ANTIBODY RESPONSE IN HEALTHCARE WORKERS VACCINATED WITH LIVE-ATTENUATED RECOMBINANT JAPANESE ENCEPHALITIS CHIMERIC VACCINE

Warunee Punpanich Vandepitte[1], Areerat Chaweethamawat[2], Lalita Panakorn[3] and Sutee Yoksan[4]

[1]Division of Infectious Diseases, Department of Pediatrics, Queen Sirikit National Institute of Child Health, [2]Division of Occupational Health, [3]Research and Technology Department, Queen Sirikit National Institute of Child Health; [4]Center for Vaccine Development, Institute of Molecular Biosciences, Mahidol University and Translational Research Unit, Chulabhorn Research Institute, Bangkok, Thailand

Abstract. A recent survey indicated 20% of urban Thai healthcare workers (HCWs) have insufficient level of Japanese encephalitis (JE) neutralizing antibody (seronegative or JE antibody titer <1:10). The study determined safety and immunogenicity after one dose of JE chimeric vaccine (JE-CV) immunization in seronegative HCWs in a single arm open trial. Blood samples for JE antibody using plaque reduction neutralization test (PRNT) as well as information on possible vaccine-associated adverse effects were obtained on day 28 post-immunization. Among participants (n = 60) seroconversion (JE antibody titer of ≥1:10 post-vaccination) occurred in 98% of subjects with a geometric mean titer (GMT) (SD) of 719 (1054). Proportion of adequate JE immunity (GMT) for subjects 21-30, 31-40, 41-50, and 51-60 years of age was 93 (744), 100 (635), 100 (GMT 717), and 100% (GMT 731), respectively. Adverse reactions were mild with self-limited fever and maculopapular rash in 8 and 2% respectively. In conclusion, a single dose of JE-CV in adults was well tolerated and provided high rate of seroconversion in adult HCWs.

Keywords: adult, health care workers, Japanese encephalitis vaccine, neutralizing antibody, Thailand

INTRODUCTION

Japanese encephalitis virus (JEV) is the most common cause of serious and life threatening infection of central nervous system in Asia (Britton *et al*, 2014) with high fatality rate of 20-30% of those who developed clinical disease (Dickerson *et al*, 1952; Kumar *et al*, 1993). Approximately 50% of those survive experience long term neurological sequelae (Solomon *et al*, 2000). Although the disease occurs primarily among children, adults are not always immune to this illness (Olsen *et al*, 2010; Vandepitte *et al*, 2016).

JEV is a zoonotic agent with natural reservoirs, which, as yet, remains unable to

Correspondence: Warunee Punpanich Vandepitte, Infectious Disease Unit, Department of Pediatrics, Queen Sirikit National Institute of Child Health, 420/8 Ratchawithi Road, Ratchathewi District, Bangkok 10400, Thailand.
Tel: +66 (08) 5515 8299;
E-mail: waruneep@gmail.com

be eradicated (Ladreyt *et al*, 2019). Without specific therapy and effective vector control program for JE, immunization is the only public health intervention available (Tsai, 2000). In Thailand, inactivated JE vaccine has been introduced into the National Immunization Program in 1990 in high risk areas (WHO, 2006). The program was introduced country wide in 2000 resulting in considerable reduction of the disease burden (Olsen *et al*, 2010). Nevertheless, the burden of sporadic cases of JE remains substantial and JE remains an important cause of encephalitis in Thailand (Olsen *et al*, 2010). Despite the availability of JE vaccine for more than two decades, the majority of new JE cases still occur in areas with existing JE vaccination program and approximately 25% of cases occur in adults or children ≥15 years of age. A recent systematic review to estimate global incidence of JE indicated an overall incidence of 1.8/100,000 population, among which only 10% are reported to the WHO (Campbell *et al*, 2011), indicating 75% of cases occur in children ≤14 years of age (incidence = 5.4/100,000 population). Approximately 80% of cases occur in areas with existing JE vaccination program (Campbell *et al*, 2011), reinforcing the need to strengthen current JE control program if the JE burden is to be minimized.

Live attenuated Japanese encephalitis chimeric vaccine (JE-CV or Imojev®) was constructed by replacing pre-membrane and envelope coding sequences from the yellow fever (YF) vaccine virus (strain 17D) with the corresponding sequences from attenuated JEV strain, SA14-14-2 (Monath *et al*, 2003). Clinical trials of JE-CV in young, otherwise healthy adults with and without pre-existing YF immunity demonstrated the vaccine is immunogenic with 100% seroconversion rate (JE antibody detectable at titer ≥1:10 after initially negative), able to neutralize wild type JE virus and well tolerated with a geometric mean titer (GMT) in a plaque reduction neutralization test (PRNT) ranging 128-327 (Monath *et al*, 2002). Another study in adults demonstrated a 94% seroconversion rate with PRNT GMT of 201 (Monath *et al*, 2003). A strong booster effect was observed after challenging a subset of JE-CV recipients with a single dose of inactivated mouse brain-derived JE vaccine, suggesting the ability of JE-CV to elicit a memory immune response (Monath *et al*, 2003). In addition, the protective durability and efficacy of a single JE-CV dose in a prospective study among 202 healthy young adults from non-endemic countries demonstrated a seroconversion rate of 99% with GMT (range) of 317 (260-385) at Month 1 post vaccination and 97% of participants remaining seroprotective (JE antibody titer ≥1:10) with GMT of 151 at Month 6 (Nasveld *et al*, 2010). A subgroup receiving a booster dose at Month 6 post-vaccination had 100% seroprotection with a GMT of 353. A follow-up after 5 years following initial vaccination showed 95% of recipients who received a single-dose vaccine remaining seroprotective compared to 97% of those who received two doses of the JE-CV (Nasveld *et al*, 2010). A Kaplan-Meier analysis indicated 87 and 96% of participants who received a single vaccine and a 2-dose schedule respectively remained seroprotective at 5 years after vaccination. A phase III trial of 820 participants, comparing JE-CV with an inactivated mouse brain-derived JE vaccine (Nakayama-NIH strain or JE-VAX®), demonstrated comparable immunogenicity of a single JE-CV dose (99.1% seroconversion rate) compared to a 3-dose regimen of JE-VAX® (95% seroconversion rate) (Torresi *et al*, 2010).

Desai *et al* (2012) employing a statistical modeling to estimate durability of protective immunity indicated the rate of antibody decline is gradual enough to confer seroprotection for up to 10-year post-vaccination. This model is based on a GMT value at 28-day post-vaccination of 1,392 in those receiving JE-CV, with estimated median duration of seroprotection of at least 20 years. The results of the model suggest booster vaccination may be unnecessary for the adult population. In addition, natural boosters from repeated exposure in JE endemic areas may also contribute the long-term protective immunity (Chin and Torresi, 2013; Monath *et al*, 2003; Nasveld *et al*, 2010). Given the limitation of long term effectiveness and reactogenicity of existing mouse-brain derived vaccines, live attenuated JEV vaccine conferring a simpler immunization schedules and with better acceptability has become more utilized on a wide scale (Halstead and Thomas, 2011).

Many countries where JE is endemic including Thailand have insufficient information to enable decision-making regarding JE immunization in adults (Hedge and Gore, 2017). Part of this uncertainty stems from insufficient knowledge on seroepidemiology, disease burden and the immunogenicity of the new vaccines in the adult population. In particular, there are limited data available on the seroprevalence of JE among adults born before 2000. A previous epidemiological data in Thailand indicated a significant proportion of JE cases among adults (Olsen *et al*, 2010). A more recent epidemiological survey in Bangkok revealed 19.5% of adult healthcare workers (HCWs) do not have sufficient level of neutralizing JE antibody, and thus, being at risk of symptomatic infection (Vandepitte *et al*, 2016). The highest rate (23%) of JE seronegativity is among those 21-30 and lowest (17.6%) among 31-40 years of age. Among those with negative JE serology, JE vaccine may be beneficial for protection. A live attenuated JE-CV (Imojev®) has recently received approval for use in the adult population in Thailand (GPO-MBP Imojev®, 2013).

Here, the study assessed the safety and immunogenicity after one dose of JE-CV (Imojev®) immunization in seronegative HCWs. The findings should help inform decision makers on the need for JE immunization of adults in Thailand.

MATERIALS AND METHODS

Recruitment of participants

JE seronegative adult HCWs ($n = 78$) previously enrolled in a JE sero-survey at Queen Sirikit National Institute of Child Health, a tertiary care center in Bangkok, Thailand were invited to participate (Vandepitte *et al*, 2016). Seronegative individuals were defined as those with undetectable JE antibody or the neutralizing antibody titer less than starting dilution of 1:10 (lower dilution than this results in cell toxicity) (Russell *et al*, 1967).

The study was registered with the Thai Clinical Trial Registry (study ID TCTR20151203001) and research protocol received approval from the Research Ethics Committee of Queen Sirikit National Institute of Child Health (approval no. 59-001). Prior written informed consent was obtained from all participants.

Sample analysis

Following a single dose of JE-CV (Imojev®; Sanofi Pasteur, France) given subcutaneously at deltoid area upon

enrollment, blood samples for PRNT and information on possible vaccine-associated adverse effects were obtained on Day 28 post-JE-CV vaccination. PRNT assay was performed as previously described (Russell *et al*, 1967). In brief, serum was heat-inactivated at 56°C for 30 minutes and 4-fold serial dilutions were made in phosphate with heat inactivated 20% Fetal Bovine Serum. An equal volume of JE virus was added to each serum dilution and the mixture was incubated at 37°C for 60 minutes. Then, a 0.2 ml aliquot was inoculated in triplicate into a well of a 6-well plates containing confluent continuous monkey kidney (LLC-MK2) cells), incubated at 37°C for 90 minutes, overlaid with a 4 ml aliquot of 3.0% carboxy methyl cellulose-minimum Eagle's medium and incubated at 37°C for 7 days under a humidified atmosphere containing 5% CO_2 followed by plaque counting.

Data were then analyzed descriptively for demographic data and level of neutralizing antibody titer. Participants without sufficient level of neutralizing antibody against JE were offered an additional dose of JE-CV and PRNT was conducted at Day 28 post-booster vaccination.

RESULTS

Of 78 seronegative participants, 60 were enrolled; average age was 40±10 years old, 8.3% (*n*=24) was male, 45% (*n* =27) being domiciled in Bangkok and 40% (*n*=24) traveled outside Bangkok for one month or more (Table 1). No participants had been vaccinated against JE virus. The vaccine appeared to be well tolerated with minor side effects, such as low grade fever in five cases (8.3%) and self-limited maculopapular rash in one case (Fig 1). Fifty-nine participants (98.3%) demonstrated sufficient level of neutralizing antibody against 3 Beijing JE virus strains (>10 reciprocal PRNT titer) with JE antibody GMT = 719 at Day 28 (Table 2). Proportion of

Table 1
Demographic data of participants in Japanese encephalitis chimeric vaccine (Imojev®) trial.

Characteristics	Number (%) (n = 60)
Gender	
Male	5 (8.3)
Female	55 (91.7)
Age group (years)*	
21-30	15 (25)
31-40	12 (20)
41-50	23 (38.3)
51-60	10 (16.7)
Past domicile	
Bangkok	27 (45)
Bangkok suburb	4 (6.7)
Central	4 (6.7)
Northern	1 (1.6)
North-Eastern	14 (23.3)
Eastern	3 (5)
Western	3 (3)
Southern	4 (6.7)
History of ≥1 month(s) stay in upcountry	23 (38.3)
Central	6 (26.1)
Northern	3 (13)
North-Eastern	8 (34.7)
Eastern	2 (8.6)
Western	1 (4.3)
Southern	3 (13)

*Mean ± SD = 40 ± 10.

adequate immunity to prevent disease and corresponding antibody level across the four age groups with GMT range = 635-744.

Only one case failed to seroconvert after JE-CV immunization, and thus, it was not possible to determine associated factors (*eg* age, gender, past and / or present domicile) for the lack of seroconversion after one dose of JE-CV. This case was given an additional JE-CV dose and JE serology re-evaluation at Day 28 post-second vaccination showed PRNT level = 23. Thus, 100% seroconversion was achieved after one dose or two doses of JE-CV.

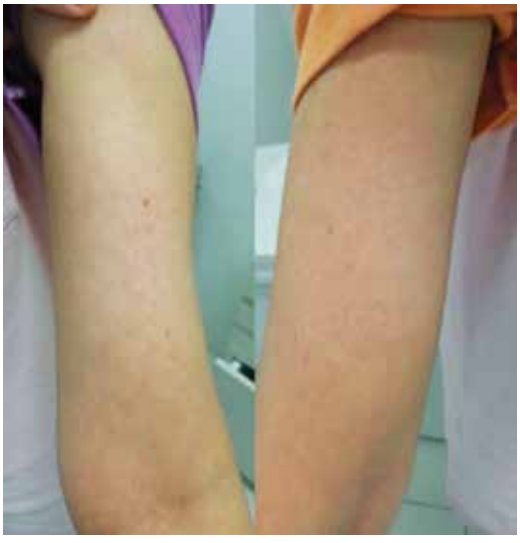

Fig 1-Generalized maculopapular rash occurring within 24 hours following Japanese encephalitis chimeric (Imojev®) vaccination.

DISCUSSION

Despite the availability of inactivated JE vaccine in Thailand National Immunization Program since 2001 (Pongpairoj *et al*, 1989; WHO, 2006), JE remains one of the most common causes of vaccine preventable cause of encephalitis in the country (Olsen *et al*, 2010). Many adults born before the implementation of nationwide JE immunization did not receive JE catch-up immunization, thus remaining at risk for insufficient protection against this serious illness (Vandepitte *et al*, 2016). With the advent of a safe and effective live JE vaccine, it is essential to have information regarding immunogenicity and reactogenicity of this type of immunization before considering recommendation for high risk adults residing in endemic or traveling

Table 2
Plaque reduction neutralization test (PRNT) level at Day 28 post-vaccination by age group of participants in Japanese encephalitis chimeric vaccine (Imojev®) trial.

Age group (years)	Seroconversion (PRNT level on day 28)			
	PRNT ≥10 Number (%)	Geometric mean of test (SD)	PRNT >1,000 Number (%)	Geometric mean of test (SD)
21-30 (n = 15)	14 (93.3)	744.2 (1,586.4)	2 (13.3)	4,477.0 (132.9)
31-40 (n = 12)	12 (100)	635.1 (510.9)	2 (16.7)	1,359.0 (48.1)
41-50 (n = 23)	23 (100)	716.6 (822.0)	2 (8.7)	3,158.0 (427.1)
51-60 (n = 10)	10 (100)	731.5 (799.9)	2 (20)	2,135.5 (65.8)
Total (n = 60)	59 (98.3)	718.9 (1,053.7)	8 (13.3)	2,789.9 (1,250.8)

to an endemic area. Although the most recognizable illness caused by JE virus is a severe form of encephalitis, JE infection is also responsible for 19.2% of adults presenting with acute undifferentiated fevers without neurologic deficits in a northern province of the country (Watt and Jongsakul, 2003).

The present study indicates live-attenuated JE-CV is safe and well tolerated for a subgroup of JE seronegative Thai HCWs. Safety of this vaccine was shown in several clinical trials on adult population (Appaiahgari and Vrati, 2010; Guy *et al*, 2010; Nasveld *et al*, 2010). In addition, it provided high level of GMT and 98% seroconversion rate in this population after one dose of JE-CV immunization. The findings corresponded with a previous study conducted in Japanese adults showing a seroprotection rate before and after a single dose of Vero cell-derived JE vaccination is 51.9 and 94% respectively (Takeshita *et al*, 2014). The seroconversion rate among initial seronegative cases is 86.8%. Age is significantly different between those with and without seroprotection at baseline; seroconversion rate is 100% in age group 25-39 years with previous history of vaccination but only 55.6% among those without and/or unknown prior JE vaccination, while among adults ≥40 years of age, seroconversion rate is 96% after vaccination. This study demonstrated one dose of JE vaccine is sufficient to protect most Japanese adults, except those 25-39 years of age who did not receive prior JE vaccination

In a non-endemic area, a single dose of live attenuated JE-CV given to JE-naive adults provided 94, 99, 97 and 87% seroprotection at two weeks, four weeks, six months, and five years, respectively (Torresi *et al*, 2010). Five years following vaccination, 95% of those who received a single dose remained seroprotected, compared to 97% in those who received two doses of the vaccine (Nasveld *et al*, 2010). These studies indicated reactogenicity profile of JE-CV is comparable with that of placebo and its use has been recommended for international travelers to JE endemic area (Torresi *et al*, 2010). The average GMT in adults is 1,392 and over 90% of adults remain seroprotected for five years post-vaccination. Woolpert *et al* (2012) demonstrated among military personnel in the United States a single dose of Vero cell culture JE vaccine adequately boosts neutralizing antibody in those previously vaccinated with at least three doses of mouse brain-derived JE vaccine, and those without prior JE immunization have 93% seroconversion after the two doses of JE-CV.

A statistical model employed by Desai *et al* (2012) to predict neutralizing antibody titer and seroprotection up to 25 years post-vaccination demonstrated a predicted seroprotection rate at year 10 is 85.5% with an estimated median duration of seroprotection of 21.4 years in non-endemic area. This analysis suggests one dose of JE-CV confers to most adults a high level of protection against JE for at least 10 years. For countries where JE is endemic, natural booster may confer a much longer duration of protection. Thus, only one dose of this chimeric JE vaccine is currently recommended for at risk adults (Appaiahgari and Vrati, 2010).

One important consideration regarding the use of JE-CV is the possible emergence of non-vaccine genotype of JE virus (Li *et al*, 2011). JEV is classified into five genotypes (GI-GV) (Solomon *et al*, 2003) Japanese encephalitis has spread across Asia and has become the most important

cause of epidemic encephalitis worldwide. Four genotypes of Japanese encephalitis virus (JEV. Currently available vaccines are derived from JEV GIII, formerly the predominant strain in Asia (Mackenzie *et al*, 2004). From the 1990s, JEVGI has become more commonly isolated and is a leading genotype replacing GIII in several endemic regions (Ma *et al*, 2003; Mackenzie *et al*, 2004; Nitatpattana *et al*, 2008; Chen *et al*, 2011). This phenomenon raises an important concern for the cross-protective capacity of current GIII-based JE vaccines (Mackenzie *et al*, 2004; Nitatpattana *et al*, 2008; Chen *et al*, 2011). Although existing report demonstrated both JE-CV and mouse brain-derived JE vaccine elicit a protective immunity to both vaccine (GIII) and non-vaccine genotypes, GMT against GI is low, raising concern regarding the duration of the cross-protection (Erra *et al*, 2013). The present study did not examine levels of heterotypic antibody against other genotypes. Thus, information regarding changing epidemiological trends remains essential when considering use of GIII-based JE vaccines in prevention and control of JE.

In conclusion, JE-CV (Imojev®) appears to have a favorable safety and high immunogenicity profile in a small cohort of Thai adults. This vaccine may help reduce the burden of JE among seronegative adults living in or traveling to JE endemic areas. Further studies using a larger cohort and conducted in other regions of the country will be needed to support the results from the current study.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the assistance of Fontip Buttremee for sample collection and study coordination and thank Supoth Rajakum, Institute of Molecular Biosciences, Mahidol University for assistance in JE serology assay and Dr Piriya Poonnoi for research support and critical review of the manuscript. The study was supported in part by Sanofi Pasteur (Thailand). The opinions expressed herein are those of the authors and do not necessarily reflect those of the Queen Sirikit National Institute of Child Health.

CONFLICTS OF INTEREST

The funder has no role in data analysis and manuscript preparation. Dr Vandepitte occasionally received honoraria as speaker on Imojev® vaccine from Sanofi Pasteur. These statements are made in the interest of full disclosure and not because the authors consider this to be a conflict of interest.

REFERENCES

Appaiahgari MB, Vrati S. IMOJEV(®): a Yellow fever virus-based novel Japanese encephalitis vaccine. *Expert Rev Vaccines* 2010; 9: 1371-84.

Britton PN, Khandaker G, Booy R, Jones CA. The causes and consequences of childhood encephalitis in Asia. *Infect Disord Drug Targets* 2014;14: 78-88.

Campbell GL, Hills SL, Fischer M, *et al*. Estimated global incidence of Japanese encephalitis: a systematic review. *Bull World Health Organ* 2011; 89: 766-74.

Chen YY, Fan YC, Tu WC, *et al*. Japanese encephalitis virus genotype replacement, Taiwan, 2009-2010. *Emerg Infect Dis* 2011; 17: 2354-6.

Chin R, Torresi J. Japanese B encephalitis: an overview of the disease and use of chimerivax-JE as a preventative vaccine. *Infect Dis Ther* 2013; 2: 145-58.

Desai K, Coudeville L, Bailleux F. Modelling the long-term persistence of neutralizing

antibody in adults after one dose of live attenuated Japanese encephalitis chimeric virus vaccine. *Vaccine* 2012; 30: 2510-5.

Dickerson RB, Newton JR, Hansen JE. Diagnosis and immediate prognosis of Japanese B encephalitis; observations based on more than 200 patients with detailed analysis of 65 serologically confirmed cases. *Am J Med* 1952; 12: 277-88.

Erra EO, Askling HH, Yoksan S, *et al*. Cross-protective capacity of Japanese encephalitis (JE) vaccines against circulating heterologous JE virus genotypes. *Clin Infect Dis* 2013; 56: 267-70.

GPO-MBP Imojev®. Package Insert. Confidential/Proprietary Information Version 08 dated December 2013 [Cited 2020 Apr 06]. Available from: URL: http://www.fda.moph.go.th/sites/drug/Shared%20Documents/Vaccine/U1DR1A10B2530000111C-SPC.pdf

Guy B, Guirakhoo F, Barban V, Higg S, Monath TP, Lang J. Preclinical and clinical development of YFV 17D-based chimeric vaccines against dengue, West Nile and Japanese encephalitis viruses. *Vaccine* 2010; 28: 632-49.

Halstead SB, Thomas SJ. New Japanese encephalitis vaccines: alternatives to production in mouse brain. *Expert Rev Vaccines* 2011; 10: 355-64.

Hegde NR, Gore MM. Japanese encephalitis vaccines: Immunogenicity, protective efficacy, effectiveness, and impact on the burden of disease. *Hum Vaccin Immunother* 2017;13: 1-18.

Kumar R, Mathur A, Singh KB, *et al*. Clinical sequelae of Japanese encephalitis in children. *Indian J Med Res* 1993; 97: 9-13.

Ladreyt H, Durand B, Dussart P, Chevalier V. How central is the domestic pig in the epidemiological cycle of Japanese encephalitis virus? A review of scientific evidence and implications for disease control. *Viruses* 2019; 11(10). pii: E949.

Li MH, Fu SH, Chen WX, *et al*. Genotype v Japanese encephalitis virus is emerging. *PLoS Negl Trop Dis* 2011; 5: e1231.

Ma SP, Yoshida Y, Makino Y, Tadano M, Ono T, Ogawa M. Short report: a major genotype of Japanese encephalitis virus currently circulating in Japan. *Am J Trop Med Hyg* 2003; 69: 151-4.

Mackenzie JS, Gubler DJ, Petersen LR. Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nat Med* 2004; 10(12 Suppl): S98-109.

Monath TP, Guirakhoo F, Nichols R, *et al*. Chimeric live, attenuated vaccine against Japanese encephalitis (ChimeriVax-JE): phase 2 clinical trials for safety and immunogenicity, effect of vaccine dose and schedule, and memory response to challenge with inactivated Japanese encephalitis antigen. *J Infect Dis* 2003; 188: 1213-30.

Monath TP, McCarthy K, Bedford P, *et al*. Clinical proof of principle for ChimeriVax: recombinant live, attenuated vaccines against flavivirus infections. *Vaccine* 2002; 20: 1004-18.

Nasveld PE, Ebringer A, Elmes N, *et al*. Long term immunity to live attenuated Japanese encephalitis chimeric virus vaccine: randomized, double-blind, 5-year, phase II study in healthy adults. *Hum Vaccin* 2010; 6: 1038-46.

Nitatpattana N, Dubot-Pérès A, Gouilh MA, *et al*. Change in Japanese encephalitis virus distribution,Thailand. *Emerg Infect Dis* 2008; 14: 1762-5.

Olsen SJ, Supawat K, Campbell AP, *et al*. Japanese encephalitis virus remains an important cause of encephalitis in Thailand. *Int J Infect Dis* 2010; 14: e888-92.

Pongpairoj S, Choakouychai B, Boonrueng C, *et al*. A test production of inactivated mouse brain JE vaccine in Thailand. *Southeast Asian J Trop Med Public Health* 1989; 20: 647-52.

Russell PK, Nisalak A, Sukhavachana P, Vivona S. A plaque reduction test for dengue virus

neutralizing antibodies. *J Immunol* 1967; 99: 285-90.

Solomon T, Dung NM, Kneen R, Gainsborough M, Vaughn DW, Khanh VT. Japanese encephalitis. *J Neurol Neurosurg Psychiatry* 2000; 68: 405-15.

Solomon T, Ni H, Beasley D, Ekkelenkamp M, Cordosa MJ, Barrett AD. Origin and evolution of Japanese encephalitis virus in southeast Asia. *J Virol* 2003; 77: 3091-8.

Takeshita N, Lim CK, Mizuno Y, *et al*. Immunogenicity of single-dose Vero cell-derived Japanese encephalitis vaccine in Japanese adults. *J Infect Chemother* 2014; 20: 238-42.

Torresi J, McCarthy K, Feroldi E, Méric C. Immunogenicity, safety and tolerability in adults of a new single-dose, live-attenuated vaccine against Japanese encephalitis: randomised controlled phase 3 trials. *Vaccine* 2010; 28: 7993-8000.

Tsai TF. New initiatives for the control of Japanese encephalitis by vaccination: minutes of a WHO/CVI meeting, Bangkok, Thailand, 13-15 October 1998. *Vaccine* 2000; 18 (Suppl 2): 1-25.

Vandepitte WP, Yoksan S, Wannachart M. The seroprevalence of neutralizing antibody against Japanese encephalitis virus in health care workers. *Int J Infect Dis* 2016; 45(Suppl 1): 425-6.

Watt G, Jongsakul K. Acute undifferentiated fever caused by infection with Japanese encephalitis virus. *Am J Trop Med Hyg* 2003; 68: 704-6.

World Health Organization (WHO). Japanese encephalitis vaccines. *Wkly Epidemiol Rec* 2006; 81: 331-40.

Woolpert T, Staples JE, Faix DJ, *et al*. Immunogenicity of one dose of Vero-cell culture-derived Japanese encephalitis (JE) vaccine in adults previously vaccinated with mouse brain-derived JE vaccine. *Vaccine* 2012; 30: 3090-6.