

# FOCAL DENGUE VIRUS TRANSMISSION IN KAMPHAENG PHET, THAILAND AND IMPLICATIONS FOR MANAGEMENT

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**Abstract.** Dengue is the most globally prevalent vector-borne viral disease. However, our understanding of dengue virus (DENV) transmission is incomplete. Prospective longitudinal cohort and cluster studies in Kamphaeng Phet, Thailand have contributed much to our understanding of DENV transmission. These studies have demonstrated the spatiotemporal heterogeneity of DENV transmission with highly focal transmission at small scales in a rural setting. Geographic cluster studies have suggested the presence of small “hotspots” of transmission at the house level that may have a disproportionately high impact on local spread. These hotspots should be considered when planning overall vector control interventions. The combined cohort and cluster design have shown that clinically inapparent DENV infections from prospective longitudinal cohorts likely consist of a clinical spectrum of infections from asymptomatic to mildly symptomatic with and without fever. The proportion of all DENV infections that are completely asymptomatic may be substantially lower than those considered to be inapparent in cohort studies. In addition, some of these inapparent infections from cohort studies have viable DENV and may potentially contribute to virus transmission. These findings require further validation in other settings and in adults and children. A more comprehensive understanding of DENV transmission will be critical to inform prevention, prognostication and management strategies.

**Keywords:** asymptomatic, cluster, cohort, dengue, focal, inapparent, spatiotemporal, transmission

## INTRODUCTION

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Dengue is the most common vector-borne viral disease globally with approximately 2.5 billion people living in areas at-risk for infection. In the past several decades, dengue has expanded rapidly, with dengue virus (DENV) recently estimated to cause 390 million infections per year worldwide and with 96 million symptomatic cases (Bhatt *et al*, 2013). Four closely related, but antigenically distinct DENV serotypes

(DENV 1-4) from the genus *Flavivirus* in the family Flaviviridae are known to cause human disease (Gubler, 2002; Lindenbach and Rice, 2003).

These serotypes often co-circulate in endemic regions leading potentially to both disease mitigation and disease enhancement among different serotypes (Reich *et al*, 2013). However, despite the widespread circulation and disease burden caused by DENV, our understanding of transmission dynamics is incomplete but is critical to inform prevention, prognostication, and management strategies. In order to improve our knowledge of DENV transmission, the Armed Forces Research Institute of Medical Sciences (AFRIMS) in close partnership with other collaborating institutions has conducted prospective dengue studies in Kamphaeng Phet, Thailand since the 1990's (Gibbons *et al*, 2013).

Prospective longitudinal cohort studies conducted over multiple years can provide useful information about the true incidence of infections within a defined cohort, elucidate the full clinical spectrum of infections including clinically inapparent infections, and clarify the spatiotemporal distribution of infections (Endy *et al*, 2010). Geographic cluster studies can additionally indicate finer scale spatiotemporal dimensions of transmission and can be more sensitive in detecting mildly symptomatic and asymptomatic infections (Yoon *et al*, 2013). Cohort and cluster studies, therefore, provide useful platforms from which to study the different elements of DENV transmission. Here, we present some of the contributions to our understanding of DENV transmission from two sequential cohort/cluster studies conducted in Kamphaeng Phet, Thailand.

## DESCRIPTION OF TWO COHORT/ CLUSTER STUDIES IN KAMPHAENG PHET, THAILAND

Two sequential prospective longitudinal cohort studies were conducted from 1998-2002 (called KPSI) (Endy *et al*, 2002a,b; Endy *et al*, 2011) and 2004-2007 (called KPSII) (Mammen *et al*, 2008; Yoon *et al*, 2012a) in Mueang District of Kamphaeng Phet Province in rural north-central Thailand (Fig 1). Dynamic cohorts of approximately 2,000 primary school children in grades 2-6 (KPSI) or kindergarten to grade 6 (KPSII) were followed by active school absence-based surveillance for acute febrile illnesses from June to November each year.

Acute blood samples were collected from cohort subjects who reported fever in the previous seven days; convalescent blood samples were collected two weeks later. Acute samples were tested by semi-nested reverse transcriptase polymerase chain reaction (RT-PCR) to detect DENV RNA (Lanciotti *et al*, 1992; Klungthong *et al*, 2007). Acute/convalescent sample pairs were tested by an in-house dengue/Japanese encephalitis (JE) IgM/IgG capture enzyme-linked immunosorbent assay (ELISA) (Innis *et al*, 1989) and dengue/JE hemagglutination inhibition (HAI) assay (Clarke and Casals, 1958).

Virus isolation was attempted in *Aedes albopictus*-derived C6/36 cells from selected DENV PCR-positive samples (Jarman *et al*, 2011). Cohort subjects also underwent scheduled phlebotomy in January, May, August and November of each year (KPSI), or May and December/January of each year (KPSII). These scheduled blood samples

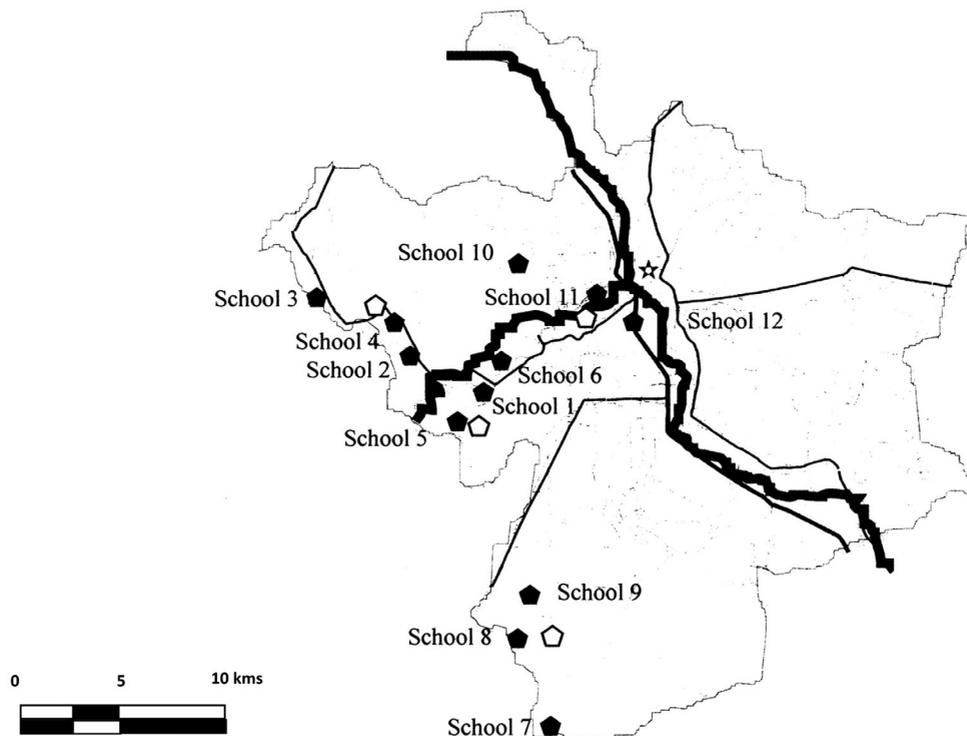


Fig 1—Map of Mueang District, Kamphaeng Phet Province, Thailand. Black pentagons indicate the 12 schools that participated in the first prospective cohort study (KPSI). Adapted from: Endy *et al*, 2002a.

were tested by dengue/JE HAI for four-fold rise in dengue HAI titers, and, if positive, were confirmed by dengue/JE plaque reduction neutralization test (PRNT) (Russell and Nisalak, 1967; Salje *et al*, 2014).

‘Symptomatic’ DENV infection in a cohort subject was defined as an acute febrile illness with positive DENV PCR in the acute sample and/or positive dengue ELISA/HAI in the acute/convalescent sample pair. “Inapparent” DENV infection was defined as four-fold rise in dengue HAI and PRNT titers between scheduled blood samples but without symptomatic infection during the intervening surveillance period.

In conjunction with the second cohort study (KPSII), geographic cluster investi-

gations were additionally performed (Yoon *et al*, 2012a; 2013). KPSII cohort subjects with DENV PCR-positive acute samples served as index cases for ‘positive’ geographic cluster investigations around the index house. DENV PCR-negative cohort subjects served as index cases for ‘negative’ cluster investigations. Contact subjects aged six months to 15 years living within 100 meters of the index case were clinically evaluated at days 0, 5, 10, and 15. Blood samples were also collected on days 0 and 15 and tested for DENV infection by DENV PCR and ELISA/HAI.

Contact subjects with DENV infection could have had fever history, no fever history but other non-fever symptoms, or

no detectable symptoms at all (asymptomatic). In addition, adult female *Aedes aegypti* mosquitoes were collected on day 1 of each cluster investigation from all houses within a cluster, and immature *Aedes aegypti* (larvae and pupae) were collected from water-holding containers. The heads/thoraces of captured adult mosquitoes were tested for DENV infection by DENV PCR (Yoon *et al*, 2012b).

#### HETEROGENEITY OF DENGUE VIRUS INFECTION

Dengue incidence in the cohort subjects at different schools participating in KPSI and KPSII demonstrated marked heterogeneity in both space and time (Endy *et al*, 2002a,b; Yoon *et al*, 2012a). Dengue incidence was cyclical in each school. However, these cycles did not necessarily coincide among different schools even when the schools were in close proximity (within 5 km). This heterogeneity included not just dengue incidence but also the predominant circulating serotypes, which could be quite variable among different schools during the same year. The ratios of inapparent-to-symptomatic infections were also highly variable among schools.

An analysis of these ratios from KPSI showed that the most important determinants at a given school were the incidence of DENV infection in a given year and the incidence in the preceding year suggesting serotype cross-reactive immunity with disease mitigating effects at the school level (Endy *et al*, 2011). When geographic cluster investigations were added in KPSII, the heterogeneous pattern of DENV infections was demonstrated to occur within individual villages.

Contact subjects in positive clusters had a mean DENV infection rate of 16.0% compared to only 1.1% in negative clusters despite the fact that paired positive and negative clusters were typically performed within the same village and within 5 days of each other (Yoon *et al*, 2012b). Even among the positive clusters, the infection rates varied widely among individual clusters ranging from 0-to-65% (Fig 2).

#### SPATIOTEMPORAL DIMENSIONS OF FOCAL TRANSMISSION

Given the heterogeneity of DENV infections among different schools and within individual villages, the spatiotemporal dimensions of transmission risk were further evaluated within the boundaries of the positive clusters. By analyzing infections in contact subjects living at various distances from DENV PCR-positive index cases, infection rates were noted to differ substantially even within the 100 meter radius of the clusters (Mammen *et al*, 2008; Yoon *et al*, 2012a).

The DENV infection rate was approximately 35% among contact subjects living in the same house as an index case and about 30% in houses within 20 meters of the index case. However, the infection rate decreased to less than 10% in houses 80-100 meters away (Fig 3). Within the same positive clusters, the DENV infection rate in adult female *Aedes aegypti* mosquitoes was greater than 8% in index houses, 2%-3% in houses within 40 meters of the index case, and under 1% in houses >40 meters away.

Taken together, these findings indicated that DENV transmission between humans and mosquitoes largely took place within a 40 meters radius during the two-

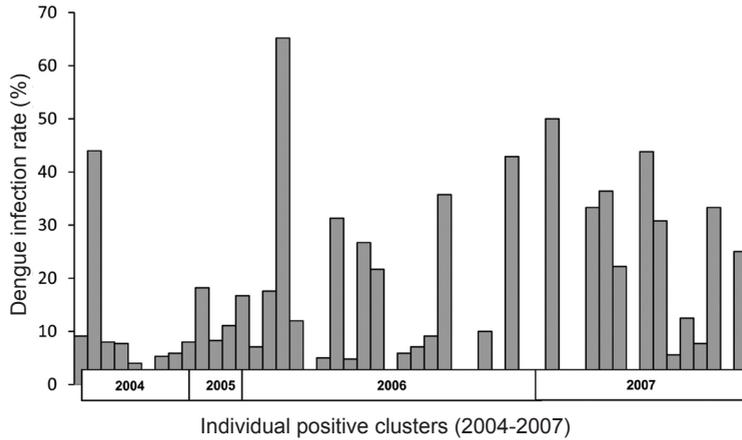


Fig 2–Dengue virus infection rates in individual positive clusters. Fifty positive cluster investigations were conducted from 2004-2007 in the second prospective cohort/cluster study (KPSII). Adapted from: Yoon *et al*, 2012b.

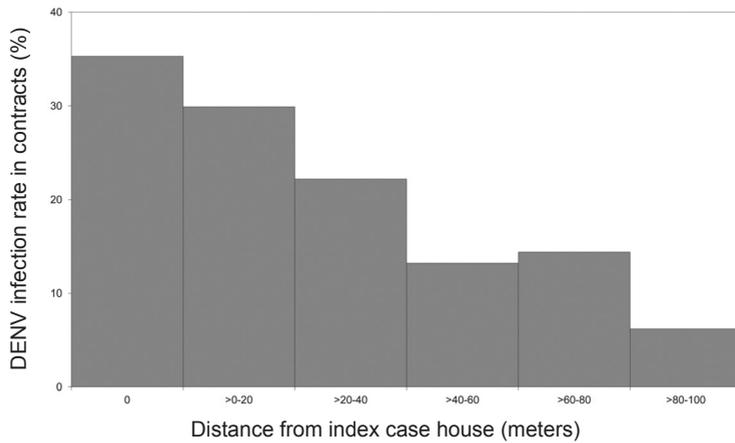


Fig 3–Dengue virus (DENV) infection rates in contact subjects living in houses at various distances from a DENV PCR-positive index case. Adapted from: Yoon *et al*, 2012a.

week interval of each cluster investigation. Therefore, within this dengue hyperendemic rural setting in Thailand, DENV transmission was found to be very highly focal in space and time.

PRESENCE OF DENGUE VIRUS TRANSMISSION “HOTSPOTS”

The data available from the KPSII geographic cluster investigations allowed for

further detailed evaluation of DENV infection risk in different houses. Entomological indices and human infection rates were analyzed in houses from which DENV-infected mosquitoes were captured, and in houses from which no infected mosquitoes were captured but were located in clusters either with or without infected mosquitoes (Table 1). The infection rate was about 47% in contact subjects in houses with infected mosquitoes, 28% in houses with-

Table 1  
Characteristics of houses with and without DENV-infected mosquitoes in positive clusters.

Parameter	Houses with infected mosquitoes	Houses without infected mosquitoes within clusters with infected mosquitoes	Houses in positive clusters without infected mosquitoes	p-value
Houses, <i>n</i>	16	158	324	-
Adult female <i>Aedes aegypti</i> per person (SD)	1.57 (2.05)	0.49 (1.00)	0.45 (0.89)	<0.001
<i>Aedes aegypti</i> pupae per person (SD)	5.84 (11.59)	1.86 (3.76)	1.22 (2.56)	<0.001
Human adults per house (SD)	2.94 (1.18)	2.92 (1.31)	2.70 (1.44)	0.239
Children per house (SD)	1.75 (0.86)	1.68 (0.88)	1.77 (1.04)	0.598
DENV-infected child contacts/all enrolled contact subjects (%)	9/19 (47.4)	56/195 (28.7)	64/591 (10.8)	<0.001

DENV, dengue virus; *n*, number; SD, standard deviation. Adapted from: Yoon *et al*, 2012a.

out infected mosquitoes located in clusters with infected mosquitoes, and 10% in positive cluster houses without infected mosquitoes.

Interestingly, the numbers of adult female mosquitoes and pupae per person were elevated only in houses with infected mosquitoes despite the fact that the human infection rate was quite high (28%) in houses without infected mosquitoes located in clusters with infected mosquitoes. This suggested that houses with elevated entomological indices served as DENV transmission hotspots that increased the risk of human infection not only in the same house but also in neighboring houses without elevated entomological indices.

Given the relatively small spatial scale of these hotspots (house level), it may be difficult to identify these hotspots in the course of wide scale vector control efforts. Because hotspots may have a disproportionately high impact on local transmission, vector control interventions that miss these hotspots may have limited effectiveness in reducing overall DENV transmission.

#### ROLE OF INAPPARENT INFECTIONS IN DENGUE VIRUS TRANSMISSION

The clinical spectrum of DENV infection can vary from asymptomatic, or inapparent, infection to mild dengue fever, dengue hemorrhagic fever, dengue shock syndrome, and other forms of severe dengue. Inapparent infections can account for varying proportions of all infections depending on the specific cohort study design, location and year (Endy *et al*, 2010). Much of this variability is affected by the method of surveillance and how inapparent infections are defined (Yoon *et al*, 2013;

Grange *et al*, 2014). In KPSII, approximately 65% of all DENV infections in the cohort were inapparent using the school absence-based active surveillance cohort design. However, in the geographic cluster investigations, only 40% of all DENV infections in contact subjects were 'inapparent' if this was defined as having no fever history (with or without other symptoms).

Alternatively, if inapparent was defined as being completely asymptomatic, then a mere 20% of all infections in contact subjects could be considered "inapparent". Because cluster investigations are generally more sensitive in detecting symptoms (and assuming that the KPSII cohort and clusters had similar dengue epidemiology as they were done concurrently), then about 40% of inapparent infections in the cohort may, in fact, have had fever history. Alternatively, about 70% of cohort inapparent infections may have had some symptoms (with or without fever history). These percentages are merely hypothetical since DENV infections in contact subjects did not correspond to the actual same inapparent infections from the cohort.

However, because the cohort and clusters investigations were conducted in the same area during the same time period, some inapparent infections in the cohort were, in fact, detected as contact subject infections in the cluster investigations. Sixteen such overlapping cases were identified in KPSII, with 12 of these 16 reporting fever histories as a contact subject. Despite the fever history, these 12 infections did not lead to school absence resulting; therefore, in characterization of the infection as inapparent in the cohort. Of the 16 overlapping cases, nine also

had detectable DENV RNA by PCR with mean viremia of 4.84 log RNA copies/ml (range, 2.80-7.14). All nine had viable virus by culture and fever history. These nine cases demonstrated that at least some inapparent infections in the cohort had the potential to contribute to DENV transmission. To what degree they might play such a role in actuality would depend on other factors to include the level and duration of viremia (Nguyet *et al*, 2013).

#### INCREASING AGE OF DENGUE IN KAMPHAENG PHET

Because KPSI and KSP II were studied in children, the direct applicability to adults is not clear. In particular, the proportion of inapparent infections in adults and the role of adult inapparent and symptomatic infections in DENV transmission require more study. The mean age of dengue in Thailand has increased over the past 10-20 years with decreasing force of infection possibly due to decreasing birth and death rates (Cummings *et al*, 2009). Despite this decreasing force of infection, the potential for infection (as indicated by the basic reproduction number) may, in fact, have changed very little as supported by a comparison of age-stratified dengue seroprevalence assessments conducted in Rayong, Thailand in 1980 and 2010 (Rodriguez-Barraquer *et al*, 2014). As in other provinces of Thailand, the mean age of dengue in Kamphaeng Phet has also increased over the past 20 years (Yoon, submitted). Further cohort and cluster studies in Kamphaeng Phet are being planned in order to clarify the clinical spectrum of dengue in adults and to investigate the role of adults in DENV transmission.

## CONCLUSION

Prospective longitudinal cohort and cluster studies in Kamphaeng Phet have contributed significantly to our understanding of DENV transmission. These studies have demonstrated the spatiotemporal heterogeneity of DENV transmission with highly focal transmission at small scales in this rural setting. Small easily missed hotspots of transmission may exist which can have a disproportionately high impact on local spread. Therefore, these hotspots should be taken into account when planning overall vector control strategies. Inapparent DENV infections as defined by prospective longitudinal cohort studies consist of a clinical spectrum of infections from asymptomatic to mildly symptomatic with and without fever. The proportion of all DENV infections that are completely asymptomatic may be substantially lower than those considered to be inapparent. Some of the inapparent infections from cohort studies have viable DENV and may, therefore, potentially contribute to virus transmission. These findings require further validation in other settings (for example, urban areas) and in adults and children to develop a more comprehensive understanding of DENV transmission.

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