

# OUTBREAK OF CHIKUNGUNYA FEVER IN THAILAND AND VIRUS DETECTION IN FIELD POPULATION OF VECTOR MOSQUITOES, *Aedes aegypti* (L.) AND *Aedes albopictus* Skuse (DIPTERA: CULICIDAE)

Usavadee Thavara<sup>1</sup>, Apiwat Tawatsin<sup>1</sup>, Theerakamol Pongsakul<sup>1</sup>, Payu Bhakdeenuan<sup>1</sup>, Sumalee Chanama<sup>1</sup>, Surapee Anantapreecha<sup>1</sup>, Chusak Molito<sup>2</sup>, Jakkrawarn Chompoonsri<sup>1</sup>, Suwich Thammapalo<sup>2</sup>, Pathom Sawanpanyalert<sup>1</sup> and Padet Siriyasatien<sup>3</sup>

<sup>1</sup>National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi; <sup>2</sup>Office of Disease Control 12, Songkhla; <sup>3</sup>Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

**Abstract.** We investigated chikungunya fever outbreak in the southern part of Thailand. Human plasma specimens obtained from suspected patients and adult wild-caught mosquitoes were detected for chikungunya virus employing reverse transcriptase-polymerase chain reaction technique. Chikungunya virus was detected in about half of the blood specimens whereas a range of 5.5 to 100% relative infection rate was found in both sexes of the vector mosquitoes, *Aedes aegypti* (L.) and *Ae. albopictus* Skuse. The infection rate in *Ae. albopictus* was higher than in *Ae. aegypti*, with relative infection rate in male of both species being higher than in female. The appearance of chikungunya virus in adult male mosquitoes of both species reveals a role of transovarial transmission of the virus in field population of the mosquito vectors. These findings have provided further understanding of the relationship among mosquito vectors, chikungunya virus and epidemiology of chikungunya fever in Thailand.

## INTRODUCTION

Chikungunya fever is an arthropod-borne disease caused by chikungunya virus (Family: *Togaviridae*, Genus: *Alphavirus*) and is transmitted to humans by the bite of infected mosquitoes. Chikungunya virus was first isolated from man and mosquito during an epidemic of fever in Newala, Tanzania between 1952 and 1953 (Ross, 1956). Two species of *Aedes* mosquitoes, *Ae. aegypti* (L.) and *Ae. albopictus* Skuse, are well recognized

as vectors of this disease (McIntosh and Gear 1981; Gratz, 2004; Vazeille *et al*, 2007; Reiskind *et al*, 2008). However, many studies have revealed that *Ae. albopictus* shows a higher susceptibility to chikungunya virus and more efficiency to transmit the virus than *Ae. aegypti* (Mangiafico, 1971; Turell *et al*, 1992; Yamanishi, 1999; Schuffereinecker *et al*, 2006; Vazeille *et al*, 2007, 2008). Chikungunya fever is rarely life-threatening and milder than dengue infection as it has no severe hemorrhage manifestations or shock (Nimmannitya and Mansuwan, 1966). When compared with dengue infection, chikungunya fever seems to be more acute (short onset of illness) and predominant in high fever (with short duration), erythematous maculopapular eruption, headache and

---

Correspondence: Dr Padet Siriyasatien, Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

Tel: 66 (0) 2256 4387; Fax: 66 (0) 2252 5944  
E-mail: padet.s@chula.ac.th

muscle pain (Nimmannitya and Mansuwan, 1966). The disease also frequently causes rash and severe arthralgia (joint pain without inflammation) or arthritis (joint pain with inflammation), which sometimes persisted for weeks to months (Thaikruea *et al*, 1997). Until now, no vaccine is available against chikungunya viral infection.

Sporadic outbreaks of chikungunya fever were reported from many countries in Asia since late 1950s. Since then, chikungunya virus has been isolated or detected in Thailand in 1958 (Hammon *et al*, 1960), and subsequently in Cambodia (Chastel, 1963) and India (Sarkar *et al*, 1964) in 1963, Vietnam (Dai and Kim-Thoas, 1967) and Sri Lanka (Hermon, 1967) in 1967, the Philippines in 1969 (Campos *et al*, 1969), Myanmar in 1970 (Khai *et al*, 1974), Malaysia in 1978 (Marchette *et al*, 1978) and Indonesia in 1982 (Slemons *et al*, 1984). Since 2000, indigenous and imported cases of chikungunya fever have been reported from several countries in various continents, including Africa (Congo, Gabon and Kenya), Asia (India, Sri Lanka, Indonesia, Malaysia, Singapore and Thailand), Europe (Italy, France, Germany, Norway and Spain) and some islands in the Indian Ocean (Comoros, Madagascar, Mauritius, Mayotte, La Reunion and Seychelles) (WHO, 2007). In 2004, the disease affected almost 500,000 people in Africa (Epstein, 2007). Recently, chikungunya fever affected 266,000 cases (approximately one third of total population) in La Reunion Island during the period from February 2005 to June 2006, and about 1.42 million cases in India during the epidemic between January 2006 and August 2007 (WHO, 2007).

The first record of chikungunya fever in Thailand as well as in Southeast Asia was found in Bangkok in 1958 by virus isolation from blood specimens collected from patients during the epidemic of dengue fever and dengue hemorrhagic fever (Hammon

*et al*, 1960). In 1962, 160 blood specimens out of 815 patients with hemorrhagic fever admitted to the Children's Hospital, Bangkok, were randomly selected for virus isolation and serological studies and 135 cases were confirmed as dengue (98 cases), chikungunya (29) and possible double infection (8) (Nimmannitya and Mansuwan, 1966). Chikungunya fever disappeared from Thailand for about 14 years until some cases were reported from Prachin Buri in 1976. The disease re-emerged in the country with reported cases of chikungunya fever in 1988 from Surin, in 1991 from Khon Kaen, in 1993 from Loei and Phayao, and in 1995 from Nong Khai and Nakhon Si Thammarat. Since August 2008, chikungunya fever has re-emerged again in Thailand with several thousands of reported cases from at least 47 provinces of Thailand. This paper provided information of a recent incidence of chikungunya fever in Thailand together with data of viral infection in vector mosquitoes conducted in Songkhla Province, a particular area with high incidence of the disease in southern Thailand.

## MATERIALS AND METHODS

### **Incidence of chikungunya fever in Thailand**

The data of reported cases of chikungunya fever in Thailand between January 1, 2008 and June 30, 2009 were obtained from the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand.

### **Collection of blood specimens**

Blood specimens were taken from suspected patients who were admitted to hospitals in various provinces of Thailand, including Songkhla Province. Blood samples were drawn into test tubes containing EDTA as anticoagulant, and centrifuged to obtain plasma, which were kept in liquid nitrogen and then transported to the Arbovirus Section, National Institute of Health, Department

of Medical Sciences, Nonthaburi, Thailand for determination of chikungunya infection.

#### **Virus detection in blood specimens**

Viral RNA was extracted from 100 µl of patient plasma using the QIAamp viral RNA mini kit (QIAGEN, Germany) following the manufacturer's protocol. The procedures for chikungunya virus (CHIKV) detection in plasma followed the methods described by Parida *et al* (2007) with minor modifications. In brief, one-step reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using a primer pair of CHIKV E1 gene [CHIK-F3 (ACGCAATTGAGCGAAGCAC) (genome position 10294 to 10312) and CHIK-B3 (CTGAAGACATTGGCCCCAC) (10498 to 10480)]. Amplification was carried out in a 25 µl total reaction volume using Superscript III one-step RT-PCR kit (Invitrogen, USA) with 50 pmol of each primer and 2 µl of RNA. Thermal cycling of RT-PCR was 48°C for 30 minutes and 94°C for 2 minutes, followed by 35 cycle of 94°C for 1 minute, 54°C for 1 minute and 72°C for 1 minute and a final extension cycle at 72°C for 10 minutes. RT-PCR products were detected by electrophoresis in 2% agarose gel.

#### **Study site for mosquito collection**

Songkhla, a province of southern Thailand was selected as the study site. This province has been recognized as an area with high incidence of chikungunya since 2008 and it also has two species of mosquito vectors, *Ae. aegypti* and *Ae. albopictus*. Eight villages from two districts, Mueang and Hat Yai (four villages from each district), were randomly selected. At least 20 houses from each village were randomly chosen for collection of adult mosquitoes.

#### **Mosquito collection**

Mosquito collection was carried out at the study sites during late January 2009. The dwellings for mosquito collection were randomly selected from the eight villages of the

study sites. Eight volunteers collected mosquitoes indoors for 20 minutes in each dwelling. The collectors usually situated themselves in dark areas of the room where most mosquito landing and biting activities occur. The collectors bared their legs between knee and ankle and collected all landing mosquitoes individually in vials capped. Using a similar procedure, mosquito collecting was also conducted outdoors (approximately 5 - 10 m away from dwellings) to catch *Ae. albopictus* in the same environment. Mosquito collection was usually carried out from 9:00 AM to 5:00 PM. The collected mosquitoes were visually identified, as there were only two species, *Ae. aegypti* and *Ae. albopictus*, present. These live mosquitoes were inactivated by placing in a refrigerator, and then separated by species, sex and locality. Pools were stored in liquid nitrogen for subsequent chikungunya viral detection. A maximum of 5 mosquitoes were placed in each pool.

#### **Virus detection in mosquitoes**

In each pool, mosquito wings and legs were removed and the remaining bodies were ground in the lysis solution provided with the test kit and centrifuged. The supernatant was then processed for RNA extraction as described above.

## **RESULTS**

In 2008, a total of 2,233 cases of chikungunya fever were reported from 4 provinces of southern Thailand, Narathiwat, Pattani, Yala and Songkhla. The first official report of chikungunya fever in Thailand in 2008 was at week 33 (August 10 - 16, 2008) from Narathiwat. The reported cases of chikungunya fever increased gradually week by week until the end of 2008 with the highest incidence of about 400 cases per week (Fig 1). However, chikungunya fever has increased dramatically from the first week of 2009 with an incidence of about

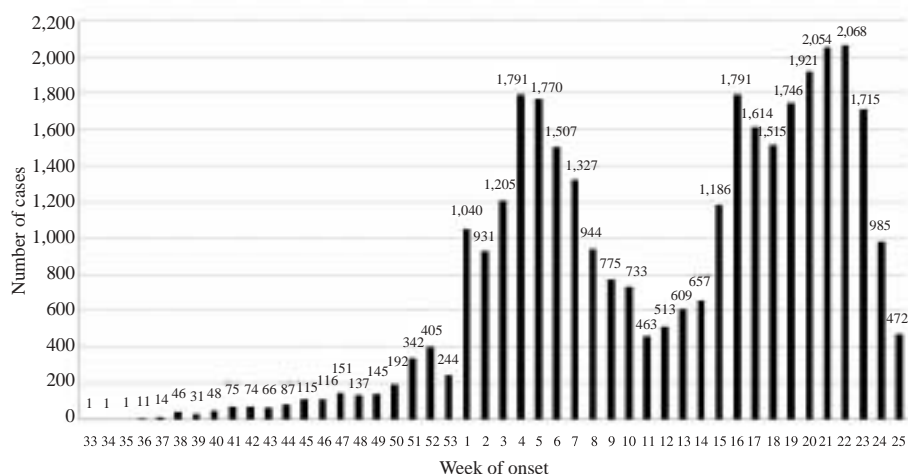


Fig 1—Number of reported cases of chikungunya fever in Thailand by week of onset between August 2008 and June 2009.

1,040 cases. The incidence of chikungunya fever in 2009 has fluctuated between 463 and 2,068 cases per week with 3 peaks appearing at week 4 (1,791 cases), week 16 (1,791 cases) and week 22 (2,068 cases) (Fig 1). A total of 32,102 cases of chikungunya fever were reported from 47 out of 76 provinces of Thailand during January 1 and June 30, 2009. Among these, 31,768 cases (98.96%) were from the southern region (14 provinces) whereas those from central (14 provinces), north (9 provinces) and northeastern region (10 provinces) were 137 cases (0.43%), 129 cases (0.40%) and 68 cases (0.21%), respectively. The top-ten highest incidence were reported from Songkhla (9,451 cases), followed by those from Narathiwat (7,735 cases), Pattani (4,219 cases), Yala (2,735 cases), Phatthalung (2,212 cases), Phuket (2,154 cases), Trang (1,370 cases), Surat Thani (473 cases), Chumphon (434 cases) and Nakhon Si Thammarat (397 cases), all located in southern Thailand (Fig 2). The reported cases of chikungunya fever from the other 37 provinces ranged from 1 to 262 cases. Interestingly, only 72 cases of chikungunya fever were reported from

Bangkok during this period. Regarding age distribution, most reported cases were frequently found in patients aged 35 - 44 (19.1%), 25 - 34 (18.0%), 15 - 24 (15.9%) and 45 - 54 years (15.2%), and less than 10% were found in other age groups (Table 1). The lowest percentage of 0.4% was found in children aged less than 1 year. There was no mortality among all reported cases of chikungunya fever during the current outbreak.

From 1,756 blood specimens collected from patients admitted in hospitals from various provinces throughout Thailand during the period from October 2008 to June 2009 and determined for presence of chikungunya virus, 964 specimens (54.9%) were positive for chikungunya infection (Table 2). The relative infection rate obtained in 2008 (54.4%) was almost equal to that detected in 2009 (55.6%). Some 76 out of 169 specimens obtained from Songkhla were positive for chikungunya virus, but the infection rate (36%) in 2008 was substantially lower than that (58%) in 2009.

Mosquito collections were carried out randomly in eight villages of two districts of Songkhla Province, Mueang and Hat Yai

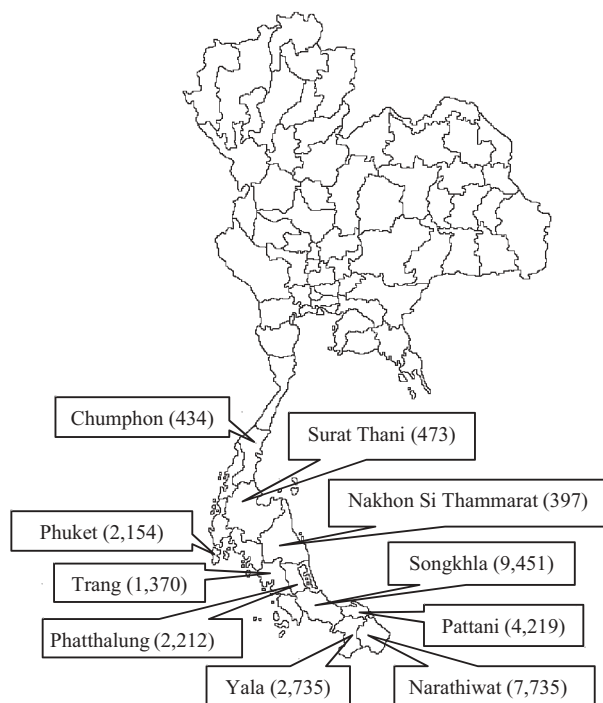


Fig 2—Map of Thailand showing 10 provinces with high reported cases of chikungunya fever from January 1 to June 30, 2009.

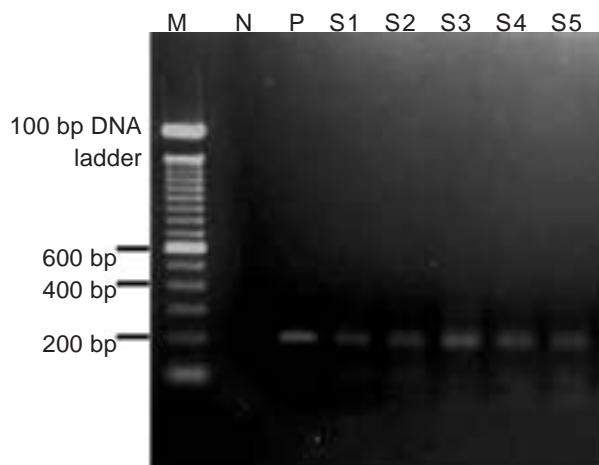


Fig 3—Gel electrophoresis of RT-PCR amplicon of chikungunya virus. RT-PCR was carried out as described in Materials and Methods. M, molecular weight marker; N, negative control; P, positive control; S1 – S5, samples positive for chikungunya virus.

(four villages from each one) during late January. A total of 169 houses from the eight villages were surveyed in this study. Of 192 adult mosquitoes collected from the study sites 165 were *Ae. aegypti* (41 males and 124 females) and 27 *Ae. albopictus* (2 males and 25 females), and subsequently they were pooled according to species, sex and locality of collection. There were 101 pools of *Ae. aegypti* (28 pools of males and 73 pools of females) and 17 pools of *Ae. albopictus* (2 pools of males and 15 pools of females), and all 118 pools were identified for chikungunya virus by RT-PCR assay (Fig 3 and Table 3). Nineteen out of 118 pools (16%) were positive for chikungunya virus. Of these positive pools, 10 pools were *Ae. aegypti* (6 males and 4 females) and 9 pools were *Ae. albopictus* (2 males and 7 females). Overall, the relative infection rate of chikungunya virus in *Ae. albopictus* (53%) was higher than in *Ae. aegypti* (10%). In addition, the relative infection rate of the former species in both males (100%) and females (47%) was higher than those of the latter species, 21% and 5%, respectively.

## DISCUSSION

After the first appearance in Thailand in 1958, epidemics of chikungunya fever have re-occurred many times (in 1962, 1976, 1988, 1991, 1993, 1995 and 2008). Apparently, chikungunya fever has shown remarkable epidemiological appearance, *ie* epidemics occur and disappear periodically, with inter-epidemic periods of a few years and sometimes as long as more than 10 years. A long silence of 10 years or more was also observed in other countries, such as India and Malaysia. The reason for a long period of disappearance of chikungunya fever in these places is still unknown. It may be due to a broken transmission cycle of the disease between infected humans and vector mosquitoes. The



Table 1  
Age distribution of totally 32,102 reported cases of chikungunya fever in Thailand from January 1 to June 30, 2009.

Age (Year)	<1	1 - 6	7 - 9	10 - 14	15 - 24	25 - 34	35 - 44	45 - 54	55 - 64	>65
Proportion (%)	0.4	4.5	3.5	8.7	15.9	18.0	19.1	15.2	8.4	6.3

Table 2  
Prevalence of chikungunya infection detected in blood specimens collected from patients admitted to hospitals from various provinces of Thailand and Songkhla, between October 2008 and June 2009.

Area	Year	Total test specimens	PCR result		Relative infection rate (%)
			Positive	Negative	
Thailand	2008	1,011	550	461	54.4
	2009	745	414	331	55.6
	Total	1,756	964	792	54.9
Songkhla	2008	100	36	64	36.0
	2009	69	40	29	58.0
	Total	169	76	93	45.0

Table 3  
Prevalence of relative infection of chikungunya virus in *Ae. aegypti* and *Ae. albopictus* mosquitoes collected from Songkhla Province, Thailand, during late January 2009.

Mosquito species		Total test mosquitoes	Total test pools	Total positive pools	Relative infection rate (%)
<i>Ae. aegypti</i>	Male	41	28	6	21.4
	Female	124	73	4	5.5
	Total	165	101	10	9.9
<i>Ae. albopictus</i>	Male	2	2	2	100
	Female	25	15	7	46.7
	Total	27	17	9	52.9
Grand total		192	118	19	16.1

chikungunya virus appears in blood circulation of the infected person only for a few days or so, and if no vector mosquito takes a blood meal during this viremic period, the transmission cycle then will be broken.

Chikungunya fever could re-emerge when a carrier person travels and stays in a place which already has the vector mosquitoes, namely, *Ae. aegypti* and *Ae. albopictus*.

The recent outbreaks of chikungunya

fever in India (2006 - 2007) and Thailand (2008 - 2009) similarly affected a larger number of people than previous epidemics. These could be due to variations in genotypic and antigenic characteristics of chikungunya virus in the regions. Formerly, the Asian genotype of chikungunya virus was responsible for the epidemics in the whole continent of Asia, whereas the other two genotypes of the virus, West African (WA) and East Central South African (ECSA) strains, were responsible for the African countries (Arankalle *et al*, 2007). However, the ECSA genotype of chikungunya virus has been introduced into the Asian continent and was responsible for the explosive outbreak in India in 2006 (Yergolkar *et al*, 2006) and Singapore in 2008 (Leo *et al*, 2009). During the current epidemic in Thailand, similar strains of chikungunya virus, as isolated from the outbreaks in India in 2007 and in Singapore in 2008, were also found in clinical specimens collected from patients in Narathiwat (Theamboonlers *et al*, 2009), and both species of mosquito vectors were collected from Prachuab Khiri Khan (unpublished data). Regarding the variation in antigenic characteristics of chikungunya virus, a mutation at position 226 of the E1 gene with the substitution of alanine by valine was observed from virus isolated in La Reunion in 2006 (Schuffenecker *et al*, 2006) and in India in 2007 (Arankalle *et al*, 2007). This mutant strain of chikungunya virus, is also suspected to be in Thailand during the current outbreak, and studies on genomic sequencing of chikungunya virus are required.

Regarding the prevalence of chikungunya infection in blood specimens, the positive rates found in this study were relatively low, ranging from 36% to 58%. This could be due to at least three main factors affecting the results, namely, quality of blood specimens, sensitivity of detection method and misdiagnosis. The most appropriate blood specimen

for viral detection should be collected from suspected patient during the acute phase of onset. Beyond this period, the possibility to detect virus from clinical specimens appears to be low, although the patient is infected with chikungunya virus. In this study, we used a conventional RT-PCR method, which has a detection limit of about 200 copy numbers, to determine chikungunya virus in blood specimens. The sensitivity of this method, however, may be insufficient to detect chikungunya virus in some specimens having low concentration of virus. Therefore, a more sensitive method to detect chikungunya virus, such as reverse transcription loop-mediated isothermal amplification (RT-LAMP) that has a detection limit of about 20 copy numbers (Parida *et al*, 2007) should be used in future studies of chikungunya infection in clinical specimens. Among the specimens that were negative for chikungunya infection, there were also some samples positive for dengue infection. This could be due to a misdiagnosis between chikungunya and dengue infection, which has similarly clinical symptoms as it was found that there were some specimens positive for dengue infection among the specimens negative for chikungunya. However, co-infection of both viruses was also observed in this study as some specimens were positive for both dengue and chikungunya infections (data not shown). A recent report from India also reveals the co-infections with chikungunya virus and dengue virus occurred in Delhi areas in 2006 during a dengue outbreak, and these concurrent infections might result in overlapping clinical symptoms, making diagnosis and treatment difficult for physicians (Chahar *et al*, 2009).

The first reported case of chikungunya fever in Thailand in the current outbreak was found in mid-August 2008 in Narathiwat, the southernmost province of Thailand-Malaysia border. Prior to this report, there

was no evidence of chikungunya fever within any area of the southern provinces or elsewhere in Thailand. During that period, a total of 1,703 cases of chikungunya fever were already present in 5 states of Malaysia and 117 cases also occurred in Singapore (Bureau of Epidemiology, 2008a,b). It is possible that the chikungunya virus was introduced into Thailand from people who traveled between the epidemic areas and Thailand during that period. Afterwards, the incidence of chikungunya fever had increased and spread to 4 southern provinces along Thailand-Malaysia border (Narathiwat, Yala, Pattani and Songkhla) in 2008 and 47 provinces throughout Thailand in 2009. This indicates the Thai people are highly susceptible to the current strain of chikungunya virus, and that the disease could spread rapidly within a short period. As seen in Fig 2, high incidences of this disease were in the southern provinces of Thailand. This could be due to the prevalent of vector mosquitoes, *Ae. aegypti* and *Ae. albopictus*, especially the latter. We have found that *Ae. albopictus* is abundant in all 14 provinces of southern Thailand, especially in such habitats as rubber plantations, palm plantations, orchards, waterfalls and public parks. Besides the southern region, *Ae. albopictus* is also found in other provinces throughout Thailand, possibly with lower abundance than in the southern provinces (Huang, 1972; Benjaphong and Chansang, 1998; Thavara, 2001).

In this study, we could collect only small numbers of *Ae. aegypti* and *Ae. albopictus*, especially the latter, because it was during the dry season with no rain when the mosquito collection was carried out. It was documented previously that *Ae. albopictus* populations are markedly suppressed during the dry season when their natural breeding sites are mostly dry, whereas *Ae. aegypti* could be present all year round since their breeding

sites are human-made water-storage containers that are usually filled with water, even in the dry season (Thavara *et al*, 2001). Although the collected numbers of both mosquito species were low, the relative infection rates of chikungunya virus of the two species were quite high, especially in *Ae. albopictus*. This may imply that *Ae. albopictus* in Thailand has a higher potential to transmit chikungunya virus than *Ae. aegypti*. This is supported by a number of studies (Mangiafico, 1971; Turell *et al*, 1992; Reiskind *et al*, 2008). Genetics is likely to be one factor that controls the susceptibility of *Ae. albopictus* to chikungunya virus infection (Tesh *et al*, 1976). Recently, it was reported that the mutant strain of chikungunya virus (E1: A226V) shows a shorter incubation period in *Ae. albopictus*, which enables the mosquito to transmit the virus as early as two days after an infected blood-meal (Vazeille *et al*, 2007). However, more subsequent studies would be needed to demonstrate this capability of *Ae. albopictus* in Thailand.

It is interesting to note that chikungunya virus was also detected in males of *Ae. aegypti* and *Ae. albopictus* collected in our study from different locations. It is, therefore, an obvious evidence for the phenomenon of transovarial transmission occurring in the natural environment in the study sites in Thailand. This phenomenon is similar to that of dengue virus as described previously by Thavara *et al* (2006). Based on laboratory studies, the positive rates of infection in larvae and adult females of their progeny in *Ae. albopictus* are higher than those of *Ae. aegypti* and the infected females of *Ae. aegypti* and *Ae. albopictus* are capable of vertically transmitting chikungunya virus to their offsprings to at least the third generation (Zhang *et al*, 1993).

To control the epidemic of chikungunya fever, many measures have to be carried out by public health officers with active commu-



nity participation. These measures include active case management, space spraying of insecticides, larval source reduction of the vector mosquitoes, public health education and personal protection. The viremic patients, especially during the high fever period, should be treated in screened ward at hospital or under mosquito net at home in order to prevent biting by vector mosquitoes (Townson and Nathan, 2008). Space spraying of insecticides, employing thermal fogging or ultra low volume (ULV) spraying, has to be carried out thoroughly in the village as soon as possible after a case of chikungunya fever is detected, and at least one more spraying should also be repeated 7 days after the first application. Larval source reduction of *Aedes* mosquitoes in the village also have to be implemented concurrently with space spraying of insecticides. Effective larvicides against *Ae. aegypti* larvae containing various active ingredients, such as temephos (Mulla *et al*, 2004; Thavara *et al*, 2004; Tawatsin *et al*, 2007), novaluron (Mulla *et al*, 2003; Arredondo-Jimenez and Valdez-Delgado, 2006), diflubenzuron (Thavara *et al*, 2007; Chen *et al*, 2008), and *Bacillus thuringiensis israelensis* (Bti) (Mulla *et al*, 2004; Lee *et al*, 2008) should be applied in water-storage containers in and around houses whereas the natural habitats of *Ae. albopictus* could be applied with Bti (Lee *et al*, 1996) employing ULV applicator. In addition, the integration of larvicides and adulticides could provide the possibility of achieving both larvicidal and adulticidal effects against targeted mosquitoes, such as *Ae. aegypti* and *Ae. albopictus* when applied as ULV spraying (Yap *et al*, 1997).

Public health education is an important measure that could prevent epidemic of chikungunya fever. The relevant information about chikungunya fever, such as etiology of the disease, disease prevention, vector mosquitoes and their control should be supplied

to the public in an understandable format, especially in the high risk areas. Personal protection from biting mosquito is also one of the critical measures that could minimize the expansion of chikungunya fever in the epidemic areas. This measure requires mosquito net for infants or children who always sleep during daytime and mosquito repellents, for instance, mosquito coils, vaporizers and topical repellents. Effective topical repellents containing such active ingredients as deet (diethyl methyl benzamide), IR3535 (ethyl butylacetylaminopropionate), and essential oils extracted from plants, have demonstrated a high degree of repellency against *Ae. aegypti* and *Ae. albopictus* (Thavara *et al*, 2001; Tawatsin *et al*, 2001, 2006a,b).

In summary, chikungunya fever has re-emerged in Thailand after a disappearance of about 13-14 years. The current outbreak of this disease in 2009 has infected some 32,000 people, mostly in the southern provinces of Thailand. Chikungunya virus was detected in blood specimens obtained from suspected patients and in both sexes of wild-caught vector mosquitoes, *Ae. aegypti* and *Ae. albopictus*. The presence of chikungunya virus in adult male mosquitoes of both species revealed a role of transovarial transmission of the virus in field population of the mosquito vectors. As no vaccine is currently available for chikungunya infection, vector control employing various measures and personal protection from biting mosquito still remain the main effective strategies to control the disease.

#### ACKNOWLEDGEMENTS

The authors are grateful to the Department of Medical Sciences, Ministry of Public Health, Thailand, for budget allocation to support this study. We acknowledge the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand

for providing data regarding incidence of chikungunya fever in Thailand. We thank the staff of the Office of Disease Control 12, Songkhla for their valuable assistance in collecting mosquitoes from the study sites.

## REFERENCES

- Arankalle VA, Shrivastava S, Cherian S, *et al*. Genetic divergence of chikungunya viruses in India (1963-2006) with special reference to the 2005-2006 explosive epidemic. *J Gen Virol* 2007; 88: 1967-76.
- Arredondo-Jimenez JI, Valdez-Delgado KM. Effect of novaluron (Rimon 10EC) on the mosquitoes *Anopheles albimanus*, *Anopheles pseudopunctipennis*, *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* from Chiapas, Mexico. *Med Vet Entomol* 2006; 20: 377-87.
- Benjaphong N, Chansang U. A minor mosquito vector of dengue haemorrhagic fever in Thailand: *Aedes (Stegomyia) albopictus* (Skuse), Diptera: Culicidae. *Com Dis J* 1998; 24: 154-8.
- Bureau of Epidemiology. Outbreak verification summary, 33<sup>rd</sup> week, August 10 - 16, 2008. Weekly epidemiological surveillance report, 33<sup>rd</sup> week. Nonthaburi: Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand, 2008a.
- Bureau of Epidemiology. Outbreak verification summary, 36<sup>th</sup> week, August 31-September 6, 2008. Weekly epidemiological surveillance report, 36<sup>th</sup> week. Nonthaburi: Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand, 2008b.
- Campos LE, San Juan A, Cenabre LC, Almagro EF. Isolation of chikungunya virus in the Philippines. *Acta Med Phil* 1969; 5: 152-5.
- Chahar HS, Bharaj P, Dar L, Guleria R, Kabra SK, Broor S. Co-infections with chikungunya virus and dengue virus in Delhi, India. *Emerg Infect Dis* 2009; 15: 1077-80.
- Chastel C. Infections humaines au Cambodge par le virus chikungunya ou un agent  
etroitement apparente. III. Epidemiologie. *Bull Soc Pathol Exot Filiales* 1963; 1: 65-82.
- Chen CD, Seleena B, Chiang YF, Lee HL. Field evaluation of the bioefficacy of diflubenzuron (Dimilin®) against container-breeding *Aedes* sp mosquitoes. *Trop Biomed* 2008; 25: 80-6.
- Dai VQ, Kim-Thoas NT. Enquete sur les anticorps anti-chikungunya chez des enfants Vietnamiens de Saigon. *Bull Soc Pathol Exot Filiales* 1967; 4: 353-9.
- Epstein PR. Chikungunya fever resurgence and global warming. *Am J Trop Med Hyg* 2007; 76: 403-4.
- Gratz NG. Critical review of the vector status of *Aedes albopictus*. *Med Vet Entomol* 2004; 18: 215-27.
- Hammon W McD, Rudnick A, Sather GE. Viruses associated with epidemic haemorrhagic fevers of the Philippines and Thailand. *Science* 1960; 131: 1102-3.
- Hermon YE. Virological investigation of arbovirus infections in Ceylon, with special reference to the recent chikungunya fever epidemic. *Ceylon Med J* 1967; 12: 81-92.
- Huang YM. Contributions to the mosquito fauna of Southeast Asia. XIV. The subgenus *Stegomyia* of *Aedes* in Southeast Asia. I - The *Scutellaris* group of species. *Contrib Amer Ent Inst* 1972; 9: 13-7.
- Khai MC, Thien S, Thaug U, *et al*. Clinical and laboratory studies of haemorrhagic fever in Burma, 1970-72. *Bull WHO* 1974; 51: 227-35.
- Lee HL, Chen CD, Masri SM, Chiang YF, Chooi KH, Benjamin S. Impact of larviciding with a *Bacillus thuringiensis israelensis* formulation, Vectobac WG, on dengue mosquito vectors in a dengue endemic site in Selangor State, Malaysia. *Southeast Asian J Trop Med Public Health* 2008; 39: 601-9.
- Lee HL, Gregorio ER Jr, Khadri MS, Seelena P. Ultra low volume application of *Bacillus thuringiensis* ssp *israelensis* for the control of mosquitoes. *J Am Mosq Control Assoc* 1996; 12: 651-5.
- Leo YS, Chow ALP, Tan LK, Lye DC, Lin L, Ng LC. Chikungunya outbreak, Singapore, 2008.

- Emerg Infect Dis* 2009; 15: 836-7.
- Mangiafico JA. Chikungunya virus infection and transmission in five species of mosquito. *Am J Trop Med Hyg* 1971; 20: 642-5.
- Marchette NJ, Rudnick A, Garcia R, Mac Vean S. Alphaviruses in Peninsular Malaysia. I. Virus isolations and animal serology. *Southeast Asian J Trop Med Public Health* 1978; 9: 317-29.
- McIntosh BM, Gear JHS. Arboviral zoonoses in southern Africa: Chikungunya fever. In: Steele JH, Beran GW, eds. Section B: Viral zoonoses. CRC Handbook Series in Zoonoses. Boca Raton, FL: CRC Press, 1981: 217-20.
- Mulla MS, Thavara U, Tawatsin A, Chomposri J, Zaim M, Su T. Laboratory and field evaluation of novaluron, a new acylurea insect growth regulator against *Aedes aegypti* (Diptera: Culicidae). *J Vector Ecol* 2003; 28: 241-54.
- Mulla MS, Thavara U, Tawatsin A, Chomposri J. Procedures for evaluation of field efficacy of slow-release formulations of larvicides against *Aedes aegypti* in water-storage containers. *J Am Mosq Control Assoc* 2004; 20: 64-73.
- Nimmannitya S, Mansuwan P. Comparative clinical and laboratory findings in confirmed dengue and chikungunya infections. *Bull WHO* 1966; 35: 42-3.
- Parida MM, Santhosh SR, Dash PK, *et al.* Rapid and real-time detection of chikungunya virus by reverse transcription loop-mediated isothermal amplification assay. *J Clin Microbiol* 2007; 45: 351-7.
- Reiskind MH, Pesko K, Westbrook CJ, Mores CN. Susceptibility of Florida mosquitoes to infection with chikungunya virus. *Am J Trop Med Hyg* 2008; 78: 422-5.
- Ross RW. The Newala epidemic. III. The virus: isolation, pathogenic properties and relationship to the epidemic. *J Hyg* 1956; 54: 177-91.
- Sarkar JK, Pavri KM, Chatterjee SN, Chakarvarty SK, Anderson CR. Virological and serological studies of cases of haemorrhagic fever in Calcutta. *Indian J Med Res* 1964; 52: 684-91.
- Schuffereenecker I, Iteman I, Michault A, *et al.* Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. *PLoS Med* 2006; 3: e263.
- Slemons RD, Haksohusodo S, Suharyono W, Harundriyo H, Laughlin LW, Cross J. Chikungunya viral disease in Jogjakarta, Indonesia. Presented at the 33<sup>rd</sup> Annual Meeting of the American Society of Tropical Medicine and Hygiene, December 2-6, 1984.
- Tawatsin A, Wratten SD, Scott RR, Thavara U, Techadamrongsin Y. Repellency of volatile oils from plants against three mosquito vectors. *J Vector Ecol* 2001; 26: 76-82.
- Tawatsin A, Asavadachanukorn P, Thavara U, *et al.* Repellency of essential oils extracted from plants in Thailand against four mosquito vectors (Diptera: Culicidae) and oviposition deterrent effects against *Aedes aegypti* (Diptera: Culicidae). *Southeast Asian J Trop Med Public Health* 2006a; 37: 915-31.
- Tawatsin A, Thavara U, Chansang U, *et al.* Field evaluation of deet, Repel Care<sup>®</sup>, and three plant-based essential oil repellents against mosquitoes (Diptera: Culicidae), black flies (Diptera: Simuliidae) and land leeches (Arhynchobdellida: Haemadipsidae) in Thailand. *J Am Mosq Control Assoc* 2006b; 22: 306-13.
- Tawatsin A, Thavara U, Chomposri J, Bhakdeenuan P, Asavadachanukorn P. Larvicidal efficacy of new formulations of temephos in non-woven sachets against larvae of *Ae. aegypti* (L.) (Diptera: Culicidae) in water-storage containers. *Southeast Asian J Trop Med Public Health* 2007; 38: 641-5.
- Tesh RB, Gubler DJ, Rosen L. Variation among geographic strains of *Aedes albopictus* in susceptibility to infection with chikungunya virus. *Am J Trop Med Hyg* 1976; 25: 326-35.
- Thaikruea L, Charearnsook O, Reanphumkarnkit S, *et al.* Chikungunya in Thailand: A re-emerging disease? *Southeast Asian J Trop*

- Med Public Health* 1997; 28: 359-64.
- Thavara U. Ecological studies of dengue mosquito vectors in four provinces of southern Thailand. Thesis: Faculty of Tropical Medicine. Bangkok: Mahidol University, 2001; 134 pp.
- Thavara U, Tawatsin A, Chansang U, *et al.* Laboratory and field evaluations of insect repellent 3535 (Ethyl Buthylacethylaminopropionate) and deet (*N,N*-diethyl-3-methylbenzamide) against mosquito vectors in Thailand. *J Am Mosq Control Assoc* 2001; 17: 190-5.
- Thavara U, Siriyasatien P, Tawatsin A, *et al.* Double infection of heteroserotypes of dengue viruses in field populations of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) and serological features of dengue viruses found in patients in Southern Thailand. *Southeast Asian J Trop Med Public Health* 2006; 37: 468-76.
- Thavara U, Tawatsin A, Chansang C, Asavada-  
chanukorn P, Zaim M, Mulla MS. Simulated field evaluation of the efficacy of two formulations of diflubenzuron, a chitin synthesis inhibitor against larvae of *Aedes aegypti* (L.) (Diptera: Culicidae) in water storage containers. *Southeast Asian J Trop Med Public Health* 2007; 38: 269-75.
- Thavara U, Tawatsin A, Chansang C, *et al.* Larval occurrence, oviposition behavior and biting activity of potential mosquito vectors of dengue on Samui Island, Thailand. *J Vector Ecol* 2001; 26: 172-80.
- Thavara U, Tawatsin A, Chompoonsri J, *et al.* Efficacy and longevity of a new formulation of temephos larvicide tested in village-scale trials against *Aedes aegypti* larvae in water-storage containers. *J Am Mosq Control Assoc* 2004; 20: 176-82.
- Theamboonlers A, Rianthavorn P, Praiananta-  
thavorn K, Wuttirattanakowit N, Poovorawan Y. Clinical and molecular characterization of chikungunya virus in South Thailand. *Jpn J Infect Dis* 2009; 62: 303-5.
- Townson H, Nathan MB. Resurgence of chikungunya. *Trans R Soc Trop Med Hyg* 2008; 102: 308-9.
- Turell MJ, Beaman JR, Tammareillo RF. Susceptibility of selected strains of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) to chikungunya virus. *J Med Entomol* 1992; 29: 49-53.
- Vazeille M, Moutailler S, Coudrier D, *et al.* Two chikungunya isolates from the outbreak of La Reunion (Indian Ocean) exhibit different patterns of infection in the mosquito, *Aedes albopictus*. *PLoS ONE* 2007; 11: e1168.
- Vazeille M, Moutailler S, Pages F, Jarjaval F, Failloux A. Introduction of *Aedes albopictus* in Gabon: what consequences for dengue and chikungunya transmission? *Trop Med Int Health* 2008; 33: 1176-9.
- WHO. Outbreak and spread of chikungunya. *WHO Weekly Epidemiol Rec* 2007; 82: 409-15.
- Yamanishi H. Susceptibility of Chinese strains of *Aedes albopictus* and *Aedes aegypti* to chikungunya virus. *Med Entomol Zool* 1999; 50: 61-4.
- Yap HH, Chong AS, Adanan CR, *et al.* Performance of ULV formulations (Pesguard 102/Vectobac 12AS) against three mosquito species. *J Am Mosq Control Assoc* 1997; 13: 384-8.
- Yergolkar PN, Tandale BV, Arankalle VA, *et al.* Chikungunya outbreaks caused by African genotype, India. *Emerg Infect Dis* 2006; 12: 1580-3.
- Zhang H, Zhang Y, Mi Z. Transovarial transmission of chikungunya virus in *Aedes albopictus* and *Aedes aegypti* mosquitoes. *Chin J Virol* 1993; 9: 222-7.