RESEARCH NOTE

IDENTIFICATION OF DENGUE VIRUS IN *AEDES* MOSQUITOES AND PATIENTS' SERA FROM SI SA KET PROVINCE, THAILAND

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Abstract. Dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are the re-emerging infectious diseases caused by dengue (DEN) virus, transmitted by *Aedes* mosquito. There are more than 100,000 cases of dengue infection and more than 100 deaths annually in Thailand. Virological surveillance for DEN viruses is used as an early warning system to predict outbreaks. The seroprevalence of infection and serotypes of DEN virus in 116 pediatric patients at Si Sa Ket Province, Thailand were analyzed during June to September 2004. At the same period, *Aedes* mosquitoes were caught from patients' and their neighbors' houses, from control houses, located in villages with no report of dengue infection during the previous 3 years. The majority of DHF cases were secondary infections of DEN-2 and DEN-4 serotypes. Of the 1,652 *Aedes* mosquitoes collected 1,583 were *Ae. aegypti* and 69 *Ae. albopictus*. Ten mosquitoes from each house were pooled and dengue viruses were determined using RT-PCR assay; only 1 positive pooled was found. Although the dengue infection rate in the field caught mosquitoes was low, the existing dengue virus control program in transmission areas by aerial spraying to destroy the larva breeding sites should be continued.

Keywords: dengue virus, mosquito, RT-PCR, surveillance

INTRODUCTION

Dengue virus infection is a serious health problem in Southeast Asia and worldwide (Gubler, 1998). Annually, there are 20 million cases of dengue virus infection, and around 24,000 cases are fatal. There were more than 60,000 cases of dengue virus infection annually reported in Thailand (Dengue Hemorrhagic Fever Section, 2011). Dengue viruses can cause a variety of symptoms ranging from asymptomatic fever, which may lead to undifferentiated fever (viral infection), to dengue fever (DF), dengue hemorrhagic

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fever (DHF) with plasma leakage that may lead to hypovolemic shock (dengue shock syndrome, DSS) (WHO, 1997). The clinical features of dengue virus infection depend on the age of the patient, immunological condition, as well as presence of antibody-enhancement of dengue virus replication and differences in the dengue virus strains (WHO, 1997; WHO SEARO, 1989).

Dengue virus infection can lead to hospitalization and death. However, there has been a decrease in incidence due to people's awareness of the disease and changes in their risk behavior. This behavior is difficult to establish, so new or improved methods of surveillance of dengue virus infected-mosquitoes is necessary to decrease incidence of the disease. Virological surveillance provides an early warning sign for the risk of transmission in an area and for the specific predominant circulating serotype in the vector population (Rodhain and Rosen, 1997). Dengue viruses have four antigenically distinct serotypes: dengue virus types 1-4 (DEN-1-4) (Innis, 1995; WHO 1997). *Aedes aegypti* is considered the main vector because this species is closely associated with human habitation, but in some regions other *Aedes* species, such as *Ae. albopictus* and *Ae. polynesiensis*, are also involved. Transovarial transmission is considered to be important in maintaining dengue virus at a low level of transmission within the human population (Rigau-Perez *et al*, 1998).

The objectives of this study were: 1) to identify dengue serotypes in the field caught mosquitoes from Si Sa Ket Province, Thailand, 2) to determine the infection rate in these feild caught mosqitoes; and 3) to compare the relationship between serotypes detected in mosquitoes and in serum samples collected from dengue patients at Si Sa Ket hospitals.

MATERIALS AND METHODS

Mosquitoes samples

Female *Ae. albopictus* mosquitoes were collected by using landing and resting collection techniques according to WHO protocol (WHO, 1997) during the epidemic period of June to October, 2004. The *Aedes* mosquitoes were collected from the houses of 15 cases of dengue patients and their neighbor's houses, which had not been sprayed with insecticide for at least 3-4 weeks previously, which reflect the first case of village in the year. Each mosquito collection was performed within 24 hours after dengue infection report and before aerial spray on the particular area. The distances between dengue patients' houses and neighbores' houses were less than 200 meters. Control mosquitoes were collected from houses located in villages, where there has been no report of dengue cases for 3 years. The mosquitoes were separated by sex and species (Rueda, 2004) and then were stored at -70ºC until used.

Patient's samples

A total of 116 paired sera were collected during febrile stage and 7-10 days after the first blood drawn from dengue suspected patients, enrolled in this study at Si Sa Ket provincial hospitals during the period June-September, 2004. These paired sera were used for determination of dengue infection using IgM and IgG capture ELISA and PCR assay. All serum samples were kept in liquid nitrogen tank and transported to laboratory of the Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand and kept at -70ºC until analyzed.

Serological assay

In order to confirm the diagnosis of dengue infection in the patients based on WHO criteria (WHO, 1997), serum sample was tested for anti-dengue virus IgM and IgG antibodies by IgM and IgG capture ELISA (Innis *et al*, 1989). In brief, 50 l of patients' sera were added to 96-well microtiter plate coated with 1:800 diluted goat anti-human IgM or IgG antiserum in 6mM carbonate buffer at pH 9.0. After incubation for 2 hours and washed with PBS-T (phosphate buffer saline-Tween) 3 times, 50 l of pooled sucrose acetone extracted dengue antigen in PBS plus 20% acetone extracted normal human serum was added and incubated at 37ºC for 2 hours then washed 3 times with PBS-T. Twenty-five microliters of 1:1,500 horseradish peroxidase conjugated human anti-flavivirus IgG in PBS plus 20% acetone extract normal human sera and 0.5% bovine serum albumin were added, incubated at 37ºC for 1 hour and washed 3 times with PBS-T. The reaction was followed by the addition of 50 l of o-phenyline diamine in 0.1M citrate buffer and terminated with 50 l of 4N H_2SO_4. Optical density was measured at 492 nm using an ELISA reader.

Interpretation of results. A ratio of anti-dengue Ig M to Ig G (if Ig M > 40 units) > 1.8 was the criterion for primary dengue infection. A ratio of Ig M to Ig G <1.8 was the criterion for secondary infection.

PCR assay

The acute phase serum from each patient and mosquito samples were screened for dengue virus by reverse transcriptase-polymerase chain reaction (RT-PCR). For mosquito samples, 10 mosquitoes from each house were pooled and subjected to RNA extraction using commercial viral RNA extraction kit (Invitrogen, Carlsbad,

Table 1

Species of *Aedes* mosquitoes from the collection sites.

	Ae. aegypti	*Ae. albopictus*
Control houses	206 (12.5%)	0 (0%)
Patients' houses	1,377 (83.4%)	69 (4.2%)

CA). RT-PCR assay followed by the nested PCR assay, was performed as described by Lanciotti *et al* (1992) using primers according to Reynes *et al* (2003). RT-PCR and nested-PCR amplicons were analyzed by 1.5% agarose gel-electrophoresis and stained with ethidium bromide (0.5 g/ml).

RESULTS

Patients samples

The final clinical diagnosis of each patient indicated that 102/116 having dengue infection and of these 31% (36/102) and 57% (66/102) can be classified as DF and DHF, respectively. Serum samples of 116 dengue suspected in-patients were analysed in this study. All serum samples were tested for anti-dengue virus IgM and IgG antibodies by IgM and IgG capture ELISA and febrile stage serum samples were determined for dengue serotype by PCR. Capture ELISA assay revealed 61/116 (53%) had no antibodies against dengue virus, while 55/116 (47%) had secondary infection (data not shown). No primary infection was found in this study. As for the PCR assay, 54/116 (46%) were positive for dengue viral RNA, classified as 13 (24%) DEN-1, 19 (35.19%) DEN-2, 3 (5.56%) DEN-3, and 19 (35.19%) DEN-4 (data not shown). The clinical diagnosis for DF and DHF among the serotypes revealed that the most DHF cases caused

by DEN-2 and DEN-4 and all of them were secondary infections.

Mosquito samples

A total of 1,652 *Aedes* mosquitoes were caught; 1,583 (95%) were *Ae.egypti* and 69 (4.2%) were *Ae. albopictus* (Table 1). Only 1 pooled sample of *Ae. aegypti* from patients' houses revealed the positive PCR result for DEN-2 viral RNA with 0.52% infection rate. Dengue viral RNA was not detected from all pooled *Ae. albopictus* samples. All pooled *Aedes* mosquitoes in control houses gave negative PCR results.

DISCUSSION

In this study, *Aedes* mosquitoes were collected from dengue patients' houses during the rainy season in Si Sa Ket Province, one of the highest epidemic areas of DHF transmission in northeastern Thailand (Dengue hemorrhagic Fever Section, 2011). This study was conducted only in the rainy season due to the increasing of dengue virus transmission during this period, although dengue can be spread throughout the year. It is speculated that temperature and humidity favor the survival of adult mosquitoes beyond their normal period, increasing the probability that viral transmission occurs (McBride and Ohmann, 2000).

The single dengue RNA positive pooled was obtained from the house of a patient who also gave a positive result for dengue viral RNA DEN-2 serotypes in both instances.

The low infectivity rate of *Ae. aegypti* is similar to that reported in Singapore of 0.51% and 0.59% of dengue virus infection rate in *Ae. aegypti* and *Ae. albopictus*, respectively (Chan *et al*, 1971). The infection rate depends on various factors: the virus strain, susceptibility of the mosquito species, sensitivity of virus detection technique, study sites, and the period of the investigation (Tuksinvaracharn *et al*, 2004).

Clinical diagnosis was relatively high (88%) when compared with the capture ELISA assay (47%). For IgM test results using ELISA assay the critical factor is the time from the onset of symptoms and the point of specimen collection. Specimens taken earlier than 6 days after onset will have some false negatives due to insufficient time for the production of detectable IgM antibody. If paired serum samples are available, they can be titrated, and a rising, stable, or falling titer will indicate more accurately antibody production during infection. One complicating factor is that a small percentage of secondary dengue virus infections shows a low or negative IgM reaction (Kuno *et al*, 1991; Ruechusatsawat *et al*, 1994). If negative results are shown because of contradictory clinical or epidemiologic data, an IgG determination should be undertaken. An early rising and high IgG titer is observed if the case is a recent secondary infection (Gubler, 1997).

Although, low dengue infection rate in the field caught mosquitoes was found in this study the dengue virus control in transmission areas by aerial spraying to destroy mosquito larva breeding sites should be continued (Chareonviriyaphap *et al*, 2003).

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