# SAFETY AND IMMUNOGENICITY OF TETRACTHIB (A VACCINE COMBINING DTP VACCINE AND *HAEMOPHILUS INFLUENZAE* TYPE B CONJUGATE VACCINE) ADMINISTERED TO INFANTS AT 6, 10 AND 14 WEEKS OF AGE

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The safety and immunogenicity of TETRActHIB (a vaccine combining diphtheria and tetanus toxoids-pertussis vaccine (DTP) with *Haemophilus influenzae* type b (Hib) conjugate vaccine (polyribosyl ribitol phosphate conjugated to tetanus protein) (PRP-T)) was assessed in 131 Cape Town infants immunised at 6, 10 and 14 weeks of age. Serological responses to all component antigens were measured before the first dose and at 18 weeks of age. In addition, anti-PRP antibodies were measured at 9 and 18 months of age to determine long-term immunogenicity. The vaccine was well tolerated by infants and no significant side-effects were reported. Responses to Hib at 18 weeks of age were good in that most infants achieved a level of anti-PRP antibodies ≥ 0.15 μg/ml, indicative of short-term protection, and 70% achieved a level ≥ 1 μg/ml, indicative of long-term protection. The proportions of children with protective levels ≥ 0.15 μg/ml and ≥ 1 μg/ml were similar at 9 and 18 months of age, i.e. approximately 75% and 45%, respectively. Responses to tetanus and diphtheria toxoids were excellent and all infants achieved protective serological levels. Responses to pertussis were moderate in that approximately 65% achieved 'protective' serum levels of pertussis agglutinins, i.e. titres ≥ 320. In conclusion, this study has shown that the DTP/PRP-T vaccine is safe, immunogenic and well tolerated in infants immunised at 6, 10 and 14 weeks of age. TETRActHIB is therefore suitable for inclusion in the World Health Organisation Expanded Programme on Immunisation (WHO EPI) schedule.

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*Haemophilus influenzae* type b (Hib) is one of the most common causes of serious bacterial infections in children under 5 years of age. Serious Hib infections manifest as meningitis, epiglottitis, pneumonia, arthritis, cellulitis, and other forms of bacteraemic disease. In Europe and the USA the annual incidence of invasive Hib disease varied from 25 to 80/100 000 children under 5 years before the introduction of Hib conjugate vaccines.[1,2] In Alaskan native or Australian native populations, the incidence reached 500 cases/100 000 children.[3,4]

In South Africa, Hib is a major cause of childhood morbidity and mortality. Approximately 70% of cases occur in children under 1 year of age. The incidence of disease in these children is 169/100 000, and overall case fatality is approximately 9%. One study estimated that 1 in 250 children will develop invasive disease in the first year of life and that 1 in 11 children with the disease will die.[5]

Since the introduction of the conjugate vaccines in the early 1990s, the incidence of Hib disease has declined dramatically in developed countries. In fact, the disease has been virtually eliminated in parts of the USA and Europe.[6-8] In developing countries, however, experience with conjugate vaccines is limited. Studies from the Gambia[9,10] and Chile[11,12] have indicated that the vaccines are immunogenic and protect against Hib disease when administered to children from the age of 2 months. Nevertheless, few studies have evaluated the safety and efficacy of these vaccines when given at an early age, i.e. at 6, 10 and 14 weeks, the current World Health Organisation (WHO) Expanded Programme on Immunisation (EPI) schedule of vaccination. Recent studies from the Philippines[13] and Cape Town[14] have indicated that such vaccines are safe and immunogenic when administered according to this schedule.

Hib vaccine can be administered in combination with DTP vaccine. One such combination vaccine is TETRActHIB, which consists of DTP extemporaneously combined with polyribosyl ribitol phosphate conjugated to tetanus protein (PRP-T) (capsular polysaccharide extracted and purified from Hib and conjugated to a tetanus toxoid carrier). Studies of the various combined vaccines indicate that they are immunogenic and safe when administered to infants from the age of 2 months.[15-18] However, there are no published data from Africa for infants immunised at 6, 10 and 14 weeks of age, which is the WHO's recommended schedule for DTP immunisation. Data from the Philippines indicate that the vaccines are immunogenic when administered according to this schedule.[19]

The objective of the present study was to assess the safety and immunogenicity of TETRActHIB vaccine (lyophilised PRP-T vaccine reconstituted with the liquid DTP vaccine) in 6-week-old South African infants.

## METHODS AND STUDY POPULATION

### Study design and ethical considerations

This study was an open, non-comparative, phase IV trial conducted at two primary care clinics in Cape Town, South Africa. The Research and Ethics Committee of the Faculty of Medicine, University of Cape Town, and the Medicines Control Council, South Africa, approved the study protocol. Study procedures complied with Good Clinical Practice and the Declaration of Helsinki. Written informed consent was obtained from a parent or legal guardian for each child enrolled in this study.

### Subjects

Healthy 6-week-old infants of either sex, born after 37 weeks of pregnancy with a birth weight of > 2 500 g, were considered for this study. Non-inclusion criteria were prior administration of Hib, DTP or hepatitis B vaccine, an acute disease, known congenital or acquired immunodeficiency (including HIV infection), family history of sudden infant death syndrome (SIDS) or receiving immunosuppressive drug therapy. Any child presenting with fever at the time of enrolment was not eligible for inclusion.

From February 1997 to September 1997, 131 infants were included in this study. Upon inclusion, each infant was assigned an identification number that corresponded to a package containing the study vaccine. Each infant received TETRActHIB vaccine, according to the EPI immunisation schedule, i.e. at 6, 10 and 14 weeks of age. Children were given oral polio vaccine (Polioral, Chiron, Italy) and hepatitis B vaccine (Hepaccine, Cheil Foods and Chemicals, South Korea) concomitant with the study vaccine.

### Vaccines

TETRActHIB (batch no. 652, Aventis Pasteur (formerly, Pasteur Mérieux Connaught) Lyon, France) comprises a lyophilised Hib capsular polysaccharide tetanus conjugate vaccine (ActHIB, Aventis Pasteur) that is reconstituted with liquid DTP (Aventis Pasteur).

Each 0.5 ml dose of DTP, presented in a pre-filled syringe fitted with a 16 mm needle, contains purified inactivated diphtheria toxoid (≥ 30 IU), purified tetanus toxoid (≥ 60 IU) and inactivated *Bordetella pertussis* suspension (≥ 4 IU). The vaccine also contains aluminium hydroxide, thiomersal, and buffer solution. The lyophilised Hib conjugate vaccine contains 10 µg of Hib capsular polysaccharide, PRP, conjugated to a tetanus toxoid carrier protein. Each dose also contains 0.6 mg tris (HCl) and 42.5 mg sucrose.

Vaccine was reconstituted immediately before administration and was given by intramuscular injection into the anterolateral aspect of the thigh. Hepatitis B vaccine was administered by intramuscular injection into the opposite thigh.

### Procedures

Immediately before the first dose of vaccine, a blood sample (4 ml) was taken from each infant. The vaccine was then administered and the infant was kept under supervision during the next 30 minutes to detect any immediate reaction to vaccination. The parents were instructed on how to use a digital thermometer and were given a diary card to record any local or systemic reactions during the 3 days following each dose of vaccine. Infants were brought back to the study centre at 10 and 14 weeks of age for their second and third vaccinations. At each visit, a medical history was taken to ensure that there were no contraindications to continuation in the trial. Parents were asked to bring their child back to the centre for further blood sampling at 9 and 18 months of age.

### Laboratory investigations

All serological analyses were performed by the Immunology Laboratory of the Institute of Child Health (ICH), University of Cape Town. The anti-PRP responses were measured by means of a radio-immunological assay (RIA) derived from the Farr method, using an iodine$^{125}$-labelled polysaccharide.[20] Anti-PRP antibody levels were expressed in µg/ml. Antibodies to diphtheria and tetanus toxoids were measured using an enzyme-linked immunosorbent assay (ELISA) sandwich assay.[21,22] Anti-pertussis antibodies were measured using a conventional agglutination reaction.[23] All measurements were performed on samples taken just before vaccination and 1 month after the third vaccine dose. Additional, anti-PRP antibodies titres were measured at 9 and 18 months of age.

For quality control purposes the anti-PRP antibody measurements were repeated on the first 25 samples obtained at 6 and 18 weeks of age at the Clinical Sero-immunology Laboratory, Aventis Pasteur, Val de Reuil, France, using the same RIA method as that used in the ICH laboratory.

### Data analysis

The main objective of this study was to confirm that the Hib conjugate vaccine, in combination with DTP vaccine, elicited a satisfactory immune response when given at 6, 10 and 14 weeks of age.

PRP antibody levels were described for the study population as geometric mean titres (GMT) before vaccination (6 weeks of age), 1 month after the third vaccine dose (18 weeks), and at 9 and 18 months of age. The proportions of infants with PRP

antibody levels ≥ 0.15 µg/ml, indicative of short-term protection, and ≥ 1.0 µg/ml, indicative of long-term protection, were calculated at each blood sampling time. GMTs for tetanus, diphtheria and pertussis antibodies were calculated at 6 and 18 weeks of age, as were the proportions of infants with seroprotective levels of antibodies (0.01 IU/ml for tetanus and diphtheria antitoxins, and ≥ 320 for pertussis agglutinins ).

Safety data were described in terms of the percentage of children who reported adverse reactions at any time during the first 72 hours following immunisation. Results are shown here for each sign/symptom reported.

Data were collated and analysed using Epi-Info v6.04 (Centers for Disease Control, Atlanta) and Statistica packages (StatSoft, USA).

## RESULTS

One hundred and thirty-one children were enrolled in the study; 2 children were withdrawn subsequently for personal reasons (mother refused to let child give second blood sample). The safety data are presented for 129 children. The serological data are presented for the 112 children for whom serum samples were obtained at all time points.

The vaccine was well tolerated by all the infants. No immediate adverse events were noted within the first 30 minutes following immunisation. Local and systemic reactions within 72 hours following immunisation are reported in Table I. Most reactions were mild, resolving within 72 hours, and none had any sequelae. Pain at the injection site and crying for a short period following injection were the most frequently reported events. Redness and swelling at the injection site exceeding 2.5 cm occurred only in 3 and 4 children respectively. A high fever (> 39.5°C) was reported in only 1 instance after a third vaccine dose.

No deaths were reported during the study period. Three children were hospitalised (more than 14 days following immunisation) during the study period. The reason for hospitalisation in each instance was diarrhoea, bronchiolitis and pneumonia. No hospitalisation was considered to be related to the immunisation.

Anti-PRP antibody results obtained at 6 weeks of age (before immunisation), 18 weeks (4 weeks after the third vaccination), 9 months and 18 months of age are given in Table II. Responses to Hib after the third vaccine dose were good in that most infants achieved a level of anti-PRP antibodies > 0.15 µg/ml and 69% achieved a level > 1 µg/ml. Although antibody titres tended to drop during the first 5 months after immunisation, the proportions of children with levels ≥ 0.15 µg/ml and ≥ 1.0 µg/ml at 9 and 18 months of age remained more or less stable (78 - 73% and 46 - 43%, respectively).

**Table I. Percentage of children (*N* = 129) with reported adverse reactions at any time during the first 72 hours following immunisation with DTP/PRP-T combination vaccine at 6, 10 and 14 weeks of age**

| | Post-vacc 1 | Post-vacc 2 | Post-vacc 3 |
|---|---|---|---|
| Local reactions | | | |
| Pain* | | | |
| Little | 40.8 | 27.7 | 23.9 |
| Significant | 12.3 | 8.5 | 7.7 |
| Redness | 21.6 | 18.5 | 21.6 |
| Swelling | 24.6 | 20.8 | 16.9 |
| Systemic reactions | | | |
| Temp > 37.5°C | 7.7 | 6.2 | 7.8 |
| Crying | | | |
| < 1 hour | 50.8 | 31.6 | 32.3 |
| > 1 hour | 10.1 | 4.7 | 5.5 |
| Reduced activity | 11.6 | 12.3 | 13.1 |
| Poor feeding | 13.9 | 10.9 | 12.3 |
| Vomiting | 8.4 | 3.1 | 3.9 |
| Diarrhoea | 14.6 | 13.1 | 13.9 |

*Little and significant were respectively defined as baby crying briefly or crying a lot when the injection site was touched.
Vacc = vaccination.

**Table II. Anti-PRP antibody levels in children immunised with three doses of DTP/PRP-T vaccine given at 6, 10 and 14 weeks of age; serological examination was done at 6 and 18 weeks, and at 9 and 18 months of age (*N* = 112)**

| Age | 6 wks | 18 wks | 9 mo. | 18 mo. |
|---|---|---|---|---|
| Geometric mean titre | 0.14 | 1.76 | 0.61 | 0.62 |
| Minimum | 0.04 | 0.08 | 0.01 | 0.01 |
| Maximum | 7.41 | 69.12 | 217.6 | 563.2 |
| % with level > 0.15 µg/ml | 43.7 | 95.5 | 78.6 | 75.9 |
| % with level > 1.0 µg/ml | 13.6 | 69.1 | 46.4 | 43.7 |

Responses to tetanus, diphtheria and pertussis at 6 and 18 weeks of age are shown in Table III. All infants achieved protective serological levels to tetanus and diphtheria. Responses to pertussis were moderate in that approximately 65% achieved 'protective' serum levels of pertussis agglutinins, i.e. a titre > 320.

The anti-PRP data from the ICH and Aventis Pasteur laboratories are shown in Table VI. The GMT results from the Aventis Pasteur laboratory were significantly higher than those from the ICH laboratory ($P < 0.001$). However, there was a good correlation between the two laboratories; $r = 0.96$ (0.91 - 0.98) for the first specimen and $r = 0.83$ (0.64 - 0.92) for the second specimen. In addition, the proportions of children with post-immunisation levels ≥ 0.15 and ≥ 1.0 ug/ml were identical.

## DISCUSSION

This study was done to determine the safety and immunogenicity of a combination Hib-DTP vaccine when

**Table III. Tetanus, diphtheria and pertussis antibody levels in children immunised with three doses of DTP/PRP-T vaccine given at 6, 10 and 14 weeks of age; results are shown before immunisation at 6 and 18 weeks of age**

| Age | 6 weeks | 18 weeks |
|---|---|---|
| Anti-tetanus toxin antibody | | |
| Geometric mean titre | 0.06 | 6.32 |
| Minimum | < 0.001 | 0.60 |
| Maximum | 4.91 | 71.3 |
| % with level > 0.01 | 71.8 | 100 |
| Anti-diphtheria toxin antibody | | |
| Geometric mean titre | 0.02 | 2.94 |
| Minimum | < 0.01 | 0.46 |
| Maximum | 0.75 | 14.56 |
| % with level > 0.01 | 67.4 | 100 |
| Anti-pertussis antibody | | |
| Geometric mean titre | 48.71 | 422.77 |
| Minimum | 10 | 10 |
| Maximum | 2 550 | 5 110 |
| % with level ≥ 320 | 13.7 | 64.3 |
| % with 4-fold increase | NA | 54.6 |

NA = not applicable.

**Table IV. Comparison between ICH and AP laboratory data — anti-PRP antibody results before immunisation at 6 (pre-dose 1) and 18 weeks (post-dose 3), in children immunised with three doses of a DTP/PRP-T vaccine at 6, 10 and 14 weeks of age**

| | GMT | % ≥ 0.15 µg/ml | % ≥ 1 µg/ml |
|---|---|---|---|
| ICH (6 wks) | 0.11 | 31.1 | 9.7 |
| AP (6 wks) | 0.19 | 52.2 | 13.o |
| ICH (18 wks) | 1.45 | 95.7 | 65.2 |
| AP (18 wks) | 2.22 | 95.7 | 65.2 |

ICH = Institute of Child Health, University of Cape Town; AP = Aventis Pasteur.

administered according to the WHO EPI schedule. In addition, persistence of Hib antibodies was evaluated.

A PRP antibody level ≥ 0.15 µg/ml is regarded as being a conservative threshold level of protection at any single time,[24] while a level ≥ 1 µg/ml is regarded as providing long-term protection.[25] In this study, 1 month after three doses of vaccine (i.e. at 18 weeks of age), almost all the children (95.5%) had an anti-PRP antibody titre greater than 0.15 µg/ml — indicative of good short-term protection — and 69.1% had a titre greater than 1 µg/ml . The results are similar to those of a previous study from the same area, which evaluated the immunogenicity of two other Hib vaccines (HibTITER, Wyeth, USA and Pedvax, Merck Research Laboratories, USA) administered as a monovalent vaccine[14] (Table V). The proportion of children in this study with anti-PRP-T levels greater than 1.0 µg/ml following the primary immunisation series was, however, slightly less than that reported from the Philippines,[13] Israel[15] and Chile[18] with the same vaccine given at 2, 4, and 6 months of age (Table V).

**Table V. Comparative Hib immunogenicity data 4 weeks after three doses of either monovalent or combined Hib conjugate vaccine**

| | % > 0.15 µg/ml | % > 1 µg/ml |
|---|---|---|
| Vaccination at 6, 10, 14 weeks of age | | |
| This study | | |
| DTP-PRP-T (TETRAct-HIB) | 96 | 69 |
| Hussey *et al.*[14] | | |
| PRP-OMP (PedvaxHIB) | 94 | 79 |
| PRP-HbOC (HibTITER) | 92 | 72 |
| Capeding[13] | | |
| PRP-OMP (Pedvax) | 92 | 44 |
| PRP-HbOC (HibTITER) | 88 | 62 |
| PRP-T (ActHib) | 97 | 92 |
| Vaccination at 2, 4, 6 months of age | | |
| Watemberg *et al.*[15] | | |
| DPT-PRP-T | 98 | 94 |
| Avendano *et al.*[18] | | |
| DPT-PRP-T | 100 | 98 |

A decline in the anti-PRP GMT and the proportion of infants with anti-PRP antibody levels ≥ 0.15 and ≥ 1 µg/ml during the first 5 months post-immunisation (Table II) has been reported previously.[26] The significance of these findings is, however, unclear. If an antibody level of ≥ 0.15 µg/ml is regarded as protective, then the data suggest that at least 25% of immunised children would be susceptible to Hib at about 9 months of age. Since approximately 50% of cases of invasive Hib diseases in the Cape Town area occur in children over the age of 9 months,[5] the data would suggest that a booster Hib vaccine at this age would be appropriate. On the other hand, this may not be necessary. Low levels of anti-PRP antibody (below 15 µg/ml) may in fact still be protective.[27] In a recent study[27] in which a reduced response to PRP-T was observed on combination with an acellular pertussis vaccine, a vigorous response was observed when a dose of unconjugated PRP was administered to children at the age of 12 - 14 months, proving that these children had been immunologically primed. This anamnestic response, observed even in children with anti-PRP titres < 0.15 µg/ml, certainly suggests that such children are nevertheless protected against subsequent exposure to the Hib pathogen. In addition, routine Hib immunisation may protect a significant proportion of the 'susceptibles' indirectly through herd immunity by reducing circulation of the organism.[28]

All infants given three doses of TETRActHIB at 6, 10 and 14 weeks of age achieved protective serum concentrations of anti-tetanus and anti-diphtheria antibodies 1 month after the last dose of the vaccine. Approximately 65% of infants developed 'immunity' to pertussis (i.e. level ≥ 320). This is slightly less than reported in other studies.[15-18] One reason for this may have been the high pre-immunisation level of anti-pertussis antibodies, probably because of passively derived maternal

antibodies, which may have interfered with the immune response to the vaccine;[29] the GMT (48) for pertussis agglutinins observed in this study cohort before immunisation was higher than that observed in other studies (generally less than 20) in which immunisation was started at 2 months of age.[15,18] The implications of these observations are not clear. The precise relationship between pertussis antibody level and protection against disease is not known.[30,31] Nevertheless, the agglutination response following immunisation could be viewed as constituting a rough correlate of vaccine-induced immunity to pertussis.

The vaccine was well tolerated by the infants. Observed reactions at the site of injection occurred in approximately 20% of children, while significant systemic reactions did not exceed 15%. These adverse events rates were similar to those reported in a previous study from Cape Town using other Hib vaccines.[14]

In conclusion, this study has shown that the DTP/PRP-T vaccine is safe, immunogenic and well tolerated in infants immunised at 6, 10 and 14 weeks of age. TETRActHIB is therefore suitable for inclusion in the WHO EPI schedule.

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