# THE STUDY ON EFFICACY AND SAFETY OF SA-14-14-2 VACCINE AGAINST JAPANESE ENCEPHALITIS VIRUS AT VIRUDHUNAGAR DISTRICT

***DISSERTATION SUBMITTED FOR***

## M.D. (BRANCH – IV)

## MICROBIOLOGY

**THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY**

**CHENNAI – TAMILNADU**

**MARCH - 2009**

# CERTIFICATE

This is to certify that the dissertation entitled **"THE STUDY ON EFFICACY AND SAFETY OF SA -14-14-2 VACCINE AGAINST JAPANESE ENCEPHALITIS VIRUS AT VIRUTHUNAGAR DISTRICT"** by **Dr. G.VAZHAVANDAL** for M.D. Microbiology Examination, March 2009 under The Tamil Nadu Dr. M.G.R. Medical University is a bonafide work carried out under my direct supervision and guidance.

**Director**

Institute of Microbiology
Madurai Medical College
Madurai

# DECLARATION

I, **Dr. G.VAZHAVANDAL** declare that the dissertation titled **"THE STUDY ON EFFICACY AND SAFETY OF SA-14-14-2 VACCINE AGAINST JAPANESE ENCEPHALITIS VIRUS AT VIRUDHUNAGAR DISTRICT"** has been prepared by me.

This is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfilment of the requirement for the award of M.D. Degree, Branch IV (MICRO BIOLOGY) degree Examination to be held in MARCH 2009.

Place : Madurai
Date :

**Dr.G. VAZHAVANDAL**

# ACKNOWLEDGEMENT

I am grateful to **The Dean,** Madurai Medical College , Madurai for permitting me to carry out this study.

I wish to place on the records my deep sense of gratitude and sincere thanks to Professor **Dr. A. Uma, M.D.,** Director i/c, Institute of Microbiology, Madurai Medical College, for her constant help, guidance and encouragement given to me throughout this study.

I express my sincere thanks to our Additional Professor **Dr. P.A.T. Jegadheeswari, M.D.,** for her valuable suggestions and moral support.

I am highly indebted to **Dr. Jhansi Charles, M.D.,** Additional Professor, whose keen interest, sincere guidance and encouragement were always there as a source of strength, right from selecting the topic till the dissertation is brought to print.

I also express my thanks to **Dr. Vibhushanan M.D.,** Additional Professor, and **Dr. S. Chandrasekar ., Ph.D.** (Non Medical Professor) for their valuable help and guidance given to me throughout the study.

I express my sincere thanks to Assistant professors **Dr. B. Shanthi, M.D., Dr. S. Radhakumari, M.D., Dr.N. Mythili, M.D. and Tutors Dr. J .Suryakumar, MD, Dr. S. Ganesan, MD, Dr.S. Lallitha, MD, Dr. C.Sugumari, MD, Dr.N.Rammurugan, MD, and Dr.K.Lavanya, MD** Tutor and Senior Entomologist **Mr. M. Ismail,** for their valuable suggestions given to me.

My sincere thanks are to **Dr. A. Maheswaran, Dr. M. Padmini** Veterinary

surgeons of this college, **Mrs.Annie magdelin**, Lab superviser, for their immense and timely help in carrying out the animal studies.

I am thank full to my colleagues **, Dr.M. Sudha , Dr. J.Preeti, Dr.N. Anuradha, Dr. N. Therese mary, and Dr.S. Ramalatha** for their moral support and cooperation rendered during the work.

At last but not the least, I extend my thanks to all staff members, Institute of Microbiology for giving full cooperation and timely help in carrying out the laboratory studies.

# CONTENTS


| TITLE | PAGE NO |
|---|---|
| INTRODUCTION | 1 |
| REVIEW OF LITERATURE | 12 |
| AIM AND OBJECTIVES | 30 |
| MATERIALS AND METHODS | 31 |
| RESULTS | 46 |
| DISCUSSION | 61 |
| SUMMARY | 66 |
| CONCLUSION | 67 |
| APPENDIX | |
| BIBLIOGRAPHY | |

# INTRODUCTION

**Background**

Japanese encephalitis is the leading viral cause of Acute Encephalitis Syndrome (AES) in Asia. The disease primarily affects children under the age of fifteen. 70% of those who develop illness either die or survive with a long term neurological disability. Since the first Case of JE was documented in the late $19^{th}$ century; the disease has spread beyond its early domain- traveling as far as Australia by 2000.Over the past 60 years it is estimated that JE has infected~ 10 million children globally, killing 3 million and causing long-term disability in 4 million.

**Current Scenario in India**

JE virus transmission has been widespread in India. The first evidence of presence of JE virus dates back to 1952 in Nagpur subdivision of Maharashtra. JE was clinically diagnosed for the first time in 1955 at Vellore, North Arcot district of Tamil Nadu. In subsequent years, outbreaks have occurred in various states and Union Territories ( UTs) in the country. The first major JE epidemic was reported from Burdwan and Bankura districts of West Bengal in 1973 followed by another outbreak in 1976. Outbreaks have been reported from States like Uttar Pradesh, West Bengal, Assam, Andhra Pradesh, Karnataka, Bihar, Tamil Nadu, Haryana and other states through the years.

The Directorate of National Vector Borne Disease control Programme (NVBDCP) formerly known as the National Anti Malaria Programme (NAMP) has been monitoring JE incidence in the country since 1978. Though cases of JE have been reported from 26

states and UT's occasionally in some years since 1978, repeated outbreaks have been reported only from 12 States.

The table below highlights the case load in some of the states during last 5 years (2001- 2005). However, the disease reporting has been passive without adequate diagnostic support. These are reported cases through passive surveillance without adequate diagnostics support.

Table 1: **State wise JE cases and deaths from 2001 – 2005**

| S. No. | States | 2001 | | 2002 | | 2003 | | 2004 (P)* | | 2005 (P)* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | D | C | D | C | D | C | D | C | D |
| 1. | Andhra Pradesh | 33 | 4 | 18 | 3 | 329 | 183 | 7 | 3 | 0 | 0 |
| 2. | Assam | 343 | 200 | 472 | 150 | 109 | 49 | 235 | 64 | 145 | 52 |
| 3. | Bihar | 48 | 11 | - | - | 6 | 2 | 85 | 28 | 192 | 64 |
| 4. | Chandigarh | - | - | 4 | - | 0 | 0 | 0 | 0 | 0 | 0 |
| 5. | Delhi | - | - | 1 | - | 12 | 5 | 17 | 0 | 2 | 0 |
| 6. | Goa | 6 | 2 | 11 | - | 0 | 0 | 0 | 0 | 5 | 0 |
| 7. | Haryana | 47 | 22 | 59 | 40 | 104 | 67 | 37 | 27 | 38 | 31 |
| 8. | Karnataka | 206 | 14 | 152 | 15 | 226 | 10 | 181 | 6 | 108 | 8 |
| 9. | Kerala | 128 | 5 | - | - | 17 | 2 | 9 | 1 | - | - |
| 10. | Maharashtra | 126 | 1 | 27 | 12 | 475 | 115 | 22 | 0 | 66 | 30 |
| 11. | Manipur | - | - | 2 | 1 | 1 | 0 | 0 | 0 | 1 | 0 |
| 12. | Punjab | - | - | 10 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13. | Tamil Nadu | - | - | - | - | 163 | 36 | 88 | 9 | 8 | 1 |
| 14. | Uttar Pradesh | 1005 | 199 | 281 | 69 | 1124 | 237 | 1030 | 228 | 5978 | 1458 |
| 15. | West Bengal | 119 | 21 | - | - | 2 | 1 | 3 | 1 | 6 | 1 |
| | Grand Total | 2061 | 479 | 1037 | 292 | 2568 | 707 | 1714 | 367 | 6550 | 1645 |

C - Cases ; D - Deaths Source: NVBDCP data; P* - Provisional
Source : NVBDCP

The Case Fatality Rate (CFR) due to JE in India has been around 24 % with wide variations in states. Annual reported cases due to JE ranges between 1037 and 6550 while deaths due to JE range between292 and 1645.

## THE EPIDEMIOLOGY OF JAPANESE ENCEPHALITIS

### The JE Virus

JE virus (JEV) is a member of the genus Flaviviridae, together with Yellow Fever virus and Dengue virus. With the help of genome sequencing studies, it has been possible to determine the various genotypes of JEV in circulation in different geographic areas. The two common Indian Genotypes [GP78 and Vellore P20778] show genetic similarity to Chinese SA14 and Beijing Genotypes.

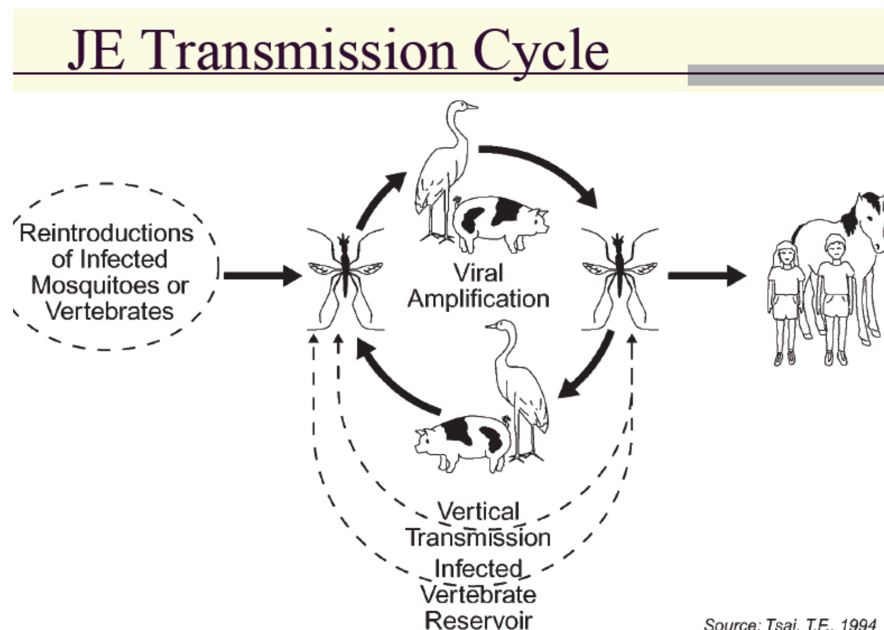
### Communicability and transmission

JE is a Zoonotic viral disease. JE virus has a complex life cycle. In nature, JE virus is maintained in animals and birds, particularly pigs and Ardied birds (e.g., Cattle egrets, pond herons etc.).The virus does not cause any disease among its natural hosts and the transmission continues unnoticed through mosquitoes. It is carried by female mosquitoes from infected pigs or water birds like pond herons and ducks to susceptible children. The main vector, Culex Mosquitoes (Culex tritaeniorhynchus, C.vishnui, C.pseudovishnui and others totally 8 species) live in rural rice growing and pig-farming regions. The mosquito breeds in flooded rice fields, marshes, and standing water around planted fields. This is the reason why JE is mostly a rural disease.

JE is a seasonal disease. Epidemics coincide with the monsoon and post monsoon period (August to December) in Tamilnadu & agricultural pratices, during this period, due to high density of the mosquito vector because of stagnant water and presence of reservoir host (pigs).

Pigs are the most important reservoirs. Though they do not manifest the disease, they develop very high titers of virus in circulating blood and infect mosquitoes. Thus pigs are the amplifying hosts. Susceptible children are infected by infected mosquito bites. After mosquito bite, disease appears in 5-16 days. The virus then invades the central nervous system and cause disease. Although infection in human is incidental, the virus can cause serious neurological disease with high morbidity and mortality. Infection during the first six months of pregnancy may result in infection of the fetus and miscarriage.

JE does not spread from child to child or from cattle to humans because of the low and transient viremia. This is the reason why an increase in cattle and pig ratio may reduce the risk of JE in humans [mosquito bites are shared by cattle and pigs].



## Clinical manifestation

The incubation period in man following a mosquito bite varies from 4-14 days. Not all individuals bitten by mosquitoes develop disease. The ratio of overt disease to inapparent infection varies from 1:300 to 1:1000. Encephalitis due to JE shows a scattered distribution. The course of disease in man may be divided into prodromal, acute, late stage and sequele phase. The fatality varies between 20-40 percent, but may reach over 58 percent. The average period between the onset of illness and death is 9 days.

An etiological diagnosis of JE is based on serological testing using Elisa that detects specific IgM in the CSF or in blood of almost all patients within 4-7 days of onset of disease.

## Protective immune response

Protection is associated with the development of neutralizing antibodies. Although no internal standard has yet been established, neutralizing antibody titers of 1:10 or more are commonly accepted as evidence of protection.

## Treatment

Once a person becomes sick with JE, there is no treatment that can be used to cure the patient. Antibiotics do not work against viruses, and no anti virals are effective. Because there is no specific therapy for JE, attention is given to prevention of complications.

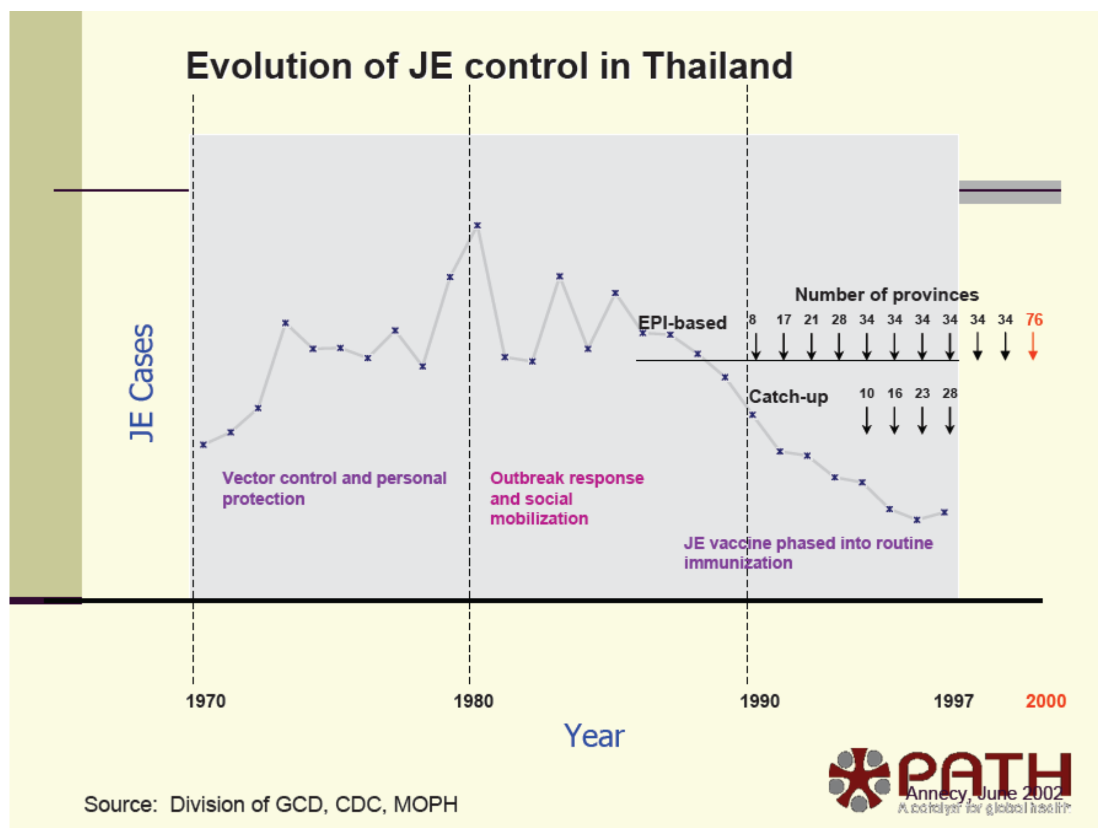
## Control programs

Spraying mosquito habitates with insecticides, use of bed nets, and pig control have not yielded the desired results. Human vaccination is the only method that has proven effective in the control of JE.

## JE IMMUNIZATION

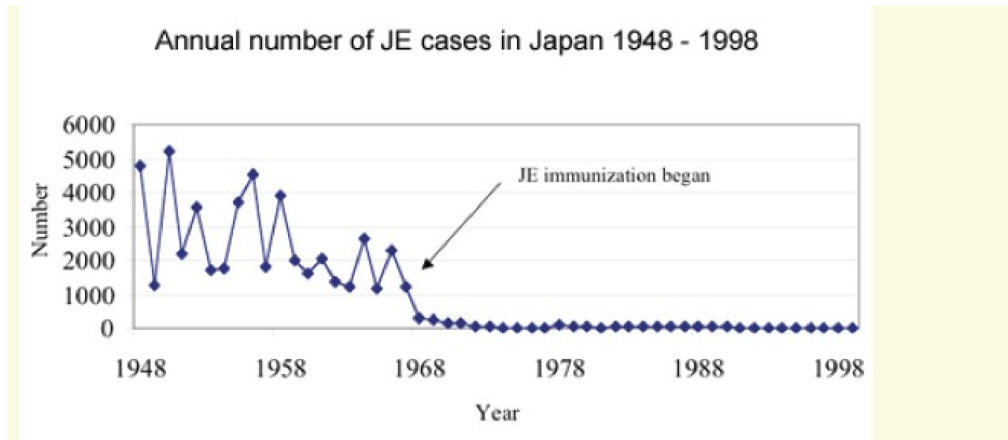
### Scientific evidence for JE immunization

Human vaccination has been the only reliable tool to control JE. Thailand, for example, for many years attempted to control mosquitoes and to respond to outbreaks, but it was not until JE vaccine was introduced into the country that the incidence of JE fell dramatically.



There is compelling evidence that human immunization is effective for controlling JE.

Studies in Korea, Thailand and Japan showed >99% reduction in JE cases after immunization.




## JE vaccination in India

In the past attempts have been made in India to vaccinate children against JE. However inadequate availability of JE vaccine has limited the campaigns to small geographical areas. Though there has been a dramatic reduction in the case load immediately following these vaccination campaigns, however due to lack of sustained vaccination program there has not been any major impact in controlling the disease.

Following sustained JE vaccination in Perambulur district of Tamil Nadu since 1995 and in the high risk villages of Andhra Pradesh since 1999 there has been some impact in reducing the case load and disease incidence in these areas.

Following the massive outbreak of JE in 2005 in the districts of Eastern Uttar Pradesh and adjoining districts of Bihar, Vaccination campaigns were carried out in 11 highest risk districts of the country in 2006. Children between the age group of 1 to 15

years were vaccinated with a single dose of SA 14-14-2 vaccine.

**Summary of JE vaccines available or under development**

**Source; WHO, October 2006**

**Cumulative district JE vaccination coverage was as follows**

The second year of the government's five year strategy targets 23 districts of 9 states. JE vaccination Campaigns kicked off in March 2007 and by July2007, 20 million more children and adolescents were targetted to be immunized against JE.

Since during 1997, the mosquito pool from Virudhunagar District was confirmed to be positive for JE virus and the past history had shown that very high number of cases and deaths due to JE were reported in this district. Hence there was every threat to roll back the virus activities in the younger generation who were not protected now in the area for JE virus. So, Virudhunagar District was selected for Japanese Encephalitis vaccination programme during the year 2007.

**VIRUDHUNAGAR DISTRICT**

**INCIDENCE OF JE**

| YEAR | 91 | 92 | 93 | 94 | 95 | 96 | 97 & 98 | 99 | 2000 To 2005 | 2006 | 2007 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of Cases | 18 | 1 | 11 | 7 | 10 | 48 | 0 | 4 | 0 | 1 | 3 |
| No. of Deaths | 8 | 1 | 9 | 7 | 8 | 18 | 0 | 2 | 0 | 1 | 1 |

The vaccination was completed in Virudhunagar District during August 2007.

# REVIEW OF LITERATURE

## PREVALENCE AND GEOGRAPHICAL DISTRIBUTION

Igarasi .A, 1992,,Burke et al 1998,Umenai et al 1985, in their articles stated that Japanese encephalitis (JE), a mosquito-borne flaviviral infection, is the leading recognized cause of childhood encephalitis in Asia. Approximately 35,000 cases and 10,000 deaths are reported annually, but in many locations the disease is not under systematic surveillance, and official reports undoubtedly under estimate the true number of cases. [1-3]

The mosquito-borne mode of JE transmission was elucidated with the isolation of JE virus from Culex tritaeniorhynchus mosquitoes in 1938, and subsequent field studies established the role of aquatic birds and pigs in the viral enzootic cycle.

Hiroyama T, 1962, in his article mentioned that summer-fall encephalitis outbreaks consistent with JE were recorded in Japan as early as 1871, of which the largest, in 1924, led to more than 6000 cases, 60% of them fatal.[4]

Carey DE, et al 1969, Banerjee K, 1994, in their articles stated that JE transmission was first recognized in Southwest Asia after an outbreak occurred in 1948 in Sri Lanka. Sporadic cases and later epidemics were recognized on the Indian subcontinent around Vellore. [8,9] The disease is currently recognized to be hyperendemic in Northern India and Southern Nepal, Central India (Andhra Pradesh), and Southern India (Goa, Karnataka, and Tamil Nadu)

Service MW, 1991 in his article described that the apparent spread to or amplification of JE in new areas has been correlated with agricultural development and intensive rice cultivation supported by irrigation schemes.[10]

## CLINICAL ILLNESS

Halstead SB et al, 1962, Gajanana et al, 1995 in their articles stated that the great majority of infections are not apparent, and only 1 in 250 infections results in symptomatic illness. [11,12] The principal clinical manifestation of illness is encephalitis, and milder clinical presentations, such as aseptic meningitis and simple febrile illness with headache, usually escape recognition. [13,22]

## CONGENITAL INFECTION

Bhonde RR et al 1985, in their study observed that human placental organ cultures, obtained from medically terminated pregnancies at 8 to 12 weeks' gestation, supported JE viral replication, but tissues from full-term pregnancies were resistant to infection.[26]

Ogata A et al 1991, Kimura-Kuroda J 1993, in their experimental studies have shown that JE viral neurotropism is related to neuronal immaturity. [27,28]

## VIROLOGY

Kuno G et al 1998, Chambers TJ et al 1990, stated that JE virus is one of 70 viruses in the Flavivirus genus of the Flaviviridae family[29,30]

Murphy PA 1980, in his article mentioned that the complete genomic sequences of

JE virus and several other flaviviruses have been determined, including yellow fever (YF) virus, the prototype virus in the family. Morphologically, flaviviruses are spherical, approximately 40 to 50 nm in diameter, with a lipid membrane enclosing an isometric 30-nm-diameter nucleocapsid core comprising a capsid (C) protein and a single-stranded messenger (positive) sense viral RNA.[31]

Sumiyoshi H, et al 1987, Nitayaphan S et al,1990, in their articles stated that membrane surface projections are composed of a glycosylated envelope (E) and membrane (M) protein, a mature form of the premembrane (prM) protein. JE viral RNA, 10,976 bases in length, encodes an uninterrupted open reading frame (ORF), flanked by 95 and 585 base untranslated regions at the 5' and 3' ends, respectively. [32,33]

The E glycoprotein is the major virion antigen responsible for virion assembly, receptor binding, membrane fusion and elicitation of virus-neutralizing, haemagglutination inhibiting, and anti-fusion antibodies. The E protein is the principal target for neutralization in vitro and in vivo by specific antibodies (Gould et al, 1986; Hawkes et al, 1988;Kimura -Kiroda and Yasui, 1988).

NS1(Non structural 1 protein) is believed to be involved in the assembly and release of virions (Lee et al, 1989).NSI protein evokes a strong antibody response that protects the host against challenge with flavivirus (Schlensinger et al,1987).

Rupinderjeet kaur et al, 2003 in their study found that the prM, E, NS1 proteins are the most effective candidates for inducing protective immunity against JEV.[144].

**PATHOGENESIS**

Johnson RT,et al,1985,Hase T,et al,1990,Dropulic B,et al,1990, in their articles

stated that after an infectious mosquito bite, viral replication occurs locally and in regional lymph nodes. Virions disseminate to secondary sites, where further replication contributes to an augmented viremia. Invasion of the CNS probably occurs from the blood by antipodal transport of virions through vascular endothelial cells. [23,40-42].

The pathogenesis of JE encephalitis appears to be a combination of direct, virally mediated damage and the host inflammatory response. The host immune response comprises antibody mediated immunity, particularly against the E and NS1 proteins, cell mediated immunity, including cytotoxic T lymphocytes (Johnson et al,1985;Konishi et al,1995),and apoptosis(Jan et al,2000; Liao et al ,1997; Parquet et al 2002).[146,147]

**DIAGNOSIS**

Inada R,et al,1937,Innis B, et al,1995,Burke D Set al, 1985,Kedarnath N et al,1984,in their article mentioned that although a history of exposure to an endemic area and certain clinical features may suggest JE, clinical diagnosis is unreliable, and laboratory confirmation, usually by serological tests, is necessary. JE virus occasionally can be recovered from blood in the pre neuro invasive phase (up to 3 to 7 days after onset), but patients presenting with encephalitis usually are no longer viremic[13, 24,67]

The text book VACCINES 3rd vol by Plotkin,Orenstein states that JE virus produces cytopathic effects in Vero, $LLCMK_2$, and PS cells and kills suckling mice inoculated intracerebrally. C6/36 and Ap61 mosquito cell lines and T. splendens, inoculated intrathoracically, also are sensitive systems for viral isolation. Viral isolates are readily identified by immunofluorescent (IF) techniques using virus specific

monoclonal antibodies or by neutralization.

Burke DS et al, 1985, Innis BL,et al,1989 in their studies found that the most widely used diagnostic method is IgM-capture enzyme-linked immunosorbent assay (ELISA) [25, 69] Specific IgM can be detected in CSF, serum, or both in approximately 75% of patients within the first 4 days after onset of illness , and nearly all patients are positive 7 days after onset. Both fluids should be tested to maximize sensitivity. A specific diagnosis also can be confirmed by demonstrating fourfold or greater changes in antibody titer by conventional serological procedures (e.g., hemagglutination inhibition, complement fixation, IF antibody, ELISA, or neutralization). Heterologous flaviviral antibodies (e.g., to dengue and West Nile viruses) are a potential source of false-positive reactions.

Konishi E et al 1996, in his study demonstrated that synthetic antigens, including recombinant virus-like particles expressing the viral E protein, are a potential source of standardized antigen with improved specificity.[70]

**EPIDEMIOLOGY**

IgarashiA,1992, BurkeDS,et al, InnisB, 1995, RosenL, 1986, SchererWF, et al, 1959, in their articles showed that JE is transmitted in epidemics or in an endemic pattern, or both, in virtually every country of Asia. JE is principally a disease of rural areas in which vector mosquitoes proliferate in close association with pigs, wading birds, and ducks, the principal vertebrate amplifying hosts (Fig. 27-9) [1,2,1371,72].

Gould DJ et al 1964, Wang YJ etal 1982 in their studies found that humans and

horses may become ill after infection, but such illness is incidental to the transmission cycle[73,74]

RosenL,1986, SchererWF et al,1959, in their article mentioned that Culex tritaeniorhynchus is the principal JE vector in most areas of Asia, but various principally ground pool and rice paddy-breeding species, including Culex vishnui, Culex pseudovishnui, Culex gelidus, Culex fuscocephala, Culex bitaeniorhynchus, Culex infula, Culex ubitmorei, and Culex annulus, are also important locally. [71,72].

Bista MB et al,2001 in their articles stated that the Haem-agglutination inhibition test was done on human and animal serum during 1978 to 1980 in Nepal .It has shown that pigs and ducks are main reservoir hosts.[143].

Takahashi K et al 1972, in his article mentioned that JE mosquito vectors are zoophilic; Consequently, cows and certain other animals can reduce risk to humans by diverting vector mosquitoes (zooprophylaxis). [75].

J. Commun .Dis.35(1)2003:40 in a study on a focal outbreak of Japanese Encephalities among horses in Pune District India also confirmed the zoophilic nature of the vectors.

Takahashi K et al1972, Sasaki O et al 1982, Vaughn DW et al 1992, in their studies found that immunizations of pigs prevents abortion and stillbirths and also may reduce viral transmission by nullifying the role of pigs as viral amplifiers. [75-77]

Takahshi K et al 1972, in their study explained that experimental immunization of nearly the entire pig inventory on one island led to a significant reduction in human

cases.[75]

**ACTIVE IMMUNISATION**

**INACTIVATED MOUSE BRAIN DERIVED JE VACCINE**

The text book VACCINES 3rd vol by Plotkin, Orenstein, states that Mouse brain-derived vaccines are produced in Japan and elsewhere using a similar sequence of centrifugation, ultrafiltration, protamine sulfate precipitation, and formalin inactivation in the cold, followed by further purification by ultrafiltration, ammonium sulfate precipitation, and continuous zonal centrifugation on sucrose density gradients.

Oya A 1998 in his article mentioned that in most areas of Asia, vaccine produced from the Nakayama strain is given subcutaneously in two 0.5/mL doses 1 to 4 weeks apart (1.0 mL for people >3 years of age) usually beginning at the age of 12 to 36 months, with a booster dose at 1 year and additional booster doses thereafter at 1- to 3-year intervals.[83]

Immunogenicity studies in subjects from areas without endemic transmission (Western countries and areas of India) indicate that three doses are necessary for an adequate antibody response. [80,92-94]

The U.S. Public Health Service (Advisory Committee on Immunization Practices) (ACIP) recommends three doses, on days 0, 7, and 30. [95]

**INACTIVATED PHK CELL DERIVED JE VACCINE**

Gu PW,et al ,1987 , in their article reviewed that inactivated JE vaccine prepared

from the P3 strain in PHK cells is produced exclusively in the PRC and has been that country's principal JE vaccine since 1968. [82]

Zhang XC et al, 1990, in their article explained that concurrent JE vaccine administration did not lead to significant changes in responses to measles or DPT antibody titers or in the size of BGC reactions.[96]

**LIVE ATTENUATED JE VACCINE**

Attenuated JE viral strains have been sought by passaging wild strains serially in various cell culture systems, including PHK, chick embryo, and embryo mouse skin cells. [97-100].

Ni H , et al, 1998 in their article found that loss of neurovirulence in mice, hamsters, or pigs, or any combination of the three, initially suggested the possibility of safe use in humans. Attenuation may be correlated with decreased binding to mouse brain cell receptors. [101]

Wang SG ,et al 1990 in their studies described that several hundred ampoules of seed virus, prepared from the seventh passage level of SA14-14-2 virus, are maintained in lots at the NICPBP in Beijing.[107]

Wang SG et al 1990 in their studies showed that the infectious titer of lyophilized vaccine is not appreciably changed after storage at 37°C for 7 to 10 days, at room temperature for 4 months, or at 4 to 8°C for at least 1.5 years. After reconstitution with sterile saline or distilled water and storage at 23°C, the vaccine's infectious titer is stable for 2 to 4 hours or 2 hours, respectively.[107]

Yu YX , et al ,1989, in their studies found that attenuated vaccine provided more effective protection than inactivated P3 vaccine against a spectrum of JE strains isolated in China.[108]

Chen BQ et al 1982 in their article stated that the virus did not revert to a neurovirulent phenotype after mosquito passage. [120.] A 0.5 mL dose is administered subcutaneously to children at 1 year of age and again at 2 years. In some areas, a booster dose is given at 6 years.

Zheng-Le Liu et al, 1997 in their article stated that the short term safety of both the SA -14-14-2 virus strain and of hamster kidney cells as a substrate for the manufacture of live attenuated vaccines has been well documented. [141]

Tsai TF et al 1998 in their studies mentioned that a more conventional administration schedule in which the two primary doses were given at intervals of 1 or 2.5 months was shown to produce immunity in 94 to 100% of immunized school-aged children[122.]

**EXPERIMENTAL VACCINES**

Several candidate JE vaccines are in various stages of early clinical and preclinical development or research.[123]

The most extensively evaluated candidate vaccines have been a recombinant virus pair engineered by inserting four JE viral genes (prM, E, NS1, and NS2a) into attenuated vaccinia (NYVAC) or canarypox (ALVAC) viruses, Yellow Fever 17D viruses. These recombinants expressed the encoded JE structural and nonstructural gene products and

stimulated JE protective antibodies in mice.

Fu DW,etal, 1996 in their studies stated that adaptation of the SA14-14-2 strain to insect cells has been reported to improve propagation.[124]

Konishi E, et al 1997 in their studies found that the best-characterized candidate subunit vaccine is a vaccinia-JE virus recombinant that releases extracellular particles (EPs) composed of JE, prM, and E proteins in an apparently more authentic configuration than when presented as simple peptides. [125]

**PROTECTIVE EFFECTS OF IMMUNIZATION**

C.V.R.Mohan Rao et al, 1993 in their study mentioned that the serum antibody titres were determined by mouse neutralization test (MNT), plaque reduction neutralization test (PRNT) and haemagglutination inhibition test (HI). From the results ,MNT appeared to be the most sensitive of the three tests followed by PRNT, HI.

**INACTIVATED MOUSE BRAIN DERIVED JE VACCINE:**

A neutralizing antibody titer of more than 1:10 generally is accepted as evidence of protection and postvaccination seroconversion. Passively immunized mice that acquire this level of neutralizing antibody are protected against challenge from $10^5$ $LD_{50}$ of JE virus, a typical dose transmitted by an infectious mosquito bite. Among Asian children immunized with two doses of Nakayama or Beijing-1 strain-derived vaccines, neutralizing antibody responses to the respective homologous vaccine strains are in the range of 94 to 100%; Responses to strains representing a heterologous antigenic group are lower . [126-128]

The proportion of vaccinees retaining detectable neutralizing antibodies and their Geometric Mean Titres ( GMTs ) declined rapidly in the year after the primary two-dose series, so that only 78 to 89% of Nakayama vaccine recipients and 88 to 100% of Beijing-1 vaccine recipients still had protective levels before the scheduled 1-year booster. Antibody persistence was greater among Beijing-1 vaccine recipients. After booster immunization (third vaccine dose) antibody response rates were uniformly high (100%). Where the influence of previous flaviviral infections was unlikely, vaccinees receiving two doses produced lower seroconversion rates and lower GMTs [20, 221-223]. Moreover, as rapidly as 6 to 12 months after primary immunization with two doses, neutralizing antibody titers declined below 1:8 in 90% of vaccinees.[181] A three-dose primary schedule was more immunogenic, resulting in seroconversion rates exceeding 90% and significantly higher neutralizing antibody titers. [9, 92-94]

Hoke CH ,et al 1988 in their article stated that results of the efficacy trial comparing a monovalent Nakayama strain vaccine with a bivalent vaccine also containing Beijing-1 antigen showed that the two were equally efficiacious. [129]

Neutralizing activity may be present below the threshold of detection in in vitro assays, and T-cell memory may have been established in vaccinees who appear to be seronegative, providing sufficient help to clear infections upon reexposure.

Hoke et al immunized 43,708 Thai children with JE vaccine (Biken) and administered tetanus toxoid vaccine to another 21,516 chilren, who served as a control group .Protective efficacy in the JE vaccine group was 91%, and the group receiving JE

vaccine also showed a trend toward fewer and less severe cases of dengue fever.[129]

Studies in Asia to determine the persistence of vaccine-derived immunity are complicated by natural infections with dengue, West Nile virus, or other flaviviruses and reexposure to JE virus itself, all of which act to reinforce and broaden vaccine-derived immunity to JE virus.[38,39,43-66,69]

Poland JD et al 1990, Kanamitsu M, et al 1970, in their studies found that even with the potential for these reinforcing infections, several studies in Asian and in Western subjects indicate a progressive decline in antibody levels in the first year after primary immunization with two doses. [80, 84]

Cross-sectional sero surveys in Japan and Taiwan indicate a rapid decline of immunity in childhood.  Observations of vaccine efficacy in the Taiwan field trial parallel these results: In the second year after immunization, protective efficacy declined from 80 to 55% (95% confidence interval (CI) = 39-75%).[130]

Gowal D, et al 1995 , in a small study of vaccinees receiving an Indian-manufactured JE vaccine found that 34 of 35 (97%) retained neutralizing antibodies 3 years after a primary series of three doses, and 31 of 34 (91%) retained antibodies at 4.5 years, with GMTs of 71 and 32, respectively.  However, the boosting effect of naturally acquired flaviviral infections in these subjects cannot be ruled out. [131]

**INACTIVATED PHK CELL DERIVED VACCINE**

Gu PW et al 1987, Ren YL 1982 in their studies showed that Extensive raindomized field trails among 480,000 children have demonstrated vaccine efficacies in

the range of 76 to 95%. [82, 133.] These observations suggest that clinical efficacy is better than the reported protective efficacy. [82]

## L IVE ATTENUATED JE VACCINE

More than 200 million doses of this SA-14-14-2 vaccine have been delivered since 1988 with excellent record of safety and efficacy (Xin et al,1988;Liu et al,1997;Bista et al 2001). Other countries that have licensed and used the vaccine are South Korea( since 2001),Nepal (since 1999), and India (since 2006).(PATH).

### Prequalification and licensing

The manufacturer is preparing a file for submission to WHO for prequalification of the SA-14-14-2-vaccine.WHO prequalification enables procurement of product by United Nations agengies. It is anticipated that the vaccine will be prequalified by 2009. WHO prequalification is not a requirement for use of the vaccine; A country can individually license the vaccine through its own national regulatory authority. (PATH-April 2007).

After a single dose, antibody response are produced in 85 to 100% of non immune 1 to 12year old children, with a response gradient that parallels progressive vaccine dilution. [105,106,134,135]

Because of variable immune response rates after one dose, SA14-14-2 vaccine is given in a schedule of two doses separated by a year, according to the custom of administering JE vaccines in annual spring compaigns. The immunogenicity of two

doses given at intervals of either 1 or 2.5 months was shown in 12 to 15year old children. Response rates were similar: 75 to 100% after one dose and 94 to 100% after two doses (two vaccine lots were compared), but there was a trend toward better seroconversion with the longer interval, and GMTs were approximately two-fold higher (65-89 versus 115-158, respectively). If these results can be confirmed in infants, the SA 14-14-2 vaccine could be integrated into a routine childhood immunization schedule, potentially improving vaccine coverage.[122.]

Efficacy trials in children 1 to 10 years old have consistently yielded high protection rates above 98%. [114,136,137]

## SIDE EFFECTS OF IMMUNISATION

### Inactivated Mouse Brain Derived JE Vaccine

Local tenderness, redness, or swelling at the injection site occur in approximately 20% of individuals immunized with inactivated mouse brain-derived vaccines. Mild systemic symptoms, chiefly headache, low-grade fever, myalgias, malaise, and gastrointestinal symptoms, are reported by 10 to 30% of vaccinees. [80, 93,94, 126]

In a prospective study among United States military personnel in Japan , the overall allergic reaction was 0.6% (Japanese encephalitis vaccine. Weekly Epidemiological Record,1998).

An important feature of the reactions is the potential for delayed onset, particularly after a second dose. In a prospective study of 14,249 U.S. Marines, the median interval between immunization and onset was 18 to 24 hours after the first dose,

with 74% of reactions occurring within 48 hours. [80,81, 139].

## LIVE ATTENUATED JE VACCINE

LiuZL, et al, 1997,in their study found that a block randomized cohort study of 13,266 vaccinated and 12,951 nonvaccinated 1 to 2 year-old children followed prospectively for 30 days confirmed the vaccine's safety. No cases of encephalitis or meningitis were detected in either group, and rates of hospitalization; new onset of seizures; fever lasting more than 3 days; and allergic, respiratory, and gastrointestinal symptoms were similar in the two groups. The observations excluded a vaccination-related encephalitis risk above 1 in 34..[141]

## INDICATIONS FOR IMMUNISATION

The text book VACCINES 3rd vol by Plotkin,Orenstein states that Universal Primary immunization is indicated for children between 1 and 2 years of age. The peak risk of infection is in children between 1 and 4 years of age, which may reflect the waning protective effects of maternal immunity and patterns of outdoor activity that place young children at risk. However, cases occur in children through the first decade of life, and in most areas with risk of enzootic transmission, immunity should be maintained by boosters through the age of 10 years. Immunization is recommended for visitors to epidemic or endemic areas during the transmission season, especially when there will be an extended period of exposure (more than 30 days) or the individual is at high risk of exposure to vectors.

Ramakrishna and colleagues, 1999, showed that oral immunization of mice with live JEV induced a brisk protective immune response (76.7% protection with mouse brain derived immunogen and 70% with cell culture derived immunogen) against subsequent intra cranial challenge.[148].

# AIMS & OBJECTIVES

"**The study on Efficacy and Safety of SA-14-14-2 vaccine against Japanese Encephalitis Virus at Virudhunagar District"** conducted from October 2007 to December 2007 in 60 school going children in the age group of 6-10 years was aimed.

- To know the prevaccination titre of JE antibodies in the study population.
- To study the efficacy of the vaccine
  - a) By demonstrating seroconversion in the vaccinees by standard techniques like Haemagglutination Inhibition Test and Mouse Neutralisation Test.
  - b) By Case Study.
- To study the safety of the vaccine by observing systemic toxicity and Local reaction.

# MATERIALS AND METHODS

A total of 60 children in the age group of 1to 15yrs, belonging to 5 schools in 5 blocks (12 from each) of Virudhunagar District of Tamilnadu were vaccinated after a Physical examination for fitness, and after ruling out contraindications. A total of 178 blood samples were collected from them, 3 samples from each child, one sample prior to vaccination, one sample 4 weeks after vaccination and one sample 12 weeks after vaccination. Two postvaccination samples from one child was not collected .

This is an interventional type of study, in a randomized sample collection with an aim to study the prevention, Safety and Efficacy to determine the outcome of immunogenicity of JE live attenuated SA-14-14-2 Vaccine.

This study was conducted at the WHO Acute Meningo Encephalitis Syndrome Laboratory, Institute of Microbiology, Madurai Medical College, Madurai. The study period was 3 months from October 2007 – Dec. 2007. The study population included 60 children vaccinated against Japanese Encephalitis, of 6-10 years age groups. The total sample size was 178 serum samples.

Written informed consent was obtained from the Parents/Guardian of vaccinated children. Ethical Clearance was obtained from the college Human and Animal Ethical Committees.

**Eligibility Criteria**

**Inclusion Criteria**

- 1-15 years of age group in both sexes.
- Person without fever / malnutrition / acute infectious disease.
- Person without active untreated tuberculosis
- Person without any systemic disease.
- Person without any allergy & Convulsions.
- Person not on any immunosuppressive therapy.
- Person without any H/o hypersensitivity.
- Person without a history of immunization with any other live attenuated vaccine within the past one month.

**Exclusion Criteria**

- Acute infectious disease
- Tympanitis
- Cardiac, liver, Kidney diseases.
- Person with a proven or suspected hypersensitivity .
- Person on immuno suppressive therapy
- Known or suspected impairement of immunologic function.
- History of serious chronic disease.
- Acute medical illness with or without fever within last 72 hours or an axillary temperature > 37.5ºC at the time of inclusion.
- History of documented and suspected encephalitis, encephalopathy, meningitis.

- Immunization with JE vaccine prior to enrollment.
- If received any vaccine, other than the study vaccine within 4 weeks prior to or scheduled to receive a non study vaccination during the conduct of this trial.
- History of seizures including history of febrile seizures, or any other neurologic disorder.

**Vaccine**

Live attenuated SA 14-14-2 JE Vaccine manufactured in Chengdu Institute of Biological Products, Chengdu, CHINA, was used for vaccination. This vaccine was supplied in 5 dose vials as a lyophilized powder and was reconstituted with 2.5ml of sterile diluent (PBS ). After reconstitution, it turns into a transparent Orange, red or light pink liquid. The reconstituted vaccine was used within 2 hours of reconstitution. Vaccine was stored and transported between 2°C to 8°C and protected from light.

**Schedule**

One dose (0.5ml) contains not less than 5.4 log PFU of live JE virus. The vaccine was injected subcutaneously in the upper arm of children of 1-15 years of age. Sterile water was used for cleansing the skin before injection. The vaccine was administered in the 1st week of August 2007. The children were followed up for 5 days for side effects or reactions.

**Blood Specimens**

Blood samples of 60 children were collected approximately 1 week prior to the

vaccine to determine their antibody status to JE virus. These samples were treated as prevaccination samples. The samples were again collected approximately 4 weeks & 12 weeks after the vaccination.

**Collection & Storage of Blood Specimens**

All the blood specimens (5-10 ml each) were collected by vene puncture. Serum was separated within 48 hours and the sera were stored on wet ice till they were transported to Madurai Medical College,(MMC), Institute of Microbiology, WHO AMES LAB, Madurai which is situated at a distance of 50 km from Virudhunagar. At MMC, the sera were stored at minus 20°C till testing.

**Processing**

I. Pre and post vaccination samples were screened for antibodies to the JE virus by MAC ELISA by NIV PUNE KIT.

II. The antibody titre in the pre and postvaccination samples were determined by the haemagglutination inhibition test using suckling mice brain as antigens which was prepared inhouse.

III. The samples were also tested for presence of neutralizing antibodies by the Neutralization test in mice (MNT)

**Test Procedures**

**A) MAC ELISA**

**Procedure of the assay**

IgM antibodies in the vaccinees blood are captured by Anti-human IgM (µ chain specific) that are coated on to the solid surface (wells). In the next step, JE antigen is added which binds to captured IgM, if the IgM and antigen are homologous. Unbound antigen is removed during the washing step. In the subsequent step, Biotinylated flavivirus cross reactive monoclonal antibody (Hx-B) is added followed by Avidin-HRP. Subsequently, substrate / chromogen is added and watched for development of colour. The reaction is stopped by I N $H_2SO_4$. The intensity of color / optical density is monitored at 450 nm. OD readings were directly proportional to the amount of JE virus specific IgM antibodies in the sample.

**Test Protocol**

1) The serum samples were diluted 1:100 with sample dilution buffer.
2) The coated wells were washed thrice with wash buffer.
3) 50µl of diluted samples were transferred to the appropriate wells. 50 µl of positive & negative controls were added to respective wells.
4) The plate was kept in humidified box & incubated at 37°c for 1 hour.
5) At the end of incubation, the plate was washed 5 times with wash buffer. The plate was tapped after last wash on a tissue paper.
6) 50 µl of JE antigen was added to each well. The step 4 & 5 were repeated.
7) 50 µl of biotinylated monoclonal antibody was added to each well. The step 4 & 5 were repeated.

8) 50 µl Avidin-HRP was added to each well. The plate was kept in a humidified box and incubated for 30 minutes at 37$^{o}$c. The step 5 was repeated.

9) 100 µl of diluted substrate was added to each well.

10) The plate was incubated at room temperature for 10 minutes.

11) The reaction was stopped with 100 µl of 1 N $H_2SO_4$.

12) The absorbance was measured at 450nm within 10 minutes.

**Quality Control**

Each kit was supplied with one Positive control and one Negative control. These were mainly used for validation of the kit.

Expected values are given below

Positive : OD values $\geq 0.5$

Negative : OD value $\leq 0.18$

**Interpretation of the results**

1. If OD value of sample tested exceeded OD of Negative control by a factor 2.1 (Sample OD $\geq$ Negative OD x 2.1), the <u>sample was considered as "Positive</u>".

**B) HAEMAGGLUTINATION AND HAEMAGGLUTINATION INHIBITION TESTS**

**Principle**

Various animal viruses have the property to adsorb to red blood cells. It usually results in agglutination of these cells. This haem agglutination (HA) test provides a

fairly quantitative and easy method of detection, identification and titration of virus. This haemagglutination can be inhibited by specific antiviral antibody. This haem agglutination inhibition (HI) has been employed as an assay method for detecting viral antibody titres.

The antigen used in this test was prepared in the lab by inoculating the seed virus into the brain of suckling mice.

**Procedure for JE antigen extraction**

5 litters of suckling mice were inoculated each by intracerabral route with 0.02ml of the JE seed virus using a 0.25ml tuberculin syringe and ¼” 27 G needle. The mice were observed daily for 21 days. The mouse with symptoms were recorded using standardized abbrevations as follows :

M - Missing

Pr - Prostrate

E - Eaten by mother

W - Weak

D - Dead

S - Sick

Pa - Paralysed

Co - Convulsions

The mice showing definite signs of infection were sacrificed by brief exposure to chloroform fumes.

The brains of the sick and dead mice were harvested & stored at minus $70^0$C.

The brains were ground with borate saline by using mortar and pestle and was centrifuged at 10,000 rpm for 15 mts.

The antigen was sonicated and to this, protamine sulphate (for 1ml antigen – 3mg protamine sulphate) was added & refrigerated overnight.

This antigen was centrifuged for 15 mts at 3000 rpm in the cold.

This antigen was used for HA & HI tests.

**PREPARATION OF GOOSE ERYTHROCYTES AND THEIR WORKING SUSPENSION**

i) 0.5ml of goose blood was collected aseptically from wing vein in 1.5ml of acid citrate dextrose (ACD) solution.

ii) One volume of whole blood was mixed with 2.5 volume of dextrose gelatin veronal (DGV) solution and was centrifuged at 1,500 rpm for 15 minutes at +4ºC.

iii) Packed cells were resuspended in normal saline 3 times the original volume of blood and was centrifuged. This procedure was repeated three times.

iv) Packed cells were suspended in normal saline in 3 times the original blood volume and stored at +4ºC.

v) This was Centrifuged at 2,500 rpm for 6 minutes at room temperature or +4ºC.

vi) The supernatant was discarded. To packed cells, 0.9 percent NaCl was added for suspension and was centrifuged at 2,500 rpm for 3 minutes twice and for 6

minutes once .

vii) Packed cells were suspended in 0.9 percent NaCl to get a 10 percent suspension. The optical density was checked with a spectrophotometer.

viii) The packed cell suspension was stored at +4ºC.

**PROCEDURE FOR HAEMAGGLUTINATION TEST**

1) The microtiter plate was marked with a glass marking pencil giving different dilutions of antigen and was prechilled.
2) 25µl BABS (Bovine Albumin in Borate Saline) was added upto $10^{th}$ well, $11^{th}$ well was left blank, and to the $12^{th}$ well , 50 µl BABS was added.
3) The plate was refrigerated for half an hour.
4) With the microdilutor, 25 µl of JE antigen was transferred to the $1^{st}$ well, mixed and transferred 25µl from the $1^{st}$ well to $2^{nd}$ well, and further 2 fold dilutions were made upto 10th well.
5) Refrigerated for 1 hour.
6) 50 µl of 0.4% goose RBCs was added to all except $11^{th}$ well.
7) The plate was shaken gently to mix the reagents and was kept at room temperature or 37ºC.
8) The test results were recorded after about 60 minutes.
9) Complete agglutination = +

   Partial agglutination = (+)

   Trace agglutination = (±)

No agglutination = 0

10) 16 units of this antigen was used in HI test.

## PROCEDURE FOR HAEMAGGLUTINATION INHIBITION TEST

### Kaolin Treatment and Erythrocyte Adsorption of Serum

i) 2 volumes of serum was mixed with 3 volume of borate saline in pH 9 & 5 volumes of 25% Kaolin.

ii) This mixture was kept at room temperature for 20 minutes with occasional shaking.

iii) This was centrifuged at 2,500 rpm for 30 minutes at room temperature. The supernatant fluid is 1:5 dilution.

iv) The kaolin treated sera were chilled in ice bath and 0.1ml packed washed goose RBC , was added per 5ml of a 1:5 diluted sera.

v) This was kept in ice bath for 20 minutes with occasional shaking.

vi) This was centrifuged for 10-15 minutes at 2000 rpm in the cold.

vii) The supernatant was collected as 1:5 dilution.

### Procedure for Haemagglutination inhibition Test

1. The microtitre U plate was Prechilled & marked.
2. 25µl BABS was added upto 10$^{th}$ well. 11$^{th}$ well was left blank, and to the 12$^{th}$ well , 50 µl of BABS was added.
3. Kept at 4$^{o}$C for half an hour.
4. 25 µl of 1 in 5 diluted sera were added to the 1$^{st}$ well with the microdilutor.

5. Serial 2 fold dilutions. (1:10 to 1:5120) of sera were made with the microdilutor upto 10th well.
6. Kept at 4ºC for half an hour.
7. 25 µl of 16 units HA antigen was added to each well.
8. Kept at 4ºC overnight.
9. 50 µl of 0.4% goose RBCs was added to each well including cell control well (12th)
10. Kept at room temperature for half an hour.
11. The titre was read as the highest dilution of the serum which completely inhibited agglutination.

**Neutralization Tests in mice (MNT)**

**Titration of JE virus in vivo by intracerebral inoculation**

The infectivity titre of the virus was determined in vivo by inoculating increasing dilutions of the virus material to laboratory infant mice and based on mortality seen in different dilutions, the infectivity titre which is the reciprocal of highest dilution showing 50% mortality in the inoculated mice and expressed as $LD_{50}$/ml was calculated by using Reed-Muench formula.

**Procedure**

1) Working in microbiological safety cabinet, the diluting fluid PBS containing 2% serum was prepared & dispensed as 9ml in test tubes labelled $10^{-1}$ to $10^{-7}$. The test tubes were kept in rack immersed in plenty of ice.

2) To make 10 fold (log) dilutions of the virus material, 1 ml of virus was diluted in 9ml of diluent to get the initial dilution i.e. $10^{-1}$. Subsequently, 1ml of previous virus dilution was transferred to next dilution by using at each step a fresh pipette, to achieve serial 10 fold dilutions.

3) 0.03ml of each virus dilution was inoculated intra cerebrally into infant mice, starting from the highest dilution ($10^{-7}$). At least 6 mice per dilution were used and these were transferred into cages appropriately labelled.

4) The mice were observed for 14 days for specific signs and symptoms of JE. Any death occurring within 5 days should be considered non-specific. The total number of specific deaths were noted in each dilution and the virus titre was calculated by using Reed-Muench formula.

Neutralization tests were carried out using a fixed dose of virus (P20778 strain of JE virus) and undiluted serum. Equal quantities of serum and virus suspension were mixed and were incubated at 37°C for one hour and were then inoculated into infant mice (0.03 ml) by the intra peritoneal (IP) route. The virus dose varied between 85 to 360 LD in IP route. A serum was considered positive for neutralizing (N) antibody if the survival ratio in the mouse group inoculated with the respective serum virus mixture was between 70 and 100 per cent, partially positive if it was between 31 and 69 per cent and negative if it was below 30 per cent.

The reports of IgM capture ELISA, HI antibody titre and MNT antibody titres were analysed in the pre immunized and post immunized samples to find out whether the JE vaccine given in this district had any immunological effect in the vaccinated children.

The efficacy of the vaccine was further confirmed by the case study. The cases of JE and deaths reported prior to vaccination and post vaccination were analysed and efficacy determined by noting the decrease in the incidence of cases and deaths.

Further the safety of vaccine was studied by analysing various local reactions and systemic toxicity. In local reactions, symptoms like local tenderness, redness, swelling, and itching were noted. In systemic toxicity, fever, headache, rash, malaise, nausea, vomitting, diarrhoea, and sorethroat were noted. These signs and symptoms were analysed to note if they adversely affected the immunised children.

# RESULTS

During the year 2007, 94% of 1-15 year population of Virudhunagar District was immunized with live attenuated JE SA 14-14-2 vaccine amounting to 4,83,537 beneficiaries.

A total of 178 serum samples were collected from 60 children, selected from 5 Block PHCs, 12 from each Block PHC, and 3 samples from each child., one sample prior to vaccination, one sample 4 weeks after vaccination and one sample 12 weeks after vaccination. As one female child from Mallanginar Block PHC refused to give post vaccination blood samples, only 178 samples were collected.

The distribution of samples is given in Table – I.

**TABLE – 1**

**BLOCKWISE DISTRIBUTION OF SAMPLES**

| Sl.No. | Name of Block PHC | No. of children selected | No. of Blood samples collected |
|---|---|---|---|
| 1) | Narikudi | 12 (20%) | 36 (20.22%) |
| 2) | Reddiapatti | 12 (20%) | 36 (20.22%) |
| 3) | Pandalkudi | 12 (20%) | 36 (20.22%) |
| 4) | Kanniseriputhur | 12 (20%) | 36 (20.22%) |
| 5) | Mallanginar | 12 (20%) | 34 (19.10%) |
| | **Total** | 60 (100%) | 178 (100%) |

It was found that except from Mallanginar Block PHC, equal number of samples

were collected from all other Block PHCs.

The distribution of samples were analysed agewise and sexwise and out of 60 samples collected, 36(60%) were from male children and 24(40%) were from female children .All the samples were from the age group 6-10 years.

This is given in Table – 2.

**TABLE – 2**

**AGE & SEX WISE DISTRIBUTION OF SAMPLES**

| **Gender** | **Age in years** | | |
|---|---|---|---|
| | **0-5** | **6-10** | **11-15** |
| Males | - | 36 (60%) | - |
| Females | - | 24 (40%) | |
| **Total** | - | 60 (100%) | - |

Thus it was found that all the samples collected were in the age group of 6-10 years, all school going and more number of samples were collected from males(60%).

All the 178 blood samples, 60 pre-vaccinated and 118 post vaccinated were screened for IgM antibodies to JE virus with NIV PUNE MAC ELISA KIT. Among the 60 pre vaccinated samples, only 2 (3.33%) showed IgM antibodies to JE virus. Among the samples collected after 4 weeks of vaccination, 24 (40.68%) showed sero conversion to JE and among the samples collected after 12 weeks of vaccination, 58 (98.30%) showed sero conversion to JE virus.

This is given in Table –3

## TABLE –3

## SEROCONVERSION IN PRE & POST VACCINATION SAMPLES

| Block PHC | Pre vaccinated IgM (No. of positives/ Total No. of samples %) | Samples collected after 4 weeks of vaccination | Samples collected after 12 weeks of vaccination |
|---|---|---|---|
| 1. Narikudi | 0 | 0 | 12(20.34%) |
| 2. Reddiapatti | 0 | 10(16.95%) | 12(20.34%) |
| 3. Pandalkudi | 1 (1.66%) | 2(3.39%) | 12(20.34%) |
| 4. Kanniseriputhur | 0 | 4(6.78%) | 11(18.64%) |
| 5. Mallanginar | 1 | 8(13.56%) | 11(18.64%) |
| **Total** | 2/60 (3.33%) | 24/59 (40.68%) | 58/59 (98.30%) |

It was found that 3.33% showed IgM antibody to JE virus prior to vaccination., 40.68% showed seroconversion after 4 weeks of vaccination and 98.3% showed seroconversion after 12 weeks of vaccination.

The IgM positive samples were **analysed sexwise**, in the prevaccinated, 4 weeks and 12 weeks after vaccination samples in the 5 Block PHCs and it was noted that **in pre vaccinated**, a male child (12.5%) from Pandalkudi PHC and a female child (12.5%) from Mallanginaru PHC showed sero conversion. **In the samples collected after 4 weeks of vaccination** 4 males each from Reddiapatti PHC (100%) and Kanniseriputhur PHC (40%), 2 males each from Pandalkudi PHC (25%) and Mallanginar PHC (50%) showed sero conversion and 6 females each from Reddiapatty

PHC (75%) and Mallanginar PHC (85.71%) showed sero conversion.

This is given in Tables 4 & 5

**TABLE – 4**

**IgM ANTIBODY PROFILE- PRE VACCINATION SAMPLES**

| Place | Number of samples tested | IgM Positives | | | | | |
|---|---|---|---|---|---|---|---|
| | | Male | | | Female | | |
| | | Total No. collected | Sero converted | % | Total No. collected | Sero converted | % |
| Narikudi | 12 | 10 | - | - | 2 | - | - |
| Reddiapatti | 12 | 4 | - | - | 8 | - | - |
| Pandalkudi | 12 | 8 | 1 | 12.5 | 4 | - | - |
| Kanniseri puthur | 12 | 10 | - | - | 2 | - | - |
| Mallanginaru | 12 | 4 | - | - | 8 | 1 | 12.5 |

**TABLE – 5**

**IgM ANTIBODY PROFILE- 4 WEEKS POST VACCINATION.**

| Place | Number of samples tested | IgM Positives | | | | | |
|---|---|---|---|---|---|---|---|
| | | Male | | | Female | | |
| | | Total No. collected | Sero converted | % | Total No. collected | Sero converted | % |
| Narikudi | 12 | 10 | 0 | 0 | 2 | 0 | 0 |
| Reddiapatti | 12 | 4 | 4 | 100 | 8 | 6 | 75 |

| Pandalkudi | 12 | 8 | 2 | 25 | 4 | 0 | 0 |
|---|---|---|---|---|---|---|---|
| K.S.Puthur | 12 | 10 | 4 | 40 | 2 | 0 | 0 |
| Mallanginar | 11 | 4 | 2 | 50 | 7 | 6 | 75 |

It was found that there was seroconversion in both sexes *equally* in the prevaccinated samples suggesting the presence of subclinical cases *in both sexes* in the vaccinated area. In samples collected after 4 weeks of vaccination, more male children seroconverted in Pandalkudi, Kanniseriputhur whereas more females seroconverted in Reddiapatty & Mallanginar. *No seroconversion* was seen in Narikudi in either sex.

Similarly in the **samples collected after 12 weeks of vaccination,** among the seroconverted 35(97.22%) were males and 23 were (100%) females. In these samples 10 males (100%) from Narikudi, 4 males (100%) from Reddiapatti, 8 males (100%) from Pandalkudi, 9 males (90%) from Kanniseriputhur, 4 males (100%) from Mallanginar showed seroconversion and 2 females (100%) from Narikudi, 8 females (100%) from Reddiapatti, 4 females (100%) from Pandalkudi, 2 females (100%) from Kanniseriputhur, 7 females (100%) from Mallanginar showed seroconversion.

This is given in Table 6

**TABLE – 6**

**IgM ANTIBODY PROFILE- 12 WEEKS POST VACCINATION**

| **Place** | **Number of samples tested** | **IgM Positives** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Male** | | | **Female** | | |
| | | **Total No.** | **Sero converted** | **%** | **Total No.** | **Sero converted** | **%** |

| | | collected | | | collected | | |
|---|---|---|---|---|---|---|---|
| Narikudi | 12 | 10 | 10 | 100 | 2 | 2 | 100 |
| Reddiapatti | 12 | 4 | 4 | 100 | 8 | 8 | 100 |
| Pandalkudi | 12 | 8 | 8 | 100 | 4 | 4 | 100 |
| Kanniseri puthur | 12 | 10 | 9 | 90 | 2 | 2 | 100 |
| Mallanginaru | 11 | 4 | 4 | 100 | 7 | 7 | 100 |

It was found that there was 100% seroconversion in females in all block PHCs whereas in males except Kanniseriputhur, all other block PHCs showed 100% seroconversion.

The antibody titre of all the 178 samples were also determined by HI technique. In the prevaccinated IgM positive samples, one male child from Pandalkudi PHC, one female from Mallanginar PHC , showed HI titre of 1 in 160 confirming the presence of subclinical case prevalence in these two Blocks.

This is given in Table – 7

## TABLE – 7

## HI TITRE PROFILE -PRE VACCINATION SAMPLES.

| Place | Number of samples tested | IgM Positives | | | | | |
|---|---|---|---|---|---|---|---|
| | | Male | | | Female | | |
| | | Total No. collected | Sero converted | % | Total No. collected | Sero converted | % |
| Narikudi | 12 | 10 | - | - | 2 | - | - |
| Reddiapatti | 12 | 4 | - | - | 8 | - | - |
| Pandalkudi | 12 | 8 | 1 | 12.5 | 4 | - | - |
| Kanniseri puthur | 12 | 10 | - | - | 2 | - | - |
| Mallanginaru | 12 | 4 | - | - | 8 | 1 | 12.5 |

In the samples collected after 4 weeks of vaccination, out of 59 vaccinated, 24 showed HI titre (40.68%). In Narikudi, out of 12 vaccinated , no cases was shown with antibody titre, in Reddiapatty 4 showed antibody titre 1 in 160, 6 showed 1 in 320 and two sample were negative. In Pandalgudi, 2 samples showed antibody titre 1 in640. In Kanniseriputhur, 2 showed 1 in 160, 2 showed 1 in 320, In Mallanginar, out of 11, 2 showed 1 in 80, 5 showed 1 in 320, 1 showed 1 in 2560.

This is given in Table 8

## TABLE – 8

## LOCALE & SEX WISE DISTRIBUTION OF HI TITRE OF 4 WEEKS POST VACCINATION SAMPLES.

| Area | No. of samples Tested | HI TITRE | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nil | | 1 : 20 | | 1 : 40 | | 1 : 80 | | 1:160 | | 1:320 | | 1:640 | | 1:1280 | | 1:2560 | | Total | |
| | | M | F | M | F | M | F | M | F | M | F | M | F | M | F | M | F | M | F | M | F |
| Narikudi | 12 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Reddiapatti | 12 | - | - | - | - | - | - | - | - | - | 4<br>50% | 4<br>100% | 2<br>25% | - | - | - | - | - | - | 4<br>100% | 6<br>75% |
| Pandalgudi | 12 | - | - | - | - | - | - | - | - | - | - | | - | 2<br>25% | - | - | - | - | - | 2<br>25% | - |
| K.S.puthur | 12 | - | - | - | - | - | - | - | - | 2<br>20% | - | 2<br>20% | - | - | - | - | - | - | - | 4<br>40% | - |
| Mallanginar | 11 | - | - | - | - | - | - | - | 2<br>28.6% | - | - | 2<br>50% | 3<br>42.9% | - | - | - | - | - | 1<br>12.3% | 2<br>50% | 6<br>85.7% |

Thus among the seroconverts, 2 females (8.7%) showed 1 in 80, 2 males (5.56%) and 4 females (17.4%) showed 1 in 160, and 8 males (22.2%) and 5 females (21.7%) showed 1 in 320, 2 males (8.7%) showed 1in 640,and 1 female (4.35%) showed 1 in 2560 after 4 weeks of vaccination. After 4 weeks of vaccination, both males and females showed HI titres and 1 in 2560 titre was seen in 1 female.

**In the samples collected after 12 weeks of vaccination**, out of 59 vaccinated, 1 male (2.78%) from Kanniseriputhur showed no HI titre, and 58 showed HI titre(98.30%). Out of the 58 HI titre positives samples, 5 males (13.89%) showed 1 in 20, 10 males (27.78%) and 6 females (26.08%) showed 1 in 40, 6 males (16.67%) and 6 females (26.08%) showed 1 in 80, 8 males (22.22%) and 2 females (8.7%) showed 1 in 160, 4 males (11.11%) and 3 females (13.04%) showed 1 in 320, 2 males (5.56%) and 6 females (26.08%) showed 1 in 640.

Further HI titre analysis of the samples collected after 12 weeks of vaccination, showed that in **Narikudi Block PHC** all the samples who had no antibody titre in the sample collected after 4 weeks of vaccination, showed titre in the range of 1 in 20 to 1 in 320. In **Reddiapatti Block PHC** 4 females showed four fold increase in HI titre in the sample collected after 12 weeks of vaccination, . 2 females showed 2 fold increase in titre, 2 males showed no increase in titre, 2 females showed 1 in 320 titre, only in the sample collected after 12 weeks of vaccination, 2 males showed decrease in titre,

In **Pandalgudi Block PHC** 2 females and 4 males showed 1 in 80 titre only in the sample collected after 12 weeks of vaccination, 2 females showed 1 in 40 in the sample collected after 12 weeks of vaccination, 1 male showed 1 in 160 in prevaccinated, 1 in 640 titre in the sample collected after 4 weeks and 12 weeks of vaccination, 2 males showed 1 in 160 only in the sample collected after 12 weeks of vaccination, 1 male showed two fold increase in titre.

In **Kanniseriputhur Block PHC** 3 males showed 1 in 20, only in the sample collected after 12 weeks of vaccination, 2 males showed 1 in 80, only in the sample collected after 12 weeks of vaccination, 2 males showed four fold decrease in titre, 2 females showed 1 in 40, only in the sample collected after 12 weeks of vaccination, 2 males showed 2 fold decrease in titre, 1 male who had no titre in pre vaccinated, and the sample collected after 4 weeks of vaccination also showed no titre in the sample collected after 12 weeks of vaccination.

In **Mallanginar Block PHC** 3 females showed four fold decrease in titre, 2 males showed two fold decrease in titre, 2 females showed a titre 1 in 40, only in the sample collected after 12 weeks of vaccination, 1 female who had 1 in 160 in prevaccinated sample, 1 in 2560 in the sample collected after 4 weeks of vaccination, showed a decrease in titre 1 in 160, 2 females showed two fold decrease in titre, 2 females showed 1 in 160 only in the sample collected after 12 weeks of vaccination.

This is given in Table-9

## TABLE – 9

## LOCALE & SEX WISE DISTRIBUTION OF HI TITRE 12 WEEKS AFTER VACCINATION

| Area | No. of samples Tested | HI TITRE | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nil | | 1 : 20 | | 1 : 40 | | 1 : 80 | | 1:160 | | 1:320 | | 1:640 | | 1:1280 | | 1:2560 | | Total positives | |
| | | M | F | M | F | M | F | M | F | M | F | M | F | M | F | M | F | M | F | M | F |
| Narikudi | 12 | - | - | 2 20% | - | 4 40% | - | - | 2 100% | 2 20% | - | 2 20% | - | - | - | - | - | - | - | 10 100% | 2 100% |
| Reddiapatti | 12 | - | - | - | - | 2 50% | - | - | - | - | - | 2 50% | 2 25% | - | 6 75% | - | - | - | - | 4 100% | 8 100% |
| Pandalgudi | 12 | - | - | - | - | - | 2 50% | 4 50% | 2 50% | 2 25% | - | - | - | 2 25% | - | - | - | - | - | 8 100% | 4 100% |
| K.S.puthur | 12 | 1 10% | - | 3 30% | - | 2 20% | 2 50% | 2 20% | - | 2 20% | - | - | - | - | - | - | - | - | - | 9 90% | 2 100% |
| Mallanginar | 11 | - | - | - | - | 2 50% | 2 28.6% | - | 2 28.6% | 2 50% | 2 28.6% | - | 1 14.3% | - | - | - | - | - | - | 4 100% | 7 100% |

**Among the 24 samples, who had HI titre in the sample collected after 4 weeks of vaccination, 14 showed a decrease in titre in the sample collected after 12 weeks of vaccination 7 showed a increase in titre, 3 showed no change in titre after 12 weeks of vaccination. 34 childrens who had no HI titre in the sample collected after 4 weeks of vaccination also showed HI titre in the range of 1 in 20 to 1 in 640. A male from Kanniseriputhur showed no titre in all 3 samples. Thus there is evidence that antibody titre increased in all the samples except one from Kanniseriputhur.**

The samples which showed antibody titre by HI were further confirmed with Mouse Neutralisation Test. Out of 59 samples inoculated in mice **all the samples except the one from Kanniseriputhur showed Neutralizing antibodies, thus confirming the presence of JE antibody in these samples. The mice survival ratio was less than 30% in the sample from Kanniseriputhur.**

This is given in Table 10

**TABLE – 10**

**NEUTRALIZING ANTIOBODIES VS MNT OF THE SAMPLES COLLECTED AFTER 12 WEEKS OF VACCINATION**

| **Area** | **No. of Sample Tested** | **Mice Survival ratio** | | |
|---|---|---|---|---|
| | | **70-100%** | **31-69%** | **< 30%** |
| Narikudi | 12 | 12 + | - | - |
| Reddiapatti | 12 | 12 + | - | - |
| Pandalgudi | 12 | 12 + | - | - |
| Kanniseripudur | 12 | 11 + | - | 1+ |
| Mallanginar | 11 | 11 + | - | - |
| **Total** | 59 | 58 + | - | 1+ |

Thus the efficacy of the vaccine was proved by the seroconversion in the samples collected in all 5 Block PHCs at Virudhunagar by both HI and MNT.

The efficacy of the vaccine was further confirmed by the case study also. The number of cases & deaths reported in the year 2006-07 before vaccination were compared with the number of cases & deaths in the year 2007-08 and it was found that there were 3 cases and 1 death from September 2006 to August 2007 in the age group of 1-15 years at Virudhunagar District,. whereas there was no case and no deaths reported from September 2007 to August 2008 in Virudhunagar District after

vaccination showing that this vaccine has protected the population of 1-15 years in this district with marked rise in antibody titre in the vaccinated individuals.

This is given in Table 11

**TABLE – 11**

**YEAR WISE COMPARISON OF CASE FATALITY RATE**

| | **From September 2006 to August 2007** | | **From September 2007 to August 2008** | |
|---|---|---|---|---|
| | **No. of samples with Acute encephalitis manifestations** | **No. of positives %** | **No. of samples with Acute encephalitis manifestations** | **No. of positives %** |
| Cases | 6 | 3**(50%)** | 9 | - |
| Deaths | 6 | 1 (16.67%) | 9 | - |

Further evaluation of safety of this JE SA 14-14-2 vaccine was analysed by noting the local reaction and systemic toxicity if any, which occured after the vaccination and it was shown that swelling was present in 1 child (1.67%) in Narikudi, headache was present in 1 child (1.67%) in Pandalgudi. **No cases of encephalitis / meningitis, seizure were detected in any Block**.

This is given in Table 12

**TABLE – 12**

**REPORTED SIDE EFFECTS OF LIVE ATTENUATED JE SA 14-14-2 VACCINE**

| Area | No. of children | Local reaction | Systemic toxicity | Encephalitis |
|---|---|---|---|---|
| Narikudi | 12 | *1 (1.67%) | - | - |
| Reddiapatti | 12 | - | - | - |
| Pandalgudi | 12 | - | **1 (1.67%) | - |
| Kanniseripudur | 12 | - | - | - |
| Mallanginar | 12 | - | - | - |
| **Total** | 60 | 1 (1.67%) | 1 (1.67%) | - |

* Swelling present.

** Headache present.

Thus it was proved to be safe for the age group 6-10 years in all the block PHCs at Virudhunagar in all the vaccinated children.

# DISCUSSION

**The study on Efficacy and Safety of SA 14-14-2 vaccine against Japanese Encephalitis virus at Virudhunagar District** was conducted in 5 Block areas from October 2007 to December 2007.

As the population selected in the study was only the **school going children, the age group involved in the study was from 6-10 years**. Similar study by **C.V.R. Mohan Rao et al 1992** also involved school going children of this age group for the study of efficacy of JE vaccination at South Arcot District in 1992. As the ratio of school going males exceeded females in this District, the sample collection was also more in the males in this study.

In this study, it was shown that **3.33% subclinical cases were present in the population** with IgM antibody in prevaccinated samples. Similar findings were also found in the study by **C.V.R. Mohan Rao et al** which showed 42.48% subclinical cases in South Arcot District. In Tamil Nadu, Virudhunagar District, Vilupuram, Thiruvarur, Thanjavur and Perambalur Districts have already proved to have JE cases and deaths reported from 1991. Similar study by **A.Gajanana et al 1989** showed that in the non transmission season there may be considerable decline in seropositivity of general population. The samples in this study were collected before the transmission season and this may be the reason for

the decline in the number of subclinical cases.

In this study, it was found that **40.68% sero converted after 4 weeks of vaccination** and **98.3% seroconverted after 12 weeks of vaccination**. A similar study by **Young Mo Sohn et al 1998** showed that 16% seroconversion was seen in their vaccinees after 4 weeks of vaccination. **Indian Paediatrics 2001** reported that IgM antibodies after JE infection arises by $3^{rd}$ day of onset and maximum at 7th day and last upto 3 months, but the studies on immunization by **Theodar et al** showed that 94 – 100% seroconversion can be expected only after 2 primary doses of SA 14-14-2 vaccine at intervals of 1 month and 2.5 month. In this study also eventhough only one dose was given, the seroconversion was 98.3% after 12 weeks. Thus there is variation in seroconversion after viral infection and immunisation. The viral vaccines are prepared by serial cell culture passages and neuro attenuation, hence the nucleotide sequence of the vaccine gets varied. So the immune response in the disease may not be similar to that after vaccination.

In this study, it was found that **Narikudi Block PHC was the only one where there was no seroconversion after 4 weeks**. The article by **Theodar F Tsai** showed that lower seroconversion rates were mainly due to the vaccine dilutions. In this vaccine, the expected infectious titre, for the minimal standard of vaccine infectivity should not be less than $10^{6.7}$

$TCID_{50}$ per ml. As Narikudi is the only block which showed no seroconversion after 4 weeks, it is obvious that there might be some technical deficiencies in vaccine dilutions.

After 12 weeks, it was noted that 7 children showed marked increase in titre, whereas 14 showed decrease in titre and 3 showed no change in titre. It was already shown in **studies in Asia** that the persistence of vaccine-derived immunity are complicated by natural infections with dengue, West Nile virus, or other flaviviruses and reexposure to JE virus itself, all of which act to reinforce and broaden vaccine-derived immunity to JE virus.[101,102,122-124,153]. Perhaps this might have occured in this population also because this area is also endemic for Dengue and Westnile and other flavi viruses.

In this study it was shown that **one child had no seroconversion at all**. It is already shown by **Mohammed Zeeshan et al** that failure of immune response in their vaccination studies was due to nutritional status, site of administration of vaccine and genetic factors. These factors might be the reason for no immune response in one child in Kanniseriputhur.

In this study, seroconversion was **diagnosed by identifying both HI antibodies and Neutralizing antibodies.** Thus these 2 tests were used to study the efficacy of the vaccine. This demonstration of HI

antibodies for seroconversion was already done by **C.V.R. Mohan Rao et al** and also proved by **Gajanana et al** that HI has several advantages making it particularly appropriate for subclinical infections as well as post infection phase. It is a sensitive accurate indicator needing only a small quantity of serum or plasma and also HI antibodies develop faster than the neutralizing antibodies and previously infected person may show measurable antibody responses. In this study also HI technique detected subclinical cases and post vaccinated cases in different periods that is after 4 weeks and 12 weeks.

The serum antibody determination by mouse neutralization test after JE vaccine was detected by **C.V.R. Mohan Rao et al** in a study at South Arcot District and proved mouse survival ratio between 70-100% as positive and below 30 as negative. In this study it was shown that there was only one sample which showed survival rate below 30% giving negative neutralization test. Thus in this study, there was only one sample which was negative for both HI and neutralizing antibodies. All the other samples showed HI titres varying from 1 in 80 to 1 in 2560 and mice survival rate between 70-100%. Thus the study of seroconversion by 2 methodologies confirm the presence of serum antibodies for JE virus.

In this study, the **efficacy of the vaccine was proved by**

**comparing** the fatality rate of AES cases in 2007 prior to vaccination and in 2008 , after vaccination. It was found that prior to vaccination, Virudhunagar reported 3 JE positive cases and one death due to JE in 2007 and there were no JE positive cases and deaths due to JE in 2008. Thus JE vaccine is proved to be highly efficient in preventing JE virus. A similar study by **Bista M.R. et al** also showed that JE vaccine was highly efficient in preventing JE when administered before exposure to virus. His study with 20 children confirmed the present study. Similar study by **Konishi E et al 2002** also showed and evaluated the protective capacity of the approved inactivated JE vaccine by comparing the ratio with those reported for unvaccinated population.

**The safety of the vaccination was evaluated by observing the local reaction and systemic toxicity .** It was shown that except for one case with swelling, and one with mild headache no other symptoms were observed, proving the safety of the vaccine. Similar study by **Ma F B, Zeng L et al** revealed that 1.6% local reaction alone was seen in the vaccinees and the vaccine was proved to be effective and safe. Similarly **KuZuhara S et al 2003** also demonstrated that the advanced reactions, headache and malaise were mild occuring at a rate of 6.7% proving that this vaccine was safe and effective.

# SUMMARY

"**The study on Efficacy and Safety of SA-14-14-2 vaccine against Japanese Encephalitis Virus at Virudhunagar District**" revealed the following.

- There were sublclinical cases in the area selected for the vaccine study which was demonstrated by the presence of IgM antibodies to JE virus.
- After 4 weeks of vaccination, 40.68% vaccinated children seroconverted and after 12 weeks of vaccination 98.3% seroconverted.
- The efficacy of the vaccine was proved by the seroconversion in both males and females equally and increase in titre in all the samples except one at Kanniseriputhur. This child was a malnourished child.
- In Narikudi Block PHC there was slow seroconversion probably due to improper vaccine dilutions.
- The two confirmatory tests used for the seroconversion, were HI test and MNT. The efficacy of the vaccine was also proved by case study. There was no JE case / deaths after the vaccination in the study area.
- The safety of the vaccine was evaluated by observing systemic toxicity and local reaction which proved to be of negligence rate in the vaccinated children, hence the safety of the vaccine was confirmed.

# CONCLUSION

**The study on "Efficacy and Safety of SA 14-14-2 vaccine against Japanese Encephalitis Virus at Virudhunagar District"** showed

- There was subclinical cases (3.33%) in the study population at Virudhunagar District.
- 98.3% samples showed seroconversion after 12 weeks of vaccination equally in both sexes.
- Only one sample from a malnourished child at Kanniseriputhur showed no seroconversion.
- Narikudi Block PHC showed slow seroconversion.
- The eifficacy of the vaccine SA-14-14-2 was confirmed by the seroconversion methods. (HI & MNT) and also case study.
- The absence of systemic toxicity & local reactions proved the safety of the vaccine.

# ANNEXURE - I

## CONSENT FORM FOR SERUM COLLECTION FROM CHILDREN FOR JE ANTIBODY DETERMINATION

Name :

Address:

Age: Date of birth:

Sex:

Vaccination histry: Vaccination under EIP covered or not:

Whether previously immunized for JE, if so, any complications if any:

Whether willing to collect blood samples:

Whether agree to accept any minor complications like fever:

(to explain about the vaccination procedure, complications and follow up in the local language )

Whether accept for all explanations : YES/NO

Signature of blood collector Signature of parent/ guardian

## ANNEXURE - II

Ref. No.15950 / E1 (4) /2007

**ETHICAL CLEARANCE CERTIFICATE**

DR.S.VIJAYALAKSHMI.M.D., ( Pharmacology ), Dean i/c & Chairman Animal Ethical committee, Madurai Medical College, Madurai, hereby endorse ethical clearance to the proposal.

**"THE STUDY OF JE VACCINE POTENCY IN THE IMMUNISED CHILDREN OF VIRUDHUNAGAR"**

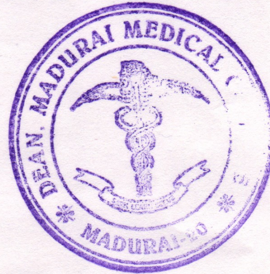
Submitted by

Dr.G.Vazhavandal,
Post graduate student,
Institute of Microbiology,
Madurai Medical College,
Madurai.

The study did not violate the regulations and guidelines prescribed by ICMR and are within the permitted norms of animal experimentation in this country. The out come of the study may be beneficial to the human and animals.

Date : 6.5.2008
Place: Madurai

Office seal:




Dean i/c & Chairman

## ANNEXURE - III

# PREPARATION OF REAGENTS

**I. Borate Saline pH 9.0**

| | | |
|---|---|---|
| 1.5M NaCl | - | 160 ml |
| 0.5M $H_3BO_3$ | - | 200 ml |
| 1N NaOH | - | 47 ml |

Add double D.W. to make 2 litre, pH 9.0 (if pH is not 9.0 then add 0.5M $H_3BO_3$or IN NaOH to adjust)

**II. 0.4% percent Bovine Albumin in Borate Saline (BABS) pH 9.0**

| | | |
|---|---|---|
| Bovine Albumin | - | 4.0 gm |
| Borate Saline pH 9 | - | 100 ml |

Keep overnight in cold and adjust pH to 9.0 with 1M NaOH. Take 100 ml of this solution and add to 900 ml of borate saline solution, pH 9.0.

**III. 25% Percent Kaolin in Borate Saline pH 9.0**

| | | |
|---|---|---|
| Kaolin (acid washed) | - | 25 gm |
| Borate Saline pH 9.0 | - | 100 ml |

Stir Borate Saline on magnetic stirrer and add Kaolin powder slowly till uniform suspension is obtained.

**IV. 0.9% % NaCl**

| | | |
|---|---|---|
| NaCl | - | 9 gm |
| Double distilled water (DDW) | - | 1000 ml |

Autoclave at 15 Ibs for 15 minutes.

**V. Acid Citrate Dextrose (ACD)**

| | | |
|---|---|---|
| Sodium Citrate | - | 11.26 gm |
| Citric Acid | - | 4.0 gm |
| Dextrose | - | 11.0 gm |
| DDW | - | 500 ml |

Autoclave at 15 Ibs for 10 minutes.

**VI. Dextrose Gelatin Veronal**

| | | |
|---|---|---|
| A. Veronal (Diethyl barbituric acid) | - | 0.580 gm |
| Gelatin | - | 0.600 gm |
| Hot D.D.W. | - | 250 ml |
| B. Sodium Barbitone | - | 0.380 gm |
| $MgSO_4 7H_2O$ | - | 0.120 gm |
| $CaCl_2$ (anhydrous) | - | 0.020 gm |
| NaCl | - | 8.5 gm |
| Dextrose | - | 10.0 gm |
| Merthiolote | - | 0.1 gm |
| D.D.W | - | 750 ml |

Mix A and B solutions. Autoclave at 10 Ibs for 10 minutes.

## VII. METHOD FOR PREPARATION OF PHOSPHATE BUFFER SALINE(PBS).

**I. PBS(10x)**

| | | |
|---|---|---|
| Nacl | - | 80.0 gms |
| Kcl | - | 2.0 gms |
| Na2H PO4 | - | 11.5 gms |
| KH2PO4 | - | 2.0 gms |
| H2O to make | - | 1000 ml. |

**Store at $4^0$c**

**II Mgcl2.6H2O (100x)**

| | | |
|---|---|---|
| Mgcl2.6H2O | - | 1.0 gms . |
| H2O | - | 100 ml |

Autoclave at 15 Ibs for 15 minutes .Store at $4^0$c

**III Cacl2.2H2O(100x)**

| | | |
|---|---|---|
| Cacl2.2H2O | - | 1.8 gms . |
| H2O | - | 100.0 ml . |

PBS(Dilute) for actual use

| | | |
|---|---|---|
| A | PBS(10x) | - 100.0 ml |
| | H2O | - 880.0 ml |

Autoclave at 15 Ibs for 15 minutes.Wait until cool and then add (b) and (c) as mentioned below.

| | | | |
|---|---|---|---|
| (b) Mgcl2.6H2O(100x) | II | - | 10.0 ml. |
| (C) Cacl2.2H2O(100x) | III | - | 10.0 ml . |

Store at $5^0$c.

# BIBLIOGRAPHY

1. Igarashi A. Epidemiology and control of Japanese encephalitis. World Health Stat Q 45 (2-3): 299-305, 1992.
2. Burke DS, Leake CJ. Japanese encephalitis. In Monath TP (ed). The Arboviruses: Epidemiology and Ecology. Vol.3. Boca Raton, FL. CRC Press, 1988, pp 63-92.
3. Umenai T, Krzysko R, Bektimirov TA, Assaad FA. Japanese encephalitis: Current worldwide status. Bull World Health Organ 63:625-631, 1985.
4. Hiroyama T. Epidemiology of Japanese encephalitis (in Japanese) Saishin-Igaku 17:1272-1280, 1962.
5. Kuttner AG, T'sun T. Encephalitis in north China. Results obtained with neutralization tests. J Clin Invest 15:525-530, 1936.
6. Sohn YM. Control of Japanese encephalitis through immunization in Korea: Past, present and future. In preparation; Seoul, Korea; 1997.
7. Yu YX. Japanese encephalitis in China. Southeast Asian J Trop Med Public Health 26S3:17-21, 1995.
8. Carey DE, Myers RM, Reuben R, Webb JKG. Japanese encephalitis in South India. A summary of recent knowledge. J Indian Med

Assoc 52:10-15, 1969.

9. Banerjee K. Japanese encephalitis in India (a country report). Proceedings of a regional workshop on control strategies for Japanese encephalitis; Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand; October 4-6, 1994.
10. Service MW. Agricultural development and arthropod-borne disease-a review. Revista Saude Publica 25:165-178, 1991.
11. Halstead SB, Grosz, CR. Subclinical Japanese encephalitis. I. Infection of Americans with limited residence in Korea. Am J Hyg 75:190-201, 1962.
12. Gajanana A, Thenmozhi V, Samuel P, Reuben R. A community based study of subclinical flavivirus infections in children in an area of Tamil Nadu, India, where Japanese encephalitis is endemic. Bull World Health Organ 73: 237-244, 1995.
13. Innis B. Japanese encephalitis. In porterfield J (ed). Exotic Viral Infections. Oxford, Chapman & Hall, 1995.
14. Kalayanarooj S. Japanese encephalitis: Clinical manifestations, outcome and management. Southeast Asian J Trop Med Public Health 26S3:9-10, 1995.
15. Lincoln AF, Sivertson SE. Acute phase of Japanese B encephalitis. Two hundred and one cases in American soldiers, Korea, 1950.

JAMA 150 : 268-273, 1952.

16. Benenson MW, Top FH, Gresso W, et al. The virulence of Japanese B encephalitis virus in Thailand. Am J Trop Med Hyg 24:974-980, 1975.

17. Bu'lock FA. Japanese B virus encephalitis in India – a growing problem. Q J Med 233:825-836, 1986.

18. Misra UK, Kalita J. Movement disorders in Japanese encephalitis. J Neurol 244:299-3030, 1997.

19. Kamala CS, Venkatwshwara Rao M, George S, Prasanna NY. Japanese encephalitis in children in Bellary Karnataka. Indian Pediatr 26:445-452, 1989.

20. Rathi AK, Kushwaha KP, Singh YD, et al. JE virus encephalitis: 1988 epidemic at Gorakhpur. Indian Pediatr 30:325-333, 1993.

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

22. Kumar R, Mathur A, Kumar A, et al. Clinical features and prognostic indicators of Japanese encephalitis in children in Lucknow (India). Indian J Med Res 91:321-327, 1990.

23. Johnson RT, Burke DS, Elwell M, et al. Japanese encephalitis : Immunocytochemical studies of viralantigen and inflammatory cells in fatal cases. Ann Neurol 18:567-573, 1985.

24. Burke DS, LorsomrudeeW, Leake CJ, et al. Fatal outcome in Japanese encephalitis. Am J Trop Med Hyg 34:1203-1210, 1985.

25. Burke DS, Nisalak A, Ussery MA, et al. Kinetics of IgM and IgG responses to Japanese encephalitis virus in human serum and cerebrospinal fluid. J Infect Dis 151:1093-1099, 1985.

26. Bhonde RR, Wagh UV. Susceptibility of human placenta to Japanese encephalitis virus in vitro. Indian J Med Res 82:371-373, 1985.

27. Ogata A, Nagashima K, Hall WW, et al. Japanese encephalitis virus neurotropism is dependent on the degree of neuronal maturity. J Virol 65:880-886, 1991.

28. Kimura-Kuroda J, Ichikawa M, Ogata A, et al. Specific tropism of Japanese encephalitis virus for developing neurons in primary rat brain culture. Arch Virol 130:477-484, 1993.

29. Kuno G, Chang G-JJ, Tsuchiya KR, et al. Phylogeny of the genus Flavivirus. J Virol 72:73-83, 1998.

30. Chambers TJ, Hahn CS, Galler R, Rice CM. Flaviviruses genome organization, expression and replication. Annu Rev Microbiol 44:649-688, 1990.

31. Murphy PA. Togavirus morphology and morphogenesis. In

Schlesinger RW (ed). The Togaviruses: Biology, Structure and Replication. New York, Academic Press, 1980, pp 241-316.

32. Sumiyoshi H, Mori C, Fuke I, et al. Complete nucleotide sequence of the Japanese encephalitis virus genome RNA. Virology 161:497-510, 1987.
33. Nitayaphan S, Grant JA, Chang GJ, Trent DW. Nucleotide sequence of the virulent SA-14 strain of Japanese encephalitis virus and its attenuated vaccine derivative, SA-14-14-2. Virology 177:541-552, 1990.
34. Gollins SW, Porterfield JS. Flavivirus infection enhanement in macrophages: An electron microscopic study of viral cellular entry. J Gen Virol 66:1969-1982, 1985.
35. Hase T, Summers PL, Dubois DR. Ultrastructural changes of mouse brain neurons infected with Japanese encephalitis virus. Int J Exp Pathol 71:493-505, 1990.
36. Ng ML, Hong SS. Flavivirus infection: Essential ultrastructural changes and association of Kunjin virus NS3 protein with microtubules. Arch Virol 106:103-120, 1989.
37. Konishi E, Mason P.Proper maturation of the Japanese encephalitis virus envelope glycoprotein requires cosynthesis with the premembrane protein. J Virol 67:1672-1675, 1993.

38. Calisher CH, Karabatsos N, Dalrymple JM, et al. Antigenic relationships among flaviviruses as determined by cross-neutralization tests with polyclonal antisera. J Gen Virol 70:37-43, 1989.

39. Porterfield JS. The flaviviruses (group B arboviruses): A crossneutralization study. J Gen Virol 23:91-96, 1974.

40. Hase T, Summers PL, Ray P. Entry and replication of Japanese encephalitis virus in cultured neurogenic cells. J Virol Methods 30:205-214, 1990.

41. Dropulic B, Masters Cl. Entry of neurotropic arboviruses into the central nervous system: An in vitro study using mouse brain endothelium. J INfect Dis 161:685-691, 1990.

42. Johnson RT, Intralawan P, Puapanwatton S. Japanese encephalitis: Identification of inflammatory cells in cerebrospinal fluid. Ann Neurol 20:691-695, 1986.

43. Sather GE, Hammon WM. Protection against St. Louis encephalitis and West Nile arboviruses by previous dengue virus (types 1-4) infection. Proc Soc Exp Biol Med 135:573-578, 1970.

44. Tarr GC, Hammon WM. Cross-protection between group B arboviruses: Resistance in mice to Japanese B encephalitis and St. Louis encephalitis viruses induced by dengue virus immunization. Infect Immunol 9:909-915, 1974.

45. Edelman R, Nisalak A, Pariyanonda A, et al. Immunoglobulin response and viremia in dengue-vaccinated gibbons repeatedly challenged with Japanese encephalitis virus. Am J Epidemiol 97:208-218, 1973.

46. Goverdhan MK, Kulkarni AB, Gupta AK, et al. Two-way cross protection between West Nile and Japanese encephalitis viruses in bonnet Macaques. Acta Virol 36:277-283, 1992.

47. Jia LL, Zheng Z, Yu YX. Study on the immune mechanism of JE attenuated live vaccine (SA-14-14-2 strain). Chin J Immunol Microbiol 12:366-367, 1992.

48. Nathanson N, Cole GA. Fatal Japanese encephalitis virus infectionin immunosuppressed spider monkeys. Clin Exp Immunol 6:161-166, 1970.

49. Gould EA, Buckley A. Antibody-dependent enhancement of yellow fever and Japanese encephalitis virus neurovirulence. J Gen Virol 70:1605-1608, 1989. s

50. Ghosh SN, Prasad SR, Thakare JP, et al. Evidence for synthesis of immunoglobulins within central nervous system of Japanese encephalitis cases. Indian J Med Res 86:276-283, 1987.

51. Burke DS, Nisalak A, Lorsomrudee W, et al. Virus-specific antibody-producing cells in blood and cerebrospinal fluid in acute Japanese encephalitis. J Med Virol 17:283-292, 1985.

52. Konishi E, Kurane I, Mason PW, et al. Japanese encephalitis virus-specific proliferative responses of human peripheral blood T lymphocytes. Am J Trop Med Hyg 53:278-283, 1995.

53. Desai A, Ravi V, Chandramuki A, Gourie-Devi M. Proliferative response of human peripheral blood mononuclear cells to Japanese encephalitis virus. Microbiol Immunol 39:269-273, 1995.

54. Desai A, Ravi V, Guru SC, et al. Detection of autoantibodies to neural antigens in the CSF of Japanese encephalitis patients and correlation of findings with the outcome. J Neurol Sci 122:109-116, 1994.

55. Desai A, Ravi V, Chandremuki A, Gourie-Devi M. Detection of immune complexes in the CSF of Japanese encephalitis patients: Correlation of findings with outcome. Intervirology 37:352-355, 1994.

56. Lad VJ, Gupta AK, Goverdhan MK, et al. Susceptibility of BL6 nude (congenitally athymic) mice to Japanese encephalitis virus by the peripheral route. Acta Virol 37:232-240, 1993.

57. Murali-Krishna K, Ravi V, Manjunath R. Protection of adult but not newborn mice against lethal intracerebral challenge with Japanese encephalitis virus by adoptively transferred virus-specific cytotoxic T lymphocytes: Requirement for $L3T4^{+}$ cells. J Gen Virol 77:705-714, 1996.

58. Yu YX, Wang JF, Zheng GM, Li HM. Response of normal and athymic mice to infection by virulent and attenuated Japanese encephalitis viruses. Chin J Virol 1:203-209, 1985.

59. Jia LL, Zheng Z, Yu YX. Pathogenicity and immunogenicity of attenuated Japanese encephalitis vaccine (SA14-14-2) in immune inhibited mice. Virol Sinica 8:20-24, 1993.

60. Jia LL, Zheng Z, Yu YX. Study on the immune mechanism of JE attenuated live vaccine (SA14-14-2 strain). Chin J Immunol Microbiol 12:364-366, 1992.

61. Brooks TJ, Lukaszewski, Phillpotts R. Cytokine prophylaxis for St. Louis encephalitis virus infection in mice (abstract G-15). 37th Interscience Conference on Antimicrobial Agents and Chemotherapy; Toronto; Sept. 28-Oct. 1, 1997.

62. Lin Y, Huang Y, Ma S, et al. Inhibition of Japanese encephalitis virus infection by nitric oxide: Antiviral effect of nitric oxide on RNA virus replication. J Virol 71:5227-5235, 1997.
63. Kreil T, Eibl MM. Nitric oxide and viral infection: No antiviral activity against a flavivirus in vitro, and evidence for contribution to pathogenesis in experimental infection in vivo. Virology 219:304-306, 1996.
64. Khanna N, Mathur A, Chaturvedi UC. Regulation of vascular permeability by macrophage-derived chemotactic factor produced in Japanese encephalitis. Immunol Cell Biol 72:200-204, 1994.
65. Liu YF, Teng CL, Liu K. Cerebral cysticercosis as a factor aggravating Japanese B encephalitis. Chin Med J 75:1010-1017, 1957.
66. Das SK, Nityanand S, Sood K. Japanese B encephalitis with neurocysticercosis. J Assoc Physicians India 39:643-644, 1991.
67. Kedarnath N, Prasad SR, Dandawate CN, et al.Isolation of Japanese encephalitis and West Nile viruses from peripheral bloodof encephalitis patients. Indian J Med Res 79:1-7, 1984.
68. Gajanana A, Samuel PP, Thenmozhi V, Rajendran R. An appraisal of some recent diagnostic assays for Japanese encephalitis. Southeast Asian J Trop Med Public Health 27:673-679, 1996.

69. Innis BL, Nisalak A, Nimmannitya S, et al. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis cocirculate. Am J Trop Med Hyg 40:418-427, 1989.

70. Konishi E, Mason PW, Shope RE. Enzyme-linked immunosorbent assay using recombinant antigens for serodiagnosis of Japanese encephalitis. J Med Virol 48:76-79, 1996.

71. Rosen L. The natural history of Japanese encephalitis virus. Annu Rev Microbiol 40:395-414, 1986.

72. Scherer WF, Buescher EL. Ecologic studies of Japanese encephalitis virus in Japan. I. Introduction. Am J Trop Med Hyg 8:644-650, 1959.

73. Gould DJ, Byrne RJ, Hayes DE. Experimental infection of horses with Japanese encephalitis virus by mosquito bite. Am J Trop Med Hyg 13:742-746, 1964.

74. Wang YJ, Gu PW, Liu PS. Japanese B encephalitis virus infection of horses during the first epidemic season following entry into an infected area. Chin Med J 95:63-66, 1982.

75. Takahashi K, Matsuo R, Kuma M, et al. Use of vaccine in pigs. A. Effect of immunization of swine upon the ecological cycle of Japanese encephalitis virus. In Hammon WM, Kitaoka M, Downs

WG (eds). Immunization for Japanese Encephalitis. Amsterdam, Excerpta Medica, 1972, pp. 292-303.

76. Sasaki O, Karoji Y, Kuroda A, et al. Protection of pigs against mosquito-borne Japanese encephalitis virus by immunization with a live attenuated vaccine. Antiviral Res 2:355-360, 1982.
77. Vaughn DW, Hoke CH. The epidemiology of Japanese encephalitis: Prospects for prevention. Epidemiol Rev 14:197-221, 1992.
78. Gajanana A, Rajendran R, Samuel PP, et al. Japanese encephalitis in south Arcot District, Tamil Nadu: A three year longitudinal study of vector density and vector infection frequency. J Med Entomol 34:651-659, 1997.
79. Chaudhuri N, Shaw BP, Mondal KC, Maity CR. Epidemiology of Japanese encephalitis. Indian Pediatr 297:861-865, 1992.
80. Poland JD, Cropp CB, Craven RB, Monath TP. Evaluation of the potency and safety of inactivated Japanese encephalitis vaccine in U.S. inhabitants. J Infect Dis 161:878-882, 1990.
81. Berg SW, Mitchell BS, Hanson RK, et al. Systemic reactions in US Marine Corps personnel who received Japanese encephalitis vaccine. J Infect Dis 24:265-266, 1997.

82. Gu PW, Ding ZF. Inactivated Japanese encephalitis (JE) vaccine made from Hamster cell culture (a review). JE HFRS Bull 2:15-26, 1987.

83. Oya A. Japanese encephalitis vaccine. Acta Pediatr Jpn 30:175-184, 1988.

84. Kanamitsu M, Hashimoto N, Urasawa S, et al. A field trial with an improved Japanese encephalitis vaccine in a nonendemic area of the disease. Biken J 13:313-328, 1970.

85. Shope RE. The potency test for inactivated Japanese encephalitis (JE) vaccines. JE HFRS Bull 2:27-32, 1987.

86. World Health Organization. Requirements for Japanese encephalitis vaccine (inactivated) for human use. WHO Tech Rep Series 77:1133-1156, 1988.

87. Kobayashi Y, Hasegawa H, Oyama T, et al. Antigenic analysis of Japanese encephalitis virus by using monoclonal antibodies. Infect Immunol 44:117-123, 1984.

88. Hashimoto H, Nomoto A, Watanabe K, et al. Molecular cloning and complete nucleotide sequence of the genome of Japanese encephalitis virus Beijing-1 strain. Virus Genes 1:305-317, 1988.

89. Kitano T, Yabe S, Kobayashi M, et al. Immunogenicity of JE Nakayama and Beijing-1 vaccines. JE HFRS Bull 1:37-41, 1986.

90. Kitano T. Immunogenicity and field trial of Beijing-1 vaccine. Working group on vaccine development and vaccination strategies for Japanese encephalitis, Osaka. 1-8, 1985.

91. The Research Foundation for Microbial Diseases of Osaka University. Japanese encephalitis vaccine lyophilized, "Biken." Unpublished report, 1991, pp 1-149.

92. Gambel JM, DeFraites R, Hoke C, et al. Japanese encephalitis vaccine: Persistence of antibody up to 3 years after a three-dose primary series. J Infect Dis 171:1074, 1995.

93. Henderson A. Immunization against Japanese encephalitis in Nepal: Experience of 1152 subjects. J R Army Med Corps 130:188-191, 1984.

94. Sanchez JL, Hoke CH, McCowan J, et al. Further experience with Japanese encephalitis vaccine. Lancet 335:972-973, 1990.

95. Immunization Practices Advisory Committee (ACIP). Inactivated Japanese encephalitis virus vaccine: Recommendations of the ACIP. MMWR Morb Mortal Wkly Rep 42(RR1):1-15, 1993.

96. Zhang XC, Nie SX, Din CS, Wang MJ, Observations on the efficacy of inactivated Japanese encephalitis vaccine in combination with other vaccines. Chin J Pub Health 6:203, 1990.

97. Inour YK. An attenuated mutant of Japanese encephalitis virus. Bull World Health Organ 30:181-185, 1964.

98. Yoshida I, Takagi M.Inokuma E, et al. Establishment of an attenuated ML-17 strain of Japanese encephalitis virus. Biken J 24:47-67, 1981.

99. Kodama K, Sasaki N, Inoue YK. Studies of live attenuated Japanese encephalitis vaccine in swine. J Immunol 100: 194-200, 1968.

100. Hammon WM, Darwish MA, Rhim JS, et al. Studies on Japanse B encephalitis virus vaccines from tissue culture. V. Response of man to live, attenuated strain of OCT 541 virus vaccine. J Immunol 96:518-524, 1966.

101 . Ni H, Barret A. Attenuation of Japanse encephalitis virus by selection of its mouse brain membrane receptor preparation escape variants. Virology 241:30-36, 1998.

102. Yu YX, Ao J, Chu YG, et al. Studies on mutation of JE virus V. Biological characteristics of the attenuated vaccine strain. Acta Microbiol Sinica 13:16-24, 1973.

103. Yu YX, Fang C, Wu PF, Li HM, Studies on the variation of JE virus VI. The changes in virulence and immunity after passaging subcutaneously in suckling mice. Acta Microbiol Sinica 15:133-138, 1975.

104. Yu YX, Wu PF, Ao J, et al. Selection of a better immunogenic and highly attenuated live vaccine virus strain of JE. I. Some biological characteristics of SA14-14-2 mutant. Chin J Microbiol Immunol 1:77-84, 1981.
105. Ao J, Yu Y, Tang YS, et al. Selection of a better immunogenic and highly attenuated live vaccine strain of Japanese encephalitis. II, Safety and immunogenicity of live JBE vaccine SA14-14-2 observed in inoculated children. Chin J Microbiol Immunol 3:245-248, 1983.
106. Yu YX, Ming AG, Pen GY, et al. Safety of a live –attenuated Japanese encephalitis virus vaccine (SA14-14-2) for Children. Am J Trop Med Hyg 39:214-217, 1988.
107. Wang SG, Yang HJ, Den YY, et al. Studies on the production of SA14-2 Japanese encephalitis live vaccine. Chin Virol 6:38-43, 1990.
108. Yu YX, Zhang GM, Zheng Z. Studies on the immunogenicity of live and killed Japanese encephalitis (JE) vaccines to challenge with different Japanese encephalitis virus strains. Chin J Virol 5:106-110, 1989.
109. Wills MR, Sil BK, Cao JX, et al. Antigenic characterization of the live attenuated Japanese encephalitis vaccine virus SA14-14-2: A

comparison with isolates of the virus covering a wide geographic area. Vaccine 10:861-872, 1992.

110. Sil BK, Wills MR, Cao JX, Immunogenicity of experimental live attenuated Japanese encephalitis vaccine viruses and comparison with wild- type strains using monocolonal and polyclonal antibody-ies. Vaccine 10:329-333, 1992.

111. Hase T, Dubois DR, Summers PL, et al. Comparison of replication rates and pathogenicities between the SA14 parent and SA14-14-2 vaccine strains of Japanese encephalitis virus in mouse brain neurons. Arch Virol 130:131-143, 1993.

112. Jia LL, Zhong Z, Yu YZ. Study on the stability of viral strains of live-attenuated Japanese encephalitis vaccine. Chin J Biolog 5:174-176, 1992.

113. Eckels KH, Yu XY, Dubois DR, et al. Japanese encephalitis virus live-attenuated vaccine, Chinese strain SA 14-14-2; adaptation to primary canine kidney cell cultures and preparation of a vaccine for human use, Vaccine 6:513-518, 1988

114. Regional Antiepidemic Station, Hueyang, Guangtong: Prelimi-nary observation on epidemiological effectiveness of JE live vaccine. Bull Biol Prod 7:111-114, 1978.

115. Ling JP, Zhu YG, Du GZ, et al. Comparative susceptibilities of rhesus monkeys and mice to Japanese encephalitis virus. Beijing, Institute for Control of Pharmaceutical and Biological products, unpublished report, 1996.

116. Jia LL, Zheng Z, Wang SW, Yu YZ. Protective effects and antibody responses in guinea pigs immunized with Japanese encephalitis live-attenuated vaccine SA14-14-2 after challenge with virulent JE virus. Adv Microbiol Immunol (China) 23:73-75, 1995.

117. Ni H, Chang GJ, Zie H, et al. Molecular basis of attenuation of neurovirulence of wild-type Japanese encephalitis virus strain SA14. J Gen Virol 76:409-413, 1995.

118. Ni HL, Barrett ADT. Molecular differences between wild-type Japanese encephalitis virus strains of high and low mouse neuro-virulence. J Gen Virol 77:1449-1455, 1996.

119. Indian Pediatrics 2001;38:1252-1264 Japanese encephalitis. P.Nagabhushana Rao.

120. Chen BQ, Beaty BJ. Japanese encephalitics vaccine (28 strain) and parent (SA 14 strain) viruses in Culex tritaenior byncbus mosquitoes. Am J Trop Med Hyg 31:403-407, 1982.

121. Ao J, Yu YX, Wu PF, Zhang GM. Further observations on JBE attenuated lie vaccines used for prevention of stillbirths in swine. Acta Microbiol Sinica 21:174-179, 1981.

122. Tsai TF, Yu YX, Jia LL, et al. Immunogenicity of live attenuated SA14-14-2 Japanese encephalitis vaccine –a comparison of 1-and 3 month immunization schedules. J Infect Dis 177:221-223, 1998.

123. Chambers TJ, Tsai TF, Pervivkov Y, Monath TP. Vaccine development against dengue and Japanese encephalitis: Report of a World Health Organization meeting. Vaccine 15:1494-1502, 1997.

124. Fu DW, Zhand PF. Establishment and characterization of Japanese B encephalitis virus persistent infection in the Sf9 cell line. Biologicals 24:225-233, 1996.

125. Konishi E, Win KS, Kurane I, et al. Particulate vaccine candidate for Japanese encephalitis induces long-lasting virus-specific memory T lymphocytes in mice. Vaccine 15:281-286, 1997.

126. Rojanasuphot S, Charoensook OA, Ungchusak K, et al. A field trialon inactivated mouse brain Japanese encephalitis vaccines produced in Thailand. Mosquito-Borne Dis Bull 8:11-16, 1991.

127. Wu YC. Neutralizing antibody responses to Nakayama and Beijing strain JE vaccine in children of Taipei City, 1993-4. Taipei, National Institute of Preventive Medicine, unpublished report, 1994.

128. Nimmannitya S, Hutami S, Kalayanarooj S, Rojanasuphot S. A field study on Nakayama and Beijing strains of Japanese encephalitis vaccines. Southeast Asian J Trop Med Pub Health 26:689-693, 1995.
129. Hoke CH, Nisalak A, Sangawhipa N, et al. Protection against Japanese encephalitis by inactivated vaccines. N Engl J Med 319:608-614, 1988.
130. Okuno T, Tseng PT, Hsu ST, et al. Japanese encephalitis surveillance in China (Province of Taiwan) during 1958 – 1971. II. Age specific incidence in connection with Japanese encephalitis vaccination program. Jpn J Med Sci Biol 28:255-267, 1975.
131. Gowal D, Tahlan AK, Evaluation of effectiveness of mouse brain inactivated Japanese encephalitis vaccine produced in India. Indian J Med Res 102:267-271, 1995.
132. Wang SG, Yang HJ, Den YY, et al. Improved inactivated Japanese encephalitis vaccine produced in primary hamster kidney cells. National Serum and Biologics Institute, Hebei Health Station, Funien County Health Station. Biol Prod Comm 8:283-285, 1979.
133. Ren YL, Biol Prod Com (Chinese) 7:111, 1978. Cited inHuang CH. Studies of Japanese encephalitis in China. Adv Virus Res 27:72-101, 1982.

134. Ao G, Yu YX, Wu PF, et al. Studies on mutation of Japanese B encephalitis virus. VII. An observation of persistence of immunity in children inoculated with JBE attenuated live vaccine (SA-14-5-3 mutant). Acta Microbiol Sinica 21:501-505, 1981.

135. Jia LL, Yu YX, Zheng Z. Neutralizing antibody response of Japanese encephalitis live vaccine in children residing in JE endemic area. Chin J Zoonoses 11:343-344, 1995.

136. Chengdu Biologics Institue. Report on epidemiologic results of lyophilized Japanese encephalitis vaccine. Unpublished report, 1991.

137. Wang JL, NA JC, Zhao SS, et al. An epidemiologic study of the efficacy of live Japanese encephalitis vaccine. Chin J Biolog 6:36-37, 1993.

138. Hennessy S, Liu ZL, Tsai TF, et al. Effectiveness of live-attenuated Japanese encephalitis vaccine (SA14-14-2): a case-control study. Lancet 347:1583-86, 1996.

139. Plesner AM, Ronne T. Allergic mucocutaneous reactions to Japanese encephalitis vaccine. Vaccine 15:1239-1243, 1997.

140. Ma X, Yu YX, Wang SG. Observations on safety and serological efficacy from a large-scale field trial of Japanese encephalitis vaccine. Chin J Biolog 6:188-191, 1993.

141. Liu ZL, Hennessy S, Strom BL, et al. Short-term safety of live attenuated Japanese encephalitis vaccine (SA 14-14-2): Results of a 26,239-subject randomized trial. J Infect Dis 176:1366-1369, 1997.

142. C.V.R.Mohan Rao, A.R.Risbud , C.N.Dandawate, U.B.Umarani,
V.M.Ayachit, F.M.Rodrigues , and K.M.Pavri .NIV Pune 1992 Serological response to Japanese encephalitis vaccine in agroup of school children in South Arcot District of Tamil Nadu.

143. Bista MB and Shrestha JM .Japanese encephalitis in Nepal,2000.: Analysis of 2000 data and review of literature: Epidemiology and Disease Control Division, Department of Health Services ,Ministry of Health, Kathmandu, Nepal 2001.

144. Rupinderjeet Kaur and Sudhanshu Vrati ; Development of recombinant vaccine against Japanese encephalitis :National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi, India.

145. Bista MB, Banerjee MK, Shin SH, Tanden JB, et al 2001,.Efficacy of single dose of SA-14-14-2 vaccine against Japanese encephalitis: a case control study. Lancet 358:791-795.

146. Jan JT, Chen BH, Ma SH, Liu CI, Tsai HP, Wu HC, Jiang SY, Yang KD, Shaio MF 2000.Potential dengu virus triggered apoptotic pathway in human neuroblastoma cells :arachdonic acid ,superoxide anion, and NF- Kappa B and sequentially involved . J Virol 74: 8680-8691.

147. Parquet MC ., Kumotori A, Hasebe F, et al 2002. St .Louis encephalitis induced pathology in cultured cells. Arch Virol 147:1105-1119.

148. C,.Ramakrishna, Anita Desai, S.K.Shankar, A.Chandramuki and V.Ravi Oral immunisation of mice with live Japanese encephalitis virus induces a protective immune response. Department of Neurovirology, National Institute of Mental Health and Neurosciences (NIMHANS) ,Bangalore 560029, India.