Am. J. Trop. Med. Hyg., 77(5), 2007, pp. 893–896

Autochthonous Hepatitis E Infection in a Slaughterhouse Worker

Maria Teresa Pérez-Gracia,* Maria Luisa Mateos, Carolina Galiana, Salceda Fernández-Barredo, Angel García, Maria Teresa Gómez, and Victor Moreira

Departamento de Atención Sanitaria, Salud Pública y Sanidad Animal, Facultad de Ciencias Experimentales y de la Salud, Universidad CEU Cardenal Herrera, Moncada, Valencia, Spain; Servicio Microbiología, Hospital Ramón y Cajal, Madrid, Spain; Servicio de Gastroenterología, Hospital Ramón y Cajal, Madrid, Spain

Abstract. We report the first hepatitis E infection case detected in a slaughterhouse worker. The identified strain belonged to genotype 3, subtype 3f. Partial sequence analysis of the strain isolated from his serum showed a percentage of nucleotide homology ranging from 83.4% up to 97.3% compared with European human and swine strains, respectively. These findings point strongly to hepatitis E virus as a vocationally acquired illness by means of the manipulation of infected organs from pigs.

INTRODUCTION

Hepatitis E virus (HEV) is a positive-sense, single-stranded RNA virus without an envelope. This virus is classified in the family *Hepeviridae,* genus *Hepevirus,* as the sole member.[1] Based on the extensive genomic variability among HEV isolates, HEV sequences have been classified into four genotypes: genotype 1 is comprised of epidemic strains found in Asian and African countries; genotype 2 has been described in Mexico and several African countries; genotype 3 is widely distributed and has been isolated from sporadic cases of acute hepatitis E (HE) and in domestic pigs throughout the world, except in Africa. Genotype 4 is made up of human and swine strains found in Asia.[2]

HE has been traditionally thought to be enterically transmitted. This epidemiologic pattern has been recorded in developing countries where epidemic outbreaks linked to contaminated drinking water have been reported. However, the epidemiology of HE in industrialized countries may be changing. When it was first reported in developed countries, it was related to travel to endemic areas.[3] The rise in acute autochthonous cases in developed regions with no history of traveling to endemic regions[4,5] has led HE to be thought as an infection linked to an animal reservoir.[6]

The origin of sporadic forms in industrialized countries might be caused mainly by the infection by swine HEV.[7] The high percentage of nucleotide homology between human and swine strains from the same area supports this assumption. In the same way, the low number of HE cases reported in industrialized countries might be because of the fact that swine HEV strains would be less efficient for infecting humans because they are not as well adapted to humans as the epidemic strains.

This paper describes the infection by HEV of a slaughterhouse worker with a previous history of chronic hepatopathy and the genetic characterization of the strain isolated.

MATERIALS AND METHODS

A 62-year-old male, type 2 diabetic, slaughterhouse worker was admitted to Ramon y Cajal Hospital (Madrid, Spain) with severe jaundice and dark urine of 3-day evolution. In the anamnesis, he reported asthenia, hypopexy, and general weakness during the days previous to the onset of the symptoms. Alcohol consumption was 7 L of beer and 1.5 L of cognac weekly during the last years. On physical examination, the patient was deeply jaundiced and showed a marked right upper and lower back quadrant tenderness. Abdominal computed tomography (CT) scanning was indicative of chronic hepatopathy. Biochemical parameters on admission were as follows: aspartate aminotransferase, 4,320 U/L (reference value < 19 U/L); alanine aminotransferase, 5,727 U/L (reference value < 23 U/L); total bilirubin, 10.4 mg/dL (reference value < 1.3 mg/dL). Serologic markers for infection by hepatitis A (HVA; IgM anti-HAV), B (HBV; HBsAg and anti-HBc), and C (HCV; anti-HCV) viruses (ASXYM; Abbott Laboratories, North Chicago, IL) were negative. Polymerase chain reaction (PCR) for HCV and HBV (Cobas Amplicor; Roche Laboratories, Branchburg, NJ) was also negative. Moreover, Cytomegalovirus, Epstein-Barr virus (ASXYM; Abbott Laboratories and IFA; Oxoid, Hampshire, UK, respectively) and Q fever infections (IFA; Biomerieux, Lyon, France) tested negative.

Finally, immunoglobulin levels of anti-HEV IgM and IgG were detected by a commercial ELISA (Bioelisa HEV IgG and Bioelisa IgM; Biokit, Barcelona, Spain) and were confirmed by Western blot analysis (RecomBlot HEV IgG/IgM; Mikrogen, Martinsried, Germany). In addition, HEV RNA was amplified by reverse transcriptase (RT)-nested PCR[8] in two serum samples taken at the moment of admission to the hospital and 3 days later, respectively. Stool samples were not taken.

The patient recovered normal liver function uneventfully within 45 days after his admission to the hospital. No further serum samples were available for the tracking of HEV markers because he was sent to his general practitioner.

RESULTS

The partial sequence of the HEV strain isolated from this patient was obtained and compared with other known human and swine strains of HEV. Phylogenetic analysis of a 260-bp fragment belonging to the ORF2 revealed that this HEV isolate showed a high percentage of homology (87.3–97.3%) with some Spanish swine strains, followed by other Spanish human HEV strains (91.5–96.9%). Compared with other human European strains, the closest homology of this strain was observed with some British HEV strains (83.4–91.5% nucle-

* Address correspondence to Maria Teresa Pérez-Gracia, Departamento de Atención Sanitaria, Salud Pública y Sanidad Animal, Facultad de Ciencias Experimentales y de la Salud, Universidad CEU Cardenal Herrera, Avenida Seminario s/n 46113, Moncada, Valencia, Spain. E-mail: teresa@uch.ceu.es

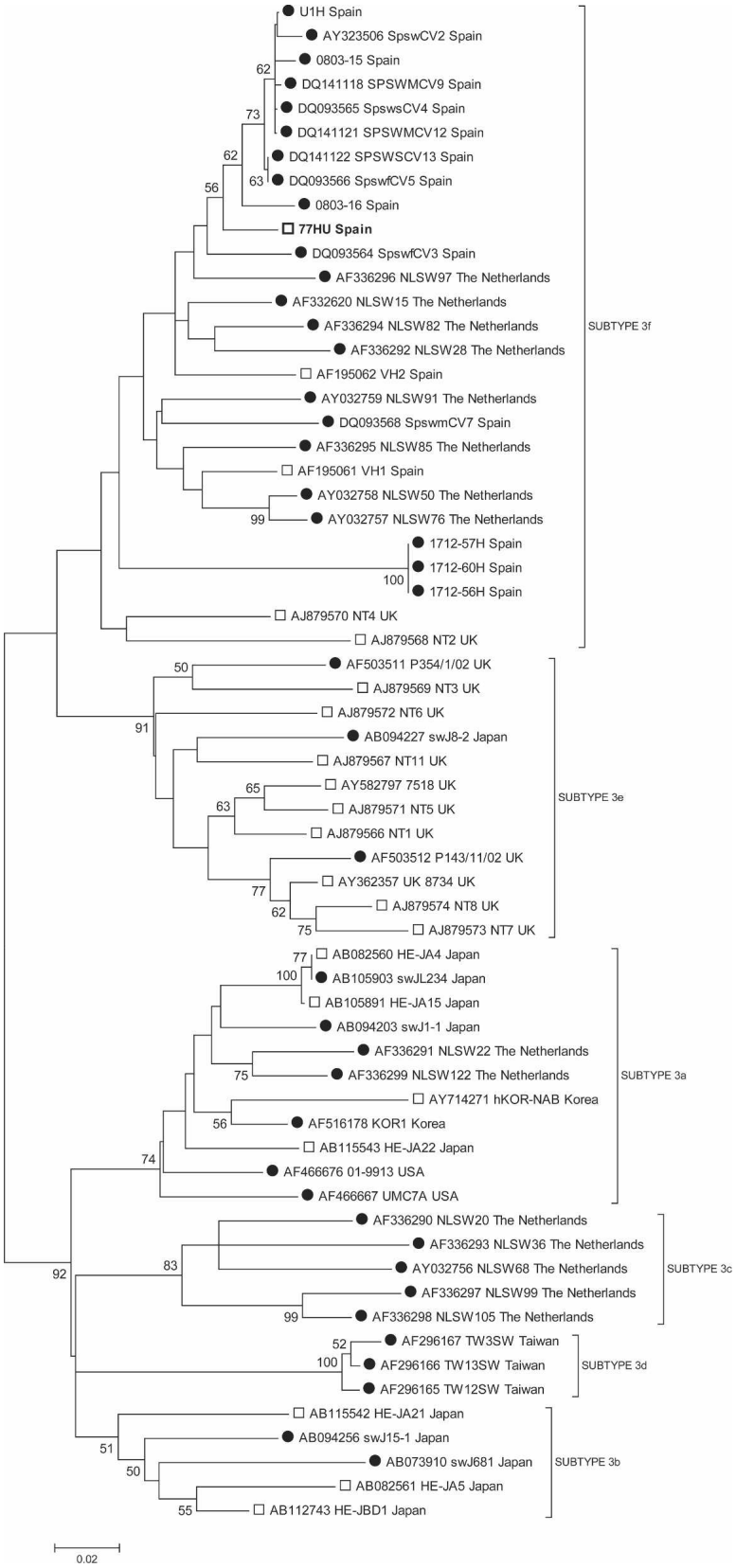

FIGURE 1. Phylogenetic tree based on nucleotide sequences of a 260-bp region within the *ORF2* gene of HEV. 77HU is the case described in this paper, and it is shown in bold. Bootstrap values were determined on 1,000 resamplings of the data sets. The sequence reported in this work was compared with 63 selected sequences from the genotype 3 available in the GenBank database, including strains from human (□) and swine (●) origin.

otide identity). Regarding the comparison to other European swine strains, the highest homology was recorded for Dutch strains (87.3–97.3%), followed by British strains (84.2–86.5%). Most of the nucleotide mutations were found to be silent and did not result in significant differences at the amino acid level. The HEV strain identified in this work showed a 100% amino acid sequence homology compared with other swine and human HEV strains from genotype 3, subtype 3f. A phylogenetic analysis of these sequences is shown in Figure 1.

The HEV sequence identified in this work was deposited in the GenBank database with accession number EF523421.

DISCUSSION

This is the first report of HE infection in a slaughterhouse worker. This patient had no history of traveling to endemic areas, and he had not consumed raw meat or seafood. Moreover, he had not received any blood transfusion or had history of intravenous drug use. The only risk factor he showed seemed to be his occupation. In light of these epidemiologic data, it is reasonable to think that the most probable route of transmission could have been the fecal–oral route after manipulating HEV-infected organs from pigs in the slaughterhouse where he works.

In the literature, there is a case reported in United Kingdom about a butcher suffering from acute HE. This patient spent much of his time butchering pork carcasses imported from the European Community and the Far East. Moreover, another worker from the same butchery was found to have hepatitis antibodies.[9]

HE infection after consumption of poorly cooked or raw swine liver has been reported.[10] Additionally, cases of HE infection by deer and wild boar meat ingestion have been reported.[11,12]

Recent studies have reported that HE can be a zoonotic illness,[13] and the pigs represent the most probable reservoir for human population. In Spain, 25.65% of HEV RNA-positive pigs and 66.66% of HEV RNA-positive pig farms[8,14] were found. The high HEV presence detected in this study indicates that this virus is widespread among the swine population in Spain. In this way, it has been observed that anti-HEV IgG seroprevalence in pig handlers and in veterinarians in contact with pigs is elevated in Spain (Galiana and others, unpublished data). All these data support the potential of HEV for spreading among the human population through contact with contaminated organs, crops, or in personnel that handle swine manure and spread this waste on agricultural fields.

The sequence identified in this patient clustered into genotype 3, subtype 3f, following the classification by Lu and others,[15] and it was located close to swine Spanish strains (97.3% homology). These data are similar to the data recorded in other industrialized countries between human and swine strains from the same area.[6,16–18]

Phylogenetic analysis showed that the HEV isolate identified in this study clustered in the same genotype with Spanish swine and human HEV and two Dutch strains of swine HEV. The importation of piglets from The Netherlands to Spain seems to be the most probable cause of the close genetic relationship observed between this strain and our patient's sequence. In Spain, the importation of live piglets is usual, because there is not enough production of them to cover the free vacancies in grower-to-finisher farms. Thus, Spain is the third importer from Europe, and piglets are mainly bought from The Netherlands.

A high seroprevalence in a healthy population without previous history of hepatitis in industrialized countries has been reported.[19] These data suggest that HEV infection is subclinical in an undetermined proportion of the population. This case agreed with the observations reported by other authors[20,21] who suggest that age and underlying disease may make symptomatic presentation more likely. In the same way, it has been reported that patients who suffer HEV superinfection of chronic hepatopathy normally show a poor prognosis,[22] although in this case, the patient recovered successfully.

In conclusion, HEV could constitute an important public health problem, especially for swine workers (veterinarians, butchers, slaughterhouse workers, pig handlers) who are at a higher risk to be infected; therefore, HE should be considered an occupational disease. Our findings suggest that HEV infection in industrialized countries, like Spain, is an emerging disease. HE diagnosis should be considered in any patient who displays clinical symptoms of acute hepatitis and has been in contact with pigs or its organs.

Received May 11, 2007. Accepted for publication July 14, 2007.

Financial support: This work was supported by a grant from CEU Cardenal Herrera University (PRUCH 06/21).

Authors' addresses: Maria Teresa Pérez Gracia, M.T. Pérez-Gracia, C. Galiana, S. Fernández-Barredo, and A. García, M.T. Gómez, Departamento de Atención Sanitaria, Salud Pública y Sanidad Animal, Facultad de Ciencias Experimentales y de la Salud, Universidad CEU Cardenal Herrera, Avenida Seminario s/n 46113, Moncada, Valencia, Spain. M.L. Mateos, Servicio de Microbiología, Hospital Ramón y Cajal, Ctra de Colmenar Km 9.1, Madrid 28034, Spain. V. Moreira, Servicio de Gastroenterología, Hospital Ramón y Cajal, Ctra de Colmenar Km 9.1, Madrid 28034, Spain.

REFERENCES

1. Mayo MA, 2005. Changes to virus taxonomy. *Arch Virol 150:* 189–198.
2. Okamoto H, 2007. Genetic variability and evolution of hepatitis E virus. *Virus Res 127:* 216–228.
3. Piper-Jenks N, Horowitz HW, Schwartz E, 2000. Risk of hepatitis E infection to travelers. *J Travel Med 4:* 194–199.
4. Buti M, Clemente-Casares P, Jardi R, Formiga-Cruz M, Schaper M, Valdes A, Rodriguez-Frias F, Esteban R, Girones R, 2004. Sporadic cases of acute autochthonous hepatitis E in Spain. *J Hepatol 41:* 126–131.
5. Mateos ML, Molina A, Ta TH, Moreira V, Milicua JM, Bárcena R, 2006. Acute hepatitis E in Madrid: description of 18 cases. *Gastroenterol Hepatol 29:* 397–400.
6. Ijaz S, Arnold E, Banks M, Bendall RP, Cramp ME, Cunningham R, Dalton HR, Harrison TJ, Hill SF, Macfarlane L, Meigh RE, Shafi S, Sheppard MJ, Smithson J, Wilson MP, Teo CG, 2005. Non-travel-associated hepatitis E in England and Wales: demographic, clinical, and molecular epidemiological characteristics. *J Infect Dis 7:* 1166–1172.
7. Perez-Gracia MT, Garcia-Valdivia MS, Galan F, Rodriguez-Iglesias MA, 2004. Detection of hepatitis E virus in patients sera in southern Spain. *Acta Virol 48:* 197–200.
8. Fernández-Barredo S, Galiana C, Garcia A, Vega S, Gómez MT, Perez-Gracia MT, 2006. Detection of hepatitis E virus shedding in feces of pigs at different stages of production using reverse transcription-polymerase chain reaction. *J Vet Diag Invest 18:* 462–465.
9. Jary C, 2005. Hepatitis E and meat carcasses. *Br J Gen Pract 55:* 557–558.

10. Yazaki Y, Mizuo H, Takahashi M, Nishizawa T, Sasaki N, Gotanda Y, Okamoto H, 2003. Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. *J Gen Virol 84:* 2351–2357.
11. Takahashi K, Kitajima N, Abe N, Mishiro S, 2004. Complete or near complete nucleotide sequences of hepatitis E virus genome recovered from a wild boar, a deer, and four patients who ate the deer. *Virology 330:* 501–505.
12. Tei S, Kitajima N, Takahashi K, Mishiro S, 2003. Zoonotic transmission of hepatitis E virus from deer to human beings. *Lancet 362:* 371–373.
13. Meng XJ, Halbur PG, Shapiro MS, Govindarajan S, Bruna JD, Mushahwar IK, Purcell RH, Emerson SU, 1998. Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. *J Virol 72:* 9714–9721.
14. Fernández-Barredo S, Galiana C, García A, Gómez-Muñoz MT, Vega S, Rodríguez-Iglesias MA, Pérez-Gracia MT, 2007. Prevalence and genetic characterization of Hepatitis E virus in paired samples of feces and serum from naturally infected pigs. *Can J Vet Res 71:* 236–240.
15. Lu L, Li C, Hagedorn CH, 2006. Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. *Rev Med Virol 16:* 5–36.
16. Meng XJ, Purcell RH, Halbur PG, Lehman JR, Webb DM, Tsareva TS, Haynes JS, Thacker BJ, Emerson SU, 1997. A novel virus in swine is closely related to the human hepatitis E virus. *Proc Natl Acad Sci USA 18:* 9860–9865.
17. Herremans M, Vennema H, Bakker J, van der Veer B, Duizer E, Benne CA, Waar K, Hendrixks B, Schneeberger P, Blaauw G, Kooiman M, Koopmans MP, 2007. Swine-like hepatitis E viruses are a cause of unexplained hepatitis in the Netherlands. *J Virol Hepatol 14:* 140–146.
18. Ahn JM, Kang SG, Lee DY, Shin SJ, Yoo HS, 2005. Identification of novel human hepatitis E virus (HEV) isolates and determination of the seroprevalence of HEV in Korea. *J Clin Microbiol 7:* 3042–3048.
19. Meng XJ, Wiseman B, Elvinger F, Guenette DK, Toth TE, Engle RE, Emerson SU, Purcell RH, 2002. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. *J Clin Microbiol 1:* 117–122.
20. Ramachandran J, Eapen CE, Kang G, Abraham P, Hubert DD, Kurian G, Hephzibah J, Mukhopadhya A, Chandy GM, 2004. Hepatitis E superinfection produces severe decompensation in patients with chronic liver disease. *J Gastroenterol Hepatol 2:* 134–138.
21. Amon JJ, Drobeniuc J, Bower WA, Magana JC, Escobedo MA, Williams IT, Bell BP, Armstrong GL, 2006. Locally acquired hepatitis E virus infection, El Paso, Texas. *J Med Virol 78:* 741–746.
22. Dalton HR, Hazeldine S, Banks M, Ijaz S, Bendall R, 2007. Locally acquired hepatitis E in chronic liver disease. *Lancet 369:* 1260.

Am. J. Trop. Med. Hyg., 77(5), 2007, pp. 897–902

Application of Mosquito Sampling Count and Geospatial Methods to Improve Dengue Vector Surveillance

Chitti Chansang and Pattamaporn Kittayapong*
Center for Vectors and Vector-Borne Diseases and Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand

Abstract. Dengue hemorrhagic fever is a major public health problem in several countries around the world. Dengue vector surveillance is an important methodology to determine when and where to take the control action. We used a combination of the Global Positioning System (GPS)/Geographic Information System (GIS) technology and the immature sampling count method to improve dengue vector surveillance. Both complete count and sampling count methods were used simultaneously to collect immature dengue vectors in all houses and all containers in one village in eastern Thailand to determine the efficiency of the sampling count technique. A hand-held GPS unit was used to record the location of surveyed houses. Linear regression indicated a high correlation between total immature populations resulting from the complete count and estimates from sampling count of immature stages. The immature survey data and the GPS coordinates of house location were combined into GIS maps showing distribution of immature density and clustering of immature stages and positive containers in the study area. This approach could be used to improve the efficiency and accuracy of dengue vector surveillance for targeting vector control.

Dengue hemorrhagic fever (DHF) is a major public health problem in Thailand[1] and many tropical regions of the world.[2] Controlling the principal vector, *Aedes aegypti* (L.), is the only known method to reduce disease incidence. In Asia and the Americas, primary breeding sites of dengue vectors are man-made containers in and around houses. In Thailand, many types of containers such as water jars, ant traps, tires, bath basins, and metal drums serve as *Ae. aegypti* breeding sites.[3] Surveying immature stages of *Ae. aegypti* is necessary for control plans. Visual larval survey[4] is a standard method promoted for *Ae. aegypti* larval surveillance, but this approach does not provide an accurate estimate of the relationship between *Ae. aegypti* larval and adult densities,[5] and indices from this survey and epidemic risk are hard to define.[6] Other sampling approaches involve sampling immature *Ae. aegypti* using a net in metal drums[7] and other containers such as tires.[8] The premise condition index[9] and pupae index obtained from *Ae. aegypti* surveys have been found to be the most accurate method for estimating dengue risk.[10] The survey of *Ae. aegypti* immature stages from houses in villages has been reported.[11] The complete count method (total number of larvae and pupae in a container) for estimating *Ae. aegypti* immature stages by sampling 10 houses in each month in 1 year has been studied.[12] However, thus far, there has been no study to find the relationship between the complete count and sampling count to evaluate the accuracy of immature sampling.

Geographic Information Systems (GISs) were used for studying *Ae. aegypti* vectors by creating a map of the study areas[13–15] and also for evaluation of a sampling count methodology for rapid assessment of *Ae. aegypti* infestation levels.[16] These methods are likely to be used more and more in *Ae. aegypti* control programs in the future.[17] The main objective of our study was to improve the surveillance and control of *Ae. aegypti* by combining the GIS technology and the immature sampling methodology to create a spatial density distribution maps and to identify the clusters of immature stages and the main breeding sources that should be the targets for vector control. Therefore, in this study, we surveyed immature stages of *Ae. aegypti* in all houses and all containers in a Thai village, and Global Positioning System (GPS) coordinates of houses were recorded at the same time. GIS was used to estimate the spatial distribution of *Ae. aegypti* immature stages and evaluate the relationship between the complete count and the sampling count methods.

MATERIALS AND METHODS

Study areas and data collection. Village 10 (13°38′18″ N, 101°17′32″ E) in Hua Samrong Subdistrict, Plaeng Yao District, Chachoengsao Province, Thailand, was selected as the study area because there were high dengue infections from a serologic survey of anti-dengue immunoglobulin IgM and IgG in primary school children conducted by Kittayapong and others (unpublished data) in September 2001. Village 10 is located in a low-lying (~10 m above the sea level) flat area surrounded by rice fields. Housing in the village was not crowded and was interspersed with small orchards and rice fields. There were two basic types of dwellings made from local materials: wood and a combination of wood and cement. The dwellings were divided into four groups separated by rice fields, irrigation canals, and small roads. Water for household use came from subsurface wells, tap water, and rain water. In addition, residents stored water in various types of containers during the year. These containers included ones for human and livestock drinking water.

A survey of *Ae. aegypti* immature stages was conducted in Village 10. All containers in 151 houses were examined at the beginning of the hot and dry season in March 2002. The number of containers with or without *Aedes* immature stages was surveyed and recorded following standard WHO procedures.[4] The complete count and the sampling count methods of *Ae. aegypti* immature stages were conducted following the methods of Strickman and Kittayapong.[11,12] For the sampling count method, containers were sampled with a very fine round mosquito dip net with the diameter of 24 cm by dipping the net in the water, starting at the top of the container, and continuing to the bottom in a swirling motion. For the com-

* Address correspondence to Pattamaporn Kittayapong, Center for Vectors and Vector-Borne Diseases, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok 10400, Thailand. E-mail: grpkt@mahidol.ac.th

plete count method, the containers with the remaining immature stages from the sampling count method were emptied, and all larvae and pupae were collected with a strainer. The total numbers of larvae and pupae from the complete count method were calculated by adding the number of larvae and pupae from the sampling count method in the containers. For small containers, e.g., ant traps and small plastic containers, an immature survey was conducted by the complete count method. Only drinking water containers were carefully examined with a flashlight to count the number of larvae and pupae. The numbers of containers, the number of positive containers, and the number of larvae and pupae from both methods were recorded. The linear regression was used for finding the relationship between the two methods. The number of immature stages from the complete count method was set as the dependent variable, and the number of the immature stages from the sampling count method was set as the independent variable.

The geographic coordinates of each survey house were determined by GPS observations made with a Leica GS5+ (Leica Geosystems, Torrance, CA), which had an accuracy of ±3 m. The GPS unit linked to the pocket PC iPAQH 3850 (Compaq Information Technologies Group, Palo Alto, CA); ArcPad (ESRI, Redlands, CA) software was used to manipulate field data with maximum setting of the position dilution of precision (PDOP) = 2, and the field data were recorded in shape files that were transferred to a personal computer. ArcView software (ESRI) was used to create a GIS map and to analyze the data obtained.

The density of immature stages in each house from both methods was mapped using GIS. The degree of association between the two methods was analyzed using Pearson correlation.[18] Getis and others[15] reported an application of the local spatial statistic $Gi^*(d)$ to find clustering of *Ae. aegypti* in Peru. This statistic was used to test whether a particular location i and its surrounding regions constitute a cluster of higher values than average values of a variable (x) of interest[18] and is written as

$$Gi^*(d) = \left[\sum_j w_{ij}(d)x_j - W_i^*\overline{X}\right]\{s[(NS_{1i}^* - W_i^{*2})/(N-1)]^{1/2}\}, \text{ all } j$$

where s is the sample SD of the x values (larvae, pupae, and positive containers), and $w_{ij}(d)$ is equal to 1 if region j is within a distance of d from region i and 0 otherwise. The sum is calculated over all regions, including region i. Also, $W_i^* = \sum_j w_{ij}(d)$ and $S_{1i}^* = \sum_j w^2_{ij}$. The terms "location" and "region" refer to the ith house and surrounding areas (at distance < d) of the jth house. In this study, $N = 151$, and the local spatial statistic $Gi^*(d)$ was used under ArcView's Arctoolbox to identify the houses that showed clustering of *Ae. aegypti* immature stages and positive containers. The local spatial statistic $Gi^*(d)$ was performed by setting conceptualization of spatial relationships = fixed distance band, distance method = Euclidean distance, and distance band or threshold distance = 0, 10, 20, and 30 m. The coordinate system and datum for GPS link with ArcPad, ArcView and the local spatial statistic $Gi^*(d)$ were Universal Transverse Mercator and Indian 1975, respectively. The units of measure were in meters. SPSS version 9.0 software package (SPSS, Chicago, IL) was used for statistical analysis.

RESULTS

Surveys of *Ae. aegypti* immatures were conducted in all houses in Village 10 (Figure 1). *Ae. aegypti* was the dominant species (99.88%), and *Ae. albopictus* represented 0.12% of the 2,374 pupae identified. The House Index (HI), Container Index (CI), and Breteau Index (BI) in Village 10 were 84.77, 31.21, and 327.15, respectively. The containers for the survey were classified into 12 types. For drinking water containers, mainly standard water jars, there were only 1,692 larvae and 172 pupae in 361 containers that were directly counted by flashlight. The prevalence of *Aedes* immature stages in each container type resulting from the complete count method is shown in Table 1. The main breeding sources were water jars of various types, especially standard water jars, and cement bath basins.

Linear regression was used to find the relationship between the complete count and the sampling count methods. The total numbers of 494 positive containers were surveyed by the complete count, from which 269 containers were surveyed by the sampling count method. The results are shown in Table 2. There is a high correlation between these two methods. Linear regression is also used to find the relationship between the complete count and the sampling count methods in each important container type for larvae and pupae. The results are shown in Table 3.

The results from the complete count and the sampling count method including the locations of surveyed houses by GPS were entered into the GIS program to create a map to study the density distribution of *Ae. aegypti* immature stages between these two methods. The maps showing the number of larvae and pupae in each house are shown in Figures 2 and 3, respectively. These maps show similar results between the complete count and sampling count methods. Pearson correlations between the two methods for larvae and pupae survey were 0.95 ($P < 0.001$) and 0.94 ($P < 0.001$), respectively. The map of the number of positive containers in each house is shown in Figure 4. The houses that were members of significant clustering of larvae are shown in Figure 2. From the complete count method, three houses were members of significant clustering of larvae within 10 m. From the sampling

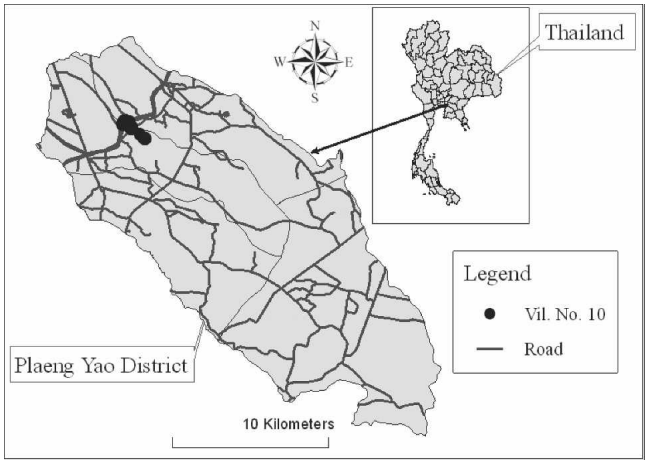

FIGURE 1. Location of the study area in Village 10, Plaeng Yao District, Chachoengsao Province, Thailand.

TABLE 1
Prevalence of *Aedes* immature stages in each container types from the complete count method

Container type	Total	Positive container		Immature stage		Total
		With larvae	With pupae	Number of larvae	Number of pupae	
Standard water jar (~200 L)	760	251	134	12,556	1,069	13,625
Small water jar (~100 L)	136	62	38	2,797	225	3,022
Large water jar < 1.5 m (~400 L)	146	23	10	1,036	20	1,056
Large water jar > 1.5 m (~1000 L)	190	0	0	0	0	0
Tire	18	5	2	291	46	337
Metal drum (~200 L)	2	1	0	37	0	37
Ant trap (~0.5 L)	20	11	9	390	38	428
Large cement bathroom basin (1,000–2,000 L)	107	54	24	4,078	307	4,385
Small cement bathroom basin (50–100 L)	79	44	30	2,913	298	3,211
Misc. cement	36	7	4	834	169	1,003
Small plastic container	17	8	6	384	38	422
Misc. not cement	72	28	18	1,149	164	1,313
Total	1,583	494	275	26,465	2,374	28,839

count method, five houses were members of significant clustering of larvae within 10 m and one within 20 m. The houses that were members of significant clustering of pupae are shown in Figure 3. From both methods, six houses are members of significant clustering of pupae, three houses within 10 m, and three houses within 20 m. The houses that were members of significant clustering of positive containers are shown in Figure 4. Three houses showed significant clustering of positive containers within 10 m.

DISCUSSION

From the complete count methods, the immature number of *Ae. aegypti*, especially pupae, in each container type indicated that the main breeding sources were standard water jars and cement bath basins, which accounted for 70.51% of total pupae in the study sites. Nearly the same results were obtained from the study of Strickman and Kittayapong.[12] From this study, 72% of the breeding sources were standard and small water jars. Control of immature mosquitoes in these containers should have an impact on > 70% of pupae of *Ae. aegypti* in the village.

In Trinidad, Focks and Chadee[10] showed that adult density could be estimated from pupae that were able to be counted in the containers. In Thailand, it was quite difficult to count all the number of pupae in all containers because there were many types of containers and a high number of immature stages. Kittayapong and Strickman[3] reported that there were 10 types of breeding sources, whereas this study had 12 types of breeding sources. In a more practical approach, the sampling count method should be used. The surveyed data were analyzed using linear regression to find the relationship between the two methods. Some positive containers that were surveyed by the complete count method had low water levels. These containers could not be surveyed by the sampling count method using the mosquito dip net. Therefore, the positive container numbers for the sampling count method in Table 3 were less than those for the complete count in Table 1. Results showed that the sampling count method was highly correlated with the complete count method in both the numbers of larvae and pupae. However, it is proposed to take into account the small container that can be more important in other areas and for which only the complete count method can be applied. Our study supported the idea that the sampling count method could be used to accurately estimate the density of immature stages of *Ae. aegypti*.

The GIS/GPS technology together with *Ae. aegypti* immature surveys at a village scale were used to create the GIS map and clustering of immatures that could be used for targeting control efforts to be more efficient and more cost effective. GIS and the local spatial statistic $Gi^*(d)$ were applied to study the density distribution of *Ae. aegypti*. Our result showed that this approach had a good potential for future use in the surveillance and control of *Ae. aegypti*. Maps of the density distribution of immature stages from the two survey methods were created for visual comparison. Results from both methods showed significant Pearson correlation, and the GIS maps created were quite similar. Both methods could characterize the densities distribution of *Ae. aegypti* mosquitoes in the village. Clustering of *Ae. aegypti* immature stages and positive containers in our study indicated that both surveillance methods yielded similar results, except that the sampling count method showed higher degree of clustering of larvae than the complete count method. Clustering of larvae, pupae, and positive containers was detected within the same house up to 20 m. One house showed clustering of larvae and pupae and one house showed clustering of larvae and positive containers. The groups of houses that had high *Aedes* densities could be characterized and be the target for prioritizing activities of the vector control plan. The $Gi^*(d)$ statistic is in part biased

TABLE 2
Regression models for estimating total larvae and pupae from larvae sampling and pupae sampling

Type	Range of x	Equation	R^2	F	P
Larvae sampling (x)	1–60	Total larvae (y) = –0.73 + 1.83x	0.63	126.73	< 0.001
	62–111	Total larvae (y) = 48.1 + 1.29x	0.81	315.18	< 0.001
Pupae sampling (x)	1–4	Total pupae (y) = 0.54 + 1.71x	0.33	39.38	< 0.001
	5–92	Total pupae (y) = 1.82 + 1.44x	0.83	351.54	< 0.001

TABLE 3

Regression models for estimating total larvae and pupae from larvae sampling and pupae sampling for various container types

Container type	Stage	Equation	r^2	F	P
Standard water jar (n = 165)	Larvae	$y = 13.5 + 1.65x$	0.85	964.89	< 0.001
	Pupae	$y = 0.60 + 1.97x$	0.85	950.69	< 0.001
Small water jar (n = 33)	Larvae	$y = 4.85 + 1.76x$	0.89	267.60	< 0.001
	Pupae	$y = 2.05 + 1.74x$	0.56	42.05	< 0.001
Large water jar < 1.5 m (n = 14)	Larvae	$y = 9.44 + 1.50x$	0.88	95.79	< 0.001
	Pupae	$y = 0.41 + 0.91x$	0.69	30.41	< 0.001
Large cement bath basin (n = 24)	Larvae	$y = 19.3 + 1.46x$	0.94	374.05	< 0.001
	Pupae	$y = 0.10 + 1.46x$	0.96	480.01	< 0.001
Small cement bath basin (n = 33)	Larvae	$y = 32.3 + 1.27x$	0.53	36.83	< 0.001
	Pupae	$y = 4.88 + 1.24x$	0.25	11.83	< 0.001

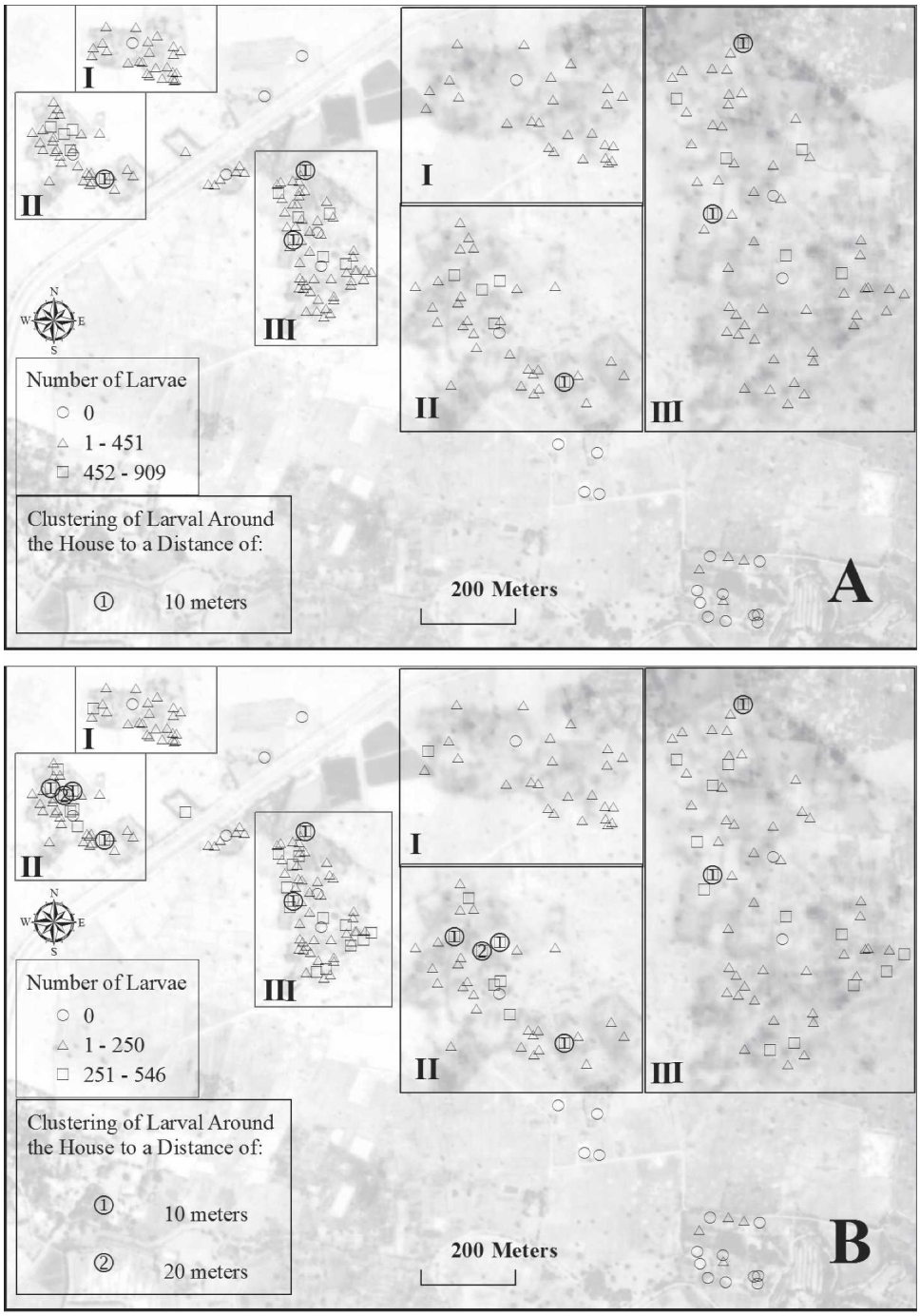

FIGURE 2. Maps of the number of larvae and the clustering of larvae based on the number of larvae in each house. **A**, From the complete count. **B**, From the sampling count method.

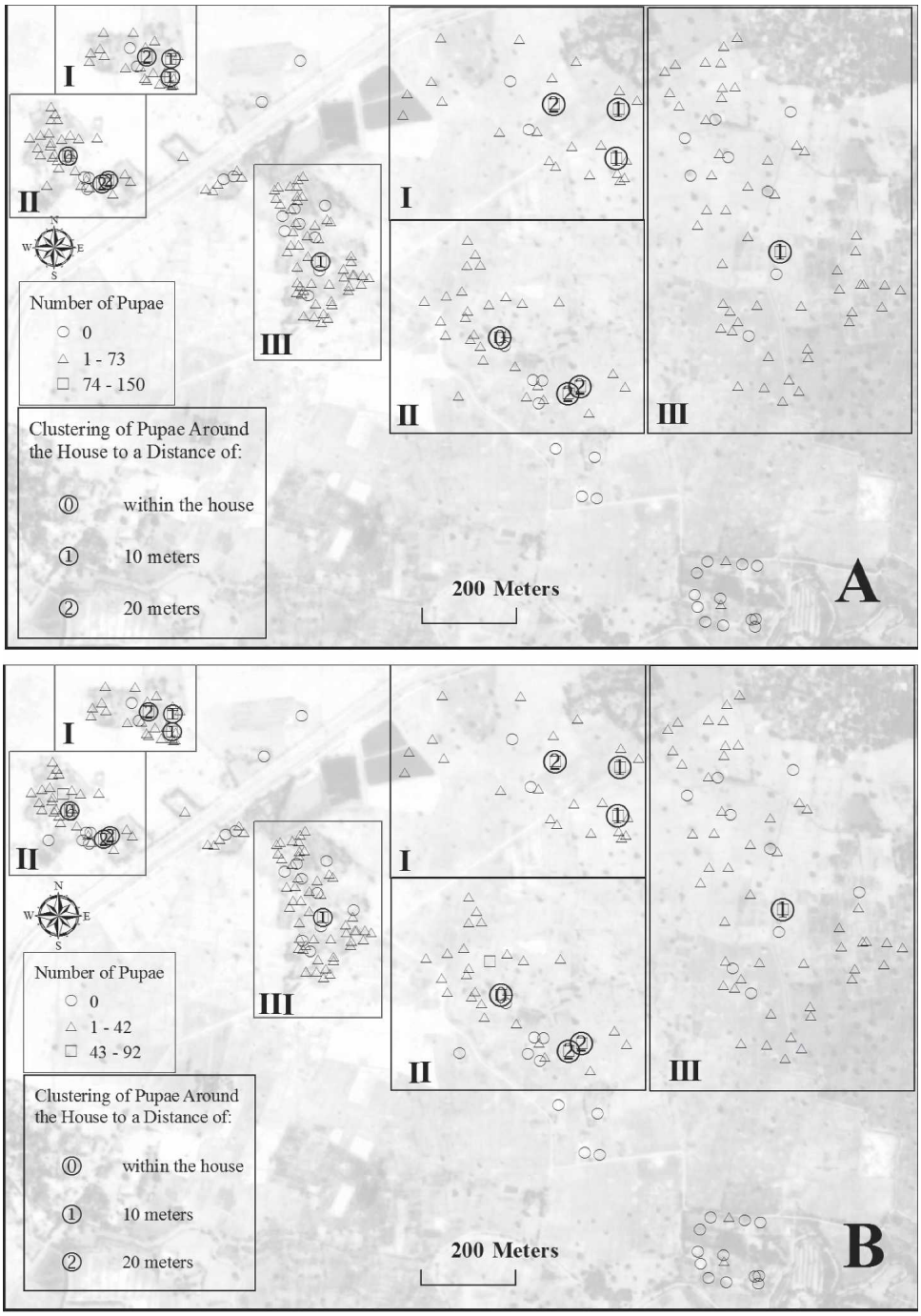

FIGURE 3. Maps of the number of pupae and the clustering of pupae based on the number of pupae in each house. **A**, From the complete count. **B**, From the sampling count method.

by the heterogeneous density of houses in the four areas. Our results indicate spatial clustering of *Ae. aegypti* immature stages and positive containers, which were different from the study in Peru where no clustering of larvae or pupae was found beyond individual households and only limited clustering of adult *Ae. aegypti* occurred.[15] The GIS maps of the density distribution and Pearson correlation support the use of sampling count method for *Ae. aegypti* survey, which could give nearly the same information as that from the complete count.

The visual larval survey method was commonly used for *Ae. aegypti* survey. The number of containers with or without *Ae. aegypti* immature stages in houses was recorded, and the indices such as HI, CI, and BI were calculated to show *Aedes* indices in the study area. From this study, GIS/GPS could be used to analyze and show *Ae. aegypti* immature stages and positive containers in the houses. Our result showed that there were variations in *Ae. aegypti* density from house to house. Individual houses and a group of houses that had high *Ae. aegypti* density could be characterized. Therefore, individual households were an appropriate spatial unit for entomologic surveys.[15] In addition, maps resulting from GIS and

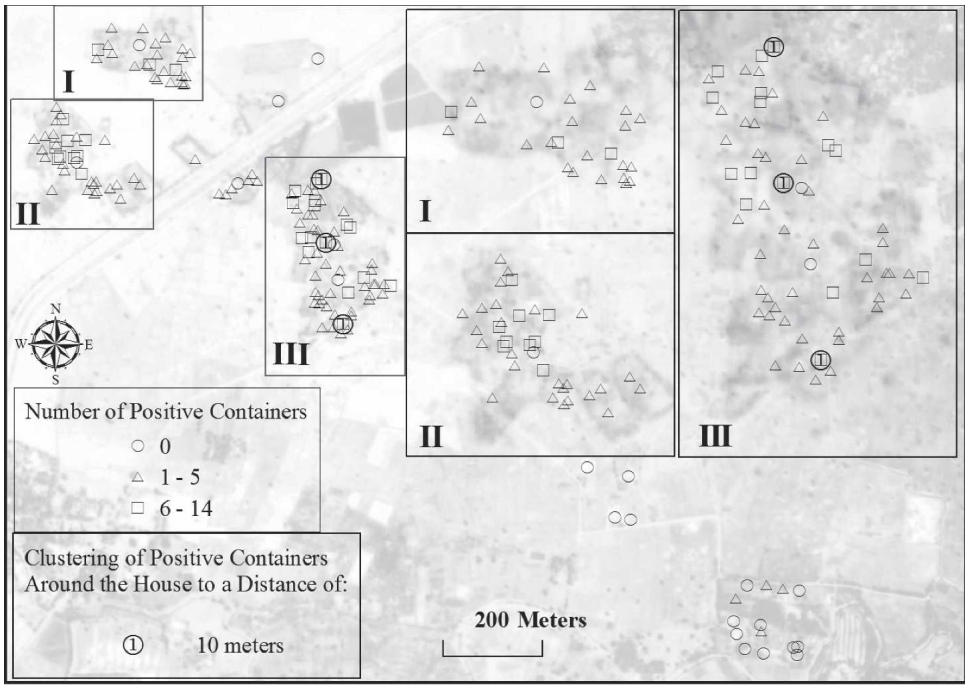

FIGURE 4. Map of number of positive containers and the clustering of positive containers based on the number of positive container in each house.

the local spatial statistic $Gi^*(d)$ could be used for developing an effective *Aedes* control plan by monitoring and controlling *Ae. aegypti* in the targeted groups of houses with clustering of immature stages and positive containers.

Received October 26, 2005. Accepted for publication April 7, 2007.

Acknowledgments: The authors thank Dr. John D. Edman for help in reviewing and editing the final manuscript; Dr. Laura C. Harrington for critical review and helpful comments; Somboon Srimarat, Tanong Aimmak, Sumas Jantamas, Suwanna Austthapornrungroj, Damrongrith Vinij, and Uruyakorn Chansang for field assistance; and residents of surveyed houses, local public health authorities, and local administrative authorities in Hua Sam Rong Subdistrict, Plaeng Yao District for cooperation.

Financial Support: This study was supported by the Thailand Research Fund (RGJ/PHD/0051/2544), the Mahidol University Research Grant (SCBI-47-T-217), and the UNICEF/UNDP/World Bank/WHO Special Programme for Tropical Diseases Research and Training (TDR/RCS/A00786).

Authors' addresses: Pattamaporn Kittayapong and Chitti Chansang, Center for Vectors and Vector-Borne Diseases, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok 10400, Thailand, Telephone: 662-201-5935, Fax: 662-201-5923, E-mail: grpkt@mahidol.ac.th.

Reprint requests: Pattamaporn Kittayapong, Center for Vectors and Vector-Borne Diseases, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok 10400, Thailand. E-mail: grpkt@mahidol.ac.th.

REFERENCES

1. Kantachuvessiri A, 2002. Dengue hemorrhagic fever in Thai society. *Southeast Asian J Trop Med Pub Hlth 33:* 56–62.
2. World Health Organization, 1995. *Vector Control for Malaria and Other Mosquito-Borne Diseases*. Geneva: World Health Organization.
3. Kittayapong P, Strickman D, 1993. Distribution of container-inhabiting *Aedes* larvae (Diptera: Culicidae) at a dengue focus in Thailand. *J Med Entomol 30:* 601–606.
4. World Health Organization, 1972. An international system for the surveillance of vectors. *Wkly Epidemiol Rec 47:* 73–80.
5. Tun-Lin W, Kay BH, Barnes A, Forsyth S, 1996. Critical examination of *Aedes aegypti* indices: correlations with abundance. *Am J Trop Med Hyg 54:* 543–547.
6. Reiter P, Gubler DJ, 1997. Surveillance and control of urban dengue vectors. Gubler DJ, Kuno G eds. *Dengue and Dengue Hemorrhagic Fever,* New York: CAB, 425–462.
7. Tun-Lin W, Kay BH, Burkot TR, 1994. Quantitative sampling of immature *Aedes aegypti* in metal drums using sweep net and dipping methods. *J Am Mosq Cont Assoc 10:* 390–396.
8. Zhen TM, Kay BH, 1993. Comparison of sampling efficacy of sweeping and dipping for *Aedes aegypti* larvae in tires. *J Am Mosq Cont Assoc 9:* 316–320.
9. Tun-Lin W, Kay BH, Barnes A, 1995. The premise condition index, a tool for streamlining surveys of *Aedes aegypti. Am J Trop Med Hyg 53:* 591–594.
10. Focks DA, Chadee DD, 1997. Pupal survey: An epidemiologically significant surveillance method for *Aedes aegypti*, an example using data from Trinidad. *Am J Trop Med Hyg 56:* 159–167.
11. Strickman D, Kittayapong P, 2002. Dengue and its vectors in Thailand: Introduction to the study and seasonal distribution of *Aedes* larvae. *Am J Trop Med Hyg 67:* 247–259.
12. Strickman D, Kittayapong P, 2003. Dengue and its vectors in Thailand: calculated transmission risk from total pupal counts of *Aedes aegypti* and association of wing-length measurements with aspects of the larval habitat. *Am J Trop Med Hyg 68:* 209–217.
13. Sithiprasasna R, Linthicum KJ, Lerdthusnee K, Brewer TG, 1997. Use of Geographic Information System to study the epidemiology of dengue haemorrhagic fever in Thailand. *Dengue Bull 21:* 68–72.
14. Ali M, Wagatsuma Y, Emch M, Breiman RF, 2003. Use of a Geographic Information System for defining spatial risk for dengue transmission in Bangladesh: role for *Aedes albopictus* in an urban outbreak. *Am J Trop Med Hyg 69:* 634–640.
15. Getis A, Morrison AC, Grey K, Scott TW, 2003. Characteristics of the spatial pattern of the dengue vector, *Aedes aegypti*, in Iquitos, Peru. *Am J Trop Med Hyg 69:* 494–505.
16. Morrison AC, Astete H, Chapilliquen F, Ramirez-Prada G, Gloria D, Getis A, Gray K, Scott TW, 2004. Evaluation of sampling methodology for rapid assessment of *Aedes aegypti* infestation levels in Iquitos, Peru. *J Med Entomol 41:* 502–510.
17. Nelson MJ, 1994. The role of sampling in vector control. *Am J Trop Med Hyg 50:* 145–150.
18. Rogerson PA, 2001. *Statistical Methods for Geography*. London: SAGE Publications.