Vaccine 37 (2019) 4407–4413

Contents lists available at ScienceDirect

Vaccine

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

A randomized, open-labelled, non-inferiority phase 4 clinical trial to evaluate the immunogenicity and safety of the live, attenuated, oral rotavirus vaccine, ROTAVAC® in comparison with a licensed rotavirus vaccine in healthy infants

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ARTICLE INFO

Article history:
Received 11 January 2019
Received in revised form 11 April 2019
Accepted 6 May 2019
Available online 6 June 2019

Keywords:
Rotavirus vaccine
nHRV
ROTAVAC
Immunogenicity
Oral vaccine
Diarrhoea
Rotarix
RV1

ABSTRACT

Background: ROTAVAC® (nHRV), derived naturally from the human 116E rotavirus (RV) neonatal strain, was licensed in India in 2015 based on promising results of a phase 3, safety and efficacy vaccine trial. As a pre-requisite for WHO prequalification, we compared the immunogenicity and safety of ROTAVAC® to those of a WHO-prequalified, Rotarix®.

Methods: We conducted a multicentre, open-labeled, randomized phase 4 clinical trial where 464 infants, 6–8 weeks of age were equally randomized to receive as licensed, the complete regimen of ROTAVAC® (3 doses; Group I) or Rotarix® (2 doses; Group II). Antibody responses (serum anti-RV Immunoglobulin A [IgA]) were measured by enzyme-linked immunosorbent assay (ELISA). The primary analysis was an assessment of non-inferiority of ROTAVAC® to Rotarix® for geometric mean concentration (GMC) for infants who received the complete regimen of either vaccine.

Results: The GMC for Group I was 20.4 (95%CI: 17.6, 23.6) and that for Group II was 24.8 (95%CI: 20.3, 30.3), the GMC ratio was 0.82 (95% CI: 0.64, 1.05), thus meeting the non-inferiority criterion. Site-wise analysis of GMC titres revealed that one site had a peculiar pre-vaccination titre affecting only ROTAVAC® post-vaccination GMCs. Seroconversion rates were 35.3% (95%CI: 29.0, 41.9) and 31.0% (95%CI: 25.1, 37.4) for Groups I and Group II, respectively. There was no substantive difference in safety profiles between both vaccines.

Conclusions: The complete regimen of ROTAVAC® demonstrated immunological non-inferiority to the complete regimen of Rotarix® with a clinically acceptable safety profile. Because the demand for RV vaccines is increasing as more countries are expanding their immunization schedules, the lack of need of a buffering agent, low dose volume (0.5 mL), non-interference with other concomitantly administered vaccines, and conformance with WHO-prequalification requirements provide ROTAVAC® the potential for widespread global usage. Post completion of this study, ROTAVAC® is now a WHO-prequalified vaccine.

Clinical Trials Registration: (CTRI Number: CTRI/2015/12/006428).

1. Introduction

The World Health Organization (WHO) recommends that rotavirus (RV) vaccines be included in all national immunization programs and should be considered a priority, particularly in countries in south and south-eastern Asia and sub-Saharan Africa with high mortality rates due to RV gastroenteritis (RVGE) [1]. ROTAVAC® is a neonatal human RV vaccine (nHRV) which is derived from the naturally attenuated and reassorted RV strain, 116E, which has a bovine rotavirus VP4 gene (G9P [11]) [2]. The 3-dose, oral vaccine is manufactured by Bharat Biotech, Hyderabad, India [3,4]. ROTAVAC® was licensed in India in 2015 and introduced into four early adopter states in 2016; it is now implemented in ten states as part of a national scale-up for rotavirus immunization [5]. ROTAVAC® was WHO pre-qualified in 2018,

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https://doi.org/10.1016/j.vaccine.2019.05.069
0264-410X/

enabling the procurement of the vaccine by Gavi, The Vaccine Alliance and UNICEF [6].

In addition to ROTAVAC®, there are currently three other live-attenuated, oral vaccines globally available to prevent RVGE: (i) Rotarix® (GlaxoSmithKline Biologicals, Rixensart, Belgium) containing a human G1P [8] strain, and administered as a 2-dose regimen [7]; and (ii) RotaTeq® (Merck Co., Inc, Whitehouse Station, NJ, USA) containing 5 human-bovine reassortant strains expressing 5 different human surface proteins (G1, G2, G3, G4, and P1A [8]) and administered as a 3-dose regimen [8,9]. (iii) ROTASIIL® (Serum Institute of India Pvt. Ltd., Pune, India) containing 5 bovine-human VP7 reassortants (G1, G2, G3, G4, and G9) is a lyophilized vaccine requiring reconstitution with a liquid diluent [10].

Both Rotarix® and RotaTeq® have been WHO prequalified for several years and introduced in approximately 42 Gavi -eligible countries [11]. Despite financial support from Gavi, The Vaccine Alliance, these vaccines are not affordable for the developing world and supplies are limited [12–14]. Therefore, the timely development of a mature RV vaccine market that includes multiple manufacturers which provide WHO prequalified RV vaccines in sufficient quantities, and competitive fashion is of the uttermost importance.

Post-licensure of ROTAVAC® in India, which was based on a large Phase 3 efficacy study [4], and as required by WHO for prequalification, the clinical trial reported herein was conducted to evaluate non-inferiority of the immunogenicity and safety of the licensed, 3-dose series of ROTAVAC® as compared to those of the 2-dose series of Rotarix® when administered orally to healthy infants in India.

2. Methods

2.1. Clinical trial design and randomization of subjects

An open-labelled, multicentre, phase 4 clinical trial to compare the immune response and safety of ROTAVAC® and Rotarix® was conducted from December 2015 to May 2016 across the following sites in India: (1) Kore Hospital and Medical Research Centre, Belgaum; (2) King George Hospital, Visakhapatnam; (3) Sant Dnyaneshwar Medical Educational Research Centre, Pune; and (4) Cheluvamba Hospital, Mysore. The trial was approved by the National Regulatory Authority – Central Drugs Standard Control Organisation (CDSCO) (India), respective Ethical Committees at all sites and was conducted in compliance with Good Clinical Practices, Schedule Y (Drugs and Cosmetics Act, 2005) and ethical guidelines for biomedical research on human participants (Indian Council of Medical Research, 2006). Infants were screened for eligibility and enrolled after obtaining written informed consent. Exclusion criteria included the presence of diarrhoea or vomiting in the previous 72 h, prior history of fever, and acute or chronic illness. Details of the inclusion and exclusion criteria can be found on (www.ctri.nic.in) as (CTRI Number: CTRI/2015/12/006428). A total of 464 healthy infants (6–8 weeks of age, weighing ≥2.5 kg, and recipients of all indicated EPI vaccines at birth) were randomly allocated in a 1:1 ratio to receive (1) three doses of ROTAVAC® (Group I, n = 232), administered approximately at 6, 10 and 14 weeks of age or (2) two doses of Rotarix® (Group II, n = 232) at approximately 6 and 10 weeks of age, in accordance to the licensed regimen of each vaccine. The randomization list was generated by a third-party agency, Sensaas™, India. Vaccines were labeled with a combination of subject ID, a treatment code, and specific dose number. A copy of the computer-generated randomization list of subject numbers and a decode key were sent to the biostatistician at the end of the study for statistical analysis. The laboratory staff performing the serologic assays remained blinded to all treatment assignments.

2.2. Vaccines

The study vaccine, ROTAVAC®, containing at least 10^5 Focus Forming Units (FFU) of RV strain 116E in 0.5 mL per dose was administered orally without buffering agent (batch number 61DA13002). Approximately 30 min before administration, the liquid, frozen vaccine vials were shifted from −20 °C to room temperature for thawing. The comparator vaccine, Rotarix®, contained a suspension of at least 10^6 median Cell Culture Infective Dose ($CCID_{50}$) of RV strain 89–12 (G1P [8]) per 1 mL dose (batch numbers were XROTA318B3 and XROTA317A2). A vial of the lyophilized vaccine was reconstituted with a liquid diluent in a prefilled oral applicator (provided by the manufacturer) before oral administration.

2.3. Clinical trial objectives/endpoints

The primary objective of the study was to show non-inferiority in antibody response (assessed by measuring serum anti-rotavirus IgA) of the licensed study vaccine (ROTAVAC®) in Group I compared to the comparator vaccine (Rotarix®) in Group II. The comparison of the licensed, complete regimen of ROTAVAC® was done at 28 days post-dose (PD) 3 (at day 84) versus the complete regimen of Rotarix® at 28 days PD2 (day 56). As a secondary objective, the comparison of the partial regimen of ROTAVAC® was done at 28 days post-dose PD2 (at day 56) versus the complete regimen of Rotarix® at 28 days PD2 (day 56). We also compared the safety and tolerability through solicited adverse events (AEs), unsolicited AEs and serious AEs after vaccine administration of each dose in both groups.

The primary endpoints were measurements of geometric mean concentration (GMCs) and seroconversion rates (SCRs). SCR was achieved if the following criteria were met: (1) Infants with a pre-vaccination concentration <20 U/mL achieving a post-vaccination concentration ≥ 20 U/mL, and, (2) Infants with pre-vaccination concentration ≥20 U/mL achieving a 2-fold rise in their post-vaccination concentration. Seropositivity was defined as an anti-RV IgA ≥ 20 U/mL. Serum anti-RV IgA concentration were estimated using an enzyme-linked immunosorbent assay (ELISA) at the Wellcome Trust Research Laboratories, Christian Medical College (CMC), Vellore, India [15]. Briefly, 96 well plates (Costar, Corning) coated with rabbit hyper-immune serum to RV were incubated with purified cell culture lysates (WC3) or mock-infected MA104 cells. Serial dilutions of standard pool of human serum and test sera were added followed by biotinylated rabbit anti-human IgA (Jackson ImmunoResearch Laboratories, West Grove, PA), thereafter developed and absorbance was read at 492 nm. Background corrected optical density values from sample wells were compared with the standard curve and anti-RV IgA concentration was determined based on derived units of IgA arbitrarily assigned to the standard curve. Seropositivity was defined as an anti-RV IgA concentration ≥20 U/ml. This method was used for both ROTAVAC® and Rotarix® immunogenicity studies in the past [16,17].

2.4. Procedures

Infants were screened at 6–8 weeks of age for eligibility into the study after obtaining a written informed consent from the parents/legally acceptable representative. Once enrolled, infants received ROTAVAC® (Group I) on days on days 0, 28 ± 2, and 56 ± 2, or Rotarix® (Group II) on days 0 and 28 ± 2. All other concomitant childhood EPI vaccines were administered accordingly under the supervision of the study personnel. There were no specific instructions to mothers regarding breastfeeding, before or after vaccine administration. Peripheral venous blood samples (3 mL) were

obtained from all infants before vaccination at day 0 (pre-vaccination), day ± 2 (28 days after the second dose of both Groups) and day 84 ± 2 (28 days after the third dose of ROTAVAC®) for Group I only. The sponsor was not involved in the conduct of this trial.

2.5. Reactogenicity and safety

All infants were followed up for safety after each vaccination by documentation of adverse events (AEs) between the administration of the first dose and 28 days after the last dose of vaccine. Infants were observed for 30 minutes post vaccine administration for any signs of vomiting or spitting and immediate adverse reactions. Detailed safety information for any AE, was collected via subject diary card, telephone contact by the study staff and was reviewed at each visit.

Vaccine reactogenicity was documented as events reported within seven days following vaccination. Since all infants received concurrent childhood EPI vaccines, the local solicited AEs included those related to the site of parenteral vaccine administration and General solicited AEs included fever, crying, refusal to feed, diarrhoea, and vomiting. Any 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 the Brighton Collaboration Intussusception Working Group [18]. Serious AEs were reported to the CDSCO and the Ethics Committee and reviewed by an independent Data and Safety Monitoring Board within the stipulated timeline. Medical expenses and hospital visits were covered by the sponsor.

2.6. Statistical considerations

A two-sided *t*-test was used to test for differences in continuous variables between groups. Differences in categorical variables were tested using a chi-square test or Fisher's exact test. The primary analysis was an assessment of non-inferiority of ROTAVAC® (as licensed) to Rotarix®; the criterion for non-inferiority was that a two-sided 95% confidence interval (CI) for the ratio of GMCs (ROTAVAC® GMC divided by Rotarix® GMC) have lower limit >0.5, a criterion which has frequently been used in vaccine trials [19]. The CI for the GMC ratio was estimated by first obtaining a CI for the difference between the means of natural log-transformed concentration and then taking exponentials of the resulting limits. A CI for a SCR was obtained by exact binomial calculations; a CI for the difference between SCRs (ROTAVAC® - Rotarix®) was estimated by a likelihood scores method [20]. A third-party Contract Research Organization, Croissance Clinical Research™, and an independent biostatistician conducted all aspects of the trial including randomization, data management and analysis. Statistical analysis was done using SAS version 9.3 (SAS Institute, Cary, NC, USA) and NCSS 10 (Number Cruncher Statistical Systems, Kaysville, Utah, USA).

For the non-inferiority criterion used in this study, a sample size 432 (216 per group) gives approximately 90% power to show non-inferiority of ROTAVAC® to Rotarix®, assuming a true GMC ratio of 0.8 and standard deviation of 1.5 for ln(concentration). Thus, the study size of 464 would allow for almost 7% loss of data due to dropout, etc. PASS 13 software (Number Cruncher Statistical Systems, Kaysville, Utah, USA) was used for sample size calculation.

3. Results

In total, 464 infants were equally randomized and allocated to receive either vaccine across four sites. Among them, 453 (97.6%) infants were included in the immunogenicity cohort (Fig. 1). There were no known significant ($p < 0.05$) differences in demographic characteristics or before vaccination seropositivity (anti-RV IgA ≥ 20 U/mL) between the groups (Table 1).

3.1. Immunogenicity

The immunogenicity cohort comprised of 453 infants (224 and 229 in Groups I and II, respectively) who completed vaccination and provided post-vaccination sera. The primary objective assessed non-inferiority of ROTAVAC® to Rotarix® as measured by the ratio of post-vaccination GMCs. The GMCs were 20.4 (95% CI: 17.6, 23.6) and 24.8 (95% CI: 20.3, 30.3) for ROTAVAC® and Rotarix®, respectively. The GMC ratio was 0.82 (95% CI: 0.64, 1.05). Since the lower limit of the 95% confidence interval for the GMC ratio was higher than 0.5, we conclude that ROTAVAC® was non-inferior to Rotarix® (Table 2). Proportion of infants achieving seropositivity were 97 (43.3%) for ROTAVAC® and 95 (41.5%) for Rotarix® ($p = 0.70$) (Fig. 2). The SCR was 35.3% (95% CI: 29.0, 41.9) and 31.0% (95% CI: 25.1, 37.4) for ROTAVAC® and Rotarix®, respectively. The SCR difference (ROTAVAC®- Rotarix®) was 4.3% (95% CI: −4.4, 12.9). The SCR difference provide supporting, but not definitive, evidence of non-inferiority.

Among seronegative infants (anti-RV IgA < 20 on day 0), post-vaccination GMCs were 19.6 (95%CI: 16.7, 23.0) and 19.8 (95%CI: 15.9, 24.6) for ROTAVAC® and Rotarix®, respectively. The GMC ratio was 0.99 (95%CI: 0.76, 1.29). SCRs were 42.4% (95%CI: 34.8, 50.2) for ROTAVAC® and 33.9% (95%CI: 26.8, 41.7) for Rotarix®. The SCR difference was 8.4 (95%CI: −2.0, 18.6) (Table 2).

The proportions of infants who achieved fourfold or higher rises in antibody concentration in serum anti-RV IgA from baseline to 28 days PD3 (ROTAVAC®) or 28 days PD2 (Rotarix®) were 25.9% (95% CI: 20.3, 32.1) and 22.3% (95% CI: 17.1, 28.2), respectively. The percentages showing a fourfold were higher in subjects receiving ROTAVAC®, but the difference between groups were not significant by chi-square test.

We also evaluated a partial dose regimen of the study vaccine and compared the post-vaccination immunogenicity of two doses of ROTAVAC® (n = 231) to that noted above of the complete regimen of two doses of Rotarix®, with measurements at 28 days PD2 (day 56) for both vaccines. The GMCs were 17.8 (95% CI: 15.1, 21.0) and 24.8 (95% CI: 20.3, 30.3) for ROTAVAC® and Rotarix®, respectively. The respective SCRs were 24.7% (95% CI: 19.3, 30.8) and 31.0% (95% CI: 25.1, 37.4). No statistical difference was observed.

Since India is a vast country, it is imperative that clinical trial sites be spatially well distributed and as homogenous as possible. To confirm this aspect, we routinely carry out a site-wise analysis of our ROTAVAC® clinical trials. Following a site-wise analysis, we observed a specific difference in pre- and post-vaccination anti-RV IgA responses at one site, Mysore, compared to those for the other three sites. Among infants who completed the study, those at the Mysore site (n = 28 and 29 receiving ROTAVAC® and Rotarix®, respectively), had considerably higher pre-vaccination anti-RV IgA concentration when compared to those at other sites. At the Mysore site, pre-vaccination GMCs were 31.1 (95%CI: 16.8, 57.9) and 46.5 (95%CI: 24.4, 88.5) for ROTAVAC® and Rotarix®, respectively, while the pre-vaccination GMC's at the other sites were in the range 10–13 (Supplementary, Table 1). The GMC at day 84 for Mysore infants who received ROTAVAC® was 12.6 (95%CI: 8.4, 18.9) and was much lower than in infants from the remaining pooled cohort (GMC = 21.8 (95%CI: 18.6, 25.6)). Additional laboratory analysis using different serum aliquots from randomly selected samples from two sites, including the Mysore site, did not reveal any differences in results. Upon re-analysis of serum samples, no errors in the original readings were identified.

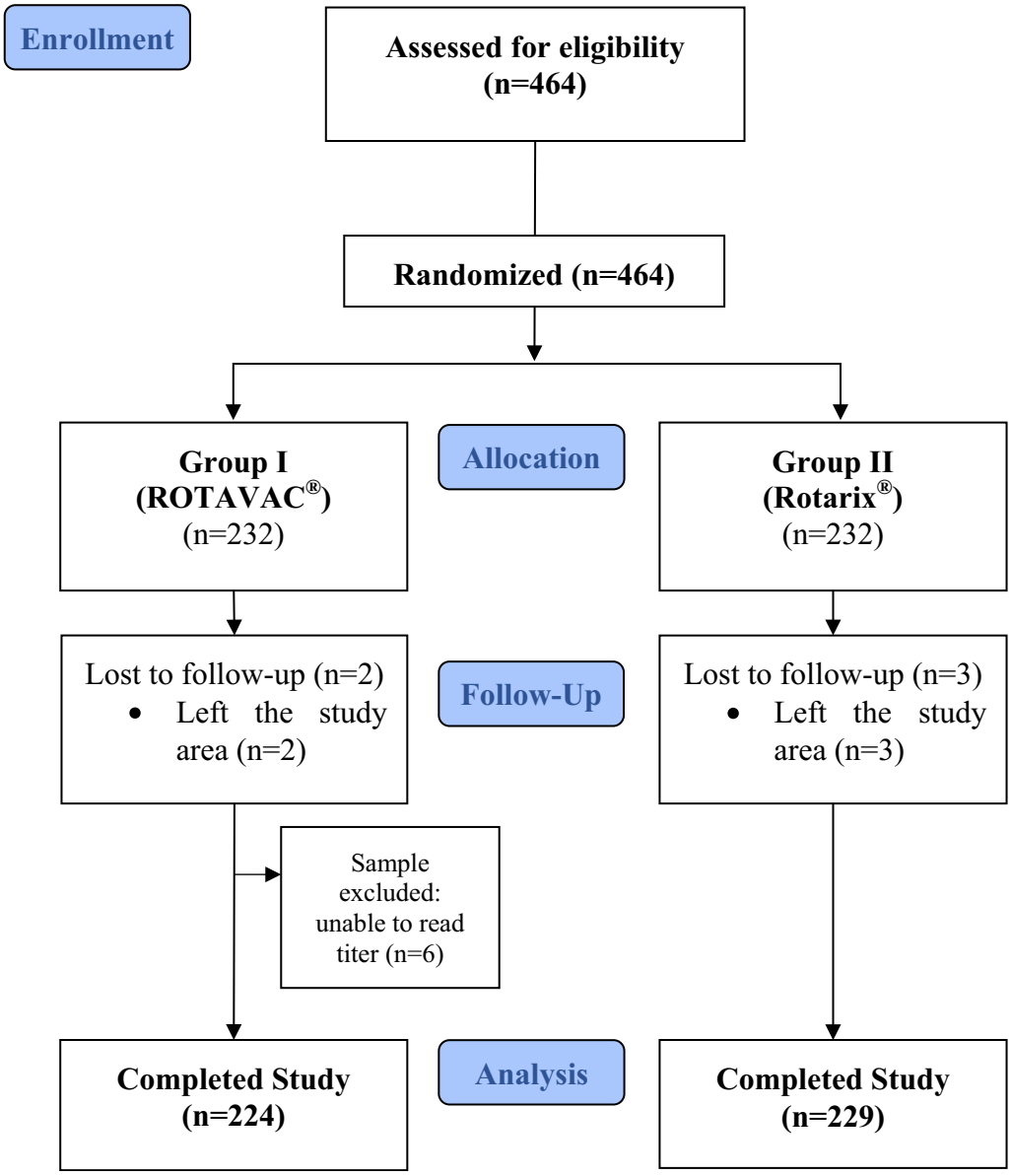

Fig. 1. Enrollment flow chart.

Table 1
Demographics of study participants.

Variable	Measure	ROTAVAC® (n = 232)	Rotarix® (n = 232)	p
Sex (male)	n (%)	108 (46.6)	102 (44.0)	0.5
Age (weeks) (at enrolment)	mean (SD)	6.8 (0.7)	6.8 (0.7)	0.5
Weight (kg) (at enrolment)	mean (SD)	4.2 (0.7)	4.1 (0.6)	0.5
Seropositivity (at day 0)	n (%)	56 (24.1)	64 (27.6)	0.4

Seropositivity was defined as *serum anti-rotavirus Immunoglobulin A* (anti-RV IgA) $\geq$ 20 U/mL; *day 0, serum collected before vaccination of the first dose. Student's t-test (two-sided) was used to calculate differences in continuous variables between Groups. Chi-square test was used to calculate differences in categorical variables.*

3.2. Adverse events

Within the 28-day follow-up after receipt of any dose, a total of 124 and 118 solicited AEs was reported in Groups I and II, respectively. Fever was the most common AE reported followed by tenderness, swelling, and redness at the injection site (from concomitant parenteral EPI vaccine administration). The number of infants reporting at least one solicited adverse event was 96 in each group (p = 1.00). There were no unsolicited AEs, serious AEs, cases of intussusception or deaths reported during the conduct of the clinical trial. The two vaccines had similar safety profiles.

Table 2
Post-vaccination Geometric mean concentration (GMC) and Seroconversion (SCR) comparison of Complete Dose Regimen of ROTAVAC® with Complete Dose Regimen of Rotarix®.

Primary Measure	ROTAVAC® (n = 224)	Rotarix® (n = 229)	GMC ratio/SCR Difference[2](95% CI)	Non-inferiority achieved
GMC	20.4	24.8	0.82	Yes
(95% CI)	(17.6, 23.6)	(20.3, 30.3)	(0.64, 1.05)	
SCR[1] n (%)	79 (35.3) (29.0. 41.9)	71 (31.0)	4.3	–
(95% CI)		(25.1,37.4)	(−4.4, 12.9)	
Baseline seronegative infants	(n = 170)	(n = 165)		
GMC	19.6	19.8	0.99	Yes
(95% CI)	(16.7, 23.0)	(15.9, 24.6)	(0.76, 1.29)	
SCR n (%)	72 (42.4)	56 (33.9)	8.4	–
(95% CI)	(34.8, 50.2)	(26.8, 41.7)	(−2.0, 18.6)	

GMC, geometric mean concentration; CI, confidence interval; Baseline seronegative infants (anti RV IgA GMC < 20 on Day 0). The comparison of the complete regimen of ROTAVAC® was done at 28 days post-dose 3 (PD3) (day 84) versus the complete regimen of Rotarix® at 28 days post-dose 2 (PD2) (day 56). Non-inferiority of the study vaccine was achieved if the lower limit of the two-sided 95% CI for the GMC ratio (i.e., study vaccine divided by comparator vaccine) was higher than 0.5.
[1] SCR was achieved if the following criteria were met: (1) Infants with a pre-vaccination concentration of <20 U/mL achieving a post-vaccination concentration of ≥ 20 U/mL, and,
[2] Infants with pre-vaccination concentration of ≥ 20 U/mL achieving a 2-fold rise in their post-vaccination concentration.

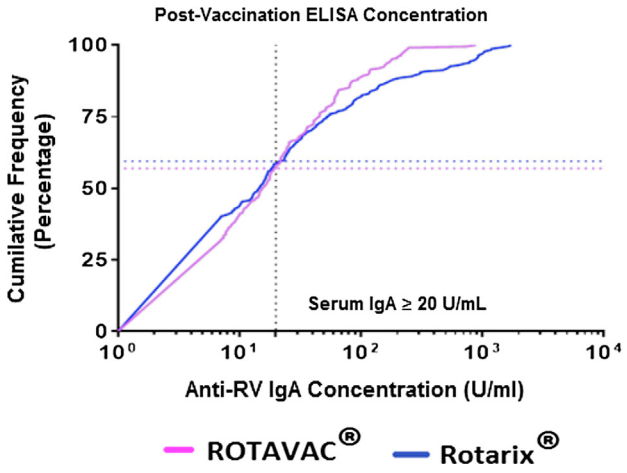

Fig. 2. Post-vaccination cumulative frequency plot comparing serum anti-rotavirus IgA* (log_{10} scale) titers in the two vaccine groups. *Anti-RV IgA, serum anti-rotavirus Immunoglobulin A; The dotted line indicates a seropositivity titer (anti-RV IgA titer ≥ 20 U/mL); Proportion of infants achieving post-vaccination anti-RV IgA titer ≥ 20 U/mL was 97 infants (43.3%) in the ROTAVAC® Group and 95 infants (41.5%) in the Rotarix® Group (p = 0.69). The comparison of the complete regimen of ROTAVAC® was done at 28 days post-dose (PD) 3 (day 84)* versus *the complete regimen of Rotarix® at 28 days PD2 (day 56).*

4. Discussion

Group I (ROTAVAC®) and Group II (Rotarix®) showed comparable immunogenicity and safety profiles, as evaluated under licensed schedule. Regarding antibody response (anti-RV IgA GMC), ROTAVAC® was found to be non-inferior to Rotarix®, and SCRs were fairly equal between ROTAVAC® and Rotarix®.

Unlike our previously conducted ROTAVAC® clinical trials, in this study, we observed a heterogeneity in the seroprevalence data among sites. Because the additional analysis of the serum samples from the Mysore site revealed no laboratory errors at either pre- or post-vaccination time points, to explain the high pre-vaccination concentration we postulate that there may be an increased prevalence of neonatal RV infection. Previous reports from Mysore and surrounding areas have shown the presence of neonatal RV infection (G10P [11] infections [21]). Though there have been no recent reports showing the persistence of neonatal RV infections, the higher pre-vaccination concentration suggests the infants from the Mysore site have had earlier exposure to RV compared to the other sites in the study. Additionally, the enrollment period was simultaneous across all sites and between December-May. Various studies carried out across India have reported peaks in RV diarrhea cases during the winter and spring season [22,23]. The remaining sites did not observe such high pre-vaccination anti-RV IgA concentrations. Thus, to estimate the immune responses to vaccines, we excluded the Mysore cohort in the post-hoc analysis. The post-vaccination GMC was 21.8 (95%CI: 18.6, 25.6) at PD3 and 18.8 (95%CI: 15.9, 22.3) at PD2 in Groups I and II respectively for all 3 sites, excluding Mysore.

A limitation of this study is that we have not followed up the anti-RV IgA antibody concentration, post the required 28 days of PD3 and PD2 for ROTAVAC® and Rotarix®. This would have given us an idea of the antibody decay rates post the mandatory final serum collection time point to assess the immunogenicity of the vaccines. Also, this would have been of value in comparing the decay profiles at different trial sites. Infants in Group I may have had additional exposure to wild type infection in the period 56 days of age to 84 days of age. This may account for the slightly higher GMC in the Group I subjects. Partial dose regimen of ROTAVAC® was less immunogenic than a full dose Rotarix®, albeit no statistical difference was observed. We postulate this difference to be attributable to the difference in log viral vaccine titers.

Low immune responses to oral RV vaccines observed in developing countries may be due in part to high levels of pre-existing RV IgG antibodies transferred to the infant from the mother via the placenta [24]. High concentration of RV IgG have been found to diminish the immune responses of infants to ROTAVAC® (with initial doses) [25]. To address the peculiar finding at the Mysore site, we evaluated maternally transferred RV IgG in a randomly selected subset (n = 16 each) of samples from 2 trial States, Mysore and Belgaum (a city located in the same State as Mysore). Both the sites reported high and similar IgG levels, pre- and post-vaccination.

WHO Immunization guidelines state that RV vaccines are to be administered concomitantly with EPI vaccines: oral polio vaccine (OPV) and diphtheria, tetanus, pertussis, hepatitis B, haemophilus influenza type b (DTP-HepB-Hib) pentavalent vaccine [26,27]. A recent trial showed that ROTAVAC® did not interfere with the immune responses to OPV or the pentavalent vaccine [28]. Our current study protocol ensured infants were vaccinated concomitantly with EPI vaccines. There was no meaningful difference in the number of adverse events reported in the two groups. No confirmed cases of intussusception were identified.

Trials of both Rotarix® and RotaTeq® have shown that vaccine efficacy is higher in developed countries (approximately 85–98%) than in developing countries (approximately 40–50%) [29–35]. Similarly, in India efficacy of ROTAVAC® was 56.4% and 48.9% in the first and second years of life, respectively [3,4]. Co-morbidities, malnutrition, trans-placental maternal IgG (immunoglobulin G) and neutralizing antibodies in breast milk are known factors that affect the

development of a robust RV immune response [25,31,36–39]. Previous immunogenicity studies with Rotarix® in India resulted in post-vaccination GMC of 26.0 (95%CI: 19.3, 34.9) [40] and SCR of approximately 26% [27]. In this study, antibody response in terms of GMC and SCR of infants receiving Rotarix® were comparable to those in previous immunogenicity studies completed in India [17,27,40,41].

In 2016, Gavi funded 40 countries in RV vaccine introductions [42]. Recently, Bangladesh, Nigeria, Democratic Republic of Congo and Pakistan have applied for Gavi support. The Government of India has introduced the vaccine in ten States (as part of their national immunization program) in preparation for a national roll-out [43]. An adequate supply of affordable and efficacious RV vaccines is essential to prevent this disease. In January 2018, ROTAVAC® (nHRV) was granted WHO prequalification status, making it available for purchase by Gavi eligible counties and United Nations agencies [6]. Hence, future large-scale post-introduction studies of the vaccine in other countries could be beneficial to determine the public health benefit.

5. Financial disclosure

Bharat Biotech International Limited was the funding source and was not involved in the analysis of the trial (CTRI Number: CTRI/2015/12/006428). Bharat Biotech International Limited took charge of all the costs associated with the development and publishing of the present manuscript.

Declaration of Competing Interest

The author declares that there is no conflict of interest.

Acknowledgements

All listed authors meet the criteria for authorship set forth by the International Committee for Medical Editors. The authors wish to thank the infants and their families who participated in this trial, all investigators, study coordinators and nurses, and other staff members for contributing in many ways to this study. We are grateful for the significant contributions from by Mr. Siddharth Reddy and, Vamshi Krishna and Mrs. Sandhya Nandala. We thank Dr. Harry Greenberg, Senior Associate Dean, Research, Stanford University, Palo Alto and Dr. Roger Glass, Director Fogarty International Center, Bethesda, U.S.A for insightful discussions and guidance on the analysis of the study results. We thank Dr. Gagandeep Kang, Executive Director, THSTI, Faridabad, India for several discussions and & staff of the Wellcome Trust Research Laboratories, Christian Medical College, Vellore for specimen analysis.

Conflict of Interest Disclosures

E.R and K.M., are employees of Bharat Biotech International Limited. All authors have nothing to disclose according to the ICMJE guidelines for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.05.069.

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