WORLD HEALTH ORGANIZATION
REGIONAL OFFICE FOR THE WESTERN PACIFIC

REPORT

WORKSHOP ON THE EPIDEMIOLOGY AND LABORATORY DIAGNOSIS
OF DENGUE FEVER/DENGUE HAEMORRHAGIC FEVER
AND JAPANESE ENCEPHALITIS

Nagasaki, Japan
9-15 August 1994

Manila, Philippines
November 1994
REPORT

WORKSHOP ON THE EPIDEMIOLOGY AND LABORATORY DIAGNOSIS OF DENGUE FEVER, DENGUE HAEMORRHAGIC FEVER AND JAPANESE ENCEPHALITIS

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

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9-15 August 1994

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NOTE

The views expressed in this report are those of the participants in the Workshop on the Epidemiology and Laboratory Diagnosis of Dengue Fever/Dengue Haemorrhagic Fever and Japanese Encephalitis and do not necessarily reflect the policies of the World Health Organization.

This report has been prepared by the Regional Office for the Western Pacific of the World Health Organization for governments of Member States in the Region and for the participants in the Workshop on the Epidemiology and Laboratory Diagnosis of Dengue Fever/Dengue Haemorrhagic Fever and Japanese Encephalitis, Nagasaki, Japan, 9-15 August 1994.
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Keywords:

| Dengue - epidemiology, diagnosis, prevention & control | Encephalitis, Japanese-epidemiology, diagnosis, prevention & control | Diagnosis, Laboratory / Western Pacific / Japan |
SUMMARY

The Workshop on the Epidemiology and Laboratory Diagnosis of Dengue Fever/Dengue Haemorrhagic Fever (DF/DHF) and Japanese encephalitis (JE) met in Nagasaki, Japan, from 9 to 15 August 1994.

The objectives of the workshop were:

1. To review the epidemiological situation on DF/DHF and JE in selected countries of the WPR in which the diseases are endemic.

2. To exchange information and share experiences on DF/DHF and JE control activity between selected countries in which the diseases are endemic.

3. To review the most recent approaches to the prevention and control of DF/DHF and JE, including laboratory diagnosis and case management.

Eleven participants from Cambodia, Fiji, Lao People's Democratic Republic, Philippines and Viet Nam, as well as observers from 11 countries, three temporary advisers, four resource persons and three secretariat members participated in the workshop. Two participants from China did not participate due to unforeseen circumstances.

The members selected Professor Akira Igarashi as a Facilitator, and Dr Josefa Koroivueta and Dr James N.C. Piad as rapporteurs.

Dr S.T. Han, Regional Director for the Western Pacific Region, delivered the opening and closing remarks. In the opening speech, he outlined the importance of DF/DHF and emphasized that the exchange of information and sharing of knowledge and experiences on DF and JE activities between countries with similar situations are necessary.

The WHO staff presented the DF/DHF situation in the Western Pacific Region and the global situation on DF/DHF as a general introduction to the workshop. The lectures on epidemiology, vector control virological examination including advanced means such as PCR method, vector control and case management were given by the temporary advisers and resource persons. The participants from the countries presented their country reports on DF/DHF and JE.

Bench work practice on virus isolation, ELISA and PCR were done.

The workshop drew several conclusions and made several recommendations as follows:

1. All participants recognized that DF/DHF is one of the most important communicable diseases in the Region. They noted its special significance in China, the countries of the Indo-China Peninsula, and presumably the Philippines.

2. The participants noted that the exchange of information and sharing of experiences on a regular basis are important in the development of prevention and control strategies for DF/DHF and JE.
(3) Epidemiological surveillance of DF/DHF and JE should be strengthened where these diseases are endemic. Both diseases should be officially reportable. Laboratory diagnostic capabilities should be enhanced to assure the accuracy of epidemiological data and to improve appropriate case management.

(4) The trainees of this workshop should become the trainers for national training courses in their own countries to disseminate knowledge regarding the most recent approaches to the prevention and control of DF/DHF and JE, including clinical and laboratory diagnosis and case management.

(5) Countries of the Region should work closely with WHO collaborating centres for arbovirus reference and research to exchange information, improve laboratory diagnostic capabilities, and to conduct collaborative research studies.

(6) Efforts to enhance the knowledge of the general population regarding DF/DHF and JE should be encouraged. Specific objectives include instilling an understanding of the basic epidemiology of these diseases (i.e. mosquito-borne), the principles of community-based vector control for dengue vectors (source reduction), and the clinical warning signs of shock.

(7) DF/DHF mosquito control activities should be coordinated with filariasis and/or malaria control activities, where appropriate.

(8) All participants recognized that JE immunization is the most effective means to control JE virus infections in humans.

(9) A quality assurance programme for locally-produced diagnostic reagents for DF/DHF and JE should be established, and regular proficiency testing of national diagnostic laboratories begun. Regional or international centres of excellence may contribute to these efforts.

(10) National control programmes on DF/DHF and/or JE should be strengthened, or established if none exists. Programmes should include sustainable vector control, routine surveillance and reporting, clinician training, health education and enhanced laboratory capacity.

(11) Information from epidemiological surveillance should be regularly reported to WHO/WPRO for publication in the Dengue Newsletter (WPRO/SEARO) and the Weekly Epidemiological Record (Headquarters).
I. INTRODUCTION

Dengue fever (DF)/dengue haemorrhagic fever (DHF) is recognized as a global public health problem. The 46th World Health Assembly which met in 1993 called for global action to control DF/DHF. It has been recognized as one of the serious communicable diseases particularly in Cambodia, China, the Lao People's Democratic Republic, Philippines and Viet Nam. It is also a problem of concern in the South Pacific. There is no cure or vaccine against DF/DHF, so the disease surveillance and virological/serological diagnosis have to be improved in the countries where these diseases is endemic to upgrade the quality of information and ensure proper case management.

Japanese encephalitis (JE) is under control in Japan and the Republic of Korea. It is still serious in China and Viet Nam where, because of its high mortality rate and severe sequelae, local production of JE vaccine has been initiated.

Endemicity of JE has been suspected in Cambodia and Lao PDR; however, due to shortage of laboratory diagnosis and lack of experienced personnel, reporting is poor. The presence of JE in the Philippines was confirmed in 1985; however, the situation is not clear since JE is not a reportable disease there.

As countries with similar endemic situations have had few opportunities so far to exchange information and share their experience and knowledge on prevention and control measures of DF/JE, including laboratory diagnosis and case management, this workshop was conducted on a regional basis.

1.1 Objectives

The objectives of the workshop were:

1) to review the epidemiological situation with regard to DF/DHF and JE in selected countries in which the diseases are endemic;

2) to exchange information and share experiences on DF/DHF and JE control activity between selected countries in which the diseases are endemic;

3) to review the most recent approaches to the prevention and control of DF/DHF and JE, including laboratory diagnosis and case management.

1.2 Participants

Participants numbered 11 experts from Cambodia, Fiji, Lao PDR, Philippines, and Viet Nam who are heads of communicable disease control units in Ministries of Health and/or experienced persons involved in the control of communicable diseases and laboratory workers in charge of DF/JE; three temporary advisers; four resource persons; 15 observers from 11 countries; and three secretariat members. Two participants from China and one temporary adviser from Malaysia were unable to attend owing to unforeseen circumstances. The list of the participants is attached as Annex 1. The workshop took place from 9 to 15 August 1994 at the Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan. The agenda and the schedule of the workshop are attached as Annexes 2 and 3.
1.3 Opening ceremony

Dr S.T. Han, Regional Director of WHO, Western Pacific Region, welcomed the participants. He outlined the importance of DF/DHF which is now recognized as a global public health problem. The Forty-sixth World Health Assembly in May 1993 called for global action to control the disease. He also mentioned that the disease surveillance and virological diagnosis must be improved to gather more accurate information and ensure proper case management. For JE, Dr Han also mentioned that the disease is still considered a serious problem in China and Viet Nam, and endemicity is suspected in Cambodia and Lao PDR. The situation in the Philippines has not been clarified.

Dr Han emphasized that exchange of information and sharing of knowledge and experience on DF and JE activities between the countries with similar situations is important. Dr Han expressed appreciation for the collaboration and contribution of
Professor Igarashi and his staff for making the WHO workshop possible to take place at the Department of Virology, Institute of Tropical Medicine, Nagasaki University, WHO Collaborating Centre for Reference and Research on Tropical Virus Diseases.

Professor Igarashi was appointed as facilitator; Dr J. Koroivueta and Dr J. Piad were appointed as rapporteurs to this workshop.

2. PROCEEDINGS

2.1 Outline of daily sessions

The first day included a general introduction to the workshop, a presentation on the DF/DHF and JE situation in the Western Pacific Region, and the global situation of DF/DHF.

The second day started with a lecture on DF/DHF/JE vectors, virological examination, case management and control. This was followed by the country report from Cambodia, Lao People's Democratic Republic, Philippines, Viet Nam and Fiji on DF/DHF and JE.

The third day was devoted to start the lecture on principles of laboratory diagnosis and epidemiological surveillance on dengue fever and Japanese encephalitis viruses and bench work practices on virus isolation using Aedes albopictus clone C6/36 cells. The afternoon session started with the lectures on JE vector and mosquito control and was followed by bench work practices on sandwich ELISA (enzyme-linked immunosorbent assay) to detect virus antigen and IgM-capture ELISA.

During the fourth day the participants all attended the continuous laboratory practices on ELISA to detect virus antigen and IgM-capture ELISA. Before the end of the fourth day session, a plenary session was held to discuss some important points in the laboratory practices.

The fifth day started with a lecture on the principle of PCR (polymerase chain reaction) and its application for the diagnosis of dengue fever and Japanese encephalitis and followed by bench work practices on PCR for the diagnosis of JE.

The sixth day had the final lecture on DHF/DHF/JE case management followed by a general discussion on the workshop’s conclusions and the finalization of the draft conclusions.

2.2 Summary of the country reports on DF/DHF and JE

2.2.1 DHF in Cambodia: Epidemiology, ecology and strategy for control

The first case of DHF in Cambodia was recorded in 1962. Since 1980, this disease has become an important public health problem, and has spread from the most densely populated parts of the country (Phnom Penh, Kandal, Prey Veng) to all other provinces, except some sparsely populated provinces in the north-east. Outbreaks occurred in 1983, 1985, 1987 and 1990. In 1992 and 1993, outbreaks occurred only in Battambang Province, not in Phnom Penh. In June 1992, the Ministry of Health established the DHF control committee, comprising four subcommittees (epidemiology, vector control, health education and clinical management).
The DHF control committee successfully responded to an epidemic of DHF in progress in Battambang province in 1992 and prevented an expected epidemic in Phnom Penh in 1993.

Although JE is known to exist in adjacent countries, this disease has never been formally reported in Cambodia.

2.2.2 DF/DHF in Fiji

Dengue fever is not a new public health problem in Fiji. Retrospective evidence suggests the disease was present in Fiji as early as the 1880s. Dengue epidemics have occurred periodically in Fiji since 1885, and include major outbreaks in 1930, 1944 and 1971. The more serious form of the disease, DHF first appeared in 1975, with subsequent outbreaks in 1979-1980 and 1989-1990. In the last epidemic, more than 3,600 confirmed cases were reported, with 30 deaths due to DHF (case fatality rate of 6%). The epidemic was due to dengue virus type 1. Epidemics due to dengue serotype 2 and 4 have also been recognized, but no dengue type 3 epidemic has been documented. Sporadic cases of DF have been seen in the last three years. Fiji is now the only country with four known dengue vectors (or possibly even more if *Ae. rotundus* and *Ae. horrescens* are proved): *Ae. aegypti*, *Ae. albopictus*, *Ae. polynesiensis* and *Ae. pseudoscutellaris*. In response to the 1989-1990 epidemic, a National Dengue Task Force was formed by the Ministry of Health in March 1990 to coordinate efforts to prevent and control dengue. Health education and community participation are vital components of a source reduction strategy now being promoted in the prevention and control programme. A national contingency plan is being developed to ensure the sustainability of the control programme at all levels of the health care delivery system. Diagnostic capability exists at the Wellcome Virus Laboratory, but it requires strengthening to allow more rapid confirmation of cases, specific identification of dengue viruses, and improved epidemiological surveillance.

Japanese encephalitis is not known to exist in Fiji.

2.2.3 Country report on DF/DHF and JE in Lao PDR

DF/DHF is now recognized as one of the major public health problems of Lao PDR. DHF was first recognized in Laos in 1979, when 37 cases of DHF were reported to the Ministry of Health. The first epidemic occurred in 1985, and a larger outbreak occurred two years later, with 9,699 cases and 295 deaths. Most cases were detected in the Vientiane Municipality area. Serotype 2 seemed to be most prevalent among four serotypes of dengue virus in the 1987 outbreak. The peak season for DF is in June and July, the country’s monsoon season. Fatal haemorrhagic complications are usually confined to persons under 15 years of age, with a peak incidence in the 3-6-year-old age group. Haemagglutination inhibition test (HI) is used as a standard laboratory technique to detect dengue virus infection. ELISA tests are also used to confirm the diagnosis. At the end of 1988, an Aedes Control Unit (ACU) was set up in the Vientiane Municipality to reduce the Aedes larva density by strengthening health education of the residents, and enhancing community health participation.

JE was not reported in Lao PDR until 1989, when five cases of JE were confirmed by the Armed Forces Research Institute of Medical Sciences (AFRIMS), Thailand. Subsequent research on JE antibody in swine sera showed a high distribution of JE virus in Vientiane Municipality. In 1993, two human cases were found positive by IgM capture ELISA. Unlike DF, there is no specific control unit responsible for JE virus infection, and the public health significance of JE in Lao PDR is not known.
2.2.4 National prevention and control programme for DF and JE in the Philippines

DF/DHF is a major public health concern in the Philippines. It is endemic all over the country, with epidemics observed every 3-5 years. The incidence of the disease is highest in Mindanao, the National Capital Region and Central Visayas. The National Dengue Prevention and Control Programme, which addresses the problem of dengue in the country, is now being piloted in three areas namely, Dumaguete City, Navotas and Manila. Strategies of the programme include integrated vector control, case diagnosis and management, surveillance and research projects, training, and rapid response emergency control. The policies of the programme are that the activities should be community-based; chemical management is confined to areas with confirmed dengue outbreaks; the Department of Health (DOH) supports an integrated vector control approach and the decentralization of decision-making; the DOH should conduct basic and operational research regarding dengue prevention and control; and the DOH should be responsible for the development of the necessary health manpower for sustained dengue control.

At present, the country has no programme on JE. The presence of JE in the Philippines was confirmed by the US Naval Medical Research Unit-2 in 1985, and the disease is known to occur in areas with rice fields, where a greater percentage of children, especially in the 1-10-year age group, are affected. Because the disease is not considered reportable, little information is available on its distribution and impact.

2.2.5 Viet Nam

2.2.5.1 DF/DHF situation in northern Viet Nam

DF/DHF problem in Viet Nam was first recorded in 1959. Since then it has become endemic throughout the country. The biggest outbreaks occurred in 1983, 1987 and 1991. All four types of viruses were isolated but only type 2 and type 1 viruses are predominant. There are two species of mosquitoes identified as the transmission vectors of dengue viruses, i.e., *Ae. aegypti* and *Ae. albopictus*. The main vector, however, is the *Ae. aegypti*.

Only traditional preventive measures are used for larvae and mosquito control such as cleaning the water cisterns, applying larvivorous fishers, and using nets, curtains, and mosquito coils/sticks. For outbreak control, the combination of traditional measures with spraying insecticides (malathion, permethrin, sumithion, etc.) are applied to control the *Ae. aegypti* population. A national DF control activity is now being formulated.

2.2.5.2 DF/DHF situation in southern Viet Nam

In the south of Viet Nam, cases of DHF have been recorded since 1975. The DHF caseload in Viet Nam is known to be the biggest in the world with 77,087 cases in 1983 and 83,905 cases in 1987. In Ho Chi Minh City, the mortality rate per 100,000 population is 1.05. The mean mortality rate/cases is 0.55%. The majority of confirmed cases were among children aged from five to nine.

Patients are given fluids to drink on the first day to prevent shock. Hepatomegaly was observed to occur with severe grades of DHF with shock.
2.2.5.3 JE situation in northern Viet Nam

Japanese encephalitis, a mosquito-transmitted flavivirus (groups A & B) has been one of the serious public health problems in Viet Nam, especially the northern part. The disease was focused in seven provinces of the midlands with large areas of rice cultivation, watered paddy fields, pig-breeding, high temperature and heavy rainfall.

Viral encephalitis syndrome (VES) has been reported every year. Since 1960, serodiagnosis on VES cases and virus isolations from patients, birds and mosquitoes have shown the presence of JE in an endemic form. The highest morbidity rates were observed in May, June and July with a peak in July with an average of two cases per year. The main age groups affected by JE comprised children under 15. Endemicity has been reported in 40.8% of the district provinces in the north of Viet Nam. The mortality rate is under 10%. About 40% of severe sequelae have nervous paralytic and mental impairment.

The principal means for protection is mainly systematic vaccination of susceptible children in areas of endemicity. This involves about 2 million doses/year. Local vaccine production in Viet Nam has been supported by WHO with technology from Japan.

2.2.5.4 JE situation in southern Viet Nam

Cases of acute encephalitis syndrome (AES) and deaths in southern Viet Nam have been reported every year. The highest morbidity/mortality was 936/257 in 1980, the lowest was 197/71 in 1990. Sporadic cases have been reported throughout the year but small outbreaks with low peaks were seen in February and July annually. Twenty-five strains of JE virus were isolated during 1978-1992: eight from patients’ blood, five from cerebrospinal fluid (CSF), nine from *Culex fatigans*, three from *Aedes aegypti*.

Serologically confirmed JE cases were scarce, because most of the human sera sent to the Pasteur Institute, Ho Chi Minh City for testing were used for differential diagnosis of pernicious malaria.

The anti-JE antibody prevalence among humans in 13 of 17 provinces was found to be extremely high, especially in adults.

The antibody positive rate among swine to JE was found to be high: 82% with GMT 65.2 in 189 sera taken at My Tho Tien Giang in March 1978 and 77.3% with GMT 49.7 in 261 sera taken in the vicinity of Ho Chi Minh City in September 1992.

From the above data, the southern part of Viet Nam is classified an endemo-epidemic area of JE virus infection.

2.3 The lectures

As a general introduction to the workshop, Dr N. Okabe presented an epidemiological picture of DF/DHF and JE in the Western Pacific Region and Dr J. LeDuc presented the global situation on DF/DHF.

The lectures were given on epidemiological analysis, virological examination, vector control, insecticides and case management on DF/DHF and JE by temporary advisers and resource persons.
The abstracts of the lectures are attached as Annex 4 a-j.

2.4 Summary of laboratory practices

2.4.1 Virus isolation

Participants were exposed to inoculation of C6/36 cell lines using homogenate suspension of field-caught mosquitoes. Inoculated cells were incubated at 28°C before microscopic examination for evidence of any cytopathic effect.

2.4.2 Enzyme-linked immunosorbent assay (ELISA)

Participants were grouped for better supervision during hands-on experience of two different ELISA formats. Protocols and introductory lectures were delivered by Professor Igarashi and Dr Nawa.

ELISA is an in-house sandwich-type immunological assay for the detection of dengue-specific antigen in cell cultures. The use of sodium thiocyanate enhanced test specificity (which was quite evident in the test).

ELISA is an IgM-capture assay using goat anti-human IgM as the capture antibody. Test serum, viral antigen and enzyme-conjugated anti-viral IgG were added in sequence before adding substrate to detect any colour changes.

Test results were available in 4-6 hours. The session ended with a discussion by Professor Igarashi and Dr Nawa.

2.4.3 Polymerase chain reaction (PCR)

Under close supervision and using a strict aseptic technique, participants carried out PCR. Dr Morita lectured on the principles and the applications of this technology in diagnosing DF and JE. The set of primers used showed 100% sensitivity and good specificity in their study. PCR was used to detect RNA specific to dengue virus and its antigenic type.

2.5 Closing ceremony

The workshop concluded with remarks from Dr N. Okabe, on behalf of Dr S.T. Han, Dr Nguyen Thuy Hoa, on behalf of the participants, and Professor Igarashi, Facilitator of the workshop. Dr Okabe formally closed the workshop and thanked everybody for their participation.

3. CONCLUSIONS

The following conclusions were drawn as a result of discussions in the workshop:

(1) All participants recognized that DF/DHF is one of the most important communicable diseases in the Region. They noted its special significance in China, the countries of the Indo-China peninsula, and presumably in the Philippines.
(2) The participants noted that the exchange of information and sharing of experiences on a regular basis are important in the development of prevention and control strategies for DF/DHF and JE.

(3) Epidemiological surveillance of DF/DHF and JE should be strengthened where these diseases are endemic. Both diseases should be officially reportable. Laboratory diagnostic capabilities should be enhanced to assure the accuracy of epidemiological data and to improve appropriate case management.

(4) The trainees of this workshop should become the trainers for national training courses in their own countries to disseminate knowledge regarding the most recent approaches to the prevention and control of DF/DHF and JE, including clinical and laboratory diagnosis and case management.

(5) Countries of the Region should work closely with WHO collaborating centres for arbovirus reference and research to exchange information, improve laboratory diagnostic capabilities, and to conduct collaborative research studies.

(6) Efforts to enhance the knowledge of the general population regarding DF/DHF and JE should be encouraged. Specific objectives include instilling an understanding of the basic epidemiology of these diseases (i.e. mosquito-borne), the principles of community-based vector control for dengue vectors (source reduction), and the clinical warning signs of shock.

(7) DF/DHF mosquito control activities should be coordinated with filariasis and/or malaria control activities, where appropriate.

(8) All participants recognized that JE immunization is the most effective means to control JE virus infections in humans.

(9) A quality assurance programme for locally-produced diagnostic reagents for DF/DHF and JE should be established, and regular proficiency testing of national diagnostic laboratories begun. Regional or international centres of excellence may contribute to these efforts.

(10) National control programmes on DF/DHF and/or JE should be strengthened, or established if none exists. Programmes should include sustainable vector control, routine surveillance and reporting, clinician training, health education and enhanced laboratory capacity.

(11) Information from epidemiological surveillance should be regularly reported to WHO/WPRO for publication in the Dengue Newsletter (WPRO/SEARO) and the Weekly Epidemiological Record (Headquarters).
INFORMATION BULLETIN NO. 2

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WORKSHOP ON THE EPIDEMIOLOGY
AND LABORATORY DIAGNOSIS OF
DENGUE FEVER/DENGUE HAEMORRHAGIC
FEVER AND JAPANESE ENCEPHALITIS

Nagasaki, Japan
9 - 15 August 1994

PROVISIONAL AGENDA

1. Opening ceremony
2. Presentation of the WHO programme on the control of dengue fever/dengue
   haemorrhagic fever and Japanese encephalitis
3. Lectures on dengue fever/dengue haemorrhagic fever/Japanese encephalitis
   vector, virological examination, case management and control
4. Country reports on dengue fever and dengue haemorrhagic fever
5. Country reports on Japanese encephalitis
6. Practicals of virological examinations
7. Demonstration of virological examination
8. General discussions
9. Conclusion
10. Closing ceremony
ANNEX 3

WORLD HEALTH ORGANIZATION

REGIONAL OFFICE FOR THE WESTERN PACIFIC
BUREAU RÉGIONAL DU PACIFIQUE OCCIDENTAL

WORKSHOP ON THE EPIDEMIOLOGY AND LABORATORY DIAGNOSIS OF DENGUE FEVER/DENGUE HAEMORRHAGIC FEVER AND JAPANESE ENCEPHALITIS

WPR/OCD/CDS(O)/2/94.1A
2 AUGUST 1994
ENGLISH ONLY

Nagasaki, Japan
9 - 15 August 1994

PROGRAMME OF ACTIVITIES

Tuesday, 9 August 1994

1400 Registration
1430 Opening ceremony
   Opening remarks by Regional Director
   Self-introduction of participants
   Designation of Chairman, Vice-Chairman and Facilitators
   Group photograph

1530 Coffee Break

1600 Presentation of the WHO Programme on the control of dengue fever/dengue haemorrhagic fever (DF/DHF) and Japanese encephalitis (JE)
1610 DF/DHF/JE situation in the Western Pacific Region
   - Dr N. Okabe
1630 Global situation on DF/DHF
   - Dr J. LeDuc

Wednesday, 10 August 1994

0830 Lectures on DF/DHF vector, virological examination, case management and control
   DF/DHF/JE virological examination
   - Dr Y. Makino
0900 DF/DHF/JE virological examination
   - Dr M. Nawa
0930 Country report on DF/DHF
   - Cambodia
Annex 3

1000 Coffee Break
1030 Country report on DF/DHF
   - China
1100 Country report on DF/DHF
   - Laos
1130 Country report on DF/DHF
   - Philippines
1200 Lunch Break
1330 Country report on DF/DHF
   - Viet Nam (Part I)
1400 Country report on DF/DHF
   - Viet Nam (Part II)
1430 Country report on DF/DHF
   - Fiji
1500 Country report on JE
   - China
1530 Coffee Break
1600 Country report on JE
   - Viet Nam (Part I)
1630 Country report on JE
   - Viet Nam (Part II)
1700 Country report on JE
   - Laos
1730 Country report on JE
   - Cambodia/Philippines

Thursday, 11 August 1994

0830 Lecture and explanation on practicals of virological examination
   - Dr A. Igarashi
0900 Practical of virological examination
   - Virus isolation
1000 Coffee Break
1030 Practical of virological examination
   - Virus isolation (cont’d.)
1200 Lunch Break
1330 Lecture on DF/DHF vector
   - Dr H.H. Yap
1400 Lecture on JE vector
   - Dr Y. Wada
1430 Lecture on Mosquito control
   - Dr T. Itoh
1500 Lecture and explanation on practicals of virological examination
   - Dr K. Morita
1530 Coffee Break
1600 Practicals of virological examination
   - IgM-ELISA

Friday, 12 August 1994
0830 Practicals of virological examination
   - IgM-ELISA (cont'd.)
1000 Coffee Break
1030 Practicals of virological examination
   - IgM-ELISA (cont'd.)
1200 Lunch Break
1330 Practicals of virological examination
   - IgM-ELISA (cont’d.)
1530 Coffee Break
1600 Practicals of virological examination
   - IgM-ELISA (cont’d.)

Saturday, 13 August 1994
0830 Practicals of virological examination
   - Antigen-ELISA
1000 Coffee Break
1030 Practicals of virological examination
   - Antigen-ELISA (cont’d.)
1200 Lunch Break
1330 Practicals of virological examination
   - Antigen-ELISA (cont’d.)
1400 Demonstration of virological examination
   - PCR
1530 Coffee Break
1600 Practicals of virological examination
   - Antigen-ELISA (cont’d.)
1630 Demonstration of virological examination
   - PCR (cont’d.)

Sunday, 14 August 1994
FREE DAY

Monday, 15 August 1994
0830 Lecture on DF/DHF/JE case management
   - Dr S. Nimmanitya
Annex 3

1000 Coffee Break

1030 General discussion
    Preparation for conclusion

1200 Lunch Break

1330 Conclusion

1400 Closing ceremony
SITUATION ON DENGUE FEVER AND DENGUE HAEMORRHAGIC FEVER
AND JAPANESE ENCEPHALITIS IN THE WESTERN PACIFIC REGION

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STP for Communicable Diseases
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The Western Pacific Region (WPR) of World Health Organization (WHO) is spread over a vast area, extending from China, Japan and the Republic of Korea in the north to Australia and New Zealand in the south, from China, Malaysia and Singapore in the west to Cook Islands and French Polynesia in the east. Covering nearly one-third of the world’s population, the WPR is perhaps the most culturally and socially diverse of the 6 regions of WHO. The Region embraces some of the world’s least developed countries as well as its most rapidly growing economies. Geographically, most the countries in our Region are located in tropical or subtropical zone.

Relatively little information is available, the presence of dengue fever (DF) or DF like illness was known prior to the World War II in the countries and areas in the WPR such as South China, Indo-China Peninsula, Philippines, Fiji, New Caledonia and some other Pacific Islands. It spread widely during the war throughout the Pacific area and appears to have occurred almost everywhere that An. aegypti or another suitable Stegomyia vector was present.

DF/DHF is recognized as one of the major public health problems in most of the countries in the Western Pacific Region such as China, Philippines, Malaysia, Singapore and the countries in Indo-China Peninsula. In the South Pacific, several countries had an outbreak of DF/DHF from 1970s and it is now endemic. There is no cure nor practical using vaccine against the disease at the time. Disease surveillance and virological diagnosis must be improved so that endemic countries in the Region have more accurate information and proper case management. However, the shortage of trained personnel, facility and equipment for clinical and virological diagnosis is a big obstacle in the most of the DF/DHF endemic countries in the Region.
Annex 4a

The forty-sixth World Health Assembly, WHO, in May 1993 called for global action to control DF/DHF and urges Member States to strengthen national and local programmes for prevention and control of DF/DHF.

In the WPR, 28/35 countries and areas experienced DF/DHF outbreak in the past two decades. DF/DHF is now recognized as one of the major public health problem in most of the countries in the WPR.

Japanese encephalitis (JE) is a serious public health problem with significant mortality and severe sequelae for the children and old age in Asia. Immunization of JE is recognized as the most effective mean to control JE virus infections and it has been under control in Japan and Republic of Korea where better agricultural practices, vector control and immunization have been applied. Immunization with locally produced JE vaccine has also been carried in China. Local vaccine production in Viet Nam has been supported by WHO and technology on JE vaccine production has been transferred from Japan.

Although existence of JE in the countries in the Indo-China Peninsula is suspected, accurate epidemiological record has not been available yet due to shortage of laboratory diagnosis facility and lack of experienced personnel in hospitals and laboratories. Due to severity of the disease, importance of the disease in the public health field should be emphasized and the control activity of JE in the endemic countries in the Region should be encouraged.
GLOBAL SITUATION ON DENGUE FEVER

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Dengue fever, and its serious complication dengue haemorrhagic fever (DHF) and dengue shock syndrome, are the most important arbovirus diseases of the world today. An estimated 1.8 billion people are potentially at risk of dengue infection, with approximately 500,000 or more cases occurring annually. The incidence of dengue and DHF is increasing, due in part to increased international travel, which serves to introduce new dengue serotypes into susceptible populations more rapidly than previously experienced, and to invasion of the primary mosquito vector, *Aedes aegypti* into urban, suburban and rural settings throughout the tropics. Over the past three to four decades, DHF has evolved from an infrequent disease to one that is endemic in many parts of Asia, with virtual continuous transmission resulting in thousands of cases annually in many major cities. The Americas are currently experiencing a similar phenomenon, with outbreaks of dengue occurring more frequently in many parts of Latin America, with increasing numbers of DHF cases. This trend, if left uncontrolled, will evolve to a situation similar to that experience in Asia. At present, the only approaches to dengue control are through sustainable vector control to reduce the risk of infection, and improved clinical management, to reduce the risk of death. Efforts to develop an effective tetravalent vaccine to protect against dengue and DHF are in progress, with live, attenuated candidate vaccines in human safety and immunogenicity trials in Thailand, and several efforts to produce engineered subunit or recombinant vaccines in progress under the direction of the World Health Organization Global Programme for Vaccines. There is a critical need to improve surveillance efforts of dengue and DHF to fully document the extent of the problem, quantitate the global burden of these diseases, and to monitor the success of control intervention. To address this need, the World Health Organization is attempting to improve surveillance efforts for dengue worldwide, as part of a larger initiative on emerging infectious diseases.
Since 1993, virological and seroepidemiological survey of Japanese encephalitis (JE), dengue (DEN) and chikungunya (CHIK) virus infections was conducted in pilot areas in Laos under the WHO/JICA Laos trilateral Primary Health Care Project. Human sera were obtained at a pilot village (Sok Yai) in Vientiane Municipality and at the laboratory in Khammouane Provincial Hospital, and specific antibodies were assayed by focus-reduction neutralization (N) test.

Overall positive rates of antibodies to DEN-1, -2, -3 and -4 were 45.3%, 79.1%, 67.6% and 29.7% respectively in Sok Yai Village, while 90.0%, 87.9%, 73.8% and 67.4% respectively in Khammouane province. The positive rate tend to increase with age. CHIK antibody was detected in 16.9% of the sera in Sok Yai Village and in 22.7% of those in Khammouane province. Since DEN and CHIK show similar symptom, diagnosis of these two virus infections may be mixed up.

Overall positive rate of JE antibody was 30.4% in Sok Yai Village and 44.7% in Khammouane province. Swine sera obtained at a slaughterhouse in Vientiane Municipality and from baited swine indicated that the seroconversion to JE was observed during rainy season.

On the primary health care basis, in Sok Yai Village, health education to the villagers and environmental sanitation are being conducted. The project team has organized several training courses on the diagnosis and treatment of JE and DEN for junior doctors, health workers, and laboratory technicians.
Dengue fever (DF) and Dengue haemorrhagic fever (DHF) have been the most common urban diseases in Southeast Asia since the 1950s. More recently, the diseases have spread to Central and South America and are now considered as worldwide diseases. Both Aedes aegypti and Aedes albopictus are involved in the transmission of DF/DHF in Southeast Asian region. The paper discusses the basic ecology of the above Aedes species as well as the present status and future prospects of Aedes control. Both Ae. aegypti and Ae. albopictus are container-breeders and prefer unpolluted water with darker substrate for oviposition. In general, Ae. aegypti breeds in man-made artificial containers in the urban area, whereas Ae. albopictus are ubiquitous and prefer natural containers. Both species have relatively short life cycle and complete immature stages within seven days. Both species bite throughout day light with major biting peaks at change of light intensity especially before sunset. The flight distance of both species is relatively short and is within 200 meters. Vector control approaches which include source reduction and environmental management, larviciding with the use of chemicals (synthetic insecticides and insect growth regulators and microbial insecticide), and adulticiding which include personal protection measures (household insecticide products and repellents) for long-term control and space spray (both thermal fogging and ultra low volume sprays) as short-term epidemic measures are discussed. The potential incorporation of IGRs and Bacillus thuringiensis14 (Bti) as larvicides in addition to insecticides (temephos) is discussed. The advantages of using water-based spray over the oil-based (diesel spray and the use of spray formulation which provide both larvicidal and adulticidal effects that would consequently have greater impact on the overall vector and disease control in DF/DHF are highlighted.
It has been reported that Japanese encephalitis (JE) virus is transmitted by mosquito vectors such as Culex fuscoccephala, Cx. gelidus, Cx. pseudovishnui, Cx. tritaeniorhynchus, and Cx. vishnui. Among them Culex tritaeniorhynchus is the most important throughout the areas where JE virus is distributed in East, Southeast and South Asia.

The larvae of JE vectors including Cx. tritaeniorhynchus breed abundantly in rice fields, therefore the ecology of these mosquitoes is very much influenced by the practice of rice cultivation. The increase in number coincides with the time when the area of irrigated fields increases by rice transplanting. Moreover, the water becomes suitable for the development of larvae by the fertilizer added for the growth of rice plants. Thus, the epidemic of JE is closely related to the rice cultivation. Therefore, due consideration of JE is required when the development of rice fields, for example by deforestation, is attempted. In fact, great epidemic of JE in recent years in Sri Lanka, Nepal and probably in Thailand is thought to be associated with such development of rice fields.

In the field as well as in the laboratory, various methods of chemical control for Cx. tritaeniorhynchus were tested in Japan and other countries. Owing to the great dispersal ability of Cx. tritaeniorhynchus, the aerial spray of insecticides around human habitation and inside animal sheds and dwellings is not enough to control this mosquito. The residual spray of insecticides on the inside walls is not effective to suppress the population of Cx. tritaeniorhynchus probably owing to the exophilic habit of this mosquito.

On the other hand, the application of insecticides to rice fields, which are the main breeding place of Cx. tritaeniorhynchus and other JE vectors, is effective only for one or two weeks in usual cases. Thus, if we wish to keep the mosquito density low enough to interrupt the transmission of JE throughout the epidemic season, at least several applications are required. This means that larviciding will be too costly to control JE vectors breeding in the vast area of rice fields.

The chemical control of Cx. tritaeniorhynchus became more difficult in recent years, owing to the development of insecticide resistance. Cx. tritaeniorhynchus populations collected in 1984 from many localities in Japan all showed surprisingly high resistance to organophosphorus and carbamate insecticides. This high level of insecticide resistance was caused apparently by agricultural chemicals, because insecticides were never used against mosquitoes in rice fields. This strongly suggests the possibility of rice field mosquitoes to develop insecticide resistance wherever agricultural insecticides are used.
Annex 4f

There is evidence that natural enemies play important roles in reducing the number of JE vectors. Fishes, dytiscid beetles, dragonfly larvae, and spiders are particularly important. However, artificial introduction of natural enemies does not have operational value in usual circumstances. For example, it was found that the release of Gambusia affinis, a predator of mosquitoes, to some parts of Japan drove away the Oryzias latipes, a native fish with the same ecological niche in rice fields. Gambusia, therefore, added little to the role of natural enemies in mosquito control.

Mosquito control by using light traps has been a great concern of entomologists. The light trap set at pigsties attracts and kills many JE vectors, while their natural enemies are much less affected. However, the efficiency of the light trap in collecting mosquitoes was revealed to be so low that strong impact on vector populations is hardly expected.

In the control of JE, reduction of mosquito contacts with pigs (amplifier of JE virus) and man should be taken into serious consideration. This could be done, for example, by air-conditioning the house and by using the mosquito screen or the mosquito net with or without insecticide impregnation. It is also advised that human dwellings and pigsties be settled at a far distance from rice fields where JE vectors breed.

Hundred of JE cases were recorded annually in Japan in the 1960s, but the number decreased to tens or less in the 1970s. During these periods, there was clear positive relation between the number of JE cases and the density of vector mosquitoes. There was, however, no appreciable increase in the number of JE cases in the 1980s and on, despite the increase of vector mosquitoes by the development of insecticide resistance. That discrepancy between the number of JE cases and the density of vectors seems to be due to the improvement of human living style and the movement of pigsties to areas far from rice fields during the 1970s, thus reducing mosquito contacts with man and pigs.

In conclusion, most of the measure against JE vectors need further studies and evaluation. Instead, in the control of JE the priority should be given to the improvement of human living style so as to prevent mosquito bites and the vaccination of man.
Utilization of bloodfed females of *Aedes aegypti* as a vehicle for the transfer of the insect growth regulator, pyriproxyfen, to larval habitats

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Bloodfed females of *Aedes aegypti* were exposed to a surface treated with pyriproxyfen at 1.0 g/m² for 30 minutes and then allowed to lay eggs in cups of water containing 4th instar larvae in a cage. Adult emergence from the immatures was highly inhibited, and transmission of pyriproxyfen from the females to the water was revealed. The transfer of the chemicals to the water decreased time before the blood meal. Chemical analysis for pyriproxyfen on the exoskeleton of treated females demonstrated the rapid disappearance of the compound. Pyriproxyfen obviously affected egg maturation of females treated before blood meals, as the number of eggs deposited decreased in proportion to the number of days before the blood meals. Utilization of adults of *Aedes aegypti* as a vehicle of pyriproxyfen was examined at a house in Thailand. The black-colour nettings treated with the chemical 1.5 g/m² and ovitraps containing water were arranged inside the house. The ovitraps were collected after four days to count the number of eggs deposited. The 4th instar larvae of *Aedes aegypti* were inoculated in the water in the trap. Adult emergence from the larvae was highly inhibited at certain ovitraps. This experimental result suggests that the adults which inhabited the house came into contact with the treated nettings and carried pyriproxyfen to the water of the ovitraps.
DENGE FEVER/DENGE HAEMORRHAGIC FEVER CASE MANAGEMENT

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The management of DHF during the febrile phase is similar to that of DF. Survival depends on frequent monitoring of patients for a drop in platelet count and a rise in haematocrit and early clinical recognition of plasma leakage. Early volume replacement as the haematocrit rises sharply with plasma leakage can prevent the development of shock. The critical period for the development of shock is the transition from the febrile to the afebrile phase, which occurs from approximately the third day. Patients who are restless and who have cool extremities, acute abdominal pain and oliguria should be admitted to a hospital.

A drop in the platelet count to < 100 000/cumm or less than 1-2 platelets/oil field (average of 10 OF counts) usually precedes a rise in haematocrit and the onset of shock. A rise in haematocrit of 20% or more indicates significant plasma loss, and fluid therapy is indicated. In mild DHF, intravenous therapy can be given for a period of 12-24 hours at an outpatient clinic.

**Management of dengue shock syndrome**

1) Immediate rapid volume replacement with 5% D/NSS or 5% D/Ringer lactate (RL) or 5% D/Ringer acetate (RA) 10-20 ml/kg/hr or as bolus infusion in case of severe profound shock. Oxygen therapy may be needed.

2) Adjust rate of IV fluid when improvement from shock is apparent:

   reduce the rate to 7 -> 5 -> 3 ml/kg/hr. respectively if the vital signs are stable and there is clinical improvement.

   if the vital signs are unstable and there is no clinical improvement, check the Hct.:

   if Hct ↑ change the IV fluid to colloid solution (Dextran 40 or plasma) 10-20 ml/kg.

   if Hct ↓ give the fresh whole blood transfusion (10 ml/kg/dose at the time and re-evaluate).

3) Continue replacement of plasma loss for the period of leakage (12-48 hrs.) with isotonic solution 5% RL, RA, or 5% D/NSS/2.
Annex 4h

- Check vital signs q 15-30 minutes until stable and then q 1 hour.

4) Correct electrolyte and metabolic disturbance (hyponatremia, metabolic acidosis, hypocalcemia,...)

5) Blood transfusion in case with significant bleeding. Fresh blood transfusion should be given, and occasionally platelet rich plasma should also be given.

6) Other unusual manifestations:
   - CNS involvement - hyponatremia, hypocalcemia, hypoglycemia
   - Intracranial haemorrhage/DIC
   - Control convulsion, if any
   - Correct Na, Ca.
   - Restrict volume - early use of colloid in cases with hepatic failure
   - Exchange transfusion for hepatic failure, DIC

   Fluid replacement must be stopped when the haematocrit becomes stable, after the 24-48 hour period of leakage, and vital signs return to normal and a diuresis ensues. Overtransfusion of fluid at this stage carries the risk of heart failure or pulmonary edema when a return of extravasated plasma takes place.

Prognosis

Case fatality rate of DHF complicated by shock at the Children’s Hospital has declined from 2% in 1984 to less than 1%. There is no evidence that corticosteroids are beneficial. The efficacy of heparin in the treatment of a severe bleeding resulting from disseminated intravascular coagulation remains debatable.

Patients with unusual manifestations, most importantly acute hepatic failure and renal failure (which usually follows prolonged shock), require appropriate treatment. Early exchange blood transfusion in a case with Reye’s syndrome is life saving.
Laboratory diagnosis on viral diseases relies on two principles: (1) virus isolation and (2) serology, which can be applied also to mosquito-borne arboviruses such as dengue and Japanese encephalitis (JE). Events over time in typical acute viral diseases show a viremic phase corresponding to the acute febrile period, followed by antibody production and clearance for the virus, leading to recovery from the diseases. Therefore, virus isolation can be applied only during the acute viremic period, and serology only after antibody production.

Although virus isolation is the most direct way in laboratory diagnosis of viral diseases to detect the presence of infectious agent(s) in the patient. It requires skillful techniques and relatively long time to obtain the final results. Historically, virus isolation was carried out by inoculating test materials into susceptible host animal, for example intracerebral inoculation into mice. When abnormal clinical symptoms or pathological changes were observed in these animals, the infectious agent was assumed to have multiplied and isolated. The animal was then replaced by embryonated eggs, and cell cultures, for ease and to save space and expense. In the case of dengue viruses which were relatively difficult to isolate by inoculation into mouse brains or vertebrate cell cultures, the application of mosquito cell culture was a breakthrough. The time required for virus isolation procedures depends not only on the incubation period for the progeny virus to multiply, but also on the identification of the isolated agents. Classical identification was carried out by the neutralization (N) test, and the procedure was greatly simplified by the development of type-specific monoclonal antibodies (MAB). In some cases, demonstration of viral antigen can be used as an alternative to virus isolation, for example, demonstration of JE antigen in post-mortem brain specimens by immunofluorescent staining.

A recent major advance was the introduction of viral genome detection using polymerase chain reaction (PCR). The principle and detailed procedures will be explained by another lecturer. Further analysis of the nucleotide and deduced amino acid sequences of viral genome have provided more detailed information on the genotype of the virus strains. Such molecular epidemiological studies have given valuable information on the route of disease transmission. Studies on the molecular structure of viral genome will reveal the disease mechanisms, for example, by identifying the virulent viral gene in the future. This information will ultimately be used in the development of genetically-engineered viral vaccines.
The second method of serology in diagnostic virology relies on the principle of immunology, particularly its specificity. Three methods have been used in viral serology for a long time: complement-fixation (CF); hemagglutination-inhibition (HI), and N-tests. The HI was most widely used because it is relatively sensitive, simple and rapid. Problem in the HI test, however, is the cross-reactions, which are the highest of the three methods. Although the N-test is most specific, it was not used as often because of the time and skill required. The CF test is not sensitive enough and relatively complicated in procedure, and was therefore not widely used. All these three serological methods require paired sera which are collected at the acute and then convalescent phase after an appropriate interval to demonstrate a significant rise in the number of antibodies. Since IgM-class antibodies are produced earlier persist transiently and are more specific than the IGM antibodies, the assay on IgM-antibodies was incorporated in the viral serology to provide results even with a single serum specimen. The application of enzyme-linked immunosorbent assay (ELISA) on IgM-antibodies has gradually replaced or supplemented the traditional HI test. In addition to these quantitative antibody assay methods, qualitative or semi-quantitative antibody detection by dot-blot tests were recently introduced as a simple test. In the traditional HI test or the ELISA on dengue or JE, assay antigen is prepared from infected mouse brains by sucrose-acetone-ether extraction. Recently, such antigen was also prepared from infected cell cultures. One of the future directions of viral serology is the development of genetically-engineered assay antigens in place of current antigens.

The principle of virus isolation, demonstration of viral antigen or viral genome detection by PCR, and the assay of antiviral antibodies is also applicable to epidemiological surveillance of dengue and JE virus infections. In these mosquito-borne diseases, surveillance of disease vectors should be supplemented with virological detection of viral agents in the vectors, to obtain more precise information. Virus isolation from vector mosquitoes has been coupled with the antibody surveillance among most important amplifier vertebrates, such as swine, has been used in the surveillance on JE viruses in Japan. Seroepidemiological surveillance of antiviral antibodies in healthy human beings can provide information past exposure to or prevalence of viral agents in the environment, or even using the IgM-antibody assay recent exposure to the disease agents. This information is required to determine the levels of natural infection, and is indispensable for designing a national control strategy on the viral diseases in general.
PRINCIPLE OF PCR AND ITS APPLICATION FOR THE DIAGNOSIS OF DENGUE AND JAPANESE ENCEPHALITIS

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Four antigenetically related but distinct serotypes of dengue virus (D1, D2, D3 and D4) are circulating in many countries of Southeast Asia and cause dengue fever and dengue haemorrhagic fever, both of which are highly prevalent in the countries. The identification and typing of dengue virus, which are essential for epidemiological and clinical investigations, have been performed by immunological methods such as hemagglutination inhibition test, virus neutralization test, complement fixation test and immuno-straining with type-specific dengue virus monoclonal antibodies, after virus isolation from field-caught mosquitoes and from patients.

Although these immunological virus identification methods are reliable and promise to produce results, PCR identification possesses several advantages over conventional immunological procedures. For example, one mutation of nucleic acid can alter an amino acid sequence on an epitope and results in the lack of reactivity with monoclonal antibody for the epitope, while on the contrary, a couple of nucleotide variation does not strongly affect the PCR results. Actually, type-specific monoclonal antibody escape variants are sometimes observed. Furthermore, PCR identification can be completed faster than immunological methods. The PCR procedure, which will be demonstrated in this lecture, is direct rapid RT-PCR procedure enabling simultaneous identification and typing within three hours.

Virus genome could be amplified and detected directly from the acute phase patient's serum without virus isolation procedure. This enables a rapid virological diagnosis in clinical area prior to the elevation of anti-dengue IgM and IgG antibodies, thus enormously facilitating the rapid diagnosis of dengue infection. For example, when the sera specimen obtained at day 2 from the onset of the disease were subjected to the PCR, almost 100% PCR positive result was obtained. However, as viremia is cleared quickly after the elevation of antibodies, the combination of PCR and ELISA diagnosis is always necessary. The precise relationship between PCR detectable viremia and the level of antibodies will be demonstrated in the lecture.
Annex 4j

On the other hand, PCR is not an effective diagnostic tool of Japanese encephalitis (JE), based on our examination. Only one case out of 17 Japanese encephalitis patients showed PCR positive from his cerebrospinal fluid (CSF) before sero-conversion by IgM-capture ELISA. On the contrary, sero-conversion was already observed in 15 cases of PCR negative patients when the patients showed the apparent encephalitis symptoms. It is supposed that the virus in the CSF had been cleared at that time. Thus, IgM-capture ELISA is a realistic tool for rapid diagnosis of JE.