

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. cullicomyia*) (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 15° South to 45° 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).

MATERIALS AND METHODS

Study site

Samut Songkhram Province is located in the central plain of Thailand. It covers 417 km² with a coastal area on the Gulf of Thailand and has a population of 205,346 (2002).

Phuket Province is an island, covering 543 km² with a population of 246,043 (2002), and is

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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 4°C, tube filter (Spin-x, 0.22 µl Costar 8160). Supernatant was aliquoted and stored at -70°C. 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-cm² flask and incubated in CO₂ incubator at 32°C for 90 minutes. Then 4 ml of MEM media 4 was added to the flask and incubated in a CO₂ incubator at 32°C 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 Khao 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.

<i>Culex</i> species	Samut Songkhram		Phuket			
	Ban Brong		Mai Khao		Ban Don	
<i>quinquefasciatus</i>	7 ^a (77.8) ^b	1/1 ^c	191 (98.0)	1/10	825 (92.1)	0/14
<i>gelidus</i>	0	0	2 (1.0)	0/1	23 (2.6)	0/1
<i>tritaeniorhynchus</i>	0	0	1 (0.5)	0/1	45 (0.1)	0/1
<i>whitmorei</i>	0	0	1 (0.5)	0/1	1 (0.1)	0/1
Vishnui complex (<i>Culicomyia</i>)	0	0	0	0	1 (0.1)	0/1
	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

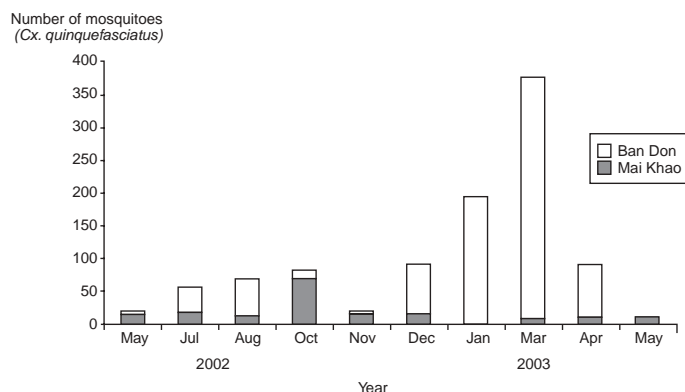


Fig 1—JE virus isolation from female *Cx. quinquefasciatus* trapped by CDC gravid trap in Mai Khoa and Ban Don sub-districts in Talang district in the Phuket Province, by month.

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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REFERENCES

- Banerjee K, Deshmukh PK, Ilkal MA, Dhanda V. Experimental transmission of Japanese encephalitis virus through *Anopheles tessellatus* and *Culex fatigans* mosquitoes. *Indian J Med Res* 1977; 65: 746-52.
- Buescher EL, Scherer WF. Ecologic studies of Japanese encephalitis virus in Japan. IX. Epidemiologic correlations and conclusions. *Am J Trop Med Hyg* 1959; 8: 719-22.
- Burke DS, Leake CJ. Japanese encephalitis. In: Monath TP, ed. *The Arboviruses: epidemiology and ecology*. Vol 3. Boca Raton: CRC Press,

- 1988: 63-92.
- Burke DS, Tingpalapong M, Ward GS, Andre R, Leake CJ. Intense transmission of Japanese encephalitis virus to pigs in a region free of epidemic encephalitis. *Southeast Asian J Trop Med Public Health* 1985;16: 199-206.
- Endy TP, Nisalak A. Japanese encephalitis virus: ecology and epidemiology. *Curr Top Microbiol Immunol* 2002; 267: 11-48.
- Henchal EA, McCown JM, Seguin MC, Gentry MK, Brandt WE. Rapid identification of dengue virus isolates by using monoclonal antibodies in an indirect immunofluorescence assay. *Am J Trop Med Hyg* 1983; 32: 164-9.
- Kohn M. A survey on indoor resting mosquito species in Phnom Penh, Kampuchea. *Folia Parasitol (Praha)* 1990; 37: 165-74.
- Michael E, Ramaiah KD, Hoti SL, Barker G, *et al.* Quantifying mosquito biting patterns on humans by DNA fingerprinting of bloodmeals. *Am J Trop Med Hyg* 2001; 65: 722-8.
- Mourya DT, Ilkal MA, Mishra AC, Jacob PG, *et al.* Isolation of Japanese encephalitis virus from mosquitoes collected in Karnataka state, India from 1985 to 1987. *Trans R Soc Trop Med Hyg* 1989; 83: 550-2.
- Reuben R, Thenmozhi V, Samuel PP, Gajanana A, *et al.* Mosquito blood feeding patterns as a factor in the epidemiology of Japanese encephalitis in southern India. *Am J Trop Med Hyg* 1992; 46: 654-63.
- Rosen L. The nature history of Japanese encephalitis virus. *Ann Rev Microbiol* 1986; 40: 395-414.
- Rosen L, Lien JC, Shroyer DA, Baker RH, *et al.* Experimental vertical transmission of Japanese encephalitis virus by *Culex tritaeniorhynchus* and other mosquitoes. *Am J Trop Med Hyg* 1989; 40: 548-56.
- Scherer WF, Moyer JT, Izumi T, Gresser I, *et al.* Ecologic studies of Japanese encephalitis virus in Japan. VI. Swine infection. *Am J Trop Med Hyg* 1959; 8: 698-706.
- Solomon T, Dung NM, Kneen R, Gainsborough M. Japanese encephalitis. *J Neurol Neurosurg Psychiatry* 2000; 68: 405-15.
- Thao NTK, Vien NT, Mai TT, Xuan NTN. Japanese encephalitis vectors: isolation of virus from culicine mosquitoes in the Saigon area. *Southeast Asian J Trop Med Public Health* 1974; 5: 408-12.
- Yamada T, Rojanasuphot S, Takagi M, Wungkobkiat S, Hirota T. Studies on an epidemic of Japanese encephalitis in the northern region of Thailand in 1969 and 1970. *Biken J* 1971; 14: 267-96.

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