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Effectiveness of an Indigenous Pentavalent, Live Attenuated, Oral Rotavirus Vaccine ROTASIIL® among Children Younger than 2 Years of Age Hospitalized with Acute Gastroenteritis in Seven States of India: 2019-2023

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Abstract

ROTASIIL®, a pentavalent oral rotavirus vaccine licensed in India in 2017, was introduced into the Universal Immunization Programme in 2018. We assessed vaccine effectiveness (VE) against moderate-to-severe rotavirus gastroenteritis (RVGE) in children <2 years using pooled data from two multi-centric observational studies across 32 hospitals in seven states (July 2019–December 2023). A test-negative case–control design estimated adjusted VE (aVE) for three ROTASIIL® doses. Among 2,243 children aged 4–23 months hospitalized with acute gastroenteritis, 638 were ELISA-positive. Overall aVE was 52% (95% CI, 32–66), highest in <6 months was 88%, (CI, 53–97). The aVE against severe and moderate RVGE was 54% (CI, 31-70) and 56% (CI, 11-79), respectively and for at least one dose was 45% (CI, 26–60). Highest genotype-specific aVE was 62% (CI, 29-80) against G1P[8]. A completed series of ROTASIIL® effectively reduced RVGE hospitalizations; protection waned with age, suggesting benefits of timely immunization.

Keywords: pentavalent, oral rotavirus vaccine, effectiveness, ROTASIIL, India

Main text

Rotavirus is the predominant etiological agent of acute gastroenteritis (AGE) in infants and young children worldwide, contributing to approximately one-third of the severe diarrhea cases that result in hospitalization [1]. Children in low- and middle-income countries face a higher risk of diarrhea-associated mortality due to inadequate access to - safe water, improved sanitation, and urgent medical care [2]. According to the Indian National Rotavirus Surveillance Network, prevalence of rotaviral diarrhea among Indian children aged younger than 5 years was 39.6% between the year 2012 to 2014 and, among those with rotavirus infection, 64% had severe or very severe disease [3].

In a systematic review analysing data from 73 countries, the number of deaths from diarrhea in children younger than 5 years of age had reduced by 83%, from an estimated 2.7 million in 1980 to approximately 0.4 million in 2021 [4, 5]. This reduction has been attributed to improved oral rehydration [6] and routine use of rotavirus vaccines [2]. The World Health Organization (WHO) has identified the development and global introduction of vaccines against rotavirus as a strategy to reduce rotaviral diarrhea mortality [7].

RotaTeq® and ROTARIX® were among the first vaccines to receive global prequalification by WHO in 2008 and 2009, respectively. High-income countries with low child mortality have shown effectiveness of 86% for both vaccines in children younger than 1 year of age [7]. In 2009, the WHO recommended introducing rotavirus vaccination in the national immunization schedule for children worldwide. The countries that adopted the vaccine saw a significant reduction in rotaviral diarrheal infections [8]. Among children hospitalized for AGE, the positivity rate for rotavirus dropped from 38% to 23% between 2008 and 2016 in countries with a rotavirus vaccination program, compared to no change in positivity in countries without the vaccine. Additionally, the median age of children with hospitalized rotavirus gastroenteritis (RVGE) shifted from 12 (7-20) months to 15 (9-25) months, following the introduction of the vaccine [9]. However, in low-income countries with high child mortality, rotavirus vaccine effectiveness (VE) for ROTARIX® and RotaTeq® was 63% and 66%, respectively, among children younger than 1 year of age while RVGE-related healthcare encounters decreased by 79%-83% in low-mortality, 58% in medium-mortality, and 48%-69% in high-mortality countries [10].

Two additional rotavirus vaccines from India, ROTAVAC® and ROTASIIL®, were prequalified by WHO in 2018 [11]. ROTASIIL®, a freeze-dried/lyophilized pentavalent rotavirus vaccine produced by Serum Institute of India Private Limited., received WHO prequalification in September 2018 [12]. ROTASIIL® was first introduced in the Universal Immunization Programme (UIP) in India starting with Jharkhand state on April 7, 2018, followed by Gujarat on July 1, 2019, Maharashtra on July 6, 2019, Karnataka on August 26, 2019, and Kerala on September 6, 2019 [13]. Under the directions of the Ministry of Health and Family Welfare (MoHFW), the states of Odisha and Tamil Nadu switched from ROTAVAC® to ROTASIIL® in January 2022.

The recommended dosing schedule of ROTASIIL® under the UIP is 6, 10, and 14 weeks of age for the first, second, and third doses, respectively, four weeks apart, concomitantly with other childhood vaccines [14]. Among Indian children younger than 2 years of age, during the phase III trial of ROTASIIL®, the efficacy over the entire follow-up period was found to be 39.5% against rotavirus-positive diarrhea with a higher efficacy of 54.7% noted against very severe rotavirus cases [15].

Prior to this study, no other studies have assessed the effectiveness of ROTASIIL® in India. Findings from this study will improve our understanding of the impact of this rotavirus vaccine on rotavirus gastroenteritis and support its use in India and worldwide.

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Results

A total of 8,105 children younger than 5 years of age were admitted with AGE at the study hospitals in 32 sites across seven states of India (Figure 1, Supplementary Table S1). Of the 2,660 that were excluded, 2,426 did not meet the specified inclusion criteria, 225 could not be screened, and 9 had incomplete case report forms (CRFs). In total, 5,445 (67.2%) children younger than 5 years of age were enrolled in the surveillance (Figure 2) and 2243 (41.2%) children younger than 2 years were eligible to be included in the VE analysis for a completed series of ROTASIIL® (Figure 3).

Among all the enrolled children younger than 5 years, with a sufficient stool sample to be tested by ELISA for rotavirus, 1,396 (27.9%) tested positive, while 3,600 (72.1%) tested negative. The majority, 2,224 (40.8%) of the enrolled children were infants with a median (IQR) age of 14 (8 to 24) months. The proportion of children who received a completed series of any brand of rotavirus vaccine was 3,349 (61.5%), while 363 (6.7%) and 430 (7.9%) received one and two doses, respectively. About 1,303 (23.9%) of the children were unvaccinated for rotavirus. Most mothers (78.5%) had attained at least a secondary level of education, while 212 mothers (4%) had no formal education. The primary source of drinking water was tap-to-house (47%) among the enrolled children. About 716 (13.1%) were moderately stunted, and 1,016 (18.7%) had severe stunting (Table S2).

Of the 5,445 children enrolled, 1,380 (25.3%) were more than 23 months of age and hence were not included in the VE analysis for completed series of ROTASIIL, along with 1,822 (33.5%), who were excluded for other reasons. A total of 2,243 (41.2%) children were eligible for the primary analysis (Figure 3). Of these, 638 (28.4%) tested positive for rotavirus by ELISA while 1,605 (71.6%) were negative. The overall median (IQR) age in months was 12 (9 to 16), while the same was 13 (9 to 16) and 12 (8 to 16) for cases and controls, respectively. About half, 1,191 (53.1%) of the eligible cohort belonged to the age group of 12-23 months while 1,457 (65%) were males. More than three-fifths, 1,443 (64.3%) belonged to the lower socio-economic status while 995 (44.4%) had tap-to-house as the primary source of drinking water. Most mothers, 1,754 (78.2%) had attained secondary and above level of education while 77 (3.4%) had no formal education. A similar pattern was observed for paternal education. The proportion of children who received a completed series of ROTASIIL was 2,081 (92.8%), while 162 (7.2%) of the children were unvaccinated. About 301 (13.5%) were moderately stunted, and 398 (17.7%) had severe stunting. Among the eligible children, 2,182 (97.3%) were discharged well after treatment, 48 (2.1%) left against medical advice, 5 (0.2%) were transferred out and 8 (0.4%) died due to causes other than AGE (Table 1).

VE for completed series

Among the children aged between 4 and 23 months and vaccinated with a completed series of ROTASIIL® (Figure 3), the unadjusted VE when compared with the unvaccinated children, was 42% (95% CI, 19-58). Adjusting for the age of children, maternal education, stunting, and source of drinking water, the adjusted VE was 52% (95% CI, 32-66) for the same age group. The overall adjusted VE in the children with moderate and severe rotavirus AGE was found to be 56% (95% CI, 11-79) and 54% (95% CI, 31-70), respectively. Children between 4 and 6 months of age had the highest adjusted VE of 88% (95% CI, 53-97), while those aged between 4 and 11 months had a VE of 66% (95% CI, 43-80) (Table 2).

VE for partial series

Children between 3 and 11 months of age who were immunized with a partial series of ROTASIIL® showed a lower aVE of 39% (95% CI, 1-67) when compared to those who were unvaccinated in the same age group (Tables S3). In the other age groups, a partial series of the vaccine did not offer significant protection against the disease.

VE against specific genotypes

The most common circulating genotype observed was G3P[8] (34%), followed by G2P[4] (32%) and G1P[8] (16.9%). The highest adjusted VE of 62% (95% CI, 29-80) was observed against G1P[8], followed by G3P[8] at 56% (95% CI, 28-73) (Table S5).

VE for at least one dose and any severity

The adjusted VE among children between 2 and 23 months of age for at least one dose against hospitalized rotavirus AGE of any severity was 45% (95% CI, 26-60). The highest adjusted VE of 56% (95% CI, 33-71) was observed among children between 2 and 11 months of age (Table S4).

Discussion

With over 2200 vaccine-eligible children enrolled from 32 sites in seven states in India, this study found that ROTASIIL® was 52% effective in preventing moderate-to-severe RVGE hospitalizations among children 4 to 23 months of age. These findings from the first study of ROTASIIL® field effectiveness in India are in line with the pre-licensure evaluations and covers a wide geography with larger number of enrolments. The VE estimates from the study are consistent with the findings for the monovalent ROTARIX® against severe diarrhea from low-middle income countries, like Malawi and Kenya at 64% each [30, 31]. Similarly, VE for ROTARIX® in Zimbabwe, South Africa, and Tanzania was reported between 57% to 61% [32-34]. A 2006 systematic review by Jonesteller et al. found that rotavirus vaccines had a VE of 75% in countries with medium child mortality rates [35]. In contrast, studies conducted in high-income countries with a completed three-dose RotaTeq® and a two-dose ROTARIX® vaccination course reported higher VE values, providing 80% protection against rotavirus hospitalizations [36].

From the present data, a stratified analysis of VE by age showed higher protection in children less than six months of age (88% [95% CI, 53-97]), when compared to other age groups. The lowest protection was noted in the age group of 12-23 months (35% [95% CI, 0-60]). These findings may be due to the waning protective effect of rotavirus vaccine [37]. A lower VE in the same age-group was also noted in countries with medium and high child mortality [35]. The VE in children aged younger than 12 months is predicted to be higher than that in 12-23-month age group as younger children are exposed to fewer infections and receive the rotavirus vaccine which provides an added immunity [8].

The VE estimates for a completed series of the vaccine were higher than for a partial series in this study, as was found in a systematic review of 15 articles but were not reported to be statistically significant in any of these studies [35]. Wider confidence intervals seen in the VE estimates of partial dose(s) of vaccination compared to the completed series were likely due to the smaller sample size, indicating that most children who initiate rotavirus vaccination complete the recommended schedule, also evidenced in the systematic review by Hungerford et al. [39]. In a reanalysis of pooled vaccine effectiveness data from 24 countries with medium to high under 5 mortality rates, VE for a complete series of rotavirus vaccine was 53% compared with 42% for a partial series [40].

The point estimates for VE against severe (54%) rotavirus infection were comparable with those observed for moderate (56%) infection. In a parallel study conducted in Mozambique [10], VE against severe rotavirus infections was notably higher. Likewise, longitudinal data spanning 13 years (2009–2022) from Diallo et al. [41] in the United States reported VE estimates of 80% for moderate and 94% for severe rotavirus infections. Taken together, these observations suggest the significant protective effect of rotavirus vaccination in preventing moderate to severe clinical outcomes.

In our analysis, the highest VE value was reported for G1P[8] (62%) followed by G3P[8] (56%) and both of these G types are included in the vaccine. In the clinical trial of ROTASIIL® in Niger, vaccine efficacy against severe and very severe disease caused by G types included in the vaccine was 61% and

67%, respectively [42]. A pooled analysis conducted across 22 countries with ROTARIX® in their national immunization schedules in Europe, the Americas, Asia, and Africa also showed genotype-specific vaccine efficacy of 96% against G1P[8] [43]. In a systematic review and meta-analysis covering 32 countries spanning low-, middle-, and high-income settings, Cates et al. [44] reported VE for ROTARIX® ranging from 80% to 90% against the G1P[8] strain, which is the strain included in the vaccine and for RotaTeq VE against homotypic strains ranged from 55% to 88%. A study carried out in Taiwan between 2014 and 2017 evaluating the long-term effectiveness of pentavalent and monovalent rotavirus vaccines found no significant differences in the VE across rotavirus genotypes [45].

This multi-centric analysis provides the first estimates of the VE of ROTASIIL® in the UIP of India. By combining data from two similarly designed studies, we leveraged the breadth of this approach to explore associations between various confounders that could influence the VE. This stands as one of the key strengths of the study. The data collected from three different regions of the country helped achieve a comprehensive view of VE across a diverse population. Given the relatively high accuracy of rotavirus diagnostics and the broad similarity of clinical features across multiple enteric pathogens, we were able to employ a test-negative design, using children with rotavirus-negative AGE as the comparison group.

There were limitations to this study. This analysis used data from two studies with different criteria for calculating sample size. However, the classification of cases and controls, as well as methods for vaccination status verification were identical. The study only enrolled children who visited the study sites to seek medical care, which may not be a complete representation of rotavirus disease prevalent in the community. Children who were vaccinated but lacked reconfirmed vaccination information were excluded. These children may differ in other ways from those with documented vaccination; however, their small numbers are unlikely to significantly impact the overall findings.

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Methods

Study design

This analysis of two multi-centric observational studies includes pooled data from the "ROTASIIL® vaccine effectiveness, rotavirus gastroenteritis and intussusception surveillance study in Jharkhand (Study A)" and the "ROTASIIL® vaccine impact assessment study in Kerala, Karnataka, Maharashtra, Gujarat, Odisha, and Tamil Nadu, India (Study B)" conducted across 32 hospitals in seven states within three regions (South, East, and West) of India from July 2019 to December 2023 using harmonised protocols. To ensure adequate statistical power and sample size, the datasets from the two test-negative case-control studies were pooled to achieve a robust estimate of VE. Each surveillance hospital, equipped with in-patient facilities, was responsible for recruiting participants and collecting clinical information and stool samples for testing. Trained staff collected data from parent interviews and clinical documents using similar paper and electronic case report forms at the sites. The treatment for acute gastroenteritis remained unaffected by participation in the study. Details of all sites have been included in the supplementary material (Supplementary Table S1).

Eligibility criteria and data collection

The surveillance included children younger than 5 years of age with AGE admitted to the wards or emergency departments of the study hospitals for a minimum of six hours. AGE was defined as the occurrence of ≥3 episodes of loose stools (stools of a less formed character than usual) within a 24-hour period [16]. For eligible children, sociodemographic, clinical and vaccination data were collected in a pre-designed and structured paper and/ or electronic format by interviewing the parent or caregiver after obtaining a written informed consent. Children with loose stools for more than seven days and/ or those admitted at another hospital for >24 hours before being transferred to the study site, and / or those with onset of illness within two weeks of rotavirus vaccination were excluded from being enrolled. Information on immunization of the child was verified by trained staff at all the surveillance sites using vaccination cards to validate the vaccination status. Queries generated due to the insufficient vaccination information were resolved and updated by reconfirming the status from the source of vaccination, e.g., state immunization portals and registers of the concerned field staff. For the primary VE analysis, children between 16 weeks (2 weeks older than the eligible age to receive all three doses of ROTASIIL®) and 23 months of age who were age eligible to have received ROTASIIL® at the time of its introduction in each participating state were considered. A stool sample was collected within 48 hours of admission, along with a copy of the vaccination card from each enrolled child.

Definition of covariates

A 20-point Vesikari clinical severity scoring system was used to define severity at admission, with a score of 11 or more classified as severe and 15 or more classified as very severe [17, 18]. Anthropometric measurements of the enrolled children were recorded according to the guidelines set by WHO. Their weight was measured using a standardized Salter weighing scale or an analogue weighing machine. For children older than 2 years of age who could stand without support, height was measured using a stadiometer, and for those younger than 2 years old or those unable to stand, their recumbent length was measured using an infantometer. Wasting was assessed using the Anthro Survey Analyzer tool, developed by the WHO's Department of Nutrition for Health and Development [19]. Height for age (HAZ) Z score of ≥-2 standard deviations SD was classified as no stunting, scores between <-2 and ≥-3 SD indicated moderate stunting and a Z-score of < -3 SD was classified as severe stunting. Similarly, wasting (WHZ) and malnutrition (MUAC) Z scores were calculated.

Sample collections and lab procedure

A minimum of 5gm or 5ml stool sample was collected in a screw capped sterile container from each enrolled child and stored at -20 degree Celsius at the study sites before being transported on a regular

basis to the reference laboratory which was blinded to the clinical data including vaccination status. The samples were tested for the presence of rotavirus by validated enzyme-linked immunosorbent assay (ELISA) kits (Rotaclone; Meridian Biosciences, USA). According to the manufacturer's guidelines, the assay has >90% sensitivity and specificity [20]. Genotyping was performed using reverse transcriptase polymerase chain reaction (RT-PCR) on all ELISA-positive samples to identify the G (VP7) and P (VP4) types of rotaviruses [21, 22]. Sanger sequencing was used to evaluate strains that could not be typed for G and P types using standard PCR-based methods [23, 24]. Any sample that tested positive by ELISA but was not typed for both G and P strains and negative for VP6 was considered rotavirus-negative and the child was classified as a control in the study.

Exposure and Outcome Definitions

Among all enrolled children younger than 2 years who were hospitalized with AGE, and with verified vaccination status, those classified as rotavirus positive by ELISA were defined as cases while those classified as rotavirus negative were considered as controls. Children who did not receive any dose of ROTASIIL® were deemed unvaccinated, while those receiving one or two doses were considered partially vaccinated. Children who received three doses were regarded as vaccinated with a completed series and were included in the primary analysis for VE.

Sample size

Jharkhand was the first state to introduce ROTASIIL® in their UIP on April 7, 2018 [25], and the RVEIS study was initiated in December 2018 with approval from the Institutional Review Board (IRB) of the coordinating institute. As the vaccine was introduced in the UIP of other states of India, the RIAS study with the same hypotheses was planned and approved by the IRB of the coordinating institute, to be conducted in the states of Kerala, Karnataka, Maharashtra, Gujarat, Odisha, and Tamil Nadu in September 2020. The selected sites were approached to initiate local ethics committee approvals at their institutes. Since the primary and secondary objectives, as well as the endpoints of both studies, were similar, it was decided to pool the collected data for a combined analysis.

The initial sample size for the study A was based on 80% vaccine coverage for a completed series, a case-to-control ratio of 1:2, and a target VE of 40%. However, due to reduced admissions during the pandemic, a revised sample size was calculated using updated parameters: a detectable odds ratio of 0.6 (VE of 40%), a 1:1 case-to-control ratio, and 90% vaccine coverage among eligible controls. To demonstrate a VE of ≥40%, approximately 437 case patients and an equal number of rotavirus-negative controls were required.

The data collection for the study B was based on a sample size designed to achieve a two-sided 95% confidence interval (CI) with a precision width of 50%. The sample size calculation detected an odds ratio of at least 0.6 (VE of 40%), a 1:2 case-control ratio, and 80% vaccine coverage for age-eligible controls. Using these parameters, the study required approximately 242 case patients and 484 rotavirus-negative controls to demonstrate a VE of ≥40%.

Statistical analysis

As noted in a previous study for ROTARIX® and RotaTeq® [8], the vaccine efficacy against laboratory-confirmed rotavirus diarrhea was higher after completing the full series of the vaccines than receiving one or two doses respectively. So, our study aimed to examine the VE of a completed and partial series of the vaccine. Analyses to describe socio-demographic characteristics, clinical severity of AGE, nutritional status, and outcome were performed for the enrolled children younger than 5 years and children younger than 2 years eligible for VE analysis. Since it has also been noted that maternal antibodies may affect the VE in children younger than 6 months of age [26], we included children between 4 and 6 months of age in the analyses.

A logistic regression model was used to assess the effectiveness of ROTASIIL® against moderate and severe rotavirus gastroenteritis. VE was calculated using the formula VE= (1-Odds ratio) *100. The outcome variable, as described earlier, was binary, indicating the case-control status of the enrolled children. The primary exposure variable was verified ROTASIIL® vaccination status (unvaccinated, partially vaccinated, and completely vaccinated). The analysis included both unadjusted and adjusted models. The adjusted multivariate logistic regression analysis included the following covariates: age of the child [27], maternal education [28], stunting [10], and source of drinking water [29] as observed from previous studies. The estimated adjusted odds ratio was used to calculate VE for completed, partial series, and at least one dose of ROTASIIL® against specific circulating genotypes. 95% CI was calculated for all VE estimates. A *p*-value <0.05 was considered statistically significant. All analyses were performed using Stata version 16.1 (Stata Corp, College Station, Texas, USA).

Ethical consideration

These studies were conducted in accordance with the ethical principles set forth in the World Medical Association Declaration of Helsinki concerning medical research in humans (Revised Brazil, 2013) and under the auspices of the IRB of the coordinating institute. Ethical Committee at XXXX acted as the central ethics committee. Approval from the local ethics committee of each participating site was obtained before the first enrolment.

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Conclusions

This analysis represents the first publication regarding the real-world effectiveness of ROTASIIL®, providing critical evidence to support countries as they decide on rotavirus vaccine introduction or optimization. These findings confirm that the field performance of the vaccine is consistent with pre-licensure evaluations. As noted with the other rotavirus vaccines, a completed series of ROTASIIL® is more effective against severe rotavirus gastroenteritis than partial vaccination. Vaccinated children younger than 6 months of age experienced greater protection against RVGE, with the level of protection decreasing as they got older, but that protection nevertheless persists through the second year of life. Timely vaccination would aid in the reduction of morbidity and mortality caused by rotavirus in the very youngest population. Continuous community-based surveillance is vital to understand rotavirus transmission and vaccine effectiveness.

Tables and figures

Figure 1: Surveillance sites for the vaccine effectiveness evaluation of ROTASIIL® among children younger than 2 years of age hospitalized with acute gastroenteritis in seven states of India: 2019-2023

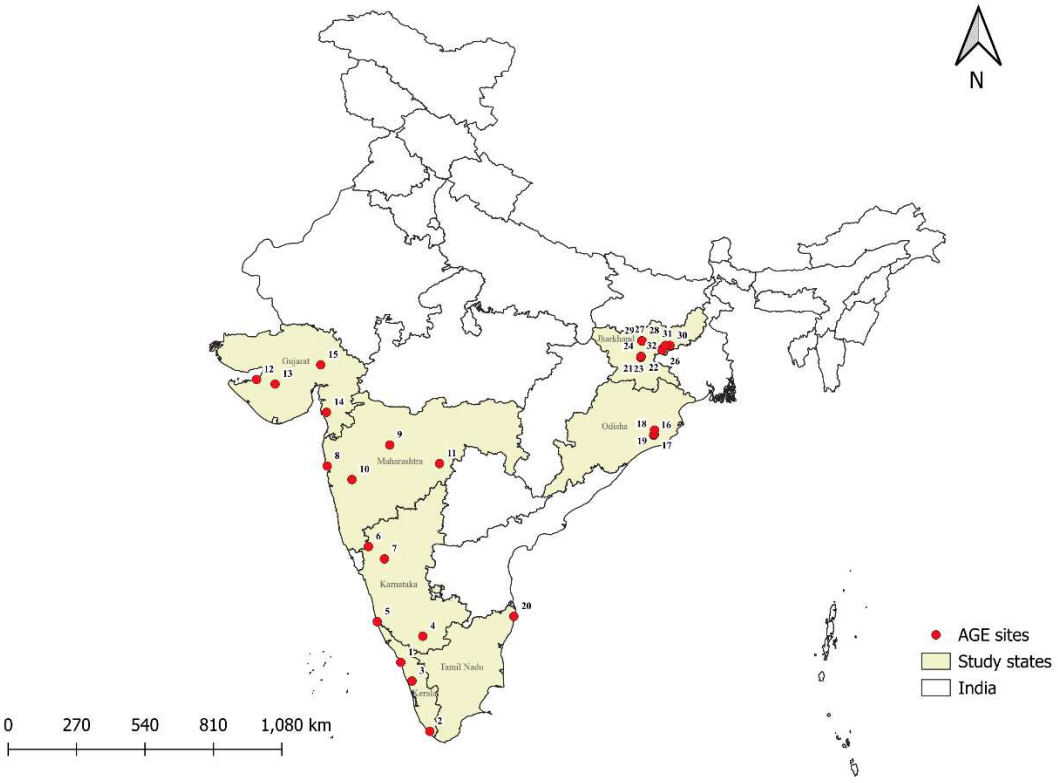


Figure 2: Combined (RVEIS+RIAS) AGE surveillance for rotavirus acute gastroenteritis

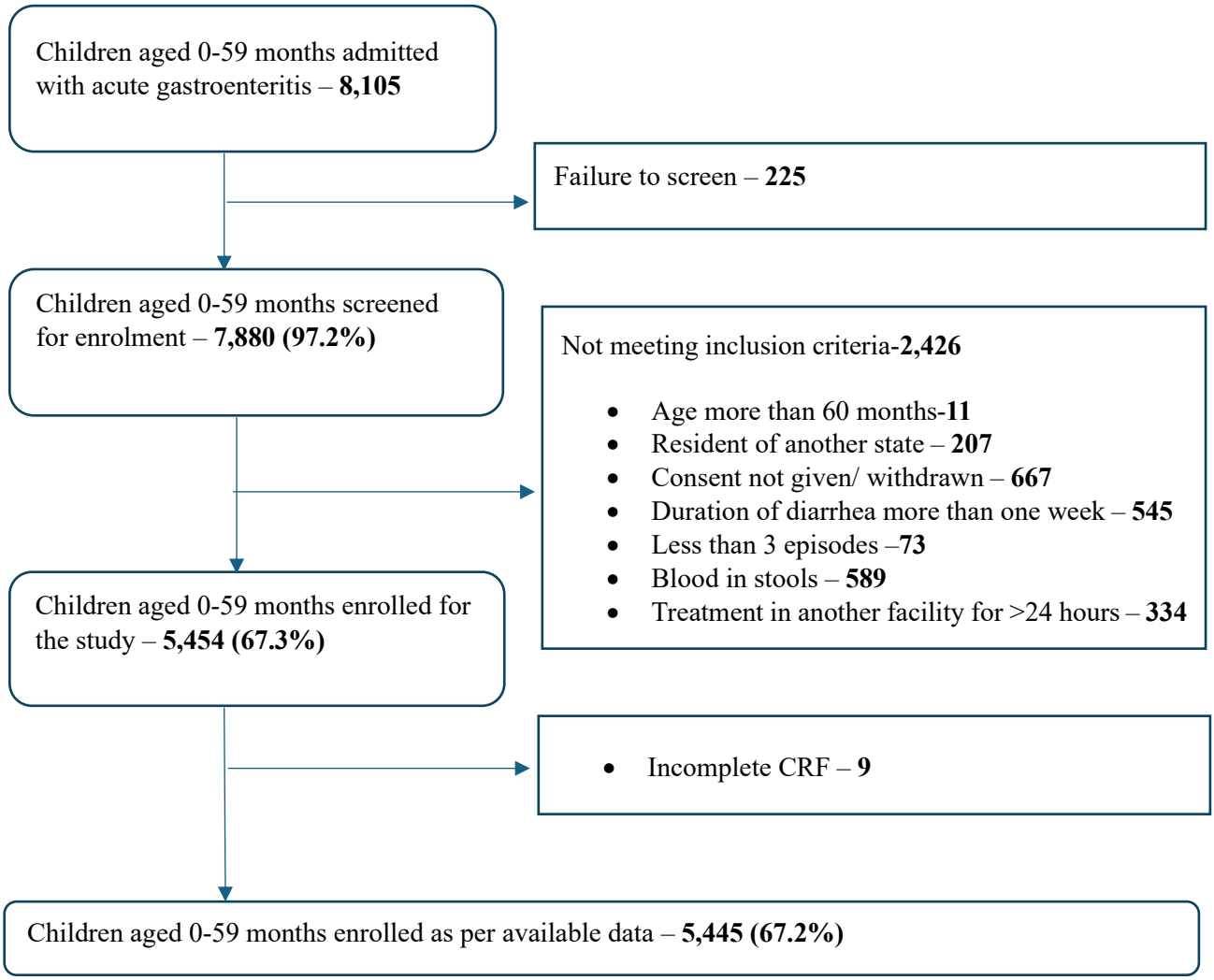


Table 1. Baseline characteristics of children younger than 2 years admitted with AGE eligible for vaccine effectiveness analysis for completed series N=2243

Variable	Number of participants, n (%)	Rotavirus positives, n= 638 (28.4%)	Rotavirus negatives, n=1,605 (71.6%)
Age group			
Median (IQR) age in months	12, (9 to 16)	13, (9 to 16)	12, (8 to 16)
<12 months	1,052 (46.9)	267 (41.9)	785 (48.9)
12-23 months	1,191 (53.1)	371 (58.1)	820 (51.1)
Sex			
Male	1,457 (65)	409 (64.1)	1,048 (65.3)
Female	786 (35)	229 (35.9)	557 (34.7)
Wealth tertile			
Lower	1,443 (64.3)	407 (63.8)	1,036 (64.5)
Middle	469 (20.9)	130 (20.4)	339 (21.2)
Upper	331 (14.8)	101 (15.8)	230 (14.3)
Rotavirus vaccination status			
Unvaccinated	162 (7.2)	64 (10)	98 (6.1)
Vaccinated with three doses	2,081 (92.8)	574 (90)	1,507 (93.9)
Severity of diarrhea based on clinical Vesikari scoring system[a]			
Moderate (7-10)	623 (27.8)	123 (19.3)	500 (31.2)
Severe (11-20)	1,620 (72.2)	515 (80.7)	1,105 (68.8)
Stunting (HAZ Score)			
No stunting (>-2SD)	1,544 (68.8)	434 (68)	1,110 (69.2)
Moderate stunting (<-2 to -3)	301 (13.5)	81 (12.7)	220 (13.7)
Severe stunting (<-3)	398 (17.7)	123 (19.3)	275 (17.3)
Malnutrition (MUACZ score)			
No malnutrition (-2 to 2)	1,782 (79.5)	527 (82.6)	1255 (78.2)
Moderate malnutrition (<-2 to -3)	310 (13.8)	73 (11.4)	237 (14.8)
Severe malnutrition (<-3)	151 (6.7)	38 (6)	113 (7)
Wasting (WHZ scores)			
No wasting (>-2SD)	1,613 (71.9)	479 (75.1)	1,134 (70.7)
Moderate wasting (<-2 to -3)	307 (13.7)	82 (12.9)	225 (14)
Severe wasting (<-3)	323 (14.4)	77 (12)	246 (15.3)
Maternal education (n=2238)			

No formal education	77 (3.4)	21 (3.3)	56 (3.5)
Primary school (up to 5th standard)	407 (8.2)	113 (17.7)	294 (18.4)
Secondary school (up to 10th standard)	601 (26.9)	178 (28)	423 (26.4)
Higher secondary and college (11th & 12th standard)	587 (26.2)	166 (26.1)	421 (26.3)
University and above (Graduate / Postgraduate)	566 (25.3)	159 (25)	407 (25.4)
Paternal education (n=2238)			
No formal education	50 (2.2)	13 (2)	37 (2.3)
Primary school (up to 5th standard)	275 (12.3)	65 (10.2)	210 (13.1)
Secondary school (up to 10th standard)	616 (27.5)	179 (28.1)	437 (27.3)
Higher secondary and college (11th & 12th standard)	612 (27.4)	169 (20.5)	443 (27.7)
University and above (Graduate / Postgraduate)	685 (30.6)	211 (33.1)	474 (29.6)
Source of drinking water (n= 2241)			
Tap to house	995 (44.4)	281 (44)	714 (44.6)
Community tap	330 (14.7)	78 (12.2)	252 (15.7)
Any kind of well	916 (40.9)	279 (43.8)	637 (39.7)
Final outcome			
Survived	2,182 (97.3)	625 (98)	1,557 (97)
Transferred out	5 (0.2)	2 (0.3)	3 (0.2)
Left Against Medical Advice	48 (2.1)	10 (1.6)	38 (2.4)
Died	8 (0.4)	1 (0.2)	7 (0.4)

[a]198 Vesikari scores unavailable

Figure 3: Participants with completed series of ROTASIIL® in the study with available ELISA results and vaccine information included in the vaccine effectiveness analysis

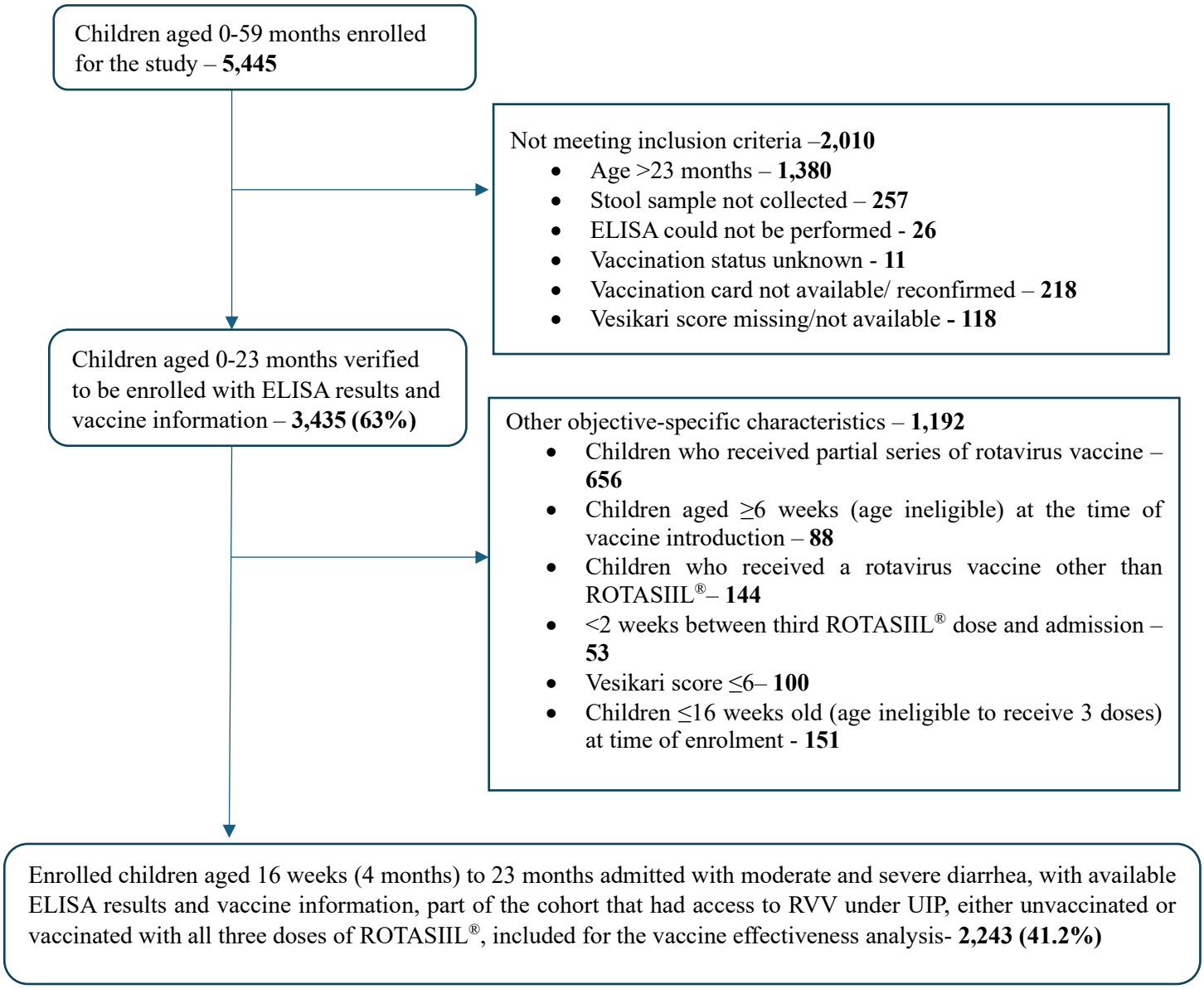


Table 2. ROTASIIL® vaccine effectiveness for completed series against moderate and severe diarrhea (N=2243)

Age groups	Rotavirus positive n (%) 638 (28.4)	Rotavirus negative n (%) 1,605 (71.6)	Unadjusted VE (95% CI)	Adjusted* VE (95% CI)
4–23 months				
0 doses	64 (10.0%)	98 (6.1%)	ref	ref
3 doses	574 (90.0%)	1,507 (93.9%)	42% (19%, 58%)	52% (32%, 66%)
4– 6 months				
0 doses	8 (57.1)	21 (18.4)	ref	ref
3 doses	6 (42.9)	93 (81.6)	83% (46%, 95%)	88% (53%, 97%)
4-11 months				
0 doses	33 (12.4%)	52 (6.6%)	ref	ref
3 doses	234 (87.6%)	733 (93.4%)	50% (20%, 68%)	66% (43%, 80%)
6-23 months				
0 doses	56 (9%)	77 (5.2%)	ref	ref
3 doses	568 (91%)	1,411(94.8%)	45% (21%, 61%)	46 % (22%, 63%)
12-23 months				
0 doses	31 (8.4%)	46 (5.6%)	ref	ref
3 doses	340 (91.6%)	774 (94.4%)	35% (0%, 59%)	35% (0%, 60%)
Moderate diarrhea (Vesikari score 7-10)				
0 doses	15 (12.2%)	38 (7.6%)	ref	ref
3 doses	108 (87.8%)	462 (92.4%)	41% (0%, 69%)	56% (11%, 79%)
Severe diarrhea (Vesikari score 11-20)				
0 doses	49 (9.5%)	60 (5.4%)	ref	ref
3 doses	466 (90.5%)	1,045 (94.6%)	45% (19%, 63%)	54% (31%, 70%)

Funding statement

The study A was wholly supported by the XXXX. The conclusions and opinions expressed in this work are those of the authors alone and shall not be attributed to the Foundation. Under the grant conditions of the Foundation, a Creative Commons Attribution 4.0 License (CCA 4.0) has already been assigned to the Author Accepted Manuscript (AAM) version that might arise from this submission. Please note works submitted as a preprint have not undergone a peer review process.

The study B was supported by XXXX through its internal research fund at XXXX.

Role of the funding source

The funders had no role in study design, collection, analysis, and interpretation of data, writing of the report, or the decision to submit the paper for publication. The corresponding author had full access to all the data from the study and had final responsibility for the decision to submit for publication.

Declaration of interests

All authors declare no conflicts.

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Contributors

Collaborators of the vaccine effectiveness of ROTASIIL®

SUPPLEMENTARY TABLES

Table S1. Details of all participating sites from seven Indian states

State & Date of vaccine introduction in UIP	Site Code	Site Name	Timeline of surveillance	Number of children screened	Number of children enrolled n (%)	Vaccination cards collected n (%)	Stool samples tested n (%)	Samples positive for rotavirus n (%)
Jharkhand April 7, 2018	JH01	Deleted to keep the document anonymised	July 2019 - December 2023	137	63	55	58	15
	JH02		July 2019 - December 2023	843	358	310	313	120
	JH03		July 2019 - August 2021	281	245	240	244	62
	JH04		September 2019 - August 2021	110	102	96	102	62
	JH05		December 2019 - December 2023	588	368	367	363	129
	JH06		February 2020 - August 2021	125	89	89	89	30
	JH07		January 2020 - March 2020	10	9	9	9	6
	JH08		December 2019 - December 2023	130	89	84	73	28
	JH09		December 2019 - December 2023	656	461	412	396	183
	JH10		December 2019 - August 2021	43	41	41	41	26
	JH11		December 2019 - August 2021	128	82	70	72	38
	JH12		January 2021 - July 2021	43	30	26	27	12

Kerala September 6, 2019	KL01		July 2021 - December 2023	655	408	400	392	61
	KL02		February 2021 - December 2022	117	71	63	66	12
	KL03		November 2020 - December 2023	370	187	184	185	42
Karnataka August 26, 2019	KA01		July 2021 - December 2023	345	310	264	284	83
	KA02		March 2021 - January 2022	9	8	8	7	1
	KA03		March 2021 - September 2021	28	28	28	27	0
	KA04		March 2023 - December 2023	131	87	81	87	13
Maharashtra July 6, 2019	MH01		July 2021 - May 2022	62	30	20	20	9
	MH02		December 2020 - December 2023	477	421	355	406	139
	MH05		August 2022 - December 2023	92	90	83	73	20
	MH07		February 2023 - December 2023	48	30	29	30	5
Gujarat July 1, 2019	GJ01		November 2020 - December 2023	739	537	528	534	89
	GJ02		February 2021 - December 2023	401	318	303	288	66
	GJ03		November 2022 - December 2023	315	257	237	234	45
	GJ04		December 2022 - December 2023	163	108	101	93	6
Odisha	OR01		January 2023 - December 2023	262	211	196	160	36

January 2022 (Switch from Rotavac)	OR02		June 2023 - December 2023	224	154	138	128	31
Tamil Nadu January 2022 (Switch from Rotavac)	TN05		March 2023 - December 2023	348	253	238	195	27
Total				**7880**	**5445 (69.1%)**	**5055 (92.8%)**	**4996 (91.8%)**	**1396 (27.9%)**

Table S2. Baseline characteristics of children younger than five years of age admitted with AGE (N=5,445)

Variable	Number of participants, n (%)	Rotavirus positive n=1,396 (27.9%)[a]	Rotavirus negative n=3,600 (72.1%)[a]
Age group			
<12 months	2,224 (40.8)	499 (35.7)	1,583 (44)
12-23 months	1,841 (33.8)	577 (41.3)	1,123 (31.2)
24-59 months	1,380 (25.4)	320 (23)	894 (24.8)
Sex			
Male	3,427 (62.9)	894 (64)	2,249 (62.5)
Female	2,018 (37.1)	502 (36)	1,351 (37.5)
Wealth tertile			
Lower	3,439 (63.2)	845 (60.5)	2,309 (64.1)
Middle	1,130 (20.7)	287 (20.6)	731 (20.3)
Upper	876 (16.1)	264 (18.9)	560 (15.6)
Rotavirus vaccination status			
Unvaccinated	1,303 (23.9)	353 (25.3)	814 (22.6)
Vaccinated with one dose	363 (6.7)	85 (6.1)	259 (7.2)
Vaccinated with two doses	430 (7.9)	120 (8.6)	287 (8)
Vaccinated with three doses	3,349 (61.5)	838 (60)	2,240 (62.2)
Severity of diarrhea based on Vesikari clinical scoring system[b]			
Mild (0-6)	181 (3.4)	20 (1.5)	146 (4.1)
Moderate (7-10)	1,505 (28.7)	263 (20.2)	1,123 (31.9)
Severe (11-20)	3,561 (67.9)	1,020 (78.3)	2,255 (64)
Stunting (HAZ Score)			
No stunting (>-2SD)	3,713 (68.2)	945 (67.7)	2,490 (69.2)
Moderate stunting (<-2 to -3)	716 (13.1)	177 (12.7)	476 (13.2)
Severe stunting (<-3)	1,016 (18.7)	274 (19.6)	634 (17.6)
Malnutrition (MUACZ score)			
No malnutrition (-2 to 2)	4,294 (78.9)	1,156 (82.8)	2,785 (77.4)
Moderate malnutrition (<-2 to -3)	750 (13.8)	161 (11.5)	530 (14.7)
Severe malnutrition (<-3)	401 (7.4)	79 (5.7)	285 (7.9)
Wasting (WHZ scores)			

No wasting (>-2SD)	3,735 (68.6)	992 (71.1)	2,429 (67.5)
Moderate wasting (<-2 to -3)	801 (14.7)	222 (15.9)	519 (14.4)
Severe wasting (<-3)	909 (16.7)	182 (13)	652 (18.1)
Maternal education (n=5426)		**n=1,393**	**n=3,585**
No formal education	212 (3.9)	47 (3.4)	149 (4.2)
Primary school (up to 5th standard)	956 (17.6)	218 (15.7)	684 (19.1)
Secondary school (up to 10th standard)	1,462 (26.9)	383 (27.5)	956 (26.7)
Higher secondary and college (11th & 12th standard)	1,386 (25.5)	365 (26.2)	892 (24.9)
University and above (Graduate / Postgraduate)	1,410 (26)	380 (27.3)	904 (25.2)
Paternal education (n=5425)		**n=1,393**	**n=3,584**
No formal education	160 (2.9)	40 (2.9)	104 (2.9)
Primary school (up to 5th standard)	672 (12.4)	145 (10.4)	487 (13.6)
Secondary school (up to 10th standard)	1,540 (28.4)	383 (27.5)	1,028 (28.7)
Higher secondary and college (11th & 12th standard)	1,423 (26.2)	359 (25.8)	946 (26.4)
University and above (Graduate / Postgraduate)	1,630 (30)	466 (33.5)	1,019 (28.4)
Source of drinking water (n= 5437)		**n=1,395**	**n=3,593**
Tap to house	2,541 (46.7)	624 (44.7)	1,719 (47.8)
Community tap	828 (15.2)	172 (12.3)	549 (15.3)
Any kind of well	2,068 (38.1)	599 (43)	1,325 (36.9)
Final outcome			
Survived	5,255 (96.5)	1,369 (98)	3,484 (96.8)
Transferred out	17 (0.3)	4 (0.3)	11 (0.3)
Left Against Medical Advice	145 (2.7)	22 (1.6)	86 (2.4)
Died	28 (0.5)	1 (0.1)	19 (0.5)

[a]449 stool samples were either not collected or were insufficient for ELISA, [b]198 Vesikari score unavailable

Figure S1. Participants with partial series of ROTASIIL® in the study with ELISA results and vaccine information available

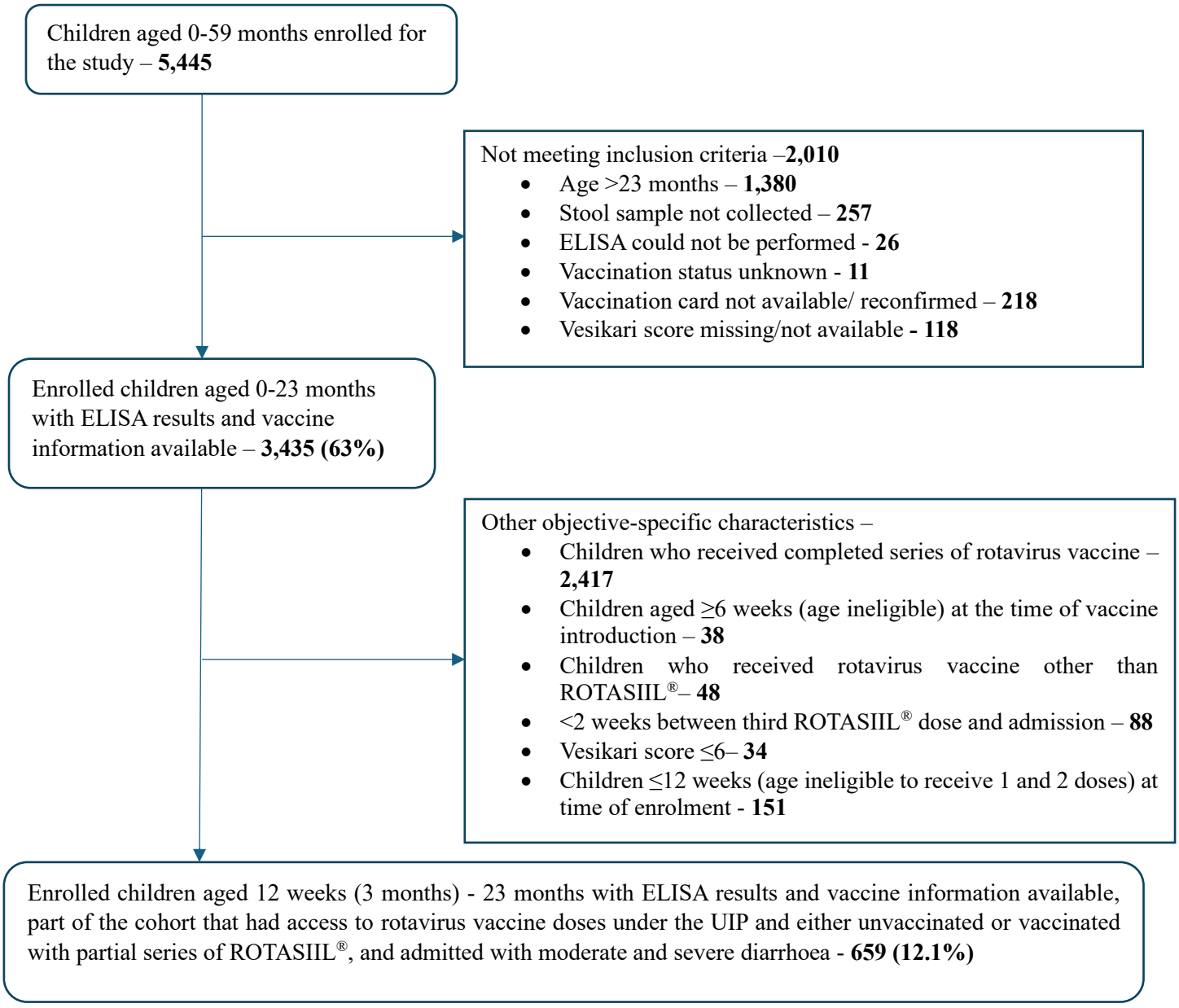


Table S3. Vaccine effectiveness of enrolled children aged between 3 and 23 months admitted with moderate-severe AGE (Vesikari ≥7) who are either unvaccinated or vaccinated with one or two doses or partial series of ROTASIIL® (N=659)

Age groups	Rotavirus positive n (%)	Rotavirus negative n (%)	Unadjusted VE (95% CI)	Adjusted VE (95% CI)
3 – 23 months				
0 dose	69 (34)	123 (27)	ref	ref
1 dose	53 (26.1)	144 (31.6)	34% (0%, 57%)	25% (0%, 53%)
2 doses	81 (39.9)	189 (41.4)	24% (0%, 49%)	26% (0%, 52%)
Partial series	134 (66)	333 (73)	28% (0%, 50%)	26% (0%, 50%)
3 – 6 months				
0 dose	13 (25.5)	46 (24.2)	ref	ref
1 dose	20 (39.2)	72 (37.9)	2% (0%, 56%)	0% (0%, 53%)
2 doses	18 (35.3)	72 (37.9)	12% (0%, 60%)	24% (0%, 67%)
Partial series	38 (74.5)	144 (75.8)	7% (0%, 54%)	9% (0%, 57%)
3 – 11 months				
0 dose	38 (30.4)	77 (22.3)	ref	ref
1 dose	39 (31.2)	117 (33.8)	33% (0%, 60%)	30% (0%, 60%)
2 doses	48 (38.4)	152 (43.9)	36% (0%, 62%)	45% (6%, 68%)
Partial series	87 (69.6)	269 (77.7)	35% (0%, 59%)	39% (1%, 67%)
12-23 months				
0 dose	31 (39.7)	46 (41.8)	ref	ref
1 dose	14 (18)	27 (24.5)	23% (0%, 66%)	32% (0%, 71%)
2 doses	33 (42.3)	37 (33.6)	0% (0%, 32%)	0% (0%, 35%)
Partial series	47 (60.3)	64 (58.2)	0% (0%, 40%)	0% (0%, 46%)
6-23 months				
0 dose	56 (36.8)	77 (28.9)	ref	ref
1 dose	33 (21.7)	72 (27.1)	37% (0%, 63%)	38% (0%, 65%)
2 doses	63 (41.5)	117 (44)	26% (0%, 54%)	29% (0%, 57%)
Partial series	96 (63.2)	189 (71.1)	31% (0%, 55%)	32% (0%, 57%)
Moderate Vesikari clinical score (7-10)				
0 dose	18 (38.3)	51 (30.7)	ref	ref
1 dose	11 (23.4)	45 (27.1)	31% (0%, 71%)	19% (0%, 70%)
2 doses	18 (38.3)	70 (42.2)	27% (0%, 66%)	32% (0%, 73%)
Partial series	29 (61.7)	115 (69.3)	29% (0%, 64%)	27% (0%, 67%)
Severe & very severe Vesikari clinical score (11-20)				
0 dose	51 (32.7)	72 (24.8)	ref	ref
1 dose	42 (26.9)	99 (34.2)	40% (1%, 64%)	34% (0%, 63%)
2 doses	63 (40.4)	119 (41)	26% (0%, 54%)	36% (0%, 62%)
Partial series	105 (67.3)	218 (75.2)	33% (0%, 56%)	36% (0%, 60%)

Figure S2. Participants either unvaccinated or vaccinated with at least one dose of ROTASIIL® with available ELISA results and vaccine information

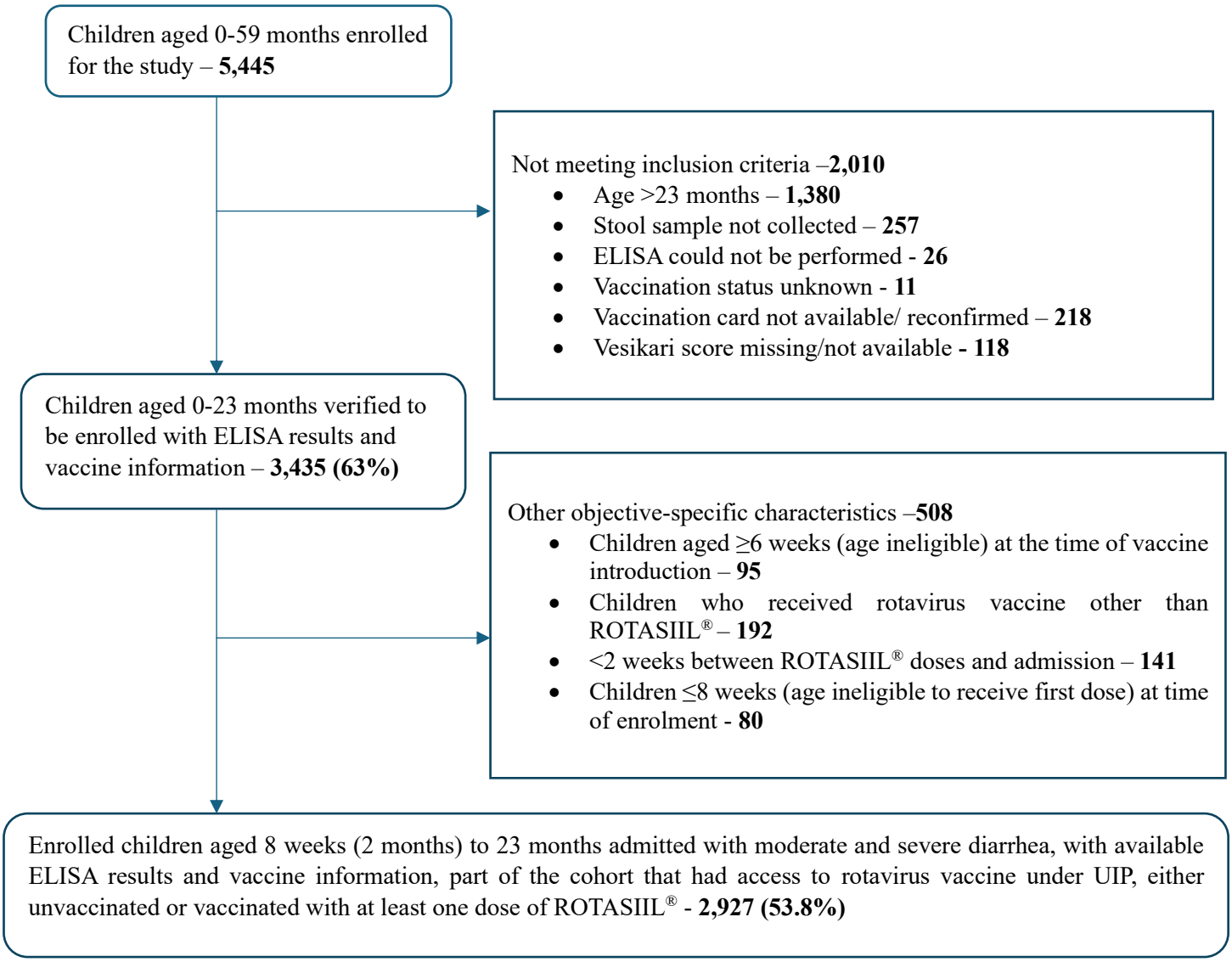


Table S4. Vaccine effectiveness of ROTASIIL® among enrolled children aged between 2 and 23 months admitted with any severity of AGE (Vesikari ≥7) who are either unvaccinated or vaccinated with at least one dose (N=2,927)

Age groups	Rotavirus positive cases n (%)	Rotavirus negative controls n (%)	Unadjusted VE (95% CI)	Adjusted VE (95% CI)
2 - 23 months				
0 doses	78 (9.8%)	174 (8.2%)	ref	ref
At least one dose	720 (90.2%)	1955 (91.8%)	18% (0%, 38%)	45% (26%, 60%)
2 – 6 months				
0 doses	18 (28.6)	93 (25.1)	ref	ref
At least one dose	45 (71.4)	277 (74.9)	16% (0%, 54%)	39% (0%, 69%)
2 – 11 months				
0 doses	47 (12.5%)	124 (10.4%)	ref	ref
At least one dose	328 (87.5%)	1064 (89.6%)	19 (0%, 43%)	56% (33%, 71%)
12-23 months				
0 doses	31 (7.3%)	50 (5.3%)	ref	ref
At least one dose	392 (92.7%)	891 (94.7%)	29% (0%, 56%)	29% (0%, 56%)
6-23 months				
0 doses	60 (8.2%)	81 (4.6%)	ref	ref
At least one dose	675 (91.8%)	1678 (95.4%)	46% (23%, 62%)	47% (25%, 63%)

Figure S3. Participants unvaccinated or vaccinated with all three doses of ROTASIIL® with available genotyping data

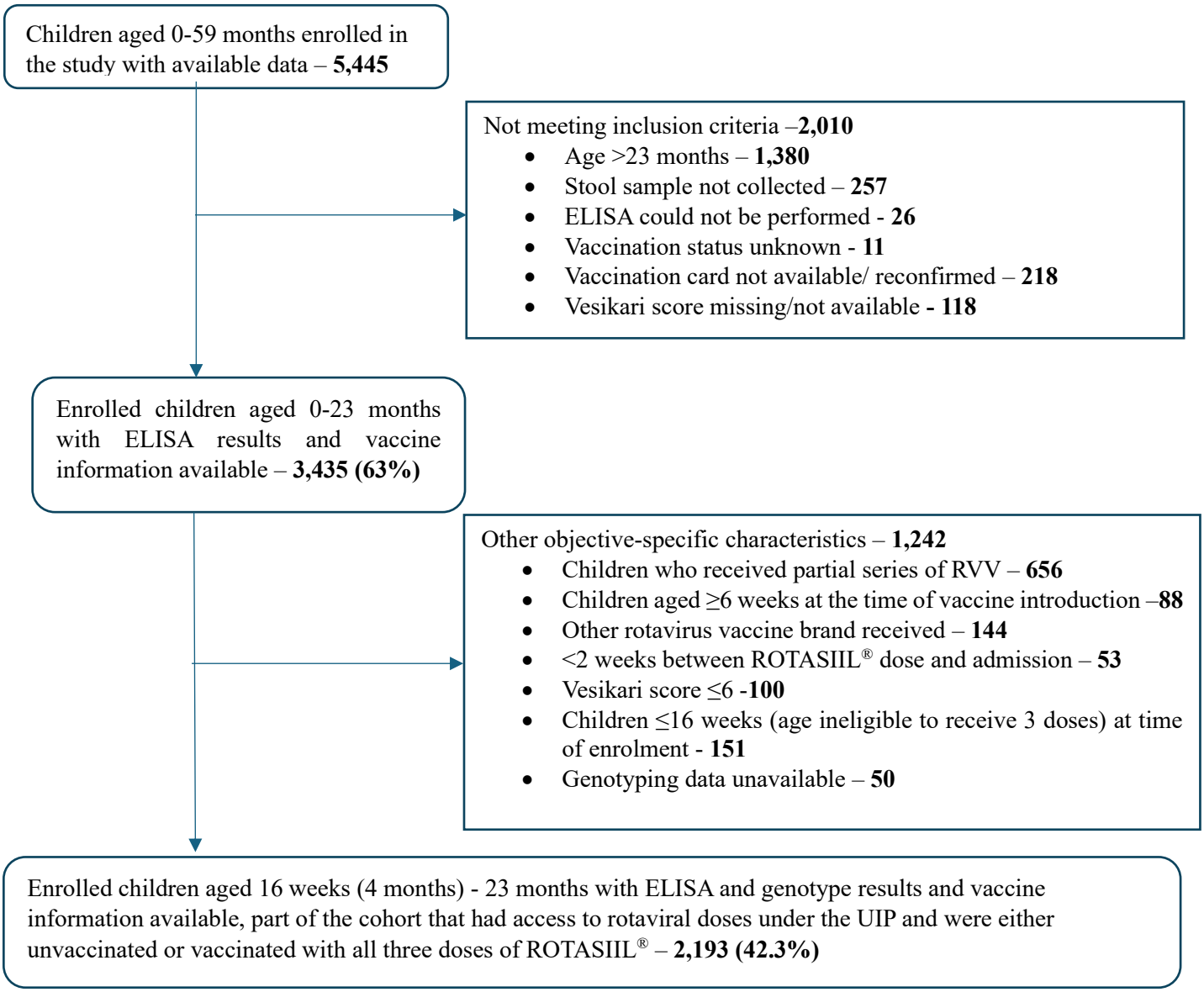


Table S5. Vaccine effectiveness for completed series of ROTASIIL® against specific genotypes in children admitted with moderate and severe AGE (N=2,193)

Circulating genotypes	Rotavirus positive cases n (%)	Rotavirus negative controls n (%)	Unadjusted VE (95% CI)	Adjusted VE (95% CI)
G3P8 (N= 215)				
0 doses	24 (11.2)	97 (6.2)	ref	ref
3 doses	191 (88.8)	1467 (93.8)	47% (16%, 67%)	56% (28%, 73%)
G2P4 (N=201)				
0 doses	18 (9)	97 (6.2)	ref	ref
3 doses	183 (91)	1467 (93.8)	33% (0%, 60%)	43% (2%, 67%)
G1P8 (N= 106)				
0 doses	14 (13.2)	97 (6.2)	ref	ref
3 doses	92 (86.8)	1467 (93.8)	57% (21%, 76%)	62% (29%, 80%)