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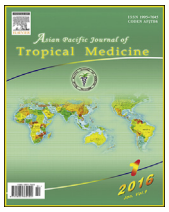

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Surveillance of dengue and chikungunya infection in Dong Thap, Vietnam: A 13-month study

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ABSTRACT

Objective: To establish a surveillance in Dong Thap, at the border with Cambodia by assessing the presence of DENV serotypes and CHIKV among patients hospitalized at Dong Thap general hospital.

Methods: Cross-sectional descriptive analysis was conducted on a cohort of 131 patients hospitalized with acute fever and symptoms compatible with dengue or chikungunya. The study was conducted from January 2012 to February 2013. The full clinical picture was established as well as serological and molecular detection. Serological analysis was sequentially performed on blood samples collected on admission and an average of seven days after admission. The detection of IgM antibody to DENV was performed by IgM capture ELISA and the detection of DENV and CHIKV RNA was done by reverse-transcription multiplex PCR.

Results: 101 patients out of 131 (77%) were confirmed with dengue. All four dengue serotypes were detected with a predominance of DENV2 and DENV4. No chikungunya infection was detected although reported in neighboring Cambodia. A differential efficiency of serological dengue detection was observed. Efficiency was 29% upon admission and 53% after seven days on the same patients. 30 patients out of 131 (23%) were negative with both DENV and CHIKV.

Conclusions: Dengue is at risk of being underestimated and chikungunya is not systematically detected. Changes in detection and surveillance procedures are therefore discussed to increase efficiency of dengue detection and continue the monitoring the emergence of CHIKV in Dong Thap province and in Vietnam.

1. Introduction

Arthropod-borne viral infections (or arboviral infections) are common causes of fever syndromes worldwide and more than 100 kinds of arboviruses are known to cause disease in humans [1–3]. Dengue fever is caused by a flavivirus belonging to the family of Flaviviridae [4] while the chikungunya virus (CHIKV) is an alphavirus from the family *Togaviridae* [5–7]. Both dengue and chikungunya diseases are transmitted by *Aedes aegypti* and *Aedes albopictus* [6,8] and can cause potentially severe and or debilitating chronic disease [9]. While dengue has been recorded as the most rapidly spreading mosquito-borne viral disease in the world [10,11], chikungunya has recently re-emerged after an interval of several decades. It represents a risk for millions of people in the Indian Ocean areas, Africa, Southeast Asia and more recently has spread to the Caribbean, Pacific and Europe [12–14]. Coinfection with dengue virus (DENV) and CHIKV has been reported on patients from Asian, African and Pacific countries [15–19].

Vietnam is a hyperendemicity country with all four serotypes being present all year long throughout the country [20], but affecting mostly the southern part with major seasonal outbreaks

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during the rainy season from June to December [21]. Since 1960, dengue fever epidemics have become more frequent and widespread with an increasing number of cases and deaths over the past 15 years [21,22]. From 1963 to 1995, 1 518 808 dengue hemorrhagic fever (DHF) cases and 14 133 deaths were reported [22,23]. The dengue surveillance program in the Southern Vietnam has demonstrated the occurrence of epidemic peaks of higher magnitude approximately every 5 years from 1975 to 1987 [24]. Following an 11-year gap a major outbreak of 119 429 DHF cases and 342 fatalities occurred in 1998 [24]. 592 938 dengue cases were reported during the 2001–2010 decade in 19 southern Vietnam provinces, which corresponds to a median annual incidence of 232 cases per 100 000 [20].

DENV and CHIKV are both transmitted by the same mosquito species, *A. aegypti* and *A. albopictus*. Although chikungunya was first described in Vietnam in the 1960's [25], serological evidence of its presence remain scarce and is mainly associated to the Vietnam War era. In 1966, ten American soldiers were identified to be infected with CHIKV [26] and serological surveys among children have detected anti-CHIKV antibodies as early as 1967 [27]. Cambodia which has a long and extensive border with Vietnam, is not only endemic for dengue, but also for chikungunya which has developed recently [28]. Both diseases can easily be imported by travelers, spread rapidly through common vectors and result in social, economic and healthcare system impacts.

Vietnam is at risk to be like Cambodia affected both by dengue and chikungunya and be an overlapping area of distribution for both viruses. Furthermore, owing to the similarity in clinical manifestations and differences in clinical management, clinicians should be aware of the need to include CHIKV in the differential diagnosis of dengue fever. The aim of the study was therefore to assess, through a dual screening of clinical samples of acute febrile episode patients in Dong Thap general hospital in Southern Vietnam, the respective prevalence of dengue and chikungunya.

2. Material and methods

2.1. Cohort design and ethical clearance

The study was approved by the Institutional Review Board of National Institute of Hygiene and Epidemiology, Hanoi, Vietnam (No: 14IRB July 23, 2012) in charge of ethical clearance. Patients were eligible for recruitment if they were admitted to the infectious diseases department of Dong Thap general hospital between January 1, 2012 and February 28, 2013. All hospitalized patients with suspected arbovirus infection were eligible for participating in this study provided they displayed acute fever in addition to two of any of the following symptoms: headache, rash, myalgia, joint pain and arthralgia.

2.2. Study setting

Dong Thap general hospital is located in Cao Lanh city, Dong Thap province. The province is located in the Mekong delta region in southern Vietnam, and bordered with Cambodia to the north (Figure 1). Dong Thap is characterized by a typical tropical climate with two distinctive seasons: The rainy season from May to November and the dry season from December to April. The annual average temperature is around 26 °C. Dong Thap is one of the provinces of southern Vietnam with people movement from Cambodia and display a high rate of dengue infection.

2.3. Patient enrollment, clinical sample and data management

After obtaining informed consent from patients, a total of 131 paired blood samples were collected from January 2012 to February 2013 from acute fever cases suspected to be infected by dengue within 1–14 d from the day onset of illness according to WHO guidelines [2,29]. The collection of clinical samples was performed twice and 3 mL or 5 mL of blood were collected each

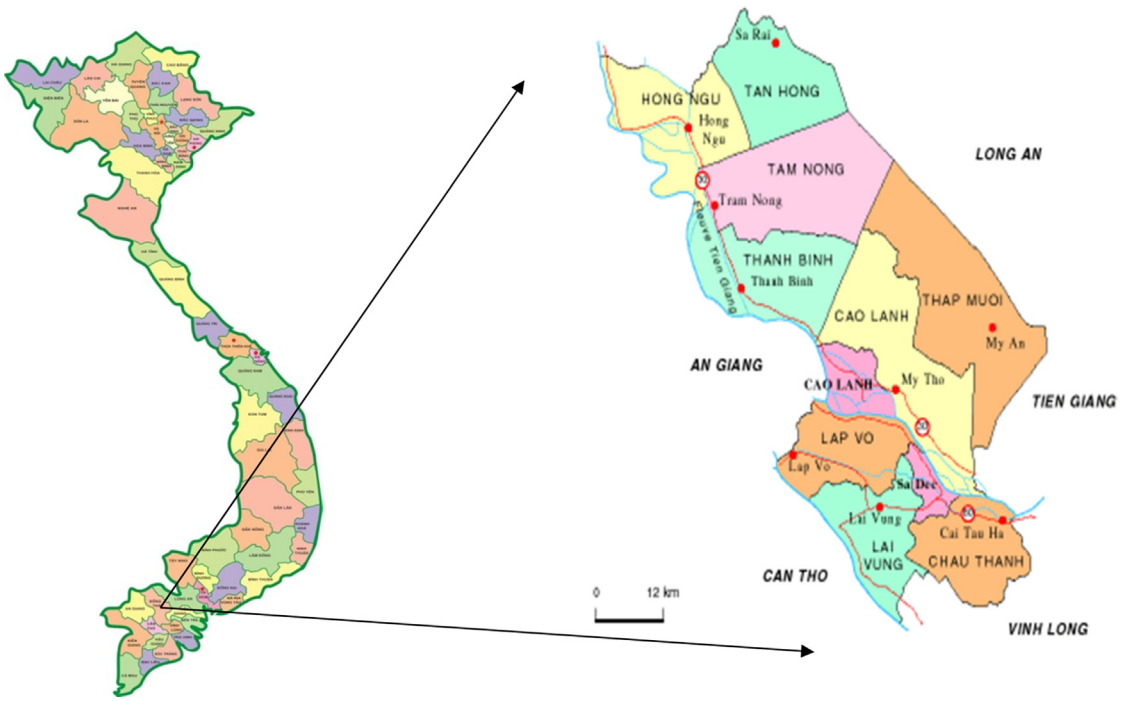

Figure 1. Location and map of the Dong Thap province.

phase: (1) In acute phase, blood samples were collected <7 d from onset of illness and then divided into two tubes: One tube for serum analysis and the other one for plasma analysis; (2) Ten to fifteen days later, in convalescent phase, a second blood sample was collected from the same patients. Samples were kept at −20 °C and kept at −80 °C for virological diagnosis by ELISA and PCR. For each patient, the collected information included a unique identification number and demographic data such as full name, age, gender, residential address, day of onset, date of first and second sample collection. Signs and symptoms were recorded on the day of admission. Samples were coded prior to laboratory analysis.

2.4. RNA extraction and cDNA synthesis

The viral RNA was extracted from 140 μL of sample using the QIAamp viral RNA Mini kit (QIAgen, Hildden, Germany). Elution was performed in 60 μL according to the supplier and RNA stored at −80 °C until further use. RNA was reverse transcribed into cDNA using Super transcript III reverse transcriptase (RT – Invitrogen). RNA template was mixed with DNase, incubated at 37 °C for 30 min and then 75 °C for 15 min, reverse transcription mix was then added and incubated at 65 °C for 5 min and then transferred in ice immediately. To prepare double-stranded cDNA, annealing was performed at 25 °C for 5 min, followed by extension at 42 °C for 60 min, and inactivation by holding the mixture at 75 °C for 15 min.

2.5. Laboratory confirmation of dengue and chikungunya

Detection of DENV and CHIKV was conducted both by a Dengue IgM capture ELISA in first samples (plasma and serum) and second samples (serum) and by a multiplex PCR amplification for both first and second samples. IgM antibody-capture enzyme linked immunosorbent assay (MAC-ELISA) was conducted using the Capture DxSelect™ kit made in CDC Fort Collins, United States according to the supplier. PCR detection of DENV and CHIKV was conducted in a one-step, single tube serotype specific assay using double-stranded cDNA templates as previously described [30,31]. The amplification was carried out in 50 μL reaction volume with DENV a group-specific consensus forward primer and four serotype-specific reverse primers. Nonstructural protein 2 (nsP2) primers were used for the detection of CHIKV. All relevant aspects of the PCR reaction (Master Mix, Primer, Tag polymerase, number of cycles and annealing temperature) were initially optimized using a quantitated purified DENV ds-cDNA to achieve a maximum level of sensitivity. Target RNA was amplified in 50 μL volume containing 5 μL of DNA was combined with 10 pmol of each specific primer DENV 1–4. PCR was conducted with 35 cycles under the following conditions: denaturation at 94 °C for 2 min, annealing at 57 °C for 45 s and extension at 72 °C for 1.30 min with a final extension at 72 °C for 10 min. PCR products were analyzed by Agarose gel electrophoresis in Tris-acetate – EDTA (TAE) buffer. The expected size of amplicons were 492 bp (DENV1), 119 bp (DENV2), 290 bp (DENV3), 392 bp (DENV4) ad 120 bp (CHIKV).

2.6. Data analysis

All the results were summarized in terms of medians and ranges for continuous data, odds ratio (*OR*), *Chi* square and Fishers exact tests were used as appropriate. Data for study clinical symptom of patients was compared, which includes age, gender, province and district.

3. Results

3.1. Clinical features

A total of 131 eligible DHF suspected cases were enrolled at Dong Thap general hospital over 13 months starting in January 2012. 114 patients were from 11 districts in the Dong Thap province, 16 patients from neighboring provinces such as An Giang and 1 case from Ho Chi Minh City. The cohort comprised 62 females and 69 males, ranging in age from 5 months to 49 years with a median age of 15 years. The mean body temperature of patients on the day of admission was 39 °C, and fever was observed from 37.5 to 40.5 °C of patients. The most common clinical features observed were: headache (88.5%), myalgia (72.0%), arthralgia (40.5%), rash (12.2%) a positive tourniquet test (16.8%), and nausea/vomiting (3.8%) The mean length of time to admission was 4 d after reported onset of fever ranging from 1 to 7 d. The first blood sample occurred in a median time of 4 d (1–7 d) after onset (time of admission) while the second blood sample was taken at median time of 7 d after admission (7–14 d) (Table 1).

3.2. Prevalence of dengue and chikungunya in Dong Thap hospital

Among the 131 acute fever patients enrolled in the cohort, none of them were found to have been infected with chikungunya. All CHIKV PCR tests proved to be negative. However, results were totally different with respect to dengue and were dependent upon the detection method implemented and on

Table 1
Frequency of symptoms on admission to Dong Thap general hospital (n = 131).

Signs and symptoms	Value	Odds ratio (*OR*)	<18 years (n = 98)	>18 years (n = 33)	*P*-value
Headache	88.5%	12.10	84.7	97.0%	0.062 5
Myalgia	72.0%	2.54	75.5	60.6%	0.090 0
Arthralgia	40.5%	0.70	31.6	66.6%	0.003 9
Rash	12.2%	0.14	10.0	18.0%	0.220 0
Petechiae	19.0%	0.20	20.4	15.0%	0.500 0
Nausea/Vomit	3.8%	0.03	3.0	6.0%	0.430 0
Positive Tourniquet test	16.8%	0.25	14.3	24.2%	0.180 0

Note: Value are the mean (range) or number (%). *P* calculated by *Chi* square test and fisher test.

Table 2
Results of diagnostic tests for suspected dengue patients in Dong Thap, Vietnam.

Diagnostic test	Phase	Type of sample	No. of samples tested	No. of positive samples	% Positive cases
IgM capture ELISA	Acute	Plasma	131	38	29
		Serum	131	18	14
	Convalescent	Serum	131	70	53
Multiplex PCR	Acute	Plasma	131	97	74
		Serum	131	95	73

Table 3
Distribution of dengue serotypes among positive patients in Dong Thap hospital, Vietnam.

Age	No. of DENV patients	No. of males	No. of females	Dengue serotypes			
				DENV1	DENV2	DENV3	DENV4
0–4	8	3	5	0	3	5	0
5–14	55	25	30	7	20	11	17
15–24	19	8	11	1	6	2	10
25–34	14	8	6	2	6	0	6
>35	5	2	3	1	1	1	2
Total	101	46	55	11	36	19	35

the time of blood sampling when using serology. Out of the 131 paired serum/plasma samples collected on the day of admission (first sample collection) during the acute phase, 38 patients (29%) were dengue-positive when analyzing sera samples whereas 18 (14%) patients only were positive when using the plasma fraction (Table 2). When analyzing sera from convalescent patients, *i.e.* second collection time, the number of IgM positive samples rose to 70 (53%) (Table 2).

3.3. Distribution of dengue serotypes in Dong Thap positive dengue cases

101 samples (77%) out of 131 collected were positive for dengue. All four dengue serotypes were identified and the respective number of positive was 11 (11%) for DENV1, 36 (36%) for DENV2, 19 (19%) for DENV3, and 35 (35%) for DENV4 (Table 3). Males were more affected than females (Table 3). Age was another discriminative criterion with the 5–14 year class comprising 54.5% of all cases (55 out of 101) (Table 3).

4. Discussion

The initial objective of this work was to assess within 13 months the occurrence of dengue and chikungunya among patients from Dong Thap general hospital admitted for acute fever and dengue/chikungunya-related symptoms. The most common symptoms on the day of admission were headache, fever, myalgia, arthralgia, and a positive tourniquet test. Although the majority of clinical manifestations were similar between adult and pediatric patients, adults were significantly more affected by arthralgia than pediatric patients. More males were found to be affected than females which correspond to other reports from Nepal, China and Vietnam [32–34]. The most affected age class was the 5–14 years class which in agreement with other studies which have reported dengue mainly in children [33–39].

The co-circulation of four serotypes of DENV is in agreement with the status of region of hyperendemicity of both southern Vietnam and Cambodia [38,40,41]. However, if the overall predominance recorded in this work is for DENV2 and DENV4, reports from Cambodia have stressed the predominance of DENV2 and DENV3 with a rotation and regular replacement of serotypes [40,41]. Dong Thap is located along the Mekong River at the Cambodian border where cases of chikungunya have been described along the major northwest to southwest routes and in provinces bordering Vietnam [28]. This dynamic of expansion might lead to emergence in neighboring Vietnam provided that the Cambodian–Vietnamese border in Dong Thap is a highly active zone of transboundary movements. Although, the 13-months surveillance described in this work did not show any presence of CHIKV, but the risk is still present and this surveillance should be maintained.

An important outcome from this work is the differential efficiency of detection of dengue through serology. DENV infection was hardly detected in acute phase through serological tests with only 29% of plasma and 14% of sera to be positive. Conversely, DENV infection was detected at 53% in clinical samples obtained during the convalescence phase and up to 74% when using PCR. Time of seroconversion should be taken into account when implementing detection procedures. Indeed there is no control on the time between onset and admission and this time might vary greatly from one patient to another. Time for seroconversion should be taken into account when implementing detection procedures. However, for practical reasons, admission is the only time when blood samples can be taken since patients can hardly be followed up after leaving the hospital. The best solution would be therefore to implement multiplex PCR detection on blood sample taken at admission. Cost of PCR is nowadays not higher than that of serological tests and the possibility to combine several detection tests, i.e. dengue and chikungunya, at the same time in a one-step single tube procedure as well as the higher efficiency of PCR make it a highly cost-effective option. A recommendation from this work would therefore be to replace current procedures for serological detection of dengue by a standard operating procedure for DENV–CHIKV multiplex single step PCR.

26% of patients hospitalized with acute fever symptoms were negative for both DENV and CHIKV. This unknown etiology may need further work to identify what the causative pathogens involved. Other limitations of this study is the limited number of patients enrolled and therefore of samples available. Owing to the dynamics of both chikungunya and dengue and the potential of emergence for chikungunya in Vietnam it is of importance to implement a larger surveillance system which will provide valuable information on the prevalence and incidence of DENV infection and CHIKV circulation which are essential for planning an appropriate public health strategy.

Conflict of interest statement

We declare that we have no conflict of interest.

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