

RESEARCH PAPER



Immunogenicity and safety of a combined DTPa-IPV/Hib vaccine administered as a three-dose primary vaccination course and a booster dose in healthy children in Russia: a phase III, non-randomized, open-label study

Victor Romanenko^a, Irina Osipova^b, Anna Galustyan^c, Michael Scherbakov ^d, Nathalie Baudson^e, Déborah Farhi^f, Luis Anaya^f, Sherine O. Kuriyakose^g, Nadia Meyer^f, and Winnie Janssens ^f

^aChildren's City Hospital №11, Ekaterinburg, Russian Federation; ^bOOO "ASKO-MED-PLUS", Barnaul, Russian Federation; ^cMedical Technologies Ltd, St. Petersburg, Russian Federation; ^dGSK, Moscow, Russian Federation; ^eGSK, Rixensart, Belgium; ^fGSK, Wavre, Belgium; ^gGSK Asia Private Ltd., Bengaluru, India

ABSTRACT

We assessed the immunogenicity and safety of the combined diphtheria-tetanus-acellular pertussis-inactivated poliovirus/*Haemophilus influenzae* type b vaccine (DTPa-IPV/Hib) in children in Russian Federation aiming to support the registration of the vaccine in Russia. In this phase 3, non-randomized, open-label study (NCT02858440), healthy children received three primary doses at 3, 4.5, and 6 months of age (N = 235) and a booster dose at 18 months of age (N = 225). Seroprotection rates against diphtheria, tetanus, Hib, and poliovirus 1–3, seropositivity rates against pertussis antigens, and antibody geometric mean concentrations/titers for all antigens were evaluated one month post-primary and post-booster vaccinations. Solicited local and general adverse events (AEs) were collected during a 4-day period and unsolicited AEs during a 31-day period post-vaccination. Serious AEs were recorded throughout the study. At post-primary vaccination, all infants were seroprotected against diphtheria, tetanus, and poliovirus 1 and 2, 99.3% against poliovirus 3, and 98.4% against Hib. At least 98.9% of participants were seropositive for the three pertussis antigens. At post-booster vaccination, all toddlers were seroprotected/seropositive against all vaccine components. The most frequent local and general solicited AEs were redness, reported for 52.6% and 44.9% of children, and irritability, reported for 64.7% and 39.1% of children, post-primary and post-booster vaccination, respectively. Unsolicited AEs were reported for 20.4% (post-primary) and 5.8% of children (post-booster vaccination). Most AEs were mild or moderate in intensity. Six serious AEs were reported in three (0.4%) children; none were fatal or assessed as vaccination-related. DTPa-IPV/Hib proved immunogenic and well tolerated in the Russian pediatric population.

ARTICLE HISTORY

Received 15 October 2019
Revised 2 January 2020
Accepted 19 January 2020

KEYWORDS

DTPa-IPV/Hib; acellular pertussis; diphtheria; tetanus; poliovirus *Haemophilus influenzae* type b; pediatric population; immunogenicity; safety; reactogenicity

Introduction

Although routine infant vaccination significantly decreased the morbidity and mortality associated with previously common childhood infectious diseases, including diphtheria, tetanus, pertussis, *Haemophilus influenzae* type b (Hib) and poliomyelitis, disease burden remains substantial, affecting populations worldwide.^{1–6} The crowded childhood immunization routine schedules might be a potential deterrent for parents and providers to comply with recommendations and this can result in decreased vaccine coverage, and ultimately, disease outbreak.⁷ Introducing combination vaccines to replace complex immunization schedules has several benefits, such as ease of storage, simplified administration, fewer injections, increased patient and health care acceptance, higher rates of compliance to vaccination schedules, improved coverage rates, reduced shipping and administration costs, reduced confusion over labeling in the medical office, and reduced number of visits.^{8–11}

A pentavalent diphtheria-tetanus-acellular pertussis-inactivated polio and Hib conjugate vaccine (DTPa-IPV/Hib;

Infanrix-IPV/Hib, GSK) has been widely used in several countries across the world since its first license in 1997. The vaccine was shown to be immunogenic, with an acceptable safety profile, when administered as primary and/or booster vaccination according to different schedules.^{12–17} In the Russian Federation, a 3-dose primary vaccination schedule (with doses administered at 3, 4.5, and 6 months of age), and a booster dose at 18 months of age are currently recommended against the following diseases: diphtheria, tetanus, pertussis, poliomyelitis and diseases caused by Hib.^{18,19} DTPa-IPV/Hib combines all antigens in one single formulation and its use can therefore complement the current standard of care for hepatitis B immunization in Russian children.

The aim of the present study was to evaluate the immunogenicity and safety of the combined DTPa-IPV/Hib vaccine when administered as a 3-dose primary vaccination course at 3, 4.5, and 6 months of age and as a booster dose at 18 months of age in healthy children according to the Russian immunization schedule to support the registration of the combination vaccine in this country.

CONTACT Winnie Janssens  winnie.x.janssens@gsk.com  GSK Vaccines, Avenue Fleming 20, Wavre 1300, Belgium

 Supplemental data for this article can be accessed at <https://doi.org/10.1080/21645515.2020.1720437>

© 2020 The Author(s). Published with license by Taylor & Francis Group, LLC.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Methods

Study design and participants

This phase 3, single group, non-randomized, open-label study was conducted in five centers in the Russian Federation between September 2016 and November 2018. Healthy infants, born full-term, aged 3–4 months (90–120 days) at the time of the first vaccination, for whom written informed consent was obtained from their parents/adoptive parents were enrolled in the study. Infants were not eligible if they had received immunosuppressant or immune-modifying drugs or previous DTP, poliovirus or Hib vaccination. A full list of exclusion criteria is provided in the Supplementary text.

Participants received four doses of combined DTPa-IPV/Hib as a 3-dose primary vaccination course at 3, 4.5 and 6 months of age and a booster dose at 18 months of age. At each vaccination, a 0.5 mL dose was administered intramuscularly in the upper side of the thigh.

An internet-based central randomization system was used to allocate treatment numbers by dose and to track enrollment in the study. Laboratory personnel performing sample testing were blinded to the treatment allocation.

In total, three commercial lots of both DTPa-IPV (liquid) and Hib (lyophilized) components were used in the study. Each dose of DTPa-IPV/Hib contained ≥ 30 International Units (IU) diphtheria toxoid, ≥ 40 IU tetanus toxoid, 25 μg pertussis toxoid (PT), 25 μg filamentous hemagglutinin (FHA), 8 μg pertactin (PRN), 40 D-antigen units (DU) inactivated poliovirus type 1, 8 DU inactivated poliovirus type 2, 32 DU inactivated poliovirus type 3, 10 μg purified Hib polyribosyl-ribitol phosphate (PRP) capsular polysaccharide conjugated to tetanus toxoid (~ 25 μg), and 500 μg aluminum hydroxide as adjuvant. Co-administration of a vaccine against hepatitis B virus (HBV) and other vaccines given as part of the national immunization schedule and as part of routine vaccination practice were allowed at any time during the study period.

The study was conducted in compliance with the Declaration of Helsinki, the International Conference on Harmonization Guideline for Good Clinical Practice, and all applicable local regulations. The study protocol and informed consent were reviewed and approved by Independent Ethics Committees/Institutional Review Boards at each center. The trial is registered at <http://www.clinicaltrials.gov> (NCT02858440) and the full protocol is available at <http://www.gsk-clinicalstudyregister.com/study/4677>.

Study objectives

The primary objective was to evaluate the immune responses to the vaccine components in terms of seroprotection rates for diphtheria, tetanus, Hib, and poliovirus serotypes 1–3 antigens, and in terms of seropositivity rates for pertussis antigens in infants one month after the third dose of the primary vaccination.

Secondary objectives were to assess the immune responses to the vaccine components in terms of seroprotection rates for diphtheria, tetanus, Hib, and poliovirus serotypes 1–3

antigens, and in terms of seropositivity rates for pertussis antigens in toddlers one month after the booster vaccination; antibody concentrations or titers against diphtheria, tetanus, Hib, poliovirus types 1–3, and pertussis antigens in children one month after both primary and booster vaccinations; as well as to evaluate vaccine safety and reactogenicity.

Immunogenicity assessments

Blood samples (3.5 mL) were collected one month after the third dose of the primary vaccination and one month after the booster vaccination. Antibodies against diphtheria,²⁰ tetanus,²¹ Hib PRP, and pertussis components^{22,23} were measured using standard in-house enzyme-linked immunosorbent assays. 96-well microplate coated with the corresponding purified antigen were incubated with dilutions of serum samples, controls, and standard. Microplate were washed and mouse horseradish peroxidase (HRP)-conjugated anti-human IgG monoclonal antibodies (diphtheria, tetanus, pertussis) or goat HRP-conjugated anti-human Ig polyclonal antibodies (Hib) were added. Enzyme activity was revealed spectrophotometrically using tetramethylbenzidine. Concentrations were calculated from the reference standard curve using a four parameters logistic fitting algorithm and expressed in IU/mL (diphtheria, tetanus, pertussis) or microgram (μg)/mL. Assay cutoffs (equals to the lower limit of precision and linearity) were 0.057 IU/mL (diphtheria), 0.043 IU/mL (tetanus), 0.066 $\mu\text{g}/\text{mL}$ (anti-PRP), 2.693 IU/mL (PT), 2.046 IU/mL (FHA), and 2.187 IU/mL (PRN). Antibodies against poliovirus 1–3 antigens were measured by a standard in-house neutralizing antibody assay adapted from the WHO Guidelines for WHO/EPI Collaborative Studies on Poliomyelitis.²⁴ All analyses were performed at the Clinical Laboratory Sciences (GSK, Rixensart or Wavre, Belgium) applying validated laboratory tests.

Seroprotection was defined as antibody concentrations ≥ 0.1 IU/mL for diphtheria and tetanus, ≥ 0.15 $\mu\text{g}/\text{mL}$ (indicative of short-term protection) and 1.0 $\mu\text{g}/\text{mL}$ (indicative of long-term protection) for PRP, and antibody titers ≥ 8 ED₅₀ (titers expressed in terms of the reciprocal of the dilution resulting in 50% inhibition; samples with a titer greater than or equal to 1:8 is considered seroprotective) for poliovirus types 1–3.^{25–27} A generally accepted correlate of protection for *Bordetella pertussis* is not yet established since not only PT antibodies play an important role, but also other antibodies, such as FHA and PRN, as well as cellular immune responses seem to contribute to protection.^{26,27} In this study, participants with anti-PT, anti-FHA, and anti-PRN antibody concentrations above the assay cutoffs were considered seropositive.

Antibody geometric mean concentrations (GMCs) and geometric mean titers (GMTs), and seroprotection/seropositivity rates were calculated one month following both primary and booster vaccination.

Safety and reactogenicity assessments

Participants were observed for at least 30 minutes following the administration of the study vaccine for any immediate reactions. Solicited local (injection site pain, redness, swelling)

and general (drowsiness, fever, irritability, loss of appetite) adverse events (AEs) occurring within the 4-day (days 0–3) period and unsolicited AEs occurring within the 31-day (days 0–30) period after each vaccine dose administration were recorded on diary cards by the participants' parents/adoptive parents. All solicited local AEs were considered as related to vaccination. The causality of other AEs was assessed by the investigator. The intensity of all AEs was evaluated on a 3-grade scale from mild to severe. Severe (grade 3) AEs were defined as crying when a limb is moved (for pain), diameter >20 mm (for redness and swelling), axillary temperature >39.0°C (for fever), preventing normal everyday activities (for irritability and drowsiness), and as not eating at all (for loss of appetite). Large injection site reactions (swelling with a diameter >50 mm, noticeable diffuse swelling or noticeable increase of limb circumference) were recorded for up to 4 days (days 0–3) after the booster vaccination. Related and medically-attended AEs were also recorded. Serious AEs (SAEs) were collected during the entire study period. All unsolicited AEs and SAEs were classified using the Medical Dictionary for Regulatory Activities (MedDRA) Primary System Organ Class and Preferred Terms.²⁸

Statistical analyses

A sample size of 200 evaluable infants was requested by the local regulatory authorities; assuming a 15% drop-out rate, a total of approximately 235 infants were to be enrolled in the study.

For each vaccination course (primary and booster), immunogenicity analyses were performed on the according-to-protocol (ATP) cohort for immunogenicity, which included all vaccinated participants who met all eligibility criteria, complied with the protocol, and for whom assay results were available post-vaccination for at least one study vaccine antigen. Seroprotection rates, seropositivity rates, and antibody GMCs and GMTs were calculated with 95% confidence intervals (CIs) one month after the primary and booster vaccinations. GMC/GMT calculations were performed by taking the anti-log of the mean of the log₁₀ concentration/titer transformations. Antibody concentrations/titers below the cutoff of the assay were given an arbitrary value of half the cutoff.

For each vaccination course, safety analyses were performed on the total vaccinated cohort, which included all participants who received at least one dose of the study vaccine. The percentage of infants with at least one solicited (local and general) and unsolicited AE was calculated after each vaccine dose and overall per participant, with exact 95% CIs.

Results

Demographics

In total, 235 children were enrolled and vaccinated with three primary doses; 225 of them received the booster dose (Figure 1). The ATP cohort of the primary and booster vaccination courses included 183 and 190 participants, respectively. Reasons for

exclusion from the ATP cohort, as well as reasons for withdrawal from the study are presented in Figure 1. The mean age was 14.1 weeks at the receipt of the first primary dose and 17.7 months at booster dose. All participants were of European heritage (Table 1). Vaccination against HBV was documented for 162 out of the 235 children, 115 of them received the vaccine at the same time as the study vaccine.

Immunogenicity

One month after the third dose of primary vaccination, all infants had antibody levels above the seroprotective threshold for diphtheria, tetanus, and poliovirus types 1 and 2, and 99.3% of participants (all but one) were seroprotected against poliovirus type 3 (Table 2). Against Hib, 179 (98.4%) participants had anti-PRP antibody concentrations ≥ 0.15 $\mu\text{g/mL}$. At least 98.9% of participants were seropositive for each of the three pertussis antigens (Table 3).

One month after the booster vaccination, all infants had seroprotective antibody levels against diphtheria, tetanus, and poliovirus types 1–3. All participants had anti-PRP antibody concentrations ≥ 1.0 $\mu\text{g/mL}$ and were seropositive for each of the PT, FHA and PRN antigens.

Between primary and booster vaccinations, considerable increases in antibody concentrations and titers were observed for all vaccine antigens (Tables 2, 3).

Safety and reactogenicity

The most commonly reported local AE was redness after both primary (52.6%) and booster (44.9%) vaccinations. Irritability was the most frequent general AE, reported for 64.7% of children following the primary and 39.1% following the booster vaccination; any fever was recorded for 22.8% and 11.6% of children, respectively (Figure 2). The most common local AE of grade 3 intensity was pain, reported by 3 (1.3%) infants after the primary and 4 (1.8%) toddlers after the booster vaccination. Grade 3 irritability was reported by 19 (8.2%) participants following the primary and 11 (4.9%) participants following the booster vaccination. One child experienced grade 3 fever after the booster dose, that was considered vaccination-related (Figure 2).

One child experienced a large swelling reaction the day after the booster vaccination, with a maximum diameter of 80 mm. The swelling resolved within seven days.

During the 30-day post-vaccination period in the primary phase, at least one unsolicited AE was reported for 48 (20.4%) infants; 2 (0.9%) infants experienced an unsolicited AE considered vaccination-related (agitation and erythema) (Table 4). One (0.4%) infant experienced a grade 3 unsolicited AE (rhinitis) considered unrelated to vaccination by the investigator. During the 30-day post-booster period, unsolicited AEs were recorded for 13 (5.8%) children; for one child (0.4%), one event (nightmare) was assessed by the investigator to be vaccination-related. No grade 3 unsolicited AEs were reported in the 30-day period after the booster dose. Medically attended AEs were recorded for 23 (9.8%) participants after the primary and 6 (2.7%) participants after booster vaccination (Table 4).

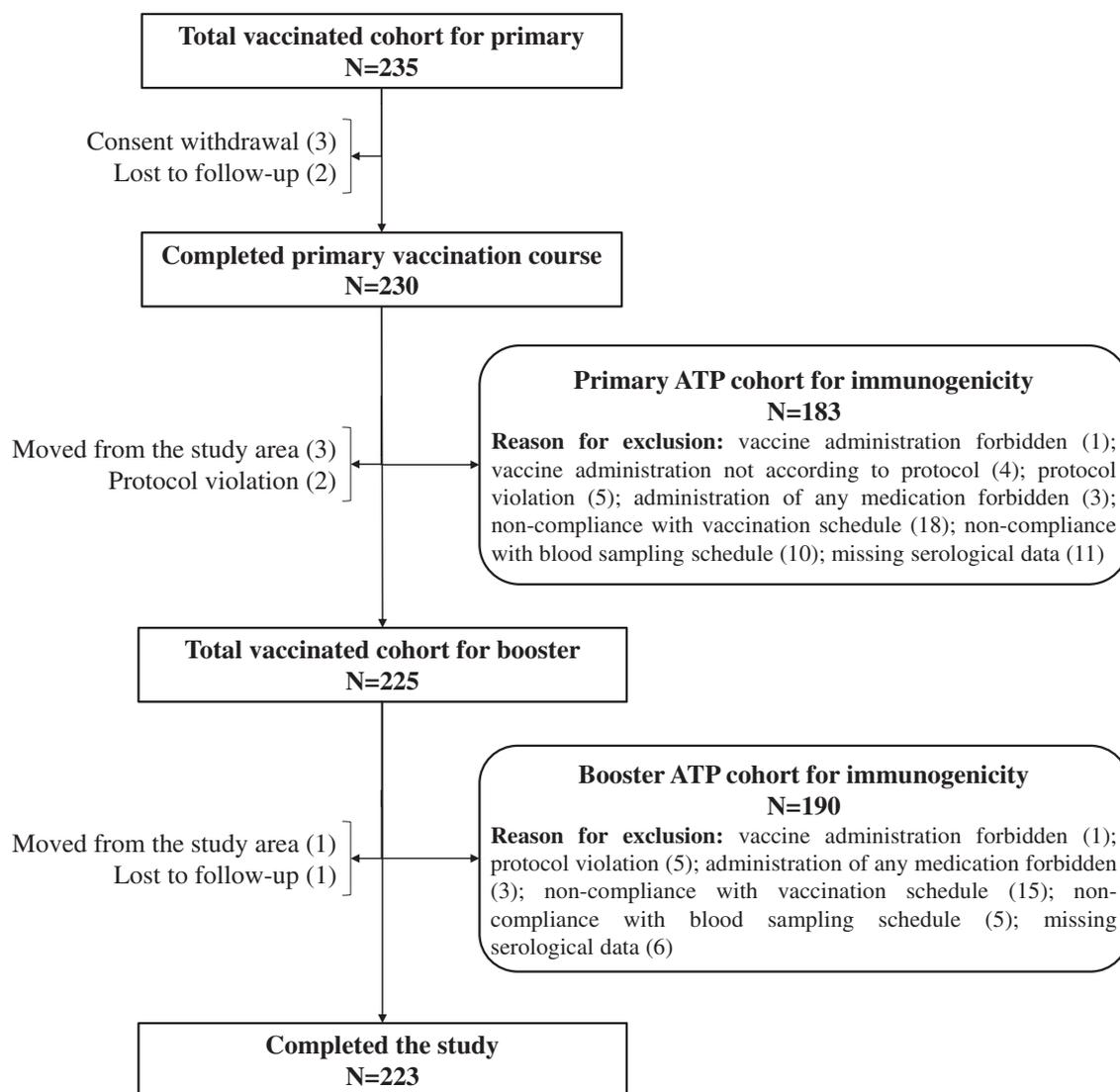


Figure 1. Flow of participants N, number of participants; ATP, according-to-protocol. Note: participants may have more than one reason for exclusion.

Table 1. Summary of demographic characteristics.

	Primary vaccination		Booster vaccination	
	TVC	ATP	TVC	ATP
N	235	183	225	190
Mean age at first dose (\pm SD), weeks/months*	14.1 \pm 1.2	14.1 \pm 1.2	17.8 \pm 0.5	17.7 \pm 0.4
Male, n (%)	124 (52.8)	94 (51.4)	118 (52.4)	101 (53.2)
White-Caucasian/European heritage, n (%)	235 (100)	183 (100)	225 (100)	190 (100)

TVC, total vaccinated cohort; N, number of participants; ATP, according to protocol; SD, standard deviation; n (%), number (percentage) of children in a given category. *Mean age expressed in weeks for primary vaccination and months for booster vaccination.

A total of six SAEs were reported for three (1.3%) infants: one infant experienced gastric infection, one infant experienced anal fistula and proctitis, and one infant experienced a circulatory collapse, congenital heart disease, and patent ductus arteriosus. All SAEs were considered by the investigator as unrelated to vaccination and all infants recovered by study end. No fatalities were reported during the study.

Discussion

The combined DTPa-IPV/Hib vaccine induced robust immune responses to all vaccine antigens after three primary doses and a booster dose and had an acceptable safety profile when administered to healthy Russian children before their second year of life. A booster effect on antibody concentrations was observed for all vaccine antigens.

All participants achieved seroprotective levels against diphtheria and tetanus one month after the primary and booster vaccinations. This is in line with data from previous studies conducted in healthy children in Asia and Europe.^{15,29,30} When infants received DTPa-IPV and Hib vaccines, administered separately or combined as a single injection, according to a 2-4-6-months primary schedule followed by a booster dose at 16-19 months of age, seroprotection rates for diphtheria and tetanus were 100% one month after the primary series, and declined in time, but returned to 100% one month after the booster dose.¹³ In another study evaluating the immune responses of DTPa-IPV/Hib

Table 2. Seroprotection rates and antibody GMCs/GMTs post-primary and post-booster vaccination against diphtheria, tetanus, *H. influenzae* type b, and poliovirus 1–3 antigens (ATP cohorts).

	Threshold	Time point	N	Seroprotection rate (95% CI)	GMC/GMT (95% CI)
Anti-diphtheria	0.1 IU/mL	Post-primary	176	100.0 (97.9–100.0)	3.24 (2.84–3.68)
	0.1 IU/mL	Post-booster	188	100.0 (98.1–100.0)	12.11 (10.82–13.56)
	1 IU/mL		187	99.5 (97.1–100.0)	
Anti-tetanus	0.1 IU/mL	Post-primary	176	100.0 (97.9–100.0)	3.14 (2.81–3.51)
	0.1 IU/mL	Post-booster	188	100.0 (98.1–100.0)	8.18 (7.35–9.11)
	1 IU/mL		188	100.0 (98.1–100.0)	
Anti-PRP	0.15 µg/mL	Post-primary	179	98.4 (95.3–99.7)	2.97 (2.48–3.54)
	0.15 µg/mL	Post-booster	188	100.0 (98.1–100.0)	28.72 (24.70–33.40)
	1.0 µg/mL		188	100.0 (98.1–100.0)	
Anti-poliovirus 1	8 ED ₅₀	Post-primary	151	100.0 (97.6–100.0)	613.9 (505.5–745.5)
		Post-booster	176	100.0 (97.9–100.0)	2185.4 (1901.1–2512.3)
Anti-poliovirus 2	8 ED ₅₀	Post-primary	151	100.0 (97.6–100.0)	591.6 (487.3–718.3)
		Post-booster	169	100.0 (97.8–100.0)	2944.1 (2601.3–3332.2)
Anti-poliovirus 3	8 ED ₅₀	Post-primary	151	99.3 (96.4–100.0)	827.4 (674.7–1014.6)
		Post-booster	167	100.0 (97.8–100.0)	3684.6 (3225.3–4209.3)

ATP, according-to-protocol; GMC, geometric mean concentration; GMT, geometric mean titer; N, number of participants with available results; CI, confidence interval; IU, International Units; Post-primary, one month after the primary course of vaccination; Post-booster, one month after the booster vaccine dose; PRP, polyribosyl-ribitol phosphate; ED₅₀, median effective dose.

Assay cutoffs were 0.057 IU/mL (diphtheria), 0.043 IU/mL (tetanus), 0.066 µg/mL (anti-PRP), and a titer ≥8 (poliovirus 1–3).

Table 3. Seropositivity rates and antibody GMCs post-primary and post-booster vaccination against pertussis antigens (ATP cohorts).

	Assay cutoff	Time point	N	Seropositivity rate (95% CI)	GMC (95% CI)
Anti-PT	2.693 IU/mL	Post-primary	176	98.9 (96.0–99.9)	65.0 (57.7–73.2)
		Post-booster	188	100.0 (98.1–100.0)	107.9 (96.5–120.7)
Anti-FHA	2.046 IU/mL	Post-primary	176	99.4 (96.9–100.0)	120.2 (107.0–135.1)
		Post-booster	188	100.0 (98.1–100.0)	268.4 (242.4–297.2)
Anti-PRN	2.187 IU/mL	Post-primary	176	99.4 (96.9–100.0)	166.1 (146.8–187.8)
		Post-booster	187	100.0 (98.0–100.0)	563.4 (495.6–640.5)

ATP, according-to-protocol; GMC, geometric mean concentration; N, number of participants with available results; CI, confidence interval; PT, pertussis toxoid; Post-primary, one month after the primary course of vaccination; Post-booster, one month after the booster vaccine dose; FHA, filamentous hemagglutinin; PRN, pertactin.

Seropositivity rates were defined as the percentage of participants with antibody concentrations above the assay cutoff.

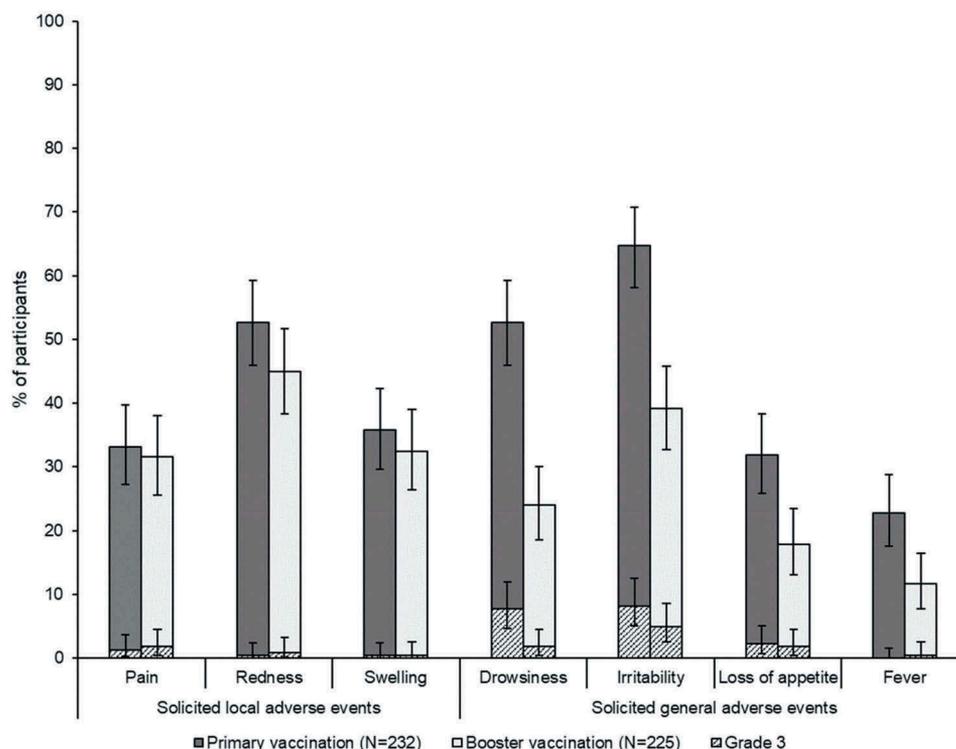
**Figure 2.** Solicited adverse events (total vaccinated cohorts) N, number of participants for primary and booster vaccination course with at least one documented dose.

Table 4. Percentage of participants with reported unsolicited adverse events and serious adverse events (total vaccinated cohorts).

	Primary vaccination (N = 235)		Booster vaccination (N = 225)	
	n	% (95% CI)	n	% (95% CI)
Any unsolicited AEs	48	20.4 (15.5–26.2)	13	5.8 (3.1–9.7)
Grade 3	1	0.4 (0.0–2.3)	0	0.0
Related to vaccination	2	0.9 (0.1–3.0)	1	0.4 (0.0–2.5)
Medically attended AEs SAEs up to study end, n (%)	23	9.8 (6.3–14.3)	6	2.7 (1.0–5.7)

N, number of participants; n (%), number (percentage) of participants reporting at least one AE; AE, adverse event; CI, confidence interval; SAE, serious AE.

co-administered with a rotavirus vaccine, 97.3% of participants were seroprotected against diphtheria and 100% against tetanus after three vaccine doses administered at 3, 4 and 5 months of age.¹⁷

In the present study, 98.9% of participants were seropositive for PT and 99.4% for FHA and PRN antigens one month after the primary vaccination. Similar results have been observed in trials conducted in European infants, comparing the immune responses to pertussis vaccination following vaccination with either an HBV-containing hexavalent combination (DTPa-HBV-IPV/Hib) or the DTPa-IPV/Hib and HBV vaccines separately.^{29,31} In several studies conducted in Asian infants, seropositivity rates of 100% were also reported for all three pertussis antigens following primary vaccination with DTPa-IPV/Hib according to different schedules.^{14,15,30} In the current study, at one month after the booster dose, all participants were seropositive for all three pertussis antigens, in line with previous reports.^{15,30} As different assays and seropositivity thresholds were used across studies, seropositivity rates cannot be directly compared. However, data indicate the mounting of robust immune responses against pertussis antigens following vaccination with DTPa-IPV/Hib.

Almost all children (98.4%) achieved anti-PRP antibody levels ≥ 0.15 $\mu\text{g/mL}$ one month following the primary vaccination. While several studies reported seroprotective levels for 100% of study participants after completing the primary vaccination course,^{17,29,30} in other reports seroprotective rates ranged between 96.4% and 98.7%.^{13,15} One month post-booster dose, all study participants achieved seroprotective levels indicative of long-term protection (≥ 1.0 $\mu\text{g/mL}$) against Hib. In other studies, lower anti-PRP antibody levels were observed in children receiving the combined DTPa-IPV/Hib vaccine as compared with those who received the Hib vaccine as a separate injection.^{13,14,16} Nevertheless, the lower anti-PRP antibody responses to combined DTP-Hib vaccines were previously shown not to be associated with an impaired function of the induced antibodies, nor with impaired immune memory against Hib.^{32,33} The successful long-term Hib disease control in Europe achieved with a DTPa-HBV-IPV/Hib combination vaccine also confirms that these immunological findings have no clinical impact.³⁴

The immune responses observed in the current study against poliovirus types 1–3 one month following the primary vaccination were in line with previous reports. In Caucasian infants who completed a 3-dose primary schedule with either DTPa-HBV-IPV/Hib or DTPa-IPV/Hib + HBV vaccines, seroprotection rates were achieved by almost all participants;

antibody levels ranged between 481.6–1590.3 ED_{50} (poliovirus 1), 350.6–1961.2 ED_{50} (poliovirus 2), and 1152.3–2425.5 ED_{50} (poliovirus 3).^{29,35}

The three primary doses of DTPa-IPV/Hib vaccine administered at 3, 4.5, and 6 months of age and the booster dose administered at 18 months of age were well tolerated, with an acceptable reactogenicity profile. The frequencies of the observed solicited local and general AEs were similar to those reported from other studies, with redness and irritability being the most frequent solicited local and general AEs (of all grades), respectively, although the vaccination schedules differed across studies.^{14,29,30,36} In line with previous reports,^{15,37} the most commonly reported grade 3 local and general solicited AEs were pain and irritability, respectively. While the incidences of solicited local AEs remained similar, the incidence of solicited general AEs in the current study tended to be lower after the booster dose as compared with primary vaccination. The frequencies of AEs observed following booster vaccination were nevertheless similar with those reported from a phase 3 trial evaluating the safety of the booster dose of DTPa-IPV/Hib in Vietnamese toddlers.¹²

Consistent with previous reports,^{14,15} the occurrence of vaccine-related unsolicited AEs was not frequent. No SAEs that occurred during the study were considered related to the study vaccine. The observed 0.4% frequency of large swelling reactions is comparable with literature data.^{36,37}

The strengths of this study included the successful enrollment of a large number of children from 5 different sites distributed over the Russian Federation, the laboratory testing conducted in one central laboratory, and the use of validated laboratory tests. The open-label and non-randomized design might be one of the limitations of the study. The number of evaluable participants for the primary endpoint of the study on immunogenicity was not derived from a sample size powered computation but based on the required number of participants as requested by the Russian Regulatory Authorities. The trial was conducted in one country, though the results could be generalized to other populations with similar disease prevalence and immunization practices. The lack of data on pre-primary and pre-booster immune responses in infancy did not allow the assessment of the fold-change of antibody levels from pre- to post-vaccination for the primary and booster immunization series. Co-administration of DTPa-IPV/Hib with an HBV vaccine was not envisaged in the study protocol, however, concomitant administration with HBV or any other vaccine as part of the national immunization schedule and as part of routine vaccination practice was allowed. Additionally, broad literature data exist to support the concomitant injection of DTPa-IPV/Hib and HBV vaccines^{30,35,38,39} and many countries use these vaccines as standard of care.

The coverage of pediatric DTP immunization in the Russian Federation has remained high for more than a decade, with 97% of children receiving the third DTP dose.^{40,41} However, resurgences of childhood diseases still occur. Therefore, a high uptake of pediatric vaccinations remains paramount and the administration of combination vaccines has been shown to improve vaccination compliance.¹⁰ Moreover, in the Russian Federation whole-

Focus on the Patient

What is the context?

- Combination vaccines protect against several diseases in one single injection. They are therefore widely used in the routine pediatric practice.
- The combination vaccine DTPa-IPV/Hib (Infanrix-IPV/Hib) is indicated for immunization against five childhood diseases: diphtheria, tetanus, pertussis, poliomyelitis, and diseases caused by *Haemophilus influenzae* type b (Hib).
- Three doses plus one booster dose against these diseases are currently recommended for infants in the Russian Federation.

What is new?

- We investigated the immunogenicity and safety of the combination vaccine in healthy Russian infants.
- This vaccine induced a robust immune response.
- After the three first doses we found that:
 - all infants had protective antibody levels against diphtheria and tetanus
 - more than 98% of infants had protective antibody levels against poliomyelitis and Hib antigens
 - more than 98% of infants were seropositive for three pertussis antigens
- All toddlers had protective or positive antibody concentrations for all 5 diseases after the booster dose.
- There are no safety concerns and the vaccine is well tolerated.

What is the impact?

- This study shows that this combination vaccine has an acceptable immunogenicity and safety profile and could thus replace standalone vaccines reducing the number of injections needed to follow the immunization recommendation of the Russian Federation.

Figure 3. Plain language summary.

cell pertussis vaccination predominates over the acellular pertussis vaccines.⁴² The use of an acellular pertussis containing combination vaccine like DTPa-IPV/Hib might therefore contribute to improved vaccination acceptance, as this vaccine is less reactogenic than whole-cell pertussis vaccines, enhancing compliance and coverage.⁴³

In conclusion, the combined DTPa-IPV/Hib vaccine administered as a 3-dose primary vaccination at 3, 4.5 and 6 months of age and a booster dose at 18 months of age induced robust immune responses to all vaccine antigens and was well tolerated in healthy Russian infants. For the benefit of healthcare professionals, a summary contextualizing the results and relevance of this clinical research is displayed in the Focus on Patient Section (Figure 3).

Abbreviations

AE	adverse event
ATP	according-to-protocol
CI	confidence interval
DTP	diphtheria-tetanus-pertussis
DTPa-IPV/Hib	

DU	diphtheria-tetanus-acellular pertussis-inactivated polio and <i>Haemophilus influenzae</i> type b conjugate vaccine
ED ₅₀	D-antigen units
FHA	median effective dose
GMC	filamentous hemagglutinin
GMT	geometric mean concentration
Hib	geometric mean titer; HBV, hepatitis B virus
IU	<i>Haemophilus influenzae</i> type b
PRP	International Units; PRN, pertactin
PT	polyribosyl-ribitol phosphate
SAE	pertussis toxoid
	serious adverse event

Acknowledgments

The authors thank the children and their parent(s)/adoptive parent(s) for participating in this study, and the study staff for their contribution to the study. The authors are grateful to Narcisa Mesaros (GSK, Wavre, Belgium), Madhura Saralaya (GSK, Bangalore, India), and Galina Karpova (GSK, Moscow, Russian Federation) for their contributions to the study. The authors would also like to thank the Modis platform (c/o GSK) for editorial assistance and manuscript coordination. Botond Nagy provided medical writing support and Sonja Sachse coordinated manuscript development and provided editorial support.

Author contributions

SOK, MS, and IO were involved in the conception and design of the study; VR, WJ, and AG collected the data; MS, VR, IO, and WJ performed the study; VR, NB, and WJ contributed materials/analysis/reagent tools, and NM, SOK, VR, WJ, LA, and DF were involved in data analysis and interpretation. All authors had full access to the data, revised the work critically, approved the final version to be published, and take full accountability for all aspects of the work.

Disclosure of potential conflicts of interest

MS, NB, DF, LA, SOK, NM, and WJ are employed by the GSK group of companies.

WJ, LA, and MS hold restricted shares in the GSK group of companies as part of their employee remuneration. VR, IO, and AG have nothing to declare.

Funding

This work was supported by GlaxoSmithKline Biologicals SA, including all costs associated with the development and publication of this manuscript.

Data sharing statement

The results summary for this study (GSK study number 116194, gcp02858440) is available on the GSK Clinical Study Register and can be accessed at www.gsk-clinicalstudyregister.com.

For interventional studies that evaluate our medicines, anonymized patient-level data will be made available to independent researchers, subject to review by an independent panel, at www.clinicalstudydataquest.com within six months of publication.

To protect the privacy of patients and individuals involved in our studies, GSK does not publicly disclose patient-level data.

Trademark statement

Infanrix-IPV/Hib is a trademark of the GSK group of companies.

ORCID

Michael Scherbakov  <http://orcid.org/0000-0002-9189-4729>

Winnie Janssens  <http://orcid.org/0000-0003-1314-3468>

References

- Gentile A, Bhutta Z, Bravo L, Samy AG, Garcia RD, Hoosen A, Islam T, Karimi A, Salem M, Simasathien S, et al. Pediatric disease burden and vaccination recommendations: understanding local differences. *Int J Infect Dis*. 2010;14:e649–658. doi:10.1016/j.ijid.2009.11.006.
- WHO. *Haemophilus influenzae* type b (Hib) vaccination position paper - July 2013. *Wkly Epidemiol Rec*. 2013;88:413–26.
- WHO. Pertussis vaccines: WHO position paper - September 2015. *Wkly Epidemiol Rec*. 2015;90:433–58.
- WHO. Polio vaccines: WHO position paper - March, 2016. *Wkly Epidemiol Rec*. 2016;91:145–68.
- WHO. Diphtheria vaccine: WHO position paper - August 2017. *Wkly Epidemiol Rec*. 2017;92:417–35.
- WHO. Tetanus vaccines: WHO position paper - February 2017. *Wkly Epidemiol Rec*. 2017;92:53–76.
- Sealey KL, Belcher T, Preston A. Bordetella pertussis epidemiology and evolution in the light of pertussis resurgence. *Infect Genet Evol*. 2016;40:136–43. doi:10.1016/j.meegid.2016.02.032.
- Kalies H, Grote V, Verstraeten T, Hessel L, Schmitt H-J, von Kries R. The use of combination vaccines has improved timeliness of vaccination in children. *Pediatr Infect Dis*. 2006;25:507–12. doi:10.1097/01.inf.0000222413.47344.23.
- Maman K, Zollner Y, Greco D, Duru G, Sendyona S, Remy V. The value of childhood combination vaccines: from beliefs to evidence. *Hum Vaccin Immunother*. 2015;11:2132–41. doi:10.1080/21645515.2015.1044180.
- Marshall GS, Happe LE, Lunacsek OE, Szymanski MD, Woods CR, Zahn M, Russell A. Use of combination vaccines is associated with improved coverage rates. *Pediatr Infect Dis J*. 2007;26:496–500. doi:10.1097/INF.0b013e31805d7f17.
- Skibinski DA, Baudner BC, Singh M, O'Hagan DT. Combination vaccines. *J Glob Infect Dis*. 2011;3:63–72. doi:10.4103/0974-777x.77298.
- Anh DD, Van Der Meeren O, Karkada N, Assudani D, Yu TW, Han HH. Safety and reactogenicity of the combined diphtheria-tetanus-acellular pertussis-inactivated poliovirus-Haemophilus influenzae type b (DTPa-IPV/Hib) vaccine in healthy Vietnamese toddlers: an open-label, phase III study. *Hum Vaccin Immunother*. 2016;12:655–57. doi:10.1080/21645515.2015.1084451.
- Halperin SA, King J, Law B, Mills E, Willems P. Safety and immunogenicity of Haemophilus influenzae-tetanus toxoid conjugate vaccine given separately or in combination with a three-component acellular pertussis vaccine combined with diphtheria and tetanus toxoids and inactivated poliovirus vaccine for the first four doses. *Clin Infect Dis*. 1999;28:995–1001. doi:10.1086/514741.
- Kim KH, Kim CS, Kim HM, Kim JD, Ma SH, Kim DH, Hwang PH, Han JW, Lee TJ, Kim JH, et al. Immunogenicity and safety of a combined DTPa-IPV/Hib vaccine administered as a three-dose primary vaccination course in healthy Korean infants: phase III, randomized study. *Hum Vaccin Immunother*. 2019;15:317–26. doi:10.1080/21645515.2018.1536588.
- Li Y, Li RC, Ye Q, Li C, Liu YP, Ma X, Li Y, Zhao H, Chen X, Assudani D, et al. Safety, immunogenicity and persistence of immune response to the combined diphtheria, tetanus, acellular pertussis, poliovirus and Haemophilus influenzae type b conjugate vaccine (DTPa-IPV/Hib) administered in Chinese infants. *Hum Vaccin Immunother*. 2017;13:588–98. doi:10.1080/21645515.2016.1239670.
- Lin TY, Wang YH, Chang LY, Chiu CH, Huang YC, Tang H, Bock HL. Safety and immunogenicity of a diphtheria, tetanus, and acellular pertussis-inactivated poliovirus vaccine/Haemophilus influenzae type B combination vaccine administered to Taiwanese infants at 2, 4, and 6 months of age. *Chang Gung Med J*. 2003;26:315–22.
- Phua KB, Quak SH, Lim FS, Goh P, Teoh YL, Datta SK, Han HH, Bock HL. Immunogenicity, reactogenicity and safety of a diphtheria-tetanus-acellular pertussis-inactivated polio and Haemophilus influenzae type b vaccine in a placebo-controlled rotavirus vaccine study. *Ann Acad Med Singapore*. 2008;37:546–53.
- Order of the Ministry of Health of the Russian Federation N252n. On approval of the national immunization schedule and immunization schedule due to epidemic indications. Registered in the Ministry of Justice of the Russian Federation, registration number 32115 (as of June 20, 2013). 2014.
- WHO vaccine-preventable diseases: monitoring system. 2018 global summary. [accessed 2019 July 2]. http://apps.who.int/immunization_monitoring/globalsummary/schedules.
- Camargo ME, Silveira L, Furuta JA, Oliveira EP, Germek OA. Immunoenzymatic assay of anti-diphtheric toxin antibodies in human serum. *J Clin Microbiol*. 1984;20:772–74. doi:10.1128/JCM.20.4.772-774.1984.
- Melville-Smith ME, Seagroatt VA, Watkins JT. A comparison of enzyme-linked immunosorbent assay (ELISA) with the toxin neutralization test in mice as a method for the estimation of tetanus antitoxin in human sera. *J Biol Stand*. 1983;11:137–44. doi:10.1016/s0092-1157(83)80038-9.

22. Granstrom M, Thoren M, Blennow M, Tiru M, Sato Y. Acellular pertussis vaccine in adults: adverse reactions and immune response. *Eur J Clin Microbiol.* 1987;6:18–21. doi:10.1007/bf02097184.
23. Karpinski KF, Hayward S, Tryphonas H. Statistical considerations in the quantitation of serum immunoglobulin levels using the enzyme-linked immunosorbent assay (ELISA). *J Immunol Methods.* 1987;103:189–94. doi:10.1016/0022-1759(87)90289-4.
24. Immunization WHO/EPI. Guidelines for WHO/EPI collaborative studies on poliomyelitis: standard procedure for determining immunity to poliovirus using the microneutralization test. Geneva, Switzerland: World Health Organization; 1993.
25. Kayhty H, Peltola H, Karanko V, Makela PH. The protective level of serum antibodies to the capsular polysaccharide of *Haemophilus influenzae* type b. *J Infect Dis.* 1983;147:1100. doi:10.1093/infdis/147.6.1100.
26. Plotkin SA. Correlates of protection induced by vaccination. *Clin Vaccine Immunol.* 2010;17:1055–65. doi:10.1128/cvi.00131-10.
27. Plotkin SA. Updates on immunologic correlates of vaccine-induced protection. *Vaccine.* 2019; doi:10.1016/j.vaccine.2019.10.046.
28. Medical Dictionary for Regulatory Activities. [accessed 2019 Aug 14]. <https://www.meddra.org/how-to-use/support-documentation/english>.
29. Aristegui J, Dal-Re R, Diez-Delgado J, Mares J, Casanovas JM, Garcia-Corbeira P, De Frutos E, Van Ezzo D, Verdaguer J, De la Flor J, et al. Comparison of the reactogenicity and immunogenicity of a combined diphtheria, tetanus, acellular pertussis, hepatitis B, inactivated polio (DTPa-HBV-IPV) vaccine, mixed with the *Haemophilus influenzae* type b (Hib) conjugate vaccine and administered as a single injection, with the DTPa-IPV/Hib and hepatitis B vaccines administered in two simultaneous injections to infants at 2, 4 and 6 months of age. *Vaccine.* 2003;21:3593–600. doi:10.1016/S0264-410X(03)00420-1.
30. Shao PL, Lu CY, Hsieh YC, Bock HL, Huang LM. Immunogenicity and reactogenicity of DTPa-IPV/Hib vaccine co-administered with hepatitis B vaccine for primary and booster vaccination of Taiwanese infants. *J Formos Med Assoc.* 2011;110:415–22. doi:10.1016/s0929-6646(11)60061-2.
31. Schmitt HJ, Knuf M, Ortiz E, Sanger R, Uwamwezi MC, Kauffhold A. Primary vaccination of infants with diphtheria-tetanus-acellular pertussis-hepatitis B virus- inactivated polio virus and *Haemophilus influenzae* type b vaccines given as either separate or mixed injections. *J Pediatr.* 2000;137:304–12. doi:10.1067/mpd.2000.107796.
32. Eskola J, Ward J, Dagan R, Goldblatt D, Zepp F, Siegrist CA. Combined vaccination of *Haemophilus influenzae* type b conjugate and diphtheria-tetanus-pertussis containing acellular pertussis. *Lancet.* 1999;354:2063–68. doi:10.1016/s0140-6736(99)04377-9.
33. Poolman J, Kauffhold A, De Grave D, Goldblatt D. Clinical relevance of lower Hib response in DTPa-based combination vaccines. *Vaccine.* 2001;19:2280–85. doi:10.1016/s0264-410x(00)00517-x.
34. Wang S, Tafalla M, Hanssens L, Dolhain J. A review of *Haemophilus influenzae* disease in Europe from 2000–2014: challenges, successes and the contribution of hexavalent combination vaccines. *Expert Rev Vaccines.* 2017;16:1095–105. doi:10.1080/14760584.2017.1383157.
35. Avdicová M, Prikazský V, Hudecková H, Schuerman L, Willems P. Immunogenicity and reactogenicity of a novel hexavalent DTPa-HBV-IPV/Hib vaccine compared to separate concomitant injections of DTPa-IPV/Hib and HBV vaccines, when administered according to a 3, 5 and 11 month vaccination schedule. *Eur J Pediatr.* 2002;161:581–87. doi:10.1007/s00431-002-1079-5.
36. Heining U, Sanger R, Jacquet JM, Schuerman L. Booster immunization with a hexavalent diphtheria, tetanus, acellular pertussis, hepatitis B, inactivated poliovirus vaccine and *Haemophilus influenzae* type b conjugate combination vaccine in the second year of life: safety, immunogenicity and persistence of antibody responses. *Vaccine.* 2007;25:1055–63. doi:10.1016/j.vaccine.2006.09.060.
37. Lim FS, Phua KB, Lee BW, Quak SH, Teoh YL, Ramakrishnan G, Han HH, Van Der Meeren O, Jacquets JM, Bock HL. Safety and reactogenicity of DTPa-HBV-IPV/Hib and DTPa-IPV/I-Hib vaccines in a post-marketing surveillance setting. *Southeast Asian J Trop Med Public Health.* 2011;42:138–47.
38. Avdicova M, Crasta PD, Hardt K, Kovac M. Lasting immune memory against hepatitis B following challenge 10–11 years after primary vaccination with either three doses of hexavalent DTPa-HBV-IPV/Hib or monovalent hepatitis B vaccine at 3, 5 and 11–12 months of age. *Vaccine.* 2015;33:2727–33. doi:10.1016/j.vaccine.2014.06.070.
39. Zepp F, Schmitt HJ, Cleerhout J, Verstraeten T, Schuerman L, Jacquet JM. Review of 8 years of experience with Infanrix hexa (DTPa-HBV-IPV/Hib hexavalent vaccine). *Expert Rev Vaccines.* 2009;8:663–78. doi:10.1586/erv.09.32.
40. WHO and UNICEF estimates of national routine immunization coverage, 2017 revision (completed July 2018). [accessed 2019 July 2]. <http://data.unicef.org/topic/child-health/immunization/>.
41. Organisation for Economic Co-operation and Development (OECD) Data. Child vaccination rates. [accessed 2019 Aug 6]. <https://data.oecd.org/healthcare/child-vaccination-rates.htm>.
42. Kurova N, Timofeeva EV, Guiso N, Macina D. A cross-sectional study of *Bordetella pertussis* seroprevalence and estimated duration of vaccine protection against pertussis in St. Petersburg, Russia. *Vaccine.* 2018;36:7936–42. doi:10.1016/j.vaccine.2018.11.007.
43. WHO. Pertussis vaccines: WHO position paper, August 2015. *Wkly Epidemiol Rec.* 2016;90:433–60.