

# A Single Dose of Vero Cell–Derived Japanese Encephalitis (JE) Vaccine (Ixiaro) Effectively Boosts Immunity in Travelers Primed With Mouse Brain–Derived JE Vaccines

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(See the Editorial Commentary by Hatz, on pages 835–6.)

**Background.** A significant part of the world population lives in areas with endemic Japanese encephalitis (JE). For travelers from nonendemic countries, Vero cell–derived vaccine (JE-VC; Ixiaro) has replaced traditional mouse brain–derived vaccines (JE-MB) associated with safety concerns. The 2 vaccines are derived from different viral strains: JE-VC from the SA14-14-2 strain and JE-MB from the Nakayama strain. No data exist regarding whether JE-VC can be used to boost immunity after a primary series of JE-MB; therefore, a primary series of JE-VC has been recommended to all travelers regardless of previous vaccination history.

**Methods.** One hundred twenty travelers were divided into 4 groups: Volunteers with no prior JE vaccination received primary immunization with (group 1) JE-MB or (group 2) JE-VC, and those primed with JE-MB received a single booster dose of (group 3) JE-MB or (group 4) JE-VC. Immune responses were tested before and 4–8 weeks after vaccination using plaque reduction neutralization test (PRNT) against both vaccine strains.

**Results.** In vaccine-naïve travelers, the vaccination response rate for test strains Nakayama and SA14-14-2 was 100% and 87% after primary vaccination with JE-MB and 87% and 94% after JE-VC, respectively. Antibody levels depended on the target virus, with higher titers against homologous than heterologous PRNT<sub>50</sub> target strain ( $P < .001$ ). In travelers primed with JE-MB, vaccination response rates were 91% and 91%, and 98% and 95% after a booster dose of JE-MB or JE-VC, respectively. Subgroup analysis revealed that a higher proportion of primed (98%/95%) than nonprimed (39%/42%) volunteers responded to a single dose of JE-VC ( $P < .001$ ).

**Conclusions.** A single dose of JE-VC effectively boosted immunity in JE-MB–primed travelers. Current recommendations should be reevaluated.

**Clinical Trials Registration.** NCT01386827.

Received 30 December 2011; accepted 19 April 2012; electronically published 13 June 2012.

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**Clinical Infectious Diseases** 2012;55(6):825–34

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DOI: 10.1093/cid/cis542

Japanese encephalitis virus (JEV), a mosquito-borne flavivirus, is a significant cause of encephalitis in Asia with an estimated 50 000 cases of clinical disease annually [1]. Genotypes I and III are the most widely distributed types, although a more divergent genotype V appears to be emerging [2, 3]. The case fatality rate can be as high as 30% among persons with symptomatic disease, and approximately 50% of survivors suffer long-lasting neuropsychiatric sequelae [4]. No effective antiviral therapy exists.

For most travelers from nonendemic countries, the risk of Japanese encephalitis (JE) is generally very low, but varies depending on season, destination, duration of travel, and activities of the traveler [5–7]. Disease severity and lack of antiviral therapy support recommendations that travelers at increased risk for JE infection be vaccinated before travel [7–9].

Until 2009, inactivated mouse brain–derived JE vaccines (JE-MB; JE-VAX and Japanese Encephalitis Vaccine-GCC) were the only products available to travelers from nonendemic countries. JE-MBs are prepared by inoculating mice intracerebrally with the JEV strain Nakayama or Beijing-1 (the latter only in endemic areas). Rare but serious hypersensitivity reactions and neurological complications have been reported following immunization with JE-MB [10–14], potentially brought about by gelatin and murine neural proteins in the vaccines [11, 12, 15]. Consequently, JE-VAX vaccine production was discontinued, and a need for a safer alternative was recognized.

In 2009, an inactivated Vero cell–derived alum-adsorbed JE vaccine (JE-VC; Ixiaro) was licensed in Europe, the United States, and Australia. JE-VC is prepared from the JEV strain SA14-14-2. It does not contain gelatin or murine neural proteins; therefore, it is free from substances associated with safety concerns in JE-MBs. JE-VC was immunogenic and well tolerated in clinical trials evaluating primary immunization and booster dosing [16–21]. Postmarketing surveillance has also confirmed a favorable safety profile of JE-VC [22].

Until now, no studies have explored the potential of JE-VC to boost immunity after a primary series of JE-MB. For that reason, the Centers for Disease Control and Prevention has recommended a 2-dose primary series of JE-VC for all adults needing JE vaccine, regardless of previous immunization status [23]. Moreover, data on the administration of JE-VC simultaneously with other vaccines are scarce. The present study explored whether a single dose of JE-VC is sufficient to boost immunity in JE-MB–primed subjects. Protective efficacy of the 2 vaccines was compared by analyzing neutralizing antibodies against both of the JEV strains in the vaccines. The study was conducted at travel clinics in Finland and Sweden in travelers receiving JE-MB or JE-VC as a primary immunization series or as a booster dose after a primary series of JE-MB.

## METHODS

This was a single-blind (serologic analysis), prospective, non-randomized study conducted in a real-life setting at 2 travel clinics in Europe.

The study (EudraCT:2010-023300-27) was registered in required databases and performed in accordance with the principles outlined in the Declaration of Helsinki. Study

documents were approved by the appropriate ethics committee at each study site and all volunteers provided written informed consent.

### Study Population

The study population consisted of adult volunteers planning to travel to a JEV-endemic area in Asia, who would need protection against JE during their stay. Vaccine-naïve travelers were eligible to receive a primary series of JE-MB or JE-VC. Those with prior history of JE-MB vaccination were considered to need a booster dose if the time since previous JE-MB vaccination exceeded 3 years; the traveler planned to stay in Asia until after the recommended 3-year booster point; or the traveler had previously received only a 2-dose primary series of JE-MB. Exclusion criteria included age <18 years, acute disease at the time of enrollment, pregnancy or lactation, clinically significant immunodeficiency or immunosuppressive treatment, known history of JE, history of alcohol or drug abuse, or history of known or suspected anaphylaxis or hypersensitivity to any of the vaccine components.

### Study Procedures

The study groups are shown in Figure 1A. Travelers were enrolled into 1 of 4 groups based on their previous vaccination status and the vaccine received. Travelers with no previous JE vaccination history received a primary vaccination series either with JE-MB (group MB) or JE-VC (group VC). Travelers with a prior history of receiving a primary series of JE-MB received 1 booster dose of either JE-MB (group MB-MB) or JE-VC (group MB-VC). The choice of vaccine type (JE-MB or JE-VC) depended on the availability of the vaccines; when both were on hand, the travelers were given the choice.

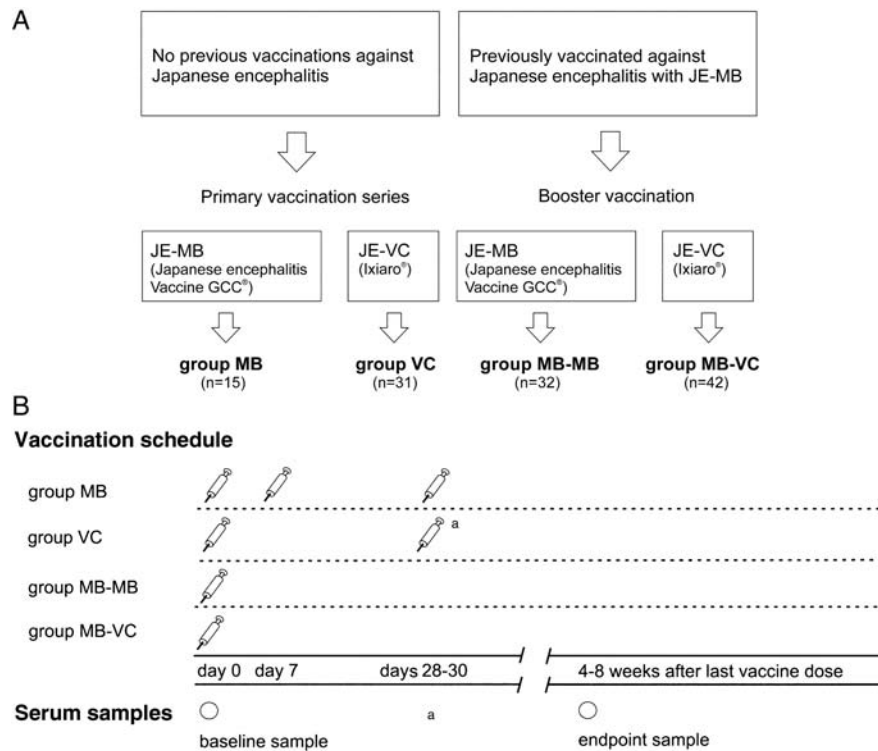
JE-MB (Japanese Encephalitis Vaccine GCC; Green Cross Corp, South Korea) was administered as 1-mL doses subcutaneously into the upper arm, and JE-VC (Ixiaro; Intercell, AG, Vienna, Austria) was injected as 0.5-mL doses into the deltoid muscle.

The time points for vaccinations and blood samples are shown in Figure 1B. Group MB received JE-MB on days 0, 7, and 28–30 (3 doses) and group VC received JE-VC on days 0 and 28 (2 doses). Booster groups received 1 dose of JE-MB or JE-VC on day 0.

Serum samples were collected before vaccination on day 0 (baseline sample) and 4–8 weeks after the last vaccine dose (endpoint sample). In group VC, a subgroup of 26 volunteers provided an extra blood sample 1 month after the first dose of JE-VC prior to receiving the second vaccine dose.

### Determination of the Neutralizing Antibody Response

Immune responses were evaluated using the plaque reduction neutralization test (PRNT) previously described [24]. As the



**Figure 1.** A, Study groups. Group MB and group VC consisted of JE vaccine-naïve travelers who received a primary vaccination series of JE-MB or JE-VC, respectively. Groups MB-MB and MB-VC consisted of travelers previously primed with JE-MB who were administered a single booster dose of JE-MB or JE-VC, respectively. The numbers of subjects enrolled in each group are indicated in the figure. A total of 38 subjects were excluded before analyses due to protocol violations: 11 in group MB, 14 in VC, 6 in MB-MB, and 7 in MB-VC. B, Timing of vaccinations and serum samples. <sup>a</sup>A subgroup of group VC provided an extra serum sample 1 month after the first vaccine dose. Abbreviations: JE, Japanese encephalitis; MB, mouse brain-derived vaccine; VC, Vero cell-derived vaccine.

study vaccines contain different JEV strains (Nakayama [JE-MB] and SA14-14-2 [JE-VC]), all serum samples were tested against both vaccine strains in order to avoid potential bias in favor of either vaccine. All the serological analyses were carried out blinded. Plaque count was determined by using the LLC-MK<sub>2</sub> plaque assay single overlay technique. In brief, sera were thawed, diluted, and heat-inactivated by incubation at 56°C for 30 minutes. Serial dilutions (1:10, 1:100, and 1:1000) of serum were made and an equal volume of diluted JE virus (Nakayama and SA14-14-2 strains), containing about 40–60 plaque-forming units/0.2 mL, was added to each serum dilution tube. Following incubation at 37°C for 60 minutes, 0.2 mL was removed from each tube and inoculated in duplicate on 6-well plates with confluent LLC-MK<sub>2</sub> cells. Each plate was incubated at 37°C for 90 minutes and the monolayers were overlaid with 4 mL of 3.0% carboxymethyl cellulose/minimum essential medium. Plates were incubated for 7 days at 37°C with 5% carbon dioxide. Plaques were counted and PRNT<sub>50</sub> titers (the reciprocal of the serum dilution that reduced the virus plaque count by 50% compared with the virus-only controls) were determined by SPSS (IBM

SPSS, Chicago, Illinois). A PRNT<sub>50</sub> titer of  $\geq 10$  was considered protective [25].

For each vaccination group, geometric mean titers (GMTs), protection rate (percentage of volunteers with protective PRNT<sub>50</sub> titers), and response rate were calculated. Protection rate was determined both at baseline and at endpoint. As some travelers had a PRNT<sub>50</sub> titer  $\geq 10$  already at baseline, the vaccination response rate was also recorded. Responders were (1) subjects with PRNT<sub>50</sub> titers  $< 10$  at baseline who achieved PRNT<sub>50</sub> titer  $\geq 10$  postvaccination or (2) those with protective titers at baseline who achieved at least a 2-fold increase in postvaccination titer. The PRNT results are reported separately for both target strains.

#### Statistical Analysis

Statistical analysis was performed with the R 2.13.0 software (R Development Core Team 2011). The level of statistical significance was set at  $\alpha = .05$ . Two-sided  $\chi^2$  tests were used to compare the vaccination response and protection rates between the groups. The differences in antibody levels were assessed using 2-sided Wilcoxon exact tests.

**Table 1. Characteristics of the 120 Travelers Included in the Study**

Characteristic	Group MB (n = 15)	Group VC (n = 31)	Group MB-MB (n = 32)	Group MB-VC (n = 42)
Age, years, median (range)	32 (18–49)	26 (18–69)	33 (19–72)	32 (19–63)
Sex				
Male	9 (60%)	12 (39%)	13 (41%)	14 (33%)
Female	6 (40%)	19 (61%)	19 (59%)	28 (67%)
Ethnic origin				
Finnish/Swedish	13/13 <sup>a</sup> (100%)	31 (100%)	29/31 <sup>b</sup> (94%)	41 (98%)
Other	0 <sup>a</sup> (0%)	0 (0%)	2/31 <sup>b</sup> (6%)	1 (2%)
Chronic diseases	0 (0%)	1 (3%)	1 (3%)	5 (12%)
Previous flavivirus vaccines				
JE vaccination	0 (0%)	0 (0%)	32 (100%)	42 (100%)
YF vaccination	2 (13%)	10 (32%)	9 (28%)	14 (33%)
TBE vaccination	4 (27%)	6 (19%)	0 (0%)	9 (21%)
Any of the 3	5 (33%)	14 (45%)	32 (100%)	42 (100%)
Previous dengue infection	0 (0%)	0 (0%)	1 (3%)	1 (2%)
Concomitant vaccines <sup>c</sup>	13 (87%)	22 (71%)	21 (66%)	20 (48%)
Previous JE-MB schedule				
2 doses	...	...	9 (28%)	16/41 <sup>b</sup> (39%)
3 doses	...	...	23 (72%)	25/41 <sup>b</sup> (61%)
Time since preceding JE-MB dose, years, median (range)	...	...	4 (1–16)	5.2 (1–20.5)

All data are No. (%) unless otherwise specified. There were no differences in the background characteristics between groups MB and VC or groups MB-MB and MB-VC (as analyzed with 2-sided  $\chi^2$  tests and Wilcoxon exact tests), except that in the distribution of previous TBE vaccinations in booster groups: 9 of 42 (21%) in group MB-VC versus none in group MB-MB.

Abbreviations: JE, Japanese encephalitis; JE-MB, mouse brain-derived Japanese encephalitis vaccine; MB, mouse brain-derived vaccine; TBE, tick-borne encephalitis; VC, Vero cell-derived vaccine; YF, yellow fever.

<sup>a</sup> Data missing for 2 subjects.

<sup>b</sup> Data missing for 1 subject.

<sup>c</sup> Other vaccines given or prescribed simultaneously with JE vaccine, including 2 simultaneous TBE vaccinations in group MB and 1 simultaneous TBE vaccination in group VC.

## RESULTS

### Study Group Characteristics

One hundred fifty-eight travelers enrolled and 38 were excluded before analyses due to protocol violations (2 failed the eligibility assessment, 5 did not receive all vaccine doses, and 31 failed to provide follow-up samples). Thus, 120 travelers were included in the final analyses (82 from Finland, 38 from Sweden).

Data on baseline demographic characteristics, previous flaviviral contacts, and concomitant vaccinations are shown in Table 1. The study population included 72 female (60%) and 48 male (40%) travelers between the ages 18 and 72 years (median, 31.0 years). Most subjects (97%) were of Finnish or Swedish origin. Ninety-four percent of participants were generally healthy and none of those with chronic diseases were considered to have clinically significant immunosuppression.

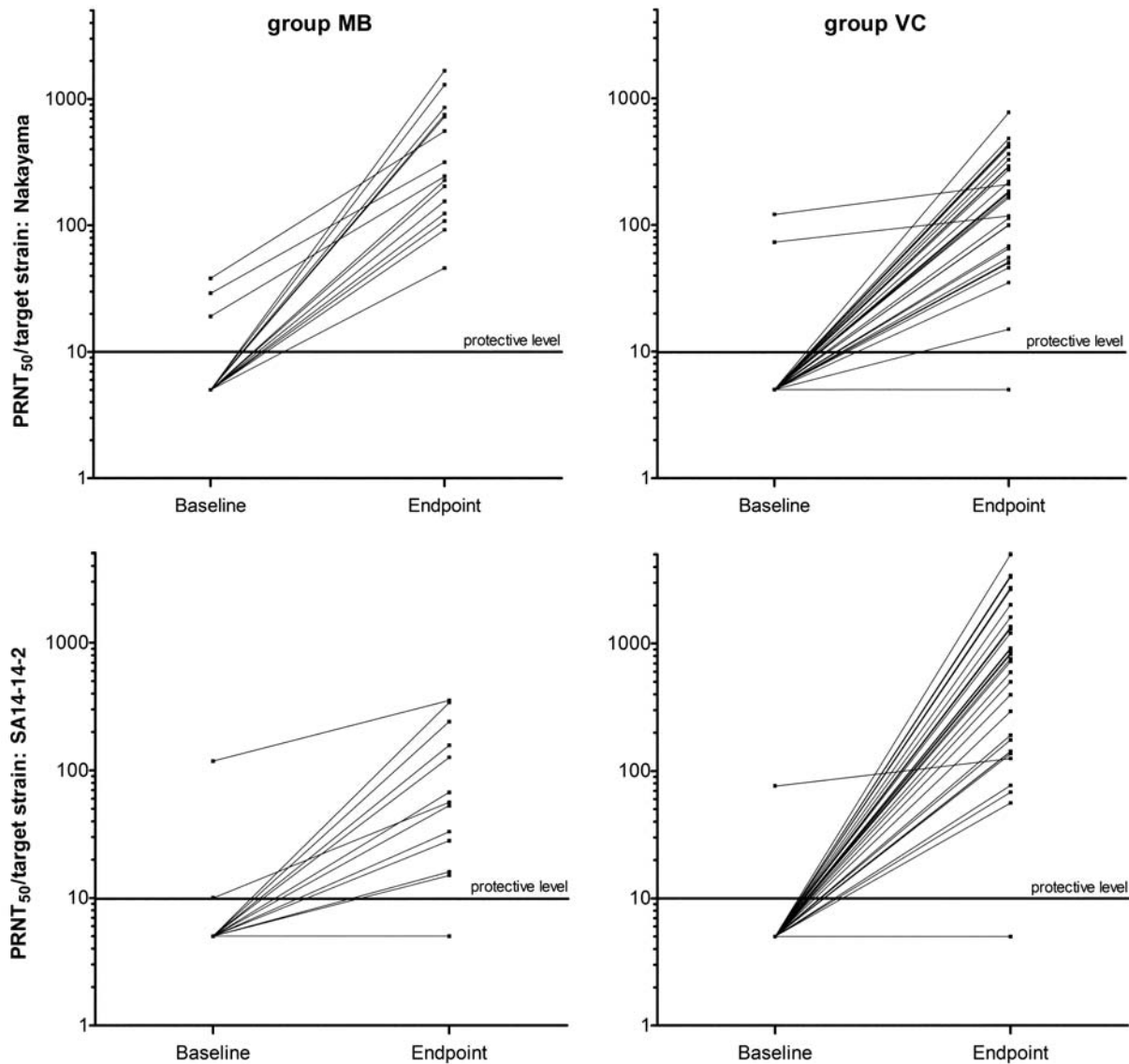
All subjects in the booster groups had received a 2-dose (39%) or 3-dose (61%) schedule of JE-MB from 1 to 20.5 years previously (median, 5.0 years).

Overall, 63% of the travelers received other vaccines (or a prescription for them) at the same visit, most commonly typhoid fever vaccine (48% of travelers).

### Serological Analyses

#### Primary Vaccination Groups

PRNT<sub>50</sub> titers in the primary vaccination groups are shown for each individual in Figure 2 and summarized in detail in Table 2. The vaccination response rates were 100% and 87% in group MB and 87% and 94% in group VC for strains Nakayama and SA14-14-2, respectively. The endpoint protection rates were 100% and 87% in group MB and 94% and 97% in group VC for target strains Nakayama and SA14-14-2, respectively. There were no significant differences between the groups. Notably, the endpoint PRNT<sub>50</sub> titers differed significantly between the 2 groups depending on the JEV strain used in the assay. When Nakayama was the target strain, the group vaccinated with Nakayama-based vaccine (JE-MB) reached higher titers than the group receiving SA14-14-2-based vaccine (JE-



**Figure 2.** Immune response to primary vaccination with JE vaccines in adult travelers: PRNT<sub>50</sub> titers (reciprocal of the serum dilution that reduced the virus plaque count by 50% compared with the virus-only controls) are shown before and 4–8 weeks after a vaccination series of JE-MB (group MB; n = 15) or JE-VC (group VC; n = 31). Abbreviations: JE, Japanese encephalitis; MB, mouse brain–derived vaccine; VC, Vero cell–derived vaccine.

VC) ( $P < .05$ ; Table 2). Similarly, when SA14-14-2 was the target strain, PRNT<sub>50</sub> titers were higher in the group receiving SA14-14-2–based vaccine ( $P < .001$ ; Table 2). Furthermore, an analysis within each primary vaccination group showed higher endpoint PRNT<sub>50</sub> titers against the homologous than the heterologous target strain ( $P < .001$  in both groups).

#### Booster Vaccination Groups

The PRNT titers for the booster groups are shown for each individual in Figure 3 and summarized in more detail in Table 3. Endpoint protection rates for the target strains Nakayama and SA14-14-2 were 100% and 97% in the MB-MB

group and 100% and 98% in the MB-VC group, respectively. One subject per group failed to reach a protective PRNT<sub>50</sub> titer against SA14-14-2, whereas all subjects had protective endpoint titers against Nakayama. Vaccination response rates for the target strains Nakayama and SA14-14-2 were 91% and 91% in the MB-MB group and 98% and 95% in the MB-VC group, respectively.

Due to slight differences in baseline antibody levels between booster groups, a subanalysis of individuals with baseline PRNT<sub>50</sub> titers  $< 10$  was performed, which showed a 100% response rate for both booster groups against both target strains (9 of 9 in group MB-MB and 17 of 17 in group MB-VC).

**Table 2. Vaccination Response Rates, Protection Rates, and Geometric Mean Titers on Day 0 and 4–8 Weeks After Completing Primary Vaccination Series of Mouse Brain–Derived Japanese Encephalitis Vaccine<sup>a</sup> or Vero Cell–Derived Japanese Encephalitis Vaccine<sup>b</sup>**

Group	Vaccination Response Rate		Protection Rate				Geometric Mean Titer			
			Baseline		Endpoint		Baseline		Endpoint	
	MB	VC	MB	VC	MB	VC	MB	VC	MB	VC
PRNT Nakayama <sup>c</sup>	100% (15/15)	87% (27/31)	20% (3/15)	6% (2/31)	100% (15/15)	94% (29/31)	<10	<10	304	120
PRNT SA14-14-2 <sup>c</sup>	87% (13/15)	94% (29/31)	13% (2/15)	3% (1/31)	87% (13/15)	97% (30/31)	<10	<10	46	499

Abbreviations: MB, mouse brain–derived vaccine; PRNT, plaque reduction neutralization test; VC, Vero cell–derived vaccine.

<sup>a</sup> Group MB: 3 doses.

<sup>b</sup> Group VC: 2 doses.

<sup>c</sup> The titers of neutralizing antibodies were analyzed with the PRNT<sub>50</sub> (reciprocal of the serum dilution that reduced the virus plaque count by 50% compared with the virus-only controls) using Nakayama and SA14-14-2 as target strains.

Another subanalysis among subjects with baseline PRNT<sub>50</sub> titers <10 revealed that a single dose of JE-VC induced more frequently protective levels of neutralizing antibodies in primed (group MB-VC) than in nonprimed (group VC) volunteers (100% vs 40%,  $P < .001$ ). Consistently, the responses were also significantly higher in the primed than in the nonprimed group (GMTs, 236 and 236 in the primed vs 9 and 12 in the nonprimed groups for the target strains Nakayama and SA14-14-2, respectively,  $P < .001$ ) (Table 4).

No significant differences were observed between the booster vaccination groups in response or endpoint protection rates. However, differences were observed in endpoint PRNT<sub>50</sub> titer levels depending on the PRNT target strain both in within- and between-group analyses.

Within group MB-MB, significantly higher PRNT<sub>50</sub> titers were observed against the homologous than the heterologous target strain ( $P < .001$ ). Within group MB-VC (ie, following a heterologous booster vaccine), the endpoint PRNT<sub>50</sub> titers were similar against both target strains. Between-group comparisons with Nakayama as the target strain showed significantly higher PRNT<sub>50</sub> titers for the homologous (MB-MB) than for the heterologous (MB-VC) booster group ( $P < .05$ ). When SA14-14-2 was the target strain, no significant difference in PRNT<sub>50</sub> titers was observed between groups.

#### Subanalysis Based on Previous Vaccinations Against Other Flaviviruses

When subgroups of volunteers with a history of previous vaccination against tick-borne encephalitis (TBE) or yellow fever (YF) were compared to those without such history, no differences were observed for 3 of the 4 vaccine groups (data not shown). In group VC, travelers with a previous YF vaccination history had higher endpoint antibody titers than subjects without a history of YF vaccination (GMTs, 197 vs 95 for travelers with or without a history of previous YF vaccination,

respectively;  $P < .05$ ). This difference was only found when Nakayama was the PRNT target strain.

## DISCUSSION

### Justification of the Study

The new Vero cell–derived inactivated JE vaccine (JE-VC) has replaced the former mouse brain–derived vaccines (JE-MB) in Europe, the United States, Canada, and Australia. A key question in clinical practice is whether the immunity in those primed with JE-MB can be boosted with JE-VC. Lack of data has resulted in recommendations suggesting 2 doses of JE-VC for all vaccinees, regardless of previous vaccination history. We present the first data to address this question.

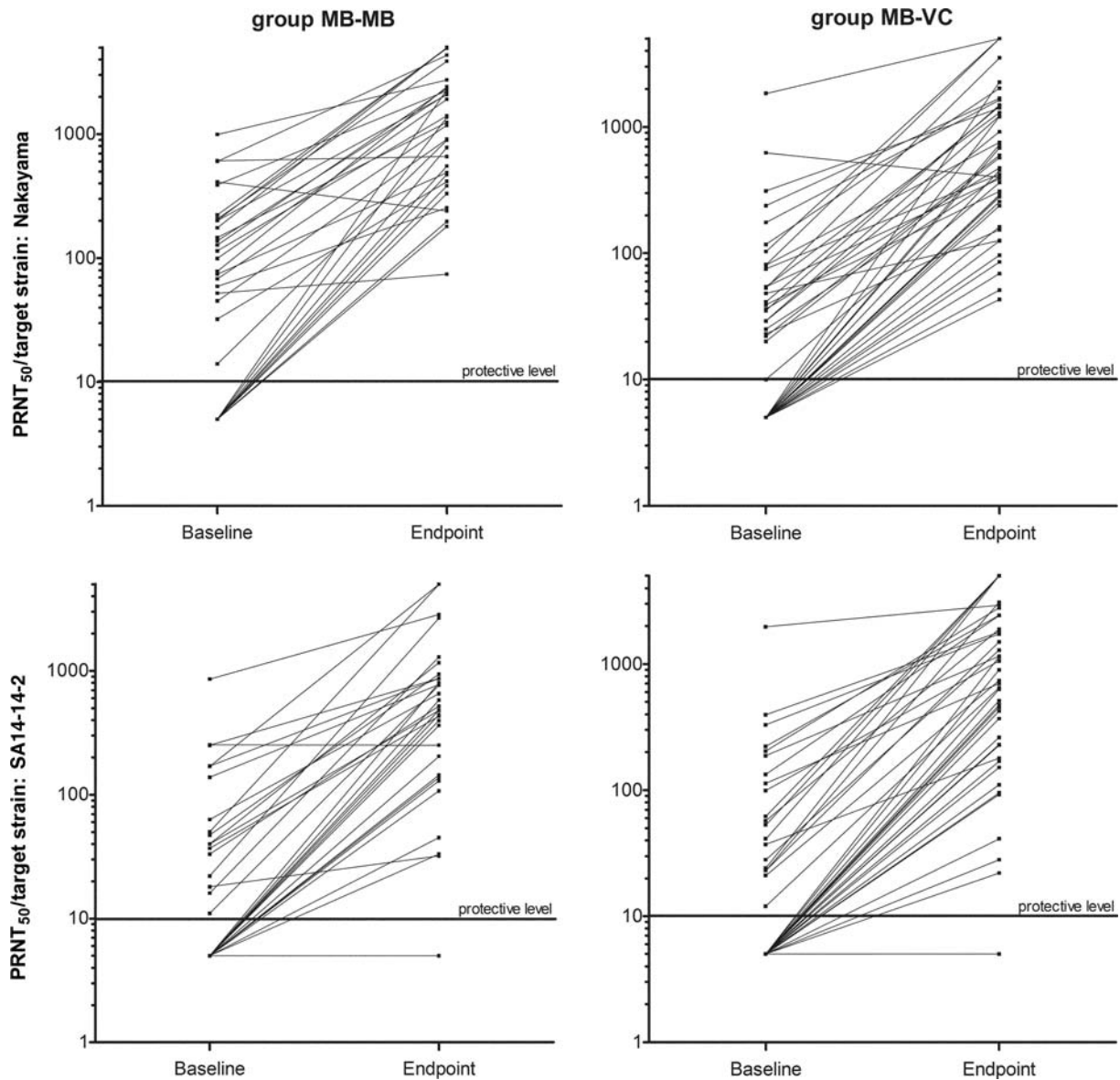
### Immunogenicity of Primary Vaccination

The efficacy of purified JE-MB vaccines was first demonstrated in a large placebo-controlled randomized trial in Thailand in 1984–1985 [26]. Thereafter, placebo-controlled JE vaccine efficacy trials were considered unethical, and the accepted correlate of protection became a PRNT<sub>50</sub> titer of  $\geq 10$  [25]. Later, a 2-dose primary schedule with JE-MB was found suboptimal for subjects from nonendemic areas, whereas a 3-dose schedule resulted in satisfactory seroconversion rates [27–30]. The response rates in the current study after 3 doses of JE-MB are in line with previous reports for adults in nonendemic settings [16, 27–29].

The immunogenicity of a 2-dose primary series of JE-VC has been demonstrated in several company-coordinated, randomized controlled noninferiority trials [16, 18, 19]. The present study was the first investigator-initiated study of JE-VC. Immune responses were consistent with previous studies using JE-VC vaccine strain SA14-14-2.

### Immunogenicity of Booster Doses

JE-MB and JE-VC vaccines are prepared from different virus strains, which raises the question whether the viral strains



**Figure 3.** Immune response to booster vaccination with JE vaccines in adult travelers previously primed with JE-MB: PRNT<sub>50</sub> titers (reciprocal of the serum dilution that reduced the virus plaque count by 50% compared with the virus-only controls) are shown before and 4–8 weeks after a booster dose of JE-MB (group MB-MB; n = 32) or JE-VC (group MB-VC; n = 42). Abbreviations: JE, Japanese encephalitis; MB, mouse brain-derived vaccine; VC, Vero cell-derived vaccine.

Nakayama and SA14-14-2 are immunologically similar enough to elicit significant cross-reactive immune responses with booster dosing. Lack of proper anamnestic immune response would prevent the use of heterologous vaccine for boosting. As no data have been available to address this question, the only possibility has been to also recommend primary series of 2 doses of JE-VC to JE-MB-primed travelers requiring a booster dose. We observed significant cross-reactivity between the 2 vaccines: 95%–98% of JE-MB-primed travelers had a protective response after a single booster dose of JE-VC.

Protection and response rates were similar regardless of which of the 2 vaccines was used for boosting. Moreover, a significantly higher proportion of JE-MB-primed than nonprimed volunteers responded to a single dose of JE-VC.

Our results indicate that only 1 dose of JE-VC is needed to boost immunity in travelers primed with JE-MB. This finding has important practical consequences because both costs of vaccination and time required to reach protective immunity are reduced (eg, in Finland and Sweden the price for 1 dose is more than €100). There is an ongoing follow-up study to

**Table 3. Vaccination Response Rates, Protection Rates, and Geometric Mean Titers on Day 0 and 4–8 Weeks After a Single Booster Dose of Mouse Brain–Derived Japanese Encephalitis Vaccine (JE-MB; Group MB-MB) or Vero Cell–Derived Japanese Encephalitis Vaccine (JE-VC; Group MB-VC) in Volunteers Previously Primed With JE-MB**

Group	Vaccination Response Rate		Protection Rate				Geometric Mean Titer			
			Baseline		Endpoint		Baseline		Endpoint	
	MB-MB	MB-VC	MB-MB	MB-VC	MB-MB	MB-VC	MB-MB	MB-VC	MB-MB	MB-VC
PRNT Nakayama <sup>a</sup>	91% (29/32)	98% (41/42)	72% (23/32)	60% (25/42)	100% (32/32)	100% (42/42)	54	23	1017	523
PRNT SA14-14-2 <sup>a</sup>	91% (29/32)	95% (40/42)	53% (17/32)	48% (20/42)	97% (31/32)	98% (41/42)	19	19	398	504

Abbreviations: MB, mouse brain–derived vaccine; PRNT, plaque reduction neutralization test; VC, Vero cell–derived vaccine.

<sup>a</sup> The titers of neutralizing antibodies were analyzed with the PRNT<sub>50</sub> (reciprocal of the serum dilution that reduced the virus plaque count by 50% compared with the virus-only controls) assay using Nakayama and SA14-14-2 as target strains.

address the longevity of the immune response in the same traveler population.

### Strain Specificity in the PRNT<sub>50</sub> Assay

Five genotypes of JE virus are considered to exist in nature [3]. All currently available JE vaccines are prepared from genotype III strains. In most of the previous JE vaccine immunogenicity studies, only the homologous virus strain was used in PRNTs for each vaccine [16, 18–20, 31, 32]. In contrast, we tested all serum samples from all vaccinees against both of the vaccine strains included in JE-MB and JE-VC (Nakayama and SA14-14-2).

Significant differences were observed in the titers of neutralizing antibodies in a target virus–dependent fashion. The titers were significantly higher when the PRNT target strain was homologous to the vaccine JEV strain, consistent with some previous studies [33, 34]. In the primary vaccination groups, this effect was seen both in the between-group and within-group analyses. In the booster groups, the levels of neutralizing antibodies depended on the target strain only in the MB-MB group. In the MB-VC group, by contrast, similar

responses were found to both target strains, which is logical, since the volunteers within this group had been vaccinated with both vaccine types. To sum up, these data clearly indicate that assessing immunogenicity of heterologous JE vaccines requires testing against both strains to avoid a bias favoring either vaccine.

### Limitations of the Study

Had it been possible, a randomized controlled trial would have been ideal for addressing the question of JE boosting. The fact that the present study was conducted at travel clinics in a real-life setting can be considered as a strength, as the results will mostly be applied to travelers in similar situations. However, this setting also poses some confounding factors, including fairly small group sizes, receipt of other simultaneous vaccines at the same visit, and the fact that some of the participants visited Asia before providing the postvaccination sample. A natural booster to JE immunity acquired during the trip cannot be excluded, yet it appears improbable.

Previous and simultaneous vaccinations against other flaviviruses were not restricted, resulting in some heterogeneity in

**Table 4. Subgroup Analysis of Response Rates, Protection Rates, and Geometric Mean Titers After a Single Dose of Vero Cell–Derived Japanese Encephalitis Vaccine in Previously Primed (Group MB-VC) and Nonprimed (Group VC) Travelers**

	Response Rate After 1 Dose of JE-VC <sup>a</sup>		Protection Rate After 1 Dose of JE-VC <sup>b</sup>		Geometric Mean Titers After 1 Dose of JE-VC <sup>b</sup>	
	Nonprimed	Primed	Nonprimed	Primed	Nonprimed	Primed
PRNT Nakayama	39% (10/26)	98% (41/42)	40% (10/25)	100% (17/17)	<10	236
PRNT SA14-14-2	42% (11/26)	95% (40/42)	40% (10/25)	100% (17/17)	12	236

Responders were defined as (1) subjects with PRNT<sub>50</sub> (reciprocal of the serum dilution that reduced the virus plaque count by 50% compared with the virus-only controls) titers <10 at baseline who achieved PRNT<sub>50</sub> titer ≥ 10 postvaccination or (2) those with protective titers at baseline who achieved at least a 2-fold increase in titers postvaccination.

Abbreviations: JE, Japanese encephalitis; MB, mouse brain–derived vaccine; PRNT, plaque reduction neutralization test; VC, Vero cell–derived vaccine.

<sup>a</sup> All subjects included, regardless of preceding baseline PRNT<sub>50</sub> titers.

<sup>b</sup> Only subjects with baseline PRNT<sub>50</sub> titers ≤10 included.



the TBE vaccination status between the groups (cf Table 1). Data on the influence of preexisting TBE immunity on immune responses to JE vaccines are scarce. In one study, vaccine-induced TBE immunity enhanced neutralizing JEV-specific antibody responses after a single dose of JE-VC [35]. Moreover, seroprotection elicited by 2 doses of JE-VC appears to last longer in populations with a high coverage of preceding TBE vaccinations than in those without such coverage [18, 19]. We observed no differences in JEV antibody responses with respect to TBE vaccination status, yet the uneven distribution and small number of previous or simultaneous TBE vaccinations limits firm conclusions based on these data.

When considering concomitant use of nonflaviviral vaccines, one clinical trial reported no influence on the immune response to either JEV or hepatitis A when these vaccines were administered concomitantly [36].

Notably, immune responses to JE vaccines were satisfactory in all groups, implying that simultaneous administration of other vaccines should not be a major concern with respect to JEV immunity induced.

## Conclusions

The PRNT assay favored the vaccine homologous to the target strain used, indicating that future studies comparing the immunogenicity of JE-MB and JE-VC should always include assays for both strains. The present study was the first to explore the use of heterologous JE vaccine in boosting. A single dose of JE-VC was found to have the potential to elicit protective levels of neutralizing antibodies in JE-MB-primed travelers. This implies that it is time to reevaluate the current vaccination recommendations requiring 2 doses for all travelers regardless of previous JE vaccine history.

## Notes

**Acknowledgments.** The authors thank the personnel in Travel Clinic, Lääkärikeskus Aava, Postitalo, Finland, and Cityakuten/Wasavaccination, Sweden, for help in collecting blood samples and recruiting patients; Lisa DeTora (Novartis Vaccines and Diagnostics) for linguistic revisions; and Katrin Dubischar-Kastner and Shailesh Dewasthaly (Intercell) for comments on a late-stage draft of the manuscript.

**Financial support.** This work was supported in part by Novartis Vaccines and Diagnostics (assay expenses), by a specific Finnish governmental subsidy for health science research (to E. E.), and by Wiipurilaisen Osakunnan Stipendisäätiö (to E. E.). The funding sources had no involvement in study design, data collection, analysis, interpretation of data, writing of the report, or the decision to submit the article for publication.

**Potential conflicts of interest.** A. K. and L. R. have participated as members in an advisory board for and received honoraria from Novartis. A. K. has acted as a consultant on vaccination immunology to and has received research funding from Crucell. A. K., L. L., J. R., and L. R. have received honoraria for lectures from Crucell, GlaxoSmithKline, and Pfizer. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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