Brief report

Immunogenicity and safety of 3-dose primary vaccination with combined DTPa-HBV-IPV/Hib vaccine in Canadian Aboriginal and non-Aboriginal infants

David W. Scheifele, Murdo Ferguson, Gerald Predy, Meena Dawar, Deepak Assudani, Sherine Kuriyakose, Olivier Van Der Meeren, Htay-Htay Han

**A R T I C L E   I N F O**

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**A B S T R A C T**

This study compared immune responses of healthy Aboriginal and non-Aboriginal infants to *Haemophilus influenzae* type b (Hib) and hepatitis B virus (HBV) components of a DTaP-HBV-IPV/Hib combination vaccine, 1 month after completing dosing at 2, 4 and 6 months of age. Of 112 infants enrolled in each group, 94 Aboriginal and 107 non-Aboriginal infants qualified for the immunogenicity analysis. Anti-PRP concentrations exceeded the protective minimum (≥0.15 μg/ml) in ≥97% of infants in both groups but geometric mean concentrations (GMCs) were higher in Aboriginal infants (6.12 μg/ml versus 3.51 μg/ml). All subjects were seroprotected (anti-HBs ≥10 mIU/ml) against HBV, with groups having similar GMCs (1797.9 versus 1544.4 mIU/ml, Aboriginal versus non-Aboriginal, respectively). No safety concerns were identified. We conclude that 3-dose primary vaccination with DTaP-HBV-IPV/Hib combination vaccine elicited immune responses to Hib and HBV components that were at least as high in Aboriginal as in non-Aboriginal Canadian infants.

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1. **Introduction**

In Canada, new vaccines can be licensed without domestic clinical trial data. A recent example was a hexavalent combination vaccine (*Infanrix hexa*, GlaxoSmithKline [GSK] Vaccines) that had been evaluated mainly in Europe [1–7]. The studied populations did not include Aboriginal children, who comprise about 5% of the Canadian pediatric population, mainly as First Nations members. Recognizing the diminished immune responses of certain Aboriginal populations elsewhere [8–10] to the *Haemophilus influenzae* b (Hib) polyribosylribitol phosphate (PRP) component of some vaccines and not knowing if this applied to Canadian Aboriginals, we wished to assess anti-PRP responses to the new vaccine. Responses to the hepatitis B virus (HBV) component were also of interest, as the immunogenicity of this antigen in children was reduced in a different hexavalent vaccine [11]. The study reported here provides new, relevant data for Canadian Aboriginal families and communities.

2. **Methods**

This was a phase IV, open-label study (NCT00753649) with two parallel groups, including Aboriginal infants (potentially First Nations, Métis, and Inuit) and non-Aboriginal infants. It began as a single center study (in Vancouver, British Columbia) but limited enrollment of Aboriginal infants prompted extension to centers in Edmonton, Alberta and Truro, Nova Scotia. Eligible infants were healthy, 6–12 weeks old at study entry and born between 36 and
42 weeks gestation. Exclusion criteria included immunosuppression, receipt of any blood products since birth or during the study, serious chronic illness, major congenital defect, prior vaccination or suspected allergy to any vaccine component. Informed consent was obtained from a parent or legal guardian prior to enrolling each infant. Prior approval of the study was obtained from the research ethics board for each center and the participating Aboriginal communities. Guidelines for research involving Aboriginal participants were followed [12].

Aboriginal infants were recruited at health centers serving their communities, with study vaccine given by suitably trained local staff. Non-Aboriginal infants were enrolled in Vancouver, where recruitment was community-based and vaccines were given by study nurses.

The study vaccine (Infanrix hexa®) contained per 0.5 ml dose diphtheria toxoid ≥30 IU (25 Lf), tetanus toxoid ≥40 IU (10 Lf), pertussis toxoid 25 μg, fimbrial hemagglutinin 25 μg, pertactin 8 μg, recombinant HBV surface antigen (HBs) 10 μg, poliovirus type 1– 40 D antigen units, type 2 – 8 D antigen units, type 3 – 32 D antigen units, PRP 10 μg conjugated to tetanus toxoid 20–40 μg and aluminum salts 0.82 mg. Study vaccinations were scheduled at 2, 4 and 6 months of age. The vaccine was administered intramuscularly in the anterolateral thigh. Rotavirus vaccine (Rotarix®, GSK) was offered as a course at 2 and 4 months of age. Most participants also received routine 7-valent pneumococcal conjugate (all centers) and meningococcal C conjugate (except in Nova Scotia) vaccinations concurrently with the study vaccine in the opposite thigh. EMLA® topical anesthetic could be given prior to venipuncture. Vaccines were stored at 2–8°C.

Parents were asked to report promptly to the investigator any medically attended adverse event (MAAE) occurring within 31 days after vaccination and any serious adverse event (SAE) occurring while enrolled in the study. Parents were questioned about any such events at each visit.

A single blood sample (2–5 ml) was obtained at 7 months of age, 30–48 days after the final dose of study vaccine. Serum was promptly harvested and stored frozen until tested. All serologic assays were performed at GSK Biologicals central laboratory or designated alternative, using standardized, validated procedures. Testing was limited to measurement of anti-PRP by enzyme-linked immunosorbent assay (ELISA) and anti-HBs by ELISA and a chemiluminescence immunoassay (CLIA)(Centaur, Siemens). Anti-PRP responses ≥0.15 μg/ml were taken as the minimum protective level [13] and responses ≥1.0 μg/ml as indicative of longer-term protection [14]. Anti-HBs concentrations ≥10 mIU/ml were considered protective [15]. The primary study outcome was the percentage of participants with anti-PRP concentrations ≥0.15 μg/ml. Secondary outcomes included the percentage of participants with anti-PRP concentrations ≥1.0 μg/ml, anti-HBs concentrations ≥10 and ≥100 mIU/ml, and the geometric mean concentration (GMC) of anti-PRP and anti-HBs antibodies. An exploratory outcome was the difference in seroprotection rate and GMC ratio between groups for anti-PRP and anti-HBs, with 95% confidence intervals. The safety outcome was occurrence of MAAE and SAE.

The study was powered to detect a decrease in the anti-PRP seroprotection rate in Aboriginal as compared to non-Aboriginal infants of greater than 10% (one-sided equivalence test of the difference in proportions). Assuming an anti-PRP seroprotection rate of 95% in the non-Aboriginal infants [6,11], a sample size of 95 subjects per group was needed to provide 81% power to rule out a seroprotection rate lower by ≥10% in Aboriginal versus non-Aboriginal infants (alpha 0.025, one-sided). Allowing for a 15% drop-out rate and non-evaluable subjects, 112 subjects were to be included in each study group.

### 3. Results

Study visits occurred from September, 2008, to March, 2013. Enrollment of non-Aboriginal infants (N = 112) was completed in Vancouver; enrolment of Aboriginal infants (N = 112) was slower and completed at 3 centers (center totals 88, 5 and 19). Table 1 describes protocol completion rates for each group. The protocol-compliant cohorts for immunogenicity assessment included 94 Aboriginal and 107 non-Aboriginal infants, representing 84% and 96% of enrollees, respectively. Demographic characteristics of the study participants are presented in Table 2. Groups were similar in terms of age at study entry, gender mix and concomitant vaccinations. All Aboriginal infants were First Nations; no Mètis or Inuit infants were recruited.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Aboriginal infants</th>
<th>Non-Aboriginal infants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>ATP cohort</td>
</tr>
<tr>
<td>N</td>
<td>112</td>
<td>94</td>
</tr>
<tr>
<td>Age (weeks), dose 1</td>
<td>9.3</td>
<td>9.2</td>
</tr>
<tr>
<td>Minimum</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Maximum</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Female</td>
<td>62</td>
<td>49</td>
</tr>
<tr>
<td>Male</td>
<td>50</td>
<td>45</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Asian</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>African</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Other</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Aboriginal</td>
<td>112</td>
<td>94</td>
</tr>
<tr>
<td>Concomitant vaccinations</td>
<td>110</td>
<td>–</td>
</tr>
<tr>
<td>Men C conjugate</td>
<td>93</td>
<td>–</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>82</td>
<td>–</td>
</tr>
</tbody>
</table>

a According-to-protocol immunogenicity cohort.

b Based on first doses administered.

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**Table 1**

Protocol completion rates for study groups.

<table>
<thead>
<tr>
<th></th>
<th>Aboriginal infants</th>
<th>Non-Aboriginal infants</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number enrolled</td>
<td>112</td>
<td>112</td>
<td>224</td>
</tr>
<tr>
<td>Number immunized</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose 1</td>
<td>112</td>
<td>112</td>
<td>224</td>
</tr>
<tr>
<td>Dose 2</td>
<td>109</td>
<td>112</td>
<td>221</td>
</tr>
<tr>
<td>Dose 3</td>
<td>106</td>
<td>112</td>
<td>218</td>
</tr>
<tr>
<td>ATP cohort for safety</td>
<td>111</td>
<td>112</td>
<td>223</td>
</tr>
<tr>
<td>Attended final visit (serology)</td>
<td>105a</td>
<td>112</td>
<td>217</td>
</tr>
<tr>
<td>Invalid, missing or off schedule serum sample</td>
<td>11</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>ATP cohort for immunogenicity</td>
<td>94</td>
<td>107</td>
<td>201</td>
</tr>
</tbody>
</table>

a Withdrawals included 4 lost to follow-up, 1 relocation, 2 protocol violations.

b ATP: according-to-protocol.
Immunogenicity data are presented in Table 3 for the according-to-protocol (ATP) cohorts. Results for the total vaccinated cohorts were not appreciably different (data not presented). The GMC values were 6.12 and 3.51 µg/ml for anti-PRP responses of Aboriginal versus non-Aboriginal infants, yielding a GMC ratio of 1.74 (95% CI 1.18, 2.57). For anti-HBs responses the corresponding GMC values were 1797.9 and 1544.4 µIU/ml, yielding a ratio of 1.16 (95% CI 0.83, 1.63).

Safety data were available following 327 vaccinations of Aboriginal infants and 336 vaccinations of non-Aboriginal infants. Rates of MAAEs that occurred within 30 days after vaccinations were higher in Aboriginal than other infants (Table 4) but most events were unrelated to immunization. SAE reports were limited to Aboriginal infants: 5 were hospitalized for infections (3) or seizures (2) and two others were attended as outpatients, one for seizures and one for pyrexia. Only the latter was possibly vaccine-related, with onset shortly after vaccination. All infants recovered.

4. Discussion

This study was the first to our knowledge to assess responses of Canadian Aboriginal infants to Hib and HBV antigens in any vaccine formulation, including the present hexavalent study vaccine. Despite early evidence that some Canadian native populations were at high risk of Hib meningitis [16], similar to Alaska Natives [17], vaccination programs for them were implemented without studies of immunogenicity. Likewise, northern Aboriginal communities were known to have high rates of HBV infection [18] but vaccination programs were undertaken without specific trials. Such studies are challenging to organize given the dispersal of the native populations among small, widely separated, often remote communities and the necessary precautions for culturally responsible research [12]. Having met those challenges, we found that participating First Nations infants responded robustly to the Hib component of a hexavalent DTPa combination vaccine, besting the GMC of non-Aboriginal infants. Anti-HBs responses were similar between groups. The reason for the greater Hib responses of the Aboriginal infants is not known but is a welcome opposite to the study hypothesis. Code-labeled sera were all tested at the end of the study so testing-related bias is unlikely. Most infants received concurrent 7-valent pneumococcal and meningococcal C conjugate vaccinations, which did not appear to interfere with the measured responses. Few other vaccine response data exist for this population: First Nations and non-Aboriginal adults had similar mean titers after receiving a pandemic (H1N1) influenza vaccine [19]. High Hib titers (≥ 5 µg/ml) could afford greater resistance to Hib colonization and reduce transmission to other Aboriginal infants [20].

There were no safety concerns attributed to study vaccine administration in either group. Aboriginal infants had more medically attended AE and SAE but almost all were unrelated to immunization, reflecting higher rates of various infections during the study (Table 4).

The study had several important limitations. Concurrent recruitment of non-Aboriginal infants occurred at only one center, possibly introducing bias to comparisons, but only 21% of Aboriginal participants were recruited at other centers. Fewer Aboriginal than other infants provided a final blood sample, mainly due to accessing difficulties in small communities. The number of Aboriginal infants was relatively small and limited to a sample of First Nations, not necessarily representative of all First Nations. Inuit and Métis infants were not available in the study communities and may also differ from those studied. Responses to other study vaccine antigens were not assessed but have shown little variability among other studied populations [1–7].

Hexavalent DTPa combination vaccine was well tolerated and immunogenic for Aboriginal and non-Aboriginal infants in this study, for both antigens assessed. Additional studies of Canadian Aboriginal populations are warranted to determine which, if any, require evaluation of specific vaccine responses.

Acknowledgements

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EMLA is a trademark of AstraZeneca.

Conflict of interest statement: O.V.D.M., H.H.H., D.A. and S.K. are employees of GSK Vaccines; O.V.D.M., H.H.H. and D.A. have stock options from the sponsoring company. D.S. has received a grant from GSK for the current and other clinical trials. M.F. received funding (to the Colchester Research Group) for this trial from GSK and from other companies for other, unrelated clinical trials. G.P. and M.D. declared having no conflicts of interest.

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Contributors’ statement: D. Scheifele and M. Dawar conceptualized and designed the study and served as site investigators for data acquisition in Vancouver. D.S. led manuscript development. Both reviewed and revised the manuscript and approved the final manuscript as submitted.

M. Ferguson (Truro, NS) and G. Predy (Edmonton, AB) served as site investigators for data acquisition, reviewed and revised the manuscript and approved the final manuscript as submitted.

D. Assudani, S. Kurijakose, O. Van Der Meeren and H.H. Han oversaw study management as sponsor (HHH), data analysis and interpretation (D.A., S.K.) and laboratory testing (OVDM). Each reviewed and revised the manuscript and approved the final manuscript as submitted.

References


