



Safety and immunogenicity of a freeze-dried, cell culture-derived Japanese encephalitis vaccine (Inactivated) (JEBIK®V) in children

Kenji Okada^a, Toshiaki Iwasa^b, Junko Namazue^{c,*}, Masateru Akechi^c, Shigeharu Ueda^c, JE Vaccine Clinical Study Group¹

^a Department of Pediatrics, Fukuoka National Hospital, Fukuoka, Japan

^b Iwasa Pediatrics Clinic, Mie, Japan

^c The Research Foundation for Microbial Diseases of Osaka University, Osaka, Japan

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ABSTRACT

Freeze dried, cell culture-derived Japanese encephalitis vaccine (Inactivated) (JEBIK®V) is approved for a three-dose primary immunization followed by a one-dose booster immunization in Japan. We conducted a multicenter, double-blinded, randomized controlled trial of the safety and immunogenicity of the vaccine in 370 healthy children who received three doses of 5, 2.5 or 1.25 µg of virus protein per 0.5 mL formulation subcutaneously. Children received two doses of test vaccine 7–28 days apart followed by a dose 6–12 months after the second vaccination. The three-dose regimen showed a good safety profile with no serious vaccine-related adverse events. Fever and reactions at the injection site were common adverse reactions at each dose of vaccine. The seroconversion rates were 100%, 99.2% and 95.0% after two doses in the 5, 2.5 and 1.25 µg groups, respectively, and 100.0% after three doses in all groups. The geometric mean titers were high for all three formulations after the second and third doses, with a very clear dose–response relationship. These results indicate that JEBIK®V is likely to be a useful vaccine.

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1. Introduction

The Japanese encephalitis virus (JEV) is estimated to cause at least 50,000 cases of clinical disease every year, mostly in children younger than 10 years old [1–4]. JEV is detected in mosquitoes and pigs at high frequency in Japan, although only a few human cases are reported annually. JE has an incubation period of 4–14 days and is mostly characterized by sudden onset of fever, chills, myalgia, mental confusion and sometimes cervical rigidity, with a mortality of up to 30–50% and a high percentage of neurological sequelae in survivors [1]. There is no specific antiviral treatment for JE, but vaccination is the single most important control measure. Formalin-inactivated, mouse-brain derived vaccines have been supplied for decades, with a change in JE virus strain from the Nakayama-NIH strain to the Beijing-1 strain in 1988 in Japan [5–10]. Use of the mouse-brain derived vaccine has reduced the incidence of JE in a lot of countries, including Japan, with a rate of serious side effects such as anaphylaxis and acute disseminated encephalomyelitis (ADEM) after vaccination of less than 1 per

100,000 people [11]. However, the mouse neural tissue content of the vaccine has raised concerns about safety and the possibility of vaccine-related neurological side effects, although the exact cause of ADEM is unknown.

We have developed a novel inactivated JE vaccine produced on Vero cell culture. The vaccine has less potential risk of serious neurological adverse reactions, since it does not contain impurities from mouse-brain-derived proteins. The vaccine also has less potential risk of including an unknown mouse-derived virus. Here, we report clinical data for a purified, inactivated JE vaccine produced using a certified Vero-cell culture substrate for virus propagation.

2. Subjects and methods

2.1. Participants

The subjects were healthy infants or children aged 6 to <90 months. The main exclusion criteria were hypersensitivity to vaccine components or excipients; immunosuppression or immunomodulatory treatment; administration of immunoglobulin or infusion of blood (within 3 months before the start of the study); participation in a clinical study (within 6 months before the start of the study); and previous vaccination with a JE vaccine. Written informed consent was obtained from each parent or guardian before study enrolment.

* Corresponding author at: The Research Foundation for Microbial Diseases of Osaka University, 3-1 Yamadaoka, Suita, Osaka 565-0871, Japan. Tel.: +81 6 6877 4812; fax: +81 6 6876 5815.

E-mail address: jnamazue@mail.biken.or.jp (J. Namazue).

¹ See Source Information.

2.2. Vaccines and viruses

Inactivated lyophilized JE vaccine was prepared using the Beijing-1 strain of JE. Three doses of vaccine containing 1.25, 2.5 or 5.0 µg of virus protein per 0.5 mL were formulated without adjuvant or preservative. The Beijing-1 strain of JE virus was derived from seed virus for mouse-brain derived JE vaccine used in Japan and was propagated in Vero cells. The JE virus was concentrated, inactivated with formalin, and then purified by protamine treatment and sucrose-gradient centrifugation. After dilution at the defined protein contents, bulk vaccines were combined with lactose and sodium L-glutamate as stabilizers, filled into vials, and freeze-dried.

2.3. Procedures

The study was conducted in accordance with protocols approved by institutional review boards at the participating sites. A randomized, dose comparison study design was used with 1:1:1 randomization in each age strata based on the indications for different volumes of vaccine: 0.5 mL for children aged ≥3 years old and 0.25 mL for those aged ≥6 months to <3 years old. The vaccine was administered subcutaneously in the upper arm. The children were given two doses of vaccine 7–28 days apart, followed by a third dose at 6–12 months after the second dose, based on the recommended schedule for conventional JE vaccine in Japan.

The children were observed for 30 min after inoculation of each dose by the investigators and then parents or guardians recorded the presence and severity of local and systemic symptoms and daily body temperature for the next 14 days. Investigators and parents or guardians used a standard scale to grade adverse events over the observation period. Fever was defined as mild if body temperature was 37.5–38.0 °C, moderate if 38.1–39.0 °C, and severe if >39.1 °C. Other systemic symptoms were considered mild if they did not interfere with normal daily activities, moderate if they resulted in some interference with normal daily activities, and severe if they prevented subjects from engaging in normal daily activities. Erythema and swelling at the injection site were defined as mild if the diameter was ≤5 cm, moderate if 5–10 cm, and severe if >10 cm. Observations of the parents or guardians and the medical history of the subjects were reviewed by the investigators of the study. Serum samples for a virus neutralization assay were obtained before the first and third vaccinations, and 28–48 days after the second and third vaccinations.

2.4. Determination of the neutralizing antibody response

Serum neutralizing antibody to the Beijing-1 strain of the JE virus was assayed using a 50% plaque reduction test (PRNT) in Vero cells [12]. The challenge virus fluid contained the Beijing-1 strain of the JE virus derived from mouse brain. Serum samples were heat inactivated for 30 min at 56 °C and then initially diluted by 10-fold in medium followed by serial two-fold dilutions. An aliquot (0.5 mL) of each diluted sera was mixed with an equal volume of 200 plaque forming units (PFU)/0.1 mL JE virus suspensions, and then incubated at 37 °C for 90 min. These mixtures were then plated (0.1 mL/well) on Vero cells cultured in 6-well plates in triplicate and incubated at 37 °C for 90 min. Each well was then overlaid with 1.5% methylcellulose at 3 mL/well. After 6–8 days incubation at 37 °C, the viral plaques were fixed with 10% formalin, stained with 0.04% methylene blue, and counted. PRNT₅₀ titers were calculated from the serum dilution giving a 50% plaque reduction compared to plaque formation in virus-only controls. Serum samples were tested at a starting dilution of 1:10 and negative samples were assigned a value of 1:5 for calculation purposes.

2.5. Statistical analysis

The prespecified primary immunologic primary endpoint in the trial was the seroconversion rate based on neutralizing antibody titer. Seroconversion was defined as an increase in antibody titer from <20 before vaccination to ≥20 after the second vaccination. The seroconversion rate was calculated with an exact 95% confidence interval (CI). Geometric mean titers of antibodies (GMTs) and their 95% CIs were computed by transforming the results to a logarithmic scale. Rates of reactogenicity after each vaccination were based on the most severe response reported. For the overall comparison between doses for reactogenicity, a 95% CI was calculated. All data manipulations and statistical analyses were conducted with SAS v. 8.2.

The sample size for the study of 120 children in each dose group was selected based on immunogenicity data from previous studies. Given enrolment of 100 children in each dose group, the lower limit of the 95% CI for the percentage of children with seroconversion did not extend to <90%, assuming a seroconversion rate of approximately 96%. In addition, the 95% CI for rates of reactogenicity did not extend to >10% from the observed rate.

3. Results

3.1. Study population

A total of 370 children were enrolled in the study and randomized to each dose group (Fig. 1). Written informed consent was obtained from the parents of each child. All enrolled children were included in the safety population. Over 97.8% (362/370) of the children completed the study schedule. Eight children did not complete the study schedule. Children aged ≥3 years old (0.5 mL dose) accounted for 68.0%, 68.0% and 68.3% of the subjects in the groups given 5, 2.5 and 1.25 µg of virus protein, respectively; and 8.2–12.0% and 9.6–14.2% of children in the three groups were ≥5 and <2 years of age, respectively. The three groups in the immunogenicity population had similar demographics and baseline characteristics. The mean ages ± SD of the children who received 5, 2.5 and 1.25 µg of virus protein were 42.4 ± 14.2 (range 11–80), 40.5 ± 13.5 (range 13–71), and 41.4 ± 14.6 (range 9–73) months, respectively. There were no significant differences in sex in each dose group. The first two doses were given 7–13 days apart in 103 children, 14–20 days apart in 174 children, and 21–28 days apart in 90 children. Most children (352/364) received the third dose at 6–7 months (within 240 days) after the second dose.

3.2. Adverse events

The vaccines were well tolerated at all doses, with no withdrawal due to vaccine-related adverse events. The rates of occurrence of the most frequently reported local and systemic vaccine-related adverse events after administration are shown in Table 1. Vaccine-related adverse events occurred in 169/370 children, including in 71 (56.3%), 49 (39.8%), and 49 (40.5%) who received 5, 2.5 and 1.25 µg of virus protein, respectively. The rates of vaccine-related systemic adverse events were similar for the 2.5 and 1.25 µg doses, but higher for the 5 µg dose (2.5 µg vs 5 µg, $p=0.0112$). The most frequently reported injection site reactions were erythema and swelling. These events were not dependent on the viral protein content and all the reactions at the injection site were graded as mild. The most frequently reported vaccine-related systemic event was fever (body temperature ≥ 37.5 °C), followed by rhinorrhea and cough. There was an increase in the frequency of fever with higher antigen content, with fewer children having fever after receiving 2.5 µg of viral protein compared with those

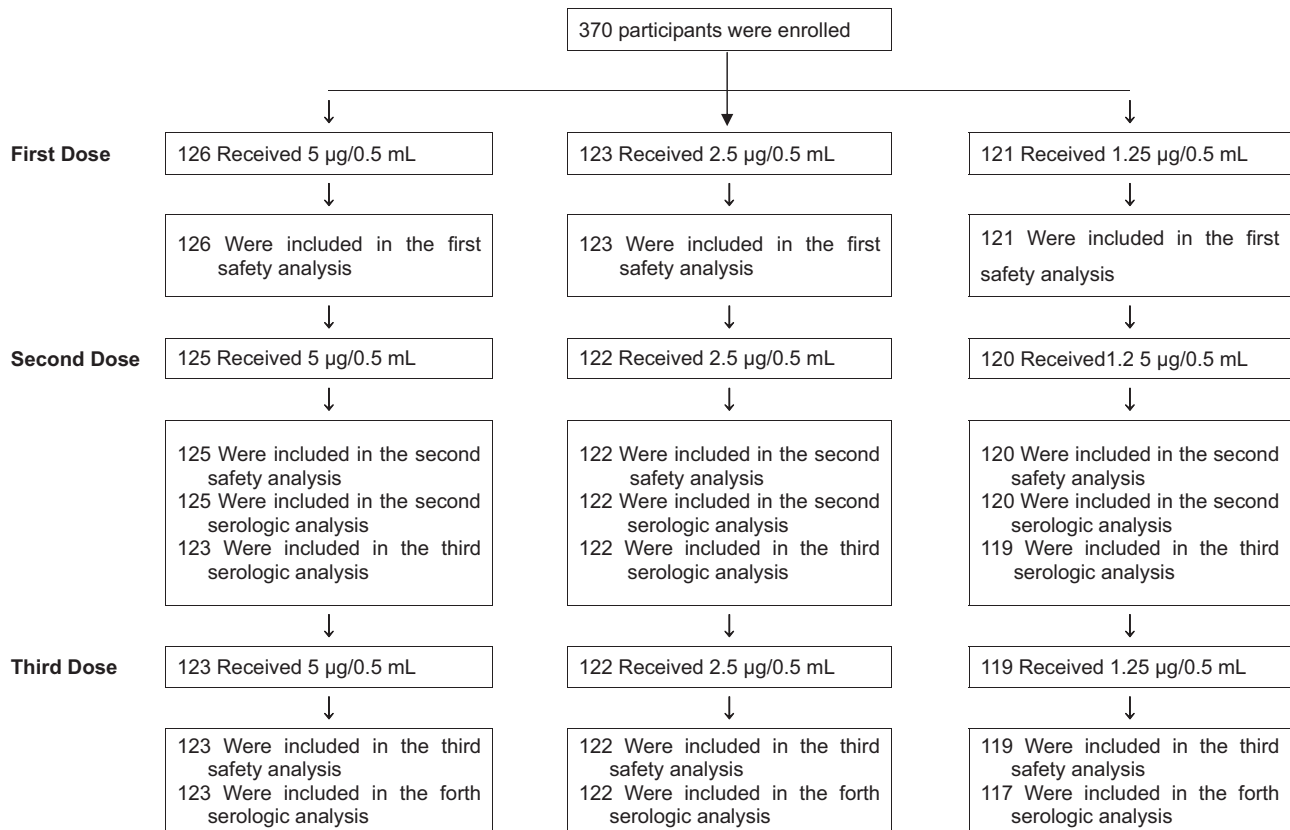


Fig. 1. Flowchart showing the disposition of the patients in the trial.

receiving the 5 µg dose ($p=0.0065$). However, the frequency of fever in children receiving the 2.5 µg dose did not differ significantly from that in children who received the 1.25 µg dose. There was no clear relationship between age and the frequency of vaccine-related adverse events. The interval between the first and

second doses did not have a marked influence on the frequency of vaccine-related adverse events after the second dose.

Most reported systematic reactions were graded as mild or moderate. Only 17 children had severe fever ($\geq 39.1^\circ\text{C}$) after vaccination, including 9 (7.1%), 4 (3.3%), and 4 (3.3%) after administration

Table 1

Vaccine-related adverse events after one or more vaccinations.

No. of subjects	5 µg		2.5 µg		1.25 µg		Fisher's exact test		
	N	n	N	n	N	n	5 µg vs 2.5 µg	5 µg vs 1.25 µg	2.5 µg vs 1.25 µg
Any symptom	126	71 (56.3%; 47.2–65.2)	123	49 (39.8%; 31.1–49.1)	121	49 (40.5%; 31.7–49.8)	$p=0.0112$	$p=0.0155$	$p=1.0000$
<3 years	41	19 (46.3%)	40	12 (30.0%)	39	16 (41.0%)	$p=0.1714$	$p=0.6586$	$p=0.3524$
≥ 3 years	85	52 (61.2%)	83	37 (44.6%)	82	33 (40.2%)	$p=0.0442$	$p=0.0085$	$p=0.6374$
Local reactions									
Erythema	126	16 (12.7%; 7.4–19.8)	123	11 (8.9%; 4.5–15.4)	121	16 (13.2%; 7.8–20.6)	$p=0.4164$	$p=1.0000$	$p=0.3135$
<3 years	41	3 (7.3%)	40	3 (7.5%)	39	5 (12.8%)	$p=1.0000$	$p=0.4764$	$p=0.4814$
≥ 3 years	85	13 (15.3%)	83	8 (9.6%)	82	11 (13.4%)	$p=0.3519$	$p=0.8266$	$p=0.4752$
Swelling	126	7 (5.6%; 2.3–11.1)	123	6 (4.9%; 1.8–10.3)	121	7 (5.8%; 2.4–11.6)	$p=1.0000$	$p=1.0000$	$p=0.7834$
<3 years	41	3 (7.3%)	40	2 (5.0%)	39	1 (2.6%)	$p=1.0000$	$p=0.6158$	$p=1.0000$
≥ 3 years	85	4 (4.7%)	83	4 (4.8%)	82	6 (7.3%)	$p=1.0000$	$p=0.5301$	$p=0.5346$
Systemic reactions									
Fever									
Any grade	126	43 (34.1%; 25.9–43.1)	123	23 (18.7%; 12.2–26.7)	121	19 (15.7%; 9.7–23.4)	$p=0.0065$	$p=0.0011$	$p=0.6119$
<3 years	41	13 (31.7%)	40	5 (12.5%)	39	7 (17.9%)	$p=0.0600$	$p=0.1998$	$p=0.5458$
≥ 3 years	85	30 (35.3%)	83	18 (21.7%)	82	12 (14.6%)	$p=0.0609$	$p=0.0024$	$p=0.3132$
Mild	126	23 (18.3%)	123	15 (12.2%)	121	12 (9.9%)	$p=0.2185$	$p=0.0692$	$p=0.6841$
Moderate	126	16 (12.7%)	123	8 (6.5%)	121	5 (4.1%)	$p=0.1322$	$p=0.0212$	$p=0.5706$
Severe	126	9 (7.1%)	123	4 (3.3%)	121	4 (3.3%)	$p=0.2544$	$p=0.2552$	$p=1.0000$
Rhinorrhea	126	16 (12.7%)	123	12 (9.8%)	121	17 (14.0%)	$p=0.5489$	$p=0.8522$	$p=0.3281$
Cough	126	15 (11.9%)	123	14 (11.4%)	121	11 (9.1%)	$p=1.0000$	$p=0.5371$	$p=0.6739$

Data are shown as a number (%; 95% CI).

Fever grades: mild, ≥ 37.5 to $\leq 38.0^\circ\text{C}$; moderate, ≥ 38.1 to $\leq 39.0^\circ\text{C}$; severe, $\geq 39.1^\circ\text{C}$.

Table 2

Vaccine-related fever after vaccinations at each dose.

	5 μ g		2.5 μ g		1.25 μ g		Fisher's exact test		
	N	n	N	n	N	n	5 μ g vs 2.5 μ g	5 μ g vs 1.25 μ g	2.5 μ g vs 1.25 μ g
Any dose	126	43	(34.1%; 25.9–43.1)	123	23	(18.7%; 12.2–26.7)	$p=0.0065$	$p=0.0011$	$p=0.6119$
First dose	126	30	(23.8%; 16.7–32.2)	123	12	(9.8%; 5.1–16.4)	$p=0.0038$	$p=0.0010$	$p=0.8238$
Second dose	125	9	(7.2%; 3.3–13.2)	122	13	(10.7%; 5.8–17.5)	$p=0.3781$	$p=0.5968$	$p=0.1503$
Third dose	123	14	(11.4%; 6.4–18.4)	122	6	(4.9%; 1.8–10.4)	$p=0.1005$	$p=0.0541$	$p=1.0000$

Data are shown as a number (%; 95% CI).

of 5, 2.5 and 1.25 μ g of viral protein, respectively. In most children with fever (71/85), the fever disappeared within two days. There was no indication that the frequency of fever was greater after the second or third dose compared to that after the first dose (Table 2). Fever after the first dose was significantly more frequent in children receiving the 5 μ g dose compared to the 2.5 ($p=0.0038$) or 1.25 μ g dose ($p=0.0010$). Of 42 children with fever who received three doses of 5 μ g of viral protein, 7 had fever after two vaccinations, and only one child had fever after all three vaccinations.

There were no serious vaccine-related adverse events, although one serious adverse event of hospitalization due to a viral infection was reported 13 days after the second dose in the 2.5 μ g group. Also, one child in the 5 μ g group did not receive the third dose due to diagnosis of Panayiotopoulos syndrome (focal seizures) about six months after receipt of the second dose. However, this symptom was judged to be unrelated to vaccination. There was no vaccine-related hypersensitivity or neurological adverse event that led to suspicion of ADEM.

3.3. Immune response

Before vaccination, most children did not have detectable antibodies against JE virus. Three children had titers ≥ 20 (two in the 5 μ g group and one in the 1.25 μ g group), of whom only one had a titer >100 . The results of immunogenicity testing using neutralizing antibody assays are shown in Table 3. After the first two doses, the titer increased to ≥ 20 in all 123 children (100%) who received 5 μ g of viral protein and in 121/122 (99.2%) and 113/119 (95.0%) of the children who received doses of 2.5 and 1.25 μ g, respectively. The seroconversion rate in children who received 5 μ g of viral protein was significantly higher than that in children who received a 1.25 μ g dose ($p=0.0132$), but not significantly higher than that in children who received a 2.5 μ g dose. Of 7 children with a titer $<1:20$ after the second dose (one in the 2.5 μ g group and 6 in the 1.25 μ g group), 6 (including the child in the 2.5 μ g group) had positive titers before receiving the third dose. All children in all three groups had positive titers after receiving the third dose.

GMTs are shown in Table 3 and Fig. 2. There was a substantially higher GMT in all three groups after the second and third doses, with a dose–response relationship. Before the third vaccination, children showed slightly reduced GMTs compared to those after the second dose, but satisfactory GMTs were obtained for all the formulated vaccines. After the third vaccination, the neutralizing antibody titer increased in all children and the GMTs in all three groups were higher than the respective GMTs after the second vaccination.

4. Discussion

Mouse-brain derived vaccine has been used for many years to reduce the incidence of JE in a number of countries, including Japan. Two doses are given subcutaneously to children 7–28 days apart, usually beginning at the age of 3 years old, with a booster dose at 6–12 months after the second dose in a primary three-dose

schedule, and an additional booster dose is given at 9–10 years old in Japan. Three doses of the Beijing-1 strain of the mouse-brain derived vaccine elicited neutralizing antibodies in 100% of Japanese children [7]. A correlation of PRNT titers with protection has been shown in non-clinical studies and an antibody threshold of 1:10 in PRNT is regarded as protective [7,13,14]. In this study, we defined PRNT₅₀ $\geq 1:20$ as a positive response based on the definition in our previous study of the conventional Beijing-1 strain of the mouse-brain derived vaccine [7].

In this study, all except one of the children who received 2.5 μ g of viral protein had titers ≥ 20 after receiving the second dose and all children showed satisfactory seroconversion rates before the third vaccination (about 6 months after the second dose), although GMTs were reduced slightly. We have previously compared the immunogenicity of Vero cell-derived and mouse brain-derived vaccines (both at doses of 5 μ g of virus protein per 0.5 mL). The results showed that the Vero cell-derived vaccines were more immunogenic and that GMTs were higher than the mouse-brain derived vaccine (data not shown). Thus, the 2.5 μ g dose of Vero cell-derived vaccine is expected to have similar clinical efficacy to the conventional vaccine, since our new vaccine at doses of 2.5 μ g has immunogenicity similar to the mouse brain-derived vaccine at doses of 5 μ g. The transitory predominant features of JE virus infection include fever, headache, and myalgia. It is likely that JE virus infection is an under-recognized cause of flu-like symptoms and our new JE vaccine also has the potential to decrease these symptoms.

There were no serious adverse reactions in the study and almost all children completed the three-dose regimen. The three-dose schedule of our new JE vaccine will probably have an acceptable tolerability profile in healthy children with a lower frequency of

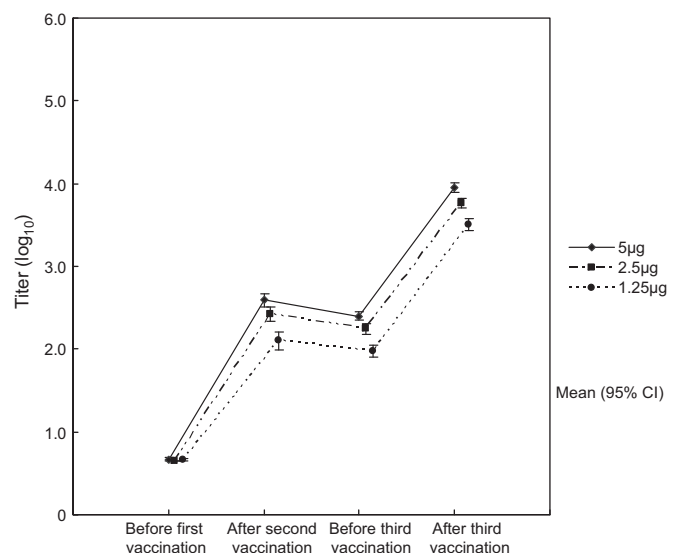
**Fig. 2.** Antibody titers before and after vaccination.

Table 3
Seroconversion rate (95% CI) and geometric mean titer (95% CI) after vaccination.

	5 μ g		2.5 μ g		1.25 μ g		Fisher's exact test			
	Seroconversion	GMT (log 10)	Seroconversion	GMT (log 10)	Seroconversion	GMT (log 10)	5 μ g vs 2.5 μ g	5 μ g vs 1.25 μ g	2.5 μ g vs 1.25 μ g	
After second dose	100% (97.0–100)	2.592 (2.512–2.671)	99.2% (95.5–100)	2.420 (2.330–2.511)	95.0% (89.3–98.1)	2.109 (2.000–2.217)	p = 0.4980	p = 0.0132	p = 0.0637	
Before third dose	100% (97.0–100)	2.402 (2.350–2.455)	100% (97.0–100)	2.244 (2.183–2.305)	98.3% (94.0–99.8)	1.982 (1.909–2.056)	–	p = 0.2427	p = 0.2407	
After third dose	100% (97.0–100)	3.957 (3.897–4.018)	100% (97.0–100)	3.766 (3.706–3.825)	100% (96.9–100)	3.498 (3.428–3.568)	–	–	–	

vaccine-related adverse reactions in children who receive a 1.25 or 2.5 μ g dose compared to those who receive a 5 μ g dose. GMTs after the third vaccination were higher than those after the second vaccination. A high antibody titer is important for persistence of immunity. Children are administered a booster dose (the fourth dose) at 9–10 years old in the public immunization program in Japan. In a clinical study, a booster dose of JEBIK®V has been shown to be safe and immunogenic in subjects who had received three doses of mouse brain-derived vaccine or Vero cell-derived vaccine. The clinical study also showed that all children had a protective antibody titer before receipt of a booster dose and that an antibody persisted for a long period of time, for at least 5 years.

Rare cases of ADEM have been reported among children who received the mouse-brain derived vaccine, and the neural tissue content of the vaccine has raised concerns about safety and possible vaccine-related neurological side-effects. Recent cases of ADEM in Japanese children have been suggested to be related to the mouse-brain derived vaccine, which led to a suspension of the recommendation for use of this vaccine in public health programs and cessation of mouse-brain derived vaccine production. JEBIK®V has less risk of serious neurological adverse reactions, since it does not contain impurities from mouse brain-derived proteins or unknown mouse-derived viruses, and does not include thimerosal as preservative. However, further investigation of the vaccine is required in large-scale surveillance studies to detect possible rare adverse events, such as hypersensitivity and neurological events.

Mouse-brain derived vaccine has been used in children in several Asian trials. A live attenuated vaccine derived from the SA 14-14-2 viral strain was licensed in China in 1989 [15–17]. The SA 14-14-2 strain of JE virus was grown in primary hamster kidney cells. This vaccine is routinely used at eight months and has contributed to reducing the incidence of JE in China. The strain is thought to be genetically stable and to have no neuroreactogenicity. However, potentially dangerous adverse events associated with reverse mutation of this virus have not been ruled out. A new inactivated Vero cell-derived, whole-virus JE vaccine derived from the SA 14-14-2 strain, IXIARO, has been licensed in several countries [18–21]. The immunogenicity of IXIARO has been shown to be at least non-inferior to that of JE-VAX, and IXIARO has a safety and tolerability profile similar to that of a placebo in healthy adults. Thus, IXIARO is indicated for prevention of JE in persons aged ≥ 17 years old, but it is not currently recommended for use in children and adolescents. The safety and immunogenicity of IXIARO in children older than 6 months of age is currently under evaluation [22]. JEBIK®V is indicated for prevention of JE in persons aged ≥ 6 months old.

We have developed a novel inactivated JE vaccine produced on Vero cell culture. The results of our study in children provide safety and immunogenicity data. Three doses of 2.5 μ g of viral protein were immunogenic and well-tolerated in this population. JEBIK®V was approved in Japan in 2009.

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