

# Immunogenicity and safety of an enterovirus 71 vaccine in children aged 36-71 months: A double-blind, randomised, similar vaccine-controlled, non-inferiority phase III trial

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## Summary

**Background** The enterovirus 71 (EV71) vaccine produced by Wuhan Institute of Biological Products Co., Ltd. (WIBP) (B-EV71) has been given to children aged 6-35 months, and it has shown good safety, immunogenicity and efficacy. However, the administration of EV71 vaccine in children aged 36-71 months, which is another target population, needs further exploration.

**Methods** We conducted a double-blind, randomised, controlled, non-inferiority phase III clinical trial in children aged 36-71 months, with a further comparison group of children aged 6-35 months in China. Children aged 6-71 months with no history of hand, foot and mouth disease or prior-vaccination of EV71 vaccine were eligible and recruited. Eligible participants aged 36-71 months were randomly assigned (1:1) to receive two doses of the B-EV71 vaccine (Older-B group) or the control EV71 vaccine (C-EV71 vaccine, produced by Institute of Medical Biology, Chinese Academy of Medical Sciences) (Older-C group), administered at a 30-day interval. Eligible participants aged 6-35 months were enrolled consecutively to receive two doses of the B-EV71 vaccine (Younger-B group) at a 30-day interval. Participants, investigators and those assessing outcomes were masked to the vaccine received. Non-inferiority analyses were conducted to compare the immunogenicity of EV71 vaccine in the Older-B group with that in the Older-C and Younger-B groups. Non-inferiority margins were 10% for seroconversion rate differences and 0.5 for geometric mean titre (GMT) ratios. The primary endpoints were the GMT level and seroconversion rate of anti-EV71 neutralising antibody 30 days after the second dose of vaccination. The primary analysis was performed in the per-protocol population. Safety analyses were conducted amongst participants receiving at least one dose of vaccine. This trial was registered at [Chinadrugtrials.org.cn](http://Chinadrugtrials.org.cn) (#CTR20192345).

**Findings** Between June 3 and June 30, 2020, 1600 participants were enrolled and assigned, including 625 participants in the Older-B group, 625 participants in the Older-C group and 350 participants in the Younger-B group. The seroconversion rate of anti-EV71 neutralising antibody in the Older-B group (99.66%; 95% CI: 99.18%–100.00%) was non-inferior to that of the Older-C (99.32%; 95% CI: 98.65%–99.98%) and Younger-B groups (100.00%; 95% CI: 100.00%–100.00%). The differences in seroconversion rates in the Older-B group to those in the Older-C and Younger-B groups were 0.34% (95%CI: -2.17%–2.86%) and -0.34% (95%CI: -2.78%–2.09%). The GMT of the anti-EV71 neutralising antibody in the Older-B group (693.87) was also non-inferior to that in the Older-C (289.37) and Younger-B groups (634.80). The ratios of GMTs in the Older-B group to those in the Older-C and Younger-B

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groups were 2.67 (95%CI: 2.00–3.00) and 1.00 (95%CI: 0.75–1.00), respectively. The incidence of any adverse event (AE) related to vaccination was similar amongst the three groups (34/625 [5.44%] in the Older-B group, 32/623 [5.14%] in the Older-C group, and 26/349 [7.45%] in the Younger-B group), with only 2 (0.57%) participants having grade 3 AEs in the Younger-B group. Fifteen (0.94%) participants from these three groups had reported serious AEs (SAEs), all of which were unrelated to vaccines.

**Interpretation** EV71 vaccine produced by WIBP could extend to be administered to children aged 36–71 months against EV71 infection. However, the persistence of vaccine-induced immunities needs to be further investigated.

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**Keywords:** Enterovirus 71 vaccine; Hand, foot and mouth disease; Immunogenicity; Safety

### Research in context

#### *Evidence before this study*

Previous studies have shown that the enterovirus 71 (EV71) vaccine produced by Wuhan Institute of Biological Products Co., Ltd (B-EV71 vaccine) had good immunogenicity and safety in infants aged 6–35 months, who are deemed to be the priority of the target population. Nevertheless, children 3–5 years of age accounted for approximately 30% of hand, foot and mouth disease (HFMD) cases and are also susceptible to EV71 infection. Further exploration is needed for the B-EV71 vaccine application in older children. But there are no clinical trials or studies of the B-EV71 vaccine among children aged 36–71 months.

#### *Added value of this study*

This is the first phase III trial to assess the safety, immunogenicity, and persistence after vaccination of the B-EV71 vaccine among children 36–71 months of age. The B-EV71 vaccine administered to children aged 36–71 months was proven to be immunologically non-inferior to that in infants aged 6–35 months. Meanwhile, the immunogenicity of the B-EV71 vaccine was non-inferior to that of the control EV71 vaccine (C-EV71 vaccine, produced by the Institute of Medical Biology, Chinese Academy of Medical Sciences) in children aged 36–71 months. Additionally, antibody responses 12 months after two doses of vaccination were higher in participants of 36–71 months received the B-EV71 vaccine than the C-EV71 vaccine. No safety concerns associated with the B-EV71 vaccine occurred in both children aged 3–5 years and infants aged 6–35 months during the follow-up.

#### *Implications of all the available evidence*

The B-EV71 vaccine has got licensed for children aged 6–35 months by the former China Food and Drug Administration in 2016. Our findings confirm that

the B-EV71 vaccine is also safe and immunogenic among children aged 36–71 months, who would still be susceptible to EV71 infection. Results from the current trial will be critical for supporting licensure of the B-EV71 vaccine in childhood immunization of children aged 36–71 months.

### Introduction

Hand, foot and mouth disease (HFMD) is an acute infectious disease in children under 5 years old, which is mainly caused by coxsackievirus A6, coxsackievirus A16 and enterovirus 71 (EV71).<sup>1,2</sup> EV71 accounts for the majority of severe and fatal cases of HFMD. HFMD infected with EV71 could lead to severe central nervous system pathology and other complications.<sup>3–5</sup> Although most cases are mild and self-limited, HFMD remains a serious public health problem for children throughout Asia.<sup>6</sup> Since the discovery of the first HFMD case in China in 1981, it has broken out and spread across the country, leading to a serious public health threat and economic burden in China.<sup>7–10</sup> Given the nationwide HFMD epidemic of HFMD in China, HFMD was classified as a category C notifiable infectious disease by the Chinese Ministry of Health on 2 May, 2008. HFMD cases must be reported through the surveillance system within 24 h.<sup>11</sup> From 2008 to 2017, a total of 17,945,308 HFMD cases had been reported in China, 151,194 (0.85%) of which were severe cases and 3623 (0.02%) were fatal cases, and most of these cases were children under 5 years old.<sup>12</sup> Amongst the category C notifiable infectious diseases reported in 2018, HFMD accounted for 30.28%.<sup>13</sup> Given that HFMD is a serious public health problem that threatens children's health, the EV71 vaccine is of great importance to young children.

The inactivated EV71 vaccine produced by Wuhan Institute of Biological Products Co., Ltd. (WIBP) (B-EV71) is suitable for preventing HFMD caused by EV71.<sup>14,15</sup> In phase III clinical trials, the efficacy of EV71 vaccine on HFMD caused by EV71 was above 90%, with a 100% protective effect on hospitalised or severe cases.<sup>14</sup> In 2016, the China Food and Drug Administration approved the registration of the B-EV71 vaccine with a target population of children aged 6 months to 3 years, which had been widely used in Chinese children. However, leaving children aged 3-5 years uncovered by EV71 immunisation may cause the peak incidence to shift from young children to older children.<sup>16-18</sup> The older ones would still be susceptible to EV71 infection. In addition, studies have reported that EV71 vaccines could be administered to children aged 3 years or older to protect them from HFMD.<sup>19,20</sup> For example, a phase IV study of licenced inactivated EV71 vaccine (C-EV71 vaccine, produced by the Institute of Medical Biology, Chinese Academy of Medical Sciences) applied to children aged 6-71 months revealed overall level of 89.7% protection effectiveness against EV71 infection and a 4.58% rate of reported adverse events. Therefore, we aim to evaluate whether the B-EV71 vaccine could be adapted for children aged 3-5 years.

In this phase III clinical trial, we investigated the safety and immunogenicity of the B-EV71 vaccine in children aged 36-71 months, compared with (i) that of the control EV71 vaccine (C-EV71 vaccine) in the same age group, and (ii) that of the B-EV71 vaccine in children aged 6-35 months. The C-EV71 vaccine made from human diploid cells was developed and licenced in mainland China. Phase III and IV clinical trials have verified that the C-EV71 vaccine could elicit EV71-specific immune responses and protect against EV71-associated HFMD in children aged 6-71 months.<sup>21,22</sup>

## Methods

### Study design

This study was a double-blind, randomised, controlled, non-inferiority, phase III clinical trial investigating the safety and immunogenicity of the B-EV71 vaccine (Vero Cell) in children aged 36-71 months, with a further comparison group of children aged 6-35 months in Xiangzhou District, Xiangyang City, Hubei Province, China. Blood samples were collected from participants aged 36-71 months who received the B-EV71 or C-EV71 vaccine 12 months after two doses of vaccination to test anti-EV71 neutralising antibody and evaluate the immunity persistence of the B-EV71 vaccine. The protocol and informed consent form were approved by the ethics committee of Hubei Provincial Centre for Disease Control and Prevention. All parents or guardians of the participants provided a written informed consent. The trial was performed in compliance with the Declaration of

Helsinki, the good clinical practice (GCP) guidelines, and the Chinese regulatory requirements. The trial was also reported in adherence to CONSORT reporting guidelines. The protocol is available in Appendix Text 1.

### Participants

After the parents or guardians of the participants signed the written informed consent, physical examination, consultation, screening, and enrollment were carried out. Healthy children aged 6-71 months at the research site could be enrolled. Children who had prior EV71 vaccination, history of HFMD, history of a severe allergy to any vaccine or vaccine ingredient, autoimmune disease or immunodeficiency, acute disease or acute stage of chronic disease within 7 days prior to the enrollment or other things that may affect the evaluation of the trial assessed by the investigators were excluded. The additional eligibility criteria are available in Appendix Text 1.

### Randomisation and masking

Computer-generated randomisation lists were prepared by an independent study statistician using SAS software (version 9.4; SAS Institute, Cary, NC). Participants aged 36-71 months were randomly assigned in a 1:1 ratio to receive the B-EV71 vaccine (Older-B group) or the C-EV71 vaccine (Older-C group). Each eligible participant aged 36-71 months was assigned a randomisation code and received the vaccine. All participants, investigators and those assessing outcomes were masked to the vaccine received. Eligible participants aged 6-35 months were enrolled consecutively to receive the B-EV71 vaccine (Younger-B group) by the investigators. All experimental vaccines were placed in a packaging box with the same appearance and only marked with the random number of the vaccine, which was the research number.

### Procedures

The experimental vaccine was a vero cell-based inactivated EV71 vaccine (subgenotype C4, Ho7 strain; lot 201902003; produced by WIBP) (B-EV71 vaccine), which has been licenced for children aged 6-35 months in 2016. The control vaccine is a human diploid cell-based inactivated EV71 vaccine (subgenotype C4, FY-23K-B strain; lot 201810082Q; produced by the Institute of Medical Biology, Chinese Academy of Medical Sciences) (C-EV71 vaccine) which has been licenced for children aged 6-71 months in 2015. Both the B-EV71 and C-EV71 vaccines were packaged in the syringe with at least 3.0 efficacy units of neutralisation antibody titre for EV71 per dose (0.5 mL/vial) and stored and transported at 2°C-8°C in the dark. In this study, vaccines were tested and approved for lot release by the National Institutes for Food and Drug Control (NIFDC). All participants in the Older-B, Older-C and Younger-B groups would receive two doses of the B-EV71 or C-EV71 vaccine on a 0 and 30 day schedule.

The administration was performed via intramuscular injection into the upper arm. Vaccines were administered appropriately by the trained trial staff at the trial site. Blood samples were collected on day 0 (pre-vaccination) and day 60 (30 days after the second dose of vaccination) for immunogenicity analyses. Immediate local and systemic AEs were observed on-site for 30 min after each dose of vaccination. Diary cards were provided to record solicited AEs within 7 days after each dose of vaccination with instructions. Local AEs included pain, tenderness, induration, swelling, rash, flushing and pruritus. Systemic AEs included fever ( $\geq 37.5^{\circ}\text{C}$ ), diarrhea, constipation, dysphagia, anorexia, vomiting, nausea, muscle pain (not at the vaccination site), arthritis, arthralgia, headache, new convulsions and cough, etc. On the 8th day after each dose of vaccination, the diary cards were collected and reviewed by the investigators, and the contact cards were issued to record unsolicited and serious AEs. Weekly telephone visits were assigned until the 30th day after each administration to review the contact cards. Monthly follow-up and the active reporting of the participants' caregivers were combined to collect the SAEs observed from the first dose of vaccination to 6 months after the second dose of vaccination. During the visits, the caregivers collected information on AEs and assessed the grade of AEs and the relationship between AEs and vaccinations. Then, investigators checked the classification of possible AEs and reports to assure completeness and accuracy. The grade of AEs was determined on the basis of the Guidelines for Adverse Event Classification Standards for Clinical Trials of Preventive Vaccines (2019)<sup>14</sup> issued by the National Medical Products Administration. Blood samples were collected from the participants aged 36-71 months at 12 months after the second dose of vaccination for immune persistence analyses.

### Outcomes

The primary outcomes included the GMT level and seroconversion rate of anti-EV71 neutralising antibody 30 days after the second dose of vaccination for immunogenicity assessment and the incidence of systemic and local AEs 0-30 days after each dose for safety assessment. Secondary outcomes included the seropositivity rate and GMT of the anti-EV71 neutralising antibody during 12 months of follow-up for immune persistence assessment and the incidence of SAEs collected from the first dose of vaccination to 6 months after the second dose of vaccination to evaluate the long-term safety.

Intravenous blood was separated after collection and stored below  $-20^{\circ}\text{C}$ . NIFDC was responsible for neutralising antibody detection. Neutralising antibody titre of clinical serum samples was determined by using a cytopathogenic effect assay.<sup>14,23</sup> Simply, the sample was diluted 1:8 and then serially diluted twofold, mixed with 100 TCID<sub>50</sub> of EV71 and neutralised for 2 h. The rhabdomyosarcoma cell suspension was added, cultured at

$37^{\circ}\text{C}$  and incubated with 5% CO<sub>2</sub> for 7 days to determine the result. The highest dilution that could inhibit 50% of the cytopathic changes was determined as the anti-EV71 neutralising antibody titre. In this study, a reciprocal neutralising antibody titre of 1:8, 1:16, 1:32 and 1:64 was used as the different criteria for seroconversion. Seroconversion was defined as (1) participants with a reciprocal neutralising antibody titre  $<1:8$  before vaccination and  $\geq$  the criterion value (1:8, 1:16, 1:32 or 1:64) post-vaccination or (2) participants with a reciprocal neutralising antibody titre  $\geq 1:8$  before vaccination and at least fourfold increase post-vaccination.

### Statistical analysis

The sample size was calculated using NCSS-PASS (version 11) based on a non-inferiority design. Non-inferiority would be claimed when (i) the B-EV71 vaccine was non-inferior to the C-EV71 vaccine in children aged 36-71 months, and (ii) the B-EV71 vaccine in children aged 36-71 months was non-inferior to that in children aged 6-35 months. With regard to the seroconversion rate, the sample size calculation assumed a non-inferiority margin of 10% difference between the seroconversion rate in the Older-B group and the Older-C or Younger-B group, a seroconversion rate of 90% and a true difference in seroconversion rate of 0%. The study need recruit 304 participants in each arm to achieve 90% power at a one-sided 1.25% significance level, after adjusting for a drop rate of 20%. With regard to the GMT, the sample size calculation assumed a non-inferiority margin of a 0.5-fold difference between GMT in the Older-B group and the Older-C or Younger-B group, a standard deviation of 0.8 on a log<sub>10</sub> scale and a true difference in GMT on a log<sub>10</sub> scale of zero.<sup>18</sup> The study need recruit 222 participants in each group to achieve 90% power at a one-sided 1.25% significance level, after adjusting for a drop rate of 20%. Considering the baseline seropositivity of participants (20% in children aged 6-35 months and 60% in children aged 36-71 months), the non-inferiority of seroconversion rate and GMT, and the sample size of phase III clinical trial in relevant regulations, 625 participants were enrolled in the Older-B group and Older-C group, respectively, and 350 participants were enrolled in the Younger-B group with a total of 1,600 participants.

Descriptive statistical analysis was used for demographic characteristics. Age, height, weight, sex, ethnicity, etc. were compared using the Student's *t* test or Chi-square test/Fisher's exact test. AEs were calculated in the safety set (SS), including participants who received at least one dose of vaccine. Immunogenicity analysis was conducted in the full analysis set (FAS) and per-protocol set (PPS). FAS included participants who received at least one dose of vaccine and collected at least one blood sample. PPS included participants who met the eligibility criteria, complied with the study protocol and had valid immunogenicity results. Neutralising

antibody titres were log<sub>10</sub> transformed to calculate geometric mean titre (GMT) and 95% CIs. Student's *t* test or Wilcoxon rank sum test was conducted to compare GMT and geometric mean increase (GMI). All statistical analyses were conducted using SAS, version 9.4 (SAS Institute, Cary, NC).

### Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all data. YT and XC had primary responsibility and the final decision to submit for publication.

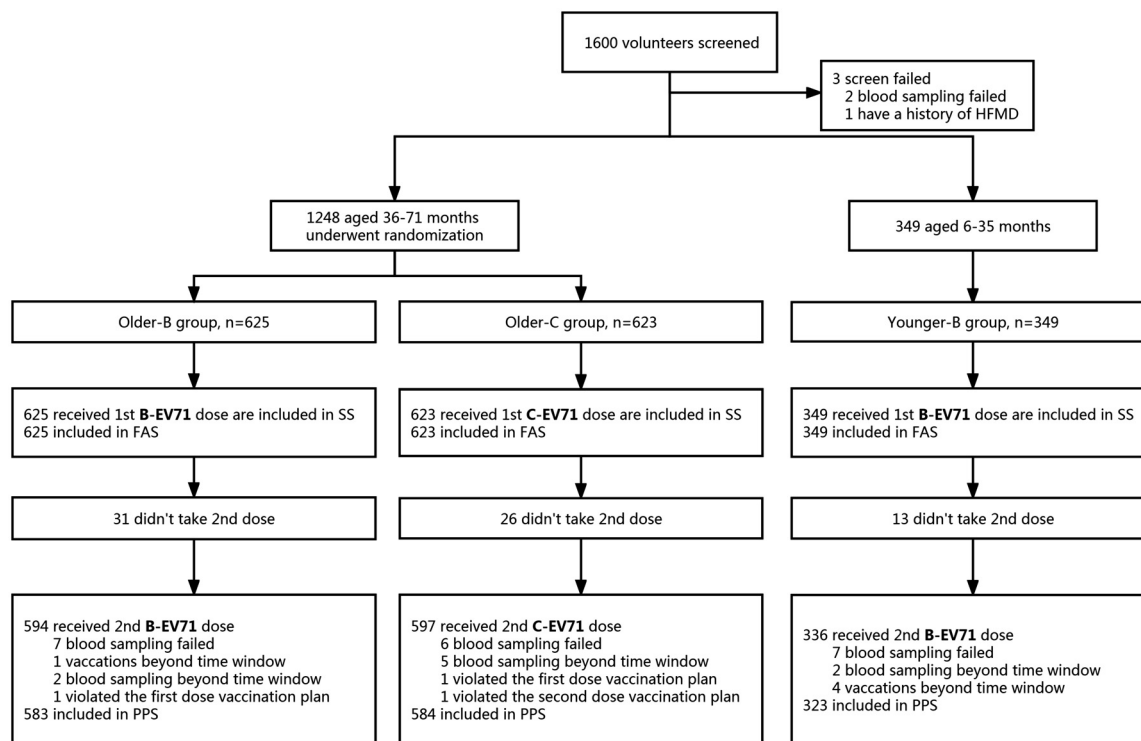
## Result

### Study individuals

Figure 1 shows the study process. Between June 3 and June 30, 2020, 1,600 participants were enrolled and

assigned, including 1,250 participants aged 36-71 months and 350 participants aged 6-35 months. Participants aged 36-71 months were equally randomised to receive two doses of the B-EV71 vaccine (Older-B group, n=625) or two doses of the C-EV71 vaccine (Older-C group, n=625). All participants aged 6-35 months enrolled were assigned to receive two doses of the B-EV71 vaccine (Younger-B group, n=350).

A total of 1,597 participants received the first dose of the B-EV71 or C-EV71 vaccine (2 cases in the Older-C group and 1 case in the Younger-B group withdrawn before the first injection). They were involved in the SS and FAS. Seventy participants did not take the second dose, including 31 cases in the Older-B group, 26 cases in the Older-C group, and 13 cases in the Younger-B group. 1,527 participants received the second dose, of which 1,490 cases were included in the PPS for immunogenicity analysis, including 583 cases in the Older-B group, 584 cases in the Older-C group, and 323 cases in the Younger-B group.



**Figure 1. Flow chart of participants screening, assignment, and analysis.** Abbreviations: HFMD, hand, foot and mouth disease; Older-B group, participants aged 36-71 months vaccinated with B-EV71 vaccine; Older-C group, participants aged 36-71 months vaccinated with C-EV71 vaccine; Younger-B group: participants aged 6-35 months vaccinated with B-EV71 vaccine; B-EV71 vaccine: EV71 vaccine produced by Wuhan Institute of Biological Products Co., Ltd.; C-EV71 vaccine: control EV71 vaccine produced by the Institute of Medical Biology, Chinese Academy of Medical Sciences; SS, safety set, including participants who received at least one dose of vaccine; FAS, full analysis set, including participants who received at least one dose of vaccine and collected at least one blood sample; PPS, per protocol set, including participants who met the eligibility criteria, complied with the study protocol and had valid immunogenicity results.



### Baseline demographic

In the SS, FAS, and PPS population, baseline characteristics and physical examination results before the first dose of vaccination were similar between the two older groups. There were no statistically significant differences between the Older-B group and the Younger-B group in sex, ethnic classification, and physical examination (cardiopulmonary auscultation, skin examination, throat examination, and nutrition and development status) before the first dose of vaccination (Table 1).

### Immunogenicity

In the PPS population, the baseline seropositivity rates of the Older-B and Older-C groups were 9.43% (95%CI: 7.06%–11.81%) and 9.42% (95%CI: 7.05%–11.79%), respectively. Their baseline GMTs were 5.44 (95%CI: 4.99–5.92) and 5.39 (95%CI: 4.95–5.87), respectively. There were no significant differences in seropositivity rates and GMTs between these two groups. Compared to the Older-B group, the Younger-B group had lower baseline seropositivity rate of 1.55% (95%CI: 0.20%–2.89%) and lower baseline GMT of 4.07 (95%CI: 4.00–4.14). All participants in each group were tested positive for anti-EV71 neutralising antibody after two doses of vaccination. The seroconversion rate after two doses of vaccination was 99.66% (95%CI: 99.18%–100.00%) of the Older-B group, 99.32% (95%CI: 98.65%–99.98%) of the Older-C group, and 100% (95%CI: 100.00%–100.00%) of the Younger-B group. The difference was 0.34% (95% CI: –2.17%–2.86%) between the Older-B and Older-C groups, and –0.34% (95% CI: –2.78%–2.09%) between the Older-B and Younger-B groups, which showed that the seroconversion rate of the Older-B group was non-inferior to that of the Older-C and Younger-B groups. The GMT after two doses of vaccination was 693.87 (95%CI: 626.14–768.9) of the Older-B group, 289.37 (95%CI: 259.62–322.5) of the Older-C group, and 634.80 (95%CI: 574.62–701.20) of the Younger-B group. The ratio was 2.67 (95% CI: 2.00–3.00) between the Older-B and Older-C groups, and 1.00 (95% CI: 0.75–1.00) between the Older-B and Younger-B groups, which showed that the GMT after two doses of vaccination in the Older-B group was also non-inferior to that of the Older-C and Younger-B groups (Table 2). The reverse cumulative curves of anti-EV71 neutralising antibody titres of participants in these three groups post-vaccination in the PPS population are shown in Figure 2. Participants with anti-EV71 neutralising antibody titer <1:8 before vaccination were defined as the susceptible population. Similar findings were observed in the susceptible participants (Appendix Table 1).

The persistence of vaccine immunity was assessed by monitoring anti-EV71 neutralising antibody 12 months after two doses of vaccination in the Older-B

and Older-C groups. The seropositivity of the participants in the Older-B group (100%; 95% CI: 100.00%–100.00%) was slightly higher than that in the Older-C group (97.54%; 95% CI: 96.22%–98.86%) ( $P=0.0003$ ). The GMT of the participants in the Older-B group (138.6; 95% CI: 123.29–155.8) was also higher than that of the Older-C group (58.36; 95% CI: 51.95–65.57) ( $P<0.0001$ ) (Table 3). All susceptible participants in the Older-B group were tested positive for anti-EV71 neutralising antibody after 12 months of vaccination, whose seropositivity was higher than the Older-C group (97.28%; 95% CI: 95.82%–98.74%) ( $P=0.0003$ ). The GMT of the Older-B group (119.57; 95% CI: 107.04–133.5) was higher than that of the Older-C group (47.98; 95% CI: 43.23–53.24) ( $P<0.0001$ ) (Appendix Table 2).

### Safety

The incidences of overall AEs within 0–30 days were similar between the Older-C group (103/623[16.53%]) and the Older-B group (123/625[19.68%]), which is lower than that in the Younger-B group (92/349 [26.36%]). A total of 15 (0.94%) participants had reported with SAEs (4 in the Older-B group, 8 in the Older-C group, and 3 in the Younger-B group), all of which were judged by the investigators to be unrelated to vaccines (Appendix Table 3). The incidences of overall AEs related to vaccination were similar among these 3 groups, including 34 (5.44%) cases in the Older-B group, 32 (5.14%) cases in the Older-C group, and 26 (7.45%) cases in the Younger-B group, respectively. None of the participants were reported with grade 3 AEs related to vaccination in both the Older-B and Older-C groups. Only 2 (0.94%) participants in the Younger-B group were reported with grade 3 AEs related to vaccination. The most common local AEs related to vaccination were pain in the Older-B group (0.32%), redness in the Older-C group (0.64%), and pruritus in the Younger-B group (0.29%). Fever (3.04% in the Older-B group, 1.61% in the Older-C group, and 3.44% in the Younger-B group) was the most frequently reported in systemic AEs related to vaccination (Table 4).

### Discussion

Previous studies have shown that the B-EV71 vaccine has an effective protective effect on preventing HFMD caused by EV71 in children aged 6–35 months. In this study with an expanded population, our results indicated that the EV71 vaccine also had good immunogenicity, safety and immune persistence amongst children aged 36–71 months.

This study found that the B-EV71 vaccine was non-inferior to the C-EV71 vaccine in children aged 36–71 months in both the seropositivity rate and GMT of the anti-EV71 neutralising antibody 30 days after the second

| Characteristics                         | Older-B Group | Older-C Group | Younger-B Group |
|---|---------------|---------------|-----------------|
| <b>FAS/SS</b>                           |               |               |                 |
| Number of participants                  | 625           | 623           | 349             |
| <b>Sex</b>                              |               |               |                 |
| Male                                    | 323 (51.68%)  | 322 (51.69%)  | 165 (47.28%)    |
| Female                                  | 302 (48.32%)  | 301 (48.31%)  | 184 (52.72%)    |
| <b>Ethnic</b>                           |               |               |                 |
| Han                                     | 622 (99.52%)  | 620 (99.52%)  | 344 (98.57%)    |
| Others                                  | 3 (0.48%)     | 3 (0.48%)     | 5 (1.43%)       |
| Age (months)                            | 49.53 (9.76)  | 49.61 (9.86)  | 22.5 (8.06)     |
| Height (cm)                             | 105.31 (6.72) | 105.54 (7.26) | 86.78 (8.24)    |
| Weight (kg)                             | 16.56 (2.50)  | 16.71 (2.71)  | 11.79 (2.13)    |
| <b>Cardiopulmonary auscultation</b>     |               |               |                 |
| Normal                                  | 625 (100.00%) | 623 (100.00%) | 349 (100.00%)   |
| Abnormal                                | 0 (0.00%)     | 0 (0.00%)     | 0 (0.00%)       |
| <b>Skin examination</b>                 |               |               |                 |
| Normal                                  | 622 (99.52%)  | 621 (99.68%)  | 346 (99.14%)    |
| Abnormal                                | 3 (0.48%)     | 2 (0.32%)     | 3 (0.86%)       |
| <b>Throat examination</b>               |               |               |                 |
| Normal                                  | 590 (94.40%)  | 594 (95.35%)  | 336 (96.28%)    |
| Abnormal                                | 35 (5.60%)    | 29 (4.65%)    | 13 (3.72%)      |
| <b>Nutrition and development status</b> |               |               |                 |
| Normal                                  | 625 (100.00%) | 623 (100.00%) | 349 (100.00%)   |
| Abnormal                                | 0 (0.00%)     | 0 (0.00%)     | 0 (0.00%)       |
| <b>PPS</b>                              |               |               |                 |
| Number of participants                  | 583           | 584           | 323             |
| <b>Sex</b>                              |               |               |                 |
| Male                                    | 294 (50.43%)  | 298 (51.03%)  | 153 (47.37%)    |
| Female                                  | 289 (49.57%)  | 286 (48.97%)  | 170 (52.63%)    |
| <b>Ethnic</b>                           |               |               |                 |
| Han                                     | 580 (99.49%)  | 581 (99.49%)  | 318 (98.45%)    |
| Others                                  | 3 (0.51%)     | 3 (0.51%)     | 5 (1.55%)       |
| Age(months)                             | 49.76 (9.76)  | 49.56 (9.97)  | 22.36 (8.03)    |
| Height(cm)                              | 105.39 (6.72) | 105.57 (7.39) | 86.63 (8.19)    |
| Weight(kg)                              | 16.57 (2.50)  | 16.70 (2.74)  | 11.76 (2.11)    |
| <b>Cardiopulmonary auscultation</b>     |               |               |                 |
| Normal                                  | 583 (100.00%) | 584 (100.00%) | 323 (100.00%)   |
| Abnormal                                | 0 (0.00%)     | 0 (0.00%)     | 0 (0.00%)       |
| <b>Skin examination</b>                 |               |               |                 |
| Normal                                  | 580 (99.49%)  | 582 (99.66%)  | 320 (99.07%)    |
| Abnormal                                | 3 (0.51%)     | 2 (0.34%)     | 3 (0.93%)       |
| <b>Throat examination</b>               |               |               |                 |
| Normal                                  | 549 (94.17%)  | 557 (95.38%)  | 311 (96.28%)    |
| Abnormal                                | 34 (5.83%)    | 27 (4.62%)    | 12 (3.72%)      |
| <b>Nutrition and development status</b> |               |               |                 |
| Normal                                  | 583 (100.00%) | 584 (100.00%) | 323 (100.00%)   |
| Abnormal                                | 0 (0.00%)     | 0 (0.00%)     | 0 (0.00%)       |

**Table 1: Baseline characteristics of the study participants (FAS/SS, PPS).**

Data are mean (SD), or n (%).

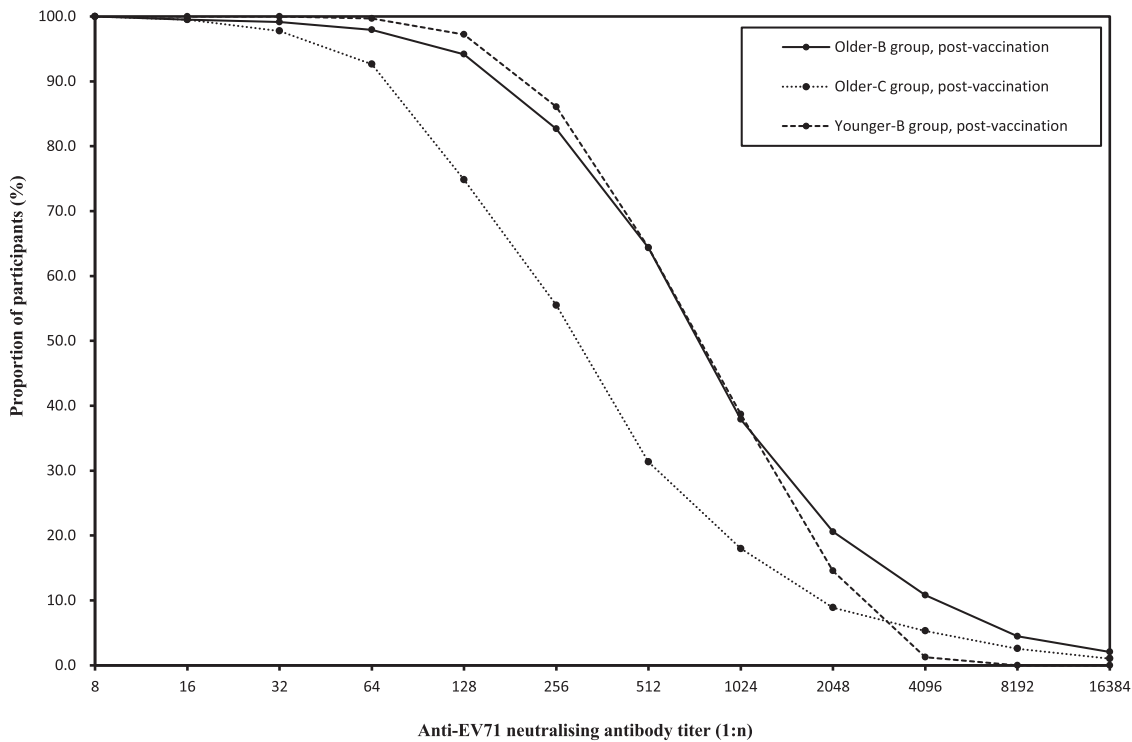
Abbreviations: Older-B group, participants aged 36-71 months vaccinated with B-EV71 vaccine; Older-C group, participants aged 36-71 months vaccinated with C-EV71 vaccine; Younger-B group: participants aged 6-35 months vaccinated with B-EV71 vaccine; FAS, full analysis set; SS, safety set; PPS, per protocol set.

| Time                             | Parameter                          | Older-B Group              | Older-C Group             | Younger-B Group            | Older-B vs Older-C |                                   |                 | Older-B vs Younger-B |                                   |                 |
|----------------------------------|------------------------------------|----------------------------|---------------------------|----------------------------|--------------------|-----------------------------------|-----------------|----------------------|-----------------------------------|-----------------|
|                                  |                                    |                            |                           |                            | P-value            | Difference (%)/<br>Ratio (95% CI) | Non-inferiority | P-value              | Difference (%)/<br>Ratio (95% CI) | Non-inferiority |
| Before<br>vaccination<br>(day 0) | Number of participants             | 583                        | 584                       | 323                        | -                  | -                                 | -               | -                    | -                                 | -               |
|                                  | Seropositivity, (%) (95%CI)        | 9.43<br>(7.06, 11.81)      | 9.42<br>(7.05, 11.79)     | 1.55<br>(0.20, 2.89)       | 0.99               | -                                 | -               | <0.0001              | -                                 | -               |
|                                  | GMT (95%CI)                        | 5.44<br>(4.99, 5.92)       | 5.39<br>(4.95, 5.87)      | 4.07<br>(4.00, 4.14)       | 0.98               | -                                 | -               | <0.0001              | -                                 | -               |
| After<br>vaccination<br>(day 60) | Seropositivity, (%) (95%CI)        | 100.00<br>(99.35, 100.00)  | 100.00<br>(99.35, 100.00) | 100<br>(98.82, 100.00)     | -                  | -                                 | -               | -                    | -                                 | -               |
|                                  | Seroconversion (1:8), (%) (95%CI)  | 99.66<br>(99.18, 100.00)   | 99.32<br>(98.65, 99.98)   | 100.00<br>(100.00, 100.00) | 0.68               | 0.34<br>(-2.17, 2.86)             | Yes             | 1.00                 | -0.34<br>(-2.78, 2.09)            | Yes             |
|                                  | Seroconversion (1:16), (%) (95%CI) | 99.31<br>(98.64, 99.98)    | 98.8<br>(97.92, 99.68)    | 100.00<br>(100.00, 100.00) | 0.37               | 0.51<br>(-2.06, 3.09)             | Yes             | 0.33                 | -0.69<br>(-3.12, 1.75)            | Yes             |
|                                  | Seroconversion (1:32), (%) (95%CI) | 98.97<br>(98.15, 99.79)    | 97.09<br>(95.73, 98.45)   | 100.00<br>(100.00, 100.00) | 0.02               | 1.88<br>(-0.88, 4.64)             | Yes             | 0.16                 | -1.03<br>(-3.46, 1.41)            | Yes             |
|                                  | Seroconversion (1:64), (%) (95%CI) | 97.77<br>(96.57, 98.97)    | 91.95<br>(89.75, 94.16)   | 99.69<br>(99.08, 100.00)   | <0.0001            | 5.82<br>(2.56, 9.08)              | Yes             | 0.05                 | -1.92<br>(-4.40, 0.56)            | Yes             |
|                                  | GMT (95%CI)                        | 693.87<br>(626.14, 768.9)  | 289.37<br>(259.62, 322.5) | 634.80<br>(574.62, 701.20) | <0.0001            | 2.67<br>(2.00, 3.00)              | Yes             | 0.61                 | 1.00<br>(0.75, 1.00)              | Yes             |
|                                  | GMI (95%CI)                        | 127.62<br>(115.29, 141.20) | 53.68<br>(48.55, 59.34)   | 155.94<br>(140.69, 172.80) | <0.0001            | -                                 | -               | 0.0077               | -                                 | -               |

**Table 2: Immune response of total population after two doses of vaccination (PPS).**

Abbreviations: CI, confidence interval; GMT, geometric mean titer; GMI, geometric mean increase; Older-B group, participants aged 36-71 months vaccinated with B-EV71 vaccine; Older-C group, participants aged 36-71 months vaccinated with C-EV71 vaccine; Younger-B group: participants aged 6-35 months vaccinated with B-EV71 vaccine; PPS, per protocol set.





**Figure 2. Reverse cumulative curves of anti-EV71 neutralising antibody titers at post-vaccination (day 60) of participants in PPS population.** Abbreviations: PPS, per protocol set, including participants who met eligibility criteria, complied with the study protocol, and had valid immunogenicity results; Older-B group, participants aged 36-71 months vaccinated with B-EV71 vaccine; Older-C group, participants aged 36-71 months vaccinated with C-EV71 vaccine; Younger-B group: participants aged 6-35 months vaccinated with B-EV71 vaccine.

| Time                         | Parameter                 | Older-B Group              | Older-C Group           | P-value |
|------------------------------|---------------------------|----------------------------|-------------------------|---------|
| After vaccination (month 12) | Number of participants    | 526                        | 528                     | -       |
|                              | Seropositivity(%) (95%CI) | 100.00<br>(100.00, 100.00) | 97.54<br>(96.22, 98.86) | 0.0003  |
|                              | GMT (95%CI)               | 138.60<br>(123.29, 155.80) | 58.36<br>(51.95, 65.57) | <0.0001 |

**Table 3: Immune persistence of total population after two doses of vaccination (PPS).**

Abbreviations: CI, confidence interval; GMT, geometric mean titer; Older-B group, participants aged 36-71 months vaccinated with B-EV71 vaccine; Older-C group, participants aged 36-71 months vaccinated with C-EV71 vaccine; PPS, per protocol set.

dose of vaccination. Previous studies showed that the C-EV71 vaccine had conveyed good immunogenicity in children aged 36-71 months in phase III and IV clinical trials.<sup>24,25</sup> Additionally, the B-EV71 vaccine in children aged 36-71 months was also non-inferior to that in children aged 6-35 months. The anti-EV71 neutralising antibody of children aged 6-35 months in this study was similar to that reported in the lot-to-lot consistency study after the B-EV71 vaccine was available on the market.<sup>26</sup> These results indicated the good immunogenicity of the B-EV71 vaccine amongst children aged 36-71 months. In addition, we evaluated the immunity persistence of the B-EV71 vaccine by monitoring the anti-

EV71 neutralising antibody 12 months after two doses of vaccination in the Older-B and Older-C groups. The seropositivity and GMT of the participants in the Older-B group were higher than those in the Older-C group. Although the antibody level decreased at 12 months after two doses of vaccination, the GMT of the anti-EV71 neutralising antibody was much higher than the protection threshold level of 1:16/1:32. Moreover, the GMT of the Older-B group in 12 months after two doses of vaccination was significantly higher than that of placebo individuals reported in the previous studies, although EV71 vaccine-induced immunity could also be strengthened by natural infection in the epidemic region.<sup>14,24</sup> This

|  | Older-B Group (N=625) | Older-C Group (N=623) | Younger-B Group (N=349) | P-value            |                      |
|--|-----------------------|-----------------------|-------------------------|--------------------|----------------------|
|  |                       |                       |                         | Older-B vs Older-C | Older-B vs Younger-B |
| AEs related to vaccination                         | 34 (5.44%)            | 32 (5.14%)            | 26 (7.45%)              | 0.81               | 0.21                 |
| Grade $\geq$ 3                                     | 0 (0.00%)             | 0 (0.00%)             | 2 (0.57%)               | -                  | 0.13                 |
| Solicited AEs related to vaccination               | 34 (5.44%)            | 32 (5.14%)            | 26 (7.45%)              | 0.81               | 0.21                 |
| Injection-site AEs                                 | 2 (0.32%)             | 7 (1.12%)             | 1 (0.29%)               | 0.18               | 1.00                 |
| Pain   | 2 (0.32%)             | 2 (0.32%)             | 0 (0.00%)               | 1.00               | 0.54                 |
| Redness  | 0 (0.00%)             | 4 (0.64%)             | 0 (0.00%)               | 0.13               | -                    |
| Swelling   | 0 (0.00%)             | 1 (0.16%)             | 0 (0.00%)               | 0.50               | -                    |
| Pruritus   | 0 (0.00%)             | 0 (0.00%)             | 1 (0.29%)               | -                  | 0.36                 |
| Systemic AEs                                       | 33 (5.28%)            | 26 (4.17%)            | 26 (7.45%)              | 0.36               | 0.17                 |
| Fever  | 19 (3.04%)            | 10 (1.61%)            | 12 (3.44%)              | 0.09               | 0.73                 |
| Cough  | 4 (0.64%)             | 7 (1.12%)             | 1 (0.29%)               | 0.36               | 0.79                 |
| Diarrhea   | 4 (0.64%)             | 0 (0.00%)             | 4 (1.15%)               | 0.14               | 0.64                 |
| Hypersensitivity                                   | 2 (0.32%)             | 2 (0.32%)             | 1 (0.29%)               | 1.00               | 1.00                 |
| Nausea/vomiting                                    | 2 (0.32%)             | 1 (0.16%)             | 1 (0.29%)               | 1.00               | 1.00                 |
| Constipate   | 1 (0.16%)             | 1 (0.16%)             | 0 (0.00%)               | 1.00               | 1.00                 |
| Stomach ache                                       | 0 (0.00%)             | 2 (0.32%)             | 0 (0.00%)               | 0.25               | -                    |
| Erythema   | 0 (0.00%)             | 0 (0.00%)             | 2 (0.57%)               | -                  | 0.13                 |
| Rash   | 1 (0.16%)             | 3 (0.48%)             | 1 (0.29%)               | 0.61               | 1.00                 |
| Fatigue  | 2 (0.32%)             | 0 (0.00%)             | 0 (0.00%)               | 0.50               | 0.54                 |
| Decreased appetite                                 | 2 (0.32%)             | 0 (0.00%)             | 2 (0.57%)               | 0.50               | 0.94                 |
| Maculopapular rash                                 | 0 (0.00%)             | 0 (0.00%)             | 1 (0.29%)               | -                  | 0.36                 |
| Swelling of the ear                                | 0 (0.00%)             | 0 (0.00%)             | 1 (0.29%)               | -                  | 0.36                 |
| Allergic dermatitis                                | 1 (0.16%)             | 0 (0.00%)             | 0 (0.00%)               | 1.00               | 1.00                 |
| Myalgia  | 1 (0.16%)             | 0 (0.00%)             | 0 (0.00%)               | 1.00               | 1.00                 |
| Headache   | 1 (0.16%)             | 0 (0.00%)             | 0 (0.00%)               | 1.00               | 1.00                 |
| Papular urticaria                                  | 0 (0.00%)             | 1 (0.16%)             | 0 (0.00%)               | 0.50               | -                    |
| Hives  | 0 (0.00%)             | 0 (0.00%)             | 1 (0.29%)               | -                  | 0.36                 |
| Unsolicted AEs related to vaccination              | 1 (0.16%)             | 1 (0.16%)             | 1 (0.29%)               | 1.00               | 1.00                 |
| Respiratory system, chest and mediastinal diseases | 1 (0.16%)             | 1 (0.16%)             | 0 (0.00%)               | 1.00               | 1.00                 |
| Skin and subcutaneous tissue diseases              | 0 (0.00%)             | 4 (0.64%)             | 1 (0.29%)               | 1.00               | 0.36                 |

**Table 4: AEs related to vaccination within 30 days in the safety set.**  
 Abbreviations: Older-B group, participants aged 36-71 months vaccinated with B-EV71 vaccine; Older-C group, participants aged 36-71 months vaccinated with C-EV71 vaccine; Younger-B group: participants aged 6-35 months vaccinated with B-EV71 vaccine; AEs: adverse events.

finding indicated the good persistence of vaccine-induced immunogenicity.

No significant difference in the incidence of AEs was observed between the Older-B group (19.68%) and Older-C group (16.53%), but it was lower than that of the Younger-B group (26.36%) within 30 days after two doses of vaccination. Most AEs related to vaccination were mild and resolved within a few days. The incidence of AEs reported in this study in children aged 6-35 months old was lower than that reported in the previous phase III clinical trial.<sup>14</sup> These two clinical trials referred to different classification standards for AEs. In this trial, AEs were classified in accordance with the ‘Guidelines for Adverse Event Classification Standards for Clinical Trials of Preventive Vaccines (2019)’ issued by the National Medical Products Administration. However, AEs in the previous phase III clinical trial were classified on the basis of the ‘Guidelines for Adverse Event

Classification Standards for Clinical Trials of Preventive Vaccines (2015)’. Given the COVID-19 pandemic in 2020, the decrease in AEs may be due to the promotion in the public’s awareness of the prevention and control of infectious diseases, including washing hands, wearing masks and social distancing. Although the overall incidence of AEs was reduced, the types of AEs collected in this study were not significantly different from those in the previous phase III clinical trial. Therefore, the sensitivity of the AE monitoring system was good.

With regard to solicited AEs, the AEs at the injection site primarily included pain, and the systemic AEs primarily included fever, cough and diarrhea in the Older-B group. This result was slightly different from the phase III clinical trial,<sup>14</sup> in which cough was reported in a relatively high proportion of solicited AEs. Respiratory tract infections were reported to be the common unsolicited AEs in both trials. The incidence of AEs in the

Older-B group (19.68%) was less than that in the Younger-B group (26.36%), which may be related to the higher immunity and stronger resistance of children of older age. Consistent with the results in the clinical trial of the Sinovac vaccine in children aged 36-71 months,<sup>27</sup> the B-EV71 vaccine showed good safety.

In conclusion, this study showed that the B-EV71 vaccine had good immunogenicity, safety and immune persistence in children aged 36-71 months. The immunogenicity of the EV71 vaccine produced by different manufacturers was different, whereas the immune response of the B-EV71 vaccine performed better to a certain degree. However, this study is a single-centre clinical trial with a sample size of 1,597. Expanding the sample size nationwide is necessary to further explore the safety and immunogenicity of the B-EV71 vaccine in children aged 36-71 months.

#### Contributors

YT, XG, SW, XL, XC, and KD developed the design. JC, WC, ZW, QL, BY, XQ, JL, LH, SD, XL, CG, HS, YL, PD, TX, QLL, LL, and HD did the clinical visits. QM, FG, and WL did the laboratory assays and technique support. All authors had access to data and YT and XZ verified the data. YT, XZ, and JH analysed data. YT, XZ, JC, WC, ZW, and QL drafted the manuscript. JH, SW, XG, XL, and XC were responsible for reviewing and editing the manuscript. All coauthors approved the final version of the manuscript.

#### Data sharing statement

Anonymised participant data will be made available when the trials are complete, upon requests directed to the corresponding author (XC). Proposals will be reviewed and approved by the sponsor, investigator, and collaborators on the basis of scientific merit. After approval of a proposal, data can be shared through a secure online platform after signing a data access agreement. All data will be made available for a minimum of 5 years from the end of the trial.

#### Declaration of interests

YT reported receiving grants from Hubei Province's young medical talent program (20191229), Hubei Province's young talent program (2021), and Hubei Province's young public health talent program (2021) during the conduct of the study. JC, WC, KD, XL, CG, TX, QLL, LL, HD, and XC report being employees of Wuhan Institute of Biological Products Co Ltd. All other authors report no potential conflicts of interest.

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The opinions expressed in this article are those of the authors and are not necessarily to reflect those of Wuhan Institute of Biological Products Co., Ltd.

#### Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2022.101596.

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