

Original Research Article

A multi-center, randomized, controlled, phase-2/3 study to evaluate the safety and immunogenicity of inactivated Japanese encephalitis vaccine in healthy ≥ 1 to < 3 years old Indian children

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ABSTRACT

Background: Japanese encephalitis (JE) is endemic in various parts of India. Due to the limited treatment options available, a new treatment option that is safe and effective is highly expected for the existing medical needs.

Methods: In the present randomized Phase-2/3 study, safety, immunogenicity, and reactogenicity of JE-vaccine manufactured by Biological E (BE-JE), given in 2-dose schedule, 28-days apart was assessed in 456 healthy ≥ 1 to < 3 -year-old children compared to a licensed JE-vaccine with 3-dose schedule manufactured by Green-cross-corporation (GCC-JE).

Results: The BE-JE group demonstrated non-inferiority compared to GCC-JE group at day-56 as the lower confidence limit of the group difference was not $\geq -10\%$. At day-56, the geometric mean titre values were significantly higher in BE-JE group compared with the GCC-JE group (217.97 vs 125.85; $p=0.0023$). No apparent difference was seen in the safety-profile between both vaccine groups, with all events reported being either mild or moderate in intensity. Also, no significant difference ($p=0.2198$) was reported in the number of subjects experiencing at least one adverse-event (AE) between both groups.

Conclusions: The present study demonstrated BE-JE's Vero-cell-based inactivated JE-vaccine, administered in 2-dose schedule to be immunogenic, well-tolerated, and non-inferior, compared to GCC-JE-vaccine, administered in a 3-dose schedule in the pediatric Indian-population.

This study was prospectively registered with clinical trial registry of India bearing no CTRI/2011/091/000108 on 14/02/2011.

Keywords: Children, Incidence, India, JE, Pediatric, Vaccine, Vaccine trial

INTRODUCTION

Japanese encephalitis (JE) virus (JEV) is antigenically related to other flaviviruses like dengue, yellow fever, and West Nile virus and is the leading cause of viral encephalitis in Asia.¹ This virus was first isolated in Japan in 1933.² JE is a vector borne disease caused by Flavivirus, which is an emerging threat globally, majorly in the south, South-East Asia, and Australia.³ The Culex

mosquito, known to breed in stagnant water of rice paddy fields, transmits the JE infection in humans.⁴ Humans are the dead-end hosts because of low viremia and become infected when bitten by an infected mosquito.⁵ It is difficult to eradicate the virus because it is transmitted from natural reservoirs.⁶ Being neuroinvasive, this virus easily crosses the blood-brain barrier resulting in acute encephalitis.^{7,8} The severe infection can lead to paralysis, coma, and death. One-third of patients who recover from JE also have long-term effects with residual neurological

disability including memory loss, impaired cognition, behavioral disturbances, and tone and coordination abnormalities.⁹

The JEV primarily affects children resulting in acquiring of active immunity to the disease in adulthood in countries where the disease is endemic.¹⁰ The infection rate of JE in children is 5-10 times more than those aged 15 and above. JE is attributed to an estimated 50,000-70,000 cases worldwide, with a high case fatality rate of 30-50%.⁷ Over 100,000 cases and 25,000 deaths were attributed to JE in 2015 globally.¹¹ The JEV is the leading cause of all acute encephalitis diagnosed in India.¹² The clinical cases of JE were first reported in 1955 in Vellore and Pondicherry in south-India.⁴ The first major epidemic caused by the JEV was reported from Burdwan and Bankura districts of West Bengal in 1973, which led to death in 300 patients. Outbreaks of JE were reported from Uttar Pradesh in 1978, 1988, and 2005 and West Bengal in 2014.¹²⁻¹⁴ A higher incidence of JE cases was reported in Assam from 2012 to 2014.¹⁵ In 2016, a total of 11,651 cases led to 1,301 deaths reported to the National Vector Borne Diseases Control Program with a case fatality rate of 11%. The majority of fatalities were reported from Uttar Pradesh, West Bengal, Assam, and Bihar.¹² At present, JE is endemic to 171 districts and 19 states in India.¹² As per world health organization (WHO), India has reported 2,496 cases of JE in the year 2019 alone.¹⁶

The JE is a vaccine preventable disease.^{9,12} As there are no effective antiviral therapies available for this disease, vaccination is the only way of preventing JE.¹⁴ Vaccination programs have averted around 45,000 JE cases worldwide in 2015 and provide long-term control measures.^{11,17} The underlying basis for using vaccines against JE is that they help the body produce and maintain antigen-specific immune-response, leading to long-lasting humoral immunity.⁷ The 3 major types of vaccines against JE that are currently available include mouse brain-derived inactivated (no longer in use due to associated AEs), cell-culture derived inactivated, and cell-culture derived live-attenuated JE-vaccines. The live-attenuated vaccines offer better prospects for future vaccine development since they require fewer viruses to mount satisfactory immune-response.⁷

The JE-vaccine is recommended for residents of endemic areas and visitors to these areas.¹⁸ In India, JE vaccination was introduced in the year 2006. Since 2014, the JE-vaccine was a part of the national immunization program in 179 districts in 9 states where the disease is highly prevalent.¹² However, it is also spreading to non-endemic areas in India, because of ecological changes and extensive traveling of people seeking for employment and social reasons.¹⁹ In such a scenario, it is pertinent to vaccinate all individuals of pediatric age group. The vaccination against JE could only reduce the morbidity and mortality associated with the disease. However, there are issues about the effectiveness of the currently employed SA₁₄-14-2 JE-vaccine in India. Despite using

this vaccine in routine immunization, there has been no appreciable change in the epidemiology of JE. According to a post-marketing surveillance study in India conducted by the Indian Council of Medical Research, the efficacy of the vaccine was not as high as seen in Nepal. The study showed that the protective efficacy of the vaccine at one year was 43.1% overall and 35% for those who were non-immune at pre-vaccination.²⁰ Hence, a safe and effective JE-vaccine is needed to protect residents of endemic region and travelers. The growing risk of transmission to travelers will increase the need for pre-travel immunization with a safe, convenient, and immunogenic vaccine.²¹

Intercell AG, Austria and Biological E Limited, India have collaborated for the development of inactivated JE SA₁₄-14-2 virus vaccine in South-East Asia. Intercell's vaccine is already approved in various parts of the world with the brand names "IXIARO®" in the US and as "JESPECT®" in Australia. BE-JE vaccine is manufactured in India with the technical collaboration with Intercell. This vaccine has successfully completed its preclinical and phase-1 study in Indian adult population at the time of this study. In view of the above, this phase-2/3 study was carried out in 2-parts. Phase-2 part assessed the safety 7-days post vaccination in healthy ≥ 1 to < 3 years old Indian children. Phase-3 part assessed the non-inferiority of the BE-JE-vaccine compared with the licensed JE-vaccine manufactured by Green Cross Corporation, South Korea (GCC-JE) in terms of immunogenicity and safety in the same target population. During this study, the GCC-JE-vaccine was the acceptable choice for comparison as it was the only inactivated JE-vaccine that was earlier licensed for marketing in India.

METHODS

Study population and study design

Study population

Healthy subjects of either gender between ≥ 1 to < 3 years of age were included in the study. Children with a previous history of JE vaccination, severe hypersensitivity reaction to vaccinations, chronic administration (> 14 days) of immuno-suppressants or immune-modifying therapy up to 6-months prior to vaccination, evidence of previous JE infection, dengue or yellow fever, and any cardiovascular disorders or any other medical condition that would make intramuscular (IM) injection unsafe, were excluded from the study.

Institutional ethics committee or institutional review board approved the study protocol at all study sites. The study was conducted in accordance with the ethical principles defined in the declaration of Helsinki, international council for harmonization-good clinical practices (ICH-GCP) guidelines, and applicable regulatory requirements. Written informed consent was

obtained from parents/legally acceptable representatives of all children included in the study before the enrolment.

Study design

This was a multicenter, open-label, parallel, randomized phase-2/3 study designed to evaluate the immunogenicity and safety of vero cell-derived inactivated BE-JE-vaccine (BE-JE) in comparison with a licensed mouse brain-derived inactivated JE-vaccine (GCC-JE). The laboratory samples were appropriately coded and the central laboratories performed blinded analysis. This ensured avoiding any identification that would differentiate a separate group or sequential testing by a group.

In phase-2 part of the study, safety and reactogenicity were assessed for 35% (n=160) of the study subjects enrolled until day-7. Following the data and safety monitoring board (DSMB) review, all 160 subjects who completed their 7th day following the first-dose continued to be part of phase-3 (part-2) of the study by receiving their subsequent doses as per the dosing schedule of the respective vaccine groups. In the Phase-3 part of the study, comparative immunogenicity and safety of both study vaccines were evaluated assessing non-inferiority based on difference in the proportion of subjects seroconverted between investigational (BE-JE) and control groups (GCC-JE). Visit schedule and assessments were presented as supplementary information.

Immunogenicity evaluations

Immunogenicity was the primary objective of the phase-3 part of this study. For this purpose, the proportion of subjects' seroprotected with anti-JEV neutralizing antibodies at day-56 in both the treatment groups was compared. JEV neutralizing antibodies were measured by plaque reduction neutralization test (PRNT), which was carried out for all subjects in both groups for whom the serum samples were available following the method described by Russell et al. with several modifications.²²

As a means of quality control in each assay, the serum dilution giving a 50% reduction in plaques in a PRNT (PRNT50) of the positive control sample was determined. Probit analysis utilizing SPSS software (Version) assessed a 50% reduction in plaque counts (PRNT50 titre). PRNT50 titre of $\geq 1:10$ is defined as seroconversion, also known as seroprotection.

Center of vaccine development, Mahidol university, at Salaya, Nakhonpathom, Thailand carried out all validated PRNT assay by adhering to their standard operating procedures in biological safety level 2 environment in conformance with Good Laboratory Practice.

Secondary objectives in the phase-3 part of this study included the comparison of the proportion of subjects seroprotected with anti-JEV neutralizing antibodies at Day-28, and comparison of the geometric-mean-titres

(GMTs) of anti-JEV IgG neutralizing antibodies in both treatment groups and fold increase (from day-0 to day-56) in anti-JEV neutralizing antibody titres against JEV antigen in both treatment groups.

Safety evaluations

Following vaccination, the subjects were observed for 60-minutes for any allergic reactions. A seven-day follow-up (Day-0 to day 6) of solicited local and systemic AEs along with the assessment of its intensity was performed by the subject's parents or legally acceptable representative/guardian, 60-minutes after vaccination and recorded in the diary provided. Solicited AEs were assessed as local tolerability that included local pain, redness, swelling, tenderness at injection site, itching and hardening; and systemic tolerability that included fever, diarrhea, unusual crying, drowsiness, loss of appetite, vomiting, and rash. Any other unsolicited AEs reported during the study period were also recorded. The AEs were recorded and followed-up for the entire duration of the study starting immediately following vaccine administration until the 28th-day after the last dose (Day-56).

Statistical methods

Sample size determination

The study was designed to have a power of 90%. Based on an earlier phase-2 study (IC51-221) conducted in India, the GCC-JE-vaccine was known to offer a seroconversion rate of 90.9% in ≥ 1 to < 3 years old healthy children. The BE-JE-vaccine was also expected to offer no less than 90% of the seroconversion rate. Details of statistical analyses performed in the study were summarized as supplementary information.

RESULTS

Subject disposition

The study was carried out at 8 centers in India from 28 February 2011 to 11 July 2011. Of the 506 subjects screened at baseline, 456 subjects (304 in BE-JE and 152 in GCC-JE) were randomized into the study and received the first-dose of the study vaccine. Of the 456 randomized subjects, 421 (92.3%) subjects completed the study and 35 (7.7%) subjects discontinued the study due to the following reasons: lost to follow-up (32 subjects), SAE, migration from the study area and missed visit (1 subject each) (Figure 1).

In total, 506 subjects were screened and 456 subjects were enrolled to participate in the study. All 456 participants received vaccination. Out of 456 participants, 421 subjects completed the study, 34 subjects dropped out due to occurrence of SAE or lost to follow up or migrated from study area and 1 subject missed the visit.

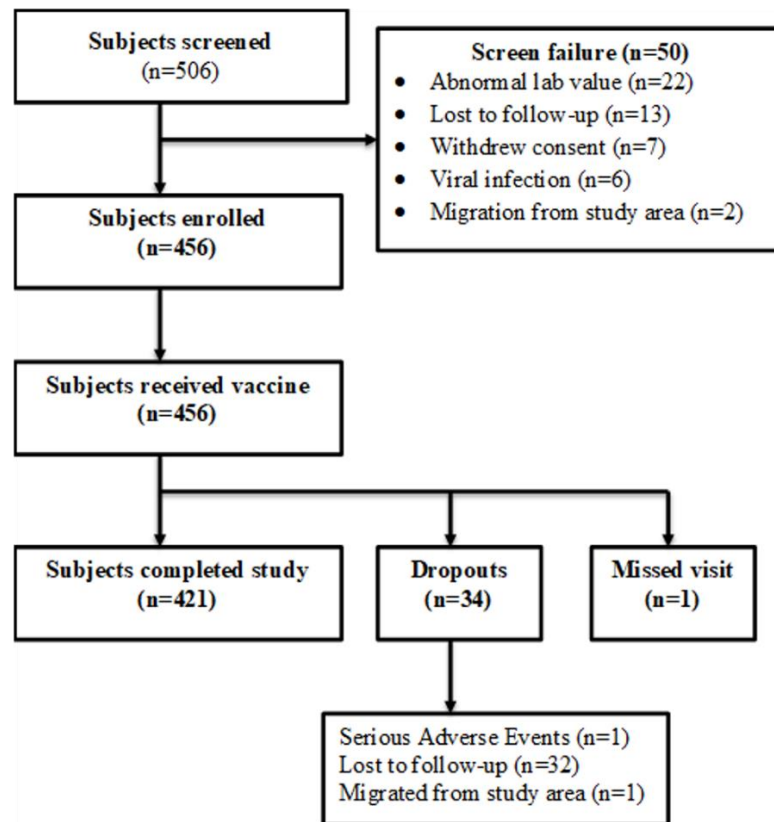


Figure 1: Subject disposition.

Table 1: Demographics characteristics-intent-to-treat analysis population.

Parameter	BE-JE (n=304), (%)	GCC-JE (n=152), (%)	All (n=456), (%)
Male	161 (52.96)	89 (58.55)	250 (54.82)
Female	143 (47.04)	63 (41.45)	206 (45.18)
Median age (Years)	1.11	1.10	1.11
Range (min, max)	(1.00, 3)	(1.00, 3)	(1.00, 3)
95% CI of mean	(1.49, 1.61)	(1.43, 1.6)	(1.49, 1.58)
Median height (Feet)	2.11	2.10	2.11
Range (min, max)	(0.73, 3.34)	(1.08, 3.3)	(0.73, 3.34)
95% CI of mean	(2.28, 2.38)	(2.28, 2.4)	(2.30, 2.37)
Median weight (kgs)	10.10	10	10
Range (min, max)	(6.20, 15)	(3.90, 16)	(3.90, 16)
95% CI of mean	(9.95, 10.30)	(9.80, 10.4)	(9.96, 10.27)

BE-JE=Biological E Japanese encephalitis vaccine, CI=Confidence interval, GCC-JE=Green cross corporation Japanese encephalitis vaccine, ITT=Intent-to-treat, Kgs=Kilograms.

Demographics and baseline characteristics

Demographics characteristics were analyzed in the ITT population in both BE-JE (n=304) and GCC-JE (n=152) groups. There were more male subjects (n=250, 54.82%) in study compared to female subjects (n=206, 45.18%).

The median (range) age, height, and weight of the subjects were 1.11 (1.00 to 3.00) years, 2.11 (0.73 to 3.34) feet, and 10.00 (3.90 to 16.00) Kgs, respectively. Overall, the demographic characteristics were comparable between the BE-JE and GCC-JE groups

(Table 1). Paracetamol was the commonly used concomitant medication in the fever management of 7/304 (2.3%) subjects in the BE-JE group and 12/152 (7.89%) subjects in the GCC-JE group.

Immunogenicity findings

Immunogenicity assessments were primarily based on the PP population that consisted of 418/456 (91.6%) subjects, with 277 in the BE-JE group and 141 in the GCC-JE group. However, immunogenicity analysis was assessed in both ITT and PP populations. Overall, the proportion

of subjects seroconverted at day-56 were 92.42% in the BE-JE group and 98.58% in the GCC-JE group in PP population (Figure 2). The difference in seroconversion rates between the groups at Day-56 was 6.16%, with the lower limit of 95% CI -0.098 and upper limit of -0.025%. The BE-JE-vaccine demonstrated non-inferiority compared to GCC-JE as the lower confidence limit of the group difference was not below -10.0%. Not much difference was observed in the seroconversion rates in both groups as per ITT and PP analysis sets.

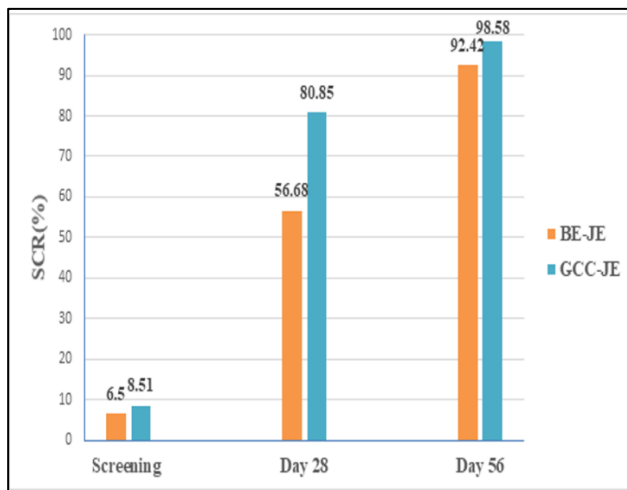


Figure 2: Seroconversion rates at screening, day-28 and day-56 by vaccine group in per-protocol population.

Percent seroconversion rate after vaccination with BE-JE or GCC-JE at day 28 and day 56 in comparison to baseline values were shown in Figure 2.

The proportion of subjects seroconverted at Day-28 was 56.68% in the BE-JE group and 80.85% in the GCC-JE group in PP population. Similar results were observed for both groups in the ITT population. The GMT values were significantly higher in the BE-JE group compared with the GCC-JE group and day-56 (217.97 vs 125.85; $p=0.0023$) in the PP population (Table 2). Similar results were observed for both groups in the ITT population.

On day-28, the proportion of subjects who achieved ≥ 2 , ≥ 3 and a ≥ 4 -fold increase in anti-JEV neutralizing antibody titres from baseline were significantly higher ($p<0.0001$) in the GCC-JE group compared with the BE-JE group in the PP population. However, the proportion of subjects who achieved a ≥ 4 -fold increase in anti-JEV neutralizing antibody titres at Day-56 from baseline was similar in the BE-JE and GCC-JE groups (78.70% [218/277 subjects] vs 78.01% [110/141 subjects]). Whereas, the proportion of subjects who achieved a ≥ 10 -fold increase in anti-JEV neutralizing antibody titres at Day-56 from baseline was higher in the BE-JE group compared with the GCC-JE group (63.90% [177/277 subjects] vs 56.03% [79/141 subjects]) (Table 3).

Table 2: Summary of GMTs between vaccine groups-per-protocol population.

Variables	BE-JE (n=277), (%)	GCC-JE (n=141), (%)	P*
Day-0			
Geometric mean titre ± SD	9.70± 1.44	9.84± 1.50	0.7177
95% CI of GMT	(9.29, 10.13)	(9.20, 10.52)	
Day-28			
Geometric mean titre ± SD	27.20± 3.62	55.68± 4.30	<0.0001
95% CI of the GMT	(23.37, 31.68)	(43.68, 70.98)	
P value** (Day- 0 to day-28)	<0.0001	<0.0001	
Day-56			
Geometric mean titre ± SD	217.97± 6.93	125.85±4 .86	0.0023
95% CI of the GMT	(172.55, 275.33)	(96.67, 163.85)	
P value** (Day- 28 to day-56)	<0.0001	<0.0001	

BE-JE=Biological E Japanese encephalitis vaccine, CI=confidence interval, GCC-JE=Green cross corporation Japanese encephalitis vaccine, GMT=Geometric mean titre, PP=Per-protocol, SD=Standard deviation. *P value was obtained using un-paired t test (two tailed, $\alpha=0.05$) between groups. **P value was obtained using paired t test (two tailed, $\alpha=0.05$) between visits.

Table 3: Fold increase in antibody titres by vaccine group-per-protocol population.

Fold raise	BE-JE, (n=277) (%)	GCC-JE, (n=141) (%)	P value*
From day-0 to day-56			
≥ 2 fold	235 (84.84)	129 (91.49)	0.0552
≥ 3 fold	226 (81.59)	119 (84.40)	0.4745
≥ 4 fold	218 (78.70)	110 (78.01)	0.8718
≥ 8 fold	188 (67.87)	84 (59.57)	0.0926
≥ 10 fold	177 (63.90)	79 (56.03)	0.1184
From day-0 to day-28			
≥ 2 fold	130 (46.93)	100 (70.92)	<0.0001
≥ 3 fold	109 (39.35)	90 (63.83)	<0.0001
≥ 4 fold	95 (34.30)	81 (57.45)	<0.0001

BE-JE=Biological E Japanese encephalitis vaccine, GCC-JE=Green cross corporation Japanese encephalitis vaccine, PP=per-protocol. *P value was calculated using Chi-square test (two tailed, $\alpha=0.05$).

Safety findings

Phase 2

An interim safety report was generated to assess the safety and reactogenicity of BE's inactivated vaccine 7 days after first-dose administration (the primary objective

of phase 2) on 160 subjects. Further, enrolment was kept on hold until vaccine safety was established in these subjects. An independent DSMB monitored and reviewed the interim safety report. The AEs experienced by subjects were found to be either mild or moderate in nature. There were 2 SAEs reported (gastroenteritis [verbatim term: vomiting] in the BE-JE group and bronchitis in the GCC-JE group) that were moderate in severity and not related to the study vaccine as per the investigator.

Phase 3

The study was further opened for phase 3 part of the study after DSMB concluded that the study vaccine was safe and well-tolerated with no safety concerns. The 160 subjects enrolled in Phase-2 continued to be in part 3 of the study. The secondary safety analyses were based on the safety population, which included 456 subjects (100%) who received at least one dose of study vaccination.

Overall, 211 AEs (128 in the BE-JE group and 83 in the GCC-JE group) were reported during the entire study

period by 70/304 (23.03%) subjects in the BE-JE group and 43/152 (28.29%) subjects in the GCC-JE group (Table 4). All the events were either mild or moderate in intensity. There was no statistically significant difference ($p=0.2198$) in the number of the subject experiencing at least one AE between BE-JE and GCC-JE groups. The most common system organ class for TEAEs ($>10\%$ in any group) was general disorders and administration site conditions with 64/304 (21.05%) and 39/152 (25.66%) subjects in the BE-JE and GCC-JE groups, respectively.

The most common AEs observed ($>5\%$ of subjects in any group) in BE-JE and GCC-JE groups were pyrexia (34/304 [11.18%] vs 24/152 [15.79%] subjects), injection site pain (26/304 [8.55%] vs 13/152 [8.55%] subjects), and injection site swelling (10/304 [3.29%] vs 8/152 [5.26%] subjects). The incidence of local AEs was higher in the BE-JE (59/128 [46.09%] AEs) group compared with the GCC-JE (33/83 [39.76%] AEs) group. The most common local AEs ($>5\%$ of subjects in any group) in BE-JE and GCC-JE groups were injection site pain (26/304 [8.55%] vs 13/152 [8.55%] subjects) and injection site swelling (10/304 [3.29%] vs 8/152 [5.26%] subjects) (Table 5).

Table 4: Summary of AEs-safety population.

Particulars	BE-JE, (n=304)		GCC-JE, (n=152)	
	N (%)	95% CI	N (%)	95% CI
Number of AEs	128 (100)	-	83 (100.00)	-
Number of SAEs	1 (0.78)	(0.74, 2.31)	1 (1.20)	(1.14, 3.55)
Number of subjects with at least 1 AE	70 (23.03)	(18.29, 27.76)	43 (28.29)	(21.13, 35.45)
Number of subjects with at least 1 SAE	1 (0.33)	(0.31, 0.97)	1 (0.66)	(0.63, 1.94)
Subjects discontinued due to AE/SAE	1 (0.33)	(0.31, 0.97)	0 (0.00)	(0.00, 0.00)
Number of subjects with medically attended AEs	16 (5.26)	(2.75, 7.77)	16 (10.53)	(5.65, 15.41)
Number of subjects with at least 1 related AE	63 (20.72)	(16.17, 25.28)	37 (24.34)	(17.52, 31.16)
Number of subjects with at least one related serious AE	0 (0.00)	(0.00, 0.00)	0 (0.00)	(0.00, 0.00)

AE=adverse event, BE-JE=Biological E Japanese encephalitis vaccine, CI=Confidence interval, GCC-JE=Green cross corporation Japanese encephalitis vaccine, SAE=Serious adverse event.

Table 5: Summary of local and systemic AES by soc and pt in $>1\%$ subject in any group-safety population particulars.

Variables	BE-JE, (n=304)		GCC-JE, (n=152)	
	N (%)	95% CI	N (%)	95% CI
Number of AEs	128 (100)	-	83 (100)	-
Number of local AEs	59 (46.09)	(37.46, 54.73)	33 (39.76)	(29.91, 50.52)
Number of systemic AEs	69 (53.91)	(45.27, 62.54)	50 (60.24)	(49.48, 70.09)
Local AEs				
General disorders and administration site conditions				
Injection site pain	26 (8.55)	(5.41, 11.70)	13 (8.55)	(4.11, 13.00)
Injection site swelling	10 (3.29)	(1.28, 5.29)	8 (5.26)	(1.71, 8.81)
Tenderness	14 (4.61)	(2.25, 6.96)	5 (3.29)	(0.45, 6.13)
Skin and subcutaneous tissue disorders				
Injection site erythema	8 (2.63)	(0.83, 4.43)	6 (3.95)	(0.85, 7.04)
Systemic AEs				
Gastrointestinal disorders				
Vomiting	2 (0.66)	(0.25, 1.57)	2 (1.32)	(0.50, 3.13)

Variables	BE-JE, (n=304)		GCC-JE, (n=152)	
General disorders and administration site conditions				
Pyrexia	34 (11.18)	(7.64, 14.73)	24 (15.79)	(9.99, 21.59)
Infections and infestations				
Nasopharyngitis	6 (1.97)	(0.41, 3.54)	4 (2.63)	(0.09, 5.18)
Metabolism and nutrition disorders				
Decreased appetite	8 (2.63)	(0.83, 4.43)	5 (3.29)	(0.45, 6.13)
Nervous system disorders				
Crying	4 (1.32)	(0.03, 2.60)	2 (1.32)	(0.50, 3.13)
Somnolence	5 (1.64)	(0.21, 3.07)	2 (1.32)	(0.50, 3.13)
Respiratory, thoracic and mediastinal disorders				
Cough	4 (1.32)	(0.03, 2.60)	5 (3.29)	(0.45, 6.13)
Skin and subcutaneous tissue disorders				
Rash	4 (1.32)	(0.03, 2.60)	2 (1.32)	(0.50, 3.13)

AE=adverse events BE-JE=Biological E Japanese encephalitis vaccine, CI=Confidence interval, GCC-JE=Green cross corporation Japanese encephalitis vaccine, SOC=System organ class, PT=Preferred term.

Table 6: Overview of medically attended AEs -safety population.

SOC, local AE	BE-JE, (n=304)		GCC-JE, (n=152)	
	N (%)	95% CI	N (%)	95% CI
Number of subjects with at least 1 medically attended AE	16 (5.26)	(2.75, 7.77)	16 (10.53)	(5.65, 15.41)
Gastrointestinal disorders				
Diarrhoea	1 (0.33)	(0.31, 0.97)	1 (0.66)	(0.63, 1.94)
Vomiting	2 (0.66)	(0.25, 1.57)	2 (1.32)	(0.50, 3.13)
General disorders and administration site conditions				
Pyrexia	8 (2.63)	(0.83, 4.43)	11 (7.24)	(3.12, 11.36)
Infections and infestations				
Dysentery	0 (0.00)	(0.00, 0.00)	1 (0.66)	(0.63, 1.94)
Gastroenteritis viral	0 (0.00)	(0.00, 0.00)	1 (0.66)	(0.63, 1.94)
Hordeolum	1 (0.33)	(0.31, 0.97)	0 (0.00)	(0.00, 0.00)
Nasopharyngitis	5 (1.64)	(0.21, 3.07)	3 (1.97)	(0.24, 4.18)
Respiratory, thoracic and mediastinal disorders				
Cough	4 (1.32)	(0.03, 2.60)	4 (2.63)	(0.09, 5.18)

AE=Adverse event, BE-JE=Biological E Japanese encephalitis vaccine, CI: confidence interval, GCC-JE=Green cross corporation Japanese encephalitis vaccine, SOC=System organ class.

The incidence of systemic AEs was lower in the BE-JE (69/128 [53.91%] AEs) group compared with the GCC-JE (50/83 [60.24%] AEs) group. The most common systemic AE (>5% of subjects in any group) in the BE-JE and GCC-JE groups was pyrexia (34/304 [11.18%] vs 24/152 [15.79%] subjects) (Table 5). The proportion of subjects with at least one related AE was 20.72% (63/304 subjects) in the BE-JE group and 24.34% (37/152 subjects) in the GCC-JE group. No subjects had related SAEs in any of the groups (Table 4).

No death was reported due to AEs in the study. Overall, the SAEs reported during the entire study period were very low. Only 2 subjects reported SAEs of gastroenteritis (n=1 in the BE-JE group) and bronchitis (n=1 in the GCC-JE group) during the entire study. The subject who had experienced an SAE of gastroenteritis discontinued from the study. Both the SAEs required medical attention, which were usually either a medication or an investigation. Both SAEs were resolved and were

considered as not related to the study vaccine by the investigator. However, for SAE of gastroenteritis, the DSMB could not rule out the possibility of a causal relationship with the study vaccine.

The incidence of medically attended AEs was higher in the GCC-JE (16/152 [10.53%] subjects) group compared with the BE-JE (16/304 [5.26%] subjects) group. The most common medically attended AE (>5 subjects in any group) was pyrexia in both BE-JE (8/304 [2.63%] subjects) and GCC-JE (11/152 [7.24%] subjects) groups (Table 6).

DISCUSSION

At present, JE is not only endemic to many parts of India but has also spread to non-endemic areas.^{16,19} Vaccination against JEV, therefore, remains the first choice of protecting the vulnerable population. The mouse brain-derived JE-vaccines are no longer available due to the associated AEs.⁷ As advised by WHO, several nations

have transitioned from mouse brain-derived vaccines to newer, less reactogenic vaccines with easier dose regimens.²³

However, despite the usage of the SA₁₄-14-2 JE-vaccine, there were contradictory reports regarding its efficacy in India.²⁰ The present study compared the safety and immunogenicity between BE's Vero cell-derived inactivated JE-vaccine administered IM in a 2-dose schedule and GCC's mouse brain-derived inactivated JE-vaccine administered in a 3-dose schedule administered subcutaneously to ≥ 1 to < 3 years old healthy Indian subjects of both genders.

In this multicenter, open-label, randomized, phase-2/3 study, the primary safety objective at phase-2 was attained through a favorable safety recommendation from DSMB. The study then progressed to phase-3 after getting a go-ahead from the board.

The primary objective of the phase-3 (part-2) study was to demonstrate the non-inferiority of BE's Vero cell-derived inactivated JE-vaccine against GCC's mouse brain-derived inactivated JE-vaccine with respect to the difference in the proportion of subjects seroconverted at day-56.

As antibodies play a key role in protection against infection, these are considered as correlates of protection. The neutralization test is the most specific measure of antibody. To measure neutralizing antibody titres PRNT is usually used.²⁴ BE-JE-vaccine demonstrated this primary objective by achieving PRNT₅₀ $\geq 1:10$ criteria set at 95% CI (-0.098, -0.025) at day-56. A statistically significant difference was observed in the proportion of subjects seroconverted at day-56 ($p < 0.05$). The priming effect of a single dose of BE-JE vs two doses of GCC-JE was demonstrated with the statistically significant difference in proportion of subjects seroconverted at Day-28 (56.68% vs 80.85% in BE-JE group and GCC-JE group, respectively; $p < 0.0001$). Furthermore, at Day-56, the BE-JE-vaccine demonstrated a significantly higher GMT compared with the GCC-JE-vaccine group (217.97 vs 125.85). An adequate ≥ 4 -fold rise in GMT was observed in subjects vaccinated with the BE-JE-vaccine at Day-56, and the ≥ 4 -fold rise in GMT were comparable in both the vaccine groups. Immunogenicity results observed in this study were consistent with a previous Phase-2 study of Intercell's IXIARO vaccine conducted in Indian children between ≥ 1 to < 3 years of age.²⁵

In the present study, the most common AEs observed were pyrexia, injection site pain, and injection site swelling. The most frequently observed AEs were common and consistent after all other types of vaccination in children.²⁶ Most of the related events were mild in nature. Overall, a comparable safety profile was observed in both study groups at day-56 with no significant difference in terms of proportion of subjects reporting events. The safety findings of this study were

also in line with a previous study comparing the safety of Intercell's IXIARO vaccine with the GCC-JE-vaccine.²⁵

This study has limitation that the immunogenicity and safety data were collected and evaluated only until Day-56. A longer follow-up in study participants would have been more insightful in terms of providing more robust safety and immunogenicity profile of the study.

The BE-JE-vaccine demonstrated non-inferiority in terms of immunogenicity by the proportion of subjects seroconverted and GMT as compared to the GCC-JE-vaccine at day-56. The 2-dose immunization schedule of the BE-JE-vaccine revealed a similar probability of vaccine-associated AEs when compared to a 3-dose schedule of GCC's JE-vaccine.

CONCLUSION

In conclusion, the current study demonstrated that BE's vero cell-based inactivated JE-vaccine, administered in a 2-dose schedule (Day-0 and day-28), was immunogenic, well-tolerated, and non-inferior when compared to GCC-JE-vaccine, administered in a 3-dose schedule (Day-0, day-7, and day-28).

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