Combined diphtheria, tetanus, pertussis, and *Haemophilus influenzae* type b vaccines for primary immunisation

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Abstract

A total of 146 infants were immunised at ages 2, 3, and 4 months with a combined diphtheria, tetanus, pertussis (DTP) - *Haemophilus influenzae* type b (Hib) tetanus toxoid conjugate (PRP-T) vaccine (Pasteur Merieux) to assess the antibody response and adverse events associated with immunisation. Adverse events, including fever, were recorded by parents in a diary for three days following each injection. Blood was taken before the first immunisation and four weeks after the third immunisation to assess antibody response. Data were compared with those from historical controls who had received DTP and PRP-T vaccines by separate injection. The combined vaccine was well tolerated. Rates of local and general reactions were similar to those reported for infants immunised by separate injection. All infants achieved protective antibody titres (> 0.01 IU/ml) for diphtheria and tetanus; 98% acquired Hib (PRP) antibody > 0.15 μ g/ml and 82.5% > 1.0 μ g/ml. Pertussis antibody titres (pertussis toxin, filamentous haemagglutinin, total agglutinins, and agglutinins 2 and 3) showed appreciable rise following immunisation. DTP and PRP-T vaccines provide similar antibody responses and adverse effects whether mixed in the same syringe or administered by separate injection. The vaccines could be combined for use in the United Kingdom primary immunisation schedule.

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The introduction of *Haemophilus influenzae* type b (Hib) immunisation to the universal schedule in the United Kingdom in 1992 has led to a dramatic reduction in reported rates of invasive haemophilus disease in children.¹ However, extending the protection offered by immunisation doubled the number of injections given to infants in the primary schedule as Hib and diphtheria, tetanus, and pertussis (DTP) vaccines have been given separately at each immunisation visit. Combining the injected antigens of the primary schedule into a single injection would reduce discomfort for infants, so might improve parental acceptabil-

ity and vaccine uptake. In the United Kingdom a combined DTP-Hib vaccine would reduce the number of immunising injections by two million each year. Healthcare staff would welcome the time saved by carrying out the simpler procedure. Demonstrating the safety and immunogenicity of vaccine combinations will become increasingly important as new antigens are introduced into the universal primary immunisation schedule.

Several conjugate vaccines derived from Hib capsular polysaccharide have been developed and considered for primary immunisation of infants. Previous studies, using polyribosylribitol phosphate conjugated with tetanus toxoid (PRP-T) given in the same syringe as DTP, have shown the combination to be well tolerated.²⁻⁵ However, some reports have suggested a reduction in vaccine immunogenicity,^{6,7} particularly in the response to pertussis agglutinins.⁸⁻¹⁰ It is unclear how much variation is attributable to the mixing of antigens and still more difficult to assess the clinical significance of minor changes in mean antibody titre. A previous study mixing these vaccines identified lower levels of PRP antibody in the group receiving the combined vaccine.⁶ Mean titres in the mixed group were still several times higher than the level thought to confer long term protection, and all infants achieved levels above this threshold. Protective thresholds have not been established for pertussis, which makes interpreting differences in immune response more difficult.

Concerns about impaired antibody response may be particularly relevant in the United Kingdom in view of the earlier completion of the accelerated schedule at 4 months, which may itself interfere with optimal immune response,^{11,12} and in the absence of booster doses of pertussis or Hib vaccines beyond this age.

We sought to establish whether combined administration of DTP with PRP-T (a Hib conjugate vaccine in current use in the United Kingdom) would provide satisfactory protection for infants with an acceptable profile of adverse events. Accepted protective antibody thresholds exist for Hib,¹³⁻¹⁶ diphtheria, and tetanus,¹⁷⁻¹⁹ allowing comparisons to be made with previous studies of infants immunised in the United Kingdom accelerated schedule.^{11,12,20-22}

We immunised infants with a vaccine combining DTP and Hib (PRP-T) mixed in a

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'bypass' syringe given at age 8, 12, and 16 weeks, assessed the frequency and severity of adverse events and examined the antibody response to immunisation.

Methods

Parents of healthy infants were approached on the postnatal wards of the two obstetric units serving the city of Sheffield, England and given information about the study. Families who expressed interest by returning a reply slip were seen later at home, where written informed consent for the study was obtained. Babies born at less than 37 weeks' gestation or weighing less than 2500 g at birth were excluded. Infants of families taking part were scheduled to receive immunisations at home at age 8, 12, and 16 weeks. All received a single injection at each visit combining Hib-tetanus toxoid conjugate (PRP-T) and adsorbed diphtheria, tetanus, and whole cell pertussis vaccines (Pasteur Merieux). This was given by intramuscular injection in the right anterolateral thigh using a 25G needle. Antigens were mixed immediately before administration using a 'bypass' syringe which allows liquid DTP vaccine in the upper barrel to reconstitute dried PRP-T in the lower portion of the syringe, resulting in an injected volume of 0.5 ml. Single batches of PRP-T and DTP vaccines were supplied by the manufacturer for the study. Oral polio vaccine (Wellcome) was given at each immunisation visit.

Parents were given a surface activated liquid crystal thermometer (Body-temp, Tommee Tippee, Cramlington, UK) and a diary in which to record temperature twice daily, with details of other adverse events for the three days following immunisation. They were asked to note redness or swelling at the injection site (greater than 2.5 cm diameter), to assess infant crying, activity, and feeding, and to record any use of paracetamol. Parents were advised to give paracetamol if their baby seemed to be in discomfort or had a temperature of 38°C or above. We contacted parents by telephone at one and three days following immunisation to ask about unwanted effects and to confirm diary information.

Venous blood was taken before the first injection and again four weeks after the third immunisation to assess antibody responses. A local anaesthetic cream (EMLA, Astra Pharmaceuticals, Kings Langley, UK) was applied before blood sampling at 5 months. Sera were analysed for antibody to diphtheria and tetanus toxoids, PRP, and pertussis antigens (pertussis toxin (PT), filamentous haemagglutinin (FHA), total agglutinins and agglutinins 2 and 3 (fimbrial antigens)). Antibody to PRP was measured by radioimmunoassay. Diphtheria and tetanus antitoxins, antibodies to PT, FHA, and agglutinins 2 and 3 were measured by enzyme linked immunosorbent assay (ELISA). Total pertussis agglutinins were determined by serial dilution of serum until precipitation with pertussis suspension was no longer observed.

All assays except for pertussis agglutinins 2 and 3 were performed at Val de Reuil, France (laboratory 1). There are no universally

accepted standards or methods for pertussis antigen assays, so postimmunisation sera were additionally assayed (ELISA)²³ for pertussis toxin and FHA at the Centre for Applied Microbiology and Research, Porton Down (laboratory 2) to allow comparison with previous reports from the United Kingdom. This laboratory was also responsible for preimmunisation and postimmunisation assays for agglutinins 2 and 3.²³ Where results lay below the limit of detection for the assay, a value of one half the lower limit was used to calculate geometric mean titres.

STATISTICS

Antibody levels following immunisation were compared in groups of infants with low and high preimmunisation titres by the Mann-Whitney U test for non-parametric data. Analyses were performed using SPSS for Windows Release 6.0 (SPSS, Chicago, Illinois, USA). Categorical data were compared using the χ^2 test or Fisher's exact test where fewer than five infants were expected in any group. Approval for the study was obtained from the North and South Sheffield research ethics committees.

Results

One hundred and forty six infants (71 girls) were recruited to the study. Twenty four per cent of the parents approached agreed to take part. All immunisations and blood tests took place on or within two weeks of the dates when these were due. Median ages at immunisation and at final blood sampling were 8.1, 12.4, 16.6, and 20.9 weeks. Two infants did not complete the study: temporary loss of contact with one family led to a delay between second and third immunisations, and a second family moved away before the final blood sample was taken.

One infant developed swelling involving the major part of the thigh following second immunisation, precluding administration of a third dose of the combined vaccine. She remained otherwise well; swelling and erythema had resolved by four days. Immunisation was completed with DT, Hib, and polio vaccines and this infant excluded from serological analysis. No other adverse effects met Department of Health criteria for severe reactions to immunisation.²⁴ A minority of infants developed appreciable erythema or swelling at the immunisation site (table 1). Modest rates were reported for prolonged crying and for disturbed feeding (table 1). Up to one half of all infants were febrile at some time in the three days following immunisation. Temperatures $\geq 38^\circ\text{C}$ were recorded less frequently with succeeding doses (table 1).

Serum was available from 143 infants following immunisation with three doses of combined vaccine. Insufficient blood was obtained from one infant for preimmunisation analysis. All achieved protective antibody titres (over 0.01 IU/ml) for diphtheria and tetanus (table 2). Three infants failed to achieve protective titres for PRP (table 2). Geometric mean antibody titres for these antigens before

Table 1 Number (percentage) of infants experiencing adverse events in the 3 days following immunisation (n=140-146)

	Dose		
	1	2	3
Erythema $\geq$ 2.5 cm	6 (4)	12 (8)	9 (6)
Swelling $\geq$ 2.5 cm	16 (11)	15 (10)	13 (9)
Crying			
> 1 hour	16 (11)	9 (6)	14 (10)
Inconsolable > 4 hours	0 (0)	2 (1)	1 (1)
Disturbed feeding			
Fed less than usual	59 (41)	30 (21)	33 (23)
Missed feeds	17 (12)	6 (4)	10 (7)
Fever $\geq$ 38°C			
On first evening	52 (36)	26 (18)	17 (12)
Any (over 3 days)	77 (53)	44 (31)	35 (24)

and after immunisation are shown in table 3. There were marked increases in titres of antibody to the four pertussis antigens measured (table 4).

The influence of maternally acquired antibody was assessed by comparing antibody titres before and after immunisation. Infants with titres above protective threshold for tetanus and diphtheria before immunisation achieved lower titres at 5 months than those with initial levels of antibody below these titres (table 5). This was more clearly seen for tetanus, 38 infants (27%) experiencing a fall in titre between 2 and 5 months of age despite immunisation. Similarly, infants with low antibody levels before immunisation achieved higher titres for pertussis toxin, FHA, and agglutinins 2 and 3. No effect of preimmunisation titre on antibody levels at 5 months was shown for PRP or for total pertussis agglutinins.

Discussion

Combining the antigens administered by separate injections at one visit would be welcomed by infants, parents, health workers, and administrators. As new vaccines are proposed for inclusion in the primary immunisation schedule, it will continue to be necessary to demonstrate the safety and effectiveness of vaccine combinations in order to maximise protection against infectious diseases without an unacceptable increase in immunisation visits or injections.

Several studies have addressed the immunogenicity and tolerance of a number of DTP vaccines mixed with PRP-T in other settings, given by more extended schedules.²⁻¹⁰ Those that have considered PRP-T in the United Kingdom schedule, either combined or as a separate injection, have used the Wellcome (Evans) DTP vaccine.²⁰⁻²² Certain DTP/Hib conjugate vaccine combinations have been licensed, are in use in the USA,²⁵ and have recently been recommended in the United Kingdom.²⁶

There was no obvious bias in maternal age, race, social class, or geographical distribution within the city in the sample recruited to this study. The combined PRP-T/DTP vaccine given at 2, 3, and 4 months was well tolerated by these infants. Only one child failed to complete immunisation for all antigens because of a local reaction following the second dose of

the combined vaccine. Rates of redness (4-8%) and swelling (9-11%) at the injection site were similar to those reported for PRP-T combined with Wellcome DTP²² and lower than those reported for DTP when given either in a separate limb from PRP-T (redness 12-27%, swelling 12-24%),²² or given alone without Hib vaccine (redness 10-26%, swelling 10-32%).²¹⁻²⁷

No serious systemic reactions were encountered, although over 50% of infants had fever $\geq$ 38°C occurring at any time during the three days after the first dose of vaccine compared with the low rates (3-10%) reported previously.²² However, direct comparisons are difficult as the methods for analysis of temperature data in that study are unclear. In another study, 6% of infants receiving vaccines as two separate injections had fever after the first dose when parents measured temperatures once daily.²⁰ Rates of fever for subsequent doses in our study (31% following the second and 24% after the third) compare with reports of 16% for both doses for combined vaccine recipients²² or 5-16% and 6-24% respectively, for infants receiving vaccine by separate injection.²⁰⁻²²

Our results may have been influenced by the use of a forehead thermometer reading to the nearest whole degree (allowing interpolation to nearest 0.5°C) which may have overestimated the number of infants with temperature above 38°C as temperatures were rounded up. One previous study has reported a higher rate of fever in infants receiving one of two batches of PRP-T combined with DTP,¹⁰ but no other has shown an increase in febrile responses among combined vaccine recipients, and an earlier United Kingdom study found similar rates in historical controls given vaccine by separate injection.²² Differences in response might also be explained by the use of DTP from different manufacturers,²⁸ or by greater use of paracetamol in other studies. Paracetamol was given by parents after 55% of immunisations in this study, but these data have not been recorded in previous reports. None of the febrile infants in this study were seriously unwell, and no child required admission to hospital or intervention other than with simple antipyretics.

Several studies have addressed the immunogenicity of mixed PRP-T/DTP in more extended schedules. Some have identified modest reductions in antibody titre to PRP,⁶ tetanus,⁷⁻⁹ and pertussis,⁸⁻¹⁰ while others have found no evidence for reduced immunogenicity.²⁻⁵ One study showed an enhanced response to diphtheria for combined vaccine recipients,³ and in another, infants immunised on a 2, 3, 4 month schedule showed higher antibody titres to tetanus.²²

Infants in our study acquired satisfactory antibody titres for each vaccine constituent. All achieved diphtheria and tetanus titres above protective threshold. Geometric mean titres (GMT) for diphtheria and tetanus were similar to those reported for United Kingdom infants immunised with DTP in the accelerated schedule (table 3).¹¹⁻¹²⁻²¹⁻²²

The mean titre for PRP in this group of infants is similar to the figure reported by Begg

Table 2 Number (percentage) of infants with antibody levels above protective threshold compared with those reported previously for infants receiving vaccines by separate injections^{11 20}

	Preimmunisation PRP-T/DTP combined	Postimmunisation PRP-T/DTP combined	Postimmunisation PRP-T and DTP* separate sites ^{11 20}
Diphtheria			
> 0.01 IU/ml	46/134 (34)	143/143 (100)	† (100)
> 0.1 IU/ml	7/134 (5)	142/143 (99)	
Tetanus			
> 0.01 IU/ml	133/142 (94)	143/143 (100)	† (100)
> 0.1 IU/ml	109/142 (77)	143/143 (100)	89/91 (98)
PRP			
> 0.15 µg/ml	43/142 (30)	140/143 (98)	105/107 (98)
> 0.1 µg/ml	1/142 (1)	118/143 (82)	97/107 (91)

* Wellcome (Evans) DTP.
† Numbers not given in report.
DTP = absorbed diphtheria, tetanus, pertussis vaccine; PRP-T = polyribosylribitol phosphate tetanus toxoid conjugate vaccine; PRP = polyribosylribitol phosphate.

Table 3 Geometric mean antibody titres (95% confidence limits for mean) for diphtheria, tetanus, and polyribosylribitol phosphate (PRP), compared with those reported previously for infants receiving vaccines by separate injections^{11 20}

	Preimmunisation PRP-T/DTP combined (n=142)	Postimmunisation PRP-T/DTP combined (n=143)	Postimmunisation PRP-T and DTP** separate sites ^{11 20} (n=103)
Diphtheria (IU/ml)	0.007* (0.006 to 0.009)	1.07 (0.95 to 1.21)	3.87
Tetanus (IU/ml)	0.25 (0.19 to 0.33)	1.01 (0.87 to 1.16)	0.70
PRP (µg/ml)	0.12 (0.11 to 0.14)	3.65 (2.92 to 4.56)	5.01

* 134 samples available for preimmunisation diphtheria serology.
** Wellcome (Evans) DTP.
DTP = absorbed diphtheria, tetanus, pertussis vaccine; PRP-T = polyribosylribitol phosphate tetanus toxoid conjugate vaccine; PRP = polyribosylribitol phosphate.

Table 4 Geometric mean antibody titres (95% confidence limits for mean) for pertussis antigens compared with those reported previously for infants receiving vaccines by separate injections¹¹

	Preimmunisation PRP-T/DTP combined laboratory 1 (n=142)	Postimmunisation PRP-T/DTP combined laboratory 1† (n=143)	Postimmunisation PRP-T/DTP combined laboratory 2† (n=143)	Postimmunisation PRP-T and DTP** separate sites laboratory 2 ¹¹ (n=102)
Pertussis toxin (EIAU/ml)	1.2 (1.0 to 1.5)	41 (31 to 55)	6700 (5250 to 8540)	850
FHA (EIAU/ml)	5.7 (4.7 to 7.0)	40 (35 to 47)	6110 (5270 to 7080)	6200
Total agglutinins (reciprocal of dilution)	20 (16 to 24)	420 (360 to 480)	—	—
Agglutinins 2 and 3 (reciprocal of dilution)	195* (140 to 270)	—	33 490* (26 610 to 42 150)	38 900

* Preimmunisation and postimmunisation serology for agglutinins 2 and 3 performed in laboratory 2.
** Wellcome (Evans) DTP.
† Different assays used for antibody to pertussis toxin and FHA in laboratories 1 and 2. Results cannot be compared between laboratories.
DTP = absorbed diphtheria, tetanus, pertussis vaccine; PRP-T = polyribosylribitol phosphate tetanus toxoid conjugate vaccine; FHA = filamentous haemagglutinin; EIAU = enzyme immunoassay (that is, arbitrary) units.

et al from Gloucester, mixing PRP-T with DTP.²² Both are a little lower than titres reported for PRP-T administered in a separate limb: infants in our study achieved a mean titre of 3.65 µg/ml compared with 3.4 µg/ml for the combined vaccine in Gloucester.²² Infants given vaccine by separate injection achieved GMT of 4.5 µg/ml in that study and 5.0 µg/ml in the first United Kingdom study of the PRP-T vaccine in the Oxford region.²⁰ Three infants in our study failed to achieve minimum protective antibody titres four weeks after the primary course was complete. The same proportion (2%) remained unprotected in Oxford,²⁰ an expected response to Hib conju-

gate vaccines in infancy. Follow up from a large trial of the earlier Hib polysaccharide (unconjugated) vaccine found that PRP titres above 1 µg/ml three weeks after immunisation were associated with continued protection against invasive Hib disease in an immunised population.¹⁴⁻¹⁶ Eighty two per cent of our infants achieved titres above 1.0 µg/ml, while 91% given vaccine by separate injections reached this threshold in Oxford.²⁰ The proportion achieving this titre in our study—although lower than that reported from Oxford—is consistent with experience in industrialised countries of administering PRP-T by separate injection.²⁹ Two studies have shown equivalent GMT for PRP following immunisation with DTP and PRP-T vaccines when combined or given at separate sites^{5 10}; several others have found lower mean values in the combined vaccine group,^{3 9 22} although this only achieved statistical significance in one study from Chile.⁶

Infants completing the current study with PRP titres below 0.15 µg/ml were offered an additional dose of vaccine at 8-10 months of age. All three subsequently achieved antibody levels above 1 µg/ml, suggesting effective priming by earlier immunisation.

There were substantial rises in antibody titre to each of the pertussis antigens measured. Mean titres following immunisation were higher (pertussis toxin) or close (FHA, agglutinins 2 and 3) to figures from previous reports from the accelerated schedule (table 4).^{11 21 22} Those studies used a different DTP vaccine; the response to pertussis antigens, particularly pertussis toxin, may vary between vaccines from different manufacturers.^{30 31}

The 'accelerated' United Kingdom primary schedule is unusual in beginning immunisation at age 2 months with completion by 4 months in the absence of subsequent reinforcing doses of pertussis or Hib vaccines. The timing of the schedule has raised concerns about interference from maternal antibody still present at the time of first immunisation.¹¹ We found no evidence for substantial interference in this population, although infants with high levels of maternal antibody to tetanus and diphtheria achieved lower titres at 5 months than those with lower initial levels of antibody (table 5). The clinical significance of this inhibition is uncertain. In the short term at least, all infants achieved levels of tetanus and diphtheria antitoxin held to confer protection against disease.¹⁷⁻¹⁹ The effect on response to diphtheria was less marked, with preimmunisation titres much lower than for tetanus, reflecting maternal immunisation experience. Until recently the last dose of diphtheria toxoid has been given in the United Kingdom at age 4 years (school entry). Since 1994, combined tetanus/diphtheria immunisation has been recommended for school leavers. As diphtheria antibody levels rise in the young adult population, interference with the response to primary immunisation may become more important.

Inhibition from passive antibody has previously been demonstrated in the accelerated schedule for tetanus, pertussis toxin, and for

Table 5 Effect of preimmunisation titres on subsequent antibody levels. Infants were divided into two groups (above and below threshold indicated) on the basis of initial antibody titre. Postimmunisation titres for these groups were compared by Mann-Whitney test

	Threshold preimmunisation	n _{low pre-}	n _{high pre-}	Mean titre _{low pre-}	Mean titre _{high pre-}	p Value
Diphtheria	0.01 IU/ml	85	49	1.25	0.83	< 0.01
Tetanus	0.1 IU/ml	33	109	1.62	0.88	< 0.001
PRP	0.15 µg/ml	99	43	3.52	3.91	0.68
Pertussis toxin*	Median	71	71	91.5	18.0	< 0.001
FHA*	Median	71	71	50.3	32.5	< 0.01
Total	≤ 10/ ≥ 20	62	80	438	408	0.91
agglutinins						
Agglutinins 2 and 3	Median	71	71	48 591	22 504	< 0.01

* Assays performed in laboratory 1.

n_{low pre-} = number of infants with initial antibody below threshold indicated; n_{high pre-} = number of infants with initial antibody above threshold indicated; mean titre_{low pre-} = final geometric mean antibody titre in group with 'low' initial titres; mean titre_{high pre-} = final geometric mean antibody titre in group with 'high' initial titres; PRP = polyribosylribitol phosphate; FHA = filamentous haemagglutinin.

pertussis agglutinins 2 and 3.¹¹ High initial titres of antibody to tetanus, the PRP-T carrier protein, have also been associated with lower antibody response to PRP in infants immunised with two doses of PRP-T.³² In contrast, we found higher PRP responses in infants with preimmunisation tetanus antibody above 0.1 IU/ml ($p < 0.001$, Mann-Whitney test). These infants were more likely to achieve protective thresholds for PRP; three infants with low initial tetanus titres failed to reach 0.15 µg/ml for PRP, while no infant with higher initial titres remained unprotected ($p = 0.01$, Fisher's exact test). Similarly, 61% of those with low preimmunisation tetanus titres achieved PRP antibody of 1.0 µg/ml or more, compared with 89% for those with high initial tetanus titres ($p < 0.001$, χ^2 test).

Factors influencing the antibody response to the primary series—such as age at immunisation, the mixing of antigens, or the nature of the conjugate vaccine carrier protein—may all have implications for continued vaccine protection. Concerns about impaired antibody response to vaccines may increase as new antigens, for example acellular pertussis vaccines, are included and mixed in the primary schedule. This may require reappraisal of the need for booster doses of vaccine in the second year. We intend to investigate the persistence of protective antibody in a study measuring antibody levels at age 13 months and at school entry.

Despite these observations, immunisation in the manner described conferred high levels of protection with few adverse effects. Combining the injected antigens of the primary schedule in the same syringe provides similar antibody responses to those achieved by administering DTP and Hib (PRP-T) vaccines by separate injection. The combined vaccine improves acceptability and provides a basis for including new immunising antigens in the future.

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