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

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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 tritaeniorynchus (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 many 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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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”N, E100°11’02.1”E). 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”N, E100°17’38.6”E), 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 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 Swamphen, Little Grebe, Chinese Pond Heron, Pheasant–tailed Jacana and Bronze–winged Jacana[17].

Bungchawak zoo, Suphan Buri Province (N14°54’42.5”N, E100°02’51.1”E) 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”N, E100°31’56.63”E), 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”N, E100°39’22.0”E) 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, Terms, 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 Rattanarithikul 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×, 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 nucleic acid extraction kit (Geneaid Biotech Ltd., Taiwan). Viral RNA was extracted from mosquitoes by using 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, 70 °C for 45 seconds, 72 °C for 90 seconds and final extension at 72 °C for 10 minutes. Positive and negative controls were included in each run. Positive controls for WNV and JEV 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 for WNV was 408 base pairs.

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 screening both WNV and JEV infection. The reaction mixture contained 0.125 μL of forward primer JE/WN−OF 5’ GAG GGT CTC TCT TGG CGT TCTT 3’ , 0.125 μL of reverse primer JE/WN−OR 5’ CGG GGT CTC TCT 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 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, Coquillettidia, Culex, Latzia and Mansonia. The six most abundant mosquito species collected were Culex tritaeniorhyncha (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 tritaeniorhyncha 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.

| Mosquito species            | Light traps | Gravid traps | Total | Percent |
|-----------------------------|-------------|--------------|-------|---------|
| Aedeomyia catastica         | 2           | 0            | 2     | <1.0    |
| Aedes aeegypti              | 21          | 8            | 29    | <1.0    |
| Aedes mediolineatus         | 1           | 0            | 1     | <1.0    |
| Aedes vexans                | 6           | 0            | 6     | <1.0    |
| Anopheles argyropus         | 3           | 0            | 3     | <1.0    |
| Anopheles barbirostris      | 12          | 0            | 12    | <1.0    |
| Anopheles campestris        | 2           | 0            | 2     | <1.0    |
| Anopheles nigerrimus        | 57          | 0            | 57    | <1.0    |
| Anopheles peditaeniatus     | 748         | 1            | 749   | 1.1     |
| Anopheles sinensis          | 2           | 0            | 2     | <1.0    |
| Anopheles sondaicus         | 40          | 0            | 40    | <1.0    |
| Anopheles vagus             | 1           | 8            | 9     | <1.0    |
| Anopheles tessellatus       | 5           | 0            | 5     | <1.0    |
| Armigeres subalbatus        | 4           | 29           | 33    | <1.0    |
| Coquillettidia crassipes    | 6           | 0            | 6     | <1.0    |
| Culex bitaenioryhynchus     | 19          | 3            | 22    | <1.0    |
| Culex fascocephala          | 4           | 0            | 4     | <1.0    |
| Culex gelidus               | 516         | 31           | 547   | <1.0    |
| Culex pseudowishnui         | 186         | 126          | 312   | <1.0    |
| Culex quinquefasciatus      | 978         | 1 226        | 2 204 | 3.3     |
| Culex sitiens               | 3 965       | 5            | 3 970 | 6.0     |
| Culex tritaeniorhyncha      | 50 193      | 2 613        | 52 806| 79.3    |
| Culex vishnui               | 4 547       | 928          | 5 475 | 8.2     |
| Latzia fuscans             | 0           | 83           | 83    | <1.0    |
| Mansonia indiana           | 49          | 2            | 51    | <1.0    |
| Mansonia uniformis         | 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 |
|-----------------------------------|------------------|
|                                   | Dusit zoo | Bung chawak | Bang pu | Bung Boraphet | Ban Wang Pet | Total |
| Aedes vexans                      | 0         | 0           | 0       | 2             | 0           | 2     |
| Aedes aegypti                     | 27        | 1           | 0       | 0             | 1           | 29    |
| Aedes medineolusitus              | 0         | 0           | 0       | 1             | 0           | 1     |
| Anopheles argyrosus               | 0         | 0           | 0       | 6             | 0           | 6     |
| Anopheles barbipalpus             | 0         | 1           | 0       | 0             | 11          | 12    |
| Culexquisquefasciatus             | 0         | 2           | 0       | 55            | 0           | 57    |
| Culex gelidus                     | 1         | 71          | 0       | 333           | 344         | 774   |
| Anopheles sinensis                | 0         | 0           | 0       | 2             | 0           | 2     |
| Anopheles sondaicus               | 0         | 0           | 0       | 40            | 0           | 40    |
| Anopheles vago                    | 1         | 8           | 0       | 0             | 0           | 9     |
| Anopheles tessellatus             | 0         | 5           | 0       | 0             | 0           | 5     |
| Armigeres subalbatus              | 6         | 0           | 0       | 0             | 27          | 33    |
| Coquillettidia crassipes          | 1         | 0           | 0       | 0             | 5           | 6     |
| Calex bitaeniorynchus             | 0         | 3           | 0       | 18            | 1           | 22    |
| Calex fasciiceps                  | 0         | 0           | 0       | 0             | 4           | 4     |
| Calex gelidus                     | 169       | 49          | 125     | 11            | 193         | 547   |
| Calex pseudovishnui               | 0         | 91          | 0       | 40            | 181         | 312   |
| Calex quisquefasciatus            | 1 661     | 318         | 104     | 10            | 1 111       | 2 204 |
| Calex sitiens                     | 0         | 10          | 3 875   | 65            | 20          | 3 970 |
| Calex tritaeniorynchus            | 93        | 12 575      | 0       | 14 788        | 25 350      | 52 806|
| Calex vishnui                     | 34        | 1 681       | 0       | 2 241         | 1 519       | 5 475 |
| Lutia fusca                      | 18        | 4           | 0       | 30            | 31          | 83    |
| Mansonia indiana                 | 2         | 3           | 0       | 22            | 24          | 51    |
| Mansonia uniformis               | 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. Bung Chawak 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 tritaeniorynchus followed by Culex vishnui. Culex vishnui is a common and a widespread species frequently found in association with Culex tritaeniorynchus[28]. Culex quisquefasciatus 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 quisquefasciatus 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 quisquefasciatus; 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 quisquefasciatus 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 tritaeniorynchus and Culex bitaeniorynchus 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 Nakon 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.

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