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journal homepage: <http://ees.elsevier.com/apjtm>Original research <http://dx.doi.org/10.1016/j.apjtm.2015.06.009>Role of *Aedes aegypti* and *Aedes albopictus* during the 2011 dengue fever epidemics in Hanoi, VietnamPham Thi Kim Lien<sup>1,2</sup>, Vu Trong Duoc<sup>1\*</sup>, Laurent Gavotte<sup>3</sup>, Emmanuel Cornillot<sup>2</sup>, Phan Thi Nga<sup>1</sup>, Laurence Briant<sup>2</sup>, Roger Frutos<sup>2,4</sup>, Tran Nhu Duong<sup>1\*</sup><sup>1</sup>National Institute of Hygiene and Epidemiology, 1 Yersin Street, 10000 Hanoi, Viet Nam<sup>2</sup>CPBS, UMR 5236, CNRS-UM1-UM2, 1919, route de Mende 34293 Montpellier Cedex 5, France<sup>3</sup>ISEM, UMR 5554, CNRS-UM2-IRD, Université Montpellier 2 CC065, Place E. Bataillon, 34095 Montpellier Cedex 5, France<sup>4</sup>Intertryp, UMR 17, Cirad-IRD, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France

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## ABSTRACT

**Objective:** To record the human cases of dengue fever (DF) and investigate the *Aedes* mosquito species circulating during the Hanoi 2011 DF epidemics.**Methods:** 24 different outbreak points were recorded in 8 districts between August and December 2011.**Results:** 140 patients were hospitalized following dengue diagnostic with a predominance of males (59.3%) and the 15–34 age class. Only DENV-1 (11.27%) and DENV-2 (88.73%) serotypes were detected in human samples. Mosquito sampling performed in and around patients households revealed the predominance of *Aedes aegypti* (*A. aegypti*) (95.15%) versus *Aedes albopictus* (4.85%).**Conclusions:** There is a positive correlation between the population density of *A. aegypti* and the number of human cases and duration of outbreaks. This was not observed for *Aedes albopictus*. Three pools of *A. aegypti* were positive with dengue virus, two with DENV-1 and one with DENV-2.

## 1. Introduction

Four distinct DENV serotypes are currently described which cause dengue fever in humans resulting in a range of clinical symptoms including fever, headache, muscle, joint pains, and a characteristic skin rash similar to measles [1–4]. Dengue fever (DF) can also evolve into severe forms such as dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), which could result in death [5]. An estimated 390 million dengue infections occur every year, of which 96 million are asymptomatic [6], which could make the burden of dengue three times higher than considered [7,8]. More than 100 countries are affected by outbreaks of dengue and more than 60 have reported the occurrence of DHF [9]. Southeast Asia is among the regions most affected by dengue and Vietnam is

one of the five countries in this region with the highest burden [10]. DF was first described in northern Vietnam in 1958 and expanded to the southern area in the 1960s [11–13]. DHF was first described in Hanoi during the rainy season of 1958 [14,11] with a mortality rate of 7% [15]. During the 1998–2009 decade, two large outbreaks occurred in the central urban area of Hanoi, which resulted in a total of 25983 cases mostly among young adults [16,17].

Dengue virus (DENV) is transmitted to humans by two mosquito species, *Aedes aegypti* (*A. aegypti*) and *Aedes albopictus* (*A. albopictus*). *A. aegypti* is considered the most important vector of DENV while *A. albopictus* is generally believed to be a less competent vector resulting in milder epidemics [18]. However, dengue outbreaks have been attributed to both *A. aegypti* and *A. albopictus* in different regions of the world including Asia [1,11,18,19]. Each species displays a specific ecology, behaviour and geographical distribution. *A. aegypti* prefers urban habitats, whereas *A. albopictus* is primarily a forest species that has become adapted to rural, suburban and urban human environments [18,20,21]. Owing to the increasing presence of *A. albopictus* and the co-circulation of both mosquito species in

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Vietnam [22,23], this study was undertaken to investigate their respective potential role in DENV transmission in the recent epidemics and correlation between mosquito abundance and size and duration of outbreaks.

## 2. Materials and methods

### 2.1. Ethics and enrolment of patients

The study protocol was cleared and approved by the Scientific and Ethical Committee of the National Institute of Hygiene and Epidemiology, Vietnam. The study was conducted as part of the Vietnamese National Dengue Prevention and Control Program. All patients considered in the analysis gave a written informed consent of participation to the study.

### 2.2. Location of sampling

Mosquitoes and blood samples from hospitalized patients were collected during outbreaks from August 2011 to December 2011 in eight districts of Hanoi: Ba Dinh, Hai Ba Trung, Dong Da, Ha Dong, Thanh Xuan, Thanh Oai, Thanh Tri, and Tu Liem (Figure 1). The population of the Northern city of Hanoi was estimated in 2009 to be 2.6 million for urban districts, and 6.5 million for the metropolitan jurisdiction [24]. Climate is contrasted with hot and humid summers, a rainy season from May to September with temperatures from 38 °C to 40 °C. Winters are relatively dry and cool from November to March with temperatures as low as 6 °C. Spring is marked by light rain. The period from June to September is suitable for the development of the mosquitoes [25]. The DF outbreak areas were defined according to Ministry of Health guidelines as geographic areas (town/village/hamlet, population groups or equivalent) where patients were tested positive for DENV and simultaneous detection of mosquitoes was confirmed [26]. Small outbreaks were defined as occurrences with less than 20 positive patients, medium outbreaks comprised 21 to 100 positive patients and large outbreaks were considered as involving more than 100 patients. Outbreaks were considered terminated when no case was reported for at least 14 days.

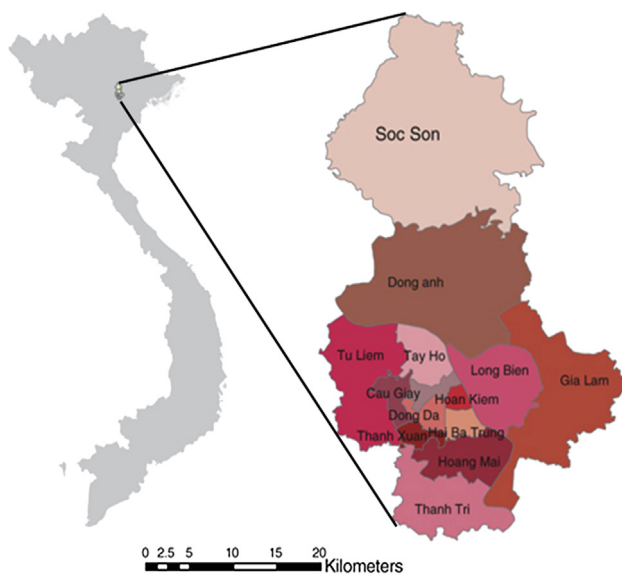


Figure 1. Map of sampling locations.

### 2.3. Case definition and sampling

Patients admitted to the National Hospital for Tropical Diseases in Hanoi between August 1, 2011 and December 21, 2011 were considered in the study when presenting dengue symptoms as defined both by WHO and Vietnamese Ministry of Health guidelines on surveillance, diagnosis, treatment of dengue. These symptoms were a continuous fever for 2–7 days in an individual from an endemic area and displaying two or more of the following clinical manifestations of DF: nausea, vomiting, rash, aches and pains; positive tourniquet test, leukopenia and any warning sign [7,27]. Blood samples were systematically collected from patients corresponding to the above-mentioned criteria. Acute phase serum sample was collected after fever onset from day 1 to 7 and a follow-up 3–5 ml serum sample was taken, stored at 4 °C until being sent each day to arbovirus laboratory, NIHE for RNA extraction then stored at –80 °C. Description of cases included the onset date and place, age and gender of patients with notified cases of DF infection. The serum obtained was subjected to serological and molecular testing to determine the presence of dengue virus and identify the serotype. With the patient's consent, the following data were collected: full name, residence address, gender, time of onset and intensity, and location of symptoms. After data collection, contacts were taken with Preventive Medicine Centres in each district to implement captures of mosquitoes in patient's house and households.

### 2.4. Mosquito collection and identification

Adult mosquitoes were collected from patient's house, and 15 households around the patient's household located within a radius of approximately 20–50 m using a backpack aspirator. For each outbreak area, 50–100 households were randomly selected for daily collection. For each household, sampling was performed indoor and outdoor for approximately 15 min during the day. Mosquito collection were carried out by 4 groups volunteer with 2 sessions per day between 5–10 AM and 4–8 PM. Collected mosquitoes were stored in RNA later solution (Qiagen) and kept refrigerated at 2 °C–8 °C. Identification of *A. aegypti* and *A. albopictus* was performed according to morphological criteria following binocular examination. Mosquito samples were sorted according to species, gender, date of collection, geographical coordinates and number of mosquitoes for each location, and then stored at –80 °C in RNA later solution until further use.

### 2.5. RNA extraction and RT-PCR amplification

Viral RNA was extracted from 140 µL patient blood serum and from 970 mosquitoes (923 *A. aegypti* and 47 *A. albopictus* individuals) by pools of up to 10 mosquitoes depending upon sample size. Males and females were pooled separately. Viral RNA was extracted using QIAamp viral RNA Mini kit (Qiagen) according to the supplier and stored at –80 °C until further use. DENV RNA was detected and typed using a single tube multiplex RT-PCR according to an experimental protocol adapted from previously published procedures [28,29]. Both reverse transcription and PCR were conducted using the Access Quick RT-PCR kit (Promega). Reverse transcription was conducted at 45 °C for 30 min using random primers

(Invitrogen). PCR was then performed in a 50 µL reaction volume using a set of five primers (25 pmol each) comprising a dengue virus consensus reverse primer and four serotype-specific forward primers (Table 1). PCR was conducted for 35 cycles under the following conditions: denaturation at 94 °C for 2 min, annealing at 55 °C annealing for 45 s and extension at 72 °C for 90 s followed by a final extension for 10 min at 72 °C. PCR products were analysed in a 2% agarose gel electrophoresis using 10% SYBR safe DNA dye (Invitrogen) in 1% TAE buffer. The expected size of the amplicons was 492 bp, 119 bp, 290 bp and 392 bp for DENV-1, DENV-2, DENV-3 and DENV-4, respectively.

## 2.6. Data analysis

Data were analyzed using STATA 10.0. Spearman's Rank correlation coefficient analysis was used to investigate the association between the density of Aedes mosquitoes, number of confirmed dengue cases and duration of outbreaks.

## 3. Results

### 3.1. Outbreaks location, size and duration

During the study period, a total of 24 infectious foci were detected within the eight districts in Hanoi, all of them being small or medium outbreaks (Figure 1). The mean duration of an individual outbreak was 69.3 days, ranging from 17 to 123 days (median duration 76 days). Samples were collected from a total of 140 hospitalized patients confirmed with dengue by serology. The number of confirmed cases in each district varied from 2 to 42 (mean = 16, and median = 23) (Table 2). No shock or haemorrhage characteristic of severe dengue was reported among cases included in the study. Men (59.3%) were more affected than women (40.7%) (Table 3). The youngest patient was 3 years old and the oldest was 88 (mean age = 33 years, median = 29 years) (Table 3).

### 3.2. Collection of mosquitoes in and around patients' households

A total of 1200 households were sampled during the study and 970 mosquitoes were collected (Table 2). All mosquitoes collected belonged to the genus Aedes. 923 (95%) belonged to the *A. Aegypti* species whereas 47 (5%) were *A. albopictus*. For each district, the total number of Aedes collected ranged from 5 to 322. *A. aegypti* largely predominated in each district with the exception of Thanh Oai and Thanh Tri where the number of *A. albopictus* was higher. Hai Ba Trung district recorded the highest number of captured *A. albopictus* (17/47 or 36%). The

**Table 2**

Mosquito and human samples collected from outbreaks in Hanoi, 2011.

District	Case	A. <i>aegypti</i>	A. <i>albopictus</i>	Time outbreaks (day/month/year)	Duration of outbreak in days
Ba Dinh	23	180	0	1/8–13/10/2011	74
Hai Ba Trung	31	204	17	12/8–13/12/2011	123
Dong Da	42	313	9	18/8–30/11/2011	104
Thanh Xuan	6	68	4	11/9–28/11/2011	78
Thanh Oai	6	1	4	1/10–25/10/2011	25
Thanh Tri	2	1	5	12/10–28/10/2011	17
Tu Liem	2	13	0	1/9–30/9/2011	20
Ha Dong	28	143	8	12/8–3/12/2011	113
Total	140	923	47	1/8–13/12/2011	564

observed density of Aedes mosquitoes was higher during outbreaks. However, due to the small size of mosquito samples collected in Thanh Tri, Thanh Oai and Tu Liem districts, we cannot exclude that this result was biased as mosquitoes may have been collected under different meteorological conditions or time periods.

### 3.3. Detection and identification of dengue virus

Out of the 140 dengue serology-positive blood samples, 71 were tested by PCR. Only DENV-1 and DENV-2 serotypes were detected. Only 8 patients (11.27%) distributed over four districts, i.e. Ba Dinh, Hai Ba Trung, Dong Da, and Thanh Tri, tested positive for DENV-1 (Table 3). 63 patients (88.73%) tested positive for DENV-2 which was present in all the districts investigated with the exception of Thanh Tri. In three districts, i.e. Dong Da, Hai Ba Trung and Ba Dinh, the presence of both DENV-1 and DENV-2 was recorded.

### 3.4. Correlation between Aedes population size and outbreak intensity

A positive correlation was observed between the population density of *A. aegypti* and the number of human cases recorded during an outbreak ( $R = 0.57$ ,  $P = 0.003$ ). A similarly positive association was observed between the number of *A. aegypti* mosquitoes collected at outbreak sites and the duration of outbreaks. *A. aegypti* population density was found higher when outbreak was longer ( $R = 0.57$ ,  $P = 0.003$ ). The number of *A. aegypti* individuals collected in the early period of outbreaks was higher than the number collected towards the end of the outbreaks. Conversely, no correlation was found between the number *A. albopictus* collected in outbreak areas and the number of confirmed dengue cases in the same areas ( $R = -0.62$ ) or with

**Table 1**

Oligonucleotide primers used to amplify and type dengue viruses.

Primer	Sequence	Genome position	Size, in bp, of amplified DNA product (primers)
D1	5'-TCAATATGCTGAAACGCGAGAA ACCG-3'	616–644	511
TS1	5'-CGTCTCAGTGATCCG GGGG-3'	568–586	492 (D1 and TS 1)
TS2	5'-CGC CAC AAG GGC CAT GAA CAG-3'	232–252	119 (D1 and TS 2)
TS3	5'-TAA CAT CATCAT GAG ACA GAGC-3'	400–421	290 (D1 and TS 3)
TS4	5'-CTCTGTGTCTTAAACAA GAGA-3'	506–527	392 (D1 and TS 4)

Note: D1 is reverse primer, and forward primers are TS1 (DENV1), TS2 (DENV2), TS (DENV3) and TS 4 (DENV4).

**Table 3**

Distribution of gender and age in dengue case.

Gender	Dengue case (%)	Age of dengue case (%)				Rate ratio
		<15	15–34	35–44	>45	
Male	83 (59.3)	9 (10.8)	47 (56.6)	10 (12.0)	16 (19.3)	1.46
Female	57 (40.7)	5 (8.7)	27 (47.4)	10 (17.5)	16 (28.0)	1.00
Total	140 (100%)	14 (10%)	74 (52.9%)	20 (14.3%)	32 (22.9%)	

the duration of outbreaks ( $R = -0.67$ ). All *A. albopictus* samples were negative for dengue virus (Table 4). Only *A. aegypti* female mosquitoes collected in the districts of Ba Dinh, Hai Ba Trung and Dong Da were positive for DENV-1 and DENV-2 (Table 5).

#### 4. Discussion

This study conducted in Hanoi, Vietnam, reported 140 confirmed cases of DF in 24 outbreaks over eight districts. The patients were mainly males over 15 with the highest number of dengue cases found in the 15–34 years age class. Only 10% of patients were under 15. The age distribution reported in this study differs from other works which reported dengue mainly in children [30–37]. However, increasing dengue incidence in older age groups was also reported in Latin America and Southeast Asia [38–41]. During the study period, we also found that more males were affected than females. Others have reported similar findings in Vietnam, China and Nepal [30,42,43]. There is no clear explanation for this bias which might be associated to an occupational factor.

**Table 4**

Distribution of serotypes among confirmed dengue positive patients.

District	Case	DENV serotype in patient			
		DENV-1	DENV-2	DENV-3	DENV-4
Ba Dinh	23	1	11	0	0
Hai Ba Trung	31	5	9	0	0
Dong Da	42	1	24	0	0
Thanh Xuan	6	0	1	0	0
Thanh Oai	6	0	3	0	0
Thanh Tri	2	1	0	0	0
Tu Liem	2	0	1	0	0
Ha Dong	28	0	14	0	0
Total	140	8	63	0	0

**Table 5**

Dengue virus serotypes detected in Aedes mosquitoes collected in outbreaks areas.

District	No. of mosquito pools		No. of positive pools		% Positive pools	
	A.	A.	A.	A.	A.	A.
	<i>aegypti</i>	<i>albopictus</i>	<i>aegypti</i>	<i>albopictus</i>	<i>aegypti</i>	<i>albopictus</i>
Ba Dinh	18	0	1 (D1)	0	5.53%	0.00%
Hai Ba Trung	21	3	1 (D1)	0	5.90%	0.00%
Dong Da	32	4	1 (D2)	0	3.10%	0.00%
Thanh Xuan	7	2	0	0	0.00%	0.00%
Thanh Oai	1	1	0	0	0.00%	0.00%
Thanh Tri	1	1	0	0	0.00%	0.00%

The detection of only DENV-1 and DENV-2 in mosquitoes along with the predominance of DENV-2 reported in this work is in agreement with previous reports on from Hanoi in recent years [16,42]. This suggests that DENV-1 and DENV-2 were already co-circulating during previous outbreaks in the same area and probably maintained over time through vertical transmission in mosquito populations. The overwhelming presence of *A. aegypti* in the captured mosquitoes, its widespread distribution and the positive correlation of higher local density with the number of human cases reported in outbreaks, altogether strongly suggest that *A. aegypti* is involved in dengue virus transmission in Hanoi, with a pivotal role during the 2011 dengue outbreaks and before as a maintenance host.

Despite the fact that it is considered to be a less efficient vector, *A. albopictus* is adapted to urban domestic environments and was described as a vector in DENV outbreaks in different regions including China, Gabon or Madagascar [44–46]. However, in this work, DENV were not detected in *A. albopictus* and no correlation was found between the number of *A. albopictus* captured and the number of human cases. These data corroborate reports suggesting that *A. albopictus* is not an important vector in urban environment when compared to *A. aegypti* [21,47–50]. Despite its growing importance and presence it remains a secondary dengue vector, indicating that control actions must remain directed towards *A. aegypti* habitats.

The importance of mosquito population densities in the dynamic of dengue fever highlighted by this work might, however, be underestimated. Indeed, a limitation to this study is that it only covered the latter part of the rainy season, considered an ideal time for dengue transmission. Additionally, no information was available for artificial containers that might have acted as breeding habitats for Aedes mosquitoes. There may also have been case ascertainment biases as we relied on patients presenting themselves to health services, thus milder and asymptomatic cases may not have been identified. Nevertheless, this work strongly supports the need of further investigation in other geographic areas and environment, i.e. suburban and rural, to determine the distribution and relative role of *A. aegypti* and *A. albopictus* in the transmission of DF and DHF in Vietnam and a potential evolution linked to the spread of *A. albopictus*. This work also underlines the need of a better understanding of the dynamics of mosquito populations to implement efficient and adapted mosquito population monitoring and mosquito control strategies, which is at the moment the best way of controlling dengue and other mosquito-borne diseases.

#### Conflict of interest statement

We declare that we have no conflicts of interest.

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