# Immunogenicity and safety of inactivated enterovirus A71 vaccines in children aged 6-35 months in China: a non-inferiority, randomised controlled trial

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### ARTICLE INFO

### ABSTRACT

*Background:* China's three inactivated enterovirus A71 (EV-A71) vaccines are the first and currently world's only EV-A71 vaccines approved by a national regulatory authority and used to prevent EV-A71 associated diseases. The three vaccines vary by vaccine strain, manufacturing cell substrate, and antigen dose, but no head-to-head comparisons of these vaccines have been done. We compared immunogenicity of the vaccines in children 6-35 months old.

*Methods:* We recruited healthy children aged 6-35 months who lived in a study site county into a multicentre, open-label, non-inferiority, three-group, randomised controlled trial that was conducted in five counties in China. Enrolled children were randomly assigned (1:1:1) to receive two doses of one of the three EV-A71 vaccines. The primary outcome was the proportion of children with EV-A71 neutralizing antibody seroconversion 4 weeks after the second dose; a secondary outcome was adverse events in the 4 weeks after each dose. Analyses of immunogenicity included all children who completed the study (per-protocol analysis). Safety analysis included all children completed safety follow-up after at least one. We used a 10% margin to establish non-inferiority. This trial was registered on a World Health Organization platform: Chinese Clinical Trial Registry (ChiCTR1900026663).

*Findings:* 1631 children were assessed for eligibility between Nov 4 and Nov 20, 2019. Of 1500 (92%) enrolled children, 500 were assigned to vaccine group A, B, or C; 483 in group A,484 in group B, and 487 in group C completed the study. Before dose one, the seropositive rates in groups A, B, and C were 9.7%, 7.2%, and 7.0%. Four weeks after the second dose, seroconversion rates of groups A, B, and C were 98.8%, 99.4% and 99.8% - mutually non-inferior in all two-group comparisons. There were no serious adverse events in any group and no evidence of a difference among the three groups in the incidence of local adverse event or systemic adverse event. Fever was the most common adverse event. All children with reported adverse events recovered.

*Interpretation:* Non-inferior and high seroconversion rates and equivalent safety of three EV-A71 vaccines supports use any of these vaccines to prevent EV-A71-associated diseases. These results may be useful for regulators, vaccine policy makers, and immunization programmes in China and in countries where EV-A71 is endemic.

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Research in context

**Evidence before this study**

Phase III clinical trials of the three now-licensed EV-A71 vaccines were conducted in different study sites and among different populations; the vaccines are made with different strains and grown on different cell substrates; all three vaccines, individually, showed good immunogenicity and safety in the pivotal Phase III trials. EV-A71 vaccines have been shown to provide good cross-reactivity against eleven other EV-A71 subgenotypes (A, B0-B5, C1, C2, C4, and C5).

**Added value of this study**

We report a study to directly compare immunogenicity of three EV-A71 vaccines using unified methods and a common study population. Our study found that seroconversion rates of the three EV-A71 vaccines were all very high and were non-inferior to each other in all pairwise comparisons. The safety profiles of the vaccines were similar, with no serious safety signals identified.

**Implications of all the available evidence**

Non-inferior and high seroconversion rates and equivalent safety of three EV-A71 vaccines supports selection any of the vaccines to prevent EV-A71-associated diseases. These results may be useful for regulators, vaccine policy makers, and immunization programmes in China and in countries where EV-A71 is endemic.

## 1. Introduction

Enterovirus A71(EV-A71) causes several diseases, the most common of which is hand, foot, and mouth disease (HFMD). HFMD is an intestinal infectious disease characterized by fever, painful sores in the mouth, and rash or blisters on the hands, feet, and buttocks. HFMD can be caused by several enteroviruses, including Enterovirus A, including EV-A71, CV-A6, CV-A10, and CV-A16. [1] Most severe and fatal HFMD cases are caused by childhood EV-A71 infections - especially among children less than 3 years old. In China between 2009 and 2018, nearly 70% of laboratory confirmed severe HFMD cases and more than 90% of fatal cases were caused by EV-A71. HFMD outbreaks caused by EV-A71 have been reported worldwide, but are prominent in the Asia-Pacific region. [2-5] China reports the majority of HFMD cases globally, and from 2010 through 2018, HFMD has been the top-ranked notifiable disease in China. [6]

Three manufactures in China - Sinovac Biotech Ltd.; Institute of Medical Biology, Chinese Academy of Medical Sciences; and Wuhan Institute of Biological Products – began development of inactivated EV-A71 vaccines in 2008; all obtained regulatory approval between December 2015 and July 2016, [7] and EV-A71 vaccines became available for use in China in 2016. [8] The three vaccines are uniquely licensed and available in China.

Chinese National Standards for both EV-A71 antigen content and neutralizing antibody assays were developed by China's National Institutes for Food and Drug Control (NIFDC) in 2010. [9] The first international standards (IS) for anti EV-A71 serum (Human) and the first IS for EV-A71 inactivated vaccines to be used globally were established in 2015. [10] These standards ensure that methods used to measure serum neutralizing activity against EV-A71 are accurate, sensitive, and reproducible. In 2019, vaccine potency assays were harmonized globally, identical to the Chinese standards. [11]

Based on phylogenetic analysis of VP1 genes, EV-A71 viruses are divided into 8 genogroups, A to H. [12] Genogroup B and C, which have been causing large-scale epidemics in Asia since 1997, can be further divided into major genotypes B1-B5 and C1-C5. [12] Genotype C4 can be further classified as subgenotype C4a and C4b. All

three vaccines were made using the C4a subgenotype virus strain prevalent in China. All are inactivated by formaldehyde and include an aluminum hydroxide adjuvant. Differences among the vaccines include the vaccine strain (gene mutation rate, the amino acid mutation rate, and virus titres), manufacturing cell substrate (Vero or diploid cells), cell culture system (cell factories or microcarrier bioreactor systems), production process, protective agent in final container products (FCP), and FCP with alum adjuvant(Table 1). [13-14] These differences in the vaccines may lead to differences in immunogenicity. [15-16] The Phase IIand III clinical trials differed by protocol, study site, and investigator, making it difficult to directly compare immunogenicity. Comparing immunogenicity in a unified way can provide evidence to optimize the EV-A71 vaccine immunization strategy in China and fulfill a requirement for post-market-authorization review. A head-to-head comparison of immunogenicity and safety can help procurement agencies and policy makers make wise decisions on vaccine selection and recommendations.

We report a study to compare immunogenicity of three EV-A71 vaccines using unified methods and a common study population to provide evidence for policy makers, immunization programmes, and regulators to better understand these vaccines and help standardize evaluations for future EV-A71 vaccines.

## 2. Methods

### 2.1. Study design

We conducted a multicentre, open-label, non-inferiority randomized, controlled clinical trial in five counties/districts in Hebei, Zhejiang, and Yunnan provinces in China to compare seroconversion with two-dose schedules of three EV-A71 vaccines. The three provinces were selected to be exemplars of high (Yunnan), middle (Zhejiang), and low (Hebei) HFMD incidence provinces. We assessed the per-dose safety and tolerance of each vaccine.

### 2.2. Participants

Eligible participants were healthy children 6 to 35 months old who lived in a study site county/district, whose parents or legal guardians stated willingness to participate for the entire study period, and who had no contraindications to any of the three EV-A71 vaccines. Exclusion criteria were history of HFMD or herpangina, previous receipt of EV-A71 vaccine, receipt of blood products or immunosuppressive therapy within the previous 6 months, and receipt of any vaccine within the previous 2 weeks. Parents or legal guardians gave written informed consent before enrolment and study group assignment.

### 2.3. Randomisation and masking

To control for possible influence of age on immunogenicity, children were enrolled into five age groups: 6-11 months, 12-17 months, 18-23 months, 24-29 months, and 30-35 months. Children in each age group were randomly assigned to receive one of three vaccines (groups A, B, and C). To achieve a balance of study group assignment by participating clinic, children in the same clinic were randomly assigned to one of the three groups using a 1:1:1 block randomisation scheme with a block size of 60 (four per group per age group). Randomisation lists were completed before enrolment by China CDC and given to participating clinics. Group information for each participant was concealed in a separate envelope, and only after enrolment was group allocation revealed to the field investigators and parents or legal guardians. Investigators and parents/guardians were not masked to study group assignment because vaccination practices in China do not allow

**Table 1**
Characteristics of the three inactivated EV-A71 vaccines.

| Manufacture | Strain genotype | GenBank accession number | Cell substrate | Mode of production | Inactivation | Adjuvant | Antigen content, Number of doses |
|---|---|---|---|---|---|---|---|
| A | C4 | EU 812515.1 | Human diploid cell (KMB17) | Cell factory | Formalin | Aluminum hydroxide | 125 U/dose, 2 doses |
| B | C4 | HQ328793.1 | Vero cell | Cell factory | Formalin | Aluminum hydroxide | 480 U/dose, 2 doses |
| C | C4 | JX025561 | Vero cell | Bio-reactor | Formalin | Aluminum hydroxide | 420 U/dose,2 doses |

masking for post-market-authorization vaccine studies. Laboratory staff analysing sera were masked to group assignment; investigators analysing data and assessing outcomes were not masked.

### 2.4. Procedures

After enrolment, infants received vaccines according to their group assignment. Infants in each group received two doses of vaccine produced by one of three manufactures with an interval of 28-35 days between doses. At the initial visit following enrolment, study staff used a standardized questionnaire to obtain basic information about the participants, including date of birth, sex, addresses, and telephone number.

Vaccines used in this study were domestically-produced single-dose presentations that had been lot-released by the National Institute for Food and Drug Control in China (NIFDC). Vaccine strains were FY-23K-B, H07, AHFY087VP5 EV-A71 strains. GenBank accession numbers are EU812515.1, HQ328793.1, and JX025561.

We obtained two blood samples from each subject - before dose one to measure baseline EV-A71 neutralizing antibody titre and four weeks (28–35 days) after dose two to assess EV-A71 antibody response.

EV-A71 neutralizing antibody titres were determined using the cytopathogenic effect (CPE) method. [15] Serum samples were inactivated at 56°C for 30 minutes, serially diluted two-fold from 1:8 to 1:16384 in 96-well microplates, mixed with equal volumes of 100 $CCID_{50}$/0.05ml of an EV-A71 strain (EV-A71-523 strain, GenBank number EU 753398.2) , and incubated at 37°C for two hours. Rhabdomyosarcoma (RD) cells ($1$–$2 \times 10^5$cells/mL) were added to the mixture. Plates were placed in a $CO_2$ incubator at 35°C for seven days. CPE was observed by microscopy. Neutralizing antibody titres were defined as reciprocals of the highest serum dilution that inhibited 50% of CPE. National standard neutralizing antibody (N12:1914 IU/ml) was used to control quality of each test. Only when the results of N12 test were within the allowable range could the assay continue. Samples were standardized to IU according to N12 titres. [17] Standardized IU = neutralization titre of the sample divided by the average neutralization titre of the National Standards for EV-A71 neutralizing antibody multiplied the value of N12.

EV-A71 seropositivity was defined as an antibody titre no less than 1:8. Seroconversion was defined as a change from seronegative to seropositive or a four-fold or more increase in antibody titre between samples collected at baseline and 4 weeks after the second dose. There is no globally standardized protective antibody titre for EV-A71, precluding assessment of protection. When calculating median titres or geometric mean titres (GMT), values below 1:8, which was the beginning dilution multiple, were assigned as 1:4, and values above 1:16384, which was the highest dilution multiple, were assigned as 1:32768. We used an average value (22 IU/ml) to substitute for seronegative results and 175588 to substitute for results above 87794 when calculating geometric mean concentrations (GMC) of standardized IUs.

### 2.5. Outcomes

Laboratory testing was conducted by NIFDC, which is the government's statutory and Supreme Technical Arbitration Institution for quality inspection of pharmaceutical biological products. The primary outcome was non-inferiority of seroconversion. Non-inferiority was defined as a lower confidence interval above an inferiority margin of–10%. Secondary outcomes were adverse events within 4 weeks following each dose. We also conducted a post-hoc analysis of antibody titres. Primary and additional outcomes were assessed among children completing the study - per-protocol analyses. Secondary outcomes were assessed among children completing the safety assessment.

We actively monitored adverse events following immunisation (AEFIs) based on the WHO Global Manual on Surveillance of AE-FIs. [18] Participants were observed for 30 minutes after vaccination. A diary card was given to parents or guardians to record observed local or systemic reactions for 4 weeks after each dose. Parents or guardians were prompted to look for local reactions of redness, swelling, induration, rash, and pain, and systemic reactions of fever, allergy, vomiting, diarrhea, cough, runny nose, and crying. Parents or guardians were asked to report any other possible reactions, and to report medications and other vaccinations received. Parents or guardians were contacted by telephone or home-visit on the 3rd day, the 7th day, and the 14th day after vaccination to assess their record-keeping, to follow up on reported AEFIs, and to answer any questions. At the 4-week visit, diary cards were reviewed with the parent or guardian to ensure completion and address any questions about the recordings. Each AEFI was classified as mild, moderate, severe, or very severe according to the guidelines for adverse event classification standards for vaccine clinical trials, released by the China Food and Drug Administration in 2005 (Appendix 1).

### 2.6. Statistical analysis

For sample size calculations, based on results from clinical trials, we assumed 93% seroconversion as a minimum and 98% as a maximum. [19-21] A sample size of 425 subjects per group was required to achieve a power of 0.90 with a one-sided $\alpha$ of 0.025 and a non-inferiority margin of –10% and with a Z test using pooled variance. Assuming a 15% loss-to-follow-up rate, we aimed to enroll 500 children per group.

We used a standardized questionnaire to obtain information and establish a database of subjects using Epidata software. To ensure data quality, we requested field staff to input each questionnaire twice, independently by two different individuals. After final data cleaning, personal identifying information was removed to create an analytic database.

We compared distributions (medians and IQRs) using rank sum testing for non-normal, continuous variables (e.g., vaccination interval, blood collection interval after the second dose, and antibody titre). We compared age distributions (means and standard deviation) using F-tests. We compared other normally distributed, continuous variables (e.g., GMT and GMC) using F-test (means with
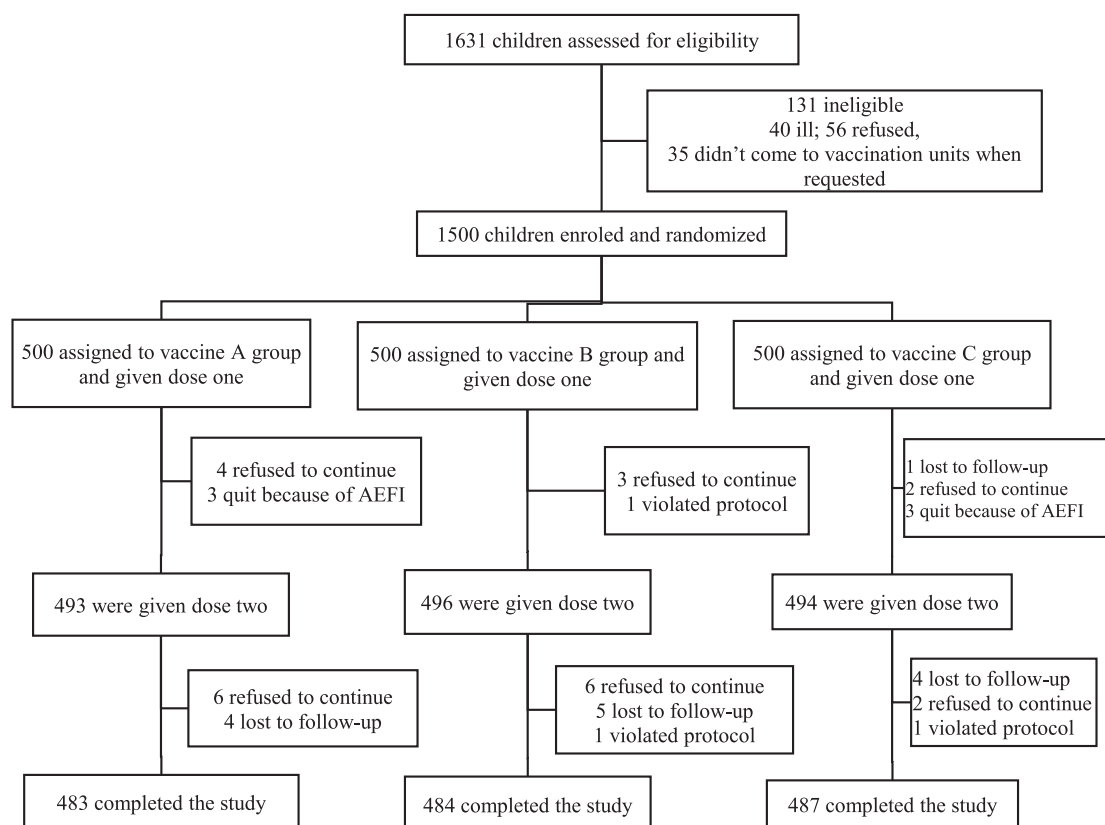
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┌─────────────────────────────────────┐
│  1631 children assessed for eligibility │
└─────────────────────────────────────┘
                │
                │        ┌──────────────────────────────────┐
                │        │          131 ineligible           │
                ├────────│  40 ill; 56 refused,              │
                │        │  35 didn't come to vaccination units when │
                │        │  requested                        │
                │        └──────────────────────────────────┘
                │
┌─────────────────────────────────────┐
│  1500 children enroled and randomized │
└─────────────────────────────────────┘
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| 500 assigned to vaccine A group and given dose one | 500 assigned to vaccine B group and given dose one | 500 assigned to vaccine C group and given dose one |
|---|---|---|
| 4 refused to continue<br>3 quit because of AEFI | 3 refused to continue<br>1 violated protocol | 1 lost to follow-up<br>2 refused to continue<br>3 quit because of AEFI |
| 493 were given dose two | 496 were given dose two | 494 were given dose two |
| 6 refused to continue<br>4 lost to follow-up | 6 refused to continue<br>5 lost to follow-up<br>1 violated protocol | 4 lost to follow-up<br>2 refused to continue<br>1 violated protocol |
| 483 completed the study | 484 completed the study | 487 completed the study |

**Figure 1.** Study flow chart.

95% CI). For categorical variables (e.g., gender, seropositivity, seroconversion), we compared frequencies and proportions using Pearson's $\chi^2$ test or Fisher's exact test. For non-inferiority testing, we calculated a two-sided, 95% CI (Farrington-Manning) of the difference between each pairwise groupings. All data analyses were conducted with SAS 9.4.

### 2.7. Ethical review and clinical trial registration

The study was approved by the Ethical Review Committee of the Chinese Center for Disease Control and Prevention (approval number 201934).

This study is registered on a World Health Organization platform: Chinese Clinical Trial Registry (ChiCTR), number ChiCTR1900026663.

### 2.8. Role of the funding source

### 3. Results

Between November 4 and November 20, 2019, 1631 infants were assessed for eligibility; 131 were excluded (Figure 1). Of the 1500 infants enroled, 500 were assigned at random to one of the three study groups. A total of 1454 (96.9%) subjects completed the study: 483 in group A, 484 in group B, and 487 in group C (Figure 1); 532 children were from Hebei province, 451 were from Zhejiang province, and 471 were from Yunnan province. Characteristics of the participants who completed the study are shown in Table 2. There were no statistically significant differences among the three groups.

Before vaccination, 47 (9.7%) Group A subjects, 35 (7.2%) Group B subjects, and 34 (7.0%) Group C subjects were seropositive. Four weeks after the second dose, seropositivity increased to 99.8% in all three groups (482 of 483 children in group A; 483 of 484 in group B; 486 of 487 in group C), yielding a non-inferior proportion of seroconversion of 98.8% (477 of 483 children) in group A, of 99.4% (481 of 484 children) in group B and of 99.8% (486 in 487 children) in group C (Figure 2, Table 3).

The GMTs of EV-A71 neutralizing antibody were similar among the three groups and by age group before vaccination. After vaccination, GMTs increased to 1:149.03 in Group A, to 1:157.00 in Group B, and to 1:377.06 in Group C, with significant difference among groups, as Group C GMT was higher than the other two groups in all age groupings (Table 4). Median titres were similar with the GMT analysis (Appendix 2). GMCs in standardized IUs before vaccination were similar among the three groups and by age grouping. GMCs after vaccination were significantly different, with Group C GMC higher than the other two groups (Table 5).

AEFI data were obtained for participants who completed the safety evaluation of any dose. Mild local reactions included redness, swelling, and pain; systemic reactions included fever, diarrhea, and vomiting. There were no serious AEFIs (Table 6 and Table 7, Appendix 3). In group A, 10 (2%) of 500 children were reported to have one or more local AEFI after the first dose, and 1 (0.2%) of 492 after the second dose; in group B, 11 (2.2%) of 500 children were reported to have one or more local AEFI, and 2 (0.4%) of 496 after second dose; in group C, 17 (3.4%) of 499 children were reported to have one or more local AEFI, and 4 (0.8%) of 494 after second dose. There was no evidence of a difference in the incidence of any local AEFI among the three groups after the first dose or the second dose. Systemic AEFI were reported for the four

**Table 2**
Characteristics of subjects who completed the study.

| Variable | Value | A(N=483) | B(N=484) | C(N=487) |
|---|---|---|---|---|
| Age group | | | | |
| 6-11[1] | (months) | 8.79(1.80) | 9.16(1.82) | 9.22(1.71) |
| 12-17[1] | (months) | 14.80(3.00) | 14.33(2.44) | 14.32(2.46) |
| 18-23[1] | (months) | 20.32(2.67) | 20.89(2.83) | 20.94(1.78) |
| 24-29[1] | (months) | 26.69(1.78) | 26.22(1.77) | 26.56(1.77) |
| 30-35[1] | (months) | 32.74(1.54) | 32.94(1.78) | 32.93(1.65) |
| Total[1] | (months) | 20.70(8.76) | 20.78(8.71) | 20.76(8.67) |
| Interval | | | | |
| 2nd and 1st dose[2] | (days) | 30(28-31) | 30(28-31) | 30(28-31) |
| 2nd blood draw and 2nd dose[2] | (days) | 30(29-31) | 30(29-31) | 30(29-31) |
| Age group[3] | 6-11 | 97(20.1) | 96(19.8) | 100(20.5) |
| | 12-17 | 94(19.5) | 96(19.8) | 94(19.3) |
| | 18-23 | 98(20.3) | 96(19.8) | 99(20.3) |
| | 24-29 | 97(20.1) | 98(20.2) | 97(19.9) |
| | 30-35 | 97(20.1) | 98(20.2) | 97(19.9) |
| Sex[3] | Male | 241(49.9) | 246(50.8) | 249(51.1) |
| | Female | 242(50.1) | 238(49.2) | 238(48.9) |
| In preschool[3] | No | 475(98.3) | 482(99.6) | 479(98.4) |
| | Yes | 8(1.7) | 2(0.4) | 8(1.6) |
| Study province[3] | Hebei | 177(36.6) | 177(36.6) | 178(36.6) |
| | Zhejiang | 150(31.1) | 149(30.8) | 152(31.2) |
| | Yunnan | 156(32.3) | 158(32.6) | 157(32.2) |

[1] Mean(SD)
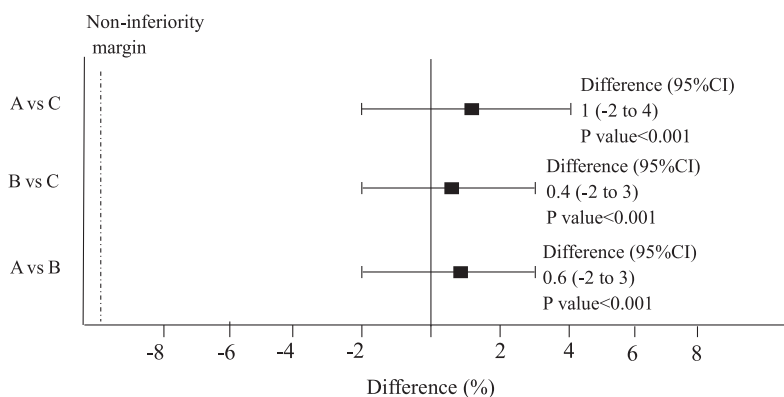[2] Median (25th, 75th percentile)
[3] n (%)



**Figure 2.** seroconversion comparisons
Non-inferiority was assessed by calculating the difference in proportion seroconverting in each two groupings, using a two-sided 95% CI (Farrington-Manning) method. We defined the non-inferiority margin as −10%.

weeks following the first dose and the second dose for 110 (22.0%) and 83(16.9%) children in Group A, 97 (19.4%) and 93(18.8%) children in Group B, and 110 (22.0%) and 95 (19.2%) children in Group C. There was no evidence of a difference in the incidence of any systemic AEFI among the three groups after the first dose (Table 6) or the second dose (Table 7). Fever was the most common systemic AEFI. All children with reported AEFIs recovered fully.

## 4. Discussion

Our study found that EV-A71 neutralizing antibody seroconversion rates were non-inferior between vaccines in all pairwise comparisons, showing that immunogenicities of the three EV-A71 vaccines used in China are similar. Seropositivity and seroconversion after two vaccine doses were nearly 100% for each of the three vaccines.

Although there is no globally agreed upon EV-A71 neutralizing antibody correlate of protection, in Phase III clinical trials, investigators explored protective titres and found that neutralizing titres of 1:16 and 1:32 were associated with protection against EV-A71 associated diseases. [20-21] Although these two thresholds have

the advantage of being known to be associated with protection, they may be more conservative than needed. We used these two titres as protective titres and found that the protective rates after vaccination were 99.0%, 98.6%, 99.8%, and 95.4%, 92.4%, 98.6%, respectively.

Taken together, our study provides confidence that children can be protected from EV-A71 associated diseases after two doses of any of the three EV-A71 vaccines licensed and in use in China.

To the best of our knowledge, our study is the first to show GMC results in standardized IUs, allowing comparison with future studies. Zhu and colleagues suggested that long-term protection is afforded when a standardized IU is no less than 70.2IU/ml. [22] Using this criterion, 98.96%, 99.17%, and 99.79% subjects in our study may have long-term protection from EV-A71 related diseases, regardless of which vaccine is used.

We note that GMTs and standardized IUs after two doses were different among the three groups, with Group C having higher GMTs and standardized IUs compared with groups A and B. The significant differences in GMTs and standardized IUs induced by the three vaccines may be related to the antigenic and immunogenic properties of the vaccines. An animal study found that neu-

**Table 3**

EV-A71 neutralizing antibody seropositivity and seroconversion rates before and after vaccination.

| | | Age group (months) | A(N=483) % (95%CI) | B(N=484) % (95%CI) | C(N=487) % (95%CI) | $\chi^2$ | P |
|---|---|---|---|---|---|---|---|
| Pre-vaccination seropositivity | | 6-11 | 13.4(7.3-21.8) | 8.3(3.7-15.8) | 8.0(3.5-15.2) | 2.00[1] | 0.37[2] |
| | | 12-17 | 5.3(1.8-12.0) | 4.2(1.2-10.3) | 6.4(2.4-13.4) | - | 0.76[3] |
| | | 18-23 | 8.2(3.6-15.5) | 1.0(0.03-5.7) | 5.1(1.7-11.4) | - | 0.052[3] |
| | | 24-29 | 9.3(4.3-16.9) | 6.1(2.3-12.9) | 7.2(3.0-14.3) | 0.72[1] | 0.70[2] |
| | | 30-35 | 12.4(6.6-20.6) | 16.3(9.6-25.2) | 8.2(3.6-15.6) | 2.94[1] | 0.23[2] |
| | | Total | 9.7(7.2-12.7) | 7.2(5.1-9.9) | 7.0(4.9-9.6) | 3.05[1] | 0.22[2] |
| Post-vaccination seropositivity | pre-positive | 6-11 | 100.0(75.3-100.0) | 100.0(63.1-100.0) | 100.0(63.1-100.0) | - | - |
| | | 12-17 | 100.0(47.8-100.0) | 100.0(39.8-100.0) | 100.0(54.1-100.0) | - | - |
| | | 18-23 | 100.0(63.1-100.0) | 100.0(2.50-100.0) | 100.0(47.8-100.0) | - | - |
| | | 24-29 | 100.0(66.4-100.0) | 100.0(54.1-100.0) | 100.0(59.0-100.0) | - | - |
| | | 30-35 | 100.0(73.5-100.0) | 100.0(79.4-100.0) | 100.0(63.1-100.0) | - | - |
| | | Total | 100.0(92.5-100.0) | 100.0(90.0-100.0) | 100.0(89.7-100.0) | - | - |
| | Pre-negative | 6-11 | 100.0(95.7-100.0) | 100.0(95.9-100.0) | 100.0(96.1-100.0) | - | - |
| | | 12-17 | 100.0(95.9-100.0) | 100.0(96.1-100.0) | 100.0(95.9-100.0) | - | - |
| | | 18-23 | 100.0(96.0-100.0) | 100.0(96.2-100.0) | 100.0(96.2-100.0) | - | - |
| | | 24-29 | 100.0(95.9-100.0) | 98.9(94.1-99.97) | 98.9(94.0-99.97) | - | 1.00[3] |
| | | 30-35 | 98.8(93.6-99.97) | 100.0(95.6-100.0) | 100.0(95.9-100.0) | - | 0.65[3] |
| | | Total | 99.8(98.7-99.99) | 99.8(98.8-99.99) | 99.8(98.8-99.99) | - | 1.00[3] |
| | Total | 6-11 | 100.0(96.3-100.0) | 100.0(96.2-100.0) | 100.0(96.4-100.0) | - | - |
| | | 12-17 | 100.0(96.2-100.0) | 100.0(96.2-100.0) | 100.0(96.2-100.0) | - | - |
| | | 18-23 | 100.0(96.3-100.0) | 100.0(96.2-100.0) | 100.0(96.3-100.0) | - | - |
| | | 24-29 | 100.0(96.3-100.0) | 99.0(94.5-99.97) | 99.0(94.4-99.97) | - | 1.00[3] |
| | | 30-35 | 99.0(94.4-99.97) | 100.0(96.3-100.0) | 100.0(96.3-100.0) | - | 0.66[3] |
| | | Total | 99.8(98.9-99.99) | 99.8 (98.9-99.99) | 99.8(98.9-99.99) | - | 1.00[3] |
| Seroconversion rate | Pre-positive | 6-11 | 92.3(64.0-99.8) | 100.0(63.1-100.0) | 100.0(63.1-100.0) | - | 1.00[3] |
| | | 12-17 | 100.0(47.8-100.0) | 100.0(39.8-100.0) | 100.0(54.1-100.0) | - | - |
| | | 18-23 | 100.0(63.1-100.0) | 100.0(2.50-100.0) | 100.0(47.8-100.0) | - | - |
| | | 24-29 | 77.8(40.0-97.2) | 100.0(54.1-100.0) | 100.0(59.0-100.0) | - | 0.31[3] |
| | | 30-35 | 83.3(51.6-97.9) | 87.5(61.7-98.5) | 100.0(63.1-100.0) | - | 0.66[3] |
| | | Total | 89.4(76.9-96.5) | 94.3(80.8-99.3) | 100.0(89.7-100.0) | - | 0.15[3] |
| | Pre-negtive | 6-11 | 100.0(95.7-100.0) | 100.0(95.9-100.0) | 100.0(96.1-100.0) | - | - |
| | | 12-17 | 100.0(95.9-100.0) | 100.0(96.1-100.0) | 100.0(95.9-100.0) | - | - |
| | | 18-23 | 100.0(96.0-100.0) | 100.0(96.2-100.0) | 100.0(96.2-100.0) | - | - |
| | | 24-29 | 100.0(95.9-100.0) | 98.9(94.1-99.97) | 98.9(94.0-99.97) | - | 1.00[3] |
| | | 30-35 | 98.8(93.6-99.97) | 100.0(95.6-100.0) | 100.0(95.9-100.0) | - | 0.65[3] |
| | | Total | 99.8(98.7-99.99) | 99.8(98.8-99.99) | 99.8(98.8-99.99) | - | 1.00[3] |
| | Total | 6-11 | 99.0(94.4-99.97) | 100.0(96.2-100.0) | 100.0(96.4-100.0) | - | 0.66[3] |
| | | 12-17 | 100.0(96.2-100.0) | 100.0(96.2-100.0) | 100.0(96.2-100.0) | - | - |
| | | 18-23 | 100.0(96.3-100.0) | 100.0(96.2-100.0) | 100.0(96.3-100.0) | - | - |
| | | 24-29 | 97.9(92.8-99.8) | 99.0(94.5-99.97) | 99.0(99.4-99.97) | - | 0.85[3] |
| | | 30-35 | 96.9(91.2-99.4) | 98.0(92.8-99.8) | 100.0(96.3-100.0) | - | 0.29[3] |
| | | Total | 98.8(97.3-99.5) | 99.4(98.2-99.9) | 99.8(98.9-99.99) | -- | 0.11[3] |

[1] $\chi^2$ value
[2] Pearson Chi-square test
[3] Fisher's Exact test

tralizing antibody titres induced by V2 (Group C in our study) were higher than observed in the other two vaccines, but titres decreased faster in serial dilutions. [13] EV-A71 vaccines contain empty and full virus particles which are known to have structural differences. Full virus particles are more immunogenic than empty particles. [23-24] Therefore, the ratios of empty particles and full particles of vaccines produced by different manufacturers may be a reason for different immunogenicity. The relative contribution of empty and full virus particles in EV-A71 vaccines to the antigenic and immunogenic properties of different EV-A71 vaccine products should be further investigated. Whether higher GMTs and standardized IUs lead to longer antibody persistence in humans should also be determined in future studies.

Because these are the first licensed EV-A71 vaccines globally, there are no similar studies from other countries. The only other evidence of EV-A71 vaccine immunogenicity comes from clinical trials in China. Laboratory testing for our study was performed by NIFDC with the same methods as the licensure clinical trials, making these results comparable. In our study, seropositive rates, proportions of titres $\geq$1:16 or 1:32, and GMTs after two doses were similar with the clinical trials data for each vaccine (vaccine B: 98.8%, 97.1%, 91.4%, 1:165.8; vaccine C: 99.7%, 99.5%, 95.3%, 1:325.3). [20-21] However, in our study, seroconversion rates were

higher than in the clinical trials (vaccine A: 97.7%; vaccine B: 95.5%; vaccine C:91.7%), [19-21] and seropositive rates and proportions of titres $\geq$1:16 or 1:32, and GMT before vaccination were all lower than in the clinical trials (vaccine B: 15.0%, 14.3%,13.8%, 1:7.5; vaccine C: 27.2%, 26.2%, 24.5%, 1:11.5), [20-21] which may be a possible reason that seroconversion was higher in our study.

We found that seropositivity varied by age group. The lowest seroconversion rates were in children aged 12-17 months, and seroconversion increased with age. It is possible that this finding may be due to an increased cumulative incidence of EV-A71 asymptomatic infections as children age. Higher seroconversion in infants aged 6-11 months may have been related with maternal immunity. In general, EV-A71 antibody levels before vaccination were much lower than what was seen in the clinical trials, which is consistent with the changing prevalence of EV-A71 in China. According to HFMD reports in the National Notifiable Diseases Reporting System, among all HFMD cases, the proportion due to EV-A71 decreased annually, [25] showing a decreasing prevalence of EV-A71 in China. It is also possible that our study participants had fewer chances to be exposed to EV-A71 virus than participants in earlier clinic trials.

The per-dose safety profiles of each vaccine were similar. The only difference was the incidence of diarrhea, which was higher

**Table 4**

EV-A71 neutralizing antibody Geometric Mean Titers (1:x) before and after vaccination.

|  |  | Age group (months) | A (N=483)[1] | B (N=484)[1] | C(N=487)[1] | F | P[2] |
|---|---|---|---|---|---|---|---|
| Pre-vaccination | Pre-positive | 6-11 | 8.51(7.77-9.34) | 10.01(7.65-13.10) | 15.11(5.00-45.65) | 1.62 | 0.22 |
|  |  | 12-17 | 12.41(4.74-32.54) | 8.00(-) | 8.56(7.19-10.18) | 1.28 | 0.31 |
|  |  | 18-23 | 8.00(-) | 8.00(-) | 8.68(6.93-10.87) | 0.88 | 0.44 |
|  |  | 24-29 | 27.79(4.14-186.60) | 8.00(-) | 8.00(-) | 1.60 | 0.23 |
|  |  | 30-35 | 37.52(11.89-118.40) | 42.43(13.99-128.63) | 38.62(6.39-233.31) | 0.01 | 0.99 |
|  |  | Total | 16.06(10.23-25.22) | 18.05(10.41-31.32) | 13.78(8.68-21.89) | 0.28 | 0.76 |
|  | Total | 6-11 | 4.43(4.20-4.67) | 4.32(4.09-4.56) | 4.45(4.02-4.92) | 0.19 | 0.83 |
|  |  | 12-17 | 4.25(3.99-4.52) | 4.12(4.00-4.24) | 4.20(4.04-4.37) | 0.49 | 0.61 |
|  |  | 18-23 | 4.23(4.07-4.40) | 4.03(3.97-4.09) | 4.16(4.02-4.31) | 2.52 | 0.08 |
|  |  | 24-29 | 4.79(3.98-5.75) | 4.17(4.04-4.32) | 4.21(4.06-4.36) | 1.96 | 0.14 |
|  |  | 30-35 | 5.28(4.35-6.40) | 5.88(4.62-7.48) | 4.82(4.06-5.73) | 0.93 | 0.39 |
|  |  | Total | 4.58(4.33-4.84) | 4.46(4.24-4.70) | 4.36(4.18-4.55) | 0.92 | 0.40 |
| Post-vaccination | Pre-negtive | 6-11 | 134.31(110.26-163.61) | 166.86(135.11-206.08) | 412.51(338.93-502.07) | 34.87 | <0.0001 |
|  |  | 12-17 | 116.41(91.39-148.27) | 101.85(79.91-129.81) | 322.32(254.01-409.02) | 26.79 | <0.0001 |
|  |  | 18-23 | 127.50(100.18-162.27) | 182.24(142.32-233.34) | 437.16(352.93-541.49) | 28.70 | <0.0001 |
|  |  | 24-29 | 153.49(121.46-193.98) | 143.77(112.19-184.24) | 339.33(258.82-444.88) | 14.27 | <0.0001 |
|  |  | 30-35 | 180.62(146.57-222.59) | 145.70(112.34-188.98) | 322.91(258.59-403.23) | 12.77 | <0.0001 |
|  |  | Total | 140.46(127.00-155.34) | 145.38(130.45-162.03) | 364.84(329.58-403.87) | 106.06 | <0.0001 |
|  | Pre-positive | 6-11 | 114.13(68.62-189.84) | 341.91(145.88-801.40) | 587.37(134.34-2568.07) | 5.05 | 0.01 |
|  |  | 12-17 | 241.69(89.68-651.35) | 206.32(34.89-1220.06) | 387.35(271.22-553.20) | 0.97 | 0.41 |
|  |  | 18-23 | 318.18(202.40-500.20) | 384.00(-) | 467.22(163.67-1333.76) | 0.51 | 0.61 |
|  |  | 24-29 | 208.58(65.23-666.89) | 221.70(93.24-527.13) | 570.62(189.66-1716.80) | 1.45 | 0.26 |
|  |  | 30-35 | 655.02(214.44-2000.78) | 714.10(361.68-1409.91) | 932.91(221.58-3927.77) | 0.13 | 0.88 |
|  |  | Total | 258.08(173.06-384.87) | 420.96(279.67-633.64) | 584.90(369.09-926.88) | 4.06 | 0.02 |
|  | Total | 6-11 | 131.41(109.66-157.49) | 177.14(144.29-217.48) | 424.34(346.04-520.36) | 38.05 | <0.0001 |
|  |  | 12-17 | 121.02(95.79-152.90) | 104.89(82.71-133.01) | 326.13(260.81-407.80) | 27.96 | <0.0001 |
|  |  | 18-23 | 137.38(109.31-172.68) | 183.66(143.74-234.66) | 438.63(357.01-538.90) | 28.22 | <0.0001 |
|  |  | 24-29 | 157.92(125.45-198.81) | 147.63(116.54-187.02) | 352.30(271.79-456.65) | 15.69 | <0.0001 |
|  |  | 30-35 | 211.83(167.51-267.87) | 188.87(144.65-246.62) | 352.44(279.56-444.31) | 7.23 | 0.001 |
|  |  | Total | 149.03(134.89-164.64) | 157.00(141.03-174.78) | 377.06(341.21-416.69) | 100.51 | <0.0001 |

[1] Mean (95%CI)

[2] F-test

**Table 5**

EV-A71 antibody Geometric Mean Concentration, standardized IU before and after vaccination.

|  |  | Age group (months) | A (N=483)[1] | B (N=484)[1] | C(N=487)[1] | F | P[2] |
|---|---|---|---|---|---|---|---|
| Pre-vaccination | Pre-positive | 6-11 | 25.59(20.62-31.75) | 28.01(18.41-42.62) | 50.94(13.36-194.30) | 1.73 | 0.20 |
|  |  | 12-17 | 38.75(8.06-186.26) | 23.00(14.74-35.90) | 25.79(19.14-34.74) | 0.36 | 0.70 |
|  |  | 18-23 | 24.22(18.01-32.57) | 20.00(-) | 25.28(16.47-38.80) | 0.57 | 0.58 |
|  |  | 24-29 | 81.87(10.72-625.22) | 24.94(17.31-35.95) | 26.75(18.79-38.07) | 0.63 | 0.55 |
|  |  | 30-35 | 130.03(32.67-517.46) | 141.67(44.42-451.82) | 130.83(15.57-1099.23) | 0.52 | 0.60 |
|  |  | Total | 50.13(30.02-83.71) | 55.79(31.00-100.39) | 44.56(25.91-76.64) | 0.36 | 0.70 |
|  | Total | 6-11 | 22.45(21.84-23.08) | 22.45(21.77-23.15) | 23.53(21.38-25.90) | 0.78 | 0.46 |
|  |  | 12-17 | 22.67(21.36-24.07) | 22.04(21.82-22.27) | 22.22(21.88-22.57) | 0.65 | 0.53 |
|  |  | 18-23 | 22.17(21.74-22.62) | 21.98(21.93-22.02) | 22.15(21.82-22.49) | 0.44 | 0.65 |
|  |  | 24-29 | 24.85(20.92-29.52) | 22.17(21.80-22.55) | 22.31(21.83-22.80) | 1.61 | 0.20 |
|  |  | 30-35 | 27.41(22.67-33.14) | 29.82(23.91-37.18) | 25.48(21.49-30.22) | 0.64 | 0.53 |
|  |  | Total | 23.84(22.61-25.13) | 23.53(22.48-24.64) | 23.11(22.22-24.04) | 0.43 | 0.65 |
| Post-vaccination | Pre-negtive | 6-11 | 710.18(582.83-865.36) | 861.91(704.39-1054.65) | 2136.13(1755.42-2599.40) | 35.15 | <0.0001 |
|  |  | 12-17 | 645.13(509.49-816.87) | 563.44(458.89-691.81) | 1791.07(1442.39-2224.04) | 32.56 | <0.0001 |
|  |  | 18-23 | 689.45(545.95-870.67) | 957.74(755.82-1213.59) | 2306.92(1884.97-2823.33) | 30.42 | <0.0001 |
|  |  | 24-29 | 754.90(602.98-945.09) | 707.44(559.81-894.00) | 1691.95(1301.80-2199.03) | 16.07 | <0.0001 |
|  |  | 30-35 | 912.80(745.16-1118.17) | 732.48(571.22-939.28) | 1603.59(1297.29-1982.21) | 13.35 | <0.0001 |
|  |  | Total | 735.87(667.42-811.34) | 753.08(680.78-833.06) | 1892.84(1717.59-2085.98) | 116.21 | <0.0001 |
|  | Pre-positive | 6-11 | 426.40(261.61-694.99) | 1361.73(551.25-3363.81) | 2184.06(487.52-9784.51) | 1.54 | 0.23 |
|  |  | 12-17 | 848.85(224.28-3212.69) | 786.86(200.88-3082.13) | 1411.19(857.36-2322.77) | 0.61 | 0.56 |
|  |  | 18-23 | 1112.79(582.47-2125.95) | 1680.09(-) | 1907.32(890.44-4085.48) | 0.19 | 0.83 |
|  |  | 24-29 | 892.24(261.59-3043.34) | 915.56(472.30-1774.81) | 2073.06(802.49-5355.27) | 1.17 | 0.33 |
|  |  | 30-35 | 2498.12(751.62-8302.87) | 2943.36(1371.17-6318.26) | 3649.29(722.54-18431.40) | 0.01 | 0.99 |
|  |  | Total | 977.18(639.18-1493.91) | 1709.82(1113.04-2626.57) | 2212.75(1376.39-3557.33) | 0.15 | 0.86 |
|  | Total | 6-11 | 663.25(552.04-796.86) | 895.39(736.21-1088.98) | 2139.93(1745.66-2623.24) | 38.92 | <0.0001 |
|  |  | 12-17 | 654.61(521.06-822.40) | 571.33(468.04-697.43) | 1764.02(1438.44-2163.30) | 33.54 | <0.0001 |
|  |  | 18-23 | 716.93(575.58-892.99) | 963.36(761.95-1218.01) | 2284.87(1883.1-2772.35) | 30.87 | <0.0001 |
|  |  | 24-29 | 766.70(612.90-959.08) | 718.69(575.79-897.06) | 1716.94(1339.1-2201.39) | 17.27 | <0.0001 |
|  |  | 30-35 | 1033.88(821.99-1300.39) | 919.22(710.27-1189.63) | 1716.12(1369.46-2150.52) | 7.64 | 0.0006 |
|  |  | Total | 756.46(686.52-833.53) | 799.08(723.04-883.12) | 1913.59(1738.83-2105.92) | 110.14 | <0.0001 |

[1] Mean (95%CI)

[2] F-test

**Table 6**
Reported adverse events following immunization (AEFIs) per subject after the first dose.

| | Adverse event | A(N=500) No (%) | B(N=500) No. (%) | C(N=499) No. (%) | $\chi^2$ | P[1] |
|---|---|---|---|---|---|---|
| Local adverse events | Redness | 2 (0.4) | 6 (1.2) | 4 (0.8) | | 0.39[2] |
| | Swelling | 3 (0.6) | 0 | 2 (0.4) | | 0.30[2] |
| | Induration | 1 (0.2) | 0 | 4 (0.8) | | 0.05[2] |
| | Pain | 4 (0.8) | 0 | 4 (0.8) | | 0.13[2] |
| | Rash | 4 (0.8) | 6 (1.2) | 5 (1.0) | | 0.86[2] |
| | Any event | 10 (2.0) | 11 (2.2) | 17 (3.4) | 2.34 | 0.31 |
| Systemic adverse events | Fever | 47 (9.4) | 45 (9.0) | 54 (10.8) | 1.04 | 0.59 |
| | Allergy | 3 (0.6) | 3 (0.6) | 6 (1.2) | | 0.55[2] |
| | Vomiting | 16 (3.2) | 6 (1.2) | 9 (1.8) | 5.20 | 0.07 |
| | Diarrhea | 16 (3.2) | 8 (1.6) | 14 (2.8) | 2.81 | 0.25 |
| | Cough | 37 (7.4) | 26 (5.2) | 26 (5.2) | 2.87 | 0.24 |
| | Runny nose | 26 (5.2) | 24 (4.8) | 21 (4.2) | 0.55 | 0.76 |
| | Crying | 12 (2.4) | 5 (1.0) | 15 (3.0) | 5.06 | 0.08 |
| | Upper respiratory infection | 2 (0.4) | 8 (1.6) | 5 (1.0) | 3.63 | 0.16 |
| | Decreased feeding | 5 (1.0) | 3 (0.6) | 10 (2.0) | 4.40 | 0.11 |
| | Any event | 110 (22.0) | 97 (19.4) | 110 (22.0) | 1.35 | 0.51 |

Subjects can have more than 1 reported event.
[1] Pearson Chi-square test
[2] Fisher's exact test

**Table 7**
Reported adverse events following immunization (AEFIs) per subject after the second dose.

| | Adverse event | A(N=492) No (%) | B(N=496) No. (%) | C(N=494) No. (%) | $\chi^2$ | P[1] |
|---|---|---|---|---|---|---|
| Local adverse events | Redness | 1 (0.2) | 1 (0.2) | 1 (0.2) | | 1.00[2] |
| | Swelling | 0 | 0 | 3 (0.6) | | 0.07[2] |
| | Induration | 0 | 0 | 1 (0.2) | | 0.67[2] |
| | Pain | 0 | 0 | 1 (0.2) | | 0.67[2] |
| | Rash | 0 | 1 (0.2) | 0 | | 1.00[2] |
| | Any event | 1 (0.2) | 2 (0.4) | 4 (0.8) | | 0.42[2] |
| Systemic adverse events | Fever | 43 (8.7) | 46 (9.3) | 53 (10.7) | 1.23 | 0.54 |
| | Allergy | 1 (0.2) | 4 (0.8) | 4 (0.8) | | 0.41[2] |
| | Vomiting | 3 (0.6) | 7 (1.4) | 5 (1.0) | | 0.50[2] |
| | Diarrhea | 13 (2.6) | 3 (0.6) | 6 (1.2) | | 0.03[2] |
| | Cough | 22 (4.5) | 26 (5.2) | 19 (3.8) | 1.12 | 0.57 |
| | Runny nose | 12 (2.4) | 3 (0.6) | 12 (2.4) | 6.16 | 0.05 |
| | Crying | 5 (1.0) | 8 (1.6) | 5 (1.0) | 0.99 | 0.61 |
| | Upper respiratory infection | 4 (1.2) | 7 (1.4) | 12 (3.2) | 4.32 | 0.12 |
| | Decreased feeding | 2 (0.4) | 5 (1.0) | 4 (0.8) | | 0.65[2] |
| | Any event | 83 (16.9) | 93 (18.8) | 95 (19.2) | 1.06 | 0.59 |

Subjects can have more than 1 reported event.
[1] Pearson Chi-square test
[2] Fisher's Exact test

in the vaccine A group after the second dose, but whether diarrhea was casually associated with EV-A71 vaccination is not known and likely not clinically significant. Our study used the same AEFI assessment methods and standardized grading of AEFIs as in the clinical trials. The spectrum of AEFIs in our study was similar to results from clinical trials, [19-21] with the most frequent systematic AEFI being fever. One difference we found is that the AEFI incidences in our study were lower than the clinic trials. However, since we had no placebo group, the AEFI incidence could only be compared among three vaccines. We found that incidences of AEFIs after the second dose were lower than after the first dose. It is unclear why the second dose was less reactogenic than the first dose.

Overall, immunogenicity and safety profiles of the three EV-A71 vaccines licensed in China are similar and qualified for use in China's immunization programme. EV-A71 associated diseases such as HFMD occur not only in China, but also in other countries - especially countries in the Asia-Pacific region, including Singapore, Malaysia, Japan, and Vietnam. [2-5] In China, the predominant EV-A71 sub-genotype is C4, and these vaccine were developed based on C4 sub-genotype. In other countries, the predominant sub-genotype is different [5,26-28]. However, two post-hoc

studies based on clinical trial specimens tested cross-reactivity for three of the vaccines against eleven other EV-A71 sub-genotypes (A, B0-B5, C1, C2, C4, and C5) and found good cross-reactive responses. [29-30] Therefore it is likely that China's EV-A71 vaccine can protect not only people in China, but also people globally - a valuable public health product for safe and effective prevention of EV-A71 associated diseases. Our study will also provide evidence for WHO prequalification and EV-A71 vaccine global use.

Our study had strengths and limitations. One strength is that this was a non-inferiority randomized controlled study to compare the immunogenicity of three EV-A71 vaccines in same geographic region and population. A second strength is that the completion rate was high (96.9%), as only 46 (3.1%) children did not complete the study. The proportion of participants lost to follow-up was similar in each group and there was no substantive difference in basic characteristics among the groups (Appendix 4). Loss to follow-up was much lower than the 15% that we used in the sample-size calculation, indicating that study power was not compromised. A limitation of our study is that there is no global criteria for protective titres. Therefore the immunogenicity results can not directly equate to effectiveness against disease. Potentially protective titres were published and can be used to roughly eval-

uate our results. Once global criteria are established, our results can be used to evaluate protection against disease. Another limitation is that we did not test cross-reactivity against other genotypes prevalent in other countries. However, there are published studies demonstrating good cross-reactivity against eleven other subgenotypes. We could not blind parents to study group assignment. Although all parents knew which vaccine their children received, no parent requested to change study group assignment. Exclusion criteria were histories of HFMD and herpangina, so our study does not generalize to children having these conditions. Finally, we cannot determine antibody persistence from our study because it was of short duration. We plan to follow these subjects over time to assess antibody persistence.

In conclusion, we found that children 6-35 months of age seroconverted equally well to three EV-A71 vaccines used in China and that immunogenicity of the vaccines is nearly the same. We found no safety concerns for any of the vaccines. Differences in antibody titres could be evaluated in future research. The three EV-A71 vaccines should be considered for WHO prequalification and more widespread use.

## Contributors

YL, YW, HW, ZY,ZA and ZF conceptualized and designed the study; YL, ZA and ZF designed the data collection database; JL, YZ, HL, SW, HY and XL did and supervised the field investigation and collected the data; QM,FG and ZL carried out the laboratory test;YL,FG,QM and ZF carried out the data analyses and YL, QM, FG,ZA, ZF drafted the initial manuscript.

All authors revised the manuscript, approved the submitted version, and agree to be accountable for all aspects of the work.

## Data Sharing Statement

De-identified individual data that underlie the results in this article can be made available for meta-analysis upon request to the corresponding author. The data will be available beginning 9 months and ending 36 months following article publication. The data requestor will need to sign a data access agreement.

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## Declaration of Competing Interest

We declare no competing interests.

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## Supplementary materials

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