Long Term Follow up of the Lived Attenuated Japanese Encephalitis Vaccine (SA-14-14-2) Efficacy in Children of the four hyper-endemic Provinces of Northern Thailand, 2016.

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Abstract

Background. In Thailand, Japanese encephalitis appears with a higher incidence of the Northern region (0.0/100,000 cases/year) as compared to the central southern region of the country (0.01/100,000/year). Also, all Japanese encephalitis virus (JEV) recently isolated strains in country belong to the genotype I and III.

Methods. In order to evaluate the long-term efficacy of the newly developed live attenuated SA 14-14-2 Japanese encephalitis vaccine in Thailand, the immune response in children was studied after a second immunization campaign. Following 2012 immunization campaign, a second JEV immunization vaccine was delivered to children among four provinces of the Northern Region of Thailand. For each province, the recipients were identified accordingly to the two vaccine campaigns time including the children who received the second dose of vaccine: 1/ less than one year before the present study (N=30 by province); 2/ more than one year before the present study (N=70 by province). Altogether, a total of 400 children were enrolled in this project. After receiving the second vaccine dose, blood samples were collected and tested for JEV (Genotypes I and III) neutralizing antibodies following a standard procedure of LLC-MK2 infected cells.

Results. All recipient presented neutralizing antibodies cross reacting against the prevalent JEV **genotypes I (SM1 JEV strain) and genotype III (**SA 14-14-2 and Beijing JEV strains). Most of the children from the first group had a seroconversion rate of 94.2% against homologous (i.e. GIII). Among them, an optimal rate of 100% seroconversion was find at the Phayao Province against both heterologous (GI) and homologous (GIII) JEV strains. Geometric Mean Titer (GMT**)** of neutralizing antibody against SM1, SA 14-14-2 JEV, and Beijing JEV strains were consistently high, respectively as 239±50, 323±201.2 and 177±36.7. For the second group, seroconversion rate was lower against SM1, SA 14-14-2 and Beijing JEV strains with a rate of 94.3, 90% and 86.8% with respectively a GMT of 154±32, 90±23 and 82±13.6.

Conclusion. Although, the immunity appears to decrease within the period of 2.5 years, neutralizing antibody rate are consistent to protect against the JEV infection. Nevertheless, a long-term follow up is suitable to evaluate an eventual third dose opportunity.

Background

Japanese encephalitis (JE) is the leading cause of viral encephalitis in the World including 50,000 cases of acute nervous system infections leading to over 15,000 of deaths yearly, while 23% of survivors develop serious permanent neurological sequelae (Solomon, 2000). The mosquito, *Culex tritaeniorhynchus,* transmits the virus to humans and other amplifying hosts, such as swine, birds, and other vertebrates (Gould et al., 1974).

Though JE occurred within a variety of environment in Asia, Japanese Encephalitis epidemics in Thailand are confined to the Northern region of the country (Yamada et al, 1971). More than ten encephalitis cases

are still registered annually in Thailand and prompted (Annual Epidemiological Surveillance Report 2015 Bureau of Epidemiology, Ministry of Public Health). Thai Ministry of Public Health developed strategic immunization campaign prioritizing high endemic areas stating in 1992 (Muangchana et al., 2010).

Although inactivated, mouse brain-derived JE vaccine (MBDV) was found to be highly effective and was included in the expanded program of immunization (EPI) for children in several countries including Thailand (Muangchana et al., 2012), due to safety concerns and the need for multiple injections, new vaccine generation were expected to replace MBDV (Lindsey et al,. 2010). In this matter, the SA14-14-2 live-attenuated JE vaccine (Chengdu Institute of Biological Products, People's Republic of China) was licensed in Thailand. The safety was demonstrated (Xin et al., 1988), while several field trials implemented in China and Thailand suggested the efficacy of a single dose of the SA14-14-2 live-attenuated JE vaccine was expected to reach 95% protection, and a two doses with one year interval may exceed 98% (Tsai and Yu, 1995, Chotpitayasunondh et al., 2011). However, these results came from the neutralization test using a JEV strain genotype III homologous to the derived SA14-14-2 live-attenuated JE vaccine. Moreover, it has been reported that JEV genotype I strain actively circulate in Thailand and was isolated from the neighboring country of Lao People's Democratic Republic (Lao PDR) from an infected patient in Vientiane (Aubry et al., 2013). In 2012, the Ministry of Public Health of Thailand decided to administer the live-attenuated JE vaccine SA14-14-2 as a two doses campaigns of immunization in children of Northern Thailand among eight JE potentially endemic Provinces. Then, in 1992, the National Vaccine Institute Department of Communicable Diseases, Ministry of Public Health requested a JE vaccine SA14-14-2 vaccine efficacy study before including it in the present vaccine in EPI program.

In 2014 to 2015, the first dose vaccination was extended to four Northern provinces (Chiang Rai, Phayao, Mae Hong Son, Nan), while a second dose was administered in 2015 to 2016 followed by the present study on the efficacy and immune response.

Methods

Study population and area

The children who received 2 doses of Live attenuated SA 14-14-2 Japanese encephalitis (JE) vaccine (Chengdu Institute of Biological Products, People's Republic of China) where selected among the targeted 4 provinces of Chiang Rai, Phayao, Mae Hong Son and Nan among the Northern Region of Thailand (table1, figure1).

For each province, the children cohort (Retrospective study) was separated into two arms: The first arm of 30 children who received a first vaccine dose less than 1 year before the study. The second arm of 70

children who receive the first vaccine dose more than 1 year before the study. A total of 400 children were enrolled. Both sexes and age above 2.5-3.5 year were represented blood samples of 2 ml taken. All samples were tested for JEV antibody by Plaque Reduction Neutralization Test (PRNT) against JEV genotype I and III.

Oral consent was obtained from each child assisted by their parents or relative under the guidance of the Mahidol University Ethical Committee (MU: 2016-006).

Plaque Reduction Neutralization Test (PRNT)

The PRNT is one of the most specific serological tests for alphavirus and correlates with serum levels of protection from virus infection (Russell et al., 1967). PRNT involves virus–antibody interaction in a test tube, and then measures antibody effects on viral infectivity by plating the mixture on virus-susceptible cells. As previously described, LLC-MK2 cells were used for virus production of the SM1 JEV (genotype I) strain, SA14-14-2 live-attenuated JE vaccine and, and Bejing JEV (genotype III) strains (Nitatpattana et al., 2015). Data were interpreted using the Probit model with the SPSS program, and PRNT end point titers were expressed as the reciprocal of the last serum dilution calculated on a 50% reduction in plaque counts (PRNT50). Geometric Mean Titer (GMT) was function in Microsoft Office 2010, (Excel program).

Results

The children from the first group (first vaccine dose less than 1 year) had a seroconversion rate of 90 - 100% (Table 2). The participants from the Phayao province had a high seroconversion rate of 100% against both heterologous (SM1 JEV) and homologous (SA 14-14-2) strains. Most of GMT for SM1, SA 14-14-2 and Beijing strains were consistently high about from 239±50, 323±201.2 and 177±36.7 respectively. Phayao province had highest GMT (707) against SA 14-14-2 strain.

The second group (first vaccine dose more than 1 year), when compared to the first group, showed a slightly reduced conversion rate (Table 2) between 86.9 to 94.3%, but nevertheless showing a consistent seroconversion rate corresponding to a GMT of 154±32, 90±23 and 82±13.6 respectively against SM1, SA 14-14-2 and Beijing JEV strains (Table 2).

This first study confirmed this vaccine safety while the administration of two doses provided a strong crossing immunogenicity against JE virus **genotype I (SM1) and genotype III** (SA 14-14-2 and Beijing).

Discussion

Accordingly, with previous immunization campaigns all the 400 children from the four Northern provinces of Thailand had a seroconversion rate against JEV above 93.9% with a GMT unit higher than 151 units after the first round of immunization campaign (Kwon et al., 2015). Moreover after they received a second JEV vaccine dose they showed a consistent and efficient seroconversion rate (> 87%) against homologous as well as heterologous JEV genotypes I and III circulating in Thailand. Although, the immunity decrease, antibody neutralizing titer and GMT appears efficiently protective against JE virus infection while, at the national level, Thai children less than 2 years-old had a 32% natural immunity and 69% for the 2-9 years old children JEV seroprevalence (Yoocharoan et al., 2009). Nevertheless, a long term follows up of the protective immune response is needed to evaluate the necessity of a third dose to protect all children these class of age particularly vulnerable to JE.

Abbreviations

GMT: Geometric Mean Titer

JEV: Japanese encephalitis virus

MBDV: mouse brain-derived JE vaccine ()

PRNT: Plaque Reduction Neutralization Test

Declarations

Ethics approval and consent to participate. Oral consent was obtained from each child assisted by their parents or relative under the guidance of the Mahidol University Ethical Committee (MU: 2016-006). As for any minors' participant (age 16>) involved in the study, consent forms were applied and obtained from their accompanying parent or guardian and signed, on behalf of all of the minors, by their parent or guardian.

Consent for Publication: Not Applicable

Availability of data and materials. All data and material of the present study are available upon request to the authors including all field and laboratory original data anonymized in an excel format.

Competing interests. All authors declare no financial and/or non-financial competing interests.

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Authors' contributions. All authors have read and approved the present manuscript.

N.N. and S.Y. conceived and developed the project and wrote the manuscript; K.N. performed the laboratory tests. S.J., S.R., S.R., S.A. performed the field work and all field data collection and computation; FV and JPG equally gave guidance for the data analysis and interpretation and actively participate to wrote the manuscript.

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Tables

Table 1. Selected study areas among four districts of four Provinces endemic for Japanese Encephalitis Virus of the Northern Region of Thailand, 2016

District	Province	LAT	LONG
Mae Suai	Chiang Rai	19.630	99.524
Dok Khamtai	Phayao	19.195	100.034
Sop Moei	Mae Hong Son	18.113	98.166
Ban Luang	Nan	18.907	100.425

Table 2. Neutralizing antibody among Children sequentially immunized with two doses of the Japanese Encephalitis virus vaccine (SA14-14-2) within four highly endemic provinces of Thailand, 2015.

Previous dose [(1)]	Site	PRNT (2)		
		SM1	SA14-14-2	Beijing
<1 year	Chiang Rai	28/30 (93.3)	27/30 (90.0)	27/30 (90.0)
	Phayao	30/30 (100)	30/30 (100)	27/30 (90.0)
	Mae Hong Son	27/30 (90.0)	27/30 (90.0)	27/30 (90.0)
	Nan	29/30 (96.7)	29/30 (96.7)	27/30 (90.0)
Total		114/120 (95.0)	113/120 (94.2)	108/120 (90.0)
>1 year	Chiang Rai	67/70 (95.7)	67/70 (95.7)	67/70 (95.7)
	Phayao	65/70 (92.9)	63/70 (90.0)	58/70 (82.9)
	Mae Hong Son	66/70 (94.3)	60/70 (86.0)	59/70 (84.3)
	Nan	66/70 (94.3)	62/70 (88.6)	59/70 (84.3)
Total		264/280 (94.3)	252/280 (90.0)	243/280 (86.8)

Legend: [(1)] Blood sampling time after receiving the 2 vaccine doses;; (2) Number positive/Totals (Percent)

Table 3. Geometric Mean Titer (GMT) of Japanese Encephalitis virus neutralizing antibody among Children sequentially immunized with two doses of the Japanese Encephalitis virus vaccine (SA14-14-2) within four highly endemic provinces of Thailand, 2015.

Previous dose [1]	Site	Geometric Mean Titer (GMT)		
		SM1	SA14-14-2	Beijing
<1 year	Chiang Rai	249	326	198
	Phayao	170	707	232
	Mae Hong Son	311	218	139
	Nan	246	217	154
Total±SD[2]	239±50	323±201.2	177±36.7	
>1 year	Chiang Rai	168	71	83
	Phayao	103	81	105
	Mae Hong Son	180	86	68
	Nan	180	131	77
Total±SD	154±32	90±23	82±13.6	

Legend: [1] Bleeding time after received 2 doses; [2] SD = Standard Deviation

Figures

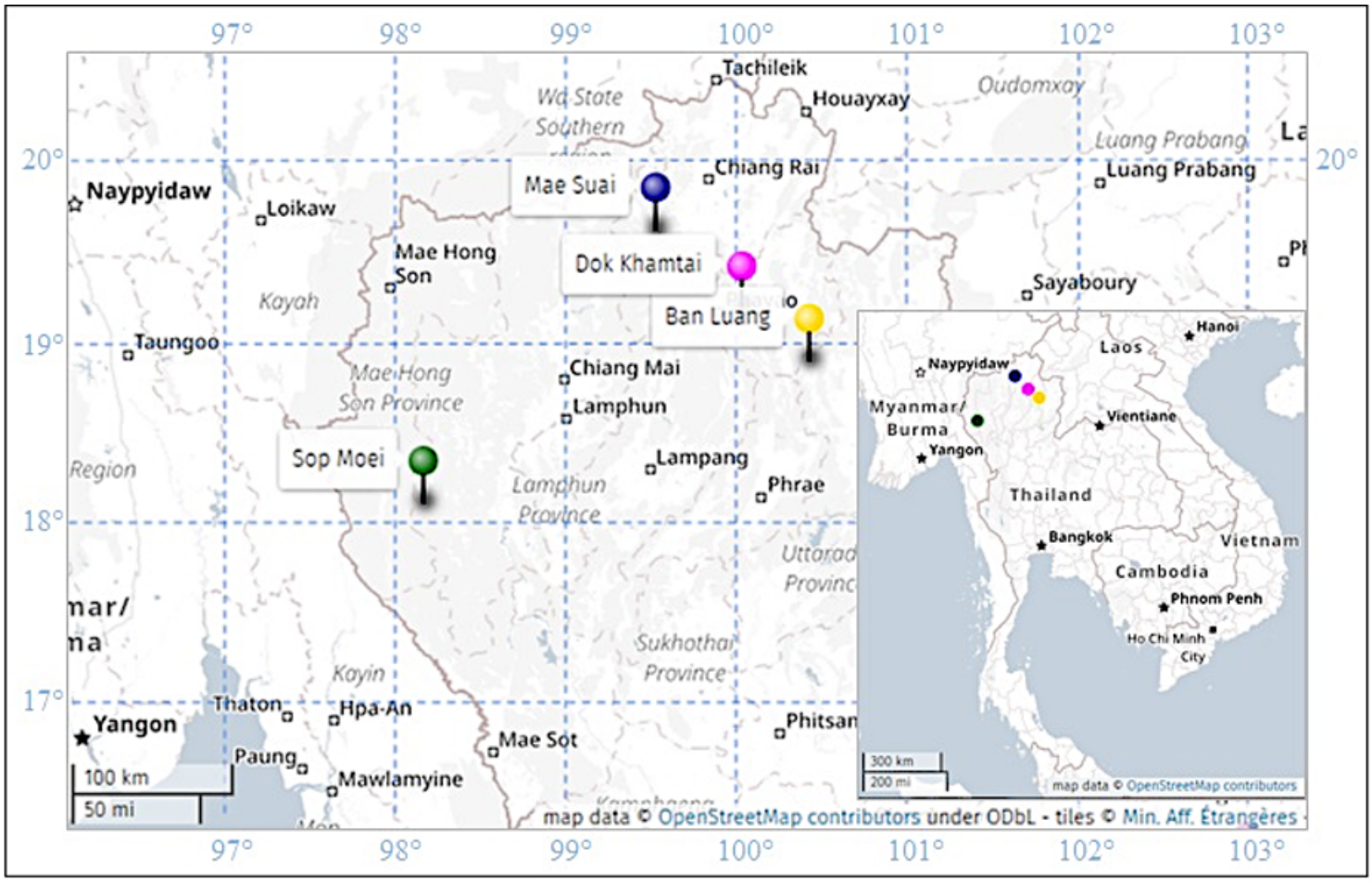

Figure 1

Study area of the Northern Provinces of Thailand. Legend: Each colored circle/pin refers to the centroid of each province