Vaccine 36 (2018) 2378–2384

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

A randomized, open label trial to evaluate and compare the immunogenicity and safety of a novel liquid hexavalent DTwP-Hib/Hep B-IPV (EasySix™) to licensed combination vaccines in healthy infants

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ARTICLE INFO

Article history:
Received 27 March 2017
Received in revised form 7 September 2017
Accepted 8 September 2017
Available online 23 March 2018

Keywords:
DTwP-HepB-Hib-IPV
Combination vaccines
Hexavalent
Immunogenicity
Infants

ABSTRACT

Immunogenicity and safety of a newly developed liquid DTwP-Hib/HepB-IPV hexavalent vaccine (EasySix™) was evaluated and compared with administration of commercially licensed Pentavac SD® (DTwP-HepB/Hib) and Imovax Polio® vaccine in an open-label, randomized multi-centric trial. 284 participants, aged 6–10 weeks, randomized in a 1:1 allocation, received three doses of test or comparator vaccines, administered 4 weeks apart. Immunogenicity of the vaccines was determined by measuring the baseline and post-vaccination antibody responses and comparing the proportions of subjects achieving seroprotection against the vaccine antigens; safety was evaluated in terms of solicited (local and systemic) and unsolicited incidences in the follow up phase. Post-vaccination, seroprotection was achieved against all six vaccine antigens in both vaccine groups. The seroresponse rate as well as geometric mean titers of antibody for all vaccine components were comparable between EasySix™ and Pentavac SD®-Imovax Polio® group. Both vaccines had similar reactogenicity profiles and were well tolerated; all adverse events resolved completely without any sequelae. Only one serious adverse event was reported that completely resolved; it was regarded unconnected to the vaccine administered. This study demonstrated that immunogenicity and safety profiles of EasySix™ vaccine, manufactured by Panacea Biotec Ltd, are non-inferior to the commercially available vaccines.

Clinical trial registration: CTRI/2015/02/005578.

1. Introduction

Over the years, progressive use of vaccines has led to the prevention of multiple diseases [1]. However, continuous inclusion of new antigens to routine vaccination has added complexity to an existing congested childhood immunization program. American Academy of Pediatrics (AAP) and US Advisory Committee on Immunization Practices (ACIP) recommends vaccination against 14 diseases through the first 2 years of childhood, achieved by 17–20 injections administered during multiple health care visits; this adds burden on both the health care provider and patients [2]. Combination vaccines- composite of vaccines targeting different diseases are being preferred over monovalent vaccines because they are relatively economical, convenient to use, require fewer injections, and thereby have improved compliance [3–6].

Since the first trivalent DTP (Diphtheria, Tetanus, Pertussis) vaccine licensed in US in 1949 [1,7], several DTP combination vaccine have been introduced in vaccination programs globally [8]. Similar immunization schedules facilitated consolidation of vaccines against *Haemophilus influenza* type b (Hib), hepatitis B (Hep B) and polio virus with DTP using different combination strategies [9–20].

Hexavalent combination (DTaP-HepB/Hib-IPV) combining six vaccines targeting diphtheria, tetanus, acellular pertussis (aP), IPV, Hib and Hep B have been developed, tested and licensed (Infanrix®, Hexaxim®) for vaccination in several countries, including India [19,21–29]. However, WHO has hypothesized that substitution of whole cell pertussis (wP) by less responsive acellular pertussis (aP) in DTP combinations [4,30–32] may be related to decrease in protection [33,34]. Currently, DTwP based hexavalent vaccine are commercially unavailable.

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https://doi.org/10.1016/j.vaccine.2017.09.029
0264-410X/

The objective of the present study is to assesses the immune response (primary outcome) and safety profile (secondary outcome) of a completely liquid, novel DTwP based hexavalent combination vaccine, EasySix™ (DTwP-Hib/Hep B-IPV) and compared to co-administration of two commercially available licensed products (DTwP-Hib/Hep B-Pentavac SD®; IPV-Imovax Polio®) comprising of same vaccine antigens.

2. Methods

A randomized, open label trial was conducted at four independent centers across India, to assess non-inferiority of a wholly liquid hexavalent DTwP-Hib/HepB-IPV vaccine (EasySix™; Panacea Biotec Ltd.) to immunization with same vaccine antigens using commercially licensed vaccines. Protocols and related pertinent documents were pre-approved by Indian regulatory and Independent Ethics Committees. The study was conducted as per guidelines laid in Declaration of Helsinki, GCP (Central Drug Standard Control Organization) and Ethical Guidelines for Biomedical Research on Human Subjects (Indian Council of Medical Research). Signed informed consent from parents or legally acceptable representatives (LAR) was prerequisite.

2.1. Subjects

Healthy, full term (>36 weeks gestation period) infants of either sex, aged 6–10 weeks, weighing >3.3 kg, whose parents/Legally acceptable Representative (LAR) gave prior consent, and who complied with all trial procedures, were considered eligible. Exclusion criteria included known HBsAg positivity in mother; immunizations apart from the study vaccine (except zero polio, BCG and birth dose of HBV); history of infection (potentially related to pathogens targeted by the DTwP-Hib/Hep B-IPV vaccine); presence of neurological disorder; history of seizures before trial; temperature >38 °C in last 3 days; indication of acute illness/infection within past 7 days; surgery during the study, known or suspected immune dysfunction (congenital or hereditary); record of anaphylaxis or allergy to vaccine components; evidence of bleeding disorder, any clinically significant chronic disease (such as cardiac, pulmonary, renal, gastrointestinal, hepatic, endocrine, cancer, skin or autoimmune) or basic congenital defects; post-natal administration of immunoglobulin, blood products, cytotoxic agents or radiotherapy and use of any trial or unlisted drug before the commencement or during the trial.

2.2. Study vaccines

A block randomization method was used to randomly allocate 284 eligible subjects in a 1:1 ratio to receive 3-doses primary vaccination series of either EasySix™ or PentavacSD® co-administered with Imovax Polio® (Sanofi Pasteur India Pvt. Ltd.). Treatment groups were assigned to eligible subjects by investigators at their respective site as per the randomization list prepared by the biostatisticians at Panacea Biotec Ltd. using computerized codes (generated by Sealed Envelope Ltd. 2014). To prevent biasness, details of block size and randomization codes were not disclosed to investigators until intervention allocation.

Vaccines were administered to subjects at approximate age of 6, 10 and 14 weeks via intramuscular route. A single injection (0.5 ml) of EasySix™ comprised of diphtheria toxoid (≥30 IU), tetanus toxoid (≥60 IU), inactivated whole cell pertussis (≥4 IU), *H. influenzae* type b conjugated (PRP-TT) (10 μg), recombinant hepatitis B surface antigen (HBsAg) (≥10 μg), inactivated Salk Polio Virus (Type 1 = 40DU, Type 2 = 8DU, Type 3 = 32DU); aluminum phosphate gel (≤1.25 mg) and 2-phenoxyethanol (3.3 mg),or of Pentavac SD® that comprised of HepB/Hib vaccine Pentavac SD® (Serum Institute of India Ltd.) diphtheria Toxoid (≥20 Lf to ≤30 Lf), tetanus Toxoid (≥2.5 Lf to ≤10 Lf), inactivated whole Cell Pertussis (≥4 IU), H. Influenza type b Conjugated (PRP-TT) (10 μg), rec-hepatitis B surface antigen (≥10 μg) and of Imovax Polio® comprised of inactivated Salk Polio Virus (Type 1 = 40 DU, Type 2 = 8 DU, Type 3 = 32 DU); aluminum phosphate gel (≤1.25 mg) and thiomersal (0.005%).

2.3. Serological analysis

For immunogenicity analysis, blood samples (4 ml) were collected before first dose and 4 weeks after conclusion of three dose immunization schedule. The trial sera, stored at −20 °C until analysis, were labeled with a unique 5-digit subject enrollment ID (along with protocol number, parent/LAR initial and sample collection date) that permitted to conduct all serological assays at Drug Discovery Lab, Mohali in a blinded fashion. Immunogenicity was evaluated by measuring antibodies specific for diphtheria and tetanus toxoids, pertussis (Anti PT and Pertussis IgG), Hib (PRP) and Hepatitis B (Anti HBsAg) by specific Enzyme Linked Immunosorbent Assay (ELISA) kits (Demeditec Diagnostics GmbH, Germany). The cut off value for sero-protection against Diphtheria & Tetanus was ≥0.1 IU/ml, for Hib was ≥0.15 μg/ml for short term protection and ≥1 μg/ml for long term protection. For Pertussis, no serological correlate of protection is recognized. The range of testing with upper and lower limits of detection with the use of ELISA kits have been indicated below:

For Diphtheria, range of testing is 0.01–1 IU/ml with limit of detection as 0.01 IU/ml. Whereas, for Tetanus, range of testing is 0.01–5 IU/ml with limit of detection as 0.01 IU/ml. Pertussis IgG, can be quantified in the range of 1–150 U/ml with limit of detection as 1 U/ml. In case of Pertussis PT range of testing is 0.01–200 IU/ml with limit of detection as 0.01 IU/ml. Range of testing for HIB 0.1–10 μg/ml with limit of detection as 0.1 μg/ml. While, Hepatitis can be analyzed from 10 to 1000 mIU/ml and detection limit is 10 mIU/ml. 5 calibrators have been used for all antigens except for Pertussis IgG in which 4 Calibrators have been used.

Neutralizing antibodies against all polio virus types were quantified using seroneutralization assay; the results were computed as the reciprocal of dilution. Defined correlates of seroprotection [35,36] have been established for diphtheria, tetanus, poliovirus, Hib, and Hep B (Table 2). Seroconversion against polio is defined as change in post-vaccination concentration of detectable poliovirus type-specific antibodies from baseline (≥8 reciprocal titer). In absence of universally acceptable correlate of seroprotection for pertussis, seroconversion rate was predefined as a ≥4-fold increase in post vaccination antibody level, in subjects that were initially seropositive for pertussis antigen. In case of subjects that were seronegative to begin with, the response was evaluated as per instruction manual of the Kit (Pertussis IgG ≥ 18 U/ml; anti-PT ≥ 100 μg/ml). Proportion of subjects achieving seroprotection and, antibody geometric mean titers (GMTs) against all six vaccine antigens were evaluated.

2.4. Safety assessment

Subjects were monitored for minimum of 30 min to identify and resolve any adverse response to vaccination. Parents or LAR recorded adverse events (AEs) during four days following immunization (solicited; local and systemic) and incidents occurring till 4 weeks post-vaccination (unsolicited) in the provided diary card. Vital examination (axillary temperature, heart rate and respiration) and clinical examination were done on each visit, follow up visit or at any time during the study, if necessary.

Solicited (local and systemic) and unsolicited AEs (Table 4) were graded as mild (grade 1), moderate (grade 2) or severe (grade 3) based on intensity. Occurrence of serious adverse events (SAEs) and unexpected AEs that required medical intervention were promptly informed to the investigators by parents/LARs; investigators were further reported to the licensing authority, sponsor and the ethics committee within twenty four hours of their occurrence.

2.5. Statistical analysis

The prime objective of the study was to validate the non-inferiority of the hexavalent test vaccine, EasySix™ to the comparator vaccines (Pentavac SD® and Imovax Polio®) with respect to immunogenicity, reactogenicity and safety, 4 weeks after the conclusion of 3-dose vaccination schedule. Descriptive statistics (mean, standard deviation and range) were used to evaluate demographic characteristics such as age, weight, and length; count and percentages represented discrete variable like gender. Proportion of subjects achieving seroprotection (seroprotection rate; SPR), difference in the SPRs between treatment groups, pre- and post-immunization antibody titers represented as geometric mean titers (GMTs) were calculated and compared using (*t*-test or Fischer's exact test). Asymptotic standardized 95% confidence intervals (CIs) were calculated for the difference in SPRs and antibody GMTs. A clinically acceptable difference of <10% between the 95% CI (upper limits) of the SPRs was considered to establish non-inferiority of EasySix™ vis-à-vis the comparator vaccines.

Assuming that the test vaccine has the same efficacy as the pentavalent comparator vaccine, a minimal sample size of 128 participants each group (a total of 256 subjects) were determined essential to corroborate non-inferiority of EasySix™ with 90% power and alpha set at 0.05. Considering an attrition rate of approximately 10%, 284 eligible infants (142 subjects each group) were recruited.

Immunogenicity of the vaccine was assessed on the per-protocol cohort that included all participants who completed the study and provided samples for serological analysis. Safety assessment was performed on every subject who received at least one dose of any vaccine (intent-to-treat cohort). Statistical investigations were done with SAS® software version 9.1 (SAS Institute, Cary, NC). P-values calculated on two-tailed tests with values $\leq$0.05 were deemed statistically significant.

3. Results

284. infants in good health, 6–10 weeks old, were enlisted and randomized at a 1:1 ratio (142 subjects per group) to receive three injections of either the test (EasySix™) or comparator vaccine Pentavac SD® co-administered with Imovax Polio®, at an interval of 4 weeks (Fig. 1). Subject recruitment commenced in April 2015 and the trial ended successfully in October 2015. Of these, 276 subjects (138 in each group) received minimum one dose of either vaccine and were selected for safety assessment. In the end 272 subjects (136 subjects in each group) got all three vaccine doses and were assigned in the per protocol immunogenicity cohort. Twelve recipients discontinued before the trial culmination either due to loss to follow-up after 1st (n = 8; 4 subjects per group), 2nd (n = 2 in Pentavac-SD®-Imovax Polio® group) or 3rd (n = 1 in EasySix™ group) visit or for non-compliance (n = 1, EasySix™ group) (Fig. 1). No participant withdrew due to adverse events. No clinically important distinction between groups, concerning the demographic/baseline characteristics (age, gender, weight and length), was observed (Table 1).

3.1. Immunogenicity

Seroprotection rates (SPR)/seroresponsiveness (Table 2) as well as the antibody Geometric mean titers (GMTs) (Table 3) were estimated against all antigens in both treatment groups, one month after administration of the last vaccine dose. Subsequent to vaccination, recipients in both the groups presented good and comparable responses.

The SPRs for tetanus, Hib (PRP-T), diphtheria, polio and hepatitis B in both immunogenicity cohorts were high (>85%) (Table 2). 100% subjects in the EasySix™ and Pentavac SD®-Imovax Polio® group achieved seroprotection against tetanus toxoid (anti-tetanus toxoid antibodies $\geq$0.1 IU/ml) and PRP-T (anti-PRP antibody titer of $\geq$0.15 μg/ml) ($p > 0.05$). Post-immunization long-term PRP SPR ($\geq$1.0 μg/ml) for EasySix™ and Pentavac SD®-Imovax Polio® were 92.65% and 88.97%, respectively ($p = 0.294$). With a difference in response of −3.68 and the upper limit of 95% CI (−10.529, 3.176) lower than the pre-defined 10% non-inferiority (NI) margin, EasySix™ was found comparable to Pentavac SD®-Imovax Polio® group for Hib immune response (Table 2).

Seroprotection against diphtheria (anti-diphtheria antibody titer $\geq$0.1 IU/ml) was reported in 94.85% subjects in EasySix™ and 95.59% in comparator group ($p = 0.776$) with a treatment difference of 0.74 (95%CI, −4.3 to 5.8). As the upper 95% CI limit for the treatment difference was within the NI margin of 10, EasySix™ was considered non-inferior to Pentavac-SD®-Imovax Polio® with respect to anti-diphtheria antibody (Table 2).

In EasySix™ group, percentage seroprotection for Hepatitis B (anti-HBsAg antibody $\geq$10 mIU/ml) was 97.79% as compared to 97.06% in the Pentavac SD®-Imovax Polio® group ($p = 0.701$). The difference in response of −0.73 and the upper limit of the 95% CI (−4.49, 3.02) lower than the pre-specified 10% NI margin, demonstrated that EasySix™ was non-inferior to licensed vaccines in terms of protection against Hepatitis B (Table 2).

Antibody titers against all poliovirus types was quantitated using standardized neutralization assay. A change from non-detectable (<8 reciprocal titer) to detectable ($\geq$8 reciprocal titer) poliovirus type specific antibodies defined seroconversion. The seroconversion rate against polio type 1, 2 and 3 was 89.71%, 93.38% and 88.24% in the EasySix™ group as compared to 91.91%, 94.12% and 90.44% in the Pentavac SD®-Imovax Polio® group, respectively ($p = 0.528$, 0.802, 0.555); the difference in response between the groups 2.2 (95%CI, −4.65 to 9.06), 0.74 (95%CI, −5.01 to 6.48), 2.2 (95%CI, −5.12 to 9.53). Since the upper 95% CI limit was within the pre-specified NI margin, EasySix™ met the non-inferiority criteria for all poliovirus types (Table 2). SPR against pertussis antigens were comparable between both the groups. 75.74% subjects in EasySix™ as compared to 72.79% in comparator group ($p = 0.578$) developed seroprotective pertussis IgG antibody titers. Similarly, anti-PT antibody titers above the specified threshold were observed in 68.38% in the EasySix™ and 66.18% in the Pentavac SD®-Imovax Polio® arm ($p = 0.698$). The seroresponse of EasySix™ against pertussis was non-inferior to the comparator vaccines with a difference in response of −2.95 (95%CI, −13.32 to 7.44) for pertussis IgG and −2.2 (95%CI, −13.55 to 8.94) for anti-PT and the upper 95% CI limit lower than the previously defined 10% NI margin (Table 2).

Baseline and post-vaccination GMTs for all antigens in both vaccine groups were analyzed. Both vaccine groups had similar GMTs for all components (Table 3).

Reverse cumulative distribution curves (RCDCs), that facilitate visual inspection of variability and central tendencies of the antibody data, were plotted for post-vaccination antibody level against tetanus toxoid (Fig. S1), diphtheria (Fig. S2), Hep B (Fig. S3), Hib (Fig. S4), pertussis IgG (Fig. S5), PT (Fig. S6), and poliovirus types 1, 2 and 3 (Figs. S7–9); the distributions were comparable.

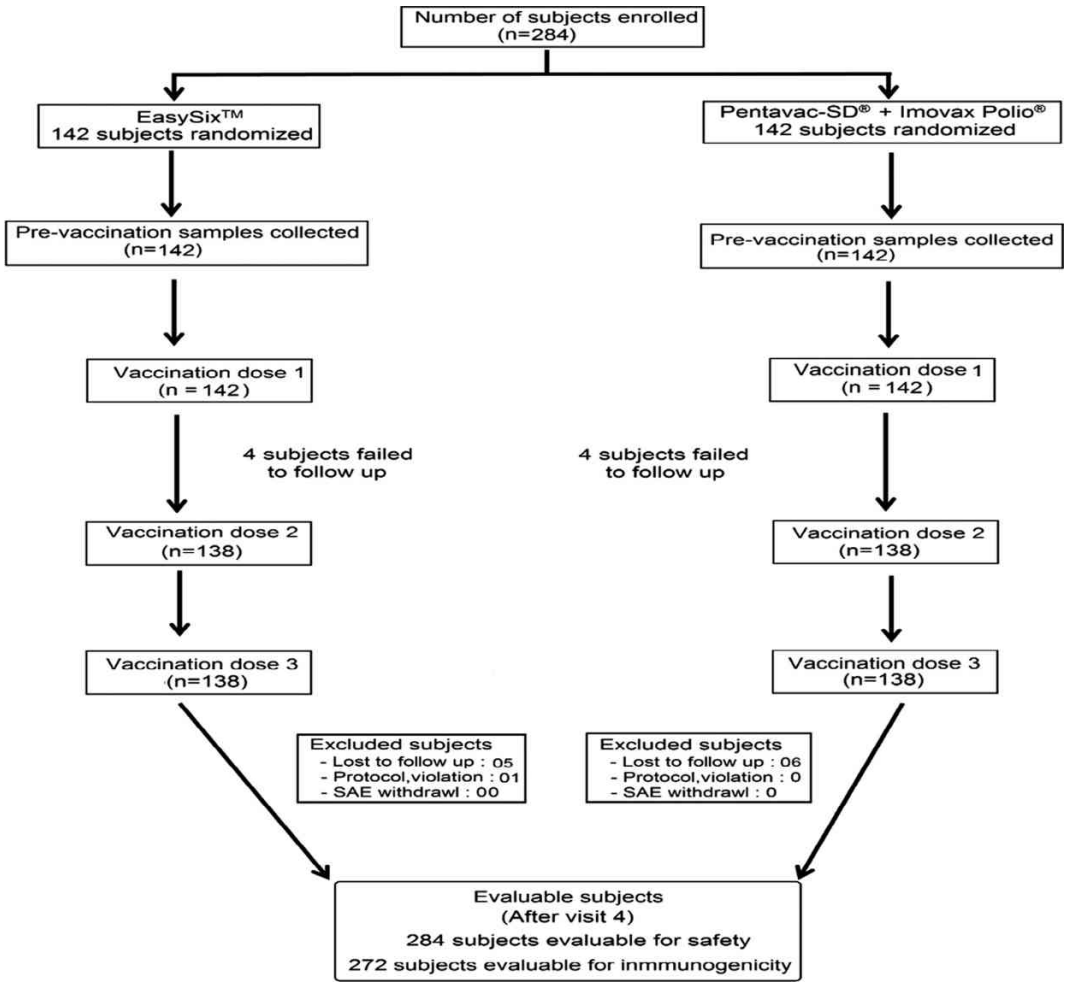

Fig. 1. Subject allocation and participation in the completion of the trial. Flow chart explaining subject participation (n = number of subject) and exclusions during different phases of the study.

Table 1
Summary of demographics and baseline characteristics of the study subjects.

Parameters (at vaccination)		Easysix™ (n = 142)	Pentavac SD® co administered with Imovax Polio® (n = 142)	P-value
Gender	Male (%)	65 (45.8%)	80 (56.3%)	0.075
	Female (%)	77 (54.2%)	62 (43.7%)	
Age (days)	Mean ± SD	48.8 ± 6.2	50.3 ± 7.7	0.084
Weight (kg)	Mean ± SD	4.2 ± 0.6	4.3 ± 0.6	0.120
Length (cm)	Mean ± SD	54.9 ± 2.9	55.6 ± 3.1	0.046

Overall, the results established non-inferiority of EasySix™ over licensed vaccines (Pentavac SD® and Imovax Polio®) with respect to immunogenicity against all targeted antigens (Table 2).

3.2. Safety

284. subjects (142 subjects each in the EasySix™ and Pentavac SD®-Imovax Polio® group) who were administered minimum of one vaccine dose were incorporated for safety analyses. During the trial, frequencies of total adverse events (AEs) were comparable between the groups (70.42% and 74.64% in EasySix™ and Pentavac SD®/Imovax Polio® groups, respectively). Overall, 203 subjects reported solicited AEs whereas 3 subjects reported unsolicited AEs in the total vaccinated cohort. No subject was removed from the study owing to an AE (Table 4).

All solicited reactions (local and systemic) were documented from immunization up to 4 days (Table 4). Fewer number of subjects reported solicited local reactions in the EasySix™ group (99/142, 55.63%) when compared to Pentavac SD®- Imovax Polio® (84/142, 59.15%); pain/tenderness at the injection site being the most common, followed by erythema and swelling (Table 4). Frequency of subjects reporting any solicited systemic AE was similar amongst EasySix™ (57.04%) and comparator group (Table 4). Commonly reported systemic events included fever, irritability, drowsiness and vomiting (Table 4). Majorly the subjects experienced mild to moderate solicited AEs that resolved within 4 days.

Three unsolicited AEs were reported in the 4 week extended follow up interval subsequent to each immunization. Two instances of nasopharyngitis and one incident each of pharyngitis and pyrexia were reported in Pentavac SD®-Imovax Polio® group. All unsolicited AEs resolved completely, following treatment. One non-fatal

Table 2
Seroprotection/Seroconversion rates (SPR) 4 weeks post-vaccination.

Antibody	Threshold[a]	% Seroprotection/Seroconversio (95% CI)		Difference in SPR[c]% (95% CI)	P-value
		EasySix™ (N = 136)	Pentavac SD®co-administered with Imovax Polio® (N = 136)		
Anti-Diphtheria	≥0.1 IU/ml	94.85(89.68, 97.91)	95.59(90.64, 98.36)	0.74 (−4.34, 5.805)	0.776
Anti-Tetanus	≥0.1 IU/ml	100 (97.32, 100)	100 (97.32, 100)	0	–
Anti-PRP	≥0.15 μg/ml	100 (97.32, 100)	100 (97.32, 100)	0	–
	≥1 μg/ml	92.65 (86.89, 96.42)	88.97(82.46, 93.69)	−3.68 (−10.5, 3.176)	0.294
Anti-HBs (Hep B)	≥10 mIU/ml	97.79(93.69, 99.54)	97.06(92.64, 99.19)	−0.73 (−4.5, 3.02)	0.702
Anti-PT	≥100 μg/ml and ≥4 fold increase[b]	68.38(59.86, 76.08)	66.18(57.56, 74.06)	−2.2 (−13.3, 8.94)	0.698
Pertussis IgG	≥18 IU/ml and ≥4 fold increase[b]	75.74(67.64, 82.67)	72.79(64.50, 80.07)	−2.95 (−13.32, 7.44)	0.578
Anti-Polio Type 1	≥8 (1/dilution)	89.71(83.33, 94.26)	91.91(85.99, 95.89)	2.2 (−4.65, 9.06)	0.528
Anti-Polio Type 2	≥8 (1/dilution)	93.38(87.81, 96.93)	94.12(88.74, 97.43)	0.74 (−5.01, 6.48)	0.802
Anti-Polio Type 3	≥8 (1/dilution)	88.24(81.60, 93.12)	90.44(84.21, 94.81)	2.2 (−5.12, 9.54)	0.555

All data are represented as percentage of subjects (95% CI), based on per protocol analysis set.
CI = confidence interval, PRP = polyribosyribitol phosphate, Anti-HBs Ag = Hepatitis B surface antigen; PT = pertussis toxin.
[a] Pre-defined correlates of seroprotection/seroconversion.
[b] For subjects that were seropositive for pertussis antibodies in pre-vaccination samples, seroresponsiveness was defined as ≥4 fold increase in antibody titer level over baseline (pre-vaccination). For subjects that were initially seronegative an assay cut-off for ≥100 μg/ml or ≥18 IU/ml was used for anti-PT or pertussis IgG antibodies.
[c] Pentavac SD® co-administered with Imovax Polio® minus EasySix™.

Table 3
Pre and post vaccination Geometric mean titer (GMT)* each vaccine component and 95% CI.

Antibody	GMT (95% CI)			
	EasySix™ (n = 136)		Pentavac SD® coadministered With Imovax Polio® (n = 136)	
	Pre-vaccination	Post vaccination	Pre-vaccination	Post vaccination
Anti-diphtheria (IU/ml)	0.04 (0.03, 0.05)	0.77 (0.62, 0.96)	0.03 (0.02, 0.04)	0.98 (0.78, 1.22)
Anti-tetanus (IU/ml)	1.18 (1.01, 1.38)	1.16 (1.02, 1.31)	1.02 (0.85, 1.23)	1.12 (1.00, 1.25)
Anti-PRP (μg/ml) (≥0.15 μg/ml)	0.81 (0.67, 0.98)	12.33 (9.29, 16.36)	0.69 (0.58, 0.82)	8.97 (6.87, 11.71)
Anti-HBs Ag (mIU/ml)	0.00 (0.00, 0.01)	328.57 (214.01, 504.44)	0.00 (0.00, 0.02)	244.97 (150.53, 398.68)
Anti-PT (μg/ml)	4.65 (3.59, 6.03)	45.97 (36.29, 58.24)	3.14 (2.02, 4.91)	47.33 (35.82,62.55)
Pertussis IgG (IU/ml)	12.21 (10.50, 14.21)	25.64 (23.33, 28.18)	10.31 (8.56, 12.42)	21.69 (19.55, 24.07)
Anti-Polio Type 1 (1/dilution)	1.00 (0.78, 1.29)	155.92 (106.90, 227.44)	0.79 (0.64, 0.97)	221.05 (152.69, 320.01)
Anti-Polio Type 2 (1/dilution)	1.13 (0.87, 1.47)	243.52 (174.07, 340.68)	0.82 (0.66, 1.03)	354.13 (250.05, 501.52
Anti-Polio Type 3 (1/dilution)	1.03 (0.80, 1.33)	197.10 (131.11, 296.31)	0.79 (0.64, 0.99)	260.18 (173.62, 389.91)

Data represent GMT (95% CI) calculated from Intent to treat analysis set.
CI = confidence interval, PRP = polyribosyribitol phosphate, Anti-HBs Ag = Hepatitis B surface antigen; PT = pertussis toxin.
* Comparison between post-vaccination GMT of the treatment groups; for individual vaccine components reflects study data.

Table 4
Summary of different adverse events (AEs) reported during the trial.

	Number and percentage of subjects reporting AEs [n (%)][a]		Total subjects in vaccinated cohort[b] (N = 284)
	EasySix™(N = 142)	Pentavac SD® co-administered with Imovax Polio® (N = 142)	
Total AEs reported	100 (70.42)	106 (74.64)	206 (72.53)
Unsolicited AEs	1 (0.70)	2 (1.40)	3 (1.05)
Solicited AEs	99 (69.71)	104 (73.23)	203 (71.47)
Solicited local AEs	79 (55.63)	84 (59.15)	163 (57.39)
Pain/Tenderness	72 (50.70)	74 (52.11)	146 (51.40)
Redness	27 (19.01)	13 (9.15)	40 (14.08)
Swelling	35 (24.64)	22 (15.49)	57 (20.07)
Solicited systemic AE	81 (57.04)	74 (52.11)	155 (54.57)
Fever	81 (57.04)	74 (52.11)	155 (54.57)
Acute allergic reaction	1 (0.70)	0 (0)	1 (0.35)
Sleepiness/drowsiness	1 (0.70)	2 (1.4)	3 (1.05)
Irritability/restlessness/fussiness	11 (7.74)	11 (7.74)	22 (7.74)
Loss of appetite	0 (0)	2 (1.4)	2 (0.70)
Vomiting	2 (1.4)	1 (0.70)	3 (1.05)
Diarrhea	0 (0)	1 (0.70)	1 (0.35)
Any SAE reported	1 (0.70)	0 (0)	1 (0.35)

[a] Safety assessment data represented as number (n) and% (in parentheses) of subjects reporting adverse events (AEs) during the entire study.
[b] Safety analyses was performed on vaccinated study cohort (Intent to treat analyses set) which consisted of participant who received at least one dose of either vaccine.

Serious Adverse Event (SAE) after third dose of EasySix™ was reported. The subject was diagnosed for pneumonia and completely recovered without sequelae, after treatment. The incidence was deemed unrelated to the vaccination; the participant was not withdrawn from the study.

Overall, the reactogenicity profiles for both vaccines were similar and were found to be safe and well tolerated by the subjects. There was no fatality reported during the trial.

4. Discussion

Major challenges associated with continuous addition of new vaccines in current childhood immunization programs are to lessen the injection count and simplify the immunization plan [1,3,7]. Introduction of combination vaccines targeting multiple diseases in one injection, in vaccination programs has proven to be an efficient strategy in overcoming these challenges leading to their global acceptance [4–6]. However, prior to licensure and inclusion in immunization programs, a combination vaccine has to prove that its reactogenicity and immunogenicity is at par with separate administration of same antigens or a previously licensed combination vaccine. Potential issues that can compromise the efficiency of the combination include antigenic interference, consolidation of different immunization schedule and stability [3,4,37,38].

Addition of vaccines that are also a part of routine immunization, for instance inactivated polio vaccine (IPV), Hep B vaccine and Hib, to the DTP base is being studied extensively [9–20,26,39–44]. In order to reduce the reactogenicity profile of pertussis antigen, whole cell pertussis (wP) part of DTwP vaccine was substituted by an acellular element of pertussis (aP), promoting DTaP based vaccines [3,4,30–32]; however, this transition posed certain setbacks. Lower antibody titers to Hib (PRP-T), due to antigenic interference were reported in DTaP based vaccines; same was not observed with the DTwP/PRP-T combined vaccines [45,46]. A WHO report states that for primary vaccination series, national programmes currently administering wP vaccination should continue administering the same [34]. Accordingly, the Indian Academy of Pediatrics (IAP) also prefers wP vaccines [47].

In this study we have demonstrated that the newly, developed fully liquid hexavalent vaccine EasySix™ is safe, & effective against childhood diseases as accredited comparator vaccines (Pentavac SD® and Imovax Polio®). All subjects assessed for immunogenicity achieved seroprotection against tetanus and Hib (Table 2). The observed seroprotective rates (SPRs) against polio (types 1, 2 and 3), Hepatitis B, Diphtheria and Hib ($\geq$1 μg/ml threshold), in either group was also high (>85%) (Table 2). The vaccine response against Pertussis in both cohorts was also comparable. The upper limit of the CIs for the difference in SPRs for all six antigens met with non-inferiority criteria (<10%) demonstrating non-inferiority of EasySix™ to the comparator vaccines. The frequency and intensity of reported adverse events were also comparable amongst the test and comparator groups (Table 4), highlighting the safety of the EasySix™.

There have been some success with hexavalent combination vaccines in past. Infanrix Hexa® (GlaxoSmithKline), the first DTaP based hexavalent vaccine to be marketed in 2000 as a lyophilized powder-liquid vaccine composite that required reconstitution, was shown to be safe and immunogenic in pediatric population of various countries including India [21,22,24]. Concomitantly another hexavalent liquid formulation, Hexavac™ (Sanofi Pasteur India Pvt. Ltd) was approved for use in European countries [26]; however, it was discontinued in 2005 following reports of reduced serological response to HBV [48]. This was followed by development of vaccine Hexaxim® (also known as Hexyon®) in 2013 with improved immune response against HBsAg. Studies in various countries accredited Hexaxim® as a safe and efficient combination vaccine, recommending its inclusion in primary immunization series of infants [23,25,27–29]. However, all the presently licensed vaccines are DTaP based and no DTwP based vaccine is commercially available yet.

In conclusion, the present study establishes that DTwP based hexavalent vaccine EasySix™ of Panacea Biotec Ltd. is safe, immunogenic and non-inferior to Pentavac SD®-Imovax Polio®, a month subsequent to conclusion of the 3 dose immunization series.

Acknowledgements

The authors acknowledge the following principal investigators and their staff who conducted the study at their respective centers: A. Swamy Naidu (King George Hospital, Vishakhapatnam, India), Ram Dhongade (Sant Dnyaaneshwar Medical Education Research Centre, Pune, India), Venkateswara Rao (Gandhi Medical College & Hospital, Hyderabad, India), S. M. Prasad (Dr. B. R. Ambedkar Medical College, Bengaluru, India). The authors would also like to express their gratitude to the trial participants and their families for cooperating. The authors would also like to recognize the contributions of Clinical data management team member Satyender Kumar.

Author contributions

LM, SS, SP and CP conceived and designed the study. LM, AS, BB, SK and CP contributed to data analyses and interpretation. All authors assisted in the initial preparation of the manuscript. LM, AS, SS and BB composed the final manuscript. AG and DC have helped in the preparation of revised manuscript.

Funding sources

This study was funded by Panacea Biotec Limited.

Conflict of interest

LM, AS, SK, CP, BB, AG, DC and SP are employees of Panacea Biotec. Drs. AS, RD, JVR and SM had no financial interest in the product or the sponsors but obtained funding to execute the study. Conflicts deemed relevant by the editors regarding the manuscript have been disclosed.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2017.09.029.

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