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A Phase I, randomized, open-label study to evaluate the safety and immunogenicity of an enterovirus 71 vaccine

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ABSTRACT

Background: Large-scale outbreaks of enterovirus 71 (EV71) infections have occurred in Asia-Pacific regions. Severe complications include encephalitis and poliomyelitis-like paralysis, cardiopulmonary collapse, and death, necessitating an effective vaccine against EV71.
Methods: In this randomized Phase I study, we evaluated the safety and immunogenicity of an inactivated alum-adjuvanted EV71 whole-virus vaccine produced on Vero cell cultures. Sixty healthy volunteers aged 20–60 years received two doses of vaccine, administered 21 days apart. Each dose contained either 5 μg of EV71 antigen with 150 μg of adjuvant (Group A05) or 10 μg of EV71 antigen with 300 μg of adjuvant (Group B10). Serologic analysis was performed at baseline, day 21, and day 42.
Results: There were no serious adverse events. Mild injection site pain and myalgia were the most common adverse events with either vaccine formulation. The immunogenicity data showed that 90% of vaccine recipients have a 4-fold or greater increase in neutralization antibody titers (NT) after the first dose, without a further increase in NT after the second dose. The seroconversion rates on day 21 and day 42 were 86.7% and 93.1% respectively, in Group A05, and 92.9% and 96.3%, respectively, in Group B10. Thus, 5 μg and 10 μg of the EV71 vaccine can induce a remarkable immune response in healthy adults after only the first vaccination.
Conclusion: The 5 μg and 10 μg adjuvanted EV71 vaccines are generally safe and immunogenic in healthy adults. (ClinicalTrials.gov number, NCT01268787).

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1. Introduction

Enterovirus 71 (EV71), a small RNA virus often found in the gastrointestinal and respiratory tracts, was first recognized in California in 1969 and subsequently reported worldwide [1–4]. It is a major causative agent of hand-foot-and-mouth disease (HFMD) [5,6]. HFMD is a common rash illness in children and infants associated with severe neurological complications such as encephalitis and poliomyelitis-like paralysis [7,8]. EV71 can also cause fatal infections, most often as a result of rhombencephalitis and cardiopulmonary collapse [9–11].

In the last decade of the 20th century, EV71 outbreaks were observed mainly in the Asia-Pacific regions [12]. In 1998, EV71 caused an unprecedented outbreak in Taiwan. During this 1998 outbreak, among the 129,106 reported cases of HFMD or herpangina, there were 405 severe cases and 78 deaths [9]. Furthermore, most patients were less than 5 years old, and up to 91% of deaths occurred in this age group. Following this large outbreak, smaller outbreaks recurred in 2000 and 2001 as more than one-half of the Taiwanese children was found to lack seroprotection to EV71 [11]. Thus, EV71 remains a public health concern in Taiwan and countries of the Asian-Pacific. For this reason, developing an effective vaccine for EV71 is a high priority for high-prevalence areas.

2. Methods

2.1. Study design and objective

From September 2011 through February 2012, we enrolled 60 healthy volunteers between the ages of 20 and 60 years in a randomized, open-label clinical trial at 2 sites in Taiwan:

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National Taiwan University Hospital and Taipei Veterans General Hospital. This study was designed by the investigators and sponsored by the National Health Research Institute (NHRI). The investigators collected the data and the clinical research organization—Qualitix—analyzed the data. All authors contributed to the content, had full access to the data, and vouched for the completeness and accuracy of the data and data analysis.

The appropriate hospital and national review boards and ethics committees approved the study protocol, which was conducted in compliance with the Good Clinical Practice guidelines and the provisions of the Declaration of Helsinki.

The objectives of this Phase I trial were to assess the immunogenicity and safety of 2 doses of formaldehyde-inactivated whole-virus vaccine formulated with an aluminum phosphate (AlPO4) adjuvant. After the first and second administration of 5 μg and 10 μg doses, the primary safety outcomes were solicited and unsolicited adverse events. At 21 days after the first and second doses of the vaccine, the primary immunogenicity outcome of the vaccine strain was changes in the titers of IgG and neutralizing antibodies (NT) in terms of seroconversion factors (SCFs, defined as the fold increase in serum antibody titers as against the baseline level).

2.2. Vaccine

The Vaccine Research and Development Center (VRDC) at the NHRI produced the vaccine, which was derived from the whole virion of the E59 strain subgenotype B4 of EV71 that was obtained from the Centers for Disease Prevention (Taiwan); it was manufactured in Vero cell culture through a cell-culture-based method by using roller bottle technology, and fully characterized according to US FDA guidelines as previously described [13–16]. The EV71/E59 vaccine is harvested, ultra-concentrated, purified and inactivated with formaldehyde, and formularized with an aluminum adjuvant (AlPO4). It contains 20 μg/ml of viral protein with 600 μg/ml of the AlPO4 adjuvant. Preclinical immunogenicity studies in mice has proven that inactivated whole E59 virion has a strong ability to elicit mice antibody against EV71 in a dose-dependent manner [14,17].

2.3. Randomization and follow-up

Subjects were eligible to participate if they were aged between 20 and 60 years inclusive, clinically healthy as determined by a physician through history taking and physical examination and by laboratory investigations at screening, understood the study procedures, provided written informed consent, and agreed to keep a daily record of symptoms. Major exclusion criteria included women who were pregnant or at risk of becoming pregnant, BMI $\geq$ 35 kg/m^2, known or potential exposure to EV71, history of herpangina, HFMD, acute hemorrhagic conjunctivitis, or any enterovirus associated illnesses in the preceding 3 months, known immunodeficiency or immunosuppressant use, history of hypersensitivity to vaccines, or the concomitant use of any other investigational/unlicensed product (see Appendix for full details).

Subjects were recruited in two phases: Part I and Part II. Following a blocking factor of safety, subjects in Part I were sequentially recruited in 3 blocks—Block A, Block B, and Block C, in which 1, 2, and 7 subjects, respectively, were enrolled. Part II was initiated after evaluation by the Data and Safety Monitoring Board (DSMB) wherein the remaining 50 subjects were recruited. Part II was conducted 14 days after the second vaccination of the subjects in Block C. The subjects were randomly assigned to receive 2 intramuscular injections into the deltoid muscle. The injections were separated by an interval of 21 days (the interval ranged from 21 to 23 days). The injection consisted of one of the following dosages: 0.25 mL (Group A05; 5 μg protein + adjuvant 150 μg AlPO4) or 0.5 mL (Group B10; 10 μg protein + adjuvant 300 μg AlPO4) (Fig. 1).

Subjects were monitored throughout the study for adverse events. A thermometer and a self-evaluation diary card were dispensed to the subjects after each vaccination to record any events for the 21 day period post vaccination. The first diary cards were collected at visit 3 (day 21) and the second diary cards were collected at visit 5 (day 42). In addition, the solicited symptoms were sought by non-directive questioning of the subject at each visit (on days 7, 14, 42) and by telephone follow-up (on days 126 and 210). All adverse events were reviewed by the investigators and severe adverse events were reviewed by the DSMB.

2.4. Virus neutralization assay

The cytopathogenic effect (CPE), based on in vitro assay measurements, was used to evaluate the virus neutralization titer. The virus neutralization titer (NT) was determined by using a microplate assay system, as reported previously [11]. In brief, 50 μL of serial two-fold diluted sera (beginning with 1:8) were mixed with 50 μL of 100 $TCID_{50}$ EV71 in a 96-well plate. Human rhabdomyoscarcoma cell suspensions (with a final concentration of 2×10^5 cells/mL) were added to the mixture. After incubation at 37 °C ± 0.5 °C with 5% CO_2 for 4 days, the CPE was observed under a light microscope. The neutralization titer was defined as the reciprocal of the highest dilution capable of inhibiting 50% of the CPE, which was calculated in accordance with the formula of the Reed–Muench method [11,13].

2.5. Specific anti-EV71 IgG antibody titer

Specific IgG titer against EV71/E59 was determined by enzyme-linked immunosorbent assay (ELISA) [18]. Inactivated EV71/E59 (1 μg/mL) was first coated onto 96-well microplates and allowed to stand overnight. The microplates were then washed with 0.5% Tween 20 in phosphate buffer saline (PBST) and blocked with 1% bovine serum albumin (BSA) in PBS for 2 h at room temperature. After washing the microplates with PBST for a second time, 100 μL of two-fold serial diluted clinical serum specimens (beginning with 1:1000) were added into each well. The microplates were incubated at room temperature for 2 h, and then washed with PBST. One hundred microliters of the detection antibody (i.e., goat anti-human IgG-Fc antibody conjugated with HRP; 1:5000 diluted) was added into each well and incubated on the microplates at room temperature for 1 h. After washing the microplates with PBST, 100 μL the chromogenic substrate 2,2-azino-di-(ethylbenzthiazoline-sulfonic acid) (ABTS) was added into each well and incubated at room temperature for 20 min. A spectrophotometer measured the absorbance at 405 nm. The IgG titer was defined as the endpoint of serial dilution at which the optical density (OD) value was two-fold more than the background value.

2.6. Statistical analysis

Since the primary purpose of this study was to explore the clinical safety of the study drug, no formal power calculation or sample size estimation was performed. The sample size was selected on the basis of previous experience with other Phase I studies.

All statistical hypothesis testing was assessed with a significance level of 0.05. For descriptive statistics, the continuous variables included the number of observations, mean, median, standard deviation, minimum, maximum, and 95% confidence intervals. Inter-group differences were analyzed by using the two-sample *t*-test or by the Wilcoxon rank sum test. The change from the baseline parameters was presented descriptively and assessed by the paired *t*-test or by the Wilcoxon signed rank test. For categorical variables,

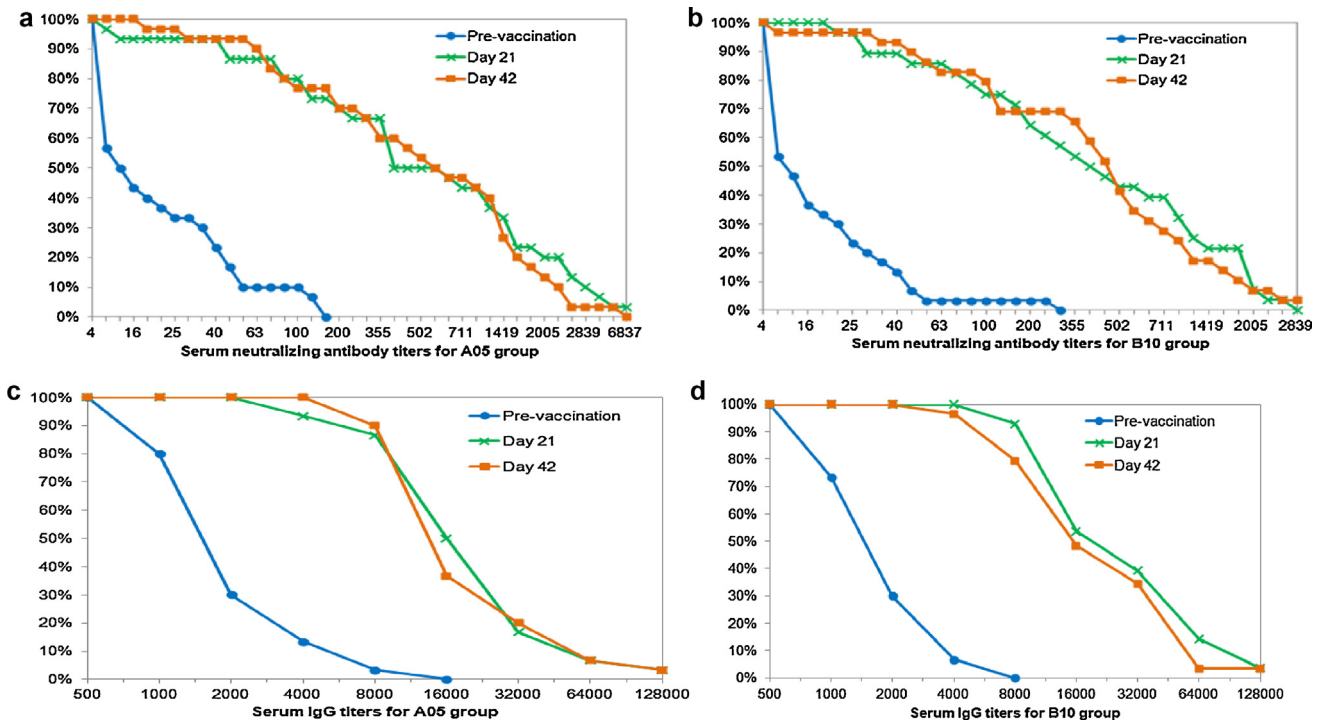

Fig. 1. Reverse cumulative distribution of serum neutralizing antibody titers (Panels A and B) and serum-specific IgG antibody titers (Panels C and D) in Group A05 and Group B10.

the count and percentages were summarized descriptively. The differences between groups were analyzed by the Chi-square test or the Fisher's exact test.

3. Results

3.1. Study population

Sixty subjects (22 men and 38 women) between the ages of 20 and 60 years were enrolled. All 60 subjects received the first dose of vaccine; 57 subjects received the second dose. Three subjects (1 in Group A05 and 2 in Group B10) did not receive the second dose of the vaccine due to a non-serious adverse event: an asymptomatic transiently elevated alanine aminotransferase (ALT) level that occurred 7 days after the first dose. Their levels individually were 39, 54, and 70 U/L. (The upper limit of normal is 37 U/L). Their plasma ALT levels normalized within 1 week. These three subjects continued to be followed up. Hence immunogenicity data in the intention-to-treat analysis was obtained from 58 subjects (two missing data) at day 21 and 59 subjects at day 42 (one missing data) and the per-protocol analysis from 57 subjects overall. All vaccinated subjects were included in the safety analysis.

3.2. Safety

No serious adverse events were recorded. The most common injection-site reaction after vaccination was pain. The most frequently reported systemic reaction was myalgia. All adverse events were mild to moderate in intensity. There were no statistically significant differences between the 2 groups after the first and second vaccination ($P = 0.49$ and $P = 0.90$, respectively, for all comparisons) with respect to solicited adverse events occurring within 7 days after each vaccination. The rate of injection-site and systemic reactions during the first 7 days after each dose of vaccine are summarized in Table 1.

The most frequent adverse event detected by laboratory tests was increased ALT. Two subjects in Group A05 and 3 subjects in Group B10 (6.7% and 10%, respectively) experienced mild elevations in ALT. One subject in each group (3.3% and 3.3%, respectively) had elevations in serum creatine phosphokinase [CPK]), one subject in Group A05 had a mild elevation in gamma-glutamyltransferase [GGT], one subject in Group B10 had a mild elevation in AST. All these asymptomatic biochemical elevations were considered to be related to the EV71 vaccine. Similar to solicited events, no severe unsolicited adverse events were reported. Two subjects (6.7%) in Group B10 reported skin rash; abdominal pain upper, procedural dizziness and oropharyngeal pain were reported once in the low-dose group; diarrhea, dry mouth, flatulence, nausea, fatigue, influenza, decreased appetite, muscular weakness and nasal obstruction were reported once in the high-dose group.

3.3. Neutralizing antibody titer (NT)

Table 2 summarizes the neutralizing antibody reactogenecity of the two study groups. The GMT and geometric standard deviation of the NT at baseline were similar between Group A05 and B10 ($P = 0.67$). After vaccination, Group B10 had higher values of NT and higher SCFs at day 21 and day 42 compared to Group A10; however, the differences were not statistically significant. Seroconversion was defined as a baseline NT of less than 8 with a conversion to an NT greater than 16; it is also defined as a baseline NT greater than 8 with a four-fold increase. The proportions of seroconversion on day 21 and day 42 were 86.7% and 93.1%, respectively, in Group A05, and 92.9% and 96.3%, respectively, in Group B10.

3.4. Specific anti-EV71 IgG antibody titer by ELISA

Table 3 shows the summary of IgG antibody titers of the 2 study groups. The geometric mean titer (GMT) and geometric standard

Table 1
Solicited mild and moderate local and systemic adverse events (AEs).

Variable\Preferred term	A05 ($N=30$)			B10 ($N=30$)			
	n	(%)	95% CI	*n*	(%)	95% CI	*P*-value
1st Vaccination	$N=30$			$N=30$			
Solicited local AEs							
Total	23	(76.7)	[61.5–91.8]	24	(80.0)	[65.7–94.3]0.75	
Injection site pain	23	(76.7)	[61.5–91.8]	24	(80.0)	[65.7–94.3]	0.75
Injection site erythema	2	(6.7)	[0.0–15.6]	5	(16.7)	[3.3–30.0]	0.42
Injection site swelling	1	(3.3)	[0.0–9.8]	4	(13.3)	[1.2–25.5]	0.35
Solicited systemic AEs							
Total	14	(46.7)	[28.8–64.5]	14	(46.7)	[28.8–64.5]	1.00
Myalgia	12	(40.0)	[22.5–57.5]	12	(40.0)	[22.5–57.5]	1.00
Fatigue	4	(13.3)	[1.2–25.5]	2	(6.7)	[0.0–15.6]	0.67
Decreased appetite	0	(0.0)	NA	3	(10.0)	[0.0–20.7]	0.24
Headache	1	(3.3)	[0.0–9.8]	0	(0.0)	NA	1.00
Diarrhea	1	(3.3)	[0.0–9.8]	0	(0.0)	NA	1.00
Vomiting	1	(3.3)	[0.0–9.8]	1	(3.3)	[0.0–9.8]	1.00
Arthralgia	1	(3.3)	[0.0–9.8]	0	(0.0)	NA	1.00
Pyrexia	1	(3.3)	[0.0–9.8]	0	(0.0)	NA	1.00
2nd Vaccination	$N=29$			$N=28$			
Solicited local AEs							
Total	12	(41.4)	[23.5–59.3]	14	(50.0)	[31.5–68.5]	0.51
Injection site pain	12	(41.4)	[23.5–59.3]	14	(50.0)	[31.5–68.5]	0.51
Injection site erythema	3	(10.3)	[0.0–21.4]	3	(10.7)	[0.0–22.2]	1.00
Injection site swelling	1	(3.4)	[0.0–10.1]	2	(7.1)	[0.0–16.7]	0.61
Solicited systemic AEs							
Total	10	(34.5)	[17.2–51.8]	4	(14.3)	[1.3–27.2]	0.08
Myalgia	6	(20.7)	[5.9–35.4]	2	(7.1)	[0.0–16.7]	0.25
Fatigue	2	(6.9)	[0.0–16.1]	0	(0.0)	NA	0.49
Decreased appetite	0	(0.0)	NA	1	(3.6)	[0.0–10.4]	0.49
Headache	2	(6.9)	[0.0–16.1]	0	(0.0)	NA	0.49
Diarrhea	2	(6.9)	[0.0–16.1]	1	(3.6)	[0.0–10.4]	1.00
Vomiting	2	(6.9)	[0.0–16.1]	0	(0.0)	NA	0.49

Table 2
Summary of serum neutralizing antibody titers.

Visit	Status	A05	B10	*P*-value
Baseline	GMT ± GSD	12.7 ± 3.3	11.7 ± 3.5	0.67
	(95% CI)	(8.2, 19.8)	(7.3, 18.7)	
Day 21	GMT ± GSD	408.6 ± 5.9[a]	517.9 ± 4.2[b]	0.82
	(95% CI)	(210.3, 794.0)	(297.1, 903.0)	
Day 42	GMT ± GSD	428.5 ± 4.4	486.3 ± 4.5[b]	0.78
	(95% CI)	(246.6, 744.5)	(275.6, 858.3)	
SCF on Day 21	Mean ± SD	113.8 ± 182.9[a]	126.2 ± 218.7[b]	0.82
SCF on Day 42	Mean ± SD	70.6 ± 88.2	120.9 ± 193.3[b]	0.21

Note: This is the intention-to-treat analysis. See Appendix for the per-protocol analysis.
[a] One blood sample was misplaced and lost.
[b] One subject withdrew from the study.

Table 3
Summary of serum specific IgG antibody titers based on ELISA.

Visit	Status	A05	B10	*P*-value
Baseline	GMT ± GSD	1203.0 ± 2.0	1071.8 ± 1.8	0.37
	(95% CI)	(925.1, 1564.4)	(852.4, 1347.6)	
Day 21	GMT ± GSD	11,848.8 ± 2.4[a]	16,401.0 ± 2.5[b]	0.31
	(95% CI)	(8511.7, 16,494.2)	(11,506.0, 23,378.6)	
Day 42	GMT ± GSD	11,848.8 ± 2.2	12,598.4 ± 2.6[b]	0.78
	(95% CI)	(8764.1, 16,019.1)	(8779.0, 18,079.5)	
SCFs on Day 21	Mean ± SD	15.3 ± 14.1[a]	26.6 ± 33.0[b]	0.10
SCFs on Day 42	Mean ± SD	14.6 ± 13.6	20.1 ± 26.3[b]	0.32

Note: This is the intention-to-treat analysis. See Appendix for the per-protocol analysis.
[a] One blood sample was misplaced and lost.
[b] One subject withdrew from the study.

deviation of a specific IgG antibody titer at baseline were similar between Group A05 and B10 ($P=0.37$). After the vaccinations, Group B10 subjects tended to have higher values of specific antibody titers and higher SCFs on day 21 and day 42 than group A05 but the difference was statistically insignificant. All vaccines (100% of Group A05 and B10) seroconverted following the first vaccination. No significant change in the anti-EV71 IgG titer was seen after the second vaccination.

4. Discussion

Hand-foot-and-mouth disease (HFMD) is a common infectious disease of infants and children that can be caused by several enteroviruses such as the coxsackieviruses A16 and EV71. EV71 infection is of particular concern in children since it is more frequently associated with serious neurological complications and fatalities [11,19]. During the 1998 outbreak in Taiwan, 91% of patients died who were below the age of 5 years and EV71 was present in 92% of patients who died [9]. According to a seroepidemiology study in Taiwan, the maternal EV71 neutralizing antibodies were at undetectable levels in nearly all 6-month-old infants [20]. These data suggest that children, especially those aged 6 months through 5 years, should be targeted. However since this particular whole-virus EV71 vaccine has never been tested in humans, healthy adults were chosen as the subjects of this Phase I study.

The safety of subjects participating in this study was of paramount consideration. The 10 μg dose of the study vaccine was expected to induce immune activation, based on scientific experience with the polio vaccine; however, the lower dose (i.e., 5 μg) was tested in the first subject. After that, all recruited subjects were randomly assigned to either a low-dose or a high-dose group. At the time the study was designed and conducted, the results of the recent clinical trials indicating that a 1 μg dose itself might be sufficient had not been published [21,22]. In retrospect, it is thus unsurprising that there were no significant differences between the low-dose and high-dose groups in terms of immunogenicity, and that a 5 μg dose was adequate in healthy adults since sufficient immunogenicity could be achieved after a dose as little as 0.25 μg when repeated once in naïve infants [23].

Another differences of note was that unlike the adult subjects studied by Li et al. of whom only 8.4% did not have detectable baseline neutralizing antibody, 63.2% (36/57) of our subjects had no detectable baseline neutralizing antibody (detection limit < 1:8). After one vaccination dose, the geometric mean titers of the neutralizing antibody for those without pre-existing NT were significantly lower than those obtained from volunteers with pre-existing NT in both Group A (285.1 vs. 746.0) and Group B (390.7 vs. 860.4) ($P < 0.0001$)(data in Appendix). However a further increase in NT after the second dose was not observed, not even for those who were seronegative at baseline. This phenomenon suggests that the increased neutralizing antibody level after the first vaccination may be caused by recall immunity in our adult subjects who unlike truly naïve infants may be cross-primed by other enteroviral infections. Consistent with this, Zhu et al. found that the NT of those seropositive at baseline was boosted by the first but not the second dose [23]. Irrespective of baseline serostatus, the first vaccination alone elicited a good immune response with over 90% of vaccinees demonstrating over a 4-fold increase in NT after the first dose, increasing to 95% after the second dose.

Despite the higher dose of vaccine used in this study compared to previously published Phase I trials, the safety profile was excellent without any severe adverse effects, whereas severe adverse effects (i.e. high fever) were reported for 3.1% of the youngest subjects and mild fever for 22.2% of adult subjects in the study by Li et al. [21]. By contrast, the most common adverse effect we found was mild local injection reaction, perhaps attributable to the aluminum adjuvant used [24,25] and only one febrile reaction in the A05 group considered unrelated to vaccination. A study with a larger sample size will be required to determine whether these adverse events are dose-dependent.

With regard to laboratory assessments, no clinically significant symptoms were reported and, although 2 subjects in Group A05 and 3 subjects in Group B10 had a mild increase in ALT, no other laboratory changes were observed in other liver function parameters. There were also no clinically significant changes in renal function or in hematological parameters.

The major limitation of this study was the fact that it was an open-label study lacking a placebo arm and an arm without the adjuvant. Although foreknowledge of the dosing group does not interfere with objective laboratory determined events and immunological responses, it may bias the recording of subjective events as well as the interpretation of the data. Fortunately, no significant differences in the incidence or severity of adverse effects between groups were found.

In conclusion, this inactivated whole-cell EV71/E59 vaccine is safe and immunogenic after the first dose in healthy adults. These promising results should be followed by an evaluation of

Table A.1
Summary of serum neutralizing antibody titers in the per-protocol analysis.

Visit	Status	A05	B10	*P*-value
Baseline	GMT ± GSD	11.7 ± 3.1	12.2 ± 3.6	0.4617
	(95% CI)	(7.7, 18.0)	(7.4, 20.1)	
Day 21	GMT ± GSD	410.6 ± 6.1	517.9 ± 4.2	0.7615
	(95% CI)	(206.3, 817.5)	(297.1, 903.0)	
Day 42	GMT ± GSD	431.2 ± 4.5	470.1 ± 4.5	0.8597
	(95% CI)	(243.2, 764.6)	(261.8, 844.1)	
SCF on Day 21	Mean ± SD	117.6 ± 184.9	126.2 ± 218.7	0.8740
SCF on Day 42	Mean ± SD	72.9 ± 88.8	114.0 ± 193.2	0.3112

Note: Fifty-seven subjects were included for the per-protocol analysis.

Table A.2
Summary of serum specific IgG antibody titers by ELISA in the per-protocol analysis.

Visit	Status	A05	B10	*P*-value
Baseline	GMT ± GSD	1154.2 ± 2.0	1104.1 ± 1.9	0.5976
	(95% CI)	(892.0, 1493.6)	(869.0, 1402.8)	
Day 21	GMT ± GSD	12,010.4 ± 2.5	16,401.0 ± 2.5	0.3444
	(95% CI)	(8533.9, 16,903.0)	(11,506.0, 23,378.6)	
Day 42	GMT ± GSD	12,010.4 ± 2.3	13,125.4 ± 2.6	0.7569
	(95% CI)	(8797.5, 16,396.6)	(9114.2, 18,901.8)	
SCFs on Day 21	Mean ± SD	15.8 ± 14.1	26.6 ± 33.0	0.1161
SCFs on Day 42	Mean ± SD	15.0 ± 13.6	20.7 ± 26.6	0.3162

Note: Fifty-seven subjects were included for the per-protocol analysis.

Table A.3
Summary of serum neutralizing antibody titers against EV71 stratified by baseline serological status.

Variable	Status	A05 positive ($N = 11$)	A05 negative ($N = 18$)	B10 positive ($N = 10$)	B10 negative ($N = 18$)
Baseline	GMT ± GSD	40.7 ± 1.8	5.5 ± 1.7	51.7 ± 2.5	5.5 ± 1.7
	95% CI for GMT	(27.8, 59.6)	(4.2, 7.2)	(27.2, 98.1)	(4.2, 7.2)
Day 21	GMT ± GSD	746.0 ± 4.1	285.1 ± 7.1	860.4 ± 2.5	390.7 ± 5.0
	95% CI for GMT	(290.4, 1916.6)	(107.2, 757.9)	(444.3, 1666.5)	(175.9, 867.8)
Day 42	GMT ± GSD	1040.8 ± 3.2	251.7 ± 4.3	844.6 ± 2.0	339.5 ± 5.7
	95% CI for GMT	(474.7, 2281.9)	(122.0, 519.2)	(512.8, 1391.0)	(142.5, 808.7)
SCFs on Day 21	Mean ± SD	36.4 ± 48.8	167.3 ± 219.1	34.7 ± 40.0	177.0 ± 259.7
	Median (Min, Max)	22.5 (2.0, 171.8)	58.4 (1.0, 709.6)	16.9 (2.0, 126.8)	88.7 (5.0, 1004.8)
	95% CI	(3.7, 69.2)	(58.3, 276.2)	(6.0, 63.3)	(47.9, 306.2)
SCFs on Day 42	Mean ± SD	38.8 ± 35.6	93.7 ± 105.0	28.8 ± 34.1	161.3 ± 228.0
	Median (Min, Max)	31.8 (3.2, 127.1)	42.2 (4.0, 316.2)	15.2 (3.3, 113.0)	49.9 (1.0, 798.1)
	95% CI	(14.9, 62.7)	(41.5, 146.0)	(4.4, 53.2)	(47.9, 274.7)

the cross-neutralization immune responses to other EV71 strains such as subgenotypes B1, B5 and C4 that are also prevalent in this region and prompt further clinical studies in young children.

Appendix A.

Tables A.1, A.2 and A.3.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2013.03.015.

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