



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Document heading doi: 10.1016/S2222-1808(12)60059-0 © 2012 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

Mosquito distribution and West Nile virus infection in zoos and in important sites of migratory and resident birds, Thailand

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ARTICLE INFO

Article history:

Received 15 March 2012

Received in revised form 27 April 2012

Accepted 28 May 2012

Available online 28 August 2012

Keywords:

Mosquito
West Nile virus
Vector
Surveillance
Zoo
Migratory bird
Resident bird

ABSTRACT

Objective: To investigate the distribution of mosquito species in the zoos and in important sites of migratory and resident birds and evaluate West Nile virus (WNV) infection in mosquito species. **Methods:** Mosquitoes distribution investigation was carried out bimonthly from January 2009 to December 2010 in five areas of birds, Thailand by using Centers for Disease Control, light traps, and gravid traps. Mosquitoes were identified, pooled into groups of up to 50 mosquitoes by species, places and time of collection and tested for WNV infection by viral isolation and reverse transcriptase polymerase chain reaction. **Results:** A total of 66 597 mosquitoes comprising 26 species in 8 genera were collected. The five most abundant mosquito species collected were *Culex tritaeniorhynchus* (79.3%), *Culex vishnui* (8.2%), *Culex sitiens* (6%), *Culex quinquefasciatus* (3.3%) and *Anopheles peditaeniatus* (1.1%). All 1 736 mosquito pools were negative for viral isolation and reverse transcriptase polymerase chain reaction. **Conclusions:** This study provides new information on number of mosquito species present and their relative abundance. Although our study found no evidence of WNV in the avifaunal sources of Thailand, mosquito active surveillance should be continuously conducted. The cooperation between related organizations is needed for early detection of WNV disease and development of effective veterinary and public health policies in this region.

1. Introduction

West Nile virus (WNV) is a mosquito-borne virus in the genus *Flavivirus* of the family *Flaviviridae* that affects mostly birds but also other animal species and humans. This virus belongs to the Japanese encephalitis virus (JEV) serogroup viruses. The other members of these serogroup are Japanese encephalitis virus, St. Louis encephalitis virus, Murray Valley encephalitis virus and Kunjin virus (subtype of WNV)[1]. WNV was first isolated from the blood of a sick woman in the West Nile District of Uganda in 1937[2]. WNV is widely distributed in several countries of Africa, Europe, North America, Australia, and Asia (especially in India)[3]. Although it has been

suggested that this virus can be classified in seven lineages[4], molecular phylogenetic studies have shown that isolates of the virus can be just divided into two lineages. WNV lineages I strains are widely distributed in most continents, whereas lineages II have mostly been found in Africa[5,6].

Transmission cycle of WNV involves birds serving as the natural reservoir hosts and *Culex* species mosquitoes serving as the enzootic and/or epizootic vectors[7]. The infection of most wild bird species is asymptomatic, while some species of birds, especially corvids (*e.g.* crows), can be severely affected and have high mortality rates[8,9]. There are among 64 mosquito species and 326 bird species found with WNV positive[10,11]. The viruses can infect humans and domestic animals, such as horses, which are generally thought to be incidental hosts that can develop diseases from moderate flu-like symptoms to fatal encephalitis[12].

Zoo, migratory and resident birds are potential sources for monitoring WNV[13–16]. In Thailand, there are many species of captive and resident birds in the zoos. Especially at Dusit zoo, Bangkok, there is a large number of crows (*Corvus*

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macrorhynchos), species that may be used as sentinels for WNV outbreak in Thailand. Furthermore, several places of Thailand such as Bung Boraphet, Nakorn Sawan Province are a common for migratory and residents birds^[17] that may be a potential source of WNV. Every year many species of birds escape the cold weather by migrating into Thailand^[18], and these birds may play an important role for WNV. Few data are available on the current WNV situation in Thailand. In a recent study, antibodies to WNV were found in horses^[15], but WNV-specific RNA was not detected. Therefore, there is no evidence in the past indicating the presence of indigenous WNV infections to our knowledge.

Centers for Disease Control (CDC) published guidelines for monitoring WNV and other arboviruses in the U.S. The goal of these comprehensive surveillance programs is to identify WNV activity in birds, mosquitoes and horses as sentinels for potential outbreaks in humans. Mosquito surveillance is one of programs widely used in several countries^[19–22]. The important goals of mosquito surveillance are to identify potential mosquito vectors in a particular area, monitoring population densities of those vectors, and determining infection rates^[23]. Our study was conducted to investigate the distribution of mosquito species in zoos, important areas of migratory and resident birds and to evaluate WNV infection in these mosquito species. The information from this study will be useful for future research on epidemiological studies, detection and prevention of WNV in Thailand.

2. Materials and methods

2.1. Study areas

Five localities including zoos and sources of migratory and resident birds in Thailand were used in this mosquito distribution (Figure 1).

Ban Wang Pet is located in the Bang Rakam District of Phitsanulok Province (N16°41'58.2'', E100°11'02.1''). This location is the large source of egrets in Thailand. Birds are found near the Yom River, which has an abundant food supply for birds: wild fish in the area over 0.128 km². Bird species at the site include Cattle Egret, Little Egret, Intermediate Egret, Great Egret, Chinese Egret, Black Crowned Night Heron, Little Cormorant, Oriental Darter and Asian Open billed Stork.

Bung Boraphet Water Bird Park, located in Nakorn Sawan Province (N15°43'21.8'', E100°17'38.6''), is the largest wetland in Thailand with about 212.38 km² in freshwater. This location has a large collection of migratory birds during the winter season (November to March) and is an important area for several resident bird species. The total number of found bird species at the site is approximately of 250. At the site water birds, shore birds and accipiters birds can be observed. Among often sighted migratory birds are Grey Heron, Purple Heron, Spot-billed Pelican, Black-headed Ibis, Asian Open bill Stork, Lesser Whistling-Duck, Garganey, Baer's Pochard and Northern Pintail. Whereas the resident birds include Cotton Pygmy-goose, Little Cormorant, Common Coot, Great Egret Watercock, Purple Swampen, Little Grebe, Chinese Pond

Heron, Pheasant-tailed Jacana and Bronze-winged Jacana^[17].

Bungchawak zoo, Suphan Buri Province (N14°54'42.5'', E100°02'51.1'') exhibits a wide variety of animal species. This zoo is abundant in bird species, possessing more than 45 species in 8 000 m² of aviary, especially Pheasant, Peacock, Painted Stork, Lesser Whistling-Duck, Parrot groups. Moreover, outside the cages, there are many species of resident birds flying around the area.

Dusit zoo, located at Khao Din Park in the Bangkok city (N13°46'29.23'', E100°31'56.63''), is the oldest and most popular zoo in Thailand^[24]. It covers a total area of 0.189 km² and has more than 1 600 animal species. This zoo is classified as a full-function animal park completed with facilities like an animal hospital, a zoo museum, an educational centre, a cafeteria, and a relaxation area. Besides the zoo animals, there are huge numbers of large-billed crows (*Corvus macrorhynchos*) that live and fly within the zoo.

Bangpu, Samut Prakarn Province (N13°31'04.2'', E100°39'22.0'') has a high diversity in shore and water bird species that visit its mangrove swamps in winter^[25]. Example of these birds are Brown-headed Gull, Asian Dowitcher, Little Heron, Terns, Ruddy Shelduck, Black-winged Stilt, Lesser Sand-Plover, Whimbrel, Spotted Redshank, Curlew Sandpiper, and Ruddy Turnstone.

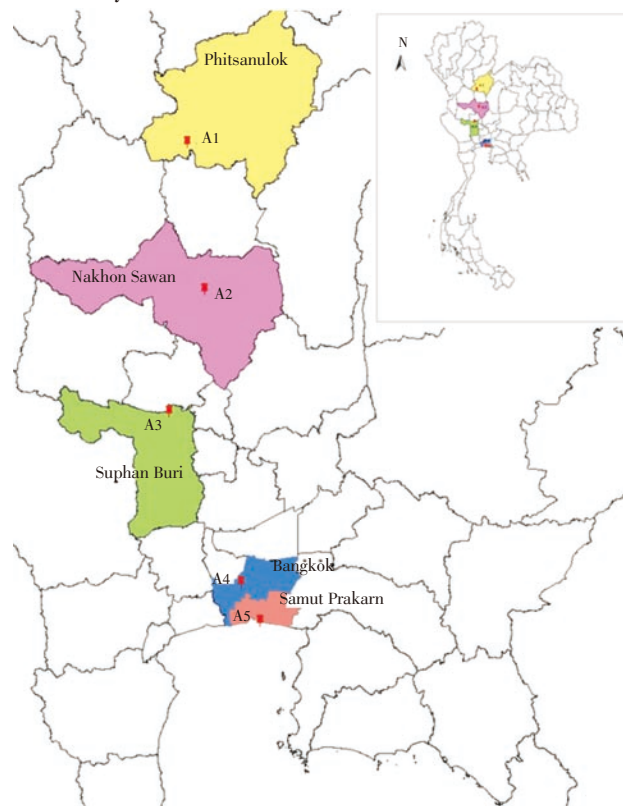


Figure 1. Map of mosquito collected sites in Thailand.

A1: Ban Wang Pet; A2: Bung Boraphet; A3: Bungchawak zoo; A4: Dusit zoo; A5: Bangpu.

2.2. Mosquito collection

Mosquitoes were collected on various occasions bimonthly by using CO₂-baited CDC light traps which dry ice was used as a source of CO₂ to attract mosquitoes and CDC gravid traps with fermented hay (John W. Hock Company, Gainesville, USA)

from January 2009 to December 2010. Each five traps operated from 6 p.m. until 6 a.m. on each study day. The mosquitoes were transported alive to laboratory for species identification by using description and keys of Rattanakul et al.^[26–29]. They were pooled by species, place, and time of collection. The number of mosquitoes per pool ranged from 1 to 50. They were stored at -80°C until tested for virus.

2.3. Viral isolation

Pools of mosquitoes were homogenized in 1 000 μL of minimum essential medium (MEM 10 \times , Penicillin G, Streptomycin and Fungizone) in 1.5 mL appendorf tube by using plastic pestle. The homogenated mosquito was centrifuged at 4 000 rpm for 10 min and supernatant was passed 0.45 μm syring filter. The 200 μL of samples were inoculated into baby hamster kidney (BHK–21) cells and incubated at 37°C in a 5% CO_2 incubator for 2 hours. 150 μL of the samples of BHK–21 cells were discarded and added 500 μL of maintenance medium (2% fetal bovine serum in MEM) and then incubated at 37°C in 5% CO_2 . We checked the presence of cytopathic effect (CPE) daily for 3 days. Positive CPE was confirmed by reverse transcription polymerase chain reaction (RT–PCR).

2.4. RNA extraction and RT–PCR

Viral RNA was extracted from mosquitoes by using a viral nucleic acid extraction kit (Geneaid Biotech Ltd., Taiwan). The RT–PCR was performed using a one–step RT–PCR kit (QIAGEN Ltd., Germany) for the screening both WNV and JEV infection. The reaction mixture contained 0.125 μL of forward primer JE/WN–OF 5' GRA ARM GDG ARG ACA TYT GGT GTG G 3', 0.125 μL of reverse primer JE/WN–OR 5' CGG GGT CTC CTC TAA CCT CTA GTC C 3', 2 μL of template DNA, 5 μL of 5X OneStep RT–PCR buffer (QIAGEN), 1 μL of 10 mM dNTP mix (QIAGEN), 1 μL of OneStep RT–PCR enzyme (QIAGEN) and RNase–free water was added to a total volume of 25 μL . PCR cycling conditions were as follows: 1 cycle at 50°C for 30 minutes; 1 cycle at 95°C for 15 minutes; 35 cycles at 94°C for 45 seconds followed by 70°C for 45 seconds, 72°C for 90 seconds and final extension at 72°C for 10 min. Positive and negative controls were included in each run. Positive controls for WNV were obtained from the United States Geological Survey while positive controls for JEV were obtain from JE vaccine strain Beijing–1. Negative controls consisted of master mix minus RNA templates. PCR product were separated by gel electrophoresis and visualized under UV light. The specific size of PCR product for WNV and JEV was 580 and 591 base pairs, respectively. If the RT–PCR positive specimens will be confirmed for WNV by using specific primer including WN233 (5' TTG TGT TGG CTC TCT TGG CGT TCTT 3') and WN640c (5' CAG CCG ACA GCA CTG GAC ATT CATA 3'). The RT–PCR cycling conditions will be used 1 cycle at 50°C for 30 minutes; 1 cycle at 95°C for 15 minutes; 35 cycles at 94°C for 45 seconds followed by 55°C for 45 seconds, 72°C for 30 seconds and the final extension at 72°C for 10 minutes. The specific size for WNV was 408 base pairs.

3. Result

There were 66 597 mosquitoes collected. They were subdivided into 61 534 mosquitoes for CDC light and 5 063 mosquitoes for CDC gravid traps (Table 1). A total of eight genera (26 species) of mosquitoes were collected: *Aedeomyia*, *Aedes*, *Anopheles*, *Armigeres*, *Coquillettia*, *Culex*, *Lutzia* and *Mansonia*. The six most abundant mosquito species collected were *Culex tritaeniorhynchus* (79.3%), *Culex vishnui* (8.2%), *Culex sitiens* (6.0%), *Culex quinquefasciatus* (3.3%) and *Anopheles peditaeniatus* (1.1%). Mosquito species distribution for the five collected areas is shown in Table 2. The species distribution was markedly different among study areas. *Culex tritaeniorhynchus* and *Culex vishnui* were the predominant species at Bung Chawak zoo, Bung Boraphet and Ban Wang Pet, whereas *Culex quinquefasciatus*, *Culex gelidus* were at Dusit zoo, and *Culex sitiens* and *Culex gelidus* at Bangpo. When the number of mosquito densities was compared among the five areas, Ban Wang Pet had the highest mosquito densities (41.8%) followed by Bung Boraphet (26.7%), Bung Chawak zoo (22.3%), Bangpo (6.2%) and Dusit zoo (3.0%), respectively (Table 2).

The total 1 736 mosquito pools were negative isolation for WNV. These results were confirmed by using RT–PCR and all of them were negative for WNV.

Table 1

Total number of mosquito species collected at five areas in Thailand, 2009–2010.

Mosquito species	Number collected			
	Light traps	Gravid traps	Total	Percent
<i>Aedeomyia catasticta</i>	2	0	2	<1.0
<i>Aedes aegypti</i>	21	8	29	<1.0
<i>Aedes mediolineatus</i>	1	0	1	<1.0
<i>Aedes vexans</i>	6	0	6	<1.0
<i>Anopheles argyropus</i>	3	0	3	<1.0
<i>Anopheles barbirostris</i>	12	0	12	<1.0
<i>Anopheles campestris</i>	2	0	2	<1.0
<i>Anopheles nigerrimus</i>	57	0	57	<1.0
<i>Anopheles peditaeniatus</i>	748	1	749	1.1
<i>Anopheles sinensis</i>	2	0	2	<1.0
<i>Anopheles sondaicus</i>	40	0	40	<1.0
<i>Anopheles vagus</i>	1	8	9	<1.0
<i>Anopheles tessellatus</i>	5	0	5	<1.0
<i>Armigeres subalbatus</i>	4	29	33	<1.0
<i>Coquillettia crassipes</i>	6	0	6	<1.0
<i>Culex bitaeniorhynchus</i>	19	3	22	<1.0
<i>Culex fascocephala</i>	4	0	4	<1.0
<i>Culex gelidus</i>	516	31	547	<1.0
<i>Culex pseudovishnui</i>	186	126	312	<1.0
<i>Culex quinquefasciatus</i>	978	1 226	2 204	3.3
<i>Culex sitiens</i>	3 965	5	3 970	6.0
<i>Culex tritaeniorhynchus</i>	50 193	2 613	52 806	79.3
<i>Culex vishnui</i>	4 547	928	5 475	8.2
<i>Lutzia fuscana</i>	0	83	83	<1.0
<i>Mansonia indiana</i>	49	2	51	<1.0
<i>Mansonia uniformis</i>	167	0	167	<1.0
Total	61 534	5 063	66 597	100.0

Table 2

Species distribution of mosquitoes collected at five areas in Thailand, 2009–2010.

Mosquito species	Number collected					Total
	Dusit zoo	Bung chawak	Bang pu	Bung Boraphet	Ban Wang Pet	
<i>Aedeomyia catasticta</i>	0	0	0	2	0	2
<i>Aedes aegypti</i>	27	1	0	0	1	29
<i>Aedes mediolineatus</i>	0	0	0	1	0	1
<i>Aedes vexans</i>	0	0	0	6	0	6
<i>Anopheles argyropus</i>	0	0	0	1	2	3
<i>Anopheles barbirostris</i>	0	1	0	0	11	12
<i>Anopheles campestris</i>	0	0	0	2	0	2
<i>Anopheles nigerrimus</i>	0	2	0	55	0	57
<i>Anopheles peditaeniatus</i>	1	71	0	333	344	749
<i>Anopheles sinensis</i>	0	0	0	2	0	2
<i>Anopheles sudaicus</i>	0	0	40	0	0	40
<i>Anopheles vagus</i>	1	8	0	0	0	9
<i>Anopheles tessellatus</i>	0	5	0	0	0	5
<i>Armigeres subalbatus</i>	6	0	0	0	27	33
<i>Coquillettidia crassipes</i>	1	0	0	5	0	6
<i>Culex bitaeniorhynchus</i>	0	3	0	18	1	22
<i>Culex fascocephala</i>	0	0	0	0	4	4
<i>Culex gelidus</i>	169	49	125	11	193	547
<i>Culex pseudovishnui</i>	0	91	0	40	181	312
<i>Culex quinquefasciatus</i>	1 661	318	104	10	111	2 204
<i>Culex sitiens</i>	0	10	3 875	65	20	3 970
<i>Culex tritaeniorhynchus</i>	93	12 575	0	14 788	25 350	52 806
<i>Culex vishnui</i>	34	1 681	0	2 241	1 519	5 475
<i>Lutzia fuscana</i>	18	4	0	30	31	83
<i>Mansonia indiana</i>	2	3	0	22	24	51
<i>Mansonia uniformis</i>	1	0	1	156	9	167
Total	2 014 (3.0%)	14 822 (22.3%)	4 145 (6.2%)	17 788 (26.7%)	27 828 (41.8%)	66 597 (100%)

4. Discussion

Active mosquito surveillance was carried out in five important bird sites in Thailand. This survey provides new information on the number of mosquito species present and their relative abundance at different sites. More than 60 000 mosquitoes were collected and included 26 different species. They are the mosquito species that occur in Thailand[27]. The relative species distribution in these five study areas was clearly different and was in agreement with the differences in habitat characteristics in each study site. BungChawak zoo and Bung Boraphet, Ban Wang Pet are found in areas near rivers, wetlands, and are surrounded by rice fields. In these two sites, the predominant mosquito species was *Culex tritaeniorhynchus* followed by *Culex vishnui*. *Culex vishnui* is a common and a widespread species frequently found in association with *Culex tritaeniorhynchus*[28]. *Culex quinquefasciatus* was the predominant species of mosquito at Dusit zoo followed by *Culex gelidus*. Both these two species are common species associated with humans and animals[28]. *Culex quinquefasciatus* occurs abundantly in houses and in practically all types of human and animal shelters in urban communities. Breeding sites of both these two mosquito species have a wide range of habitats including clear, turbid or polluted fresh to blackish water in ground pools, ditches, pits, wells, drains and containers for *Culex quinquefasciatus*; while *Culex gelidus* can be found in pounds, swamps, marshy depression, ditches, pits, wells, stream margins, seepage pools, rice fields, wheel tracks, and footprints[28]. At Bangpo, *Culex sitiens* was the predominant mosquito species that was mostly found in the brackish water or coastal areas, *Culex gelidus* is also found in this area.

The relative proportion of mosquito species varied according to collection methods and study area[22]. In this study, CDC light traps allowed us to collect a higher number and species of mosquitoes than CDC gravid traps, in agreement with the study of Williams *et al*[30]. For example, gravid traps were not as efficient as CDC light traps to collect several species of mosquito such as *Mansonia* sp., *Anopheles* sp., and *Coquillettidia* sp. Although CDC gravid traps collected fewer individuals and fewer species than light traps, the infection rate of mosquitoes infected was higher in the gravid traps presented by Williams *et al*[30].

In the present study, we found two species of mosquitoes (*Culex quinquefasciatus* and *Culex vishnui*) that could act as potential vectors of WNV in Thailand as they have been isolated the strains of WNV in Asia[3,31]. Furthermore, *Culex tritaeniorhynchus* and *Culex bitaeniorhynchus* presented in this study could also act as potential vectors of WNV in Thailand since these species reported from experiment studies in India[3].

Although our study found no evidence of WNV in the avifaunal sources of Thailand, the mosquito active surveillance should be continuously conducted. The first project surveying WNV in Thailand was during 2005–2006[15] and also found WNV negative in mosquitoes collected at bird areas including Bung Boraphet; Nakonsawan province, Wat phailom, Pathum Thani province and Nong Han Lake, Sakon Nakhon Province. Our study also confirms the situation of WNV infection in mosquitoes at Bung Boraphet area.

In Thailand, WNV could be introduced in near future under the current circumstances of many people and materials entering and leaving the country within short periods of time, the development of transportations, the importation of illegal

birds and other domestic pets, unintentional introduction of virus-infected mosquitoes or other vector species. Moreover, some bird species such as crows, which are the major WNV amplifier and other birds especially ardeid birds, important vertebrate host of WNV in Asia^[3] are presented in Thailand. Finally, several species of mosquitoes with the ability to transmit WNV can be found in the potential source of WNV including zoos and important sites of migratory and resident birds from our study.

Additional mosquito surveillance, the other guidelines for WNV surveillance were recommended by CDC^[23] including avian, horses and human encephalitis cases surveillance. For future study, all of those should be considered. Finally, the cooperation between the government and other agencies, such as wildlife conservation organizations, veterinarians and private sectors, is needed for early detection of WNV disease and development of effective veterinary and public health policies in this region.

Conflict of interest statement

We declare that we have no conflict of interest.

References

- [1] Nash D, Mostashari F, Fine A, Miller J, O'Leary D, Murray K, et al. The outbreak of West Nile virus infection in the New York City area in 1999. *N Engl J Med* 2001; **344**:1807–1814.
- [2] Smithburn KC, Hughes TP, Burke AW, Paul JH. A neurotropic virus isolated from the blood of a native of Uganda. *Am J Trop Med* 1940; **20**: 471–492.
- [3] Paramasivan R, Mishra AC, Mourya DT. West Nile virus: the Indian scenario. *Indian J Med Res* 2003; **118**: 101–108.
- [4] Mackenzie JS, Williams DT. The zoonotic flaviviruses of southern, south-eastern and eastern Asia, and Australasia: the potential for emergent viruses. *Zoonoses Public Health* 2009; **56**(6–7): 338–356.
- [5] Jiménez-Clavero MA, Agüero M, Rojo G, Gómez-Tejedor C. A new fluorogenic real-time RT-PCR assay for detection of lineage 1 and lineage 2 West Nile viruses. *J Vet Diagn Invest* 2006; **18**(5): 459–462.
- [6] Venter M, Swanepoel R. West Nile virus 2 as a cause of zoonotic neurological disease in humans and horses in southern Africa. *Vector Borne Zoonotic Dis* 2010; **10**(7): 659–664.
- [7] Gubler DJ. The continuing spread of West Nile virus in the western hemisphere. *Clin Infect Dis* 2007; **45**(8): 1039–1046.
- [8] Marra PP, Griffing S, Caffrey CL, Kilpatrick AM, McLean RG, Brand C, et al. West Nile virus and wildlife. *BioScience* 2004; **54**(5): 393–402.
- [9] McLean RG. West Nile virus in North American birds. *Ornithological Monographs* 2006; **60**: 44–64.
- [10] Centers for Disease Control and Prevention (CDC). West Nile virus [Online] Available from <http://www.cdc.gov/ncidod/dvbid/westnile/mosquitospecies.htm>. [Accessed on 2011 Oct 1]
- [11] Centers for Disease Control and Prevention. West Nile virus [Online] Available from <http://www.cdc.gov/ncidod/dvbid/westnile/birdspecies.htm>. [Accessed on 2011 Oct 1]
- [12] Dauphin G, Zientara S, Zeller H, Murgue B. West Nile: worldwide current situation in animals and humans. *Comp Immunol Microbiol Infect Dis* 2004; **27**(5): 343–355.
- [13] Ludwig GV, Calle PP, Mangiafico JA, Raphael BL, Danner DK, Hile JA, et al. An outbreak of West Nile virus in a New York City captive wildlife population. *Am J Trop Med Hyg* 2002; **67**(1): 67–75.
- [14] Reed KD, Meece JK, Henkel JS, Shukla SK. Birds, migration and emerging zoonoses: west Nile virus, Lyme disease, influenza A and enteropathogens. *Clin Med Res* 2003; **1**(1): 5–12.
- [15] Thammapalo S, Kanjanopas K, Nitatpatana N, Krataithong K, Pawaputanun A, Vonghirun D, et al. West Nile Virus Surveillance Project in Thailand. *J Vector Borne Dis (In Thai)* 2007; **4**(1): 12–21.
- [16] Seidowski D, Ziegler U, von Rönn JA, Müller K, Hüppop K, Müller T, et al. West Nile virus monitoring of migratory and resident birds in Germany. *Vector Borne Zoonotic Dis* 2010; **10**(7): 639–647.
- [17] Uptan N. Bueng Boraphet Non-hunting Area. 2009. [Online] Available from <http://www.thaibirding.com/locations/central/bboraphet.htm>. [Accessed on 2011 Oct 10]
- [18] Chantawong V, Chaipakdee M. Migratory birds of Thailand. *Wildlife Yearbook (In Thai)* 2006; **7**: 206–235.
- [19] Andreadis TG, Anderson JF, Vossbrinck CR. Mosquito surveillance for West Nile virus in Connecticut, 2000: isolation from *Culex pipiens*, *Cx. restuans*, *Cx. salinarius*, and *Culiseta melanura*. *Emerging Inf Dis* 2001; **7**: 670–674.
- [20] Meece JK, Henkel JS, Glaser L, Reed KD. Mosquito Surveillance for West Nile Virus in Southeastern Wisconsin – 2002. *Clin Med Res* 2002; **1**: 37–42.
- [21] Tabei Y, Hasegawa M, Iwasaki N, Okazaki T, Yoshida Y, Yano K. Surveillance of mosquitoes and crows for West Nile virus in the Tokyo metropolitan area. *Jpn J Infect Dis* 2007; **60**: 413–416.
- [22] Almeida AP, Freitas FB, Novo MT, Sousa CA, Rodrigues JC, Alves R, et al. Mosquito surveys and West Nile virus screening in two different areas of southern Portugal, 2004–2007. *Vector Borne Zoonotic Dis* 2010; **10**(7): 673–680.
- [23] Centers for Disease Control and Prevention. Epidemic/epizootic West Nile virus in the United States: Guidelines for surveillance, prevention, and control. [Online] Available from <http://www.cdc.gov/ncidod/dvbid/westnile/resources/wnv-guidelines-aug-2003.pdf>. [Accessed on 2011 Oct 1]
- [24] The Zoological Park Organization (ZPO). 2011 sep 29. [Online] Available from <http://www.dusitzoo.org>. [Accessed on 2011 Oct 10]
- [25] Uptan N. Bang Poo. 2008. [Online] Available from http://www.thaibirding.com/locations/central/bang_poo.htm. [Accessed on 2011 Oct 10]
- [26] Rattanarithikul R, Panthusiri P. Illustrated keys to the medically important mosquitoes of Thailand. *Southeast Asian J Trop Med Public Health* 1994; **25**(Suppl 1): 1–66.
- [27] Rattanarithikul R, Harrison BA, Panthusiri P, Coleman RE. Illustrated keys to the medically important mosquitoes of Thailand I. Background; geographic distribution; lists of genera, subgenera, and species; and a key to the genera. *Southeast Asian J Trop Med Public Health* 2005; **36**(Suppl 1): 1–80.
- [28] Rattanarithikul R, Harbach RE, Harrison BA, Panthusiri P, Jones JW, Coleman RE. Illustrated keys to the mosquitoes of Thailand II. Genera *Culex* and *Lutzia*. *Southeast Asian J Trop Med Public Health* 2005; **36**(Suppl 2): 1–97.
- [29] Rattanarithikul R, Harrison BA, Harbach RE, Panthusiri P, Coleman RE, Panthusiri P. Illustrated keys to the mosquitoes of Thailand. IV. Anopheles. *Southeast Asian J Trop Med Public Health* 2006; **37**(Suppl 2): 1–128.
- [30] Williams GM, Gingrich JB. Comparison of light traps, gravid traps, and resting boxes for West Nile virus surveillance. *J Vector Ecol* 2007; **32**(2): 285–291.
- [31] Khan SA, Dutta P, Khan AM, Chowdhury P, Borah J, Doloi P, et al. West Nile virus infection, Assam, India. *Emerg Infect Dis* 2011; **17**(5): 947–948.