

Chikungunya and dengue virus infections during pregnancy: seroprevalence, seroincidence and maternal–fetal transmission, southern Thailand, 2009–2010

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Received 20 July 2014; Final revision 21 April 2015; Accepted 29 April 2015;

first published online 26 June 2015

SUMMARY

Limited information is available on the seroprevalence of chikungunya virus (CHIKV) infection and maternal–fetal transmission incidence of CHIKV and dengue virus (DENV) infections during the 2008–2009 CHIKV outbreak in southern Thailand. A community-based post-epidemic seroprevalence study was conducted in parturient women admitted to the Thepa District Hospital in Songkhla Province, Thailand, for delivery from November 2009 to May 2010. The women were tested for chikungunya (CHIK) IgM/IgG and dengue (DEN) IgM/IgG. Cord blood samples were also tested for CHIK IgM or DEN IgM in women who tested positive for CHIK IgM or DEN IgM, respectively. The seroprevalence of CHIKV infection (CHIK IgM or IgG positive) was 227/319 (71·2%) with pre-outbreak seroprevalence (IgM–/IgG+) of 43·6% and the seroprevalence of DENV infection was 288/319 (90·3%). Complications during pregnancy, newborn outcomes and congenital anomalies were not different in those who had recent, remote or no CHIKV infections. None of the newborns whose mothers were CHIK or DEN IgM positive had cord blood positive for both CHIK and DEN IgM. In conclusion, both CHIKV and DENV are endemic in southern Thailand; during the recent CHIKV outbreak CHIK seroprevalence increased from 43·6% to 71·2%.

Key words: CHIK, chikungunya, dengue viral infection, seroprevalence.

INTRODUCTION

Chikungunya virus (CHIKV) outbreaks have been recorded in Africa and Asia since the disease was first observed in 1952 [1]. The East/Central/South African

strain of CHIKV, first noted in Asia in 2006 in the Indian subcontinent, spread to Singapore and Malaysia in 2008 [2, 3], and southern Thailand in 2009 [4, 5].

In the recent outbreak of CHIKV around the Indian Ocean, post-CHIKV outbreak seroprevalences varied from 38·2% in La Reunion Island to 62–68% in outbreak districts in India [6, 7] and 55·6% in Bagan Panchor village, Malaysia [8]. During this outbreak, 70–80% of CHIKV-infected participants were symptomatic [8, 9]. Later studies reported that during the

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outbreak, CHIKV had had no observable effect on pregnancy outcomes and no effect on newborns if the mother was infected before the peripartum period [10, 11]. However, another study found that if the mother was symptomatic between 2 days before and 2 days following delivery, the neonates of these symptomatic mothers also developed chikungunya (CHIK) symptoms [12].

The *Aedes* mosquito is the vector for both CHIKV and dengue virus (DENV). In Thailand, DENV is endemic, while to date there have been only occasional outbreaks of CHIKV. During 1998–1999 there were no reports of CHIKV outbreaks, but one study noted the seroprevalences of CHIKV and DENV in cord blood serum were 33.6% and 96.9%, respectively [13]. This same study also suggested that CHIKV might have actually been endemic at the time, with CHIKV quite widely present in Bangkok, but underreported due to the victims being asymptomatic or misdiagnosed as DENV infections; also during this time, the serology test for CHIKV was not routinely performed, possibly resulting in further underreporting [13, 14].

During the outbreak of CHIKV in southern Thailand in 2008–2009, the incidence of CHIKV infection was 534/100 000 with a peak of 1130/100 000 during December 2008–July 2009 [15]. In 2010, in contrast to the outbreak levels, the incidence was only 15.3/100 000 [15]. During 2008–2010, the incidence of dengue (DEN) in southern Thailand was 100–300/100 000 population [16].

Information on congenital infections and complications during pregnancy with CHIKV infection or with both CHIKV and DENV infections during pregnancy is lacking, especially in DEN endemic areas.

The purpose of this paper was to study the seroprevalence and seroincidence of both CHIKV and DENV in parturient women in southern Thailand, and the intrauterine infection rate of both viruses in this patient population.

METHOD

This community-based epidemiological prospective study was performed at the Thepa District Hospital in Songkhla Province, Thailand, one of the provinces affected by the 2009–2010 CHIKV outbreak. The hospital normally has 60–80 deliveries per month in the district it serves with ~70 000 inhabitants.

The finite population proportion equation with values of proportion (p) = 0.7 and 0.9 for CHIKV and DENV, respectively, and error (d) = 0.05 was used to estimate the

required sample sizes in our study which, in order to determine the seroprevalences of CHIKV and DENV infections, were 323 and 139 patients, respectively. Parturient women admitted at Thepa Hospital for delivery from November 2009 to May 2010, a total of 7 months, were enrolled in the study. Obstetric history and neonatal outcome data were recorded, including maternal complications during pregnancy, gestational age, mode of delivery, birth asphyxia, birth weight, length, and head circumference. Clinical symptoms associated with CHIK such as arthralgia and/or fever and with DEN such as fever, abnormal bleeding, abdominal pain, and/or myalgia, were noted. If the overall clinical profile was inconclusive in terms of providing enough information to make a firm diagnosis of either CHIKV or DENV, the patient was classified as acute febrile illness (AFI). Risk factors for CHIKV and DENV infections such as frequency of mosquito bites, occupation, and the use of a wire mosquito screen over windows in the home were also recorded. The women were tested for CHIK IgM/IgG and DEN IgM/IgG. Cord blood samples were also tested for CHIK IgM or DEN IgM in women who tested positive for CHIK IgM or DEN IgM, respectively, using a commercial immunofluorescence assay and indirect ELISA for CHIK and DEN, respectively (EUROIMMUN AG, Germany) [17]. The seroprevalence rate included both positive and weakly positive blood tests of any IgM or IgG. Mothers or infants who had AFI without a clear source identified during the peripartum period and suspected of having CHIKV or DENV infection had a blood sample taken to test for CHIKV and/or DENV infections using real-time reverse transcriptase–polymerase chain reaction (direct one-step TaqMan RT–PCR) [18]. CHIK IgM+/IgG– or IgM+/IgG+ were defined as recent infection (seroincidence), and IgM–/IgG+ was defined as remote infection.

Ethics statement

Permission from the Institutional Review Board of the Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand, was obtained prior to conducting the study (Ethics review no. 52-331-01-1-2). Each enrolled woman provided written, informed consent on behalf of herself and her fetus before any procedures were begun. The purpose of the study, the freedom to decline without prejudice to their care, and the guarantee of anonymity of each subject were

explained to each potential participant before requesting written consent.

Statistical analysis

We conducted a descriptive analysis of the main characteristics of the study population. Mean and standard deviation, median and interquartile range (IQR), or frequencies and percentages were calculated as appropriate. Statistical differences in the frequency distributions of DENV and CHIKV infections according to the characteristics of the study population were tested using χ^2 or Fisher's exact tests. ANOVA *F* test or Kruskal–Wallis tests were used to test differences for normally distributed and non-normally distributed continuous variables, respectively. Age-specific seroprevalences in the study population were used to estimate overall seroprevalence in women of reproductive age in the Thai population [19] by the method of direct standardization. Statistical significance was set at $P < 0.05$. Seroprevalences, CHIK IgM or IgG, only CHIK IgG, and any CHIK IgM were plotted against calendar time to show trends. Stata version 10 (StataCorp, USA) was used for statistical analysis.

RESULTS

Clinical characteristics

During the 7-month study period 340 parturient women were enrolled in the study. Seventeen cases with no cord blood samples and four cases with incomplete important data were excluded, giving a total of 319 cases for inclusion in the statistical analysis. All subjects had a history of mosquito bite, and 264/319 (82.8%) said they received mosquito bites every day. Only two of the subjects said they lived in a house which had some form of wire mosquito screen over the windows. More than half (53.9%) of the women worked in agriculture, with the largest number (38.6%) working on rubber plantations. Most (313/319, 98.1%) had normal delivery; the remainder (6/319) had vacuum extraction. Complications arose in 16 cases: preterm delivery, premature rupture of the membrane, and abnormal vaginal bleeding in 11, three, and two cases, respectively.

Maternal clinical profiles, except for CHIKV history, were similar in the recent, remote, and no CHIKV infection groups (Table 1).

None of the newborns had birth asphyxia or a congenital anomaly, and all were discharged with their

mother. Most were full term (308/319, 96.6%) with normal birth weight (2500–4000 g) and head circumference (Table 2).

CHIKV infection

CHIK IgM or IgG was detected in 227/319 (71.2%) of the patients; of which the number of patients with IgM+/IgG– (recent infection 5–15 days), IgM+/IgG+ (recent infection assumed), and IgM–/IgG+ (remote infection) were four, 84 and 139, respectively. Of the four women who had IgM+/IgG–, all had asymptomatic infection; two each were enrolled in March and April 2010. Symptomatic CHIKV infection was reported in 15 patients, of whom 13 (86.7%) had confirmed serology. Of these 13 women with confirmed symptomatic CHIK, eight and five women had CHIK before and during pregnancy, respectively (Table 1). Of the five women who had CHIK during pregnancy, two, two and one women had had CHIKV infection at gestational ages of 2, 5, and 7 months, respectively; none of this group had had complications during pregnancy and none of their newborns had any congenital anomaly. None of the 319 women was positive for both CHIK IgM and DEN IgM, and none reported having had CHIKV and DENV infections simultaneously.

None of the newborns whose mothers were CHIK or DEN IgM positive had cord blood positive for CHIK IgM or DEN IgM, respectively.

CHIK seroprevalence

The peak of the CHIK outbreak was December 2008–July 2009. We began enrolling the patients 4 months after the end of the peak outbreak, November 2009, and continued enrolling to the end of May 2010. The seroprevalence rates (CHIK IgM or IgG+) increased, the seroincidence (CHIK IgM+) decreased, and the rates of remote infection (only CHIK IgG+) increased by enrolled month, but none of the changes was statistically significant (Fig. 1).

After adjustment by direct standardization to the age structure of women of reproductive age in the population, CHIK seroprevalence was 72.3% [95% confidence interval (CI) 52.4–84.6], seroincidence was 30.6% (95% CI 14.8–48.6), and proportion of remote infection was 45.7% (95% CI 27.6–63.9) (Table 3).

Table 1. *Clinical characteristics of the study population with recent, remote or no chikungunya viral infection*

Clinical profile	Recent infection (N = 88)	Remote infection (N = 139)	No infection (N = 92)	P*
Maternal age (years), median (IQR)	24·7 (21·3–31·1)	25·8 (21·7–31·2)	23·9 (21·3–28·4)	0·22
At least one mosquito bite every day, n (%)	74 (84·1)	115 (82·7)	75 (81·5)	0·90
Occupation, n (%)				0·22
Housewife	14 (15·9)	13 (9·4)	19 (20·7)	
Rubber tapper	32 (36·4)	62 (44·6)	29 (31·5)	
Other agriculture	13 (14·8)	23 (16·5)	13 (14·1)	
Other	29 (32·9)	41 (29·5)	31 (33·7)	
History of chikungunya infection in a family member, n (%)	11 (12·5)	11 (7·0)	7 (7·6)	0·42
History of having chikungunya infection, n (%)	11 (12·5)	2 (1·4)	2 (2·2)	<0·01
Prior to pregnancy	6	2	2	
During pregnancy	5	0	0	
History of having dengue infection, n (%)	0	1	1	0·46
Acute febrile illness, n (%)	3 (3·4)	7 (5·0)	4 (3·5)	0·96
Complication during pregnancy, n (%)	8 (9·1)	2 (1·4)	2 (2·2)	0·12
Preterm (gestational age <37 weeks)	5 (5·7)	4 (2·9)	2 (2·2)	
Premature rupture of membrane	0	3 (2·2)	0	
Abnormal vaginal bleeding	1 (1·1)	0	1 (1·1)	
Gestational age at delivery (weeks), median (IQR)	38·9 (38·0–40·0)	39·0 (38·0–40·1)	39·0 (38·0–40·1)	0·39
Mode of delivery, n (%)				0·36
Normal vaginal delivery	86 (97·7)	138 (99·3)	89 (96·7)	
Vacuum extraction	2 (2·3)	1 (0·7)	3 (3·3)	

IQR, Interquartile range.

* Kruskal–Wallis test was used for continuous variables. χ^2 or Fisher's exact test was used for comparisons of categorical data.

Table 2. *Clinical characteristics of newborns delivered from mothers with recent, remote or no chikungunya viral infection*

Clinical profile	Recent infection (N = 88)	Remote infection (N = 139)	No infection (N = 92)	P*
Male, n (%)	45 (51·1)	74 (53·2)	46 (50·0)	0·92
Body temperature (°C), median (IQR)	37·0 (36·8–37·2)	37·0 (36·8–37·2)	37·0 (36·8–37·2)	0·40
Heart rate (beats/min), median (IQR)	141 (136–148)	142 (140–150)	143 (138–150)	0·26
Respiratory rate (time/min), median (IQR)	54 (52–60)	56 (52–59)	56 (52–59)	0·34
Birth weight of term neonate (g), n (%)				0·66
<2500	3 (3·6)	5 (3·7)	3 (3·3)	
2500–4000	79 (95·2)	129 (95·6)	84 (93·3)	
>4000	1 (1·2)	1 (0·7)	3 (3·3)	
Head circumference (cm), median (IQR)	33·0 (32·0–33·5)	33·0 (32·0–34·0)	33·0 (32·0–33·6)	0·73
Duration of admission (days), median (IQR)	2 (2–3)	2 (2–3)	2 (2–3)	0·57

IQR, Interquartile range.

* Kruskal–Wallis test was used for continuous variables. χ^2 or Fisher's exact test was used for comparisons of categorical data.

Concordance of CHIK IgG in maternal blood and in cord blood

Maternal blood and cord blood CHIK IgG were initially tested in 190 samples; 176/190 (92·6%) mother/fetus pairs were in concordance; 51 of these were positive concordance, 123 were negative concordance, and two were weakly positive concordance (Table 4).

DENV infection

DEN IgM and IgG were detected in 4/319 (1·3%) and 288/319 (90·3%) women, respectively, and the monthly DEN IgG seroprevalences were not significantly different during the study period.

All DEN IgM-positive women were also DEN IgG positive and were asymptomatic; one case had preterm

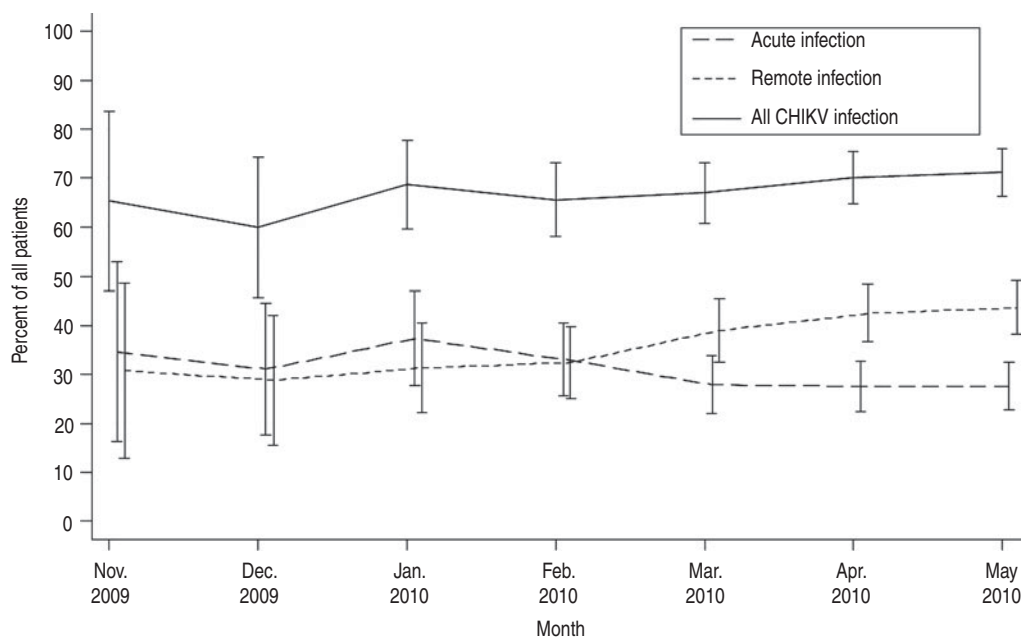


Fig. 1. Cumulative seroprevalence of chikungunya virus (CHIKV) infection.

delivery without abnormal vaginal bleeding. Two women reported having DENV infection prior to pregnancy and DEN IgG was detected in both cases but not IgM. Two women who had pre-eclampsia were CHIK and DEN IgG positive but also CHIK and DEN IgM negative; both had normal delivery without complications.

DISCUSSION

The seroprevalences of CHIKV and DENV in the study population were 71.2% and 90.3%, respectively. None of the newborns contracted transplacental CHIKV or DENV infections. During the CHIKV outbreak in this DENV-endemic area, none of the study population was both DEN and CHIK IgM positive.

The seroprevalence of CHIKV in pregnant woman in Thepa district in our study was 71.2%. In previous studies, seroprevalences have varied from 10% to 75%, depending on the outbreak area, the time the serosurveillance studies were performed (during the peak period, or after the outbreak) and the pre-outbreak seroprevalence [7, 9, 13, 20–23]. Our high seroprevalence of CHIKV (71.2%) was similar to previous studies from India (68%) and Kenya (75%) [7]. These findings suggest that a CHIKV outbreak will stop when around 70% of the population has the antibody to CHIKV.

The high seroprevalence of CHIKV in our study could be related to the fact that we had high pre-outbreak seroprevalence (43.6%) and the main occupation of most women in the study area was rubber plantation worker, where natural and man-made holes in the trees, and the rain that collects in the cups used for collecting rubber latex, are good breeding areas for *A. albopictus* [24, 25]. In addition, our serosurveillance was performed 4 months after the peak of the CHIKV outbreak, so seroprevalence would be higher than seroprevalence during the outbreak, which is confirmed by a previous study that found during the epidemic upsurge and the post-epidemic era that CHIK seroprevalences were 18.2% and 38.2%, respectively [26].

We found pre-outbreak CHIKV in 43.6% of pregnant women. A previous study done in Bangkok, Thailand, found a CHIK seroprevalence during a period of no CHIKV outbreak of 33.6%, lower than in our study [13]. We also found that pregnant women aged 35–39 years had a higher proportion of CHIK IgG than younger age groups (Table 3), which supports our belief that CHIKV was probably endemic in Thepa district before this outbreak, so the older age group had a higher proportion of remote infections.

We cannot confirm that recent CHIKV infections (IgM+27.6%) in this outbreak represent the true sero-incidence of CHIKV infection, since CHIK IgM

Table 3. *Expected numbers of positive women in each age group in 100 pregnant women*

Age (years)	Seroincidence (IgM+)		Pre-outbreak seroprevalence (IgG+/IgM-)		Seroprevalence (IgG+ or IgM+)	
	No.	95% CI	No.	95% CI	No.	95% CI
15–19	4.1	2.3–6.2	5.8	3.8–8.0	9.0	6.8–11.0
20–24	4.4	3.1–5.8	6.4	4.9–7.9	10.7	9.3–12.0
25–29	4.4	2.8–6.3	6.7	4.9–8.7	11.1	9.2–12.7
30–34	4.8	2.7–7.4	7.4	4.9–10.0	12.1	9.5–14.4
35–39	3.7	1.7–6.6	10.2	7.2–13.0	13.9	10.1–16.1
40–44	9.2	2.2–16.3	9.2	2.2–16.3	15.4	6.6–18.4
Total	30.6	14.8–48.6	45.7	27.6–63.9	72.3	52.4–84.6

CI, Confidence interval.

can last for years in patients following a CHIKV infection [27, 28]. We could confirm 15 cases of recent CHIKV infection (4.7%), which included four cases with IgM+/IgG- and 11 cases who had symptomatic CHIKV infection with IgM+/IgG+.

We also found a high seroprevalence of DENV infection (90.3%), a rate similar to a previous study from Bangkok, Thailand (96.9%) [13]. However, the seroprevalence of DENV in pregnant women in other countries has been found in previous studies to range from 17% to 53% [20, 29]. These findings suggest that most adult Thais have had DENV infection at some point in their lives and the 2008–2009 CHIKV outbreak did not correspond to an epidemic upsurge of DENV infection. It is known that Thailand is a DENV-endemic area, and these studies confirm a very high proportion of the Thai population has been exposed to DENV.

Previous studies have found that 70–80% of both the general population and pregnant women who had laboratory-confirmed CHIKV infection were symptomatic [7, 9, 20, 26]. We found that of the 227 patients who had a CHIKV seropositive reaction, only 11/88 (12.5%) had clinical symptoms of CHIKV infection. The higher asymptomatic CHIKV rate in our study could be explained by recall bias, and many or most of these patients may have had mild symptoms.

We also found, as in previous studies, that a fairly large number of our patients, 13.3%, who thought that they had CHIKV infection, did not actually have the infection [7, 9, 20, 26]. In 2009, the estimated incidence of symptomatic CHIK patients in the highest outbreak area in southern Thailand was around 1000/100 000 population [15]. This information is

consistent with our finding that most of the patients who had CHIKV infection were asymptomatic.

The number of symptomatic DENV infection patients in our study was 2/288 (0.7%). In Southern Thailand, during 2008–2010, the symptomatic DENV infection rate was 100–300/100 000. This information is consistent with our finding that <1% of the population had symptomatic DENV infection (if DENV seroprevalence was 90%).

We do note a possible concern about the possibility that the high DENV seroprevalence we found could be related to a cross-antibody reaction from a previous Japanese encephalitis virus (JEV) infection or a JE vaccination, as both DENV and the JEV are in the Flaviviridae family [30, 31]. However, we do not believe this is a serious concern. Since 1990, JE vaccinations have been provided in JEV-endemic areas, and since 2001 to all Thai children, and the reported rate of JEV infection in Thailand is currently very low. Thus we believe it is highly likely that at least most, if not all, of our DENV serology positive cases resulted from a previous DENV infection and not from JEV infection or from JE immunization, as JEV is not endemic in southern Thailand and all the parturient mothers were aged >13 years old, and thus had not received the JE vaccination.

We found, as in a previous study, that CHIKV vertical transmission did not occur if the woman developed symptoms before the intrapartum period, i.e. before ~2 days before delivery [26]. Although complications during pregnancy in those who had a recent CHIKV infection (9.1%) were higher than in those who had a remote CHIKV infection (1.4%) or no history of CHIKV infection (2.2%), the differences were not significant. Another previous study also found, in

Table 4. Concordance of chikungunya IgG in maternal blood and cord blood

Maternal blood	Cord blood		
	Positive	Negative	Weakly positive
Positive	51	5	0
Negative	3	123	3
Weakly positive	0	3	2

agreement with our study, that CHIKV had no observable effect on pregnancy outcome if the women had had a CHIKV infection other than in the peripartum period [10].

A limitation of our study is that it began after the outbreak, so none of our subjects had CHIKV infection during the intrapartum period. In addition, the histories of previous CHIKV or DENV infections were based on recalled information, with no serology tests to confirm any previous infections, nor any indication as to whether untested mild symptoms were actually CHIK or DEN or some other AFI. In addition, pregnant women are not representative of the general population.

In conclusion, 4 months after the CHIKV outbreak in our study area, the seroprevalences of CHIKV and DENV infections were quite high, at 71% and 90%, respectively, most of the pregnant women studied had only asymptomatic infections, and co-infection was very rare. None of the infants born to infected mothers developed congenital infections, either CHIKV or DENV, during the antepartum period.

ACKNOWLEDGEMENTS

The authors thank Miss Walailuk Jitpi boon, the study research assistant, for assistance with data analysis, and David Patterson, an English teacher and consultant, for help with the English.

DECLARATION OF INTEREST

None.

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