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Occurrence of Dengue and Japanese Encephalitis Viruses in Oriental Mindoro, Philippines

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Introduction

Dengue and Dengue Hemorrhagic Fever are caused by viruses of the family Flaviviridae (former group B or Togaviridae), genus *Flavivirus,* subgroup no. 7 (DE MADRID and PORTERFIELD [5]). At present there are about 70 members of this genus at least half of which are established human pathogens. By ecological and epidemiological criteria dengue viruses are arboviruses (arthropod-borne) since they are transmitted by various members of the mosquito genus *Aedes (Aedes aegypti, Aedes albopictus, Aedes scutellaris* complex). Four clearly defined types (Dengue 1 to 4) are identified on the basis of serologic differences. The virus is spherical, approximately 50 nm in diameter and possesses a lipidcontaining envelope. After an incubation period of 2 to 7 days, dengue fever syndrome is characterized by a biphasic fever and rash for one to five days with pharyngeal inflammation, rhinitis, mild cough and headache. Occasionally back pain precedes or accompanies the fever. Dengue hemorrhagic fever and shock syndrome are followed after some days by rapid deterioration and physical collapse. Frequently there are scattered petechiae on the forehead and extremities with weak pulse. Death occurs in 10 - 40% of patients, depending on availability of supportive therapy in a hospital. The geographic distribution comprises S. E. Asia, Sri Lanka, the Philippines, New Guinea, Tahiti, as well as Nigeria and the states and islands of Central America.

Japanese (B) Encephalitis (JE) is caused by another member of the genus *Flavivirus*. Subgroup no. 3 also includes Murray Valley Encephalitis, West Nile, St. Louis Encephalitis, Kunjin, Usutu, Kokobera, Stratford and Alfuy viruses. The main vectors of this arbovirus are mosquitoes of the genus *Culex (Culex tritaeniorhynchus, C. pipiens* complex, *C. gelidus, C. annulus, C. vishnui.* Isolates of this virus are also reported from Anophelinae mosquitoes *(Anopheles barbirostris, A. hyrcanus, A. sinensis, A. vagus* and *A. annularis).*

JE virions are spherical, lipoprotein-enveloped particles of about 20 nm diameter. The genomes are single stranded RNA. Human infections vary from clinically inapparent to acute fatal encephalitis disease. After an incubation period of 7 to 10 days clinical disease is usually abrupt with fever, headache and meningeal irritation. Manifestations of upper motor neuron and extrapyramidal tract involvement are frequent. Prognosis varies with the age of patients, ranging from 20% or below in children to nearly 50% in patients over 50 years of age. Sequelae remain in 30 to 40% of convalescent patients.

JE occurs in epidemics in people in maritime areas of the western Pacific region from eastern Siberia southwards to Taiwan and in tropical areas of Southeast Asia in India, Thailand, Malaysia, Indonesia and the Philippines.

During the last decade a number of outbreaks of Dengue Fever and Dengue Hemorrhagic Fever could be observed in the Philippines. Between 1983 and 1986, 336 strains of dengue virus could be isolated from 1,911 patients who had been hospitalized with clinically suspected dengue infection in Manila and surrounding areas. Multiple serotypes were present every

year (MANALOTO and HAYES, [11]). HAYES et al. (1989) reported dengue virus infections in 24 American military personnel in 1984. Another study was conducted from May, 1983 to January, 1984 in a hospital in Manila, where 517 patients were found to be infected by dengue viruses. Dengue 2 was the predominant serotype, followed by Dengue 3 and Dengue 1, respectively. Dengue 4 was found only in a few isolates (HAYES et al. [8]). A total of 377 Filipino children out of a total of 5,427 admissions from October, 1983 to March, 1984 were found to have dengue fever or dengue hemorrhagic fever (SONGCO et al. [13]). Few studies have shown that Japanese Encephalitis does occur in the Philippines with the majority of the cases affecting the one to ten year old age group in places where rice fields abound. According to the author the morbidity rate is 15 - 17%, with a mortality rate of about 7 - 30% (BARZAGA [1]).

Until today all investigations on the activity of the two arboviruses were conducted in and around Manila, the capital of the Philippines, and on a few other places of the main island of Luzon. Our present study on Mindoro, an island close to Luzon should demonstrate the presence of dengue and JE viruses.

Material and Methods

0.5 ml of untreated serum specimen were obtained from outpatients visiting the hospital for different reasons, most of them had fever. Serum collection started in November 1991 and was finished in March, 1992. The frozen sera were stored in Calapan until dispatch to Vienna. In order to avoid unspecific reactions, the sera were treated with acetone and afterwards tested in a dilution of 1 : 10 against 4 to 8 antigen units of Dengue 2 and 4 and also of JE in the hemagglutination inhibition (HI) test (CLARKE and CASALS [4]) in U-Nunc-Microtiterplates. Further dilutions were done geometrically to 1 : 1.280.

Results and Discussion

45 of a total number of 129 sera tested were not reactive with one of the three antigens. 46 sera reacted positive against D2 and D4 antigens. Most of the sera showed the same titers against both antigens, in some cases the antibody titers against D2 were higher than those against D4. 38 sera were positive against JE (Tab. 1).

Many serum specimens showed cross reactions in the HI test between D2/D4 and JE antigen. Better results were obtained by BURKE et al. (3) using epitope-blocking immunoassays for antibody detection in human sera. The authors found the results superior to the HI test and comparable to the plaque reduction neutralization for identifying subjects immune to DF and JE or both viruses. Antibody response in JE and DHF patients was measured by the indirect micro ELISA by BUNDO et al. (2). The authors reported that responses of JE were rather monospecific in contrast to DHF cases whose antibody responses were cross-reactive to JE and D1 antigens even in the primary infection. GUNASEGARAN et al. (7) developed a highly specific, sensitive and economical hemadsorption immunosorbent technique for the detection of dengue-specific immunoglobulin M (IgM) antibody. The technique is based on the reaction of human sera with anti-human IgM immobilized onto a solid phase followed by the detection of dengue-specific IgM by the addition of a known quantity of dengue virus hemagglutinin and goose erythrocytes. Dengue-specific IgG did not interfere with the results, nor was there any cross-reactivity between dengue-hemagglutinins and IgM specific for other viruses.

JOO and WADA (10) reported on prevalence of the vector mosquitoes of JE in Korea. They could demonstrate the role of three species of culicide mosquitoes, *Culex tritaeniorhynchus, C. pipiens pallens,* and *Anopheles sinensis,* transmitting JE. The seasonal peak occurrence of the three species was different as well as the night-time biting activity. *C. tritaeniorhynchus* is probably the most important vector of JE virus in towns, and both, *C. tritaeniorhynchus* and *C. p. pallens* would be important in rural localities. OLSON et al. (12) isolated JE virus from *Anopheles annularis* and *A. vagus* in Lombok, Indonesia. The frequencies of JE viral infection

Table 1:
Antibody titers of the 84 positive sera

Dilution 1:	20	40	80	160	320	640	1280	total
Dengue 2 and 4	2	8	15	16	3	2	–	46
Japanese Encephalitis	5	5	5	7	10	4	2	38

in zoophilic Anopheles species was higher than in *C. tritaeniorhynchus,* the principal vector of JE virus in Asia. According to the authors the low frequency of infection in *C. tritaeniorhynchus* and the relatively infrequent raising of pigs (in an Islamic country) may account for the low prevalence of JE neutralizing antibodies in the human populations of Lombok.

The natural foci of DF and JE viruses are towns on the one hand, and rural areas on the other hand. Different vector species transmit the viruses. The main vector of the urban cycle of DF Virus in *Aedes aegypti,* in rural areas other members of the subgenus *Stegomyia* of the genus *Aedes* transmit the DF virus, i. e. *A. albopictus* and the *A. scutellaris* complex. JE virus is mainly transmitted by *C. tritaeniorhynchus* in urban as well as in rural areas. A large number of other species of culicine and anopheline mosquitoes are also involved in the natural cycle of the virus, as can be seen above. The vectors of both viruses are rice paddy breeding mosquito species with a marked preferance for animal feeding.

Field trials with experimentally developed live vaccines were conducted in Thailand against DF virus. Some vaccines against JE virus have been available for many years and are produced mainly in Japan. DENNING and KANEKO (6) suggest that all visitors to rural areas should receive vaccination against JE, particularly during the summer monsoon months in Southeast Asia.

Summary 129 human serum specimens were obtained from the Provincial Hospital in Calapan, Oriental Mindoro, in order to gather information about the occurrence of Dengue, Dengue Hemorrhagic Fever and Japanese Encephalitis in Oriental Mindoro. In the hemagglutination inhibition test 45 sera were negative, 46 positive against Dengue virus, and 38 positive against Japanese Encephalitis virus.

Key words Dengue, Japanese Encephalitis viruses, Mindoro/Philippines.

Zusammenfassung

Verbreitung von Dengue und Japan B-Enzephalitis in Ost-Mindoro, Philippinen

129 Serumproben aus dem Provinz-Spital in Calapan, Ostmindoro wurden auf den Gehalt an Antikörpern gegen Dengue (Typ 2 und 4) und Japonica B Enzephalitis untersucht. Im Haemagglutinationshemmungs-Test zeigten sich 45 Proben negativ gegen beide Antigene, 46 Proben zeigten positive Titer gegen Dengue-Viren und 38 solche gegen Japonica B Enzephalitis Virus.

Schlüsselwörter Dengue Virus, Japan B Enzephalitis Virus, Mindoro/Philippinen.

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