'07 03/09 VEN 15:04 FAX 450 [redacted] 002
McAfee [redacted] its Software and Internet Security For [redacted] P.02/09 Page 1 of 2

Pediatr Infect Dis J, 1994;13:348-55
0891-3668/94/1305-0348$03.00/0
Copyright © 1994 by Williams & Wilkins
Vol. 13, No. 5
Printed in U.S.A.

Hib-PRP-T
DPTP 24007-11
Changed Polio from MK to MRC-5
Fyfe'nd recommended 0,4,8 mos
Study funded by Connaught
48 mos F/U
6 left study
13 Sites

Safety and immunogenicity of *Haemophilus influenzae* vaccine (tetanus toxoid conjugate) administered concurrently or combined with diphtheria and tetanus toxoids, pertussis vaccine and inactivated poliomyelitis vaccine to healthy infants at two, four and six months of age

RONALD GOLD, MD, DAVID SCHEIFELE, MD, LUIS BARRETO, MD, SHARON WILTSEY, MPH, GORDEAN BJORNSON, MA, WILLIAM MEEKISON, MD, ROLAND GUASPARINI, MD AND LORNA MEDD, MD

The safety and immunogenicity of *Haemophilus influenzae* vaccine (tetanus toxoid conjugate (PRP-T)) administered concurrently in separate sites or mixed in the same syringe with diphtheria and tetanus toxoids, pertussis vaccine and inactivated poliomyelitis vaccine were assessed in 439 infants at 2, 4 and 6 months of age. The proportions with local redness, tenderness and swelling in the separate and combined groups were 18% *vs.* 11% ($P < 0.001$), 27% *vs.* 24% and 15% *vs.* 13%, respectively. Systemic reactions occurred at similar rates in both groups. The combined vaccine induced tetanus and diphtheria antitoxin titers ≥0.01 IU/ml in 99.5 and 99.1% of infants, pertussis agglutinin titers ≥64 in 92.4%, anti-polyribosylribitol phosphate titers ≥0.15 μg/ml in 93.8% and ≥1.0 μg/ml in 75% and polio-neutralizing titers ≥8 in >98% of infants. However, antibody concentrations to PRP-T, some pertussis antigens and tetanus toxoid were significantly lower after combined than after separate injections of DPT/diphtheria and tetanus toxoids, pertussis vaccine and inactivated poliomyelitis vaccine and PRP-T. The clinical significance of these differences is not known, but the interactions observed among the components of the pentavalent vaccine may be of concern because they might influence antibody persistence until the fourth dose is administered.

Accepted for publication Jan. 27, 1994.
From the Division of Infectious Diseases, The Hospital for Sick Children and the University of Toronto, Toronto, Ontario (RGo); the Vaccine Evaluation Center, BC's Children's Hospital and the University of British Columbia, Vancouver, British Columbia (DS, GB); Connaught Laboratories, Ltd., Willowdale, Ontario (LB, SW); Simon Fraser Health Unit, Coquitlam, British Columbia (WM); Central Fraser Valley Health Unit, Maple Ridge, British Columbia (RGu); and the Boundary Health Unit, Surrey, British Columbia (LM), Canada.
Key words: Vaccine, *Haemophilus influenzae* type b, PRP-T, diphtheria-tetanus-pertussis vaccine, diphtheria, tetanus, pertussis.
Address for reprints: Dr. Ronald Gold, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada.

INTRODUCTION

Before the introduction and widespread use of *Haemophilus influenzae* type b (Hib) conjugate vaccine in infants starting at 2 months of age, Hib had been the major cause of meningitis and epiglottitis in children as well as being responsible for many cases of bacteremia, septic arthritis, cellulitis and pneumonia.[1,2] The safety and efficacy of the conjugate Hib vaccines in infants have been well-established.[3-18] Mass immunization has resulted in remarkable declines in the incidence of Hib disease.[19-23] Immunization of infants requires a primary series of two to three doses starting at 2 months of age followed by a booster in the second year of life.[24] In order to minimize the number of visits required for immunization, it is recommended that Hib vaccine be administered concurrently with the primary doses of diphtheria-pertussis-tetanus (DPT) vaccine or with DPT/inactivated poliomyelitis vaccine adsorbed (IPV) in those jurisdictions using IPV rather than oral polio vaccine. Because such practice results in two separate injections per visit, a preparation that combines Hib vaccine with DPT or DPT/IPV into one injection would be highly desirable.

The combination of DPT with PRP-T is prepared by using the dose of DPT as the diluent for lyophilized PRP-T. The safety and immunogenicity of this combination have been demonstrated in a number of

https://secure.mcafee.com/Document/DocumentOrder.aspx 2/7/2007

tudies.[16, 17, 19, 25-27] Although antibody responses to all acciné components were judged to be adequate in erms of the proportions of infants achieving titers onsidered to be protective, lower antibody responses o PRP-T were observed in infants receiving the com-ined vaccines in two studies in Chile.[16, 25, 26] In a 'ancouver study anti-polyribosylribitol phosphate PRP) concentrations were higher after the combined accine.[27] However, reduced responses to tetanus tox-id and to some pertussis antigens were observed in ll three studies.

The present study was undertaken to compare the afety and immunogenicity of PRP-T when adminis-ered separately or in combination with DPT/IPV to ifants at 2, 4 and 6 months of age.

ETHODS

Infants were recruited from the birth lists provided / three participating public health units in suburban ancouver which immunize 40 to 60% of children in eir jurisdictions.[27] Eligibility criteria for enrollment ito the study were: 8 to 15 weeks of age at entry; eedom from acute illness or conditions for which use DPT, IPV, or PRP-T vaccines are contra-dicated[28]; availability of home telephone; ability of arents to converse in English; and absence of plans relocate within 5 months. Written informed consent as obtained from parents at the first immunization sit. The study protocol was approved by the ethics ommittee of the University of British Columbia.

The study was conducted by two field research eams. Infants were vaccinated at 2, 4 and 6 months f age with diphtheria and tetanus toxoids and per-ussis and inactivated poliomyelitis vaccines adsorbed DPT/IPV) and *H. influenzae* type b tetanus conju-ate vaccine (PRP-T). Vaccines were supplied by Con-aught Laboratories, Ltd., Willowdale, Ontario, Can-da. One lot of DPT/IPV vaccine was used (Con-aught Lot 24007-11) which contained 25 Lf iphtheria toxoid, 5 Lf tetanus toxoid, 4 to 12 protec-ve units of pertussis vaccine and 40, 8 and 32 D ntigen units of types 1, 2 and 3 of IPV. Types 1 and were enhanced IPV produced on MRC-5 cells, hereas type 2 was standard IPV produced on monkey idney cells. Two lots of PRP-T (S2240 and S2241), anufactured by Pasteur Mérieux Sérums et Vaccins, yon, France, were used. The PRP-T was supplied in yophilized form in single dose vials, each containing 0 μg of PRP covalently linked to 20 μg of tetanus oxoid. The two lots will be referred to subsequently t Lots 1 and 2. All vaccines were stored according to ie manufacturer's recommendations.

Participants were randomly assigned to receive ither a single injection of combined DPT/IPV/PRP-in a single syringe (combined group) or concurrent jections of DPT/IPV and PRP-T in opposite thighs (separate group). Infants within each group were randomly assigned to receive either Lot 1 or Lot 2 of PRP-T, resulting in 4 equal groups. Randomization sequences were prepared for each field team and were based on a table of randomly generated numbers in balanced (1:1 assignment ratio) blocks of 12. Assignments were incorporated into each individual's study number which were serially assigned at entry and linked to numbered boxes containing the appropriate diluent for the method of immunization and all 3 doses of the PRP-T lot to be used, labeled only with the subject number.

Preparation and administration of vaccines were performed in a standardized fashion by research nurses, as described previously.[28] All vaccines were administered intramuscularly in the anterolateral thigh using a 25-gauge, 22-mm needle. Parents of infants in the separate group were not told which vaccine was which. DPT/IPV and DPT/IPV/PRP-T were given on the right side and PRP-T on the left at all visits in order to minimize recording errors. Acetaminophen prophylaxis (15 mg/kg at 0, 4 and 8 hours postimmunization) was recommended to parents after each vaccination.

Each infant was observed for 15 minutes postimmunization to detect and treat any immediate allergic reactions. Parents were asked to look for and record in a simple diary any changes at the injection site(s) or in their child's health or behavior for 48 hours after each vaccination. Digital thermometers were supplied to parents with instructions on their use. Parents were free to use either the axillary or rectal method. Temperature was to be measured 1 or 2 times daily and whenever parents suspected fever. Parents were supplied with celluloid rulers with a linear scale and with circles 10 to 50 mm in diameter to measure redness at the injection site at 24 and 48 hours after vaccination. Parents also were provided with and instructed on the use of plastic tapes to measure the circumference of each thigh at the level of the injection site(s) at 24 and 48 hours. The change in circumference before and after vaccination was determined to assess local swelling. Because parents of infants receiving combined vaccine routinely measured both the injected and uninjected thighs, it was possible to determine the variability of this method of measurement; 95% of the changes in circumference of the uninjected thigh before and after vaccination were <1.5 cm. Therefore swelling was defined as a ≥1.5 cm change in circumference.

Parents were telephoned by research staff at 24 and 48 hours to review any adverse events, using a structured interview with specific questions about irritability, crying, drowsiness, changes in appetite, vomiting and changes in bowel habits and open-ended questions about any other parental concerns. Parents were also asked if they saw the child's physician or made an

'07 03/09 VEN 15:05 FAX 450 [redacted] 004
JAN 16 2007 16:32 FR [redacted] TO [redacted] P.04/09

emergency room visit during the 48 hours postvaccination. Parents were also encouraged to report to study nurses any severe adverse event during the period of the study. Nurses were authorized to extend follow-up for 7 to 10 days by telephone or home visit if severe adverse events occurred.

Blood was obtained from infants by means of heel or finger punctures with the use of Microtainer® collector-separator devices (Becton Dickinson, Rutherford, NJ) before the first and third and 1 month after the third dose (i.e. at 2, 6 and 7 months of age). Serum was separated promptly and stored at −20°C before testing for anti-PRP antibody using a Farr-type radioimmunoassay. Only sera obtained 1 month after the third dose were tested for diphtheria antitoxin by microneutralization assay; tetanus antitoxin by enzyme-linked immunoabsorbent assay; pertussis agglutinins by microagglutination assay; IgG antibodies to pertussis toxin, filamentous hemagglutinin, 69-kDa protein and fimbrial agglutinogens 2, 3 by enzyme-linked immunoabsorbent assay; and polio antibody by neutralization inhibition assay. All antibody assays were performed on code-labeled sera at Connaught Laboratories, Ltd.

Case report forms were checked for accuracy and completeness on receipt at the Vaccine Evaluation Center and corrections effected if necessary. Accuracy of data entry into the custom-designed database was verified through programming checks and manual verification of key data for all data files. All results were assembled and analyzed by the principal investigator.

Antibody responses were analyzed in terms of geometric means, with nondetectable values being assigned one-half the lower detection limit of the assay, and by the proportions exceeding predetermined threshold titers associated with protective efficacy for each vaccine. Means were compared using Student *t* tests and 95% confidence intervals (CI_{95}). The chi square test was used to analyze difference in proportions. Two-tailed tests were used throughout. Antibody responses of the separate and combined groups were analyzed for possible interactions between individual vaccine components by applying the Friedman test to the ranked geometric mean titers (GMTs). The Friedman test is a nonparametric two-way analysis of variance by ranks of matched samples. All calculations were performed using Statview 4.01 FPU® (Abacus Concepts, Inc., Berkeley, CA) on a MacIntosh IIsi.

RESULTS

Four hundred forty infants were enrolled and randomly allocated to one of four groups: Group S1, separate injections of DPT/IPV and PRP-T Lot 1; Group S2, separate injections of DPT/IPV and PRP-T Lot 2; Group C1, combined injection of DPT/IPV and PRP-T Lot 1; Group C2, combined injection of DPT/IPV and PRP-T Lot 2. One infant was withdrawn after becoming pale after the preimmunization blood sample was obtained. The numbers of infants in each group were: S1, 110; S2, 110; C1, 110; and C2, 109. The total numbers of infants completing follow-up after doses 1, 2 and 3 were 439, 431 and 426, respectively. Completion rates for dose 3 were 95.8 and 98.2% for combined and separate vaccine groups, respectively. Participants were remarkably similar in terms of sex ratio (M:F 1.15:1), ethnicity (97.7% Caucasian), gestational age (mean, 39.4 weeks), birth weight (mean, 3492 g), and ages at immunization. Parents provided follow-up information after 99.4% of the 1298 vaccinations.

Thirteen infants were withdrawn from the study: 6 because the family moved; 1 because the family could not be located; 1 because of maternal request following adverse symptoms after dose 2; 1 because of physician request after unconsolable crying following dose 1; 1 because of developmental delay with onset unrelated temporally to vaccination; and 3 after severe adverse events leading to hospitalization within 48 hours of vaccination (see below).

Local adverse reactions. Local adverse reactions were more common with vaccines containing DPT/IPV than with PRP-T given separately and were also more common after DPT/IPV alone than after DPT/IPV/PRP-T (Table 1). Redness occurred significantly less often after the combined vaccine than after DPT/IPV without PRP-T, at 11.3% *vs.* 18.2%, respectively, after 24 hours (chi square, 12.238; $P < 0.0005$). Both of these rates of redness were significantly greater than the 2.6% reported after PRP-T alone (chi square, 65.539; $P < 0.00001$). Redness ≥25 mm occurred in approximately 2% of infants after DPT/IPV with or without PRP-T but in only 0.4% after PRP-T alone.

Tenderness was reported with almost equal frequency after combined vaccine and DPT/IPV alone (23.8 and 26.6%, respectively) but significantly more often than after PRP-T alone (17.3%; chi square, 16.429; $P < 0.0001$). Moderate to severe tenderness was reported in 8.6 and 10.8% of infants receiving combined vaccine and DPT/IPV, respectively, compared with 4.6% after PRP-T by itself (chi square, 30.912; $P < 0.00001$).

The frequency of swelling (i.e. an increase in circumference of ≥1.5 cm) was similar in those receiving DPT/IPV with or without PRP-T, at 13.5% *vs.* 15.0%, respectively, rates only slightly larger than the 11.4% reported with PRP-T alone. Among those infants who did have swelling, the mean increase in circumference of the thigh was 2.1 ± 1.0 (SD) cm. Increases greater than 5 cm occurred after only two injections of DPT-containing vaccine (0.2%).

Local reactions were almost always more frequent after Lot 2 than after Lot 1 with each dose of PRP-T. There was a trend for local reactions to increase with successive doses of vaccine.

'07 03/09 VEN 15:06 FAX 450 [redacted]
JAN 15 2007 16:32 FR [redacted] TO [redacted] P.05/09 005

ABLE 1. Summary of local and systemic adverse events after vaccination of infants at 2 to 6 months of age with PRP-T given separately or combined with DPT/IPV

Reaction	Dose	Combined	Separate	PRP-T Alone
Redness: any at 24 hours	1	17/217* (8.2)	25/217 (11.5)	3/216 (1.4)
	2	27/213 (12.7)	35/217 (16.5)	6/212 (2.8)
	3	28/210 (13.3)	58/216 (26.9)	8/215 (3.8)
Swelling ≥1.5 cm	1	29/211 (13.7)	25/201 (12.4)	23/200 (11.5)
	2	25/206 (12.0)	28/208 (13.5)	15/208 (7.2)
	3	30/208 (14.8)	39/203 (19.2)	32/209 (15.3)
Tenderness: any at 24 hours	1	56/217 (25.8)	48/216 (22.3)	35/216 (16.2)
	2	44/214 (20.6)	61/216 (28.2)	40/212 (18.9)
	3	54/211 (25.1)	63/215 (29.3)	36/215 (16.7)
Maximum fever ≥38.5°C during first 24 hours	1	63/214* (29.4)	65/210 (31.0)	
	2	54/209 (25.8)	53/202 (26.2)	
	3	44/208 (21.4)	52/205 (25.9)	
Irritability during first 24 hours	1	121/217 (55.8)	120/216 (56.1)	
	2	117/214 (54.7)	118/216 (55.3)	
	3	106/211 (50.2)	112/216 (56.5)	
Increased crying during first 24 hours	1	80/217 (36.9)	87/216 (39.9)	
	2	68/214 (31.8)	77/217 (34.6)	
	3	56/211 (26.5)	68/216 (31.6)	
Altered sleeping habits during first 24 hours	1	100/217 (46.1)	117/216 (53.7)	
	2	75/214 (35.1)	81/217 (37.3)	
	3	73/211 (34.6)	77/216 (35.7)	
Changes in eating habits during first 24 hours	1	72/217 (33.2)	73/216 (33.8)	
	2	38/214 (17.8)	49/217 (22.6)	
	3	43/211 (20.4)	51/216 (23.6)	
Changes in bowel habits during first 24 hours	1	23/217 (10.6)	22/216 (10.1)	
	2	18/214 (8.4)	29/217 (13.4)	
	3	12/211 (5.7)	12/216 (5.6)	

*Denominators for all rate calculations are the number of reports obtained in each group at each point in time. Reports with missing data are omitted. Results for PRP-T alone are liable only for local reactions.

Systemic adverse events. Assessment of systemic adverse events was influenced by the occurrence of intercurrent illnesses (mostly cold and cough symptoms) after 65 immunizations (1.6%) and near-universal use of prophylactic acetaminophen (98.1% of immunizations). Moreover additional doses of acetaminophen were administered to 45.3% of children during the first 24 hours and to 25.6% during the second 24 hours postimmunization.

Nearly all parents obtained temperature measurements during the initial 24 hours after vaccination. Fever >39.0°C was detected 10 times more often among infants whose temperature was measured rectally than those with axillary measurements, at 26 of 7 (8.0%) compared with 7 of 936 (0.8%), respectively (chi square, 49.415; $P < 0.00001$). The incidence and height of fever did not differ between the vaccine groups (Table 1).

Reports of irritability (54.1%), more crying than usual (38.7%), altered sleeping habits (40.4%), altered eating habits (25.2%) and changes in bowel habits (10.0%) were almost identical in all vaccine groups (Table 1). The frequencies of these common systemic adverse events tended to be higher after the first than after subsequent doses.

Three severe adverse events temporally associated with vaccination resulted in hospitalization: (1) an infant (Group C2) developed supraventricular tachycardia 48 hours after Dose 1 which responded promptly to digoxin therapy; (2) an infant (Group S1) was hospitalized after waking 3 hours after Dose 1 with cough, respiratory distress and cyanosis. Recovery occurred within 4 hours. Aspiration or gastroesophageal reflux was suspected; (3) an infant (Group S2) was hospitalized after an episode of screaming with apnea or breath-holding 4 hours after Dose 1. Other adverse reactions reported in these infants included minimal local redness (≤10 mm) and slight irritability. Infant 1 had fever of 38.9°C on Day 1 and 38.3°C on Day 2 postvaccination, but Infants 2 and 3 were afebrile. All 3 infants recovered completely. Although the fever may have triggered the cardiac arrhythmia in Infant 1, the cause of the other two reactions is not clear.

Overall parents rated the adverse events experienced as moderate to severe after 17.0% of first doses, 15.3% of second doses and 14.3% of third doses (chi square, 12.966; $P < 0.01$). The decline in rating of adverse events suggests that the parents perceived that the infants tolerated the vaccination better with successive doses and/or increasing age. The reaction ratings were similar in all vaccine study groups.

Serologic responses to PRP-T vaccine. Serum samples were available in sufficient volume for serologic testing against all antigens from 422 subjects of the 427 who received all 3 doses of vaccine (98.8%). Omissions were the result of withdrawal from the study or inadequate or no sample. The distribution of anti-PRP titers at study entry (2 months of age), reflecting maternally derived antibody, was comparable among the four groups (Table 2). The proportion of all infants with detectable anti-PRP antibody (≥0.06 μg/ml) was 37% (range among groups, 35.5 to 40.6%). The GMT ranged from 0.11 to 0.12 μg/ml. There were no statistically significant differences in GMTs among the 4 groups.

'07 03/09 VEN 15:07 FAX 450 [redacted] 006
JAN 16 2007 16:33 FR [redacted] TO [redacted] P.06/09

TABLE 2. Concentrations of anti-PRP antibody in infants vaccinated at 2, 4 and 6 months of age with PRP-T given separately or combined with DPT/IPV

	n	Anti-PRP											
		Age 2 months				Age 4 months				Age 6 months			
		GMT (μg/ml)	$CI_{95\%}$ (μg/ml)	No. < 0.15 μg/ml	No. ≥ 1.00 μg/ml	GMT (μg/ml)	$CI_{95\%}$ (μg/ml)	No. < 0.15 μg/ml	No. ≥ 1.00 μg/ml	GMT (μg/ml)	$CI_{95\%}$ (μg/ml)	No. < 0.15 μg/ml	No. ≥ 1.00 μg/ml
Separate													
Lot 1	104	0.11	0.09–0.04	70 (67.3)*	6 (5.8)	0.48	0.39–0.57	27 (26.0)	26 (25.0)	2.73	2.11–3.55	2 (1.9)	83 (79.8)
Lot 2	107	0.11	0.09–0.14	73 (68.2)	5 (4.7)	0.41	0.31–0.55	26 (24.3)	26 (24.3)	2.79	2.13–3.68	2 (1.9)	86 (80.4)
Combined													
Lot 1	106	0.12	0.09–0.15	69 (65.1)	5 (4.7)	0.36	0.29–0.34	40 (39.7)	14 (13.7)	2.25	1.74–3.00	5 (4.7)	82 (77.4)
Lot 2	105	0.11	0.09–0.14	71 (67.6)	6 (5.7)	0.33	0.27–0.30	45 (9.9)	13 (17.1)	1.84	2.36–2.42	8 (7.6)	69 (65.7)

At 6 months of age, i.e. 2 months after the second dose of vaccine, there were statistically significant differences in the responses to PRP-T. After separate and combined vaccines, respectively, the GMTs were 0.42 vs. 0.24 μg/ml ($P < 0.05$), the proportions of infants with anti-PRP concentrations ≥1.0 μg/ml were 25.1 and 15.2% (chi square; 6.497, $P < 0.025$), and the proportions of infants with no detectable anti-PRP antibody (<0.06 μg/ml) were 20.9 and 33.2% (chi square, 8.125; $P < 0.005$).

One month after the third dose of PRP-T, 98.1% of subjects had detectable anti-PRP antibody and 75% had ≥1.00 μg/ml. The GMT of anti-PRP antibody overall was 2.37 μg/ml ($CI_{95\%}$ 1.96 to 2.87 μg/ml). Antibody responses to PRP-T were greater after separate than after combined vaccines. Although the differences in GMT were not statistically significant, they were consistent with the lower GMTs seen in sera obtained after the second dose.

Pertussis responses. Serologic assays were performed for the components of DPT/IPV on sera obtained approximately 4 weeks after the last 3 dose of DPT/IPV vaccine had been administered (blood taken at 7 months).

Pertussis agglutinins were detected in all but 2 subjects and 95.3% had titers ≥64. The groups given separate and combined vaccine had similar responses to pertussis in terms of distribution of titers and GMTs (Table 3). However, the proportion of infants attaining high titers (≥256) after combined vaccine was significantly lower than after separate vaccine: 39.6% *versus* 50.2% (chi square, 9.920, $P < 0.005$). No significant differences in GMTs of antibodies to pertussis toxin, filamentous hemagglutinin, 69 kD protein, or fimbrial agglutinogens 2, 3 were observed among infants in the 4 vaccine groups. As with the difference in pertussis agglutinins, there was a trend for lower GMTs in response to pertussis toxin, filamentous hemagglutinin, 69-kDa protein, and fimbrial agglutinogens 2, 3 among infants receiving combined vaccine. None of these differences was statistically significant except that for 69-kDa protein (chi square, 9.153; $P < 0.025$).

Diphtheria antitoxin. There was no difference in diphtheria antitoxin responses after combined or separate vaccines. Only five infants (1.2%) did not develop the minimum protective level of diphtheria antitoxin (≥0.01 IU/ml) after the third dose of vaccine (Table 3). The overall proportion with ≥0.1 IU/ml of diphtheria antitoxin was 73.6%.

Tetanus antitoxin. Only 1 infant (0.02%) did not develop a protective titer of tetanus antitoxin (≥0.01 IU/ml) after the third dose of vaccine (Table 3). The proportions with antitoxin concentrations ≤ 0.1 IU/ml were similar after separate and combined vaccine, 1.9 and 4.3% ($P = 0.259$, Fisher's exact test). However, the geometric mean antitoxin concentrations were significantly lower after combined than after separate vaccine. The lot of PRP-T did not affect tetanus antitoxin titers. The GMT ($CI_{95\%}$) after separate vaccine was 0.76 IU/ml (range, 0.68 to 0.86 IU/ml) compared with 0.50 IU/ml (range, 0.42 to 0.60 IU/ml)

TABLE 3. Concentrations of antibody to pertussis, diphtheria and tetanus in infants at 7 months of age, 1 month after vaccination with three doses of PRP-T given separately or combined with DPT/IPV

Antibody assay	n	Geometric Mean Titer						
		Reciprocal of geometric mean agglutinin titer	Pertussis toxin (AU)	Filamentous hemagglutinin (AU)	69-kDa protein (AU)	Fimbrial agglutinogens (AU)	Diphtheria toxin (IU/ml)	Tetanus antitoxin (IU/ml)
Separate								
Lot 1	104	187 (158–200)*	48.3 (36.4–6.7)	6.5 (5.2–8.1)	3.81 (3.08–4.73)	406 (331–498)	0.24 (0.18–0.32)	0.76 (0.68–0.84)
Lot 2	107	191 (160–229)	54.6 (40.8–73.5)	8.3 (6.6–10.3)	4.13 (3.34–5.11)	450 (367–550)	0.27 (0.21–0.36)	0.62 (0.71–0.96)
Combined								
Lot 1	106	130 (108–155)	35.5 (27.0–46.4)	5.7 (3.4–8.4)	3.68 (2.50–5.83)	309 (252–379)	0.25 (0.18–0.34)	0.49 (0.41–0.59)
Lot 2	103	132 (107–162)	44.0 (32.5–59.6)	6.4 (3.1–8.0)	3.16 (2.57–3.94)	340 (278–417)	0.27 (0.21–0.34)	0.51 (0.44–0.61)

* Numbers in parentheses, $CI_{95\%}$.
AU, enzyme-linked immunosorbent assay units/ml.

ar combined vaccine. The lower GMT appears to e been the result of a smaller proportion of children aining high titers (>2.0 IU/ml) after the combined cine. The proportions of infants with ≥2.0 μg/ml antitoxin after separate and combined vaccines re 12.3 and 3.3%, respectively (chi square, 11.867; < 0.001).

Polio-neutralizing antibody. The results for an-ody to type 1, 2 and 3 poliovirus are presented in ble 4. The proportions of infants with titers ≥8 ar 3 doses of type 1, 2 and 3 poliovirus vaccines, pectively, were 99.3, 97.9 and 100%, significant ferences were seen in responses to separate or com-ned vaccines.

Analysis of immunologic interactions between iccines. In order to assess the antibody responses the combined and separate groups for possible teractions among antigens, the GMT responses were alyzed by the Friedman test. The mean ranks of the tibody responses in C1, C2, S1 and S2 were 1.6, 1.9, 3 and 3.9 (1 being lowest and 4 highest ranking). te differences in ranks were highly significant (chi uare, 13 of 783; $P = 0.0032$), suggesting that signif-ant interactions among vaccine components oc-rred in the combined group.

ISCUSSION

This was a well-controlled comparison in terms of e uniformity of subjects, balanced randomization, icellent adherence to the intended schedule of rents, full compliance with adverse events monitor-g and very high rates of protocol completion.

Local adverse reactions to both lots of PRP-T vac-ne were infrequent and mild. A slight increase was en with successive doses. Local redness, swelling id tenderness were significantly more common after iccines containing DPT/IPV than after PRP-T one. Unexpectedly the reaction rates were lower ter the combined DPT/IPV/PRP-T than after PT/IPV; the reasons for this were unknown. No gnificant differences in systemic adverse events were en after separate and combined vaccines. The rates f adverse events reported in this study were similar

TABLE 4. Concentrations of antibody to polio types 1 to in infants at 7 months of age, 1 month after vaccination with three doses of PRP-T given separately or combined with DPT/IPV

Antibody Assay	Neutralization titer (Reciprocal of Geometric Mean Titer			
	Separate		Combined	
	Lot 1 (n = 104)	Lot 2 (n = 107)	Lot 1 (n = 106)	Lot 2 (n = 105)
Polio type 1	264 (174–342)*	325 (235–452)	226 (163–318)	279 (199–390)
Polio type 2	181 (128–254)	249 (172–359)	197 (141–275)	251 (179–351)
Polio type 3	1017 (726–1428)	1031 (739–1437)	894 (643–1250)	940 (672–1315)

* Numbers in parentheses, 95%

to those observed in trials of DPT/PRP-T without IPV.[13, 15, 16, 26, 27]

Although no differences in antibody responses to diphtheria toxoid or IPV were observed between the combined and separate groups, significant interactions did occur in the combined group in relation to pertussis vaccine, tetanus toxoid and PRP-T. The proportion of infants with pertussis agglutinin titers ≥256 was significantly lower after combined than after separate vaccine (39.8% *vs.* 50.2%, $P < 0.005$) even though the GMTs were similar. The tetanus antitoxin responses after both separate and combined vaccines were significantly lower than those observed in the Vancouver-1 study of DPT/PRP-T which did not contain IPV.[27] In the latter study the overall GMT ($CI_{95\%}$) was 0.9 IU/ml (range, 0.8 to 0.9 IU/ml) and 46.1% had ≥1.0 IU/ml. In contrast the GMTs ($CI_{95\%}$) in this study were 0.76 IU/ml (range, 0.68 to 0.86) after separate and 0.50 IU/ml (range, 0.44 to 0.57) after combined vaccine. The proportions with ≥1.0 IU/ml were 37.4 and 21.3%, respectively.

The results of published clinical trials comparing administration of PRP-T in combined or separate injections are summarized in Table 5 in terms of GMT and the ratio of GMTs achieved in infants receiving combined *vs.* separate vaccines (C:S ratio). The C:S ratio was used in order to compare studies in spite of the variability arising from the use of different lots of vaccines and the performance of antibody assays in different laboratories. The DPT vaccines used in the Chile-1, Chile-2 and Vancouver-1 studies were manufactured by Merieux,[15] Connaught Laboratories, Inc. (Swiftwater, PA)[26] and Connaught Laboratories, Ltd., respectively.[27] Different lots of PRP-T were used in all four studies. Only in the Vancouver-1 study were the GMTs of anti-PRP higher after combined than separate injections. In the other studies the C:S ratios were all <1.0. The mean C:S ratio for all the studies was 0.82 with a $CI_{95\%}$ of 0.51 to 1.13.

The GMTs of anti-PRP antibody observed in the current study using DPT/IPV were the lowest reported in any published study of PRP-T. In the Vancouver-1 study the overall GMT of anti-PRP antibody was 4.28 μg/ml ($CI_{95\%}$ 3.79 to 4.84), significantly greater than the overall GMT of 2.37 μg/ml ($CI_{95\%}$ 1.96 to 2.97 μg/ml) observed in the current study. In the earlier study with DPT/PRP-T, 88% of infants had anti-PRP concentrations ≥1.0 μg/ml compared with 75% with DPT/IPV/PRP-T vaccines (chi square, 23.233, $P < 0.0001$).

The results of the Chile-1 and -2 and Vancouver-1 and -2 studies in terms of C:S ratios of antibody to diphtheria, tetanus, pertussis and PRP-T antigens are summarized in Table 5. Although no differences in responses to diphtheria toxoid were observed in combined and separate groups in any study, the mean C:S ratios were less than 1.0 for all other antigens. The

'07 03/09 VEN 15:08 FAX 450 [redacted]
JAN 16 2007 16:34 FR [redacted] TO [redacted] P.09/09 008

TABLE 5. Comparison of antibody responses of infants immunized in 4 different clinical trials at 2, 4 and 6 months of age with DPT, OPV or IPV and PRP-T vaccines administered separately or combined

Place	Vaccines	GMT Anti-PRP (μg/ml)		C:S Ratios					
		Combined	Separate	PRP-T	D	T	P-Agg	PT	FHA
Chile-1[25]	DPT, OPV, PRP-T	4.83	11.33	0.43	1.00	0.83	0.47	0.87	1.1
Chile-2[26]	DPT, OPV, PRP-T	6.94	9.93	0.70	1.32	0.78	1.50	1.10	0.86
Vancouver-1[27]	DPT, OPV, PRP-T (Lot 3)	4.50	3.56	1.26	1.33	1.00	0.88	0.50	1.00
	DPT, OPV, PRP-T (Lot 4)	4.37	4.25	1.03	1.33	0.78	0.68	0.80	0.75
Vancouver-2*	DPT, OPV, PRP-T (Lot 1)	2.25	2.72	0.84	1.04	0.70	0.78	0.74	1.03
	DPT, OPV, PRP-T (Lot 2)	1.84	2.79	0.66	1.00	0.62	0.50	0.61	0.77
Mean				0.80	1.17	0.79	0.85	0.87	0.92
CI_{95}				0.53–1.08	0.95–1.35	0.65–0.93	0.49–1.22	0.77–1.07	0.78–1.01

* Current study.
D, diphtheria toxoid; T, tetanus toxoid; P-Agg, pertussis agglutinin; PT, pertussis toxoid; FHA, filamentous hemagglutinin.

reduction in mean C:S ratio reached statistical significance only for tetanus antitoxin: mean 0.79; 99% confidence interval; 0.63 to 0.95.

The causes of the interactions among antigens in both the quadrivalent and pentavalent vaccines are not known. The hypothesis that the tetanus toxoid conjugate is involved in producing the interactions is supported by the lack of such interactions after vaccination with combined DPT *H. influenzae*-CRM conjugate (HbOC) vaccines.[30,31] However, the latter product is a true liquid combination vaccine with optimal formulation conditions for each monovalent component so that results with HbOC cannot be compared directly with those of the current study in which liquid DPT/IPV was used as the diluent for lyophilized PRP-T. A separate role for IPV in the interaction has been reported in terms of depression of pertussis responses in infants vaccinated with DPT concurrently or combined with IPV.[32] The effects were similar with both lots of PRP-T used in this study although titers to all vaccine components were almost always lower after Lot 1 than after Lot 2 of PRP-T, whether injected separately or in combination. Whether other lots of DPT/IPV or PRP-T would behave in a similar way cannot be addressed by these data. Given the wide range of potency permitted by regulation for pertussis vaccine (i.e. 4 to 12 mouse protection units/dose), it will be important to determine in postlicensure studies whether other lots of DPT and DPT/IPV produce similar or greater depressions of antibody responses.

The clinical relevance of the reduced antibody concentrations in relation to protection against disease is unknown. The combined DPT/IPV/PRP-T vaccine induced tetanus and diphtheria antitoxin titers ≥0.01 IU/ml in 99.5 and 99.1% of infants, respectively; pertussis agglutinin titers ≥64 in 92.4%; anti-PRP seroresponses ≥0.15 μg/ml in 93.8%; and anti-PRP ≥1.0 μg/ml in 75.1% and neutralizing antibody titers of ≥8 against 98.1 to 100% of poliovirus types. Although it may be of concern that one-third of infants had no serologic response to PRP-T after the second dose of combined vaccine, almost all infants did respond to the third dose. These results suggest that the interactions observed with the combined vaccine are unlikely to reduce the short term protective efficacy of any of the components of the vaccine. However, the reduced titers of tetanus antitoxin, pertussis antibodies and anti-PRP could affect the duration of protection after completion of the primary series, particularly in the several months preceding the booster dose at 18 months of age. Since protection against tetanus requires the presence of ≥0.01 IU/ml of antitoxin, infants whose titers fall below this value would be at risk. If the reduced antibody responses are shown to have no significant impact on protective efficacy, the pentavalent vaccine appears to offer a substantial increase in convenience of vaccine delivery.

ACKNOWLEDGMENTS

This study was funded by a grant from Connaught Laboratories, Ltd. We are grateful to the staff of the participating health units for their cooperation and to the field research teams for their expert assistance. Teams were led by T. Arcand and K. Jacobsen. Participants from Connaught Laboratories included P. Lavigne and J. Sparkes (review of manuscript and data) and W. Schaurt, J. Bevilaqua and L. Young (Serology Testing and Validation).

REFERENCES

1. Broome C. Epidemiology of *Haemophilus influenzae* type b infections in the United States. Pediatr Infect Dis J 1987;6:779–82.
2. Varughese PV, Invasive *Haemophilus influenzae* disease in Canada. Can Med Assoc J 1988;138:1033.
3. Eskola J, Peltola H, Takala AK, et al. Efficacy of *Haemophilus influenzae* type b polysaccharide-diphtheria conjugate vaccine in infancy. N Engl Med J 1987;317:717–22.
4. Käyhty H, Peltola H, Eskola H, et al. Immunogenicity and reactogenicity of four *Haemophilus influenzae* type b capsular polysaccharide vaccines in Finnish 24-month-old children. Pediatr Infect Dis J 1988;7:674–7.
5. Weinberg G, Granoff D. Polysaccharide-protein conjugate vaccines for the prevention of *Haemophilus influenzae* type b disease. J Pediatr 1988;113:621–30.
6. Käyhty H, Peltola H, Eskola J, et al. Immunogenicity of *Haemophilus influenzae* oligosaccharide-protein and polysaccha

ride-protein conjugate vaccination of children at 4, 6, and 14 months of age. Pediatrics 1989;84:995–9.

7. Eskola J, Käyhty H, Takala A, et al. A randomized, prospective field trial of a conjugate vaccine in the protection of infants and young children against invasive *Haemophilus influenzae* type b disease. N Engl J Med 1990;323:1381–7.

8. Black S, Shinefield H, Lampert D, et al. Safety and immunogenicity of oligosaccharide conjugate *Haemophilus influenzae* type b (HbOC) vaccine in infancy. Pediatr Infect Dis J 1991;10:92–6.

9. Black S, Shinefield H, Lampert D, et al. Efficacy in infancy of oligosaccharide-conjugate *Haemophilus influenzae* type b (HbOC) vaccine in a United States population of 61 080 children. Pediatr Infect Dis J 1991;10:97–104.

10. Ferreccio C, Clemens J, Avendano A, et al. The clinical and immunologic response of Chilean infants to *Haemophilus influenzae* type b polysaccharide tetanus protein conjugate vaccine coadministered in the same syringe with diphtheria-tetanus toxoid-pertussis vaccine at two, four and six months of age. Pediatr Infect Dis J 1991;10:761–71.

11. Holmes S, Murphy T, Anderson R, et al. Immunogenicity of four *Haemophilus influenzae* type b conjugate vaccines in 17- to 19-month-old children. J Pediatr 1991;118:364–71.

12. Käyhty H, Eskola J, Peltola H, et al. Antibody responses to four *Haemophilus influenzae* type b conjugate vaccines. Am J Dis Child 1991;145:223–7.

13. Santosham M, Hill J, Wolff M, et al. Safety and immunogenicity of a *Haemophilus influenzae* type b conjugate vaccine in a high risk American Indian population. Pediatr Infect Dis J 1991;10:173–7.

14. Turner R, Cimino C, Sullivan B. Prospective comparison of the immune response of infants to three *Haemophilus influenzae* type b vaccines. Pediatr Infect Dis J 1991;10:108–12.

15. Watemberg N, Dagan R, Arbeli Y, et al. Safety and immunogenicity of *Haemophilus influenzae* type b tetanus protein conjugate vaccine, mixed in the same syringe with diphtheria-tetanus toxoid-pertussis vaccine in young infants. Pediatr Infect Dis J 1991;10:758–63.

16. Fritzell B, Plotkin S. Efficacy and safety of a *Haemophilus influenzae* type b capsular polysaccharide-tetanus conjugate vaccine. J Pediatr 1992;121:356–62.

17. Scheifele D, Bjornson G, Barreto L, et al. Controlled trial of Haemophilus influenzae type b diphtheria, tetanus and pertussis vaccines in 18-month-old children, including comparison of arm versus thigh injection. Vaccine 1992;10:455–60.

18. Deckar M, Edwards K, Bradley R, Palmer P. Responses of children to booster immunization with their primary conjugate *Haemophilus influenzae* type b (Hib) vaccine or with polyribosylribitol phosphate conjugated with diphtheria toxoid. J Pediatr 1993;122:410–3.

19. Jonsdottir K, Steingrimsson O, Olafsson O. Immunization of infants in Iceland against *Haemophilus influenzae* type b. Lancet 1992;340:252–3.

20. Nelson J, McCracken GH Jr. Bacterial meningitis. Pediatr Infect Dis J 1992;11:661.

21. Black SB, Shinefield HR, Kaiser Permanente Pediatric Vaccine Study Group. Immunization with oligosaccharide conjugate *Haemophilus influenzae* type b (HbOC) vaccine on a large health maintenance organization population: extended follow-up and impact on *Haemophilus influenzae* disease epidemiology. Pediatr Infect Dis J 1992;11:610–3.

22. Michaels R, Ali O. A decline in *Haemophilus influenzae* type b meningitis. J Pediatr 1993;122:407–9.

23. Scheifele D, Gold R, Law B, et al. Decline of *Haemophilus influenzae* type b invasive infections at five Canadian pediatric centres. Can Commun Dis Rep 1993;19:85–91.

24. National Advisory Committee on Immunization. Statement on *Haemophilus influenzae* type b conjugate vaccine for use in infants and children. Can Med Assoc J 1993;148:199–204.

25. Clemens J, Ferreccio C, Levine M, et al. Impact of *Haemophilus influenzae* type b polysaccharide-tetanus protein conjugate vaccine on responses to concurrently administered diphtheria-tetanus-pertussis vaccine. JAMA 1992;267:673–8.

26. Avendano A, Ferreccio C, Lagos R, et al. *Haemophilus influenzae* type b polysaccharide-tetanus toxoid protein conjugate vaccine does not depress serologic responses to diphtheria, tetanus, or pertussis antigens when coadministered in the same syringe with diphtheria-tetanus-pertussis vaccine at two, four and six months of age. Pediatr Infect Dis J 1993;12:630–43.

27. Scheifele D, Barreto L, Mee'kisen W, et al. Can *Haemophilus influenzae* type b-tetanus toxoid conjugate vaccine be combined with diphtheria toxoid-pertussis vaccine-tetanus toxoid? Can Med Assoc J 1993;149:1103–16.

28. National Advisory Committee on Immunization. Canadian Immunization Guide. 3rd ed. Ottawa: Health and Welfare Canada, 1993:82–3.

29. Baker JD, Halperin SA, Edwards K, Miller B, Decker M, Stephens D. Antibody response to *Bordetella pertussis* antigens after immunization with American and Canadian whole-cell vaccines. J Pediatr 1992;121:523–7.

30. Black SB, Shinefield HR, Kaiser Permanent Pediatric Vaccine Study Center. Conjugate Hib vaccines and their combinations: present success and future possibilities. JAMA Southeast Asia 1993;3(Suppl):20–3.

31. Paradiso P, Hogerman D, Madore D, et al. Safety and immunogenicity in infants of a tetravalent vaccine composed of HbOC (HibTITER) and DTP (TRI-IMMUNOL) [Abstract 1028]. Pediatr Res 1992;31(Part 2):174A.