Introduction

With increasing use of the capsular group B meningococcus vaccine 4CMenB (Bexsero, GlaxoSmithKline, Rixensart, Belgium) in infant schedules, co-administration with hexavalent vaccines containing diphtheria, tetanus, acellular pertussis, polio, *Hemophilus influenzae* type and Hepatitis B is becoming increasingly common.[1] While co-administration with one of the hexavalent vaccines for use in Europe, Infanrix Hexa (Hex-IH, GlaxoSmithKline, Rixensart, Belgium) has been extensively studied,[2,3] no data are available on concomitant use of 4CMenB and the hexavalent vaccine, Vaxelis (Hex-V, MCM Vaccines, Leiden, Netherlands). Previous head-to-head studies of these vaccines conducted in the absence of 4CMenB suggested that a primary immunization course of Hex-V was more immunogenic than Hex-IH against *Hemophilus influenzae* type b (Hib), while after a fourth (booster) dose the reverse was true [4–6]

Comparing Hex-IH and Hex-V when used alongside 4CMenB is therefore crucial to inform the design of immunization schedules that allow the flexible use of these vaccines while optimizing their immunogenicity and reactogenicity profiles.[4,5]

These vaccines differ in key aspects, with additional pertussis antigens (fimbriae types 2 and 3) in Hex-V compared with Hex-IH[7] and the use of different carrier proteins for conjugation to the Hib polysaccharide, i.e. tetanus-toxoid for Hex-IH and a meningococcal outer membrane complex (OMPC) for Hex-V. The latter difference is especially relevant to co-administration with 4CMenB as this vaccine also contains meningococcal outer membrane proteins. There is therefore a theoretical risk of carrier induced epitopic suppression of the immune response to the Hib component of Hex-V when given concurrently with 4CMenB. This in turn could potentially lead to a cohort of infants with sub-optimal immune responses to the Hib antigen with a risk of a recurrence of a Hib outbreak similar to that seen in the UK from 1999-2003.[8] Increasing the total dose of meningococcal outer membrane proteins by

concurrent administration of Hex-V with 4CMenB may also lead to an increase in adverse vaccine reactions both locally and systemically compared to Hex-IH.

Accordingly, we conducted a non-inferiority unblinded randomized trial comparing the immunogenicity and reactogenicity at 5 and 13 months of both licensed DTaP-Hib-IPV-HepB vaccines when administered at 2, 3 and 4 months of age alongside the current UK vaccination schedule (including 4CMenB).

Materials and Methods

Study design and participants

In this single center, open label, non-inferiority randomized clinical trial, we recruited healthy infants born at term gestation living in the UK aged between 8 and 13 weeks who had not yet received their primary immunizations. Exclusion criteria were confirmed or suspected immunodeficiency, allergy to any constituents or excipients of the vaccines used in the trial, latex hypersensitivity, contraindications to vaccination as defined by Department of Health guidelines[9] or participation in another interventional clinical trial. Following recruitment, maternal pertussis immunization status was determined by maternal recall or by request to primary care providers.

The study received ethical approval from the South Central – Oxford A Research Ethics Council (reference number: 19/SC/0052) and is registered on the ISRCTN clinical trials register (ISRCTN85819697).

Randomization and masking

Infants were randomly assigned in a 1:1 ratio using computer generated block randomization (random block sizes of 2 and 4), to receive either Hex-IH or Hex-V at 2, 3 and 4 months. Study visits were conducted in participants' homes with randomization occurring at the study center prior to the first study visit by study staff not involved in this visit, who placed the vaccines in a sealed envelope. This was opened by research nurses after parental consent and participant screening, and immediately prior to vaccine administration, thereby maintaining vaccine allocation concealment. Following vaccine administration the trial became open label.

Procedures

Hex-IH is produced as a lyophilized Hib powder and is reconstituted with a solution containing DTaP-IPV-HBV to a total volume of 0.5 ml. Hex-V is produced in a ready to use liquid form of 0.5 ml volume. Both vaccines are administered intramuscularly. The antigen composition of each vaccine is summarized in a supplementary table (Table S1).

Participants also received their other routine vaccinations as per the UK routine childhood immunization schedule: PCV 13 (Prevenar 13, Pfizer, New York, USA), oral rotavirus vaccine (Rotarix, GlaxoSmithKline, Rixensart, Belgium), 4CMenB, Hib-MenC-TT (Menitorix, GlaxoSmithKline, Rixensart, Belgium) and MMR (Priorix, GlaxoSmithKline, Rixensart, Belgium) (Table S2). Vaccination visits occurred at 2, 3, 4 and 12 months of age. Blood (serum) samples were taken at 5 and 13 months of age.

Outcomes

Antigen-specific IgG concentrations were measured by ELISA at the ImmunoAssay Group UKHSA Porton Down laboratory (Salisbury, UK; validated assay methods published previously)[10] using serum samples collected at 5 and 13 months of age for Hib polysaccharide (polyribosylribitol phosphate

[PRP]), pertussis antigens (pertussis toxin, pertactin, filamentous haemagglutinin, fimbriae 2 and 3) and tetanus and diphtheria toxoids. The samples were also analyzed for human complement dependent serum bactericidal antibody (hSBA) against reference strains for three key 4CMenB vaccine antigens: factor H binding protein 1 (fHbp) by 44/76-SL, NadA by 5/99, and OMV (porin A [PorA]) by NZ98/254. These assays, as well as an assay for rabbit complement SBA (rSBA) titers for the recommended MenC reference strain C11 (C:16:P1.7-1,1) were done at the Vaccine Evaluation Unit, UKHSA, Manchester, UK, by means of a previously published methodology.[11,12] Analysis of IgG concentrations against Hepatitis B surface antigen was conducted at the Oxford University Hospitals NHS Foundation Trust Laboratories. Analysis of IgG concentrations against vaccine-serotype pneumococcal capsule antigens was conducted at the University College London laboratory.[13]

Participants' parents or legal guardians were asked to keep an electronic diary or paper diary card of reactions (both solicited and unsolicited) after each vaccination visit (at 2, 3, 4 and 12 months). This included measuring a temperature at 6 hours after vaccination or before the participant settled for their night-time sleep (whichever was earliest) and then daily for the next 5 days. Solicited events included local (erythema, induration, swelling and tenderness at the vaccination site) and systemic reactions (change in feeding, drowsiness, vomiting, diarrhea and irritability/fussiness). Severity of reactions were categorized as mild, moderate, and severe as outlined in the study protocol (supplementary file). Unsolicited adverse events in days 0 to 5 following vaccination were also recorded. Serious adverse events were recorded for the duration of the study.

Statistical analysis

The original sample size planned to give 85% power at a two-sided 5% significance level, incorporating a 10% attrition rate and further allowances for protocol violations and unexpected dropouts, was 240 (n=120 in each arm). Disruptions to clinical activities due to the COVID-19 pandemic from March 2020

led to a re-evaluation of the study size. Recruitment was stopped at 194 participants, of which 172 participants had blood samples available for primary endpoint evaluation in the mITT analysis. To retain a study power of 85%, the type I error was increased from two-sided 5% (one-sided 2.5%) to one-sided 5%.

Immune responses at 5 and 13 months of age are summarized as medians and interquartile ranges (IQRs), and geometric means (GMs) with 95% CIs for log-transformed data. Geometric mean ratios (GMRs; Hex-V/Hex-IH) with 95% CIs are presented to compare the GMs of each antigen between the arms; one-sided 95% CI for the primary outcome and two-sided 95% CIs for all secondary outcomes. GMRs produced for pertussis antigens were adjusted for receipt of pertussis vaccination during pregnancy by fitting linear regression models with both vaccine and maternal pertussis vaccination (yes/no) terms. Non-inferiority was claimed if the lower bound of the one-sided 95% CI of the GMR was >0.5 for the 5-month Hib antibody response. Where non-inferiority was confirmed, superiority of Hex-V over Hex-IH was tested using a Student's t-test and presented with a two-sided 95% CI.

Although the study was not powered based on specific thresholds of anti-PRP IgG concentrations, the difference between arms was reported with a one-sided 95% Yates' continuity corrected CI, and a non-inferiority margin of ≥-10%. Difference in proportions between arms and accompanying two-sided 95% CIs were presented for secondary outcomes for pathogens with accepted correlates of protection (Table S3). Values equal to half the lower limit of detection (LLOD) were imputed for immune responses reported as lower than the LLOD. For assays with an upper limit of detection (Hep B), the value of the upper limit was used for results higher than this value.

All safety analyses are descriptive, with solicited adverse reactions presented as frequencies with 95% binomial exact CIs. Safety was evaluated in all participants who received at least one 6-in-1 vaccination.

The primary outcome was assessed in the per-protocol cohort as a sensitivity analysis. The per-protocol cohort consisted of participants who received all three 6-in-1 vaccinations within pre-defined windows, slightly relaxed due to COVID-19 disruptions, and gave a blood sample at the 5-month timepoint, within the relaxed visit window.

Role of the funding source

The funders (MCM Vaccine) had no role in study design, data collection, data analysis, data interpretation, write up of the report or the decision to submit the manuscript for publication. The corresponding author had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

Results

Participants

Between July 2019 and April 2020, 204 infants were randomized to the 6-in-1 study, of whom 194 were eventually enrolled (96 randomized to Hex-V and 98 Hex-IH). (Figure 1). Baseline characteristics in the enrolled participants were similar between the arms: 54% and 51% of the Hex-V and Hex-IH arms, respectively, were female; median age at enrolment was 60 days in both arms; median birth weights were 3.5kg (Hex-V) and 3.4kg (Hex-IH) (Table 1). Antenatal pertussis vaccination was received by 89% of mothers on the trial. There were 4 (2%) withdrawals after enrolment before the primary endpoint. The primary modified intention-to-treat (mITT) cohort consisted of 85 and 89 participants in the Hex-V and Hex-IH arms, respectively; and the per-protocol cohort was comprised of 73 and 75

participants in the Hex-V and Hex-IH arms, respectively. The mITT cohort for analysis at the secondary endpoint, at 13 months of age, consisted of 84 participants from the Hex-IH arm, and 80 from the Hex-V arm. Adequate blood samples were unable to be processed for one Hex-IH participant at the 5-month timepoint, and one Hex-V participant at the 13-month timepoint.

Immunogenicity

Anti-PRP IgG geometric mean concentrations (GMCs) at 5 months of age in the Hex-V arm were 23-times higher than concentrations in the Hex-IH arm, demonstrating non-inferiority of Hex-V compared to Hex-IH (GMR 23.25; one-sided 95% CI 16.21, -) (Table 2). Results were similar within the per-protocol cohort (GMR 24.08; one-sided 95% CI 16.19, -), and superiority of Hex-V over Hex-IH in anti-PRP IgG GMCs at 5 months was also demonstrated ($p<0.0001$). Over 90% of infants in the Hex-V arm had anti-PRP IgG concentrations at the ≥1.0 μg/ml correlate of protection, with a between group difference of 42.3% (95% CI 29.1%-55.5%), meeting the non-inferiority criteria for this threshold (Figure 2, Table S4). More participants had anti-PRP IgG GMCs ≥1.0μg/ml at 5 months of age after vaccination with Hex-V than after receiving Hex-IH (difference of 42.34%; 95% CI 29.15-55.52%). At 13 months of age, GMCs were almost 6-times greater in the Hex-V arm than in the Hex-IH arm (GMR 5.79; 95% CI 3.75-8.94), with 100% of Hex-V recipients achieving anti-PRP IgG concentrations above the 0.15 and 1.0μg/ml correlates of protection. However, there was no statistically significant difference between cohorts in the percentage of participants achieving anti-PRP GMC titers above the correlates of protection.

At 5 months of age, hSBA geometric mean titers (GMTs) against the 5/99 strain of MenB in the Hex-V arm were statistically significantly higher in the Hex-V arm compared to the Hex-IH arm (GMR 1.56; 95% CI 1.13-2.14). The point estimates of hSBA GMTs against the NZ98/254 and 44/76-SL MenB strains were also higher in participants receiving Hex-V *versus* Hex-IV at this timepoint, but this was

not statistically significant. IgG GMCs against pertussis fimbriae were over 63-times higher in the Hex-V arm than in the Hex-IH arm at 5 months (GMR 63.40; 95% CI 49.94-85.63), which remained high at 13 months (GMR 30.27; 95% CI 22.65-40.44). IgG GMCs against diphtheria and pertussis FHA were lower in Hex-V recipients than in those receiving Hex-IH, with upper bounds of the GMR 95% CIs below 1. IgG GMCs against pertussis FHA after Hex-V vaccination remained lower than those reported in the Hex-IH arm at 13 months of age, however GMCs against diphtheria were similar between arms by this timepoint (GMR 1.01; 95% CI 0.75-1.35).

At both 5 and 13 months of age, IgG GMCs against tetanus were statistically significantly higher in participants receiving Hex-V compared to those receiving Hex-IH (5 months: GMR 1.88; 1.50-2.36; 13 months: GMR 2.46; 1.74-3.48). IgG GMTs against MenC were similar between the Hex-V and Hex-IH arms at 5 months, but were statistically significantly higher in the Hex-V arm at 13 months (GMR 1.69; 95% 1.15-2.48). No evidence of a statistical difference in immune response to the 13 pneumococcal strains was observed between Hex-V and Hex-IH arms at either the 5 or 13 month timepoint (Table S4).

Reactogenicity and Safety

No obvious differences in occurrence or severity of solicited adverse reactions between the two 6-in-1 vaccines was apparent. During the five days after the first dose of the study vaccines, the most commonly reported systemic symptoms were irritability/fussiness (81% Hex-V, 77% Hex-IH) and drowsiness (73% Hex-V, 79% Hex-IH) (Table S5). Similar reactogenicity was observed after each study visit at which a 6-in-1 vaccine was administered, with few reports of severe reactions (Figure 3, Figures S1-4). Local reactogenicity was mostly mild in both vaccine arms across all study visits (Figure 3). During the study period, 6/98 (6%) participants in the Hex-IH arm reported SAEs, compared to 8/96 (8%) receiving Hex-V (Table S6). One SAE in the Hex-IH arm was considered a SAR, where the participant was admitted to hospital following their first immunization with a fever of 39°C,

tachycardia and tachypnoea. This was felt to be an expected but uncommon post-vaccination event. No other SAEs were considered related to the study vaccinations.

Discussion

Here we present data from the first immunogenicity and reactogenicity study comparing two hexavalent vaccines administered in infancy alongside 4CMenB. These demonstrate non-inferiority of Hex-V compared to Hex-IH for Hib immunogenicity, with anti-PRP IgG GMCs over 20-fold higher at 5 months after Hex-V than Hex-IH. No increase in reactogenicity was observed, supporting the introduction of Hex-V as an alternative to Hex-IH in the routine childhood immunization schedule of the UK and other countries deploying 4CMenB in infancy.

These data are important given the widespread use in infant schedules of hexavalent (DTaP-IPV-Hib-HepB) vaccines and, increasingly, 4CMenB, which is now licensed in over 40 countries and routinely recommended in ten European countries and South Australia. [1,14,1517] Of particular concern was the possibility of carrier-induced epitopic suppression, in which antibody responses against the target (polysaccharide) antigen of protein-polysaccharide conjugate vaccines are impacted by concomitant administration with vaccines containing the same protein.[16,17] For example, priming with Diphtheria toxoid can suppress responses to Diphtheria-Men A conjugates[17], whereas priming with CRM_{197} does not seem to suppress subsequent antibody responses to Meningitis A conjugate vaccines in which it, or Diphtheria Toxoid, is used. Although this study was not specifically designed to assess the immunogenicity of Hex-V with and without co-administered 4CMenB, the impressive anti-PRP IgG concentrations observed here suggests that the shared meningococcal outer membrane proteins between 4CMenB and Hex-V in no way impaired the immunogenicity of the Hib component in the latter vaccine.

Instead, immunization with Hex-V generated anti-PRP IgG GMC's more than 20-fold higher than Hex-IH after early infant immunization. This is consistent with previous studies comparing these two vaccines without concomitant 4CMenB[4,5], which demonstrated that this difference persisted to 12 months, a timepoint not evaluated in this study. The higher concentration of tetanus toxoid in Hex-IH raises the possibility that the lower anti-PRP IgG concentrations (at 5 and 13 months) and MenC SBA titers (at 13 months) in those immunised with Hex-IH could be due to carrier induced epitopic suppression reducing the immune response to the Hib component of Hex-IH, and to Hib and MenC components of Hib-MenC-TT. This enhanced immunogenicity for Hib may take on particular relevance for the UK owing to the imminent withdrawal from this country's schedule of the Hib-MenC-TT vaccine currently given at 12 months of age, with the potential for an additional dose of DTaP-IPV-Hib-HepB at 18 months of age.[18] Of note is that the previous studies suggest that following administration of the toddler booster dose the course of Hex-IH ultimately generates higher anti-PRP IgG GMC's than Hex-V, and the possibility of heterologous boosting with Hex-IH after Hex-V at 18 months warrants further study.[18]

With regard to 4CMenB immunogenicity, the results show higher bactericidal antibody titers against the 5/99 strain in Hex-V participants compared to the Hex-IH group at 5 months, although all participants in both groups had SBA titers ≥1:4. While the reason for this is unclear, one biologically plausible explanation is a contribution from the meningococcal OMPC in Hex-V to this immune response. No convincing additional immunogenicity was seen for the other MenB strains, however.

It is notable that anti tetanus-toxoid IgG concentrations were significantly higher in Hex-V recipients than Hex-IH at both the 5- and 13-month time points, despite the higher overall tetanus-toxoid content in the latter vaccine. Given the excellent control of tetanus achieved in countries deploying either Hex-IH or Hex-V[19,20] this difference is unlikely to be clinically significant. The differences in antibody concentrations against pertussis antigens observed are expected given a

comparison between a vaccine containing three pertussis antigens (Hex-IH) and five antigens (Hex-V), however a World Health Organization review of acellular pertussis vaccines found no convincing evidence of a difference in the effectiveness of 3 versus 5 component vaccines.[21]

There were a number of limitations to our study. Recruitment to our study was affected by the COVID-19 pandemic, however the robust evidence of non-inferiority demonstrated suggests that this did not affect the study integrity. A further result of the COVID-19 pandemic was that we were also unable to obtain results for Poliovirus neutralizing antibodies, as initially planned in the study protocol owing to laboratory constraints. An additional potential limitation was randomization occurring prior to formal enrolment in the study due to study visits being conducted in participant's homes. While this created a potential recruiting bias, this was minimized as both participants and study staff conducting the visit were unaware of group allocation until immediately prior to administration of the study intervention.

In conclusion, our study has shown that with regards to Hib immunogenicity, Hex-V is non-inferior to Hex-IH. Additionally, Hex-V is safe and well-tolerated and is therefore a potential candidate vaccine for use in the increasing number of countries deploying 4CMenB in their infant immunization schedule.

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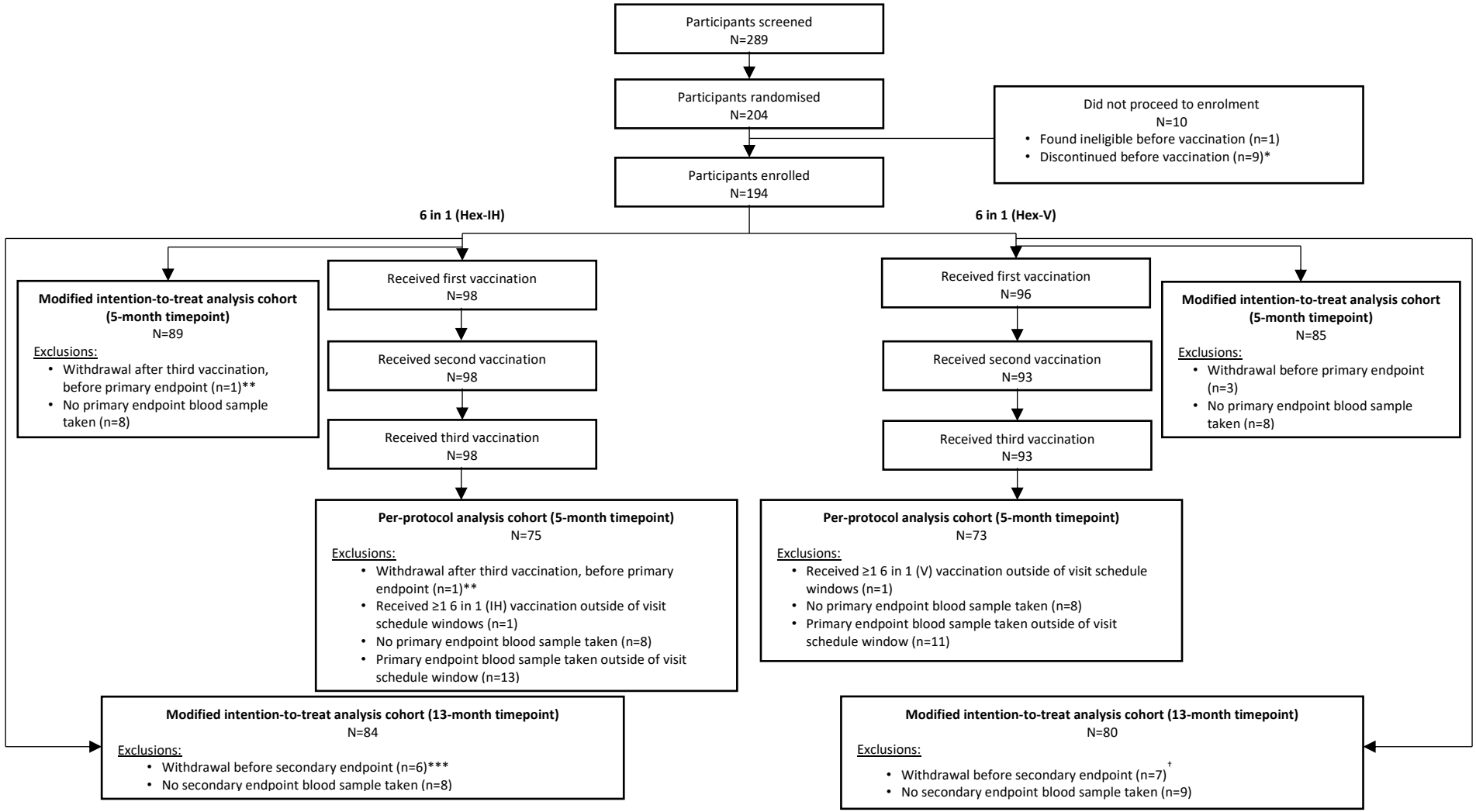
Figure 1 – CONSORT



Note that randomization took place prior to enrolment, with enrolment to the trial defined as infants receiving at least one dose of the study vaccinations.

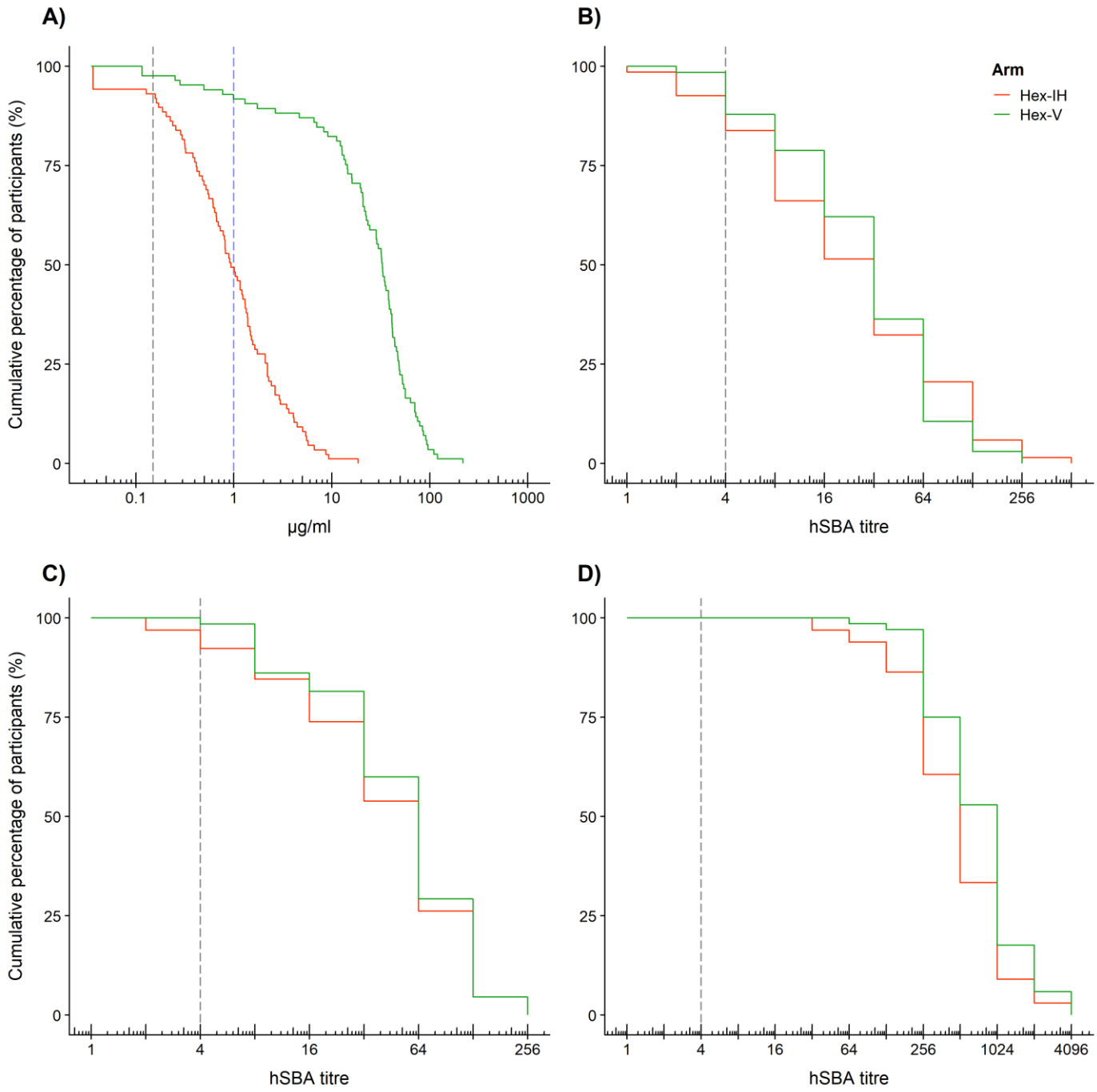
*Reasons for discontinuation included parents changing their minds, parents cancelling enrolment visits and unable to arrange a further suitable date, and participant already having first dose of vaccine. Neither team nor parents were aware of allocation to randomization arm before their decision not to proceed with enrolment. N=4 participants were randomized to the Hex-IH arm, and n=6 to the Hex-V arm.

**Refers to the same participant

***Refers to all withdrawals in the Hex-IH arm before the secondary endpoint. Withdrawal reasons are: withdrawal of consent (n=2), moved out of area (n=3), and parent not wanting infant to undergo blood test (n=1).

†Refers to all withdrawals in the Hex-V arm before the secondary endpoint. Withdrawal reasons are: withdrawal of consent (n=4), moved out of area (n=3).

Figure 2 – Reverse cumulative distribution curves of anti-PRP concentrations and hSBA titers at 5 months of age



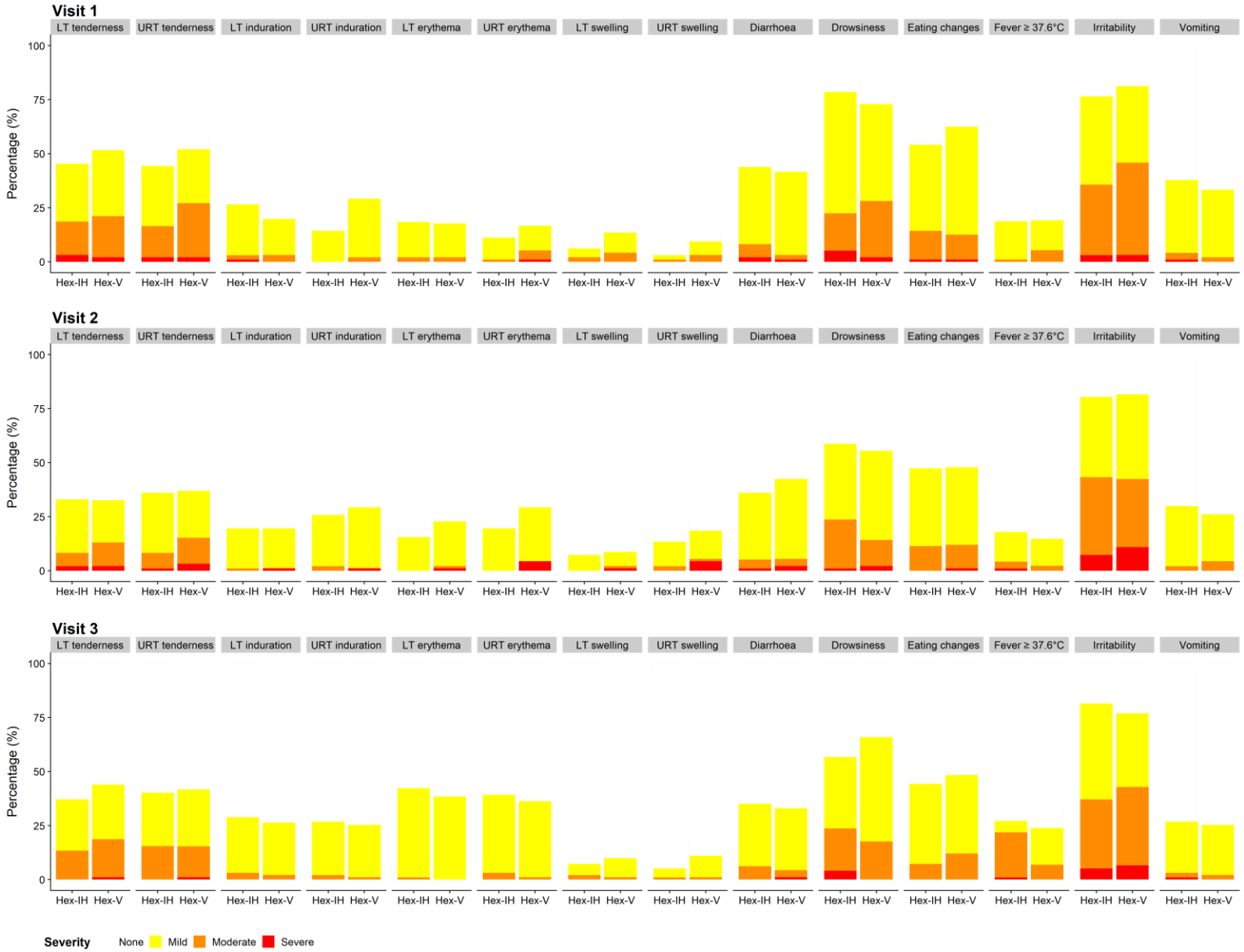
0) Hib. Dashed black line shows 0.15 µg/ml threshold, dashed blue line shows 1.0 µg/ml threshold.

B) MenB NZ98 254. Dashed black line shows 4 hSBA titer threshold.

C) MenB 44/76. Dashed black line shows 4 hSBA titer threshold.

D) MenB 5/99. Dashed black line shows 4 hSBA titer threshold.

Figure 3 – Maximum severity of solicited local and systemic adverse events over days 0-5 following vaccinations with Hex-V or Hex-IH



6 in 1 vaccine was given in the upper right thigh (URT) at all visits. MenB vaccine was given in the left anterolateral thigh (LT) at visits 1 and 3, and PCV13 vaccine was given in the LT at visit 2.

Table 1 – Baseline demographics of the enrolled participants

	Hex-IH	**Hex-V**
Number enrolled	98	96
Sex (female)	50 (51.0%)	52 (54.2%)
Age at enrolment (days), median [IQR]	60.0 [57.2-63.0]	60.0 [57.0-63.0]
Age range (days)	56-94	56-79
Birth weight (kg), mean (SD)	3.4 (0.5)	3.5 (0.5)
Weight range (kg)	2.5-4.5	2.2-4.5
Mother received pertussis vaccine in pregnancy	90 (91.8%)	82 (85.4%)

Table 2 – Immunology results at 5 (primary outcome) and 13 (secondary outcome) months of age, following vaccination with Hex-V or Hex-IH

Antigen	Component/ serotype	5 months			13 months		
		Hex-V: GM (95% CI) [n]	**Hex-IH: GM (95% CI) [n]**	**GMR† (95% CI)**	**Hex-V: GM (95% CI) [n]**	**Hex-IH: GM (95% CI) [n]**	**GMR† (95% CI)**
Hib		20.34 (14.58, 28.37) [n=85]	0.87 (0.66, 1.16) [n=87]	**23.25 (15.11, 35.78)***	88.07 (66.38, 116.85) [n=79]	15.21 (10.89, 21.25) [n=84]	**5.79 (3.75, 8.94)***
MenB	NZ98/254	27.34 (20.83, 35.88) [n=66]	23.09 (16.42, 32.48) [n=68]	1.18 (0.77, 1.82)	23.22 (16.57, 32.53) [n=67]	26.53 (18.32, 38.43) [n=74]	0.88 (0.53, 1.44)
	44/76-SL	48.5 (37.91, 62.05) [n=65]	40.03 (30.01, 53.4) [n=65]	1.21 (0.83, 1.76)	35.8 (28.11, 45.59) [n=68]	39.72 (31.74, 49.71) [n=77]	0.90 (0.65, 1.25)
	5/99	709.47 (575.1, 875.23) [n=68]	456.14 (356.77, 583.19) [n=66]	**1.56 (1.13, 2.14)***	2173.36 (1718.22, 2749.08) [n=70]	1560.29 (1150.23, 2116.53) [n=79]	1.39 (0.95, 2.04)
MenC		2.5 (2.12, 2.93) [n=72]	2.42 (2.03, 2.88) [n=73]	1.03 (0.82, 1.30)	977.15 (772.31, 1236.32) [n=74]	578.03 (425.1, 785.98) [n=80]	**1.69 (1.15, 2.48)***
Diphtheria		0.24 (0.19, 0.29) [n=85]	0.47 (0.39, 0.56) [n=87]	**0.51 (0.39, 0.67)***	0.86 (0.69, 1.08) [n=79]	0.86 (0.7, 1.05) [n=84]	1.01 (0.75, 1.35)
Tetanus		2.81 (2.38, 3.31) [n=85]	1.49 (1.27, 1.75) [n=87]	**1.88 (1.50, 2.36)***	7.83 (6.25, 9.81) [n=79]	3.19 (2.44, 4.17) [n=84]	**2.46 (1.74, 3.48)***
HepB		244.96 (165.52, 362.52) [n=52]	341.41 (263.35, 442.60) [n=53]	0.72 (0.45, 1.14)	75.00 (51.07, 110.14) [n=60]	148.90 (102.07, 217.23) [n=62]	**0.50 (0.30, 0.86)***
Pertussis	Fimbriae	196.85 (160.29, 241.75) [n=83]	3.11 (2.50, 3.87) [n=83]	**63.40 (46.94, 85.63)***	31.40 (25.05, 39.37) [n=79]	1.07 (0.88, 1.29) [n=84]	**30.27 (22.65, 40.44)***
	Pertactin	37.42 (31.10, 45.03) [n=83]	48.54 (40.35, 58.39) [n=85]	0.77 (0.59, 1.00)	8.68 (6.92, 10.89) [n=79]	6.87 (5.49, 8.59) [n=84]	1.28 (0.93, 1.76)
	Pertussis Toxin	54.19 (45.73, 64.21) [n=85]	35.69 (31.17, 40.86) [n=86]	**1.49 (1.20, 1.84)***	8.01 (6.56, 9.78) [n=79]	9.10 (7.55, 10.97) [n=84]	0.88 (0.67, 1.16)
	FHA	31.76 (27.42, 36.78) [n=84]	61.51 (53.91, 70.19) [n=86]	**0.51 (0.42, 0.62)***	5.65 (4.80, 6.64) [n=79]	19.63 (16.56, 23.27) [n=84]	**0.28 (0.22, 0.36)***
Pneumococcus	1	0.42 (0.33, 0.54) [n=62]	0.45 (0.36, 0.58) [n=68]	0.93 (0.66, 1.32)	8.47 (7.01, 10.23) [n=67]	8.55 (6.87, 10.65) [n=69]	0.99 (0.74, 1.32)
	3	0.39 (0.32, 0.47) [n=60]	0.49 (0.39, 0.61) [n=65]	0.80 (0.60, 1.07)	0.97 (0.81, 1.16) [n=65]	0.94 (0.79, 1.11) [n=69]	1.04 (0.81, 1.33)
	4	0.35 (0.27, 0.45) [n=65]	0.39 (0.32, 0.49) [n=70]	0.88 (0.63, 1.23)	3.99 (3.30, 4.81) [n=67]	4.59 (3.79, 5.56) [n=69]	0.87 (0.67, 1.13)
	5	0.22 (0.17, 0.27) [n=60]	0.20 (0.16, 0.25) [n=66]	1.06 (0.76, 1.47)	1.99 (1.70, 2.33) [n=66]	1.89 (1.59, 2.24) [n=69]	1.05 (0.84, 1.33)
	6A	0.11 (0.09, 0.13) [n=60]	0.12 (0.10, 0.14) [n=66]	0.91 (0.74, 1.13)	7.18 (6.02, 8.56) [n=67]	7.88 (6.39, 9.72) [n=69]	0.91 (0.69, 1.20)
	6B	0.09 (0.08, 0.10) [n=69]	0.08 (0.08, 0.09) [n=72]	1.04 (0.92, 1.17)	2.46 (1.93, 3.14) [n=69]	2.93 (2.24, 3.84) [n=72]	0.84 (0.58, 1.20)
	7F	0.65 (0.51, 0.84) [n=60]	0.87 (0.68, 1.12) [n=67]	0.75 (0.53, 1.06)	3.64 (3.07, 4.33) [n=67]	4.02 (3.51, 4.61) [n=69]	0.91 (0.73, 1.13)
	9V	0.18 (0.14, 0.22) [n=62]	0.20 (0.16, 0.25) [n=67]	0.87 (0.65, 1.17)	3.17 (2.59, 3.87) [n=66]	3.71 (3.14, 4.38) [n=69]	0.85 (0.66, 1.11)
	14	1.04 (0.80, 1.35) [n=59]	1.09 (0.84, 1.40) [n=66]	0.95 (0.66, 1.37)	14.61 (11.38, 18.78) [n=66]	15.20 (12.20, 18.93) [n=69]	0.96 (0.69, 1.34)
	18C	0.18 (0.14, 0.22) [n=60]	0.24 (0.19, 0.30) [n=66]	0.74 (0.54, 1.01)	1.88 (1.57, 2.25) [n=67]	2.06 (1.77, 2.41) [n=69]	0.91 (0.72, 1.15)
	19A	0.36 (0.29, 0.46) [n=68]	0.41 (0.34, 0.50) [n=72]	0.88 (0.65, 1.18)	10.05 (8.20, 12.31) [n=68]	10.88 (9.10, 13.02) [n=72]	0.92 (0.71, 1.21)
	19F	0.56 (0.46, 0.68) [n=60]	0.58 (0.47, 0.71) [n=66]	0.97 (0.73, 1.28)	15.98 (12.73, 20.06) [n=67]	17.92 (15.06, 21.32) [n=67]	0.89 (0.67, 1.18)
	23F	0.09 (0.08, 0.10) [n=65]	0.08 (0.07, 0.09) [n=70]	1.14 (0.98, 1.33)	2.00 (1.63, 2.45) [n=67]	2.01 (1.65, 2.44) [n=69]	1.00 (0.75, 1.32)

†GMRs presented for pertussis are adjusted for maternal pertussis vaccination received during pregnancy (yes/no)

*Statistically significant

Supplementary Tables

Table S1 – Components of Hex-IH and Hex-V

Hex-IH			Hex-V		
Component	**Dose**	**Additional information**	**Component**	**Dose**	**Additional Information**
Diphtheria toxoid	Not less than 20 international units (IU)	Absorbed onto aluminum hydroxide	*Diphtheria toxoid*	Not less than 20 international units (IU)	Absorbed onto aluminum phosphate
Tetanus toxoid	Not less than 40 international units (IU)		*Tetanus toxoid*	Not less than 40 international units (IU)	
Bordetella pertussis antigens			*Bordetella pertussis antigens*		
- Pertussis toxoid (PT)	25 micrograms	Absorbed onto aluminum hydroxide	- Pertussis toxoid (PT)	20 micrograms	Absorbed onto aluminum phosphate
- Filamentous Haemagglutinin (FHA)	25 micrograms		- Filamentous Haemagglutinin (FHA)	20 micrograms	
- Pertactin (PRN)	8 micrograms		- Pertactin (PRN)	3 micrograms	
			- Fimbriae Types 2 and 3 (FIM)	5 micrograms	
Hepatitis B surface antigen	10 micrograms	- Produced in yeast cells (Saccharomyces cerevisiae) by recombinant DNA technology - absorbed on aluminum phosphate ($AlPO_4$)	*Hepatitis B surface antigen*	10 micrograms	- Produced in yeast cells (Saccharomyces cerevisiae) by recombinant DNA technology - Absorbed onto aluminum hydroxyphosphate sulfate
Poliovirus (inactivated) IPV			*Poliovirus (inactivated) IPV*		
- Type 1 (Mahoney strain)	40 D-antigen unit	Propagated in VERO cells	- Type 1 (Mahoney strain)	40 D-antigen unit	Propagated in VERO cells
- Type 2 (MEF-1 strain)	8 D-antigen unit		- Type 2 (MEF-1 strain)	8 D-antigen unit	
- Type 3 (Saukett strain)	32 D-antigen unit		- Type 3 (Saukett strain)	32 D-antigen unit	
Hemophilus influenzae type b polysaccharide .-.conjugated to tetanus toxoid	10 micrograms	Absorbed on aluminum phosphate ($AlPO_4$)	*Hemophilus influenzae type b polysaccharide* - conjugated to meningococcal protein	3 micrograms 50 micrograms	Absorbed onto aluminum hydroxyphosphate sulfate

Table S2 – Study visit schedule

Visit		Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
Age of participant		2 months	3 months	4 months	5 months	12 months	13 months
Visit windows		8 – 13 weeks of age	28-42 days after visit 1	28-42 days after visit 2	28-42 days after visit 3	12 months of age (+28 days)	28-42 days after visit 5
Relaxed visit windows		N/A	21-49 days after visit 1	21-49 days after visit 2	21-49 days after visit 3	350-406 days of age	21-49 days after visit 5
Median of observed visit windows [IQR] (range)*		60 [58-64] (56-94) days of age	30 [28-33] (28-45) days after visit 1	32 [28-35] (26-55) days after visit 2	34 [28-41] (26-118) days after visit 3	372 [370-377] (367-428) days of age	34 [29-40] (26-86) days after visit 5
Group	**Hex-IH**	6 in 1(Hex-IH) 4CMenB Rotavirus	6 in 1(Hex-IH) Rotavirus PCV13	6 in 1(Hex-IH) 4CMenB	Blood sampling	Hib-MenC-TT PCV13 MMR 4CMenB	Blood sampling
	Hex-V	6 in 1(Hex-V) 4CMenB Rotavirus	6 in 1(Hex-V) Rotavirus PCV13	6 in 1(Hex-V) 4CMenB			

*In participants included in the primary mITT analysis.

Table S3 – Correlates of protection used

Vaccine antigen		Assay	Level required for protection†
Hemophilus influenzaee b (Hib)		ELISA (anti-PRP IgG)	≥0.15 µg/ml (short term) ≥1.0 µg/ml (long term)
Diphtheria		ELISA (IgG to toxoid)	≥0.1 IU/ml
Tetanus		ELISA (IgG to toxoid)	≥0.1 IU/ml
Hepatitis B		ELISA (anti-HBs IgG)	≥10 IU/ml
Group B meningococcus (MenB):	44/76-SL 5/99 NZ98/254	SBA (human complement)	≥4 hSBA titer
Group C meningococcus (MenC)		SBA (rabbit complement)	≥8 rSBA titer
Polio		Neutralization	≥1/8 titer
Pneumococcus		ELISA	≥0.35 µg/ml

Table S4 – Proportion of participants achieving prespecified antibody concentrations/titers (defined correlates of protection) at 5 and 13 months of age, following vaccination with Hex-V or Hex-IH

Antigen	Component/ serotype	Threshold	5 months			13 months		
			Hex-V, n (%)	Hex-IH, n (%)	Difference in proportions (95% CI)	Hex-V, n (%)	Hex-IH, n (%)	Difference in proportions (95% CI)
Hib		≥0.15 µg/ml	83 (97.65%)	81 (93.10%)	4.54% (-2.84%, 11.93%)	79 (100.00%)	84 (100.00%)	0.00% (-)
		≥1.0 µg/ml	78 (91.76%)	43 (49.43%)	**42.34% (29.15%, 55.52%)***	79 (100.00%)	81 (96.43%)	3.57% (-1.63%, 8.77%)
MenB	NZ98/254	≥4 hSBA titer	65 (98.48%)	63 (92.65%)	5.84% (-2.52%, 14.20%)	62 (92.54%)	67 (90.54%)	2.00% (-8.59%, 12.59%)
	44/76-SL	≥4 hSBA titer	65 (100.00%)	63 (96.92%)	3.08% (-2.66%, 8.81%)	68 (100.00%)	77 (100.00%)	0.00% (-)
	5/99	≥4 hSBA titer	68 (100.00%)	66 (100.00%)	0.00% (-)	70 (100.00%)	79 (100.00%)	0.00% (-)
MenC		≥8 rSBA titer	6 (8.33%)	4 (5.48%)	2.85% (-6.77%, 12.48%)	74 (100.00%)	78 (97.50%)	2.50% (-2.22%, 7.22%)
Diphtheria		≥0.1 IU/ml	67 (78.82%)	83 (95.40%)	**-16.58% (-27.48%, -5.68%)***	76 (96.20%)	82 (97.62%)	-1.42% (-7.97%, 5.14%)
Tetanus		≥0.1 IU/ml	85 (100.00%)	87 (100.00%)	0.00% (-)	79 (100.00%)	84 (100.00%)	0.00% (-)
HepB		≥10 IU/ml	50 (96.15%)	53 (100.00%)	-3.85% (-10.98%, 3.29%)	55 (91.67%)	58 (93.55%)	-1.88% (-12.81%, 9.05%)
Pneumococcus	1	≥0.35 µg/ml	34 (54.84%)	39 (57.35%)	-2.51% (-21.13%, 16.10%)	67 (100.00%)	69 (100.00%)	0.00% (-)
	3	≥0.35 µg/ml	40 (66.67%)	44 (67.69%)	-1.03% (-18.53%, 16.48%)	62 (95.38%)	63 (91.30%)	4.08% (-5.79%, 13.95%)
	4	≥0.35 µg/ml	32 (49.23%)	44 (62.86%)	-13.63% (-31.72%, 4.47%)	67 (100.00%)	69 (100.00%)	0.00% (-)
	5	≥0.35 µg/ml	19 (31.67%)	16 (24.24%)	7.42% (-9.83%, 24.68%)	66 (100.00%)	69 (100.00%)	0.00% (-)
	6A	≥0.35 µg/ml	3 (5.00%)	3 (4.55%)	0.45% (-7.46%, 8.37%)	67 (100.00%)	69 (100.00%)	0.00% (-)
	6B	≥0.35 µg/ml	2 (2.90%)	0 (0.00%)	2.90% (-2.48%, 8.28%)	68 (98.55%)	70 (97.22%)	1.33% (-4.73%, 7.39%)
	7F	≥0.35 µg/ml	43 (71.67%)	56 (83.58%)	-11.92% (-27.94%, 4.11%)	67 (100.00%)	69 (100.00%)	0.00% (-)
	9V	≥0.35 µg/ml	9 (14.52%)	18 (26.87%)	-12.35% (-27.67%, 2.97%)	64 (96.97%)	69 (100.00%)	-3.03% (-8.65%, 2.59%)
	14	≥0.35 µg/ml	50 (84.75%)	57 (86.36%)	-1.62% (-15.58%, 12.34%)	66 (100.00%)	69 (100.00%)	0.00% (-)
	18C	≥0.35 µg/ml	16 (26.67%)	26 (39.39%)	-12.73% (-30.57%, 5.12%)	65 (97.01%)	69 (100.00%)	-2.99% (-8.53%, 2.56%)
	19A	≥0.35 µg/ml	33 (48.53%)	41 (56.94%)	-8.42% (-26.33%, 9.50%)	68 (100.00%)	72 (100.00%)	0.00% (-)
	19F	≥0.35 µg/ml	45 (75.00%)	46 (69.70%)	5.30% (-11.88%, 22.48%)	67 (100.00%)	67 (100.00%)	0.00% (-)
	23F	≥0.35 µg/ml	2 (3.08%)	1 (1.43%)	1.65% (-4.87%, 8.17%)	66 (98.51%)	67 (97.10%)	1.41% (-4.91%, 7.72%)

Note that pertussis has been omitted from this table due to no defined correlate of protection for this pathogen.

*Statistically significant

Table S5 – Breakdown of maximum severity of solicited systemic adverse events over days 0-5 following each dose of study vaccination

Visit	Symptom	Arm	None	Mild	Moderate	Severe	Any
1	Fever	Hex-IH	78 (81%, 72%-88%)	17 (18%, 11%-27%)	1 (1%, 0%-6%)	0 (0%, 0%-4%)	18 (19%, 12%-28%)
		Hex-V	76 (81%, 71%-88%)	13 (14%, 8%-22%)	5 (5%, 2%-12%)	0 (0%, 0%-4%)	18 (19%, 12%-29%)
	Eating changes	Hex-IH	45 (46%, 36%-56%)	39 (40%, 30%-50%)	13 (13%, 7%-22%)	1 (1%, 0%-6%)	53 (54%, 44%-64%)
		Hex-V	36 (38%, 28%-48%)	48 (50%, 40%-60%)	11 (11%, 6%-20%)	1 (1%, 0%-6%)	60 (62%, 52%-72%)
	Drowsiness	Hex-IH	21 (21%, 14%-31%)	55 (56%, 46%-66%)	17 (17%, 10%-26%)	5 (5%, 2%-12%)	77 (79%, 69%-86%)
		Hex-V	26 (27%, 19%-37%)	43 (45%, 35%-55%)	25 (26%, 18%-36%)	2 (2%, 0%-7%)	70 (73%, 63%-81%)
	Vomiting	Hex-IH	61 (62%, 52%-72%)	33 (34%, 24%-44%)	3 (3%, 1%-9%)	1 (1%, 0%-6%)	37 (38%, 28%-48%)
		Hex-V	64 (67%, 56%-76%)	30 (31%, 22%-42%)	2 (2%, 0%-7%)	0 (0%, 0%-4%)	32 (33%, 24%-44%)
	Diarrhoea	Hex-IH	55 (56%, 46%-66%)	35 (36%, 26%-46%)	6 (6%, 2%-13%)	2 (2%, 0%-7%)	43 (44%, 34%-54%)
		Hex-V	56 (58%, 48%-68%)	37 (39%, 29%-49%)	2 (2%, 0%-7%)	1 (1%, 0%-6%)	40 (42%, 32%-52%)
	Irritability	Hex-IH	23 (23%, 15%-33%)	40 (41%, 31%-51%)	32 (33%, 24%-43%)	3 (3%, 1%-9%)	75 (77%, 67%-85%)
		Hex-V	18 (19%, 12%-28%)	34 (35%, 26%-46%)	41 (43%, 33%-53%)	3 (3%, 1%-9%)	78 (81%, 72%-88%)
2	Fever	Hex-IH	78 (82%, 73%-89%)	13 (14%, 7%-22%)	3 (3%, 1%-9%)	1 (1%, 0%-6%)	17 (18%, 11%-27%)
		Hex-V	75 (85%, 76%-92%)	11 (12%, 6%-21%)	2 (2%, 0%-8%)	0 (0%, 0%-4%)	13 (15%, 8%-24%)
	Eating changes	Hex-IH	51 (53%, 42%-63%)	35 (36%, 27%-46%)	11 (11%, 6%-19%)	0 (0%, 0%-4%)	46 (47%, 37%-58%)
		Hex-V	48 (52%, 42%-63%)	33 (36%, 26%-47%)	10 (11%, 5%-19%)	1 (1%, 0%-6%)	44 (48%, 37%-58%)
	Drowsiness	Hex-IH	40 (41%, 31%-52%)	34 (35%, 26%-45%)	22 (23%, 15%-32%)	1 (1%, 0%-6%)	57 (59%, 48%-69%)
		Hex-V	41 (45%, 34%-55%)	38 (41%, 31%-52%)	11 (12%, 6%-20%)	2 (2%, 0%-8%)	51 (55%, 45%-66%)
	Vomiting	Hex-IH	68 (70%, 60%-79%)	27 (28%, 19%-38%)	2 (2%, 0%-7%)	0 (0%, 0%-4%)	29 (30%, 21%-40%)
		Hex-V	68 (74%, 64%-83%)	20 (22%, 14%-32%)	4 (4%, 1%-11%)	0 (0%, 0%-4%)	24 (26%, 17%-36%)
	Diarrhoea	Hex-IH	62 (64%, 54%-73%)	30 (31%, 22%-41%)	4 (4%, 1%-10%)	1 (1%, 0%-6%)	35 (36%, 27%-46%)
		Hex-V	53 (58%, 47%-68%)	34 (37%, 27%-48%)	3 (3%, 1%-9%)	2 (2%, 0%-8%)	39 (42%, 32%-53%)
	Irritability	Hex-IH	19 (20%, 12%-29%)	36 (37%, 28%-48%)	35 (36%, 27%-46%)	7 (7%, 3%-14%)	78 (80%, 71%-88%)
		Hex-V	17 (18%, 11%-28%)	36 (39%, 29%-50%)	29 (32%, 22%-42%)	10 (11%, 5%-19%)	75 (82%, 72%-89%)
3	Fever	Hex-IH	70 (73%, 63%-81%)	5 (5%, 2%-12%)	20 (21%, 13%-30%)	1 (1%, 0%-6%)	26 (27%, 19%-37%)
		Hex-V	67 (76%, 66%-85%)	15 (17%, 10%-27%)	6 (7%, 3%-14%)	0 (0%, 0%-4%)	21 (24%, 15%-34%)
	Eating changes	Hex-IH	54 (56%, 45%-66%)	36 (37%, 28%-48%)	7 (7%, 3%-14%)	0 (0%, 0%-4%)	43 (44%, 34%-55%)
		Hex-V	47 (52%, 41%-62%)	33 (36%, 26%-47%)	11 (12%, 6%-21%)	0 (0%, 0%-4%)	44 (48%, 38%-59%)
	Drowsiness	Hex-IH	42 (43%, 33%-54%)	32 (33%, 24%-43%)	19 (20%, 12%-29%)	4 (4%, 1%-10%)	55 (57%, 46%-67%)
		Hex-V	31 (34%, 24%-45%)	44 (48%, 38%-59%)	16 (18%, 10%-27%)	0 (0%, 0%-4%)	60 (66%, 55%-76%)
	Vomiting	Hex-IH	71 (73%, 63%-82%)	23 (24%, 16%-33%)	2 (2%, 0%-7%)	1 (1%, 0%-6%)	26 (27%, 18%-37%)
		Hex-V	68 (75%, 65%-83%)	21 (23%, 15%-33%)	2 (2%, 0%-8%)	0 (0%, 0%-4%)	23 (25%, 17%-35%)
	Diarrhoea	Hex-IH	63 (65%, 55%-74%)	28 (29%, 20%-39%)	6 (6%, 2%-13%)	0 (0%, 0%-4%)	34 (35%, 26%-45%)
		Hex-V	61 (67%, 56%-77%)	26 (29%, 20%-39%)	3 (3%, 1%-9%)	1 (1%, 0%-6%)	30 (33%, 23%-44%)
	Irritability	Hex-IH	18 (19%, 11%-28%)	43 (44%, 34%-55%)	31 (32%, 23%-42%)	5 (5%, 2%-12%)	79 (81%, 72%-89%)
		Hex-V	21 (23%, 15%-33%)	31 (34%, 24%-45%)	33 (36%, 26%-47%)	6 (7%, 2%-14%)	70 (77%, 67%-85%)

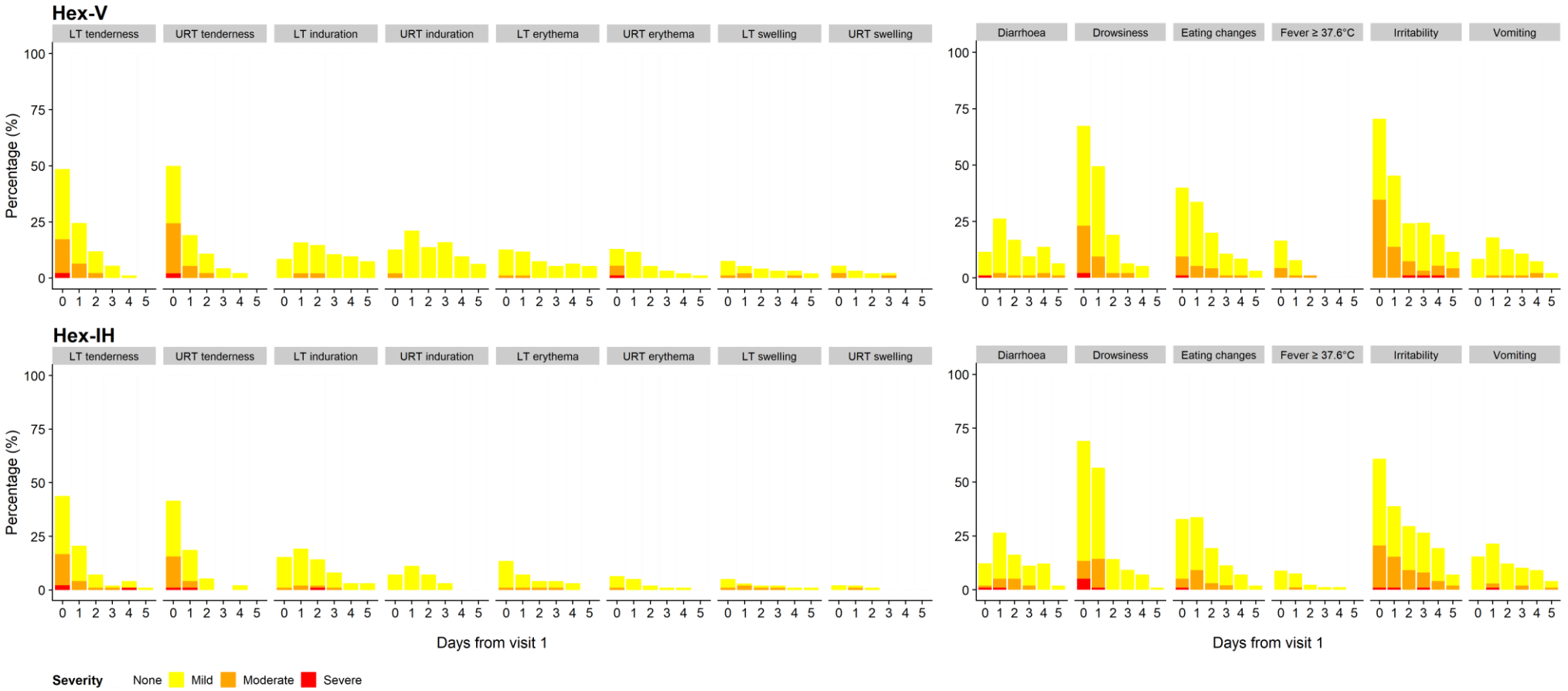
Table S6 – SAE, SAR and SUSAR line listing

ID	Arm	Duration of event (days)	Description	Severity	Related to vaccine administration	Expected (if related)	Classification
1	Hex-IH	3	Bronchiolitis	Moderate	Unrelated		SAE
2	Hex-IH	3	Bronchiolitis	Mild	Unrelated		SAE
2	Hex-IH	1	Bronchiolitis	Mild	Unrelated		SAE
3	Hex-V	5	Urinary tract infection with unilateral ureteric dilatation	Moderate	Unrelated		SAE
4	Hex-IH	2	Viral infection	Moderate	Unrelated		SAE
5	Hex-V	9	Bronchiolitis	Mild	Unrelated		SAE
5	Hex-V	1	Head injury and respiratory tract infection	Moderate	Unrelated		SAE
6	Hex-V	17	Respiratory tract infection	Mild	Unrelated		SAE
7	Hex-IH	1	Post immunization pyrexia	Severe	Related	Yes	SAR
8	Hex-IH	-	Tonsillitis and gastro-esophageal reflux disease	Mild	Unrelated		SAE
9	Hex-V	4	Bronchiolitis	Severe	Unrelated		SAE
10	Hex-V	7	Bronchiolitis	Mild	Unrelated		SAE
11	Hex-V	3	Croup	Moderate	Unrelated		SAE
12	Hex-IH	4	Bronchiolitis	Moderate	Unrelated		SAE
13	Hex-V	-	Bronchiolitis and laryngomalacia	Severe	Unrelated		SAE
14	Hex-V	32	Respiratory tract infection	Mild	Unrelated		SAE

Figure S1 – Maximum severity of solicited local and systemic adverse events over days 0-5 following vaccinations with Hex-V (V) or Hex-IH (IH) (severity scales for induration, erythema, and swelling according to trial protocol)

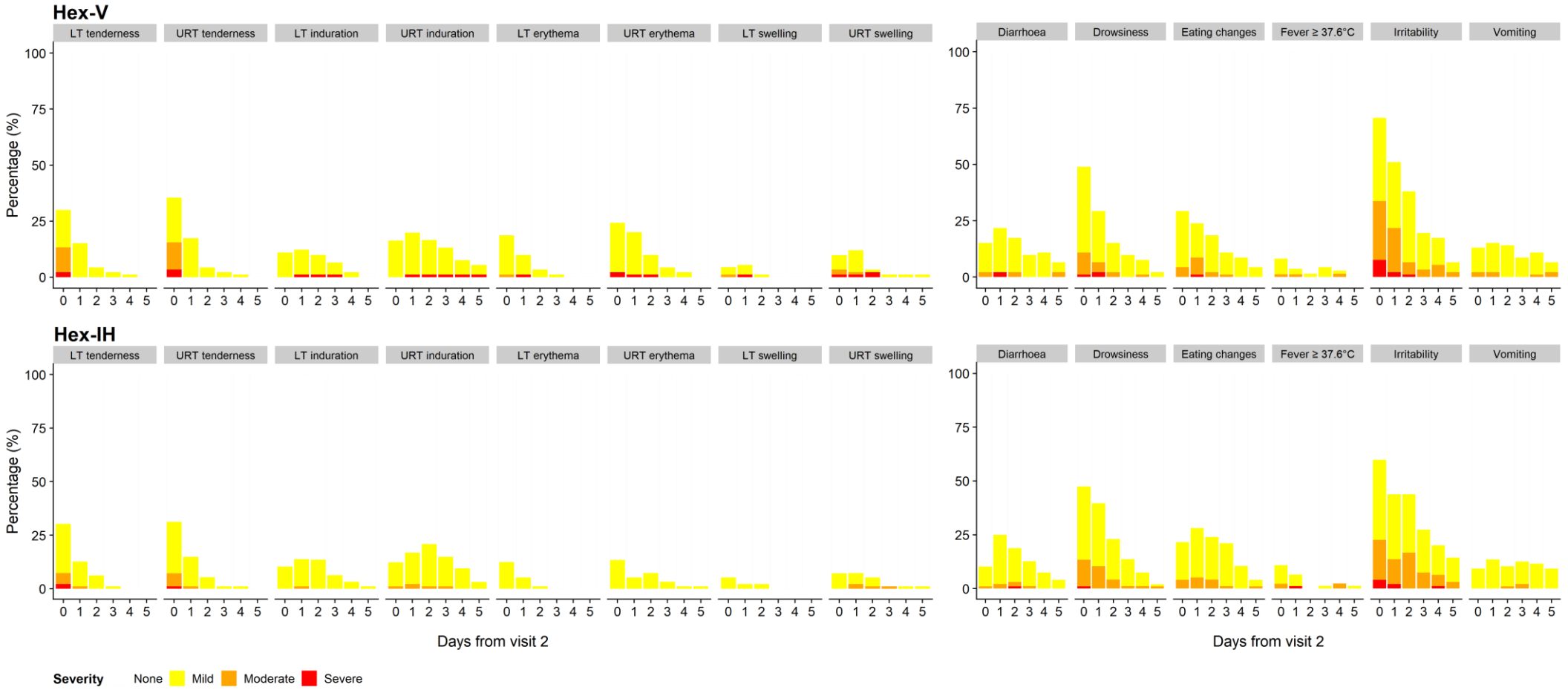
6 in 1 vaccine was given in the upper right thigh (URT) at all visits. MenB vaccine was given in the left anterolateral thigh (LT) at visits 1 and 3. PCV13 vaccine was given in the LT at visit 2.

Figure S2 – Solicited local and systemic adverse events on days 0-5 following first vaccination with Hex-V or Hex-IH



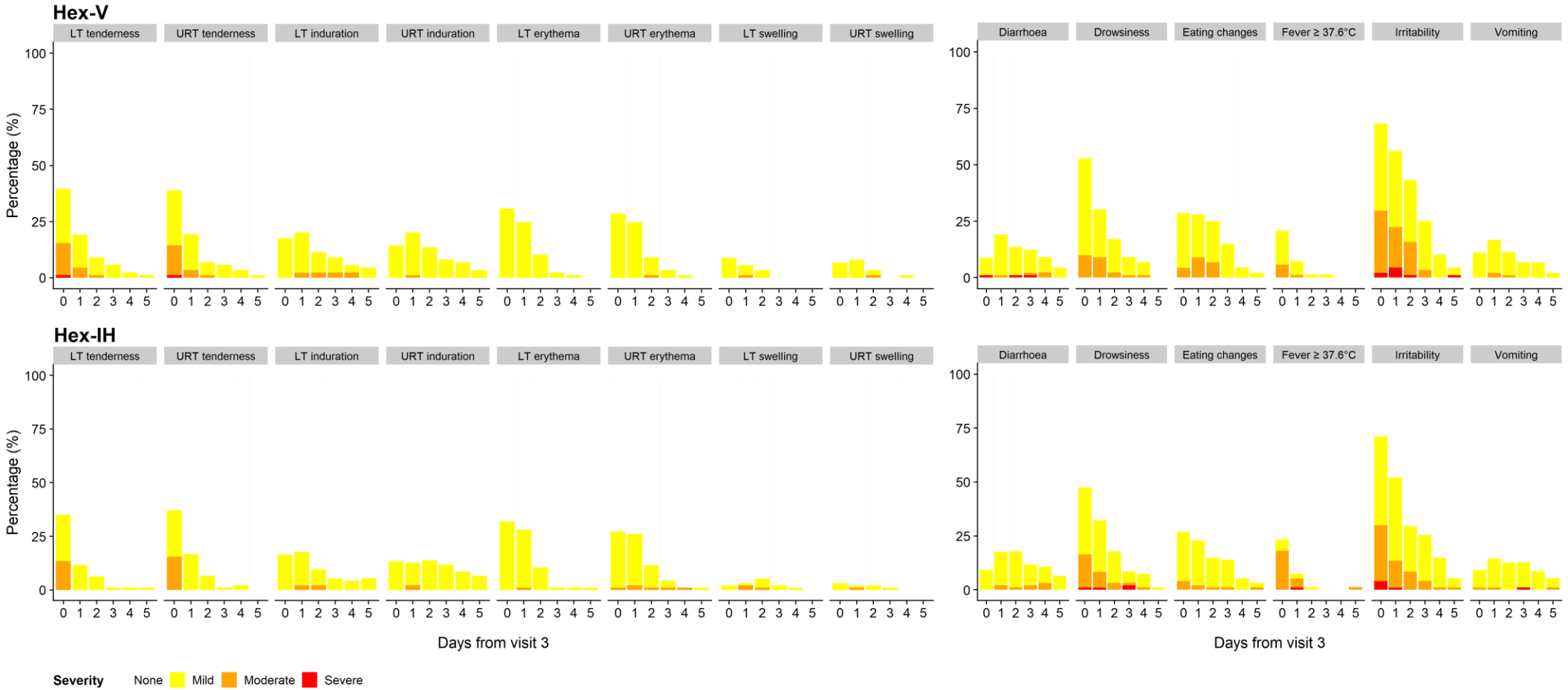
6 in 1 vaccine was given in the upper right thigh (URT) and Men B vaccine was given in the left anterolateral thigh (LT).

Figure S3 – Solicited local and systemic adverse events on days 0-5 following second vaccination with Hex-V or Hex-IH



6 in 1 vaccine was given in the upper right thigh (URT) and PCV13 vaccine was given in the left anterolateral thigh (LT).

Figure S4 – Solicited local and systemic adverse events on days 0-5 following third vaccination with Hex-V or Hex-IH



6 in 1 vaccine was given in the upper right thigh (URT) and Men B vaccine was given in the left anterolateral thigh (LT).