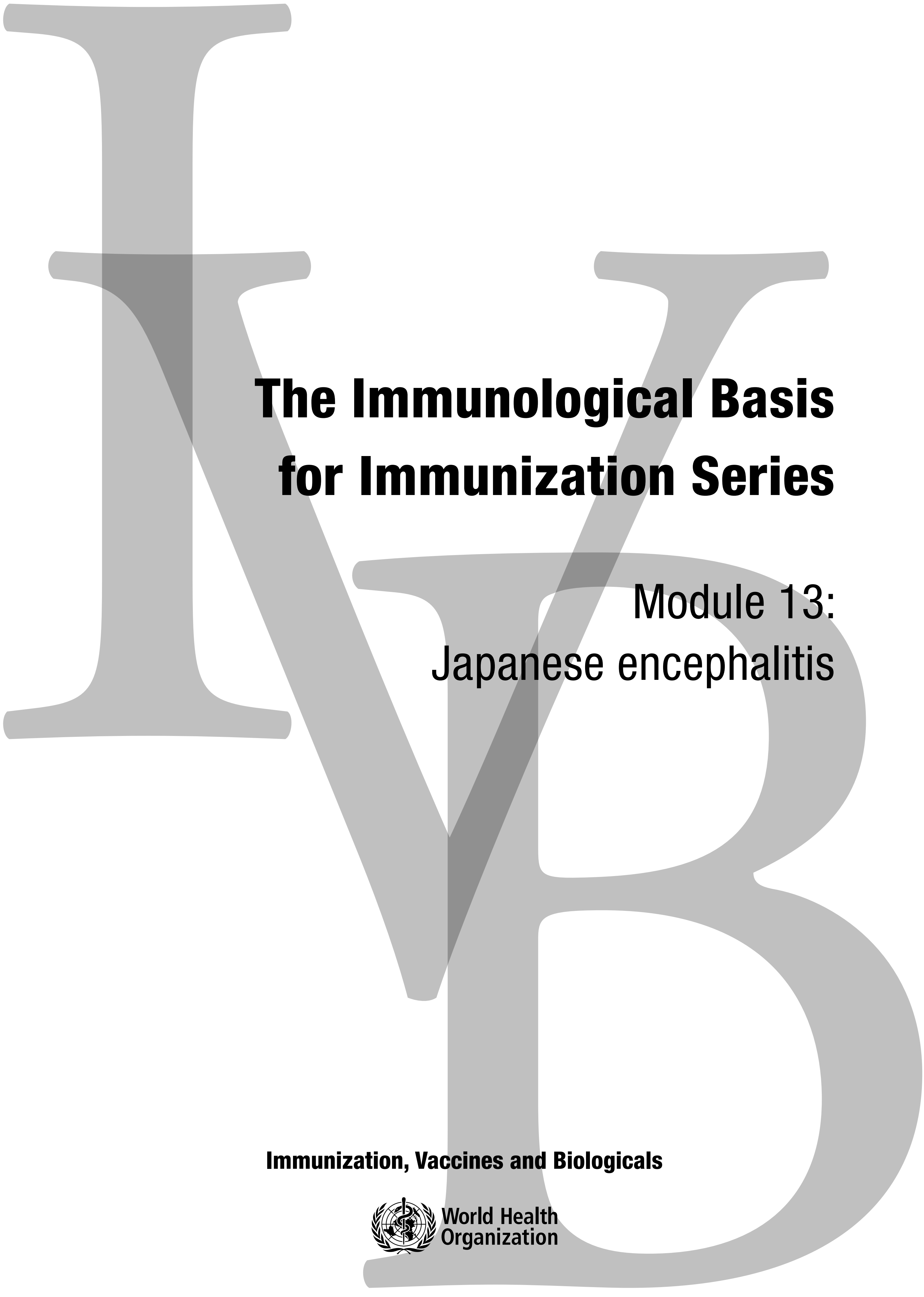
The Immunological Basis for Immunization Series

Module 13: Japanese encephalitis

Immunization, Vaccines and Biologicals

World Health Organization



The Immunological Basis for Immunization Series

Module 13: Japanese encephalitis

Immunization, Vaccines and Biologicals

World Health Organization

WHO Library Cataloguing-in-Publication Data

The immunological basis for immunization series: module 13: Japanese encephalitis virus.

(Immunological basis for immunization series ; module 13)

1.Encephalitis virus, Japanese - immunology. 2.Japanese encephalitis vaccines - therapeutic use. 3.Encephalitis, Japanese - immunology. 4.Encephalitis, Japanese - epidemiology. I.World Health Organization. II.Series.

ISBN 978 92 4 159971 9 (NLM classification: WC 542)

© World Health Organization 2010

All rights reserved. Publications of the World Health Organization can be obtained from WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel.: +41 22 791 3264; fax: +41 22 791 4857; e-mail: bookorders@who.int). Requests for permission to reproduce or translate WHO publications – whether for sale or for noncommercial distribution – should be addressed to WHO Press, at the above address (fax: +41 22 791 4806; e-mail: permissions@who.int).

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use.

The Department of Immunization, Vaccines and Biologicals thanks the donors whose unspecified financial support has made the production of this document possible.

This module was produced for Immunization, Vaccines and Biologicals, WHO, by:

Dr Janet Daly, Lecturer in Comparative Virology,
School of Veterinary Medicine and Science,
University of Nottingham, Sutton Bonington Campus,
Sutton Bonington LE12 5RD, UK
e-mail: janet.daly@nottingham.ac.uk

and

Prof Tom Solomon, Chair of Neurological Science,
Director of Institute of Infection and Global Health,
University of Liverpool, L69 3GA, UK
e-mail: tsolomon@liv.ac.uk

Printed in May 2010

Copies of this publication as well as additional materials on immunization, vaccines and biological may be requested from:

World Health Organization
Department of Immunization, Vaccines and Biologicals
CH-1211 Geneva 27, Switzerland
• *Fax:* + 41 22 791 4227 • *Email:* vaccines@who.int •

© World Health Organization 2010

All rights reserved. Publications of the World Health Organization can be obtained from WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel: +41 22 791 3264; fax: +41 22 791 4857; email: bookorders@who.int). Requests for permission to reproduce or translate WHO publications – whether for sale or for noncommercial distribution – should be addressed to WHO Press, at the above address (fax: +41 22 791 4806; email: permissions@who.int).

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use.

The named authors alone are responsible for the views expressed in this publication.

Printed by the WHO Document Production Services, Geneva, Switzerland

Contents

Abbreviations and acronyms .... *v*

Preface .... *vii*

1. **Introduction** **1**

2. **Japanese encephalitis virus structure and replication** **3**

3. **Japanese encephalitis — the disease** **5**

4. **Ecology and epidemiology** **6**

5. **The nature of immunity to Japanese encephalitis** **7**
    - *5.1 Immunity following natural infection* *7*
    - *5.2 Vaccine-induced immunity and correlates of protection* *8*

6. **Vaccines** **9**
    - *6.1 Mouse brain-derived inactivated JE vaccine* *9*
    - *6.2 Inactivated cell culture-derived vaccines* *12*
    - *6.3 Live attenuated SA14-14-2 vaccine* *13*
    - *6.4 Chimeric yellow fever–JE vaccine* *17*
    - *6.5 Poxvirus-vectored vaccines* *17*
    - *6.6 DNA vaccine* *18*

7. **Immunization strategies for Japanese encephalitis endemic countries** **19**

References **21**

Abbreviations and acronyms

ADEM	acute disseminated encephalomyelitis
AEFI	adverse events following immunization
C	capsid protein
CDC	Centers for Disease Control and Prevention (USA)
CF	complement fixation
CTL	cytotoxic T lymphocyte
DENV-2	dengue-2 virus
DNA	deoxyribonucleic acid
E	envelope protein
ELISA	enzyme-linked immunosorbent assay
EPI	Expanded Programme on Immunization
GACVS	Global Advisory Committee on Vaccine Safety (WHO)
GIVS	Global Immunization Vision and Strategy
GMT	geometric mean titre
HI	haemagglutination inhibition
IFA	indirect fluorescent antibody test
Ig	immunoglobulin
JE	Japanese encephalitis
JE-CV	chimeric yellow fever–JE (vaccine)
JEV	Japanese encephalitis virus
M	matrix protein
MLD	median lethal dose
MVA	Modified Vaccine Ankara (platform)
MVEV	Murray Valley encephalitis virus
NS	non-structural protein

P3	Peking-3 (strain)
PATH	Program for Appropriate Technology in Health
PHK	primary hamster kidney (cells)
prM	precursor of M protein
PRNT	plaque reduction neutralization test
RNA	ribonucleic acid
SLEV	Saint Louis encephalitis virus
TBEV	tick-borne encephalitis virus
UNICEF	United Nations Children's Fund
USA	United States of America
UTR	untranslated region
WHO	World Health Organization
WNV	West Nile virus
WRAIR	Walter Reed Army Institute of Research

Preface

This module is part of the series *The Immunological Basis for Immunization*, which was initially developed in 1993 as a set of eight modules focusing on the vaccines included in the Expanded Programme on Immunization (EPI)[1]. In addition to a general immunology module, each of the seven other modules covered one of the vaccines recommended as part of the EPI programme — diphtheria, measles, pertussis, polio, tetanus, tuberculosis and yellow fever. The modules have become some of the most widely used documents in the field of immunization.

With the development of the Global Immunization Vision and Strategy (GIVS) (2005–2015) (http://www.who.int/vaccines-documents/DocsPDF05/GIVS_Final_EN.pdf) and the expansion of immunization programmes in general, as well as the large accumulation of new knowledge since 1993, the decision was taken to update and extend this series.

The main purpose of the modules — which are published as separate disease/vaccine-specific modules — is to give immunization managers and vaccination professionals a brief and easily-understood overview of the scientific basis of vaccination, and also of the immunological basis for the World Health Organization (WHO) recommendations on vaccine use that, since 1998, have been published in the *Vaccine Position Papers* (http://www.who.int/immunization/documents/positionpapers_intro/en/index.html).

WHO would like to thank all the people who were involved in the development of the initial *Immunological Basis for Immunization* series, as well as those involved in its updating, and the development of new modules.

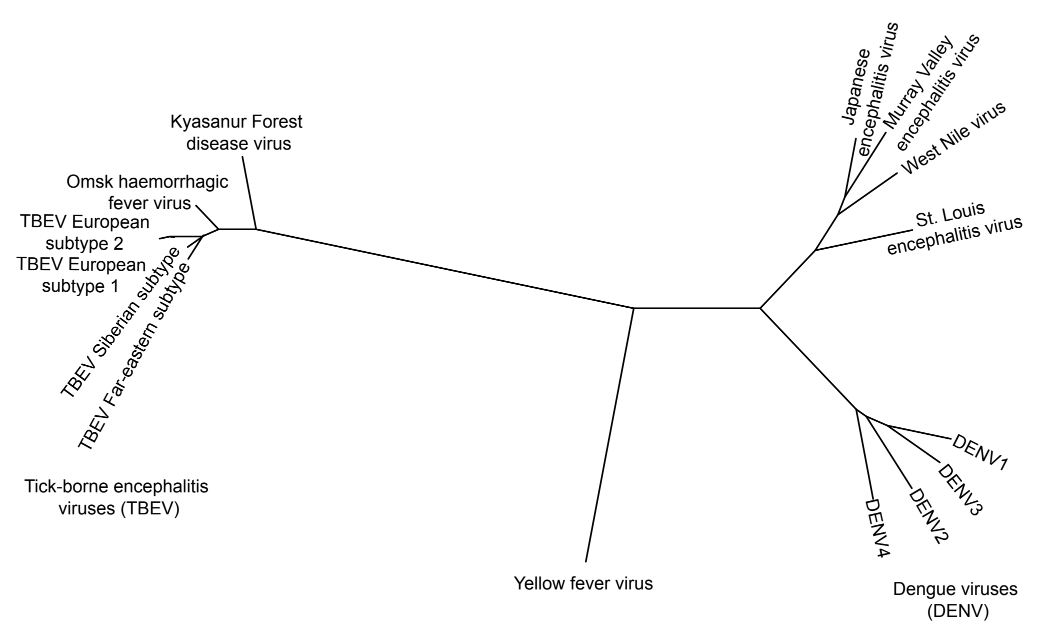
---

[1] This programme was established in 1974 with the main aim of providing immunization for children in developing countries.

1. Introduction

Japanese encephalitis (JE) is the most important recognized cause of childhood viral encephalitis in Asia, causing an estimated 50 000 clinical cases and 10 000 deaths every year (Solomon, 1997; WHO, 2008). Japanese encephalitis virus (JEV) is a mosquito-borne member of the *Flavivirus* genus in the family *Flaviviridae*. Other viruses in the same serogroup include West Nile virus (WNV), Murray Valley encephalitis virus (MVEV) and Saint Louis encephalitis virus (SLEV) (Figure 1).

Figure 1: Phylogenetic relationships of medically important flaviviruses. Phylogenetic reconstruction based on complete polyprotein sequences performed using maximum likelihood analysis (PAUP v.4.0b10). Tick- and mosquito-borne viruses are highlighted.

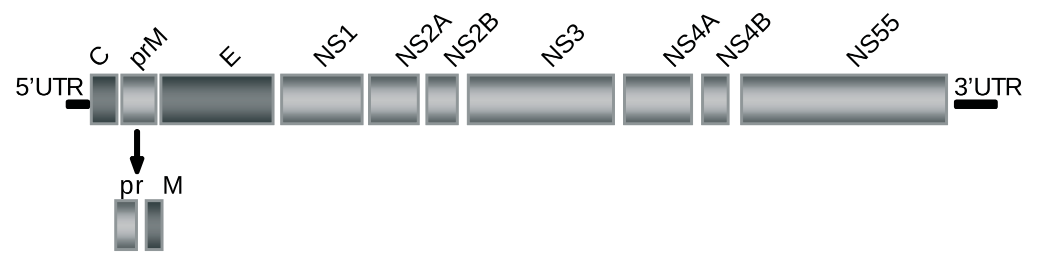

Kyasanur Forest disease virus
Omsk haemorrhagic fever virus
TBEV European subtype 2
TBEV European subtype 1
TBEV Siberian subtype
TBEV Far-eastern subtype
Tick-borne encephalitis viruses (TBEV)
Yellow fever virus
Japanese encephalitis virus
Murray Valley encephalitis virus
West Nile virus
St. Louis encephalitis virus
DENV1
DENV3
DENV2
DENV4
Dengue viruses (DENV)

Although the incidence of JE has reduced considerably in Japan, the Republic of Korea, and Taiwan, China, and to a lesser extent in China and Thailand, the distribution of epidemic JE has expanded to areas such as western India and Nepal where it has become a substantial public-health problem (Igarashi, 1992; Akiba et al., 2001; Ohrr et al., 2005; WHO, 2008). There is no specific treatment for the disease, and vector control and infection prevention in amplifying hosts are not practical in resource-poor countries (Marfin & Gubler, 2005). The World Health Organization (WHO) encourages the use of JE vaccine as the single most effective preventive measure (WHO, 2006). The high cost of the commercially available inactivated mouse brain-derived vaccine, uncertain supply and the need for multiple injections have made control of JE by vaccination difficult in many countries. More recently, other JE vaccines, in particular a live attenuated vaccine, are replacing the classical JE mouse brain-derived vaccines, bringing gains in operational and vaccine costs. Other JE vaccines, either recently licensed or in late-stage clinical development, are expected to broaden the supply base of JE vaccines.

2. Japanese encephalitis virus structure and replication

The JEV genome is a single-strand of positive-sense ribonucleic acid (RNA) almost 11 kilobases in length (Figure 2), which is associated with the highly basic capsid protein to form an isometric 30 nm diameter nucleocapsid core enclosed by a lipid membrane containing the glycosylated E and M proteins (Sumiyoshi et al., 1987; Rice, 1996).

Figure 2: Schematic of flavivirus RNA genome showing viral proteins produced by proteolytic cleavage of viral polyprotein (to scale). Structural proteins, capsid (C); envelope (E), and matrix (M) are in darker shading than non-structural (NS) proteins. Untranslated region (UTR) shown as line.



Much of what is known about the structure and replication of flaviviruses, including JEV, is derived from structural, biochemical and functional studies of tick-borne encephalitis virus (TBEV) and dengue-2 virus (DENV-2). The E protein is the largest structural protein; it is responsible for viral attachment to cellular receptors, specific membrane fusion, and elicits a protective antibody response. The E protein consists of a homodimer lying parallel to the viral surface. Each monomer consists of three domains; (i) domain I has a central beta barrel and acts as a hinge that links domains II and III; (ii) domain II is an elongated dimerisation region; (iii) domain III is a C-terminal immunoglobulin-like module (Mandl et al., 1989; Rey et al., 1995; Modis et al., 2003). It is thought that domain III, which projects from the viral surface, is responsible for the virus binding to the cellular receptor(s) (Rey et al., 1995).

Flaviviruses enter host cells by receptor-mediated endocytosis (Chambers et al., 1990) via an as-yet unidentified receptor; recent results suggest that the mechanism of entry and receptor usage of JEV is distinct from that of dengue viruses (Boonsanay & Smith, 2007). Inside the cell, the nucleocapsid is uncoated by acid-dependent fusion of the viral and endosomal membranes (Bressanelli et al., 2004). The genomic RNA is released into the cytoplasm where translation of the uncoated viral genome takes place.

The translated polyprotein is co- or post-translationally processed by a combination of virus-specific non-structural protease complex, NS2B-NS3, host cell signal peptidase and, unidentified host cell-specific protease, into the 10 viral proteins (Figure 2) and assembled into a virus-specific replication complex. Extensive proliferation of membranous organelles in the perinuclear region is seen in flavivirus-infected cells (Hase et al., 1990), and may represent virus factories in which translation, RNA synthesis and virus assembly occur. In immature virions in the cell, the glycosylated precursor of M protein (prM) is closely associated with E protein and acts as a 'chaperone' to prevent low pH-induced rearrangements of E protein, thus allowing efficient secretion and proper folding (Konishi & Mason, 1993; Heinz et al., 1994; Allison et al., 1995). Immediately before virion release, prM is cleaved by a furin-like enzyme associated with the trans-Golgi membrane, leaving only M protein associated with the mature virion (Stadler et al., 1997).

Strains of JEV can be classified into four distinct genotypes (designated I to IV) (Chen et al., 1990 and 1992; Uchil & Satchidanandam, 2001*)*. The extent of variation between genotypes is limited, and cross-reactivity between representatives of different genotypes is observed in serological assays, resulting in the classification of JEV as a single serotype (Tsarev et al., 2000). A comprehensive study of the evolution of E genes of JEV demonstrated that all four genotypes circulated in the Indonesia-Malaysia region, whereas only the more recent genotypes (I to III) circulate in other areas (Solomon et al., 2003). These results suggest that JEV originated from an ancestral virus in the Indonesia-Malaysia region where it evolved into distinct genotypes (of which genotypes I to III have since spread across Asia), whereas genotype IV strains have been isolated only since the early 1980s. Until the 1990s most isolates belonged to genotype III; more recently, the predominantly isolated strains in China, Japan, Thailand and Viet Nam belonged to genotype I (Wang et al., 2007; Nitatpattana et al., 2008) with descriptions of co-circulation of genotypes I and III, followed by more recently genotype I only. This suggests that genotype I is replacing genotype III in south-east Asia. There are relatively few isolates of genotype II. Recombination signals have been detected in the E gene of some JEV strains suggesting coinfection with viruses of different genotypes (Twiddy & Holmes, 2003) but evidence of recombination in JEV strains is a controversial area.

3. Japanese encephalitis — the disease

In areas with high JE transmission, young children most commonly develop clinical signs; older children and adults have generally developed immunity through earlier asymptomatic exposure. Symptomatic patients typically develop a few days of non-specific febrile flu-like illness, which can include diarrhoea, and rigors. In patients that progress to more serious disease, this is followed by headache, vomiting, and a reduced level of consciousness, often heralded by a convulsion. The majority of JEV infections are subclinical (Vaughn & Hoke, 1992). Encephalitis is usually severe, resulting in a fatal outcome in a quarter of cases and residual neuropsychiatric sequelae in 30% of cases (Burke & Leake, 1988; Halstead, 1992). Recently published studies have demonstrated that JE infection can result in permanent neurological disability in survivors (Ding et al., 2007; Ooi et al., 2008).

The clinical presentation of severe disease can vary. Seizures, which are a risk factor for development of sequelae (Ooi et al., 2008), occur frequently, being reported in up to 85% of children and 10% of adults (Dickerson et al., 1952; Kumar et al., 1990). The classic description of JE includes a Parkinson's disease-like dull, flat 'mask-like' face with wide, unblinking eyes, tremor, generalized hypertonia, and cogwheel rigidity. These features were reported in 70% to 80% of American service personnel and 20% to 40% of Asian children (Poneprasert, 1989; Kumar et al., 1990). In one study, a subgroup of patients presenting with acute flaccid paralysis (55%) had evidence of acute JEV infection compared with only 1% of age-matched controls (Solomon et al., 1998).

Limited data indicate that JE acquired during the first or second trimesters of pregnancy causes intra-uterine infection and miscarriage (Chaturvedi et al., 1980; Mathur et al., 1985). Infections that occur during the third trimester of pregnancy have not been associated with adverse outcomes in newborns.

4. Ecology and epidemiology

The virus is transmitted in an enzootic cycle among mosquitoes and amplifying vertebrate hosts, chiefly ardeid (wading) birds and domestic pigs (Burke & Leake, 1988). Humans are incidental hosts. Culex mosquitoes, primarily *C. tritaeniorhynchus*, are the principal vectors. These species are prolific in rural areas where their larvae breed in ground pools and especially in flooded rice fields. All elements of the transmission cycle are prevalent in rural areas of Asia, where most human infections occur. Because vertebrate amplifying hosts and agricultural activities may be situated within and at the periphery of cities, JE cases are increasingly reported from urban locations. The geographic spread of JE may be related to increasing international trade and travel, and global warming could permit overwintering in more temperate regions.

JEV is transmitted seasonally in most areas of Asia, but there are, in broad terms, two epidemiological patterns. In northern (temperate) regions, JEV is transmitted during the summer months, around May to September (Okuno, 1978; Umenai et al., 1985; Burke & Leake, 1988; Monath, 1990; Halstead, 1992). In southern (subtropical and tropical) regions, the virus is largely endemic, with sporadic outbreaks throughout the year, peaking at the start of the rainy season. A variety of explanations have been offered for this regional variation, but the generally favoured hypothesis is that climate is the underlying factor. Seasonal patterns of viral transmission are correlated with the abundance of vector mosquitoes and of vertebrate amplifying hosts which, in turn, fluctuate with rainfall, the rainy season, and the migratory patterns of avian hosts. In some tropical locations, however, irrigation associated with agricultural practices is a more important factor affecting vector abundance, and transmission may be year-round (Rosen, 1986; Sucharit et al., 1989; Thongcharoen, 1989; Service, 1991). Patterns of JE viral transmission vary regionally, within individual countries, and from year-to-year. In areas where JE is endemic, and in the absence of immunization, annual incidence ranges from 1 to 10 per 10 000 (Hoke et al., 1988). Children less than 15 years of age are principally affected. Seroprevalence studies indicate nearly universal exposure by adulthood; calculating from a ratio of asymptomatic to symptomatic infections of 200 to 1, approximately 10% of the susceptible population is infected per year. In areas where routine immunization of children has been introduced, there has been a shift in the age of JE cases to older children and teenagers (Wong et al., 2008) and a secondary increase has also been observed in the elderly, presumably due to immunosenescence (Arai et al., 2008). “Herd immunity” cannot be attained through vaccination due to the zoonotic nature of the virus — it remains in the environment.

5. The nature of immunity to Japanese encephalitis

5.1 Immunity following natural infection

Neutralizing antibodies appear during the first week after natural infection and last for many years, probably for life. The importance of antibodies to E protein in protective immunity against JE has been demonstrated in mice and pigs using different experimental systems (Kimura-Kuroda & Yasui, 1988; Mason et al., 1989 and 1991; Konishi et al., 1992 and 1998a; Jan et al., 1993). In addition, the NS1 protein evokes a strong humoral response which protects against challenge, probably through an Fc-dependent complement-mediated pathway, i.e. not neutralizing antibodies (Schlesinger et al., 1993).

Although there is good evidence for the role of cell-mediated immunity in recovery from infection by flaviviruses, the role of virus-specific T cell responses in protection against infection is not well defined. Virus-specific CD4+ and CD8+ T cells have been isolated and found to proliferate in response to JEV stimulation (Konishi et al., 1995). Vaccinees receiving inactivated virus vaccine (i.e. no non-structural proteins) have been shown to produce CD4+ T cells (Aihara et al., 1998) and those receiving a poxvirus-based vaccine based on JEV prM and E proteins have been shown to produce CD8+ T cells (Konishi et al., 1998b), both of which mediated JEV-specific cytotoxic activities.

In the murine model, JEV-specific cytotoxic T lymphocytes (CTLs) are induced by JEV infection or immunization with extracellular particle-based or poxvirus-based vaccines (Murali-Krishna et al., 1994; Konishi et al., 1997a and 1997b). Whether these specific T cell responses are protective against JEV infection is still controversial. Adoptive transfer of splenocytes or T lymphocytes has been reported to protect mice from a lethal challenge (Mathur et al., 1983; Murali-Krishna et al., 1996). However, depending on the route of transfer and age and strain of recipient animals, adoptively transferred T cells were not always protective (Miura et al., 1990; Murali-Krishna et al., 1996). DNA immunization with plasmids expressing C, NS1, NS2A, NS2B, NS3 or NS5 stimulated CTL, but only provided partial protection against lethal intraperitoneal challenge in mice (Konishi et al., 2003). On the other hand, plasmid DNA expressing prM and E gave rise to protective virus neutralizing antibody, suggesting that virus neutralizing antibody prevents virus dissemination to the brain and provides more efficient protection then CTL capable of killing virus-infected cells. These results suggest that, as with many other acute viral infections, virus neutralizing antibodies are critical for protection.

5.2 Vaccine-induced immunity and correlates of protection

As antibodies play a central role in protection against infection, these have been considered as correlates of protection (Hombach et al., 2005). A number of serological methods are used in studying antibody responses to JE including neutralization, haemagglutination inhibition (HI), complement fixation (CF), enzyme-linked immunosorbent assay (ELISA), and the indirect fluorescent antibody test (IFA). However, antibodies measured by HI, CF, ELISA and IFA do not correlate with protection. Only neutralizing antibodies correlate with protection.

The neutralization test is the most specific measure of antibody, i.e. little cross-reaction with other flaviviruses. To measure neutralizing antibody titres, the plaque reduction neutralization test (PRNT) is most often used. The essence of the test is that when JEV is grown on a cell monolayer, it causes plaques to form. The number of plaques formed is reduced if the virus has been mixed with serum containing neutralizing antibody, and this reduction in plaques gives a measure of the antibody titre. Hombach et al. (2005) recommend an end-point of 50% reduction in PRNT, but no international standard for the exact procedure or choice of end-points has been established. A recent study showed that there were some differences in neutralization titre depending on the JEV strain used in the plaque reduction neutralization test (Ferguson et al., 2008), an aspect to be considered when assessing vaccine-induced immune responses.

Determination of immunoglobulin M (IgM) antibodies by ELISA is the most useful method indicating recent infection (Burke et al., 1985; Innis et al., 1989). Heterologous antibodies (e.g. to dengue and West Nile viruses) are a potential source of false-positive reactions. To determine whether antibodies are specific to JEV, epitope-blocking ELISA (Burke et al., 1987), or analysis of ELISA absorbance ratios to the respective antigens can be used, and these are often confirmed by a plaque reduction neutralization test.

To determine the neutralizing antibody titre that is protective against JEV, mice were passively immunized with anti-JEV antibody and then challenged with 10^5 median 50% lethal dose (MLD_{50}) of JEV, which is thought to be a typical dose transmitted by an infectious mosquito bite. Mice that had neutralizing antibody (PRNT) titres greater than 1 in 10 were found to be protected against infection, whereas mice with lower titres were not (Lubiniecki et al., 1973; Oya, 1988). Neutralizing antibody titres greater than 1 in 10 are therefore extrapolated to indicate post-vaccination seroconversion and protection in humans; observations from vaccine trials in people support the adoption of this criterion (Halstead & Tsai, 2004).

6. Vaccines

6.1 Mouse brain-derived inactivated JE vaccine

Immunogenic, efficacious, and safe inactivated vaccines have been available for over 50 years and have been widely used in middle- and high-income Asian countries. Their use in low-income Asian countries has been hampered by cost, complex immunization schedules, and limited availability, among other factors. Some mouse brain-derived inactivated virus vaccines are manufactured in Asia for the domestic market only, including in Thailand and Viet Nam, but the vaccines produced by Biken in Japan and Green Cross in the Republic of Korea have also been manufactured for international distribution. However, production of the former vaccine was stopped by some manufacturers in 2006 in anticipation of the new cell-derived formula (section 6.2) becoming available for national use. In the interim, limited stocks of inactivated vaccine are available internationally.

6.1.1 Development of mouse brain-derived inactivated JE vaccines

Soon after the isolation of JEV in the 1930s, Japan and the former Soviet Union produced crude vaccines by inactivating virus grown in mouse brains (Smorodintsev et al., 1940). During World War II, a similar vaccine was developed for the American Army by Albert Sabin and colleagues. It was shown to be immunogenic and was given to 60 000 American soldiers during an encephalitis outbreak in 1945 in Okinawa, Japan (Sabin, 1947).

Since it was licensed in Japan in 1954, the production of the mouse brain-derived vaccine has been refined (Halstead & Tsai, 2004). WHO has issued written standards for production, quality control and clinical evaluation of inactivated JEV vaccines, including mouse brain-derived vaccines, and these were updated in 2007 (WHO, 2007). Previously, most formalin-inactivated vaccines were based on a derivative of the original Nakayama strain of JEV isolated in 1935, but from 1989 onwards the vaccine produced for the domestic market in Japan, and later the vaccine produced in Thailand and Viet Nam was prepared using the Beijing-1 strain (also a genotype III strain), which had been isolated in China in 1948. The Beijing-1 strain was found to induce antibodies that were superior to those induced by the Nakayama strain at neutralizing a wide variety of wild-type JEV strains.

6.1.2 *Administration of mouse brain-derived inactivated JE vaccines*

The recommended dose of the current mouse brain-derived vaccine, which contains the strain Beijing-1, is 0.5 ml delivered subcutaneously for children three years and older (half the volume for children aged 1ñ2 years). A variety of dosage regimens are used depending on the setting (Monath, 2002). In endemic parts of Asia, a two-dose primary immunization schedule is used. Children typically receive their first dose at any age between one and three years; the second dose is given seven to 30 days, ideally four weeks, later. Adequate protection throughout childhood is provided by a booster dose one year after the primary immunizations and further boosters every three years (WHO, 2006). In Japan and the Republic of Korea, where the incidence of JE has declined, the first dose is given at 18 months to three years, whereas in Thailand, vaccination is initiated at 18 months, Sri Lanka and Viet Nam at 12 months, and in Sarawak, Malaysia, at nine months.

For travellers (and military personnel) a three-dose primary schedule is recommended as a two-dose regimen fails to produce neutralizing antibody in at least 20% of subjects. The recommended immunization schedule is three doses on days 0, 7, and 28 or 30 (CDC, 1993; WHO, 2006). If there is insufficient time available before departure, an accelerated regimen (days 0, 7, and 14) is recommended by the USA Centers for Disease Control and Prevention (CDC). Both regimens produce nearly 100% seroconversion, but geometric mean antibody titres are significantly lower six months after the accelerated regimen. Although not ideal, even two doses given seven days apart produce antibody in 80% of recipients and so, under exceptional circumstances, might be better than nothing (CDC, 1993). The WHO recommends two doses, preferably given four weeks apart, or three doses given at 0, 1 and 4 weeks (WHO, 2006). For continued protection, booster doses are recommended.

Limited data are available on the requirement for administration of booster doses. Studies of American Army vaccine recipients that were based in endemic countries showed that antibody titres were maintained for up to three years in nearly 95% of recipients (Gambel et al., 1995). Boosters are currently recommended every three years up to the age of 15 years., The practice has varied over time in different parts of Asia. Annual boosters during childhood were given in the Republic of Korea until the 1980s; in Japan boosters are given approximately every five years, and in poorer countries that are able to afford only primary vaccination in a limited number of children, no boosters are given at all. WHO recommends an initial booster after one year, and thereafter every three years up to the age of 15. Other age-ranges might need to be considered in newly endemic regions. Natural boosting, by exposure to JEV or heterologous flaviviruses such as dengue, might be important in regions where these viruses circulate.

6.1.3 *Efficacy of mouse brain-derived inactivated JE vaccines*

Studies of the immune response in vaccine recipients, revealed important differences between residents of endemic areas, and travellers, which have led to the different vaccination schedules being recommended. When Asian children were vaccinated with a primary regimen of two doses of Nakayama or Beijing-1 strain-derived vaccines, 94% to 100% had neutralizing antibody to the homologous strain, although seroconversion rates were lower against the non-vaccine strain (Nimmannitya et al., 1995). Nearly 100% seroconversion was achieved after a 1-year booster dose. By contrast, seroconversion rates in travellers and military personnel from the United Kingdom and United States of America after a two-dose primary vaccination regimen were lower (33% to 80%) (Henderson, 1984;

Poland et al., 1990; Sanchez et al., 1990; Gambel et al., 1995). A three-dose primary schedule was more effective, giving seroconversion in more than 90% of recipients and higher geometric mean titres (Sanchez et al., 1990; Gambel et al., 1995). The difference in vaccine immunogenicity between the two populations is presumed to be due to a degree of naturally-acquired immunity among the Asian children because of exposure to JEV and, potentially, other flaviviruses, particularly dengue. However, the differences in the age of the populations studied (most of the travellers and military personnel were adults) might also be important.

Two randomized double-blind placebo-controlled trials have assessed the clinical efficacy of formalin-inactivated JE vaccines. In the first, conducted in 1965 and published in a non-peer-reviewed publication, nearly 134 000 children aged 3ñ7 years in Taiwan, China, were given either a single (n=22 194) or a double (n=111 749) dose of the Nakayama vaccine (a less purified form than todayís vaccine); nearly 132 000 children were given tetanus toxoid as placebo, and around 140 000 were unvaccinated (Hsu et al., 1971). The JE attack-rates per 100 000 recipients were 24.9 in the unvaccinated group, 18.2 in the placebo group, 9.0 in the single-dose group, and 3.6 in the double-dose group. Thus, a single dose of vaccine yielded 50% (95% CI: 26% to 88%) efficacy, and two doses gave 80% (95% CI: 71% to 93%) efficacy. In the second trial, the Biken vaccine was assessed in the 1980s in northern Thailand (Hoke et al., 1988). Children, aged one to 14 years, randomly allocated to three groups, received two doses (one week apart) of either the Biken monovalent Nakayama vaccine (n=21 628), the bivalent Nakayama/Beijing-1 vaccine (n=22 080), or tetanus toxoid as placebo (n=21 516). After a 2-year observation period, the JE attack rate in the placebo group was 51 per 100 000, whereas in both vaccine groups it was five per 100 000 giving a protective efficacy of 91% (95% CI: 70% to 90%). These results were accepted by the United States Food and Drug Administration as evidence for efficacy, leading to the Biken vaccine being licensed in the USA for travellers (Halstead & Tsai, 2004).

A retrospective analysis of the efficacy of the mouse brain-derived inactivated virus vaccine containing the Nakayama strain in Taiwan, China, using immunization records from 1971 to 2000, suggested that, among one to 14-year-old children, a single dose of JE vaccine gave a protective efficacy of 86%, and there was a significantly lower incidence of disease than in unvaccinated children (Yang et al., 2006). Therefore, vaccine might still be beneficial for infectious disease control at the population level for some JE epidemic- or endemic-developing countries with limited resources.

The question of whether the inactivated virus vaccines (which are based on genotype III strains) are efficacious against other genotypes of JEV has been raised. There are no clinical or epidemiological data to suggest that it is a problem, and studies have shown that virus neutralizing antibodies raised in response to immunization with inactivated virus vaccine, cross-react with different JEV strains, although neutralizing antibody titres vary and are usually less than that against the homologous virus in the vaccine (Okuno et al., 1987; Kurane & Takasaki, 2000).

6.1.4 Safety of mouse brain-derived inactivated JE vaccines

Inactivated vaccines are generally regarded as safe, and the mouse brain-derived JEV vaccines have a long history of use. Local reactions (tenderness, redness or swelling, occur in around 20% of vaccinated individuals and mild systemic side-effects (headache, low-grade fever, myalgias, malaise, and gastrointestinal symptoms) occur shortly after vaccination in around 5% to 10% of vaccinees (Hoke et al., 1988; Poland et al., 1990; Sanchez et al., 1990; Andersen & Ronne, 1991; Ruff et al., 1991; Defraites et al., 1999).

There has been a concern that, because the vaccine is grown in mouse brain, an immune response may be raised against mouse neural tissue which could attack the human nervous system causing autoimmune-type conditions such as acute disseminated encephalomyelitis (ADEM). However, no causal relationship has been established, and the WHO Global Advisory Committee on Vaccine Safety (GACVS) recently concluded that there is no evidence of increased risk of ADEM associated with administration of the inactivated JE vaccine of assured quality (WHO, 2005).

As the formalin-inactivated JE vaccine became available to travellers from Australia, Europe and North America, hypersensitivity reactions (including urticaria, angioedema, and bronchospasm) not previously reported, were described (Ruff et al., 1991; Berg et al., 1997; Plesner & Ronne, 1997; Plesner et al., 2000; Sohn, 2000). The risk of adverse reactions led to recommendations that, for travellers, the vaccine only be administered to those at high risk of infection (CDC, 1993).

6.2 Inactivated cell culture-derived vaccines

Limitations of the formalin-inactivated vaccines grown in mouse brain include the complexity and high cost of production, concerns over adverse reactions and, increasingly in some countries, reluctance to use animals in vaccine production. As a result there has been much attention focussed on the development of cell-culture systems to grow viruses for inactivated virus vaccines.

6.2.1 Inactivated vaccine containing Peking-3 or Beijing 1

Studies in China showed primary hamster kidney (PHK) cells gave the highest yield of JEV (Lee et al., 1965). A formalin-inactivated vaccine produced from the Peking-3 (P3) strain of JEV grown in PHK cells has been used in China since the 1960s and for many years was the country's principal JE vaccine (Halstead & Tsai, 2004). The vaccine, which is not purified, is stabilized with 0.1% human serum albumin and presented as a liquid formulation. The primary course consists of two 0.5 ml subcutaneous doses given one week apart to children aged 6–12 months, then boosters one year later at school entry, and again at 10 years of age. After primary immunization, only 60% to 70% of children seroconverted, and antibody levels waned rapidly, but a booster dose elicited a strong anamnestic response in 93%–100% of recipients (Monath, 2002). In five randomized field trials in China involving a total of 498 361 children, of whom 310 627 received the vaccine, the vaccine's efficacy ranged from 76% to 95% (Halstead & Tsai, 2004). The vaccine has only been licensed in China, and is gradually being replaced with the live attenuated vaccine (see below).

Vaccines grown in Vero cells (a continuous cell line derived from African green monkeys, and a conventional substrate for vaccine production), have also been developed. One such vaccine, a formalin-inactivated P3 virus grown in Vero cells in roller bottles, has been licensed in China (Ding et al., 1998). Inactivated Vero cell-grown Beijing-1 strain vaccines are being developed by several Japanese companies using microcarrier technology to increase the yield (Sugawara et al., 2002), and the vaccine developed by Biken was licensed in Japan in 2009. This lyophilized, preservative-free vaccine is given with the same schedule as the traditional mouse brain-derived vaccine.

6.2.2 Inactivated vaccine derived from Vero cells containing strain SA14-14-2

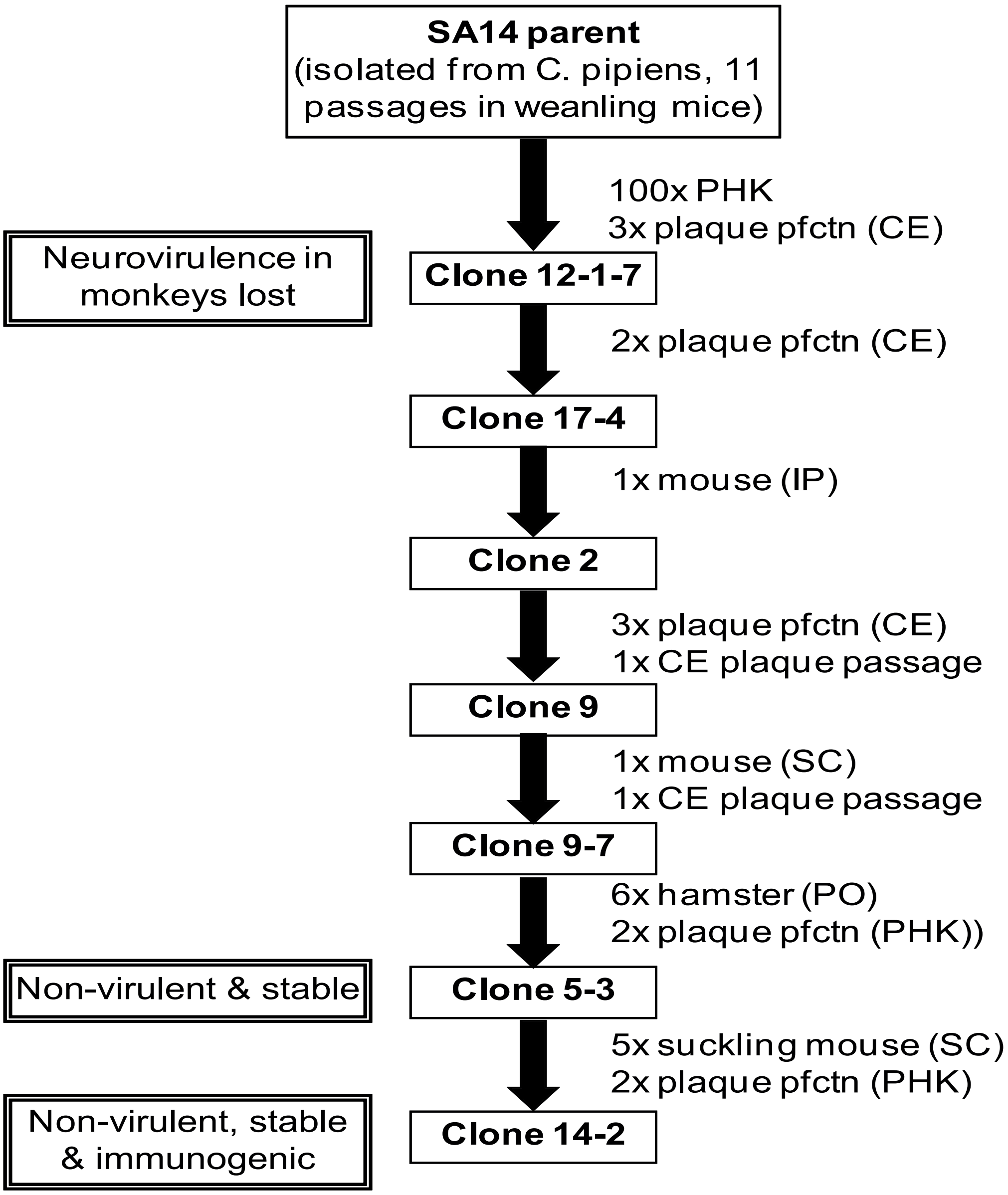
The Walter Reed Army Institute of Research (WRAIR) in the United States have adapted the live attenuated SA14-14-2 strain (see below) to growth on Vero cells, i.e. the SA14-14-2/PHK vaccine developed in China was passaged in primary dog kidney cell culture, and subsequently Vero cells, at WRAIR. Because this uses an attenuated rather than a virulent virus, production is easier, requiring biosafety level 2 rather than level 3 facilities. An inactivated vaccine based on this virus was licensed to Intercell (Vienna, Austria). In a multicentre blinded and randomized phase III clinical trial head-to-head with the Biken mouse brain-derived inactivated virus vaccine, the cell-derived vaccine had a good safety profile and a better local tolerability profile. Seroconversion rates were higher after two doses of the cell-derived vaccine (98%) than after three doses of the mouse brain-derived vaccine (95%), and geometric mean titres were higher. Persistence of titres has been shown for up to 24 months (Tauber et al., 2008; Schuller et al., 2008 and 2009). In 2009, the vaccine was licensed for adult travellers in several developed countries, and Novartis has acquired marketing and distribution rights for Europe, the United States and certain other markets including Asia and Latin America. A phase II paediatric trial of the vaccine, which began in India in 2007 as a joint venture with Biological E, has recently been completed, and a phase III paediatric study was launched in 2009.

6.3 Live attenuated SA14-14-2 vaccine

The live attenuated SA14-14-2 vaccine was developed cooperatively by two Chinese institutes (the Chengdu Institute of Biological Products, and the National Institute of Control for Pharmaceuticals and Biological Products). The vaccine has been produced in China since 1989 and over 300 million doses have been used to vaccinate Asian children. This live vaccine, manufactured by several producers in China, has also been licensed in several Asian countries; however only the Chengdu Institute of Biological Products' vaccine has obtained a licence for export.

To produce the live attenuated JEV strain, the wild-type strain SA14, isolated from *Culex pipiens* larvae collected in Xian, China, in 1954 (Xin et al., 1988), was passaged empirically in a range of cell-culture systems (Figure 3). After isolation, SA14 was passaged 11 times in weanling mice. The parent virus was then attenuated by passage 100 times in PHK cells to generate a variant, termed 12-1-7, which was no longer neurovirulent in monkeys but was not stable. To produce a stable, attenuated virus, it was then inoculated intraperitoneally into mice, harvested from the spleen, plaque-purified further in chick embryo cells, and passaged subcutaneously in mice and orally in hamsters before further purification in PHK cells (Halstead & Tsai, 2004). The resultant strain, SA14-5-3, did not revert to virulence after intracerebral passage in suckling mice and was still attenuated. It was safe in humans but in large field trials in southern China had poor immunogenicity in flavivirus-naive subjects. To increase immunogenicity, the virus was therefore passaged subcutaneously in suckling mice five times, and twice plaque-purified on PHK cells to produce SA14-14-2. Overall, SA14-14-2 is as attenuated as SA14-5-3 but is more immunogenic.

Figure 3: Schematic of empirical derivation of SA14-14-2 live attenuated vaccine strain


SA14 parent
(isolated from C. pipiens, 11 passages in weanling mice)
100x PHK
3x plaque pfctn (CE)
Neurovirulence in monkeys lost
Clone 12-1-7
2x plaque pfctn (CE)
Clone 17-4
1x mouse (IP)
Clone 2
3x plaque pfctn (CE)
1x CE plaque passage
Clone 9
1x mouse (SC)
1x CE plaque passage
Clone 9-7
6x hamster (PO)
2x plaque pfctn (PHK))
Non-virulent & stable
Clone 5-3
5x suckling mouse (SC)
2x plaque pfctn (PHK)
Non-virulent, stable & immunogenic
Clone 14-2

Key:
IP=intraperitoneal; PO=per os; SC=subcutaneous; CE=chick embryo;
PHK=primary hamster kidney; pfctn=purification

Currently, the exact molecular basis of attenuation of SA14-14-2 is not known due to the lack of studies using reverse genetics. However, several publications agree on seven amino acid substitutions in the process of passaging SA14 to generate SA14-14-2/PHK (E-138, E-176, E-315, E-439, NS2B-63, NS3-105 and NS4B-106) (Nitayapahan et al., 1990; Aihara et al, 1991; Ni et al., 1994 and 1995). Further studies showed that although residue 138 of E protein had a dominant effect, attenuation depended on at least three distinct clusters in different combinations (Arroyo et al., 2001).

The vaccine is produced from seed virus by infecting PHK cells, and is manufactured as a freeze-dried product in one- or five-dose presentations stabilized with gelatin and sorbitol. After reconstitution with normal saline, the vaccine must be used within six hours in order to comply with the WHO open vial policy — a 0.5ml dose is given by subcutaneous injection.

The SA14-14-2 vaccine based on a genotype III strain protected mice against challenge with JEV strains belonging to different genotypes from Indonesia, Thailand and Viet Nam (Xin et al., 1988).

6.3.1 Efficacy of SA14-14-2 vaccine

SA14-14-2 vaccine has undergone a series of clinical trials and observational studies in China, the Republic of Korea and Nepal. However, no randomized clinical trials comparing the efficacy of SA14-14-2 vaccine with placebo or other JE vaccines have been conducted. Several small trials demonstrated that after a single dose of SA14-14-2, 85% to 100% of children seroconvert, with a proportion seroconverting dependent on the quantity of vaccine administered in a dose (Halstead & Tsai, 2004). In China, SA14-14-2 vaccine has traditionally been administered annually as part of a spring vaccination campaign, with two doses given one year apart, and a further dose given at school entry age; this schedule was adopted based on operational criteria. However, the vaccine's efficacy was demonstrated in several open-label field studies in China between 1988 and 1993 that involved nearly 600 000 children (Halstead & Tsai, 2004). Comparisons of the incidence of JE in vaccinated and unvaccinated children showed a protective efficacy of around 98% after one or more doses. These findings were confirmed in a more rigorous, relatively simple and inexpensive post-licensure case-control study in which the prevalence of immunization was compared between 56 JE cases and 1299 age- and village-matched controls (Hennessy et al., 1996). The effectiveness of one dose was 80% (95% CI: 44%–93%) and of two doses one year apart, 97.5% (CI: 86%–99.6%). Although more rigorous and extensive, this study also has its limitations, in that vaccine histories were unreliable and when vaccinations were given was not detailed.

The efficacy of single-dose vaccine given just before the JE season, was assessed in a similar case-control study in Nepal in 1999, when approximately 160 000 children were vaccinated (Bista et al., 2001). None of 20 JE cases had received vaccine, compared with 326 of 557 age- and sex-matched village controls, giving a short-term protective efficacy of 99.3% (CI: 94.9%–100%). Subsequently, the duration of immunity after a single dose of SA14-14-2 was examined. In July 1999, Nepalese children aged one to 15 years were given a single dose of SA14-14-2. A case-control study conducted one year later gave a protective effect 12–15 months after vaccination of 98.5% (CI: 90.1%–99.2%) (Ohrr et al., 2005). A further study five years after administration of the single dose gave a protective efficacy of 96.2% (CI: 73.1%–99.9%) (Tandan et al., 2007). A recent case-control study in India corroborates these findings.

A study conducted in the Philippines demonstrated that SA14-14-2 can be safely co-administered with measles vaccine at nine months of age without significantly affecting the immune response to either vaccine (Gatchalian et al., 2008). The modest reduction observed in both seroconversion and geometric mean titre (GMT) titre for measles vaccine when co-administered with SA 14-14-2 was considered acceptable, but further studies were encouraged *(*WER, 25 January 2008*)*. Additional co-administration studies have been conducted in Sri Lanka to corroborate the findings and these are awaiting publication.

6.3.2 Safety of SA14-14-2 vaccine

The attenuation of the SA14-14-2 vaccine strain has been demonstrated in various animal models (Halstead & Tsai, 2004). As for any live virus, one safety concern is the possibility of reversion to a virulent form. Vaccine safety was assessed in a post-licensure, randomized, placebo-controlled trial in 26 000 children (Liu et al., 1997). One month after vaccination, the two groups (vaccinated and placebo control) had similar rates of hospitalization and illness. There were no cases of post-vaccination anaphylaxis or neurological disease. Numerous other smaller studies have demonstrated a very good safety record for SA14-14-2 vaccine (Xin et al., 1988; Sohn et al., 1999).

The SA14-14-2 vaccine did not immediately gain regulatory approval outside China because of concerns about the PHK substrate, which is not an accepted cell type for vaccine production (i.e. primary hamster kidney cell culture derived from baby hamsters), uncertainty about the quality-control tests for adventitious agents, and other issues related to manufacturing practices. The WHO developed guidelines to facilitate the international acceptance of the vaccine (WHO, 2002). Work is still in progress to follow-up on GACVS recommendations made in 2005. As a result of a comprehensive review on the safety of the vaccine conducted by GACVS in 2005, some specific recommendations were made requesting more detailed study of: the safety profile in special risk groups including immunocompromised people and pregnant women; whether viral shedding occurs in vaccinees and the potential implications of such shedding; further analysis of sequential or co-administration of JE and measles vaccines; the interchangeability of inactivated and live JE vaccines; the safety of vaccine administration to infants aged under one year, and the implications for the efficacy and safety of the vaccine in infants with maternal antibodies against JE virus. Also, population-based safety data from the use of the vaccine in countries with a functioning adverse events following immunization (AEFI) system were considered desirable (WHO, 2005). In the meantime acceptable results of vaccine co-administration with measles vaccine and immunogenicity in children below one year of age have been published (Gatchalian, 2008). Publication of the results of other studies (viraemia and interchangeability of vaccines) is awaited.

On balance, no specific safety signal is associated with this vaccine, and GACVS noted that reported data suggest an excellent safety and efficacy profile of the SA14-14-2 vaccine. While not WHO-prequalified, this vaccine is now licensed and used in many countries, including China, India, the Republic of Korea, Nepal, Sri Lanka and Thailand.

6.4 Chimeric yellow fever–JE vaccine

Chimeric yellow fever–JE vaccine (JE-CV) is a live, attenuated vaccine against JE, in which the prM-E genes of attenuated JEV strain SA14-14-2 were inserted into an infectious clone of the 17D-204 yellow fever vaccine strain (Chambers et al., 1999). The chimaeric virus replicated efficiently in vitro and was shown to be immunogenic, efficacious, and safe in mice and nonhuman primates, being even more attenuated than the original 17D-204 yellow fever strain (Guirakhoo et al., 1999; Monath et al., 2000). The chimaeric virus was incapable of infecting mosquitoes by oral feeding and had reduced replication after indirect intrathoracic inoculation, allaying fears of secondary transmission after vaccination and suggesting that it is unlikely to be transmitted by mosquitoes biting recently vaccinated individuals (Bhatt et al., 2000; Reid et al., 2006). In a small phase I trial, the vaccine was shown to be safe and immunogenic (Monath et al., 2002). The vaccine was also well tolerated in a larger phase 2 clinical trial (Monath et al., 2003) in which 82 (94%) of 87 subjects administered graded doses (1.8–5.8 $\log_{10}$) of chimeric yellow fever–JE vaccine developed neutralizing antibodies. The JE-CV vaccine appears to provide protective levels of neutralizing antibody after a single dose, but a second dose administered 30 days later had no booster effect. A separate study explored immunological memory, both in subjects who had received JE-CV vaccine nine months before, and in vaccine-naive subjects challenged with inactivated mouse-brain vaccine. Anamnestic responses were observed in the pre-immune individuals (Monath et al., 2003). Interestingly, in both humans and monkeys, prior yellow fever immunity did not reduce the response to the chimeric vaccine (Guirakhoo et al., 1999), but there was a suggestion, though it was not statistically significant, that JE-CV vaccine reduced the response to YF 17D vaccine administered 30 days later.

The vaccine was developed as a single-dose Japanese encephalitis vaccine primarily for people living in endemic countries of the Asian Pacific region. The clinical development is being done mainly in Australia and Thailand, and a license application was submitted in 2009. The vaccine is presented as freeze-dried powder in 1- or 4-dose presentations to be reconstituted before use.

Notably, a minimally invasive alternative route of delivery of the JE-CV vaccine has been assessed in nonhuman primates (Dean et al., 2005). Cutaneous delivery by either skin microabrasion or intradermal delivery using microneedles gave rise to higher levels of virus neutralizing antibodies than conventional subcutaneous injection.

6.5 Poxvirus-vectored vaccines

ALVAC and NYVAC, two highly attenuated recombinant poxvirus vectors, have been tested as candidates for vaccination against JE. Recombinant vaccines delivering prM-E or prM-E-NS1 genes induced protective immunity in mice and monkeys in challenge experiments, and proceeded to clinical studies (Mason et al., 1991; Konishi et al., 1992). ALVAC-JE was poorly immunogenic in all human subjects and NYVAC-JE was poorly immunogenic in those that had previously been vaccinated against smallpox (Kanesa-thasan et al., 2000). Although these results were disappointing, interest in recombinant poxviruses has been revived recently using the Modified Vaccine Ankara (MVA) platform, and preclinical studies are in progress.

6.6 DNA vaccine

DNA-mediated immunization involves the introduction into tissues of a DNA plasmid carrying an antigen-coding gene that transfects cells in vivo leading to expression of the encoded antigen and resulting in an immune response. Two doses of plasmid DNA vaccines encoding JEV PrM-E genes produced promising results in mice and pigs (Konishi et al., 1998a and 2000). Although successful in many animal models of various diseases, in general, DNA vaccines remain insufficiently immunogenic in humans. Furthermore, DNA vaccines are better at inducing cell-mediated immune responses compared to humoral responses, and all evidence to date indicates that protective immunity for JEV is induced by neutralizing antibodies. As DNA vaccines offer the advantages of ease of manipulation and simplicity of manufacture, and stability and resistance to temperature extremes so that the storage, transport, and distribution of DNA-based vaccines is more practical and less expensive, various approaches to improve the immunogenicity of DNA vaccines against JE continue to be investigated (Ashok & Rangarajan, 2002; Imoto & Konishi, 2005; Wu et al., 2006).

7. Immunization strategies for Japanese encephalitis endemic countries

The number of countries introducing JE vaccination into their routine childhood immunization schedules is increasing. With improved vaccines becoming available and a better understanding of the burden of disease, it is anticipated that, by 2012, the global demand for JE vaccines will be more than double the current demand (Marfin & Gubler, 2005). Of particular note was the introduction in India of mass vaccination campaigns aimed at vaccinating 100 million children with JE vaccine in the period 2006 to 2010. With the aid of the WHO, UNICEF and PATH, 9.3 million children were vaccinated in 2006 using the live attenuated SA14-14-2 vaccine (Biswal et al., 2007). Increased production of the SA14-14-2 vaccine together with availability of new JE vaccines (Table 1) is likely to assure the supply.

Table 1: Summary of currently licensed or submitted vaccines against Japanese encephalitis

Description (strain)	Producer	Availability	Notes
Inactivated vaccines			
Mouse brain-derived (Nakayama or Beijing-1)	Kitasota, Japan Green Cross, Republic of Korea CRI, India Vabiotech, Viet Nam GPO, Thailand	International Domestic Domestic International Domestic Domestic/India Domestic/region	3-dose primary with repeat boosters (regimen dependent on setting) Dose:1ml (Nakayama) / 0.5 ml (Beijing-1)
Primary hamster kidney (P3)	Multiple, China	Domestic	2-dose primary with repeat boosters
Vero (P3)	China	Domestic	
Vero (Beijing-1)	Biken, Japan	Domestic	3-dose primary with repeat boosters
Vero (SA14-14-2)	Intercell; Biological E, India	Traveller & military; Paediatric	2-dose primary, boosters TBD (adult); Phase II/III paediatric trials in progress in India
Live-attenuated vaccines			
Primary hamster kidney (SA14-14-2)	China, mainly Chengdu Institute of Biological Products	China, India, Nepal, Republic of Korea, Sri Lanka	Single-dose primary, boosters TBD*
Genetically-engineered vaccines			
Chimeric yellow fever – JE (SA14-14-2)	Sanofi	Licence application filed in Australia & Thailand	Single-dose primary, boosters TBD*

* TBD, to be decided.

The high protective effect of one dose of SA14-14-2 vaccine provides an argument for integration of a single dose of the live attenuated vaccine into the WHO Expanded Immunization Programme (EPI) at 12 to 15 months of age. The JE-CV vaccine has similar potential to be introduced into immunization programmes as a single-dose vaccine. The use of simpler and less expensive schemes will improve the acceptance and hence effectiveness of immunization programmes compared to the traditional mouse brain-derived vaccines.

WHO recommends as the most effective immunization strategy in JE endemic settings a one-time catch-up campaign followed by incorporation of the JE vaccine into the routine childhood immunization schedule (WHO, 2006). As yet, guidelines tailored for different endemicity patterns have not been developed.

Vaccination in response to outbreaks needs to be further explored. It may not be an efficient means of control in the absence of a routine immunization programme, as the short duration of outbreaks means that there may not be sufficient time for an adequate campaign to be conducted, and immunity established in vaccine recipients.

Finally, it is expected that JE vaccines will become WHO-prequalified in the near future, helping to make them more widely available by facilitating financial support through the international community.

References

Aihara H et al. (1998). Establishment and characterization of Japanese encephalitis virus-specific, human CD4(+) T-cell clones: flavivirus cross-reactivity, protein recognition, and cytotoxic activity. *Journal of Virology*, 72:8032–8036.

Aihara S et al. (1991). Identification of mutations that occurred on the genome of Japanese encephalitis virus during the attenuation process. *Virus Genes*, 5:95–109.

Akiba T et al. (2001). Analysis of Japanese encephalitis epidemic in Western Nepal in 1997. *Epidemiology and Infection*, 126:81–88.

Allison SL et al. (1995). Synthesis and secretion of recombinant tick-borne encephalitis virus protein E in soluble and particulate form. *Journal of Virology*, 69:5816–5820.

Andersen MM, Ronne T (1991). Side-effects with Japanese encephalitis vaccine. *Lancet*, 337:1044.

Arai S et al. (2008). Japanese encephalitis: surveillance and elimination effort in Japan from 1982 to 2004. *Japanese Journal of Infectious Diseases*, 61:333–338.

Arroyo J et al. (2001). Molecular basis for attenuation of neurovirulence of a yellow fever virus/Japanese encephalitis virus chimera vaccine (ChimeriVax-JE). *Journal of Virology*, 75:934–942.

Ashok MS, Rangarajan PN (2002). Protective efficacy of a plasmid DNA encoding Japanese encephalitis virus envelope protein fused to tissue plasminogen activator signal sequences: studies in a murine intracerebral virus challenge model. *Vaccine*, 20:1563–1570.

Berg SW et al. (1997). Systemic reactions in US Marine Corps personnel who received Japanese encephalitis vaccine. *Clinical Infectious Diseases : an official publication of the Infectious Diseases Society of America*, 24:265–266.

Bhatt TR et al. (2000). Growth characteristics of the chimeric Japanese encephalitis virus vaccine candidate, ChimeriVax-JE (YF/JE SA14-14-2), in Culex tritaeniorhynchus, Aedes albopictus, and Aedes aegypti mosquitoes. *The American Journal of Tropical Medicine and Hygiene*, 62:480–484.

Bista MB et al. (2001). Efficacy of single-dose SA14-14-2 vaccine against Japanese encephalitis: a case-control study. *Lancet*, 358:791–795.

Biswall P et al. (2007). Partnering for successful introduction of Japanese encephalitis vaccine in India [abstract]. *Eleventh Annual Scientific Conference of the International Center for Diarrhoeal Disease Research*, Bangladesh, 2007 (http://www.path.org/vaccineresources/files/Partnering_successful_intro_JE_vaccine_India_PATH.pdf, last accessed 17 February 2009).

Boonsanay V, Smith DR (2007). Entry into and production of the Japanese encephalitis virus from C6/36 cells. *Intervirology*, 50:85–92.

Bressanelli S et al. (2004). Structure of a flavivirus envelope glycoprotein in its low-pH-induced membrane fusion conformation. *The EMBO Journal*, 728–738.

Burke DS, Leake CJ (1988). Japanese encephalitis. In: Monath TP, ed. *The arboviruses: epidemiology and ecology.* FL, Boca Raton, CRC Press.

Burke DS, et al. (1985). Kinetics of IgM and IgG responses to Japanese encephalitis virus in human serum and cerebrospinal fluid. *The Journal of Infectious Diseases*, 151:1093–1099.

Burke DS, et al. (1987). Detection of flavivirus antibodies in human serum by epitope-blocking immunoassay. *Journal of Medical Virology*, 23:165–173.

CDC (1993). Inactivated Japanese encephalitis virus vaccine. Recommendations of the advisory committee on immunization practices (ACIP). *MMWR. Morbidity and Mortality Weekly Report*, 42:1–15.

Chambers TJ, et al. (1990). Flavivirus genome organization, expression, and replication. *Annual Review of Microbiology*, 44:649–688.

Chambers TJ, et al. (1999). Yellow fever/Japanese encephalitis chimeric viruses: construction and biological properties. *Journal of Virology*, 73:3095–3101.

Chaturvedi UC, et al. (1980). Transplacental infection with Japanese encephalitis virus. *The Journal of Infectious Diseases*. 141:712–715.

Chen HW, et al. (1999). Screening of protective antigens of Japanese encephalitis virus by DNA immunization: a comparative study with conventional viral vaccines. *Journal of Virology*, 73:10137–10145.

Chen WR, et al. (1990). Genetic variation of Japanese encephalitis virus in nature. *The Journal of General Virology*, 71:2915–2922.

Chen WR et al. (1992). A new genotype of Japanese encephalitis virus from Indonesia. *American Journal of Tropical Medicine and Hygiene*, 47:61–69.

Chen Y et al. (1997). Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. *Nature Medicine*, 3:866–871.

Dean CH et al. (2005). Cutaneous delivery of a live, attenuated chimeric flavivirus vaccine against Japanese encephalitis (ChimeriVax)-JE) in nonhuman primates. *Human Vaccines*, 1:106–111.

Defraites RF et al. (1999). Japanese encephalitis vaccine (inactivated, BIKEN) in US soldiers: immunogenicity and safety of vaccine administered in two dosing regimens. *American Journal of Tropical Medicine and Hygiene*, 61:288–293.

Dickerson RB et al. (1952). Diagnosis and immediate prognosis of Japanese B encephalitis; observations based on more than 200 patients with detailed analysis of 65 serologically confirmed cases. *The American Journal of Medicine*, 12:277–288.

Ding D et al. (2003). Cost-effectiveness of routine immunization to control Japanese encephalitis in Shanghai, China. *Bulletin of the World Health Organization*, 81:334–342.

Ding D et al. (2007). Long-term disability from acute childhood Japanese encephalitis in Shanghai, China. *American Journal of Tropical Medicine and Hygiene*, 77:528–533.

Ding Z et al. (1998). Production of purified Japanese encephalitis vaccine from Vero cells with roller bottles. *Zhonghua Yi Xue Za Zhi*, 78:261–262.

Ferguson M et al. (2008). Effect of genomic variation in the challenge virus on the neutralization titres of recipients of inactivated JE vaccines — report of a collaborative study on PRNT50 assays for Japanese encephalitis (JE) antibodies. *Biologicals : Journal of the International Association of Biological Standardization*, 36:111–116.

Gambel JM et al. (1995). Japanese encephalitis vaccine: persistence of antibody up to 3 years after a three-dose primary series. *The Journal of Infectious Diseases*, 171:1074.

Gatchalian S et al. (2006). Measles vaccine immunogenicity after coadministration with live attenuated Japanese encephalitis vaccine shows equivalence to that of measles vaccine given alone [abstract]. In: *American Society of Tropical and Medicine and Hygiene Fifty-fourth Annual Meeting*, Atlanta, GA, 12–16 November 2006. 2605.

Guirakhoo F et al. (1999). Immunogenicity, genetic stability, and protective efficacy of a recombinant, chimeric yellow fever-Japanese encephalitis virus (ChimeriVax-JE) as a live, attenuated vaccine candidate against Japanese encephalitis. *Virology*, 257:363–372.

Halstead SB (1992). Arboviruses of the Pacific and Southeast Asia. In: RD Feigin RD, Cherry JD, eds. *Textbook of pediatric infectious diseases*, 3rd ed. Philadelphia, PA, WB Saunders.

Halstead SB, Tsai TF (2004). Japanese encephalitis vaccines. In: Plotkin SA, Orenstein WA, eds. *Vaccines*, 4th ed. Philadelphia, PA, WB Saunders.

Hase T et al. (1990). Ultrastructural changes of mouse brain neurons infected with Japanese encephalitis virus. *International Journal of Experimental Pathology*, 71:493–505.

Heinz FX et al. (1994). Structural changes and functional control of the tick-borne encephalitis virus glycoprotein E by the heterodimeric association with protein prM. *Virology*, 198:109–117.

Henderson A. (1984). Immunization against Japanese encephalitis in Nepal: experience of 1152 subjects. *Journal of the Royal Army Medical Corps*, 130:188–191.

Hennessy S et al. (1996). Effectiveness of live-attenuated Japanese encephalitis vaccine (SA14-14-2): a case-control study. *Lancet*, 347:1583–1586.

Hoke CH et al. (1988). Protection against Japanese encephalitis by inactivated vaccines. *The New England Journal of Medicine*, 319:608–614.

Hombach J et al. (2005). Report on a WHO consultation on immunological endpoints for evaluation of new Japanese encephalitis vaccines, Geneva, World Health Organization, 2–3 September 2004. *Vaccine*, 23:5205–5211.

Hsu TC et al. (1971). A completed field trial for an evaluation of the effectiveness of mouse-brain Japanese encephalitis vaccine. In: Hammon WMcD, Kitaoka M, Downs WG, eds. *Immunization for Japanese encephalitis*. Tokyo, Igaku Shoin Ltd.

Igarashi A (1992). Epidemiology and control of Japanese encephalitis. *World Health Statistics Quarterly*, 45:299–305.

Imoto J, Konishi E (2005). Needle-free jet injection of a mixture of Japanese encephalitis DNA and protein vaccines: a strategy to effectively enhance immunogenicity of the DNA vaccine in a murine model. *Viral Immunology*, 18:205–212.

Innis BL et al. (1989). An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. *American Journal of Tropical Medicine and Hygiene*, 40:418–427.

International Rice Research Institute (2005). World rice statistics. May 2005. (http://www.irri.org/science/ricestat/index.asp).

Jan LR et al. (1993). Increased immunogenicity and protective efficacy in outbred and inbred mice by strategic carboxyl-terminal truncation of Japanese encephalitis virus envelope glycoprotein. *American Journal of Tropical Medicine and Hygiene*, 48:412–423.

Kanesa-thasan N et al. (2000). Safety and immunogenicity of NYVAC-JEV and ALVAC-JEV attenuated recombinant Japanese encephalitis virus-poxvirus vaccines in vaccinia-nonimmune and vaccinia-immune humans. *Vaccine*, 19:483–491.

Kimura-Kuroda J, Yasui K (1988). Protection of mice against Japanese encephalitis virus by passive administration with monoclonal antibodies. *Journal of Immunology (Baltimore, Md. : 1950)*, 141:3606-–3610.

Kitaoka M (1972). Shift of age distribution of cases of Japanese encephalitis in Japan during the period 1950 to 1967. In: Hammon WMcD, Kitaoka M, Downs WG, eds. *Immunization for Japanese encephalitis*. Amsterdam, Excerpta Medica.

Konishi E, Mason PW (1993). Proper maturation of the Japanese encephalitis virus envelope glycoprotein requires co-synthesis with the pre-membrane protein. *Journal of Virology*, 67:1672–1675.

Konishi E et al. (1992). A highly attenuated host range-restricted vaccinia virus strain, NYVAC, encoding the prM, E, and NS1 genes of Japanese encephalitis virus prevents JEV viremia in swine. *Virology*, 190:454–458.

Konishi E et al. (1995). Japanese encephalitis virus-specific proliferative responses of human peripheral blood T lymphocytes. *American Journal of Tropical Medicine and Hygiene*, 53:278–283.

Konishi E et al. (1997a). Poxvirus-based Japanese encephalitis vaccine candidates induce JE virus-specific CD8+ cytotoxic T lymphocytes in mice. *Virology*, 227:353–360.

Konishi E et al. (1997b). Particulate vaccine candidate for Japanese encephalitis induces long-lasting virus-specific memory T lymphocytes in mice. *Vaccine*, 15:281–286.

Konishi E et al. (1998a). Induction of protective immunity against Japanese encephalitis in mice by immunization with a plasmid encoding Japanese encephalitis virus pre-membrane and envelope genes. *Journal of Virology*, 72:4925–4930.

Konishi E et al. (1998b). Induction of Japanese encephalitis virus-specific cytotoxic T lymphocytes in humans by poxvirus-based JE vaccine candidates. *Vaccine*, 16:842–849.

Konishi E et al. (2000). Japanese encephalitis DNA vaccine candidates expressing pre-membrane and envelope genes induce virus-specific memory B cells and long-lasting antibodies in swine. *Virology*, 268:49–55.

Konishi E et al. (2003). Comparison of protective efficacies of plasmid DNAs encoding Japanese encephalitis virus proteins that induce neutralizing antibody or cytotoxic T lymphocytes in mice. *Vaccine*, 21:3675–3683.

Kulkarni AB et al. (1992). Analysis of murine major histocompatibility complex class II-restricted T-cell responses to the flavivirus Kunjin by using vaccinia virus expression. *Journal of Virology*, 66:3583–3592.

Kumar P et al. (2003). Screening for T cell-eliciting proteins of Japanese encephalitis virus in a healthy JE-endemic human cohort using recombinant baculovirus-infected insect cell preparations. *Archives of Virology*, 148:1569–1591.

Kumar P et al. (2004a). Conserved amino acids 193-324 of non-structural protein 3 are a dominant source of peptide determinants for CD4+ and CD8+ T cells in a healthy Japanese encephalitis virus-endemic cohort. *The Journal of General Virology*, 85:1131–1143.

Kumar P et al. (2004b). Cell-mediated immune responses in healthy children with a history of subclinical infection with Japanese encephalitis virus: analysis of CD4+ and CD8+ T cell target specificities by intracellular delivery of viral proteins using the human immunodeficiency virus Tat protein transduction domain. *The Journal of General Virology*, 85:471–482.

Kumar R et al. (1990). Clinical features & prognostic indicators of Japanese encephalitis in children in Lucknow (India). *The Indian Journal of Medical Research*, 91:321–327.

Kurane I, Takasaki T (2000). Immunogenicity and protective efficacy of the current inactivated Japanese encephalitis vaccine against different Japanese encephalitis virus strains. *Vaccine*, 18(Suppl. 2):S33–S35.

Lee GC et al. (1965). Growth of Japanese encephalitis virus in cell structure. *The Journal of Infectious Diseases*, 115:321–329.

Liu ZL et al. (1997). Short-term safety of live attenuated Japanese encephalitis vaccine (SA14-14-2): results of a randomized trial with 26 239 subjects. *The Journal of Infectious Diseases*, 176:1366–1369.

Lobigs M et al. (1994). The flavivirus nonstructural protein NS3 is a dominant source of cytotoxic T cell peptide determinants. *Virology*, 202:195–201.

Lubiniecki AS et al. (1973). Passive immunity for arbovirus infection. II. Quantitative aspects of naturally and artificially acquired protection in mice for Japanese (B) encephalitis virus. *American Journal of Tropical Medicine and Hygiene*, 22:535–542.

Mandl CW et al. (1989). Antigenic structure of the flavivirus envelope protein E at the molecular level, using tick-borne encephalitis virus as a model. *Journal of Virology*, 63:564–571.

Marfin AA, Gubler DJ (2005). Japanese encephalitis: the need for a more effective vaccine. *Lancet*, 366:1335–1337.

Mason PW et al. (1989). Molecular characterization of a neutralizing domain of the Japanese encephalitis virus structural glycoprotein. *The Journal of General Virology*, 70:2037–2049.

Mason PW et al. (1991). Japanese encephalitis virus-vaccinia recombinants produce particulate forms of the structural membrane proteins and induce high levels of protection against lethal JEV infection. *Virology*, 180:294–305.

Mathur A et al. (1983). Host defence mechanisms against Japanese encephalitis virus infection in mice. *The Journal of General Virology*, 64:805–811.

Mathur A et al. (1985). Japanese encephalitis virus infection during pregnancy. *The Indian Journal of Medical Research*, 81:9–12.

Miura K et al. (1990). A single gene controls resistance to Japanese encephalitis virus in mice. *Archives of Virology*, 112:261–270.

Modis Y et al. (2003). A ligand-binding pocket in the dengue virus envelope glycoprotein. *Proceedings of the National Academy of Sciences of the United States of America*, 6986–6991.

Monath TP (1990). Flaviviruses. In: Fields BN, Knipe DM, eds. *Virology*. New York, Raven Press.

Monath TP (2002). Japanese encephalitis vaccines: current vaccines and future prospects. *Current Topics in Microbiology and Immunology*, 267:105–138.

Monath TP et al. (2000). Chimeric yellow fever virus 17D-Japanese encephalitis virus vaccine: dose-response effectiveness and extended safety testing in rhesus monkeys. *Journal of Virology*, 74:1742–1751.

Monath TP et al. (2002). Clinical proof of principle for ChimeriVax: recombinant live, attenuated vaccines against flavivirus infections. *Vaccine*, 20:1004–1018.

Monath TP et al. (2003). Chimeric live, attenuated vaccine against Japanese encephalitis (ChimeriVax-JE): phase 2 clinical trials for safety and immunogenicity, effect of vaccine dose and schedule, and memory response to challenge with inactivated Japanese encephalitis antigen. *The Journal of Infectious Diseases*, 188:1213–1230.

Murali-Krishna K et al. (1994). Cytotoxic T lymphocytes raised against Japanese encephalitis virus: effector cell phenotype, target specificity and in vitro virus clearance. *The Journal of General Virology*, 75:799–807.

Murali-Krishna K et al. (1996). 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+ T cells. *The Journal of General Virology*, 77 (Pt. 4):705–714.

Ni H et al. (1994). Comparison of nucleotide and deduced amino acid sequence of the 5' non-coding region and structural protein genes of the wild-type Japanese encephalitis virus strain SA14 and its attenuated vaccine derivatives. *The Journal of General Virology*, 75: 1505–1510.

Ni H et al. (1995). Molecular basis of attenuation of neurovirulence of wild-type Japanese encephalitis virus strain SA14. *The Journal of General Virology*, 76: 409–413.

Nimmannitya S et al. (1995). A field study on Nakayama and Beijing strains of Japanese encephalitis vaccines. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 26:689–693.

Nitatpattana N et al. (2008). Change in Japanese encephalitis virus distribution, Thailand. *Emerging Infectious Diseases*, 14:1762–1765.

Nitayaphan S et al. (1990). Nucleotide sequence of the virulent SA-14 strain of Japanese encephalitis virus and its attenuated vaccine derivative, SA14-14-2. *Virology*. 177:541–552.

Ohrr H et al. (2005). Effect of single dose of SA14-14-2 vaccine 1 year after immunization in Nepalese children with Japanese encephalitis: a case-control study. *Lancet*, 366:1375–1378.

Okuno T (1978). An epidemiological review of Japanese encephalitis. *World Health Statistics Quarterly*, 31:120–133.

Okuno Y et al. (1987). Effect of current Japanese encephalitis vaccine on different strains of Japanese encephalitis virus. *Vaccine*, 5:128–132

Ooi MH et al. (2008). The epidemiology, clinical features, and long-term prognosis of Japanese encephalitis in Central Sarawak, Malaysia, 1997–2005. *Clinical Infectious Diseases : an official publication of the Infectious Diseases Society of America*, 47:458–468.

Oya A (1988). Japanese encephalitis vaccine. *Acta Paediatrica Japonica; Overseas edition*, 30:175–184.

Plesner AM, Ronne T (1997). Allergic mucocutaneous reactions to Japanese encephalitis vaccine. *Vaccine*, 15:1239–1243.

Plesner A et al. (2000). Case-control study of allergic reactions to Japanese encephalitis vaccine. *Vaccine*, 18:1239–1243.

Poland JD et al. (1990). Evaluation of the potency and safety of inactivated Japanese encephalitis vaccine in US inhabitants. *The Journal of Infectious Diseases*, 161:878–882.

Poneprasert B (1989). Japanese encephalitis in children in northern Thailand. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 20:599–603.

Reid M et al. (2006). Experimental infection of Culex annulirostris, Culex gelidus, and Aedes vigilax with a yellow fever/Japanese encephalitis virus vaccine chimera (ChimeriVax-JE). *American Journal of Tropical Medicine and Hygiene*, 75:659–663.

Rey FA et al. (1995). The envelope glycoprotein from tick-borne encephalitis virus at 2 A resolution. *Nature*, 375:291–298.

Rice CM (1996). Flaviviridae: the viruses and their replication. In: Fields BN, Knipe DM, Howley PM, eds. *Fields Virology*. Philadelphia, Lippincott-Raven.

Rosen L (1986). The natural history of Japanese encephalitis virus. *Annual Review of Microbiology*, 40:395–414.

Ruff TA et al. (1991). Adverse reactions to Japanese encephalitis vaccine. *Lancet*, 338:881–882.

Sabin AB (1947). Epidemic encephalitis in military personnel. *JAMA : the journal of the American Medical Association*, 133:281–293.

Sanchez JL et al. (1990). Further experience with Japanese encephalitis vaccine. *Lancet*, 335:972–973.

Schlesinger JJ et al. (1993). The Fc portion of antibody to yellow fever virus NS1 is a determinant of protection against YF encephalitis in mice. *Virology*, 192:132–141.

Schuller at al. (2008). Long-term immunogenicity of the new Vero cell-derived, inactivated Japanese encephalitis virus vaccine IC51. Six and 12 month results of a multicentre follow-up Phase 3 study. *Vaccine*, 26:4382–4386.

Schuller et al. (2009). Comparison of a single, high-dose vaccination regimen to the standard regimen for the investigational Japanese encephalitis vaccine, IC51. A randomized, observer-blind, controlled Phase 3 study. *Vaccine*, 27:2188–2193.

Service MW (1991). Agricultural development and arthropod-borne diseases: a review. *Revista de Saúde Pública*, 25:165–178.

Siraprapasiri T et al. (1997). Cost benefit analysis of Japanese encephalitis vaccination program in Thailand. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 28:143–148.

Smorodintsev AA et al. (1940). Etiology of autumn encephalitis in the Far East of the USSR. *Archiv für die gesamte Virusforschung*, 1:549–559.

Sohn YM (2000). Japanese encephalitis immunization in South Korea: past, present, and future. *Emerging Infectious Diseases*, 6:17–24.

Sohn YM et al. (1999). Primary and booster immune responses to SA14-14-2 Japanese encephalitis vaccine in Korean infants. *Vaccine*, 17:2259–2264.

Solomon T (1997). Viral encephalitis in Southeast Asia. *Neurol Infect Epidemiol*, 2:191–199.

Solomon T et al. (1998). Poliomyelitis-like illness due to Japanese encephalitis virus. *Lancet*, 351:1094–1097.

Solomon T et al. (2003). Origin and evolution of Japanese encephalitis virus in southeast Asia. *Journal of Virology*, 77:3091–3098.

Stadler K et al. (1997). Proteolytic activation of tick-borne encephalitis virus by furin. *Journal of Virology*, 71:8475–8481.

Su CM et al. (2001). Highly sulfated forms of heparin sulfate are involved in Japanese encephalitis virus infection. *Virology*, 286:206–215.

Sucharit S et al. (1989). Vectors of Japanese encephalitis virus (JEV): species complexes of the vectors. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 20:611–621.

Sugawara K et al. (2002). Development of Vero cell-derived inactivated Japanese encephalitis vaccine. *Biologicals*, 30:303–314.

Sumiyoshi H et al. (1987). Complete nucleotide sequence of the Japanese encephalitis virus genome RNA. *Virology*, 161:497–510.

Tandan JB et al. (2007). Single dose of SA14-14-2 vaccine provides long-term protection against Japanese encephalitis: a case-control study in Nepalese children 5 years after immunization. *Vaccine*, 25:5041–5045.

Tauber et al. (2008). Randomized, double-blind, placebo-controlled phase 3 trial of the safety and tolerability of IC51, an inactivated Japanese encephalitis vaccine. *The Journal of Infectious Diseases*, 198:493–499.

Thongcharoen P (1989). Japanese encephalitis virus encephalitis: an overview. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 20:559–573.

Tsai TF (2000). New initiatives for the control of Japanese encephalitis by vaccination. Report of a WHO/CVI meeting. Bangkok, Thailand, 13–15 October 1998. *Vaccine*, 18(Suppl. 2):S1–S25.

Tsarev SA et al. (2000). Phylogenetic analysis suggests only one serotype of Japanese encephalitis virus. *Vaccine*, 8(Suppl. 2):S36–S43.

Twiddy SS, Holmes EC (2003). The extent of homologous recombination in members of the genus Flavivirus. *The Journal of General Virology*, 84:429–440.

Uchil PD, Satchidanandam V (2001). Phylogenetic analysis of Japanese encephalitis virus: envelope gene based analysis reveals a fifth genotype, geographic clustering, and multiple introductions of the virus into the Indian subcontinent. *American Journal of Tropical Medicine and Hygiene*, 65:242–251.

Umenai T et al. (1985). Japanese encephalitis: current worldwide status. *Bulletin of the World Health Organization*, 63:625–631.

Vaughn DW, Hoke CH Jr. (1992). The epidemiology of Japanese encephalitis: prospects for prevention. *Epidemiologic Reviews*, 14:197–221.

Wang L-H et al. (2007). Japanese encephalitis outbreak, Yuncheng, China, 2006. *Emerging Infectious Diseases*, 13:1124–1125.

Wong SC et al. (2008). A decade of Japanese encephalitis surveillance in Sarawak, Malaysia: 1997–2006. *Tropical Medicine & International Health : TM & IH*, 13:52–55.

World Health Organization (2002). *Guidelines for the production and control of Japanese encephalitis vaccine (live) for human use.* Geneva, World Health Organization (WHO Technical Report Series, No. 910, Annex 3).

World Health Organization (2005). Global advisory committee on vaccine safety, 9–10 June 2005. *Weekly Epidemiological Record*, 80:242–243.

World Health Organization (2007). *Proposed revision: Recommendations for Japanese encephalitis vaccine (inactivated) for human use. Report of a WHO Expert Committee on Biological Standardization.* Geneva, World Health Organization, 8–12 October 2007. http://www.who.int/biologicals/publications/trs/areas/vaccines/jap_encephalitis/en/index.html. [Last accessed 16Feb2009].

World Health Organization (2006). Japanese encephalitis vaccines. *Weekly Epidemiological Record*, 81:331–340.

World Health Organization (2008). *The global burden of disease: 2004 update*. (http://www.who.int/healthinfo/global_burden_disease/GBD_report_2004update_full.pdf, last accessed 16 February 2009).

Wu CJ et al. (2006). Development of an effective Japanese encephalitis virus-specific DNA vaccine. *Microbes and Infection / Institut Pasteur*, 8:2578–2586.

Xin YY et al. (1988). Safety of a live-attenuated Japanese encephalitis virus vaccine (SA14-14-2) for children. *American Journal of Tropical Medicine and Hygiene*, 39:214–217.

Yang S-E et al. (2006). The efficacy of mouse-brain inactivated Nakayama strain Japanese encephalitis vaccine — results from 30 years experience in Taiwan. *Vaccine*, 24:2669–2673.

The World Health Organization has provided technical support to its Member States in the field of vaccine-preventable diseases since 1975. The office carrying out this function at WHO headquarters is the Department of Immunization, Vaccines and Biologicals (IVB).

IVB's mission is the achievement of a world in which all people at risk are protected against vaccine-preventable diseases. The Department covers a range of activities including research and development, standard-setting, vaccine regulation and quality, vaccine supply and immunization financing, and immunization system strengthening.

These activities are carried out by three technical units: the Initiative for Vaccine Research; the Quality, Safety and Standards team; and the Expanded Programme on Immunization.

The Initiative for Vaccine Research guides, facilitates and provides a vision for worldwide vaccine and immunization technology research and development efforts. It focuses on current and emerging diseases of global public health importance, including pandemic influenza. Its main activities cover: i) research and development of key candidate vaccines; ii) implementation research to promote evidence-based decision-making on the early introduction of new vaccines; and iii) promotion of the development, evaluation and future availability of HIV, tuberculosis and malaria vaccines.

The Quality, Safety and Standards team focuses on supporting the use of vaccines, other biological products and immunization-related equipment that meet current international norms and standards of quality and safety. Activities cover: i) setting norms and standards and establishing reference preparation materials; ii) ensuring the use of quality vaccines and immunization equipment through prequalification activities and strengthening national regulatory authorities; and iii) monitoring, assessing and responding to immunization safety issues of global concern.

The Expanded Programme on Immunization focuses on maximizing access to high quality immunization services, accelerating disease control and linking to other health interventions that can be delivered during immunization contacts. Activities cover: i) immunization systems strengthening, including expansion of immunization services beyond the infant age group; ii) accelerated control of measles and maternal and neonatal tetanus; iii) introduction of new and underutilized vaccines; iv) vaccine supply and immunization financing; and v) disease surveillance and immunization coverage monitoring for tracking global progress.

The Director's Office directs the work of these units through oversight of immunization programme policy, planning, coordination and management. It also mobilizes resources and carries out communication, advocacy and media-related work.

Department of Immunization, Vaccines and Biologicals

Family and Community Health

World Health Organization
20, Avenue Appia
CH-1211 Geneva 27
Switzerland
E-mail: vaccines@who.int
Web site: http://www.who.int/immunization/en/

ISBN 978 92 4 159971 9