Efficacy, safety, and immunology of an inactivated alum-adjuvant enterovirus 71 vaccine in children in China: a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial

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Summary

Background A vaccine for enterovirus 71 (EV71) is needed to address the high burden of disease associated with infection. We assessed the efficacy, safety, immunogenicity, antibody persistence, and immunological correlates of an inactivated alum-adjuvant EV71 vaccine.

Methods We did a randomised, double-blind, placebo-controlled, phase 3 trial. Healthy children aged 6–35 months from four centres in China were randomly assigned (1:1) to receive vaccine or alum-adjuvant placebo at day 0 and 28, according to a randomisation list (block size 30) generated by an independent statistician. Investigators and participants and their guardians were masked to the assignment. Primary endpoints were EV71-associated hand, foot, and mouth disease (HFMD) and EV71-associated disease during the surveillance period from day 56 to month 14, analysed in the per-protocol population. This study is registered with ClinicalTrials.gov, number NCT01508247.

Findings 10 245 participants were enrolled and assigned: 5120 to vaccine versus 5125 to placebo. 4907 (with three cases of EV71-associated HFMD and eight cases of EV71-associated disease) versus 4939 (with 30 cases of EV71-associated HFMD and 41 cases of EV71-associated disease) were included in the primary efficacy analysis. Vaccine efficacy was 90·0% (95% CI 67·1–96·9) against EV71-associated HFMD (p=0·0001) and 80·4% (95% CI 58·2–90·8) against EV71-associated disease (p<0·0001). Serious adverse events were reported by 62 of 5117 (1·2%) participants in the vaccine group versus 75 of 5123 (1·5%) in the placebo group (p=0·27). Adverse events occurred in 3644 (71·2%) versus 3603 (70·3%; p=0·33).

Interpretation EV71 vaccine provides high efficacy, satisfactory safety, and sustained immunogenicity.

Funding China's 12–5 National Major Infectious Disease Program, Beijing Vigoo Biological.

Introduction

Disease associated with enterovirus 71 (EV71) infection was first described by Schmidt and colleagues in 1974,[1] who reported 20 patients with CNS disease—including one who died—in California, USA, between 1969, and 1972. Since then, outbreaks of hand, foot, and mouth disease (HFMD) associated with EV71 infection have been reported worldwide[2–5]—particularly in infants and young children—accounting for more than 6 000 000 cases and more than 2000 deaths in the past decade.[6] EV71 infection is associated with various diseases including HFMD, herpangina, neurological signs (aseptic meningitis or encephalitis) with or without serious sequelae, and nonspecific illnesses—eg, febrile illness, viral exanthem, respiratory infection.[6–8] A vaccine for EV71 is urgently needed to prevent and control epidemics of EV71.[9,10]

Phase 1 and phase 2 trials have assessed the safety and immunogenicity of an inactivated alum-adjuvant EV71 vaccine in adults, children, and infants.[5,11,12] We did this phase 3 trial in young children and infants to assess the efficacy and safety of the vaccine for prevention of disease associated with EV71.

Methods

Study design and participants

We did this randomised, double-blind, placebo-controlled phase 3 trial in children aged 6–35 months at four centres in China (Donghai, Pizhou, and Baoying counties in Jiangsu province, and Chaoyang district in Beijing) covering 32 townships and 485 villages from January, 2012, to March, 2013. Eligibility was assessed by medical history inquiry and physical examination. Exclusion criteria included: history of HFMD, previous vaccination with EV71 vaccine, acute febrile disease at recruitment, and taking any immunosuppressive drugs, blood products, drugs under investigation, inactivated vaccines, or attenuated live vaccines within a defined period (appendix p 3 shows full criteria). The proportion of enrolled children in every township was less than 40% of the total number of children aged 6–35 months, to avoid creating herd immunity in study areas (appendix p 4).

The study was approved by the institutional review board of Jiangsu Provincial Center of Disease Control and Prevention, and done in accordance with the Declaration of Helsinki, Good Clinical Practice, and Chinese regulatory requirements. All guardians of participants provided written informed consent. The study protocol is available online.

Randomisation and masking

Three consecutive batches of EV71 vaccine (numbers 201108002, 201108003, and 201108004) and one batch of placebo (number 201108001) were used for this study in a 1:1:1:3 ratio. Each dose of vaccine and placebo was assigned a code from a randomisation list by block randomisation (block size 30). The randomisation list was generated by an external statistician with SAS (version 9.1). All packages of vaccine and placebo were identical in appearance, with the code as the only identifier. Each participant was assigned a sequential number according to their sequence of enrolment and received vaccine or placebo labelled with the same numbers. Thus, participants were randomly assigned to receive EV71 vaccine or placebo in a 1:1 ratio. Investigators involved in randomisation and masking did not participate in any other part of the trial. Allocation was masked from all participants, their guardians, and investigators.

Procedures

Inactivated alum-adjuvanted EV71 vaccine (vero cell), containing 320 U of antigen and 0·18 mg of alum, was developed and manufactured by Beijing Vigoo Biological with a seed virus of EV71 strain FY7VP5/AH/CHN/2008 (genotype C4, which is the predominant strain in mainland China).[5] Each dose of placebo contains 0·18 mg of alum adjuvant and no EV71 antigen.

Vaccine or placebo was administered intramuscularly to the anterolateral side of the thigh (for participants aged 6–11 months) or the deltoid muscle (those aged 12–35 months) at day 0 and 28. Participants were observed for immediate adverse events for 30 min after each injection. Fever or any other adverse events were recorded by their guardians on diary cards within 28 days, which were checked by investigators to assure completeness and accuracy.

We took blood samples from all participants at day 0 (immediately before the first dose) and day 56 (28 days after second dose). In addition, we took blood samples at month 8 and 14 from a subset of participants (immunogenicity cohort) by randomised cluster sampling based on township for assessment of immunogenicity and persistence (appendix p 5). Researchers at the Chinese National Institute for Food and Drug Control measured neutralising antibody against EV71 with a modified cytopathogenic effect assay.[5] A data and safety monitoring committee of nine members—including paediatricians, infectious disease doctors, epidemiologists, and an immunologist—reviewed all the clinical, epidemiological, and laboratory data to confirm EV71-associated disease, and serious adverse events attributed to vaccine. The committee was not involved in any other part of the study.

Participants were visited at home or came to the clinic at 5, 8, 11, and 14 months, when any medically significant events were recorded. Digital photographs were used to identify participants throughout immunisation, blood collection, and site visits.

We did active surveillance of EV17-associated disease in all 485 clinics in villages, all 32 hospitals in townships, and the seven biggest public hospitals in cities, of all vaccinated participants from day 56 to month 14. Guardians were encouraged to take their children to treatment in clinics or hospitals for any illness during the surveillance period. Unwell participants were screened for any of: HFMD-like illness, herpangina, acute respiratory symptoms or gastrointestinal symptoms, with or without other systemic complications—eg, CNS complications and cardiopulmonary failure—but excluding those who had disease that was confirmed not to involve EV71. All potential cases were reported by the clinics or hospitals. Throat or rectal swabs or both were taken for pathogen detection within 24 h. The ad hoc local laboratories in each study centre—operated by Jiangsu Mole Bioscience— tested for Coxsackie A virus 16, EV71, and other enteroviruses by real-time PCR (fluorescence assay) with a viral RNA diagnostic kit. Participants who were positive for EV71-specific RNA were deemed suspected cases and were visited by study staffs to assess exposure history and take acute blood samples, serial swabs, and stool samples. These samples were then shipped to the Chinese National Institute for Food and Drug Control to test for IgM antibody, EV71-specific RNA, virus isolation,[13] and *VP1* amplification. The data and safety monitoring committee classified participants with EV71-associated disease to one of three groups (EV71-associated HFMD, herpangina, or non-specific symptoms) according to signs and symptoms.

Additionally, for each confirmed case of EV71-associated disease we selected five controls (matched for location and age) without EV71-associated disease and compared their neutralising antibody titre on day 56 with that of participants. We assumed that cases and

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See **Online** for appendix

For the **protocol** see http://www.jscdc.cn/jkzt/jbkzzt/szkb/jsgf_yg/201305/t20130517_35229.html

***Panel 1:* Primary endpoint definitions**

Enterovirus 71 (EV71)-associated hand, foot, and mouth disease (HFMD) is characterised by febrile illness with papulovesicular rash (can occasionally be maculopapular without vesicular lesion) on palms and soles, with or without vesicles or ulcers in the mouth, buttocks, knees or elbows,[6] which is caused by EV71 virus (positive either for EV71 isolation or at least two consecutive EV71-specific RNA tests).

EV71-associated disease is defined as clinical symptoms including HFMD, herpangina, neurological signs (aseptic meningitis or encephalitis) with or without serious sequelae, and nonspecific illnesses—eg, febrile illness, viral exanthema, respiratory infection—which are caused by EV71 virus (positive either for EV71 isolation or at least two consecutive EV71-specific RNA tests).

their controls had similar exposure risk. All surveillance data were recorded in data management software EV71VCTSM (version 1·30). The primary endpoints were the occurrence of EV71-associated HFMD during the surveillance period in the per-protocol population and EV71-associated disease during the surveillance period in the per-protocol population (panel 1). Secondary endpoints for safety were occurrence of solicited adverse events within 7 days of injection, any adverse events within 28 days of injection, and all serious adverse events during the study period. Other secondary endpoints were geometric mean titre, seroconversion rate, seropositivity rate, and geometric mean fold increase in each group.

Statistical analysis

We calculated the required sample size with SAS (version 9·1) with the assumptions of 80% overall vaccine efficacy, EV71-associated HFMD incidence density rate of eight cases per 1000 person-years in the placebo group, and a drop-out rate of 20% by the end of surveillance. A sample size of 5000 participants per group would provide a 93·5% statistical power to show efficacy with a lower bound of the 95% CI exceeding 20%. Vaccine efficacy was defined as 100×(1−vaccine incidence density rate / placebo incidence density rate). Incidence density rate for each group was calculated as the number of cases divided by the total person-time of follow-up, censoring for participants who had confirmed EV71, died, or migrated away. Vaccine efficacy was estimated by a Cox proportional hazard model. If no participant in the vaccine group had EV71 disease, the 95% CI for efficacy was assessed by an exact conditional procedure dependent on a Poisson distribution. We used the Kaplan-Meier method to estimate event-free survival.

We analysed a subset of participants for vaccine immunogenicity and consistency between batches of vaccine. 600 participants per group would provide at least 95% power to show the superiority of vaccine over placebo according to data from previous trials.[5,12] 200 participants

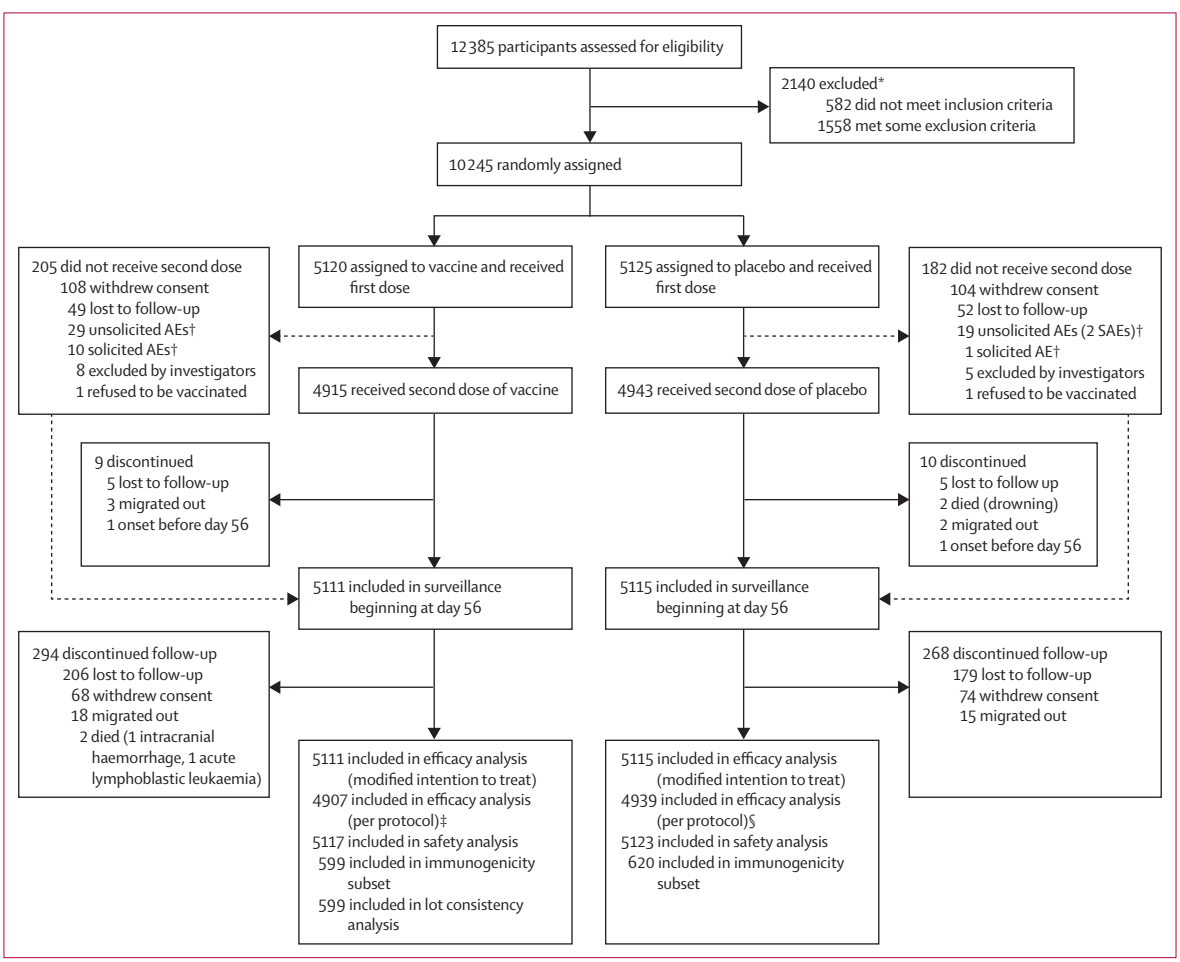

Figure 1: **Trial profile**

The modified intention-to-treat analysis included all participants who received at least one dose and entered surveillance at day 56. AE=adverse event. SAE=serious adverse event. *For details, see appendix p 6. †For details, see appendix p 39. ‡204 participants were excluded because they received only the first dose. §175 participants received only the first dose and one participant received the vaccine at second dose in the placebo group, so were excluded from the per-protocol analysis.

	Vaccine group	Placebo group
Efficacy analysis population (per protocol)		
n	5111	5115
Mean age (months)		
Overall	18·6 (8·1)	18·6 (8·1)
In those aged 6–11 months	8·7 (1·7)	8·7 (1·7)
In those aged 12–35 months	21·7 (6·6)	21·8 (6·7)
Boys	2922 (57·2%)	2805 (54·8%)
Bodyweight (kg)	12·5 (2·2)	12·5 (2·2)
BMI	18·8 (2·5)	18·7 (2·4)
Safety analysis population		
n	5117	5123
Mean age (months)		
Overall	18·6 (8·1)	18·6 (8·1)
In those aged 6–11 months	8·7 (1·7)	8·7 (1·7)
In those aged 12–35 months	21·7 (6·6)	21·8 (6·7)
Boys	2927 (57·2%)	2813 (54·9%)
Bodyweight (kg)	12·5 (2·2)	12·5 (2·2)
BMI	18·8 (2·5)	18·7 (2·4)
Immunogenicity analysis population		
n	599	620
Mean age (months)		
Overall	18·6 (8·1)	18·5 (7·9)
In those aged 6–11 months	8·6 (1·7)	8·6 (1·7)
In those aged 12–35 months	21·8 (6·6)	21·6 (6·4)
Boys	343 (57·3%)	345 (55·6%)
Bodyweight (kg)	12·3 (2·3)	12·3 (2·2)
BMI	18·4 (2·6)	18·5 (2·8)

Data are mean (SD), or n (%). BMI=body-mass index.

Table 1: **Baseline characteristics**

per vaccine batch would provide at least 80% power to detect consistency in vaccine-induced geometric mean titre with a pre-defined equivalence range (0·5–2·0) for geometric mean titre ratios.

We assessed correlates of immunity for all participants with confirmed EV71 and their controls who had data for antibody titre at day 56. We calculated sensitivity (proportion of participants with EV71-associated disease who have a titre less than the cutoff at day 56), specificity (proportion of matched controls who have a titre equal or greater than the cutoff at day 56), and corresponding Youden index ([sensitivity + specificity] − 1). The cutoff with the maximum Youden index could be thought of as a surrogate of protection for it could provide the clearest distinction between cases and controls.[14]

To compare safety and immunogenicity endpoints we used Student's *t* test for log-transformed antibody titres, and Fisher's exact or χ^2 for categorical data. Antibody titres lower than 1:8 were assigned values of 1:4 for calculation.

We did the primary analysis of vaccine efficacy in the per-protocol population, which consisted of all eligible participants who completed the two-dose regimen and entered the surveillance period at day 56. We also assessed efficacy in the modified intention-to-treat population, which consisted of all participants who received at least one dose and were followed up from day 56. We analysed safety in all participants who had safety data available, and immunogenicity analyses were done in the per-protocol population of the immunogenic subset of participants. Statistical analyses were done by external, independent statisticians with SAS (version 9.1).

This study is registered with ClinicalTrials.gov, number NCT01508247.

	Vaccine group			Placebo group			Vaccine efficacy	
	Person-years at risk	Cases (n)	Incidence density rate (cases/1000 person-years)	Person-years at risk	Cases (n)	Incidence density rate (cases/1000 person-years)	% (95% CI)	p value*
Per-protocol population†								
EV71-associated disease	4725·4	8	1·7	4742·9	41	8·6	80·4% (58·2 to 90·8)	<0·0001
EV71-associated HFMD	4725·4	3	0·6	4742·9	30	6·3	90·0% (67·1 to 96·9)	0·0001
EV71-associated other cases	4725·4	5	1·1	4742·9	11	2·3	54·3% (–31·4 to 84·1)	0·15
Modified intention-to-treat population								
EV71-associated disease	4915·5	8	1·6	4903·5	44	9·0	81·9% (61·5 to 91·5)	<0·0001
EV71-associated HFMD	4915·5	3	0·6	4903·5	33	6·7	90·9% (70·4 to 97·2)	0·0001
EV71-associated other cases	4915·5	5	1·0	4903·5	11	2·2	54·6% (–30·6 to 84·2)	0·14
Participants in per-protocol population with baseline titre <1:8								
EV71-associated disease	3347·5	8	2·4	3345·4	40	12·0	80·0% (57·2 to 90·6)	<0·0001
EV71-associated HFMD	3347·5	3	0·9	3345·4	29	8·7	89·6% (66·0 to 96·9)	0·0002
EV71-associated other cases	3347·5	5	1·5	3345·4	11	3·3	54·5% (–31·1 to 84·2)	0·14

EV71-associated other cases are EV71-associated disease with non-specific symptoms, including 14 cases of upper respiratory tract infection and two cases of diarrhoea caused by EV71 infection. EV71=enterovirus 71. HFMD=hand, foot, and mouth disease. *For comparison of incidence density rates between groups. †Three patients with EV71-associated disease were included in the modified intention-to-treat population but excluded from the per-protocol population because they did not receive the second dose.

Table 2: **EV71 vaccine efficacy against EV71-associated diseases during 1 year of surveillance**

Role of the funding source
The sponsor helped to design the trial, but had no role in data collection, statistical analysis, interpretation, or writing of the report. All authors had full access to all data. F-CZ, Z-LL, X-LS had primary responsibility and the final decision to submit for publication.

Results

Figure 1 shows the trial profile. 12 385 children were assessed for eligibility in January, 2012, of whom 10 245 (82·7%) were enrolled and randomly assigned to vaccine (n=5120) or placebo (n=5125). 9858 (96·2%) participants received the second dose of vaccine (n=4915) and placebo (n=4943). Blood samples were taken immediately before the first dose from 10 218 (99·7%; 5108 in the vaccine group *vs* 5110 in the placebo group) participants and 28 days after second dose from 9896 (96·6%; 4949 *vs* 4947) participants. The proportion of participants who withdrew from the study between days 0 and 56 did not differ significantly between vaccine and placebo groups (appendix p 7).

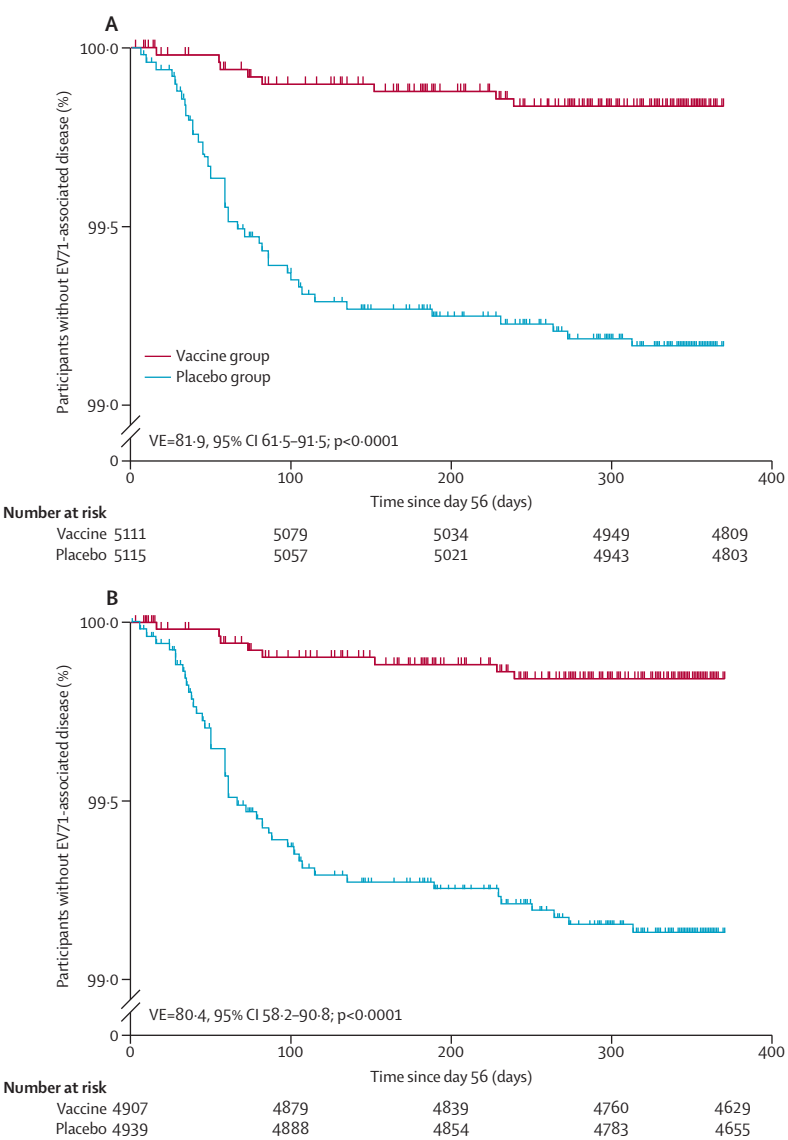

Figure 2: **Kaplan-Meier survival analysis of cumulative risk of not having EV71-associated disease**
Note, the y-axes are broken. For the modified intention-to-treat population (A) and the per-protocol population (B). VE=vaccine efficacy. EV71=enterovirus 71.

9846 (96·3%) participants were eligible for the primary efficacy analysis in the per-protocol population (4907 in the vaccine group *vs* 4939 in the placebo group). 10 226 participants (5111 in the vaccine group *vs* 5115 in the placebo group) were included in surveillance in the modified intention-to-treat population. Dropout was low during the surveillance period: only 294 of 5111 (5·8%) participants in the vaccine group and 268 of 5115 (5·2%) in the placebo group were lost to follow-up. The safety analysis population consisted of 10 240 participants (5117 in the vaccine group *vs* 5123 in the placebo group). Five participants were excluded from the safety assessment because they had lost their diary cards. For the analysis of immunogenicity and antibody persistence we assessed 1219 participants at day 56, 1134 at month 8, and 1123 at month 14. 599 participants who received EV71 vaccine and had data for antibody titre at day 56 were included in the analysis of batch consistency. Table 1 and appendix p 8 show baseline characteristics.

Surveillance lasted from March, 2012 to March, 2013, during which time 7328 participants had 17 038 episodes of illness (appendix p 9). 52 participants had laboratory-confirmed EV71-associated disease (appendix pp 10–15). 51 of these participants were seronegative, with a baseline neutralising antibody titre of less than 1:8, and one participant had a titre of 1:8 at baseline. 46 (88·5%) of the 52 participants were positive in amplification of *VP1*, and phylogenetic analysis showed that all EV71 strains were genotype C4 (appendix pp 16–17). Estimated vaccine efficacy in the per-protocol population was 90·0% (67·1–96·9) for EV71-associated HFMD and 80·4% (58·2–90·8) for EV71-associated disease (table 2). Estimated vaccine efficacy in the modified intention-to-treat population was much the same as in the primary analysis (table 2). Analysis of a modified per-protocol population only including participants with baseline titre less than 1:8, showed much the same vaccine efficacy as the normal per-protocol population. Figure 2 shows Kaplan-Meier cumulative event-free survival curves. No difference was noted between efficacy in the first 6 months and 1 year (appendix p 18). Appendix pp 19–20 shows analyses stratified by age and centre. Antibody and study centre were correlated with risk of EV71-associated disease. Appendix pp 19–21 shows the efficacy adjusted for covariates. Eight patients in the placebo group (modified intention-to-treat population) were admitted to hospital for EV71-associated HFMD (including one severe case of HFMD accompanied by encephalitis and oral herpes) compared with none in the

vaccine group, suggesting a vaccine efficacy of 100% (95% CI 41·6–100·0) for potential severe (admitted to hospital) or severe cases (appendix p 24). All patients recovered after admission to hospital. We noted no herpangina alone caused by EV71. The EV71 season peaked between April and July, and trends in prevalence of EV71 differed between the four centres with the highest incidence in Baoying county (appendix p 25). Median periods of virus shedding were 13·8 days in the vaccine group and 14·5 days in the placebo group (p=0·63; appendix p 26).

Of the 17 038 episodes of illness recorded during surveillance, 1704 were clinically diagnosed as HFMD, of which only 36 (2·1%) were finally confirmed as being associated with EV71, 577 (33·9%) were positive for Coxsackie A virus 16, 588 (34·5%) were positive in other enteroviruses, and 503 (29·5%) were not associated with enterovirus. The incidences of coxsackie A virus 16, other enteroviruses, and the overall number of clinical cases of HFMD did not differ significantly between vaccine and placebo groups (appendix pp 27–30).

Baseline immunity—as measured by geometric mean titre—was much the same in vaccine and placebo groups (table 3, appendix p 31). Two doses of EV71 vaccine elicited a substantial increase of neutralising antibody titre compared with placebo. From day 56 to month 8, the antibody titre in the vaccine group waned significantly, and then remained consistent for the next 6 months. Even by excluding potentially EV71-infected participants who had a four-fold increase of antibody titre after day 56, we noted no significant drop in titre from month 8 to month 14 (appendix p 32). However, geometric mean titre in the vaccine group was significantly higher than that in the placebo group during the whole study (appendix pp 33–34). The ratios of geotmetric mean titre between any two vaccine batches at day 56 were in the predefined range (0·5–2·0), suggesting good consistency between production batches (appendix p 35).

137 of 10 240 (1·3%) participants had 139 serious adverse events between day 0 and month 14: 62 of 5117 (1·2%) in the vaccine group and 75 of 5123 (1·5%) in the placebo group (table 4, appendix p 36). Although the incidence of serious adverse events between days 0 and 56 was higher in the placebo group than in the vaccine group (p=0·0112; table 4, appendix p 37), the difference was not significant for the whole study period. Four participants died (appendix p 38): two in the vaccine group (from acute lymphoblastic leukaemia, intracranial haemorrhage) and two in the placebo group (from drowning). None were deemed to be caused by vaccination. Within 7 days of injection, 2368 of 5117 (46·3%, 95% CI 44·9–47·7) participants receiving vaccine and 2250 of 5123 (43·9%, 42·6–45·3) receiving placebo reported solicited adverse reactions (table 4). Although the incidence of solicited adverse reactions was significantly higher in the vaccine group (p=0·0165) during days 0–7, grade 3 events did not differ significantly between groups (table 4). The most common symptoms at injection site were induration, erythema, and pain; the most common systemic symptoms were fever and diarrhoea (table 4). Overall, much the same proportions of adverse events occurred within 0–28 days after vaccination in the vaccine group (3644/5117; 71·2%) and placebo group (3603/5123; 70·3%). 59 participants did not have the second dose because of adverse events (39 in the vaccine group *vs* 20 in the placebo group; p= 0·0129).

To investigate correlates of immunity, we assessed 51 participants with EV71-associated disease and 254 matched controls (one in each group was excluded because a blood sample at day 56 was unavailable). Appendix p 40 shows the sensitivity, specificity, and Youden index for each titre. The maximum Youden index was provided by a titre of 1:16. Accounting for sensitivity, specificity, and Youden index, a titre of between 1:16 and 1:32 seems to provide the best protection against EV71-associated diseases.

	Vaccine group	Placebo group	p value
Day 0			
n	599	620	..
GMT (95% CI)	11·5 (9·9–13·3)	12·1 (10·4–14·1)	0·61
Proportion with titre ≥1:8 (n; %, 95% CI)	163 (27·2%, 23·7–31·0)	170 (27·4%, 23·9–31·1)	0·94
Proportion with titre ≥1:16 (n; %, 95% CI)	157 (26·2%, 22·7–29·9)	165 (26·6%, 23·2–30·3)	0·87
Proportion with titre ≥1:32 (n; %, 95% CI)	147 (24·5%, 21·1–28·2)	161 (26·0%, 22·6–29·6)	0·57
Day 56			
n	599	620	..
GMT (95% CI)	325·3 (284·8–371·7)	13·0 (11·1–15·2)	<0·0001
GMFI (95% CI)	28·4 (25·5–31·6)	1·1 (1·0–1·1)	<0·0001
Seroconversion (n; %, 95% CI)	549 (91·7%, 89·1–93·7)	19 (3·1%, 1·9–4·7)	<0·0001
Proportion with titre ≥1:8 (n; %, 95% CI)	597 (99·7%, 98·8–100·0)	181 (29·2%, 25·6–33·0)	<0·0001
Proportion with titre ≥1:16 (n; %, 95% CI)	596 (99·5%, 98·5–99·9)	172 (27·7%, 24·3–31·5)	<0·0001
Proportion with titre ≥1:32 (n; %, 95% CI)	571 (95·3%, 93·3–96·9)	168 (27·1%, 23·6–30·8)	<0·0001
Month 8			
n	559	575	..
GMT (95% CI)	187·3 (162·4–216·0)	18·2 (15·1–21·8)	<0·0001
GMFI (95% CI)	16·3 (14·4–18·3)	1·5 (1·4–1·7)	<0·0001
Proportion with titre ≥1:8 (n; %, 95% CI)	556 (99·5%, 98·9–100·0)	223 (38·8%, 34·8–42·8)	<0·0001
Proportion with titre ≥1:16 (n; %, 95% CI)	542 (97·0%, 95·5–98·4)	183 (31·8%, 28·0–35·6)	<0·0001
Proportion with titre ≥1:32 (n; %, 95% CI)	494 (88·4%, 85·7–91·0)	179 (31·1%, 27·4–34·9)	<0·0001
Month 14			
n	549	574	..
GMT (95% CI)	191·9 (170·8–215·6)	16·2 (13·6–19·1)	<0·0001
GMFI (95% CI)	17·2 (15·1–19·7)	1·3 (1·2–1·4)	<0·0001
Proportion with titre ≥1:8 (n; %, 95% CI)	545 (99·3%, 98·6–100·0)	220 (38·3%, 34·4–42·3)	<0·0001
Proportion with titre ≥1:16 (n; %, 95% CI)	539 (98·2%, 97·1–99·3)	190 (33·1%, 29·3–37·0)	<0·0001
Proportion with titre ≥1:32 (n; %, 95% CI)	508 (92·5%, 90·3–94·7)	181 (31·5%, 27·7–35·3)	<0·0001

Seroconversion is defined as pre-vaccination titre less than 1:8 and post-vaccination titre 1:32 or more, or pre-vaccination titre 1:8 or more and at least four-fold increase post-vaccination. GMT=geometric mean titre. GMFI=geometric mean fold increase.

Table 3: **Immune response to EV71 vaccine or placebo in the per-protocol population**

	Vaccine group (n=5117)	Placebo group (n=5123)	p value
Serious adverse events			
Within 28 days after each dose*	10 (0·2%)	25 (0·5%)	0·0112
Within 0–14 months	62 (1·2%)	75 (1·5%)	0·27
Deaths	2 (<0·1%)	2 (<0·1%)	1·00
Vaccine-related	0 (0·0%)	0 (0·0%)	..
Solicited adverse reactions within 0–7 days			
Any	2368 (46·3%)	2250 (43·9%)	0·0165
Grade 3	98 (1·9%)	78 (1·5%)	0·13
Injection-site adverse reactions			
Induration			
Any	293 (5·7%)	254 (5·0%)	0·08
Grade 3	1 (<0·1%)	0 (0·0%)	0·50
Erythema			
Any	246 (4·8%)	247 (4·8%)	0·97
Grade 3	2 (<0·1%)	3 (<0·1%)	1·00
Pain			
Any	165 (3·2%)	143 (2·8%)	0·20
Grade 3	0 (0·0%)	0 (0·0%)	..
Swelling			
Any	70 (1·4%)	65 (1·3%)	0·66
Grade 3	0 (0·0%)	0 (0·0%)	..
Pruritus			
Any	68 (1·3%)	57 (1·1%)	0·32
Grade 3	2 (<0·1%)	0 (0·0%)	0·25
Systemic adverse reactions			
Fever			
Any	1799 (35·2%)	1738 (33·9%)	0·19
Grade 3	88 (1·7%)	71 (1·4%)	0·17
Diarrhoea			
Any	309 (6·0%)	324 (6·3%)	0·55
Grade 3	1 (<0·1%)	2 (<0·1%)	1·00
Decreased feeding			
Any	299 (5·8%)	311 (6·1%)	0·63
Grade 3	1 (<0·1%)	2 (<0·1%)	1·00
Crying			
Any	290 (5·7%)	286 (5·6%)	0·85
Grade 3	2 (<0·1%)	1 (<0·1%)	0·62
Nausea or vomiting			
Any	185 (3·6%)	213 (4·2)	0·16
Grade 3	2 (<0·1%)	1 (<0·1%)	0·62
Fatigue			
Any	97 (1·9%)	119 (2·3%)	0·13
Grade 3	0 (0·0%)	2 (<0·1%)	0·50
Total adverse events within 28 days after each dose	3644 (71·2%)	3603 (70·3%)	0·33
Withdrawn due to adverse events†	39 (0·8%)	20 (0·4%)	0·0129

Data are n (%) unless otherwise indicated. Grade 3 events were those deemed severe—ie, prevented activity. *For further classifications see appendix p 37. †For further classifications see appendix p 39.

Table 4: **Serious adverse events and adverse reactions with incidence greater than 1% in the safety analysis population**

Discussion

Phase 1 and 2 trials have shown satisfactory safety and immunogenicity of an investigational EV71 vaccine in Chinese children (panel 2).[5,12] Efficacy of EV71 vaccine has not been reported worldwide. During active surveillance, vaccine efficacy was 90·0% against EV71-associated HFMD and 80·4% against EV71-associated disease: similar efficacies to other enterovirus-inactivated vaccine—eg, inactivated poliovirus vaccine.[15,16] Day 56 was planned to be the beginning of the surveillance period, when protection elicited by the EV71 vaccine should have been at its peak. In fact, only two cases of EV71-associated disease were reported between day 0 and day 56, one in each group.

We did not measure protection from EV71 infection for several reasons. First, measurement of EV71 infection is impractical because many people have asymptomatic infection, which means that all participants would need to be periodically sampled and tested for virus or RNA.[6] Furthermore, seroconversion arising from vaccination cannot be distinguished from infection. Second, because EV71 infection does not always cause symptoms, protection from infection is not necessarily the most important outcome. Furthermore, continuous circulation of EV71 might provide natural boosting and help to maintain immunity. Thus, at present, protection from EV71-associated disease rather than infection should be the priority.

Although HFMD is the most typical clinical manifestation of symptomatic EV71 infection, several other pathogens can also cause HFMD-like disease;[17,18] furthermore, EV71 infection can cause various clinical manifestations.[19] Therefore, the causal relation between EV71 and HFMD was complicated. We took several steps to improve specificity and sensitivity. The Nationwide Notifiable Infectious Diseases Reporting Information System in China is a passive surveillance system that is neither sensitive nor specific enough to detect all EV71-associated cases. We used active surveillance of many illnesses. We frequently contacted the guardians of participants and instructed them seek medical attention if any symptoms were noticed. More than 1000 staff worked on the study site during the surveillance period, especially during the HFMD epidemic season. Throat swabs, rectal swabs, and stool samples were collected for more than 94·2% episodes of illness within 24 h of onset. All specimens that were positive by real-time PCR assay in local laboratories were retested and virus isolated by the National Institute for Food and Drug Control in China.

Although EV71 was isolated from all participants with symptoms of EV71-associated disease, illness in 16 participants who had non-specific symptoms—eg, upper respiratory tract infection and diarrhoea—might have been caused by coinfection with other pathogens and not EV71. EV71 disease with non-specific signs—eg, fever, cough, diarrhoea—has been reported in

epidemiological studies, but represents only a small proportion of all EV71 infections, usually around 4–8%.[20,21] In our study, symptoms of upper respiratory tract infection occurred in 14 of 52 (25%) participants, therefore the likelihood of co-infection—at least in some patients—is high.

One limitation is that we did not test for pathogens other than EV71. If patients with non-specific disease were co-infected, specificity might have decreased, leading to underestimation of vaccine efficacy.[22] Because HFMD is a characteristic symptom of enterovirus infection, specificity for detecting EV71 might have been higher in the 36 patients with HFMD than in the 16 patients with non-specific symptoms, which could explain the higher protective efficacy against EV71-associated HFMD than against EV71-associated with non-specific symptoms. All virus strains isolated from confirmed cases in this study were genotype C4, the predominant strain in mainland China.[1,6] However, in other regions, B4, B5, C2, and C5 have been reported.[1,6,23] Variation between genotypes might compromise the efficacy of the vaccine.

Most vaccines for communicable diseases have a direct effect on the vaccinated population and an indirect effect on the unvaccinated population—ie, widespread vaccination can provide herd immunity.[24] We did not estimate the effect of herd immunity, but to reduce its effect as much as possible, we enrolled a predefined proportion of children in every township (less than 40%). Thus, no more than 20% of target children or a lower proportion of the general population would receive the vaccine, lessening the indirect effect of herd immunity. This approach also helped to maintain the chance of re-exposure to EV71 and as a result, maintain the durability of vaccine protection. A slight—although not significant—increase in geometric mean titre from month 8 to month 14 in the vaccine group suggests that some participants were re-exposed to EV71. Large-scale population immunisation would reduce the circulation of EV71 and risk of re-exposure, resulting in a fall in natural boosting and durability of immunity, as occurs for measles and mumps vaccines.[25,26]

We did not do serological screening before enrolment to exclude seropositive participants. Participants who were seropositive at baseline can be assumed to be immune and therefore an absence of infection during the trial would not be a result of vaccination. However, this effect was mitigated by taking a blood sample from participants before the first vaccination. EV71 is not the only pathogen that caused HFMD. In the 1-year surveillance period, only a small proportion of cases of HFMD were confirmed as associated with EV71, indicating that EV71 might not be the dominant pathogen in this HFMD season.[27] Despite its high efficacy for preventing EV71-associated HFMD, the EV71 vaccine might have little part in reducing the overall incidence of HFMD, even by universal mass immunisation of children. Although EV71 did not account for a high proportion of HFMD cases in this study, it has in other settings—eg, in Sarawak[28] and Taiwan.[13] Large HFMD epidemics occur almost every year, whereas EV71 epidemics occur at intervals of roughly 3 years in each region as the number of susceptible children accumulates, suggesting that different predominant pathogens might cause the yearly HFMD outbreaks. We noted differences in prevalence of EV71 associated with different regions and baseline antibody concentrations, providing an explanation for the disproportionately high event rate in Baoying; therefore, efficacy trials of EV71 vaccine should be multicentre studies.

Infection with EV71 is of particular concern because it can cause severe disease and even death in children;[6,13] the EV71 vaccine could help to prevent hospital admission and severe cases. However, few patients in our study were admitted to hospital or had severe disease, therefore the estimates of efficacy against EV71 in such patients had wide 95% CIs.

During active surveillance, incidence density of EV71 diseases in children aged 6–11 months was not substantially different to that in children aged 12–35 months (0·9 cases per 1000 person-years *vs* 2·0 cases per 1000 person-years), suggesting that children as young as 6 months should be vaccinated.[13,29] The target age of vaccination (age 6–35 months) crosses several routine vaccine times; therefore, further studies are needed to assess any effect of concomitant routine vaccination on EV71 vaccine efficacy.

The safety profile and immunogenicity of the EV71 vaccine was clinically acceptable and consistent with previous clinical trials (panel 2). Withdrawal because of adverse events was higher in the vaccine group than in the placebo group, mainly because of fever, suggesting an increased risk of fever caused by vaccine. A sample size of 5000 participants receiving EV71 vaccine is not sufficient to detect very rare adverse events after immunisation, so ongoing surveillance is needed.

***Panel 2:* Research in context**

Systematic review

We searched PubMed with the terms "enterovirus 71", "vaccine", and "trial" with no date or language restrictions on April 27, 2013. Only five immunogenicity and safety studies have been reported. Four were done in mainland China and one was done in Taiwan. No previous phase 3 study of efficacy of enterovirus 71 vaccine has been reported.

Interpretation

After a promising phase 2 trial, this study is the first report of the clinical efficacy of an inactivated enterovirus 71 vaccine. The vaccine provided significant protection against enterovirus 71-associated disease, especially EV71-associated hand, foot, and mouth disease in children aged 6–35 months. The experimental vaccine is at least as immunogenic and efficacious as traditional inactivated enterovirus vaccines.[15,16] Our data also provide the first evidence of immunological correlates of protection against enterovirus 71-associated disease.

Participants with EV71-associated disease had significantly lower antibody titres at day 56 than matched participants. Although a titre of 1:16 had the highest Youden index, a titre of 1:32 had very similar Youden index. Because a higher sensitivity provides better protection from EV71-associated disease and the antibody titre fell over time, we suggest that a titre of 1:32 be used as a surrogate of protection against EV71-associated disease. This exploratory analysis on the correlates of immunity is a substantial step forward; however, more evidence of its validity is needed.

Contributors

F-CZ, Z-LL, and X-LS designed the trial and contributed to critical review and revision of the report. F-CZ was principal investigator. Z-LL contributed to the study protocol and led the laboratory analyses. F-YM, J-XL, Y-TZ, HT, KC, and Y-MH collected data, supervised the study, and wrote and revised the report. H-MG, Z-YZ, Y-YD, Y-JC, L-YZ, Y-CL, N-MS, LL, S-GC, XA, Y-GJ, F-JL, YZ, L-WZ, and X-QC led and participated in the site work, including recruitment, and data collection and data interpretation. X-LL, Q-YM, FG, XW, XY, and Q-HC did laboratory analyses, interpreted data, and searched the published work. LP and HJ analysed data. All authors reviewed and approved the final version of the report.

Conflicts of interest

X-LL, Y-TZ, Q-HC, and X-LS are employees of the National Engineering Research Center of Innovative Vaccine of Beijing Vigoo Biological. The other authors declare that they have no conflicts of interest.

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