Original Article

High infection rate of Zika virus in mosquitoes collected from an area of active Zika virus transmission in eastern Thailand

Apiwat Tawatsin[1] Atchara Phumee[2,5] Usavadee Thavara[1] Patcharawan Sirisopa[1]

Wanapa Ritthison[3] Kitsanaphong Thammakosol[4] Proawpilart Intayot[4] Yutthana Joyjinda[2]

Supaporn Wacharapluesadee[2] Thiravat Hemachudha[2] Padet Siriyasatien[5*]

Abstract

Zika virus (ZIKV), chikungunya virus (CHIKV) and dengue virus (DENV) are emerging and re-emerging arboviral diseases. These viruses are transmitted to humans through the bites of *Aedes* mosquitoes. Recently, ZIKV infection has been described as an emerging disease in Thailand and many countries, especially in tropical and sub-tropical areas. Specific drugs and vaccines against these infections are unavailable; therefore, effective disease control relies on vector control measures only. To understand the transmission cycle of these viruses and mosquito vectors, this study is designed to investigate the natural infection of ZIKV, CHIKV and DENV in field-caught mosquitoes by molecular techniques. Adults and larvae of mosquitoes were collected in and around the patients' homes in the Klaeng District, Rayong Province, Thailand. CHIKV and DENV were detected by Multiplex Real-time RT-PCR and ZIKV was detected by Hemi-nested RT-PCR. ZIKV RNA was detected in 8 (10.3%) samples (5 (6.4%) females and 2 (2.6%) males of *Aedes aegypti* and 1 (1.3%) female *Armigeres subalbatus*) and CHIKV RNA in 5 (6.4%) (3 (3.8%) females and 2 (2.6%) larvae of *Ae. aegypti*), while DENV RNA was not detected in any samples. The Maximum Likelihood tree of nucleotide sequences of positive samples showed that ZIKV in mosquitoes were cladded within the Asian lineage. This study was a preliminary survey of the potential vectors of ZIKV, CHIKV and DENV in an affected area. Information obtained from this study helps to understand the natural infection rates in mosquitoes with ZIKV, CHIKV and DENV and may be valuable in creating the most effective mosquito vector control strategies in the future.

Keywords: Zika, Chikungunya, Dengue, Mosquitoes, Thailand

[1]*National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand*

[2]*Thai Red Cross Emerging Infectious Health Science Centre, Neuroscience Center for Research and Development & WHO-CC for Research and Training on Viral Zoonoses, King Chulalongkorn Memorial Hospital, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*

[3]*The Office of Disease Prevention and Control 6 Chonburi, Department of Disease Control, Ministry of Public Health, Chonburi, Thailand*

[4]*Medical Science Program, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*

[5]*Vector Biology and Vector Borne Disease Research Unit, Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*

***Correspondence**: Padet.S@chula.ac.th*

Introduction

Zika virus (ZIKV), dengue virus (DENV), and chikungunya virus (CHIKV) are arthropod-borne viruses or arboviruses (Waggoner and Pinsky, 2016). These viruses are primarily transmitted to humans by *Aedes* mosquitoes, and patients infected with these viruses present similar clinical symptoms (Vasconcelos and Calisher, 2016). Dengue (DEN) fever and chikungunya (CHIK) fever are commonly found in most tropical regions, whereas Zika (ZIK) fever is an emerging infectious disease in areas of Africa, Asia, the Americas, and the Pacific Islands (Pastula et al., 2016). ZIKV infections were first described in Thailand in 1954 in sera of indigenous residents by using neutralizing antibodies against several arthropod-borne viruses, such as ZIKV, West Nile virus (WNV), and Japanese encephalitis (JE) (Pond, 1963). Recently, several ZIK cases were reported among some travelers (Canadian, German, and Japanese) returning from Thailand (Fonseca et al., 2014; Tappe et al., 2014; Shinohara, 2014). Buathong et al. (2015) described seven cases (3 patients from the Ratchaburi province, 2 patients from the Phetchabun province, 1 patient from the Sisaket province, and 1 patient from the Lamphun province) of autochthonous acute ZIKV infections. This diagnosis was confirmed by molecular or serological testing. The phylogenetic tree showed that these ZIKV were of Asian lineage (Buathong et al., 2015). On the other hand, the outbreak of CHIK fever in Thailand was first reported in 1958 (Hammon et al., 1960), and it re-emerged in the southern region in 2008–2009 with more than 50,000 patients (Ungchusak, 2008; Suangto and Uppapong, 2009; Thavara et al., 2009). In 2013, CHIK fever had become established in BuengKan Province in northeastern Thailand (Wanlapakorn et al., 2014). For DENV in Thailand, the first major outbreak was in Bangkok in 1958 (Gubler, 1997). Dengue disease cases in Thailand include four DENV serotypes (DENV-1, -2, -3 or -4) (Musso et al., 2015). In 2016, the Bureau of Epidemiology (BoE) of the Thai Ministry of Public Health (MoPH) revealed that approximately 63,310 cases of dengue had been found and had resulted in 61 deaths. Mosquitoes are the main vectors of these three viruses (ZIKV, CHIKV and DENV) to humans; therefore, it may be expected that possible coinfection with two or three arboviruses may occur in mosquitoes. In this study, we determined the natural infection of ZIKV, CHIKV, and DENV in mosquitoes collected around patients' homes in the Klaeng District, Rayong Province in Thailand. CHIKV and DENV were detected using Multiplex Real-time RT-PCR. Hemi-nested RT-PCR (hn-RT-PCR) developed from this study was able to detect ZIKV in the mosquitoes effectively. Information obtained from this study provides fundamental data for the understanding of natural transmission between ZIKV, CHIKV, and DENV and mosquitoes. Data of mosquito vectors and ZIKV infection in the mosquitoes will be valuable in developing effective control strategies of ZIKV, CHIKV, and DENV infections in Thailand.

Materials and Methods

Ethics statement: The study was approved by the animal research ethics committee of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (COA No. 023/2560).

Sample collection: Larval or adult mosquitoes were collected from inside and outside patients' homes in the Klaeng District, Rayong Province, eastern Thailand. These samples were randomized and separated individually, kept in liquid nitrogen and transferred to the laboratory of Entomology, Department of Parasitology, Faculty of Medicine, Chulalongkorn University for viral detection.

Viral RNA extraction: Individual mosquito specimens were extracted for viral RNA using a viral RNA extraction kit, Invisorb® Spin Virus RNA Mini kit (STRATEC molecular GmbH, Germany) following the manufacturer's instructions. RNA concentration and purity were quantified by a Nano Drop 2000c spectrophotometer (Thermo scientific, USA).

Multiplex Real-time RT-PCR for the Detection of CHIKV and DENV: CHIKV and 4 serotypes of DENV were detected in samples in a single reaction tube [Cy5 (DEN1), FAM (DEN2), Texas Red (DEN3), Quasar 705 (DEN4), and HEX (CHIKV)] using a commercial kit. One-step Multiplex Real-time RT-PCR was performed using an *ab*TES™ DEN 5 qPCR I Kit (AIT biotech Pte. Ltd, Singapore) on a CFX96 Real-Time System (Bio-Rad Laboratories, Inc, USA). The total volume of the reaction mixture was 25 µl, including 5.4 µl of nuclease-free water, 12.5 µl of 2x RT-PCR reaction mix, 0.1 µl of PCR enhancer mix, 1.5 µl of DEN 5 primer/probe mix, 0.5 µl RT/Taq enzyme mix, and 5 µl of viral RNA template. The cDNA synthesis was performed for 10 mins at 53°C, the Taq activation was performed for 2 min 30 sec at 95°C, followed by 41 cycles of 95°C for 17 sec, 59°C for 31 sec and 68°C for 32 sec, with a final cycle of 68°C for 7 mins and a final holding at 4°C. Samples were considered as having a positive result if the specific fluorescent signals were a level higher than the threshold value. A threshold cycle of 38 was considered negative.

ZIKV detection and sequencing: The RNA samples extracted from the mosquitoes were amplified by primer sets which were modified from Moureau et al. (2007) for detecting ZIKV RNA at the nonstructural protein 5 (NS5) gene region using hn-RT-PCR. The RT-PCR amplification reaction was set up in a final volume of 25 µl using the Superscript III one-step RT-PCR kit. The RT-PCR conditions as follows; reverse transcription at 50 °C for 30 mins, denaturation at 95 °C for 15 mins, followed by 45 cycles of 94 °C for 20 sec, 55 °C for 20 sec, and 72°C for 30 sec and the final extension at 72°C for 5 mins. The nested PCR was performed with 2 µl from the first reaction using 1 unit of *Taq* DNA polymerase (Fermentas, USA) with conditions as follows; denaturation at 94 °C for 2 mins, followed by 45 cycles of 94 °C for 15 sec, 52 °C for 15 sec, and 72°C for 30 sec and the final extension at 72°C for 5 min. The PCR products were analyzed via 2% agarose gel electrophoresis, stained with ethidium bromide and visualized with Quantity One Quantification Analysis Software Version 4.5.2 (Gel DocEQ System; Bio-Rad, Hercules, CA). Positive PCR products were recovered

from the gel and purified using an Agarose Gel DNA Purification Kit: Invisorb® Fragment CleanUp (STRATEC Molecular GmbH, Germany) following the manufacturer's instructions. The purified DNA was sent for direct DNA sequencing to Macrogen Inc. (Macrogen, South Korea) to confirm the species identification. Nucleotide sequences were analyzed by comparison with the GenBank database using a BLAST search (https://blast.ncbi.nlm.nih. gov/Blast.cgi).

Phylogenetic tree construction: The sequences were aligned using BioEdit Sequence Alignment Editor Version 7.1.9. The phylogenetic trees were constructed using the Maximum Likelihood with Kimura's 2-parameter and bootstrap analysis with 1,000 replications in MEGA Version 7.0. The sequences obtained from this study were analyzed against 7 reference strains of Asian lineage (GenBank accession nos. JN860885 (FSS13025/Cambodia/2010), EU545988 (Yap/Micronesia/2007), KU312312 (Z1106033/ Suriname/2015), KX694532 (THA_PLCal_ZV_2013/ Thailand/2013), KF993678 (PLCal_ZV/Canada/2013), KU321639 (ZikaSPH2015/Brazil/2015), and HQ234499 (P6-740/Malaysia/1966)) and 7 reference strains from African lineage (NC012532 (MR_766/Uganda/2005), LC002520 (MR766-NIID/Uganda/2014), HQ234501 (ArD_41519/Senegal/1984), HQ234500 (IbH_30656/ Nigeria/1968), KF268949 (ARB15076/Central African Republic/2014), KF268950 (ARB7701/Central African Republic/2014), and KF268948 (ARB13565/Central African Republic/1976)).

Results

A total of 78 mosquito samples (males (n=20), females (n=42) and larvae (n=16)) were used for ZIKV, CHIKV, and DENV RNA detection (Table 1). The adult mosquitoes belonged to three genera, which consist of *Aedes aegypti* 69.23% (54/78), *Culex quinquefasciatus* 1.28% (1/78) and *Armigeres subalbatus* 8.98% (7/78), while larvae were identified as *Aedes spp* 20.51% (16/78). The results of virus detection showed that ZIKV RNA was detected in 6 (7.69%) females of *Ae. aegypti and Ar. subalbatus* and 2 (2.56%) male of *Ae. aegypti* using hn-RT-PCR (Figure 1A). Interestingly, ZIKV RNA was found in a female of *Ar. subalbatus*, and the sequences of the NS5 genes of ZIKV amplified in this study were 282-284 base pairs. The ZIKV sequences showed 99-100% sequence identity to the partial NS5 genes of ZIKV available in the GenBank database. The nucleotide sequences of the NS5 of ZIKV were submitted to the GenBank database, accession no MH306209-MH306216. The phylogenetic tree analysis clearly showed separate African and Asian lineages and all ZIKV sequences in mosquitoes in this study were claded within the Asian lineage (Figure 1B).

Table 1 Mosquito sample collection and molecular detection results

Area of patients' homes	Species	stage	Sex	Total Sample (n)	Results		
					CHIKV	DENV 4 serotypes	ZIKV
Indoor	*Ae. aegypti*	Adult	Male	19	0	0	1
			Female	31	3	0	4
	Cx. quinquefasciatus	Adult	Male	N/A	N/A	N/A	N/A
			Female	1	0	0	0
	Aedes spp.	Larva	N/A	1	0	0	0
Outdoor	*Ae. aegypti*	Adult	Male	1	0	0	1
			Female	3	0	0	1
	Ar. subalbatus	Adult	Male	N/A	N/A	N/A	N/A
			Female	7	0	0	1
	Aedes spp.	Larva	N/A	15	2	0	0
				78	**5**	**0**	**8**

N/A: not available

In addition, 5 samples (3 females and 2 larvae) in *Aedes spp.* were positive for CHIKV RNA by one-step Multiplex Real-time RT-PCR, *ab*TES™ DEN 5 qPCR I Kit (Figures 2A and 2B). Samples reported as positive had cycle threshold (Ct) values ≤38. In this study, 5 samples were positive with Ct values of 32.19, 34.45, and 30.32 in adult mosquitoes and 34.7 and 36.7 in larvae. However, DENV showed negative results in all samples.

Discussion

In the present study, ZIKV RNA was mostly detected in *Ae. aegypti* mosquitoes and also in a *Ae. aegypti* male. Several reports revealed that *Aedes* mosquitoes such as *Ae. africanus*, *Ae. apicocoargenteus*, *Ae. furcifer*, *Ae. luteocephalus*, *Ae. vitattus* and *Ae. aegypti* are the principle vectors of ZIKV in Africa (Weinbren and Williams, 1958; Haddow et al., 1964; Fagbami et al., 1979; McCrae and Kirya, 1982). In Southeast Asia, ZIKV was isolated from wild caught *Ae. aegypti* mosquitoes in Malaysia (Marchette et al., 1969); moreover, *Ae. aegypti* (Li et al., 2012) and *Ae. albopictus*

(Wong et al., 2013) mosquitoes were also reported as potential vectors for ZIKV transmission in Singapore. A few reports found that ZIKV can be detected in fields, including a collected male *Ae. aegypti* from Rio de Janeiro, Brazil (Ferreira-de-Brito et al., 2016) and male *Ae. furcifer* mosquitoes collected from Senegal (Diallo et al., 2014). These findings are supported by the recent experiment reported by Thangamani et al. (2016), in which they demonstrated the occurrence of vertical transmission of ZIKV in *Aedes* mosquitoes in F1 adult progeny. Surprisingly, we were able to detect ZIKV RNA in a female *Ar. subalbatus* mosquito. Several reports mentioned that other mosquito genera could transmit ZIKV. Diallo et al. (2014) reported that ZIKV was detected in *Cx. perfuscus*, *Mansonia uniformis* and *Anopheles coustani* collected from Southeastern Senegal by virus isolation and RT-PCR (Diallo et al., 2014). Recently, there have been reported that *Cx. pipiens quinquefasciatus* is a potential vector to transmit ZIKV in southern China (Thangamani et al., 2016), and field-caught *Cx. quinquefasciatus* showed positive ZIKV by RT-qPCR (Guo et al., 2016; Guedes DRD et al., 2016). These findings determined that ZIKV may have a wider range of vectors. However, vectors for ZIKV transmission in Thailand have never been investigated. This is the first report that ZIKV can be found in female *Ar. subalbatus* and in male *Ae. aegypti* mosquitoes in Thailand. This is the first study to detect ZIKV RNA in *Ar. subalbatus*, and this mosquito species is commonly found in tropical and subtropical areas. Currently, there is no study investigating *Ar. subalbatus* as a vector for ZIKV. Our results indicated that *Ar. subalbatus* could be another potential vector for ZIKV transmission in this region. Further studies to investigate the potential vectors for ZIKV transmission are required. ZIKV has been identified within two major lineages, African and Asian through phylogenetic analyses. The phylogenetic tree indicated that the ZIKV in this study belong to the Asian lineage. The Asian lineage of ZIKV had been historically reported in Malaysia, the Philippines, Pakistan, Cambodia and Thailand and caused an outbreak on Yap Island in 2007 (Gudo et al., 2016).

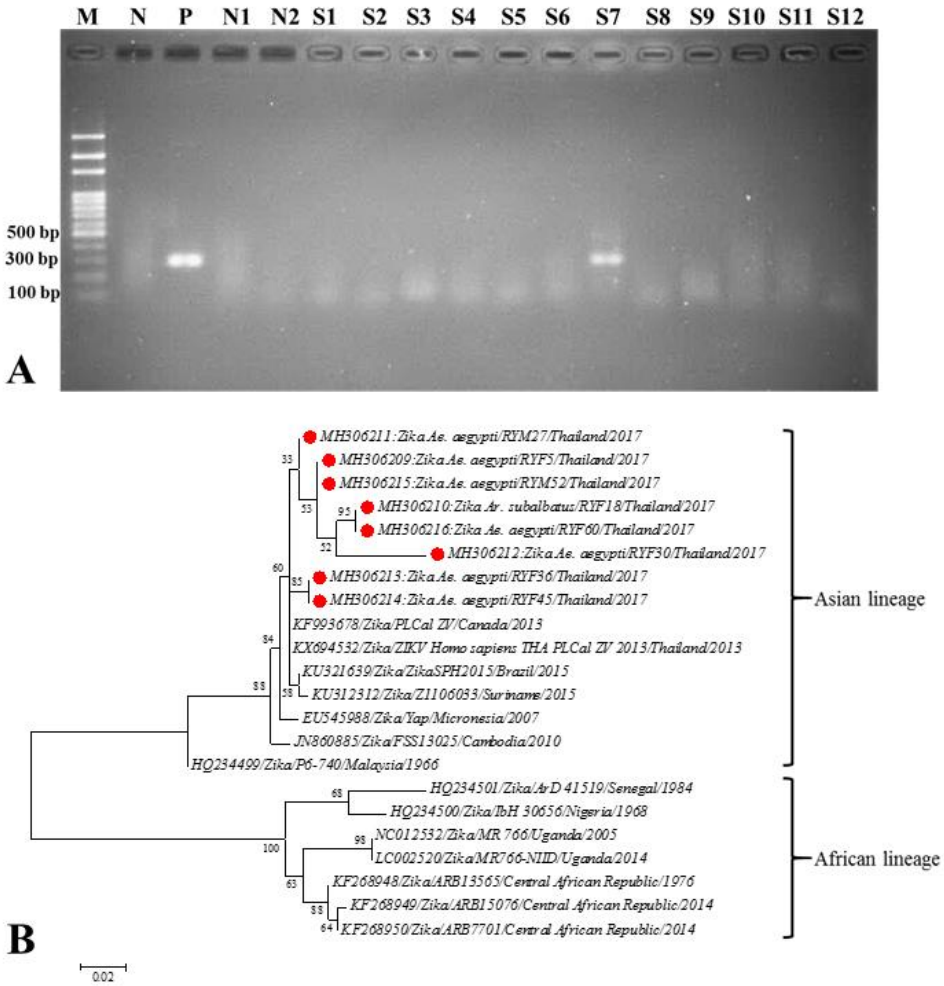

Figure 1 **A)** The 2% agarose gel images showing the amplified product of size 282 bp of ZIKV from hn-RT-PCR (Lane M: molecular mass marker (100 base pairs [bp]); lane P: positive control, lane N: negative control (no DNA template: double-distilled water); lane N1: uninfected adult mosquito; lane N2: uninfected larval mosquito: Lane S1-12: mosquito samples). **(B)** Phylogenetic tree of a NS5 gene of ZIKV from mosquitoes (indicated in a red circle) compared with reference isolates obtained from GenBank. The tree was derived using the Maximum Likelihood method based on the Kimura 2-parameter model (bootstrap 1,000 times).

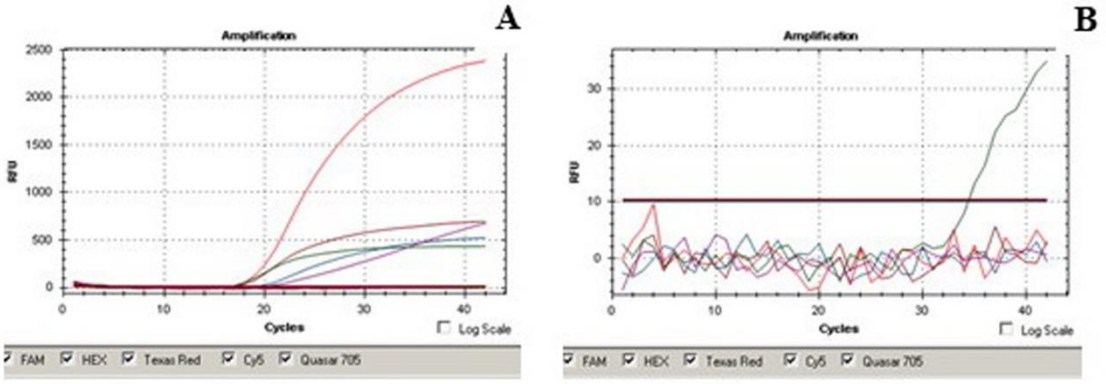

Figure 2 **(A)** Showing positive of CHIKV in mosquitoes using Multiplex Real-time RT-PCR for CHIKV and DENV [Cy5 (DEN1), FAM (DEN2), Texas Red (DEN3), Quasar 705 (DEN4), and HEX (CHIKV)]. **(B)** Mosquito sample showed positive with CHIKV.

CHIKV has three distinct genotypes based on the E1 envelope glycoprotein sequences, including the West African genotype, the East, Central and South African (ECSA) genotypes, and the Asian genotype (Sudeep and Parashar, 2008). We found CHIKV RNA in 3 samples of female *Ae. aegypti* and 2 samples of *Aedes spp.* larva. Previous reports found that CHIKV RNA present in field-caught male *Ae. albopictus* and *Ae. aegypti* from Madagascar (Ratsitorahina et al., 2008) and Thailand (Thavara et al., 2009) by CHIK-specific RT-PCR. CHIKV transmission has been studied in various species of *Aedes* mosquitoes such as *Ae. aegypti* and *Ae. albopictus* from India (Mourya, 1987) and *Ae. aegypti formosus* and *Ae. furcifer* from South Africa (Jupp et al., 1981); however, these experiments did not isolate CHIKV. Moreover, *Ae. aegypti* from India showed the evidence of vertical transmission of emerging novel Indian Ocean lineage (IOL) of the ECSA genotype of CHIKV in both natural and experimental settings (Agarwal et al., 2014). In 2016, the infection of CHIKV IOL in laboratory strains of *Ae. aegypti* and *Ae. albopictus* mosquitoes revealed that the viruses were transmitted vertically to F5 and F6 progenies in both *Ae. aegypti* and *Ae. albopictus* mosquitoes, respectively (Chompoosri et al., 2016). Our present study may support that it is possible that there is transovarian transmission of CHIKV in *Ae. aegypti* mosquitoes in the field.

Literature suggests that virus transmission depends on the mosquito species, geographical location and virus type. However, extensive survey and more precise studies of ZIKV, CHIKV and DENV infection in mosquito covering more areas and larger sample sizes must be performed in order to understand virus-vector interaction. Although we did not find evidence of co-infecting viruses in this study, the effect of co-infection between viruses (ZIKV, CHIKV and DENV) should be investigated. Competitive suppression between DENV and CHIKV has been demonstrated by Potiwat et al. (2011). Moreover, demonstration of the viability of viruses in mosquitoes collected from the field is essential to confirm that the mosquitoes are vectors of ZIKV and CHIKV.

Competing interests: The authors declare that they have no competing interests.

Acknowledgements

This study was supported by the National Research Council of Thailand and Health Systems Research Institute (Grant No. 61-003), National Science and Technology Development Agency (Thailand) (Grant No. P-16-50702) and for the Research Chair Grant, Rachadapisek Sompote Fund for Postdoctoral Fellowship Grant No. RA/MF 11/61, Chulalongkorn University.

References

Agarwal A, Dash PK, Singh AK, Sharma S, Gopalan N, Rao PV, Parida MM and Reiter P 2014. Evidence of experimental vertical transmission of emerging novel ECSA genotype of Chikungunya Virus in *Aedes aegypti*. PLoS Negl Trop Dis. 8(7): e2990.

Buathong R, Hermann L, Thaisomboonsuk B, Rutvisuttinunt W, Klungthong C, Chinnawirotpisan P, Manasatienkij W, Nisalak A, Fernandez S, Yoon IK, Akrasewi P and Plipat T 2015. Detection of Zika Virus Infection in Thailand, 2012-2014. Am J Trop Med Hyg. 93(2):380-383.

Chompoosri J, Thavara U, Tawatsin A, Boonserm R, Phumee A, Sangkitporn S and Siriyasatien P 2016. Vertical transmission of Indian Ocean Lineage of chikungunya virus in *Aedes aegypti* and *Aedes albopictus* mosquitoes. Parasit Vectors. 9:227.

Diallo D, Sall AA, Diagne CT, Faye O, Faye O, Ba Y, Hanley KA, Buenemann M, Weaver SC and Diallo M 2014. Zika virus emergence in mosquitoes in southeastern Senegal, 2011. PLoS ONE. 9(10): e10944.

Fagbami AH 1979. Zika virus infections in Nigeria: virological and seroepidemiological investigations in Oyo State. J Hyg (Lond) 83(2): 213–219.

Ferreira-de-Brito A, Ribeiro IP, Miranda RM, Fernandes RS, Campos SS, Silva KA, Castro MG, Bonaldo MC, Brasil P and Lourenço-de-Oliveira

R 2016. First detection of natural infection of *Aedes aegypti* with Zika virus in Brazil and throughout South America. Mem Inst Oswaldo Cruz. 111(10): 655-658.

Fonseca K, Meatherall B, Zarra D, Drebot M, MacDonald J, Pabbaraju K, Wong S, Webster P, Lindsay R and Tellier R 2014. First case of Zika virus infection in a returning Canadian traveler. Am J Trop Med Hyg. 91(5): 1035-1038.

Gubler DJ 1997. Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem. In: Gubler DJ, Kuno G, editors. Dengue and dengue hemorrhagic fever. Oxford: CAB International, p. 1–22.

Gudo ES, Falk KI, Ali S, Muianga AF, Monteiro V and Cliff J 2016. A Historic Report of Zika in Mozambique: Implications for Assessing Current Risk. PLoS Negl Trop Dis. 10(12): e0005052.

Guedes DR, Paiva MH, Donato MM, Barbosa PP, Krokovsky L, Rocha SWDS, Saraiva K, Crespo MM, Rezende TM, Wallau GL, Barbosa RM, Oliveira CM, Melo-Santos MA, Pena L, Cordeiro MT, Franca RFO, Oliveira AL, Peixoto CA, Leal WS and Ayres CF 2017. Zika virus replication in the mosquito *Culex quinquefasciatus* in Brazil. Emerg Microbes Infect. 6(8):e69.

Guo XX, Li CX, Deng YQ, Xing D, Liu QM, Wu Q, Sun AJ, Dong YD, Cao WC, Qin CF and Zhao TY 2016. *Culex pipiens quinquefasciatus*: a potential vector to transmit Zika virus. Emerg Microbes Infect. 5(6):e102.

Haddow AJ, Williams MC, Woodall JP, Simpson DI and Goma LK 1964. Twelve Isolations of Zika Virus from *Aedes* (*Stegomyia*) *africanus* (Theobald) Taken in and above a Uganda Forest. Bull World Health Organ. 31(1): 57–69.

Hammon W McD, Rudnick A and Sather GE 1960. Viruses associated with epidemic haemorrhagic fevers of the Philippines and Thailand. Science. 131(3407): 1102-1103.

Jupp PG, McIntosh BM and Dos Santos 1981. Laboratory vector studies on six mosquito and one tick species with chikungunya virus. Trans R Soc Trop Med. 75(1): 15-19.

Li MI, Wong PS, Ng LC and Tan CH 2012. Oral susceptibility of Singapore *Aedes* (*Stegomyia*) *aegypti* (Linnaeus) to Zika virus. PLoS Negl Trop Dis. 6(8): e1792.

Marchette NJ, Garcia R and Rudnick A 1969. Isolation of Zika virus from *Aedes aegypti* mosquitoes in Malaysia. Am J Trop Med Hyg. 18(3): 411–415.

McCrae AW and Kirya BG 1982. Yellow fever and Zika virus epizootics and enzootics in Uganda. Trans R Soc Trop Med Hyg. 76(4): 552–562.

Moureau G, Temmam S, Gonzalez JP, Charrel RN, Grard G and de Lamballerie X 2007. A real-time RT-PCR method for the universal detection and identification of flaviviruses. Vector Borne Zoonotic Dis. 7(4): 467-477.

Mourya DT 1987. Absence of transovarial transmission of chikungunya virus in *Aedes aegypti* & *Ae. albopictus* mosquitoes. Indian J Med Res. 85:593-595.

Musso D. Cao-Lormeau, VM and Gubler DJ 2015. Zika virus: following the path of dengue and chikungunya? Lancet. 386(9990): 243–244.

Pastula DM, Smith DE, Beckham JD and Tyler KL 2016. An Overview of Zika Virus Disease. Neurohospitalist. 6(3): 93-94.

Pond WL 1963. Arthropod-borne virus anti bodies in sera from residents of South-east Asia. Trans Roy Soc Trop Med Hyg. 57(5): 364-371.

Potiwat R, Komalamisra N, Thavara U, Tawatsin A and Siriyasatien P 2011. Competitive suppression between chikungunya and dengue virus in *Aedes albopictus* c6/36 cell line. Southeast Asian J Trop Med Public Health. 42(6): 1388-1394.

Ratsitorahina M, Harisoa J, Ratovonjato J, Biacabe S, Reynes J-M, Zeller H, Raoelina Y, Talarmin A, Richard V and Soares JL 2008. Outbreak of dengue and chikungunya fevers, Toamasina, Madagascar, 2006. Emerg Infect Dis. 14(7): 1135–1137.

Shinohara K. Zika Virus - Japan ex Thailand. ProMed; 2014. http://www.promedmail.org Archive no. 20140823.2716731.

Suangto P and Uppapong T. Chikungunya fever. Annual epidemiological surveillance report 2009, Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health. [cited 2014 Mar 18]. http://www.boe.moph.go.th/Annual/Annual%202552/Main.html.

Sudeep AB and Parashar D 2008. Chikungunya: an overview. J Biosci. 33: 443-449.

Tappe D, Rissland J, Gabriel M, Emmerich P, Gunther S, Held G, Smola S and Schmidt-Chanasit J 2014. First case of laboratory-confirmed Zika virus infection imported into Europe, November 2013. 19(4): pii.20685.

Thangamani S, Huang J, Hart CE, Guzman H and Tesh RB 2016. Vertical Transmission of Zika Virus in *Aedes aegypti* Mosquitoes. Am J Trop Med Hyg. 95(5): 1169–1173.

Thavara U, Tawatsin A, Pengsakul T, Bhakdeenuan P, Chanama S, Anantapreecha S, Molito C, Chompoosri J, Thammapalo S, Sawanpanyalert P and Siriyasatien P 2009. Outbreak of chikungunya fever in Thailand and virus detection in field population of vector mosquito *Aedes aegypti* and *Aedes albopictus*. Southeast Asian J Trop Med Public Health. 40(5): 951–962.

Ungchusak K 2008. Chikungunya: Thailand confirmed. Pro Med 2008 Oct 28. Available from http://www.promedmail.org,archiveno.20081028.3407.

Vasconcelos PF and Calisher CH 2016. Emergence of human arboviral diseases in the americas, 2000–2016. Vector Borne Zoonotic Dis. 16(5): 295–301.

Waggoner JJ and Pinsky BA 2016. Zika virus: diagnostics for an emerging pandemic threat. J Clin Microbiol. 54(4): 860–867.

Wanlapakorn N, Thongmee T, Linsuwanon P, Chattakul P, Vongpunsawad S, Payungporn S and Poovorawan Y 2014. Chikungunya outbreak in BuengKan Province, Thailand, 2013 [letter]. Emerg Infect Dis. 20(8): 1404-1406.

Weinbren MP and Williams MC 1958. Zika virus: further isolations in the Zika area, and some studies on the strains isolated. Trans R Soc Trop Med Hyg. 52(3): 263–268.

Wong PS, Li MZ, Chong CS, Ng LC and Tan CH 2013. *Aedes* (*Stegomyia*) *albopictus* (Skuse): a potential vector of Zika virus in Singapore. PLoS Negl Trop Dis. 7(8): e2348.

บทคัดย่อ

อัตราการติดเชื้อสูงของไวรัสซิกาในยุงที่เก็บจากแหล่งระบาดของไวรัสซิกา ณ ภาคตะวันออกของประเทศไทย

อภิวัฏ ธวัชสิน[1] อัจฉรา ภูมี[2,5] อุษาวดี ถาวระ[1] พัชราวรรณ ศิริโสภา[1] วรรณภา ฤทธิสนธิ์[3] กฤษณพงศ์ ธรรมโกศล[4] เพราพิลาศ อินตะยศ[4] ยุทธนา จ้อยจินดา[2] สุภาภรณ์ วัชรพฤษาดี[2] ธีระวัฒน์ เหมะจุฑา[2] เผด็จ สิริยะเสถียร[5]*

โรคไข้ซิกา โรคไข้ชิคุนกันยา และโรคไข้เลือดออกเดงกีเป็นโรคที่เกิดจากไวรัสที่นำโดยแมลงที่เกิดการอุบัติใหม่และอุบัติซ้ำ ซึ่งไวรัสเหล่านี้จะถูกส่งผ่านไปยังมนุษย์โดยผ่านการกัดของยุงลาย ไม่นานมานี้ไวรัสซิกาได้พบอุบัติใหม่ขึ้นในประเทศไทยและพบอีกหลายๆประเทศโดยเฉพาะประเทศในเขตร้อนและกึ่งเขตร้อน และเป็นที่ทราบกันดีว่าโรคติดเชื้อดังกล่าวเป็นโรคที่ไม่มีวัคซีนป้องกัน และไม่มียารักษาที่เฉพาะ ดังนั้นการควบคุมการเกิดโรคที่มีประสิทธิภาพจึงเป็นเพียงการควบคุมแมลงพาหะ อีกทั้งเพื่อเข้าใจวงจรการแพร่กระจายของไวรัสและยุง การศึกษานี้จึงได้ออกแบบเพื่อตรวจหาการติดเชื้อไวรัสซิกา ไวรัสชิคุนกันยา และไวรัสไข้เลือดออกเดงกีในยุงตามธรรมชาติด้วยเทคนิคทางอณูชีววิทยา โดยเก็บยุงตัวเต็มวัยและลูกน้ำยุงบริเวณรอบบ้านคนไข้ที่ตำบลแกลง จังหวัดระยอง ประเทศไทย ซึ่งไวรัสชิคุนกันยา และไวรัสไข้เลือดออกเดงกีจะตรวจสอบด้วยเทคนิควันสเต็ป มัลติเพล็ก เรียลไทม์ อาร์ที-พีซีอาร์ ส่วนไวรัสซิกาตรวจด้วยเทคนิคเนสเต็ด อาร์ที-พีซีอาร์ จากผลการทดลองพบว่ายุงที่เก็บจากธรรมชาติให้ผลบวกต่อไวรัสซิกาจำนวน 8 (10.3%) ตัวอย่าง โดยพบในยุงลายบ้าน (*Aedes aegypti*) เพศเมีย 5 (6.4%) ตัวอย่างและเพศผู้จำนวน 2 (2.6%) ตัวอย่าง และที่น่าสนใจพบว่าพบในตัวอย่างยุงแม่ไก่ (*Armigeres subalbatus*) เพศเมียจำนวน 1 ตัวอย่าง อีกทั้งยังพบการติดเชื้อไวรัสชิคุนกันยาจำนวน 5 (6.4%) ตัวอย่างในยุงลายบ้านเพศเมีย 3 (3.8%) ตัวอย่างและในระยะลูกน้ำของยุงลายบ้านจำนวน 2 (2.6%) ตัวอย่าง และไม่พบการติดเชื้อไวรัสไข้เลือดออกเดงกีในทุกตัวอย่าง เมื่อนำลำดับนิวคลีโอไทด์ของตัวอย่างที่ให้ผลบวกต่อไวรัสซิกามาสร้างแผนภูมิต้นไม้วิวัฒนาการพบว่าอยู่ในกลุ่มของเอเชีย (Asian lineage) การศึกษานี้จึงเป็นการศึกษาเบื้องต้นในการสำรวจหาเชื้อในยุงที่พบในแหล่งระบาดของโรคเพื่อศึกษาหาแมลงพาหะที่สามารถก่อโรคไข้ซิกา โรคไข้ชิคุนกันยา และโรคไข้เลือดออกเดงกี ข้อมูลที่ได้จากการศึกษานี้จะช่วยให้เข้าใจถึงอัตราการติดเชื้อของไวรัสซิกา ไวรัสชิคุนกันยา และไวรัสไข้เลือดออกเดงกีในยุงที่เก็บจากธรรมชาติได้ และอาจจะนำไปสู่การควบคุมยุงพาหะให้มีประสิทธิภาพในอนาคต

คำสำคัญ: ไวรัสซิกา ไวรัสชิคุนกันยา ไวรัสไข้เลือดออกเดงกี ยุง ประเทศไทย

[1]สถาบันวิจัยวิทยาศาสตร์สาธารณสุข กรมวิทยาศาสตร์การแพทย์ กระทรวงสาธารณสุข

[2]ศูนย์วิทยาศาสตร์สุขภาพโรคอุบัติใหม่-ศูนย์ปฏิบัติการโรคทางสมอง โรงพยาบาลจุฬาลงกรณ์ สภากาชาดไทย และ คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

[3]สำนักงานป้องกันควบคุมโรคที่ 6 จังหวัดชลบุรี กรมควบคุมโรค กระทรวงสาธารณสุข

[4]หลักสูตรวิทยาศาสตร์การแพทย์ คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

[5]หน่วยปฏิบัติการวิจัยชีววิทยาของแมลงพาหะนำโรคและโรคติดต่อนำโดยแมลง ภาควิชาปรสิตวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

***ผู้รับผิดชอบบทความ** E-mail: Padet.S@chula.ac.th*