

# Mosquito distribution and Japanese encephalitis virus infection in the immigration bird (Asian open-billed stork) nested area in Pathum Thani province, central Thailand

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Received: 24 December 2009 / Accepted: 13 January 2010  
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**Abstract** Japanese encephalitis virus infection is a mosquito-borne emerging or re-emerging infectious disease in several countries. The ecology of this virus in nature includes amplifying avian or mammal hosts and mosquito vectors. Infected immigration birds from epidemic areas may play important roles in the outbreak of the disease. The prevalence is high during the raining season in Thailand and human cases have been reported from several provinces including Bangkok suburbs. This study was conducted to investigate the mosquito distribution and Japanese encephalitis virus infection in the immigration bird (Asian open-billed stork) nested area, Pathum Thani province, central Thailand. Mosquitoes were collected by using CO<sub>2</sub>-baited Centers for disease control and prevention (CDC) light traps, and dry ice was used as a source of CO<sub>2</sub> to attract mosquitoes from March 2008 to January 2009. Eight traps were operated from 4 p.m. until 7 a.m. on each study day. There were seven genera collected: *Aedes*, *Anopheles*, *Armigeres*, *Coquillettidia*, *Culex*, *Mansonia*, and *Uranotaenia*. *Culex tritaeniorhynchus* was the most collected species in each month, except November, in which *Culex gelidus* was the most collected species. Sixty pools of *C. gelidus* and of *C. tritaeniorhynchus*, each of which had 50 mosquitoes,

were tested for Japanese encephalitis virus infection by using reverse transcription polymerase chain reactions; however, none of them was infected with the virus.

## Introduction

Japanese encephalitis virus (JEV) infection is an emerging or re-emerging infectious disease in several countries in Asia including Thailand (Gunakasem et al. 1981; Thisyakorn and Nimmannitya 1985). This virus was first isolated in 1935 in Japan and it has spread throughout Asia and Australia (van den Hurk et al. 2001). This virus belongs to the Japanese encephalitis (JE) serogroup of the genus *Flavivirus*, family Flaviviridae. Others in this serogroup are West Nile virus, St. Louis encephalitis virus, Rocio virus, and Kunjin virus which have recently been reclassified as a subtype of West Nile virus. There are five virus genotypes of JEV which have been identified according to the nucleotide sequencing of capsid/premembrane protein (C/PrM) and envelope gene. The genotype that has spread in Thailand since 1980s is genotype III; however, it has recently changed to genotype I since 2003 (Nitattattana et al. 2008).

Ecology of JEV involves avian and mammal amplifying hosts and mosquito vectors. Swine, ardeid birds, and *Culex* mosquitoes play major roles in the transmission cycle of this virus in nature (Simpson et al. 1976; van den Hurk et al. 2009). Vertebrate host was infected with JEV when bitten by an infected mosquito. This virus causes encephalitis and death in vertebrate animals and humans (Gould et al. 1964). It is considered the most important mosquito-borne viral encephalitis in humans, particularly in Asia, where it is responsible for the death of around 20,000 people annually (Jackson et al. 2007).

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Immigration birds from an endemic area play an important role for many infectious disease distributions and possibly for JEV outbreak. Several places in Thailand are the famous places for immigration birds. In Thailand, Phailom temple is one of the famous places for Asian or white open-billed storks (*Ciconia ciconia*) where birds emigrate from other countries, and it is also among the places within Thailand during the immigration season. This study area is located on the side of Chao Phraya River in Pathum Thani province, the neighboring province of Bangkok. It has been concerned that the outbreak of any vector-borne diseases in this area might reach Bangkok in a short period of time.

This study was performed to investigate the distribution of mosquito species in the immigration bird nested area and to study JEV infection in some selected mosquitoes species. The information from this study would be useful for the future research on epidemiological study, prevention, and control of JEV in Thailand.

## Materials and methods

### Mosquito collection

Mosquitoes were collected by using CO<sub>2</sub>-baited CDC light traps, and dry ice was used as a source of CO<sub>2</sub> to attract mosquitoes from March 2008 to January 2009. Eight traps were operated from 4 p.m. until 7 a.m. on each study day at the immigration bird (Asian open-billed stork) nested area (14°14'35" N, 100°31'35" E) in Pathum Thani province, central Thailand. Collected mosquitoes were identified according to Rattarithikul and Panthusiri (1994) and kept at -80°C until tested.

### Viral ribonucleic acid extraction

Five hundred *C. gelidus* and 500 *Culex tritaeniorhynchus* mosquitoes were randomly selected from each month. Viral ribonucleic acid (RNA) was extracted by using Tri Reagent LS® (Molecular Research Center, Cincinnati, OH) according to its manufacturer's recommendation with slight modification. Briefly, a pool of 50 mosquitoes was homogenized in 500 µl of Minimum Essential Medium Alpha (Invitrogen, Carlsbad, CA) by using plastic pestle. The mosquito homogenate was then centrifuged at 3,000 rpm for 10 min. Two hundred and fifty microliters of the homogenate were mixed with 750 µl of Tri Reagent LS®. Two hundred microliters of chloroform were also added and RNA was precipitated in 500 µl of isopropanol. RNA pellet was then washed with 75% ethanol and dissolved in 20 µl of ultrapure water (Invitrogen, Carlsbad, CA). RNA was kept at -80°C until tested.

### Reverse transcription polymerase chain reaction

Sixty RNA samples extracted from *C. gelidus* and 60 RNA samples extracted from *C. tritaeniorhynchus* mosquitoes were tested for JEV by using reverse transcription polymerase chain reaction (RT-PCR). The primers for C/PrM gene of JEV were forward 5' GAC TAA AAA ACC AGG AGG GC and reverse 5' CTC CCC ATG TGT TTG GAC CG. RT-PCR cycling conditions were 45 min at 48°C for the reverse transcription step and 3 min at 94°C for the initial PCR activation step followed by 35 cycles of 30 s at 94°C (denaturation), 30 s at 58°C (annealing), 30 s at 72°C (extension), and 7 min at 72°C for the final extension. RT-PCRs were performed in 25-µl reaction. One and a half microliters of RNA were mixed with 12.5 µl of master mix (AccessQuick®, Promega, Madison, WI), 0.2 µM of forward and reverse primer, 2.5 U of Avian myeloblastosis virus (AMV) reverse transcriptase (AccessQuick®, Promega, Madison, WI), and 8.5 µl of ultrapure water (Invitrogen, Carlsbad, CA). RNA was amplified by using thermocycler (Perkin Elmer Cetus 9600, Perkin Elmer, Waltham, MA). The PCR product was analyzed in 1.2% agarose gel (UltraPure™, Invitrogen, Carlsbad, CA) with expected 681-base pair band.

## Results

### Mosquito distribution and weather information

There were 7,336 mosquitoes collected in this study in March 2008 by using eight mosquito traps, and the number of mosquitoes changed to 9,737, 4,740, 4,367, 22,767, and 17,043 in May 2008, July 2008, September 2008, November 2008, and January 2009, respectively.

A total of seven genera of mosquitoes were collected: *Aedes*, *Anopheles*, *Armigeres*, *Coquillettidia*, *Culex*, *Mansonia*, and *Uranotaenia*. *Culex* was the most collected genus in each month (Table 1). The most collected species of the mosquitoes for each genus were *Aedes lineatopennis*, *Anopheles barbirostris*, *Armigeres subalbatus*, *Coquillettidia crassipes*, *C. tritaeniorhynchus*, *Mansonia uniformis* and *Uranotaenia lateralis*. *C. tritaeniorhynchus* was the most collected species in each month, except November, in which *C. gelidus* was the most collected species. Information regarding temperature, humidity, and rain in Pathum Thani province during the study period were shown in Fig. 1. The range of the average temperature, humidity, and rain were 25.1–29.7°C, 62.8–78.7%, and 0–7.6 mm, respectively.

### Japanese encephalitis virus infection in mosquitoes

Sixty samples of RNA extracted from *C. gelidus* and 60 samples of RNA extracted from *C. tritaeniorhynchus*

**Table 1** Percentage of collected mosquito species distribution in the immigration bird (Asian open-billed stork) nested area in Pathum Thani province, central Thailand during March 2008–January 2009

Mosquito species	Collected month					
	Mar 2008	May 2008	Jul 2008	Sep 2008	Nov 2008	Jan 2009
<i>Aedes albopictus</i>	0	0.04	0.34	0.09	0	0.01
<i>Aedes lineatopennis</i>	0.01	3.24	0.23	0.16	0	0
<i>Anopheles barbirostris</i>	0.53	0.08	0.51	0.71	0.57	0.17
<i>Anopheles kochi</i>	0.41	0.72	0.11	0.78	0.03	0
<i>Anopheles stephensi</i>	0.03	0.5	0.11	0	0	0
<i>Anopheles tessellatus</i>	0	0	0	0	0	0.89
<i>Armigeres subalbatus</i>	0.08	0.03	0.06	0.11	0.32	0.55
<i>Coquillettidia crassipes</i>	0.16	2.2	0.53	1.03	0.09	0.32
<i>Culex bitaeniorhynchus</i>	0.1	0.17	0	0	0	0
<i>Culex gelidus</i>	8.14	17.87	33.25	23.33	65.50	6.37
<i>Culex fuscocephala</i>	0.53	2.06	0.19	0.05	0.15	0.08
<i>Culex quinquefasciatus</i>	0.01	0.01	0	0.02	0.01	0.02
<i>Culex tritaeniorhynchus</i>	87.21	63.58	58.27	70.92	31.54	91.08
<i>Culex vishnui</i>	0	0.18	0.06	0.07	0.22	0
<i>Mansonia annulata</i>	0.01	0	0	0	0.004	0
<i>Mansonia annulifera</i>	0.01	0.03	0	0.14	0.02	0
<i>Mansonia uniformis</i>	2.64	8.98	5.82	2.52	1.53	0.51
<i>Uranotaenia lateralis</i>	0.11	0.3	0.53	0.07	0	0

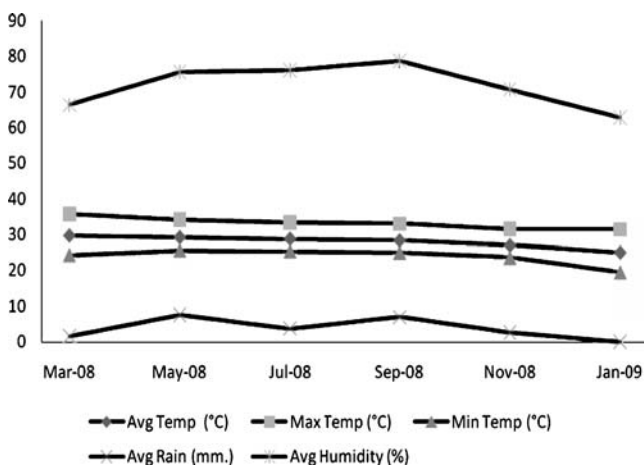
mosquitoes were tested for JEV by using reverse transcription polymerase chain reaction, and all of them were negative for JEV.

## Discussion

This study reported the collected mosquitoes from the immigrated Asian open-billed stork nested area in Pathum Thani province, central Thailand. This area is adjacent to

Chao Phraya River and surrounded by rice fields. Natural and man-made water reservoirs covered with water plants were also located in this area. These are some reasons why various species of mosquitoes were collected from this area. *C. gelidus* and *C. tritaeniorhynchus* might play important roles as vectors for any pathogen that happens in this area because they were the two most collected species of mosquitoes in this study area. The study by Tiawsirisup et al. (2008) also reported that these mosquitoes were the two most collected species during August and December in 2006. The previous study, however, did not find any *Aedes* and *Coquillettidia* in this area. In the current study, mosquito traps were operated from 4 p.m. until 7 a.m. on each study day. Some *Aedes albopictus* and *A. lineatopennis* were collected during this period of time, even though *Aedes* mosquitoes were active during the daytime. *A. lineatopennis* might be an important mosquito in this area during May, since a high number of them were collected during this time; however, no data indicate the vector competency of this mosquito for arbovirus in Thailand. In Australia, on the other hand, Ross River virus has been isolated from *A. lineatopennis* collected from northwest Queensland (van den Hurk et al. 2002).

The lowest and highest numbers of mosquitoes collected in this study were in July and November, respectively. Generally, mosquito distribution in most areas in Thailand is high during monsoon (May–October), moderate during transition (March–April and November–December), and low during dry (January–February) season. This study,



**Fig. 1** Temperature, rain, and humidity in Pathum Thani province during the study period

however, showed that the number of collected mosquitoes gradually decreased from January, May, July, and September. Temperature, humidity, and rainfall might have affected the number of mosquitoes which varies each year.

Japanese encephalitis virus infection in humans in Thailand has been reported every year; however, the incidence has decreased since the immunization program in human was developed. The report from the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand showed that the incidence of JEV infection in human were about 0.1 case per 100,000 people in 2008–2009. These human cases have been reported from the central, northern, northeastern, and southern Thailand.

Swine production is a very important agricultural industry in every part of Thailand. Even though the biosecurity of the farms has increased, it does not prevent pigs from arthropod-borne diseases including JEV infection. The intensive JEV circulation in swine farming in Thailand may be indicated by antibody seroconversion against JEV in pigs (Gingrich et al. 1987). Waste water reservoirs in the farm are famous breeding places for *C. gelidus* and *C. tritaeniorhynchus*, the major competent vectors for JEV (Gould et al. 1962; Gingrich et al. 1987; Pant et al. 1994). Recently, *C. quinquefasciatus* became another species of *Culex* mosquito that was a potential vector for JEV in Thailand (Nitapattana et al. 2005).

This study, however, could not detect any Japanese encephalitis virus (JEV) in *C. gelidus* and *C. tritaeniorhynchus* by using the sensitive and specific reverse transcription polymerase chain reactions (RT-PCR). Beijing strain of JEV was used as a positive control in this study, and the RT-PCR in this study which detects the C/PrM gene of JEV can detect the virus that has the lowest virus titer at  $10^{1.87}$  TCID<sub>50</sub>/ml. Higher sensitive RT-PCR or other techniques such as immunohistochemistry straining of infected cells might be a useful techniques for the future.

Even though no data indicated the relationship of Asian open-billed stork in the ecology of JEV in nature, the antibody against West Nile virus, another *Flavivirus*, has been shown in this wild bird species in nature in Poland (Hubalek et al. 2008). More studies need to be carried out to conclude that Asian open-billed stork may be not an important reservoir for JEV in nature in Thailand.

**Acknowledgments** This study was financially supported by the Thailand Research Fund (TRF) and Commission of Higher Education (MRG5180153). The authors would like to thank the Virology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science for Japanese encephalitis virus and the laboratory assistant. The authors would also like to thank Thai Meteorological Department for the weather information.

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