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A new DTPa-HBV-IPV vaccine co-administered with Hib, compared to a commercially available DTPw-IPV/Hib vaccine co-administered with HBV, given at 6, 10 and 14 weeks following HBV at birth

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Abstract

Three hundred and twenty eligible infants were enrolled in an open randomized clinical trial and allocated to one of two groups to receive either separate concomitant injections of a candidate combined DTPa-HBV-IPV and commercial Hib vaccine (candidate administration: DTPa-HBV-IPV + Hib) or separate concomitant injections of licensed DTPw-IPV mixed in the same syringe with Hib and HBV vaccines (comparator administration: DTPw-IPV/Hib + HBV). Vaccines were administered at 6, 10 and 14 weeks of age preceded by a monovalent dose of HBV at birth. The candidate vaccine administration was shown to be at least as immunogenic (primary objective) as the candidate administration with respect to the diphtheria, tetanus, polio, HBs and PRP seroprotection rates (primary endpoints). Post vaccination, both vaccine administrations showed an equivalent level of seroprotection with nearly all subjects ($>96\%$) acquiring seroprotective titers against diphtheria, tetanus, polioviruses, HBsAg and PRP antigens. A markedly higher anti-HBs response post dose 2 at week 14 in the group receiving the candidate vaccine, 98.6% of subjects had seroprotective titers (GMT of 505.7 mIU/ml) compared with only 88.7% (GMT of 107.5 mIU/ml) in the comparator group. There was a lower incidence of adverse events following the DTPa-based candidate administration compared with the DTPw-based comparator. Despite the early age and short interval between doses, both administrations were immunogenic, with the concomitant administration of DTPa-HBV-IPV and Hib vaccines showing an improved tolerability over the commercial vaccines DTPw-IPV/Hib and HBV.

Keywords: Combined vaccines; Hepatitis B; Early infancy; Accelerated schedule; Acellular pertussis

1. Introduction

Combination vaccines are now accepted widely as the most effective means of administering large numbers of antigens especially in the pediatric field where the number of recommended vaccines is continually increasing. This strategy enables immunization goals to be reached by increasing coverage with minimal discomfort to the vaccinee, whilst also reducing logistical costs [1]. Diphtheria–tetanus–pertussis (DTP) vaccines have been in

Abbreviations: CI, confidence interval; DTPa, diphtheria-tetanus-acellular pertussis vaccine; ELISA, enzyme-linked immunosorbent assay; EL.U, ELISA units; EPI, expanded program of immunization; FHA, filamentous hemagglutinin; GMT, geometric mean antibody titer; HbsAg, hepatitis B surface antigen; HBV, hepatitis B virus; Hib, *Haemophilus influenzae* type b; IPV, inactivated polio vaccine; OPV, oral polio vaccine; PRN, pertactin; PRP, polyribosyl-ribitol-phosphate; PT, pertussis toxoid; Pw, whole cell pertussis; SAE, serious adverse event; WHO, World Health Organisation; '+', separate concomitant administration of vaccines; '/', extemporaneously mixed administration of vaccines.

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widespread use since the 1940s. It is now estimated that DTP infant vaccine coverage exceeds 80% worldwide [2]. Consequently, DTP has become the cornerstone of pediatric combination vaccines. Over the past two decades, Pa (acellular pertussis) vaccine, to be combined to DTPa have been developed due to concerns over the reactogenicity observed with the conventional whole cell pertussis (DTPw) vaccines [3]. However, there are a number of European countries, such as the UK, Netherlands, France and most countries in Central and Eastern Europe, which still use DTPw. In addition, most of Asia and Latin America also continue to use DTPw and DTPw-based combinations.

Moldova has one of the highest hepatitis B endemicities in Europe [4,5] and with a carrier rate of 8–12%, [6] it is classified as a region of high endemicity (carrier rate > 8%). For such regions, the World Health Organization (WHO) recommends HBV at birth in order to reduce the risk of perinatal transmission and the subsequent likelihood of becoming a carrier [7]. In order to minimize possible breakthrough, the second dose is recommended within 1 month of birth [8]. The co-administration of DTP and HBV has the potential advantage of increasing infant coverage rates against hepatitis B, and consequently, the WHO has endorsed the development of combined DTP-HBV vaccines [9]. However, DTP cannot be given before 6 weeks of age [10,11], therefore, the WHO recommended DTP schedule which is given at 6, 10 and 14 weeks, is the most appropriate schedule for co-administration of DTP and HBV vaccines, following a monovalent dose of HBV at birth. Interestingly, other European DTPw-using countries, namely UK, Netherlands and France, also use a so-called accelerated schedule (8, 12 and 16 weeks). Again, the rationale is to provide coverage at an early age, however, it is also envisaged that reactogenicity may also be less in this age group, which is of particular importance when using the more reactogenic DTPw-based vaccines. The benefits to be gained from the latter have to be weighed against a possibly lower immune response, due to the early age of administration, short interval between doses and possible interference from maternal antibodies.

Recent years have also seen the use of *Haemophilus influenzae* type b (Hib) conjugate vaccine's increase and a trend to switch from live attenuated oral polio vaccine (OPV) to injectable inactivated polio vaccine (IPV). Recently, a multivalent DTPa-HBV-IPV candidate vaccine has been developed in order to address the problems associated with the growing number of injections recommended in early life. This trial compares the DTPa-HBV-IPV vaccine co-administered with a separate injection of a commercial Hib vaccine with separate concomitant administration of commercial DTPw-IPV/Hib and HBV vaccines. The primary objective was to show that, from a clinical point of view, the candidate vaccine administration (DTPa-HBV-IPV + Hib) was at least as immunogenic as the commercial comparator administration (DTPw-IPV/Hib + HBV). Comparison of the reactogenicity profile between the two study groups was a secondary objective.

2. Materials and methods

2.1. Design and subjects

This was an open, randomized, comparative study conducted at the Republican Hospital of Infectious Diseases at Chisinau, Republic of Moldova. As no ethics committee conforming to the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines was available at the time of the study, the study protocol and informed consent statement was appraised by the Medical Ethical Committee of the Medical Faculty, Free University of Brussels, Belgium. Parents/guardians granted their written informed consent prior to enrollment of their children.

Healthy male and female infants aged 6–8 weeks were eligible if they had received one dose of HBV (Engerix-B, SmithKline Beecham Biologicals) within 48 h after birth. A total of 320 eligible infants were enrolled and assigned randomly to one of the two groups to receive either the candidate vaccine or comparator vaccines as a three-dose primary schedule at 6, 10, and 14 weeks. Given the high hepatitis B endemicity in Moldova, the routinely used DTP primary schedule at 3, 4.5, and 6 months was not appropriate for using with a monovalent dose of hepatitis B at birth.

Subjects were excluded if they were participating in any other clinical trial, if they had an acute disease, a history of allergic reaction to any of the vaccine components, if they had undergone immunosuppressive therapy, had received any immunoglobulin therapy or blood products prior to the start or during the trial, or if they had received prior vaccination with any of the vaccine components (except HBV). Occurrence of any of the contra or precautionary indications for pertussis vaccination (fever ≥ 40.5°C (rectal temperature) within 48 h of vaccination, persistent inconsolable screaming or crying for more than 3 h, seizures and/or encephalopathy and hypersensitivity) [12] following any dose of trial vaccines, resulted in exclusion from the trial.

2.2. Study vaccines

Subjects received study vaccines at 6, 10 and 14 weeks of age (28–35-day interval between vaccinations). All vaccines were given by intramuscular injection in the anterolateral aspect of the thigh. Candidate administration (DTPa-HBV-IPV + Hib), a 0.5-ml dose

of the candidate vaccine (DTPa-HBV-IPV) was composed of ≥ 30 IU (25 Lf) of diphtheria toxoid; ≥ 40 IU (10 Lf) of tetanus toxoid; 25 μg of pertussis toxoid (PT); 25 μg of filamentous hemagglutinin (FHA); 8 μg of pertactin (PRN); 10 μg of recombinant HBsAg; 40 D-antigen units of type 1 (Mahoney); 8 D-antigen units of type 2 (MEF-1) and 32-D-antigen units of type 3 (Saukett) of the polio virus and 0.7 mg of aluminum as salts. The conjugate Hib vaccine (Hiberix, SmithKline Beecham Biologicals) contained 10 μg of polyribose-ribitol-phosphate (PRP) conjugated to ~ 30 μg of tetanus toxoid and 10 mg of lactose, the vaccine is prepared by reconstituting the lyophilized pellet in saline diluent (9 mg/ml NaCl). Comparator administration (DTPw-IPV/Hib + HBV), a single 0.5 ml dose of DTPw-IPV/Hib (Pentacoq, Pasteur Mérieux Connaught) comprised of ≥ 30 IU diphtheria toxoid; ≥ 60 IU tetanus toxoid; ≥ 4 IU *Bordetella pertussis* (inactivated whole cell); 40 D-antigen units of type 1 (Mahoney); 8 D-antigen units of type 2 (MEF-1) and 32-D-antigen units of type 3 (Saukett) of the polio virus, 10 μg of PRP conjugated to 24 μg of tetanus toxoid. The DTPw-IPV/Hib vaccine was obtained by mixing the freeze-dried Hib vaccine with the contents of prefilled syringe of DTPw-IPV. The HBV vaccine (Engerix, SmithKline Beecham Biologicals) contained 10 μg of recombinant HBsAg, 0.25 mg of aluminum as salts and 150 mM NaCl.

2.3. Serological analysis

Blood samples were drawn immediately prior to the first dose, 1 month after the second and third doses. Analysis was conducted at SmithKline Beecham Biologicals in a blinded fashion. Anti-diphtheria and anti-tetanus antibody titers were measured by enzyme-linked immunosorbent assay (ELISA). Titers $\geq$ 0.01 IU/ml determined by the neutralization test (NT) are generally considered to be the protective threshold. Although there is a good correlation between the NT and the ELISA [13,14], this correlation may be reduced at antibody titers below < 0.1 IU/ml, therefore, the cut off was arbitrarily and conservatively set at 0.1 IU/ml. Anti-HBs antibodies were determined using a commercial radioimmunoassay (AUSAB, Abbott), with a cut-off of 10 mIU/ml [15]. Antibodies against poliovirus were measured using a micro-neutralization assay (adapted from WHO standard assay [16]). The assay cut-off was defined as a titer of 1:8. ELISA was used to measure the anti-PRP antibodies**.** The cut-off point for this assay was 0.15 μg/ml. Antibodies against the three *B. pertussis* antigens (PT, FHA, PRN) were measured by ELISA and the assay cut-off was set at 5 EL.U/ml.

For all antibodies, except for *B. pertussis*, titers above the assay cut-offs were considered to be seroprotective. For *B pertussis*, subjects with titers above the assay cut-off were termed as seropositive. Furthermore, because there is no defined serological correlate of protection for *B. pertussis*, a vaccine response was defined. In initially seronegative subjects, a vaccine response (post dose 3) was defined as post vaccination antibody concentrations above the cut off value; and in initially seropositive subjects, as a post-vaccination titer equal to or greater than the individual's pre-vaccination titer, thereby taking into account the half-life (approximately 40 days) of maternal antibodies [17].

2.4. Reactogenicity assessment

Diary cards were used by parents or guardians to record solicited local reactions (pain, redness and swelling at each injection site) and general symptoms (fever, fussiness, vomiting, diarrhea, loss of appetite, restlessness and sleepiness) on the day of vaccination and for three subsequent days. Symptoms were graded from 1 to 3 in intensity, total incidence and grade 3 is reported here. Fever was defined as rectal body temperature ≥ 38.0°C, and grade 3 as a temperature ≥ 39.5°C. Grade 3 pain was defined as a child crying when limb was moved. Any redness or swelling at the injection site was measured. A diameter > 20 mm was defined as grade 3. Grade 3 fussiness was defined as inconsolable, persistent crying. For all other symptoms, grade 3 was defined as preventing normal daily activities. Unsolicited symptoms during a 30-day follow-up period after each vaccination and serious adverse events (SAE) during the entire study period were also recorded.

2.5. Statistical analysis

The objective of the study was to demonstrate that the candidate administration (DTPa-HBV-IPV + Hib) was at least as immunogenic as compared with the comparator administration (DTPw-IPV/Hib + HBV) (i.e. demonstrate clinical non inferiority), following the guidelines described in [18]. A pre-specified clinically acceptable limit for decrease in immunogenicity of the candidate administration was set (a priori limit of clinical non inferiority). The clinical limit was set at a 10% difference (comparator–candidate) for diphtheria, tetanus, hepatitis B and poliovirus seroprotection rates post dose 3 (primary endpoints). For post dose 3 anti-HBs GMT, the limit was set at a GMT ratio (comparator/candidate) of below 2.0 (secondary endpoint). The 90% confidence interval (CI) for differences in seroprotection rates was calculated using StatXact 3.0 and for the GMT ratio using a one-way ANOVA model on the log-transformed anti-body titers. The candidate administration was shown to be clinically-non inferior if the upper limit of the 90% CI was below the pre-specified limit defining clinical inferiority. A post hoc power calculation, using the ATP cohort study

Table 1
Seroprotection rates and GMTs for anti-diphtheria, anti-tetanus, anti-HBs and anti-poliovirus types 1, 2 and 3 and anti-PRP antibodies post primary vaccination course[a,b]

	Candidate group (DTPa-HBV-IPV+Hib)			Comparator group (DTPw-IPV/Hib+HB)			ΔSP (comparator candidate) 90% CI	Ratio GMTs (comparator candidate) 90% CI
	n/*N*	Percent SP (95% CI)	GMT (95% CI)	*n*/*N*	Percent SP (95% CI)	GMT (95% CI)		
Anti-diphtheria	148/150	98.7 (95.3–99.8)	0.54 (0.47–0.61)	142/147	96.6 (92.2–98.9)	0.47 (0.40–0.54)	−2.1[c] (−7.5; 2.8)	–[e]
Anti-tetanus	150/150	100 (97.6–100)	1.8 (1.6–2.0)	147/147	100 (97.5–100)	2.4 (2.1–2.8)	0.0 [c] (−3.6; 3.5)	–[e]
Anti-HBs	148/150	98.7 (95.3–99.8)	1016.2 (834.6–1237.2)	144/147	98.0 (94.2–99.6)	426.5 (336.5–540.7)	−0.7[c] (−5.68; 3.96)	0.42[d](0.32; 0.54)
Anti-polio type 1	148/150	98.7 (95.3–99.8)	535.1 (415.1–689.6)	143/144	99.3 (96.2–100)	170.3 (132.1–219.6)	0.64[c] (−3.3; 5.6)	–[e]
Anti-polio type 2	147/150	98.0 (94.3–99.6)	154.0 (125.5–189.1)	140/144	97.2 (93.0–99.2)	87.9 (70.0–110.5)	−0.78[c] (−6.2; 4.2)	–[e]
Anti-polio type 3	148/150	98.7 (95.3–99.8)	731.1 (590.0–906.0)	144/144	100 (97.5–100)	544.1 (443.5–667.5)	1.33[c] (−2.2; 6.1)	–[e]
Anti-PRP	144/150	96.0 (91.5–98.5)	1.9 (1.5–2.4)	146/147	99.3 (96.3–100)	4.0 (3.3–4.9)	[e]	–[e]

[a] *N*, number of subjects with available results; *n*, number of subjects with SP titers post vaccination; percent SP, seroprotection rate.
[b] The upper limit of the 90% CI exceeds the a priori limit of clinical non-inferiority.
[c] Primary endpoint-D,T and anti-polio SP rates, the a priori limit of clinical non-inferiority was set at 10% (difference in SP rates).
[d] Secondary endpoint-anti-HBs post vaccination GMTs, the a priori limit of clinical non-inferiority was set at 2.0 (GMT ratio).
[e] No limit of clinical non-inferiority was pre-defined.

size and values obtained in the trial, showed the power to reject the null hypothesis to be 99% with respect to all the primary endpoints. The 95% CI were calculated for all seroprotection, seropositivity, vaccine response rates, post vaccination GMTs and the percentage of doses followed by a report of an adverse reactions.

3. Results

Of the 320 infants who were enrolled (mean age, 6.4 weeks; range, 2–9 weeks), 312 completed the study. There were no medical reasons for the withdrawals. All infants complied with the criteria stated in the protocol for reactogenicity analysis. Seventeen infants were excluded from the immunogenicity analysis because they did not comply with protocol criteria, i.e. age outside the protocol specified range (one subject), unknown pre-vaccination serological status (one subject), deviation from vaccination (11 subjects) and blood sampling (four subjects) schedules. A re-analysis of all subjects from whom data had been collected (intention-to-treat analysis), indicated that no bias was introduced by focusing on the ATP population (data not shown).

3.1. Response to HBsAg (Tables 1 and 2)

One month after the final dose, all but two subjects receiving the candidate administration (98.7%), and all but three receiving the comparator (98.0%), had seroprotective titers. Statistical analysis demonstrated that the candidate administration was not clinically inferior to the comparator administration with respect to seroprotection rates and GMT endpoints. Immediately prior to the administration of the third dose of study vaccines (14 weeks of age) 98.6% of subjects receiving the candidate administration already had anti-HBs seroprotective titers whereas only 88.7% subjects in the comparator group had achieved this level (Table 2). This difference was also reflected in the post second and third doses GMTs.

3.2. Response to D, T and poliovirus types 1, 2 and 3

One month after the final dose, a high percentage of subjects in both groups had seroprotective anti-diphtheria, anti-tetanus, and anti-polio types 1, 2 and 3 antibody levels (Table 1). From a statistical point of view, the candidate administration was again shown to be at least as immunogenic with respect to all the above seroprotection rates. In addition, post second dose the anti-polio serotype 1 GMTs were higher (95% CI did not overlap) in subjects receiving the candidate group (Table 2) and post third dose serotypes 1 and 2 were higher following the candidate administration (Table 1).

3.3. Response to PRP

Post-primary anti-PRP titers ≥ 0.15 μg/ml were observed in 96.0% in the candidate group and 99.3% in the comparator group (Table 1). 65.3 and 82.3% of subjects in the candidate and comparator groups, respectively, had titers ≥ 1.0 μg/ml. Post-primary GMTs were also higher in the comparator group compared with those in the candidate group.

3.4. Response to pertussis antigens (Table 3)

The responses to the pertussis antigens contained in the acellular vaccine were measured. Both higher seropositivity (99.3–100%) and vaccine response (86.8–97.7%) rates were seen following the administration of the candidate vaccine. However, given the differences between whole cell and acelluar vaccine, a quantitative comparison of the antibodies measured between the two groups is precluded.

Table 2
Seroprotection rates and GMTs for anti-diphtheria, anti-tetanus, anti-HBs and anti-poliovirus types 1, 2 and 3 and anti-PRP antibodies post dose 2 of trial vaccine (week 14)

	Candidate group (DTPa-HBV-IPV + Hib)			Comparator group(DTPw-IPV/Hib + HB)		
	n/*N*	Percent SP (95% CI)	GMT (95% CI)	*n*/*N*	Percent SP (95% CI)	GMT (95% CI)
Anti-diphtheria	134/145	92.4 (86.8–96.2)	0.26 (0.23–0.30)	116/141	82.3 (74.9–88.2)	0.20 (0.17–0.23)
Anti-tetanus	145/145	100.0 (97.5–100)	0.73 (0.65–0.81)	140/141	99.3 (96.1–100)	0.79 (0.70–0.90)
Anti-HBs	143/145	98.6 (95.1–99.8)	505.7 (417–614)	125/141	88.7 (82.2–93.4)	107.5 (83.1–139.1)
Anti-polio type 1	140/140	100.0 (97.4–100)	164.3 (136–199)	133/136	97.8 (93.7–99.5)	64.6 (51.2–81.6)
Anti-polio type 2	138/141	97.9 (93.9–99.6)	43.4 (35.2–53.4)	125/137	91.2 (85.2–95.4)	29.9 (24.0–37.3)
Anti-polio type 3	135/141	95.7 (91.0–98.4)	212.4 (165.4–272.8)	130/137	94.9 (89.8–97.9)	155.2 (117.6–204.6)
Anti-PRP	97/144	67.4 (59.1–74.9)	0.38 (0.30–0.48)	132/141	93.6 (88.2–97.0)	1.3 (1.0–1.6)

Table 3
Seropositivity and vaccine response rate to PT, FHA and PRN and GMTs post primary vaccination course[a]

	Candidate group (DTPa-HBV-IPV + Hib)				Comparator group (DTPw-IPV/Hib + HB)			
	n/*N*	Percent Spo (95% CI)	Percent VR (95% CI)	GMT (95% CI)	*n*/*N*	Percent Spo (95% CI)	Percent VR (95% CI)	GMT (95% CI)
Anti-PT	143/144	99.3 (96.2–100)	97.7 (93.5–99.5)	53.1 (47.1–60.0)	115/142	81 (73.6–87.1)	78.1 (70.0–84.9)	17.9 (14.5–22.0)
Anti-FHA	146/147	99.3 (96.3–100)	86.8 (79.9–92.0)	60.8 (54.3–68.0)	118/147	80.3 (72.9–86.4)	31.6 (23.8–40.2)	8.1 (7.1–9.3)
Anti-PRN	148/148	100 (97.5–100)	93.4 (87.9–97.0)	105.1 (93.1–118.7)	147/147	100 (97.5–100)	97.0 (92.5–99.2)	83.4 (71.9–96.6)

[a] *N*, number of subjects with available results; *n*, number of subjects with titers > 5 EL.U/ml post vaccination; percent Spo, seropositivity rate-% of subjects with titers > 5 EL.U/ml; VR, vaccine response (appearance of antibodies in subjects who are initially seronegative, and at least maintenance of prevaccination antibody titers in those who are initially seropositive).

Table 4
Incidence of solicited local reactions reported (total and grade 3) per dose during the 4-day follow-up period[a]

	Candidate group ($N = 475$)		Comparator group ($N = 472$)	
	DTPa-HBV-IPV% (95% CI)	Hib% (95% CI)	DTPw-IPV/Hib% (95% CI)	HBV% (95% CI)
Pain (total)	19.4 (15.9–23.2)	12.2 (9.4–15.5)	27.1 (23.2–31.4)	11.7 (8.9–14.9)
Grade 3	1.9 (0.9–3.6)	1.7 (0.7–3.3)	3.4 (1.9–5.4)	0.4 (0.1–1.5)
Redness (total)	43.6 (39.1–48.2)	28.8 (24.8–33.1)	49.8 (45.2–54.4)	27.3 (23.4–31.6)
>20 mm	2.9 (1.6–4.9)	1.1 (0.3–2.4)	5.9 (4.0–8.5)	1.1 (0.3–2.5)
Swelling (total)	22.3 (18.6–26.3)	8.4 (6.1–11.3)	35.8 (31.5–40.3)[b]	16.9 (13.7–20.6)
>20 mm	4.6 (2.6–6.9)	1.3 (0.5–2.7)	11.7 (8.9–14.9)[b]	2.5 (1.3–4.4)

[a] *N*, number of doses with at least one symptom sheet completed; %, percentage of doses followed by a specified symptom.
[b] Significantly higher as shown by a non overlap of 95% CI (DTPa-HBV-IPV compared with DTPw-IPV/Hib sites only).

3.5. Reactogenicity

In both groups, the majority of local symptoms were associated with the DTP containing vaccine (i.e. DTPa-HBV-IPV or DTPw-IPV/Hib) rather than with the monovalent Hib or HBV (respectively) injections, as shown in Tables 4 and 5. However, the incidence of local reactions was lower at the DTPa-HBV-IPV site than at the DTPw-IPV/Hib site with the difference in the incidence of swelling being significant (as indicated by the 95% CI). Fussiness was the most frequently reported solicited general reaction, with both total and grade 3 cases occurring with a significantly higher frequency in the comparator group. A significantly lower incidence of fever and restlessness were also seen in subjects in the candidate group. In fact, a report of severe fever (rectal temperature > 39.5°C) was only recorded in two individuals (one in each group). The incidence of unsolicited symptoms were similar between groups and no SAEs were reported during the study period.

Table 5
Incidence of solicited general symptom reported (total and grade 3) per dose during the 4-day follow-up period[a]

	Candidate group ($N = 475$) (DTPa-HBVIPV + Hib)% (95% CI)	Comparator group ($N = 472$) (DTPw-IPV/Hib + HB)% (95% CI)
Fever ≥ 38°C (total)	10.3 (7.7–13.4)	17.4 (14.1–21.1)[b]
≥ 39.5°C	0.2 (0.0–1.2)	0.2 (0.0–1.2)
Fussiness (total)	21.5 (17.9–25.4)	30.5 (26.4–34.9)[b]
Grade 3	1.3 (0.5–2.7)	4.9 (3.1–7.2)[b]
Restlessness (total)	10.9 (8.3–14.1)	19.5 (16.0–23.4)[b]
Grade 3	0.8 (10.2–2.1)	1.3 (0.5–2.7)

[a] *N*, number of doses with at least one symptom sheet completed; %, percentage of doses followed by a specified symptom.
[b] Significantly higher as shown by a non overlap of 95% CI.

4. Discussion

Whole cell vaccines are still in wide use in many countries around the world, however, in recent years, acellular vaccines have been increasingly used due to their lower reactogenicity. In this trial, we compared a new DTPa-based candidate combination vaccine with a commercial DTPw-based combination vaccine, when given at the WHO accelerated recommended schedule (following a monovalent dose of HBV at birth). In both groups, the majority of subjects had seroprotective titers against diphtheria, tetanus, hepatitis B, poliovirus serotypes and Hib. Within the confines of the statistical analysis, the candidate vaccine administration was found to be at least as immunogenic as the commercial vaccines (DTPw-IPV/Hib + HBV). It is worth mentioning again that the statistical analysis of the immunogenicity data employed the non-inferiority approach (as currently requested by regulatory authorities [18]) rather than the more conventional difference comparison, therefore, no assessment of superiority of any of the responses was made. In addition, we observed a lower reactogenicity profile for the candidate vaccine.

One of the most striking differences between the two groups was in the anti-HBs response especially post dose 2 (of trial vaccines). 98.6% subjects in the candidate group had already achieved seroprotective titers at this time point, thereby fulfilling the WHO criteria for combined DTP-HB vaccines (95% of vaccinees to have seroprotective titers against hepatitis B post vaccination) [19]. Given that the trial was conducted in a region of high hepatitis B endemicity, the early protection could be of clinical importance. The response in the comparator group, where the HBV was given as a separate concomitant injection, was markedly lower, and are in fact, more typical of the response seen when administering monovalent HBV vaccines (with the first dose being given at birth) in an accelerated fashion [20–22]. Furthermore, others have reported a similar response to that seen in the comparator group, when the HBV antigen given in combination with DTPw (DTPw-HBV/Hib or DTPw-HBV + HBV) at the 6-10-14 schedules following a monovalent dose of hepatitis B at birth [23]. The low responses for vaccines administered using this schedule are generally accepted to be due to interference from maternal antibodies and the short time-interval between doses. As to why the anti-HBs response elicited by the DTPa-HBV-IPV vaccine should apparently not be affected by these factors is not known. However, it is tempting to suggest that some adjuvant property of the candidate vaccine specific for the anti-HBs response may be operating.

Although a similarly high number of subjects in the candidate and comparator groups achieved anti-PRP titers ≥ 0.15 μg/ml, a lower post vaccination GMT and number of subjects with titers ≥ 1.0 μg/ml was seen following the candidate administration. However, the anti-PRP values seen in both groups are within the range seen for already licensed monovalent Hib vaccines [24–27]. Furthermore, recent efficacy data from the UK, which uses the 8-, 12-, and 16-week accelerated schedule (with no booster recommendation in the second year of life), show that although at 1 year of age only 62% of infants have titers ≥ 0.15 μg/ml [28], the observed efficacy at 4 years of age is 94.7–99.1% [29]. This demonstrates clearly the effectiveness of the conjugated Hib vaccines, even when given at an early age. The higher response seen in the comparator group could be explained by the Pw component exerting an adjuvant effect on the Hib response [30].

There is no obvious reason for the higher anti-poliovirus titers (serotypes 1 and 2) in the candidate administration group. However, as the seroprotection rates are equivalently high, it is unlikely that these findings are of any immediate clinical significance although the impact on antibody persistence remains to be seen.

As no conclusive protective antibody concentration has been described to date for pertussis, nor is it likely that all the antibodies involved in protection have been identified (especially with respect to whole cell vaccines), no direct comparison can be made between a whole cell and acellular vaccine. However, a number of trials have demonstrated the efficacy of both vaccines from different manufactures [31–37]. In addition, some of these studies also allowed the comparison of effectiveness of DTPa and DTPw vaccines. Where such comparisons have been made, DTPa vaccines are generally considered to be as efficacious as DTPw vaccines, although some individual vaccines (of both types) have given poor results [38]. The DTPa component used in the candidate vaccine, Infanrix, was shown to be 84% (95% CI, 76–90%) and 89% (95% CI, 77–95%) efficacious in a double-blind prospective randomized trial [32] and in a prospective household contact study [36], respectively. Similarly, the DTPw component used in the comparator vaccine was shown to have an absolute efficacy 96% (95% CI, 87–94%) in an unblinded household contact study [35].

Initial comparative trials also showed all clearly the DTPa vaccines to be markedly better tolerated than the DTPw vaccines [38,39]. However, as mentioned in Section 1, the early age and short time interval between doses is thought to reduce the reactogenicity of DTPw vaccines. However, in this trial an improved tolerability DTPa vaccine was seen, although less marked than reported in the earlier trials [38,39].

The three-dose primary vaccination course of the candidate DTPa-HBV-IPV vaccine co-administered with Hib vaccine was safe and well tolerated and the incidence of symptoms following the acellular vaccine administration appeared be lower than following the whole cell vaccine administration. In conclusion, the candidate vaccine provides a viable, well tolerated DTPa-based

alternative for routine pediatric use in countries which currently use DTPw-based vaccines.

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