Acute Encephalitis Syndrome Surveillance

Field Guide

Epidemiology Unit
Department of Public Health
Ministry of Health and Sports
The Republic of the Union of Myanmar

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Chapter 1 : INTRODUCTION

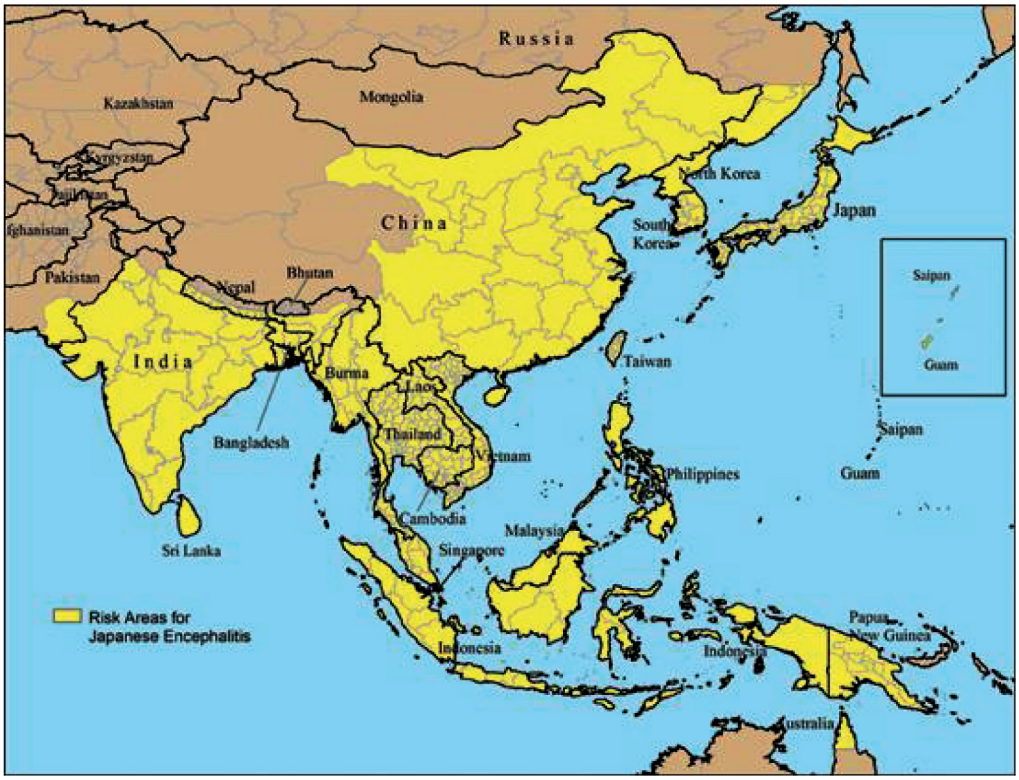
Japanese encephalitis (JE) is the leading cause of viral neurological disease and disability in Asia. JE is caused by an arbovirus in the Flaviviridae family that is similar to West Nile, Murray Valley and St. Louis encephalitis viruses. The virus is transmitted by mosquitoes. The first case of Japanese encephalitis viral disease (JE) was documented in 1871 in Japan. The global incidence of JE is unknown because the intensity and quality of JE surveillance and the availability of diagnostic laboratory testing vary throughout the world. In the late 1980s, Burke and Leake estimated that 50 000 new cases of JE occurred annually among the 2.4 billion people living in the 16 Asian countries considered endemic at the time (approximate overall annual incidence: 2 per 100 000). In the intervening two decades, despite major population growth, urbanization, changes in agricultural practices and increased use of the JE vaccine in many countries, this figure has been widely quoted, including very recently. In 2000, assuming an annual, age-group-specific incidence of 25 cases per 100 000, Tsai estimated that in the absence of vaccination 175 000 cases of JE would occur annually among Asian children aged 0–14 years living in rural areas.

The main JEV transmission cycle involves Culex tritaeniorhynchus mosquitoes and similar species that lay eggs in rice paddies and other open water sources, with pigs and aquatic birds as principal vertebrate amplifying hosts. Humans are generally thought to be dead-end JEV hosts, i.e. they seldom develop enough viremia to infect feeding mosquitoes. Less than 1% of human JEV infections result in JE.

Approximately 20–30% of JE cases are fatal and 20–30% of survivors have significant neurologic sequelae including intellectual, behavioral, and neurological sequelae. JE is primarily a disease of children and most adults in endemic countries have natural immunity after childhood infection, but all age groups are affected. In most temperate areas of Asia, JEV is transmitted mainly during the warm season, when large epidemics can occur. In the tropics and subtropics, transmission can occur year-round but often intensifies during the rainy season.

Fortunately, JE is preventable. Immunization can prevent infection. A vaccine has existed since the early 1940s.

The geographic distribution of Japanese encephalitis (in yellow)



Japanese Encephalitis in Myanmar

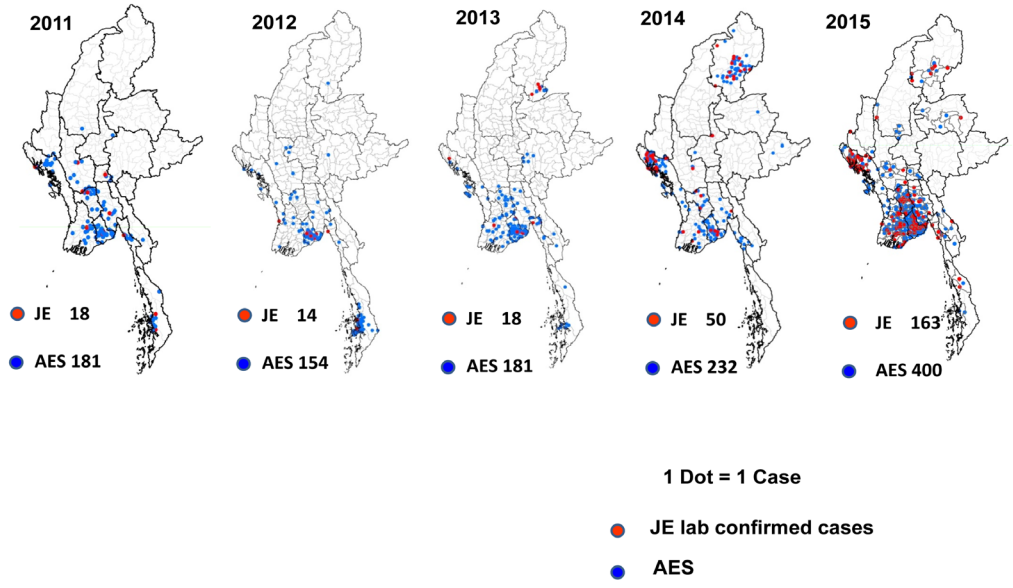
Central Epidemiological Unit first conducted serological survey for JE in 1988 and they found JE antibody in 1325 people. A study conducted from 1988-1989 shown that 19.5% of viral encephalitis admitted in Yangon Children Hospital were due to JE virus. Similarly other study in 2010 shows that 12.5% viral encephalitis cases admitted into Yangon Children Hospital were due to JE. In 2014, there were 319 CNS infection children admitted in to the hospital of which 26 dies. The outcomes of viral encephalitis cases were with only 28% complete recovery, 17% with movement disorder and 22% with motor deficit. Serological survey in 1973, among domestic herd all over the country showed about 90% of the pigs had presence of the JE HI antibody, whereas cattle and buffaloes showed a very low prevalence. JE outbreak was reported as early as in November 1977 at Animal Breeding Centre at Bahtoo Township, Southern Shan State.

There is a long history of JE/AES cases reported from Rakhine State since 1979 with alarming increasing cases in recent years (2014: 52 cases from 10 townships, 2015: up to July, 48 cases from 8 townships, 7 deaths and 16 confirmed JE case by lab). The main age group was from 1-14 years old, and most cases reported from May to August.

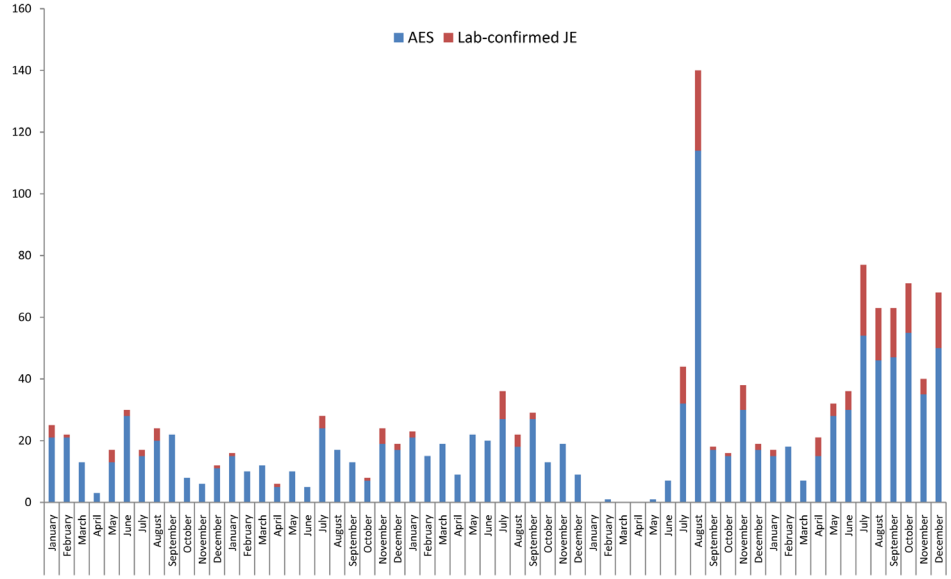
JE cases are detected in all states/regions of Myanmar. All age groups were affected but the highest group was children 5-9 years of age, then children 1-4 years of age. It's also

very alarming that in early 2015 (from Jan to June, 2015), data collected from 3 states and 4 regions shown that there were 36 JE positive cases from 211 serum samples and in Rakhine states alone, there were 45 AES cases with 5 deaths, 30 serum samples were tested with 6 samples were JE positive.

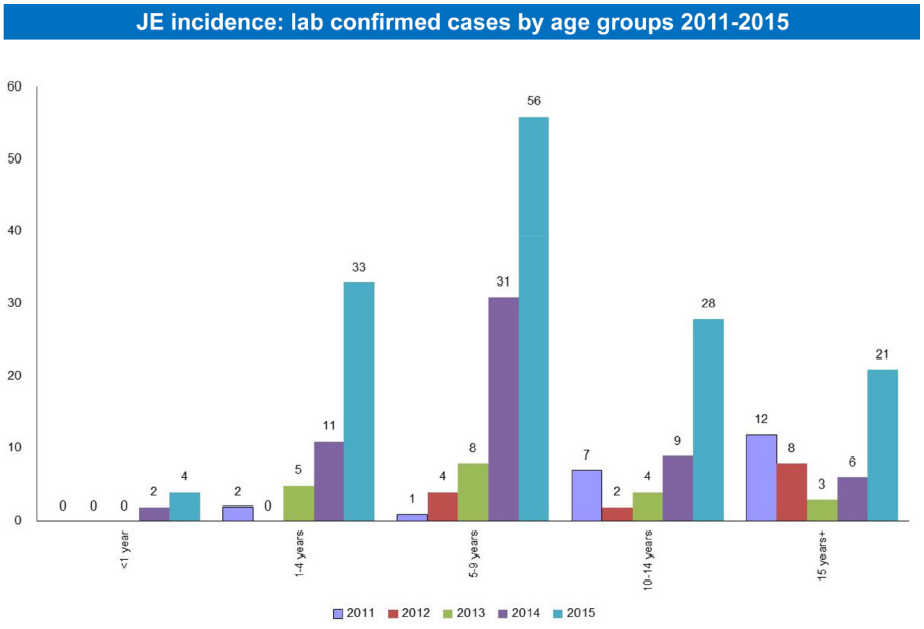
AES reported cases & JE positive cases (2011-2015)



JE incidence: lab confirmed and reported AES cases by months 2011-2015



Incidence was high in the latter half of the year, mostly in winter/cold season i.e. September, October and November



AES hospital based surveillance has been initiated in limited places since 2007. The data collected from surveillance shows that JE positive cases from total AES cases recoded were 18/181 (2011), 14/154 (2012), 18/181 (2013), 50/232 (2014), 48/198 (Jan-July 2015). The high risk age group ranged from 1-15 years old and more cases detected in the latter half of each year

The government responses included vector control (indoor residual spraying, aerial forging and breeding fishes that feed mosquito larvae), patient care and treatment, IEC activities and JE vaccination campaign was conducted in 8 townships of Rakhine state in 2015 reaching 43,896 children aged 9 months – 15 years.

Chapter 2 : Epidemiology of Japanese Encephalitis

Japanese encephalitis is caused by a parvovirus of the Flaviviridae family similar to West Nile, Murray Valley, and St. Louis encephalitis. It is a single-stranded RNA virus transmitted by a mosquito vector, most commonly of the Culex species. The pig is found to be the primary amplifying host of the virus. Birds, such as herons or ducks have also been implicated in the transmission of JE.

2.1 JE Transmission

JE virus is transmitted by mosquitoes, most commonly of the Culex species. The mosquito picks up the virus from animals (most commonly pigs and water birds), then transmits it to humans. The pig is the primary amplifying host. Birds, such as herons or ducks, have also been implicated in the transmission of JE. JE infection is not transmitted directly from person to person. Humans are generally infected "by accident", when bitten by infected mosquitoes. When an infected mosquito bites a person it transmits the virus and infection occurs.

People living in rural areas where rice is grown and pigs are raised face the highest risk of JE transmission. Staying outdoors after sunset is a risk factor since mosquitoes commonly bite in the twilight hours.

Children between the ages of 1 and 15 years are at higher risk than adults.

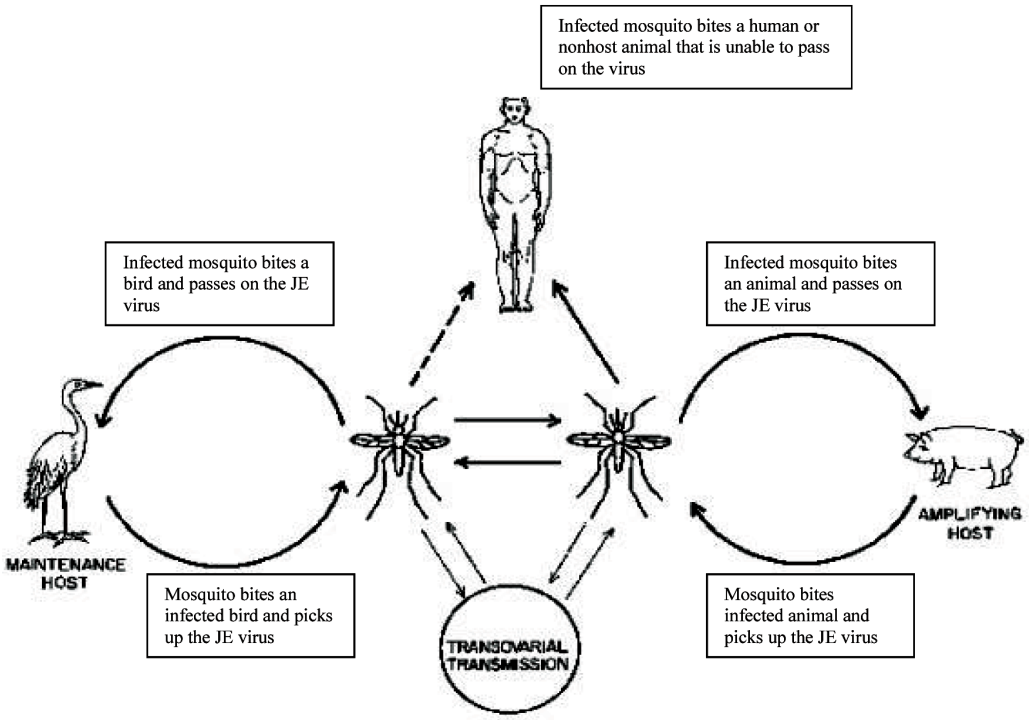
2.2 Seasonality of Transmission

In most temperate areas of Asia, JEV is transmitted mainly during the warm season, when large epidemics can occur. In the tropics and subtropics, transmission can occur year-round but often intensifies during the rainy season and pre-harvest period in rice-cultivating regions.

2.3 Age

In endemic areas, children 1-15 years old are most frequently infected with JE. In general, JE cases are infrequent among children younger than 1 year old, mainly, it is believed, because they have less exposure to mosquitoes. Adult infection most often occurs in areas where the disease is newly introduced because there is no established immunity among the population

Fig. 1 JE transmission cycle



2.4 Environmental factors

JE is typically found in rural areas with plentiful rice cultivation.

People living in rural areas where rice is grown and pigs are raised face the highest risk. Being outdoors after sunset is a risk factor since mosquitoes commonly bite in the twilight hours.

2.5 Clinical picture of JE

Less than 1% of people infected with Japanese encephalitis (JE) virus develop clinical illness. However, around 1 in every 250 people who become infected with Japanese encephalitis develops more severe symptoms, as the infection spreads to the brain. The incubation period for JE ranges from 5-15 days. The clinical picture of infection has 4 stages; prodromal, acute, subacute, and convalescence. The prodromal stage lasts from 2 to 3 days and has a high fever with severe headache. Non-specific symptoms include malaise, anorexia, nausea and vomiting. In the acute phase, lasting 3 to 4 days, the patient develops a change in the state of consciousness, which can range from mild clouding to stupor and coma. It is during this phase that patients frequently present for health care. Seizures are common and the patient remains febrile with weakness and stiff neck is frequently seen. Less commonly observed are tremor, abnormal movements and cranial nerve involvement. Fatal cases usually deteriorate rapidly at this stage and die.

The subacute phase lasts 7 to 10 days and in uncomplicated cases the fever decreases over a period of 1-2 weeks and neurological sequelae may improve. In severe cases, secondary infections are common during this phase including bladder infections, pneumonia, and bedsores. Close attention by caregivers can minimize these problems.

During the convalescence phase mild cases may recover completely over the next several weeks. Severe cases may improve somewhat but are frequently left with neurological sequelae. Late developing sequelae have also been described such as optic nerve degeneration and seizures.

The clinical presentation of JE cannot accurately be differentiated from other etiologies of meningio-encephalitis and requires laboratory diagnostic confirmation. Interestingly, in Vietnam 55% of patients identified with acute flaccid paralysis (AFP) were actually later diagnosed with JE.

2.6 Disability

Disability and sequelae have been found in 20 to 30% of surviving JE patients. Disability determinations vary depending on the type of sequelae included in the study and the timing of the follow up. Sequelae fit into 4 major categories: motor, behavioral, intellectual and other neurological. Motor deficits are common in approximately 30% of survivors with significant cognitive and language impairments in 20%. An inverse relationship exists between the percentages of survivors and the amount and severity of sequelae so that the more people survive the acute illness, the more people who are left with disabilities. There is also evidence to show that sequelae can develop as well as resolve over time. From a study in Thailand, fine motor disability, aggressiveness, uncontrolled emotion/impulsiveness, and abnormal intelligence were the most common sequelae occurring in greater than 70%.

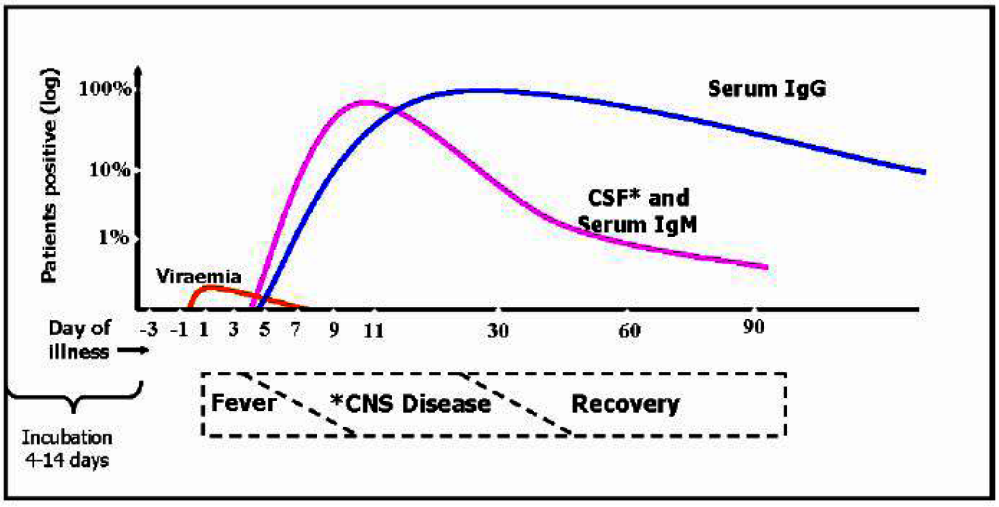
2.7 Diagnosis

Japanese encephalitis infections are asymptomatic in a majority of people infected with the JE virus. Estimates for the number of persons with asymptomatic infections compared to persons with confirmed symptomatic JE ranges from 50:1 to 300:1. A definitive diagnosis of JE can be made with viral isolation from cerebral spinal fluid (CSF) or, in fatal cases, CNS tissue; but viral isolation is not possible in most cases due to low titers and rapid neutralizing antibody. Identification of JE IgM in the CSF indicates the presence of JE virus in the central nervous system. The standard of JE diagnosis in practice is IgM-capture ELISA of a CSF specimen. Seventy-five percent of CSF and serum samples will be IgM positive at four days after onset of fever, but almost all samples drawn one week after presentation will be positive. The CSF will usually have a pattern that is consistent with a viral infection; slightly elevated protein, normal glucose, and lymphocyte pleocytosis. CSF is desired for diagnosis as it confirms a CNS infection and has higher titers than serum, however, a single serum specimen drawn at least 7 days after the

onset of fever in a patient with a clinical syndrome consistent with encephalitis can be diagnostic. Paired sera tested for total antibody can also be used for diagnosis looking for a 4-fold rise in titer between acute serum, drawn at presentation, and convalescent serum, usually drawn 2 weeks after the acute serum. Difficulty in diagnosis arises in areas where several Flaviviruses coexist. There is significant cross reactivity among Flaviviruses, particularly in IgG testing, so that Dengue and West Nile infections can give a small increase in JE titers and vice versa. It may be necessary to test for Dengue and West Nile in areas where these viruses co-circulate.

Antibodies begin to appear soon after onset, but only about 70-75% of patients have IgM antibody in specimens collected up to 4 days after onset. However, essentially all patients will have antibody 7-10 days after symptom onset.

Schematic antibody responses in JE infection



2.8 Treatment

There is no specific therapy for Japanese encephalitis. Care of JE patients is focused on supportive care. JE requires attentive care through the acute and convalescent phases and careful attention to early rehabilitation. By providing diligent care the case fatality rates can be greatly reduced. Antibiotics are not effective against viruses, and no effective antiviral drugs have been developed against JE. Supportive care, therefore, should focus on airway management, seizure control, decreasing cerebral edema, fluids and nutrition, fever control, and managing secondary infections.

2.9 Prevention

Immunization is the best way to prevent Japanese encephalitis. Personal protection against mosquito bites is also important. Mosquito control, bed nets and improved animal husbandry practices have been shown to be less effective.

Control programs for JE focus in three major areas; mosquito control, pig (amplifying host) control, and vaccination. However, neither mosquito control nor amplifying host (pig) control has been proven to be effective public health measures to control disease. Mosquito control can include spraying, draining mosquito habitats, and the use of bed nets. Such spraying is both resources intensive and expensive. While spraying is important in the control of many vector borne diseases, it is frequently ineffective in the control of JE. To be effective these control measures must cover all mosquito habitats, which include rice paddy fields, puddles and drainage areas. This is difficult anytime, but especially difficult in monsoon season and in rural rice growing areas where JE is most common. The time it takes a Culex mosquito to develop from an egg to an adult is 10-12 days. Therefore, in addition to the large area to be included in control programs, spraying must also be repeated very frequently (every 10-12 days) to control mosquito populations.

Bed nets have not been shown to be effective. The reason may be that the Culex mosquito bites in the twilight hours and only young children that are in bed in the early evening would receive benefit from their use. The at-risk population for Japanese encephalitis is 1-15 years of age so a large portion of the at-risk population will still be exposed despite the use of bed nets. Bed nets may be important for the control of many vector borne diseases besides JE and should be continued although alone cannot be relied on to control JE.

As the vector of JE is hard to control, additional efforts have been directed to the main amplifying host, the pig. Pig control has been attempted in three ways, segregation, slaughtering, or vaccination. Pigs must be segregated and contained at least 5 km from humans (the flying radius of the mosquito vector), which is not practical in most developing world settings. Slaughtering has a high economic impact and affects many families' ability to make a living. Pig vaccination has not been shown to significantly impact human cases of JE and is costly, difficult, and time consuming.

Vaccination of humans is the most effective and most cost-effective way to prevent Japanese encephalitis. Vaccination has been used to control JE in Japan, Nepal, India, Republic of Korea, Taiwan, China, and Thailand.

2.10 Vaccines

Infection with Japanese encephalitis confers lifelong immunity. There are 4 main types of JE vaccines currently in use: inactivated mouse brain-derived vaccines, inactivated Vero cell-derived vaccines, live attenuated vaccines, and live recombinant vaccines.

Inactivated JE vaccine

A formalin-inactivated mouse-brain derived vaccine was first produced in Japan in the 1930s and was validated for use in Taiwan in the 1960s and in Thailand in the 1980s. The widespread use of vaccine and urbanization has led to control of the disease in Ja-

pan, Korea, Taiwan and Singapore. The vaccine is recommended to be given as 1.0 ml injection administered one month apart for the first 2 doses and the third dose 1 year later with boosters at 3-year intervals. Hypersensitivity reactions have been described in 0.5% of recipients. Neurological complications including acute disseminated encephalomyelitis have been reported in Japan, Denmark, and Korea.

Live attenuated JE vaccine

The live attenuated JE vaccine SA 14-14-2 was developed in 1988 and used since in China. The vaccine has a 96%-98% efficacy in China. A study just completed in Nepal reports 99% efficacy with a single dose given within one week of an outbreak. SA 14-14-2 is provided in 1-dose or 5-dose vials; dosage is 0.5 mL for all ages. CD-JEV is a freeze-dried (lyophilized) vaccine that needs to be mixed with diluent before use (reconstitution). Prior to reconstitution it is a milky-white caked powder. After mixing with diluent it becomes a transparent pink liquid. The SA 14-14-2 was pre-qualified by WHO in 2013.

Inactivated Vero cell-derived vaccine

The newest vaccine in use is an inactivated SA 14-14-2 vaccine grown on Vero cells. This vaccine was approved in March 2009 for use in people aged 17 years and older and in May 2013 for use in children 2 months through 16 years of age in USA. It is now also licensed and available for use among adults in Europe and Australia. It is administered in a two dose series.

The WHO prequalified JE vaccines includes Inactivated Vero cell-based SA 14-14-2 (JEEV), Live attenuated SA 14-14-2 (CD-JEV) and Live recombinant SA 14-14-2 (IMOJEV).

2.11 Vaccine storage and handling

The live, attenuated (SA 14-14-2) vaccine and most inactivated mouse brain-derived vaccines are freeze-dried powders, which needs to be reconstituted with their diluents before use. These lyophilized or freeze-dried vaccines should be refrigerated between +2 and 8 degree C and should not be frozen at any time. Diluent must not be frozen but should be stored between +2^{o}C and +8^{o}C before reconstitution.

SA 14-14-2 vaccine comes with a vaccine vial monitor (VVM) sticker with an inner square that will darken if the vaccine has been exposed to higher temperatures long enough to affect its potency. JE vaccine has been reconstituted; opened vials can be used for up to 6 hours as long as they are kept within +2^{o}C and +8^{o}C. Any remaining reconstituted vaccine should be discarded after 6 hours or at the end of the immunization session, whichever comes first.

Chapter 3 : Surveillance for Japanese Encephalitis

3.1 Goal

Surveillance is defined as the ongoing and systematic collection, analysis, interpretation, and dissemination of data about cases of a disease and other factors influencing disease behaviour which is used as a basis for planning, implementing, and evaluating disease prevention and control activities including immunization activities.

The goal of JE surveillance is to define the disease burden and provide information to guide programmatic interventions.

3.2 Objectives

- To characterize the epidemiology and burden of JE
- To identify high-risk geographic areas & populations
- To determine the mortality and morbidity from Acute Encephalitis Syndrome (AES) and JE
- To determine the geographic and age distribution of AES and JE patients
- To determine the distribution of AES and JE over time and season.
- To determine the case fatality rates of JE and other AES cases
- To advocate for and guide programmatic interventions
- Detect early warning signals for an impending outbreak, so as to decrease mortality and morbidity due to JE by initiating timely appropriate public health measures.
- Strengthen laboratory services for serological diagnosis
- Assess the impact of control measures and vaccination

3.3 Recommended case definition

Infection with JE virus may be asymptomatic, or may cause febrile illness, meningitis, myelitis or encephalitis. Encephalitis is the most commonly recognized presentation and is clinically indistinguishable from other causes of an acute encephalitis syndrome (AES). Syndromic surveillance therefore aims to identify patients with AES and among these confirm JE infection using standard laboratory techniques.

3.4 Clinical case definition of AES

Clinically, a case of Acute Encephalitis Syndrome (AES) is defined as a person of any age, in any geographical region, at any time of year with the acute onset of fever and a change in mental status (including symptoms such as confusion, disorientation, coma, or inability to talk) AND/OR new onset of seizures (excluding simple febrile seizures).

3.5 Case classification

Suspected Case: A case that meets the clinical case definition for AES.

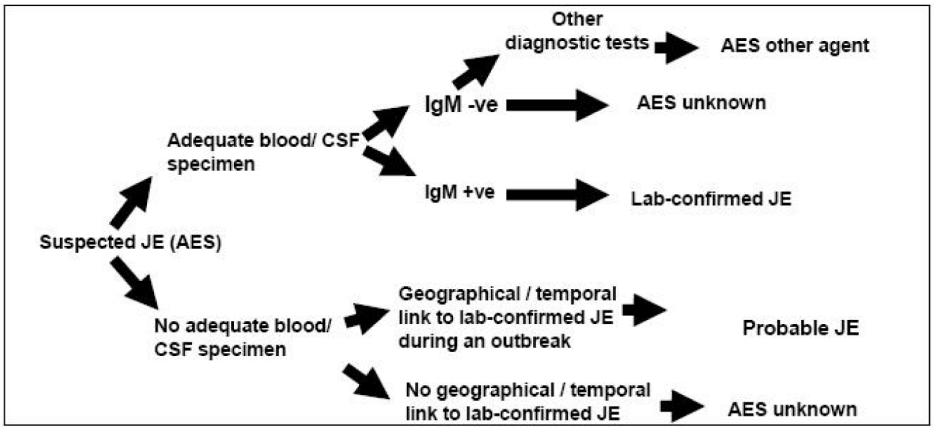
Laboratory-confirmed JE: A suspected case that has been laboratory-confirmed as JE.

Probable JE: A suspected case that occurs in close geographical and temporal relationship to a laboratory-confirmed case of JE, in the context of an outbreak.

"Acute encephalitis syndrome" – other agent: A suspected case in which diagnostic testing is performed and an etiological agent other than JE virus is identified.

"Acute encephalitis syndrome" – unknown: A suspected case in which no diagnostic testing is performed or in which testing was performed but no etiological agent was identified or in which the test results were indeterminate

Final Classification scheme for AES cases



Other early clinical findings may include an increase in irritability, somnolence or abnormal behaviour greater than that seen with usual febrile illness.

(WHO definition) – Simple febrile seizures occur in children aged 6 months to less than 6 years old whose only finding is fever and a single generalized convulsion lasting less than 15 minutes who recover consciousness within 60 minutes of the seizure.

3.6 Laboratory criteria for confirmation

Laboratory confirmation of JE virus infection includes:

1. Presence of JE virus-specific IgM antibody in a single sample of CSF or serum as detected by an IgM-capture ELISA specifically for JE virus OR any of the following:
2. Detection of JE virus antigens in tissue by immunohistochemistry; OR
3. Detection of JE virus genome in serum, plasma, blood, CSF5, or tissue by reverse transcriptase polymerase chain reaction (PCR) or an equally sensitive and specific

nucleic acid amplification test; OR

4. Isolation of JE virus in serum, plasma, blood, CSF or tissue; OR
5. Detection of four-fold or greater rise in JE virus-specific antibody as measured by haemagglutination inhibition or plaque reduction neutralization assay (PRNT) in serum collected during acute and convalescent phases of illness. The two specimens for IgG should be collected at least 14 days apart. The IgG test should be performed in parallel with other confirmatory tests to eliminate the possibility of cross-reactivity.

> The large majority of JE infections are asymptomatic. Therefore in areas that are highly endemic for JE, it is possible to have AES due to a cause other than JE virus and have JE virus-specific IgM antibody present in serum. To avoid implicating asymptomatic JE as the cause of other AES illnesses, sterile collection and testing of a CSF sample from all persons with AES is recommended when feasible.
> Because a serum sample collected on admission may not yet be positive in a JE-infected person, a second serum sample should be collected at discharge or after the 10th day of illness onset or at the time of death.

A serum sample should be obtained at admission. Because it may not yet be positive in a JE-infected person, a second serum sample should be collected at discharge or on the 10th day of illness onset or at the time of death and tested for presence of JE virus specific IgM

Further confirmatory tests (e.g. looking for cross-reactivity with other flaviviruses circulating in the geographical area) should be carried out: (a) when there is an ongoing dengue or other flavivirus outbreak; (b) when vaccination coverage is very high; or (c) in cases in areas where there are no epidemiological and entomological data supportive of JE transmission

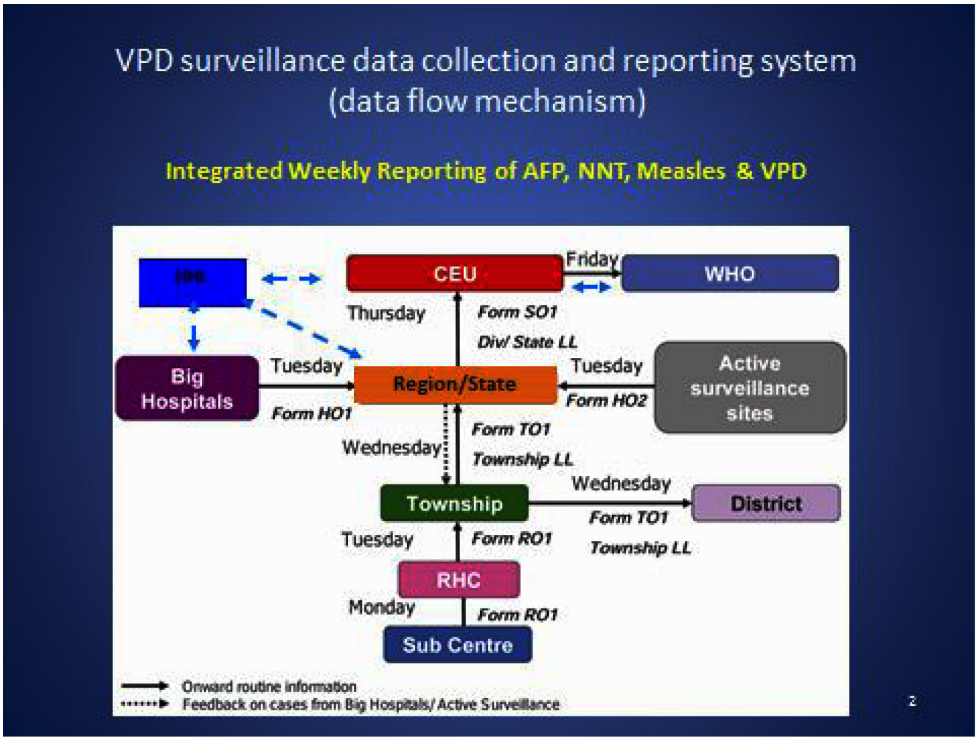
Detection of virus genome or virus isolation in serum, plasma or blood is very specific for JE diagnosis; however, it is not sensitive as virus levels are usually undetectable in a clinically ill JE case. Therefore a negative result by these methods should not be used to rule out JE in a suspected case. Similarly detection of virus genome or virus isolation in CSF is usually only found in a fatal cases and therefore not very sensitive and should not be used for ruling out a diagnosis of JE.

3.7 AES Reporting System

AES cases will be reported on weekly basis from all hospitals including Station Hospitals. All the reporting hospitals below township and at township level should report AES to the TPHO; hospitals at State and Regional level should report to State and Regional Public Health Department and after compilation of information State/Region should report to Central Epidemiology Unit as per established reporting mechanism for integrated disease surveillance. Each reporting hospital should identify one individual (and one or two alternates) who is responsible for identifying and reporting cases of AES. The TPHO,

SDCU Team Leader, RSO should make sure all necessary forms have been filled and carry out active surveillance in hospitals.

Reporting by the hospitals to TPHO, State and Regional Public Health Department and Central Epidemiology Unit should take place even when no cases of AES have been identified **("zero reporting")** – that is, even if zero cases of AES was detected during the previous week.



The components of AES case reporting include:

- **Initial Identification and Reporting of AES Cases:** Each hospital should identify one individual (and one or two alternates) who is responsible for identifying and reporting of VPD cases to TPHO or SDCU team leader.

- **Active Surveillance:** One of the most critical units in the reporting system is the hospital. Case finding through the emergency department, pediatric, medical and neurology wards, as well as through outpatient clinics, is critical to the success of this surveillance system. The TPHO, SDCU Team Leader or RSO should regularly visit these hospitals to ensure that AES cases are reported, encourage reporting from private doctors, and look for new cases. Apart from this, health workers at all levels must be encouraged to report all AES cases immediately.

- **Reporting from hospital:** If the AES case is reported/admitted in or from the hospital the responsible clinician should collect sample (CSF or serum) and inform focal

person for sample transportation to NHL. The RSO will facilitate transportation of samples from hospitals to NHL. The TPHO, SDCU Team Leader or RSO should ensure no AES cases are missed from reporting from the hospitals. The case investigation form should be filled by medical officer.

- **Follow-up:** The TPHO, SDCU Team Leader, RSO or Medical Officer must re-examine every case of laboratory confirmed JE six months after the onset of disease to confirm and report the presence or absence of disability and sequelae.

3.8 Investigation of clustering of AES cases

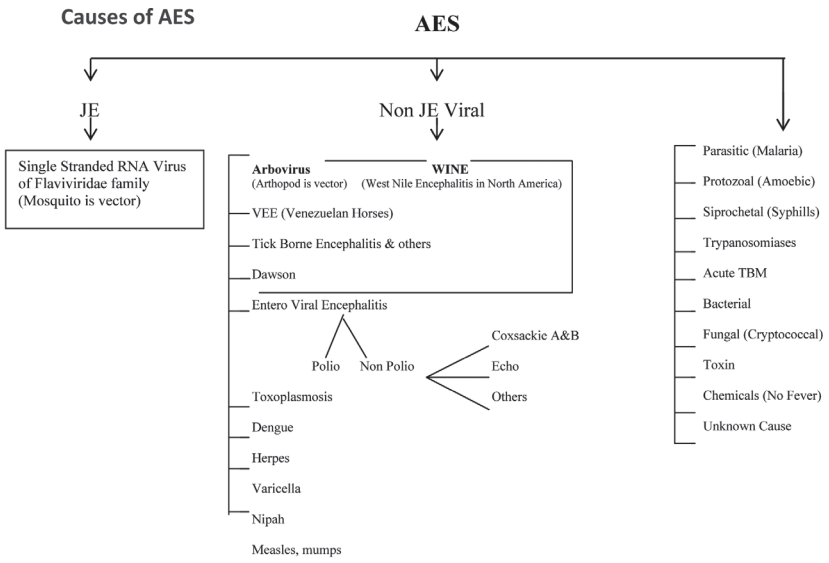
If clustering of AES cases are reported from same area outbreak should be suspected and investigated. The Rapid Response Teams (RRT) should investigate within 48 hours after receiving the information about the clustering of AES cases. Vector Borne Disease Control Officer (VBDC) or any other designated officer must personally see the case to ascertain if the case meets the AES case definition. Assess for presence of reservoir host such as pigs, cattle and poultry in the near vicinity of suspected cases. Vector surveillance should be initiated immediately, which should include collection of larvae and adult mosquitoes, identification of vector species, density and for incrimination of the vector mosquitoes. Past history of JE outbreak in that area must be reviewed

Determining the cause of clustering of AES cases

After an outbreak has been investigated the TPHO, SDCU-TL and RSO should review all reported cases and assign them a final classification based on either clinical history/signs or laboratory results.

The data should then be analyzed to determine why and where the outbreak occurred. These data will help to identify in which group the susceptible individuals have accumulated.

Outbreaks of JE are often associated with higher morbidity and mortality than found in sporadic cases. Identifying outbreaks early and ensuring that appropriate case management procedures are followed can significantly reduce associated complications and mortality



3.9 Recommended data analyses

- number and incidence of suspected cases by week, month, year, age group, and
- geographic area;
- number and incidence of confirmed cases by week, month, year, age group, and
- geographic area;
- JE vaccine coverage by year and geographical area;
- percentage of cases vaccinated and unvaccinated; and
- Completeness/timeliness of monthly reporting by geographical area.
- suspected and confirmed cases – age-specific, gender-specific, geographic area specific, and immunization status-specific incidence;
- percentage of suspected cases with CSF and/or serum specimens collected;
- percentage of cases with serum 10 or more days after onset of illness (when testing methodology is IgM-capture ELISA);
- case fatality ratio;
- final classification of all suspect cases; and
- proportion of AES attributed to JE

Performance indicators of surveillance quality

Indicator	Target
Completeness of weekly zero reporting	>90%
Timeliness of weekly zero reporting	>80%
Percentage of serum samples taken a minimum of 10 days after onset of illness	>80%
Percentage of all suspect cases for which specimens were collected	>80%
Percentage of CSF/serum samples reaching laboratory in adequate (means the specimen is transported using reverse cold chain). condition	>80%
For all tests, laboratory results reported < 1 month after receipt specimen	>80%

Chapter 4: Laboratory Aspects of JE Surveillance

Clinical signs of JE are indistinguishable from other cases of AES. Laboratory confirmation is essential for accurate diagnosis of JE.

4.1 Type of sample, collection, storage and transportation for laboratory diagnosis

Blood and cerebrospinal fluid (CSF) are the most likely specimens to be referred for detection of IgM antibodies to Japanese Encephalitis Virus. These samples (blood or CSF) should be collected as soon as possible after the admission to hospital or when patient first seen. IgM antibody rises steadily after onset of encephalitis. The percentage of patients with IgM detectable in serum increases with days after onset. As JE IgM may take up to 10 days to develop after symptoms first develop, a second serum sample should be collected at discharge or on the 10th day of illness onset or at the time of death

a) Cerebro-spinal Fluid (CSF)

- The findings of JE IgM in CSF confirms the diagnosis of JE
- IgM to JE virus rises earlier in CSF than in serum and rises to higher level in CSF than in serum (2-4 times)
- CSF is the preferred sample for the diagnosis of JE. For this purpose, about 2 ml of CSF collected in sterile vial / container.
- CSF should be transported as soon as possible (ideally within 1 hour) to the laboratory. CSF specimens for routine investigations should not be refrigerated or exposed to extreme cold, excessive heat or sunlight. However if there is likely to be delayed beyond one hour, specimens for virology should be refrigerated.
- CSF samples for virological testing should be sent to designated laboratory as soon as possible. Before the transport in the laboratory they should be kept at 4 degree C for 1-3 days or at or below -20 degree C for longer term storage. If the specimens have been frozen, they should be transported frozen. Repeated freezing and thawing of CSF should be avoided as this may lead to instability of IgM antibodies.

b) Blood

- A JE IgM positive result from the serum of a patient with encephalitis is a good indicator of acute infection (although there is a problem of some cross reactivity with other flaviviruses such as dengue)
- Blood samples should be collected by vein puncture and placed in a dry sterile vial. The volume should be approximately 5 ml for older children and adults and 1ml for infants and younger children.

- Whole blood is allowed to clot at room temperature and then stored in a cold box or refrigerator and maintained at 4–8 °C (and not frozen). It should be transported to the hospital laboratory within 24 hours.

- Once the clot is formed one can separate the serum from the clotted blood by retracting the clot by a sterile stick followed by centrifugation at 1000g for 20 minutes. Note: If there is no centrifuge, blood should be kept in the refrigerator until there is complete retraction of the clot from the serum (but no longer than 24 hours).

- Carefully remove the serum, avoiding extracting red cells, and transfer aseptically to a sterile labeled vial.

- Label vial with patients name or identifier, date of collection and specimen type. Fill in case investigation forms completely.

- Store serum at 2-8 0C until it is ready for shipment. Separated serum samples should be shipped on wet ice within 48 hours, or stored at 2-80C for a maximum period of 7 days.

- Serum samples received for IgM analysis should be tested as soon as possible after receipt in the laboratory. Short-term storage of serum (1-3 days) should be at 4oC. Longer term storage of serum should be at or below –20oC. Repeated freezing and thawing of serum should be avoided as this may lead to instability of IgM antibodies

4.2 Shipment of specimens

1. Specimens should be shipped to the referral laboratory as soon as possible. Do not wait to collect additional specimens before shipping.

2. Place specimens in ziplock, plastic bags or shipment box (Styrofoam boxes)

3. Place specimen form and investigation form in the plastic bag and tape to inner top of Styrofoam box.

4. Place four frozen ice packs in the Styrofoam box along the sides. Then place the sample box in the center.

5. Arrange shipping date and time.

6. When arrangements are finalized, inform the receiver of time and manner of transport.

AES Case Investigation Form

Acute Encephalitis Syndrome
Case Investigation Form

Case Identification Number:
AES MMR _ _ _ - _ _ _ - _ _ - _ _ _

1. Report/Investigation Information:
Date Case Reported: ____ /____ / ____
Date Case Investigated: ____ / ____ / ____

Name of Investigator(s): _____
Title/Office: _____
Notified by: _____

2. Case Identification:
Date of Birth: __ / __ / __
Father's Name: _____
Full Permanent Address: State/Region: ----------
Village/ward: _____

Patient's Name: _____
Age: years ___ months ___ Sex: ___
Mother's Name:_____
Township: _____
Street No. or Name & House No._____

3. Hospitalization: Yes/No
Name of Hospital:_____
Clinical Diagnosis:_____
Outcome: Recovered completely /Recovered with disability/ Death/Unknown

Date of Hospitalization: ___ / ___ / ___
Hospital Record Number: _____

4. Immunization History:
Vaccinated against JE? Yes / No / Unknown
Total JE doses received through campaign:_____ Date of last JE dose: ___/___/___

5. Sign and Symptoms:
Rapid Onset: Yes / No / Unknown
Headche: Yes / No / Unknown
Neck Stiffness: Yes / No / Unknown
Stupor: Yes / No / Unknown
Paresis : Yes / No / Unknown

Date of onset of sysptoms: ___/___/___
Change in mental status: Yes / No / Unknown
Seizure: Yes / No / Unknown
Travel History (2 weeks before the onset): Yes / No / Unknown
If yes, where: _____

6. Serum Specimen Collection:

	Date Collected	Date Sent to Lab	Date of Result	Laboratory Results (circle)
Serum 1	___/___/___	___/___/___	___/___/___	Positive / Negative / Un-interpretable / Pending
Serum 2	___/___/___	___/___/___	___/___/___	Positive / Negative / Un-interpretable / Pending

7. CSF Specimen Collection:

	Date Collected	**Date Sent to Lab**	**Date of Result**	**Laboratory Results (circle)**
CSF 1	**___/___/___**	**___/___/___**	**___/___/___**	**Positive / Negative / Un-interpretable / Pending**
CSF 2	**___/___/___**	**___/___/___**	**___/___/___**	**Positive / Negative / Un-interpretable / Pending**

8. Case Classification: Lab confirmed/ Probable /AES-other agent/AES-unknown

9. Signature of Investigator _____

Case Definition of AES: Clinically, a case of Acute Encephalitis Syndrome (AES) is defined as a person of any age, in any geographical region, at any time of year with the acute onset of fever and a change in mental status (including symptoms such as confusion, disorientation, coma, or inability to talk) AND/OR new onset of seizures (excluding simple febrile seizures).

Japnese Encephalitis
Laboratory Request Form
(to accompany serum/CSF specimens to laboratory)

Case Identification Number:
AES MMR _ _ _ - _ _ _ - _ _ - _ _ _

PART I: To Be Filled Out by Case Investigator:

Report/Investigation Information:

Name of Investigator:_____
Hospital:_____

Date Case Reported: ____/____/____
Date Case Investigated: ____/____/____

Case Information:

Patient's Name: _____
Sex: _____ Date of Birth: ____/____/____ Age: years____ months____
Permanent Address: State/Region:---------- Township: _____
Village: _____ Ward: _____
Street No. or Name: _____ House No.: _____
Date of onset of symptoms :____/_____/_____
Provisional clinical diagnosis: _____

Specimen Collection:

	DATES				
	Collected	Sent to Referral Lab	Rec'd in Ref. Lab	Condition*	Lab ID Number
Serum 1	___/___/___	___/___/___	___/___/___	good / poor	
Serum 2	___/___/___	___/___/___	___/___/___	good / poor	
CSF 1	___/___/___	___/___/___	___/___/___	good / poor	
CSF 2	___/___/___	___/___/___	___/___/___	good / poor	

Please send the specimens results to:
National Public Health Laboratry, Yangon E-mail: ommar swetin <ommarswetin@gmail.com>

PART II: To Be Filled Out by Receiving Lab

Name/location of laboratory: _____ Date test performed: **___/___/____**
Test performed by (name and designation): _____
Type of test: _____

Laboratory results:
Serum 1 Positive / Negative / Un-interpretable / Pending
Serum 2 Positive / Negative / Un-interpretable / Pending
CSF 1 Positive / Negative / Un-interpretable / Pending
CSF 2 Positive / Negative / Un-interpretable / Pending

Date Results sent to CEU: ____/_____/_____ Other test if done and result_____
Specimens sent to reference lab: Yes / No
Date specimens sent to reference lab: ____/____/_____
Comments: _____

PART III: To Be Filled Out byReference lab

Name/location of laboratory: _____ Date test performed: ___/___/____
Test performed by (name and designation): _____
Type of test: _____

Laboratory results:
Serum 1 Positive / Negative / Un-interpretable / Pending
Serum 2 Positive / Negative / Un-interpretable / Pending
CSF 1 Positive / Negative / Un-interpretable / Pending
CSF 2 Positive / Negative / Un-interpretable / Pending

Date Results sent to NHL / CEU/WHO: ____/_____/_____
Comments: _____

Surveillance of Acute Encephalitis Syndrome

☐ ROUTINE SURVEILLANCE ☐ OUTBREAK

RSO: _____ Region/state: ____ Duty Station: _____ Outbreak Code: OBR-___-___-___

Date outbreak reported to RSO: ___/___/___

Year: _____ Month: ___ Week: _____ Date Report Sent to CEU/WHO: ___/___/___ Outbreak reported by: _____

Source*	Case ID No. (VE-NEP-)	Name Reporting Institution	Date of patient's visit/ investigation	Patient Name	Address		Sex (M/F)	Age	Ever had JE vaccine? (Y/N/U)	Hospitalized (Y/N/U)	Provisional Diagnosis	Outcome (Cured/ Refered/ Death/ Unknown	Travel history 2 weeks before onset (Y/N/U), If yes, specify place	History pr Physical Signs (Y/N/U)							Follow up**		Lab Investigation			Case classification (Suspected/ Probable/ Confirmed)	MP Test (+Ve, -Ve, Not Done)
					Township	Village/Muni.								Data Onset of Symptoms (Fever)	Fever	Headache	Neck Regidity	Disorientation	Unconscious	Convuisions	Lost of coordination	Disability/ Sequelae at time discharge	Disability/ Sequelae after 6 months of onset	Specimen Taken (Y/N/U)	Nature of specimen (CSF/ Serum)	Intrepretation		

*1= Outbreak investigation, 2=Active Surveillance, 3=Reporting Unit, 4=Other (health institutions other than reporting units)

*If yes: 1=Motor, 2= Behavior, 3= Intellectual, 4=Other Neurologicf Sequelae; if no: 5=No Disability/Sequelae

MP=Malaria Parasite

Laboratory Request/Report Form for Diagnosis of Acute Encephalitis Syndrome

Date of Test Performed: _____/_____/_____

Date of results sent to CEU/WHO: _____/_____/_____

PATIENT DETAILS								SPECIMEN DETAILS						JE IgM CAPTURE SLISA TEST			Reference Lab	
			Address							DATES (DD-MM-YYYY)								
Case ID	Patient Name	Name of Health Facility	Township	Village/ Urban	Sex (M/F)	Age	Date onset of symptoms (Fever)	Spec. No.	Nature of specimens (CSF/ Serum)	Collection	Sent to Lab	Received at Lab	Condition (Good/ Poor)	Lab ID Number	Titre Units	Intrepretation (Positive/ Negative/ Indeterminate)	Date sample sent to RL	Results
								1st										
								2nd										
								1st										
								2nd										
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								2nd										

Name of the Laboratory : ______________________ Test Performed By : ______________________ Approved By : ______________________