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RESEARCH PAPER

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Antibody persistence upto 5 years after primary immunization and booster with an inactivated chromatographically purified Vero cell-derived Japanese encephalitis vaccine in Thai children

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ABSTRACT

Japanese encephalitis is the main cause of viral encephalitis in Asia. In a previous single-arm vaccine trial, an inactivated chromatographically purified Japanese encephalitis Vero cell vaccine (CVI-JE; JEVAC™) was safe and immunogenic in 152 Thai children aged 1–3 years receiving a 2-dose primary immunization and booster dose 1 year later. We conducted a 5-year follow-up assessment of the persistence of the immune response the 144 children remaining in this cohort after first booster dose. Immunity was assessed by 50% plaque reduction neutralization test annually for up to 5 years post-booster. Seroprotection rates (95%CI) decreased from 100% (97.1–100) at 1 year post-booster to 93% (85.0–98.3) at 5 years post-booster. No serious vaccine-related adverse events or Japanese encephalitis infections were reported. A 2-dose primary immunization and booster 1 year later with CVI-JE provided long-lasting immunity in the majority of children.

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Introduction

Japanese encephalitis (JE) is a mosquito-borne viral encephalitis caused by the Japanese encephalitis virus (JEV) (*Flavivirus: Flaviviridae*). Transmission is maintained in a natural enzootic cycle between predominately *Culex* mosquitoes and water birds with pigs as an amplifying host while humans are considered to be an incidental, dead-end host.[1,2] In Asia and the Western Pacific, JEV transmission is endemic in tropical and subtropical areas and is seasonally epidemic in temperate areas of most countries. Most infections occur in rural areas, but urban transmissions were reported.[3,4] Although vaccination programs have greatly reduced the disease burden, limited laboratory diagnostic resources and incomplete case reporting make estimated incidence uncertain, and JEV remains the leading cause of viral childhood encephalitis in Asia.[5–7]

The main control strategy is human vaccination. In Thailand, mouse-brain-derived inactivated (MBDI) vaccine was phased out by 2013 partly due to severe adverse event following immunization, including hypersensivity reactions (18–64 per 10,000 doses), and rare neurologic reactions, such as acute disseminated encephalomyelitis (1 per 50,000 to 1,000,000 vaccines).[8,9] Currently, two live attenuated vaccines based on the JE SA14-14-2 strain (CD JEVAX™ and the chimeric IMOJEV™) and one chromatographically purified Vero cell inactivated Japanese encephalitis vaccine (CVI-JE) based on Beijing-3 strain (JEVAC™) are the other licensed vaccines in Thailand. These have better safety profiles than MBDI vaccine. However, live attenuated vaccines are contraindicated in pregnant and breastfeeding mothers, individuals with HIV infection, and individuals with congenital or acquired immune deficiency impairing cellular immunity.

Studies in China and Thailand proved CVI-JE is safe and immunogenic. A study of 375 Chinese children aged 8 months to 10 years given a 2-dose primary immunization (day 0 and day 7) reported a seroconversion rate of 90.4%.[10] A study of 152 Thai children aged 1–3 years given a 2-dose primary immunization (day 0 and day 7–28) had seroprotection rates (SPRs) of 100% 28 days after completing the primary series, 89.7% at one year later pre-booster, and 100% SPR 28 days post-booster at 1 year.[11] Both studies reported self-limiting mild localized to systemic reaction adverse events following immunization (AEFI).[10,11]

CVI-JE was licensed in China in 2008 and Thailand in 2014. The current recommendation in Thailand is a 2-dose primary immunization 7–28 days apart from 6 months of age and a booster 1 year later. However, data on long-term seroprotection and antibody persistence of CVI-JE are lacking. Thus, we herein report the antibody response of CVI-JE up to 5 years after primary immunization and booster 1 year later.

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Materials and methods

Study design and subjects

The details of our previous non-randomized, open-label, single-arm trial of the safety, reactogenicity, and immunogenicity of a 2-dose primary immunization and booster 1 year later up to the 28-day post-booster response using freeze-dried CVI-JE (Liaoning Cheng Da Biotechnology Co., Ltd., Shenyang, China) have previously been reported.[11] Each dose was 0.5 mL. The study was registered with Clinical Trial registration number of NCT01408537. The combined schedule of visits for both the previous and the present study is shown in Figure 1.

In the present study, we assessed the JE antibody persistence for up to 5 years post-booster dose by annual follow-up visits for 60 months between 2011 and 2016 in all the 144 participants completing the protocol of our previous study up to the 28-day post-booster visit.[11] We recruited from two hospital sites in Bangkok, Thailand: the Well Baby Clinic, Outpatient Department, Nopparat Rajathanee Hospital and the Department of Tropical Pediatrics, Faculty of Tropical Medicine, Mahidol University. We carried out the study in accordance with the Declaration of Helsinki and the International Conference on Harmonization guidelines for Good Clinical Practice. The institutional and ethical review boards of the participating sites approved the study protocol. Signed informed consent was obtained from the parents or legal guardians of the participating children.

Procedures and assessments

At each annual visit, blood was drawn for immunogenicity assessment and physical examination was performed. Participants and parent/legal guardians were asked about any illnesses, including JE, since the previous visit to evaluate any vaccine-related serious adverse events (SAEs).[12] The study investigators evaluated whether these were related to the study vaccine.

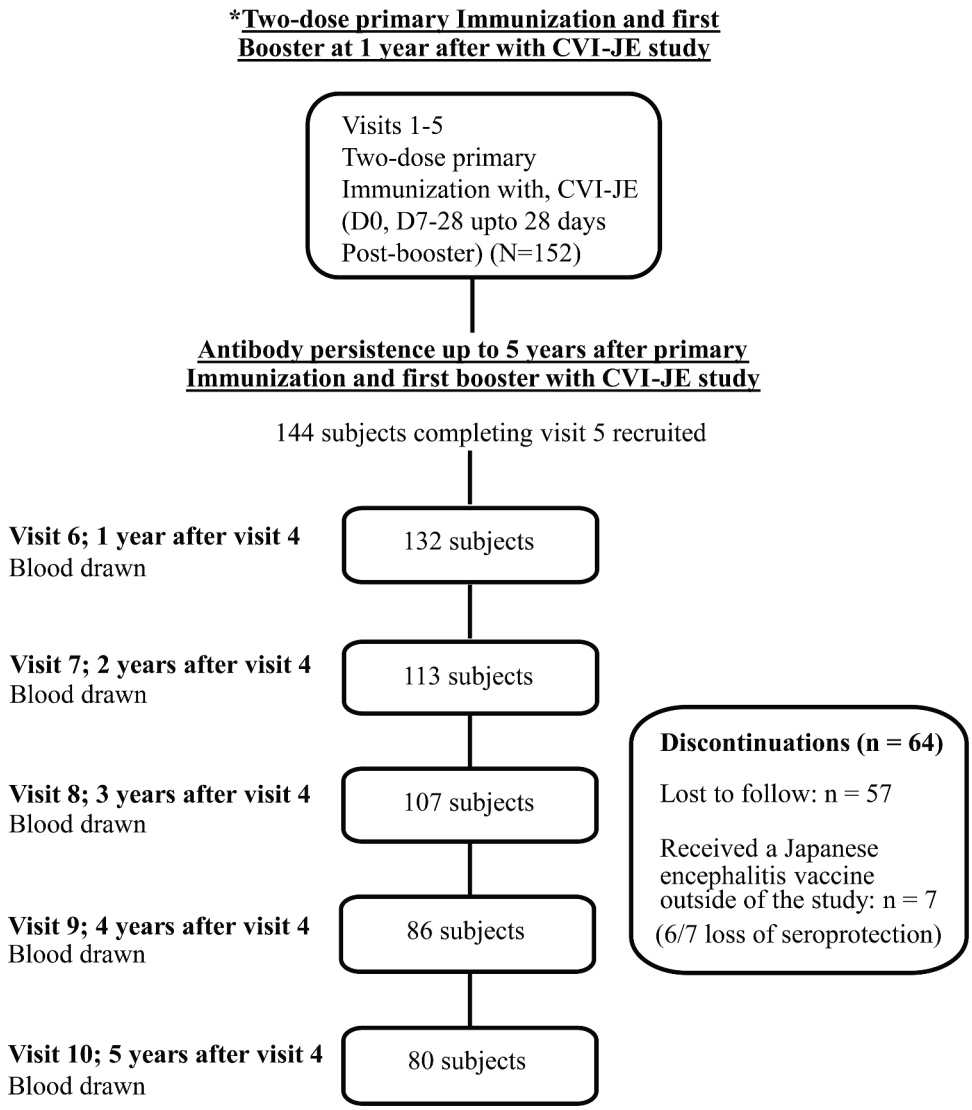

Figure 1. Disposition of participants. *This was the previous study.[11] Abbreviations: CVI-JE, chromatographically purified Vero cell inactivated Japanese encephalitis vaccine; JE, Japanese encephalitis.

JE neutralizing antibody titers were assayed with the 50% plaque reduction neutralization test ($PRNT_{50}$; Center for Vaccine Development, Mahidol University, Thailand), for which the method has previously been described.[11] This method used the homologous strain to the CVI-JE vaccine (Beijing-3) as the challenge virus. The lower limit of quantitation of the test was 10 [1/dil]. This is also the accepted threshold for seropositivity (≥10 [1/dil]) and the correlate of protection against JE (≥10 [1/dil]).[13] A titer <10 [1/dil] was defined as seronegative. Any participants who were found to have a titer measurement below the protective threshold during the 5-year follow-up were offered revaccination with a JE vaccine licensed in Thailand and were discontinued from the study.

Statistical analysis

No sample size calculation was performed as this was a 5-year follow-up of the remaining participants who completed the protocol of the previous study.[11] All analyses were descriptive without hypothesis testing. The primary analysis included all available long-term follow-up data except for participants seropositive at the baseline of the previous study.[11] Proportions and their 95%CIs were calculated by Clopper-Pearson exact binomial method.[14] GMTs and 95%CI were calculated from log_{10} titers with an assumption of normal distribution, followed by antilog transformations to report results on the original scale. $PRNT_{50}$ values below the lower limit of quantification of the test of 10 [1/dil] were replaced by 5 [1/dil] (half the limit of seropositivity).

A sensitivity analysis was performed to avoid bias in estimating the persistence of seroprotection in participants with low neutralizing titers who withdrew from the study or participants who received another JE vaccination and then were discontinued from the study. If a participant was seronegative at the last recorded visit and withdrew from the study for any reason or missed a following visit or blood sample, the participant was assumed to be seronegative at all proceeding follow-up visits. If a participant was seronegative at a visit and then was seropositive later for any reason, the participant was still considered to be seronegative at all proceeding follow-up visits. A further sensitivity analysis was performed by Kaplan–Meier analysis to estimate the proportion of participants maintaining seroprotection over the 5-year follow-up.

Table 1. Baseline characteristics of participants at 28 days post-booster (N = 144).

Characteristic	
Gender, n (%)	
Male	73 (50.7)
Female	71 (49.3)
Age (mo)	
Median (IQR)	25.8 (2.9)
Min-max	25.0–44.9
Height (cm)	
Median (IQR)	88.0 (6.00)
Min-max	75.0–104
Body weight (kg)	
Median (IQR)	12.8 (2.72)
Min-max	9.00–22.2
Body mass index	
Median (IQR)	16.3 (3.06)
Min-max	12.5–24.7

Abbreviations = n, number of participants; mo, month; IQR, interquartile range; Min-max, Minimum-maximum; cm, centimeter; kg, kilogram

Results

Study population

A total of 144 children aged 1–3 years previously receiving two primary doses and one booster dose 1 year later completed the protocol of the previous study up to visit 5 (28-day post-booster)[11] and were recruited into the present study. Of these, 5 were seropositive at the baseline of the previous study and were excluded from the primary analysis and sensitivity analysis of immunogenicity. Thus, 139 children were included in this analysis. All 144 children recruited into the present study were included safety analysis. Seventy-six participants discontinued during the 5-year follow-up, and no withdrawals occurred due to safety-related events. Their dispositions and characteristics of the study participants are summarized in Figure 1 and Table 1, respectively.

Immunogenicity assessments

Seroprotection rates

All 139 participants were seropositive at 28 days post-booster as reported in our previous study.[11] In the present study, SPRs (95%CI) in the primary analysis remained high for throughout the 5-year follow-up period (Table 2). These SPRs decreased from 100% (97.1–100) at year 1 to 93.8% (85.0–98.3) at year 5 (Table 2). Sensitivity analysis SPRs were similar to primary analysis results from years 1 to 3 of follow-up (Table 2). However, sensitivity analysis SPRs (95%CIs) at years 3 to 5 of

Table 2. Primary analysis of seroprotection rates of CVI-JE primed and boosted children aged 1–3 years up to 5 years post-booster.

Time point	n/M	% (95%CI)	n/M	% (95%CI)
	Main analysis		Sensitivity analysis	
Year 1: booster date + 12 mo ± 45 d	127/127	100 (97.1–100)	127/127	100 (97.1–100)
Year 2: booster date + 24 mo ± 45 d	101/109	92.7 (86.1–96.8)	101/109	92.7 (86.1–96.8)
Year 3: booster date + 36 mo ± 45 d	86/95	90.5 (78.5–93.9)	86/103	83.5 (74.9–90.1)
Year 4: booster date + 48 mo ± 45 d	71/81	87.7 (78.5–93.9)	71/98	72.4 (62.5–81.0)
Year 5: booster date + 60 mo ± 45 d	61/65	93.8 (85.0–98.3)	61/92	66.3 (55.7–75.8)

n = 139 at day 28 post-booster excluding 5 participants seropositive at the baseline of the previous study.[11] Abbreviations = n, number of seroprotected participants; M, total number of participants at the time point.

Table 3. Geometric mean titers of CVI-JE primed and boosted children aged 1–3 years up to 5 years post-booster.

Time point	M	[a]GMT (95%CI)	[b]M	[a]GMT (95%CI)
	Main analysis		Sensitivity analysis	
Year 1: booster date + 12 mo ± 45 d	127	264 (217–322)	127	264 (217–322)
Year 2: booster date + 24 mo ± 45 d	109	64.1 (51.1–80.5)	109	64.1 (51.1–80.5)
Year 3: booster date + 36 mo ± 45 d	95	53.1 (41.3–68.3)	103	44.2 (34.0–57.5)
Year 4: booster date + 48 mo ± 45 d	81	53.8 (40.3–71.8)	98	35.6 (26.4–48.1)
Year 5: booster date + 60 mo ± 45 d	65	40.9 (32.3–51.8)	92	22.1 (17.0–28.6)

n = 139 at day 28 post-booster excluding 5 participants seropositive at the baseline of the previous study.[11]
[a]Any $PRNT_{50}$ titer below the lower limit of quantification was replaced by 5.
[b]Sensitivity analysis includes participants seronegative at a time point in the denominator of subsequent time points and imputes their $PRNT_{50}$ value for these time points as 5.
Abbreviations = M, total number of participants at time point; GMT, geometric mean titer $PRNT_{50}$ of Japanese encephalitis neutralizing antibodies [1/dil] antilog transformed.

Table 4. Neutralizing antibody titers of 5 subjects seropositive at baseline of the previous study.[11]

	Neutralizing antibody titer				
	Year 1: booster date + 12 mo ± 45 d	Year 2: booster date + 24 mo ± 45 d	Year 3: booster date + 36 mo ± 45 d	Year 4: booster date + 48 mo ± 45 d	Year 5: booster date + 60 mo ± 45 d
Subject 1	[a]>1000	2007	2706	1150	2333
Subject 2	1352	284	199	106	70
Subject 3	3792	520	443	1084	421
Subject 4	1459	296	272	120	-
Subject 5	135	-	-	-	-

Dashed line indicates participant was lost to follow-up.
[a]$PRNT_{50}$ value unknown due to laboratory measurement error.

follow-up post-booster were lower than those of primary analysis SPRs at 83.5% (74.9–90.1), 72.4% (62.5–81.0), and 66.3% (55.7–75.8), respectively (Table 2).

Kaplan–Meier SPRs by years 1–5 of 100%, 93.7% (95%CI 89.4–98.0), 85.4% (95%CI 78.9–91.9), 75.4% (95%CI 67.2–83.6), and 70.8% (95%CI 62.0–79.6), respectively.

Persistence of neutralizing antibodies

The immune response for the primary immunization and booster 1 year later up to 28 days post-booster have been reported previously.[11] At 28 days post-booster, geometric mean titers (GMTs) (95%CI) were 622 (510–757).[11] In the present study, GMTs in both the primary analysis and sensitivity analysis remained above the seroprotection threshold for JE of ≥ 10 [1/dil] throughout the 5-year follow-up period although sensitivity analysis GMTs were lower in years 3–5 (Table 3).

The GMTs of the 5 participants seropositive at the baseline of the previous study are shown in Table 4.

Serious adverse events

Neither cases of JE nor vaccine-related serious adverse events were reported during the 5-year follow-up period.

Discussion

We report the first study of antibody persistence for up to 5 years after a 2-dose primary immunization and booster 1 year later with CVI-JE in children aged 1–3 years. A protective immune response and GMTs above the threshold considered protective were observed in the majority of the participants. No vaccine-related serious adverse events occurred, and no cases of JE were observed during the 5-year follow-up post-booster.

In the present study, primary analysis SPRs gave less conservative estimates than sensitivity analysis SPRs that more accurately estimate of the performance of the vaccine due to the including any seronegative participants in the denominator for calculating the SPR in subsequent visits, and all post-booster comparison are after the first booster dose. We compare sensitivity analysis by the same method from reports in the literature where possible. Primary analysis SPRs at 28 days post-booster reported in our previous study were 100% and remained higher than 85% throughout the 5-year follow-up period in the present study. However, estimated SPR by the sensitivity analysis in years 4 and 5 were much lower at around 72% and 66%, respectively, and the Kaplan–Meier SPRs by years 4 and 5 were around 75% and 71%, respectively. Our results are similar to comparable studies of 5-year follow-up after 1-dose primary immunization with a live, attenuated JE chimeric vaccine (JE-CV) in children aged 12–18-month in Thailand and the Philippines, which reported sensitivity analyses SPRs of 59–67% at 5 years post-booster.[15,16] The 5-year post-booster primary analysis SPRs of the present study were also comparable to studies in which JE-CV booster was given after primary immunization with either MBDI vaccine or JE-CV in children in the Philippines and Thailand, all of which had apparent SPRs of > 90%.[15,17] CVI-JE had a similar primary analysis SPR (95%CI) compared with the inactivated Vero cell vaccine IC51 (IXIARO^{TM}) in children aged 1–3 years at 2 years post-booster at 92.7% (86.1–96.8) vs 100% (94.6–100.0), respectively.[18] The primary analysis SPR (95%CI) of CVI-JE post-booster was slightly lower compared to a study in Korean children vaccinated with Beijing-1 strain inactivated Vero cell vaccine (ENCEVAC^{TM}) at 87.7% (78.5–93.9) vs 100% (91.6–100), respectively, and similar to a Nakayama strain MBDI vaccine at 4 years post-booster.[19]

GMTs of CVI-JE in the present study declined rapidly over the first 2 years of follow-up, before appearing to generally plateau. GMTs were still above the threshold considered protective at 5 years post-booster although they were considerably lower from day 28 post-booster to 2- or 5-year post-booster than any studies in which either JE-CV or IC51 was the booster.[15,17,18] A slight rise in apparent GMT in the present study was seen between years 3 and 4 of follow-up, which is

consistent with previous long-term JE vaccine immunogenicity reports in endemic settings.[15,16] This may have been caused by natural boosting from wild-type JEV or other flaviviruses, which may cross-react with the JE $PRNT_{50}$ test.[20] Natural boosting from wild-type JEV or other flaviviruses may contribute to CVI-JE antibody persistence in flavivirus-endemic settings.

There were limitations to the present study. The single-arm trial design did not allow for head-to-head comparison of the long-term immunogenicity and safety of CVI-JE with other contemporary JE vaccines. Results between testing laboratories may vary because no standardized protocol for the PRNT exists. Also, different endemic settings and study timings may be a source of heterogeneity between studies due to natural boosting from JEV or other flaviviruses. Therefore, interpretations of non-head-to-head comparisons should be treated with caution.[21,22]

Conclusion

A 2-dose primary immunization and booster 1 year later in children aged 1–3 years with CVI-JE provided long-lasting immunity for the majority of participants up to 5 years post-booster. No JE cases and no related SAEs were observed.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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