INTRODUCTION

Dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), re-emerging infectious diseases caused by the four serotypes of dengue (DEN) virus, types 1 to 4, belong to the family Flaviviridae, genus *Flavivirus*. These viruses are a major public health concern for many tropical and subtropical regions of the world, causing periodic or annual outbreaks of disease. In Thailand, dengue hemorrhagic fever was first reported in 1958 and in recent years increasingly larger outbreaks have occurred. There were 60,330, 37,929, 99,410, 126,348, 24,826 and 17,582 cases of DF/DHF reported to the Epidemiological Division, Ministry of Public Health during 1995-2000, respectively. In 2000, the number of reported cases from the central region of Thailand was the highest, followed by the northeastern, the northern and the southern regions. Dengue viruses are transmitted to humans through the bite of infective *Aedes* mosquitoes, principally *Aedes aegypti*, which breeds in stagnant water in all forms of receptacles in urban areas, especially

The Geographic Information System as an Epidemiological Tool in the Surveillance of Dengue Virus-Infected *Aedes* Mosquitos

Ratana Sithiprasasna¹, Somboon Patpoparn², Watcharee Attitipphaholkun⁴, Saravudh Suvannadabba⁵ and Mayuna Srisuphanunt³

¹Department of Entomology, US Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok; ²Roi Et Provincial Public Health Office, Roi Et; ³Faculty of Public Health, Mahidol University, Bangkok; ⁴Department of Clinical Chemistry, Faculty of Medical Technology, Mahidol University, Bangkok; ⁵Department of Diseases Control, Ministry of Public Health, Nonthaburi, Thailand

Abstract. A Geographic Information System (GIS) was used as analysis tool to study the spatial distribution of dengue virus-infected *Aedes* mosquitos in Thailand. Global Positioning System (GPS) instruments were used to map villages involved in dengue epidemiological studies in Ratchaburi Province, Thailand. Differentially processed GPS data, with a spatial resolution of approximately 1 meter, were incorporated into a GIS for analysis and mapping. Databases associated with a village GIS included village number, *Aedes aegypti* populations, and test results. Epidemiological surveillance for dengue infection through the detection of the dengue virus type(s) infecting *Aedes* mosquitos during epidemic periods constitutes a reliable sentinel system for dengue outbreaks. Various techniques were applied including: enzyme linked immunosorbent assay (ELISA), indirect immunofluorescent assay (IFA), and reverse transcriptase - polymerase chain reaction (RT-PCR) assay for the virologic surveillance of the type-specific detection of dengue viruses in artificially infected and in field-caught adult *Aedes* mosquitos. In laboratory experiments, all assays showed sufficient sensitivity to detect one virus infected mosquito and the rapid RT-PCR clearly showed serotype-specificity with very high detection sensitivity. In the field study conducted from April to September 2000, female adult *Aedes* mosquitos were collected from selected dengue-sensitive areas in Chom Bung district, Ratchaburi Province and assayed by ELISA, IFA and RT-PCR with 18.3% (44/240), 28.98% (20/69) and 15% (3/20) positive for dengue virus, respectively. Geographic distribution of the virus-infected *Aedes* mosquitos and household locations were demonstrated by the GPS and the GIS. The development of disease mapping data coupled with RT-PCR laboratory-based surveillance of dengue virus infection can successfully serve as epidemiologic tools in an early warning system for dengue hemorrhagic fever (DHF) epidemics.
following intermittent rainfall in these tropical regions (Gubler and Hayes, 1992). In Thailand, *Aedes aegypti* is the primary vector involved in dengue virus outbreaks while *Aedes albopictus*, which is also widespread and abundant in rural areas, plays an important secondary role (Ministry of Public Health, 2000). In the absence of a safe and effective mass immunization, the prevention and control of dengue outbreaks depends on the surveillance of cases and mosquito vectors (WHO, 1986; Khundsen and Sloff, 1992; Gubler and Clarke, 1994). Vector surveillance allows timely implementation of emergency mosquito control measures, such as insecticidal fogging of adult mosquitoes and destruction of breeding places, to limit an impending outbreak. In Thailand, a comprehensive mosquito control program against mosquito breeding incorporates source reduction, public health education, and community participation. The *Aedes* mosquito control strategy has focused mainly on surveillance, for the elimination of *Aedes* larval breeding habitats and emergency control of adult mosquitoes during outbreaks (Eamchan et al, 1989; Gratz, 1993; Kittayapong and Strickman, 1993). In 1999, the Ministry of Public Health initiated a dengue control program to commemorate the Sixth Cycle (72nd) Birthday of His Majesty the King by reducing the Breteau Index (BI) to less than 50% and the Container Index (CI) to less than 10% in order to prevent the emergence of progressively larger outbreaks (Kantachuvessiri, 2000).

Virologic surveillance, which involves monitoring of dengue virus infection in humans, has been used as an early warning system to predict outbreaks. Such surveillance based on the isolation and identification of dengue viruses infecting the human population provides an important means of early detection of any changes in the prevalence of dengue virus serotype(s) (Gubler, 1989; Lam, 1993). The monitoring of the dengue virus type(s) infecting *Aedes* mosquitoes during epidemic periods will complement the current virologic surveillance of dengue outbreaks (Rosen and Gubler, 1974; Lifson, 1996). Therefore, the identification and typing of dengue virus isolated from field-caught mosquitoes and clinical specimens is important for epidemiological and clinical investigations. Routine laboratory diagnosis often involves the detection of antibodies against dengue virus by the Haemagglutination Inhibition (HI) test, antibody capture enzyme-linked immunosorbent assay (IgG or IgM) ELISA, the Plaque-Reduction Neutralization Test (PRNT), Complement Fixation Test (Clarke and Casals, 1958; Bancroft et al, 1979; Kuberski and Rosen, 1997). These assays are neither rapid nor easy to manipulate and they do not identify the serotype responsible for the infection owing to the high cross-seroreactivity between dengue viruses. Serotypes of dengue virus can not be identified in individuals experiencing secondary dengue infection. The conventional method to determine the infecting serotype is by virus isolation in cell culture of mosquito, followed by immunofluorescent staining with dengue type-specific monoclonal antibodies. However, virus isolation takes from days to weeks and the success rate is often low because of factors such as inappropriate handling of specimens, formation of virus-antibody complexes and low numbers of viable virus (Gubler et al, 1984; Monath, 1990). The polymerase chain reaction (PCR) technique has been widely applied and has greatly improved the rapidity of the detection of many infectious disease agents, including dengue viruses. Several methods for detection of dengue virus in serum by reverse transcriptase-polymerase chain reaction (RT-PCR) have been recently described (Deubel et al, 1990; Henchal et al, 1991; Morita et al, 1991; Lancioti et al, 1992; Tanaka, 1993; Chunge et al, 1993; Chow et al, 1993; Seah et al, 1995), but the techniques used therein for RNA preparation are still laborious, time-consuming, and difficult when handling many samples.

Geographic Information System (GIS) databases have been recently used to monitor factors affecting disease transmission (Sithiprasasna et al, 1997, 2003a,b). Recently studies have demonstrated that satellite images digitized land use maps and global positioning data show promise for predicting changes in the habitats of mosquito vectors, as they affect disease transmission. In this study, epidemiological, digital, and GPS data have been incorporated into GIS databases to better understand the spatial distribution of DHF in Ratchaburi, Thailand. We developed a rapid, simple, and single-step RT-PCR assay for the amplification and typing of dengue virus sero-
types in field-caught Aedes mosquitos collected from April to September 2000.

MATERIALS AND METHODS

Field collection of mosquitos

Adult Aedes mosquitos were collected at weekly intervals from April to September 2000 from both indoors and outdoors in dengue-endemic areas of villages 3 and 6, Chom Bung subdistrict, Chom Bung district, Ratchaburi Province, Thailand. Specific sampling locations were selected on the basis of a history of high disease incidence, Aedes density, and human population density. Additional indoor Aedes mosquitos were caught during outbreaks where cases occurred. At each indoor station, Aedes mosquitos were captured by glass vials between 9.00-12.00 AM by collectors. At each outdoor station, Aedes mosquitos were collected by battery-operated manual aspirators for 10-15 minutes. The Aedes mosquitos were then identified by species on a chilled table. All males and females were stored at -70ºC in pools of 30 mosquitos until testing.

Methods used for dengue virus detection in mosquitos

Indirect Dengue Ag-Capture ELISA followed standard testing described by Sithiprasasna et al (1994).


Geographic information system

Provincial border boundary data for Thailand were digitized using Mapinfo software. DHF case data for 2001 were reported by provincial health offices and published by the Ministry of Public Health, Thailand. The province population data were provided by the Civil Registration Department, Ministry of Interior, Thailand. The DHF incidence was calculated as the number of cases of reported DHF/100,000 population of the province. The position of houses in villages 3 and 6, Chom Bung subdistrict, Chom Bung district, Ratchabutri Province, Thailand were mapped using a Trimble Geoexplorer III GPS instrument. Another Trimble Geoexplorer III GPS instrument was run as a base station at our laboratory in Bangkok (13º 45′ 58.878″ N Latitude, 100º 32′ 08.504″ E longitude). Both rover and base station units were run simultaneously to allow differential correction of rover data using Trimble Pathfinder software.

RESULTS

Female Bangkok strain Ae. aegypti, intrathoracically inoculated in the laboratory with DEN-1, DEN-2, DEN-3, and DEN-4, tested by ELISA, IFA, and RT-PCR methods, were positive by all three methods. This indicated that the specificity and sensitivity of these three different methods were reliable (Table 1). Adult Aedes mosquitos from the field study were divided into species, stored at -70ºC, dissected individually into head, thorax, abdomen, and tested with IFA, ELISA and RT-PCR assays. A total of 240 mosquito thorax specimens, 120 mosquitos from village 3, and 120 mosquitos from village 6, were tested by ELISA. An average of 18.3% (44 of 240) of Ae. aegypti tested were positive by ELISA, 14.16% (17 of 120) from village 3, and 22.5% (27 of 120) from village 6 (Tables 2, 3). A total of 69 mosquitos were tested by IFA. An average of 28.98% (20 of

<table>
<thead>
<tr>
<th>Methods</th>
<th>No. of mosquitos</th>
<th>No. of positive (%) of DEN virus serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DEN-1</td>
</tr>
<tr>
<td>ELISA</td>
<td>16</td>
<td>16 (100)</td>
</tr>
<tr>
<td>IFA</td>
<td>5</td>
<td>5 (100)</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>5</td>
<td>5 (100)</td>
</tr>
</tbody>
</table>
69) of *Ae. aegypti* tested were positive by IFA, 28.57% (10 of 35) from village 3 and 29.4% (10 of 34) from village 6 (Tables 2, 3). An average of 18.3% (44 of 240) of *Ae. aegypti* tested were positive for dengue virus infection by ELISA, 14.16% (17 of 120) from village 3, and 22.5% (27 of 120) from village 6 (Table 4). A total of 20 specimens, positive with both ELISA and IFA, were further tested by RT-PCR. An average of 15% (3 of 20) of *Ae. aegypti* were positive by RT-PCR, 10% (1 of 20) from village 3 and 20% (2 of 20) from village 6. The results showed that the predominant type was DEN-2 (Table 5). Pankhong et al (2002) discussed in detail the validation of specificity and sensitivity by RT-PCR.

The distribution of DHF incidence in 2001 in the provinces of Thailand is shown in Fig 1. The relative size of the yellow circle represents the incidence of DHF as indicated in the legend. Fig 2 shows a small portion of the GIS map created for the two villages that was mapped with a

Table 2

<table>
<thead>
<tr>
<th>Methods</th>
<th>No. of mosquitos</th>
<th>No. of positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>240</td>
<td>44 (18.3)</td>
</tr>
<tr>
<td>IFA</td>
<td>69</td>
<td>20 (28.98)</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>20</td>
<td>3 (15)</td>
</tr>
</tbody>
</table>

Fig 1–Distribution of dengue hemorrhagic fever/100,000 population for the year 2001 in different provinces of Thailand plotted on provincial boundary layer in a GIS.

Table 3

<table>
<thead>
<tr>
<th>Methods</th>
<th>Village 3</th>
<th>Village 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of mosquitos</td>
<td>No. of positive (%)</td>
</tr>
<tr>
<td>ELISA</td>
<td>120</td>
<td>17 (14.16)</td>
</tr>
<tr>
<td>IFA</td>
<td>35</td>
<td>10 (28.57)</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>10</td>
<td>1 (10)</td>
</tr>
</tbody>
</table>

Table 4

<table>
<thead>
<tr>
<th>Methods</th>
<th>Village 3</th>
<th>Village 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of mosquitos</td>
<td>No. of positive (%)</td>
</tr>
<tr>
<td>ELISA</td>
<td>120</td>
<td>17 (14.16)</td>
</tr>
</tbody>
</table>
GPS instrument. Locations of each house in two villages utilizing GPS data overlaid on LANDSAT 5 Thematic Mapper satellite data (spatial resolution 30 x 30 m) displayed in true color is shown in Fig 3. The GIS data were subjected to differential processing to create a map with a spatial resolution of one meter. These data were then imported into a GIS and a new database created that include village number, address, adult *Ae aegypti* population and results of ELISA, IFA, and RT PCR tests (Figs 4-6).

**DISCUSSION**

The prevention and control of DF/DHF outbreaks have been dependent upon the control of the vector mosquitoes *Ae. aegypti* and *Ae. albopictus*. In Thailand, the *Aedes* control strategy has focused mainly on surveillance for and elimination of *Aedes* larval breeding habitats and emergency control of adult mosquitos during outbreaks. Although this strategy has successfully reduced the *Aedes* mosquito population to a relatively low level, it has not prevented the emergence of progressively larger outbreaks in recent years. Outbreaks occur when a new dengue virus serotype is introduced into the human population and there are localized increases in the *Aedes* mosquito population. The reappearance of serotype(s) that have not been widely circulating in humans in the preceding years may signal an impending outbreak triggered by newly emergent dengue serotype(s).
The monitoring of the dengue virus type(s) infecting *Aedes* mosquitos during epidemic periods will complement the virologic surveillance for dengue outbreaks (Gubler, 1989; Lifson, 1996). The incidence of DF/DHF in Thailand has increased cyclically since the first recognized outbreak in 1958 (Gratz, 1993) and the most prevalent circulating serotype has been dengue 2 based on the virus serotypes isolated from patients (Ministry of Public Health, Thailand). In the 1999, dengue 2 was identified as the predominant serotype, followed by cocirculating dengue 3, which continued until 2000, when it was replaced by dengue 1 during the dengue outbreak in 2001 (Ministry of Public Health, Thailand). These findings show that the predominant virus serotype usually persists for at least two years before it is replaced by another serotype as has occurred in Malaysia (Lam, 1993) and Singapore (Goh, 1995; Chow *et al.*, 1997; 1998). Thus, a rapid, sensitive and specific tool for the diagnosis and identification of the serotypes of the virus for epidemiological purposes is needed. We tested various techniques, including ELISA, IFA and RT-PCR to determine whether

### Table 5
Identification of dengue virus serotypes using RT-PCR.

<table>
<thead>
<tr>
<th>Methods</th>
<th>No. of mosquitos positive</th>
<th>Dengue virus serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DEN-1</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig 4–Thematic map depicting numbers of female *Aedes aegypti* collected by house from April to September 2000 in village 3 (left) and village 6 (right) of Chom Bung subdistrict, Chom Bung district, Ratchaburi Province, Thailand.

Fig 5–Thematic map showing locations and number of specimens positive from 3 different laboratory methods for testing field-collected dengue virus-infected *Aedes* mosquitos from village 3.
they could be used as epidemiological tools for the virologic surveillance of the type-specific detection of dengue viruses in artificially infected and field-caught adult *Aedes* mosquitoes. In artificially infected mosquitoes, all the assays showed sufficient sensitivity to detect one virus infected mosquito. Among the three assays, RT-PCR proved to be the most rapid, simple, serotype-specific and sensitive test. ELISA is recommended for screening large numbers of mosquitos for all 4 serotypes since the use of the monoclonal antibody produced a consistent response that was optimal with broad specificity and good sensitivity. This monoclonal is known to capture all flaviviruses on immunofluorescent assay (Henchal *et al*, 1982). In this study, there was a marked difference in sensitivity for each dengue type, similar to that described by Henchal *et al* (1982) and Sithiprasansa *et al* (1994). IFA monoclonal antibodies and conjugates were used to experimentally identify dengue viruses of all four serotypes in mosquito brain tissue. The infected mosquito squashes could be examined after 14 days of inoculation, consistent with reports by Timo-

Fig 6–Thematic map showing locations and number of specimens positive from 3 different laboratory methods for testing field-collected dengue virus–infected *Aedes* mosquitoes from village 6.

The results of field caught *Aedes* mosquitos showed approximately 18.3% (44/240) positive by ELISA, 28.98% (20/69) by IFA, and 15% (3/20) RT-PCR for dengue virus. The predominant virus type was DEN 2. The infection rate of *Aedes* mosquitos in village 3 was 14.16%, and village 6 was 22.5% by ELISA. Comparative detection of dengue virus by ELISA and IFA were done blindly on 69 mosquito samples. Even though ELISA is not as specific as RT-PCR and has a higher rate of false positives, it can detect DEN antigen in infected individual mosquito vectors, it is more cost-effective and less labor-intensive than IFA and RT-PCR. ELISA can be an effective method for screening a large number of mosquitos followed by confirmation using RT-PCR.

The GIS databases have been used to study the epidemiology of dengue hemorrhagic fever in Thailand (Sithiprasansa *et al*, 1997). Recent studies have demonstrated that satellite imagery, digitized land use maps and global positioning data have shown promise for predicting changes in the habitats of mosquito vectors as they affect disease transmission (Sithiprasansa *et al*, 2003a,b). GIS allows analysis of data generated by GPS. Combined with data from surveillance and management activities, GIS and GPS provide a powerful tool for the analysis and display of areas of high disease prevalence and the monitoring of ongoing control efforts. The marrying of GIS and GPS enhances the quality of spatial and nonspatial data for analysis and decision making by providing an integrated approach to disease control and surveillance at the local, regional and/or national level. Above all, GIS should be seen as improving the set of tools to promote public health. Good epidemiologic science and good geographic information science go hand-in-hand. GIS has already played a role for the geographically literate public health expert. Epidemiologists should seize the opportunity to set their own agenda and influence technology and science toward reach-

924
GIS FOR THE SURVEILLANCE OF DENGUE INFECTED Aedes Mosquitos

In conclusion, the study findings indicated that in the development of laboratory based surveillance of dengue virus infection, ELISA should be useful as an effective method for screening large numbers of potentially infected vectors, and RT-PCR can be used for confirmation and type-specific identification, in addition to GPS/GIS. Such information can provide the epidemiological tools for an early warning system for dengue outbreaks and enable preventive mosquito control and enhance preparedness on the part of physicians, hospitals and the public.

ACKNOWLEDGEMENTS

The authors would like to thank Mr Sarawut Ninsawat of the Space Technology Applications and Research Program, Asian Center for Research on Remote Sensing, Asian Institute of Technology, Pathum Thani, Thailand for providing the LANDSAT 5 Thematic Mapper satellite data for the study area as the base map.

REFERENCES


Chow VTK, Seah CLK, Chan YC. Use of NS3 consensus primers for the polymerase chain reaction amplification and sequencing of dengue viruses and other flaviviruses. Arch Virol 1993; 133: 157-70.


Henchal EA, Polo SL, Vorndam V, Yaemsiri C, Innis BL, Hoke CH. Sensitivity and specificity of a universal primer set for the rapid diagnosis of


