



## Immunogenicity and lot-to-lot consistency of a ready to use liquid bovine-human reassortant pentavalent rotavirus vaccine (ROTASIIL - Liquid) in Indian infants



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### ABSTRACT

**Background:** A lyophilized bovine-human rotavirus reassortant pentavalent vaccine (BRV-PV, Rotasiil<sup>®</sup>) was licensed in 2016. A liquid formulation of this vaccine (LBRV-PV, Rotasiil - Liquid) was subsequently developed and was tested for non-inferiority to Rotasiil<sup>®</sup> and for lot-to-lot consistency.

**Methods:** This Phase II/III, open label, randomized study was conducted at seven sites across India from November 2017 to June 2018. Participants were randomized into four arms; Lots A, B, and C of LBRV-PV and Rotasiil<sup>®</sup> in 1:1:1:1 ratio. Three doses of study vaccines were given at 6, 10, and 14 weeks of age. Blood samples were collected four weeks after the third dose to assess rotavirus IgA antibody levels. Non-inferiority of LBRV-PV to Rotasiil was proven if the lower limit two-sided 95% confidence interval (CI) of geometric mean concentration (GMC) ratio was at least 0.5. Lot-to-lot consistency was proven if 95% CI of the GMC ratios of three lots were between 0.5 and 2. Solicited reactions were collected by using diary cards.

**Results:** Of the 1500 randomized infants, 1436 infants completed the study. The IgA GMC ratio of LBRV-PV to Rotasiil<sup>®</sup> was 1.19 (95% CI 0.96, 1.48). The corresponding IgA seropositivity rates were 60.41% (57.41, 63.35) and 52.75% (47.48, 57.97). The IgA GMC ratios among the three LBRV-PV lots were: Lot A versus Lot B: 1.34 (1.03, 1.75); Lot A versus Lot C: 1.22 (0.93, 1.60); and Lot B versus Lot C: 0.91 (0.69, 1.19). The 95% CIs for the GMC ratios were between 0.69 and 1.75. The incidence of solicited reactions was comparable across the four arms. Only one serious adverse event of gastroenteritis event in the Rotasiil<sup>®</sup> group was causally related.

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**Conclusion:** The immunological non-inferiority of LBRV-PV against Rotasiil® as well as lot-to-lot consistency of LBRV-PV was demonstrated. LBRV-PV had safety profile similar to Rotasiil®.

**Trial registration number:** Clinical Trials.Gov [NCT03474055] and Clinical Trial Registry of India [CTRI/2017/10/010104].

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## 1. Introduction

Rotavirus gastroenteritis is the commonest cause of paediatric diarrhoea and is a major cause of morbidity and mortality in children of developing countries [1]. The World Health Organization (WHO) recommends universal vaccination against rotavirus [2]. Rotavirus vaccines have shown a significant impact on rotavirus related clinic visits, hospitalizations and deaths in many countries [3–7].

A heat-stable lyophilized rotavirus vaccine made by bovine-human reassortant technology (BRV-PV, Rotasiil®) was developed in India after collaboration with the National Institute of Health (NIH), USA. Several clinical studies were conducted on BRV-PV demonstrating safety and immunogenicity of the vaccine [8–10]. The vaccine was also tested in two large studies in India and Niger which proved the efficacy of the vaccine in preventing severe and very severe rotavirus gastroenteritis [11,12]. The vaccine does not interfere with the immune response of the concomitant infant vaccines [13]. It was licensed in India in December 2016 and prequalified by WHO in September 2018.

As a ready to use option to the health care workers, a liquid formulation of the same vaccine (LBRV-PV) was developed. The manufacturing process till bulk stage, serotypes and virus titres in LBRV-PV are identical to Rotasiil®. The vaccine was tested in pre-clinical studies which showed no toxicity. A Phase I study in adults demonstrated the safety [14].

Currently four WHO prequalified rotavirus vaccines are available globally (RotaTeq®, Rotarix®, Rotavac® and Rotasiil®). As of August 2018, 96 countries have introduced rotavirus vaccines [15] and this number is expected to go up.

However, whether the global demand will be met is a big question due to the supply constraints [16]. Therefore development of this follow on vaccine of Rotasiil is encouraging news as the availability of both these vaccines will increase the supply of rotavirus vaccines globally.

The present study was undertaken to bridge the LBRV-PV with Rotasiil® on the basis of immune responses and also to demonstrate the immunological lot-to-lot consistency of LBRV-PV as per the WHO recommendations [17].

## 2. Methods

### 2.1. Ethics

Conduct of the study was in compliance with the Declaration of Helsinki, good clinical practices guidelines and the Indian regulatory rules. The study was approved by the ethics committees of the study sites and the Indian regulatory authorities. Parent(s) of all participants gave an audio-visually recorded written informed consent before their participation in the study. The study population had access to primary health care facilities and referral hospitals.

### 2.2. Study design

This was a Phase II/III multicentre, open label, randomized, active controlled study. The study had two primary objectives: to

demonstrate the immunological non-inferiority of LBRV-PV against Rotasiil® and to demonstrate manufacturing consistency of LBRV-PV by evaluating the immunogenicity of three cGMP lots. The secondary objective was to evaluate the safety of LBRV-PV. The study was conducted at seven sites across India from November 2017 to June 2018.

Three doses of LBRV-PV or Rotasiil® were administered orally at 6, 10 and 14 weeks of age. All participants were followed up till 18 weeks of age.

A total of 1500 participants were randomized in the study to one of the four groups at a 1:1:1:1 ratio to receive either LBRV-PV from one of the three lots (A, B, or C) or Rotasiil®.

### 2.3. Selection criteria

Healthy infants of 6–8 weeks of age were enrolled in the study. Participants with fever, vomiting, diarrhea, or any acute disease at the time of enrolment were temporarily excluded. History of intussusception, abdominal surgery, impairment of immunological function, persistent diarrhea, significant malnutrition or any systemic disorder, congenital abdominal disorders, or allergy to any components of the study vaccines were exclusion criteria for participation in the study.

### 2.4. Investigational products

LBRV-PV (Rotasiil - Liquid, Serum Institute of India Pvt. Ltd., SIIPL) is a liquid, ready to use formulation of live attenuated pentavalent human - bovine reassortant rotavirus vaccine available in plastic ampoules. Each dose contains  $\geq 10^{5.6}$  fluorescent focus units (FFU) each of G1, G2, G3, G4, and G9 serotypes per dose of 2.0 ml. Three batches of LBRV-PV were used: 215E6001, 215E6002 and 215E6003, all with expiry of July 2018.

Rotasiil® (BRV-PV, SIIPL) is a live attenuated, pentavalent human-bovine reassortant rotavirus vaccine. It is available as a lyophilized powder along with 2.5 ml buffered diluent. The powder contains  $\geq 10^{5.6}$  FFU each of G1, G2, G3, G4, and G9 serotypes. One batch of BRV-PV (Batch No. 145O7001, Expiry, November 2019) was used in the study. The buffer diluent contains citrated sodium bicarbonate containing 25.6 gm of sodium bicarbonate and 9.6 gm of citric acid per litre. BRV-PV was administered orally after reconstitution with 2.5 ml of buffer diluent.

If a subject vomited the study vaccine immediately after administration, a new dose was administered. All the participants received the routine Universal immunization programme (UIP) vaccines (DTwP-HepB-Hib vaccine, bOPV and IPV). LBRV-PV and Rotasiil® were transported and stored at 2–8 °C.

### 2.5. Randomization and blinding

The study design was open-label and randomized, hence investigators and parents of participants were aware of the treatment allocations though the laboratory personnel were blinded. A 1:1:1:1 randomization scheme was followed to administer LBRV-PV from each of the three lots or Rotasiil® according to a computer-generated allocation schedule. A validated interactive

web response system (IWRS) was used to assign the randomization numbers.

### 2.6. Immunogenicity assessment

Four weeks after the third dose of study vaccination, a blood sample was collected from each participant. The samples were stored and transported to the laboratory at  $-20^{\circ}\text{C}$ . Serum levels of anti-rotavirus immunoglobulin A (IgA) antibodies were measured by a validated ELISA at the Christian Medical College, Vellore [18,19]. Seropositivity was defined as IgA concentration  $\geq 20$  U/ml.

### 2.7. Safety assessment

There was a close follow up for immediate adverse events and solicited reactions. Participants were observed for 30 min after vaccination for any immediate events. For solicited reactions (diarrhea, fever, vomiting, decreased appetite, irritability, and decreased activity level), the parents recorded any occurrence in the structured diary cards for one week after each dose. Fever was defined as axillary temperature  $\geq 37.5^{\circ}\text{C}$ . There were home visits by field workers after each dose to support diary card recording and to check the participants' health. The participants were also monitored for unsolicited adverse events (AEs), intussusception, and serious adverse events (SAEs) throughout their participation.

Three committees; a protocol safety review team (PSRT), an intussusception adjudication committee (IAC), and an independent data safety monitoring board (DSMB) closely monitored safety events during the course of the study.

### 2.8. Statistical analysis

The primary immunogenicity analysis was based on the per-protocol (PP) population. Two-sided 95% CIs for the ratios of post vaccination GMCs for two groups (GMC LBRV-PV/GMC Rotasiil<sup>®</sup>) were constructed using log normal distribution. The non-inferiority was demonstrated if lower limit of two-sided 95% CI for the GMC ratio (GMC LBRV-PV/GMC Rotasiil<sup>®</sup>) was more than 0.5.

For lot-to-lot consistency, the GMC of the anti-rotavirus IgA concentrations was calculated for each lot of LBRV group. Equivalence was demonstrated if a two-sided 95% CI for the GMC ratios for each pair was within 0.5–2.0.

Number and percentage (%) of seropositivity (IgA  $\geq 20$  U/ml) along with 95% CI using Exact Binomial Method based on Clopper-Pearson method were presented for each vaccine group (three lots, combined LBRV-PV and Rotasiil<sup>®</sup>). The differences were compared using Farrington-Manning Method.

Reverse Cumulative Distribution (RCD) curves for ELISA IgA data (comparison among the three lots of LBRV-PV as well as comparison of LBRV-PV and Rotasiil) were plotted for PP Population and FA population.

All safety analyses (Immediate reactions, Solicited, Unsolicited Adverse Event and SAEs) were summarized by study group over time.

A total of 1500 infants (375 per group) were enrolled, in order to allow for approximately 20% drop out rate. The sample size of 300 evaluable participants per LBRV-PV lot provided at least 94% overall power to achieve the lot-to-lot consistency if the true difference among the three lots was up to 1.1-fold and the  $\text{Log}_{10}$  standard deviation is 0.8. The evaluable sample size of 900 for the LBRV-PV consistency lots combined and 300 for Rotasiil<sup>®</sup> provided at least 99% power to demonstrate the non-inferiority with the non-inferiority margin for GMC ratio as 0.5, one sided  $\alpha = 0.025$ , actual GMC ratio as 1 with  $\text{log}_{10}$  standard deviation as 0.8.

All the analyses were performed using SAS<sup>®</sup> Version 9.2.

## 3. Results

A total of 1570 participants were screened and 1500 eligible participants were randomized. Parents of three participants withdrew consent after randomization and before participants received first dose. 1497 (99.8%) participants received the first dose, 1458 (97.2%) participants received the second dose and 1448 (96.5%) participants received the third dose of LBRV-PV or Rotasiil<sup>®</sup> (Fig. 1).

741 (49.50%) were males while 756 (50.50%) were females. No differences in weight at birth, weight and length at baseline were observed between the combined LBRV-PV group and Rotasiil<sup>®</sup> group. The mean age (SD) at the time of the first dose was 7.0 (0.60) and 6.9 (0.59) weeks in LBRV-PV and Rotasiil<sup>®</sup> groups, respectively (Table 1).

A total of 1497 participants received study vaccine and were the population for safety data analysis. Of these, 61 participants discontinued participation, including 49 (4.4%) in LBRV-PV combined group and 12 (3.2%) in Rotasiil<sup>®</sup> group. 1436 participants were included in the full analysis (FA) and 1435 participants were included in the per protocol population.

All participants received other UIP vaccination as per the national immunization schedule (DTwP-HepB-Hib, bOPV, IPV).

### 3.1. Immunogenicity results

The IgA GMCs were 36.35 (32.57, 40.57) in the combined LBRV-PV group and 30.51 (25.12, 37.06) in the Rotasiil<sup>®</sup> group with a GMC ratio of 1.19 (0.96, 1.48). (Table 2) The non-inferiority was proven as lower limit of two-sided 95% CI for the GMC ratio was  $>0.5$ , the pre-specified clinical limit for non-inferiority.

The rotavirus seropositivity rates in the PP population were 60.41% (57.41, 63.35) in the LBRV-PV group as against 52.75% (47.48, 57.97) in the Rotasiil<sup>®</sup> group. The difference was not statistically significant. Similar results were seen in the FA population (Table 3).

For each paired comparison of the LBRV-PV vaccine groups, the IgA GMC 95% CI ratios were within the equivalence limits of 0.5 and 2. The lot-to-lot consistency between three consecutive production lots of LBRV-PV was thus demonstrated (Table 4). Similar analysis was also conducted for the FA population with the same outcome (not shown).

Reverse Cumulative Distribution (RCD) curves for IgA titres for comparison of LBRV-PV and Rotasiil as well as for comparison among the three lots of LBRV-PV for are presented here for PP Population. (Figs. 2 and 3) The curves do not show any difference in the distribution of IgA titres among different groups.

### 3.2. Safety results

There were 17 immediate adverse events (IAEs), all of vomiting in the 30 min observation period in the LBRV-PV group among at least 3246 doses (0.52%) and none in the Rotasiil<sup>®</sup> group. Of the total 17 such events, 8 happened after the first dose, 3 after the second dose and 5 after the third dose. In one case after the first dose, the subject vomited after receiving the repeat dose as well. There was no recurrence of these symptoms after subsequent doses. All events were mild in severity and brief in duration. The events were assessed as related. No acute hypersensitivity reaction was reported.

The solicited adverse events recorded within the 7 days post-vaccination were generally mild to moderate in intensity. A solicited adverse event was experienced by at least 862 (76.9%) participants in the LBRV groups and 289 (76.9%) participants in the Rotasiil<sup>®</sup> group. Fever was the most commonly reported solicited event in both, the combined LBRV-PV (64.6%) and Rotasiil<sup>®</sup>

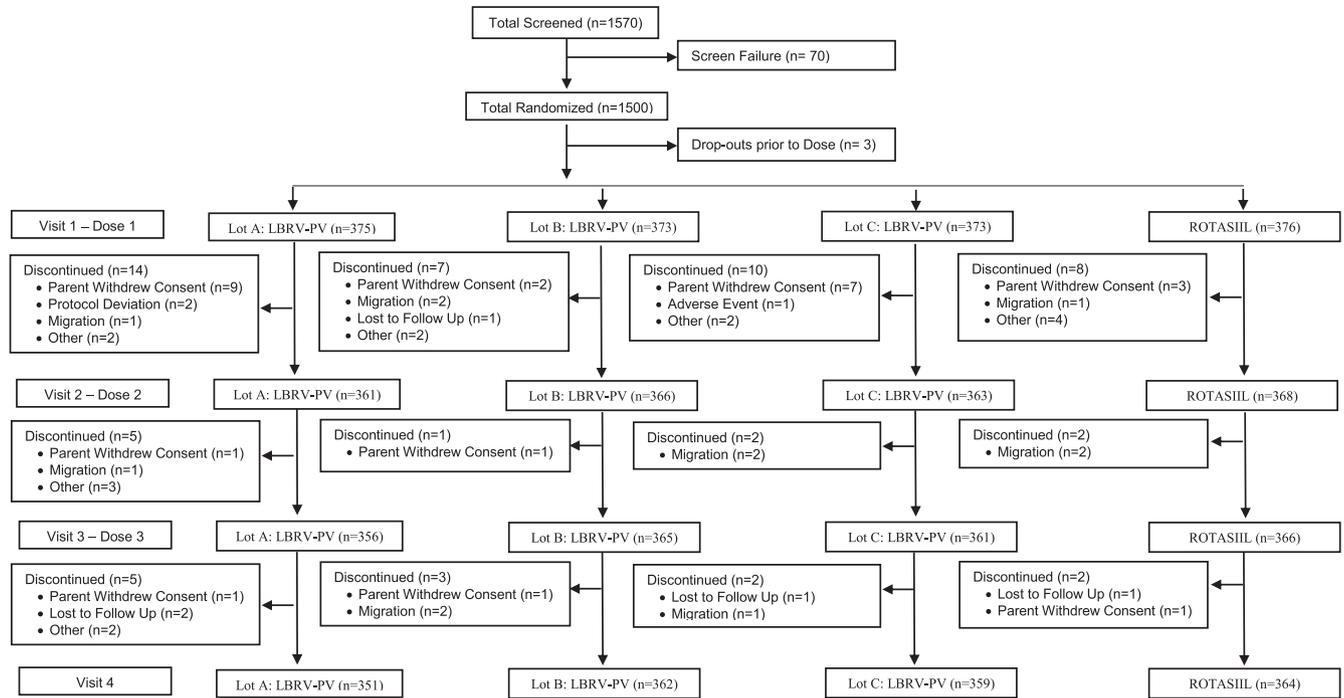


Fig. 1. Study flowchart.

Table 1 Demographics and baseline characteristics (safety population).

Characteristic	LBRV-PV Lot A (N = 375)	LBRV-PV Lot B (N = 373)	LBRV-PV Lot C (N = 373)	LBRV-PV combined (N = 1121)	Rotasiil (N = 376)
Male n (%)	193 (51.5%)	193 (51.7%)	179 (48.0%)	565 (50.4%)	176 (46.8%)
Weight at birth (Kg) Mean (SD)	2.8 (0.41)	2.8 (0.43)	2.8 (0.39)	2.8 (0.41)	2.8 (0.42)
Weight at baseline (Kg) Mean (SD)	4.3 (0.56)	4.3 (0.57)	4.3 (0.55)	4.3 (0.56)	4.3 (0.56)
Length at baseline (cm) Mean (SD)	54.5 (2.22)	54.5 (2.25)	54.6 (2.17)	54.5 (2.21)	54.4 (2.29)
Age at the time of Dose 1 (weeks)	6.9 (0.60)	6.9 (0.59)	7.0 (0.62)	7.0 (0.60)	6.9 (0.59)

Table 2 Comparison of GMCs of anti-rotavirus IgA for combined LBRV-PV and ROTASIIL groups.

Analysis population	Total of LBRV: PV (N = 1121)		ROTASIIL (N = 376)		Treatment group comparison of LBRV: PV & ROTASIIL GMC ratio (95% CI)
	n	GMC of IgA (95% CI)	n	GMC of IgA (95% CI)	
PP	1071	36.35 (32.57, 40.57)	364	30.51 (25.12, 37.06)	1.19 (0.96, 1.48)
FA	1072	36.36 (32.58, 40.58)	364	30.51 (25.12, 37.06)	1.19 (0.96, 1.48)

GMC – geometric mean concentrations, PP – Per protocol, FA – full analysis.

Table 3 Comparison of LBRV-PV and ROTASIIL in terms of seropositivity rate (Concentration ≥ 20 U/ml).

Analysis population	Total of LBRV: PV (N = 1121)		ROTASIIL (N = 376)		Treatment group difference of LBRV: PV – ROTASIIL % Difference (95% CI)
	n	% (95% CI)	n	% (95% CI)	
PP	1071	60.41 (57.41, 63.35)	364	52.75 (47.48, 57.97)	7.66 (1.80, 13.52)
FA	1072	60.45 (57.45, 63.39)	364	52.75 (47.48, 57.97)	7.70 (1.84, 13.56)

PP – Per protocol, FA – full analysis.

Table 4 GMCs and GMC ratios of Anti-Rotavirus IgA among three lots (PP population).

GMC IgA (U/ml)			GMC IgA (U/ml)			Ratio of GMCs	
Group	N	Value (95% CI)	Group	N	Value (95% CI)	Groups	Value (95% CI)
LBRV-PV – Lot A	351	42.86 (35.40, 51.90)	LBRV-PV – Lot B	362	31.94 (26.49, 38.52)	Lot A vs Lot B*	1.34 (1.03, 1.75)
LBRV-PV – Lot A	351	42.86 (35.40, 51.90)	LBRV-PV – Lot C	358	35.24 (29.06, 42.73)	Lot A vs Lot C*	1.22 (0.93, 1.60)
LBRV-PV – Lot B	362	31.94 (26.49, 38.52)	LBRV-PV – Lot C	358	35.24 (29.06, 42.73)	Lot B vs Lot C*	0.91 (0.69, 1.19)

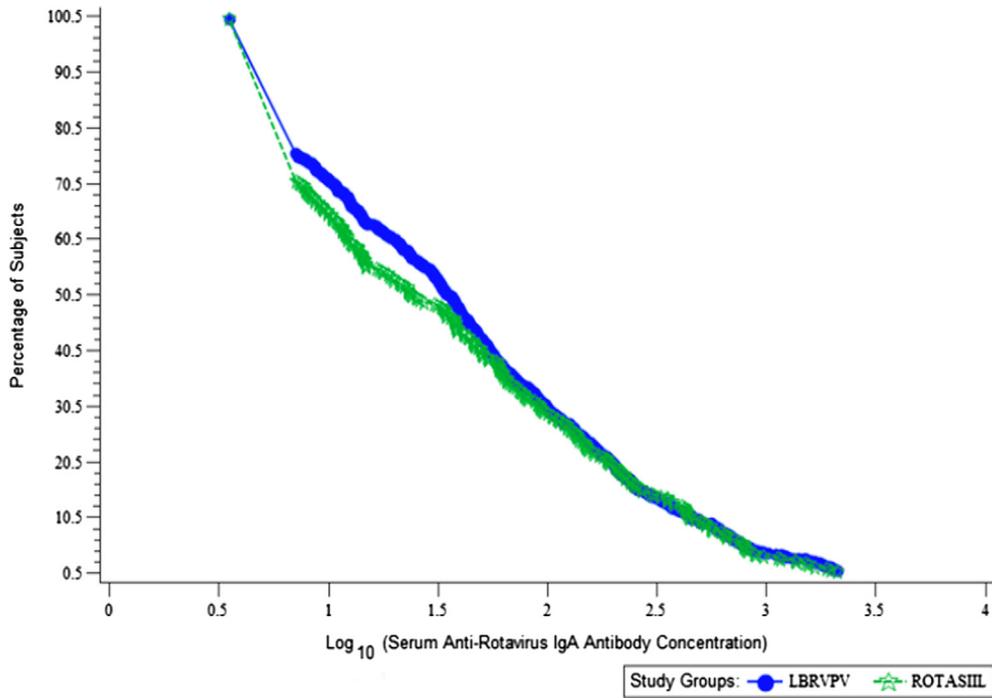


Fig. 2. Reverse cumulative distribution plots for IgA antibody immune response for the comparison of LBRVPV with ROTASIIL – PP population.

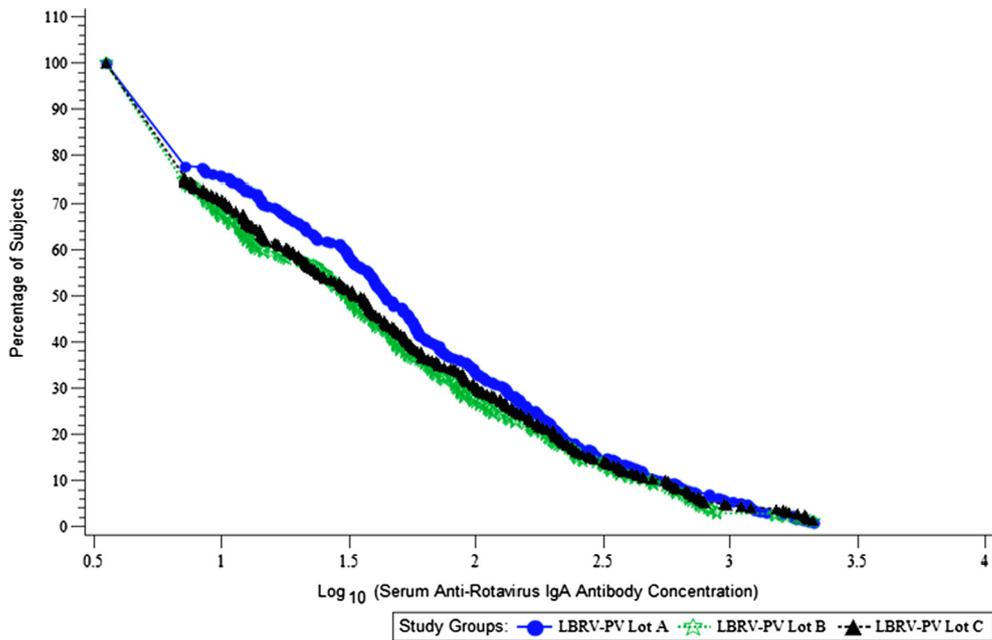


Fig. 3. Reverse cumulative distribution plots for IgA antibody immune response measured by ELISA at 28 days (+4 to +6 weeks) post third vaccination for the comparison among three lots of LBRVPV – PP population.

(66.8%) groups (overall 65.1% of participants). No statistically significant differences in incidence of solicited adverse events were observed between the three lots of the LBRV-PV vaccines ( $p > 0.05$  for all pairs) (Table 5).

The proportion of participants with solicited AEs remained similar LBRV-PV and Rotasiil® (Table 5). A total of 838 participants (55.97%) after the first dose, 758 participants (51.98%) after the second dose and 803 participants (55.45%) after the third dose had at least one solicited AE.

A total of 934 participants (62.39%) experienced 2177 unsolicited events. The incidence of unsolicited AEs was similar for the two groups, with 701 (62.5%) participants reporting 1608 events in the LBRV-PV combined group and 233 (62.0%) participants reporting 569 events in the Rotasiil® group. The most frequently reported unsolicited event was respiratory tract infection ( $n = 193$ , 17.2% in the LBRV-PV group and  $n = 66$ , 17.6% in the Rotasiil® group). Other common events included upper respiratory tract infection and injection site pain at DTWP-HepB-Hib injection site.

**Table 5**

Incidence of solicited adverse events: all three doses combined (number of participants reporting (%), Number of events).

Solicited AE details	Lot A: LBRV-PV (N = 375)	Lot B: LBRV-PV (N = 373)	Lot C: LBRV-PV (N = 373)	Total of LBRV-PV (N = 1121)	ROTASIIL (N = 376)	P value: among LBRV-PV lots	P value: LBRV-PV vs ROTASIIL
Any	285 (76.0%), 1220	294 (78.8%), 1227	283 (75.9%), 1243	862 (76.9%), 3690	289 (76.9%), 1228	0.5668	1.0000
Diarrhea	51 (13.6%), 63	43 (11.5%), 50	42 (11.3%), 48	136 (12.1%), 161	52 (13.8%), 58	0.5722	0.4183
Vomiting	68 (18.1%), 99	64 (17.2%), 81	69 (18.5%), 96	201 (17.9%), 276	52 (13.8%), 76	0.8915	0.0678
Fever	244 (65.1%), 432	240 (64.3%), 441	240 (64.3%), 426	724 (64.6%), 1299	251 (66.8%), 447	0.9777	0.4538
Decreased appetite	123 (32.8%), 165	125 (33.5%), 182	110 (29.5%), 169	358 (31.9%), 516	119 (31.6%), 171	0.4480	0.9490
Decreased activity level	89 (23.7%), 120	88 (23.6%), 133	83 (22.3%), 127	260 (23.2%), 380	84 (22.3%), 127	0.8762	0.7771
Irritability	181 (48.3%), 341	198 (53.1%), 340	198 (53.1%), 377	577 (51.5%), 1058	190 (50.5%), 349	0.3241	0.7659

The majority of events were mild to moderate. All the unsolicited events were unrelated to the study vaccines.

A total of 34 participants (2.27%) reported 35 SAEs in the study. The proportions of participants with SAEs in the study groups were similar with 22 (2.0%) participants reporting 22 events in the LBRV-PV group and 12 (3.2%) participants reporting 13 events in the Rotasiil® group. The frequently reported SAEs were bronchiolitis, lower respiratory tract infection and gastroenteritis. One gastroenteritis event in Rotasiil® group was considered to be related. All SAEs completely recovered by the end of the study. No intussusception cases and deaths were reported.

#### 4. Discussion

This was a Phase II/III, open-label, randomized study to assess non-inferiority of LBRV-PV against licensed Rotasiil® as also to evaluate the lot-to-lot consistency of LBRV-PV in infants. Three doses of LBRV-PV or Rotasiil® were administered at 6, 10 and 14 weeks of age along with UIP vaccines. Both the objectives of the study were met based on pre-defined statistical criteria.

LBRV-PV induced IgA seropositivity rate of 60.41% in the present study. Rotasiil® seropositivity figures were 56.67% in the Phase II study [8], 47% in the Phase III study [9] and 52.75% in the present study. Similar rates were reported for other vaccines when tested in India: Rotarix® 58.3% [19], RotaTaq® 83% seroconversion (the study did not include a placebo arm) [20] and 38.3% to 42.1% with three different formulations of Rotavac [21].

Initially a lyophilized formulation of Rotarix® vaccine was developed and licensed. Subsequently a liquid formulation was developed and compared with the lyophilized formulation in a Phase III non-inferiority study in 1200 infants. The immune response of liquid formulation was found non-inferior to that of lyophilized formulation [22]. The findings in our study are on the similar lines. The GMC ratio of LBRV-PV/Rotasiil® groups was 1.19 (0.96, 1.48), meeting the non-inferiority criteria. Based on the GMC and seropositivity rates, LBRV-PV has a similar immunogenicity profile as Rotasiil®, thus indicating that presentation (lyophilized or liquid) does not matter for the immunogenicity of BRV-PV.

The incidence of solicited and unsolicited AEs and SAEs was similar in LBRV-PV and Rotasiil® groups. Most of the AEs were mild to moderate in severity, transient and resolved without any sequelae. All participants had DTwP-HepB-Hib, bOPV, and IPV vaccines concomitantly. AEs like fever, irritability, decreased appetite, decreased activity, etc., are known to occur with DTwP vaccines and therefore most of the solicited events may be probably caused by this vaccine, rather than by rotavirus vaccines. It is worth noting that in the Phase III study of Rotasiil® in India, the incidence of solicited events in Rotasiil® and placebo arms was similar [12], indicating that these events were actually caused by the concomitant vaccines.

Rotasiil® is a heat stable vaccine and can be stored at or below 25 °C for 36 months. In addition it can withstand temperatures of

37 °C and 40 °C for 18 months and short term exposure to 55 °C. It can also tolerate a temperature shock of being thawed from an extreme cold temperature of –20 °C to a high temperature of 42 °C [10]. However this is possible because it is a lyophilized vaccine. LBRV-PV being a liquid vaccine will be stored between 2 and 8 °C.

To conclude, LBRV-PV was found similar to Rotasiil® in terms of safety and immunogenicity and lot-to-lot consistency of LBRV-PV has also been demonstrated. The liquid presentation will give an additional ready to use option of BRV-PV to the health care workers and will try to bridge the demand supply gap.

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#### Conflict of interest

Dr. Prasad S. Kulkarni, Dr. Sajjad Desai, Dr Dutta Gaikwad, Dr. Jagdish Zade and Mr. Abhijeet Dharmadhikari are employed by SIIPL, which manufactures the LBRV-PV and Rotasiil®.

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