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Safety and immunogenicity of human neonatal RV3 rotavirus vaccine (Bio Farma) in adults, children, and neonates in Indonesia: Phase I Trial

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ABSTRACT

Background: Despite safe and effective WHO prequalified rotavirus vaccines, at least 84 million children remain unvaccinated. A birth dose schedule of the RV3-BB vaccine was reported to be highly efficacious against severe rotavirus disease in Indonesian infants and is under further development at PT Bio Farma, Indonesia. The aim is to develop a rotavirus vaccine starting from birth that could improve the implementation, safety, and effectiveness of vaccines.

Methods: A multi-site phase I study of a human neonatal RV3 rotavirus vaccine (Bio Farma) in adults, children, neonates in Indonesia from April 2018 to March 2019. The adult and child cohorts were open-labeled single-dose, while the neonatal cohort was randomized, double-blind, and placebo-controlled three-doses at the age of 0–5 days, 8–10 weeks, and 12–14 weeks. The primary objective was to assess the safety of vaccines with the immunogenicity and vaccine virus fecal shedding as the secondary endpoints in neonates.

Results: Twenty-five adults, 25 children, and 50 neonates were recruited, and all but one in the neonatal cohort completed all study procedures. Three serious adverse events were reported (1 adult & 2 neonates), but none were assessed related to investigational product (IP). The neonatal vaccine group had a significantly higher positive immune response (cumulative seroconverted SNA and IgA) 28 days after three doses than those in the placebo group (72% vs. 16.7%, respectively). The GMT of serum IgA in the vaccine group was significantly higher at post IP dose 1 ($p < 0.05$) and post IP dose 3 ($p < 0.001$) compared to the placebo group.

Conclusion: The trial results show that the RV3 rotavirus vaccine (Bio Farma) is well tolerated in all participant cohorts (adults, children, and neonates). Three doses of this vaccine administered in a neonatal schedule were immunogenic. These promising results support further clinical development of the RV3 rotavirus vaccine (Bio Farma).

1. Introduction

Since the World Health Organization (WHO) recommendation in 2009 that all nations should include rotavirus vaccines in National Immunization Programs (NIP) [1], the morbidity and mortality caused by rotavirus have been dramatically reduced [2].

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Rotavirus-caused deaths among children under five years of age have decreased from 528,000 in 2000 to 215,000 deaths in 2013 [3]. Currently, 106 countries have introduced rotavirus vaccines in their NIP. Despite safe and effective rotavirus vaccines, at least 84 million children remain unvaccinated, even in countries that have been introduced in their NIP [4]. WHO estimated global rotavirus vaccine coverage in 2019 was 39%, which was still far behind other basic immunizations [5]. Globally, barriers to implementations of rotavirus vaccine vary, including vaccine cost, limited perception of the rotavirus severity among family, authorities, and health providers, the timing of administration, safety concerns, particularly intussusception risk, and religious barriers [6]. Among the Indonesian predominant Muslim population, the halal status of the vaccine was found to be a concern in rotavirus vaccine acceptance after the cost and knowledge barriers [7].

Rotavirus is associated with a significant burden of diarrheal disease in children < 5 years of age in Indonesia. Sixty percent of 2,240 diarrhea-related hospitalizations in children under five years of age were rotavirus-positive in a 2016 prospective surveillance study involving six provinces of Indonesia. In addition, there was a significant burden of disease in children (majority < 2 years of age) treated in the outpatient setting, with 41% of the 176 children attending with gastroenteritis testing rotavirus positive [8]. Rotavirus vaccines (RotaTeq® and Rotarix®) are licensed in Indonesia and available in the private market with low uptake; however, rotavirus vaccines are not yet available in the Indonesian NIP.

Rotavirus vaccination starting from birth may offer essential benefits, including providing early protection against severe rotavirus gastroenteritis in endemic countries, the opportunity for higher coverage when included in earlier immunization schedules, and a likely safety benefit, as intussusception is rare among newborns [9–11]. Providing an oral rotavirus vaccine at birth also avoids possible barriers to vaccine take presented by a complex gut microbiome, breast milk antibodies, and gastric acidity. A neonatal schedule for a rotavirus vaccine can be more cost-effective compared to an infant schedule [10].

The RV3 rotavirus vaccine (Bio Farma), manufactured by PT Bio Farma, Bandung – Indonesia, has been developed from the RV3-BB rotavirus vaccine under license from Murdoch Children Research Institute (MCRI) Melbourne – Australia, through technology transfer. The oral RV3-BB rotavirus vaccine was developed from the human neonatal rotavirus strain RV3 (G3P6), which was identified in the stool of a healthy newborn [12]. A Phase IIb study conducted in Yogyakarta & Central Java provinces - Indonesia, demonstrated that three doses of RV3-BB vaccine were safe, immunogenic, and efficacious in both a neonatal schedule and in an infant schedule [13]. The protective efficacy against severe rotavirus gastroenteritis provided by the neonatal schedule (dose 1 at 0–5 days of age, dose 2 8–10 weeks; dose 3 14–18 weeks) was 94% (95% CI: 56–99) at 12 months of age and 75% (95% CI: 44–91) at 18 months of age [13]. Based on this data, the RV3 vaccine produced by Bio Farma has the potential to make a significant impact on the high rotavirus disease burden and deaths in children in Indonesia.

PT Bio Farma has optimized the large-scale development of the RV3 rotavirus vaccine (Bio Farma) using a process free of porcine material. Despite modifications needed to convert to a large scale and adhere to halal requirements, the analytical comparison (genetic and antigenic/phenotypic) between RV3 rotavirus vaccine (Bio Farma) and RV3-BB vaccine (MCRI) has shown to have>99% genetic homology. The provision of a rotavirus vaccine free of porcine products in the manufacturing process is essential, especially among countries with predominantly Muslim populations. Current rotavirus vaccines manufactured using porcine trypsin are permissible in a number of countries due to the urgent need and the limited availability of halal products for use in the manufacturing process [14].

Rotavirus is a moderately acid-labile virus that can be inactivated at an acid pH [15,16]. Hence most oral rotavirus vaccines contain a component to buffering acid within the formulation or use antacid before vaccine administration to protect the vaccine virus in the passage through the stomach [15,17–19]. However, rotavirus vaccine administration without buffer agent is well tolerated and immunogenic compared to vaccine administration with a buffering agent [20,21]. Newborns have a relatively neutral gastric acidity [22]. Therefore, an oral rotavirus vaccine delivered at birth may not require acid neutralization. Pre-administered breast milk or formula feeding might provide a more practical approach to vaccine administration in developing countries. Furthermore, our previous study suggests that breast milk does not significantly impact vaccine take after 3 doses of RV3-BB [23].

The ultimate aim of this study is to develop an affordable, safe, effective, and porcine-free human neonatal rotavirus vaccine with no pre-dose antacid to protect against severe rotavirus gastroenteritis from birth. The primary objective of this phase I trial was to assess the safety of RV3 rotavirus vaccine (Bio Farma) in adults, children, and neonates, with the assessment of vaccine virus fecal shedding and immunogenicity as the secondary endpoints in neonates.

2. Materials and methods

2.1. Trial design

This study was a single-center phase I study comprising three age de-escalating stages, starting from healthy adults, progressing to children, then neonates. A Data and Safety Monitoring Board (DSMB) reviewed the results of each stage before permitting to proceed to the next stage. The adult and child cohorts were open-labeled, receiving a single dose, while the neonatal cohort was randomized, double-blind, placebo-controlled, and received three doses. The protocol was approved by the Medical and Health Research Ethics Committees at Faculty of Medicine, Public Health and Nursing of Universitas Gadjah Mada (KE/FK/0086/EC/2018), the National Agency of Drug and Food Control, Republic of Indonesia (B-PN.01.06.313.04.18.01367). It had been registered on ClinicalTrials.gov (registry number: NCT03462108).

The study was conducted from April 2018 to March 2019 at five primary health centers and five hospitals in Klaten, Central Java, Indonesia. The trial was held in compliance with International Conference on Harmonization Good Clinical Practice (ICH-GCP) guidelines and was monitored by a contract research organization. An independent DSMB regularly reviewed the safety data. PT Bio Farma funded the trial and, along with the Center for Child Health at the Universitas Gadjah Mada (CCH UGM), developed the trial design in consultation with MCRI. CCH UGM performed trial, data management, and statistical analysis. All the authors reviewed the manuscript for the accuracy and completeness of the data and analysis.

2.2. Study participants

Twenty-five adults between 18 and 40 years old, 25 children between 2 and 5 years old, and 50 neonates were enrolled. The study was explained to the participants or the parent/guardian with the written study information before recruitment, and written informed consent was sought before eligibility screening was performed. Inclusion criteria for adults and children were clinically healthy by medical history and physical exams; for adult cohort, additional screening laboratory examinations were performed, including blood biochemistry, hematology, urinalysis, chest x-ray, and electrocardiogram. Inclusion criteria for neonates were 0–

5 days of age, clinically healthy by medical history and physical exams, born full-term, and had a birth weight of 2,500 to 4,000 g. For the neonatal cohort, pregnant women were approached from approximately 32 weeks gestation and provided with written information and preliminary consent before collecting cord blood samples at birth. Final written informed consent was obtained from the parent or guardian after birth before the eligibility screening was performed.

2.3. Investigational products

Live attenuated human neonatal strain RV3 (G3P6) rotavirus vaccine, produced with a recombinant enzyme and recombinant bovine trypsin, was prepared to 1 mL dose with > $5x10^6$ fcfu/mL in a 30% sucrose/Dulbecco's Modified Eagle Medium (DMEM) cell culture media. The placebo was a 1 mL solution of 30% sucrose/DMEM and visually indistinguishable from the RV3 rotavirus vaccine (Bio Farma). The RV3 rotavirus vaccine and placebo were manufactured at PT Bio Farma (Bandung, West Java, Indonesia) following Good Manufacturing Practice (GMP) and supplied in cryovials stored and monitored at −20° C (≤−18° C) at an access-restricted trial pharmacy site. After being thawed, investigational products (IPs) were stored before administration with a maximum of 6 h at 2–8° C and a maximum of 20 min at room temperature.

2.4. Randomization and masking

Randomization was only applied to the neonatal cohort. Eligible participants were randomly assigned into either vaccine or placebo group in a ratio of 1:1 using a block randomization method performed and generated by an independent statistician before the conduct of the trial. Randomization numbers were allocated via a telephone by unblinded pharmacists. The sponsor, participating families, site staff, site monitors, data management staff, and statistician remained masked to treatment assignment throughout the study.

2.5. Procedures

A single 1 mL dose of oral RV3 rotavirus vaccine (Bio Farma) was administered to adult and child cohort participants, while 3 doses of the investigational product (IP), either oral active vaccine or placebo, were given for neonatal cohort participants at the age of 0–5 days, 8–10 weeks, and 12–14 weeks (Fig. 1). In the adult cohort, 10 mL of blood sample was obtained before receiving a dose of vaccine for screening laboratory examinations (biochemistry and hematology tests), 5 mL of blood sample was obtained on 7 days after the vaccine dose for safety parameters (liver function and hematology tests), and lastly, 4 mL of blood sample was obtained on 28 days after vaccine dose for safety parameter (hematology test). In the child cohort, blood samples (4 mL each) were obtained before and 28 days after the dose of the vaccine for safety parameters (liver function test). In the neonatal cohort, blood samples (4 mL each) were obtained from cord blood at birth, venous blood on 28 days after the first IP dose and 28 days after the third IP dose for safety parameter (liver function test).

The samples for biochemistry (including liver function test) and hematology assessments were allowed to clot at room temperature for 30 min to 2 h before being tested at the accredited central hospital's clinical pathology laboratory. Furthermore, the blood samples for immunogenicity assessments were stored at 2–8 °C for up to 24 h, then centrifuged and stored in a freezer at −20 to −80 °C. The stool samples in the neonatal cohort participants were collected between 3 and 7 days following each IP dose, then stored at 2–10 °C within 4 h and/or at ≤ -20 °C within 24 h after collection.

2.6. Safety

For safety evaluation, liver function tests including aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed. Additional hematological safety parameters were only examined in adult cohort due to safety limits of drawing blood samples in children and neonates and lack of possible hematologic abnormalities evidence due to rotavirus vaccines, including RV3-BB in the neonates [24]. The liver function tests were used for safety endpoint based on a concern that rotavirus infection may elevate hepatic transaminase [25]. The study physicians/pediatricians and independent pathologists verified the test results. The adverse events (AE) were recorded as immediate systemic events (within 30 min after each IP dose) or delayed systemic events (>0.5–24 h, 24–48 h, 48–72 h, 3–7 days, and week 2–4 following each IP dose). Adult participants, and parents of child and neonate participants, reported solicited, unsolicited adverse events (AE), temperatures, and gastrointestinal symptoms on diary cards daily for 7 days and weekly for 28 days after each dose. Research associates collected safety data via phone on day 3, at the end of week 2 and week 3 after each dose, while a study doctor or midwife collected safety data on day 7 after the IP dose.

All solicited systemic events were assessed for severity based on protocol standard criteria, while abnormal laboratory findings were evaluated based on the US Food and Drug Administration's Toxicity Grading Scale in Preventive Vaccine Clinical Trial (September 2007) [26]. Any Serious Adverse Event (SAE), defined as an adverse event that resulted in a new or prolonged hospitalization or death or life-threatening or persistent/significant disability or congenital anomaly and occurred within 28 days after a dose of vaccine or placebo, was to be reported to DSMB, sponsor, and ethics committee within 24 h after being alerted. The severity of AE was graded by the investigator, while causality was assessed by the investigator, sponsor, and DSMB. Based on WHO causality assessment of an adverse event following immunization, the causal relationship between investigational product and each AE was categorized as A). Consistent with causal association to immunization, B). Indeterminate, C). Inconsistent with causal association to immunization, and D). Unclassifiable [27].

2.7. Laboratory analysis

Among neonates, immunogenicity was be evaluated at baseline and 28 days after the first and third doses by measurement of serum IgA using enzyme-linked immunosorbent assay (ELISA) and serum neutralizing antibody (SNA) methods that were developed at MCRI, Australia [13]. SNA was tested against rotavirus strain RV3 (G3P6). ELISA method measured specific rotavirus immunoglobulin A (IgA) using rabbit anti-RV3 polyclonal antisera and tested anti-rotavirus IgA by titration starting at a dilution of 1:20. SNA titers were determined by fluorescents focus reduction neutralization assay as the reciprocal of the dilution observed at 50% reduction in fluorescence [24]. Fecal rotavirus RV3 vaccine excretions in neonates from day 3 to 7 following each dose were measured using ELISA and reverse transcription-polymerase chain reaction (RT-PCR) amplification rotavirus VP4 and VP7 genes, using gene-specific primers. RT-PCR method for vaccine virus shedding has been validated for precision based on the WHO manual of rotavirus detection and characterization methods [28]. Both serological immunogenicity and vaccine virus fecal shedding examination were performed at the clinical trial laboratory of PT Bio Farma with procedures approved and validated by the Quality Assurance Division of PT Bio Farma.

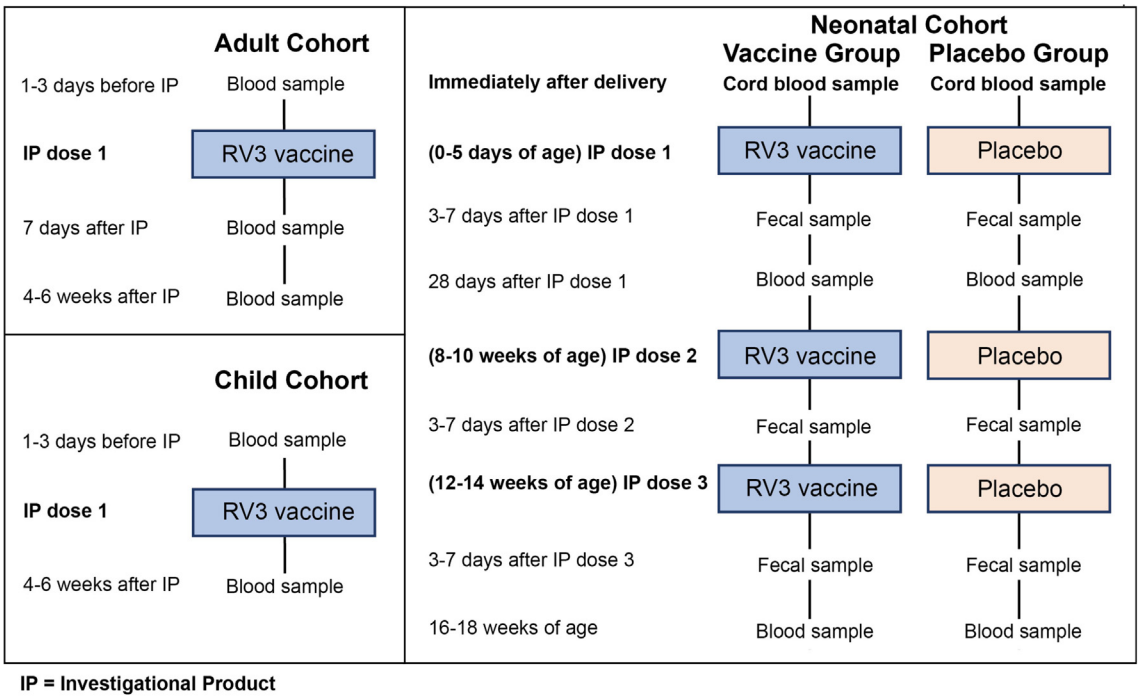

Fig. 1. Study design of the trial.

2.8. Sample size and statistical analysis

The sample size was based on WHO's a guideline on clinical evaluation of vaccines to collect sufficient safety data and to support the design of sequential age de-escalation starting from small numbers of healthy adults [29]. A total of 100 subjects were planned, divided into three age groups; 25 adults, 25 children, and 50 neonates.

Statistical calculations were performed using SAS ® version 9.4. The results were presented as mean (range) and frequency (percentage) for descriptive data. The safety analysis was conducted in the intention-to-treat population, including all enrolled participants. Safety data were presented descriptively as the number of participants experiencing each adverse event. All SAE and unsolicited AE terms were standardized at the database level using the Medical Dictionary for Regulatory Activities (MedDRA) version 21.1 (September 2018) [30].

Immunogenicity analysis (IgA and SNA) was only applied in the neonatal cohort, specifically in the per-protocol population who received all three doses of vaccine or placebo within the visit windows, for additional preliminary data. The relationships between vaccination and immune responses (IgA, SNA, or both) were assessed with a one-tailed two-sample *t*-test and bivariate Chi-square or Fischer's exact test. A significance level of 5% was used in the analysis. The geometric mean titers (GMT) of IgA at baseline, 28 days after IP dose 1 and dose 3 were compared between vaccine and placebo group and analyzed using *t*-test from natural log-transformed IgA titers. IgA and SNA seroconversions were defined as a three-fold or greater increase in titer from baseline to day 28 after IP dose 1 and dose 3. However, if the baseline level was below the limit of detection (i.e., IgA antibody titer < 10), then an arbitrary level of 10 was given in the baseline to determine the seroconversion. A positive immune response was defined if either IgA or SNA was seroconverted.

3. Results

Overall, 100 participants were recruited, 25 adults, 25 children, and 50 neonates. All adult and child participants completed the study, while 1 neonate participant discontinued dosing after IP dose 1 because the parents decided to discontinue. The participant, however, was still followed up until the end of the study period. All participants were included in the intention-to-treat and safety analysis population, while the 49 neonates who received all 3 doses of IP were included in the per-protocol analysis population (Fig. 2).

3.1. Vaccine safety

The baseline characteristics and adverse events are presented in Table 1. Adverse events (AE) were most commonly reported after 8 days or more following the IP administration in all age groups. In the neonatal cohort, 59 events were reported in the 28 days following a dose in the vaccine group compared with 79 events reported in the 28 days following a dose in the placebo group. Most events were classified as mild, while 9 moderate events were reported in the neonatal vaccine group and 14 moderate events were reported in the neonatal placebo group. There was no AE classified as severe in intensity across any cohort. Two neonates in the vaccine group were first noted to be jaundiced in the 30 min post IP dose 1; but based on the timing, this was considered unlikely to be related to IP administration. There were no significant differences in the total number of AEs between the neonatal vaccine group and neonatal placebo group. All AEs were followed up and resolved without sequelae.

All adult participants had AST and ALT levels within the normal range both at baseline and 28 days after vaccine dose. All participants had normal ALT level at baseline and 28 days post vaccine dose in the child cohort. Seven (28%) children had an AST level

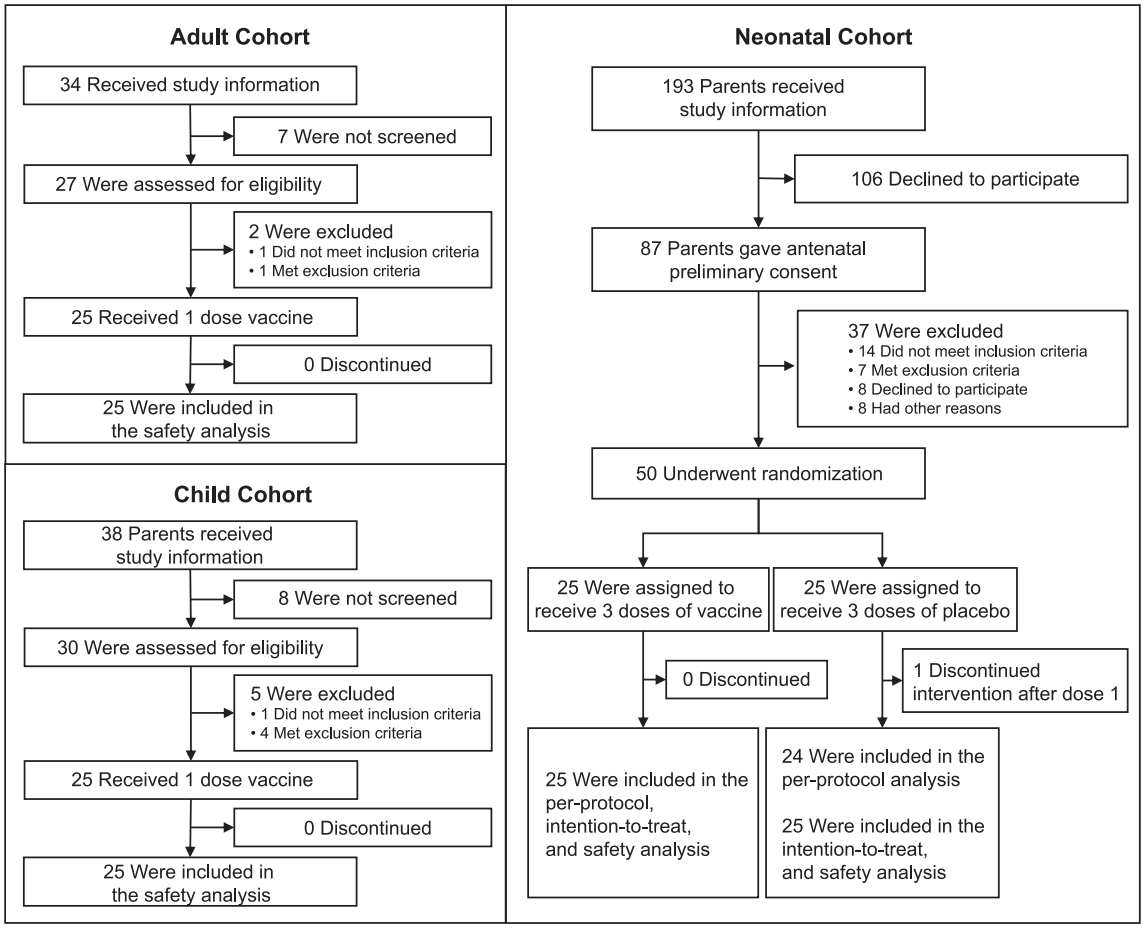

Fig. 2. Clinical trial consort diagram for each cohort.

above the normal reference range, and 8 (32%) children had an AST level above the normal reference range at 28 days post-vaccine dose. All were asymptomatic and considered unlikely related to IP. Fourteen neonates had an AST and/or ALT above the normal range in the 28 days after a dose of IP with no significant differences between vaccine and placebo groups (9/36% vs. 5/20%, $p > 0.05$). The levels of AST and ALT were also similar in the vaccine and placebo groups (AST median [interquartile range]: vaccine 41.2 [34.0–54.6], placebo 43.7 [36.2–60.35]; ALT median [interquartile range]: vaccine 28.6 [19.2–46.5], placebo 31.1 [22.5–38.9]) ($p < 0.05$ for all comparisons).

All SAEs were reviewed by the DSMB included causality assessments. During the study, there were three SAEs, one in the adult cohort (union fracture clavicula sinistra) and two in the neonatal cohort (neonatal jaundice and pneumonia). SAE of neonatal jaundice was diagnosed in a placebo group participant at 9 days of age (5 days after the first dose). It was assessed as mild severity and unlikely related to the IP. Jaundice resolved with phototherapy, and the participant was discharged after 2 days of hospitalization. The SAE of pneumonia occurred in a vaccine group participant at the age of 2 months and 6 days (53 days after the last IP dose) with an assessment of moderate severity and unlikely related to the IP. The participant was discharged after 5 days of being hospitalized and was fully recovered 6 days later.

3.2. Immunogenicity

Immunogenicity analysis showed that 18/25 (72.0%) neonates in the vaccine group had a positive immune response (cumulative seroconversion in SNA and/or IgA) after 28 days of receiving full three doses of RV3 rotavirus vaccine (Bio Farma) compared to 4/24 (16.7%) in the placebo group (Table.2). The proportions of positive immune responses in vaccine groups on 28 days post dose 3 were significantly higher than those in the placebo group (Table 2). The 18/25 (72%) neonates in the vaccine group had IgA seroconversion after receiving 3 doses, which is significantly higher than 3/24 (12.5%) neonates in the placebo group ($p < 0.001$). The Geometric Mean Titers (GMT) of serum IgA following IP dose 3 was 185.1 unit/ml in the vaccine group and 12.3 unit/mL in the placebo group. The GMTs of serum IgA in the vaccine group were significantly higher on 28 days post IP dose 1 ($p < 0.05$) and 28 days post IP dose 3 ($p < 0.001$) compared to those in the placebo group (Table 2). The GMT was high at baseline in both vaccine and placebo groups for serum neutralizing antibodies. Both SNA median and SNA GMT in the vaccine group 28 days post IP dose 3 were significantly higher than in the placebo group ($p < 0.05$). The proportion of positive SNA seroconversion in the vaccine group 28 days post-dose 3 was higher than the proportion in the placebo group but did not differ significantly (Table 2).

Fecal shedding of the vaccine virus was not detected in the stool from samples collected between 3 and 7 days after each dose of IP in the neonatal vaccine or placebo groups.

4. Discussion and conclusion

Our findings showed that the human neonatal RV3 rotavirus vaccine (Bio Farma) was well tolerated in adults, children, and neonates and immunogenic when given in a neonatal schedule in

Table 1
Characteristics of study populations & adverse events reported during the trial.

	Adults (N = 25)	Children (N = 25)	Neonates					
			Vaccine (N = 25)			Placebo (N = 25)		
	No. of participants (%), unless otherwise indicated							
Baseline characteristics								
Sex								
Female	23 (92)	13 (52)	13 (52)			11 (44)		
Male	2 (8)	12 (48)	12 (48)			14 (56)		
Mean of age (range)	30.6 (20–38) y	2.6 (2–4) y	3.6 (2–5) d			3.4 (2–5) d		
Mean of body weight (range)	63 (40–82) kg	13 (10–23) kg	2.9 (2.5–3.6) kg			3 (2.6–3.5) kg		
	No. of events							
Adverse Events			*Dose 1*	*Dose 2*	*Dose 3*	*Dose 1*	*Dose 2*	*Dose 3*
Any immediate systemic event (30 min post dose)	0	0	2	0	0	0	0	0
Any delayed systemic event (31 min-24 h post dose)	3	1	1	4	1	1	0	4
Any delayed systemic event (24 – 48 h post dose)	8	0	4	0	1	3	2	0
Any delayed systemic event (48 – 72 h post dose)	1	0	3	1	0	0	3	1
Any delayed systemic event (3 – 7 d post dose)	5	4	4	1	9	5	3	2
Any delayed systemic event (8 – 28 d post dose)	11	22	10	5	13	24	14	17
Total Adverse Events	28	27	24	11	24	33	22	24
	No. of events[*]							
Description (0 – 28 d post dose)			*Dose 1*	*Dose 2*	*Dose 3*	*Dose 1*	*Dose 2*	*Dose 3*
Solicited Adverse Event	9	10	6	11	11	14	13	15
Fever	0	2	0	1	1	1	1	6
Nausea/Vomiting	1	3	2	5	2	2	3	4
Diarrhea	0	4	1	0	1	0	2	1
Fatigue/myalgia	8	1	NA	NA	NA	NA	NA	NA
Irritability	0	0	3	3	7	11	7	4
Unsolicited Adverse Event	19	17	18	2	13	19	9	9
Nasopharyngitis	0	11	2	0	5	2	2	2
Loose stools	3	1	3	0	0	4	4	6
Rhinitis	0	2	1	0	2	4	0	0
Miliaria	0	0	3	0	1	2	0	0
Underweight	0	0	1	1	0	3	0	1
Conjunctivitis	1	0	2	0	0	2	0	0
Others	15	3	6	1	5	2	3	0
Serious Adverse Event	1	0	1	0	0	1	0	0
AE with moderate intensity	17	16	2	1	6	7	2	5
AE with severe / fatal intensity	0	0	0	0	0	0	0	0

The Abbreviations: y = years; kg = kilograms; d = days; h = hours; min = minutes; NA = not applicable;
[*] Data are for the safety analysis set (all participants who received at least one dose of vaccine or placebo). A participant could report more than one adverse event.

Indonesia. These results corresponded with the results from the phase I trial of the RV3-BB vaccine developed by MCRI, Australia. [31] The results suggest that three doses of RV3 rotavirus vaccine (Bio Farma) given at the age of 0–5 days, 8–10 weeks, and 12–14 weeks are immunogenic with a cumulative immune response of 72%.

We found that a single dose of this vaccine was well tolerated in adults and children with no safety concerns. Furthermore, neonates who received three doses of RV3 rotavirus vaccine (Bio Farma) had no difference in solicited or unsolicited adverse events than those who received placebo. Two SAEs observed in the neonatal cohort were mild-moderate in severity and unlikely related to the IP (1 neonatal jaundice occurred 5 days after receiving the first dose of placebo, 1 pneumonia occurred 53 days after receiving the first dose of vaccine). There was no episode of intussusception or death reported in this study.

Even though rotavirus is a labile acid agent, the study findings suggest that pre-administered antacid or acid neutralization in the formulation may not be needed for this live-attenuated human neonatal RV3 rotavirus vaccine (Bio Farma) to trigger an immune response in the neonatal schedule. IgA seroconversion response observed in the neonatal cohort in the RV3 rotavirus vaccine (Bio Farma) was comparable with the result observed in the Phase IIb RV3-BB vaccine trial (72% vs. 74%, respectively).[24] This study showed that serum IgA GMTs in the neonatal vaccine group on 28 days post-dose 1 and 28 days post-dose 3 were significantly higher than those in the placebo group. According to a review of other rotavirus vaccines, a high IgA GMT correlates with protection against severe rotavirus gastroenteritis.[32]

Not unexpectedly, IgA seroconversion after the first dose of RV3 vaccine was identified in only 5 of 25 (20%) participants compared to none in the placebo group, mirroring observations in the previous RV3-BB trials in Indonesian and New Zealand infants [13,24] as IgA is not transferred across the placenta and the lack of an accurate serological correlate of protection for rotavirus infection, the assessment immune response to a birth dose of a rotavirus vaccine challenging. Hence the use of other markers of immune response in this study, including serum neutralizing antibodies and vaccine virus fecal shedding. It has also been postulated that a birth dose of a viral vaccine may act to "prime" the immune system for subsequent exposure to a vaccine or wild-type infection [33,34]. After three doses of the RV3 vaccine, the cumulative IgA seroconversion in the vaccine group was significantly different from that observed in the placebo group (72.0% vs. 16.7%, respectively). This result is consistent with the previous phase IIb trial of the RV3-BB vaccine in Indonesian infants [13].

Serum neutralizing antibody seroconversion was less frequently observed. This was likely due to the high baseline SNA level observed, which reflects maternal antibodies transferred via the placental circulation. The immunogenicity analysis showed that all participants with a positive SNA seroconversion also had IgA seroconversion, corresponding to the phase IIb trial of the RV3-BB vaccine [13]. Some neonatal placebo groups showed seroconversion in either SNA or IgA levels that seem likely to result from the wild-type rotavirus. Rotavirus surveillance in Indonesia also showed 38.4% positive rotavirus among 0–5 months old children hospitalized with diarrhea [35]. The proportion of seroconversion among the vaccine group is consistent with WHO prequalified

Table 2
Summary of immune response in the neonatal cohort.

	Baseline			28 days post IP dose 1			28 days post IP dose 3		
	Vaccine (N = 25)	Placebo (N = 25)	P	Vaccine (N = 25)	Placebo (N = 24)	P	Vaccine (N = 25)	Placebo (N = 24)	P
IgA Median (IQR)	10.0 (10.0, 10.0)	10.0 (10.0, 10.0)	0.5*	10.0 (10.0, 24.5)	10.0 (10.0, 10.0)	0.008*	10.0 (10.0, 776.7)	10.0 (10.0, 10.0)	<0.001*
IgA GMT (95% CI)	10.4 (9.6–11.3)	10.0 (10.0–10.0)	0.16†	18.7 (10.9–31.9)	10.0 (10.0–10.0)	0.01†	185.1 (78.3–437.5)	12.3 (9.6–15.8)	<0.001†
SNA Median (IQR)	1611.6 (725.8–2489.3)	1065.4 (507.6–2122.1)	0.12*	1048.9 (684.2, 2154.6)	819.7 (469.1, 1512.9)	0.15*	1962.4 (650.3–2657.6)	646.8 (323.9–1101.2)	0.01*
SNA GMT (95% CI)	1418.1 (1070.5–1878.7)	1061.7 (693.9, 1624.6)	0.12†	1123.2 (822.7–1533.6)	822.3 (589.6–1146.7)	0.08†	1394.2 (963.8–2016.8)	631.0 (427.0–932.4)	0.003†
IgA seroconversion , *n(%)*	–	–	–	5 (20.0)	0 (0.0)	0.05§	18 (72.0)	3 (12.5)	<0.001‡
SNA seroconversion , *n(%)*	–	–	–	0 (0.0)	0 (0.0)	–	4 (16.0)	1 (4.2)	0.35§
Immune Response, *n(%)*	–	–	–	5 (20.0)	0 (0.0)	0.05§	18 (72.0)	4 (16.7)	<0.001‡

The Abbreviation: GMT = Geometric Mean Titres; SNA = Serum Neutralizing Antibody; IQR = Interquartile.
* Wilcoxon signed-rank test.
† Paired *t*-test from natural log-transformed.
‡ Fisher's exact test.
§ Chi-square test.

oral rotavirus vaccines when implemented in high rotavirus disease burden regions [20,32,36].

In this study, we did not detect fecal shedding of the vaccine virus from stools from neonates 3–7 days after a dose of vaccine. This was in contrast to the previous RV3-BB Phase IIb in Indonesia, where fecal vaccine virus shedding was detected in 69% of participants in the neonatal schedule group [13]. This difference is most likely attributable to critical differences in laboratory methods used in these two studies. In the Phase IIb study, a VP6 specific RT-PCR was used to detect and select samples for subsequent sequencing [13]. However, in this current study, VP4 and VP7 RT-PCR targets were used. In retrospect, this method was not ideal as it is not sensitive to detect the RV3 (G3P6) vaccine strain and likely explains the failure to detect fecal vaccine virus shedding in this study [28].

The study showed that the Bio Farma manufactured RV3 vaccine is immunogenic when three doses are administered in the absence of pre-neutralization of gastric acid in a neonatal schedule in a high child mortality region. The immunogenic observed in this study reflects that observed in the neonatal-schedule group in the previous Phase IIb study of the RV3-BB vaccine conducted in Indonesia [13]. Not needing pre-neutralization will simplify large-scale vaccine manufacture and formulation development and reduce the cost of vaccine production. It will also make the implementation of a non-formulated RV3 vaccine easier to administer from a clinical perspective. There is no need for a pre-dose of antacid and will reduce the volume of the final formulated presentation. However, since the safety endpoint becomes the primary objective of this phase I trial, the secondary immunogenicity analysis from this study needs to be confirmed in further research with larger samples.

The Bio Farma RV3 vaccine has > 99% genetic homology with the original RV3-BB rotavirus vaccine (MCRI) that is efficacious in protecting against severe rotavirus gastroenteritis in Indonesian infants in a neonatal schedule [13]. It is anticipated that the introduction of the RV3 vaccine into the NIP in Indonesia in the future has the potential to reduce rotavirus-related mortality and hospitalization in children in Indonesia significantly.

In conclusion, the trial results show that the RV3 rotavirus vaccine (Bio Farma) is well tolerated in all participant cohorts (adults, children, and neonates). Three doses of this vaccine administered in a neonatal schedule were immunogenic. These promising results support further clinical development of the RV3 rotavirus vaccine (Bio Farma).

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [J.F., R.M.S. and N.S.B. are employees of PT Bio Farma who provided funds to support this study and plan to manufacture RV3 rotavirus vaccine (Bio Farma). All other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.].

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