A fully liquid DTPw-HepB-Hib combination vaccine for booster vaccination of toddlers in El Salvador

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ABSTRACT

Objectives. *To compare the safety and immunogenicity of a booster dose of a fully liquid diphtheria-tetanus-whole cell pertussis-hepatitis B-*Haemophilus influenzae *type b (DTPw-HepB-Hib) vaccine to the separate administration of commercially available DTPw and Hib vaccines in healthy toddlers.*

Methods. *An open-label, randomized, parallel-group, Phase III study conducted at six centers in San Salvador, El Salvador, during February—June 2006. Toddlers (15–24 months of age) were eligible to participate if they had received primary immunization at 2, 4, and 6 months of age with a commercial DTPw-HepB/Hib vaccine requiring reconstitution. Participants received either one booster dose of DTPw-HepB-Hib fully liquid vaccine or DTPw and Hib vaccines administered separately. Blood samples were taken immediately prior to and at 1 month post-vaccination. For a 5-day period following vaccination, solicited adverse events were collected in subject diaries and assessed.*

Results. *The combined DTPw-HepB-Hib fully liquid vaccine was non-inferior to the separately administered DTPw and Hib vaccines, in terms of seroprotection/seroconversion rates for all antigens evaluated. The combination vaccine elicited a strong booster response as demonstrated by a large increase in antibodies against all vaccine antigens. The geometric mean concentrations (GMCs) of all antibodies in the DTPw-HepB-Hib group exceeded the seroprotection/seroconversion thresholds by very large margins, although for some antigens they were somewhat lower than the corresponding titers in the comparator group. With the combination vaccine, considerably fewer solicited local and systemic adverse events, such as fever and irritability, were reported than with the comparator vaccines.*

Conclusions. *This study demonstrates that the fully liquid combined DTPw-HepB-Hib vaccine is highly immunogenic and has a favorable safety profile when given as a booster vaccination to toddlers who have received a primary vaccination course with a different pentavalent vaccine that requires reconstitution.*

Key words Vaccines; immunization, methods; Hepatitis B vaccines; Haemophilus vaccines; Diphtheria-Tetanus-Pertussis vaccine; infant; El Salvador.

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Since the introduction of global childhood immunization programs, there has been a dramatic decline in the number of cases of childhood illnesses that were once common. The resulting mortality

reductions, averted medical costs, and productivity increases have had considerable impact on the global economy. It is estimated that, in 2003 alone, immunization efforts prevented 600 000 hepatitis B-related deaths and more than 2 million deaths from other vaccine-preventable diseases. Nonetheless, in the same year, 27 million infants worldwide did not receive immunizations (1).

Combination vaccines against diphtheria, tetanus, and pertussis (DTP) have been in use since the 1940s and represent the core childhood vaccination program around the world. Today, global DTP coverage is close to 80%. In addition to the basic DTP course given during the first year of life, optimal coverage is maintained with a booster dose at 2 or 3 years of age and a fifth dose before beginning primary school (2–4). Due to economic and logistic constraints, boosters are not administered in many developing countries.

Vaccines against hepatitis B virus have been in use since the 1980s and have been a recommended part of the World Health Organization (WHO) Expanded Program on Immunization (EPI) since 1992 (5–7). In 1996, a combined DTP-hepatitis B vaccine, better-suited for basic vaccination coverage, was recommended by WHO (8). By 2007, all countries with available delivery systems should have introduced hepatitis B vaccine (1).

In 1997, upon considering the disease burden of *Haemophilus influenzae* type b (Hib) and the vaccine efficacy and available supply, WHO recommended the addition of this vaccine to national immunization programs (9, 10). As a result, the disease has been virtually eliminated in countries where the recommendation was followed (11–13). Because Hib antibodies induced by vaccination during the first year of life tend to progressively diminish over the 2–3 years following primary vaccination (14), in many countries a booster dose is recommended for children under 4 years of age (15).

By 2004, a total of 134 of 165 developing countries had introduced hepatitis B vaccination into their national immunization schedules, and 63 had introduced Hib vaccination; however, coverage levels have stagnated or remain suboptimal in many countries. Globally, 1 of 4 children born each year will not be vaccinated (1).

Pediatric combination vaccines that include DTP with other antigens, such as hepatitis B and Hib, can simplify immunization delivery and provide multiple advantages to children, parents, and health care providers by reducing the number of injections, clinic visits, and logistic requirements (8, 16). A fully liquid diphtheria-tetanus-whole cell pertussis-hepatitis B-Hib (DTPw-HepB-Hib) CRM_{197} conjugate; combination vaccine (Quinvaxem®, Crucell, Berne, Switzerland) has been developed and shown to be safe and immunogenic when given as a three-dose primary immunization series in infancy (17). The vaccine eliminates the need for reconstitution of the commonly lyophilized Hib component of the pentavalent vaccine, thus reducing the risk of handling errors that could occur when preparing the final mix for injection. Additionally, vaccination time can be reduced when no reconstitution is required, as demonstrated by a recent time motion study in India in which vaccination with fully liquid Quinvaxem® took an average of 35% less time than a lyophilized formulation (18).

The objective of the present study was to compare safety and immunogenicity of a booster dose of the fully liquid pentavalent DTPw-HepB-Hib vaccine to the separate administration of commercially available DTPw and Hib vaccines, in toddlers who had received primary immunization with a commercial DTPw-HepB/Hib vaccine (Tritanrix-HepB/Hib,™ GlaxoSmithKline, Middlesex, United Kingdom).

MATERIALS AND METHODS

Study design and participants

This was an open-label, randomized, parallel-group, Phase III study conducted at six centers in San Salvador, El Salvador, in February—June 2006. Eligible participants were healthy children from 15–24 months of age (at the time of enrollment), previously immunized with DTPw-HepB/Hib (Tritanrix-HepB/Hib™) at approximately 2, 4, and 6 months of age as part of the routine National Immunization Program of El Salvador. The subjects had to be free from acute illness and were not allowed to participate simultaneously in other trials or vaccination programs; however, concurrent administration of oral polio vaccine, according to the local EPI schedule, was permitted. Further exclusion criteria were: immune deficiency, any current or previous immunotherapy (except inhaled or topical steroids), receipt of immunoglobulin and/or blood products within 6 months prior to study start, and a history of allergy to any of the vaccine components.

Study participants were randomly allocated in a 1:1 ratio to receive either the DTPw-HepB-Hib liquid vaccine, or separate DTPw and Hib vaccines (DTPw+Hib) administered simultaneously, but in different limbs. All vaccines were given intramuscularly in the deltoid region, with toddlers in the separate administration group receiving the DTPw vaccine in the right arm and the Hib in the left. The study was designed as an open-label trial since blinding could not be maintained due to the two separate injections in the DTPw+Hib group.

The study was approved by the National University of El Salvador Ethics Committee and by the Ministry of Health and Public Welfare of El Salvador. Parents/legal guardians gave their informed consent prior to study enrollment.

Vaccines

The DTPw-HepB-Hib fully liquid vaccine, Quinvaxem®, manufactured by Berna Biotech Korea Corporation, a Crucell subsidiary in Korea, contained ≥ 30 international units (IU) diphtheria toxoid, ≥ 60 IU tetanus toxoid, ≥ 4 IU inactivated *Bordetella pertussis*, 10 µg hepatitis B surface antigen (HBs), and 10 µg Hib polyribosylribitol phosphate (PRP) oligosaccharide conjugated to CRM_{197} per 0.5 mL dose. The comparator DTPw vaccine (Serum Institute of India Limited, Pune, India) contained ≥ 30 IU diphtheria toxoid, ≥ 60 IU tetanus toxoid, and ≥ 4 IU *B. pertussis* per 0.5 mL dose. The Hib vaccine (Vaxem-Hib®, Novartis Vaccines and Diagnostics, Basel, Switzerland) contained 10 µg PRP oligosaccharide conjugated to CRM_{197} per 0.5 mL dose.

Serology

Blood samples were taken from all children immediately prior to and 1 month after vaccination. Blood samples were analyzed at Novartis Vaccines in Marburg, Germany, and at the University of Turku in Turku, Finland (anti-*B. pertussis* antibodies only). Anti-diphtheria and anti-tetanus antibodies were measured using an indirect enzyme-linked immunosorbent assay (ELISA), with seroprotection defined as a titer level of ≥ 0.1 IU/mL. A whole-cell ELISA was

used to detect antibodies to *B. pertussis* (19). There is no international standard definition for seroprotection for *B. pertussis*. The ELISA units (EU) were calculated from a two-point method based on readings of negative and high-positive standard sera, with 2.1 EU being the lower limit of quantitation. Therefore, to determine a significant immune response to vaccination, seropositivity or seroconversion was defined as either titer levels ≥ 20 EU (19) or ≥ 4-fold increase from pre- to post-booster levels. The Hib ELISA specifically detects antibodies against PRP, the capsular polysaccharide of *Haemophilus influenzae* type B, and is set up as a competitive ELISA (20). Seroprotection rates were determined with the two commonly used cut-off levels, ≥ 0.15 µg/mL and ≥ 1.0 µg/mL. Antibodies against hepatitis B were determined using a commercial kit (Enzygnost® Anti-HBs II, Dade Behring, Marburg, Germany). Hepatitis B seroprotection was defined as concentration of anti-HepB antibodies ≥ 10 IU/L (21).

Safety and tolerability

Each subject's parent/legal guardian documented the solicited local and systemic adverse events on a diary card on the day of vaccination and for the 4 days following vaccination (5-day period). Any other unsolicited adverse events were also recorded in the subject's diary over the 5-day observation period, or—for adverse events occurring after the 5-day observation period—by the investigator during an interview with the parent/legal guardian at the study visits. Serious adverse events were recorded throughout the study period.

Solicited local reactions included tenderness, erythema, and induration at the injection site. Solicited systemic symptoms included fever—defined as body temperature ≥ 38 °C (22)—vomiting, diarrhea, irritability, change in eating habits, sleepiness, persistent crying, and rash.

All solicited adverse events at the injection site were considered vaccination-related, whereas the relationship of systemic events to the trial vaccination was determined by the investigator using the categories "related" or "unrelated." Severity grading for solicited local adverse events was done according to a 3-point scale. For injection site, erythema and induration severity were based on the diameter recorded by the parent/legal guardian with > 5 mm to ≤ 20 mm corresponding to Grade 1 and > 50 mm to Grade 3. The severity of tenderness was evaluated by the parent/legal guardian according to the discomfort caused to the child, ranging from "minor, slight reaction to touch" (Grade 1) to "crying when injected limb was moved" (Grade 3). For solicited, systemic adverse events, no assessment of severity was made.

Severity of unsolicited symptoms was graded according to a 3-point scale with Grade 1 corresponding to slight irritation and Grade 3 corresponding to considerable discomfort interfering with daily activities.

Statistical analysis

Immunogenicity data were analyzed for the according-to-protocol (ATP) population. The ATP population included all subjects who received the booster vaccination, had a post-vaccination titer for at least one antigen, and had no major protocol violations.

The study's primary hypothesis was that the DTPw-HepB-Hib vaccine is as immunogenic as the separately-administered DTPw and Hib vaccines, as demonstrated by the immune response to Hib (i.e., anti-PRP seroprotection rates at an anti-PRP titer ≥ 1.0 µg/mL) at 1 month post-vaccination. A non-inferiority limit of 10% was defined for the difference of anti-PRP seroprotection rates.

Secondary immunogenicity variables were the seroprotection/seroconversion rates for all other antigens contained in the vaccine (except hepatitis B), at 1 month post-vaccination. For these comparisons, non-inferiority of DTPw-HepB-Hib to DTPw+Hib was assessed using the same statistical method as was used for the primary variable. The study was designed so that 135 evaluable subjects per group gave 90% power to reject the null hypothesis. Since up to 10% of subjects were expected to drop-out or have unevaluable data, planned enrollment was 150 for each vaccine group.

Exact two-sided 95% confidence intervals (95%CIs; Pearson-Clopper) were calculated for the seroprotection (or seroconversion for pertussis) rates for each antigen. Calculations of geometric mean concentrations (GMCs) were performed by taking the anti-$\log_{10}$ of the mean of the $\log_{10}$ titer transformations. GMCs are presented together with the corresponding 95%CIs (normal approximation). Analysis of safety was done for the safety population and comprised descriptive statistics comparing the incidence of adverse events between the study groups. The safety population included all subjects who were vaccinated and provided follow-up safety data.

RESULTS

Study population

The disposition of subjects is shown in Figure 1. Of the 302 children enrolled and randomized, 150 were vaccinated with DTPw-HepB-Hib and 149 received DTPw+Hib vaccines. Two children allocated to DTPw+Hib were not vaccinated because their parents withdrew consent; another child in the same group was not vaccinated because a baseline blood sample could not be taken. A total of 17 vaccinated subjects discontinued the study: in 16 cases (DTPw-HepB-Hib, 9 subjects; DTPw+Hib, 7 subjects) parents withdrew their consent to the post-booster blood sampling and 1 subject (DTPw+Hib) had a protocol violation. Data from 256 children (DTPw-HepB-Hib, 127; DTPw+Hib, 129) were included in the ATP analysis of immunogenicity.

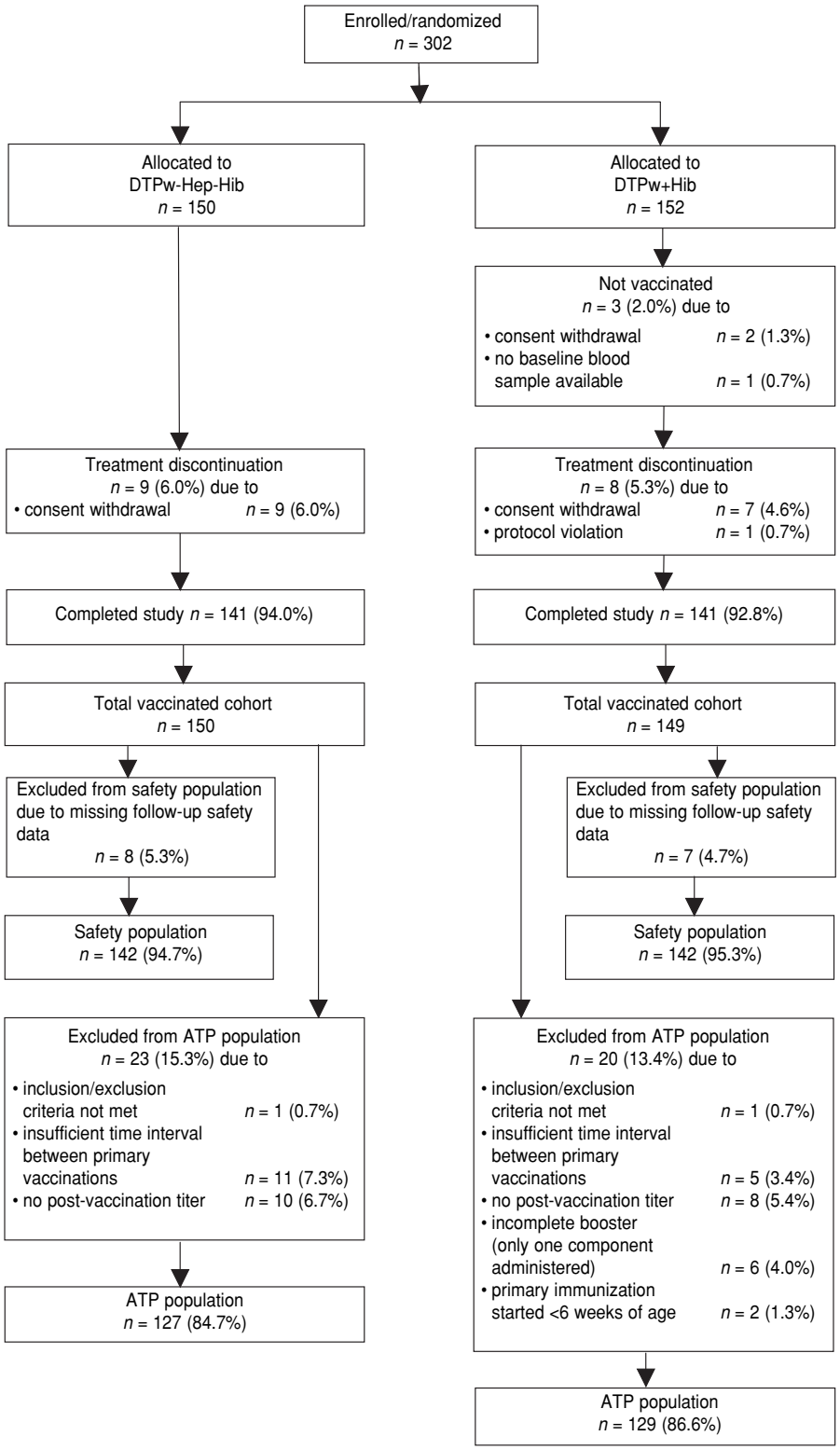
The main reasons for exclusion from the ATP population were: missing post-vaccination titer, insufficient time interval (i.e., fewer than 6 weeks) between primary vaccinations, blood sampling outside the time limit (22–57 days after booster vaccination), incomplete booster vaccination (only one component administered), and missing follow-up safety data.

A total of 284 subjects were included in the safety analyses; 15 were excluded due to missing follow-up safety data. The safety population was 56% male and had a mean age of 18.4 months at the time of the booster vaccination. There were no significant differences in the demographics of the two groups.

Immunogenicity

As shown in Table 1 and Table 2, both groups exhibited very similar seroprotection/seroconversion rates, as well as GMCs for all antibodies prior to vaccination (anti-HepB antibodies were not measured for the group that received separate administration of antigens).

FIGURE 1. Number of subjects[a] enrolled, randomized, and vaccinated with either diphtheria-tetanus-whole cell pertussis-hepatitis B-*Haemophilus influenzae* type b (DTPw-HepB-Hib) combination vaccine or separately administered DTPw and Hib (DTPw+Hib) vaccines, and included in the analysis of safety (safety population) and according-to-protocol analysis of immunogenicity (ATP population), El Salvador, February–June 2006



[a] Percentages are based on the number of randomized (top part of figure, study discontinuation/completion) or vaccinated (lower part of figure, composition of analyses sets) subjects in each group.

All subjects in both groups had anti-PRP titers of ≥ 1.0 µg/mL at 1 month post-booster (Table 1). Seroprotection rates for diphtheria, tetanus, and *B. pertussis* were similar for both groups. All subjects in both groups (except for one subject in the DTPw-HepB-Hib group) were protected against diphtheria, and all children in both groups developed protective titers against tetanus. Seroconversion to *B. pertussis* (either titer levels ≥ 20 EU or ≥ 4-fold increase in titers with respect to baseline) was achieved in 94.4% of subjects in the DTPw-HepB-Hib group and in 95.3% of subjects in the DTPw+Hib group. Regarding seroprotection rates for Hib, diphtheria, and tetanus, as well as the seroconversion rate for *B. pertussis*, statistical comparisons demonstrated that the DTPw-HepB-Hib combination vaccine was non-inferior to the separately-administered DTPw and Hib vaccines. All subjects who received the DTPw-HepB-Hib vaccine were protected against hepatitis B (not applicable for DTPw+Hib group).

Pre- to post-booster GMCs increased significantly in both groups. The boost in anti-PRP and anti-*B. pertussis* GMCs was similar in toddlers receiving the combination vaccine and those receiving the comparator vaccines (approximately 32-fold for anti-PRP and 5-fold for anti-*B. pertussis*). GMCs for anti-diphtheria antibodies increased 24 times for the DTPw-HepB-Hib group and 61 times for the DTP+Hib group. A 22-fold increase in anti-tetanus GMCs was observed after booster vaccination with DTPw-HepB-Hib, while the increase after receiving DTP+Hib was 42-fold. Comparable anti-PRP GMCs were found in both groups 1 month after receiving the booster dose (Table 2). Antibody titers against diphtheria and tetanus were significantly higher in the DTPw+Hib group than in the DTPw-HepB-Hib group (both $P < 0.001$). The same was true for anti-*B. pertussis* GMC levels ($P = 0.005$).

Safety and tolerability

The occurrence of solicited local and systemic adverse events during the 5-day period after the vaccination is shown in Table 3. The incidence of local adverse events was highest for the separately administered DTPw vaccine (79.4%), followed by the DTPw-HepB-Hib combination vaccine (68.8%), and

TABLE 1. Seroprotection (anti-PRP[a], anti-diphtheria, anti-tetanus, and anti-hepatitis B) rates, as well as baseline antibodies and seroconversion rates for anti-*B. pertussis*, before and 1 month after booster vaccination with either DTPw-HepB-Hib[b] vaccine or separately-administered DTPw[b] and Hib[b] vaccines; ATP population,[c] El Salvador, February–June 2006

	Pre-booster		Post-booster	
	DTPw-HepB-Hib	DTPw+Hib	DTPw-HepB-Hib	DTPw+Hib
Anti-PRP ≥ 0.15 µg/mL				
n[d]	124	125	125	127
% SP[e]	98.4	98.4	100[f]	100
(95% CI)[g]	(94.3–99.9)	(94.3–99.8)	(97.1–100.0)	(97.1–100.0)
Anti-PRP ≥ 1.0 µg/mL				
n	124	125	125	127
% SP	79.8	84.0	100[f]	100
(95% CI)	(71.7–86.5)	(76.4–89.9)	(97.1–100.0)	(97.1–100.0)
Anti-diphtheria ≥ 0.1 IU/mL[h]				
n	127	128	127	129
% SP	53.5	49.2	99.2[f]	100
(95% CI)	(44.5–62.4)	(40.3–58.2)	(95.7–100.0)	(97.2–100.0)
Anti-tetanus ≥ 0.1 IU/mL				
n	126	129	127	129
% SP	96.8	98.5	100[f]	100
(95% CI)	(92.1–99.1)	(94.5–99.8)	(97.1–100.0)	(97.2–100.0)
Anti-B. *pertussis* ≥ 20 EU or ≥ 4-fold increase[i,j]				
n	127	126	124	128
% BL[k]	15.7	19.0	NA[l]	NA
% SC[m]	NA	NA	94.4[f]	95.3
(95% CI)	(9.9–23.3)	(12.6–27.0)	(88.7–97.7)	(90.1–98.3)
Anti-hepatitis B ≥ 10 IU/L				
n	127	NA	127	NA
% SP	96.9	NA	100	NA
(95% CI)	(92.1–99.1)	NA	(97.1–100.0)	NA

[a] PRP = polyribosylribitol phosphate.
[b] DTPw-HepB-Hib = diphtheria-tetanus-whole cell pertussis-hepatitis B-*Haemophilus influenzae* type b.
[c] ATP = according-to-protocol.
[d] *n* = number of available observations.
[e] % SP = seroprotection rate.
[f] Non-inferiority of DTPw-HepB-Hib to DTPw+Hib demonstrated.
[g] CI = confidence interval.
[h] IU = international units.
[i] EU = ELISA units.
[j] 4-fold increase = 4-fold increase over pre-booster titers (applicable only for post-booster seroconversion rates).
[k] % BL = baseline antibody rate ≥ 20 EU
[l] NA = not applicable.
[m] % SC = seroconversion rate.

the separate Hib vaccine (56.3% of subjects). Tenderness was the most common local reaction and was reported by 63.1% of subjects in the DTPw-HepB-Hib group, and 77.2% and 54.2% of subjects in the control group (DTPw and Hib, respectively). All of the local adverse events were of mild to moderate intensity with the exception of some cases of "Grade 3" tenderness (19.1% for DTPw-HepB-Hib versus 29.4% for DTPw and 9.2% for Hib).

All solicited systemic adverse events occurred more frequently in the DTPw+Hib group than in the DTPw-HepB-Hib group (Table 3). The most common systemic adverse event in both groups of children was irritability, reported for 31.9% (DTPw-HepB-Hib) and 56.3% (DTPw+Hib). Fever (body temperature ≥ 38°C) occurred in 17.0% of the children receiving the DTPw-HepB-Hib and 44.4% receiving the DTPw+Hib. Body temperature > 39.5°C was seen in one child (0.7%) receiving the combination vaccine and in three children (2.1%) receiving the comparator vaccines.

Unsolicited adverse events were reported for 50.7% of the children in the DTPw-HepB-Hib group and 40.1% of those in the DTPw+Hib group. Only one child receiving the DTPw-HepB-Hib experienced unsolicited adverse events that were considered to be vaccination-related (viral infection and nasopharyngitis).

Most local and systemic adverse events occurred within the first day after vaccination and lasted 1–2 days. All solicited and unsolicited systemic adverse events were mild or moderate in nature. No serious adverse events were reported during the study period.

DISCUSSION

The fully liquid DTPw-HepB-Hib vaccine is immunogenic and safe when used as a booster dose in children who have received primary vaccination with another pentavalent vaccine. The possible interchangeability of different manufacturers' primary and booster immunization products is important to demonstrate because frequent shortages and changes in the vaccine availability are often encountered and could be better addressed.

Before the booster dose, almost all children (98.4%) had protective levels of anti-PRP antibodies (≥ 0.15 µg/mL);

TABLE 2. Antibody geometric mean concentrations for anti-PRP[a], anti-diphtheria, anti-tetanus, anti-*B. pertussis*, and anti-hepatitis B, before and 1 month after booster vaccination with either DTPw-HepB-Hib[b] vaccine or separately administered DTPw[b] and Hib[b] vaccines; ATP population,[c] El Salvador, February–June 2006

	Pre-booster		Post-booster	
	DTPw-HepB-Hib	DTPw+Hib	DTPw-HepB-Hib	DTPw+Hib
Anti-PRP				
n[d]	124	125	125	127
GMC[e] (µg/mL)	2.69	2.59	86.32	85.43
(95% CI)[f]	(2.12–3.42)	(2.07–3.22)	(69.57–107.1)	(68.46–106.6)
P-value[g]		0.924		0.950
Anti-diphtheria				
n	127	128	127	129
GMC (IU/mL)[h]	0.120	0.113	2.88	6.86
(95% CI)	(0.100–0.145)	(0.095–0.134)	(2.36–3.51)	(5.86–8.03)
P-value		0.614		< 0.001
Anti-tetanus				
n	126	129	127	129
GMC (IU/mL)	0.664	0.654	14.51	27.34
(95% CI)	(0.573–0.770)	(0.563–0.760)	(12.48–16.87)	(23.89–31.30)
P-value		0.848		< 0.001
Anti-*B. pertussis*				
n	127	126	124	128
GMC (EU/mL)[i]	10.94	11.63	50.53	60.38
(95% CI)	(9.68–12.35)	(10.32–13.11)	(46.01–55.49)	(54.88–66.44)
P-value		0.377		0.005
Anti-hepatitis B				
n	127	NA[j]	127	NA
GMC (IU/L)	151.4	NA	3728	NA
(95% CI)	(115.1–199.2)	NA	(2937–4731)	NA
P-value		NA		NA

[a] PRP = polyribosylribitol phosphate.
[b] DTPw-HepB-Hib = diphtheria-tetanus-whole cell pertussis-hepatitis B-*Haemophilus influenzae* type b.
[c] ATP = according–to-protocol
[d] *n* = number of available observations.
[e] GMC = geometric mean concentration.
[f] CI = confidence interval.
[g] *P*-values for between-group ratios.
[h] IU = international units.
[i] EU = ELISA units.
[j] NA = not applicable.

however, only 79.8–84.0% had maintained a level high enough to be considered adequate for long-term protection against Hib disease (anti-PRP antibodies ≥ 1.0 µg/mL). Based on data from the United Kingdom—where a change in schedule and a booster dose have been recommended following reemergence of Hib disease associated with low anti-PRP antibody titers (23, 24)—a booster dose of Hib conjugate vaccine may be considered in countries where only three doses in infancy are currently scheduled. The availability of pentavalent vaccines simplifies addition of a Hib booster (in the second year of life) to national immunization schedules that do not already recommend it.

The combination vaccine has been studied previously during a primary immunization course at 2, 3, and 4 months of age (17). In this earlier study, safety and immunogenicity profiles similar to separately-administered DTPw-Hib and hepatitis B vaccines were found. The present study demonstrated seroprotection against Hib in 100% of individuals, and non-inferiority of the DTPw-HepB-Hib vaccine to the concomitant but separate administration of DTPw and Hib vaccines at 1 month after a booster dose received in the second year of life. The DTPw-HepB-Hib vaccine also provided seroprotection or seroconversion rates for diphtheria, tetanus, and *B. pertussis* similar to the separately administered comparator vaccines.

The fully liquid DTPw-HepB-Hib vaccine generated a robust increase in antibodies against all vaccine antigens when pre- and post-booster antibody GMCs were compared. Higher GMCs for diphtheria, tetanus, and *B. pertussis* antibodies were found in the toddlers who received the separate DTPw and Hib vaccines as opposed to the combination vaccine. These results are similar to the findings of a previous study of primary immunization with the DTPw-HepB-Hib combination vaccine in which antibody GMCs were lower for tetanus, *B. pertussis*, and Hib in the group receiving the combination vaccine versus the separate vaccines (17). However, the GMCs for all antibodies in the DTPw-HepB-Hib group exceeded the seroprotection thresholds by very large margins, even when they were lower than the corresponding titers in the DTPw+Hib group. Thus, the differences observed, although statistically significant, are unlikely to have any clinical or epidemiological relevance. The strong immune response generated with the DTPw-HepB-Hib combination vaccine should provide children with long-term protection against five major childhood pathogens.

Local and systemic solicited reactions were considerably less frequent in children receiving the DTPw-HepB-Hib vaccine than when DTPw and Hib were administered separately. Given as a single

TABLE 3. Percentage of infants experiencing solicited local and systemic adverse events within 5 days after booster vaccination with either DTPw-HepB-Hib[a] vaccine or separately administered DTPw[a] and Hib[a] vaccines; safety population, El Salvador, February–June 2006

Adverse events	DTPw-HepB-Hib *n* = 141[b] % [c,d] (95%CI)	DTPw+Hib *n* = 142 % (95%CI)	
Local		DTPw[e] (*n* = 136)	Hib[e] (*n* = 142)
≥ 1 event reported	68.8 (60.5–76.3)	79.4 (71.6–85.9)	56.3 (47.8–64.6)
Tenderness	63.1 (54.6–71.1)	77.2 (69.2–84.0)	54.2 (45.7–62.6)
Erythema[f]	21.3 (14.8–29.0)	25.7 (18.6–33.9)	8.5 (4.4–14.3)
Induration[f]	18.4 (12.4–25.8)	26.5 (19.3–34.7)	7.0 (3.4–12.6)
Systemic			
≥ 1 event reported	61.0 (52.4–69.1)	79.6 (72.0–85.9)	
Irritability	31.9 (24.3–40.3)	56.3 (47.8–64.6)	
Change in eating habits	29.8 (22.4–38.1)	47.2 (38.8–55.7)	
Fever (≥ 38°C)	17.0 (11.2–24.3)	44.4 (36.0–52.9)	
Sleepiness	19.1 (13.0–26.6)	28.2 (20.9–36.3)	
Diarrhea	9.2 (5.0–15.3)	14.1 (8.8–20.9)	
Persistent crying[g]	13.5 (8.3–20.2)	18.3 (12.3–25.7)	
Vomiting	6.4 (3.0–11.8)	16.2 (10.6–23.3)	
Rash	6.4 (3.0–11.8)	7.7 (3.9–13.4)	

[a] DTPw-HepB-Hib = diphtheria-tetanus-whole cell pertussis-hepatitis B-*Haemophilus influenzae* type b.
[b] *n* = number of subjects in safety population receiving the respective vaccine who completed at least one diary.
[c] % = percentage of infants based on *n*.
[d] CI = confidence interval.
[e] Local adverse events were recorded separately for the comparator DTPw (right arm) and Hib (left arm).
[f] Recorded if diameter > 5 mm.
[g] Lasting > 3 hours.

injection, the combination vaccine appears likely to cause less pain and lead to less cumulative exposure to the preservatives and stabilizers that can contribute to adverse events (25).

A previous study revealed that an additional dose of monovalent HepB vaccine at birth did not affect the immunogenicity or tolerability of a DTPw-HepB-Hib pentavalent combination vaccine and that five doses of HepB vaccine can be given in early childhood without impact on safety (26). Thus, the new fully liquid DTPw-HepB-Hib combination vaccine could be given as both primary and booster vaccine.

The limitations of the present study were its relatively small size and the fact that a full comparison could not be made regarding the vaccines because the study group that received the fully liquid pentavalent vaccine was compared to a group receiving DTPw + Hib only. This was because although as infants all the children had been immunized with a pentavalent vaccine, at the time of the study the routine immunization schedule used in this country for children at the age group studied, had been changed to include only DTPw. According to this revised immunization schedule, these toddlers were not due to receive either a hepatitis B nor a Hib vaccine.

Conclusions

Quinvaxem® given as a booster dose in the second year of life is safe and immunogenic. The new fully liquid DTPw-HepB-Hib combination vaccine can be given as both a primary and a booster vaccine, thereby simplifying the administration of vaccines in countries that have added Hib and hepatitis B vaccination to their immunization schedules. In order to ensure long-term protection against invasive Hib disease, it is advisable to give all children in the second year of life a booster dose of Hib vaccine, preferably incorporated into a combination vaccine.

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Conflicts of interest This study was supported by a grant from Crucell (Berne, Switzerland), which produces Quinvaxem®. Christian Herzog, Ursula Aeberhard, and Christian Spyr were Crucell employees at the time of the study. Anne Katrin Hilbert is an employee of Novartis Vaccines and Diagnostics, which collaborates with Crucell in the production of Quinvaxem®. Eduardo Suárez and Edwin Asturias declare no conflicts of interest.

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RESUMEN

Vacuna líquida combinada DTPw-HepB-Hib como dosis de refuerzo en infantes de El Salvador

Objetivos. Comparar la seguridad y la inmunogenicidad en infantes saludables de una dosis de refuerzo de una vacuna líquida combinada contra la difteria, el tétanos, la tosferina (de células enteras), la hepatitis B y *Haemophilus influenzae* tipo b (DTPw-HepB-Hib), con la aplicación por separado de vacunas DTPw y Hib disponibles comercialmente.

Métodos. Se realizó un estudio de fase III abierto, aleatorizado, con grupos paralelos, en seis centros de San Salvador, El Salvador, en febrero-junio de 2006. Los infantes (de 15–24 meses) habían recibido la inmunización primaria a los 2, 4 y 6 meses de edad con una vacuna comercial DTPw-HepB/Hib que necesitaba reconstitución. Los lactantes recibieron una dosis de refuerzo con la vacuna DTPw-HepB-Hib o las vacunas DTPw y Hib por separado. Se tomaron muestras de sangre inmediatamente antes de la vacunación y un mes después. Las reacciones adversas en los cinco días siguientes a la vacunación se anotaron en diarios individuales y se evaluaron.

Resultados. Según las tasas de seroprotección/seroconversión de todos los antígenos evaluados, la vacuna DTPw-HepB-Hib no fue inferior que las vacunas DTPw y Hib administradas por separado. La vacuna combinada produjo una fuerte respuesta de refuerzo, reflejada en el gran aumento de anticuerpos contra todos los antígenos presentes. Con respecto al grupo de comparación, en el grupo vacunado con DTPw-HepB-Hib las concentraciones geométricas medias de todos los anticuerpos superaron ampliamente los umbrales de seroprotección/seroconversión —aunque con títulos menores en algunos antígenos— y hubo mucho menos reacciones adversas locales y sistémicas, como fiebre e irritabilidad.

Conclusiones. Se demostró que la vacuna líquida combinada DTPw-HepB-Hib es altamente inmunógena y satisfactoriamente segura cuando se aplica como dosis de refuerzo a infantes inmunizados primariamente con una vacuna pentavalente diferente que requiere reconstitución.

Palabras clave Vacunas; esquema de inmunización; vacunas contra la hepatitis B; vacunas contra *Haemophilus*; vacuna contra difteria, tétanos y tos ferina; lactante; El Salvador.