

RESEARCH PAPER



# Immunogenicity and safety of an inactivated enterovirus 71 vaccine co-administered with measles-mumps-rubella vaccine and live-attenuated Japanese encephalitis vaccine: a phase 4, single-center, randomized controlled trial

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## ABSTRACT

Co-administration of vaccines could be an efficient strategy to increase vaccination uptake and reduce the number of clinic visits. This randomized controlled study aimed to evaluate the immunogenicity and safety of enterovirus 71 (EV71) vaccine co-administered with measles-mumps-rubella (MMR) vaccine and live-attenuated Japanese encephalitis vaccine (LA-JEV). A total of 372 healthy infants were randomly assigned in a 1:1:1 ratio to receive simultaneous administration of EV71 vaccine (dose 1) and MMR on d 0 and EV71 vaccine (dose 2) and LA-JEV on d 30 (Group 1); administration of MMR and LA-JEV on d 0 and 30, respectively (Group 2); or administration of doses 1 and 2 of EV71 vaccine on d 0 and 30, respectively (Group 3). The non-inferiority analysis of the seroconversion for EV71 neutralizing antibody after vaccination was the primary outcome. According to per protocol set, antibody response against EV71, measles, mumps, rubella, and Japanese encephalitis (JE) virus was similar regardless of administration schedule. After vaccination, the seroconversion rate of EV71 neutralizing antibody in Group 1 (107 [97.27%] of 110) was non-inferior to that in Group 3 (109 [97.32%] of 112; difference – 0.05% [95% CI – 5.38 to 5.21]). The incidences of adverse reactions were 62.60% (77/123) in Group 1, 54.84% (68/124) in Group 2, and 37.70% (46/122) in Group 3, and most of them were mild to moderate in severity. No vaccine-related serious adverse events were reported. In total, the co-administration of combined EV71 vaccine with MMR and LA-JEV showed no interference with antibody response and demonstrated good safety profiles.

## ARTICLE HISTORY

Received 23 September 2021  
Revised 6 November 2021  
Accepted 21 November 2021

## KEYWORDS


Co-administration; EV71 vaccine; measles-mumps-rubella vaccine; live-attenuated Japanese encephalitis vaccine; immunogenicity; safety

## Introduction


Enterovirus 71 (EV71) is a small, positive-sense, single-stranded RNA virus that belongs to the *Enterovirus* genus of the *Picornaviridae* family, which was first isolated in 1969.<sup>1–3</sup> EV71 infection is associated with a wide spectrum of diseases, including hand, foot, and mouth disease (HFMD), herpangina, neurological signs, and nonspecific illnesses.<sup>4–6</sup> As one of the major causative agents of HFMD, it mainly affects infants and young children under 5 y of age.<sup>7,8</sup> From 2008 to 2017, a total of 17.9 million HFMD cases were reported in China.<sup>9</sup> In spite of most of the HFMD cases being mild or moderate, some severe HFMD cases can lead to severe central nervous system pathology and other complications.<sup>10</sup> It is worth noting that about 70% of the severe HFMD cases and approximately 90% of the fatal HFMD cases caused by EV71 infection, which has become a serious threat to public health and economic burden across some Asian countries.<sup>6,11–13</sup> In this light, it is important to develop efficacious vaccines to prevent EV71 infection.

As of 2018, three EV71 vaccines has been available in China for the prevention of EV71 infection, including a vero cell-based inactivated, alum-adjuvant EV71 vaccine developed by

Sinovac Biotech,<sup>14</sup> which has been demonstrated to have a high safety and efficiency in protecting infants aged 6–35 months from EV71-associated HFMD in a series of trials.<sup>15–17</sup> The results of sero-epidemiological study showed that the maternal EV71 antibodies waned with age after birth and reached the lowest level at 6 months of age.<sup>18</sup> Therefore, the Chinese Center for Disease Control and Prevention recommends that EV71 vaccination targets susceptible children aged 6 months or older and encourages the completion of vaccination procedures before the age of 12 months, so as to play a protective role as soon as possible.<sup>19</sup> However, there are already 14 vaccines in national immunization schedules that need to be administered during the same period, so the time window for separate two-dose of EV71 vaccine is very limited. As one of the effective measures to solve this dilemma, simultaneous administration is defined as inoculating more than one recommended vaccine through different syringes at different anatomical sites during the same visit.<sup>20</sup> Although co-administration of vaccines can increase vaccination uptake and reduce the number of clinic visits, it also raises concerns about the immunogenicity and safety of the vaccines involved, as simultaneous vaccination may impair the immune responses.<sup>21</sup>

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 Supplemental data for this article can be accessed on the publisher's website at <https://doi.org/10.1080/21645515.2021.2010428>.

In China, the busy vaccination schedule for the first two years of life has prompted the development of co-administration of vaccines with a view to increase vaccination uptake.<sup>20</sup> Measles-mumps-rubella (MMR) vaccine and live-attenuated Japanese encephalitis vaccine (LA-JEV), both of which are included in the country's Expanded Program on Immunization (EPI) schedule, have been recommended as routine vaccines. The current study aims to evaluate the immunogenicity and safety of simultaneous administration of EV71 vaccine with MMR and LA-JEV.

## Materials and methods

### Study design and participants

This study was a phase 4, single-center, open-label, randomized controlled trial conducted in Hanbin District, Ankang City, Shaanxi Province, China, from July 2020 to November 2020.

Eligible participants were healthy infants aged 8 months or older. Main exclusion criteria included prior receipt of any EV71, measles, mumps, rubella, or Japanese encephalitis vaccine; history of HFMD, measles, mumps, rubella, or Japanese encephalitis (JE); allergy or serious adverse reactions to any study vaccine components; receipt of blood products within the previous 3 months; and receipt of any live attenuated vaccines within the previous 4 weeks or of any inactivated vaccines within the previous 7 d.

After enrollment, all subjects were randomly assigned into three groups at a ratio of 1:1:1. In the co-administration group (Group 1), infants received two injections at different injection sites during the same visit, one dose each of EV71 vaccine and MMR on d 0 and one dose each of EV71 vaccine and LA-JEV on d 30. In the group where each EPI vaccine was administered separately (Group 2), infants received one dose of MMR on d 0 and one dose of LA-JEV on d 30. In the group where two doses of EV71 vaccine were administered separately (Group 3), infants received the first dose on d 0 and the second dose on d 30.

Vaccines for each group were labeled with random numbers, based on a predefined block size. Every subject was assigned a randomization code in chronological order and received the vaccine labeled with the same code.

Written informed consent was obtained from each infants' parent or guardian before enrollment. The trial was conducted in accordance with Good Clinical Practice standards and the Declaration of Helsinki. This study was approved by the ethical review committee of the Shaanxi Provincial Center for Disease Control and Prevention (SXSCDCIRB2019-001). The trial was registered with ClinicalTrials.gov (NCT04111432).

### Study vaccines

Vero cell-based inactivated alum-adsorbed EV71 vaccine (lot 201801005; Sinovac Biotech, Beijing) was packaged in a syringe with at least 3.0 Efficacy Unit (EU) of neutralization antibody titer for EV71 per dose (0.5 mL/vial). MMR vaccine (lot 201810120;

Shanghai Institute of Biological Products) was packaged in a penicillin bottle containing a median cell culture infective dose (CCID<sub>50</sub>) of  $\geq 10^{3.0}$  for measles virus,  $\geq 10^{4.3}$  for mumps virus and  $\geq 10^{3.0}$  for rubella virus in a total volume of 0.5 mL/vial. LA-JEV (lot 201806A114-2; Chengdu Institute of Biological Products) was packaged in a penicillin bottle contains not less than 5.4 log plaque-forming units (PFU) of live JE virus in a total volume of 0.5 mL/vial. All vaccines were tested and approved for lot release by the Chinese National Institute for Food and Drug Control (CNIFDC).

### Immunogenicity assessment

Blood samples were collected from all participants on d 0 (before the first vaccination) and d 60 (30 d after the second vaccination). After collection, serum samples were isolated and stored at  $-20^{\circ}\text{C}$  until assayed. The neutralizing antibody against EV71 and JE was detected in the Sinovac Biotech and Shaanxi Provincial Center for Disease Control and Prevention, respectively, and the detection methods were microcytopathogenic effect assay and microneutralization test (Supplementary method). Measles, mumps, and rubella IgG antibody concentrations were detected by an enzyme-linked immunosorbent assay (ELISA) kit produced by Virion/Serion (Wurzburg, Germany) in Shaanxi Provincial Center for Disease Control and Prevention.

The immunogenicity endpoints included seroconversion rate, geometric mean titer (GMT)/geometric mean concentration (GMC). For EV71 vaccine, the seroconversion rate was defined as the percentage of participants with a reciprocal neutralizing antibody titer of either (1)  $<1:8$  before vaccination and  $\geq 1:8$  after vaccination or (2)  $\geq 1:8$  before vaccination and at least a fourfold increase after vaccination. For LA-JEV, the seroconversion rate was defined as the percentage of participants with a reciprocal neutralizing antibody titer of either (1)  $<1:5$  before vaccination and  $\geq 1:5$  after vaccination or (2)  $\geq 1:5$  before vaccination and at least a fourfold increase after vaccination. The seropositivity of measles, mumps, and rubella was defined as the concentration of measles IgG antibody  $\geq 200$  mIU/mL, mumps IgG antibody  $\geq 100$  U/mL and rubella IgG antibody  $\geq 20$  IU/mL. Seroconversion was defined as a change from seronegative to seropositive or at least a fourfold increase in IgG antibody concentration after vaccination.

### Safety assessment

Immediate adverse events (AEs) were observed on site in 30 minutes after each vaccination. A diary card was given to parents or guardians to record solicited local or systemic AEs occurring within 14 d (or 7 d for Group 3), unsolicited AEs and SAE occurring within 30 d after each dose. Solicited systemic AEs included fever, allergic reaction, fatigue, irritability, decreased appetite, nausea/vomiting, and diarrhea; solicited local AEs included pain, induration, redness, swelling, rash, and pruritus. Face-to-face or telephone visits were assigned on the 7th day or 14th day, and 30th day after each vaccination to assure completeness and accuracy. The causal relationship between adverse events and vaccination was judged by the investigators.

## Statistical analysis

The sample size was estimated using NCSS-PASS, version 11.0 based on a non-inferiority design.<sup>22</sup> We assumed a 95% proportion of seroconversion against EV71 after administration of EV71 vaccine alone. A sample size of 95 individuals per group was required for a power (calculated as  $1 - \beta$ ) of 0.80 with a one-sided  $\alpha$  of 0.025 and a non-inferiority margin of -10% with a Z test with pooled variance. With an estimated dropout rate of  $\leq 20\%$ , a total of 372 participants finally were recruited in this trial.

The comparison of demographic characteristics was performed for the full analysis set (FAS). The immunogenicity analysis was conducted in the per-protocol set (PPS), including infants who met eligibility criteria, complied with the protocol and had immunogenicity results before and after vaccination. Safety analysis was performed for the safety set (SS), which included infants who received at least one vaccination. Categorical variables were reported as frequency and percentage, continuous variables were reported as means and standard deviation. The Student's t-test was used for comparison of

continuous data, and  $\chi^2$  test/Fisher's exact test was used for comparison of categorical data. A  $p$ -value of less than 0.05 was considered statistically significant. All analyses were done with SAS, version 9.4 (SAS Institute Inc, Cary, North Carolina, USA).

## Results

### Study subjects

The study process is shown in Figure 1. Between July 26, 2019, and Aug 25, 2019, a total of 379 infants were screened, of whom 372 were enrolled and randomly allocated to three groups in a 1:1:1 ratio. In total, 369 (99.19%, 369/372) participants received the first dose and were included in the safety population. Also, 352 (94.62%, 352/372) received the second dose and 338 (90.86%, 338/372) were included in the per-protocol population for immunogenicity analysis. The demographic characteristics of the participants were similar in terms of ethnicity, age, height, and weight among the three groups (Table 1).

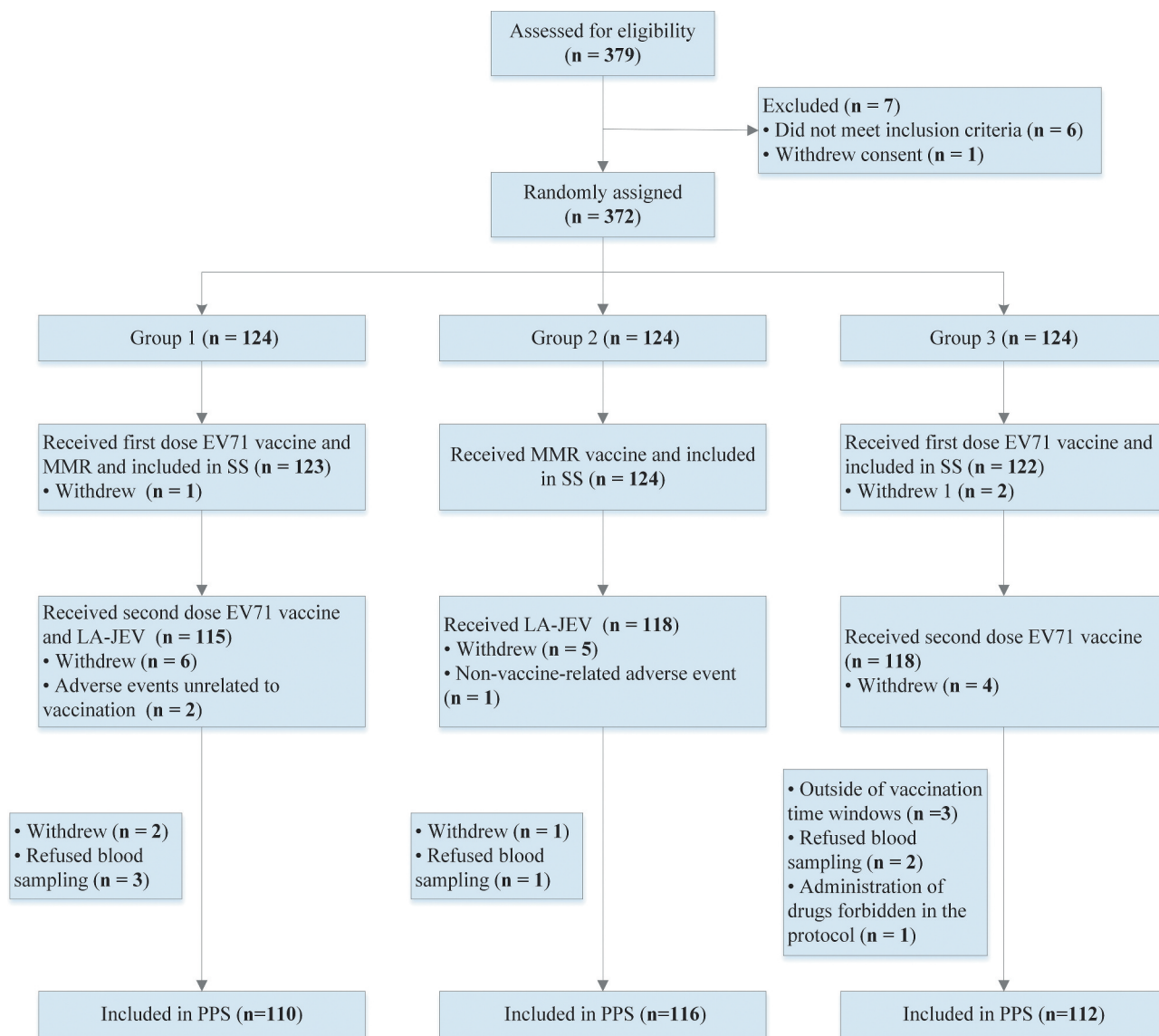


Figure 1. Trial profile.

**Table 1.** Baseline characteristics of the study participants in the full analysis set.

Characteristic	Group 1 (n = 123)	Group 2 (n = 124)	Group 3 (n = 122)	P value
Male, no. (%)	59 (47.97)	66 (53.23)	66 (54.10)	.582
Ethnic Han, no. (%)	123 (100.00)	123 (99.19)	121 (99.18)	.775
Age, months	8.30 ± 0.57	8.37 ± 0.72	8.39 ± 0.73	.891
Height, cm	71.19 ± 2.68	70.38 ± 2.42	70.68 ± 2.67	.095
Weight, kg	8.88 ± 0.99	8.82 ± 0.94	9.00 ± 1.06	.408

Data are no. (%) of participants or mean ± SD. Study groups were as follows: simultaneous receipt of enterovirus 71 (EV71) vaccine (dose 1) and measles-mumps-rubella vaccine (MMR) on d 0 and EV71 vaccine (dose 2) and live-attenuated Japanese encephalitis vaccine (LA-JEV) on d 30 (Group 1); receipt of MMR and LA-JEV on d 0 and 30, respectively (Group 2); or receipt of doses 1 and 2 of EV71 vaccine on d 0 and 30, respectively (Group 3).

### Immunogenicity

At baseline, the seropositivity and GMT of neutralizing antibody against EV71 showed no differences between Group 1 and Group 3. After vaccination, 109 (99.09%) of 110 infants in the Group 1 and 112 (100%) of 112 infants in the Group 3 developed neutralizing antibodies against EV71 ( $P = .495$ ), yielding a non-inferior proportion of seroconversion of 97 · 27% in Group 1 (107 of 110 infants) and of 97 · 32% in Group 3 (109 of 112 infants; difference – 0.05% [95% CI – 5.38 to 5.21]). Similarly, the GMTs of antibody against EV71 were comparable between Group 1 and Group 3 (96.86 vs 101.25;  $P = .901$ ) (Tables 2 and 3).

Before vaccination, seropositivity and GMC/GMT of antibodies against measles, mumps, rubella, and JE showed no differences between Group 1 and Group 2. After vaccination, the seropositivity rates for measles, rubella, and mumps and JE antibodies in Group 1 were 98.18%, 97.27%, 95.45%, and 97.27%, respectively; in Group 2 were 99.14%, 95.69%, 96.55%, and 88.79%, respectively. The seropositivity rates of measles, mumps, rubella, and JE antibodies were not significantly different between Group 1 and Group 2. Participants in

Group 1 and those in Group 2 also showed no difference in the frequency of seroconversion against measles (98.18% vs 99.14%;  $P = .963$ ), mumps (96.36% vs 93.79%;  $P = .402$ ), rubella (95.45% vs 94.83%;  $P = .827$ ), and JE (81.82% vs 86.21%;  $P = .512$ ). Similarly, the GMCs of measles, mumps, and rubella IgG antibodies, and the GMT of JE neutralizing antibodies in Group 1 were generally similar to those in Group 2 (Tables 2 and 3).

### Safety

The incidences of local and systemic adverse reactions (ARs) are presented in Table 4. All vaccination schedules were well tolerated in the three groups. Any ARs within 30 d after vaccination occurred in 77 (62.60%) of 123 participants in Group 1, 68 (54.84%) of 124 in Group 2 and 46 (37.70%) of 122 in Group 3. There was no statistically significant difference between Group 1 and Group 2 ( $P = .245$ ), but it was higher than the Group 3 ( $P < .001$ ). Most ARs were grade 1 and grade 2 in severity, and the incidence of grade 3 ARs were 4.88% (6/123) in Group 1, 5.65% (7/124) in Group 2 and 3.28% (4/122) in Group 3, respectively (Supplementary Table S1). In addition, the incidence of adverse reactions after the first dose was higher than that after the second dose (Supplementary Table S2).

Most ARs were solicited, with only one participant (0.81%) in Group 1, one (0.81%) in Group 2, and two (1.64%) in Group 3 reporting unsolicited ARs ( $P = .700$ ). Redness (4.34%, 16/369) was the most common local symptom. The most common systemic ARs were fever (43.09%, 159/369) and diarrhea (16.26%, 60/369). Except for a higher incidence of injection site swelling and fever in Group 1 and Group 2 than that in Group 3, there were no significant differences in the incidence of other solicited and unsolicited symptoms among the three groups (Table 4).

**Table 2.** Seropositive and seroconversion rates of enterovirus 71 (EV71), measles, mumps, rubella, and Japanese encephalitis (JE) antibody pre and post vaccination, per-protocol sets.

Variable	Group 1		Group 2 or Group 3		P value
	n (%)	95% CI	n (%)	95% CI	
<b>EV71 neutralizing antibody (N)</b>	110		112		
Seropositive pre-vaccination	4 (3.64)	(3.52,6.89)	6 (5.36)	(5.18,8.50)	.768
Seropositive post-vaccination	109 (99.09)	(95.04,99.98)	112 (100)	(96.76,100.00)	.495
Seroconversion	107 (97.27)	(92.24,99.43)	109 (97.32)	(92.37,99.44)	1.000 <sup>a</sup>
<b>Measles IgG antibody (N)</b>	110		116		
Seropositive pre-vaccination	0 (0.00)	(0.00,3.37)	0 (0.00)	(0.00,3.21)	-
Seropositive post-vaccination	108 (98.18)	(93.59,99.78)	115 (99.14)	(95.29,99.98)	.963
Seroconversion	108 (98.18)	(93.59,99.78)	115 (99.14)	(95.29,99.98)	.963
<b>Mumps IgG antibody (N)</b>	110		116		
Seropositive pre-vaccination	2 (1.82)	(1.76,5.13)	3 (2.59)	(2.51,5.71)	1.000
Seropositive post-vaccination	107 (97.27)	(92.24,99.43)	111 (95.69)	(90.23,98.59)	.777
Seroconversion	106 (96.36)	(90.95,99.00)	109 (93.79)	(87.96,97.54)	.402
<b>Rubella IgG antibody (N)</b>	110		116		
Seropositive pre-vaccination	0 (0.00)	(0.00,3.37)	2 (1.72)	(1.66,4.87)	.498
Seropositive post-vaccination	105 (95.45)	(89.71,98.51)	112 (96.55)	(91.41,99.05)	.935
Seroconversion	105 (95.45)	(89.71,98.51)	110 (94.83)	(89.08,98.08)	.827
<b>JE neutralizing antibody (N)</b>	110		116		
Seropositive pre-vaccination	6 (5.45)	(2.03,11.49)	3 (2.59)	(0.54,7.37)	-
Seropositive post-vaccination	96 (87.27)	(79.57,92.86)	103 (88.79)	(81.60,93.90)	.725
Seroconversion	90 (81.82)	(73.33,88.53)	100 (86.21)	(78.57,91.91)	.368

Study groups were as follows: simultaneous receipt of enterovirus 71 (EV71) vaccine (dose 1) and measles-mumps-rubella vaccine (MMR) on d 0 and EV71 vaccine (dose 2) and live-attenuated Japanese encephalitis vaccine (LA-JEV) on d 30 (Group 1); receipt of MMR and LA-JEV on d 0 and 30, respectively (Group 2); or receipt of doses 1 and 2 of EV71 vaccine on d 0 and 30, respectively (Group 3).

<sup>a</sup>Non-inferiority was achieved, as the lower bound of the two-sided 95% CI was  $> -10\%$ .

**Table 3.** Geometric mean titer (GMT)/concentration (GMC) of enterovirus 71 (EV71), measles, mumps, rubella, and Japanese encephalitis (JE) antibody pre and post vaccination, per-protocol sets.

Variable	Group 1	Group 2 or Group 3	P value
	GMT/GMC (95% CI)	GMT/GMC (95% CI)	
<b>EV71 neutralizing antibody (N)</b>	110	112	
Pre-vaccination GMT	4.13 (4.00,4.27)	4.18 (4.03,4.33)	.539
Post-vaccination GMT	96.86 (75.67,123.99)	101.25 (78.22,131.06)	.901
<b>Measles IgG antibody (N)</b>	110	116	
Pre-vaccination GMC	35.49 (32.24,39.06)	38.39 (35.44,41.57)	.087
Post-vaccination GMC	2982.53 (2545.55,3494.53)	2797.91 (2371.76,3300.64)	.260
<b>Mumps IgG antibody (N)</b>	110	116	
Pre-vaccination GMC	30.85 (28.03,33.95)	30.27 (27.40,33.43)	.703
Post-vaccination GMC	532.63 (457.18,620.53)	424.89 (367.10,491.78)	.035
<b>Rubella IgG antibody (N)</b>	110	116	
Pre-vaccination GMC	2.62 (2.45,2.80)	2.89 (2.65,3.15)	.146
Post-vaccination GMC	79.56 (68.58,92.30)	75.70 (66.16,86.61)	.415
<b>Japanese encephalitis neutralizing antibody (N)</b>	110	116	
Pre-vaccination GMT	2.60 (2.52,2.68)	2.55 (2.49,2.60)	.273
Post-vaccination GMT	7.12 (6.32,8.01)	7.33 (6.55,8.20)	.688

Study groups were as follows: simultaneous receipt of enterovirus 71 (EV71) vaccine (dose 1) and measles-mumps-rubella vaccine (MMR) on d 0 and EV71 vaccine (dose 2) and live-attenuated Japanese encephalitis vaccine (LA-JEV) on d 30 (Group 1); receipt of MMR and LA-JEV on d 0 and 30, respectively (Group 2); or receipt of doses 1 and 2 of EV71 vaccine on d 0 and 30, respectively (Group 3). GMT: geometric mean titer; GMC: geometric mean concentration.

Throughout the trial, 32 serious adverse events were reported by a total of 18 participants: 4 (3.25%) of 123 in Group 1, 6 (4.84%) of 124 in Group 2, 8 (6.56%) of 122 in Group 3, without significant difference ( $P = .448$ ). All serious adverse events were considered not related to vaccination.

## Discussion

In this phase 4, open-label, single-center, randomized controlled study, we found that co-administration of EV71 vaccine with MMR and LA-JEV did not adversely impair immune responses to any of the vaccine antigens. The high rates of seroconversion and high antibody titer against EV71 found in the co-administration group and EV71 vaccination alone group provide reassurance that infants will be protected from EV71 infection, whether the EV71 vaccine is given alone or co-administered with MMR and LA-JEV. The seropositive

post-vaccination and seroconversion rates of JE neutralizing antibody were relatively low when compared with other published results, which may be related to different laboratory detection methods. However, there were no significant differences observed in the seroconversion rate or GMT of JE neutralizing antibody in the co-administration group versus non-co-administration group, which indicated that the simultaneous administration did not affect the immune responses of JE antibody. Taken together, these data support the simultaneous administration of EV71 vaccine with those two EPI vaccines, which not only avoid the extra cost and inconvenience associated with an additional clinic visit, but also may increase the vaccine uptake.

The safety profile of co-administration group was higher than that of the EV71 vaccination alone group and was slightly higher than that in the MMR/LA-JEV vaccination alone group, but this trend was not considered a statistically significant

**Table 4.** Adverse reactions (ARs) reported following any vaccination.

Adverse reactions	Group 1 (N = 123)	Group 2 (N = 124)	Group 3 (N = 122)	Total (N = 369)	P value		
					Three groups	Group 1 vs 2	Group 1 vs 3
<b>Solicited</b>	77 (62.60)	68 (54.84)	45 (36.89)	190 (51.49)	<.001	.245	<.001
Local	6 (4.88)	6 (4.84)	7 (5.74)	19 (5.15)	.915		
Redness	5 (4.07)	4 (3.23)	7 (5.74)	16 (4.34)	.560		
Induration	1 (0.81)	2 (1.61)	4 (3.28)	7 (1.90)	.320		
Pruritus	3 (2.44)	1 (0.81)	0 (0.00)	4 (1.08)	.230		
Swelling	0 (0.00)	0 (0.00)	3 (2.46)	3 (0.81)	.036	1.000	.122
Rash	1 (0.81)	1 (0.81)	0 (0.00)	2 (0.54)	1.000		
Systemic	75 (60.98)	66 (53.23)	43 (35.25)	184 (49.86)	<.001	.248	<.001
Fever	68 (55.28)	59 (47.58)	32 (26.23)	159 (43.09)	<.001	.253	<.001
Diarrhea	25 (20.33)	18 (14.52)	17 (13.93)	60 (16.26)	.356		
Irritability	10 (8.13)	6 (4.84)	7 (5.74)	23 (6.23)	.570		
Decreased appetite	9 (7.32)	3 (2.42)	3 (2.46)	15 (4.07)	.108		
Nausea/vomiting	6 (4.88)	3 (2.42)	3 (2.46)	12 (3.25)	.576		
Allergy	4 (3.25)	4 (3.23)	1 (0.82)	9 (2.44)	.407		
Fatigue	3 (2.44)	1 (0.81)	3 (2.46)	7 (1.90)	.577		
<b>Unsolicited</b>	1 (0.81)	1 (0.81)	2 (1.64)	4 (1.08)	.700		
Cough	1 (0.81)	0 (0.00)	2 (1.64)	3 (0.81)	.219		
Abdominal distention	0 (0.00)	1 (0.81)	0 (0.00)	1 (0.27)	1.000		
<b>Overall</b>	77 (62.60)	68 (54.84)	46 (37.70)	191 (51.76)	<.001	.245	<.001

Data are no. (%) of participants. Participants can have more than 1 reported AR. Study groups were as follows: simultaneous receipt of enterovirus 71 (EV71) vaccine (dose 1) and measles-mumps-rubella vaccine (MMR) on d 0 and EV71 vaccine (dose 2) and live-attenuated Japanese encephalitis vaccine (LA-JEV) on d 30 (Group 1); receipt of MMR and LA-JEV on d 0 and 30, respectively (Group 2); or receipt of doses 1 and 2 of EV71 vaccine on d 0 and 30, respectively (Group 3).

difference. Both MMR and LA-JEV are EPI vaccines, which have been in use for decades in China and are considered safe. The vero cell-based inactivated alum-adjuvanted EV71 vaccine (Sinovac Biotech) also has been demonstrated to have significant safety and efficiency in protecting infants aged 6–35 months from EV71-associated HFMD in a series of trials,<sup>15,16,23</sup> and was licensed for infants by the China Food and Drug Administration in 2015. In the present study, compared with the EV71 vaccination alone group, the other two groups reported a higher frequency of ARs, but most of them were mild or moderate intensity and resolved within a few days. This difference was mainly related to the higher incidence of fever after the first dose of vaccination in the co-administration group and MMR/LA-JEV vaccination alone group. The underlying mechanism may be caused by the measles antigen component in the MMR vaccine.<sup>24</sup> The virus strain (191 strain) in measles vaccine still has a certain degree of toxicity.<sup>25</sup> A small number of infants will have transient high fever after vaccination, but the prognosis is good, which is significantly different from measles infection.

Our results are consistent with a randomized study done in 2017,<sup>20</sup> which showed simultaneous administration of EV71 vaccine with the recombinant hepatitis B vaccine and group A meningococcal polysaccharide vaccine did not affect EV71 neutralizing antibody seroconversion proportion compared with an EV71 vaccine only group. In addition, the investigators showed that the immunogenicity and safety of the recombinant hepatitis B vaccine and group A meningococcal polysaccharide vaccine in the co-administered group was not inferior to separate administration of each vaccine. Although those two EPI vaccines are different from this study, the consistency of the findings is reassuring.

This study has some limitations. First, a few enrolled participants withdrew or did not visit the clinic during the scheduled window, which led to the reduction of the sample size for analysis, although the final sample size was large enough to support the non-inferiority test. Another potential limitation of this trial is that it was conducted in a single center, and most of the participants were Han ethnicity. Although we cannot be sure of the differences in vaccine immune responses among different populations, we believe that further studies involving people of different ethnic backgrounds are needed. Third, since we chose only one laboratory method for testing, this may make it impossible to compare with other studies. Finally, a further limitation is the open-label design. This is unlikely to affect immunogenicity results because the laboratory personnel are blinded but could theoretically bias the safety assessment.

In conclusion, co-administration of EV71 vaccine with MMR and LA-JEV did not show significant interference in antibody responses and demonstrated good safety profiles. This provides convincing evidence that concomitant administration can be a reliable alternative and a good supplement to conventional planned vaccination program. Co-administration of these vaccines can reduce the number of needed immunization visits and economic costs, save parents and health workers' time, and alleviate the pain of infants. Furthermore, simultaneous administration has the potential of increasing the coverage of vaccination and of protecting an increased number of infants from the

morbidity and mortality associated with these diseases, so it can be considered for further promotion and application.

## Acknowledgments

We would like to express our thanks to the staff of the Hanbin District (Ankang city) Center for Disease Control and Prevention for their efforts in the implementation of the study site.

## Disclosure statement

WY, YX, and LW are employees of Sinovac Biotech Co., Ltd. All other authors declare no conflict of interest.

## Funding

This work was supported by Sinovac Biotech Co. Ltd.

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All authors participated either in the design (SZ, LW, XL), implementation (XL, WY, CZ, HW, RW, QD, YH, YX) or analysis and interpretation of data (SZ, LW, XL, WY, CZ, QD) of the study, as well as in the development of this manuscript. All authors had full access to the data and granted their final approval of the manuscript before submission.

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