npj | vaccines

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Published in partnership with the Sealy Institute for Vaccine Sciences

https://doi.org/10.1038/s41541-026-01396-x

A randomised trial of a bioreactor-produced EV-A71 vaccine for endemic control in children

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Enterovirus (EV) A71 causes endemic outbreaks of hand-foot-mouth disease (HFMD) and herpangina in young children, occasionally leading to severe complications and fatal outcomes. We evaluated EnVAX-A71, a bioreactor-produced, aluminum-adjuvanted inactivated EV-A71 (B4/E59) vaccine, in a randomized, double-blind, placebo-controlled phase 3 trial in Taiwan and Vietnam. A total of 4011 children aged 2–71 months were randomized 3:2 to vaccine or placebo; the primary endpoint was laboratory-confirmed EV-A71-associated HFMD/herpangina, evaluated from 28 days after dose two over a follow-up of 1 to 2 years (median, 490 days). In the primary analysis ($n = 3733$), one case occurred in vaccine recipients versus 70 in placebo (0.3 vs 39.2 per 1000 person-years), yielding 99.2% vaccine efficacy (95% CI: 94.3–99.9, $p < 0.001$). Seroprotection (neutralizing antibody titer ≥1:32) reached 98.7% at day 56 and remained ≥98% through one year. No vaccine-group hospitalizations occurred versus 19 in placebo (100% efficacy, 95% CI: 90.6–100.0). Safety profiles were comparable between groups. These findings support EnVAX-A71 for endemic EV-A71 control (ClinicalTrials.gov NCT05099029).

Enterovirus A71 (EV-A71) is a well-recognized etiological agent of hand, foot, and mouth disease (HFMD) and herpangina, with the potential to cause severe cardiopulmonary or neurological complications and fatality, particularly in young children[1–3]. Since its first isolation in the United States in 1969, EV-A71 has emerged as a major public health challenge across the Asia-Pacific region, including Malaysia, Taiwan, China, Singapore, and Vietnam, resulting in substantial disease burden and socioeconomic impact[4–6]. The virus has become endemic in these regions, with cyclical large-scale outbreaks occurring every two to three years, involving multiple circulating subgenogroups—B4 and B5 predominantly in Malaysia, Vietnam, Taiwan, and Singapore; C4 in China and Korea; and C1 and C2 occasionally identified in Europe[4,7].

The severity of EV-A71-associated disease disproportionately affects young children, with the most vulnerable population being infants and children under six years of age. While most infections remain mild or asymptomatic, EV-A71 can lead to severe cardiopulmonary and neurological complications including aseptic meningitis, pulmonary edema, brainstem encephalitis, acute flaccid paralysis, and potentially fatal outcomes[1,3,8]. The 1998 Taiwan outbreak exemplifies EV-A71's virulence, with 78 fatalities among 405 severe cases from a total of 129,106 HFMD or herpangina infections, with children under five years accounting for 91% of deaths[9]. These epidemiological patterns underscore the critical need for effective vaccination strategies targeting the most at-risk populations[10].

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Despite recent advances in EV-A71 vaccine development, significant gaps remain in global access, regional manufacturing capacity, and cross-genogroup protection. Several inactivated EV-A71 vaccines have been licensed recently—based on the C4 subgenogroup developed in China, and the B4 subgenogroup in Taiwan—yet accessibility remains limited to specific regions[11]. Most licensed vaccines rely on conventional roller bottle systems with inherent limitations in scalability and manufacturing consistency, which can hinder rapid deployment during endemic outbreaks[12]. An innovated bioreactor-based manufacturing platform developed for EV-A71 enables consistent large-scale production with enhanced process control, can effectively address capacity constraints during severe outbreaks.

The candidate vaccine EnVAX-A71 was developed as formalin-inactivated, aluminum-adjuvanted, purified whole viral particle vaccine. It represents the first bioreactor-produced, adjuvanted, inactivated EV-A71 vaccine developed in Taiwan using the B4(E59) strain. Comprehensive preclinical studies and early-phase clinical trials have demonstrated the immunogenicity and safety profile of EnVAX-A71[13,14]. Notably, the E59 strain exhibits broad cross-neutralization against B4, B5, and C4 subgenogroups, which are responsible for the majority of EV-A71 outbreaks in the Asia-Pacific region[15]. Phase II data supported the selection of a 1 μg dose adjuvanted with aluminum hydroxide as the optimal formulation for pediatric immunization[14].

To clinically evaluate the efficacy, immunogenicity, and safety of EnVAX-A71, we conducted a large, multinational, randomized, double-blind, placebo-controlled Phase III trial in Taiwan and Vietnam to evaluate the efficacy, immunogenicity, and safety of EnVAX-A71. The study enrolled 4011 healthy infants and young children aged 2–71 months. Based on immunogenicity and safety data obtained from Taiwan during the ongoing Phase III trial, EnVAX-A71 became the first vaccine to receive conditional regulatory approval using an immune surrogate endpoint for emergent endemic control. We present the complete results of this Phase III pivotal trial, which confirmed the clinical efficacy, safety, and immunogenicity of EnVAX-A71, and demonstrated manufacturing consistency. This study marks the first evaluation of a novel bioreactor-produced EV-A71 vaccine in a pediatric population across two endemic countries, providing critical evidence for vaccine performance in endemic settings.

Results

Study population

From June 2018 to July 2024, a total of 4832 subjects were screened across nine clinical centers in Taiwan and Vietnam, of whom 4011 were deemed eligible and subsequently randomized in a 3:2 ratio to receive EnVAX-A71 vaccine ($n = 2387$) or placebo ($n = 1624$) (Fig. 1). Among those randomized, 18 participants withdrew consent before receiving any vaccination; the remaining 3993 participants (99.6%) received at least one dose of the assigned treatment and constituted the Total Vaccinated Cohort (TVC) for safety analyses. Approximately 5% of participants in the vaccine group and 8% in the placebo group did not receive the second dose. In the vaccine group, three participants were ineligible for the second dose due to severe or serious adverse events within seven days after the first dose (predominantly respiratory or gastrointestinal illnesses), and three due to febrile episodes not resolving within the permitted dosing window. Therefore, they were excluded from receiving the second dose but were retained in safety monitoring. As a result, a total of 3735 (93.1%) completed the two-dose vaccination schedule, and 3874 (96.7%) completed the study follow-up, with similar completion rates between the EnVAX-A71 group (97.0%) and the placebo group (95.5%). Among those receiving both doses, two participants were excluded from the efficacy analyses: one withdrew before reaching

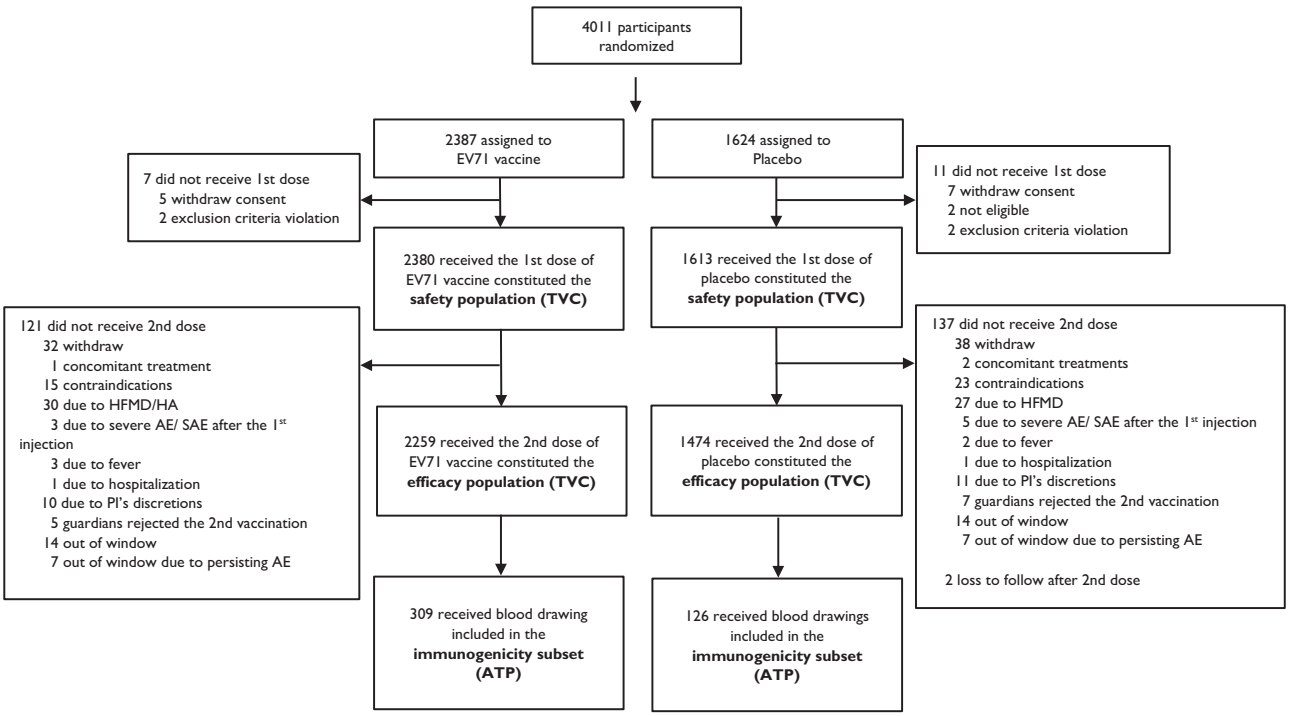

Fig. 1 | Trial profile and participant flow. CONSORT flow diagram showing participant enrollment, randomization, and follow-up through the EnVAX-A71 Phase 3 trial. Of 4832 subjects screened across nine centers in Taiwan and Vietnam (June 2018 to July 2024), 4011 were randomized in a 3:2 ratio to receive EnVAX-A71 vaccine ($n = 2387$) or placebo ($n = 1624$). The Total Vaccinated Cohort (TVC) comprised 3993 participants (99.6%) who received at least one dose and were included in safety analyses. The TVC for efficacy included 3733 participants (93.1%) who completed the two-dose vaccination schedule and were followed from 28 days after the second dose. The According-To-Protocol (ATP) immunogenicity cohort included 435 participants (309 vaccine, 126 placebo) with valid immunogenicity data and no major protocol deviations. Primary reasons for non-completion of the second dose included withdrawal of consent, protocol violations, HFMD/herpangina-related hospitalization or severe adverse events occurring between doses, and loss to follow-up. Numbers in boxes represent participant counts at each stage of the trial. TVC total vaccinated cohort, ATP according-to-protocol, HFMD hand-foot-and-mouth disease, HA herpangina, AE adverse event, SAE serious adverse event, PI principal investigator.

Table 1 | Demographic and baseline characteristics of participants by analysis population

Population	Efficacy		Safety		Immunogenicity	
	EnVAX-A71	Placebo	EnVAX-A71	Placebo	EnVAX-A71	Placebo
N	2259	1474	2380	1613	309	126
Mean age (months) (SD)	29.5 (28.73, 30.30)	29.3 (28.35, 30.26)	29.6 (28.87, 30.39)	29.6 (28.66, 30.50)	29.6 (27.50, 31.78)	28.7 (25.45, 31.90)
Age group, *n* (%)						
2–11 months	511 (22.62)	331 (22.46)	529 (22.23)	357 (22.13)	71 (22.98)	30 (23.81)
12–23 months	497 (22.00)	328 (22.25)	525 (22.06)	359 (22.26)	70 (22.65)	27 (21.43)
24–35 months	504 (22.31)	333 (22.59)	531 (22.31)	360 (22.32)	67 (21.68)	30 (23.81)
36–71 months	747 (33.07)	482 (32.70)	795 (33.40)	537 (33.29)	101 (32.69)	39 (30.95)
Gender, *n* (%)						
Male	1129 (49.98)	732 (49.66)	1193 (50.13)	810 (50.22)	162 (52.43)	66 (52.38)
Female	1130 (50.20)	742 (50.34)	1187 (49.87)	803 (49.78)	147 (47.57)	60 (47.62)
Body Weight mean (SD)	12.9 (4.8)	12.9 (5.1)	12.9 (4.8)	13.0 (5.2)	12.8 (4.7)	12.6 (4.4)
Body Height mean (SD)	88.3 (15.7)	88.6 (16.2)	88.4 (15.7)	89.0 (16.3)	88.3 (15.5)	88.1 (15.5)
BMI	16.1 (2.1)	16.0 (2.2)	16.1 (2.1)	16.0 (2.3)	16.0 (1.7)	15.9 (1.9)
Nationality, *n* (%)						
Taiwanese	992 (43.91)	248 (16.82)	1014 (42.61)	252 (15.62)	237 (76.70)	59 (46.83)
Vietnamese	1267 (56.09)	1226 (83.18)	1366 (57.39)	1361 (84.38)	72 (23.30)	67 (53.17)

Baseline demographic and clinical characteristics of participants in the EnVAX-A71 Phase 3 trial, presented for three analysis populations: Efficacy population (participants who received two doses and were followed ≥28 days post-dose 2), Safety population (all participants who received ≥1 dose), and Immunogenicity population (subset with valid antibody data and no major protocol deviations). Data are presented as *n* (%) for categorical variables or mean (95% CI) for continuous variables. Age stratification followed the protocol-specified 2:2:2:3 ratio across four age groups (2–11, 12–23, 24–35, and 36–71 months). The nationality distribution shows higher proportion of Vietnamese participants, particularly pronounced in the placebo group (83.18% Vietnamese vs. 56.09% in vaccine group), resulting from differential country-specific randomization ratios (Taiwan 4:1, Vietnam 1:1) combined with larger enrollment in Vietnam (68% of total participants, $n = 2727$). Sensitivity analyses adjusting for this nationality imbalance confirmed it did not materially affect efficacy estimates (adjusted vaccine efficacy: 98.76%, 95% CI: 91.07–99.83). All demographic characteristics were well-balanced between treatment groups within each analysis population, with no statistically significant differences in age, gender, or body measurements.
CI confidence interval, *BMI* body mass index, *TVC* total vaccinated cohort, *ATP* according-to-protocol.

28 days after the second dose, and another developed EV-A71 infection before Day 28. Recruitment and follow-up were completed as planned, and the trial was not terminated early.

Demographic and baseline characteristics were well-balanced between treatment groups across all analysis populations (Table 1). In the TVC for efficacy population ($n = 3733$), the mean age was 29.5 months (95% CI: 28.7–30.3) in the EnVAX-A71 group and 29.3 months (95% CI: 28.4–30.3) in the placebo group, with nearly equal gender distribution (~50% male in each group). Age stratification was preserved as per the study design, with roughly 22% of participants in each of the three younger age strata (2–11, 12–23, and 24–35 months) and 33% in the 36–71 months group. For the According-To-Protocol (ATP) immunogenicity population ($n = 435$), baseline demographic characteristics remained well balanced, with similar age and gender distributions between groups, and no major protocol deviations. Due to country-specific randomization ratios (4:1 in Taiwan and 1:1 in Vietnam) and the predominance of enrollment in Vietnam (~68% of participants), an imbalance in nationality distribution was observed between treatment groups. In the EnVAX-A71 group, Vietnamese participants slightly outnumbered Taiwanese participants (56.1% vs. 43.9%), whereas the difference was more pronounced in the placebo group (83.2% vs. 16.8%).

Efficacy outcomes

EnVAX-A71 vaccine demonstrated robust protection against EV71-associated HFMD/herpangina diseases in the TVC for efficacy population ($n = 3733$) (Table 2, Fig. 2). During the surveillance period, 1074 suspected HFMD/herpangina disease cases were reported and tested for EV71 (Supplementary Table S1). Of these, 71 (6.6%) were laboratory-confirmed as EV71-positive. Seventy confirmed cases occurred in the placebo group (68 from Vietnam and 2 from Taiwan), while a single case occurred in the vaccine group, in a 3.5-month-old infant from Vietnam who meeting the primary efficacy endpoint criteria.

The single breakthrough case in the vaccine group was identified in a 3.5-month-old Vietnamese infant about 3 months after completing the two-dose vaccination series. The case was confirmed as EV-A71 B5 subgenogroup infection and presented clinically as mild acute hemorrhagic conjunctivitis, which is not typical of HFMD or herpangina. The infant recovered without complications and did not require hospitalization.

Over the efficacy surveillance period, 3231.2 person-years at risk were accrued in the EnVAX-A71 group and 1786.9 person-years in the placebo group. The corresponding incidence densities of EV-A71-associated HFMD/herpangina were markedly lower in vaccine recipients (0.3 per 1000 person-years) than in placebo recipients (39.2 per 1000 person-years), yielding a vaccine efficacy of 99.2% (95% CI: 94.3–99.9; $p < 0.001$) (Table 2). Kaplan–Meier curves illustrated the cumulative incidence of EV71-associated HFMD/herpangina diseases over time with a significant separation between the EnVAX-A71 and placebo curves, indicating durable protection throughout follow-up (Fig. 2).

Protection was consistently high across all prespecified age strata (Table 2). In the youngest cohort (2–11 months), vaccine efficacy remained high at 95.7% (95% CI: 67.2–99. 5; $p = 0.002$) despite a single breakthrough case identified. Whereas, in the older age groups vaccine efficacy estimates approached or reached 100%, with no confirmed EV-A71-associated HFMD/herpangina cases observed among vaccinated children.

EnVAX-A71 also provided complete protection against EV71-associated hospitalizations. No hospitalizations were recorded in the vaccine group, whereas 19 cases occurred in the placebo group resulting a vaccine efficacy estimate of 100% (95% CI: 90.6–100.0; $p < 0.001$) for this endpoint (Table 2). All 71 EV71-confirmed cases were sequenced and determined to be B5 subgenogroup.

Additional statistical analysis was conducted to assess the impact of the nationality imbalance between the vaccine and placebo groups on the efficacy results. We conducted a sensitivity analysis using Poisson regression with covariate adjustment, and the adjusted vaccine efficacy was 98.76%

Table 2 | Vaccine efficacy against EV-A71-associated HFMD/herpangina and hospitalization by age group

Age group	EnVAX-A71			Placebo			Vaccine efficacy	
	Person-years at risk	Cases (*n*)	Incidence density rate	Person-years at risk	Cases (*n*)	Incidence density rate	% (95% CI)	*p* value
EV71-associated HFMD/HA diseases	**3231.2**	**1**	**0.31**	**1786.9**	**70**	**39.17**	**99.21 (94.31, 99.89)**	**<0.001**
2–11 months	653.81	1	1.53	334.32	12	35.89	95.74 (67.23, 99.45)	0.002
12–23 months	695.11	0	0.00	376.43	16	42.50	100.00 (88.95, 100.00)	<0.001
24–35 months	734.44	0	0.00	409.27	29	70.86	100.00 (94.03, 100.00)	<0.001
36–71 months	1147.8	0	0.00	666.92	13	19.49	100.00 (84.95, 100.00)	<0.001
EV71-associated hospitalization	**3231.2**	**0**	**0.00**	**1786.9**	**19**	**10.63**	**100.00 (90.57, 100.00)**	**<0.001**

EV-A71 Enterovirus A71, *HFMD* hand-foot-and-mouth disease, *CI* confidence interval, *M* months.
Protective efficacy of EnVAX-A71 against laboratory-confirmed EV-A71-associated hand-foot-and-mouth disease (HFMD) or herpangina from Day 28 after the second vaccination through end of follow-up in the Total Vaccinated Cohort for efficacy ($n = 3733$). Incidence density rates are expressed as cases per 1000 person-years at risk. Vaccine efficacy calculated as (1 − incidence rate ratio) × 100%. The single case in the vaccine group occurred in a 3.5-month-old Vietnamese infant who had completed vaccination and presented with mild acute hemorrhagic conjunctivitis (not typical HFMD/herpangina), recovering without hospitalization. Overall vaccine efficacy was 99.21% (95% CI: 94.31–99.89, $p < 0.001$ by exact Poisson regression). Age-stratified analyses demonstrated high efficacy across all age groups, ranging from 95.74% (95% CI: 67.23–99.45) in the 2–11 months group to 100% (95% CI: 84.95–100.00) in the 36–71 months group. The wider confidence interval in the youngest age group (67.23–99.45) reflects the smaller absolute number of cases observed (1 vaccine vs. 12 placebo) despite high point estimate of protection. Complete protection against EV-A71-associated hospitalization was achieved (0 vs. 19 cases), with 100% vaccine efficacy (95% CI: 90.57–100.00, $p < 0.001$). Person-years at risk calculated from Day 28 post-dose 2 to either event occurrence, study withdrawal, or study completion. All 71 confirmed cases were EV-A71 B5 subgenogroup by sequencing.

(95% CI: 91.1–99.8). Various statistical methods consistently confirmed the high protective efficacy of EnVAX-A71, with point estimates ranging from 98.8% to 99.4%, and the lower bounds of all 95% confidence intervals exceeded 90%.

Safety outcomes

Safety analyses were conducted on the TVC population ($n = 3993$), which comprised all participants who received at least one dose. The incidence of solicited local adverse events following any vaccination was comparable between the EnVAX-A71 group (29.9%) and the placebo group (29.5%; $p = 0.776$) (Table 3). The most frequently reported local reactions were pain (EnVAX-A71: 23.8%; placebo: 24.4%) and tenderness (EnVAX-A71: 14.8%; placebo: 13.5%). The majority of local reactions were mild (Grade 1) in intensity, with severe (Grade 3) reactions occurring in ≤0.3% of subjects in both groups.

For solicited systemic adverse events, the overall incidence was slightly lower in the EnVAX-A71 group (29.1%) compared to the placebo group (32.0%; $p = 0.051$) (Table 3). Fever was the most common systemic event, reported in 16.3% of EnVAX-A71 recipients and 20.2% of placebo recipients, followed by fatigue (EnVAX-A71: 10.3%; placebo: 10.2%) and appetite loss (EnVAX-A71: 10.3%; placebo: 9.5%). As with local reactions, most systemic events were mild to moderate in severity, with Grade 3 reactions occurring in ≤2.6% of subjects. The overall incidence and severity of local and systemic solicited adverse events were reduced significantly from the first to the second dose (Fig. 3, Supplementary Table S3).

Regarding unsolicited adverse events, 62.3% of subjects in the EnVAX-A71 group reported at least one event, compared to 66.7% in the placebo group (Supplementary Table S4). The most frequent unsolicited adverse events included upper respiratory tract infection (EnVAX-A71: 16.5%; placebo: 18.5%) and respiratory tract infection (EnVAX-A71: 8.3%; placebo: 11.7%) (Supplementary Table S4). The majority of unsolicited adverse events were mild to moderate in intensity.

Serious adverse events (SAEs) occurred less frequently in the EnVAX-A71 group (13.3%) than in the placebo group (17.9%; $p < 0.05$) (Supplementary Table S6). The most common SAEs were pneumonia (EnVAX-A71: 3.6%; placebo: 3.8%), viral infection (EnVAX-A71: 1.4%; placebo: 2.5%), and hand-foot-and-mouth disease (EnVAX-A71: 1.0%; placebo: 3.1%). The vast majority of SAEs (>98%) were assessed as not related or unlikely related to the study vaccine in both groups. Treatment-related SAEs were rare, occurring in <0.2% of participants in both groups, with gastrointestinal disorder being the most frequent. Notably, HFMD cases were reported more frequently in the placebo group than in the EnVAX-A71 group, with incidence rates of 4.3% vs 2.0% for unsolicited AEs, and 3.1% vs 1.0% for SAEs (Supplementary Tables S4, S6), consistent with the protective efficacy observed against confirmed EV-A71 infections.

One death occurred during the study in the EnVAX-A71 group, which was due to multiple injuries following a traffic accident and was considered unrelated to the study vaccine by the investigator.

Overall, the safety profile of EnVAX-A71 was favorable, with reactogenicity comparable to placebo and a lower incidence of unsolicited adverse events and SAEs.

Immunogenicity outcomes

Immunogenicity was evaluated in the ATP population ($n = 435$). At baseline, the seroprotection rates (SPR) were comparable between groups, with 7.8% (95% CI: 5.0–11.3) in the EnVAX-A71 group and 7.9% (95% CI: 3.9–14.1) in the placebo group (Table 4). Following vaccination, the SPR increased dramatically in the EnVAX-A71 group to 98.7% (95% CI: 96.7–99.7) by Day 56, and remained high at 98.7% (95% CI: 96.6, 99.6) on Day 196 and 100% (95% CI: 98.8–100.0) on Day 392. In contrast, the placebo group showed only modest increases in SPR over time, reaching 15.5% (95% CI: 9.6–23.1) by Day 392. As a result, EnVAX-A71 became the first EV-A71 vaccine to receive the conditional regulatory approval using an immune surrogate endpoint of SPR exceeding 90% on Day 56, supported by immunogenicity data from Taiwan (Supplementary Table S7).

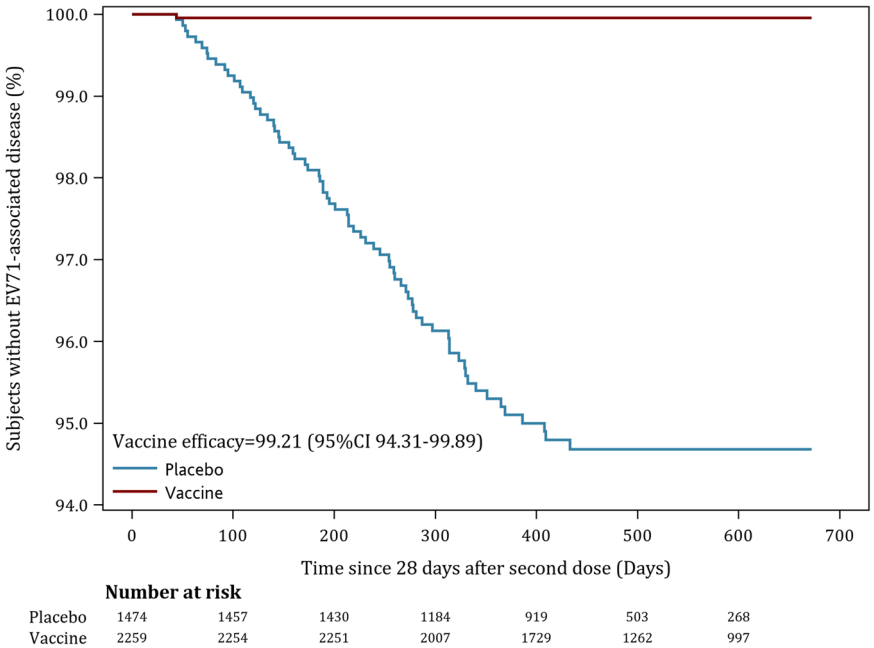

Fig. 2 | Kaplan–Meier analysis of cumulative incidence of EV-A71-associated HFMD/herpangina by treatment group. Kaplan–Meier curves showing the cumulative incidence of laboratory-confirmed EV-A71-associated hand-foot-and-mouth disease (HFMD) or herpangina from Day 28 after the second vaccination through the end of follow-up (up to 700 days) in the TVC for efficacy population ($n = 3733$). The red line represents the EnVAX-A71 vaccine group ($n = 2259$); the blue line represents the placebo group ($n = 1474$). One confirmed case occurred in the vaccine group (a 3.5-month-old infant with mild acute hemorrhagic conjunctivitis) compared to 70 cases in the placebo group, yielding a vaccine efficacy of 99.2% (95% CI: 94.3–99.9; $p < 0.001$ by log-rank test). The table below the graph shows the number of participants remaining at risk at selected time points. The sharp separation of curves beginning early in the follow-up period demonstrates consistent protective efficacy throughout the surveillance period, which coincided with substantial endemic circulation of EV-A71 B5 subgenogroup in Vietnam. EV-A71 Enterovirus A71, HFMD hand-foot-and-mouth disease, CI confidence interval.

The seroconversion rate (SCR) in the EnVAX-A71 group was 95.5% (95% CI: 92.5, 97.5) at Day 56, and remained high at 94.0% (95% CI: 90.7, 96.4) on Day 196 and 94.7% (95% CI: 91.5, 96.9) on Day 392. Conversely, the placebo group demonstrated minimal seroconversion, with SCR values of 4.9%, 5.0%, and 7.3% at the respective timepoints.

Geometric mean titers (GMT) of neutralizing antibodies in the EnVAX-A71 group increased substantially from a baseline of 6.0 (95% CI: 5.2, 7.0) to a peak titer of 1078.0 (95% CI: 950.0, 1223.2) at Day 56 after receiving two doses, representing a geometric mean titer ratio (GMTR) of 174.7 (95% CI: 153.7, 198.6). The GMT subsequently declined to 437.3 (95% CI: 383.4, 498.7) by Day 196 (GMTR: 72.1; 95% CI: 63.0, 82.5) before rising to 680.0 (95% CI: 592.2, 780.9) by Day 392 (GMTR: 112.2; 95% CI: 97.2, 129.6). In contrast, the placebo group maintained low GMT values throughout the monitoring period, ranging from 6.0 to 8.2.

Age-stratified immunogenicity analyses (Fig. 4, Supplementary Table S8) demonstrated robust immune responses across all four age groups receiving the EnVAX-A71 vaccine. At Day 56, GMT peak titers ranged from 1035.2 to 1093.7 across the four age groups, with the highest Day 56 GMT observed in the 12–23 months subgroup (1093.7; 95% CI: 875.4, 1366.3). While GMT titers declined by Day 196 in all age groups, they remained substantially above baseline. By Day 392, titers had increased again in most age groups, with the 2–11 months subgroup showing a particularly robust response (1414.6; 95% CI: 1103.2, 1813.8).

Broad cross-neutralization against B4, B5, C4, C5, and C2 strains was demonstrated using a subset of serum samples from the immunogenicity cohorts in Taiwan and Vietnam (Supplementary Table S11). Lot-to-lot consistency analysis (Supplementary Table S12) was evaluated using Day 56 GMT values from participants in Taiwan who received one of three individual EnVAX-A71 vaccine lots. The pair-wise GMT ratios across the three vaccine lots were compared, and the respective 95% confidence intervals fell within the pre-specified equivalence margins of 0.5–2.0, confirming the consistency in manufacturing.

Discussion

In this multinational, multicentred, randomized, placebo-controlled Phase III trial, EnVAX-A71 met all protocol-defined endpoint for efficacy, safety, and immunogenicity in children aged 2–71 months. Two doses of EnVAX-A71 conferred high protection against EV-A71-associated HFMD/herpangina, with vaccine efficacy exceeding 99%, and achieved 100% protection against EV-A71-associated hospitalization. These estimates are at least comparable to, and in some instances slightly higher than, those reported for currently authorized EV-A71 vaccines, which have demonstrated efficacy between about 90% and 97% in similar settings, and therefore extend the evidence base supporting EV-A71 vaccination as a key public health intervention in endemic regions. Protection was consistently high across all age strata, with robust point estimates even in the young infants under one year old, who are at the greatest risk of severe neurological and cardiopulmonary complications.

In this study, we identified a single EV-A71-confirmed case in a 3.5-month-old infant from the vaccine cohort. The symptom of the breakthrough case was manifested as mild acute hemorrhagic conjunctivitis, rather than a typical EV-A71-mediated HFMD or other related illness, and the infant recovered without complications or hospitalization. Given the limited sample size in participants aged 2–5 months (Supplementary Table S9), the vaccine efficacy for this age group may not be conclusively established. Nevertheless, immunogenicity data provided more robust supports, demonstrating that neutralizing antibody responses in both the 2–5 months and 6–11 months subgroups were comparable to those in older children. At Day 56, GMT values were 1049.7 ($n = 23$, 95% CI: 794.6–1386.7) and 1058.9 ($n = 48$, 95% CI: 815.4–1375.3), respectively (Supplementary Table S10). These immunogenicity findings indicate that the vaccine is highly immunogenic in even very young infants and could generate sustainable immunity for up to one year, suggesting that a two-dose schedule starting from 2 months of age may be a viable approach for disease protection. However, given the challenges of conducting adequately powered efficacy studies in this age group—particularly due to the relatively low incidence of EV-A71 infection—the most pragmatic and scientifically sound strategy is to evaluate the effectiveness of the EnVAX-A71 vaccine through post-marketing surveillance in real-world settings.

Another notable finding from our immunogenicity data was the decline in neutralizing antibody titers six months post the second dose (Day 196), followed by a rise at one year (Day 392) in the vaccine group. This pattern was consistent with the immunogenicity profiles observed from our Phase II study[14]. The antibody titer increase at Day 392 could be a possible natural boosting effect due to asymptomatic exposure to EV-A71. This antibody titer rise was particularly pronounced in younger age groups (Supplementary Table S8). This pattern may be linked to the elevated levels of naïve B cells characteristic of early immune development in infants[16,17],

Table 3 | Incidence and severity of solicited local and systemic adverse events within 7 days of any vaccination

Any vaccination	EV71 vaccine (*N* = 2380) *n* (%)	Placebo (*N* = 1613) *n* (%)
Any solicited local adverse event	**703 (29.9%)**	**464 (29.5%)**
Grade 1	612 (26.1%)	408 (25.9%)
Grade 2	84 (3.6%)	52 (3.3%)
Grade 3	7 (0.3%)	4 (0.3%)
Pain	**559 (23.8%)**	**384 (24.4%)**
Grade 1	497 (21.2%)	344 (21.9%)
Grade 2	59 (2.5%)	38 (2.4%)
Grade 3	3 (0.1%)	2 (0.1%)
Tenderness	**347 (14.8%)**	**213 (13.5%)**
Grade 1	317 (13.5%)	197 (12.5%)
Grade 2	27 (1.1%)	13 (0.8%)
Grade 3	3 (0.1%)	3 (0.2%)
Redness	**236 (10.1%)**	**140 (8.9%)**
Grade 1	227 (9.7%)	134 (8.5%)
Grade 2	7 (0.3%)	6 (0.4%)
Grade 3	2 (0.1%)	0 (0.0%)
Swelling	**131 (5.6%)**	**64 (4.1%)**
Grade 1	120 (9.7%)	61 (3.9%)
Grade 2	10 (0.4%)	3 (0.2)%
Grade 3	1 (0.0%)	0 (0.0%)
Ecchymosis	**73 (3.1%)**	**42 (2.7%)**
Grade 1	70 (3.0%)	41 (2.6%)
Grade 2	2 (0.1%)	1 (0.1%)
Grade 3	1 (0.0%)	0 (0.0%)
Any solicited systemic adverse event	**682 (29.1%)**	**503 (32.0%)**
Grade 1	403 (17.2%)	285 (18.1%)
Grade 2	217 (9.2%)	181 (11.5%)
Grade 3	62 (2.6%)	37 (2.4%)
Fever	**383 (16.3%)**	**318 (20.2%)**
Grade 1	165 (7.0%)	143 (9.1%)
Grade 2	163 (6.9%)	141 (9.0%)
Grade 3	55 (2.3%)	34 (2.2%)
Nausea/vomiting	**170 (7.2%)**	**135 (8.6%)**
Grade 1	137 (5.8%)	104 (6.6%)
Grade 2	32 (1.4%)	30 (1.9%)
Grade 3	1 (0.0%)	1 (0.1%)
Diarrhea	**160 (6.8%)**	**128 (8.1%)**
Grade 1	127 (5.4%)	103 (6.5%)
Grade 2	27 (1.1%)	21 (1.3%)
Grade 3	6 (0.3%)	4 (0.3%)
Appetite loss	**241 (10.3%)**	**149 (9.5%)**
Grade 1	192 (8.2%)	110 (7.0%)
Grade 2	46 (2.0%)	37 (2.4%)
Grade 3	3 (0.1%)	2 (0.1%)
Fatigue	**242 (10.3%)**	**161 (10.2%)**
Grade 1	177 (7.5%)	112 (7.1%)
Grade 2	60 (2.6%)	48 (3.1%)
Grade 3	5 (0.20%)	1 (0.1%)

Solicited adverse events reported within 7 days following either first or second vaccination in the Total Vaccinated Cohort safety population (EnVAX-A71: $n = 2380$; placebo: $n = 1613$). Data are *n* (%) of participants experiencing each event at any severity grade or by specific grade. Severity grading: Grade 1 (mild) = symptoms present but not interfering with normal daily activity; Grade 2 (moderate) = symptoms interfering with normal daily activity but not preventing it; Grade 3 (severe) = symptoms preventing normal daily activity. Local adverse events assessed at injection site included pain, tenderness, redness, swelling, and ecchymosis. Systemic adverse events included fever (≥38 °C axillary or oral temperature), nausea/vomiting, diarrhea, appetite loss, and fatigue. The overall incidence of solicited local adverse events was comparable between groups (29.94% vs. 29.50%; $p = 0.776$ by Fisher's exact test). Solicited systemic adverse events were numerically lower in the vaccine group compared to placebo (29.05% vs. 31.98%) but did not reach statistical significance ($p = 0.051$). Among individual symptoms, only swelling (5.6% vs. 4.1%; $p = 0.036$) and fever (16.3% vs. 20.2%; $p = 0.002$) showed statistically significant differences, both occurring less frequently in the vaccine group. The majority of all adverse events were mild (Grade 1), with severe (Grade 3) reactions occurring in ≤2.6% of participants for any symptom. Reactogenicity decreased significantly from first to second dose in both groups (see Supplementary Table S3). *Grade* 1 mild, *Grade* 2, moderate, *Grade* 3 severe.

which can mount to a stronger immune response than in older children. As a result, re-exposure to antigen in endemic settings may lead to a rise in neutralizing antibody titers by Day 392, reflecting the responsiveness of the infant immune system. The underlying mechanism remains to be elucidated through comprehensive analyses of B cells and T cells following vaccination in the pediatric populations. Our findings, consistent with those from other EV-A71 vaccines[18,19], indicated that the specific neutralizing antibody titers declined approximately six months after complete vaccination, but subsequently either increased or maintained without falling to unsustainable levels in the context of natural infection under endemic environments. Whether a booster dose is required beyond this period, particularly for infants vaccinated at 2–5 months of age, remains an important question that will need to be addressed through longer-term follow-up and real-world data.

The safety profile of EnVAX-A71 was very favorable and broadly comparable to that of the aluminum hydroxide adjuvant-only placebo, showing no significant increase in solicited adverse events. Unexpectedly, unsolicited adverse events and serious adverse events (SAEs) occurred less frequently in the EnVAX-A71 group. This difference is likely due to the higher incidence of HFMD cases in the placebo group (4.3% vs 2.0% for unsolicited AEs; 3.1% vs 1.0% for SAEs), which coincided with a significant EV-A71 outbreak in Vietnam during the study period. These results further support the vaccine's efficacy in preventing EV-A71-associated disease. The overall safety profile of EnVAX-A71 is comparable to or even better than other authorized EV-A71 vaccines, which have typically demonstrated acceptable reactogenicity, though there are some differences in the occurrence of fever and other systemic reactions[20,21]. The relatively low reactogenicity profile of EnVAX-A71, especially concerning systemic adverse events, is a significant advantage for vaccine acceptance and adherence among pediatric populations.

EnVAX-A71 is the first bioreactor-produced EV-A71 vaccine to complete Phase III evaluation. The theoretical advantages of bioreactor-based manufacturing—including enhanced process control and scalability—were clinically validated by our lot-to-lot consistency analysis. The pairwise GMT ratios across three independent vaccine lots all fell within prespecified equivalence margins (0.5–2.0) (Supplementary Table S12), demonstrating manufacturing reproducibility. This consistency, combined with the scalable production capacity, addresses critical challenges for vaccine deployment during endemic outbreaks, particularly in resource-constrained settings where efficient scale-up may be necessary.

There are limitations of our study that warrant acknowledgment. First, while our study demonstrated efficacy over the follow-up period of more

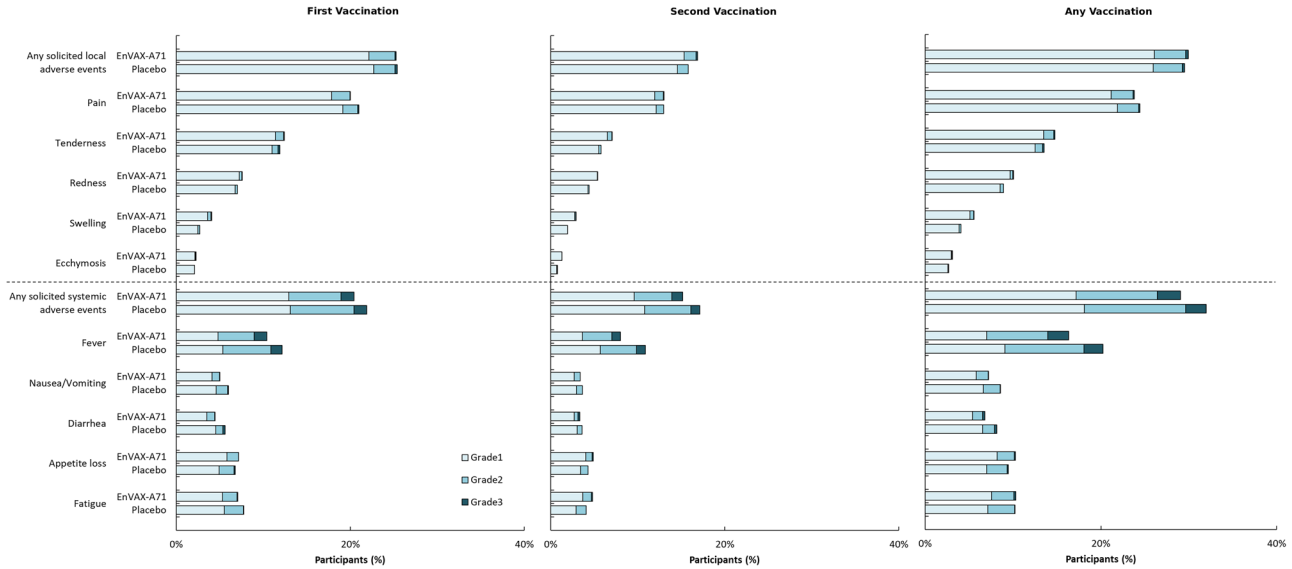

Fig. 3 | Incidence and severity of solicited local and systemic adverse events within 7 days following any vaccination. Solicited adverse events reported within 7 days after either first or second vaccination in the Total Vaccinated Cohort (TVC) safety population (EnVAX-A71: $n = 2380$; placebo: $n = 1613$). The top panel shows local adverse events; the bottom panel shows systemic adverse events. Bars represent the percentage of participants experiencing each event, stratified by severity grade. Grade 1 (light color): mild, not interfering with normal activity. Grade 2 (medium color): moderate, interfering with normal activity. Grade 3 (dark color): severe, preventing normal activity. The overall incidence of solicited local adverse events was comparable between groups (EnVAX-A71: 29.9%; placebo: 29.5%; $p = 0.776$). Solicited systemic adverse events were slightly lower in the vaccine group (29.1%) compared to placebo (32.0%; $p = 0.051$). Among individual symptoms, only swelling ($p = 0.036$) and fever ($p = 0.002$) showed statistically significant differences between groups, both occurring less frequently in the vaccine group. The majority of all reactions were mild (Grade 1), with severe (Grade 3) reactions occurring in ≤2.6% of participants for any symptom. AE adverse event.

Table 4 | Immunogenicity of EnVAX-A71 vaccine through one year post-vaccination

Immunogenicity		Baseline	Day 56	Day 196	Day 392
EV71 vaccine		**(*N* = 309)**	**(*N* = 309)**	**(*N* = 302)**	**(*N* = 301)**
SPR	*n* (%)	**24 (7.77%)**	**305 (98.71%)**	**298 (98.68%)**	**301 (100.00%)**
	(95% CI)	(5.04, 11.34)	(96.72, 99.65)	(96.64, 99.64)	(98.78, 100.00)
SCR	*n* (%)	–	**295 (95.47%)**	**284 (94.04%)**	**285 (94.68%)**
	(95% CI)	–	(92.51, 97,50)	(90.74, 96.43)	(91.51, 96.93)
GMT	Value	**6.0**	**1078.0**	**437.3**	**680.0**
	(95% CI)	(5.15, 7.03)	(949.95, 1223.2)	(383.43, 498.70)	(592.15, 780.90)
GMTR	Value	–	**174.7**	**72.1**	**112.2**
	(95% CI)	–	(153.67, 198.60)	(63.01, 82.51)	(97.16, 129.63)
Placebo		**(*N* = 126)**	**(*N* = 126)**	**(*N* = 121)**	**(*N* = 123)**
SPR	*n* (%)	**10 (7.94%)**	**13 (10.32%)**	**14 (11.57%)**	**19 (15.45%)**
	(95% CI)	(3.87, 14.11)	(5.61, 17.00)	(6.47, 18.65)	(9.56, 23.07)
SCR	*n* (%)	–	**6 (4.76%)**	**6 (4.96%)**	**9 (7.32%)**
	(95% CI)	–	(1.77, 10.08)	(1.84, 10.48)	(3.40, 13.44)
GMT	Value	**6.0**	**6.7**	**6.7**	**8.2**
	(95% CI)	(4.74, 7.71)	(5.48, 8.14)	(5.43, 8.23)	(6.62, 10.20)
GMTR	Value	–	**1.2**	**1.2**	**1.4**
	(95% CI)	–	(0.96, 1.44)	(0.93, 1.43)	(1.13, 1.78)

Neutralizing antibody responses to EV-A71 (E59 strain, B4 subgenogroup) measured in the According-To-Protocol immunogenicity population ($n = 435$) at baseline (Day 0, pre-vaccination), Day 56 (28 days post-dose 2), Day 196 (6 months post-dose 2), and Day 392 (1 year post-dose 2). Neutralizing antibody titers were measured using $TCID_{50}$-based neutralization assay. Seroprotection rate (SPR) defined as proportion of participants with neutralizing antibody titer ≥1:32. Seroconversion rate (SCR) defined as proportion achieving post-vaccination titer ≥1:32 from baseline <1:8, or ≥4-fold increase from baseline ≥1:8. Geometric mean titer (GMT) calculated with 95% confidence intervals using log-transformed values. Geometric mean titer ratio (GMTR) represents ratio of post-vaccination to baseline GMT. In the EnVAX-A71 group, SPR increased from 7.77% at baseline to 98.71% at Day 56 and remained ≥98% through Day 392. GMT peaked at 1078.0 (95% CI: 949.95–1223.2) at Day 56, representing a 174.7-fold increase from baseline, then declined to 437.3 at Day 196 before rising to 680.0 at Day 392. The antibody kinetics pattern suggests both vaccine-induced immunity and potential natural boosting from endemic exposure. In contrast, the placebo group showed minimal changes in SPR (7.94–15.45%), SCR (≤7.32%), and GMT (6.0–8.2) throughout the study period, consistent with limited natural exposure. All timepoint comparisons between vaccine and placebo groups, except at baseline, were statistically significant ($p < 0.001$ by Fisher's exact test for SPR/SCR; ANCOVA with baseline as covariate for GMT/GMTR). Based on achieving SPR > 90% at Day 56, EnVAX-A71 received conditional regulatory approval in Taiwan using neutralizing antibody as an immune surrogate endpoint while the Phase III efficacy trial was ongoing (see Supplementary Table S7 for Taiwan-specific data supporting this approval).

SPR seroprotection rate, *SCR* seroconversion rate, *GMT* geometric mean titer, *GMTR* geometric mean titer ratio, *CI* confidence interval, $TCID_{50}$ 50% tissue culture infectious dose, *ANCOVA* analysis of covariance, *EV-A71* Enterovirus A71.

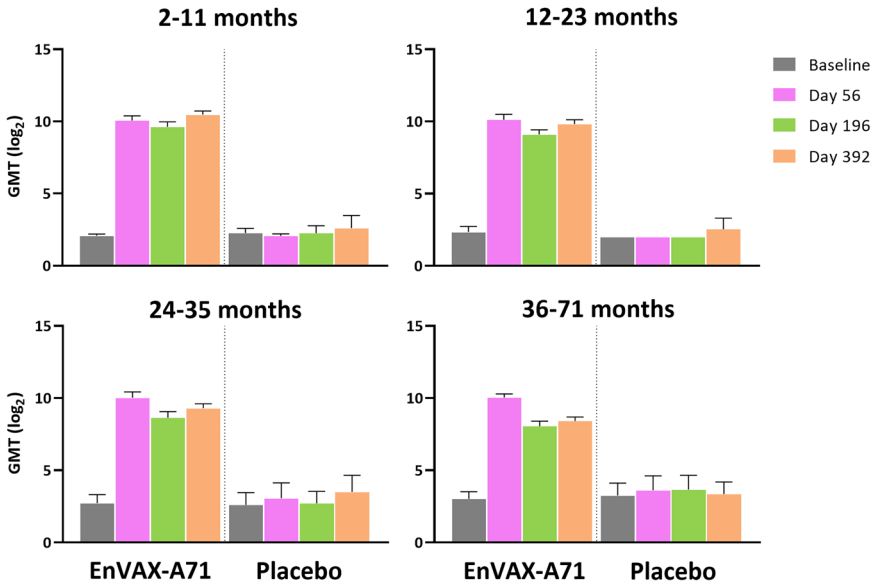

Fig. 4 | Age-stratified neutralizing antibody geometric mean titers at scheduled timepoints through one year post-vaccination. Geometric mean titers (GMT) of neutralizing antibodies against EV-A71 (E59 strain) measured by $TCID_{50}$-based neutralization assay in the According-To-Protocol (ATP) immunogenicity population ($n = 435$) at baseline (Day 0), Day 56 (28 days post-dose 2), Day 196 (6 months post-dose 2), and Day 392 (1 year post-dose 2). Data are presented separately for four age groups: 2–11 months (top left), 12–23 months (top right), 24–35 months (bottom left), and 36–71 months (bottom right). Purple bars represent baseline (pre-vaccination); pink bars represent Day 56; green bars represent Day 196; orange bars represent Day 392. Error bars indicate 95% confidence intervals. For EnVAX-A71 recipients, GMT values at Day 56 ranged from 1035.2 to 1093.7 across age groups, with the highest response in the 12–23 months subgroup (1093.7; 95% CI: 875.4–1366.3). All age groups showed substantial decline by Day 196, followed by increase by Day 392, with the most pronounced rise in the youngest age group (2–11 months: 1414.6; 95% CI: 1103.2–1813.8). Placebo recipients showed minimal GMT changes throughout the study period (range: 4.0–11.6), consistent with limited natural exposure. All timepoint differences between vaccine and placebo groups were statistically significant ($p < 0.001$ by ANCOVA with baseline as covariate). The pattern of antibody kinetics suggests both vaccine-induced immunity and potential natural boosting from asymptomatic exposure in endemic settings. GMT geometric mean titer, CI confidence interval, $TCID_{50}$ 50% tissue culture infectious dose, EV-A71 Enterovirus A71, ANCOVA analysis of covariance.

than one year post-vaccination, long-term durability of protection and potential need for booster doses remain important areas for investigation. Ongoing monitoring would be crucial for assessing vaccine protections beyond the ages that are most susceptible to EV-A71 infection and could yield important clue for the potential need for a booster shot. Second, during our surveillance period all 71 EV-A71-confirmed cases were determined to be B5 subgenogroup. This observation likely reflects the predominance of circulating strains documented during the specific surveillance period (2018–2024)[22]. While our serological cross-neutralization data demonstrated broad activity against B4, B5, C4, C5, and C2 subgenogroups (Supplementary Table S11), real-world effectiveness against non-B5 strains will require ongoing surveillance.

The public health implications of these results are significant, especially for the Asia-Pacific region. The availability of a highly effective, scalable vaccine for EV-A71, with demonstrated manufacturing consistency and local production capability, enhances regional preparedness and response capacity for endemic outbreaks. Currently, EV-A71 vaccines are predominantly available in China and Taiwan, with restrictive access in other endemic nations such as Vietnam, Malaysia, and Singapore. Expanding regional production capacity of effective vaccines could ensure broader protection coverage against EV-A71 infection. The demonstrated immunogenicity across all age groups, including young infants, potentially fills an important protection gap for the most at-risk population, with the potential to decrease the considerable illness and death linked with severe EV-A71 infections in this vulnerable group, pending confirmation through post-marketing surveillance. Furthermore, the bioreactor manufacturing platform offers feasibility for rapid scale-up capacity during epidemic situations, facilitating more efficient public health responses.

In summary, this Phase III study demonstrates that EnVAX-A71, a bioreactor-produced inactivated vaccine, provides strong protection against EV-A71-associated diseases and hospitalizations in children aged 2–71 months, with a favorable safety and immunogenicity profile. The vaccine achieved high efficacy and robust immunogenicity across all age strata up to 71 months. The compelling immunogenicity profiles observed in the younger age group indicate that EnVAX-A71 can elicit strong immune responses with the potential to confer a meaningful degree of protection against EV-A71 in the pediatric population. The validated bioreactor platform, demonstrated through lot-to-lot consistency, offers significant advantages for rapid, scalable vaccine production. These findings support EnVAX-A71 as a valuable addition to the EV-A71 prevention arsenal in endemic regions, particularly where locally manufactured and scalable solutions are critical for disease control.

Methods

Study design and participants

This was a multinational, double-blind, placebo-controlled, randomized, Phase III trial evaluating the efficacy, immunogenicity, and safety of the EnVAX-A71 vaccine in infants and young children aged 2–71 months. Participants were recruited between June 27, 2018, and June 30, 2023, from nine clinical centers (seven in Taiwan and two in Vietnam). Participating clinical sites were major hospitals and health centers with experience in pediatric clinical trials, and all interventions were administered by qualified study personnel in accordance with Good Clinical Practice (GCP) guidelines. The trial was registered at ClinicalTrials.gov (NCT05099029; October 26, 2021; https://clinicaltrials.gov/ct2/show/NCT05099029). The trial ended when the last enrolled participant completed the Day 392 (1-year) follow-up visit on July 30, 2024.

Eligible children had normal health status, a body temperature ≤38 °C at enrollment, and no prior EV71 infection or investigational EV71 vaccine administration. Major exclusion criteria were: recent enterovirus-associated disease (within 30 days), vaccine hypersensitivity, significant chronic illnesses, immunodeficiency or immunosuppressive therapy, congenital

defects, severe malnutrition, or premature birth (<34 weeks gestation or birth weight <2200 g), or receipt of immunoglobulins or blood products within three months prior to enrollment, in line with the clinical trial protocol.

The study population was stratified by age groups (2–11, 12–23, 24–35, and 36–71 months) in a ratio of 2:2:2:3. The overall study design targeted a 3:2 vaccine-to-placebo allocation ratio to maximize vaccine safety data while maintaining adequate placebo comparison. However, to accommodate different regulatory requirements and enrollment dynamics in Taiwan and Vietnam, country-specific randomization schemes were implemented. The differential allocation ratios by country (Taiwan 4:1, Vietnam 1:1) and larger enrollment in Vietnam (68% of total participants) resulted in an imbalance in nationality distribution between treatment groups. This imbalance was most pronounced in the placebo group (83% Vietnamese) compared to the vaccine group (56% Vietnamese). Among those enrolled, a subgroup of 316 subjects from Taiwan and 241 subjects from Vietnam were allocated for immunogenicity and immune persistence analysis. The immunogenicity sub-study in Taiwan was designed to provide additional assessments of immune efficacy to support accelerated regulatory approval using neutralizing antibody titers as a surrogate endpoint in the event of a serious outbreak, while the combined immunogenicity cohort was prespecified in the protocol to be broadly representative across age strata and study sites.

Randomization and masking

All eligible participants were randomized using a centralized procedure. Two separate randomization lists were created with block sizes of 16 for Taiwan and 6 for Vietnam, containing sequential codes for vaccine or placebo assignment.

A third-party statistician, independent of the study conduct, generated the random allocation sequence using SAS software (version 9.4). The sequence was implemented through a central, secure interactive web response system (IWRS), which assigned a unique randomization code to each participant upon enrollment. This centralised system ensured that site investigators and personnel enrolling participants did not have access to the allocation sequence, thereby concealing treatment assignment until the investigational product was dispensed. To maintain blinding, the vaccine and placebo were supplied in identical pre-filled syringes and packaging. All participants, caregivers, investigators, and study staff remained blinded until completion of the efficacy evaluation.

Patient and public involvement

No patients or public representatives were involved in the design, conduct, or reporting of this research. Written informed consent was obtained from parents or legal guardians of all participants.

Procedures

The investigational vaccine (EnVAX-A71) is an adjuvanted, inactivated EV71 vaccine (E59 strain, B4 subgenogroup), produced using a Vero cell bioreactor platform. Each 0.5 mL vaccine dose contained 1 μg viral antigen and 150 μg aluminum hydroxide adjuvant. The placebo consisted solely of aluminum hydroxide (150 μg per 0.5 mL). Both products were manufactured by Adimmune Corporation, Taiwan. Using aluminum adjuvant as an active placebo control enables clearer differentiation between effects attributable to the viral antigen and those related to the adjuvant itself. This approach also reduces the risk of underestimating reactogenicity associated with aluminum-adjuvanted vaccines and ensures that observed local and systemic reactions reflect real-world conditions for aluminum-adjuvanted pediatric immunization.

Participants received two intramuscular injections 28 days apart, administered in the deltoid, biceps, or quadriceps muscle, followed by clinical observation for immediate adverse reactions for 30 min post-vaccination. Solicited local and systemic adverse events (AEs) were recorded for seven days after each injection, and unsolicited AEs for 28 days. Serious AEs (SAEs) were recorded until one year All AEs were followed until resolution or stabilization. Concomitant medications were recorded throughout the trial. The protocol prohibited the use of other investigational products, systemic immunomodulators or immunosuppressants for more than 14 days, immunoglobulins or blood products, and any other vaccines administered within 14 days of a study vaccination; all other treatments were permitted as required for the participant's standard care. Surveillance for EV71-associated hand, foot, and mouth disease (HFMD) and herpangina commenced 28 days after the second dose and continued throughout the follow-up period. Guardians were instructed to report any suspected HFMD/herpangina symptoms promptly. Additional phone contacts or mobile applications were implemented at least once a month to follow up on possible occurrences of HFMD/herpangina diseases. Clinical diagnosis of suspected EV71 infection was confirmed using viral isolation or CODEHOP RT-PCR testing[23]] from throat, rectal, or stool samples collected within seven days of symptom onset.

Immunogenicity was assessed in predefined subgroups ($n = 316$ from Taiwan; $n = 241$ from Vietnam), with blood samples collected at baseline (day 0), day 56 (28 days post-dose 2), day 196 (6 months post-dose 2), and day 392 (1 year post-dose 2). EV71-specific neutralizing antibody titers were measured using a $TCID_{50}$-based neutralization assay[24], and the geometric mean titers (GMT) were calculated.

Outcomes

The primary efficacy endpoint was the incidence of laboratory-confirmed EV71-associated HFMD/herpangina occurring from the 28th day after the second vaccination through the end of the follow-up period. The primary immunogenicity endpoint wasthe seroprotection rate (SPR), defined as the proportion of participants achieving neutralising antibody titers ≥1:32 at day 56.

Secondary efficacy endpoints included the incidence of EV71-associated hospitalization and severe HFMD/herpangina, such as neurological complications, pulmonary edema, or cardiorespiratory failure. Secondary immunogenicity endpoints encompassed the seroconversion rate (SCR) — defined as the proportion of participants achieving a post-vaccination titer ≥1:32 from baseline <1:8, or ≥4-fold increase from a baseline ≥1:8— GMTs, geometric mean titer ratio (GMTRs) relative to baseline, persistence of immune response up to day 392, and vaccine lot-to-lot consistency.

Safety endpoints comprised solicited and unsolicited AEs, and serious adverse events (SAEs) monitored for the respective schedules or up to one year post-vaccination according to the protocol defined safety criteria.

Statistical analysis

Three analysis populations were defined for this study: (1) Total Vaccinated Cohort (TVC), including all participants who received at least one dose of study vaccine or placebo and was used for safety analyses, thereby including children who did not receive the second dose (for example, due to adverse events or withdrawal) but contributed safety information up to the point of discontinuation; (2) TVC for efficacy, comprised participants who received two doses of study vaccine or placebo, and were followed starting from the 28th day after the second dose untill the end of the surveillance period; this population was used for efficacy analysis; and (3) According-To-Protocol (ATP) cohort, a subset of the TVC including participants who fulfilled all inclusion/exclusion criteria, received two doses according to their random assignment, and had no major protocol deviations, used for immunogenicity.

The sample size was calculated based on the following assumptions: a vaccine efficacy of 85%, an incidence density of 5 cases of EV71-associated HFMD/Herpangina per 1000 person-years among unvaccinated children, and a 3:2 allocation ratio between vaccine and placebo groups. Using Fleiss's formula implemented in SAS software, and incorporating an event-driven design, a total enrollment of 3982 participants—upon reaching 12 confirmed cases of EV71-associated HFMD/Herpangina—would provide 80% statistical power to demonstrate that vaccine efficacy significantly exceeds that observed in the placebo group, at a two-sided significance level of 0.05.

The vaccine protective efficacy was calculated as: (incidence rate of Placebo group–Incidence rate of vaccine group)/Incidence rate of placebo group × 100%. The 95% CI for vaccine efficacy was determined using the

Poisson regression or the exact Poisson regression if any group had 0 case. The 95% confidence interval (CI) for the proportion of confirmed EV71-associated HFMD/herpangina cases was calculated using the Clopper-Pearson method, and for the incidence rate using Poisson regression.

For immunogenicity analyses, SPR and SCR were expressed with 95% Clopper-Pearson CIs for each treatment group, and comparisons between groups were performed using Fisher's exact test. The GMT and GMTR were calculated with 95% CIs for each treatment group. Intergroup differences for GMT and GMTR were assessed using analysis of covariance (ANCOVA) with baseline titer as a covariate.

Sensitivity analyses for vaccine protective efficacy were pre-specified to assess the robustness of results and address potential confounding factors. These included Poisson regression with covariate adjustment for baseline characteristics including nationality, exact Poisson regression, negative binomial regression, and the proportion method. The nationality imbalance between treatment groups, resulting from differential country-specific randomization ratios and enrollment patterns, was specifically addressed through Poisson regression models adjusting for nationality as a covariate. These sensitivity analyses were designed to confirm that the observed vaccine efficacy was not materially affected by differences in demographic distributions between treatment groups.

All statistical analyses were performed using SAS® software (version 9.4 or later) in a secure and validated environment.

No substantial changes to the trial protocol, prespecified outcomes, or statistical analyses were made after trial commencement. The primary efficacy and immunogenicity analyses were based on the According-To-Protocol (ATP) and Total Vaccinated Cohort (TVC) for efficacy populations, which included participants with complete data for the respective endpoints. For these analyses, participants with missing data were not imputed. For the analysis of unsolicited adverse events, participants who did not report an event during the follow-up period were considered as not having an event.

Ethics and regulatory compliance

This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The trial protocol was approved by Institutional Review Boards at all participating sites in Taiwan (National Taiwan University Children's Hospital, Taipei City; China Medical University, School of Medicine, Taichung City; Linkou Chang-Gung Memorial Hospital, Taoyuan City; Taipei Veterans General Hospital, Taipei City; National Taiwan University Hospital, Hsinchu branch, Hsinchu City; Taichung Veterans General Hospital, Taichung City; Changhua Christian Children's Hospital, Changhua City) and Vietnam (Nguyen Dinh Chieu Hospital, Ben Tre Province; Vinh Long City Health Center, Vinh Long Province). Specific approvals were obtained from: Research Ethics Committee, China Medical University & Hospital (CMUH106-REC2-160); Research Ethics Committee D, National Taiwan University Hospital (201801118MSD); Institutional Review Board, Taipei Veterans General Hospital (2018-03-007 AU); Institutional Review Board I & II of Taichung Veterans General Hospital (SC18044A); Institutional Review Board, Chang Gung Medical Foundation (201800261A4); Institution Review Board of National Taiwan University Hospital Hsin-Chu Branch (107-022-E); Institutional Review Board Committee Changhua Christian Hospital (180311); and Ministry of Health Pasteur Institute HCMC (18/CN_HĐĐĐ). Written informed consent was obtained from parents or legal guardians of all participants after the nature and possible consequences of the study had been fully explained. Informed consent was obtained prior to any study-related procedures and documented according to Good Clinical Practice standards. The study included representative human populations across sex, age, and ethnicity. Privacy rights of all human subjects were observed throughout the study conduct and data reporting.

Data availability

The datasets generated and/or analyzed during the current study are not publicly available due to the inclusion of sensitive individual participant data and privacy restrictions, but are available from the corresponding author on reasonable request. De-identified individual participant data, together with the study protocol, statistical analysis plan, and data dictionary, will be made available beginning 6 months and ending 5 years after publication to researchers who provide a methodologically sound proposal and sign a data access agreement. The statistical analyses were performed using SAS software (version 9.4). No custom code or mathematical algorithms were developed for this study.

Code availability

The statistical analyses were performed using SAS software (version 9.4). No custom code or mathematical algorithms were developed for this study.

Received: 10 October 2025; Accepted: 29 January 2026;
Published online: 12 February 2026

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Acknowledgements

This trial was funded by Enimmune Corporation and supported by grants from the "Fast-track Clinical Trial Plan" of the A+ Enterprise Innovation Research and Development Refinement Program, Ministry of Economic Affairs, Taiwan. The funder was the study sponsor and had a role in study design, data interpretation, as well as preparing the reports, and the decision to submit, but had no role in the data collection and data analysis. The corresponding authors had full access to all study data and held the final responsibility for the submission of the manuscript for publication. We also thank all participating study centers, investigators, site staff, and study participants and their families for their valuable contributions to this study.

Author contributions

K.P.H., L.M.H., W.T.L., C.C.L., and C.F.Y. conceived and designed the study. K.P.H., T.M.T., Y.C.H., C.C.L., P.H.L., T.M.T.T., B.F.T., P.Y.C., S.C.Y., and L.M.H. oversaw clinical evaluations and data acquisition. N.T.T.T. and P.C.L. established and conducted laboratory assays and were involved in data reporting. W.T.L., S.T.L., and C.F.Y. were involved in data curation. K.P.H., L.M.H., W.T.L., C.C.C., and C.F.Y. interpreted data and prepared the manuscript drafts. All authors critically reviewed, revised, and approved the final manuscript.

Competing interests

W.T.L., S.T.L., C.C.L., and C.F.Y. are employees of Enimmune Corporation, the funder and sponsor of the study. P.C.L. and C.C.C. are employees of Adimmune Corporation, the parent company of Enimmune Corporation. The other authors do not have a competing interest.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41541-026-01396-x.

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