guardians before the enrollment of infants.

Neonates in group 1 received aPV at birth (within 5 days) and a second dose at 1 month of age. Those in group 2 received aPV within 5 days of birth only and those in group 3 followed the routine vaccination schedule. In Australia, this includes hepatitis B vaccine at birth and, at 2, 4, and 6 months of age, diphtheria, tetanus, pertussis, hepatitis B, and *Haemophilus influenzae* type b antigens (given in this study as DTaP-HBV-IPV/Hib vaccine (*Infanrix Hexa*) as well as 7 valent pneumococcal conjugate vaccine (*Prevnar*). Thus overall, subjects in group 1, 2, and 3 received 5, 4, and 3 doses respectively of a pertussis-containing vaccine by 6 months of age.

### Subjects

Eligible subjects were healthy infants, who had completed at least 36 weeks gestation, were born after an uncomplicated pregnancy to mothers seronegative for hepatitis B surface antigen (HbsAg) and were enrolled within 120 hours of birth.

Enrollment in the study was excluded by any of the following: known contraindications to vaccination[23]; administration of immunoglobulins or blood products preceding the first dose of study vaccine or their planned administration during the study period; any confirmed or suspected immunosuppressive or immunodeficient condition in the parent or child and major congenital defects or serious chronic illness. The study was conducted in Sydney and Adelaide, Australia between February 2005 and March 2007. The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN012605000013662).

### Vaccines

A single dose of investigational aPV (0.5 mL) containing pertussis toxin (PT) 25 $\mu$g, pertactin (PRN) 8 $\mu$g, filamentous hemagglutinin (FHA) 25 $\mu$g, and 0.5 mg aluminum as hydroxide salts was supplied by GlaxoSmithKline (GSK) Biologicals, Rixensart, Belgium. All infants received 10 $\mu$g hepatitis B surface antigen (HbsAg) with 0.25 mg aluminum hydroxide adjuvant (*Engerix B*). The aPV was administered intramuscularly into the right anterolateral thigh and the HBV vaccine into the left anterolateral thigh concomitantly in Groups 1 and 2 prior to 120 hours of age. The antigen composition of the aPV used at birth and 1 month was identical to that in the combined DTaP-HBV-IPV/Hib vaccine (*Infanrix hexa*) in routine use. As indicated above, routine scheduled vaccines at 2, 4, and 6 months included *Infanrix hexa* and 7 valent pneumococcal conjugate vaccine (*Prevnar*—Wyeth pharmaceuticals), whose composition is listed elsewhere.[9] *Infanrix hexa* was administered intramuscularly in the right thigh and *Prevnar* in the left thigh at 2, 4, and 6 months of age by study nurses.

### Assessment of Immunogenicity

In total, 5 blood samples were collected. To reduce the number of blood samples required from the infant, the first sample was obtained from the mother at the same time as the infant received the first vaccination (Pa and HBV or HBV alone). Subsequent samples (n = 4) were collected from infants at 2, 4, 6, and 8 months of age. Samples were centrifuged, serum separated, stored at −80°C and shipped frozen to GSK Biologicals, Belgium (GSK) where all serologic assays were performed.

Pertussis toxin (anti-PT), pertactin (anti-PRN), and filamentous hemagglutinin (anti-FHA) IgG antibody concentrations were measured at each sampling point by enzyme linked immunosorbent assay (ELISA: cut-off 5 EL.U/mL), using standard assay methods at the GSK laboratory developed for licensure of DTPa vaccines.

Antidiphtheria (cut-off 0.1 IU/mL), antitetanus (cut-off 0.1 IU/mL), and anti-PRP (cut-off 0.15 $\mu$g.mL) IgG antibodies were measured by ELISA on the sample taken at 8 months of age (2 months after the final vaccine dose). Hepatitis B surface antibodies (anti-HBs) were measured by ELISA (AUSAB, Abbott Laboratories) as per the manufacturer's recommendations (cut-off 10 mIU/mL) on samples collected at 8 months of age. The laboratory was blind to the study assignment of subjects. There was no formal surveillance for pertussis infection.

### Assessment of Reactogenicity

After administration of each vaccine, all infants were observed for 30 minutes. Vaccine reactogenicity and safety was assessed using a 7 day diary card after each vaccination. Parents were given a thermometer, instructed in its use, and asked to record temperature and any solicited adverse reactions 3 and 6 hours after injection and at bedtime each evening for 7 days. Solicited adverse reactions included: fever, drowsiness (unusually sleepy or inactive), irritability, anorexia, vomiting, redness, and swelling at the vaccination site (each measured in millimeters) and pain. All unsolicited adverse events occurring within the time interval between vaccinations were recorded by parent/guardian and/or study physician at each study visit. Telephone contact was made with parents/guardians on days 2 and 7 to enquire about adverse events and encourage completion of the diary cards following vaccination. The total duration of safety follow-up was 2 months following the final vaccine dose at 6 months. Any serious adverse event, including hospitalization, was assessed by an independent vaccine safety committee.

### Statistical Analysis

The investigators were responsible for study design and conduct and performed all statistical analyses on individual patient data. Only subjects who had completed the vaccine schedule according to protocol and had at least 2 assay results available, including the maternal baseline sample, were included in the immunogenicity analysis. For pertussis antigens, antibody geometric mean concentrations (GMC) with 95% confidence intervals (CI) were calculated from the antilog of the mean of the log transformed values. Values below the laboratory assay cut-off were assigned a value half of the cut-off value to calculate the GMC.

The primary objective of the study was to assess if IgG antibody to PT and PRN was significantly higher in group 1 at 2 months of age (after 2 aPV doses) than after 1 dose in group 2 and no prior doses of pertussis-containing vaccine in group 3. As no universally agreed serologic correlate of protection exists for pertussis, serologic response, defined as a 4-fold increase from the prevaccination antibody titer, was examined as the variable of interest. For diphtheria, tetanus, Hib, and hepatitis B, serologic response was defined as any level above the lower limit for detection in the assay used for each antibody (0.1 IU/mL, 0.1 IU/mL, 0.15 $\mu$g/mL, and 10 mIU/mL, respectively). Comparisons of antibody responses between groups were using log-transformed data by the independent samples $t$ test with $P < 0.05$ indicating a possible group difference. The proportion of study group subjects

with a serological response and local and systemic reactions after vaccination in study groups were compared by Fisher exact test.

To detect a significant difference for the primary outcome of detectable antibody after the second dose, and to allow for drop-outs and failure to obtain some specimens by venipuncture, we aimed to recruit 25 subjects per arm for this pilot study. Our sample size calculations had indicated that this number of subjects would give 80% power to detect a 50% difference in the proportion of infants achieving detectable PT antibody.

## RESULTS

We enrolled 76 eligible newborns from February 2005 to June 2006. The mean gestational age was 39.8 weeks, 59% were male and there was no significant difference in birth weight between groups. (Table 1) Sixty-eight infants remained enrolled to completion of the vaccination schedule at 6 months and 64 infants until the completion of safety follow-up at 8 months. Eight infants, 2 from Group 1, 1 from Group 2, and 5 from Group 3 withdrew from the study after enrollment and before the first blood sample at 2 months for varied reasons including relocation (1), declining blood tests (4), and inadvertent vaccination with non study vaccines (3).

## Immunogenicity

### Antibody Responses to Pertussis Vaccination

At enrolment, the GMC of maternal IgG to both pertussis toxin (PT) and pertactin (PRN) was not significantly different among groups. However, infants randomized to group 2 had significantly higher maternal anti-PT IgG than those randomized to group 3 (GMC 6.2 vs. 3.3, $P = 0.04$).

With respect to GMCs, at 2 months, following 2 doses of aPV, Group 1 infants had statistically significantly higher GMCs for anti-PT, anti-FHA and anti-PRN IgG compared with both Group 2 and 3 infants (Tables, Supplemental Digital Content 1, 2, and 3, http://links.lww.com/INF/A249, http://links.lww.com/INF/A250, and http://links.lww.com/INF/A251). For anti-PT IgG, levels remained significantly higher in group 1 compared with groups 2 and 3 at 4 and 6 months (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A249) but not at 8 months, with little increase in IgG PT seen after a fourth or fifth dose. For anti-PRN IgG, at 4 months of age, after 3 doses of a pertussis–containing vaccine, levels were significantly higher in group 1 compared with groups 2 (2 doses) and 3 (1 dose) and although in contrast to PT, IgG to PRN increased with each successive dose of pertussis-containing vaccine, differences at 6 or 8 months of age were no longer statistically significant. (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A250). For anti-FHA, levels were significantly higher in groups 1 and 2 compared with group 3 at 4 months of age. (Table, Supplemental Digital Content 3, http://links.lww.com/INF/A251).

With respect to the proportion above the limit of detection, at 2 months old, after 2 doses of a pertussis – containing vaccine, 88% of group 1 infants had a level of IgG to PT above 5 EU/mL compared with 43% of those in group 2 (1 dose) and 15% of group 3 (no doses). Similarly, all group 1 infants had detectable antibody (>5 U/mL) to PRN 1 month after the second dose of Pa at 2 months, compared with 33% for those in group 2 who had received a dose at birth only and 30% for controls. Significantly more infants in group 1 had a 4-fold rise in anti-PT IgG from maternal values to 2 months old (56% vs. 5 and 0% respectively for groups 2 and 3, $P < 0.02$).

There was no evidence of later hypo-responsiveness to pertussis antigens in infants who received Pa vaccine within 5 days of birth. Pertussis antibody levels from 4 months to 8 months of age converged between groups, particularly for PT, and at 8 months did not significantly differ from control infants (Fig. 1).

### Influence of Maternal Pertussis Antibody Levels at Birth

At 2 months of age, antibody levels in groups 2 and 3 were slightly lower than maternal levels, consistent with loss of maternal antibodies. Of the 8 infants in Group 1 who had detectable anti-PT IgG in maternal sera (>5 EL.U/mL), 6 (75%) showed an increase in IgG PT between birth and 2 months of age compared with 1 (7%) of the infants in groups 2 and 3 combined who had detectable maternal antibody. At 8 months of age, the GMC for anti-PT and anti-PRN IgG among infants in groups 1, 2, and 3 whose mothers had detectable IgG was similar to infants in each of the 3 groups whose mothers had no detectable IgG antibodies to these antigens. However, when groups were combined after 3 doses, significantly lower anti-PRN and anti-FHA levels were found in those with detectable maternal antibody at baseline (Table 2).

### Antibody Responses to Other Vaccine Antigens

Two months after completion of the primary immunization schedule, 100% of subjects in all groups had IgG levels to diphtheria and tetanus above those usually associated with protection (0.1 U/mL), with no significant difference between the groups (Table 3). There was a nonsignificant trend to reduced hepatitis B surface antibody GMC responses in infants who received the Pa vaccine at birth (Group 1 and 2 vs. Group 3), however all were above the anti-HBs level associated with protection (10 mIU/mL). Similarly, Group 1 infants had nonsignificantly lower GMCs against Hib and a lower proportion with anti PRP IgG above 1 $\mu$g/mL, compared with Group 2 and 3 infants (26% vs. 45% vs. 47%; Table 3). Infants in groups 1 and 2 who had a 4-fold increase in anti-PT level from baseline to 4 months old had nonsignificantly higher Hib and hepatitis B surface antibody levels at 8 months compared with those with less than a 4-fold rise.

### Reactogenicity

Birth aPV was well tolerated, with no vaccine-related severe adverse events detected. After the birth dose, only 2 infants had redness or swelling >10 mm and none had fever >38C. Following the 6 month vaccination, there was no difference in the proportion

**TABLE 1.** Characteristics of Study Subjects According to Group

| Enrolled Subjects | Group 1 n=27 | Group 2 n=23 | Group 3 n=26 |
|---|---|---|---|
| Mean birthweight (g) (range) | 3454 (2840–4215) | 3306 (2575–4205) | 3560 (2600–4370) |
| Mean gestation weeks (range) | 39.8 (38–41.3) | 39.4 (37.2–41.3) | 39.7 (37–41.5) |
| % Male (n) | 68% (17) | 55% (12) | 55% (12) |
| % Vaccinated day 0–2 (n) | 36% (9) | 50% (11) | n/a |
| % Vaccinated day 3–5 (n) | 64% (16) | 50% (11) | n/a |
| Withdrew prior to 2 months old | 2 | 1 | 5 |

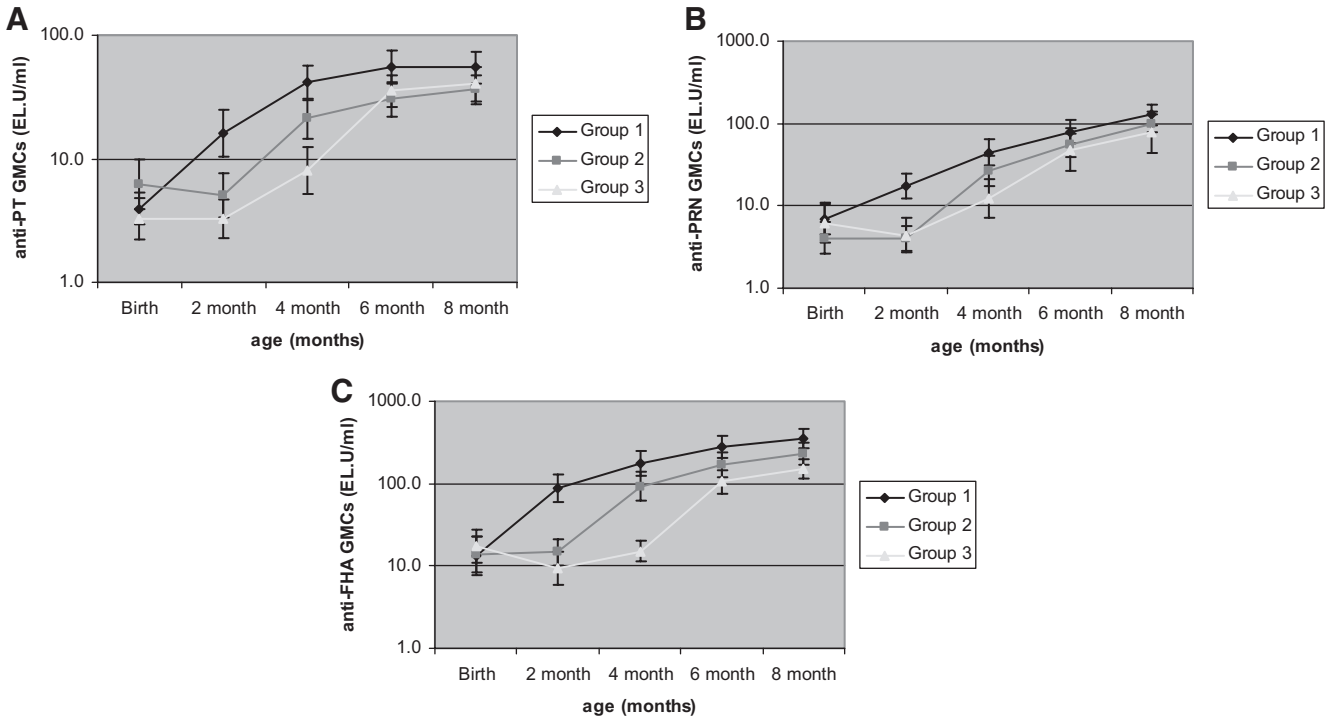


**FIGURE 1.** Anti-pertussis antibody geometric mean concentrations (GMCs) from birth until 2 months after completion of primary vaccination. A, Antibody response to pertussis toxin according to group and age; B, antibody response to pertactin according to group and age; C, antibody response to filamentous haemagglutinin according to group and age.

**TABLE 2.** Pertussis Antibody Responses After 3 Doses for Combined Group (1, 2, 3)* According to Detectable or Nondetectable Maternal Antibody at Baseline

| Baseline Pertussis Antibody | Maternal Antibody Detectable (>5 EL.U/mL) | | Maternal Antibody Not Detectable (<5 EL.U/mL) | |
|---|---|---|---|---|
| | N | GMC† (95% CI) | N | GMC† (95% CI) |
| Anti-PT | 18 | 31.8 (21.9–55.2) | 42 | 39.5 (32.1–48.7) |
| Anti-PRN | 25 | 36.4‡ (24.0–55.2) | 35 | 78.9‡ (61.7–100.8) |
| Anti-FHA | 49 | 151.2† (123.8–184.6) | 11 | 253.5† (187.2–343.4) |

*Combined groups after 3 doses:
(a) Antibody responses after 3 doses (group 1–aged 4 mo, group 2–aged 6 mo, group 3–aged 8 mo) in those with detectable maternal antibody were combined.
(b) Antibody responses after 3 doses (group 1–aged 4 mo, group 2–aged 6 mo, group 3–aged 8 mo) in those with no detectable maternal antibody were combined.
†Anti-FHA GMC significantly different between combined groups after 3 doses for detectable maternal antibody vs. non detectable antibody. ($P = 0.02$).
‡Anti-PRN GMC significantly different between combined groups after 3 doses for detectable maternal antibody vs. non detectable antibody. ($P = 0.002$).
GMC indicates geometric mean concentration (EL.U/mL).

of infants with swelling or redness >10 mm between group 1 (after 5 doses, 17% [n = 4]), group 2 (after 4 doses, 14% [n = 3]) or group 3 (after 3 doses, 22% [n=4]) ($P > 0.5$). Similarly, the proportion with reported systemic reactions or fever was similar between the groups. Two infants required hospitalization for pyloric stenosis, one aged 4 weeks in Group 2 and the other aged 6 weeks in group 3.

### Pertussis Infection

One male infant in group 1 who had received 3 doses of aPV (birth, 1 month and 2 months of age) developed symptoms of mild fever, cough and rhinorrhea at 115 days, 30 days after the third dose. Pertussis was identified by PCR from a nasopharyngeal aspirate on day 134 but pertussis culture was negative. A maternal aunt had a cough consistent with pertussis commencing approximately 14 days before onset of symptoms in the infant, with positive single titer serology. This infant had a mild clinical course and did not require hospital admission. All antipertussis antibodies at 2 months of age, measured after 2 doses and 30 days before onset of symptoms were detectable (anti-PT 15 EU/mL, anti-FHA 198 EU/mL, and anti-PRN 39 EU/mL). Convalescent antipertussis antibodies at 4 months (after 3 doses of aPV and 11 days post diagnosis of infection) increased 2-fold for anti-PT and anti-PRN and nearly 2-fold for anti-FHA. Antipertussis antibody values decreased from 6 months to 8 months after the fifth dose of an acellular pertussis-containing combination vaccine.

## DISCUSSION

This is the first study to assess the immunogenicity and reactogenicity of 2 doses of aPV (birth and 1 month) given before

**TABLE 3.** Immune Responses 2 Months After Completion of Primary Vaccination for Concomitant Antigens According to Group

| Antibody | Threshold | Group 1* | | | Group 2* | | | Group 3* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Number† | % > Threshold | GMC‡ (95% CI) | Number† | % > Threshold | GMC‡ (95% CI) | Number† | % > Threshold | GMC‡ (95% CI) |
| Hepatitis B | >10 mIU/mL | 20 | 100 | 292.9 (14.2–604.1) | 19 | 100 | 540.5 (301.8–967.8) | 15 | 100 | 821.8 (488.2–1383.3) |
| | >100 mIU/mL | | 80.0 | | | 95.0 | | | 100 | |
| *Haemophilus influenzae* b | >0.15 μg/mL | 23 | 65.2 | 0.39 (0.2–0.75) | 20 | 95.0 | 1.03 (0.47–2.22) | 19 | 89.5 | 0.8 (0.41–1.58) |
| | >1 μg/mL | | 26.0 | | | 45.0 | | | 47.4 | |
| Diphtheria | >0.1 IU/mL | 23 | 100 | 1.64 (1.2–2.24) | 20 | 100 | 1.7 (1.18–2.46) | 19 | 100 | 1.97 (1.3–2.98) |
| | >1 IU/mL | | 78.2 | | | 75.0 | | | 84.0 | |
| Tetanus | >0.1 IU/mL | 23 | 100 | 0.84 (0.55–1.28) | 20 | 100 | 1.46 (0.98–2.17) | 19 | 100 | 1.34 (0.89–2.04) |
| | >1 IU/mL | | 47.8 | | | 55.0 | | | 78.9 | |

*Group 1—Pa vaccine at birth and one month then Infanrix Hexa at 2, 4, and 6 mo of age.
*Group 2—Pa vaccine at birth then Infanrix Hexa at 2, 4, and 6 mo of age.
*Group 3—Infanrix Hexa at 2, 4, and 6 mo of age.
†Number—according to protocol number of subjects who had blood sample collected at 8 mo old for antibody measurement.
‡GMC indicates geometric mean concentration.

2 months of age. The study is also unique in that all infants received HBV vaccine at birth, thus allowing direct comparison of the potential influence of birth aPV on concomitant HBV vaccine responses.

Despite its small sample size, this study showed statistically significantly higher GMCs of anti-PT, anti-PRN and anti-FHA IgG antibody at 2 months of age in infants who received aPV at birth and 1 month of age, compared with both those receiving aPV at birth only and those who had not been vaccinated. The titers of anti PT and anti PRN IgG achieved after 3 doses of acellular pertussis-containing vaccine (birth, 1 and 2 months of age) were similar to those seen with 3 doses administered at 0, 2, 4 or the conventional 2, 4, and 6 months of age. This raises the prospect of achieving protection, particularly against severe pertussis, at least 4 months earlier than under current vaccination schedules, subject to the caveat that antibody correlates of protection against pertussis disease of different severities in infants have not been clearly established. Observational studies suggest some protection against severe pertussis from even 1 dose of vaccine, possibly due to rapid antibody production following natural exposure in a primed infant.[4,5] In Germany, estimated vaccine effectiveness against infant hospitalization was 68% after the first and >90% after the second dose of DTPa.[4] In Sweden, the incidence of pertussis fell from 230 to 235 (cases per 100,000 person years) after no or 1 dose of pertussis vaccine to 52 after 2 doses.[5] Our study also suggests that a first dose at birth primes the immune system, with a significant increase in antibody after the second dose, whether given at 1 or 2 months of age.

Four doses of acellular pertussis-containing vaccines within 4 months of birth was not associated with any major local or systemic adverse events in this small number of subjects. Similar to other studies, monovalent aPV given at birth was well tolerated with no increase in reactogenicity identified at birth or following later vaccine doses compared with infants receiving the routine vaccine schedule.[20–22] One participant, who had received 3 doses of a pertussis containing vaccine (0, 1, 2 months), developed laboratory-proven pertussis infection at 3 months of age. The illness was clinically mild and may not have been detected outside the clinical trial setting. Symptoms may have been substantially attenuated by vaccination, although pertussis infection is not universally severe in infants, and infection occurred despite documented prior antibody responses to pertussis antigens.

There are some differences in the antibody responses in our study compared with 3 other recent studies which examined administration of differing acellular pertussis- containing vaccines at birth. The study most similar to ours, which was conducted in Germany using aPV produced by the same manufacturer (GlaxoSmithKline) and the same laboratory for antibody measurement, also demonstrated a significantly higher GMC of antipertussis IgG to PT, PRN, and FHA in infants after 2 doses of Pa at birth and 2 months compared with controls, with no subsequent reduction in antibody response.[22] In an earlier Italian study, where a aPV manufactured by Chiron was given at birth and 3 months of age, higher PT IgG were also seen in these infants at 5 months.[20] By contrast, a recently reported study conducted in the United States, where a DTaP vaccine manufactured by Sanofi Pasteur was given at birth, found GMCs for both PT and FHA IgG post completion of primary vaccination were lower in the experimental group than in controls.[21] This may be related to the different composition of the pertussis antigens in the GSK (3 component) and Sanofi Pasteur (5 component) vaccine, an effect of concomitant diphtheria and tetanus toxoid or some other factor. Hyporesponsiveness, a concern of early studies[16] was not seen in our study or in Germany, with equivalent antipertussis antibody titers at 8 months with or

without a birth dose, however antibody titers converged between groups by 8 months old. This may relate to a biologic feedback phenomenon of achieving a "ceiling" of antibody level designed to protect the body from immune overload due to excessive antibody production. However, the US[21] and Italian[20] studies found that infants who received a pertussis-containing vaccine at birth had lower PT IgG at 7 to 8 months of age. In particular, the US study[21] found that the significantly lower pertussis antibody titers in infants who received DTaP at birth documented at 8 months persisted to 18 months of age, which they postulated may be due to the combination of diphtheria, tetanus, and aP in the combination vaccine resulting in interference with antigen presentation or B lymphocyte priming.

Maternal antibodies to pertussis can interfere with subsequent infant responses.[13,14,24] In our study, a small impact of maternal pertussis antibody was found when groups were combined, but this has not been adequately evaluated, particularly with respect to higher titers of maternal antibody, as our sample size was small and few mothers had detectable antibody. Larger studies, especially among women with higher pertussis antibody titers, such as would be expected following receipt of pertussis-containing vaccine as adolescents or adults or following recent natural infection, are needed. With increasing use of adult acellular pertussis booster vaccines in many countries, the potential for impact of higher maternal antibodies on infant pertussis disease and/or infant responses to pertussis-containing vaccines will become a more important issue.[3]

Other antigens included with pertussis antigens in combination vaccines include diphtheria, tetanus, polio, hepatitis B and *H. influenzae* type b (Hib). Vaccines given concomitantly in recommended national schedules in developed countries include pneumococcal conjugate and rotavirus vaccines. In the US study, infants who had received DTaP at birth had significantly lower antibody titers to diphtheria and pneumococcal serotype 14 than controls at 7 months old.[21] In the German study, attainment of anti-PRP IgG antibody responses consistent with short-term protection ($>0.15$ $\mu$g/mL) was significantly less after the first 3 doses (88% vs. 98%).[22] In our study, anti-PRP IgG appeared to be lower only in infants who received 2 doses of aPV before 2 months of age but power to detect any difference was low. Reduced anti-PRP IgG responses have been associated with DTaP-Hib combination vaccines, but this has only emerged as a clinical problem in one country, the United Kingdom, leading to introduction of a Hib booster.[25] Any such phenomenon following the primary series of vaccination might not be clinically relevant if a booster is routinely given. There was no significant difference in response to diphtheria and tetanus antibody responses. We did not measure responses to polio or pneumococcal antigens, but no significant differences in response to any of 3 polio serotypes were found by the only study measuring them following aPV at birth.[22] Hepatitis B vaccine (HBV) was given only to the control group in the German study,[22] whereas in our study, similar to routine practice in the US and as recommended by WHO, all participants received HBV vaccine at birth. Although reduced anti HBs antibody GMC was seen in infants receiving aPV at birth, all participants achieved protective titers (anti-Hbs $>10$ mIU/mL) at 8 months of age.

This study had several limitations including, small sample size, lack of data on response to all concomitant antigens (polio and pneumococcal serotypes) and has not examined persistence of antibody beyond 8 months of age.

In total, 202 infants have received monovalent aPV or DTaP vaccine at birth in recent published studies.[20–22] Despite the varying immunogenicity data referred to above, no severe adverse events have been reported. The possibility of later reductions in antibody response, and/or interference with responses to concomitantly administered antigens, necessitates larger studies. These include the timing of the second dose of pertussis-containing vaccine. A second dose at 6 weeks of age would be feasible and practical, as current combination vaccines including acellular pertussis antigens are licensed from this age and 6 weeks is consistent with the current WHO schedule. If pertussis vaccine given at birth was included in the WHO Expanded Program on Immunization schedule, infants would then receive 3 doses of a pertussis-containing vaccine by 10 weeks of age (0, 6, 10 weeks). At present, most developing countries use whole cell pertussis (Pw) vaccine in combination with diphtheria and tetanus in the primary immunization schedule and no recent data exist about the immunogenicity and reactogenicity of Pw alone at birth. Future studies with larger samples sizes are needed to address several important issues including more precise estimates of the occurrence of adverse reactions, including the magnitude of any bystander interference with responses to concomitant antigens[26] and the influence of higher levels of maternal antibodies on infant pertussis responses.

Nearly 3 quarters of a century ago, studies attempted pertussis vaccination at birth and in pregnant women, to prevent pertussis in early infancy.[15,17] Current global epidemiologic data indicate that pertussis remains a significant problem in early infancy and new strategies are needed.[27] The availability of acellular pertussis vaccines, with reduced reactogenicity, has led to renewed interest in neonatal pertussis vaccination and in maternal vaccination during pregnancy.[28,29] With respect to neonatal pertussis vaccination strategies, these antibody response data suggest that potentially protective antibody can be achieved before 2 months of age and that no more than 4 doses before 6 months of age are necessary. Larger and more detailed neonatal vaccine studies are needed to evaluate the potential of this approach to prevent death and morbidity from pertussis disease in infants under 3 months of age.

## REFERENCES


1. Crowcroft N, Stein C, Duclos P, et al. How to best estimate the global burden of pertussis? *Lancet Infect Dis*. 2003;3:413–418.
2. World Health Organization. Global Burden of Disease Estimates 2002. Available at: http://www.who.int/healthinfo/bodgbd2002revised/en/index.html. Accessed 2008.
3. Murphy T, Slade B, Broder K, et al. Prevention of pertussis, tetanus and diphtheria among pregnant and postpartum women and their infants. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep*. 2008;57:1–47.
4. Juretzko P, Fabian-Marx T, Haastert S, et al. Pertussis in Germany: regional differences in management and vaccination status of hospitalized cases. *Epidemiol Infect*. 2001;127:63–71.
5. Olin P, Gustafsson L, Barreto L, et al. Declining pertussis incidence in Sweden following the introduction of acellular pertussis vaccine. *Vaccine*. 2003;21:2015–2021.
6. World Health Organization. *WHO Immunisation Schedule. World Health Organization Immunization Policy: Global Programme for Vaccines and Immunization–Expanded Programme on Immunization. WHOGPV/GEN/95.03 Rev. 1*. Geneva, Switzerland: World Health Organization; 1996.
7. Hull B, McIntyre P. Timeliness of childhood immunisation in Australia. *Vaccine*. 2006;24:4403–4408.
8. Grant C, Roberts M, Scragg R, et al. Delayed immunisation and risk of pertussis in infants: unmatched case-control study. *BMJ*. 2003;326:852–853.
9. Dauer C. Reported whooping cough morbidity and mortality in the United States. *Public Health Rep*. 1943;58:661–676.
10. Mooi F, De Greef S. The case for maternal vaccination against pertussis. *Lancet Infect Dis*. 2007;7:614–624.
11. Healy C, Munoz F, Rench M, et al. Prevalence of pertussis antibodies in maternal delivery, cord and infant serum. *J Infect Dis*. 2004;190:335–340.
12. Van Rie A, Wendelboe A, Englund J. Role of maternal pertussis antibodies in infants. *Pediatr Infect Dis*. 2005;24:S62–S65.

13. Englund J, Anderson E, Reed G, et al. The effect of maternal antibody on the serologic response and the incidence of adverse reactions after primary immunization with acellular and whole cell pertussis vaccines combined with diphtheria and tetanus toxoids. *Pediatrics*. 1995;96:580–584.
14. Baraff L, Leake R, Burstyn D, et al. Immunologic response to early and routine DTP immunization in infants. *Pediatrics*. 1984;73:37–42.
15. Sako W, Treuting W, Witt D, et al. Early immunization against pertussis with alum precipitated vaccine. *JAMA*. 1945;127:379–384.
16. Provenzano RW, Watterlow LH, Sullivan CL. Immunization and antibody response in the newborn infant. I. Pertussis inoculation within twenty-four hours of birth. *N Engl J Med*. 1965;273:959–961.
17. Miller J, Faber H, Ryan M, et al. Immunization against pertussis during the first four months of life. *Pediatrics*. 1949;4:468–478.
18. Halsey N, Galazka A. The efficacy of DPT and oral poliomyelitis immunization schedules initiated from birth to 12 weeks of age. *Bull World Health Organ*. 1985;63:1151–1169.
19. Siegrist C. Neonatal and early life vaccinology. *Vaccine*. 2001;19:3331–3346.
20. Belloni C, De Silvestri A, Tinelli C, et al. Immunogenicity of a three-component acellular pertussis vaccine administered at birth. *Pediatrics*. 2003;111:1042–1045.
21. Halasa N, O'Shea A, Shi J, et al. Poor immune responses to a birth dose of diphtheria, tetanus and acellular pertussis vaccine. *J Pediatr*. 2008;153:327–332.
22. Knuf M, Schmitt HJ, Wolter J, et al. Neonatal vaccination with an acellular pertussis vaccine accelerates the acquisition of pertussis antibodies in infants. *J Pediatr*. 2008;152:655–660.
23. National Health and Medical Research Council. Pertussis infections. In: *The Australian Immunisation Handbook*. 9th ed. Canberra, Australia: Australian Government Department of Health and Ageing; 2008:205–216.
24. Booy R, Aitken S, Taylor S, et al. Immunogenicity of combined diphtheria, tetanus and pertussis vaccine given at 2, 3 and 4 months versus 3, 5 and 9 months. *Lancet*. 1992;339:507–509.
25. McVernon J, Andrews N, Slack MP, et al. Risk of vaccine failure after Haemophilus influenzae type b (Hib) combination vaccines with acellular pertussis. *Lancet*. 2003;361:1521–1523.
26. Rowe J, Yerkovich S, Richmond P, et al. Th2-associated local reactions to the acellular diphtheria-tetanus-pertussis vaccine in 4- to 6-year-old children. *Infect Immun.* 2005;8130–8135.
27. Forsyth K, Campins-Marti M, Caro J, et al. New pertussis vaccination strategies beyond infancy: recommendations by the global pertussis initiative. *Clin Infect Dis*. 2004;39:1802–1809.
28. Pertussis maternal immunization study. Dalhousie University, Canada. Available at: http://clinicaltrials.gov/ct2/show/NCT00553228?term=maternal+pertussis&rank=2. Accessed March 22, 2009.
29. Edwards KM. Pertussis: an important target for maternal immunization. *Vaccine*. 2003;21:3483–3486.