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Research Article

Efficacy, safety and immunogenicity of hexavalent rotavirus vaccine in Chinese infants

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ABSTRACT

A randomized, double-blind, placebo-controlled multicenter trial was conducted in healthy Chinese infants to assess the efficacy and safety of a hexavalent live human-bovine reassortant rotavirus vaccine (HRV) against rotavirus gastroenteritis (RVGE). A total of 6400 participants aged 6–12 weeks were enrolled and randomly assigned to either HRV (n = 3200) or placebo (n = 3200) group. All the subjects received three oral doses of vaccine four weeks apart. The vaccine efficacy (VE) against RVGE caused by rotavirus serotypes contained in HRV was evaluated from 14 days after three doses of administration up until the end of the second rotavirus season. VE against severe RVGE, VE against RVGE hospitalization caused by serotypes contained in HRV, and VE against RVGE, severe RVGE, and RVGE hospitalization caused by natural infection of any serotype of rotavirus were also investigated. All adverse events (AEs) were collected for 30 days after each dose. Serious AEs (SAEs) and intussusception cases were collected during the entire study. Our data showed that VE against RVGE caused by serotypes contained in HRV was 69.21% (95%CI: 53.31–79.69). VE against severe RVGE and RVGE

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hospitalization caused by serotypes contained in HRV were 91.36% (95%CI: 78.45–96.53) and 89.21% (95%CI: 64.51–96.72) respectively. VE against RVGE, severe RVGE, and RVGE hospitalization caused by natural infection of any serotype of rotavirus were 62.88% (95%CI: 49.11–72.92), 85.51% (95%CI: 72.74–92.30) and 83.68% (95%CI: 61.34–93.11). Incidences of AEs from the first dose to one month post the third dose in HRV and placebo groups were comparable. There was no significant difference in incidences of SAEs in HRV and placebo groups. This study shows that this hexavalent reassortant rotavirus vaccine is an effective, well-tolerated, and safe vaccine for Chinese infants.

1. Introduction

Rotavirus is the leading pathogen that causes severe gastroenteritis in children and infects virtually all children under five years old. Improving the safety control of water and food and implementing a better sanitation program seems unlikely to reduce the occurrence of diseases caused by rotavirus. In China, published data showed that rotavirus-associated hospitalizations accounted for 32%–50% of hospitalizations attributable to diarrhea (Fang et al., 2005). The average incidence of rotavirus infection among children under five years old was 151 cases/1000 children/year, with the highest incidence occurring among children with 12–24 months of age in Zhengding County in Hebei Province, the north of China (Wang et al., 2009).

Rotavirus vaccination should be considered as a potential cost-effective measure against rotavirus infection in China. There are two rotavirus vaccines licensed in the Chinese market: Lanzhou lamb rotavirus vaccine (LLR) consisting of rotavirus serotype G10P[12] from Lanzhou Institute of Biological Products, and RotaTeq developed from bovine WC3 strain reassorted with human strains G1, G2, G3, G4 and P [8] manufactured by Merck & Co. Inc. However, in recent years, the predominant serotype of epidemic rotavirus in China is G1, G2, G3, G4, and G9 (Liu et al., 2014; Wang et al., 2021; Zhang et al., 2022). And G8 become the predominant serotype in African countries (Ciarlet and Schodel, 2009). Based on the bovine rotavirus UK Compton strain, we developed a human-bovine reassortant hexavalent rotavirus vaccine (HRV) containing G1, G2, G3, G4, G8 and G9 serotypes to cover most serotypes of rotavirus in the world. The phase I and phase II clinical studies showed that this oral hexavalent rotavirus vaccine was well-tolerated in all adults, toddlers and infants, and was highly immunogenic in infants (Wu et al., 2021).

Here, we investigated the vaccine efficacy (VE) and safety of this oral hexavalent rotavirus vaccine in Chinese vaccinated infants by following the infants over two consecutive rotavirus epidemic seasons after vaccination. The primary objective was to evaluate the VE against rotavirus gastroenteritis (RVGE) caused by rotavirus serotypes contained in HRV at least 14 days following the third dose. The key secondary objectives included the VE against severe RVGE, VE against RVGE hospitalization caused by rotavirus serotypes contained in HRV, VE against RVGE, severe RVGE, and RVGE hospitalization caused by natural infection of any serotype of rotavirus.

2. Materials and method

2.1. Rotavirus vaccine

Oral hexavalent reassortant rotavirus vaccine was manufactured in Wuhan Institute of Biological Products Ltd. Com. This live-attenuated hexavalent bovine-human reassortant rotavirus vaccine is constructed based on the backbone of bovine rotavirus UK strain (G6P[5]) and reassorted with *VP7* fragment of human rotavirus parental strains of G1 (D strain), G2 (DS-1 strain), G3 (P strain), G4 (ST-3 strain), G8 (1280 strain) and G9 (AU32 strain) serotype respectively. The virus strains were kindly provided by Dr. Albert Kapikian from the National Institutes of Health. These reassortant rotaviruses were safe and showed 90% efficacy against severe RVGE during two rotavirus seasons (Clements-Mann et al., 2001; Vesikari et al., 2006). The vaccine strains of rotavirus were grown in Vero cells. And the viral harvest was pooled and formulated, finally filled with 2 mL/dose (with 5.5 lgFFU/dose for each serotype). The placebo was identical in content, packaging, and appearance to the vaccine but didn't contain the virus. The viral harvest, bulk, and production processes were performed following the WHO's guidelines TRS No.941 (WHO, 2007), and the products were released by National Institute for Food and Drug Control in China.

2.2. Subjects and study design

A randomized, double-blind, placebo-controlled, phase III clinical study of HRV was conducted to evaluate the protective efficacy, safety, and immunogenicity in Chinese infants from Zhengding, Daming, Yongnian, Laishui counties of Hebei Province; Yuhuan, Longyou Jiangshan counties of Zhejiang Province; Xiangtan, You counties of Hunan province; Liucheng, Rongshui counties of Guangxi Zhuang Autonomous Region within two consecutive rotavirus seasons between 2019 and 2021. Eligible healthy infants aged 6–12 weeks at the time of the first dose were enrolled. The key exclusion criteria included gastroenteritis, RVGE, immunodeficiency disease, acute disease, severe chronic disease, or in the acute phase of chronic disease, or receipt of immunoglobulins and/or blood products, frequent immunosuppressants or other immune-modifying drugs since birth, or receipt of other rotavirus vaccines. Finally, a total of 6400 healthy infants were enrolled and randomized into HRV group and placebo group with a ratio of 1:1. All the infants received three doses of vaccine by oral administration at 28 days intervals.

For the primary hypothesis, HRV was considered efficiency of $> 55\%$ (Li et al., 2014; Mo et al., 2017) against any severity of RVGE caused by G1, G2, G3, G4, G8, G9 serotype; the incidence rate of RVGE in two rotavirus seasons was 5%, the ratio of subjects in HRV and placebo group was 1:1; and at least 73 cases of acute gastroenteritis (AGE) of any severity caused by G1, G2, G3, G4, G8, G9 serotype of rotavirus are expected to be observed; HRV had a protective efficacy $> 70\%$ for severe RVGE, the cumulative incidence rate of severe RVGE in two consecutive rotavirus seasons was estimated at 1% (Chen et al., 2019; Liu et al., 2020; Zhang et al., 2020); About 20% dropout rate was considered. The sample size was calculated as 6400 subjects using the exact condition method of Chan and Bohidar under the assumption of large sample Poisson distribution (Chan and Bohider, 1998).

2.3. Safety study

All the subjects who received at least one dose without withdrawal were kept under observation. All adverse events (AEs), according to China's National Medical Products Administration (NMPA) Guidelines for grading criteria of adverse events in clinical trials of preventive vaccines published in 2005 (NMPA, 2005), within 30 min of each vaccination and AEs between day 0 and day 28 after administration of each dose were recorded. Solicited AEs including fever, irritation, somnolence, apocleisis, vomit, diarrhea, and allergy were collected from day 1 to day 14 following each vaccination. Serious adverse events (SAEs), according to China's National Medical Products Administration (NMPA) Guidelines for grading criteria of adverse events in clinical trials of preventive vaccines published in 2005 (NMPA, 2005), occurred from the first dose to the end of the study were collected.

2.4. Immunogenicity assays

About 600 of the 6400 infants were allocated to the immunogenicity subgroup, of which 300 were in the HRV group and 300 were in the placebo group. Blood samples were collected before the first dose and one month after the third dose to test anti-rotavirus IgA by ELISA. Briefly, MaxiSorp plates (Thermo Fisher Scientific) were coated with rotavirus VP6 antibody (2.5 μg/mL). Then the plates were incubated with the virus culture containing G1, G2, G3, G4, G8, and G9 vaccine strains respectively, followed by sequential incubation with human serum samples. Rabbit anti-human serum IgA-Biotin (Jacson ImmunoResearch Laboratory, INC.) was used as a detection antibody. OD values (490–620 nm) versus dilution curves were plotted, and sera IgA titers were calculated using Softmax software. The concentration of IgA in the positive serum was calculated by using a four parameters model with a standard reference. Sero-conversion was defined as IgA concentration ≧20 U/mL within one month after the third dose if anti-rotavirus IgA antibody concentration was below 20 U/mL before the first dose, or as IgA concentration increased ≧ 4 fold within one month after the third dose if anti-rotavirus IgA concentration was ≧ 20 U/mL before the first dose.

2.5. Fecal sample testing

The fecal samples from infants with diarrhea after the first dose vaccination were collected and tested for rotavirus antigen by ELISA of ProSpecT™. Viral RNAs of all the positive samples were extracted with QIAamp Viral RNA Mini Kit. *VP7* fragment was amplified with Retro Transcript-PCR. The primers of PCR were *VP7* F: 5′-GGCTTTAAAA-GAGAATTTCCGTCTGG-3′ and *VP7* R: 5′-GGTCACATCATACAATT CTAATCTAAG-3′. The size of the amplified fragment was 1062 bp. The PCR amplifications were performed with the following steps: one cycle at 50 °C for 30 min and 94 °C for 2 min, followed by 35 cycles at 94 °C for 30 s, 50 °C for 30 s, 72 °C for 1min, and a final extension step of 5 min at 72 °C. The products of PCR were sequenced with the above primers. The final sequences were analyzed with Sequencher software and genotyped using the software of RotaC V2. 0.

2.6. Efficacy study

All subjects who completed at least one dose of vaccination were involved in the observation of protective efficacy. All cases of AGE that occurred from 14 days after the first dose to the end of the study were collected. AGE cases were determined by the Clinical Endpoint Committee based on clinical symptoms, clinical diagnosis, and etiological test results.

The primary objective efficacy study was to evaluate VE against gastroenteritis caused by rotavirus serotypes contained in HRV. The key secondary objective included: VE against severe RVGE, VE against RVGE leading to hospitalization caused by serotypes contained in HRV 14 days after administration of three doses among infants within two consecutive rotavirus seasons; VE against RVGE, severe RVGE, and RVGE hospitalization caused by natural infection of any serotype of rotavirus.

The severity of gastroenteritis was finally assessed with Vesikari score by the Clinical Endpoint Committee. Vesikari Score < 7, 7–10, and ≧ 11 were divided as mild, moderate, and severe gastroenteritis respectively.

2.7. Statistic analysis

Subjects receiving at least one dose of vaccination were included in the intention-to-treat analysis. Subjects receiving three doses and 14 days of follow-up post each dose were included in the modified intention-to-treat analysis. Subjects receiving three doses without protocol violation were included in the per-protocol analysis.

All randomized participants who received at least one dose of HRV or placebo were included for safety analysis. Fisher's exact probability test was used to statistically analyze the difference between AEs and SAEs incidence between groups.

Immunogenicity assay was based on immunogenicity per-protocol analysis. The difference of seroconversion rate between the HRV and placebo group was statistically validated by Chi-test/Fisher's exact probability test. The difference in geometric mean concentration (GMC) and GMC multiple growth (GMI) of anti-rotavirus IgA against G1, G2, G3, G4, G8, and G9 serotypes between HRV and placebo groups was statistically validated by *t*-test.

Vaccine efficacy was calculated based on the modified intention-to-treat analysis which included the first episode of any specified event that occurred within 2 weeks after the third dose of the assigned study vaccine. The efficacy was primarily computed as $(1 - R_{HRV}/R_{placebo}) \times 100\%$; while R_{HRV} is the number of subjects reporting at least one specific event/total number of subjects in the HRV vaccine group, and $R_{placebo}$ is the number of subjects reporting at least one specific event/total number of subjects in the placebo group. Clopper-Pearson 95% confidence interval was computed. The person-year incidence rate and the 95% confidence interval of each group of AGE caused by G1, G2, G3, G4, G8, and G9 rotavirus infection after 14 days of the third dose of HRV/placebo were calculated respectively. The differences between the two groups were compared by Poisson regression model.

All statistical tests were conducted at $\alpha = 0.05$ (2-sided) level, and the inspection efficiency was 90%.

3. Result

3.1. Population description

A total of 6908 subjects were screened and 6400 infants were enrolled. Among them, 2300 subjects were from Hebei, 1000 from Zhejiang, 1300 from Guangxi and 1800 from Hunan Province. Besides 9 subjects who withdrew before the first dose, 3198 received HRV and 3193 received placebo. The median gestation at the birth of infants is 39 (37, 43) weeks and the median age at first dose was eight weeks (6, 13) in the HRV group and placebo group. There were 51.34% (1642/3198) males in the HRV group and 50.64% (1617/3193) males in the placebo group.

A total of 6391 subjects who received at least one dose were included in the intention-to-treat analysis. And 5932 subjects who received three doses entered in the modified intention-to-treat analysis (2950 of HRV group and 2982 of placebo group) listed in Fig. 1.

3.2. Vaccine safety

The number and incidence of AE and SAE subjects who received HRV or placebo were listed in Table 1. A total of 922 cases (466 in HRV group and 456 in placebo group) reported SAE within 14 days post each vaccination in the study period. The incidence of SAEs between those two groups had no significant difference. The most common SAEs were gastrointestinal disease and infectious disease. About 14 cases with SAEs in HRV group were considered vaccine-related, including one case of Kawasaki disease, two cases of thrombocytopenic purpura, one case of allergic dermatitis, one case of upper respiratory diseases, four cases of diarrhea, and five cases of gastrointestinal disease. Eight cases reported SAEs in placebo group were considered vaccine-related, including one case of intussusception on day 21 post the second placebo dose, two cases of diarrhea, two cases of enteritis, one case of acute RVGE, one case of gastroenteritis, and one case of allergic dermatitis. There was one death case who had chronic kidney disease and severe pneumonia in the placebo group.

The participant who developed intussusception recovered 6 days later after receiving treatment of air enema and subsequent surgical reduction. Two participants who occurred thrombocytopenic purpura on day 30 and 24 post the second dose of HRV respectively recovered 3 days later after treatment in hospitalization. One participant who developed Kawasaki

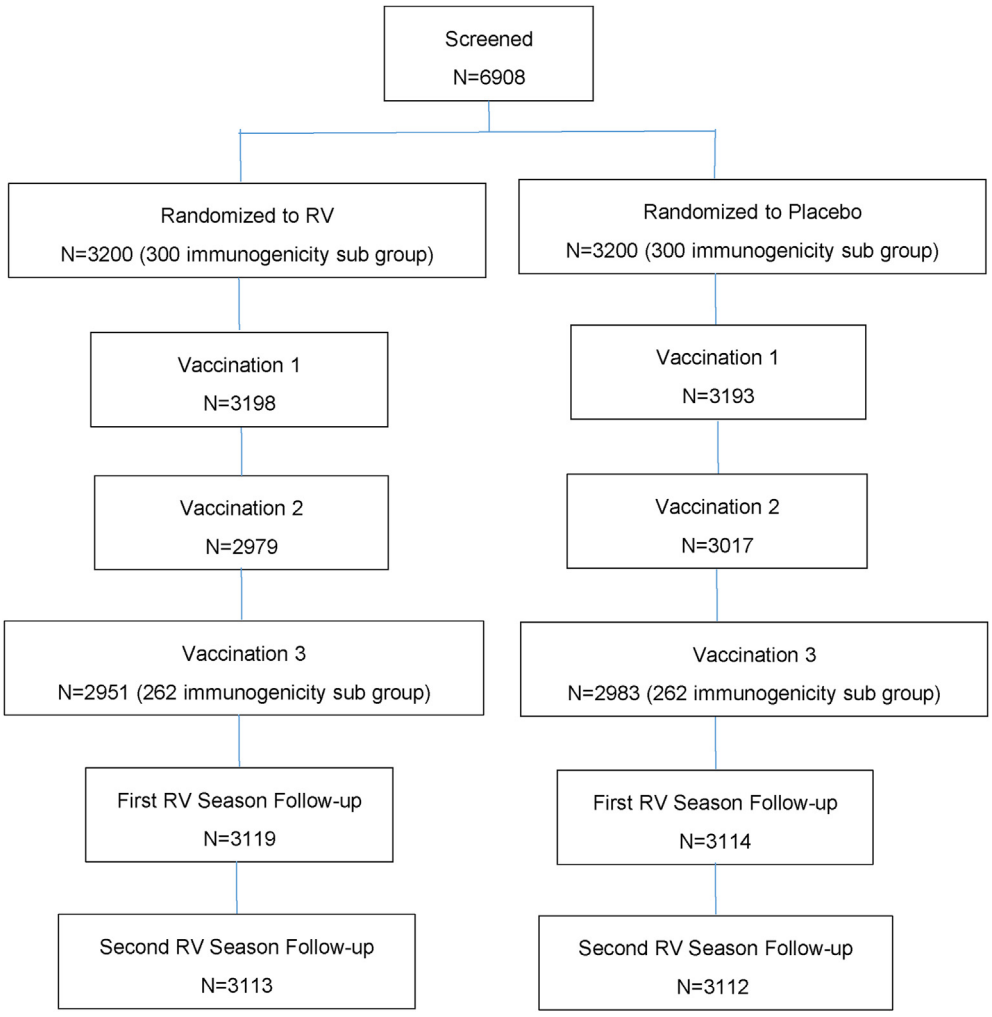

Fig. 1. Study profile.

Table 1
Number and occurrence rate of AE and SAE subjects.

	HRV, N (%)	Placebo, N (%)	*P*-value
Subject with follow up	3198	3193	
With one or more SAEs	466 (14.57)	456 (14.28)	0.7488
With SAEs related study vaccine	14 (0.44)	8 (0.25)	0.2854
With one or more AEs	2484 (77.67)	2418 (71.26)	0.0666
With AEs related study vaccine	2147 (67.14)	2062 (64.58)	0.0326
Total death	0 (0.00)	1 (0.03)	

AE: adverse events; SAE: Serious adverse events; N = number of subjects.

disease on day 21 post the second dose of HRV recovered 6 days later after treatment in hospitalization.

From the first dose to one month post the third dose, the incidences of AEs in HRV and placebo group were 77.67% (2484/3198) and 75.73% (2418/3193), which appeared comparable within those two groups (Table 2). The incidences of solicited and unsolicited AEs in the HRV group were 66.73% and 39.62%, comparable with 65.56% and 40.04% in the placebo group. The most common solicited AEs was fever above 37.1 °C, with a slightly higher incidence in HRV group (59.32%) than in placebo group (55.97%) (Table 3). Incidence of vaccine-related unsolicited AEs in HRV (2.31%, 74 cases) and placebo group (1.97%, 52 cases) were comparable. Most of them were gastrointestinal diseases. Generally, the incidence of AEs post first, second and third dose in HRV/placebo group were 58.47%/54.27%, 43.97%/42.39%, and 34.12%/35.23%, respectively, which decreased following the doses.

Table 2
Incidence of AEs post vaccination.

	HRV (3198 subjects)		placebo (3193 subjects)		
	Cases	Incidence * (%)	Cases	Incidence* (%)	*P*-value
AE	2484	77.67	2418	75.73	0.0666
Solicited AE	2134	66.73	2056	64.39	0.0514
Unsolicited AE	1267	39.62	1292	40.46	0.4908
Vaccine-related AE	2147	67.14	2062	64.58	0.0326
Solicited AE	2134	66.73	2056	64.39	0.0514
Unsolicited AE	74	2.31	52	1.63	0.0584

AE: adverse events. Incidence = number of cases/number of subjects. All events were collected within 28 days after each dose.

3.3. Vaccine immunogenicity

A total of 600 subjects were allocated to the immunogenicity subgroup, including 300 in the HRV group and 300 in the placebo group. About 262 subjects in the HRV group and 262 in the placebo group completed the vaccination of three doses. Blood samples were collected before the first dose and one month after the third dose.

The seroconversion rate of IgA and GMC of IgA antibody levels in the HRV group were significantly higher than those in the placebo group (Table 4 and Fig. 2). Among subjects who received three doses of vaccination, the seroconversion rates of IgA against G1, G2, G3, G4, G8,

Table 3
Solicited AEs related with vaccine.

AEs	HRV (N = 3198)	Placebo (N = 3193)	*P*-value
	Number of cases (%)	Number of cases (%)	
Solicited AE	2134 (66.73)	2056 (64.39)	0.0514
Fever	1897 (59.32)	1787 (55.97)	0.0068
Diarrhea	369 (11.54)	343 (10.74)	0.3204
Irritability	272 (8.51)	262 (8.21)	0.6842
Vomiting	229 (7.16)	233 (7.30)	0.8469
Drowsiness	130 (4.07)	107 (3.35)	0.1452
Decreased appetite	97 (3.03)	94 (2.94)	0.8832
Allergy	22 (0.69)	29 (0.91)	0.3295

AE: adverse events. N: number of subjects received at least one dose. The events after each dose were combined.

G9 serotype of HRV were 77.10%, 80.92%, 83.21%, 82.82%, 83.97%, 83.59%. By contrast, the seroconversion rates of IgA in placebo group were 11.83%, 13.74%, 14.50%, 12.60%, 15.65%, 14.12%.

3.4. Vaccine efficacy

After two consecutive RV seasons, a total of 2078 AGEs cases were reported (1020 cases from HRV group and 1058 cases from placebo group). The fecal samples from those cases were collected and tested for rotavirus antigen by ELISA, and a total of 200 cases were tested rotavirus positive. All the positive samples were analyzed by PCR and sequencing. The severity of AGE cases caused by rotavirus infection was recorded and classified by the Vesikari scoring system.

Among the 200 cases, 88 cases were classified as severe, while 69 and 43 were recorded as moderate and mild. VE against RVGE, severe RVGE, and hospitalization caused by rotavirus serotypes contained in HRV were 69.21% (95%CI: 53.31–79.69), 91.36% (95%CI: 78.45–96.53), and 89.21% (95%CI: 64.51–96.72). VE against RVGE, severe RVGE, and hospitalization caused by any type of rotavirus were 62.88% (95%CI: 49.11–72.92), 85.51% (95%CI: 72.74–92.30), and 83.68 (95%CI: 61.34–93.11) (Table 5).

Among the 200 ELISA-positive fecal samples, rotavirus genes were successfully amplified and sequenced in 125 samples, of which 30 were in HRV group, 95 were in placebo group. Among the 200 ELISA-positive cases, 195 were included in the modified intention-to-treat analysis, of which 53 were in HRV group and 142 were in placebo group. VE against severe RVGE induced by G1, G3 and G8 serotype were 100%, by G9 serotype was 89.97%, by untyped serotype was 66.38% (Table 6).

4. Discussion

Before the HRV phase III clinical study, the epidemic investigation was conducted in part of provinces in China. Four provinces were chosen as multi-centers to conduct phase III study of HRV, Hebei Province in the north, Hunan Province in the south, Guangxi Province in the west and Zhejiang Province in the east of China, where the epidemic serotypes of rotavirus were different (Chen et al., 2021). In the past decade, G1, G2, G3, G4, G9 serotypes of wild rotavirus were predominant in the west of China, and G1, G2, G3, G9 in the south and east of China. G9 became the prevalence serotype in the rotavirus epidemic season in 2016–2017.

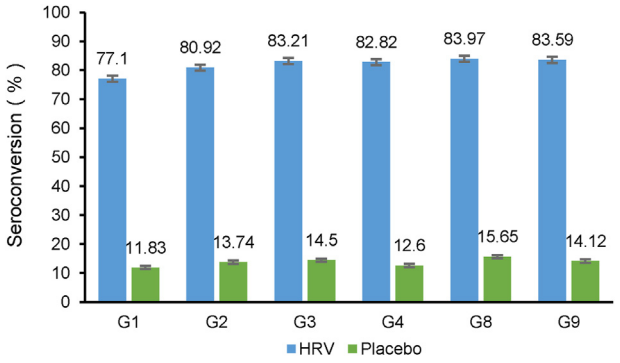

Fig. 2. Seroconversion rate of IgA among HRV and placebo groups post three doses of vaccination based on immunogenicity per protocol subjects.

In our vaccine safety analysis, the incidence of AEs of HRV and placebo were similar post any dose. The most common AEs were fever. According to China's National Medical Products Administration (NMPA) Guidelines for grading criteria of adverse events in clinical trials of preventive vaccines published in 2005 (NMPA, 2005), the incidence of fever class I (37.1–37.5 °C) within 28 days of each dose in HRV group was 53.1%, which was slightly higher than 50.08% in the placebo group. Actually, the normal body temperature of infants was 36.2–37.3 °C. The possible reason for fever was the body temperature fluctuation due to the underdeveloped infant thermoregulatory center. The new NMPA Guidelines for grading criteria of adverse events in clinical trials of preventive vaccines, which were published in Dec 2019, changed the minimal body temperature of fever to 37.5 °C (NMPA, 2019). As per the new guideline, the incidence of fever above 37.5 °C in our study was 19.17% in the HRV group and 16.29% in the placebo group, which was lower than the incidence of fever in the Rotateq clinical trial in Guangxi infants. In Rotateq clinical trial, the incidences of fever above 37.5 °C in the vaccine group and the placebo group were 21.84% and 22.83% (Mo et al., 2017). Based on the new criteria, the total incidence of solicited AEs in the HRV and placebo groups would be changed to 36.24% and 33.92%.

Moreover, the incidence of diarrhea was 11.54% in the HRV group and 10.47% in the placebo group, which was lower than Rotateq clinical trial in Guangxi infants, as 20.15% in the vaccine group and 20.11% in the placebo group. Incidence of SAE related to vaccine in HRV and placebo group were 0.44% and 0.25%, which was comparable with Rotarix clinical trial in Hong Kong, with the incidence of 0.397% in the vaccine group and 0.132% in the placebo group (Lau et al., 2013). Intussusception was observed in one case from the HRV group and one case from the placebo group. The intussusception case in the HRV group occurred five months post the third dose vaccination which was considered not vaccine-related. There was one case of Kawasaki disease (0.03%) and two

Table 4
GMC of IgA against serotype of rotavirus before first dose and one month after the third dose based on immunogenicity per protocol subjects.

Serotype	GMC of IgA (U/mL) of AbN[a] subject		GMC of IgA (U/mL) of AbP[b] subjects	
	HRV (95%CI)	Placebo (95%CI)	HRV (95%CI)	Placebo (95%CI)
G1	115.36 (94.37, 141.01)	12.25 (11.54, 13.65)	225.84 (101.20, 503.97)	30.51 (17.24, 53.97)
G2	163.47 (132.15, 202.21)	13.21 (11.95, 14.61)	380.23 (182.61, 791.74)	29.08 (17.17, 49.24)
G3	157.04 (127.11, 194.01)	13.65 (12.30,15.16)	500.69 (267.56, 936.96)	32.43 (19.62, 53.61)
G4	153.19 (124.61, 188.33)	12.80 (11.69, 14.02)	322.69 (134.58, 773.72)	33.07 (16.81, 65.08)
G8	174.05 (141.29, 214.40)	13.75 (12.39, 15.26)	529.70 (254.73, 1101.52)	28.75 (16.98, 48.68)
G9	176.90 (143.12, 218.66)	14.00 (12.53, 15.66)	371.30 (191.41, 720.25)	24.29 (15.90, 37.10)

[a] AbN: IgA antibody-negative before the first dose.
[b] AbP: IgA antibody-positive before the first dose. GMC: geometric mean concentration.

Table 5
Efficacy of HRV against RVGE and severe RVGE based on modified intention-to-treat subjects.

VE against	Case number		Efficacy (%, 95%CI)
	HRV	Placebo	
	N[a] = 2950	N[a] = 2982	
RVGE caused by G1–G4, G8–G9 serotypes	29	94	69.21 (53.31, 79.69)
First RV season	14	53	73.52 (52.28, 85.31)
Second RV season	15	41	63.57 (34.19, 79.84)
Severe RVGE caused by G1–G4, G8–G9 serotypes	5	58	91.36 (78.45, 96.53)
First RV season	4	27	85.12 (57.46, 94.79)
Second RV season	1	31	96.77 (76.34, 99.56)
Hospitalization caused by G1–G4, G8–G9 serotypes	3	28	89.21 (64.51, 96.72)
First RV season	2	11	81.69 (17.39, 95.94)
Second RV season	1	17	94.08 (55.49, 99.21)
RVGE caused by any serotype	53	142	62.88 (49.11, 72.92)
First RV season	23	73	68.47 (49.62, 80.27)
Second RV season	30	69	56.91 (33.84, 71.93)
Severe RVGE caused by any serotype	11	76	85.51 (72.74, 92.30)
First RV season	7	34	79.32 (53.35, 90.83)
Second RV season	4	42	90.49 (73.47, 96.59)
Hospitalization caused by any serotype	6	37	83.68 (61.34, 93.11)
First RV season	4	15	73.15 (19.09, 91.09)
Second RV season	2	22	90.85 (61.11, 97.85)

[a] N = subject number received HRV or placebo. RV: rotavirus. VE: vaccine efficacy. RVGE: rotavirus gastroenteritis.

cases of thrombocytopenic purpura (0.06%) in the HRV group. All the cases were treated immediately after occurring and recovered in a few days. It was noted that Kawasaki disease (0.035%) and thrmombocytopenic purpura (no data) were listed in the adverse reaction of instruction of Rotarix, and of Rotateq as well.

Exposure of placebo recipients to wild-type rotavirus attributed to the baseline of seroconversion rate even though it's much lower than the HRV group. The seroconversion rate of anti-rotavirus IgA in the placebo group was 11.83%–15.65%, which was comparable with the placebo group (11.1%) in the BRV-TV clinical test in India (Dhingra et al., 2014).

HRV efficacy against severe RVGE in the first two years of life was 85.51%, which is comparable to 72% VE of Rotarix (Li et al., 2014), 78.9% VE of Rotateq in Guangxi of China (Mo et al., 2017), and 96.9% VE of Rotarix in Hong Kong and Taiwan of China, and Singapore (Phua et al., 2012). The HRV efficacy against severe RVGE was higher than Rotasiil against severe RVGE (39.5%) and very severe RVGE (54.7%) in India (Kulkarni et al., 2017), even though HRV and Rotasiil vaccine shared the same serotypes of G1, G2, G3, G4 and G9 vaccine strains from NIH.

Table 6
Vaccine efficacy against RVGE caused by different serotypes.

Serotype	HRV (n)	Placebo (n)	Vaccine efficacy (%)	95%CI	*P* value
Severe RVGE					
G1	0	2	100.00	−437.72, 100.00	0.5049
G2	0	0	NA*	NA	NA
G3	0	1	100.00	−3838.71, 100.00	1.0000
G4	0	0	NA	NA	NA
G8	0	5	100.00	−10.20, 100.00	0.0640
G9	5	50	89.97	74.86, 96.00	<0.0001
Untyped	6	18	66.38	15.32, 86.66	0.0207
Any severe RVGE					
G1	0	2	100.00	−437.72, 100.00	0.5049
G2	0	0	NA	NA	NA
G3	3	1	−203.07	−2813.62, 68.47	0.3369
G4	0	0	NA	NA	NA
G8	1	7	85.58	−17.23, 98.23	0.0701
G9	25	84	70.29	53.57, 80.99	<0.0001
Untyped	24	48	49.67	17.85, 69.17	0.0060

NA: Not available. RVGE: rotavirus gastroenteritis.

In recent years, the predominant G serotype circulating in China was G9 (Wang et al., 2021), and G8 serotype (Wang et al., 2022), which was not contained in all the commercial rotavirus vaccines, is predominant in African countries and appears in China. This novel hexavalent rotavirus vaccine contained both G1–G4 serotypes and the new prevalence G8 and G9 serotypes in the world. Our results showed that the HRV efficacy against severe RVGE caused by G9 and G8 serotype was 89.97% (95%CI: 74.86–96.00) and 100.00% (95%CI: 10.20–100.00), slightly higher than 88.3% of Rotateq against G9 serotype (Mo et al., 2017) in China and 83.76% of Rotarix against G9 serotype (De Vos et al., 2009). Thus, HRV is hopeful to control diarrhea disease caused by G8 and G9 serotype rotavirus in infants.

5. Conclusions

In healthy Chinese infants, HRV was efficacious against natural infection of any serotype of rotavirus. It could provide high efficacy against severe RVGE and RVGE hospitalization regardless of serotype at least 14 days following the third vaccination and was well-tolerated for all AEs.

Data availability

All the data generated during the current study are included in the manuscript.

Ethics statement

The study was approved by the Institutional Review Boards of Hebei, Zhejiang, Hunan, and Guangxi Provincial Center for Disease Control and Prevention and conducted in compliance with Good Clinical Practice, the Declaration of Helsinki, and local regulations in China. Written informed consents were obtained from all the participants' parents or legal representatives. The clinical trial was registered under the number ChiCTR2100054584.

Author contributions

Zhiwei Wu: investigation. Qingliang Li: conceptualization. Yan Liu: methodology. Huakun Lv: project administration. Zhaojun Mo: project administration. Fangjun Li: project administration. Qingchuan Yu: methodology. Fei Jin: investigation. Wei Chen: supervision. Yong Zhang: investigation. Teng Huang: investigation. Xiaosong Hu: investigation.

Wei Xia: investigation. Jiamei Gao: methodology. Haisong Zhou: investigation. Xuan Bai: supervision. Yueyue Liu: methodology. Zhenzhen Liang: investigation. Zhijun Jiang: supervision. Yingping Chen: investigation. Jiuwei Zhang: supervision. Jialiang Du: methodology. Biao Yang: supervision. Bo Xing: investigation. Yantao Xing: investigation. Ben Dong: supervision. Qinghai Yang: investigation. Chen Shi: methodology. Tingdong Yan: investigation. Bo Ruan: supervision. Haiyun Shi: investigation. Xingliang Fan: methodology. Dongyang Feng: supervision. Weigang Lv: investigation. Dong Zhang: supervision. Xiangchu Kong: investigation. Liuyifan Zhou: supervision. Dinghong Que: investigation. Hong Chen: methodology. Zhongbing Chen: investigation. Xiang Guo: supervision. Weiwei Zhou: investigation. Cong Wu: supervision. Qingrong Zhou: investigation. Yuqing Liu: supervision. Jian Qiao: supervision. Ying Wang: supervision. Xinguo Li: project administration. Kai Duan: project administration. Yuliang Zhao: project administration, supervision. Xiaoming Yang: writing – review & editing, project administration. Gelin Xu: writing- original draft.

Conflicts of interest

Qingliang Li, Wei Chen, Xuan Bai, Zhijun Jiang, Jiuwei Zhang, Biao Yang, Ben Dong, Chen Shi, Bo Ruan, Dongyang Feng, Dong Zhang, Liuyifan Zhou, Hong Chen, Xiang Guo, Cong Wu, Yuqing Liu, Xinguo Li, Kai Duan, Jian Qiao, Ying Wang and Gelin Xu are currently employees of Wuhan Institute of Biological Products Co., Ltd. Xiaoming Yang is currently an employee of China National Biotec Group Co., Ltd., Beijing, China. The other authors declare they have no conflicts of interest. The sponsor had no role in data collection, analysis, or interpretation.

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