The development of a new heptavalent
diphtheria—tetanus—whole cell pertussis—hepatitis
B—Haemophilus influenzae type b—Neisseria
meningitidis serogroups A and C vaccine:
a randomized dose-ranging trial of the
conjugate vaccine components

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Neisseria meningitidis serogroup C;
Conjugate vaccine;
Haemophilus influenzae;
Immune memory

Summary
Objective: To assess immunogenicity, antibody persistence, immune memory, and reactogenicity of a novel heptavalent DTPw—HBV/Hib—MenAC (diphtheria, tetanus, whole cell pertussis—hepatitis B virus/ Haemophilus influenzae type b— Neisseria meningitidis serogroups A and C) vaccine.
Design: This was an open, randomized study in the Philippines, with DTPw—HBV/Hib—MenAC administered at 6, 10, and 14 weeks of age. Three different polysaccharide contents of the conjugate vaccine components were assessed with conjugated PRP (polyribosylribitol phosphate), MenA, and MenC polysaccharides at the following doses: 2.5 μg of each, 5 μg of each, or 2.5 μg of PRP and 5 μg each of MenA and MenC. Controls received licensed DTPw—HBV and Hib or DTPw—HBV/Hib and MenC conjugate vaccines separately. Immune memory was evaluated via plain polysaccharide challenge administered to half of the subjects at 10 months of age.
Results: After primary vaccination, at least 97.7% of DTPw—HBV/Hib—MenAC recipients had serum bactericidal antibody (SBA)—MenA and SBA—MenC titers ≥1:8, and at least 99% had anti-PRP
Introduction

Neisseria meningitidis causes endemic and epidemic meningococcal disease worldwide with at least 30,000 deaths estimated to occur each year.1 This figure does not consider the numbers who die in rural areas of Africa and Asia before reaching medical facilities. Mortality in some regions may reach 15% even with treatment, with a much higher proportion of victims left with long-term sequelae including limb loss and neurological deficit.1,2 Children under 5 years of age are most often affected by endemic disease, while infants under 3 months of age seem to be protected by maternal antibodies.2

Although outbreaks due to serogroup C (MenC) and more recently W-135 and serogroup X have been reported in Africa and Asia, serogroup A (MenA) accounts for most meningococcal epidemics and particularly affects countries within the ‘meningitis belt’ of Africa.3–8 In Asia, serogroup A has been responsible for three pandemics since the 1960s: the first began in China in the mid 1960s, the second one started in China and Nepal in the 1980s, extending to India, Bhutan, Saudi Arabia, and Yemen, and the third one began in China in 1994 and moved to Mongolia, Russia, and Africa.9,10 In addition to these pandemics, epidemics have affected Mongolia (1973–74) and Vietnam (1977). More recently, Taiwan reported a re-emergence of MenA cases in 2001; MenA outbreaks also occurred in India and the Philippines in 2005.9–11

Endemic disease rates in Africa may be as high as 20/100,000, but during epidemics may reach more than 1000/100,000.1,2,6 To place this rate in perspective, an overall disease incidence rate of approximately 10/100,000 was observed during the 1999 MenC epidemic in England and Wales prior to the introduction of MenC conjugate vaccines.12

Meningococcal polysaccharide vaccines have been available since the 1980s and are effective in preventing disease. However these vaccines do not provide long-term protection, their effect on carriage is minimal, and their use is therefore restricted to epidemic situations.1,2 Except for serogroup A polysaccharide that induces an immune response in infants under 1 year of age with some evidence of efficacy, meningococcal polysaccharide vaccines are poorly immunogenic in young children under 2 years of age. Furthermore, administration of the MenC polysaccharide is found to cause hyporesponsiveness to subsequent doses of MenC vaccine.13

The availability of MenC conjugate vaccines has fundamentally altered the epidemiology of MenC disease in countries where vaccination has been widely implemented.12,14 The development of an effective MenA conjugate vaccine for infant immunization would enlarge endemic and epidemic invasive meningococcal disease control even further. The World Health Organization (WHO) Expanded Program on Immunization (EPI) currently recommends routine vaccination against diphtheria, tetanus, pertussis, hepatitis B virus (HBV), polio, and Haemophilus influenzae type b (Hib) for all infants.15 Whole-cell pertussis vaccines are most commonly used in less industrialized countries mostly because of lower cost compared to more recent acellular pertussis vaccines. In endemic areas where poliomyelitis has not been eradicated yet, WHO recommends the use of oral polio vaccine (OPV).16 Combined diphtheria—tetanus—whole-cell pertussis—hepatitis B (DTPw—HBV) and DTPw—HBV/Hib vaccines are now widely used, and their coverage is increasing. The addition of MenA and MenC conjugates to these existing vaccines will promote rapid uptake and high coverage of the new components, while minimizing cost and logistical problems in vaccine delivery.

This study evaluated three novel heptavalent DTPw—HBV/Hib—MenAC combination vaccines that differed in the dose of the Hib—MenAC conjugate component, for use in a three-dose primary schedule during the first year of life.

Methods

Study design and subjects

The study was an open, randomized study in the Philippines, conducted in two phases: primary vaccination (study number: 759346/001) and polysaccharide challenge (study number: 759346/002). The study protocols (NCT00317174) were reviewed and approved by the relevant ethics committee and were conducted according to Good Clinical Practice Guidelines and the Declaration of Helsinki. Written informed consent was obtained from the parent/guardian of every child prior to enrolment in the study.

Healthy infants aged between 6 and 10 weeks at the time of the first vaccination were eligible for inclusion. Subjects were excluded if they had a major congenital defect or serious chronic illness, any confirmed or suspected immunosuppressive or immunodeficient condition, evidence of previous diphtheria, tetanus, pertussis, hepatitis B, Hib, MenA and/or MenC vaccination or disease, any history of allergic reactions to any vaccine component, receipt of any investigational or non-registered drug or vaccine within 30 days before or during the study, or receipt of immunosuppressive or immunoglobulin therapy or blood products before enrolment or during the study. Subjects were also excluded if they had received hepatitis B or Bacille Calmette—Guérin vaccine after the first two weeks of life.

In the primary phase of the study, 525 eligible infants were randomized to one of five groups (1:1:1:1:1). Three groups evaluated different doses of the novel heptavalent vaccine: group 2.5/2.5 received DTPw—HBV/Hib—MenAC containing 2.5 μg each of conjugated PRP (polyribosylribitol phosphate), MenA, and MenC polysaccharides, group 2.5/5...
received DTPw—HBV/Hib—MenAC with 2.5 μg of conjugated PRP and 5 μg each of conjugated MenA and MenC polysaccharides, and group 5/5 received DTPw—HBV/Hib—MenAC containing 5 μg each of conjugated PRP, MenA, and MenC polysaccharides. Two control groups received licensed vaccines: group DTPw—HBV + Hib received separate administration of TritanrixTM-HepB and HiberialTM and group DTPw—HBV/Hib + MenC received separate administration of TritanrixTM-HepB/HiberialTM and MeningitecTM. All vaccines were administered intramuscularly at 6, 10, and 14 weeks of age.

Tritanrix, Hiberial, and Mencevex are trademarks of the GlaxoSmithKline group of companies. Meningitec is a trademark of Wyeth.

Half of the subjects in each of the five primary vaccination groups were randomly selected to participate in a polysaccharide challenge study at 10 months of age, where they received 10 μg plain PRP and 10 μg of both MenA and MenC polysaccharide vaccine (one fifth dose of MencevexTM AC). All subjects received DTP booster vaccination during the second year of life and the results of this DTP booster study will be reported separately.

Vaccines

All vaccines were developed and manufactured by GlaxoSmithKline (GSK) Biologicals (Rixensart, Belgium), except MeningitecTM, which was developed and manufactured by Wyeth (Pearl River, NY, USA). The DTPw—HBV vaccine (TritanrixTM-HepB) was administered separately in the DTPw—HBV + Hib group, or was used to reconstitute lyophilized Hib—MenAC or HiberialTM vaccines in the DTPw—HBV/Hib—MenAC and DTPw—HBV/Hib + MenC groups. The composition of TritanrixTM-HepB has been described elsewhere.17 The Hib—MenAC component of the experimental heptavalent vaccines comprised PRP, MenA (PSA), and MenC (PSC) capsular polysaccharides at the dosages described above, each conjugated to tetanus toxoid (TT). The DTPw—HBV/Hib—MenAC 2.5/2.5 vaccine was prepared by injecting the full content of two monodose vials of TritanrixTM-HepB vaccine into the vial containing the lyophilized Hib—MenAC 5/5 vaccine and removing a single dose (0.5 ml) of the mixed vaccines for injection. HiberialTM contained 10 μg PRP conjugated to TT. MencevexTM AC contained 50 μg each of purified PSA and PSC. MeningitecTM contained 10 μg PSC conjugated to Corynebacterium diphtheria CRM197 protein. All DTPw-containing vaccines were administered in the left thigh. All concomitant vaccines (HiberialTM in the DTPw—HBV + Hib and MeningitecTM in the DTPw—HBV/Hib + MenC groups) were administered in the right thigh. Tritanrix, Hiberial, and Mencevex are trademarks of the GlaxoSmithKline group of companies. Meningitec is a trademark of Wyeth.

Assessment of immunogenicity

Blood samples (3.5 ml) were collected before and one month after the three-dose primary vaccination course, and prior to and one month after the polysaccharide challenge. Sera were stored at −20 °C until blinded analysis at GSK, Rixensart, Belgium.

Bactericidal dilution titers against MenA and MenC (SBA-MenA, SBA-MenC) were measured by a serum bactericidal test using baby rabbit complement.18 The cut-off of the test was a 1:8 dilution.19 Antibodies against PSA and PSC were measured by ELISA with an assay cut-off of 0.3 μg/ml.20

Antibodies against PRP, diphtheria, and tetanus toxoids, Bordetella pertussis antigens (BPT), and hepatitis B surface antigen (HBs) were measured by ELISA. The cut-off values for the assays were 0.15 μg/ml for anti-PRP, 0.1 IU/ml for diphtheria and tetanus toxoid antibodies, 15 ELISA units/ml (EL.U/ml) for BPT, and 10 mIU/ml for anti-HBs. Subjects with a post-primary anti-diphtheria antibody concentration < 0.1 IU/ml by ELISA were retested using an in vitro neutralization assay on Vero cells. The cut-off of the Vero-cell assay was 0.016 IU/ml.

Serum bactericidal titers ≥1:8 against MenA or MenC, or antibody concentrations against PRP, tetanus, diphtheria, or HBs equal to or greater than the assay cut-off (by ELISA or the in vitro neutralization assay for diphtheria antibodies) were considered to be protective. For pertussis, a vaccine response was defined in initially seronegative subjects as a post-vaccination antibody concentration equal to, or above the assay cut-off value. In initially seropositive subjects, a pertussis vaccine response was defined as a post-vaccination concentration equal to or greater than the individual’s pre-vaccination titer (thereby taking into account the half-life of maternal antibodies).

Assessment of safety

Solicited local symptoms of pain, redness, and swelling at the injection site, and general symptoms of fever (defined as axillary body temperature ≥37.5 °C), irritability/fussiness, drowsiness, and loss of appetite were recorded by the parents/guardians on the day of each vaccination and for seven subsequent days on diary cards provided by the sponsor. All adverse events occurring within one month (minimum 30 days) following administration of each vaccine dose were recorded. Serious adverse events (SAEs) were recorded from enrolment in the primary phase until one month after the booster dose in the second year of life.

The intensity of reported symptoms was scored on a three-point scale. Grade 3 pain was defined as ‘cries when limb is moved/spontaneously painful’, grade 3 redness and swelling as diameter >30 mm, grade 3 fever as axillary body temperature >39.5 °C, and for all other adverse events grade 3 intensity was defined as an event that would prevent normal everyday activities and that would cause the parents/guardians to seek medical advice. The parents/guardians were asked to contact the investigator immediately if the child manifested any signs that were perceived as serious.

Statistical methods

The main objectives of the studies were to evaluate the non-inferiority of the DTPw—HBV/Hib—MenAC 2.5/2.5 vaccine compared to DTPw—HBV + Hib with respect to the Hib response and compared to DTPw—HBV/Hib + MenC for the immunogenicity of the MenC antigen, to evaluate immunogenicity of the MenA antigen of the DTPw—HBV/Hib—MenAC 2.5/2.5 vaccine, to evaluate the persistence of antibodies against Hib, MenA, and MenC at 10 months of age as well as immune memory induced by the primary vaccination, and to assess the safety and reactivity of the vaccines.
All analyses were descriptive. The immunogenicity of primary vaccination and immune response analyses were performed on the (primary and challenge) according-to-protocol (ATP) cohorts for immunogenicity and the persistence analysis on the ATP cohort for antibody persistence, whereas the safety analysis was performed on the primary total vaccinated cohort.

The geometric mean antibody concentrations/titers (GMCs or GMTs) with 95% confidence intervals (CIs) were calculated for each antibody tested and for the serum bactericidal assay at each time point, by taking the anti-log10 of the mean of the log10 concentration/titer transformations. Seropositivity/seroprotection/vaccine response rates were calculated with exact 95% CIs.

Exploratory comparisons were performed through computation of standardized asymptotic 95% CIs for the difference in seroprotection, seropositivity, and vaccine response rates between each of the three Hib—MenAC formulations and the relevant control group one month after the primary vaccination. Additionally, 95% CIs for the GMC/T ratios between each of the study vaccine groups and the control group were calculated using a one-way ANOVA model on the log10 transformation of the titers or concentrations using the vaccine group as the only covariate. Two vaccine groups were considered significantly different if the 95% CI for the difference in rates between the two vaccine groups did not contain the value ‘0’, or if the 95% CI for the GMT/GMC ratio between the two vaccine groups did not contain the value ‘1’. For the MenA response, in the absence of a comparator group the lower limit of the exact 95% CI on the percentage of subjects with SBA-MenA titer ≥1:8 was compared to 70% for this first clinical study with a DTPw—HBV/Hib—MenAC vaccine.

The incidence and intensity of each solicited adverse event was tabulated with 95% CI. Serious adverse events and dropouts due to adverse events were described.

Results

The study was conducted at a single center in Manila between November 2002 and April 2004. There were 525 children enrolled and 524 vaccinated in the primary vaccination phase (Figure 1). A total of 217 subjects received the polysaccharide challenge at 10 months of age.

Demographic characteristics

At enrolment the mean age of subjects was 7.1 weeks (standard deviation (SD) 1.2 and range 6—10 weeks), 48.7% of subjects were female, and all were of Asian origin. Mean weight was 4.6 kg (SD 0.7) and the mean length of subjects was 56.1 cm (SD 2.4). Demographic characteristics between groups were similar (data not shown). The mean age of

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**Figure 1** Number of subjects who participated in the primary vaccination and plain polysaccharide challenge studies, and reasons for withdrawal or elimination from analyzed cohorts.
subjects at the time of the polysaccharide challenge was 43.9 weeks (SD 1.4).

Immunogenicity: MenA response

After primary vaccination with one of the three experimental heptavalent vaccines, the percentage of subjects with SBA-MenA titers ≥1:8 was at least 97.7%, compared to less than 10% of subjects in the two control groups (Table 1). The lower limit of the 95% CI for the percentage of subjects with SBA-MenA titers ≥1:8 was over 90% in the DTPw—HBV/Hib—MenAC groups, well above the pre-defined clinical limit of 70%. At least 99.0% of subjects in the DTPw—HBV/Hib—MenAC groups achieved seroprotective SBA-MenA titers, compared to 97.8% in the DTPw—HBV/Hib + MenC control group.

Prior to the polysaccharide challenge at 10 months of age, 91.3% to 94.9% of subjects primed with DTPw—HBV/Hib—MenAC continued to have seroprotective SBA-MenA titers, compared to 97.8% in the DTPw—HBV/Hib + MenC control group, and 7.3% in the DTPw—HBV + Hib control group (Table 2). Immune memory was demonstrated by a marked response to the polysaccharide C challenge in terms of SBA-MenC GMTs and anti-PSC antibody GMCs in all MenC primed groups, compared to a minimal response in subjects who received primary vaccination with DTPw—HBV + Hib (Tables 2 and 3).

A dose of plain MenA polysaccharide induced a rise in SBA-MenA titers in all groups (Table 3), with higher point estimates in the MenA-primed groups. At least 97.6% of subjects primed with a DTPw—HBV/Hib—MenAC vaccine reached titers ≥1:8 (Table 2). Significantly higher-fold increases in anti-PSA antibodies were observed in the DTPw—HBV/Hib—MenAC groups compared to controls.

Immunogenicity: MenC response

At least 99.0% of subjects had seroprotective SBA-MenC titers and were seropositive for anti-PSC antibodies after primary vaccination with each of the experimental DTPw—HBV/Hib—MenAC vaccines, with no difference compared to the MenC control vaccine. A statistically significant lower SBA-MenC GMT was observed in group 2.5/2.5, as well as a statistically significant higher anti-PSC GMC in group 2.5/5 when compared to the MenC control vaccine.

At 10 months of age, 91.3% to 94.9% of subjects primed with DTPw—HBV/Hib—MenAC continued to have seroprotective SBA-MenC titers, compared to 97.8% in the DTPw—HBV/Hib + MenC control group, and 7.3% in the DTPw—HBV + Hib control group (Table 2). Immune memory was demonstrated by a marked response to the polysaccharide C challenge in terms of SBA-MenC GMTs and anti-PSC antibody GMCs in all MenC primed groups, compared to a minimal response in subjects who received primary vaccination with DTPw—HBV + Hib (Tables 2 and 3).

Immunogenicity: PRP response

Following primary vaccination, more than 99.0% of subjects in all groups achieved an anti-PRP antibody concentration...
Table 2  SBA and antibody responses to polysaccharide challenge at 10 months of age (ATP cohort for antibody persistence for pre-polysaccharide challenge or challenge ATP cohort for immunogenicity for post-polysaccharide challenge)

<table>
<thead>
<tr>
<th>Group</th>
<th>Time-point</th>
<th>MenA</th>
<th>MenC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SBA</td>
<td>PSA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>% (95% CI)</td>
</tr>
<tr>
<td>SBA MenA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥1:8</td>
<td>Pre-PS</td>
<td>39</td>
<td>92.3 (79.1; 98.4)</td>
</tr>
<tr>
<td>≥1:128</td>
<td>Post-PS</td>
<td>37</td>
<td>94.6 (81.8; 99.3)</td>
</tr>
<tr>
<td>SBA MenC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥1:8</td>
<td>Pre-PS</td>
<td>46</td>
<td>91.3 (79.2; 97.6)</td>
</tr>
<tr>
<td>≥1:128</td>
<td>Post-PS</td>
<td>39</td>
<td>94.9 (82.7; 99.4)</td>
</tr>
<tr>
<td>Anti-PSA (µg/ml)</td>
<td>≥0.3</td>
<td>44</td>
<td>93.2 (81.3; 95.6)</td>
</tr>
<tr>
<td></td>
<td>≥2</td>
<td>44</td>
<td>50.0 (34.6; 65.4)</td>
</tr>
<tr>
<td>Anti-PSC (µg/ml)</td>
<td>≥0.3</td>
<td>44</td>
<td>90.5 (84.5; 99.4)</td>
</tr>
<tr>
<td></td>
<td>≥2</td>
<td>44</td>
<td>55.5 (41.6; 69.6)</td>
</tr>
<tr>
<td>Anti-PRP (µg/ml)</td>
<td>≥0.15</td>
<td>44</td>
<td>97.7 (88.0; 99.9)</td>
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<tr>
<td></td>
<td>≥1</td>
<td>44</td>
<td>88.6 (75.4; 96.2)</td>
</tr>
</tbody>
</table>

DTwP, diphtheria–tetanus–whole cell pertussis; HBV, hepatitis B virus; Hib, Haemophilus influenzae type b; Men, meningococci; CI, confidence interval; Pre-PS, pre-polysaccharide challenge; Post-PS, post-polysaccharide challenge; SBA, serum bacterial antibody; PSA, MenA capsular polysaccharide; PSC, MenC capsular polysaccharide; PRP, polyribosylribitol phosphate; ATP, according-to-protocol.

Table 3  SBA and antibody GMC/GMT to polysaccharide challenge at 10 months of age (ATP cohort for antibody persistence for pre-polysaccharide challenge or challenge ATP cohort for immunogenicity for post-polysaccharide challenge)

<table>
<thead>
<tr>
<th>Group</th>
<th>Time-point</th>
<th>MenA</th>
<th>PSA</th>
<th>MenC</th>
<th>PSC</th>
<th>PRP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SBA</td>
<td>GMC (µg/ml) (95% CI)</td>
<td>SBA</td>
<td>GMC (µg/ml) (95% CI)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td></td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DTPw–HBV/Hib–MenAC 2.5/2.5</td>
<td>Pre-PS</td>
<td>39</td>
<td>164.6 (99.7; 271.7)</td>
<td>44</td>
<td>1.78 (1.24; 2.55)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-PS</td>
<td>32</td>
<td>767.1 (606.3; 970.6)</td>
<td>45</td>
<td>24.66 (17.73; 34.29)</td>
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<tr>
<td>DTPw–HBV/Hib–MenAC 2.5/5</td>
<td>Pre-PS</td>
<td>35</td>
<td>142.1 (81.9; 246.6)</td>
<td>36</td>
<td>1.49 (1.05; 2.13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-PS</td>
<td>30</td>
<td>923.3 (711.6; 1196.0)</td>
<td>39</td>
<td>23.42 (16.68; 32.87)</td>
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<tr>
<td>DTPw–HBV/Hib–MenAC 5/5</td>
<td>Pre-PS</td>
<td>37</td>
<td>202.8 (134.9; 304.9)</td>
<td>39</td>
<td>2.11 (1.48; 3.00)</td>
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<tr>
<td></td>
<td>Post-PS</td>
<td>30</td>
<td>682.4 (451.4; 1031.7)</td>
<td>39</td>
<td>23.06 (16.52; 32.19)</td>
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<td>DTPw–HBV + Hib</td>
<td>Pre-PS</td>
<td>36</td>
<td>84.1 (40.6; 174.4)</td>
<td>42</td>
<td>0.16 (0.15; 0.17)</td>
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<tr>
<td></td>
<td>Post-PS</td>
<td>39</td>
<td>511.1 (353.3; 739.3)</td>
<td>38</td>
<td>0.88 (0.49; 1.56)</td>
<td></td>
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<tr>
<td>DTPw–HBV/Hib + MenC</td>
<td>Pre-PS</td>
<td>41</td>
<td>127.2 (70.5; 229.7)</td>
<td>45</td>
<td>0.16 (0.15; 0.18)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-PS</td>
<td>44</td>
<td>538.5 (367.3; 789.4)</td>
<td>45</td>
<td>0.74 (0.46; 1.20)</td>
<td></td>
</tr>
</tbody>
</table>

N, number of subjects with available results.

DTwP, diphtheria–tetanus–whole cell pertussis; HBV, hepatitis B virus; Hib, Haemophilus influenzae type b; Men, meningococci; CI, confidence interval; Pre-PS, pre-polysaccharide challenge; Post-PS, post-polysaccharide challenge; SBA, serum bacterial antibody; PSA, MenA capsular polysaccharide; PSC, MenC capsular polysaccharide; PRP, polyribosylribitol phosphate; ATP, according-to-protocol; GMT, geometric mean titer; GMC, geometric mean concentration.
immunogenicity: response to the DTPw–HBV vaccine antigens

One month after the primary vaccination course, anti-diphtheria, anti-tetanus, and anti-HBs seroprotection rates of all Hib–MenAC groups were similar to those in the DTPw–HBV + Hib group (Table 4). The anti-diphtheria seroprotection level was highest in the DTPw–HBV/Hib + MenC control group. The 5/5 group had a significantly lower anti-BPT vaccine response rate compared to the DTPw–HBV + Hib group.

Anti-diphtheria antibody concentrations were significantly higher in the DTPw–HBV/Hib + MenC group compared to the DTPw–HBV/Hib–MenAC and DTPw–HBV + Hib groups, whereas anti-tetanus antibody GMCs were significantly higher in each of the DTPw–HBV/Hib–MenAC groups compared to both control groups. Compared to the DTPw–HBV + Hib group, both Hib–MenAC formulations with 5 µg of PSA and PSC showed lower anti-BPT antibody concentrations, and the Hib–MenAC formulation containing 5 µg of Hib, PSA, and PSC also had a lower anti-HBs-antibody GMC (Table 4).

Safety

The incidence of solicited local or general symptoms that occurred after primary vaccination was in the same range across the five groups (Figure 2). Pain was the most frequently reported local symptom after primary vaccination, and irritability and fever (axillary temperature ≥37.5 °C) were most frequently reported systemic symptoms in all groups. Overall, grade 3 symptoms were reported in 16.8–22.9% of doses. Specifically, in each group, fever ≥39.5 °C (axillary) was reported after a maximum of 1% of all doses.

Seven SAEs were reported during the primary vaccination phase of the study. One case was fatal: a child developed meningitis and sepsis (no organism isolated) 23 days after the third dose of DTPw–HBV + Hib.

During the period between the end of the primary vaccination phase and the plain polysaccharide challenge at 10 months of age, 11 SAEs occurred in the study population of which three were fatal: one subject died of pneumonia that commenced 78 days after the third dose of DTPw–HBV/Hib + MenC, one died of pneumonia more than one month after the third dose of 2.5/5 (no further details available), and one died due to a cardiac arrest during an episode of meningitis and sepsis (no organism isolated) 23 days after the third dose of DTPw–HBV + Hib.
acute gastroenteritis that began 4 months after the third dose of DTPw—HBV + Hib.

Six non-fatal SAEs were reported after the administration of the polysaccharide challenge, of which one (acute gastroenteritis) occurred during the 31 days following the polysaccharide challenge. None of the SAEs were considered by the investigator to be related to vaccination.

Discussion

This study has demonstrated the feasibility of a combined, heptavalent vaccine comprising recommended EPI antigens for infant vaccination (diphtheria, tetanus, pertussis, hepatitis B) and conjugate polysaccharide components against Hib and meningococcal serogroup A and C diseases, for the primary vaccination of infants.

Three formulations of the experimental DTPw—HBV/Hib—MenAC vaccine were evaluated, and the study assessed the optimum dose of conjugated polysaccharide required to induce a satisfactory immune response to Hib, MenC, and MenA. The formulation with the lowest dose of conjugated PRP, MenA, and MenC polysaccharides (group 2.5/2.5) successfully primed against MenA and MenC when given as a three-dose primary vaccination course in infancy, while immune responses to Hib, diphtheria, tetanus, pertussis, and hepatitis B were similar to those observed after vaccination with DTPw—HBV + Hib. Both formulations with higher dosage of Hib—MenAC induced lower hepatitis B and pertussis antibody concentrations compared to the control group. The reason for this is unclear and may warrant further investigation.

For PRP there is evidence that a lower amount of PRP than that found in monovalent Hib-conjugate preparations is sufficient to induce an equivalent immune response.21–28 It was anticipated that for MenA and MenC conjugates, less than 10 μg of polysaccharide would be sufficient to induce an acceptable immune response, as was previously observed during the development of conjugated MenC vaccines28,29 and combined meningococcal vaccines including MenA conjugates30; this was confirmed in the present study. Indeed the formulation with the lowest dose (2.5/2.5 group) elicited high and persistent seropositivity rates for the three Hib, MenA, and MenC antigens with a robust anamnestic response to the polysaccharide challenge doses.

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Of note, the lowest anti-PRP antibody GMC was observed in the control group where Hib was given combined with DTPw—HepB and co-administered with the MenC conjugate vaccine (conjugated to CRM197), although seroprotection levels remained high. Interestingly, all of the DTPw—HBV/Hib—MenAC combinations (with MenC conjugated to TT) induced higher anti-PRP GMCs. Several factors might have impacted on the Hib responses in the different groups, such as mixed or separate administration of the Hib conjugate, the combination with other TT-conjugates in the Hib—MenAC groups, or a negative impact of the concomitant administration of the MenC-CRM197 with the whole-cell pertussis combination vaccine.

The first clinical trials of conjugate MenA vaccines were reported during the 1990s.31 Since then, a conjugate tetra-valent vaccine containing 4 μg PSA (MenactraTM, Sanofi Pasteur) has been licensed for use in adolescents and adults in the USA, but not in infants in whom it is only modestly immunogenic.30 Immune memory following MenA conjugate vaccination has been demonstrated for other experimental vaccines, although an experimental vaccine tested in both the UK32 and the Gambia33 was shown to induce immune memory in the first country and not in the latter.
Natural immunity against prevalent meningococcal strains is known, but it has also been observed in countries where MenA is considered not to circulate, indicating cross-reaction with other bacterial antigens. Serum bactericidal activity is known to steadily increase through childhood to 50–80% by 12 years of age.34 There is little information on the epidemiology of meningococcal disease in the Philippines but the presence of endemic MenA disease or cross-reacting bacteria contributing to the development of immunity over time cannot be ruled out. At the time the study was conducted, a MenA outbreak was under investigation in Baguio located 246 kilometers from Manila.11

It was not possible to conclude on the immune memory based on results of the serum bactericidal assays due to rapidly developing natural immunity measured by our bacterial assay in Filipino children. In this setting, anti-PSA ELISA measurement may not be the most appropriate method to assess immune memory. Alternatively, other MenA strains than the strain used in our SBA assay (F8238 of L11 immunotype) may be used as this may better discriminate between vaccine-induced protective immunity and cross-reacting natural immunity.35

Whether a booster dose of conjugate meningococcal vaccine is required during the second year of life has been a matter for debate. In the UK, primary vaccination is given in an early (2 months of age) and rapid (one month intervals between doses) schedule without a booster. Waning immunity and reduced vaccine effectiveness of Hib36 and MenC37 vaccines has been observed in the years following primary vaccination in UK infants, strongly suggesting the need for a booster dose in the second year of life. A booster dose of MenA conjugate vaccine may also be required, since at present there is no reason to suspect that the immune response to MenA will behave differently to Hib and MenC. The most appropriate timing for the booster dose, as well as the total number of vaccine doses minimally needed to induce long-term protection warrants further investigation.

In this study no increase in reactogenicity of the DTPw–HBV/Hib–MenAC vaccines during the primary vaccination course was observed when compared with the licensed DTPw–HBV + Hib vaccines. The control of meningococcal disease due to serogroup A poses particular challenges. Group A N. meningitidis is responsible for epidemics in developing regions that cause numerous deaths. From seroprevalence studies in Sudan,38 it is estimated that >80% herd immunity is necessary to prevent epidemics.38,39

Currently the WHO recommends commencement of mass vaccination against meningitis with the onset of epidemics. The success of this reactive approach depends on early recognition of an impending epidemic and efficient logistics in place to ensure rapid vaccine delivery. During the MenA epidemic in Sudan during 1998 and 1999, more than 33 000 cases and 2386 deaths occurred, despite vaccination of one-third of the population.38 It is clear that reactive vaccination policies can only be of limited success compared to preventive immunization that could have a significant impact on meningitis outbreak diffusion.40 Routine vaccination against MenA and MenC is the only way to provide lasting, widespread protection of the population against disease. Combining the Hib, MenC, and MenA vaccine components with the established DTPw–HBV vaccine in a single injection is the simplest means to ensure acceptance, rapid uptake, and high coverage.

The new heptavalent DTPw–HBV/Hib–MenAC vaccine delivers five of the currently recommended EPI antigens, as well as MenA and MenC conjugate vaccines. This heptavalent vaccine for routine infant vaccination offers the possibility to reduce deaths and long-term sequelae associated with endemic and epidemic meningococcal disease.

Conclusions

This study shows the feasibility of a novel heptavalent DTPw–HBV/Hib–MenAC vaccine for the primary vaccination of infants. The formulation of this novel vaccine with the lowest dose of conjugated PRP, Men A, and MenC polysaccharides (2.5 μg of each) was well tolerated, immunogenic, had good persistence of antibodies, and demonstrated immune memory, and consequently was selected for further development.

Further investigation is needed to provide information on the tolerability and immunogenicity of a booster dose, as well as the persistence of meningococcal bactericidal antibodies over time, in order to determine the total number of vaccine doses needed to induce long-term protection.

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Conflict of interest: Dr Palestroque has no conflict of interest to declare. Drs Gatchalian, De Vleeschauwer, Han, Poolman, Schuerman, Dobbelnaere, and Boutriau are employed by GlaxoSmithKline who is sponsoring the study. In addition Drs Schuerman, Poolman, Han, Dobbelnaere, and Boutriau have stock options.

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