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Japanese Encephalitis and Dengue Virus Infections in Northern Thailand

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INTRODUCTION

Japanese encephalitis (JE) exists in wide geographical areas of East, Southeast to South Asia. After the large epidemic in 1966, the number of JE cases in Japan decreased precipitously and annual cases never exceeded 100 during past 13 years. However, in other parts of Asia, JE is still prevalent and constitutes one of the most serious public health problems because of its high mortality and grave prognosis. In Thailand, the first JE epidemic was reported in 1969 in Northern Region around Chiang Mai. Prophylactic JE vaccine was given in a limited number of children without following up its efficacy, while the number of viral encephalitis in Thailand gradually increased and exceeded 2000 cases in 1980. Since the epidemic of JE occurs in rainy season, coincident to dengue hemorrhagic fever (DHF), conventional serological test sometimes could not give clear-cut answer because of the cross-reactions between JE and dengue viruses both belonging to Flaviviridae with common antigens. In order to understand present status of JE and DHF in Northern Thailand, virological and epidemiological studies were performed during rainy season of 1982 in collaboration with Chiang Mai University and Ministry of Public Health of Thailand, and several Japanese investigators supported by the Grant in Aid for Overseas Research from Ministry of Education, Science and Culture of Japan.

MATERIALS AND METHODS

Specimen collections

Hospitalized cases of encephalitis, DHF or other diseases were collected at Chiang Mai University Hospital, and some other Hospitals in Chiang Mai and Lamphoon Provinces.

Healthy human bloods were collected at 5 locations: Fang, Mae Taeng, Doi Saket, and Sarapee in Chiang Mai Province, and Pasang in Lamphoon Province.

Vertebrate bloods were collected as follows: swine at a slaughter house in Chiang
Mai City; horse and mule at Mae Rim Farm of Royal Thai Army Department; sheep and dog in Chiang Mai University Campus; monkey at Chiang Mai Zoo; chicken, duck, sparrow and lizard at several markets in Chiang Mai City.

Mosquitoes were collected by light traps once a week at 4 collection sites around Chiang Mai City; Mae Rim, Doi Saket, Hang Dong, and San Pathong. Mosquitoes were identified at Chiang Mai University, and Culex tritaeniorhynchus, Cx. gelidus, and Cx. fuscocephala were pooled for virus isolation.

**Virus isolation**

Isolation of JE, dengue and related viruses were performed using Aedes albopictus clone C6/36 cells, from clinical specimens of patient’s sera or postmortem brains and livers, mosquito specimens, and swine sera as described. JE virus was identified by the neutralization test, and dengue viruses were typed using dengue monoclonal antibodies and immunoperoxidase staining.

**Serological tests**

Hemagglutination–inhibition (HI) test was performed according to Clarke and Casals (1958) using sucrose-acetone extracted antigens from infected suckling mouse brains. Indirect ELISA was followed with some modifications, using formalin-inactivated and purified JE vaccine concentrate as antigen.

**RESULTS**

**Hospitalized patients**

A total of 179 cases were examined, with clinical diagnosis of 55 encephalitis, 79 DHF, 9 meningitis, 11 fever of unknown origin (FUO), and 25 other diseases or without clinical diagnosis. DHF cases were mostly observed in Chiang Mai City, 21 out of 30 cases in Chiang Mai Province, while encephalitis cases distributed more widely in the Province.

**Virus isolation**

Eleven strains of dengue viruses were isolated from 177 serum specimens of hospitalized patients. Eight of them were type 1 (D1) two were type 2 and one was type 3 dengue virus. Nine of the dengue viruses were isolated from DHF cases, and 2 of them were diagnosed as dengue infection only by virus isolation. However, one of the type 1 viruses was obtained from encephalitis, and also one of the type 2 viruses from aseptic meningitis cases, respectively, suggesting dengue encephalopathy. One strain of JE virus was isolated from one out of 3 postmortem brains from encephalitis cases. Field-collected mosquitoes totalling 15513 females, however, did not yield any JE virus, although 2 strains of unidentified flaviviruses and many filtrable agents showing cytopathic change in C6/36 cells were detected. Also one postmortem liver and 23 swine sera did not yield any virus.

**Serological examination on hospitalized patients**

Paired sera were obtained from 40 encephalitis, 54 DHF, 6 FUO, 4 meningitis, and 4 other diseases or without diagnosis. The HI test using JE and D1 antigens showed that 7 encephalitis, 1 FUO, and 1 other disease cases showed monospecific response to
JE, while response of 7 encephalitis, 10 DHF, and 3 FUO cases were monospecific to D1. However, 14 encephalitis, 29 DHF, and 2 other diseases showed antibody response to both antigens. IgG-ELISA against JE antigen also appeared to be cross-reactive. On the other hand, IgM-ELISA was more promising with diagnostic levels of antibodies among 22 encephalitis, 1 FUO and 1 other disease, but none among DHF cases. Also in the case of single serum specimens, positive JE-IgM-ELISA was observed only for encephalitis and not for DHF cases.

Seroepidemiological survey on healthy people

The HI test using JE and D1 antigen showed age-dependent increase in antibody positive rate and geometrical mean titer at all the sampling places, however, the values in Fang, the northernmost part of Chiang Mai Province, was significantly lower than in other places. In Sarapee, Doi Saket and Mae Taeng, antibody prevalence by D1 antigen was higher than that by JE antigen.

Antibody survey on vertebrates

The HI test revealed high antibody prevalence against JE virus among swine, horse, mule, sheep and dog. While, only a small percentages of monkeys, ducks and sparrows possessed anti-JE antibodies and chickens and lizards did not possess anti-JE antibodies. One-third of swine sera showed significant levels of JE-IgM-ELISA, suggesting recent infections with JE virus.

SUMMARY AND DISCUSSION

(1) DHF were observed mainly in Chiang Mai City, while encephalitis distributed more widely over the Province.
(2) Almost half of the encephalitis were JE based on IgM-ELISA, and some cases of dengue encephalopathy were observed.
(3) IgM-ELISA was useful in the differential diagnosis on JE and dengue when these 2 flavivirus infections coexist.
(4) Dengue infection apparently became more prevalent in the study area compared with the data by other investigators 15 years ago. type 1 dengue virus was most prevalent serotype in Chiang Mai 1982.
(5) Northernmost part of Chiang Mai Province showed lower prevalence of flavivirus infections compared with Chiang Mai Valley.
(6) Infection rate of vector mosquitoes with JE virus was low compared with the number of apparent JE cases although recent infection of swine with JE virus demonstrated.
(7) Anti-JE antibody positive rate was high among swine, horse, mule, sheep, and dog, but low among monkey, duck, and sparrow, and negative among chicken and lizard.
(8) Mechanism of JE virus infection among Hill Tribes remains to be investigated.
REFERENCES