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Comparison Study of Combined DTPw-HB Vaccines and Separate Administration of DTPw and HB Vaccines in Thai Children

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SUMMARY The safety, immunogenicity and tolerability of two different DTPw-HBV combination vaccines, containing 5 and 10 μg of HBsAg; were investigated in comparison with separate administration of DTPw and HBV (10 μg of HBsAg). A three dose primary vaccination course at 2, 4 and 6 months of age was followed by a booster dose at 18 months. All vaccines were safe and well tolerated. The DTPw-HBV combination vaccine containing 10 μg of HBsAg elicited significantly higher anti-HBs titres than the other two vaccines after the primary and booster vaccination course. All vaccines elicited a high response against the other components. Based on these results, DTPw-HBV (10 μg HBsAg) was the most effective vaccine at this schedule.

There is no effective treatment for hepatitis B virus infection, but well-tolerated and effective vaccines have been available since 1982.[1] All major health authorities agree that vaccination is the most effective approach to reduce the health care burden of hepatitis B virus infection. In 1992 the World Health Organization (WHO) Extended Program of Immunization (EPI) set targets for the incorporation of hepatitis B virus (HBV) vaccination into national programs by 1997. These recommendations followed the recognition that the most effective means of reducing the carrier population was infant immunization.[2] Although not all countries have been able to implement these recommendations, largely due to the financial burden, the WHO predicts that the number of new child carriers will be reduced by 80% in 2001, following infant immunization programs.

Combination vaccines are a widely accepted means of effective childhood vaccination. Not only does the reduction in the number of injections improve compliance, but there are lower administration costs and fewer logistical requirements associated with implementing the programs.[3] Following this strategy, the WHO recommends that combined diphtheria-tetanus-whole-cell-pertussis-hepatitis B (DTPw-HBV) vaccines should replace DTPw in the EPI and be preceded by a single dose of HBV at birth in countries of high endemicity.[4] In Thailand, HBV vaccination at birth became mandatory in 1992, followed by two further doses at 2 and 6 months of age. Because the DTPw vaccination schedule is 2, 4, 6 and 18 months of age, there is suitable overlap with the hepatitis B schedule for the two schedules to be combined.

In the past, there has been some debate as to the optimum HBsAg content in HBV vaccines required to confer seroprotection, balanced against the costs of vaccine production.[5] Some studies have suggested that 10 μg should

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be the recommended minimum dose for neonates, based on the observation that there was a high number of non-responders to a lower dose of 5 μg.[6] Others suggest that doses lower than 10 μg may be sufficient to confer seroprotection (titres > 10 mIU/ml).[7-9] However, data from Thailand suggest that although a low dose (2.5 μg) may be sufficient for infants of hepatitis B carrier mothers negative for e antigen, it may be inadequate for high risk infants born to carrier mothers who are also e antigen positive.[10]

The aim of this trial was to evaluate the combination of DTPw with HBV at two different doses of HBsAg (5 and 10μg), and in comparison with the separate administration of DTPw and HBV (containing 10 μg of HBsAg), in order to find the optimum HBsAg content.

MATERIALS & METHODS

Study participants and design

This was an open, randomized study with 3 groups, conducted at Chulalongkorn University Hospital, Bangkok, Thailand. Within 5 days of birth infants received a single dose (0.5 ml) of hepatitis B vaccine (Engerix™-B), as is the current practice in this hospital and thus was not considered to be a study vaccine. Subjects were randomized between three groups in the order in which they enrolled. Prior to the birth of the infant, parents were informed about the study in their native language and their written consent was then obtained. The trial received ethical approval from the Faculty of Medicine and was conducted according to Good Clinical Practice in operation at the time and the Declaration of Helsinki and amendments.

A total of 124 healthy infants with a 5 minute Apgar score ≥ 7 at birth, who were born to mothers seronegative for HBsAg and who were not receiving any immunoglobulins, were enrolled in the study. At 6 to 10 weeks of age, subjects were eligible to continue if they were not participating in other trials, had no acute disease and were not receiving immunosuppressive therapy. Other exclusion criteria were a history of allergic disease, any symptom or sign of systemic dysfunction, especially of the central nervous system and any previous vaccination with similar vaccines other than oral polio and BCG. Following the administration of vaccine, any adverse experience associated with whole cell *B. pertussis* vaccination (fever ≥ 40.5°C as measured by an axillary temperature within 48 hours of vaccination, persistent inconsolable screaming or crying for more than 3 hours within 48 hours of vaccination, seizures and/or encephalopathy within 7 days of vaccination and hypersensitivity to the vaccine) led to exclusion from the trial. Following the primary course there were 96 subjects eligible to receive the booster dose.

Vaccines

All vaccines were manufactured by SmithKline Beecham Biologicals, Rixensart, Belgium. A single dose (0.5 ml) of the DTPw vaccine contained: 7.5 Lf diphtheria toxoid, 3.25 Lf tetanus toxoid, 15 O.U. of whole cell *B. pertussis*, 0.5 mg aluminum (as aluminum salts), 150 mM sodium chloride and phenoxyethanol residue. The combined DTPw-HBV vaccine consisted of DTPw combined with 10 μg or 5 μg of hepatitis B surface antigen (HBsAg) in groups 1 and 2, respectively. Group 3 received separate injections of DTPw and Engerix ™-B (10 μg HBsAg, 0.25 mg aluminum (as aluminum salts) and phenoxyethanol residue. The primary course study vaccines were administered as a 0, 2, 4 month schedule (at approximately 2, 4 and 6 months of age). Combined vaccines (DTPw-HBV) were injected in the anterolateral thigh. Subjects in group 3 received DTPw in the left thigh and HBV vaccine in the right thigh, respectively.

A single dose of the booster vaccine (same composition as the primary vaccines) was given at approximately 18 months of age.

Reactions

After each vaccination including the booster dose, subjects were closely observed for 30 minutes for any adverse reactions. Parents were asked to report local reactions (pain, redness, swelling) indicated as either absent, mild (adverse experience which is easily tolerated) or severe (adverse experience which prevents normal everyday activities) and general (fever, irritability, unusual crying, drowsiness, feeding problems, gastrointestinal symptoms) symptoms specified and marked as present or absent on the day of vaccination and for the following 3 days. Temperature readings were not taken, so the parents subjectively ascertained the presence of fever. Any solicited symptom that occurred between 4 and 30 days post-vaccination, was recorded as an unsolicited symptom. All other symptoms or reactions occurring

within 30 days post-vaccination were recorded as unsolicited, and defined according to WHO criteria. The investigator recorded the outcome of all adverse experiences and assessed the relationship of unsolicited symptoms and general reactions to the vaccination.

Laboratory analysis

Maternal screening for hepatitis B was carried out at the investigator's site. Sera harvested one month after the primary course and booster doses were stored at -20°C until analyzed at SmithKline Beecham Biologicals in Rixensart, Belgium for antibodies against all vaccine components.

The presence of anti-HBs titers was determined using radioimmunoassay (AUSAB, Abbott) and titers were calculated in mIU/ml,[11] titrated against a WHO reference serum. The assay cut-off used for this study was 10 mIU/ml. Anti-diphtheria and anti-tetanus titers were measured by ELISA and expressed in international units per ml (IU/ml), with respect to a reference serum. The assay cut-off was 0.1 IU/ml. It is generally accepted that for both diphtheria and tetanus, titers ≥ 0.01 IU/ml, are protective. It has been previously demonstrated that a good correlation exists between *in vivo* neutralization and ELISA tests[12,13] but this correlation may be reduced at antibody titers < 0.1 IU/ml. For this reason, a titer of 0.1 IU/ml by ELISA was conservatively set as the cut-off. The percentage of subjects with titers ≥ 0.1 IU/ml was determined for each group and GMTs were calculated.

Anti-*B. pertussis* antibody titers were determined by ELISA using the IgG EIA test kit, Labsystem (ICN-FLOW) and expressed in ELISA Units (EL.U/ml), with an assay cut-off of 15 EL.U/ml. There is no serological correlate of protection for *B. pertussis*, therefore a vaccine response was defined in initially seronegative subjects as induction of antibody titer greater or equal to the assay cut-off value. In initially seropositive subjects, a post-vaccination titer at least equal to the individual pre-vaccination value was required, to take into account the 40 day half-life of maternal antibodies.[14]

Statistics

The ratio of males to females was compared between groups using Fisher's exact test. The mean ages were compared between groups and sexes, using two-way ANOVA. The seropositivity rates between groups for each of the vaccine components and the incidence of each local solicited symptom and the incidence of fever were compared between groups using Fisher's exact test. All statistical comparisons were done using two-sided statistical tests with SAS software, using a type 1 error of 5%.

RESULTS

In the primary vaccination course, out of 124 subjects that were enrolled for the study, 116 were included in the reactogenicity and 96 for the immunogenicity analysis. No child was withdrawn from the study or dropped out due to an adverse event. Ninety-six subjects who had received the primary vaccination course were eligible for the booster phase of the study. Eighty four subjects were included in the reactogenicity and 96 in the immunogenicity analysis at the booster. There was no statistically significant difference in the demographic profile between groups for either the primary or booster vaccination study cohort.

Reactogenicity

Primary vaccination course

The incidence of local and general solicited symptoms are shown in Table 1. Redness was the most frequently reported local reaction. Fever was the most frequently reported solicited general symptom in all groups (84.5%, 73.3% and 64.0% of doses in groups 1, 2 and 3, respectively). The only statistically significant parameter was a higher incidence of fever in group 1 compared with group 3 (p = 0.009). However, fever was subjectively measured by parents. The incidences of other general solicited symptoms were low (range 0-3.8%) for all groups.

A total of 254 doses of combined vaccine was administered during the trial, only 2 doses elicited an unsolicited symptom that the investigator assumed to be related or possibly related to the vaccination, neither was considered to be severe. There was one serious adverse event reported, which the investigator felt was possibly related to the vaccine. An infant in group 1 developed a fever two hours after the first dose and was given paracetamol, but later that evening she developed nausea, hematemesis. However, when the child was hospitalized a diagnosis of bleeding from low prothrombin complex was made. Following plasma and vitamin K treatment, she made a complete recovery.

Table 1 Incidence of solicited local and general symptoms following the three dose primary vaccination course

Symptom	**Group 1** (N = 97) **DTPw-HBV** (10 μg HBsAg) % (n)	**Group 2** (N = 105) **DTPw-HBV** (5 μg HBsAg) % (n)	**Group 3** (N = 111) **DTPw + HBV** (10μg HBsAg) % (n)
Local			
Pain	1.0 (1)	2.9 (3)	0.9 (1)
Redness	12.4 (12)	12.4 (13)	8.1 (9)
Swelling	12.4 (12)	17.1 (18)	9.0 (10)
General			
Fever	84.5 (82)[1]	73.3 (77)	64.0 (71)[1]
Drowsiness	0.0 (0)	1.0 (1)	0.0 (0)
GI symptoms	3.1 (3)	1.9 (2)	0.9 (1)
Irritability	2.1 (2)	3.8 (4)	1.8 (2)
Unusual crying	1.0 (1)	1.9 (2)	0.0 (0)

N = total number of doses with symptom sheets returned
n = number of doses with symptom reported
Fisher's exact test *p* values between groups for: pain ($p = 0.53$), swelling ($p = 0.19$), redness ($p = 0.50$ and between individual groups fever: groups 1 and 2 ($p = 0.059$); groups 2 and 3 ($p = 0.15$); groups 1 and 3 ($p = 0.009$)[1]
[1]statistically significant

Table 2 Incidence of solicited local and general symptoms following the booster vaccination

Symptom	**Group 1** (N = 25) **DTPw-HBV** (10 μg HBsAg) %	**Group 2** (N = 27) **DTPw-HBV** (5 μg HBsAg) %	**Group 3** (N = 32) **DTPw + HBV**[1] (10 μg HBsAg) %
Local			
Pain	8.0	3.7	3.1
Redness	12.0	11.1	6.3
Swelling	32.0	44.4[2]	9.4[2]
General			
Fever	72.0	63.0	62.5
Drowsiness	0	3.7	6.3
GI symptoms	0	3.7	0
Irritability	0	0	0
Unusual crying	0	0	3.1

[1]Solicited local symptoms were not reported for the injection site of Engerix®
n = number of symptom sheets reporting a specific symptom
Fisher's exact test *p* values for comparison between the three groups were: pain ($p = 0.681$), redness ($p = 0.721$) and swelling ($p = 0.007$)[2]: between individual groups for swelling: groups 1 and 3, $p = 0.045$; groups 1 and 2, $p = 0.404$; groups 2 and 3, $p = 0.003$[2]
[2]Statistically significant
N is the total number of symptom sheets returned since the compliance was about 90%, all subjects analyzed for reaction did not return the symptom sheet

Table 3 Percentage of seropositive subjects and Geometric Mean Titres (GMT) for anti-HBs, anti-diphtheria, anti-tetanus and anti-*B. pertussis* one month post primary vaccination

Component	**Group 1** (N = 29) DTPw-HBV (10 µg HBsAg)		**Group 2** (N = 34) DTPw-HBV (5 µg HBsAg)		**Group 3** (N = 33) DTPw + HBV (10 µg HBsAg)	
	S+ %	GMT [95% CI]	S+ %	GMT [95% CI]	S+ %	GMT [95% CI]
Anti-HBs	100.0	1,866.6[1,2] [1,122.5-3,104.1]	91.2	393.1[1] [197.9-780.9]	100.0	726.9[2] [526.9-1205.1]
Anti-diphtheria	100.0	1.970 [1.287-3.014]	97.1	1.927 [1.227-3.026]	100.0	1.632 [1.145-2.326]
Anti-tetanus	100.0	4.242 [2.867-6.277]	100.0	3.465 [2.548-4.712]	100.0	4.721 [3.355-6.643]
Anti-pertussis	100.0	171.9 [149.3-197.8]	100.0	174.8 [142.7-214.0]	100.0	189.7 [160.3-224.5]

N = number of individuals
Seropositive titers (S+) were defined as: anti-HBs ≥ 10mIU/ml; anti-diphtheria ≥ 0.10 IU/ml; anti-tetanus ≥ 0.10 IU/ml; anti-*B. pertussis* ≥ 15 EL.U/ml
Geometric mean titers (GMTs) were given in: mIU/ml for anti-HBs; IU/ml for anti-diphtheria and anti-tetanus; EL.U/ml for anti-*B. pertussis*
Student's *t* test *p* values for post GMTs for anti-HBs between: groups 1 and 2 ($p = 0.0006$)[1]; groups 2 and 3 ($p = 0.08$); groups 1 and 3 ($p = 0.01$)[2]
[1,2]Statistically significant

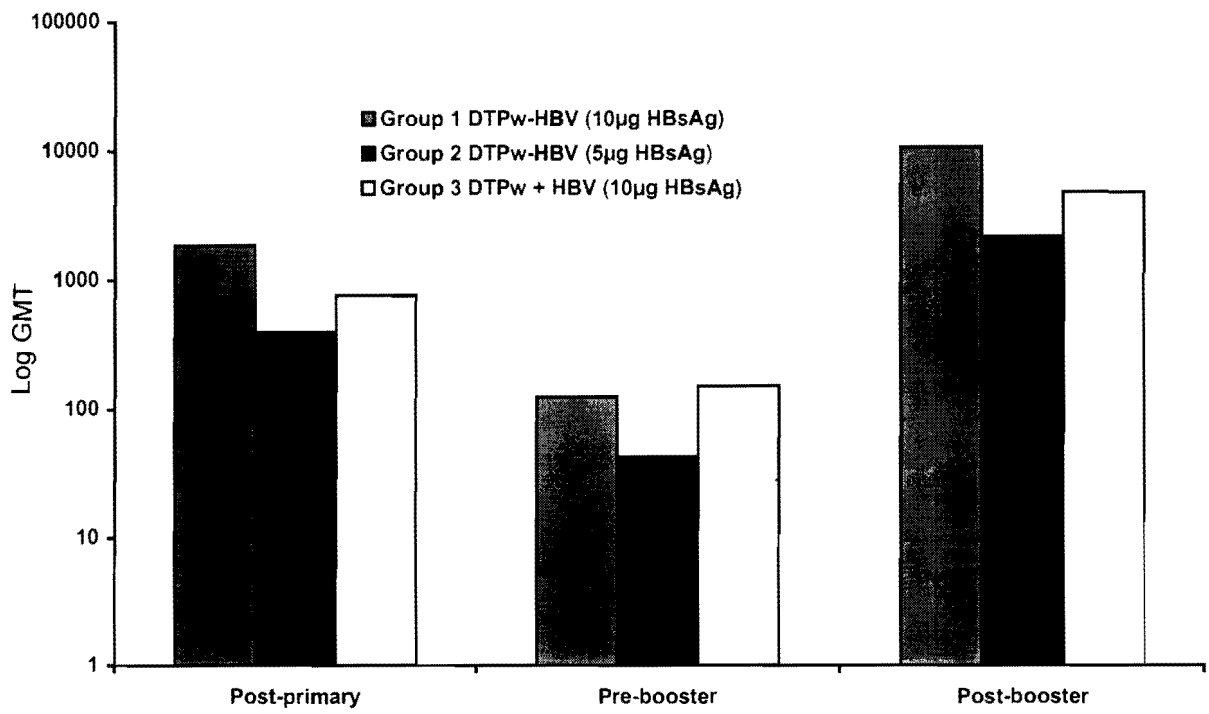

Fig. 1 Geometric mean titers (GMT) for anti-HBs before and after the primary and booster vaccination

Booster vaccination course

The incidences of local and general solicited symptoms are shown in Table 2. Swelling was the most frequently reported local reaction. The incidence of swelling was higher in the combined vaccines compared with the separate administration. There were no solicited local symptoms reported for the injection site of Engerix™-B (group 3) as shown in Table 2.

Fever was reported for 72.0%, 62.9% and 62.5% of subjects in groups 1, 2 and 3, respectively. No serious adverse event was reported.

Immunogenicity of the vaccine

Primary vaccination course

The percentages of seropositive subjects and GMTs for all vaccine components are shown in Table 3. All subjects in groups 1 and 3, and 91.2% in group 2, had protective levels of anti-HBs after the primary vaccination course. The GMT values were highest in group 1 followed by group 2. The difference was statistically significant between groups 1 and 2 ($p = 0.0006$) and groups 1 and 3, ($p = 0.01$).

After the primary vaccination course, all children in groups 1 and 3 and all except one subject in group 2 had anti-diphtheria titers greater than the cut-off value. All subjects in all groups had seroprotective anti-tetanus titers. All subjects in each group were seropositive with respect to anti *B. pertussis*, and all had an immune response to the *B. pertussis* vaccine component.

Booster vaccination

The pre- and post-booster serological data are shown in Table 4. Following the administration of

Table 4 Percentage of seropositive subjects and Geometric Mean Titres (GMT) for anti-HBs, anti-diphtheria, anti-tetanus and anti-*B. pertussis* prior to and one month post booster

Component	Timing	**Group 1** (N = 29) DTPw-HBV (10 µg HBsAg)		**Group 2** (N= 34) DTPw-HBV (5 µg HBsAg)		**Group 3** (N = 33) DTPw + HBV (10 µg HBsAg)	
		S+ %	GMT [95% CI]	S+ %	GMT [95% CI]	S+ %	GMT [95% CI]
Anti-HBs	Pre	95.0	124.5[1] [71.6-216.4]	69.6	42.7[1,2] [20.6-88.4]	100.0	150.5[2] [105.4-214.9]
	Post	100.0	10,650.6[1,2] [4,672.7-24,276.2]	100.0	2,164.1[1] [936.5-5,000.9]	100.0	4,751.6[2] [3,244.8-6,958.2]
Anti-diphtheria	Pre	70.0	0.137 [0.088-0.213]	60.9	0.131 [0.089-0.193]	62.1	0.150 [0.094-0.241]
	Post	100.0	7.288 [4.239-12.528]	100.0	5.700 [3.403-9.548]	100.0	4.107 [2.865-5.886]
Anti-tetanus	Pre	95.0	0.407 [0.282-0.589]	95.7	0.286 [0.202-0.404]	93.1	0.315 [0.228-0.435]
	Post	100.0	10.588 [7.940-14.120]	100.0	8.216 [6.526-10.344]	100.0	8.554 [6.927-10.562]
Anti-pertussis	Pre	85	28.2 [20.2-39.2]	72.7	21.5 [15.3-30.1]	96.6	40.2 [32.0-50.6]
	Post	100.0	267.9 [220.0-326.1]	100.0	252.8 [194.9-327.9]	100.0	247.0 [193.9-314.7]

N = number of individuals
[1,2]Statistically significant
Pre-blood sample taken immediately prior to booster
Post-blood sample taken 1 month after booster
Seropositive titers were defined as: anti-HBs ≥ 10mIU/ml; anti-diphtheria ≥ 0.10 IU/ml; anti-tetanus ≥ 0.10 IU/ml; anti-*B.pertussis* ≥ 15 EL.U/ml
Geometric mean titres (GMTs) were given in: mIU/ml for anti-HBs; IU/ml for anti-diphtheria and anti-tetanus; EL.U/ml for anti-*B. pertussis*
Student's *t* test *p* values for pre-booster GMTs for anti-HBs between: groups 1 and 2 ($p = 0.022$)[1]; groups 2 and 3 ($p = 0.001$)[2]; groups 1 and 3 ($p = 0.534$)
Student's *t* test *p* values for post-booster GMTs for anti-HBs between: groups 1 and 2 ($p = 0.008$); groups 2 and 3 ($p = 0.064$)[1]; groups 1 and 3 ($p = 0.0047$)[2]

the booster all subjects had titers greater than the assay cut-off values for each component of the vaccine. The vaccine response against pertussis was 100% in groups 1 and 2, and 93.1% for group 3. At the time of pre-booster blood sampling, 100% of subjects in group 3 were seropositive for anti-HBs, 95% in group 1 and 69.6% in group 2.

Fig. 1 shows the anti-HBs GMTs one month after the primary vaccination course, one month prior and one month post booster dose. A significantly higher response following the booster was seen in group 1 (10,651 mIU/ml) compared with group 2 (2,164 mIU/ml, p = 0.008) and group 3 (4,752 mIU/ml, $p = 0.0047$).

DISCUSSION

All vaccines were found to be safe, well tolerated and immunogenic when given as both primary and booster doses. The combined vaccines did not significantly alter the reactogenicity profile when compared with the separate administration of HBV and DTPw. However, DTPw combined with 10 μg HBsAg elicited higher seroprotection and significantly higher anti-HBs titers in both the primary and booster vaccination courses when compared with the other two trial vaccines.

Adverse reactions in the present study were similar to those associated with DTPw vaccine assessed in previous studies.[15] In the present study, the incidence of local symptoms was similar for all groups given the combined and separate vaccines. In this study there was a high incidence of fever however the subjective measurement was not substantiated by any objective measurement of temperature. Similarly, high incidence of fever was observed in an earlier study in both primary (64%)[16] and booster vaccination (75.6%)[17] when subjective measurements were made. However, a range of values has been observed for studies (23.7-65%) where quantitative measurements have been made.[18-20] The only SAE reported in the entire study resolved successfully following treatment.

The WHO minimum criterion for a combined vaccine is that the HBV component elicits a minimum protective level against hepatitis B in 95% of the vaccinees after the primary course.[21] In this trial, 100% of subjects receiving 10 μg of HBsAg as either a combined or separate primary course achieved this level.

One possibility for the higher anti-HBs response of the 10 μg HBsAg combined form is the adjuvant affect of the whole cell pertussis component. Furthermore, a better response was observed after the booster dose. This is a measure of the immunological memory induced by the primary course and the booster capacity. A significantly higher response was observed in group 1 compared with the other two groups (> 2-fold higher than the separate administration, group 3). It is also interesting to note that a slightly higher response to tetanus, *B. pertussis* and diphtheria was observed in the combined vaccines compared to the separate administration after the booster dose (with the exception of the tetanus response in the 5μg HBsAg combination). This might suggest that the higher concentration of protein in the vaccine brought about by the combination with either 10 or 5 μg of HBsAg has some increased priming effect. The fact that the increased response (except for the tetanus response) is dose-dependent would support this observation. However the determination of the exact mechanism is beyond the scope of this trial.

The clinical observations that all vaccinees were seropositive for all components post booster vaccination demonstrates the combined vaccines to be immunogenic. The results clearly demonstrate the 10 μg HBsAg DTPw combination to have superior immunogenicity when compared with the other two vaccines. These results match the findings of a recent study in which the immunogenicity of 5 and 10 μg of HBsAg (Engerix ™-B) were compared,[22] and again the higher content of HBsAg was shown to be more immunogenic. Our findings suggest that while the 5 μg HBsAg combination fails to reach the minimum criterion of 95% seroprotection in vaccinees (91% seroprotection after the primary course), the 10 μg combination is well in excess of this criterion. Perhaps the most clinically relevant response is the significantly larger anamnestic response seen in the 10 μg combination indicating a higher degree of priming compared with separate administration of HBV (10 μg HBsAg) and DTPw. This is because immunological memory is an indication of the capability of a vaccine to afford protection.[8]

The combination vaccine has added benefits in terms of a reduced number of injections, which will improve patient comfort and minimize costs. In summary, the

findings of this trial support the use of DTPw-HBV (containing 10 μg of HBsAg) at 2, 4 and 6 months of age, and a single dose of monovalent HBV given at birth as required in countries of high endemicity. Furthermore, the significantly higher immune response following the booster dose in the second year of life ensures long-lasting protection.

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