FIRST ISOLATION OF JAPANESE ENCEPHALITIS FROM *CULEX QUINQUEFASCIATUS* IN THAILAND

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Abstract. Isolation of Japanese encephalitis (JE) virus using C6/36 cell and immunofluorescence virus antigen detection techniques was attempted from female mosquitoes collected with CDC gravid traps in Samut Songkhram Province in the central region and in Phuket Province in southern Thailand, in 2003. One thousand and eighty female mosquitoes including 6 species of the Culicidae family (*Culex quinquefasciatus*, *Cx. gelidus, Cx. tritaeniorhynchus*, *Cx. whitmorei, Cx. vishnui* complex, *Cx. s.g. culiciomyia*) (pooled by specific specimen), were processed for virus isolation. Two pools of *Cx. quinquefasciatus* yielded a JE virus isolation. This represents the first report of JE virus isolation from *Cx. quinquefasciatus* in Thailand.

INTRODUCTION

Japanese encephalitis (JE) virus is the leading cause of viral encephalitis in unvaccinated populations of Southeast Asia with a case fatality rate of 25%. Severe clinical forms with permanent neurological sequelae have been observed in 50% of patients (Solomon *et al*, 2000). The geographical distribution of JE virus in humans extends between 15º South to 45º North latitude with a northern boundary from Japan to maritime Siberia (Rosen, 1986; Burke and Leake, 1988), south to Queensland, Australia, and expanding westward from Southeast Asia to India and eastward to Korea. JE virus is a zoonotic disease transmitted by mosquitoes to domestic pigs and wild animals, especially birds, that act as effective amplifying hosts (Buescher and Scherer, 1959; Scherer *et al*, 1959). The virus is maintained in nature by a cycle involving Culex mosquitoes (*Cx. tritaeniorhynchus*, *Cx. annulus*, *Cx. fuscocephala*, *Cx. gelidus*, and *Cx. vishnui* complex) and vertebrates.

Cx. quinquefasciatus is predominantly an urban mosquito but also the most common domestic mosquito species of semi-urban and rural areas. It is extremely abundant and geographically widespread in tropical countries, particularly in Southeast Asia. *Cx. quinquefasciatus* is a highly anthropophilic mosquito, but is also an opportunistic species since 53% to 63% feed on humans, 7% to 14% may feed on cattle, 6% to 25% and 1.5% on pigs (Reuben *et al*, 1992). Also, *Cx. quinquefasciatus* has been shown experimentally to be capable of efficiently transmitting the JE virus (Banerjee *et al*, 1977). The JE virus isolate from *Cx. quinquefasciatus* was reported for the first time in Vietnam in 1974 (Thoa *et al*, 1974).

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MATERIALS AND METHODS

Study site

Samut Songkhram Province is located in the central plain of Thailand. It covers 417 km^2 with a coastal area on the Gulf of Thailand and has a population of 205,346 (2002).

Phuket Province is an island, covering 543 km^2 with a population of 246,043 (2002), and is

located in southern Thailand. Phuket formerly derived its wealth from tin and rubber, but pig farming is increasing. It is also visited by many tourists.

Mosquito collection

Adult mosquitoes were collected within the two provinces using the CDC gravid trap (Model 1712; Centers for Disease Control, Division of Vector-Borne Disease) with fermented hay. Specimens were transported on dry ice to the laboratory for species identification and sorted into monospecific pools.

In Samut Songkhram Province *Culex* mosquitoes were collected in January 2003 in Ban Brong sub-district (Mueang district) where a human JE case was reported in December 2002. In Phuket Province, collection was performed from May 2002 to May 2003 from pig farms in Mai Khao and Ban Don sub-districts (Thalang district) that were used as JE survey sites.

Virus isolation

Mosquito females were sorted into monospecific pools of 25 to 100 mosquitoes; engorged females were not processed. Pools of mosquitoes were grinded and homogenized in 1 ml sterile phosphate buffer saline (PBS) pH 7.4 and 30% fetal calf serum. Homogenates were centrifuged at 3,000 rpm for 15 minutes at 4ºC, tube filter (Spin-x, 0.22 µl Costar 8160). Supernatant was aliquoted and stored at -70ºC. An aliquot was used for virus isolation by inoculating 0.2 ml homogenates onto a 3-day old C6/36 cell line in a 25-cm^2 flask and incubated in CO_2 incubator at 32ºC for 90 minutes. Then 4 ml of MEM media 4 was added to the flask and incubated in a CO_2 incubator at 32ºC to be harvested on Day 7.

Virus identification

Virus identification was done by indirect immunofluorescent test on C6/36 infected cells on 6 well fluorescein stained slides as previously described (Henchal *et al*, 1983).

RESULTS

Between May 2002 and May 2003, 1,091 Culex mosquitoes were collected by CDC gravid trap in Phuket Province. One thousand and sixteen were females *Cx. quinquefasciatus* and represented 98% and 92% of the catches from Mai Khoa and Ban Don, respectively (Table 1). JE

Table 1
Japanese encephalitis virus isolation attempt (cell culture C6/36) from female *Culex* mosquito trapped by CDC gravid trap in two provinces of Thailand.

Culex species	Samut Songkhram		Phuket			
	Ban Brong		Mai Khao		Ban Don	
quinquefasciatus	7[a] (77.8)[b]	1/1[c]	191 (98.0)	1/10	825 (92.1)	0/14
gelidus	0	0	2 (1.0)	0/1	23 (2.6)	0/1
tritaeniorhynchus	0	0	1 (0.5)	0/1	45 (0.1)	0/1
whitmorei	0	0	1 (0.5)	0/1	1 (0.1)	0/1
Vishnui complex	0	0	0	0	1 (0.1)	0/1
(*Culiciomyia*)	2 (22.2)	0/1	0	0	1 (0.1)	0/1
Total	9	1/2	195	1/13	896	0/19

[a] = Number of *Cx.* mosquitoes; [b] = Percent of *Cx.* species; [c] = Positive/pool of mosquitoes

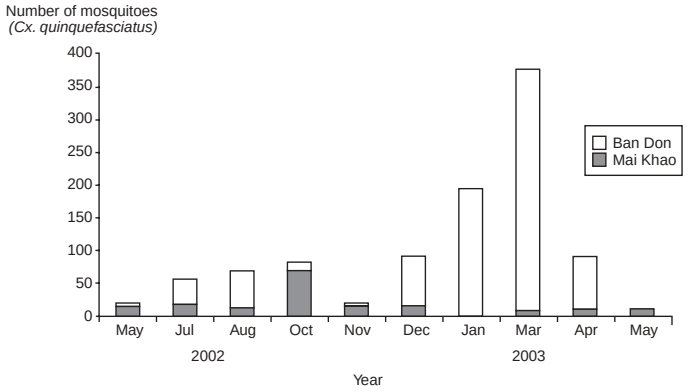

Fig 1–JE virus isolation from female *Cx. quinquefasciatus* trapped by CDC gravid trap in Mai Khoa and Ban Don sub-districts in Talang district in the Phuket Province, by month.

virus was isolated in one instance from a *Cx. quinquefasciatus* pool from Mai Khoa Sub-district (Fig 1).

Nine *Cx. quinquefasciatus* females (77.8% of the total) were collected in Samut Songkhram Province and pooled into one pool, which yielded one JE virus strain isolate.

DISCUSSION

Cx. quinquefasciatus is endemic in all tropical areas and is the predominant species residing in houses (Kohn, 1990) wherever similar species (*Cx. pipens*) are found in temperate areas. This species was rarely found during the JE epidemic in Thailand during the 1960s and 1970s (Endy and Nisalak, 2002). Meanwhile, following urbanization and the development of industrial pig farms, it has spread all over the country. It is much more anthropophilic than *Cx. tritaeniorhynchus, Cx. gelidus*, and *Cx. fuscocephala,* the historical vectors of JE during the major epidemic (1960s and 1970s) in Thailand. Reuben *et al* (1992) found that 53.2-62.7% fed on humans; 7-14.7% on cattle, and 1.5% fed on pigs. But it is also very opportunistic, and in our catches made during the night from the houses of the pig-farm owners, only 7% (3/43) had fed on humans (unpublished results).

This is the first time the JE virus was isolated from *Cx. quinquefasciatus* in Thailand. The first isolation of JE from *Cx. quinquefasciatus* was in Vietnam (Thoa *et al*, 1974). A single isolation of JE virus was made from *Cx. quinquefasciatus* in Kolar district in India, in 1986 (Mourya *et al*, 1989). Moreover *Cx. quinquefasciatus* (*Culex fatigans*) has been shown to be capable of transmitting the JE virus in the laboratory (Banerjee *et al*, 1977), and experimental vertical transmission of JE virus by *Cx. quinquefasciatus* has been demonstrated to F1 adult progeny (Rosen *et al*, 1989).

Thus in Thailand, on one hand, *Cx. quinquefasciatus* appears to extend its geographical range over areas where historically it was rarely observed. On the other hand, the development of pig farming, where JE virus circulates intensively (Yamada *et al*, 1971; Burke *et al*, 1985) in the vicinity of villages, suggests that *Cx. quinquefasciatus,* biting both humans and pigs, can be important in the potential transmission of JE virus among humans.

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