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Protocol: Assessment of long term immunogenicity of Japanese encephalitis live attenuated SA-14-14-2 vaccine in previously vaccinated Bangladeshi children and antibody response and safety to a booster dose

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	adverse event
CDIBP	Chengdu Institute of Biological Products
CD-JEV	prequalified live, attenuated SA-14-4-2 Japanese encephalitis vaccine
CI	confidence interval
CRF	case report form
CSR	clinical study report
DSMB	data safety and monitoring board
ELISA	enzyme-linked immuno assay
ERC	ethical review committee
FSC	fixed site clinic
GACVS	WHO Global Advisory Committee on Vaccine Safety
GCP	good clinical practice
GMC	geometric mean concentration
GMP	good manufacturing practice
GMT	geometric mean titer
HDSS	health and demographic surveillance system
icddr,b	International Center for Diarrheal Diseases Research, Bangladesh
ICH	International Conference on Harmonisation
ICMJE	International Committee on Medical Journal Editors
IEC	Independent or Institutional Ethics Committee
IgM	immunoglobulin M
IRB	Institutional Review Board
ITT	intent to treat analysis set
JE	Japanese encephalitis
PHK	primary hamster kidney
PP	per protocol analysis set
PRNT	plaque reduction neutralization test
RRC	research review committee
SAE	serious adverse event
SAGE	WHO Strategic Group of Experts
SOP	Standard Operating Procedure
WIRB	Western Institutional Review Board
WHO	World Health Organization

A. PROJECT SUMMARY

Principal Investigator: K. Zaman
Research Protocol Title: Assessment of long term immunogenicity of Japanese encephalitis live attenuated SA-14-14-2 vaccine in previously vaccinated Bangladeshi children and antibody response and safety to a booster dose
Proposed start date: June 2015 Estimated end date: June 2017
Japanese encephalitis (JE) virus, a mosquito-borne flavivirus, is the leading cause of viral neurological disease and disability across temperate and tropical zones of Asia. In 2012 PATH sponsored a double-blind, randomized controlled trial (icddr,b Protocol #11050, JEV05) among 818 Bangladeshi infants aged between 10 and 12 months to compare the immunogenicity of three lots of live attenuated SA-14-14-2 Japanese encephalitis vaccine (CD-JEV) manufactured in the Chengdu Institute of Biological Products' newly constructed Good Manufacturing Practice facility and one lot of CD-JEV manufactured in the pre-existing facility. Seroprotection rates (anti-JE neutralizing antibody titer $\geq$1:10) ranged from 80 to 86 percent for all four lots of vaccine, with an average geometric mean titer (GMT) of 56.[1,2] These GMT and seroprotection rates were lower than those seen in other populations, including a study conducted in Philippines.[3] This study aims to understand the persistence of the antibody response to guide the Bangladesh Ministry of Health and Family Welfare, the World Health Organization, and other ministries of health in determining if and when a booster dose of CD-JEV may be advisable. The proposed study is a phase 4 open-label trial enrolling Bangladeshi children who had previously participated in JEV05 (icddr,b Protocol #11050). Eligible participants who still live in the area will be identified using the local health and demographic surveillance system and asked to participate. A 2 ml blood sample will be collected from all JEV05 participants in 2015 and 2016, which is three and four years after initial vaccination, respectively. The sera will be tested using a 50% plaque reduction neutralization test (PRNT-50); a JE neutralizing antibody titer $\geq$ 1:10 will be considered seroprotective.[1] In 2016, four years after receiving the initial dose of CD-JEV, eligible participants will be vaccinated with a second subcutaneous dose of CD-JEV. To evaluate the persistence of immunological memory, the response to this boosting dose will be measured in a serum sample collected 7 and 28 days after vaccination. This sample will be tested for anti-JE neutralizing antibodies. Following the booster dose, children will be monitored for immediate reactions during the first 30 minutes, solicited injection site and systemic adverse reactions for 7 days, unsolicited adverse events and serious adverse events for 28 days.

B. DESCRIPTION OF THE RESEARCH PROJECT

1. HYPOTHESIS TO BE TESTED

Does this research proposal involve testing of hypothesis: ☒ No ☐ Yes (describe below)

The intention of this study is to describe the persistence of the antibody response three and four years post vaccination with live attenuated SA-14-14-2 Japanese encephalitis vaccine (CD-JEV) and to describe the kinetics of the antibody response after a booster dose. No hypotheses are defined. The results will be descriptive.

2. SPECIFIC OBJECTIVES

2.1. Primary objective

To assess the long-term antibody response three and four years after CD-JEV vaccination among children who received a single dose of vaccine between 10-12 months of age.

2.2. Secondary objectives

To evaluate the antibody response following a booster dose of CD-JEV given at five years of age, among children who previously received a single dose of CD-JEV between 10 and 12 months of age.

To determine the safety profile of a CD-JEV booster dose given to children four years after initial vaccination.

2.3. Exploratory objective

To further describe the antibody response to a booster dose by differentiating between a primary and secondary antibody response at 7 days post-booster dose.

2.4. Outcome measures

The immunologic endpoints will be:

- Seroprotection rate defined as the proportion of study participants with an anti-Japanese encephalitis (JE) neutralizing antibody titer ≥1:10 as measured by 50% plaque reduction neutralization test (PRNT-50).
- Geometric mean titer (GMT) of anti-JE neutralizing antibody as measured by PRNT-50.

The safety endpoints will be:

- Frequency counts and percentage of participants reporting immediate unsolicited adverse events (AEs) occurring within 30 minutes of booster vaccination.
- Frequency counts and percentage of participants reporting solicited local and systemic AEs occurring within 7 days of booster vaccination.
- Frequency counts and percentage of participants reporting unsolicited AEs and serious adverse events (SAEs) occurring within 28 days of booster vaccination.

The exploratory immunologic endpoint will be:

- Proportion of participants with demonstrated seropositivity for anti-JE immunoglobulin M (IgM) prior to the booster dose and 7 days post booster dose as measured by enzyme-linked immuno assay (ELISA). Seropositivity will be defined per the ELISA manufacturer's kit instructions.

3. BACKGROUND OF THE PROJECT INCLUDING PRELIMINARY OBSERVATIONS:

3.1. Disease burden

JE virus, a mosquito-borne flavivirus, is the leading cause of viral neurological disease and disability across temperate and tropical zones of Asia. The number of cases, the severity of disease, and the profound neurologic sequelae in survivors make JE the most important viral cause of encephalitis in Asia.[4-6] In Asia, more than 750 million children live in areas where they are at risk for JE virus infections.[4,5] Although the incidence in children less than 15 years of age is higher than the rate in other age groups, adult cases may also be seen in JE-endemic areas.[7]

3.2. JE in Bangladesh

The first confirmed outbreak of JE in Bangladesh was recognized in 1977.[8] However, JE was not identified again until 2003 when a new initiative was undertaken to assess the impact of JE disease.[8,9] In collaboration with the U.S. Centers for Disease Control and Prevention, the International Center for Diarrheal Diseases Research in Bangladesh (icddr,b) conducted a hospital-based surveillance study in four tertiary hospitals in Dhaka, Rajshahi, Mymensigh, and Sylhet to identify the etiology of acute encephalitis cases. Between 2003 and 2005, 492 persons with acute encephalitis were serologically evaluated for JE virus infection and 4% had evidence of recent infection. In 2007, hospital-based surveillance was established at three tertiary hospitals in Rajshahi, Chitagong, and Khulna.[9,10] Patients hospitalized with meningitis and/or encephalitis were tested for JE virus-specific Immunoglobulin M antibodies during an 11-month period. Of 632 patients with meningitis and/or encephalitis, 6% had laboratory evidence of recent JE virus infection.[10] These studies suggest that JE is an important cause of encephalitis in Bangladesh.

3.3. Previous experience with CD-JEV

CD-JEV has been used since 1988, with over 400 million doses administered internationally. The vaccine is currently licensed in twelve countries, and, in 2013, vaccine produced at a newly constructed Good Manufacturing Practice (GMP) compliant production facility was prequalified by the World Health Organization (WHO). Following prequalification, CD-JEV was also selected for use in the 2015 GAVI-supported catch-up campaigns in children less than 15-years-old.

Like other parenterally administered vaccines, vaccination with CD-JEV vaccine may cause local and systematic side effects in some children. These include pain at the injection site for a short while after injection and swelling, redness, and tenderness at the injection site. Fever may occur and, in some cases, a rash may appear in the initial days following the vaccination. Because CD-JEV is a live attenuated vaccine, there is a theoretical risk that systemic disease including encephalitis could occur if the vaccine strain replicates unchecked. To date, this has not been reported with CD-JEV. No reports of severe allergic reactions or death have been associated with CD-JEV.

From 2005-2008, the WHO Global Advisory Committee on Vaccine Safety (GACVS) and the WHO Strategic Advisory Group of Experts (SAGE) on Immunization reviewed the safety of CD-JEV and other JE vaccines.[11-14] These reviews stated that CD-JEV is well tolerated across a wide age range and can safely be given to children as young as 9 months of age. The reviewers noted that no SAEs were attributed to CD-JEV in 12 clinical studies conducted from 1992 through 2008 in China, India, the Philippines, South Korea, Sri Lanka, and Thailand.[3,15-26] In 2013, the WHO GACVS reviewed the safety of CD-JEV again and concluded that it had an excellent safety profile.[27]

Seroprotection rates following a single dose of CD-JEV ($\geq 10^{5.4}$ plaque-forming units) have been greater than 90% in most clinical trials. Several early Chinese studies have shown a dose-response gradient with 85% to 100% seroconversion after a single dose of CD-JEV vaccine was given to children 1 to 12 years of age.[15,18,19,23-25] In one of the first immunogenicity studies performed outside of China, 95% of participants aged 9 to 15 months were

seroprotected within 90 days after CD-JEV vaccination. A Korean study of 68 children one to three years of age showed high seroconversion rates (96%) four weeks after one dose of JE live attenuated CD-JEV.[21]

Although the number of clinical studies are limited, the neutralizing antibody response following immunization with vaccine remains high for up to four years after vaccination. A long-term study in Nepal demonstrated that 90% of the study participants had a high level of neutralizing antibody four years after vaccination.[28] When co-administered with measles vaccine in healthy infants in the Philippines, the long-term immunogenicity of SA 14-14-2 vaccine in infants as young as eight months was demonstrated. Specifically, seroprotective titers against JE were maintained in over 80% of study participants three years after vaccination (Table 1).[3,16]

Table 1. Seropositivity rates and anti-measles IgG geometric mean concentrations and anti-JE geometric mean titers in Philippine infants vaccinated with JE and measles vaccines.

		Group 1 (CD-JEV then MV) (N=88)		Group 2 (concomitant) (N=222)[a]		Group 3 (MV then CD-JEV) (N=180)[b]	
		% seropositive (95% CI)	GMC/GMT (95% CI)	% seropositive (95% CI)	GMC/GMT (95% CI)	% seropositive (95% CI)	GMC/GMT (95% CI)
Measles vaccine response (anti-measles IgG)	Day 0	1.1 (0.0-6.2)	12.8 (10.2-16.2)	0.0 (0.0-1.7)	7.4 (6.3-8.8)	0.0 (0.0-2.1)	7.0 (5.8-8.5)
	Day 28	88.6 (80.1-94.4)	318.9 (273.0-372.6)	91.8 (87.3-95.1)	301.9 (269.0-338.9)	86.5 (80.6-91.2)	262.5 (222.2-310.2)
JE vaccine response (anti-JE neutralizing antibody)	Day 0	3.4 (0.7-9.6)	5.7 (4.9-6.5)	5.4 (2.8-9.3)	5.7 (5.2-6.1)	6.1 (3.1-10.7)	5.9 (5.3-6.6)
	Day 28	92.1 (84.3-96.7)	202.8 (140.5-292.9)	90.5 (85.9-94.1)	155.0 (123.5-194.5)	90.6 (85.3-94.4)	139.4 (109.5-177.5)
	Year 1	90.2 (82.2-95.4)	106.8 (71.5-159.4)	84.0 (78.3-88.6)	80.7 (62.4-104.3)	85.9 (80.5-90.3)	74.9 (59.2-94.9)
	Year 2	81.7 (72.4-89.0)	68.8 (47.3-100.1)	83.0 (77.3-87.8)	61.6 (49.1-77.4)	81.7 (75.8-86.6)	70.1 (54.6-90.1)
	Year 3	79.6 (70.0-87.2)	49.9 (36.5-68.3)	81.9 (75.9-86.8)	45.7 (37.2-56.3)	82.6 (76.8-87.4)	59.3 (47.1-74.8)

IgG: immunoglobulin G; CD-JEV: live, attenuated Japanese encephalitis SA-14-14-2 vaccine; MV: measles vaccine; N: number of infants in the per protocol analysis; CI: confidence interval; GMC: geometric mean concentration; mIU/mL: milli International Units per milliliter, seroprotection defined as ≥120 mIU/mL; GMT: geometric mean titer, seroprotection defined as JE neutralizing antibody titer ≥1:10

[a] Among 222 participants in the per protocol population of Group 2, 219 had valid serology results for measles antibody during retesting.

[b] Among 180 participants in the per protocol population of Group 3, 178 had valid serology results for measles antibody during retesting.

In addition to the high seroprotection rates, CD-JEV is the only JE vaccine other than mouse brain-derived vaccine for which there is vaccine effectiveness data. A post-vaccination campaign case-control study in Nepal demonstrated that a single dose of CD-JEV was 99.3% effective (95% CI 94.9-100%) in preventing Japanese encephalitis when administered only days or weeks before exposure to infection.[29] A follow-up case-control study in Nepal showed that vaccine effectiveness was 96.2% (95% CI 73.1-99.9) five years after the initial vaccination.[30]

3.4. Study rationale

In 2012 PATH sponsored a double-blind, randomized controlled trial among 818 Bangladeshi infants aged 10 to 12 months to compare the immunogenicity of three lots of live attenuated CD-JEV manufactured in the Chengdu Institute of Biological Products' (CDIBP) newly constructed GMP facility and one control lot of CD-JEV manufactured in the pre-existing facility. The study was conducted in rural Matlab and in Mirpur, in urban Dhaka. All four lots of JE vaccine were well-tolerated by the Bangladeshi infants ("A clinical trial in healthy infants to assess lot-to-lot consistency of Japanese encephalitis live attenuated SA 14-14-2 vaccine manufactured in a new good manufacturing practice facility and non-inferiority with respect to an earlier product", icddr,b Protocol # 11050).[2] Seroprotection rates (anti-JE neutralizing antibody titer $\geq$1:10) ranged from 80% to 86% for all four lots of vaccine, with an average GMT of 56 (Table 2).[2]

Table 2. Seroprotection rates and geometric mean antibody titers measured at 28 days post vaccination by treatment group (per protocol population).

Study group (N)	Group 1 (146)	Group 2 (195)	Group 3 (192)	Group 4 (194)	New Facility Group 2-4 (581)
Number of seroprotected children (%)	126 (86.3)	160 (82.1)	154 (80.2)	164 (84.5)	478 (82.3)
95% Confidence intervals	79.8–91.0	76.1–86.8	74.0–85.2	78.8–89.0	79.0–85.2
Geometric mean antibody titer	77.3	52.8	53.4	62.8	56.2
95% Confidence intervals	59.6–100.4	42.9–65.1	42.4–67.2	50.3–78.4	49.5–63.8

Group 1 represents the existing facility lot; Groups 2, 3 and 4 the new facility lots.
Seroprotection defined as JE neutralizing antibody titer ≥1:10

This study was a requirement for WHO-prequalification; based on these data the vaccine was prequalified in October 2013. However, these GMT and seroprotection rates were lower than those seen in other study populations, including the study referenced above that was conducted in Philippines, which used the same reference laboratory to test the samples.[3] While other study factors, such as the vaccine stability or lots used, may account for the results, different populations may respond differently to vaccines. For example, Bangladeshi children have been shown to have lower immune responses to other vaccines, including oral rotavirus vaccines, as compared to children in higher-resource settings.[31]

This study aims to understand the persistence of immunity and the kinetics of the booster response. In addition, this study will determine the safety of a second dose of CD-JEV in this population.

3.5. Potential risks and benefits

3.5.1. Potential risks

The child may experience discomfort and pain from venipuncture for collection of 2 ml of blood. Venipuncture is sometimes associated with discomfort during phlebotomy, and rarely, an infection at the site of phlebotomy if improperly conducted. The child may experience discomfort, pain, redness, swelling, and/or local hardness at the injection site.

Like any vaccine, vaccination with CD-JEV may cause side effects in some children. The side effects of JE vaccination may include pain at the injection site for a short while after injection and swelling, redness, and tenderness at the injection site. Fever may occur and in some cases a rash may appear in the initial days following the vaccination. Because the vaccine is a live attenuated vaccine, there is a very small theoretical risk that encephalitis, or infection of the brain, could occur. However this has never been reported to occur with CD-JEV. Allergic reactions and death, while rare, are also possible with any vaccination.

3.5.2. Potential benefits

Children participating in this study will receive a booster dose of CD-JEV which should improve their protection against JE. Likewise, the participant will contribute information on long-term immunogenicity and response to a booster dose including needed safety information. This will facilitate potential policy decisions in Bangladesh and Southeast Asia regarding JE immunization programs. They will be significantly contributing to the advancement of this WHO-prequalified JE vaccine. By participating in this clinical trial they will help build the body of knowledge on CD-JEV and help ministries of health decide the best integrated vaccination schedule for CD-JEV to reduce and ultimately eliminate JE disease throughout Asia.

4. RESEARCH DESIGN AND METHODS

The proposed study is a phase 4 open-label trial enrolling up to 818 Bangladeshi children who had previously participated in JEV05, a PATH-supported clinical study conducted from May to December 2012 during which 10- to 12-month-old infants were given a single dose of CD-JEV. Eligible JEV05 participants who still live in the

area will be identified using the local health and demographic surveillance system (HDSS) and asked to participate. Children will be divided into two groups based on whether they received vaccine from the old or new facility in JEV05:

- Group A (up to 655 eligible): children randomized to Groups 2-4 (new facility) in JEV05.
- Group B (up to 163 eligible): children randomized to Group 1 (old facility) in JEV05.

An overview of the study activities is provided in Table 3. A 2 ml blood sample will be collected from all participants three and four years after initial vaccination. The sera will be tested using PRNT; JE neutralizing antibody titers $\geq$1:10 will be considered seroprotective.

After the blood draw at Visit 02 occurring in May to December 2016, four years after their initial vaccination, both groups will receive a single subcutaneous booster dose of CD-JEV. Additional serum samples will be collected on Day 7 and Day 28 following the booster dose for measurement of serum anti-JE neutralizing antibody titers.

Following the booster dose, children will be monitored for immediate reactions during the first 30 minutes, solicited injection site and systemic adverse reactions for 7 days, unsolicited adverse events and serious adverse events for 28 days. All safety events will be identified or observed by study staff during home visits, clinic visits, and/or reported by a parent or legal guardian. Solicited and systemic adverse reactions will be assessed through home visits 1 through 6 days following vaccination, and a fixed site clinic (FSC) visit on Day 7 following vaccination. Information regarding unsolicited AEs occurring in the 28 days following vaccination will be collected and recorded on a standard questionnaire during clinic visits. Events will be graded for severity and assessed for relatedness to vaccination by the investigator.

The study will be open-label; no placebo control will be employed and safety assessments will be unblinded.

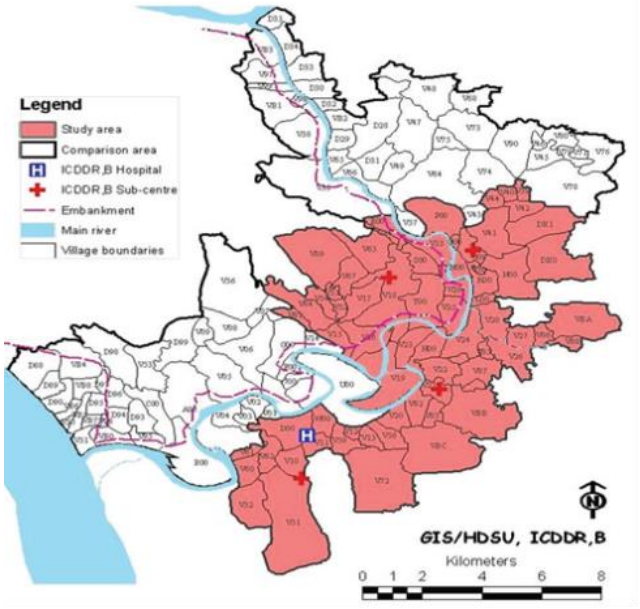
Table 3. Schedule of events

	Y3 after JEV05 ±3 months after enrollment in JEV05	Y4 after JEV05			
	Visit 1 (D0)	Visit 02 (D365±28)	Visit 03 through Visit 08 (D366-371±0)	Visit 09 (D372+3)	Visit 10 (D393±7)
Obtain informed consent	X				
Collect baseline demographic information	X				
Collect/review medical history	X	X			
Perform physical exam	X	X		X	X
Check inclusion/exclusion criteria	X	X			
Collect serum for long term response	X	X			
Collect serum for booster response				X	X
Administer booster dose of CD-JEV		X			
Observe for immediate reactions for 30 minutes		X			
Inquire about child's well being			X	X	X
Record reported adverse events		X	X	X	X
Record serious adverse events		X	X	X	X
Exit participant from study					X

4.1. Study sites

The study will be conducted in the same low risk JE sites as the JEV05 study—rural Matlab and Mirpur (urban Dhaka). Matlab Upazila is a rural site located 55 kilometers southeast of Dhaka where icddr,b has been maintaining a HDSS since 1966. The HDSS area has two parts; an icddr,b intervention area where maternal, child health, and family planning services are offered and a comparison area where these services are provided by the government of Bangladesh (Figure 1). JEV05 participants who still live in the area will be identified through the HDSS and contacted for participation. The study team will consist of the principal investigator, central administrative team, medical officers, nurses, and laboratory personnel adequately trained to conduct the study. The central administrative team will be comprised of the study coordinator, field assistants, field officers, and a data manager.

Figure 1: Matlab HDSS area (Intervention and comparison areas)



4.2. Study enrollment and withdrawal

4.2.1. Inclusion criteria at study entry (Visit 01)

Each participant must satisfy all of the following inclusion criteria at study entry:

- Participant in JEV05 study and received one dose of CD-JEV.
- Resides in the Matlab HDSS or Mirpur area.
- At least one parent or guardian willing to provide written informed consent.

4.2.2. Exclusion criteria at study entry (Visit 01)

Potential study participants will be excluded if they have:

- Received a second dose of Japanese encephalitis vaccine within the past three years.
- Received immunoglobulins and/or any blood products within 90 days prior to enrollment.
- Been diagnosed with a primary or acquired immunodeficiency, including HIV infection within the past three years.

4.2.3. Exclusion criteria for Visit 02 at the time of blood draw

Study participants will be excluded from further participation in the study if they have:

- Received a second dose of Japanese encephalitis vaccine within the past four years.
- Received immunoglobulins and/or any blood products within 90 days prior to Visit 02.
- Been diagnosed with a primary or acquired immunodeficiency, including HIV infection within the past year.

4.2.4. Exclusion criteria for Visit 02 at the time of vaccination

Study participants will be excluded from further participation in the study if they have:

- Received any other vaccine within 28 days prior to administration of the booster JE vaccine or planned vaccination during the 28 days after study vaccination.
- History of allergic disease or known hypersensitivity to any component of the study vaccine and/or following administration of vaccines included in the local program of immunization.
- Used any investigational or non-registered drug within 90 days prior to the administration of study vaccines or planned administration during the study period.
- Plan receipt of immunoglobulins and/or any blood products during the 28 days after the study vaccination.
- Received immunosuppressing or immune-modifying agents (including systemic corticosteroids equivalent to prednisone ≥0.5 mg/kg/day; topical and inhaled steroids are allowed) for 14 consecutive days or longer within 28 days prior to the administration of the booster JE vaccine.
- Acute or chronic, clinically significant pulmonary, cardiovascular, hepatic, or renal functional abnormality, as determined by medical history or physical examination, which in the opinion of the investigator, might interfere with the study objectives.
- Severe malnourishment as defined by World Health Organization weight-for-height tables (Z-score < -3).
- Acute illness at the time of vaccination (defined as the presence of a moderate or severe illness with fever [axillary temperature ≥38°C] or without fever [severity determined at the discretion of the investigator]. Acute illness is a temporary exclusion. Vaccination should be postponed until at least 7 days after recovery.
- Any condition or criterion that in the opinion of the investigator might compromise the well-being of the participant, compliance with study procedures, or interpretation of the outcomes of the study.

4.2.5. Reasons for withdrawal

After enrollment, a participant might be withdrawn from participation for several reasons:

- The investigator requests withdrawal because the participant:
    - Is not eligible or is no longer eligible;
    - Developed an AE or SAE requiring withdrawal; or
    - Is non-compliant.
- A participant voluntarily withdraws at his/her own request;
- A participant is lost to follow-up; or
- The trial is terminated prematurely by PATH.

4.2.6. Handling of withdrawals

In case of premature withdrawal for any reason, the investigator should use his/her best effort to:

- Conduct an interview to determine if the participant has had any reaction or AE (serious or non-serious) that has led to the withdrawal during the three-week period after any study vaccination. Where possible, the investigator should visibly or physically assess any reported adverse reaction or AE.
- Document the reason for premature withdrawal on the case report form (CRF).
- Complete final visit CRF.

The investigator must inform PATH within 7 days of becoming aware about of the premature termination of a volunteer's participation in the trial.

4.2.7. Termination of the trial

The trial might be suspended at any time by PATH or by any ethical review committee overseeing this study for any safety concern. This includes, for example, and without limitation, an SAE resulting in death that is viewed to be related to receipt of study vaccine or an unusually high rate of SAEs. PATH may suspend the study in the event that study conduct is found to be below Good Clinical Practice (GCP) standards.

In the event of the appearance of new data that indicate an increased level of risk to participants, the clinical trial will be suspended until PATH and all ethical review committees have reviewed relevant data and agreed that the trial may proceed.

Withdrawal of a participant by the investigator may occur only if the participant is identified to have one of the listed exclusion criteria, or if continued participation of the participant threatens the participant's well-being or the integrity of the study. Any participant with an adverse event related to involvement in the trial should continue to be followed for safety reasons until trial conclusion or resolution of the adverse event.

4.3. Study vaccine

4.3.1. Live, attenuated JE vaccine SA 14-14-2

SA 14-14-2, the vaccine virus strain, was attenuated by an empirical process of serial passage of the wild-type SA 14 virus in cell cultures of primary hamster kidney (PHK) cells and in animals (mice, hamsters) with successive plaque purifications (in primary chick embryo cells). SA 14-14-2 virus has 57 nucleotide changes compared to the parent SA 14 virus, resulting in 24 amino acid changes. Genetically-stable mutations resulting in 8 amino acid changes in the E protein gene are associated with reduced neurovirulence (attenuation).

CD-JEV is a preparation of SA 14-14-2 grown in culture on a monolayer of PHK cells. After cultivation and harvest, an appropriate stabilizer is added to the virus suspension, which is then lyophilized. Live attenuated JE virus (strain SA 14-14-2), minimum essential medium, and human serum albumin are the major components of the final vaccine.

The vaccine is prequalified by the WHO and produced in accordance with technical specifications in "Guidelines for the production and control of Japanese encephalitis vaccine (live) for human use" developed by the WHO Expert Committee on Biological Standardization, Chinese Pharmacopoeia and registered standard of Chinese State Food and Drug Administration (No.WS4-(ZB-072)-2010).[32,33]

4.3.2. Acquisition

The investigators or designee will be personally responsible for receiving the vaccine within Bangladesh and all subsequent management of study vaccines. Appropriate vaccine acquisition and handling standard operating procedures (SOPs) will be developed in collaboration with the investigator. Extra doses of vaccines will be provided and shall be used when the initially allocated dose is broken, damaged, or unusable.

PATH will coordinate direct shipment of CD-JEV from CDIBP to a central location in Bangladesh. The manufacturer, CDBIP, will donate these vaccines free of charge. The investigator or designee will receive the vaccine, provide customs clearance, and deliver the vaccine to a central facility under controlled cold-chain conditions. The investigators and their institution will receive no monetary compensation from the manufacturer for their participation in this study.

4.3.3. Packaging and labeling

The study vaccines will be provided in their commercial presentation. The commercial vial labels for these vaccines should remain intact. An additional label will be affixed by the manufacturer to each box and will bear the following information:

- Study number
- Legal information: “For Clinical Trial Use Only”

4.3.4. Product storage and stability

CD-JEV vaccine should be stored in a refrigerator or cold box at a temperature ranging from +2°C to +8°C and protected from light. Storage temperature should be monitored and documented on an appropriate form during the entire trial.

The vaccines should never be frozen. In case of deep freezing or accidental disruption of the cold chain, the vaccine should not be administered and the investigator or the responsible person should contact the study monitor to receive further instructions.

4.3.5. Dosage, preparation and administration of study vaccines

CD-JEV will be supplied in 5-dose vials as a lyophilized powder that looks like a milky-white crisp cake. After reconstitution, it turns into a transparent orange-red liquid. Each 0.5 ml human dose for subcutaneous injection contains no less than 5.4 log PFU of live JE virus. A single dose of 0.5mL should be administered by subsubcutaneous injection with the rest of the vial discarded.

The vaccines should be gently shaken prior to administration to assure homogenous suspension. CD-JEV will be administered subcutaneously into the anterolateral aspect of the right upper arm.

Standard immunization practices should be observed and care should be taken to administer the injection. As with all injectable vaccines, appropriate medical treatment, staff, and supervision will be readily available in case of rare anaphylactic reactions following administration of the study vaccine and epinephrine 1:1000, diphenhydramine, and resuscitation equipment should be available in case of any anaphylactic reactions.

4.3.6. Accountability procedures

Study vaccine must be kept in a secure place. The investigator or the person in charge of vaccine management will maintain records of the vaccine's delivery to the trial site, the inventory at the site, the dose given to each participant, and the return or disposal of unused doses to PATH. Appropriate vaccine accountability SOPs will be developed in collaboration with the investigator.

4.4. Study procedures

4.4.1. Recruitment and screening information

Participants of JEV05 will be identified from the computerized HDSS database and dataset from the previous study maintained at the field site in Matlab and Mirpur. Field workers will visit their homes and explain to the parent or legal guardian the study purpose and procedures. If the parent or legal guardian is interested in having

their child participate in the study, field staff will invite them to come to their nearby FSC on a scheduled enrollment day.

All participants from JEV05 that continue to live in the study area are eligible to participate in this study. We propose to include children that were seronegative at 28 days because there is an indication from other studies that participants seronegative at D28 may generate a detectable antibody response at a later timepoint. Furthermore, we expect that some "seronegative" participants, can generate an anamnestic response. The serum collected at Day 7 following the booster dose will be used to determine whether previously vaccinated children who did not have any antibody at 3 and 4 years post CD-JEV vaccination can generate an anamnestic response (i.e., the children were immune to JE disease). Although serum neutralizing antibody is generally considered our best a correlate of protection, there are instances where vaccinated persons without antibody level are nevertheless truly immune and they are able to mount a complete and normal immune response should they become infected. The way to determine this is to look at the anamnestic immune response, i.e., short-term antibody response. This is an antibody response that is not due to a new primary response; it depends on having primed B and T lymphocytes ready to respond quickly. The magnitude of the response measured between Day 7 and Day 28 will indicate whether the participant is mounting an anamnestic response to the booster or a primary response.

4.4.2. Enrollment (Visit 01, 3 years after JEV05 vaccination ±3 months)

When the parent or legal guardian brings their child to the FSCs on the scheduled day for enrollment, the study team will:

- Describe the study to the parents, including the study purpose, requirements, duration, and risks and benefits of the study. The parent or legal guardian will have the opportunity to ask any questions.
- Assess the family's ability to comply with study requirements.
- Obtain written informed consent. Participation in the study is strictly voluntary, and those agreeing to participate must provide a written informed consent. The consent form will be in Bangla. The parent(s) or guardian(s) will be informed that they are free to refuse to have their child participate and also to withdraw their consent at any time. One parent's or guardian's signature is acceptable as informed consent for this study. If the parent or guardian is illiterate, a left thumb impression will be taken instead of signature in the presence of an impartial literate witness who is unrelated to the study. The impartial literate witness (hierarchically independent of the PI and not specified on the list of trial contributors) will also sign the informed consent form and attest that the information in the consent form and any other written information were explained to the parents or guardian and that the information was understood. Following the informed consent process, the child will be enrolled into the study.
- Collect demographic and address information.
- Collect pertinent medical history through parent interview, including data on child's health and previous vaccinations and reaction to vaccinations and history of any chronic or recurrent medical conditions.
- Perform a physical exam (temperature, height and weight, a brief examination of the head, neck, heart, lungs, abdominal region, musculoskeletal system, and skin).
- Review study inclusion and exclusion criteria and determine eligibility.
- Assign the child a unique study participant number following confirmation of eligibility and consent.
- Collect 2 ml of blood with aseptic technique to detect anti-JE serum neutralizing antibody with the PRNT-50.
- Participants will be reminded that study staff will be contacting them in approximately one year for administration of a booster dose of CD-JEV.

4.4.3. Vaccine administration (Visit 02, 1 year after Visit 01 ±28 days)

When the parent or legal guardian brings their child to the FSCs on the scheduled day for vaccination, the study team will:

- Describe the study to the parents, including the study purpose, requirements, duration, and risks and benefits of the study. The parent or legal guardian will have the opportunity to ask any questions.
- Reassess the family's ability to comply with study requirements.
- Collect demographic and address information.
- Collect pertinent medical history through parent interview, including data on previous vaccinations and reaction to vaccinations and history of any chronic or recurrent medical and psychiatric conditions.
- Perform a physical exam (temperature, height and weight, a brief examination of the head, eyes, ears, nose, throat, neck, heart, lungs, abdominal region, lymph nodes, musculoskeletal system, and skin).
- Review exclusion criteria and determine eligibility for blood draw and booster vaccination.
- Collect 2 ml of blood from participants with aseptic technique to detect anti-JE serum neutralizing antibody and anti-JE IgM with PRNT-50 and ELISA, respectively.
- Administer CD-JEV vaccine subcutaneously in the anterolateral aspect of the right upper arm according to standard sterile techniques.
- Ask the parents to wait with the child in the clinic for 30 minutes after the vaccination(s) to assess immediate reactions. If the child experiences an adverse reaction or a SAE during this time period, he/she will be treated and the event will be recorded on the CRF.
- Inform the parents that they will be visited daily for the next 6 days in order to check on the welfare of the child and assess the occurrence of any local or systemic reactions.
- Remind the parents to seek medical care as appropriate and inform the investigator as soon as possible in the event of an adverse reaction or SAE. If the child visits a health care provider, the parents should be sure to inform him/her of the child's participation in the study and present the provider with contact information for the investigator as indicated on the study identification card.
- Give the parents a study identification card that indicates that the child has been enrolled in a research study and given a study vaccine. The card will contain the following information:
    - Name, address, and contact phone numbers of local PI.
    - Vaccination clinic location.
    - Name of vaccine given to child.
    - Schedule of visits.
    - Indication that the child is in a vaccine trial and a request that medical personnel who treat the child contact the local PI within the study duration.
- The appropriate section(s) of the CRF will be completed.

4.4.4. Home follow-up visits (Visit 03 through Visit 08, 1-6 days post vaccination ±0 day)

A field worker will visit the particpant at home to:

- Inquire about the child's health (any illnesses or medications taken or visits to a healthcare facility).
- Inquire about any reactions using a standard questionnaire to record the local and systemic reactogenicity after vaccination.

- Remind the parents that they will be need to bring their child to the FSC on the 7th day (+ 3 days) following vaccination for a blood draw and to check on the welfare of the child.
- Remind the parents to seek medical care as appropriate and inform the investigator as soon as possible in the event of an adverse reaction or SAE. If the child visits a health care provider, the parents should be sure to inform him/her of the child's participation in the study and present the provider with contact information for the investigator as indicated on the study identification card.
- Complete the appropriate section(s) of the CRF.

4.4.5. Clinic follow-up visit (Visit 09, 7 days post vaccination +3 days)

When the parent or legal guardian brings their child to the FSC/ Mirpur study clinic on the scheduled day for follow-up, the study team will:

- Inquire about the child's health (any illnesses or medications taken or visits to a healthcare facility).
- Inquire about any reactions using a standard questionnaire to record the local and systemic reactogenicity after vaccination.
- Collect 2 ml of blood from participants with aseptic technique to detect anti-JE serum neutralizing antibody and anti-JE IgM with PRNT-50 and ELISA, respectively
- Inform parents that they will need to bring their child to the FSC at Matlab or Mirpir study clinic in approximately 28 days (±7 days) in order to check on the welfare of the child and assess the occurrence of any adverse events.
- Remind parents to seek medical care as appropriate and inform the investigator as soon as possible in the event of an adverse event or SAE. If the child visits a health care provider, the parents should be sure to inform him/her of the child's participation in the study and present the provider with contact information for the investigator as indicated on the study identification card.
- Complete the appropriate section(s) of the CRF.

4.4.6. Clinic follow-up visit (Visit 10, 28 days post vaccination ±7 days)

A final study visit will be conducted 28 days after booster vaccination. The study team will:

- Inquire about the child's health (any illnesses or medications taken or visits to a healthcare facility).
- Inquire about any adverse events.
- Collect 2 ml of blood from participants with aseptic technique to detect anti-JE serum neutralizing antibody with the PRNT-50.
- Complete the appropriate section(s) of the CRF.
- Exit the participant from the study.

4.4.7. Booster Vaccine

Although studies have shown that the JE live attenuated SA 14-14-2 vaccine (CD-JEV) produces long lasting immunity, China and India currently give a boosting dose at least one year after the primary vaccination. In a recent 2015 position paper regarding JE vaccines (Weekly Epidemiological record Feb 27, 2015; http://www.who.int.wer), WHO stated that "long-term immunogenicity studies would inform optimal dosing schedules for long-term protection, which may vary by location (based on natural boosting or other factors)". As an important research need for all of the newer JE vaccines, the proposed study will provide data for guiding policy decisions with regard to the booster dose for CD-JEV, as it would be impossible for routine immunization programs to select only seronegative children for boosting. Including participants seroprotected 3 years post

vaccination at the 4 year time point of booster dose is important to understand the kinetics of the antibody response in children with existing titers and provide information for optimal schedules.

4.5. Laboratory evaluations

4.5.1. Clinical laboratory evaluations

There will be no clinical (safety) laboratory evaluations in this study. Laboratory assays specified in Section 4.5.2 are for research purposes only and are not be used for clinical diagnosis.

4.5.2. Serum assays

4.5.2.1. Anti-JE neutralizing antibody titer

Serum anti-JE neutralizing antibody titers will be measured using PRNT at the United States Army Medical Directorate, Armed Forces Research Institute of Medical Sciences in Bangkok, Thailand. The reported titer will be the PRNT-50 which is the highest serum dilution that is still capable of reducing the number of virus plaques in cell culture by 50% compared to virus growth in cell culture without any added serum. Seroprotection will be defined as a PRNT-50 titer of $\geq$1:10.[1]

4.5.2.2. Anti-JE IgM

Anti-JE IgM will be measured on specimens collected prior to the booster dose and 7 days following the booster dose using ELISA at United States Army Medical Directorate, Armed Forces Research Institute of Medical Sciences in Bangkok, Thailand. Seropositivity will be defined per the manufacturer's kit instructions.

4.5.3. Specimen preparation, handling, and shipping

4.5.3.1. Specimen collection

Blood draws of 2 ml will be collected during study visits. The identity of the child will be verified immediately prior to the blood draw using the child's study participant number. The study participant number and date of collection of the specimen will be recorded on all labels to be used in the study. One label will be affixed onto the vacutainer tube immediately prior to the blood sample drawing.

After collection, blood samples must clot for 60 minutes to 2 hours at room temperature. Samples will then be kept in a cold box for transportation until processed in the site laboratory. All samples will be centrifuged on the same day of collection.

4.5.3.2. Specimen preparation, handling, and storage

After centrifugation, the serum will be aliquoted. To insure that samples are not mixed or inaccurately labeled, the child's study participant number, date of blood collection, number of aliquots obtained, and the date and time of aliquoting will be specified on the sample identification log. A label will be affixed onto each of the aliquots. Comments may be made on the quality of samples (e.g., hemolyzed, contaminated).

All serum aliquots will be kept in the -20ºC freezer at the local study site. Study staff will monitor and record temperature on the appropriate form during the entire trial. One serum aliquot will be shipped to United States Army Medical Directorate, Armed Forces Research Institute of Medical Sciences in Bangkok, Thailand. A second aliquot will be retained at the site as back up and shipped to a long term storage facility determined by the Sponsor at the completion of the study. All unused serum specimens will be stored at their assigned location for 5 years following study completion. Samples will be destroyed after 5 years. Only authorized study staff should have access to the samples. Samples will not be used for unrelated, future studies without an institutional review board (IRB) approved amendment to the protocol.

4.5.3.3. Specimen shipment

Specimens will be shipped according to the International Air Transport Association regulations after each study year.

5. SAMPLE SIZE CALCULATION AND OUTCOME (PRIMARY AND SECONDARY) VARIABLE(S)

No sample size will be calculated for this study. However, up to 818 participants (up to 655 in Group A and up to 163 in Group B) who participated in JEV05 are expected to be enrolled in this study to evaluate the primary and secondary objectives.

5.1. Serology variables

Seroprotection for JE in this study is defined as percentage of participants with JE antibody titre ≥1:10 at Year 3, Year 4 prior to the booster dose, 7 days post-booster dose, and 28 days post-booster dose. GMTs of anti-JE neutralizing antibody will be calculated.

Evaluation of long-term anti-JE antibody response three and four years after CD-JEV vaccination among children who received the single dose of vaccine will be made at Year 3 and Year 4 prior to the booster dose in terms of JE seroprotection rates. Additionally,the analysis will stratified by the seroprotection status of participants on Day 28 post primary immunization in the previous JEV05 study.

To further describe the antibody response to a booster dose, anti-JE IgM will be measured prior to the booster dose and 7 days post booster dose. Seropositivity will be defined per ELISA manufacturer's instructions.

5.2. Safety variables

The safety profile will be evaluated in terms of frequency counts and percentage of participants experiencing the following:

- Immediate reactions occurring within 30 minutes after vaccination as observed by study staff.
- Solicited signs and symptoms occurring greater than 30 minutes after receipt of CD-JEV through 7 days post vaccination as observed by study staff or reported by the participants to study staff.
- All other adverse events occurring within one month of CD-JEV receipt as observed by study staff or reported by the participant's parents to study staff.
- SAEs occurring within 28 days of CD-JEV receipt.

6. DATA ANALYSIS

A Statistical Analysis Plan will be specified prior to the first participant first visit and finalized before locking of the database.

6.1. Immunogenicity

Long-term assessment of the immune response to CD-JEV will be evaluated in terms of percentage of participants with JE neutralizing antibody titre ≥1:10 at Year 3 and Year 4, just prior to the booster dose. Additonally, the analysis will be stratified by the seroprotection status of participants on Day 28 post primary immunization in previous JEV05 study.

Percentage of participants achieving seroprotection against JE along with 95% confidence interval (CI) will be calculated by group. The method for calculating the 95% CI will be based on the Wilson score method without continuity correction. In addition, the difference in JE seroprotection rates between Groups A and B will be

calculated along with the 95% CI of the difference using the Newcombe-Wilson method without continuity correction.

Similarly, the immune response to the booster dose following a booster dose of CD-JEV given at Year 4 will be assessed in terms of percentage of participants with JE neutralizing antibody titre ≥1:10 at 7 days post booster dose and 28 days post booster dose. Percentage of participants achieving seroprotection against JE along with 95% CI will be calculated using the Wilson score method without continuity correction.

In addition, GMTs of the anti-JE neutralizing antibody responses and their associated 95% CI will be calculated at Year 3, at Year 4 prior to the booster dose, and at 7 and 28 days post booster dose using the t-test. The GMTs will be compared between Group A and Group B in terms of ratio of the GMTs using the t-test and 95% CI of ratio of GMTs will be calculated.

The GMTs at 7 and 28 days post booster dose will be further compared to the GMTs at Year 4 prior to the booster dose using a paired t-test to evaluate the magnitude of increase in GMTs by group.

Furthermore as part of exploratory analyses to differentiate between a primary or secondary immune response, the immune response to the booster dose will be assessed in terms of the percentage of participants with demonstrated seropositivity for anti-JE IgM prior to the booster dose and 7 days post booster dose. Percentage of participants with seropositivity against JE along with 95% CI will be calculated using the Wilson score method without continuity correction.

6.2. Safety

The safety profile will be evaluated in terms of frequency counts and percentage of patients experiencing specific events falling into one of the following four categories:

- Immediate reactions occurring within 30 minutes after vaccination will be listed at the participant-level and summarized by group.
- Solicited signs and symptoms occurring greater than 30 minutes after receipt of the booster dose through 7 days post vaccination will be listed at the participant-level and summarized by severity for Groups A and B. Exact 95% CI will be provided.
- All other adverse events occurring within 28 days of the booster dose will be listed at the participant-level and summarized by severity and relationship to study vaccines for Groups A and B.
- SAEs occurring within 28 days of the booster dose will be listed at the participant-level and summarized by severity and relationship to study vaccines for Groups A and B.

6.3. Analysis sets

All immunogenicity analyses and summaries will be performed on the intention-to-treat (ITT) basis which will be considered as the primary approach to immunogenicity analyses. Per protocol (PP) analyses will also be conducted on all enrolled participants. Safety analyses will be conducted on the ITT basis. Analyses set definitions are provided below.

6.3.1. Intention-to-Treat analysis set

The ITT analysis will be conducted for analyses of serology using all enrolled participants in this study whether the participant took the booster dose or not. According to this analysis set, long-term assessment of the anti-JE antibody response to CD-JEV is evaluated using all participants enrolled in the study with at least one serology result in Year 3 or Year 4 prior to the booster dose. The ITT analysis set will be used to analyze the primary and secondary serology objectives.

6.3.2. Per-Protocol analysis set

A participant is valid for the PP analysis set if s(he) satisfies the inclusion/exclusion criteria of the study at Visit 01, has a valid serology result at Year 3 (Visit 01) and Year 4 (Visit 02) prior to vaccination, and had no significant deviation in blood collection at the two time points. The PP analysis set will be used to analyze the primary and secondary serology objectives.

6.3.3. Safety analysis set

The safety analysis set will include all enrolled participants who had the booster dose and will be used to analyze all safety measurements including solicited/ unsolicited AEs and SAEs collected in the study.

7. DATA SAFETY MONITORING PLAN

7.1. Assessment of safety

7.1.1. Specification of safety parameters

Study vaccine safety profiles will be parameterized as the proportion of participants experiencing specific adverse events categorized into one of the following four categories:

- Immediate reactions occurring within 30 minutes of booster dose, as observed by study staff or reported by the participant to study staff.
- Solicited signs and symptoms occurring greater than 30 minutes after the booster dose through 7 days post vaccination, as observed by study staff or reported by the participant to study staff.
- All other adverse events occurring within 28 days of receipt of the booster dose as observed by study staff or reported by the participant's parents to study staff.
- All SAEs occurring within 28 days of receipt of the booster dose.

7.2. Methods and timing for assessing, recording, and analyzing safety parameters

7.2.1. Adverse events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care.

Information to be collected on adverse events includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event.

Any medical condition that was present at the time that the patient was enrolled should not be reported as an AE. However, if it deteriorates during the study safety recording periods, it should be recorded as an AE.

AEs should be graded for severity and relationship to vaccine.

7.2.1.1. Severity of event

Any AE which is not a local and systemic sign or symptom occurring within 7 days post booster dose will be assessed by the clinician to quantify intensity using the following guidelines[34]:

Grade	Categorization	Definition
Grade 1	Mild	Symptoms causing no or minimal interference with usual social and functional activities.
Grade 2	Moderate	Symptoms causing greater than minimal interference with usual social and functional activities.
Grade 3	Severe	Symptoms causing inability to perform usual social and functional activities with intervention or hospitalization indicated.
Grade 4	Potentially life-threatening	Symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death.

**Intervention defined as Medical, surgical, or other procedures recommended or provided by a healthcare professional for the treatment of an adverse event*.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

7.2.2. Relationship to study vaccines

The clinician's assessment of an AE's relationship to test vaccine is part of the documentation process, but it is not a factor in determining what is or is not recorded in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be recorded. All AEs must have their relationship to study vaccines assessed using the following guidelines:

Categorization	Definition
Definitely Related	An adverse event or unanticipated problem clearly related to the research procedures.
Possibly Related	There is a reasonable possibility that the adverse event, incident, experience, or outcome may have been caused by the procedures involved in the research.
Not Related	Any adverse event or unanticipated problem clearly not related to study procedures.

7.2.3. Reactogenicity

Reactogenicity post vaccination will be assessed in the clinic for 30 minutes, during daily home visits 1 through 6 days following the booster, and during a FSC visit 7 days following the booster. During the daily home visits and FSC visit, the site staff will use a structured reactogenicity reporting form for recording reactogenicity.

Any reactogenicity will be managed in accordance with good medical practices by the clinical study site team, who will assess and treat or refer the child for medical care as appropriate. If needed to monitor or treat reactogenicity, additional study visits may be conducted. If any acute treatment or medical care is required as a result of harm caused by the study vaccine or study procedure, this will be provided by the site free of charge. All children reporting reactogenicity will be followed clinically until the reactogenicity resolves (returns to baseline) or stabilizes. Reactogenicity will be recorded as indicated in the CRF. Reactogenicity likely to be related to the product, whether serious or not, that persists at the end of the trial will be followed up by the PI until resolution or stabilization.

The parents will be instructed to contact the study site staff to report any reactogenicity their child may experience. In the case of a life-threatening event, they will be instructed to seek immediate emergency care.

Where feasible and medically appropriate, parents of children will be encouraged to seek medical care for the child where the study physician is based, and to request that the physician be contacted upon their arrival.

7.2.3.1. Immediate reactions

All participants will be observed for immediate reactions for 30 minutes after vaccination, with appropriate medical treatment readily available in case of an anaphylactic reaction following the administration of study vaccine. Immediate reactions will be assessed by a study physician or appropriately trained medical staff. All reactions that occur during this time will be recorded on the CRF. Any immediate reaction which meets the criteria for a serious adverse event must also be documented on an SAE form.

7.2.3.2. Local and systemic signs and symptoms

All participants will be monitored for assessment of local and systemic signs and symptoms occurring from 30 minutes through 7 days post vaccination. Study staff will inquire about specific (solicited) local and systemic signs and symptoms and other unsolicited adverse events.

Using a standardized data collection instrument the following local and systemic signs and symptoms will be documented and graded on predefined scales based on functional assessment or magnitude of reaction.[34] Any local and systemic reaction that meets the criteria for a serious adverse event must also be documented on an SAE form.

Local reactions (at injection site)

- Ecchymosis (bruising)
- Erythema (redness)
- Edema (swelling)
- Induration (hardness)
- Pain/tenderness

Local ecchymosis, erythema, edema, and induration, if present, will be graded as follows:

Grade 1: ≤2.5 cm in diameter.

Grade 2: >2.5 cm in diameter with <50% of surface area of extremity segment involved (upper arm or thigh).

Grade 3: ≥50% of surface area of extremity segment involved OR ulceration OR secondary infection OR phlebitis OR sterile abscess OR drainage.

Grade 4: potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue).

Injection site pain/tenderness (pain without touching or tenderness as pain when the area is touched), if present, will be graded as follows:

Grade 1: pain/tenderness causing no or minimal limitation of use of limb.

Grade 2: pain or tenderness causing greater than minimal limitation of use of limb.

Grade 3: pain/tenderness causing inability to perform usual social and functional activities.

Grade 4: pain or tenderness causing inability to perform basic self-care function OR hospitalization indicated.

Systemic reactions:
General Signs/Symptoms:

- Fever
- Change in eating habits
- Diarrhea
- Sleepiness
- Irritability
- Unusual crying
- Vomiting

Fever if present will be graded as follows (axillary temperature):

Grade 1: 37.5°C to 37.9°C

Grade 2: 38.0°C to 38.4°C

Grade 3: 38.5°C to 40.0°C

Grade 4: >40.0°C

Change in eating habits, diarrhea, sleepiness, irritability, unusual crying, vomiting, and any other unsolicited reaction occurring through 7 days post booster vaccination will be graded as follows:

Grade 1: symptoms causing no or minimal interference with usual social and functional activities.

Grade 2: symptoms causing greater than minimal interference with usual social and functional activities.

Grade 3: symptoms causing inability to perform usual social and functional activities with intervention or hospitalization indicated.

Grade 4: symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death.

7.2.4. Serious adverse events

An SAE is defined as an AE that meets one of the following conditions:

- Death.
- Life threatening (participant at immediate risk of death). *(The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.)*
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in a persistent or significant disability or incapacity.
- Important medical events that may not result in death, be life threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Medical and scientific judgment should be exercised in deciding whether reporting is appropriate.

All SAEs occurring within 28 days post vaccination must be reviewed and evaluated by a study physician (SAE relationship to study vaccine must be evaluated as outlined in Section 7.2.2) and recorded on an SAE form and reported (as specified in Section 7.3). SAEs, whether serious or not, that persist at the end of the trial will be followed up by the investigator until satisfactory resolution or until the investigator deems the event to be chronic or the participant to be stable. The investigator will document the date of final disappearance of the adverse event

on a data clarification form. All care for SAEs or care required as part of the study will be provided to study participants free of cost.

7.3. Reporting procedures

7.3.1. Serious adverse events

All SAEs occurring within 28 days post booster dose must be reported to both the local data safety and monitoring board (DSMB) and the Sponsor, even if the investigator considers that the SAE is not related to treatment. Reporting procedures to the local DSMB will follow established icddr,b policy, whereas reporting to the Sponsor will be described in detail in an SOP. To report to the Sponsor, the study clinician will complete a Serious Adverse Event Form within the following timelines of such events:

- All SAEs, regardless of relationship, will be recorded on the Serious Adverse Event Form and sent by email to the Sponsor and the contract research organization within 24 hours of site awareness, as outlined in a reporting SOP.

SAEs reported to the local DSMB will be reviewed by the committee as per established icddr,b guidelines and will be reviewed during the scheduled meetings between the icddr,b, DSMB, and the PI. Details for how review of SAEs and other unanticipated problems will be conducted will be contained in an SOP.

7.3.2. Regulatory reporting

Collected SAEs will be reported to responsible ethical review committees according to requested timelines. Collected local and systemic reactions and adverse events not meeting the criteria of an SAE will be summarized at the end of the study, and a summary report will be sent to responsible ethical review committees and CDIBP manufacturers.

7.4. Safety monitoring plan

7.4.1. Post vaccination follow-up

Parents of children will be advised about possible short-term mild reactogenicity that may occur post vaccination. Parents will have the contact numbers of the study physician and community health workers. The parent will also be reminded to contact the study team if the child experiences any illness, has received any medical care, or requires any medical care. The Sponsor and the Ethical Review Committee (ERC) will be notified within 24 hours of any SAEs.

7.4.2. Data and safety monitoring board

An independent, local DSMB will be constituted to monitor all SAEs. The DSMB chair will review all SAEs occurring 28 days post vaccination, including deaths. The DSMB will be instructed to use their clinical judgment in order to provide guidance or recommendations for stopping the study early due to a safety concern. The DSMB will evaluate the safety data from these children including SAEs. In addition, the DSMB will be asked to review in real time all SAEs suspected to be related to the product and provide recommendations to the icddr,b and PATH teams.

7.5. Halting rules

Given the experience with CD-JEV, no safety issues are anticipated which would result in the suspension of the study. However, the trial might be suspended at any time by PATH, the local DSMB, or any ethical review committee overseeing this study for any safety concern. This includes, for example, and without limitation, an SAE resulting in death, an unexpectedly high number of persons experiencing a similar event after receipt of any dose, or an unusually high rate of SAEs. PATH may suspend the study in the event that study conduct is found to be below GCP standards.

Should an SAE occur which is likely related to administration of study vaccine, the decision whether the study should continue per protocol, proceed with caution, be suspended pending further investigation, be discontinued, or be modified and then proceed will be made by PATH in consultation with the investigators, the DSMB, and/or the icddr,b Research Review Committee (RRC) and ERC. However, no rules will be prespecified to define these conditions.

If the study is halted, participants will be contacted immediately explaining why the study has been halted and the implications for their medical well-being.

In the event of the appearance of new data that indicate an increased level of risk to participants, the clinical trial will be suspended until PATH, the DSMB, and all ethical review committees have reviewed relevant data and agreed that the trial may proceed.

7.6. Data handling and record keeping

7.6.1. Data management responsibilities

The PI is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents will be completed in a neat, legible manner in black ink to ensure accurate interpretation of data and clarity of reproduced copies. The study workers will be asked not to erase, overwrite or use correction fluid or tape on the original. An SOP will be available providing details of data management responsibilities.

All source documents and laboratory reports will be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Data collection will be the responsibility of the clinical trial staff at the site under the supervision of the PI. During the study, the PI will maintain complete and accurate documentation for the study and will advise and oversee local investigators who will conduct data management, entry, and quality review. Analysis and reporting of study data will be done by the trial statistician after data lock.

7.6.2. Data capture methods

Clinical data and laboratory data will be recorded on CRFs. All children will be assigned a unique study participant number at study enrollment; this study participant number will be included on all forms and in the computer database and will serve to link study data to specific individuals. Data forms will be verified for accuracy, linked by study participant number, and managed using database management software (yet to be determined).

7.6.3. Study records

In accordance with applicable regulatory requirements, following closure of the study, the investigator/institution will maintain a copy of study documents in a secure and designated location at the study site. Essential documents shall be retained for at least five years after the completion or discontinuation of the study.

No records will be destroyed without the written consent of PATH. It is the responsibility of PATH to inform the PI when these documents no longer need to be retained.

Study data collection forms (i.e., CRFs), informed consent documents, and adverse event-related medical records are the primary source documents for this study. Only authorized study staff and representatives of the monitoring team, the sponsor, independent ethical committee, and data monitoring committee and appropriate regulatory agencies may have direct access to source documents containing study data.

7.6.4. Protocol deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or Manual of Procedures requirements. The noncompliance may be either on the part of the child or parent, the investigator(s), or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations will be promptly reported to PATH.

A completed copy of the Protocol Deviation Form will be maintained in the regulatory file. Protocol deviations will be sent to the local IRB/ERC per their guidelines. The PI and study staff are responsible for knowing and adhering to their IRB/ERC requirements.

7.7. Quality control and quality assurance

The study will be conducted in accordance with procedures identified in the protocol and SOPs. SOPs will be developed and revised as necessary. In addition, monitoring plans, data management plans, and quality management plans will be developed.

Site monitoring will be conducted to ensure that human subject protection, study procedures, laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, International Conference on Harmonisation (ICH) GCP, and regulatory standards, and that the study is conducted in accordance with the protocol and SOPs.

All site staff will be required to have training in GCP, informed consent, and protocol implementation prior to work on the project. All study staff, including temporary staff such as nurses, phlebotomists, and interviewers will attend mandatory training prior to participant enrollment.

7.8. Clinical monitoring

7.8.1. Site monitoring plan

Qualified and appropriately trained individuals from PATH or designated by PATH will carefully monitor the study. The study monitor will periodically contact the site and perform on-site visits. The extent, nature and frequency of site visits will be based on such considerations as study objectives, study design and complexity, and enrollment rate; periodicity and nature of monitoring activities will be described in the Monitoring Plan. PATH may also include representatives of the Bill & Melinda Gates Foundation (The Foundation) in site contacts and visits, as appropriate, and will keep The Foundation program officer apprised of study progress.

7.8.2. Site initiation visit

A site initiation visit will occur prior to approval to initiate the study. The study monitor and/or PATH representatives will verify and document that the study team has been properly informed about the trial, the materials to be used during the trial have been received and are appropriately stored, regulatory requirements are met and documented, and that approved study SOPs are in place. Prior to enrollment of participants at the study site, specific regulatory documents must be available, such as independent ethics committee (IEC) approvals, other IRB required approvals, and curriculum vitae for investigator and study staff.

7.8.3. Follow-up visits

Monitoring will be conducted according to PATH's requirements. The individuals responsible for monitoring the study will periodically review the progress of the study and should have access to all records necessary to ensure the ethical and safety conduct of the study and the integrity/validity of the recorded data.

During site visits and contacts, the monitor will:

- Check and assess the progress of the study.
- Review study data collected.
- Perform source data verification.
- Review trial master files for essential documents.
- Evaluate adherence to standard operating procedures.
- Identify any issues and address their resolution.

This will be done in order to verify that:

- The data are authentic, accurate, and complete.
- The safety and rights of participants are protected.
- The study is conducted in accordance with the approved protocol (and any subsequent amendment) and all applicable regulatory requirements.

As part of study conduct, the investigator agrees to allow the monitor or PATH representative direct access to all relevant documents and to allocate time to the monitor to discuss findings and any relevant issues. The investigator also agrees to allow representatives of the Foundation, authorized by PATH, to occasionally accompany the monitor or PATH representative during site visits.

7.8.4. Close-out visit

Upon completion of the study, the study monitor or PATH representative and the investigator will conduct the following activities:

- Data clarification and/or resolution.
- Accounting, reconciliation, and destruction at sites of used and unused vaccines.
- Review of site study records for completeness.
- Return copy of study data to PATH.

PATH and any regulatory body retain the right to temporarily suspend or prematurely discontinue this study at any time related to safety. If the study is stopped or suspended prematurely, PATH will inform the investigator as well as regulatory authorities about the decision and the reasons for termination or suspension. If such action is taken, all efforts must be made to ensure the safety of the participants enrolled in the study. The investigator will inform the responsible IEC and provide the reason for the suspension or termination. In case of premature study or study site closure, the monitor or PATH representative will conduct all activities as indicated above.

7.8.5. Allowable schedule of study visits

For clarity, per-protocol timing of key study visits is provided below in Table 4. No child should be dropped from the study solely on the basis of a visit falling outside of an allowable time-frame for that visit if there is good faith attempt by study staff and the child participant's parent(s)/guardian(s) to adhere to the protocol.

Table 4. Allowable Schedule

Study Visit	Should occur	May occur
Visit 01	Three years after initial vaccination	Three years ±3 months after initial vaccination
Visit 02	Day 365	One year ±28 days after Visit 01
Visit 03	Day 366	1 day after booster vaccination
Visit 04	Day 367	2 days after booster vaccination
Visit 05	Day 368	3 days after booster vaccination
Visit 06	Day 369	4 days after booster vaccination
Visit 07	Day 370	5 days after booster vaccination

Study Visit	Should occur	May occur
Visit 08	Day 371	6 days after booster vaccination
Visit 09	Day 372	7 to 10 days after booster vaccination
Visit 10	Day 393	21 to 35 days after booster vaccination

7.9. Audits and inspections

For the purpose of compliance with applicable regulatory guidelines, it may be necessary for PATH or national or foreign regulatory authorities to conduct a site audit. This may occur at any time from study start to after the conclusion of the study.

The investigator agrees to allow the auditor direct access to all relevant documents and to allocate time to the auditor to discuss findings and any relevant issues.

National and foreign regulatory authorities may conduct a regulatory inspection of this study. If a regulatory authority requests an inspection, the investigator must inform PATH immediately about this request. The investigator agrees to allow the inspector(s) direct access to all relevant documents and to allocate time and the time of staff to the inspector(s) to discuss findings and any relevant issues.

8. ETHICAL ASSURANCE FOR PROTECTION OF HUMAN RIGHTS

8.1. Ethical standard

The investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki, ICH-GCP, and/or with local regulatory requirements, whichever affords the greater protection to the participant.

8.2. Institutional review board

The protocol will be reviewed and approved by all of the following bodies before recruitment begins:

1. Western Institutional Review Board (WIRB). PATH subcontracts its ethical review for clinical trials to WIRB.
2. The icddr,b RRC and ERC.

All amendments will be approved in writing by WIRB and the icddr,b RRC and ERC before implementation and paperwork are shared between partners, as appropriate. No deviations from, or changes to, the protocol shall be initiated without prior written IRB approval by both WIRB and icddr,b of an appropriate amendment from all responsible IRBs, except when necessary to eliminate immediate hazards to the participants or when the change(s) involves only logistical or administrative aspects of the study (e.g., change of telephone number, etc.).

The PI will sign the final approved protocol.

8.3. Informed consent process

Prior to receipt of study vaccine, written informed consent will be obtained from a parent/guardian for all child participants. Informed consent documents will embody the elements of consent as described in the Declaration of Helsinki and the ICH Harmonized Tripartite Guidelines for GCP. The consent form will be in Bangla.

Original Informed Consent Forms must be kept on file by the investigator for possible inspection by regulatory authorities and PATH. The participant or the participant's legally acceptable representative must receive a copy of the signed and dated Informed Consent Form(s), and any subsequent updates or amendments. The study monitor shall check the documentation of the individual Informed Consent Form(s) during each monitoring visit.

Parents will initially be briefed about the study by a field worker, since they are known to the household and routinely inform participants and parents/guardians about vaccine trials and surveillance.

In the study clinic, the medical officer will ask if the child and parents/guardians have any questions about the study, read the informed consent form, and then conduct informed consent. One parent/guardian must sign the informed consent form. If the parent/guardian is illiterate, they may provide a thumb impression instead and an impartial literate witness (hierarchically independent of the PI and not specified on the list of trial contributors) will be present and attest to the parent/guardian consent. Consent will follow site-specific practices and be described in an SOP.

8.4. Inclusion of women and children

Children of any race/ethnicity and residing in the study area will be recruited for participation in the study. No special recruitment methods will be used to ensure certain levels of participation by any specific minorities residing in the source population. Investigators will emphasize during information sessions that girls and boys are at equal risk of JE and its complications.

8.5. Participant confidentiality

8.5.1. Confidentiality of data

By signing the protocol, the investigators agree that the study protocol, documentation, data, and all other information generated regarding the vaccines will be held in strict confidence. The investigators may divulge such information within regulatory restrictions and ethical considerations only to ethical review committees or similar expert boards or committees, and their affiliated institutions and employees, only under an appropriate understanding of confidentiality with such board or committee, and their affiliated institutions and employees. No information concerning the study or the data may be released to any unauthorized third party without prior written approval of PATH. Any regulatory agency deemed appropriate may consult study documents in order to verify case report form data. Investigators will ensure that all employees involved in the study respect the same rules.

Documents and data pertaining to the study will be kept in a locked cabinet under the responsibility of the investigator. PATH will conduct periodic monitoring visits to ensure that the data is safe and stored in this secure place and that only those authorized study staff have access to the data. Study data will be kept for five years after completion of the study.

Study participants will not be reported by name in any report or publication resulting from data collected in this study.

8.5.2. Confidentiality of patient records

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality pertains to a participant's personal and health information and extends to information on any biological specimen of the participant. Medical information about individual participants obtained during the course of this study is confidential and may not be disclosed to third parties, except authorized monitors, sponsors, auditors or inspectors. Confidentiality will be ensured by the use of participant numbers coding for the identification of each participant's personal, health, and specimen information. Access to this linkage and other confidential data will be strictly controlled.

The investigators will keep individual results confidential to the extent permitted by law. Information will not be released to anyone other than the participant, their parent or guardian, or their medical provider, unless required to do so by law.

8.5.3. Notification of primary care physician

If agreed by the participant or participant's legally acceptable representative, the investigator shall notify the participant's primary care physician, if applicable, of participation in the study. Participant's involvement in the study and study team contact numbers will be reported into the participant's identification card as applicable.

8.6. Study discontinuation

There are no expected reasons that would result in suspension and/or termination of the study. Unexpected reasons include:

- New safety data about the investigational product resulting from this or any other trial becomes available.
- The sponsor or ERC overseeing this study suspends and/or terminates the study for any justified reason, including the event that early safety data reveal an unusually high rate of SAEs or in the event study conduct fails GCP standards.

If the trial is prematurely terminated or suspended, the sponsor shall promptly inform the PI, the regulatory authorities, and the IRB/ERC of the reason for termination or suspension. Parents of children will be contacted immediately explaining why the study has been suspended or terminated and potential implications for children's safety, and provided with the opportunity to ask questions. Contacts with parents will be documented.

8.7. Future use of stored specimens

Aliquots of participants' serum specimens will be maintained for five years after the completion of the study. This is to allow the samples to be stored long enough to support any requests for additional information from WHO or other agencies required to register CD-JEV for use. Following completion of this period, all samples will be destroyed if additional studies are not requested. The specimens will be stored at a long term storage facility determined by the Sponsor . No personal identifying information associated with the study will be stored on site with the specimens. After the data analysis for the study is complete, the master linking document will be destroyed, thus removing the possibility of identifying the individual from whom the data and sample were collected. Samples will not be used for unrelated, future studies without appropriate ethics committee approvals and consent from participants (if deemed required by ethics committees and the master linking document with identifiers has not yet been destroyed).

9. USE OF ANIMALS

Not applicable. No animals will be used in this study.

10. COLLABORATIVE ARRANGEMENTS

10.1. Publication policy

A Clinical Study Report (CSR) comprised of text and results tables reflecting all safety and immunogenicity data will be generated. The CSR will be reviewed, approved, and signed by the investigator. The CSR will be compliant with ICH guidelines.

All data, documents, any recordings and information transferred by PATH to any contractor or obtained or prepared by any contractor, consultants, or persons associated by contractual relationships with any contractor during the trials, belong to icddr,b and PATH.

All confidential information communicated to the investigators by PATH shall be kept strictly confidential by them or any other person connected with the study and shall not be disclosed, either orally or in written form, to

any third party without prior written consent of the organization of which the information is the exclusive property.

Working with PATH representatives, the investigator is expected to publish the results of this research in a peer-reviewed scientific journal. In no way may anyone or any organization prohibit the public dissemination of valid results of this trial.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry. It will be the responsibility of PATH representatives to register this trial in an acceptable registry (CT.GOV). ICMJE authorship criteria will be strictly followed for publication of any manuscript(s) arising from this trial.

11. FACILITIES AVAILABLE

Icddr,b has large multi-disciplinary international and national scientific research staff. Existing field, hospital, laboratory and office facilities will be used for this study. Icddr,b scientists have conducted a variety of vaccine studies (Polio, influenza, pneumococcal, cholera, Shigella, rotavirus, Japanese encephalitis, etc.).

11.1. Field site

The study will be conducted in rural Matlab and urban Mirpur, Dhaka. Icddr,b has been maintaining a field research centre at Matlab for about fifty years. Due to the presence of ongoing HDSS, clinic and laboratory facilities, effective referral systems and well-established infrastructure at Matlab, it offers excellent research facilities for this study. The HDSS is a regularly updated information system on the approximate population of 220,000.

11.2. Laboratory facilities

Existing laboratory facilities in icddr,b will be used to process the samples.

The specimens will be tested at the United States Army Medical Directorate, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand.

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