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2-Year Efficacy, Immunogenicity, and Safety of Vigoo Enterovirus 71 Vaccine in Healthy Chinese Children: A Randomized Open-Label Study

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Background. This study evaluated the 2-year efficacy, immunogenicity, and safety of the Vigoo enterovirus 71 (EV71) vaccine.

Method. In an initial phase 3 study, we randomly assigned healthy infants and children aged 6–35 months (ratio, 1:1) to receive 2 doses of either EV71 vaccine (5120 participants) or placebo (5125 participants) at days 0 and 28, and followed them for 12 months after vaccination. In this extended follow-up study, we continued to evaluate the efficacy, immunogenicity, and safety of the EV71 vaccine for up to 2 years.

Results. Overall efficacy was 94.84% (95% confidence interval [CI], 83.53%–98.38%) during the 2-year follow-up period ($P < .0001$), and the vaccine efficacy during the second year was 100.00% (95% CI, 84.15%–100.00%) against EV71-associated hand-foot-and-mouth disease (HFMD; $P < .0001$). Geometric mean titers of neutralizing antibody in participants remained high during the 2-year follow-up period, and no vaccine-related serious adverse events were recorded.

Conclusions. Two doses of Vigoo EV71 vaccine could provide sustained protection against EV71-associated HFMD in healthy Chinese children.

Clinical Trials Registration. NCT01508247.

Keywords. Vigoo enterovirus 71 vaccine; hand, foot, and mouth disease; efficacy; immunogenicity; safety.

Enterovirus 71 (EV71) is a small, single-stranded, positive-sense RNA virus from *Enterovirus* genus in the family *Picornaviridae*, which was first isolated in California in 1969 [1–3]. Since then, EV71-associated epidemics of hand-foot-and-mouth disease (HFMD) were reported all over the world [4]. Recently, large EV71 epidemics have occurred almost exclusively in the Asia-Pacific region, including Taiwan, the mainland of China, Hong Kong, Malaysia, Vietnam, Singapore, and Thailand [5–7], which has made EV71 a serious threat to public health [8, 9]. Most of the hospitalized EV71-associated HFMD cases with complications occur in children aged <5 years, while young children <3 years of age are most susceptible to severe or fatal EV71-associated neurological diseases, which is why the pathogen has captured much attention [8, 10–14]. A vaccine against EV71 is urgently needed to prevent and control epidemics of EV71 [15, 16].

Since 2010, 3 inactivated alum-adjuvant EV71 vaccines (Vigoo, Sinovac, and CAMS) were evaluated in clinical trials in the mainland of China. In the early phase 1 and phase 2 clinical trials, Vigoo EV71 vaccine showed a satisfactory safety and immunogenicity in adults, children, and infants [17–19]. Subsequently, we performed a phase 3 trial of Vigoo EV71 vaccine in young children and infants between January 2012 and March 2013 to assess the efficacy and safety of the vaccine for prevention of disease associated with EV71. In the first year of follow-up, the EV71 vaccine was highly efficacious against EV71-associated HFMD in young children and infants, as reported previously [20]. However, evaluation of the long-term efficacy of this EV71 vaccine is required to determine the need for booster injections, for licensing, and to determine its clinical application. Here, we report the efficacy, immunogenicity, and safety of the Vigoo EV71 vaccine over the 24-month follow-up period.

METHODS

Vaccine

Vigoo EV71 vaccine is an inactivated alum-adjuvanted EV71 vaccine (Vero cell) containing 320 U of antigen and 0.18 mg of alum. The vaccine was developed and manufactured by the Beijing Vigoo Biological with a seed virus (GenBank accession number JX025561) of EV71 strain FY7VP5/AH/CHN/2008 (genotype C4, which is the predominant strain in the mainland of China). Each dose of placebo contains 0.18 mg of alum adjuvant and no EV71 antigen [17].

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Study Design and Participants

In 2012, we recruited 10 245 healthy children aged 6–35 months (at enrollment) into a randomized, double-blinded, placebo-controlled, phase 3 clinical trial (clinical trials registration NCT01508247) to evaluate the 1-year efficacy of the Vigoo EV71 vaccine [20]. In this trial, Vigoo EV71 vaccine or placebo was administered according to a 2-dose schedule (on day 0 and day 28), and recipients were followed for a 12-month surveillance period (from day 56 through month 14) for EV71-associated disease and HFMD. After the completion of the first-year follow-up study to determine the efficacy of the EV71 vaccine, we performed an extra follow-up study lasting up to 2 years, for an overall follow-up period of 24-month after vaccination. Because masking in the initial study was partially removed at month 14, at the end of the first year of follow-up, surveillance for EV71-associated HFMD during the extended second year of follow-up was open labeled; the investigators who measured antibody levels and tested for the presence of pathogen remained masked with respect to vaccine or placebo allocation. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on harmonization good clinical practice guidelines. The study protocol was approved by the ethics committee of the Jiangsu Provincial Center for Disease Control and Prevention. Written informed consent was obtained from each participant before they underwent extended follow-up.

Efficacy Assessments and Case Definition

In the second-year follow-up study, the primary outcome was the efficacy of Vigoo EV71 vaccine against HFMD. HFMD cases were identified through a well-established HFMD surveillance system covering the area of study, which has already been described previously [20]. Clinical HFMD cases were defined as participants with any suspected symptoms of clinical HFMD, such as fever, skin eruptions on hands and feet, and vesicles in the mouth. Throat, anal, and stool swab specimens were collected from these children for virological confirmation. EV71-associated HFMD was confirmed clinically on the basis of either positive results of at least 2 consecutive real-time polymerase chain reaction (PCR) analyses (ie, fluorescence assays involving an EV71 RNA diagnostic kit) or detection of EV71 in at least 1 of the throat, anal, or stool swab specimens [21]. EV71-associated disease was defined on the basis of clinical symptoms, including HFMD, herpangina, neurological signs (aseptic meningitis or encephalitis) with or without serious sequelae, and nonspecific illnesses (eg, febrile illness, viral exanthema, and respiratory infection) that are caused by EV71, and either isolation of EV71 on culture or positive results of at least 2 consecutive EV71-specific RNA tests [20].

Immunogenicity Assessments

Blood samples were collected from a subset of participants in the immunogenicity cohort, described previously, on days 0 and 56 in the initial study [20] and during months 8, 14, and 26 in the extended follow-up period. Researchers at the Chinese National Institute for Food and Drug Control measured titers of neutralizing antibodies against EV71, using a modified cytopathogenic effect assay [20, 22], which were the same as those in the initial phase 3 study. The dilution of serum ranged from 1:8 to 1:16 384.

Safety Assessments

During the extended second-year follow-up (from months 15 to 26), any serious adverse event (SAE) reported by the guardians of participants was recorded. The occurrence of SAEs in the intention-to-treat population was assessed. All SAEs were classified using the terminology specified in the Medical Dictionary for Drug Regulatory Activities. The relationship between the SAEs and vaccine was determined by the principal investigator.

Statistical Analysis

Both the overall 2-year vaccine efficacy and the second-year vaccine efficacy were estimated in the study. Vaccine efficacy was calculated as follows: [1 – (vaccine incidence density rate)/(placebo incidence density rate)] × 100. We defined 2 cohorts for the vaccine efficacy analyses: an intention-to-treat analysis cohort, which included all participants who received at least 1 dose of either the EV71 vaccine or placebo; and a per-protocol analysis cohort, which included all participants who completed 2-dose immunization with EV71 vaccine or placebo.

To evaluate the persistence of immunogenicity, we included participants in the immunogenicity cohort who received 2 doses and had serologic test results available at baseline, day 56, and at least 1 later time point (eg, months 8, 14, or 26). The proportions of participants achieving an EV71 neutralizing antibody titer of 1:8, 1:16, or 1:32 after vaccination in different treatment groups were calculated. The seronegative samples at antibody titers lower than 1:8, which was the threshold of detection, were assigned values of 1:4 for the calculation of geometric mean titers (GMTs).

A χ^2 or Fisher exact test was used to compare categorical data, while the Student t test was used to compare log-transformed neutralizing antibody values. All reported P values were 2 sided, and values of <.05 were regarded as statistically significant. Statistical analyses were done by an independent, external statistician, using SAS software, version 9.2 (SAS Institute).

RESULTS

Study Participants

A total of 10 245 participants were randomly assigned in a ratio of 1:1 to receive the vaccine (n = 5120) or placebo (n = 5125) in the initial study and were followed over 1 year after receipt of 2 doses of EV71 vaccine [20]. Of them, 10 100 (98.59%) were

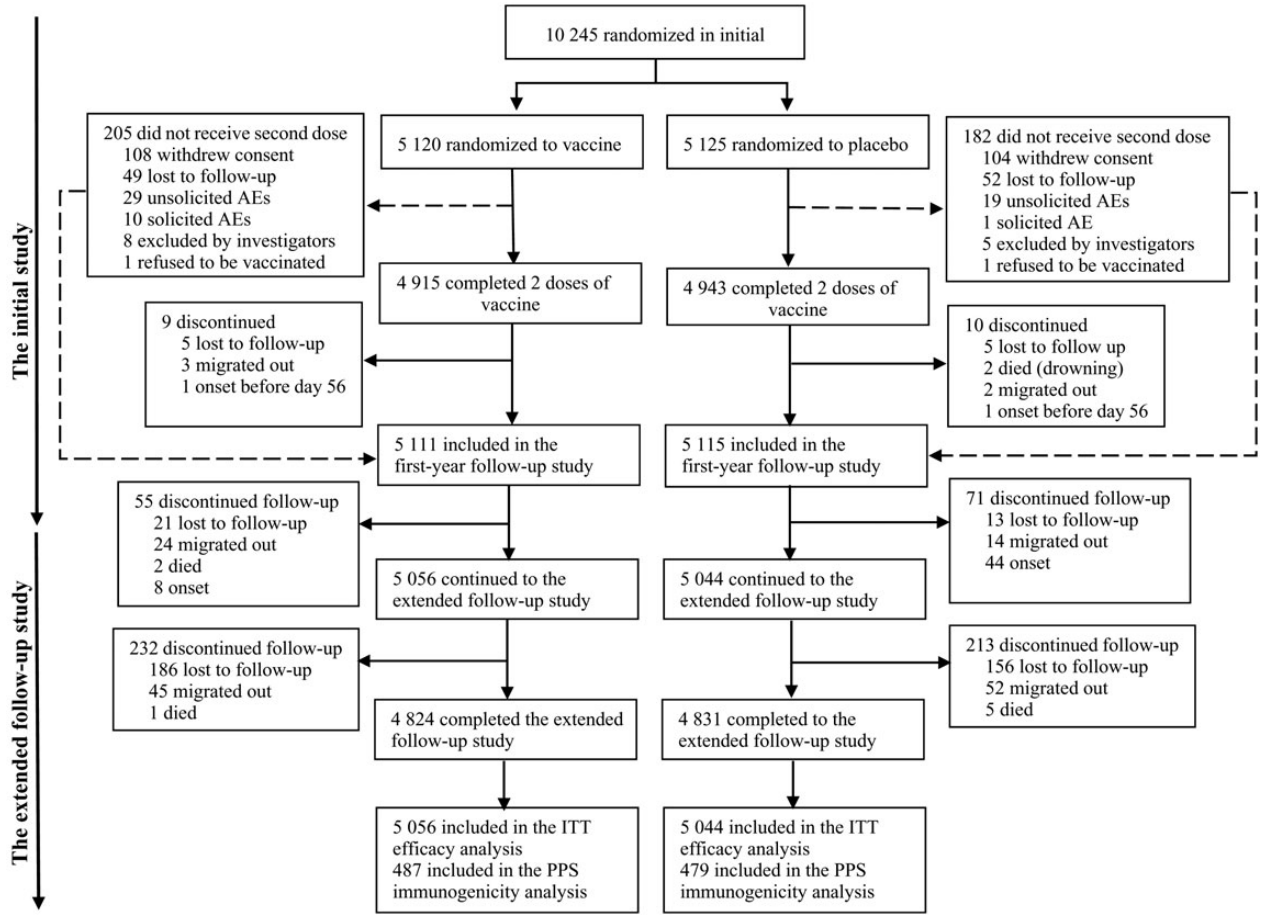

Figure 1. Participant flow through the initial and follow-up studies. The intention-to-treat (ITT) cohort efficacy analysis, including all participants who received at least 1 dose and entered into surveillance. All immunogenicity analyses were performed on the per-protocol sets (PPSs), including participants in an immunogenicity subset who received 2 doses of vaccine and had results of serologic tests at baseline, day 56, and at least 1 later time point. Numbers in parentheses indicate participants in the immunogenicity subset. Abbreviation: AE, adverse event.

enrolled at the start of the extended second-year follow-up study, and 9655 (94.24%) completed the 2-year follow-up until month 26 (Figure 1).

There were 5056 participants in the vaccine group and 5044 in the placebo group who were rerecruited and included in the intention-to-treat efficacy analysis. The mean follow-up period (±SD) of the cohort during the whole study was 23.47 ± 2.16 months in the vaccine group and 23.38 ± 2.52 months in the placebo group; no significant difference was found between the 2 treatment group ($P = .0687$).

Table 1 shows baseline demographic characteristics of the participants rerecruited in this extended follow-up study. We noted no significant difference in terms of sex, age, weight, and height between the vaccine group and the placebo group. At the end of the extended follow-up study, 487 of 636 vaccine recipients (76.57%) and 479 of 647 placebo recipients (74.03%; $P = .3009$) from the initial immunogenicity subgroup [20] underwent serologic testing and were included in the immunogenicity analysis.

Efficacy

A total of 61 cases of EV71-associated HFMD were confirmed during the 2-year study period in the intention-to-treat

Table 1. Baseline Demographic Characteristics of Participants in the Extended Follow-Up

Characteristic	Vaccine Group	Placebo Group
Efficacy analysis (intention-to-treat cohort)		
Participants, no.	5056	5044
Age, mo	18.56 ± 8.08	18.58 ± 8.13
Male sex, no. (%)	2891 (57.18)	2771 (54.94)
Height, cm	81.59 ± 8.16	81.67 ± 8.14
Weight, kg	12.46 ± 2.23	12.44 ± 2.23
Immunogenicity analysis (per-protocol cohort)		
Participants, no.	487	479
Age, mo	18.86 ± 8.06	18.70 ± 7.86
Male sex, no. (%)	285 (58.52)	264 (55.11)
Height, cm	82.19 ± 8.05	82.12 ± 7.91
Weight, kg	12.35 ± 2.26	12.34 ± 2.16

Data are means ± SD, unless otherwise indicated.

Table 2. Enterovirus 71 (EV71) Vaccine Efficacy Against EV71-Associated Hand-Foot-and-Mouth Disease (HFMD) in the Whole Surveillance Period, Combined With the Initial Study (Day 56–Month 26) and the Extended Follow-Up (Months 15–26)

	Vaccine Group			Placebo Group			Efficacy	
HFMD	Person-Years at Risk	Cases, No.	ID Rate, Cases/ 1000 Person-Years	Person-Years at Risk	Cases, No.	ID Rate, Cases/ 1000 Person-Years	Percentage (95% CI)	*P* Value
Intention-to-treat cohort[a]								
Overall follow-up (d 56–mo 26)	10 022.07	3	0.30	9993.94	58	5.80	94.84 (83.53–98.38)	<.0001
Extended follow-up (mos 15–26)	4982.45	0	0.00	4967.24	25	5.03	100.00 (84.15–100.00)	<.0001
Per-protocol cohort								
Overall follow-up (d 56–mo 26)	9628.80	3	0.31	9655.79	54	5.59	94.42 (82.16–98.25)	<.0001
Extended follow-up (mos 15–26)	4787.44	0	0.00	4800.97	24	5.00	100.00 (83.34–100.00)	<.0001

Abbreviations: CI, confidence interval; ID, incidence density.

[a] Included all participants who received at least 1 dose and entered the surveillance period.

cohort, including 36 cases that were captured in the initial study (3 in the vaccine group vs 33 in the placebo group) [20] and 25 cases in the extended follow-up study (0 in the vaccine group vs 25 in the placebo group; Supplementary Table 1). During the extended second year of follow-up, no severe or hospital-based EV71-associated HFMD events were recorded. In the intention-to-treat cohort, the 2-year efficacy of the vaccine against EV71-associated HFMD was 94.84% (95% confidence interval [CI], 83.53%–98.38%) during the overall follow-up period and 100% (95% CI, 84.15%–100.00%) during the extended second-year follow-up period (Table 2). Meanwhile, in the per-protocol cohort, 57 and 24 cases of EV71-associated HFMD were confirmed during the overall and second-year follow-up periods, respectively, resulting in vaccine efficacies against EV71-associated HFMD of 94.42% (95% CI, 82.16%–98.25%) and 100.00% (95% CI, 83.34%–100.00%), respectively. Figure 2 shows the Kaplan–Meier cumulative event survival curves of EV71-associated HFMD, which demonstrated very similar trends between the intention-to-treat cohort and the per-protocol cohort during the 2-year follow-up period after the first dose of vaccination. Furthermore, the estimated 2-year vaccine efficacy against EV71-associated disease was 95.84% (95% CI, 86.81%–98.69%) and 100.00% (95% CI, 90.11%–100.00%) during the overall and extended follow-up study periods, respectively, in the intention-to-treat cohort and 95.57 (95% CI, 85.93–98.61) and 100.00 (95% CI, 89.78–100.00), respectively, in the per-protocol population, respectively (Supplementary Table 2). The Kaplan–Meier cumulative event survival curves of EV71-associated disease also demonstrated very similar trends between the intention-to-treat cohort and the per-protocol cohort (Supplementary Figure 1).

Immunogenicity

EV71 vaccines elicited a substantial specific immune response after 2 doses, compared with placebo, with GMTs of EV71

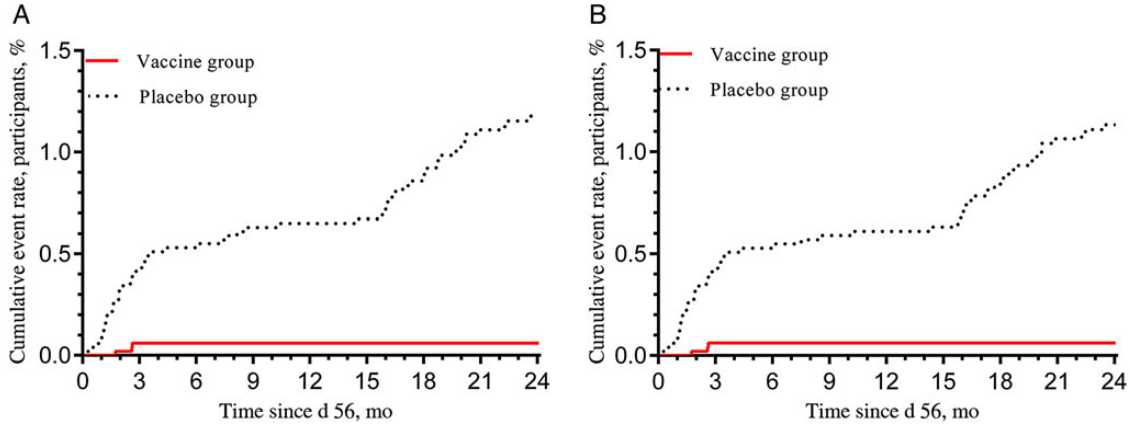

Figure 2. Kaplan–Meier survival analysis of cumulative risk of hand-foot-and-mouth disease caused by enterovirus 71 (EV71) from day 56 through month 26, according to the intention-to-treat population (*A*) and per-protocol set (*B*).

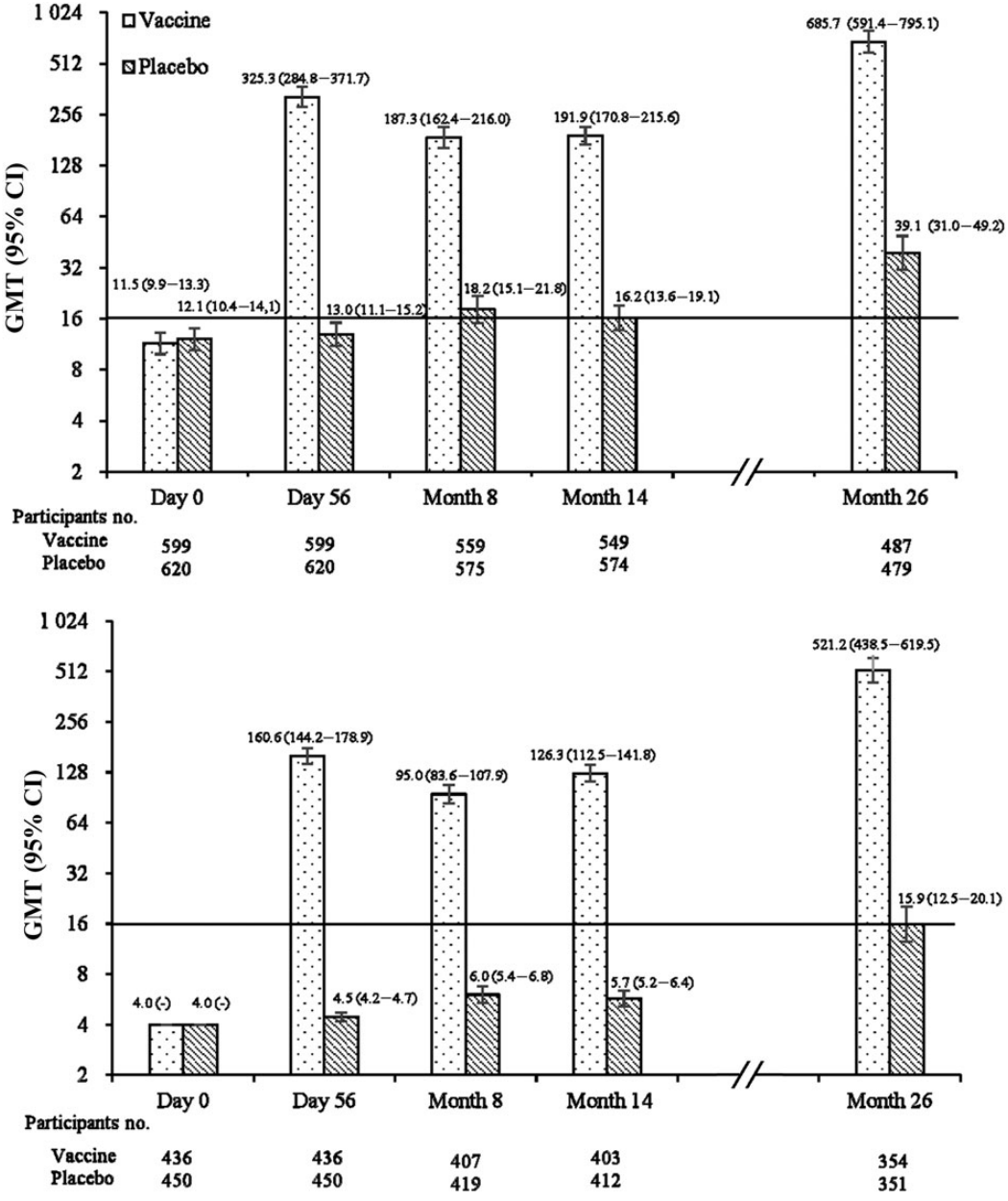

Figure 3. Geometric mean titer of enterovirus 71 (EV71) neutralizing antibodies at various time points after the first vaccination, from day 56 through month 26. Error bars and data in parentheses indicate 95% confidence intervals (CIs). The limit of detection was a titer of 1:8, and the seronegative samples were assigned values of 1:4 for the calculation of geometric mean titers (GMTs). *A*, Results for the per-protocol immunogenicity cohort. *B*, Results for the per-protocol immunogenicity cohort, in which all participants had a prevaccination titer of <1:8. Abbreviation: CI, confidence interval.

neutralizing antibody peaking at day 56. The EV71 neutralizing antibody in the vaccine group waned significantly from day 56 through month 8 and then remained stable for the next 6 months, up to month 14 [20]. We continued measuring the GMT of EV71 neutralizing antibody in participants during the extended second-year follow-up study, from months 15 to 26 (Figure 3). However, no sign of a decline in the EV71 neutralizing antibody was observed in the vaccine group during the second-year follow-up study; instead, GMTs of EV71 neutralizing antibody in vaccine recipients increased from 325.3 (95% CI, 284.8–371.7) at day 56 to 685.7 (95% CI, 591.4–795.1) at month 26. Similarly, the GMTs of EV71 neutralizing antibody in the placebo group also gradually increased during this period, from 13.0 (95% CI, 11.1–15.2) at day 56 to 39.1 (95% CI, 31.0–49.2) at month 26. However, the GMT of EV71 neutralizing antibody in the vaccine group (GMT, 685.7 [95% CI, 591.4–795.1]) was still significantly higher than that in the placebo group at the end of the follow-up period (ie, month 26; $P < .0001$). The proportions of participants in the vaccine group with an

Table 3. Neutralizing Antibody Titer Against Enterovirus 71 (EV71) in the Per-Protocol Immunogenicity Cohort (PPC) at the End of the Extended Follow-Up Period (Month 26)

Variable	Vaccine Group	Placebo Group	*P* Value
PPC participants			
Overall, no.	487	479	
Titer ≥ 1:8			<.0001
No.	472	229	
Percentage (95% CI)	96.92 (94.97–98.27)	47.81 (43.26–52.39)	
Titer ≥ 1:16			<.0001
No.	472	226	
Percentage (95% CI)	96.92 (94.97–98.27)	47.18 (42.64–51.76)	
Titer ≥ 1:32			<.0001
No.	470	225	
Percentage (95% CI)	96.51 (94.47–97.95)	46.97 (42.43–51.55)	
PPC participants with baseline titer <1:8			
Overall, no.	354	351	
Titer ≥ 1:8			<.0001
No.	341	104	
Percentage (95% CI)	96.33 (93.80–98.03)	29.63 (24.90–34.71)	
Titer ≥ 1:16			<.0001
No.	341	101	
Percentage (95% CI)	96.33 (93.80–98.03)	28.77 (24.09–33.82)	
Titer ≥ 1:32			<.0001
No.	339	100	
Percentage (95% CI)	95.76 (93.11–97.61)	28.49 (23.82–33.52)	

Abbreviation: CI, confidence interval.

EV71 neutralizing antibody GMT of ≥1:8, 1:16, or 1:32 were significantly higher than in the placebo group during the whole study period ($P < .0001$; Table 3).

A total of 333 of 1219 participants (27.32%) in the initial immunogenicity subset were seropositive for EV71 neutralizing antibody at enrollment, with a baseline neutralizing antibody titer of ≥1:8. Analysis of participants who were seronegative at baseline was also performed, and results revealed a dynamic trend in EV71 neutralizing antibody levels similar to that for the whole immunogenicity cohort.

Safety

During the 2-year follow-up period, 180 of 10 245 participants (1.76%) reported SAEs (78 of 5120 [1.52%] in the vaccine group and 102 of 5125 [1.99%] in the placebo group), with a similar incidence of occurrence between the groups ($P = .0834$; Supplementary Table 3), whereas in the second-year follow-up study, 40 of 10 245 (0.39%) reported SAEs (14 [0.27%] in the vaccine group and 26 [0.51%] in the placebo group; $P = .0801$; Supplementary Table 3). The most common SAEs during the 2-year follow-up period were infections and infestations, followed by gastrointestinal disorders, whereas the most common SAEs during the second-year follow-up period were infections and infestations, followed by injury. Six participants died in the second-year follow-up period (Supplementary Table 4), with 1 death in the vaccine group (due to unintentional injury) and 5 in the placebo group (2 due to unintentional injury, 1 due to diabetic ketoacidosis, 1 due to cerebroma, and 1 due to acute myeloid leukemia subtype M2). None of the SAEs were considered to be associated with vaccination.

DISCUSSION

The second-year extended follow-up study showed that the Vigoo EV71 vaccine provided sustained high protection against EV71-associated HFMD and persistent immunity in children aged 6–35 months for up to 2 years after the 2-dose vaccination. No safety concerns about the EV71 vaccine were identified during the 2-year follow-up study.

The capture of HFMD cases in this follow-up study was based on a previously established surveillance system in the initial study [20]. But unlike the initial study, in which we followed all potential cases with any clinical symptoms or illness and tested for pathogens to confirm EV71-associated disease, in this second-year follow-up study we conducted surveillance only for HFMD-like cases and obtained samples from affected individuals for pathogenic tests. Since HFMD is the most common and typical disease caused by EV71, with characteristic clinical symptoms for identification, capturing HFMD-like cases saved substantial time and effort in terms of sampling and laboratory analysis and was therefore a more efficient strategy for long-term surveillance [23]. Compared with the initial study, the sensitivity for HFMD surveillance in the second-year follow-up period may have declined because of possible underreporting of mild or

atypical cases of HFMD and of other non-HFMD diseases or symptoms caused by EV71. However, in the second-year follow-up period, we continued to use 2 consecutive by real-time PCR analyses and viral isolation of EV71 for laboratory case confirmation, to achieve a high specificity. Lachenbruch [24] reported that fluctuation in the specificity was more essential than fluctuation in the sensitivity for estimates of vaccine efficacy. Thus, the declining sensitivity is less likely to bias the EV71 vaccine efficacy significantly. In addition, owing to the protection of vaccine, EV71-associated HFMD in recipients of vaccine might be milder or more atypical than that in placebo recipients, which may have resulted in the underreporting of milder HFMD cases in the vaccine group, yielding a higher estimated efficacy in the second-year follow-up period.

Vaccine efficacy did not appear to be attenuated over time, and sustained vaccine efficacy observed during the entire 2-year follow-up period was associated with persistent immunity elicited by EV71 vaccine receipt. Previous studies with the EV71 vaccine (Vigoo and Sinovac) indicated that a titer of 1:16 might be correlated with the vaccine's protection against EV71-associated diseases [20, 25]. In our study, 96.33% participants in the vaccine group with a titer of $\geq$ 1:16 was observed at the end of the extended follow-up study, which was consistent with the high vaccine efficacy.

Although titers of EV71 vaccine–induced neutralizing antibodies decreased quickly during the first 6 months after peaking on day 56, a slight increase was observed during the subsequent 6 months. A more significant increase was observed during the second-year follow-up period, and the antibody titers reached a level even higher than that at day 56. At month 26, 53.75% and 24.84% of participants in the immunogenicity cohort who received EV71 vaccine and placebo, respectively, had a 4-fold increase in neutralizing antibodies levels as compared to those at month 15, which indicated that the increased antibody titers may have been associated with the prevalence of EV71 in the second-year follow-up period. The natural exposure to EV71 among the vaccine recipients might boost the specific neutralizing antibodies to levels even higher than those in the placebo recipients.

There were several limitations of this study. First, we only observed the vaccine efficacy against the subgenotype C4 strain of EV71 in this study, since the C4 type is the only predominant strain that has been found in the mainland of China [26]. However, because multiple EV71 subtypes have been reported to be circulating or cocirculating in some regions, such as Malaysia, the United States, Taiwan, and Japan [26], vaccine efficacy against EV71 subgenotypes (eg, A, B1-B5, C1-C5, D, E, and F) needs to be investigated in future studies. Second, the high protection afforded by the EV71 vaccine could reduce an individual's exposure to EV71 infection, which might delay the acquisition of natural immunity and shift the risk of EV71-associated disease to older individuals in the future. Third, we did not estimate the protection against EV71 subclinical infections, but they can contribute to the EV71 transmission as a source of infections [7]. Fourth, the sustained high efficacy against EV71-associated HFMD during the second-year follow-up period might be caused not only by vaccine-induced immunity, but also caused by boosting via natural exposure to EV71.

In conclusion, the Vigoo EV71 vaccine induced a sustained high level of antibodies and protection against EV71-associated HFMD for at least 2 years in healthy Chinese children and had a good safety profile. These findings might play an important role in its licensing, clinical application, and decisions about mass administration of EV71 vaccine for controlling EV71-associated HFMD epidemics in the future.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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All authors contributed to the implementation of the study, including substantial contributions to the trial design, data collection, study supervision, data interpretation, laboratory analyses, statistical analysis, manuscript drafting, or revision of the report. All authors had full access to all data. All authors reviewed and approved the final version of the report.

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Potential conflict of interest. L. X. and Z. Y. are employees of Beijing Vigoo Biological. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Ooi MH, Wong SC, Lewthwaite P, Cardosa MJ, Solomon T. Clinical features, diagnosis, and management of enterovirus 71. Lancet Neurol **2010**; 9:1097–105.
2. Reed Z, Cardosa MJ. Status of research and development of vaccines for enterovirus 71. Vaccine **2016**; 34:2967–70.
3. Schmidt NJ, Lennette EH, Ho HH. An apparently new enterovirus isolated from patients with disease of the central nervous system. J Infect Dis **1974**; 129:304–9.
4. Weng KF, Chen LL, Huang PN, Shih SR. Neural pathogenesis of enterovirus 71 infection. Microbes Infect **2010**; 12:505–10.
5. World Health Organization Western Pacific Region. A guide to clinical management and public health response for hand, foot and mouth disease (HFMD). Manila: World Health Organization Western Pacific Region. http://iris.wpro.who.int/bitstream/handle/10665.1/5521/9789290615255_eng.pdf. Accessed 29 August 2016.
6. Zhang Y, Zhu Z, Yang WZ, et al. An emerging recombinant human enterovirus 71 responsible for the 2008 outbreak of hand foot and mouth disease in Fuyang city of China. Virol J **2010**; 7:94.
7. Lee MS, Tseng FC, Wang JR, Chi CY, Chong P, Su IJ. Challenges to licensure of enterovirus 71 vaccines. PLoS Negl Trop Dis **2012**; 6:e1737.
8. Solomon T, Lewthwaite P, Perera D, Cardosa MJ, McMinn P, Ooi MH. Virology, epidemiology, pathogenesis, and control of enterovirus 71. Lancet Infect Dis **2010**; 10:778–90.
9. Yip CC, Lau SK, Woo PC, Yuen KY. Human enterovirus 71 epidemics: what's next? Emerg Health Threats J **2013**; 6:19780.

10. Wang Y, Feng ZJ, Yang Y, et al. Hand, foot, and mouth disease in China: patterns of spread and transmissibility. Epidemiology **2011**; 22:781–92.
11. Zhang J, Sun JL, Chang ZR, Zhang WD, Wang ZJ, Feng ZJ. Characterization of hand, foot, and mouth disease in China between 2008 and 2009. Biomed Environ Sci **2011**; 24:214–21.
12. Ooi EE, Phoon MC, Ishak B, Chan SH. Seroepidemiology of human enterovirus 71, Singapore. Emerg Infect Dis **2002**; 8:995–7.
13. Luo ST, Chiang PS, Chao AS, et al. Enterovirus 71 maternal antibodies in infants, Taiwan. Emerg Infect Dis **2009**; 15:581–4.
14. Lee TC, Guo HR, Su HJ, Yang YC, Chang HL, Chen KT. Diseases caused by enterovirus 71 infection. Pediatr Infect Dis J **2009**; 28:904–10.
15. Xu J, Qian Y, Wang SX, et al. EV71: an emerging infectious disease vaccine target in the Far East? Vaccine **2010**; 28:3516–21.
16. Qiu J. Enterovirus 71 infection: a new threat to global public health? Lancet Neurol **2008**; 7:868–9.
17. Zhu FC, Liang ZL, Li XL, et al. Immunogenicity and safety of an enterovirus 71 vaccine in healthy Chinese children and infants: a randomised, double-blind, placebo-controlled phase 2 clinical trial. Lancet **2013**; 381: 1037–45.
18. Meng FY, Li JX, Li XL, et al. Tolerability and immunogenicity of an inactivated enterovirus 71 vaccine in Chinese healthy adults and children: an open label, phase 1 clinical trial. Hum Vaccin Immunother **2012**; 8:668–74.
19. Zhu FC, Wang JZ, Li XL, et al. Reactogenicity and immunogenicity of an enterovirus 71 vaccine in Chinese healthy children and infants. Pediatr Infect Dis J **2012**; 31:1158–65.
20. Zhu FC, Meng FY, Li JX, et al. 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. Lancet **2013**; 381:2024–32.
21. Zhang Y, Tan XJ, Wang HY, et al. An outbreak of hand, foot, and mouth disease associated with subgenotype C4 of human enterovirus 71 in Shandong, China. J Clin Virol **2009**; 44:262–7.
22. Liang ZL, Mao QY, Gao Q, et al. Establishing China's national standards of antigen content and neutralizing antibody responses for evaluation of enterovirus 71 (EV71) vaccines. Vaccine **2011**; 29:9668–74.
23. Li JX, Song YF, Wang L, et al. Two-year efficacy and immunogenicity of Sinovac Enterovirus 71 vaccine against hand, foot and mouth disease in children. Expert Rev Vaccines **2016**; 15:129–37.
24. Lachenbruch PA. Sensitivity, specificity, and vaccine efficacy. Control Clin Trials **1998**; 19:569–74.
25. Zhu FC, Xu WB, Xia JL, et al. Efficacy, safety, and immunogenicity of an enterovirus 71 vaccine in China. N Engl J Med **2014**; 370:818–28.
26. Tee KK, Lam TT, Chan YF, et al. Evolutionary genetics of human enterovirus 71: origin, population dynamics, natural selection, and seasonal periodicity of the VP1 gene. J Virol **2010**; 84:3339–50.