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ROTAVAC® does not interfere with the immune response to childhood vaccines in Indian infants: A randomized placebo controlled trial

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Abstract

A phase III randomized double-blind placebo-controlled trial was conducted in the urban neighborhoods of Delhi to assess whether Oral Rotavirus Vaccine ROTAVAC® interferes with the immune response to childhood vaccines when

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coadministered. Infants aged 6 weeks were randomized to receive three doses of either ROTAVAC® or placebo along with childhood vaccines: Oral Polio Vaccine and vaccines against Diphtheria, Pertussis, Tetanus, Hepatitis B and *Haemophilus influenza* type b given as Pentavalent at 6, 10, 14 weeks of age. Blood specimens were collected from all infants at baseline and 4 weeks post dose 3 to assess the immune response to antigens in Oral Polio Vaccine, Pentavalent and ROTAVAC® vaccines. Non-inferiority of immune response to all vaccine components of the childhood vaccines when ROTAVAC® was administered concurrently was demonstrated. Non-inferior immune responses to childhood vaccines were evaluated based on the seroprotective levels of antibodies against polio types 1, 2, and 3, Diphtheria toxoid, Tetanus toxoid, *Haemophilus influenza* type b anti-polyribosyl ribitol phosphate antibodies and Hepatitis B antibodies; and the Geometric Mean Concentration for Pertussis. The proportion of infants who seroconverted (≥4 fold rise) was 38.6% in the ROTAVAC® group and 12.2% in the placebo group. The frequency and severity of immediate adverse events, adverse events and serious adverse events were similar in both groups. None of the five reported deaths were considered to be related to the ROTAVAC® and no case of intussusception meeting Brighton Diagnostic Certainty Level I criteria was reported.

This study demonstrated that ROTAVAC® can be safely administered with childhood vaccines without interfering with the immune response to the antigens contained in these vaccines.

Keywords: Pediatrics, Infectious disease, Immunology, Vaccines

1. Introduction

The World Health Organization (WHO) recommends the universal use of rotavirus vaccines in the national immunization programs of all regions of the world, due to the high disease burden especially in high child mortality settings [1].

The scale up of rotavirus vaccines in developing countries is limited by inadequate availability of high quality vaccines at affordable prices. Currently, the rotavirus vaccines available are: RotaTeq® (Merck) and Rotarix® (GSK) vaccines. These vaccines are not widely available or affordable for the developing world. The oral rotavirus vaccine (ORV), ROTAVAC® was developed and manufactured in India under the Indo-US Vaccine Action Program, through a unique collaborative Public-Private-Partnership [2]. On the basis of successful completion of Phase I, II and III studies in the country, this vaccine was licensed for use in India in January 2014 [3, 4, 5]. The national program delivers several vaccines at 6, 10 and 14 weeks of age and new vaccines including the ROTAVAC® targeted at infants can be delivered at these existing opportunities [6]. This will reduce the cost and burden to the families in terms of visits to immunization clinics and optimize

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vaccine uptake. Post licensure ROTAVAC® was launched in the private market in April 2015 and introduced in the Universal Immunization Program in a phased manner in India in March 2016.

We report the findings of a study conducted to determine whether three doses of ROTAVAC® when co- administered with other childhood vaccines i.e. Oral Polio Vaccine (OPV) and Pentavalent Vaccine against Diphtheria, Pertussis, Tetanus (DPT), Hepatitis B (HepB) and *Haemophilus influenza* type b (Hib) interferes with their immune response. These data are needed for obtaining WHO prequalification, which will allow access and equitable use of the vaccine for children in lower income countries. The immunogenicity and safety of ROTAVAC® and the clinical lot consistency of three production lots of ROTAVAC® were also assessed. The results of the clinical lot consistency will be published separately.

2. Material and methods

2.1. Study design and participants

This phase III randomized double-blind placebo-controlled study was conducted between May 2014 and August 2015 in Delhi, India. The study was conducted in compliance with the protocol, Good Clinical Practices (GCP), Schedule Y and Ethical guidelines for biomedical research on human participants [7, 8].

The study was approved by the Translational Health Science and Technology Institute (THSTI) Institutional Ethics Committee and the Western Institutional Review Board.

2.2. Study participants and procedures

This study was conducted in low resource urban neighborhoods in Delhi. The description of the site has been reported earlier [5]. Participants were identified through a household survey and infants were enrolled into the study after obtaining informed consent from the parents and screening the infant for eligibility. Infants between 42–55 days of age whose parents were willing to participate and had no plans of moving away were eligible for enrolment. Infants were excluded if they had already received the first dose of the childhood vaccines or any other rotavirus vaccine, had immunodeficiency disease or chronic gastroenteritis disease, and/or any condition warranting exclusion by the investigator. Infants were temporarily excluded if they had diarrhea or any illness requiring hospital referral on the day of screening.

Enrolled infants were given three doses of ROTAVAC® or placebo along with childhood vaccines (OPV and Pentavalent vaccine) at 6–7 weeks, 10- <14 and 14- <18 weeks of age. A minimum interval of 4 weeks was maintained between two doses of ROTAVAC® or placebo plus childhood vaccines. Infants also received

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OPV as mandated by the Government on the National Polio Immunization days. ROTAVAC® or placebo 0.5 mL, were administered approximately 5 min after administration of 2.5 mL of citrate bicarbonate buffer. Infants were kept under observation in the study clinic for 30 min after the administration of the last vaccine for any immediate adverse events (IAEs). A participant booklet was given to families which included when to contact the study team, the address of study clinic and referral hospitals, study physician contact numbers, immunization record and daily record of temperature for 14 days after each dose of ROTAVAC® or placebo. The study team made daily contacts through telephone calls or home visit for 14 days after each dose of ROTAVAC® or placebo to ascertain adverse events (AEs). Thereafter, weekly contacts were made till infants were one year of age. During this period, information was obtained on the presence of any illness, signs and symptoms of intussusception and serious adverse events (SAEs) for 4 weeks after third dose; subsequently, only presence of signs and symptoms of intussusception and events of death were ascertained. Protocol deviations were reported for all subjects to the Ethics Committees, regulatory authorities and sponsor as per the required timelines throughout the study period.

2.3. Randomization and blinding

Randomization was done by Diagnosearch Life Sciences Pvt. Ltd. and the randomization list was available with an independent biostatistician. Enrolled infants were allocated in a 1:1:1:1 ratio to one of the 4 randomization arms i.e. three vaccine production lots and placebo. The placebo was identical in content, packaging, and appearance to the vaccine. The study team received ROTAVAC® or placebo vials labeled with the subject Identification (ID) number to maintain blinding. The study team, vaccine administrators and laboratory personnel were not aware of the treatment status. For testing of serum samples for DPT, HepB and Hib antibodies, two separate lists were provided by the independent statistician to the laboratory listing the subjects IDs for whom assays were to be done; one list each for HepB and Hib antibody testing and the second for DPT antibody testing. A replacement list was also sent in case there was insufficient specimen available for those mentioned in the primary lists.

2.4. Blood specimen

Blood (1.5 mL) was collected from all infants at baseline for the Rotavirus Immunoglobulin A (RV IgA) assay and 6 mL of post immune blood specimen was collected at 28 (±5) days after the third dose of ROTAVAC® or placebo for assessing immunogenicity to ROTAVAC®, OPV and Pentavalent vaccines. Serum was separated, aliquoted, stored at −20 °C and shipped to the testing laboratories under temperature control in dry ice.

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2.5. Vaccines

ROTAVAC®, developed and manufactured by Bharat Biotech International Limited, is a liquid frozen formulation containing $10^{5.0}$ Focus Forming Unit (ffu) per dose (0.5 ml) of human live rotavirus strain G9P11.

The formulation of the placebo was also liquid frozen, consisting of the same buffered culture medium used to grow the vaccine. Three batches of ROTAVAC® and placebo were used in the study. The placebo was identical to the ROTAVAC® in packaging and appearance. ROTAVAC® or placebo was stored at −20 °C ± 5 °C. The Citrate Bicarbonate Buffer was stored at room temperature. Other vaccines administrated to all infants were; OPV (Biopolio™) which is a trivalent vaccine containing aqueous suspension of type 1, 2 and 3 attenuated poliomyelitis viruses (Sabin Strains), each dose containing not less than $10^{6.0}$ $CCID_{50}$ infectious units of each type: 2 drops orally and Com Vac5 a combined DPT, HepB and Hib vaccine: 0.5 ml intramuscularly. Bacillus Calmette Guerin (BCG), '0' dose of OPV, measles along with vitamin A syrup (100,000 IU) were offered to all as per schedule [6].

3. Laboratory assays

3.1. Assessment of immune response to childhood vaccines

Immune responses to childhood vaccines were assessed using standard criteria for seroprotective titers for anti-poliovirus (>1/8 dilution) [9, 10] Diphtheria toxoid (>0.1 IU/mL), tetanus toxoid (>0.1 IU/mL) [11], HepB (≥10 mIU/mL) [12] and Hib (≥0.15mcg/mL) [11] and Geometric Mean Concentrations (GMC) for pertussis [11]. For determining polio antibody titer in serum, polio virus infectivity neutralization assay was performed at Enterovirus Research Centre, Mumbai. Quantitative determination of antibodies against DPT and Hib was done using Enzyme linked Immuno-Sorbent Assay (ELISA) and quantitative determination of antibodies to HepB surface antigen (anti-HBs) was done using Microplate Enzyme Immuno Assay (MEIA) at SRL limited, Mumbai.

3.2. Assessment of immunogenicity of ROTAVAC®

Immunogenicity of ROTAVAC® was assessed as a ≥4-fold rise in the rotavirus specific serum IgA antibody titre at 28 (±5) days after the third dose of the vaccine in comparison to the baseline. Serum anti-rotavirus IgA was estimated by ELISA with a standard curve method, at the Clinical Investigation Laboratory, THSTI [13].

3.3. Post vaccination reactogenicity and safety of ROTAVAC®

For ascertainment of IAEs, all infants were observed at the study clinic for 30 min after each dose of the vaccines. For presence of solicited AEs of special interest:

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fever, vomiting, diarrhea, cough, listlessness or less active, runny nose, irritability and rash and unsolicited events, illness requiring hospital referral, SAEs (death, hospitalization, life threatening or medically important event) and intussusception, daily contacts were made by the team for 14 days after each dose of ROTAVAC® or placebo and thereafter weekly till one year of age.

Infants with signs and symptoms of suspected intussusception or illness requiring hospital referral were assessed and treated at the study clinic or at referral hospitals. All cases of intussusception confirmed by the treating physician were reviewed by an independent case adjudication committee to ascertain if they met the Diagnostic Certainty Level Criteria 1 developed by Brighton Collaboration Intussusception Working Group [14].

3.4. Statistical analysis

The primary analysis population for the immunogenicity endpoints was the per protocol population defined as a subset of randomized subjects who received three doses of ROTAVAC®/Placebo (any/all vaccine production lot) and childhood vaccines concomitantly (received the childhood vaccines on the same day as ROTAVAC®/Placebo), with no major protocol deviations (impact the eligibility criteria or determined to potentially interfere with the immune responses to study vaccines). The analysis population for all safety endpoints was the safety population defined as all randomized subjects who received at least one dose of ROTAVAC®/Placebo, with or without childhood vaccines, and had some safety data available.

The non interference of ROTAVAC® with childhood vaccines were tested for formal statistical non-inferiority testing with pre-specified margins using the two sided 95% confidence interval (CI) for the difference between the two treatment groups. The non-inferiority margins are 10 percentage points for the seroprotection rates for polio types 2 and 3, Diphtheria toxoid, Tetanus toxoid, Hib anti-PRP antibodies, and Hepatitis B; 15 percentage points for polio type 1 seroprotection rate; and 2-fold for Pertussis GMC.

Seroprotection rates were analyzed using the two-sided 95% CI for the absolute rate difference (Placebo − ROTAVAC®) between the treatment groups. The two-sided 95% CI for the seroprotection rates were estimated by a likelihood score method by Gart and Nam using NCSS software [15]. GMCs for Pertussis were analyzed using the two-sided 95% CI for the ratio of geometric mean titers (GMT) (Placebo/ROTAVAC®) between the treatment groups. Two-sided 95% CIs were estimated for the difference between means of log10 (concentration), under the assumption that log10 (concentration) is normally distributed, using the t-distribution. Antilogs of the log mean and the corresponding confidence limits were taken to obtain a ratio of GMCs and the CI.

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Sample size assumptions were made considering all comparisons that were part of the primary childhood vaccine immune interference objective. 1356 infants were enrolled; 339 in each of the 4 randomization arms allowing for 15% and 20% loss to follow up and giving a power of 82% and 78%, respectively, for all comparisons simultaneously. Since the loss to follow-up was much lower than 20% and the observed seroprotection rates to all 3 polio types were much higher than estimated (60–80% planned vs. ≥90% observed), this study had much higher power than planned for all analyses.

Safety results were analyzed in the intention to treat population which included all infants who had received at least one dose of ROTAVAC® or placebo with or without childhood vaccines. All events were coded using the Medical dictionary for Regulatory Activities (MedRA, Version 17.0). An independent Data and Safety Monitoring Board conducted safety review of blinded and unblinded data analyses.

Statistical analysis was performed by DiagnoSearch Life Sciences Pvt. Ltd using SAS software version 9.2.

3.5. Role of funding sources

PATH, USA; provided funding. The funder had no influence on the implementation and data collection. Analysis was done by DiagnoSearch Life Sciences Pvt. Ltd.

4. Results

4.1. Study subjects

Between 26 May 2014 and 17 September 2014, of the 1683 infants screened, 1356 infants were enrolled; 1017 were randomized to the ROTAVAC® group and 339 to the placebo group. 1327 infants completed 1 year follow up (Fig. 1). The age at first dose in both groups was mean (SD) 6.4 weeks (0.47) weeks. 1273 (93.9%) of the enrolled infants received 2 doses and 1244 (91%) received all three doses of ROTAVAC® or placebo. The baseline characteristics in both the groups were similar (Table 1).

4.2. Immune response to childhood vaccines

Post vaccination, seroprotective level of antibodies against polio virus type 1, 2, and 3 were present in 98.2%, 99.4% and 92.4%, respectively, of infants receiving OPV with ROTAVAC®, and in 99%, 98.3% and 92.7%, respectively, of infants receiving OPV with placebo. Difference in proportions that had titer ≥8 between these groups was 0.8% (95% CI −1.1%, 2.2%) for type1 strain, −1.2% (95% CI −3.3%, 0.2%) for type 2 strain and 0.3% (95% CI −3.5%, 3.6%) for type 3 polio virus strain (Table 2). Almost all infants, irrespective of the treatment group,

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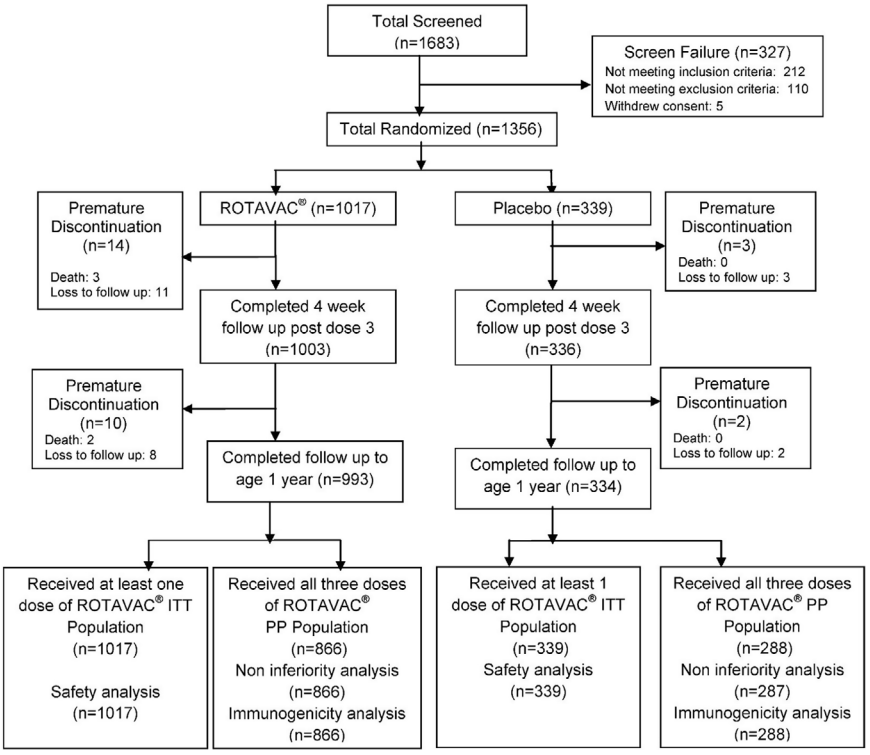

Fig. 1. Trial Profile.

developed protective antibody titer against diphtheria toxoid, tetanus toxoid and Hib (anti-PRP antibodies). Over 93% developed protective titer against HepB (anti-HBs antibodies). The difference in proportion of infants who developed protective antibody titers was 0.5% (95% CI −1.3%, 2.3%) for diphtheria toxoid, 0.9% (95% CI −0.3%, 2.4%) for tetanus toxoid, 2.2% (95% CI −1.7%, 6.0%) for anti-HBs antibodies and 0% (95% CI −1.3%, 1.1%) for anti-PRP antibodies. The ratio of

Table 1. Baseline Characteristics and Compliance to ROTAVAC® or Placebo.

Characteristics	ROTAVAC® (N = 1017)	Placebo (N = 339)
Male, n (%)	520 (51.1%)	183 (54.0%)
Age at dosing (weeks), Mean (SD)		
Dose 1	6.4(0.47)	6.4 (0.47)
Dose 2	10.7 (0.82)	10.7 (0.90)
Dose 3	15.0 (1.00)	15.0 (1.05)
Infants who received n (%)		
Dose 1	1017(100)	339 (100)
Dose 2	956 (94.0)	317 (93.5)
Dose 3	937 (91.5)	307 (90.6)

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GMCs between the placebo and ROTAVAC® groups for pertussis toxin was 1.0 (0.8, 1.1) (Table 2).

4.3. Immunogenicity

The proportion of infants who sero-converted with a ≥4 fold rise in the post vaccination rotavirus antibody titer was 38.6% in the ROTAVAC® group and 12.2% in the placebo group with the difference (placebo-ROTAVAC®) being −26.4% (95% CI −31.2%, −21.2%) (Table 3).

4.4. Reactogenicity and safety of ROTAVAC®

1356 infants (1017 in ROTAVAC® and 339 in placebo group) were monitored for 30 min after each dose for IAE. A total of 5 IAEs were observed; these were cases of vomiting, all mild and occurred at the same frequency in the two groups. One event (0.1%) was considered to be related because of temporality. Almost all infants (98.7%) had at least one AE in the 14 days period after any dose. Most (62.8%) in ROTAVAC® and 63.7% in placebo were mild, while 7.1% in ROTAVAC® group and 4.7% in placebo group were severe. None of the events in

Table 2. Immune Response to Childhood Vaccines by study group in the Per Protocol Population.

Proportion that achieved seroprotective level* (%)	ROTAVAC®	Placebo	Group Difference Placebo-ROTAVAC® (95% CI)
Polio	**N = 866**	**N = 287**	
Type 1	850 (98.2)	284 (99)	0.8 (-1.1, 2.2)
Type 2	861 (99.4)	282 (98.3)	-1.2 (-3.3, 0.2)
Type 3	800 (92.4)	266 (92.7)	0.3 (-3.5, 3.6)
Diphtheria Toxoid	**N = 338**	**N = 285**	
	334 (98.8)	283 (99.3)	0.5 (-1.3, 2.3)
Tetanus Toxoid	**N = 338**	**N = 285**	
	335 (99.1)	285 (100)	0.9 (-0.3, 2.4)
HepB (anti HBs)	**N = 338**	**N = 285**	
	314 (92.9)	271 (95.1)	2.2 (-1.7, 6.0)
Hib (anti-PRP antibodies)	**N = 338**	**N = 285**	
	338 (100)	285 (100)	0 (-1.3, 1.1)
Pertussis (GMC Titer)	**N = 338**	**N = 285**	
	18.5	17.7	GMC Ratio** 1.0 (0.8,1.1)

Per Protocol Population: Subjects who received three doses of ROTAVAC®/Placebo and childhood vaccines concomitantly with no major protocol deviations.

* Polio titer ≥ 1:8 dilution; Diphtheria Toxoid titer >0.1 IU/mL; Tetanus Toxoid titer >0.1 IU/mL; HepB (anti HBs) titer ≥10 mIU/mL; Hib (anti-PRP antibodies) titer ≥0.15 mcg/mL.

** GMC Ratio: GMC Placebo/GMC ROTAVAC®.

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both groups resulted in death. 5 deaths were reported during the follow up period and none of them were considered to be related to the vaccination. No case of intussusception meeting Diagnostic Certainty Level 1 criteria developed by Brighton Collaboration Intussusception Working Group was reported till 1 year of age (Table 4).

5. Discussion and conclusions

The market for rotavirus vaccines is in its early stage of development, with two multinational vaccine manufacturers in positions to participate in this market. ROTAVAC® manufactured by Bharat Biotech International Limited in India has been launched in the public health system in India in March 2016. The data generated from this study, i.e. non-interference of ROTAVAC® with the childhood vaccines and clinical lot consistency in the immune responses to the three production lots of ROTAVAC® along with the phase III efficacy data [5] would be used to apply for WHO prequalification. The age and sex of the study population were similar across the two treatment groups; very few infants (1.25%) did not complete 4 weeks follow up post dose 3. The loss to follow up at the end of one year was 2.1%. Protocol deviations were minimal and generally minor. Dose compliance was high with 91% of infants receiving all three doses of ROTAVAC® or placebo.

Three doses of ROTAVAC® can be safely co-administered with three doses of Pentavalent vaccine and OPV without diminishing the infant's serum antibody

Table 3. Immune Response to Rotavirus-specific Serum IgA Antibody Titers by Study Group in the Per Protocol Population.

Category	ROTAVAC® N = 866 n (%)	Placebo N = 288 n (%)	Group Difference Placebo- ROTAVAC® (95% CI)
4-fold responders	334 (38.6)	35 (12.2)	-26.4 (-31.2, -21.2)
3-fold responders	388 (44.8)	39 (13.5)	-31.3 (-36.2, -25.9)
2-fold responders	474 (54.7)	44 (15.3)	-39.5 (-44.6, -33.9)

Per Protocol Population: Subjects who received three doses of ROTAVAC®/Placebo and childhood vaccines concomitantly with no major protocol deviations.

"N": number subjects in the PP population for each study group.

Percentage: Calculated based on number of subjects for which results are available (n).

4-fold responders: ≥4 fold rise in the RV-specific serum IgA antibody titers from baseline to 28 days post third dose.

3-fold responders: ≥3 fold rise in the RV-specific serum IgA antibody titers from baseline to 28 days post third dose.

2-fold responders: ≥2 fold rise in the RV-specific serum IgA antibody titers from baseline to 28 days post third dose.

The two-sided 95% CIs were estimated by a likelihood score method [Gart 1990] using NCSS software.

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Table 4. Serious Adverse Events after Dose 1 up to 1 year of age by MedDRA Coding.

SOC	SAEs after Dose 1 up to 1 year of age			
	n (%), E			
	ROTAVAC® (N = 1017)	Placebo (N = 339)	Total (N = 1356)	P value*
Cardiac Disorders	1 (0.1%), 1	0	1 (0.1%), 1	1.000
Congenital, Familial and Genetic Disorders	3 (0.3%), 3	0	3 (0.2%), 3	0.578
Gastrointestinal disorders	1 (0.1%), 1	1 (0.3%), 1	2 (0.1%), 2	0.438
General Disorders and Administration Site Conditions	6 (0.6%), 6	1 (0.3%), 1	7 (0.5%), 7	0.688
Infections and Infestations	46 (4.5%), 61	11 (3.2%), 14	57 (4.2%), 75	0.352
Injury, Poisoning and Procedural Complications	4 (0.4%), 6	0	4 (0.3%), 6	0.578
Nervous System Disorders	5 (0.5%), 7	0	5 (0.4%),7	0.340
Reproductive System and Breast Disorders	1 (0.1%), 1	0	1 (0.1%), 1	1.000
Respiratory, Thoracic and Mediastinal Disorders	5 (0.5%), 5	3 (0.9%), 3	8 (0.6%), 8	0.421

Safety population: All randomized subjects receiving at least one dose of ROTAVAC® or placebo, with or without childhood vaccines.
SOC: System organ class.
n: Number of subject with events. Subject is counted only once per SOC or preferred term.
% Percentage is based on number of subjects in the Safety population for each study group (N).
E: Number of all reported events including multiple occurrences.
* All 2 × 2 tables (proportion of subjects with at least one event in particular category) were compared using Fisher's exact test.

response to each component of these vaccines. None of the differences in the seroprotective levels of antipolio type 1, 2 and 3 antibodies seen between the group receiving ROTAVAC® with childhood vaccines and the group receiving placebo with childhood vaccines were statistically significant. The upper limits of the 95% CI for difference in antibody levels achieved against polio viruses, diphtheria toxoid, tetanus toxoid, HepB and Hib PRP was less than 10% and met the pre-established non-inferiority criteria, demonstrating non inferiority of antibody responses to these vaccines in the two study groups. The mean antibody level of antibody to pertussis between the two groups was not significantly different and the ratio of the GMC (placebo/ROTAVAC®) groups and the corresponding upper limit of the 95% CI was less than 2. The non inferiority of ROTAVAC® group over placebo group with respect to concentration levels to pertussis toxin was established.

ROTAVAC® is moderately immunogenic as measured by serum anti rotavirus IgA. This immune response of 38.6% in the vaccine recipients is similar to 40.3% observed in the phase III efficacy trial [5].

ROTAVAC® was well tolerated when administered with other routine childhood vaccines. The five episodes of vomiting observed immediately post dosing were all mild. The solicited and unsolicited AEs in the 14 days following vaccination and

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SAEs did not show any imbalances between the groups. Most of the AEs in this period were considered related to ROTAVAC® or placebo, which is expected as a result of co-administration with routine childhood vaccines. The most common SAEs were lower respiratory tract infections and gastroenteritis.

Of the five deaths, all occurred among recipients of ROTAVAC®; none was judged related to ROTAVAC®. Four of the deaths occurred between 79–141 days after ROTAVAC® administration. One death which occurred 3 days after ROTAVAC® vaccination was an unexplained sudden death.

No case of Intussusception was identified which met Brighton Level 1 criteria.

In conclusion, ROTAVAC® can be safely co-administered with three doses of pentavalent vaccine and OPV without diminishing an infant's serum antibody responses to each component of these vaccines. It is also well tolerated when administered along with the routine childhood vaccines at 6, 10 and 14 weeks of age.

Declarations

Author contribution statement

Temsunaro Rongsen Chandola, Nidhi Goyal: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sunita Taneja, Kalpana Antony, Kiran Bhatia, Iksung Cho: Analyzed and interpreted the data.

Deepak More, Sai D. Prasad, GVJA Harshavardhan, Sudhanshu Vrati: Contributed reagents, materials, analysis tools or data.

Nita Bhandari, Maharaj Kishan Bhan: Conceived and designed the experiments; Wrote the paper.

Krishna Mohan, Tataji Surender Rao: Conceived and designed the experiments.

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Competing interest statement

The authors declare the following conflict of interests: Krishna Mohan, GVJA Harshavardhan and Sai D. Prasad are employees of Bharat Biotech International Limited. Other authors have no conflict of interest.

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Additional information

The clinical trial described in this paper was registered at Clinical Trials Registry - India (http://www.ctri.nic.in) under the registration number CTRI/2014/05/004592.

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