Fourth Hands-on Training Workshop on the Laboratory Diagnosis of Japanese Encephalitis in the Western Pacific Region

Osong, Republic of Korea
23–27 September 2013
Participants of the Fourth Hands-on Training Workshop on the Laboratory Diagnosis of Japanese Encephalitis in the Western Pacific Region, Osong, Republic of Korea, 23–27 September 2013
REPORT
FOURTH HANDS-ON TRAINING WORKSHOP ON THE LABORATORY DIAGNOSIS OF JAPANESE ENCEPHALITIS IN THE WESTERN PACIFIC REGION

Convened by:
WORLD HEALTH ORGANIZATION
REGIONAL OFFICE FOR THE WESTERN PACIFIC

Not for sale

Printed and distributed by:
World Health Organization
Regional Office for the Western Pacific
Manila, Philippines

April 2014
NOTE

The views expressed in this report are those of the participants of the Fourth Hands-On Training Workshop on the Laboratory Diagnosis of Japanese Encephalitis in the Western Pacific Region and do not necessarily reflect the policies of the World Health Organization.
SUMMARY

The Fourth Hands-on Training Workshop on the Laboratory Diagnosis of Japanese Encephalitis (JE) in the Western Pacific Region was held at the Korea Centers for Diseases Control and Prevention (Korea CDC) in collaboration with the Korea Human Resources Development Institute for Health and Welfare in Osong, Republic of Korea from 23 to 27 September 2013. The training was attended by 14 participants from JE network laboratories in Cambodia (n=2), China (n=2), Japan (n=1), the Lao People’s Democratic Republic (n=2), Malaysia (n=1), Papua New Guinea (n=1), the Philippines (n=2), the Republic of Korea (n=1) and Viet Nam (Ha Noi and Ho Chi Minh City) (n=2). In addition to the support from the WHO Secretariat, temporary advisers from the United States Centers for Disease Control and Prevention (US CDC) and the National Institute of Infectious Diseases (NIID), Japan attended the training as facilitators.

The objectives of the workshop were:

1. to enhance knowledge and skills of national JE laboratory staff by:
   (a) performing ELISA and other laboratory techniques for laboratory diagnosis of JE;
   (b) familiarizing them with the requirements for laboratory quality assurance of JE diagnosis as a WHO-network laboratory, including confirmatory testing, proficiency testing and WHO accreditation;

2. to further familiarize participants with laboratory data management using the WHO JE laboratory data reporting format for reporting to the Regional Office for the Western Pacific; and

3. to distribute 2013 proficiency panel samples to network laboratories.

The five-day training programme included lectures, country presentations, practical sessions and discussions. The hands-on activities allowed participants to perform the tests with facilitators, and thus provided better opportunities to learn. Participants were taught the recommended procedures for testing serum and CSF using the Panbio JE-Dengue IgM Combo enzyme-linked immunosorbent assay (ELISA) as well as the Inbios JE Detect IgM Capture ELISA (MAC-ELISA), which will replace the Panbio kit in the JE laboratory network in mid-2014. An algorithm for differentiating dengue cases from JE using the Inbios Dengue Detect IgM MAC-ELISA was discussed and will be in place before the Panbio assay is no longer available.

Lecture sessions comprised (1) Japanese encephalitis/acute encephalitis syndrome (JE/AES) surveillance and laboratory network, (2) quality assurance of the JE laboratory network and (3) introduction of JE IgM ELISA. Following a general introductory lecture of IgM capture assay for JE, four days of practical, hands-on training sessions were held. Fourteen participants were grouped into seven pairs for the practical sessions.

On the first and second day, practical sessions on Panbio JE-Dengue Combo ELISA were performed using serum and CSF samples, respectively. Inbios JE Detect IgM Capture ELISA was also performed using CSF samples. During the incubation period of the ELISA plates, the participants performed calibration and maintenance of the micropipettors and ELISA equipment for laboratory quality assurance. After each practical session, results were
analysed, calculated and validity of the results was determined. Results of the previous tests were reviewed by the participants and the facilitators.

On the third day, a practical session on Inbios JE IgM Capture ELISA using CSF samples was performed. Participants and facilitators reviewed the results of testing using the Inbios assay and discussed the future algorithm of JE testing. Specimen collection, preparation and shipment for virus isolation and serology were presented. Other laboratory testing methods such as plaque reduction neutralization test (PRNT) and decision algorithm used in US CDC were also presented.

On the fourth day, the participants reported the current JE surveillance and laboratory activities in their countries. Vector surveillance and pig surveillance in Japan, the Republic of Korea and China were also presented. The ELISA results performed in the duration of the workshop were extensively discussed among the participants.

On the last day of the workshop, participants completed a course assessment to measure the workshop's overall success and took a quiz. To familiarize the participants with other JE and dengue laboratory methods, PRNT and virus isolation were demonstrated. The 2013 WHO JE proficiency test samples were distributed to participating laboratories, and an additional set was given to Malaysia for the Institute for Medical Research and to the Lao Peoples Democratic Republic for Mahosot Hospital. Participants were requested to submit the results of the proficiency test within 14 days using appropriate assays in their laboratories.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. INTRODUCTION</strong></td>
<td>1</td>
</tr>
<tr>
<td>1.1 Objectives</td>
<td>2</td>
</tr>
<tr>
<td>1.2 Participants</td>
<td>3</td>
</tr>
<tr>
<td>1.3 Training programme</td>
<td>3</td>
</tr>
<tr>
<td><strong>2. PROCEEDINGS</strong></td>
<td>3</td>
</tr>
<tr>
<td>2.1 Opening session</td>
<td>3</td>
</tr>
<tr>
<td>2.2 Lecture sessions</td>
<td>3</td>
</tr>
<tr>
<td>2.3 Country reports</td>
<td>9</td>
</tr>
<tr>
<td>2.4 Vector and pig surveillance</td>
<td>17</td>
</tr>
<tr>
<td>2.5 Additional presentations</td>
<td>19</td>
</tr>
<tr>
<td>2.6 Introduction of JE IgM ELISA</td>
<td>23</td>
</tr>
<tr>
<td>2.7 Practical sessions</td>
<td>24</td>
</tr>
<tr>
<td><strong>3. CONCLUSIONS</strong></td>
<td>24</td>
</tr>
<tr>
<td>3.1 General</td>
<td>24</td>
</tr>
<tr>
<td>3.2 Evaluation of the workshop</td>
<td>25</td>
</tr>
<tr>
<td>3.3 Main outcomes of the training</td>
<td>25</td>
</tr>
<tr>
<td>3.4 Workshop follow-up</td>
<td>26</td>
</tr>
</tbody>
</table>

**ANNEXES:**

- ANNEX 1 - TIMETABLE
- ANNEX 2 - LIST OF PARTICIPANTS, OBSERVERS, TEMPORARY ADVISERS AND SECRETARIAT
- ANNEX 3 - INSTRUCTIONS AND PROTOCOLS
- ANNEX 4 - LECTURES AND PRESENTATIONS
- ANNEX 5 - COUNTRY REPORTS

**Keywords:**

Encephalitis, Japanese-prevention and control/ Laboratories-standards, utilization/ Enzyme-linked immunosorbent assay/Quality control/Vaccines/Health personnel-education
1. INTRODUCTION

Japanese encephalitis (JE) is an important cause of death and disability and is a significant public health problem for many countries in Asia. Substantial advances have been made in recent years in the development of improved JE vaccines and in the availability of high-quality commercial diagnostics that can be used for surveillance. Consequently, several countries in the Western Pacific Region have established laboratory-supported JE surveillance, and some countries have introduced JE vaccine into their routine vaccination programmes. Enhanced surveillance activities are needed to determine the disease burden and to monitor vaccination programmes.

In the Western Pacific Region, 1.74 billion people in 11 countries are at risk for JE virus (JEV) infection. So far, JE is known to be endemic in seven countries in the Region. China, Malaysia, the Republic of Korea and Viet Nam have partially or fully controlled human disease through vaccination, while Cambodia, the Lao People's Democratic Republic and the Philippines have demonstrated some evidence of endemic JEV transmission but have no vaccination programme. Transmission may be geographically limited in some countries, as in Australia and Malaysia. Australia has a JE vaccination programme only in the Torres Strait, where JE has been endemic since 1995. Malaysia has had a JE vaccination programme in Sarawak state since 2002. Papua New Guinea is a country in the Region with presumed endemic JEV transmission but without clear disease burden documentation.

Major progress has been made in JE control in the Western Pacific Region in the 1990s and 2000s. Human disease largely has been eliminated in Japan and the Republic of Korea, where sustained vaccination programmes protect 11% of the total regional population at risk. China has recorded a more than 90% decline in JE cases since the 1980s with the introduction of government-supported, low-fee JE immunization in high-risk provinces. Users fees for Expanded Programme on Immunization (EPI) vaccinations, including JE vaccine, were abolished in China in 2005, and a decision was taken in December 2007 to introduce JE vaccine nationwide for eligible children by 2010. A similar decision was taken by Viet Nam, which reports the second highest number of JE cases in the Region. Immunization programme expansions in these two countries ultimately will protect 82% of the Region's population from JE.

Disease burden has yet to be established in other low-income countries because of poor disease surveillance infrastructure. Low-income countries demonstrate the greatest need for support to control JE. In many countries, there is no routine surveillance, and in those that do have surveillance activities, there are few resources to introduce vaccine when JE disease is demonstrated. Cambodia introduced routine immunization with the live attenuated SA 14-14-2 JE vaccine in three pilot provinces since 2009, with nationwide expansion planned for the coming years. The Philippines established sentinel hospital-based surveillance. Efforts are being made to restart surveillance in the Lao People's Democratic Republic and Papua New Guinea.

The JE laboratory network (LabNet) for the Western Pacific Region was formed based on the WHO poliomyelitis and measles/rubella laboratory network models. Some WHO measles/rubella laboratories were also designated as WHO national JE laboratories. The purpose of this network is to improve and standardize the capability of JE diagnosis in countries where JE is endemic. The network consists of two global specialized laboratories in Japan and the United States of America, two regional reference laboratories (RRLs) in China and the Republic of Korea, and seven national laboratories in Cambodia, the Lao People’s Democratic Republic,
Malaysia, Papua New Guinea, the Philippines and Viet Nam (one in Ho Chi Minh City and one in Ha Noi).

Most laboratories in the JE network are using in-house or locally produced JE kits (see below), and the balance use the WHO-recommended commercial kit, Panbio JE-Dengue IgM Combo ELISA (Inverness Medical, Brisbane, Australia). However, the Panbio kit will no longer be available in 2014 and will be replaced with Inbios JE Detect IgM Capture ELISA and DENV Detect IgM Capture ELISA (Seattle, WA, USA). The Chinese Center for Disease Control and Prevention (China CDC) uses locally produced JE commercial kits (Beixi, Shanghai, China). Three JE network laboratories in the Region, namely, the National Institute of Infectious Diseases (NIID) in Japan, the National Institute of Hygiene and Epidemiology (NIHE) and Pasteur Institute in Viet Nam use their own in-house assays. The in-house assay used by NIHE is licensed in Viet Nam. Other techniques, such as reverse transcription polymerase chain reaction (RT-PCR), virus isolation, haemagglutination inhibition (HI) test and plaque reduction neutralization test (PRNT) are also being employed by JE laboratories in China, Japan, Malaysia, the Republic of Korea and Viet Nam.

For quality assurance of the JE LabNet, annual proficiency tests for JE were conducted successfully from 2009 to 2012. The fifth WHO JE proficiency test (PT) samples were distributed during this fourth hands-on training workshop and the results were finalized. A confirmatory testing mechanism was also established in the Region, and samples tested in JE national laboratories were sent to RRLs for retesting. WHO accreditation using the WHO JE laboratory checklist was initiated in 2010. To build regional laboratory capacity for JE testing, three regional hands-on training workshops were organized in 2009 and 2011 at the Korea Centers for Disease Control and Prevention (Korea CDC), and in 2010 at the Public Health Laboratory Centre in Hong Kong (China).

The Fourth Hands-on Training Workshop on the Laboratory Diagnosis of Japanese Encephalitis in the Western Pacific Region was held in Osong, the Republic of Korea from 23 to 27 September 2013 to further enhance the proficiency of laboratory staff in enzyme-linked immunosorbent assay (ELISA) testing and further improve the quality of laboratory performances. Fourteen participants from Cambodia, China, Japan, the Lao People’s Democratic Republic, Malaysia, Papua New Guinea, the Philippines, the Republic of Korea and Viet Nam attended the training.

1.1 Objectives

(1) To enhance knowledge and skills of national JE laboratory staff in:

   (a) performing ELISA for laboratory diagnosis of JE; and

   (b) carrying out laboratory quality assurance for JE diagnosis.

(2) To discuss requirements for WHO accreditation for the JE laboratories.

(3) To further familiarize participants with laboratory data management using the WHO JE laboratory data-reporting format for reporting to the Western Pacific Regional Office.

(4) To distribute 2013 PT panel samples to network laboratories.
1.2 Participants

The workshop was attended by 14 participants from WHO-designated national JE laboratories from Cambodia (n=1), China (n=2), Japan (n=1), the Lao People’s Democratic Republic (n=2), Malaysia (1), Papua New Guinea (n=1), the Philippines (n=2), the Republic of Korea (n=1) and Viet Nam (Ha Noi: n=1 and Ho Chi Minh City: n=1). In addition to support from the WHO Secretariat, temporary advisers from the United States Centers for Disease Control and Prevention (US CDC) and NIID in Japan attended the training as facilitators. The list of participants is attached as Annex 1.

1.3 Training programme

The workshop consisted of one day of lectures and country presentations followed by four days of practical sessions and discussions. A timetable of the workshop is attached as Annex 2.

The fifth PT panel samples were distributed to all participating laboratories at the end of the workshop. Flash drives with stored training documents – copies of all presentations made during the training, worksheets and all related materials – were distributed upon the completion of the workshop.

The protocol used for the training is attached as Annex 3. The presentations including the lecture sessions and country reports are attached in Annexes 4 and 5.

2. PROCEEDINGS

2.1 Opening session

Dr Myung-Chan Cho, Director of the National Institute of Health, Korea CDC, welcomed the participants and opened the hands-on training workshop with an introductory speech. The objectives of the training were presented by Dr Youngmee Jee, Regional EPI Laboratory Coordinator, WHO Regional Office for the Western Pacific.

2.2 Lecture sessions

2.2.1 JE and acute encephalitis syndrome (AES) surveillance and laboratory network

2.2.1.1 Laboratory-based JE and AES surveillance: progress, challenges and plans

Mr David Featherstone, WHO consultant, presented the strategy for JE/AES surveillance, role of laboratories in JE surveillance, development of the JE LabNet, challenges in maintaining the momentum, and prospects for maintaining sustainability. He emphasized that epidemiology and public health burden are not well understood in many JE-affected countries; thus, there is a need to characterize the epidemiology and burden of JE in those countries. In order to accomplish this, it is essential to advocate for and guide programmatic interventions, and to establish disease burden prior to introduction of JE vaccine. However, in countries where JE immunization has been introduced, it is necessary to identify high-risk populations, estimate vaccination coverage, detect new disease transmission and document impact of control measures.

A good-quality surveillance system with laboratory support is important for understanding the causes of AES and responding appropriately. AES is a clinical condition caused by infection with JEV or other infectious agents, toxic substances or increased complications of an infectious
disease. Laboratory confirmation is crucial for the differential diagnosis of JE, especially with other flaviviruses presenting antigenic cross-reactivity challenges. Therefore a definitive diagnosis of JEV infection cannot be based on clinical signs alone, but must rely on laboratory confirmation.

The JE LabNet was developed following a gradual, stepwise process and was based on the WHO poliomyelitis and measles/rubella laboratory network models. Standardized laboratory techniques are being used to confirm JEV infection. Strong quality assurance programmes, including evaluation of different assays, use of in-house control samples, proficiency testing, confirmatory testing and accreditations have been implemented among WHO JE network laboratories. It is also important for the JE LabNet to integrate with the JE programme.

To build regional capacity, a series of training workshops were held for countries in the WHO’s South-East Asian and Western Pacific regions. Six regional laboratory staff attended JE laboratory training at US CDC.

In its early stage of development, the JE LabNet is facing a number of challenges. Finding support for maintaining the laboratory network is critical because progress is fragile when support is unavailable. Where possible, countries should take responsibility for supporting JE surveillance integrated with other surveillance programmes. He also pointed out that assay assessment and quality assurance are essential because there is no perfect assay for JE confirmation yet. Support is also vital for countries using in-house assays to ensure quality. Training and accreditation reviews are necessary for maintaining quality in the JE LabNet.

Mr Featherstone announced plans for the introduction of JE vaccine manufactured by Chengdu Institute of Biological Products (China) after WHO prequalification in countries such as Cambodia, the Lao People's Democratic Republic, Malaysia and maybe the Philippines. It is critical that impact of introduction is monitored through accurate and timely laboratory-based surveillance.

2.2.1.2 Overview of JE control and progress of JE LabNet in the Region

Dr Youngmee Jee, Regional EPI Laboratory Coordinator, WHO Regional Office for the Western Pacific, presented the objectives and the timetable of the training workshop and gave an overview of JE control and progress of the JE laboratory network in the Western Pacific Region. Dr Jee stated that globally, it is estimated that approximately 67 900 JE cases typically occur annually in the 24 JE-endemic countries (incidence of 1.8 per 100 000 population). Among these, approximately 51 000 (75%) cases occurred in children under 14 years old (incidence of 5.4 per 100 000), but only 10% of cases were reported to WHO. Half of JE cases were from China, but this may be changing since JE vaccination is already implemented in China.

JE surveillance is important both before and after vaccine introduction. Before vaccine introduction, there is a need to demonstrate the presence of disease and etiology, provide evidence for decision-making, establish a system to measure impact after vaccine introduction and provide incidence data for disease burden estimation. After vaccine introduction, it is essential to assess disease trends over time, monitor impact of the vaccination programme and develop a platform for vaccine effectiveness and safety evaluation.

A global WHO surveillance network for invasive bacterial vaccine-preventable diseases (IB-VPD) was established to gather data to assess disease burden, estimate vaccine effectiveness, determine which specific serotypes of *Streptococcus pneumonia* and *Neisseria meningitides* caused the majority of disease, and monitor changes in strain prevalence over time and in response to vaccine introduction. These organisms cause diseases with a variety of clinical presentations including those of the brain (meningitis, encephalitis), lung (pneumonia) and bloodstream (sepsis). JEV is also under surveillance in areas where JE is endemic. For children under five years old, cerebrospinal fluid (CSF) samples that are JE IgM negative are also tested for invasive bacterial disease (IBD) pathogens. In the Western Pacific Region, there is ongoing IB-VPD surveillance in Cambodia, Mongolia, Papua New Guinea, the Philippines (has just started) and Viet Nam.

Dr Jee highlighted progress in JE surveillance in the Western Pacific Region. In countries with limited data on JE disease burden (for example, Lao People's Democratic Republic, Papua New Guinea, Philippines), comprehensive AES aggregate reporting and sentinel hospital surveillance with laboratory confirmation have been established to assess disease burden and characterize the distribution of disease. In one country with demonstrated disease burden and pilot vaccination (Cambodia), the purpose of expanding sentinel surveillance is to further demonstrate geographic range of JE and collect baseline data for measuring vaccine impact. An integrated meningitis and encephalitis (ME) sentinel surveillance among children under 15 years old in six sites will be established.

Dr Jee shared the results of CDC Cambodia’s ME surveillance project in Battambang and Banteay Meanchey provinces. The results showed that from June 2011 to June 2013, a total of 452 ME cases had been identified in these two provinces; 40 (17%) patients with samples collected tested positive for JEV. During April 2013–June 2013, there were 27 meningoencephalitis cases identified at the surveillance sites, with 17 (63%) cases having at least one sample collected. Cambodia introduced live, attenuated SA 14-14-2 JE vaccine into routine EPI for children 10 to 23 months of age in Kampong Chan, Takeo and Svay Rieng provinces in the third quarter of 2009. Plans for the future include catch-up campaigns and expansion of the routine immunization programme to additional provinces.

In countries with established vaccination programmes (for example, China, Malaysia and Viet Nam), ME sentinel surveillance is used to monitor vaccine impact, identify areas needing improved vaccine coverage, and detect areas with new disease transmission. It is recommended to implement surveillance on a national scale or broad geographic scale. In countries where a high level of JE control has been achieved, case-based surveillance with laboratory confirmation throughout the country is recommended. WHO support for JE surveillance includes the development of generic case investigations forms and databases and establishment of the WHO JE LabNet in the Western Pacific Region. The data on suspected meningoencephalitis cases due to JE from 2009 to mid-2012 showed that across countries (Cambodia, Philippines and Viet Nam), 13–21% of suspected meningoencephalitis cases are due to JE.

Though there is no WHO-prequalified vaccine so far, JE vaccine has been introduced in seven of 12 countries with JE risk areas, including partial introduction in one. Viet Nam has continued to expand the population covered by JE vaccination. Cambodia initiated pilot JE vaccination with live attenuated SA 14-14-2 vaccine (Chengdu Institute of Biological Products, China) during a campaign in February–March 2013 that targeted infants aged 9–10 months old in three provinces. The Lao People's Democratic Republic conducted a campaign with the same vaccine for children aged 12 months to less than 15 years old in six northern provinces in March 2013.
Dr Jee emphasized that there has been substantial progress in the establishment or strengthening of JE surveillance and in the initiation or expansion of JE vaccination programmes in the Western Pacific Region; however, JE vaccination programmes still face real constraints in terms of vaccine supply and financing.

The WHO JE LabNet in the Western Pacific Region was modelled after the polio and measles/rubella laboratory networks following the EPI TAG recommendation in 2008, with support from the Program for Appropriate Technology in Health (PATH) and Korea CDC. The JE LabNet consists of one global specialized laboratory, two RRLs, seven national laboratories and 10 subnational laboratories in China. Most JE network laboratories were designated in the national public health institute, where laboratory testing for measles and rubella is also performed (Cambodia, Lao People's Democratic Republic, Philippines, Papua New Guinea). The Papua New Guinea laboratory was the last of 10 laboratories to join the regional network in 2011 and started testing in 2012. Designation of 10 subnational JE laboratories in China (Guangdong, Sichuan, Shanghai, Zhejiang, Chongqing, Henan, Guizhou, Shandong, Guangxi, Yunnan) is ongoing. Procedures are being standardized (WHO manual), and in-house and commercial kits are being evaluated by NIID Japan and US CDC. Three JE hands-on training workshops were conducted in 2009, 2010 and 2011 to build regional capacity for JE testing, and regional JE meetings were held in 2008, 2009 and 2011. Quality assurance mechanisms (proficiency testing and confirmatory testing) and WHO accreditation using a checklist are in place. Case-based and aggregate data are received at the WHO Regional Office for the Western Pacific on a monthly basis.

Dr Jee discussed plans to replace the commercial JE Panbio kit with Inbios in 2014 due to the JE Panbio kit being no longer available from the end of 2013. Funding support through a technical services agreement (TSA) or agreement on performance of work (APW) will be provided for priority countries to support operational costs. The network laboratories will continue to implement quality assurance by establishing in-house control for the Inbios kit, sending confirmatory samples to RRL once a year (due to high shipping cost) and participating in the annual proficiency testing. WHO will provide support for two laboratories with pending accreditation and continue to conduct annual accreditation. Laboratory data reporting and analysis should also be strengthened. A joint biregional meeting on JE control in the South-East Asia and Western Pacific regions and publication of a JE bulletin are being considered.

2.2.2 Quality assurance of the JE laboratory network

2.2.2.1 Activities of the global specialized laboratory in Japan

Dr Tomohiko Takahashi from NIID, Japan, presented the activities of the JE global specialized laboratory. The laboratory performs JE confirmatory testing of samples from national JE laboratories in Cambodia, the Lao People’s Democratic Republic and Viet Nam (both NIHE and Pasteur Institute) and also evaluates in-house JE IgM ELISA kits.

From December 2011 to February 2013, a total of 481 confirmatory samples were referred from Cambodia (n=147), the Lao People’s Democratic Republic (n=90), NIHE, Viet Nam (n=111), and Pasteur Institute, Viet Nam (n=133). For JE confirmatory testing of samples, NIID used a NIID in-house assay and the PanBio kit. Some discrepancies were observed with samples from Cambodia and the Lao People’s Democratic Republic using the NIID in-house assay, while 100% concordance was obtained using the Panbio kit.

Dr Takasaki discussed the principle of IgM capture ELISA for JEV and mentioned that the kit utilizes inactivated JE virions. He also presented the results of an evaluation of the JEV antigen from kit lots 19, 20 and 21 at NIID using NIID JEV antigen; Vero cell-derived JEV
(Beijing-1) used as NIHE JEV antigen; and mouse brain-derived JEV (Nakayama). The JEV IgM capture ELISA kit in Viet Nam was evaluated to check the performance of the JEV antigen and the conjugate IgG contained in the NIHE kit. Four different sources were used to evaluate the antigen in the kit, those being: bulk JEV antigen, kit stored in NIHE, kit stored in Children’s Hospital Number 1 and NIID in-house kit. For the conjugate IgG evaluation, one kit stored in Children’s Hospital Number 1 and one kit in NIHE were compared. The evaluation results showed that JEV antigen contained in both of these kits was working. However, optical density (OD) values of the negative control were more than the OD value set for the negative control, which means that there is a problem with the quality of the conjugate in Children’s Hospital Number 1 and the conjugate should be changed. The JE kit stored in NIHE was considered to have no problem.

Laboratory confirmation is crucial for the differential diagnosis of JE especially with other flaviviruses with antigenic cross-reactivity. So, application of the dengue non-structural protein 1 (NS1) ELISA for the confirmation of dengue virus (DENV) infection was also presented. The results indicate that NS1 antigen positive rates were higher than those of RT-PCR for a longer period during the early phase of DENV infection. Thus, NS1 antigen ELISA is a useful tool for confirming DENV infection using serum samples in the acute phase or early convalescent phase, particularly when it is used in combination with RT-PCR and anti-DENV IgM ELISA.

2.2.2.2 Activities of RRL in the Republic of Korea

Dr Myung Guk Han from Division of Arboviruses, National Institute of Health, Korea CDC, presented confirmatory testing and other RRL activities in the Republic of Korea. Confirmatory samples (serum and CSF) were received from the Philippines and Malaysia. Since 2010, a total of 348 samples (serum: 199, CSF: 149) were received from the Philippines, and overall concordance rate was 94% (188/199) for serum samples and 97% (144/149) for CSF samples. A total of 204 samples (serum: 141, CSF: 63) were received from Malaysia since 2010, and overall concordance rate was 95% (134/141) for serum samples and 98% (62/63) for CSF samples.

For diagnosis of JEV, the laboratory has been using serology assays such as HI test since 1970, PRNT since 1980, in-house immunofluorescence assay (IFA) and ELISA since 2006; RT-PCR since 1990 and real-time RT-PCR since 2009 for virus detection; and virus isolation by mouse inoculation since 1970 and cell culture since 2006. Laboratory results showed no JE cases with nonspecific JE IgM reaction.

Comparison of two JE ELISA kits (Panbio and Inbios) with sera of confirmed JE cases showed 100% agreement. Sensitivity and specificity evaluation results of Panbio, Inbios and XCyton (Bangalore, India) JE kits were also discussed. Though Panbio has the lowest sensitivity among the three kits, the specificity is very high compared to Inbios and XCyton kits, as shown in Table 1.
Table 1. Sensitivity and specificity of commercial ELISA kits

<table>
<thead>
<tr>
<th>Kit</th>
<th>% Sensitivity</th>
<th>% Specificity (n=360)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PanBio</td>
<td>89</td>
<td>99 w/Dengue panel</td>
</tr>
<tr>
<td>InBios</td>
<td>99</td>
<td>56 w/o Dengue panel</td>
</tr>
<tr>
<td>Xcyton</td>
<td>97</td>
<td>65 w/o Dengue panel</td>
</tr>
</tbody>
</table>

Dr Lu Zhi from the Institute for Viral Disease Control and Prevention (IVDC), China CDC, presented the JE surveillance, JE vaccination programme and laboratory activities in China. JE is a national notifiable disease and 10 provincial CDCs are conducting JE surveillance. A JE vaccination programme has been established in China, and a two-dose schedule of live attenuated vaccine is being followed for children aged eight months and two years old with 80% protective immunity.

In China, two JE kits, Beixi (locally produced) and Panbio (commercially available), are used for the laboratory diagnosis of JE. As part of the WHO quality assurance programme, the JE RRL in China provides PT samples to 10 JE subnational laboratories. The PT results are analysed and fed back to the individual laboratories within a month of completion of the proficiency test. All laboratories scored 100% in 2011 and 2012. The PT results were also shared with WHO. Confirmatory testing is also being done, and in November 2012, the JE RRL received 826 specimens (serum: 663, CSF: 163) from nine JE subnational laboratories. Concordance rate was 100% and results were fed back within 45 days via telephone.

The first meeting of the national JE laboratory network was held in Beijing on 10 October 2012. A total of 27 participants attended the meeting, including staff from the National Immunization Programme, China CDC, IVDC and 10 JE subnational laboratories. Participants discussed progress made by the laboratory network in 2011–2012 and tasks and responsibilities of the JE laboratory network in China.

Dr Zhi also reported that there was an outbreak of JE in adults in Yuncheng, Shanxi province, China in 2006. Details of this outbreak were published in *Emerging Infectious Diseases* 2007. Also, JE occurs in low-incidence areas and this is a challenge in China.

---

2.3 Country reports

2.3.1 Cambodia

Mr Chin Savuth from the National Institute of Public Health (NIPH) presented on the JE sentinel site surveillance and JE vaccination programme in Cambodia. Hospital-based sentinel surveillance for suspected meningoencephalitis cases among children under 15 years old in six selected national and provincial hospitals was started in 2006 by Cambodia CDC, NIPH, the National Immunization Programme/Ministry of Health, PATH and WHO. NIPH used the Panbio JE-Dengue IgM Combo ELISA kits for testing.

From 2010 to July 2013, a total of 690 first serum samples, 465 second serum samples and 691 CSF samples from 706 suspected cases of meningoencephalitis were received in the NIPH laboratory for JEV confirmation. The results revealed that JE cases have declined since 2010, and two sentinel sites, Battambang and Svay Rieng, have the highest JE cases, as shown in Figures 1 and 2.

Figure 1. Results of JE laboratory confirmation of samples from ME cases from 2010 to July 2013

Figure 2. Results of JE laboratory confirmation of samples from ME cases by sentinel site
Age distribution of confirmed JE cases showed that children aged 1–10 years old were the most affected, with 34% of cases found among children 6–10 years of age and 24% among children 1–5 years of age. Meanwhile, among children aged 11–15 years old and less than one year old, only 19% and 6% of cases were JE confirmed, respectively.

A JE routine immunization programme was introduced in Kampong Cham, Svay Rieng, and Takeo provinces in October 2009. Children aged 10–24 months old were vaccinated (one month after measles vaccination) with a single dose of live attenuated SA 14-14-2 JE vaccine manufactured by the Chengdu Institute of Biological Products in China. During the 31-month period, 179,171 children under one year of age and 125,636 children from one to two years of age were immunized.

For quality assurance, the laboratory uses an in-house control, participates in annual WHO proficiency testing, and refers confirmatory testing samples to NIID twice a year. PT results from 2010 to 2012 were 100%. Confirmatory test results were 92.6% in 2010 and 100% in 2011 and 2012.

The national JE laboratory is still faced with some challenges. The laboratories in the sentinel sites need to be strengthened for basic bacteriology including culture. The quality of some samples received is poor because of haemolysis, inadequate volume of sample, problems in getting a second blood sample, specimen packaging and transport to reference laboratory. Also, inter-laboratory comparison of different assays is still an issue.

2.3.2 China

Wu Shengwei from Ghuizou provincial laboratory presented on the epidemiology and laboratory surveillance of JE in Guizhou province, China. In 2012, there were 156 reported JE cases (including clinical cases and laboratory-confirmed cases) in Guizhou province, and the incidence was 0.4497/100,000; however, there was a decrease of 28% of reported JE cases compared to 2011. There were 10 deaths in 2011 and eight deaths in 2012. JE cases were reported from nine prefectures of the province, among which, the highest JE incidence occurred in Qianxinan (1.071/100,000), and the greatest number of cases was reported from Bijie (35/156, 22%). In 2012, the number of JE cases peaked in July, August and September. Most of the JE cases were children under 6 years old, followed by 6–15 years old, and a few cases were over 15 years old. There is already a widespread application of the JE vaccine in Guizhou province with 99% coverage reported.

Laboratory testing methods include serology, virus isolation and PCR. In 2012, the provincial JE laboratory retested 110 JE samples from the prefecture network laboratories. There was 100% agreement between the results of both levels of laboratories. Since 2009, Guizhou provincial laboratory has received JE PT samples from the national JE laboratory and the results have been excellent. Similarly, the nine prefecture JE laboratories have received the PT samples from the provincial JE laboratory and the results have been good. There is a good coordination between the laboratory staff and the EPI staff.

It was mentioned that support for JE surveillance should be strengthened and the Government should increase its investment. Availability of high sensitivity and specificity serology kits, which are cheap, should be ensured.

2.3.3 Lao People's Democratic Republic

Ms Phoutsamay Vongphachanh from the National Center for Laboratory and Epidemiology (NCLE) presented the JE surveillance and immunization activities in the Lao
People's Democratic Republic. JE is a national notifiable disease reported from all 17 provinces in the Lao People's Democratic Republic. JE vaccination was introduced in the country in March 2013 for children aged 1–15 years old. The JE vaccination programme will potentially reduce mortality and morbidity in areas of known endemicity. There were 620,000 doses given in six northern provinces where there is an evidence of high transmission: Oudomxay, Luangnamtha, Boukeo, Luangprabang, Huaphanh and Xayaboury. The Lao People’s Democratic Republic uses live attenuated SA 14-14-2 vaccine produced and donated by Chengdu Institute of Biologicals in China in collaboration with PATH.

The national JE laboratory uses Panbio JE-Dengue IgM Combo ELISA kit for the diagnosis of JE. From 2010 to 2013 (August), a total of 161 samples were tested in the laboratory. The results showed JE IgM positive cases were 46.9% in 2010, 59.1% in 2011, 48.9% in 2012, and 38.1% in 2013, as shown in Figure 3.

Figure 3. Summary of JE testing and results at NCLE, 2010–2013

![Figure 3. Summary of JE testing and results at NCLE, 2010–2013](image)

It was stated that Xayaboury province reported the highest number of JE cases, with 40% JE positive among 10 cases. In 2013, JE-confirmed cases were seen most often in the months of June to August, with the highest peak in August. As of August 2013, children across all age groups with the highest number of cases were older than 15 years of age.

For laboratory quality assurance, NCLE sends all JEV samples for confirmatory testing to NIID, Japan every year and participates in the WHO JEV IgM proficiency test. NCLE started testing for JE in 2009, and laboratory data are reported on a monthly basis to the WHO Regional Office for the Western Pacific. Data are also shared with the surveillance unit and EPI. Based on NCLE data, EPI selects provinces for targeted JE immunization.

Challenges remain in the immunization programme. There is difficulty in the transportation of vaccines to the target places, and the people did not collaborate well with the vaccination group.

Amphay Phyaluanglath from Mahosot Hospital in Vientiane, Lao People's Democratic Republic presented the status of JE laboratory diagnosis in Mahosot Hospital. It was reported that Mahosot Hospital uses three methods for the diagnosis of JE, namely, serology, cell culture and RT-PCR. The laboratory is using Panbio JE-Dengue IgM Combo ELISA to diagnose JE. In 2012, a total of 546 patients admitted in Mahosot Hospital had CSF and/or blood (serum) collected and tested in the laboratory. The JE test results showed 3.48% positivity when using
both CSF and serum samples, 1.1% positivity when using CSF alone, and 9.9% when using serum samples alone, as shown in Figure 4.

Figure 4. Number of positive cases by type of sample from Mahosot Hospital, 2012

It was concluded that JEV is a cause of AES in the central, northern and southern regions of the Lao People's Democratic Republic. Out of 546 AES patients tested, only 79 (14.5%) were JEV IgM positive. The study suggests that the major burden of AES is caused by other agents that need to be further investigated.

2.3.4 Malaysia

Norazimah Tajudin from the National Public Health Laboratory (NPHL), Ministry of Health, Malaysia, presented the JE surveillance and JE vaccination programme in Malaysia. The first confirmed case of JE in Malaysia was reported in 1952. Viral encephalitis has been a notifiable disease since 1988 (syndromic-based surveillance). Increased JE cases were detected following a Nipah/JE outbreak in the early 2000s. A JE vaccination programme has been operational since 2002 in the high-risk province of Sarawak. The percentage of laboratory-confirmed JE cases from 1993 to 2012 was around 1–5%; however, as of August 2013, the proportion of JE-confirmed cases is 7.1% (23/324).

Prior 2007, there was no designated national JE laboratory. Today, testing is mainly done at the University Malaya Medical Centre (UMMC) in Klang Valley, the University Malaysia Sarawak (UNIMAS) in Sarawak and the Institute of Medical Research (IMR) in Kuala Lumpur. During 2007–2011, IMR functioned as National JE Laboratory (NJEL) and other laboratories including NPHL continued with diagnostic testing on JE. In 2012, NPHL was designated as the NJEL. A Memorandum Circular from the Ministry of Health dated 3 February 2012 was prepared to conduct national surveillance on JE in 16 sentinel sites in Malaysia.

JE vaccination started in Sarawak in 2002 and covers children aged 9–10 months. A booster dose is given to children at 18 months of age, and then every three years until 15 years of age. The Government is in the middle of tendering for single-dose vaccine in 2014. Immunization coverage in Sarawak during 2012–2013 is reported to be 100%.

For quality assurance, NPHL is MS ISO 9001:2008 certified and also undergoing ISO 15189 certification. The laboratory also participates in the WHO external quality assurance programme. NPHL has had a consistent 100% score in the WHO JE proficiency testing from
2009 to 2012. Confirmatory testing results showed 100% (first batch) and 98.5% (second batch) concordance in 2012. An in-house control is also being implemented. An on-site accreditation visit was completed in April 2013 by WHO.

It was reported that in 2013, the percentage of CSF samples increased to 54.9%, while serum collection was 45.1%, compared to 2012, when CSF collection was 49.4% and serum collection was 50.1%. However, JE positivity in serum is higher than CSF, as shown in Figure 5.

Figure 5. Percentage of JE-positive samples by sample type, 2009–2013

In 2013, out of 204 cases, 17 cases were JE laboratory-confirmed. Age distribution was as follows: less than 1 year old (n=1); 2–5 years old (n=2); 6–20 years old (n=12); and adult (n=2). Laboratory results are reported to health-care facilities (hospital/clinic/district health office) and data/line listing to the NPHL epidemiologist who in turn, submits the data to Epidemiology (state), and Disease Control Division (Ministry of Health). The JE surveillance programme is faced with a few challenges in getting samples, and the JE kits are expensive. The laboratory is planning to develop in-house kits.

Case-based reporting is implemented through a manual system. Specimens must be submitted along with the case investigation form that can be downloaded online at http://mkak.moh.gov.my. All suspected cases with samples collected would be confirmed by NPHL using JE IgM ELISA method. The Ministry of Health is in the middle of preparing a guideline for JE prevention and control activities in Malaysia and will be ready by 2014.

2.3.5 Papua New Guinea

Mr Eric Bilo from the Central Public Health Laboratory (CPhL), Port Moresby, presented JE surveillance in Papua New Guinea. In 2011, AES surveillance was initiated in four sentinel sites upon discussion with the Pediatric Society. The CPhL is designated as the national JE laboratory. Port Moresby General Hospital started surveillance in 2012, and national surveillance guidelines were developed. Currently, there is no JE vaccination programme implemented in Papua New Guinea.

The laboratory is using IgM Capture ELISA for JE diagnosis, and the Panbio JEV-Dengue IgM Combo ELISA kits are supplied by WHO. During 2012–2013, there were 24 cases reported and samples were tested. The results showed that all cases were negative for JE IgM; however,
four cases were positive for dengue IgM in 2013. Among the four dengue-positive cases, two cases were aged one to five months old, one case was aged three to five years old, and one case was aged four to seven years old.

For laboratory quality assurance, CPHL implements in-house control, participates in WHO proficiency testing, and sends samples to RRL for confirmatory testing. The laboratory is also being assessed for possible WHO accreditation. Laboratory data including zero reporting is shared to the Regional Office for the Western Pacific by the 10th of every month as aggregate data and as a case-based line-list.

Challenges and issues were also presented. Sentinel sites are not reporting AES cases. All staff in the surveillance unit were recruited in 2012 and need training. Transportation of samples from provinces under appropriate conditions is a challenge. The volume of CSF is inadequate and a second sample is not often collected. Kit reagent wash buffer has a short life span (one week) and supplementary kits are not available as for measles.

There are plans to strengthen sentinel sites, train CPHL staff, introduce IHC and prepare graphic display of controls. JE laboratory accreditation will be pursued in collaboration with WHO. The laboratory will continue to send samples for reconfirmation to the designated JE RRL in the Republic of Korea.

2.3.6 The Philippines

Ms Ava Kristy Sy from the Research Institute for Tropical Medicine (RITM) presented the updates on AES surveillance in the Philippines. There are limited data on JE infection in the Philippines. In 2005, an AES surveillance project was conducted in San Lazaro Hospital in collaboration with the Armed Forces Research Institute of Medical Sciences (AFRIMS), and the results showed that among 15 suspected AES cases, six (40%) AES cases were due to JE. During 2009–2011, a study on ME surveillance in five sentinel hospitals was initiated by RITM and the Department of Health, funded by WHO. The results of the study revealed that 40% of AES cases were due to JE. JE vaccination is not yet included in the EPI in the Philippines, and more evidence of disease burden is needed to justify introduction.

In 2008, the Philippine Integrated Disease Surveillance and Response (PIDS) programme was implemented in the country. PIDS is symptom-based and selected diseases are notified weekly from 1664 hospitals. AES is one of the 26 diseases, syndromes and conditions included in PIDS, so JE is captured through the AES surveillance. Other central nervous system infections in PIDS include meningococcal disease, which requires immediate notification, and bacterial meningitis, which is weekly notifiable. However, few cases are referred to the laboratory for confirmation.

To strengthen AES surveillance, pilot AES sentinel surveillance in five hospital sentinel sites was re-established under the category of new and underutilized vaccine surveillance of the National Epidemiology Center (NEC). These five hospital sentinel sites were the same sites where ME surveillance was conducted. An AES surveillance supplemental manual was prepared, and advocacy and orientation meetings were completed in four hospitals in June 2013. The integration of AES with bacterial meningitis (BM) surveillance has been considered. The AES-BM surveillance will be expanded into eight sentinel hospitals. An AES-BM integrated surveillance supplemental manual was drafted, but orientation of sentinel sites personnel has yet to commence.

For JE laboratory diagnosis, sera and CSF samples are collected from suspected AES cases and sent to RITM where they are analysed using a Panbio JE-Dengue IgM Combo ELISA.
From January to August 2013, a total of 159 cases were referred for confirmation. Among these cases, 93 were referred by hospitals for routine diagnosis and 66 were from four hospital sentinel sites. The results showed that only 17 cases (11%) were due to JEV and 28 cases (18%) were due to DENV infection, as shown in Table 2.

Table 2. Summary of results from hospital referrals and sentinel sites, January to August 2013

<table>
<thead>
<tr>
<th></th>
<th>Donque</th>
<th>JE</th>
<th>Negative</th>
<th>Grand Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital Referrals</td>
<td>18</td>
<td>10</td>
<td>85</td>
<td>93</td>
</tr>
<tr>
<td>(15%)</td>
<td>(11%)</td>
<td>(70%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sentinel Sites</td>
<td>10</td>
<td>7</td>
<td>49</td>
<td>66</td>
</tr>
<tr>
<td>(15%)</td>
<td>(11%)</td>
<td>(74%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Philippine Children’s Medical Center</td>
<td>5</td>
<td>1</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>(25%)</td>
<td>(5%)</td>
<td>(70%)</td>
<td></td>
<td>(30%)</td>
</tr>
<tr>
<td>San Lazaro Hospital</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(100%)</td>
<td>(2%)</td>
</tr>
<tr>
<td>Bicol Medical Center</td>
<td>3</td>
<td>5</td>
<td>27</td>
<td>35</td>
</tr>
<tr>
<td>(9%)</td>
<td>(14%)</td>
<td>(77%)</td>
<td></td>
<td>(53%)</td>
</tr>
<tr>
<td>Western Visayas Medical Center</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>(20%)</td>
<td>(10%)</td>
<td>(70%)</td>
<td></td>
<td>(15%)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>26</strong></td>
<td><strong>17</strong></td>
<td><strong>114</strong></td>
<td><strong>159</strong></td>
</tr>
<tr>
<td></td>
<td>(16%)</td>
<td>(11%)</td>
<td>(72%)</td>
<td></td>
</tr>
</tbody>
</table>

Based on 2013 data, the greatest number of affected cases were in the age group 0–2 years old, followed by 6–10 years old, 11–15 years old, >15 years old and 2–5 years old. A few cases were reported with incomplete data fields.

The JE laboratory is implementing quality assurance measures that include training of staff, calibration of equipment, validation of each test run, use of IHC, participation in the WHO proficiency testing and sending of samples to the Korea CDC RRL for confirmatory testing. Laboratory data and results are submitted to WHO, NEC and EPI monthly. Results are provided to sentinel hospitals through regional surveillance units monthly, and to physicians as soon as results are available.

Ms Sy presented challenges in the implementation of the surveillance. There is low sensitivity in the detection of cases, difficulty in getting consent for lumbar puncture (LP), and insufficient volume of CSF at times. There is incomplete information in some case investigation forms. Training sentinel hospital staff on integrated AES-BM surveillance is essential. There is a need to strengthen the relationship with bacteriology laboratories of the sentinel hospitals and advocate for physicians' support. The feedback mechanism can be improved through bulletins, email transmittals, etc. Rapid turnover of laboratory staff is still a challenge. Also, there is a lack of laboratory surge capacity during outbreaks of other diseases (e.g. dengue, chikungunya).

2.3.7 Viet Nam

2.3.7.1 Northern Viet Nam

Mr Nguyen Ngoc Linh from NIHE, Ha Noi, presented the JE surveillance in northern Viet Nam and JE vaccination programme in Viet Nam. There are four JE surveillance sites in
Viet Nam: Thai Binh in the north; Quang Ngai in the central region; and Binh Duong and Ben Tre in the south. There are also 28 provinces in the north that refer samples from suspected cases for routine confirmation to NIHE.

NIHE is using antigen and antibody detection in serum and CSF samples for the laboratory diagnosis of JE. Antigen detection includes RT-PCR and real-time RT-PCR, virus isolation using the C6/36 cell line. For the antibody detection, MAC-ELISA is performed using an in-house kit, which was licensed by the Ministry of Health, as well as the Panbio kit. From 2009 to 2013, a total of 1261 cases have had samples tested in the laboratory. A total of 2088 samples consisting of first serum samples (n=870), second serum samples (n=322) and CSF (n=896) were tested. The results showed that out of 1261 cases, 151 cases (12%) were JEV positive. During 2011–2013, a total of 496 cases with samples were tested from the four JE surveillance sites. Out of these samples tested, 27 (5.4%) were JEV positive.

Geographically, the highest numbers of encephalitis cases reported in 2013 were from the provinces of Thai Binh (n=91), Hai Phong (n=89) and Hai Phong (n=44). However, provinces with the highest percentage of laboratory-confirmed JE cases were Quang Ninh (1/1, 100%), Ha Giang (2/5, 40%) and Lai Châu (1/5, 20%). It was noted that an increase in JE cases occurred from April to September. The data showed that children aged 1–5 years old were most affected (23%), followed by those 6–10 years old (15.2%), 11–15 years old (11%), <1 year old (6.7%) and >15 years old (5%). JE vaccine coverage, from 1999 to 2012, for the targeted provinces, was reported as 93.9% for two doses and 94.8% for three doses.

Quality assurance is well implemented in the laboratory. The WHO JE PT scores in 2010 and 2011 were both 100%, and a 90% score was achieved in 2012. Concordance rates for confirmatory testing were 80% in 2010, 100% in 2011 and 96.15% in 2012.

The laboratory is still faced with a few challenges that include: a lack of some equipment (refrigerator and ELISA microplate washer), detection of cases is missed, revision and finalization of JE database is needed, instability of NIHE JE test kits, and calibration of equipment. The laboratory is requesting that WHO support the purchase of equipment and provision of JE kits.

2.3.7.2 Southern Viet Nam

Nguyen Thi Cong Dung from the Pasteur Institute, Ho Chi Minh City, presented the AES surveillance, JE vaccination status and the laboratory capacity for JEV diagnosis in southern Viet Nam. During 2008–2013 (July), a total of 1929 AES cases were reported with a mortality rate of 1.8–5.4%. JE vaccination was introduced in 2001 in three provinces and three districts in Viet Nam. In 2012, 20 provinces and 164 districts with JE vaccines reported 94.2% coverage for the first dose, 93% coverage for the second dose and 96% coverage for the third dose. In 2011, Ho Chi Minh reported the highest number of AES cases, followed by Can Tho and Bac Lieu. However, in 2012, Dong Thap had the highest number of reported AES cases, followed by Ho Chi Minh, Bac Lieu and Lam Dong.

The laboratory is using Panbio kits and in-house JE-Dengue IgM kits, virus isolation and real-time RT-PCR for the diagnosis of JEV infection. Twenty provincial laboratories are also performing tests for JEV and DENV diagnosis for the dengue national programme. A total of 1146 cases were tested for JEV and DENV from 2011 to 2013 (August): 310 in 2011, 494 in 2012, and 342 in 2013. In 2011, 39 cases (12.6%) were positive for JEV and 17 cases (5.5%) were positive for DENV; in 2012, 67 cases (13.6%) were positive for JEV and 26 cases (5.3%) were positive for DENV; and in 2013, 52 cases (15.2%) were positive for JEV and seven cases (2.0%) were positive for DENV.
There are two JE sentinel sites in southern Viet Nam, namely: Binh Duong Provincial Hospital and Ben Tre Provincial Hospital. Cases are also reported from the Hospital of Tropical Disease and Paediatric No. 2 Hospital in Ho Chi Minh City.

For laboratory quality assurance, the laboratory participates in WHO proficiency testing and confirmatory testing. The PT score using the in-house kit was 90% in 2011 and 100% in 2012. Confirmatory testing was 95% in 2011 and 100% in 2012. The Pasteur Institute laboratory has been ISO 15189 certified from November 2010.

It was indicated that the laboratory staff need training on new techniques and biosafety. The late shipment of samples to the reference laboratory in Japan also needs to be addressed.

2.4 Vector and pig surveillance

2.4.1 Vector and pig surveillance in Japan

Dr Tomohiko Takasaki from NIID gave a presentation on vector and pig surveillance in Japan. Vector surveillance is conducted by quarantine stations to detect the establishment of invasive vector animal species at an early stage, and to support the rapid implementation of control measures to eliminate the vector animals.

Two JE cases, a 73-year-old female from Fukuoka and a 75-year-old male from Kumamoto, were reported in September 2012. Only two cases were reported in 2012 since *Culex tritaeniorhynchus* mosquitoes were scarce during the previous summer in Japan. In previous years, three JE cases were reported in 2008, three in 2009, four in 2010 and eight in 2011, with an imported JE case that was detected in Tokyo but came from India in January 2011.

Since 2000, Japan has monitored JEV activity in pigs by performing antibody tests using HI tests. The results showed that most of the JEV activity in pigs occurred in Honshu, Shikoku and Kyushu islands with <50% to >80% antibody titre against JEV, while Hokkaido island had less JEV activity with 0 to <50% antibody titre against JEV. Compared to 2011, 2012 had less JEV activity in pigs. Testing of JEV infection in pigs was delayed in 2013 due to unavailability of goose red blood cells used in HI test, as there was a rubella outbreak that also needed goose red blood cells for testing.

A JEV neutralizing antibody test was performed among Japanese people of all ages. Comparing the neutralizing antibody titre among Japanese people from 2006 to 2011, the results showed that children under two years old had no or very low neutralizing antibodies, and more than 50% of people aged 3–34 years had neutralizing antibodies against JE.

2.4.2 Vector and pig surveillance in the Republic of Korea

Mr Young Eui Jeong from the National Institute of Health, Korea CDC, presented on vector and pig surveillance in the Republic of Korea. JE surveillance, also known as the “epidemic forecast program”, was initiated in 1975 in collaboration with the Division of Arboviruses, Division of Medical Entomology, and Division of National Immunization Programme. During the month of April, JE attention is given when the vector mosquito is first detected; from July to August, JE alarm is signalled when a patient is reported, when vector mosquitoes make up over 50% of total mosquitoes caught in a night, and pig sero-positivity rate is over 50% at a given place.

Monitoring of vector-mosquito population density is done on a weekly basis by the Entomology Department. A nested RT-PCR kit is used for the detection of JEV in mosquitoes,
but it will be replaced in 2013. This kit is specific to genotypes 1 and 3 and is used for both
diagnosis and surveillance. A TaqMan probe real-time RT-PCR kit (2010) and primer/probe set
from US CDC group (Journal of Clinical Microbiology, 2007) were used, but they were
discarded due to their high cost. SYBR Green 1 real-time RT-PCR was used from 2011 to 2013
and is flavivirus group specific. Through monitoring, the genotypic diversity of JEV in the
Republic of Korea was revealed. Until the early 1990s, genotype 3 was dominant. From 1994 to
2009, only genotype 1 was detected, and in 2010, genotype 5 was detected near the Demilitarized
Zone (DMZ). Later, genotype 5 was detected in the western and southern regions, while
genotype 1 was detected in the southern provinces. In the Republic of Korea, the dissemination
route of a new genotype is monitored along with its correlation with JE outbreak.

A seroprevalence study in pigs is also being implemented. Antibody tests have been
carried out in slaughtered pigs since the 1980s, first with the HI test (1980s–2006), then IFA
(2007–2008), and now immunochromatographic assay (ICA) (2009–present). The HI test was
replaced because it needs over 10 reagents, it is time consuming (four hours) and quality control
is difficult, while IFA needs a fluorescent microscope, two to three hours of experiment time and
experienced laboratory staff. ICA is preferred because facilities or instruments are not needed, it
requires only 5 to 20 minutes of experiment time, any laboratory staff can read results, and a
commercial company manufactures it. Test results serve as a guide to find the exact place where
JE virus was transmitted during the season. Addresses of all pig farms are collected and only
unvaccinated pigs are included. Together with patient location, an annual JE risk map can be
made using Google Earth.

2.4.3 Vector surveillance in China

Dr Lu Zhi from IVDC, China CDC, presented on vector surveillance in China, whereby
mosquitoes are collected in the field and processed in the laboratory for testing. The methods
used in the detection of vector-borne viruses are RT-PCR, real-time RT-PCR, IFA and cell
culture. In 2012–2013, mosquito specimens were collected from six provinces (Yunnan, Gansu,
Henan, Shaanxi, Shandong and Shanxi) for detection and monitoring of JEV and vector-borne
viruses. JEV, Getah virus (GETV), Banna virus (BAV), Culex Flavivirus (CxFV), Liaoning virus
(LNV), Culex pipiens pallens densovirus (CppDNV) and some unidentified strains were
obtained.

In Yunnan province from 11 July to 5 August 2012, 18 714 mosquitoes were collected and
tested. Ten isolates of JEV, GETV, BAV and CxFV were detected. During 10–20 August 2012,
3732 mosquitoes were collected and tested in Gansu province. Among these, 3095 were Culex pipiens pallens (83.93%). Of the 36 virus strains isolated, 32 strains were identified as CxFV and
four strains as LNV by molecular biological methods. In Henan province, 7149 mosquitoes were
collected and consisted of Culex pipiens pallens (55.71%, 3983/7149), Armigeres subalbatus
(28.75%, 2055/7149) and Culex tritaeniorhynchus (12.72%, 909/7149). Six strains of JEV and
one strain of CppDNV were identified. In Shaanxi province, 5305 mosquitoes were tested by
RT-PCR for 27 JEV sequences, one GETV sequence and four CxFV sequences. Further study
showed that among the 27 positive JEV sequences, 16 JEV sequences belonged to genotype 1
and 11 JEV sequences belonged to genotype 3. There were also eight CxFV and seven GETV
detected. A total of 4983 mosquitoes were collected from Rizhao, Linyi and Jining in Shandong
province in 2012. The results showed that among seven JEV sequences, five JEV sequences
belonged to genotype 1 and two JEV sequences belonged to genotype 3. Also, four CxFV
sequences and eight CxFV were identified. In Shanxi from 6 to 12 August 2012, 10 455
mosquitoes were collected in Yuncheng city. Testing revealed four genera and seven species,
including Culex pipiens pallens (47.02%, 4916/10 455), Culex tritaeniorhynchus (46.43%,
4854/10455), Anopheles sinensis (5.53%, 578/10455) and others. Twenty-one strains of JEV
were identified, of which, 18 strains were from the mosquito pools of Culex tritaeniorhynchus
and three strains from *Culex pipiens pallens*. Also, four strains of CxFV, four strains of CppDNV and one strain of GETV were detected.

2.5 Additional presentations

2.5.1 US CDC diagnostic laboratory testing methods and development of algorithms to improve accuracy of existing assays

Dr Barbara W Johnson from the JE global specialized laboratory at US CDC in Fort Collins, presented CDC diagnostic laboratory testing methods and development of algorithms to improve accuracy of existing assays. The Division of Vector-Borne Infectious Diseases works on JEV, West Nile virus (WNV), Saint Louis encephalitis virus, tick-borne encephalitis viruses, yellow fever virus, DENV (through the Dengue Branch in San Juan, Puerto Rico), Zika virus, Eastern, Western and Venezuelan equine encephalitis viruses (EEEV, WEEV, VEEV), Chikungunya virus, LaCrosse virus and other bunyaviruses. The US CDC Arboviral Diseases Diagnostic Laboratory provides reference and confirmatory diagnostic testing for suspected arboviruses. Specimens are tested for all possible arboviruses from geographical region, based on clinical information and volume of sample. Serological assays such as IgM ELISA, microsphere immunoassay, IgG ELISA and PRNT are performed on CSF and serum samples. Virus detection assays such as viral RNA detection, nucleotide sequencing, virus isolation, IFA and dipsticks are performed on mosquito pools, tissues, serum and CSF.

A first priority testing method based on characteristics of the virus infection and immune response is implemented. Viraemia is brief, of low titer, and cleared soon after onset of illness, so collection during the acute phase is crucial for antigen/virus detection. IgM antibodies are first to be detected in primary infections, but may be low response in secondary infection. The CDC testing algorithm for JE, West Nile, dengue and Chikungunya infection was discussed. Samples with IgM-positive results are confirmed by PRNT. For differential diagnosis, a complete serological analysis is performed (Table 3) showing high JE neutralizing antibody titer in convalescent serum samples.

Table 3. Complete serological analysis for JE differential diagnosis

<table>
<thead>
<tr>
<th>Complete Serological Analysis: Differential diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>CSF</td>
</tr>
<tr>
<td>S1</td>
</tr>
<tr>
<td>S2</td>
</tr>
<tr>
<td>Positive Control</td>
</tr>
</tbody>
</table>

Interpretation of ELISA results:
- PRNT titer > 10 = positive antibody
- PRNT titer > 4-fold over heterologous flavivirus = positive for specific antibody
- PRNT titer = 4-fold between acute and convalescent = evidence of acute infection

Dr Johnson emphasized that new approaches for improving accuracy of assays should be developed to include confirmatory testing with more specific or sensitive assays, differential diagnostic testing, testing for other aetiologies, improved performance of existing assays or reagents and development of better assays.
2.5.2 Testing algorithm using Inbios Detect assays for differential diagnosis of JE

Dr Barbara W Johnson from US CDC discussed the testing algorithm using Inbios Detect assays for differential diagnosis of JE.

Inbios JE Detect IgM Capture ELISA (MAC-ELISA) has low specificity (33%), with eight out of 24 DENV IgM positive serum specimens also being confirmed JEV IgM positive by the Inbios test. The Inbios JE Detect MAC-ELISA has 90% sensitivity, with 18 out of 20 US CDC JEV IgM positive serum specimens also being confirmed JEV IgM positive with the Inbios test. However, Inbios DENV Detect MAC-ELISA has 100% specificity and 100% sensitivity. Inbios DENV Detect non-structural protein 1 (NS1) ELISA has 100% specificity, similar to Inbios DENV Detect MAC-ELISA, but sensitivity is lower compared to Inbios DENV Detect MAC-ELISA on limited testing. The Inbios DENV Detect NS1 Rapid Test has 100% specificity, similar to DENV Detect MAC-ELISA and DENV Detect NS1 ELISA, but has lower sensitivity compared to both tests on limited testing.

Comparing the three DENV assays, DENV Detect IgM Capture ELISA requires the smallest sample volume, but sensitivity and specificity are unknown for AES cases; DENV Detect NS1 ELISA has the lowest cost but requires a high volume of sample, and sensitivity and specificity are unknown for AES cases; and Dengue NS1 Detect Rapid Test has the highest cost, requires the most sample volume, and has lower sensitivity on limited sample set. Inbios JE Detect NS1 ELISA and rapid test sensitivity depends on the timing of specimen collection after disease onset.

Dr Johnson highlighted the importance of timing in sample collection, comparing AES and fever cases, when using DENV Detect assays. Patients with AES are hospitalized within the first few days of illness and samples are collected soon after onset of illness. In the early stages of illness, DENV IgM may not be detectable when using DENV Detect IgM Capture ELISA; however, DENV Detect NS1 ELISA and Dengue NS1 Detect Rapid Test may have high sensitivity. Thus, it is important to collect convalescent samples. While with fever, patients may not seek treatment until numerous days of fever have elapsed. During the two weeks after onset of illness, DENV Detect IgM Capture ELISA may have higher sensitivity. DENV Detect assays are designed to detect dengue fever cases not dengue meningoencephalitis cases. Dengue NS1 Detect Rapid Test has lower sensitivity on a limited sample set.

A differential diagnostic testing algorithm using Inbios JE and DENV Detect MAC-ELISA kits is proposed. All samples should be tested by Inbios JE Detect MAC-ELISA. Samples with JE positive results should tested by Inbios DENV Detect MAC-ELISA. JEV-positive and DENV-negative results will be interpreted as JE positive. JEV-positive and DENV-positive results will be interpreted as dengue positive. The Inbios DENV Detect MAC-ELISA needs to be validated with the WHO JE reference panel from AES cases.

Not all of the JE and DENV Detect ELISA kit reagents be used interchangeably. The following reagents are different in each kit and cannot be used across tests: sample dilution buffer, positive and negative controls, normal control antigen and monoclonal antibody/horse radish peroxidase (Mab/HRP) conjugate. However, the following reagents are the same in both kits and can be used across both tests: washing buffer concentrate, enwash solution, tetramethylbenzidine (TMB) substrate and stop solution.

2.5.3 Confirmatory testing methods and testing algorithm of JE in the Republic Korea

Dr Young Eui Jeong from Division of Arboviruses, National Institute of Health, Korea CDC presented confirmatory testing methods and the decision algorithm of JE in the Republic of Korea. JEV was first isolated in the 1940s and the epidemic season is August to October (late
Tick-borne encephalitis virus was first isolated in 2005. A nationwide virus surveillance programme was implemented in 2005 to detect mosquito-borne flaviviruses. Chaoyang virus was detected in 2011, but no other flavivirus was identified. In 2013, nearly 100 sites were used for mosquito collection. Considering the results of the surveillance, the laboratory mainly focuses on JE testing, though, if there is a physician's request, other pathogens such as WNV are also tested.

For JE testing, both acute and convalescent samples are tested by serology such as IgM ELISA, IFA and PRNT. RT-PCR is also being performed on acute phase specimens (normally within five days from onset of illness); however, RT-PCR positivity is rare in serum or CSF. PRNT is done to confirm diagnosis. Thus, PRNT is carried out when the acute phase serum is positive for both JE and WNV by IgM ELISA and when there is a four-fold rise in both acute and convalescent sample (paired sample) for JE and WNV by IFA. JEV infection is easily differentiated from WNV infection in convalescent phase serum by PRNT: for example, JEV antibody titer (PRNT50) is >1:1000, WNV antibody titer (PRNT50) is >1:10. PRNT is also performed on cases with dengue diagnosis when samples from suspected dengue cases are dengue IgM positive using the Panbio kit but RT-PCR negative, and also dengue IgM negative using Inbios IgM Capture ELISA. Dengue PRNT results on convalescent phase serum showed antibody titer (PRNT50) of <1:10, while JE PRNT showed antibody titer (PRNT50) of 1:100. These results show that most Korean people have neutralizing antibody to JEV through previous vaccination or natural infection.

2.5.4 Procedures of PRNT for JE in Japan

Dr Tomohiko Takasaki from NIID described the procedures in performing PRNT in Japan. The test uses JE viruses from mouse brain-derived seed viruses of Beijing-1 (JEV/Hu/Beijing/1/1949) or Nakayama-NIH (JEV/Hu/Nakayama/NIH/1935). Vero JCRB 9013 cell line was purchased from the Health Science Research Resources Bank, Japan. Cell growth medium is Eagle’s minimum essential medium (EMEM) plus 10% heat-inactivated fetal bovine serum (FBS) and 60 µg/ml of Kanamycin. Overlay medium is EMEM containing 1% methyl cellulose medium with 2% heat-inactivated FBS. Serum samples and the virus are diluted using EMEM containing 2% heat-inactivated FBS. Step-by-step procedures for preparation of the overlay medium, cell preparation, serum samples serial dilutions, dilution for virus plaque dose, plaque assay and counting plaques were presented. This test requires five to seven days of incubation for virus plaques to develop.

Dr Takasaki also discussed the focus reduction neutralization test (FRNT). This is an improved test using a microtiter system. The 96-well tissue culture plates are used for preparation of cell monolayers, and the peroxidase-anti-peroxidase (PAP) staining technique is used for visualization of foci of infected cells. The test takes only three days to perform. In comparison, PRNT requires more space (6-well plate) and longer turn-around time (seven days), but the plaque is larger and easy to count and the test is cheaper. FRNT is more expensive as it needs antibodies and the focus is smaller and more difficult to count, but the test saves space in the carbon dioxide (CO2) incubator (96-well plates), facilitates testing of many samples and takes only three days for one assay.

2.5.5 Results of JE proficiency tests

Dr Barbara W Johnson, Division of Vector-Borne Diseases, US CDC, presented an analysis of results of the 2011 and 2012 JE proficiency tests as part of the quality assurance programme for the WHO JE laboratory network. Preparation and composition of the 2011 and 2012 PT panels were discussed. The 2011 PT panel consisted of six sera and four CSF samples (seven JE IgM positive, one dengue IgM positive, two JE/dengue IgM negative). Laboratories
were instructed to include their JE IgM positive in-house control sample during the PT testing. The panel was not tested with the Panbio kit before distribution due to unavailability of this assay in the United States; however, Panbio kit test results from the Korea CDC RRL were used as the reference standard. Serum sample 2 was low JE IgM positive; however, equivocal results from participating laboratories were accepted. There was a discrepant result for serum sample 4 between reference testing, so it was removed from analysis.

For the 2011 PT panel, seven laboratories used the Panbio JE-Dengue IgM Combo ELISA kit; four laboratories used JE and dengue IgM in-house assays, and three laboratories used the Beixi kit (JE IgM only). It is noted that all laboratories scored 100% (14/14 JE laboratories). Dr Johnson highlighted a few deficiencies in the submission of results. Papua New Guinea’s JE calibrator ODs were high, resulting in false negatives; thus, the PT score was 89%. Mahosot Hospital laboratory in the Lao Democratic People's Republic had a typographical error in the calibration factor, and after correction, the PT score was 100% instead of 89%. Though the laboratories were asked to include their in-house control during testing of the PT samples, not all laboratories included it due to the unavailability of JE IgM positive samples for in-house control purposes.

In 2012, the fourth PT panel samples were distributed to 13 JE laboratories. The 2012 PT panel consisted of six sera and four CSF samples (seven JE IgM positive, one dengue IgM positive, two JE/dengue IgM negative); laboratories were requested to test their in-house control sample with the panel. Nine laboratories used Panbio JE-Dengue IgM Combo ELISA, three laboratories used JE and dengue IgM in-house assays, and one laboratory used the Beixi kit (JE IgM). It is noted that the overall PT score for all 13 JE laboratories was 92% (12/13 JE laboratories). Although the laboratories were asked to include their in-house control samples during testing of the PT samples, not all laboratories included them. The worksheet and instructions were sent out after the receipt of the PT panel and resulted in delayed reporting.

Future directions for the JE LabNet were presented. The 2013 PT panel will be distributed at the end of the JE hands-on training workshop. It was recommended that all the laboratories use their in-house control and submit the trend in titre of the in-house control along with the PT panel results. WHO/US CDC will provide an in-house control sample to laboratories without JE IgM positive samples and also provide technical support to laboratories with less than 80% proficiency.

2.5.6 Specimen collection, preparation and shipment

Dr Tomohiko Takahashi from NIID presented on specimen collection, preparation and shipment for virus isolation and serology. Various types of clinical samples for testing were discussed. CSF is the preferred specimen and CSF collected upon onset of encephalitis is best for the JE diagnosis. If CSF samples are sent within one day, it is not necessary to keep the samples frozen at -80 °C, and they can be shipped cold with frozen ice packs to maintain the temperature at 4–8 °C. Upon receipt in the laboratory, CSF samples are frozen prior to virus isolation attempts.

Pathogen risk groups and categories of infectious substances were introduced in relation to the shipment of samples. Clinical samples are shipped in accordance with the International Air Transport Association (IATA) guidelines. The most important responsibility of the shipper – classification – requires knowledge of the materials being shipped in addition to the regulations. Classification establishes whether or not an item is a dangerous good. The shipper should contact the consignee before sending samples to confirm that they can be accepted, and after sending samples to share shipment details, e.g. airway bill number and flight details. Shipment during public holidays and weekends should be avoided. The materials being shipped must be properly packaged (triple packaging) according to the classification. Proper labelling of the packages for
shipment is a very important step. The labels affixed to the outside of the box communicate the hazards that are contained within the package. The shipper is responsible for making sure all regulations are met and that labels are correct. Dr Takasaki also cautioned that dry ice should not be put inside the secondary container so as to prevent an explosion.

NIID Japan has prepared a ribonucleic acid (RNA) stabilizer that stabilizes samples, protects them from degradation at room temperature and ensures total sample recovery. It was shown that dengue RNAs were stable at room temperature for several months, while at 30 °C and 40 °C, RNAs were stable for several weeks.

Dr Takasaki gave a reminder that human sera and pig sera (mammalian sera) are toxic to c6/36 cells, so, if C6/36 cells are damaged, they cannot be used for viral isolation.

2.6 Introduction of JE IgM ELISA

2.6.1 General Introduction to capture IgM assays

Mr David Featherstone discussed the principle of IgM capture ELISA. The 96-well microplate is coated with capture antibody (anti human-IgM) and blocked. Proper blocking of the unoccupied areas of the plate wells is vital to reducing background noise and attaining an accurate signal. As per Panbio assay, antigens and conjugate are mixed first. Diluted serum/CSF samples are added onto the designated wells and incubated. The plate is washed to remove unbound IgG and any extraneous substances. Premixed (antigens with HRP enzyme conjugate) are added into the test wells and incubated. Again, the plate is washed to remove unbound HRP conjugate, and then substrate (colour developer) is added into the wells. A colour will be developed when the samples are positive and enzymatic reaction should be stopped by adding acid to all test wells. OD is then measured at a wavelength prescribed in the kit.

There are possibilities of flavivirus cross-reactivity in both JE and dengue IgM. However, the Panbio kit has separate wells for JE and dengue to identify any cross-reactivity problems, and a presumptive JE or dengue diagnosis can be made. In comparison, the Inbios JE MAC-ELISA has a separate well for the JE recombinant antigen (JERA) and another well for normal cell antigen (NCA) to identify any cross-reactivity.

To validate the results using Inbios, the mean absorbance of the duplicates of the positive control and negative control are calculated. The immune status ratio of the positive control and negative control is also calculated. A set of criteria to determine the validity of the test was given. If a test is not valid, the test should not be further calculated and staff should attempt to resolve the problem before repeating the test. Result calculation and interpretation were also discussed.

2.6.2 Introduction to the ELISA practical: Panbio and Inbios

Dr Barbara Johnson from US CDC demonstrated the Panbio JE-Dengue IgM Combo ELISA procedure. The Panbio kit can test up to 43 samples per plate. The principle of the test was described. Dr Johnson emphasized that a worksheet should be prepared before testing to set up the plate, indicating the position of the controls and samples in the plate. Step-by-step assay procedures including quality control and calculation of validity, calculation of Panbio units and interpretation of results were described. When a result reveals no detectable IgM, a second sample is required if the first sample was collected less than 7–10 days post-illness. For troubleshooting: when there is a background OD, this might be due to reagents not equilibrated to room temperature, wells not washed properly or incorrect incubation temperature/time. When results are inconsistent, this might be due to inaccurate pipetting, incorrect dilutions of sample/reagents, invalid test, or kits’ reagents past expiry date. The participants were reminded to have a checklist before performing the test. The checklist should include kit expiration date,
plate washer working properly, pipettes calibrated correctly, plate reader working, kit equilibrated to room temperature and in-house control included.

Dr Johnson also demonstrated the Inbios JE Detect MAC-ELISA procedure. The test kit can test up to 44 samples/plate: samples can be tested singly or in duplicate (22 samples/plate) but must be assayed for both JERA and normal cell antigen (NCA). Preparation of the worksheet and set-up of the test are almost the same as Panbio. Step-by-step assay procedures including quality control and calculation of validity, calculation of immune status ratio and interpretation of results were described. The test is valid if JE positive and negative controls are all within acceptable ranges (provided in the kit) and expiration date has not passed. Troubleshooting and checklist are also similar to Panbio.

2.7 Practical sessions

Hands-on training session was conducted in the Korea Human Resource Development Institute for Health and Welfare, Osong, the Republic of Korea from Day 2 to Day 5. Fourteen participants were grouped into seven pairs for the practical sessions. Instructions and protocols for the practical sessions are provided in Annex 3.

On the second day, practical sessions on Panbio JE-Dengue IgM Combo ELISA and Inbios JE Detect MAC-ELISA were performed using serum samples. During the incubation time, calibration of micropipettes and maintenance of ELISA equipment were demonstrated and then performed by participants.

On the third day, the Panbio JE-Dengue and Inbios JE IgM ELISA results using serum samples were reviewed. Practical sessions on Panbio JE-Dengue IgM Combo ELISA and Inbios JE Detect MAC-ELISA were performed using CSF samples.

On the fourth day, results of the practical sessions on Day 3 were analysed and reviewed. A demonstration of Inbios Dengue NS1 Rapid Test for JE-dengue differential testing was conducted.

On the fifth day, ELISA results were consolidated, participants completed a course assessment and quiz, and PRNT and virus isolation were demonstrated. Mr Featherstone discussed the workshop assessment. At the end of the training, the participants were provided with their training certificates.

The JE PT panel samples and kits were distributed to participants (two sets for Malaysia and the Lao People’s Democratic Republic). Participants were asked to submit their results within 14 days using appropriate assays in their own laboratory.

3. CONCLUSIONS

The main conclusions of the training workshop were as follows:

3.1 General

The four main objectives of the training were fully achieved during five days of intensive lectures, hands-on practical sessions and discussions. By the end of the workshop, participants had the technical capacity and knowledge to perform ELISA for laboratory diagnosis of JE,
understood laboratory quality assurance for JE diagnosis, and were fully familiarized with the requirements for WHO accreditation for the JE laboratories.

3.2 Evaluation of the workshop

3.2.1 Overall, the participants were positive in their feedback (Figure 6). The workshop met its objectives, and the schedule and administrative arrangements were well organized by the Korea Human Resource Development Institute for Health and Welfare. The workshop participants were encouraged to follow up with each other, the facilitators and the WHO Regional Office for the Western Pacific on practical issues such as quality assurance, confirmatory testing and data reporting in order to further strengthen JE laboratory capacities in the Region.

3.2.2 The training ran smoothly throughout the practical and lecture sessions. The participants were keen to complete all the tasks and to understand topics addressed during the training. The topics covered throughout the workshop were relevant to the needs of the participants.

Figure 6: Participant satisfaction with Workshop

![Participant Satisfaction Index](image)

3.3 Main outcomes of the training

3.3.1 All participants became more familiar with the Panbio JE-Dengue IgM Combo ELISA and the Inbios JE Detect MAC-ELISA, analysis and validation of results, calibration and maintenance of micropipettes and ELISA equipment, laboratory quality assurance and quality control, and laboratory data reporting and management. The InBios JE Detect MAC-ELISA test will be used in place of the Panbio assay in 2014, as the Panbio assay will no longer be manufactured at the end of 2013. As the Inbios assay tests for JE IgM only, an algorithm for
differentiating JE from dengue infections is currently being developed and is likely to involve additional testing with a specific dengue IgM assay.

3.3.2 Participants were further familiarized with the requirements for WHO accreditation for the JE laboratories and laboratory data management using the WHO JE laboratory data-reporting format for reporting to the Western Pacific Regional Office.

3.3.3 The training also provided a chance for participants to be familiarized with PRNT.

3.3.4 At the end of the workshop, the 2013 WHO JE PT panel samples were distributed to participants. Participants from Malaysia (for NPHL and IMR) and the Lao People’s Democratic Republic (for NCLE and Mahosot Hospital) received two sets of PT samples, respectively. Participants were requested to submit their results within 14 days using appropriate assays in their respective laboratory.

3.4 Workshop follow-up

The results of 2013 JE proficiency testing from 13 JE network laboratories were submitted to the WHO Regional Office for the Western Pacific Region and US CDC within 14 days after the samples arrived in the laboratory. The JE PT panel consisted of six serum samples and four CSF samples. Most of the laboratories used Panbio kits, except for China, Japan and Viet Nam. China used the Beixi kit, while Japan and Viet Nam used in-house assays. During the analysis of the PT results, it was noted that there were discrepancies in the result of CSF sample 1 among the laboratories that performed the testing. It was decided by WHO and US CDC that the result of CSF sample 1 would not be included and only the results of nine samples would be used for analysis. All participating laboratories passed the 2013 JE proficiency test with 100% score.
Day 1, Monday, 23 September 2013

08:30  Registration
08:45  Completion of pre-assessment questionnaire
09:00  Welcoming remarks
       NIH Director
09:10  Workshop objectives
       WPRO Youngmee Jee
09:15  Self-introduction of participants and administrative announcements
       Group Photo

Session 1  Japanese encephalitis/acute encephalitis syndrome (JE/AES) surveillance and Laboratory Network (LabNet)

09:30  Laboratory-based JE/AES surveillance: progress, challenges and plans for sustain the JE LabNet
       David Featherstone
10:00  JE control and lab net progress in the Western Pacific Region
       Youngmee Jee
10:30  Coffee break

Session 2  Quality assurance of the JE LabNet

11:00  Proficiency testing 2012
       Barbara Johnson
11:15  Confirmatory testing 1 and GSL/RRL activities
       Tomohiko Takasaki
       Korea CDC
       Myung-guk Han
       China CDC

Session 3  Introduction of JE virus-specific immunoglobulin M (JEV IgM) enzyme-linked immunosorbent assay (ELISA)

12:00  General introduction to IgM assays
       David Featherstone
12:15  Introduction to the ELISA practical: Panbio and Inbios
       Barbara Johnson
13:00  Lunch break
14:00  Practical 1: JEV IgM ELISA (serum, Panbio)
       (during incubation time: calibration, maintenance of ELISA equipment, micropipettes, etc.)
15:30  Coffee break
16:00 Continuation: laboratory practice-JEV IgM ELISA
17:00 Review of Day 1 results
18:00 Adjourn for the day

Welcoming reception by WHO

Day 2, Tuesday, 24 September 2013

08:30 Discussion of Day 2 activity
Barbara Johnson and David Featherstone

09:00 Practical 2: JEV IgM ELISA (Serum, Inbios)
(during incubation time: calibration, maintenance of ELISA equipment, micropipettes, etc.)
Barbara Johnson
Facilitators & participants

10:30 Coffee break

11:00 Continuation: laboratory practice - JEV IgM ELISA

13:00 Lunch break

14:00 Review of Day 2 (morning ) result

15:00 Practical 3: JEV IgM ELISA(CSF, Panbio)

15:30 Coffee break

16:00 Continuation: laboratory practice-JEV IgM ELISA

18:00 Adjourn for the day

Reception by Korea CDC

Day 3, Wednesday, 25 September 2013

08:30 Discussion of Day 2 afternoon practical 3 (Panbio, in CSF)

09:00 Practical 4: ELISA (CSF, Inbios)
Facilitators & participants

10:00 Specimen collection, preparation and shipment for virus isolation and serology
Tomohiko Takasaki

10:30 Coffee break

11:00 Continuation: laboratory practical-JEV IgM ELISA

13:00 Lunch break

14:00 Review of Practical 4 results
Participants

15:00 Global specialized laboratory testing methods-ELISA, plaque reduction neutralization testing (PRNT) and decision algorithm
US CDC
NIID
KCDC

16:00 Coffee break

16:30 Demonstration of Inbios Dengue NS1 rapid test for JE Dengue differential testing
Barbara Johnson
JohnsonLaboratory tour to Korea CDC
### Day 4, Thursday, 26 September 2013

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:30</td>
<td>Country reports (Cambodia, China provincial CDC (2), Laos NCLE and Mahosots hospital, Malaysia and PNG)</td>
</tr>
<tr>
<td>10:30</td>
<td>Coffee break</td>
</tr>
<tr>
<td>11:00</td>
<td>Country report-continued</td>
</tr>
<tr>
<td>11:45</td>
<td>Philippines, Vietnam NIHE and PI</td>
</tr>
<tr>
<td></td>
<td>Vector surveillance and pig surveillance</td>
</tr>
<tr>
<td></td>
<td>NIID</td>
</tr>
<tr>
<td></td>
<td>KCDC</td>
</tr>
<tr>
<td></td>
<td>China CDC</td>
</tr>
<tr>
<td>13:00</td>
<td>Lunch break</td>
</tr>
<tr>
<td>14:00</td>
<td>Discussion of ELISA results</td>
</tr>
</tbody>
</table>

### Day 5, Friday, 27 September 2013

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00</td>
<td>Consolidation of ELISA results</td>
</tr>
<tr>
<td>10:00</td>
<td>Course assessment and quiz</td>
</tr>
<tr>
<td>10:30</td>
<td>Coffee break</td>
</tr>
<tr>
<td>11:00</td>
<td>Demonstration of PRNT and virus isolation</td>
</tr>
<tr>
<td>12:30</td>
<td>Lunch</td>
</tr>
<tr>
<td>13:30</td>
<td>Demonstration of PRNT and virus isolation</td>
</tr>
<tr>
<td>15:00</td>
<td>Summary of assessment</td>
</tr>
<tr>
<td>15:30</td>
<td>Coffee break</td>
</tr>
<tr>
<td>16:00</td>
<td>Distribution of proficiency panels and kits to network laboratories (two sets for Malaysia, Laos)</td>
</tr>
<tr>
<td>17:00</td>
<td>Presentation of training certificates and Closing</td>
</tr>
</tbody>
</table>
LIST OF PARTICIPANTS, TEMPORARY ADVISERS, AND SECRETARIAT

1. PARTICIPANTS

CAMBODIA

Mr CHIN Savuth, Molecular Laboratory Supervisor, National Institute of Public Health, Lot 2 Kim YL Sung Blvd., Sangkat Boeungkak II, Khan Tuol Kork, Phnom Penh.
Tel. No.: (855-97) 8626 552.
E-mail : Savuth_Chin@yahoo.com

Dr UNG Serey Sopheak, Deputy, Immunology Unit, National Institute of Public Health, Lot 2 Kim YL Sung Blvd., Sangkat Boeungkak II, Khan Tuol Kork, Phnom Penh.
Tel. No.: (855) 12 669045.
E-mail : Sopheak_niph@yahoo.com

CHINA

Dr LU Zhi, Associate Professor, Department of Viral Encephalitis, Institute for Viral Disease Control and Prevention, 155 Changbai Road Changping District, Beijing 102206.
Tel. No.: (86) 10 5890084464. E-mail : liflit@hotmail.com

Dr WU Shengwei, Laboratory Examination, Guizhou Centers for Disease Control and Prevention, No. 101 Bageyanlu, Yunyan District Guiyang 550004. Tel. No.: (86851) 6826218.
E-mail : joneswu-6@163.com

JAPAN

Dr Eri NAKAYAMA, Researcher, Laboratory of Vector-Borne Viruses, Virology I, National Institute of Infectious Diseases, Toyama, 1-23-1, Shinjuku-ku, Tokyo 162-8640.
Tel. No.: (813) 5285 111. E-mail : nakayama@nih.go.jp

LAO PEOPLE'S DEMOCRATIC REPUBLIC

Ms Amphay PHYALUANGLATH, Chief of Clinical Laboratory Department, Mahosot Hospital, Ministry of Health, Fa Ngoum Road, Vientiane. Tel. No.: (856) 21 214024.
E-mail : phayluang62@gmail.com

Miss Phoutsamay VONGPHACHANH, Staff, Sero-Virology Laboratory Center for Laboratory and Epidemiology, Km3 Thadeua Road, Sisattanak District, Vientiane.
Tel. No.: (856) 20 76380094.
E-mail : ny.vongphachanh@hotmail.com

MALAYSIA

Ms NORAZIMAH Binti Tajudin, Science Officer, Serology Unit, Virology Section, National Public Health Laboratory, Ministry of Health, Lot 1853 Kg Melayu Sg Buloh, 47000 Sungai Buloh Selangor. Tel. No.: (603) 6126 1200.
E-mail : norazimah.tajudin@mkak.moh.gov.my; norazimah.tajudin@gmail.com
Annex 2

**PAPUA NEW GUINEA**

Mr Eric BILO, Surveillance Scientific Officer, Central Public Health Laboratories c/o Port Moresby General Hospital, Private Mail Bay 1, Boroko III. Tel. no.: (675) 3248199.
E-mail: bmakuri@gmail.com

**PHILIPPINES**

Ms. Ava Kristy SY, Science Research Specialist I, Virology Department, Research Institute for Tropical Medicine, 9002 Research Drive, FCC Compound, Alabang, Muntinlupa City.
Tel. No.: 632 809 7120. Fax No.: 632 809 7120.
E-mail: avakristysy@gmail.com

Ms Herma BASE, Laboratory Technician 1, Virology Department, Research Institute for Tropical Medicine, 9002 Research Drive, FCC Compound, Alabang, Muntinlupa City.
Tel. No.: 632 809 7120. Fax No.: 632 809 7120.
E-mail: herma1212@yahoo.com

**REPUBLIC OF KOREA**

Mr Sang-gu Yeo, Staff Scientist, National Institute of Health, Center for Disease Control and Prevention, 187 Osong Sangmyeong2-ro, Osong-eup, Cheongwon-gun, Chungbuk 363-951. Tel. No.: 82 43 719 8154.
Fax No.: 82 43 719 8189. E-mail: sg10003@snu.ac.kr

**VIET NAM**

Mr NGUYEN NGOC Linh, Dengue Laboratory, Virology Department, National Institute of Hygiene and Epidemiology, No 1 Yersin Street, Hanoi. Tel. No.: (844) 39726857.
E-mail: sheva2310@gmail.com

Ms NGUYEN Dung Thi Cong, Researcher, Pasteur Institute 167 Pasteur Street, District 3, Ho Chi Minh City.
Tel. No. (84-8) 38296351.
E-mail: cong dung@hotmail.com

**2. TEMPORARY ADVISERS**

Dr Barbara JOHNSON, Research Microbiologist, Diagnostic and Reference Laboratory, Arboviral Diseases Branch, Division of Vector-Borne Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases Centers for Disease Control and Prevention, Fort Collins, Colorado, United States of America. Tel. No.: (970) 266 3543. E-mail: bfj9@cdc.gov

Mr David Alexander FEATHERSTONE, Consultant Scientist, Former WHO Global Vaccine-Preventable Disease Laboratory Network Coordinator, 471 Napier Road, RD 10 Hastings, 4180 New Zealand. E-mail: featherstoned@gmail.com

Dr Tomohiko TAKASAKI, Chief, Laboratory of Vector-Borne Viruses, Virology I, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162 8640, Japan.
Tel. No.: (813) 5285 1188. E-mail: takasaki@nih.go.jp
3. SECRETARIAT

Dr Youngmee Jee, Scientist, Expanded Programme on Immunization, World Health Organization, Regional Office for the Western Pacific, U. N. Avenue, 1000 Manila, Philippines.
Tel. No.: 632 52 89744. Fax No.: 632 521 036. E-mail: jeey@wpro.who.int