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LRH: THOMAS AND OTHERS

RRH: ENHANCED SURVEILLANCE FOR DENGUE

Improving Dengue Virus Capture Rates in Humans and Vectors in Kamphaeng Phet Province, Thailand, Using an Enhanced Spatiotemporal Surveillance Strategy

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Abstract:

Dengue is of public health importance in tropical and sub-tropical regions. DENV transmission dynamics was studied in Kamphaeng Phet Province, Thailand using an enhanced spatiotemporal surveillance of 93 hospitalized subjects with confirmed dengue (initiates) and associated cluster individuals (associates) with entomologic sampling. A total of 438 associates were enrolled from 208 houses with household members with fever history located within a 200 meter radius of an initiate case. Of 409 associates, 86 (21%) had laboratory-confirmed DENV infection. A total of 63 (1.8%) of the 3,565 mosquitoes collected were dengue PCR+. There was a significant relationship between spatial proximity to the initiate case and likelihood of detecting DENV from associate cases and *Aedes* mosquitoes. The viral detection rate from human hosts and mosquito vectors in this study was higher than previously observed by the study team in the same geographic area using different methodologies. We propose that the sampling strategy used in this study could support surveillance of DENV transmission and vector interactions.

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Introduction

Dengue illness is a disease of increasing public health importance [1]. Available data and modeling estimate that there are 390 million dengue virus (DENV) infections annually with 96 million manifesting clinically [2]. International travel, population growth, increasing urbanization, and a changing global ecology foster an increasingly favorable environment for the expanding dengue endemic areas and the peridomestic *Aedes aegypti* which transmit the viruses [3]. There are currently no licensed drugs or vaccines to treat or prevent dengue. When applied properly, vector control and personal protective measures have successfully disrupted epidemic and endemic DENV transmission [4,5]. Unfortunately, successful vector control programs have been the exception and difficult to sustain [6]. The strategic use of safe and efficacious dengue vaccines in combination with appropriately targeted and sustained vector control measures is increasingly being considered as the optimal approach to produce a sustained reduction in dengue's global burden [7].

Once a vaccine is available, numerous questions will remain about how to most effectively target and co-implement vaccination and vector control programs. The prospect of implementing large-scale, control programs raises a number of questions:

1) How will vaccination and vector control affect the complex, dynamic, and evolving interactions between vector, virus, and host occurring at the macro (i.e., country or region) and micro (i.e., province, district, village or neighborhood) spatial scales?
2) How will vaccination and vector control affect the complex, dynamic, and evolving interactions between vector, virus, and host at the population (i.e., *Aedes* species, DENV serotypes and genotypes, and people of various ethnic backgrounds) level?

3) How will "herd immunity" be affected and how will this influence DENV evolution at the micro and macro population levels and the associated observed clinical phenotypes?
4) What effect, if any, will existing herd immunity (due to vaccination or natural infection) to non-dengue flaviviruses (e.g. Japanese encephalitis and yellow fever viruses) have on DENV transmission and the observed clinical phenotypes following infection?

The overarching study objective was to explore DENV transmission dynamics and virus-vector-host interactions prior to, during, and following the introduction of dengue vaccines into central Thailand. The authors pursued this objective by building upon observations from previous prospective studies such as the focality of DENV transmission, presence or history of fever increasing the likelihood of isolating virus from a household, and the significance of year-round DENV transmission [8-10]. Study methods were modified in an effort to maximize DENV isolation rates from human hosts and mosquito vectors and further explore earlier observations, which included:

1) Enrolling initiate and associate cases throughout the year (i.e., high dengue season and low dengue season) to explore trends in seasonal and spatial DENV transmission;
2) Only enrolling DENV PCR+ initiate cases to increase the likelihood of capturing active transmission;
3) Only enrolling associates with fever or a history of fever within the last 7 days or sharing a household with someone meeting these criteria to increase the likelihood of identifying associates with recent infection;
4) Reassessing associates for the occurrence of fever between the acute and convalescent blood collection to capture additional viremic cases;

5) Expanding the age of enrollment to include children above the age of 6 months and adults to improve understanding of transmission inside the home; and
6) Extending the enrollment of associates and mosquito collection from a 100 to 200m (meter) radius around the initiate.

In this report the authors describe initiation of the baseline phase (i.e. prior to vaccine introduction) and include detailed accounts of study methodology and the entomologic, clinical, epidemiologic, virologic, serologic, and molecular characterization of human cases captured by active and passive surveillance methods between November 2009 and December 2010.

Methods:

Ethics Statement

The study protocol was approved by the Institutional Review Boards (IRB) of the Thai Ministry of Public Health (MOPH), Walter Reed Army Institute of Research (WRAIR), and the State University of New York (SUNY), Upstate Medical University. The IRB's of the University of California, Davis (UCD), University of Rhode Island (URI) and University at Buffalo established relying agreements with WRAIR IRB.

All study subjects engaged in documented informed consent or assent process, as applicable, prior to participating in any study activities. In the event the subject was unable to participate in the informed consent/assent process, a recognized health care proxy represented them in the process and documented consent. From this point forward, when the authors discuss consent, assent is also implied as applicable.

Role of the Funding Source

Funding sources for this project included National Institutes of Health grants R01 GM083224-01 and P01 AI034533. Additional funding was provided by the US Military Infectious Diseases Research Program. The funding sources had no involvement in study design, data collection, analysis or interpretation, report writing, or publication submission. The corresponding author had full access to all study data and final responsibility for the decision for publication.

Study Location

The study was conducted in Kamphaeng Phet (KPP) province in north-central Thailand. There were 725,846 registered residents of KPP in 2009 (Thailand, Department of Provincial Administration, 2010). The KPP Provincial Hospital (KPPPH) is located in the province's

central district. The Armed Forces Research Institute of Medical Sciences (AFRIMS), Department of Virology field site (KPP AFRIMS Virology Research Unit [KAVRU]) is located on the KPPPH grounds. The Department of Entomology, AFRIMS field site is located a short distance from KPPPH [11].

Demographics of house residents were collected and house spatial coordinates were identified using a Geographic Positioning System hand held unit (Trimble(r) GeoXH(tm), GeoExplorer(r) 2008 series, Trimble Navigation Limited, CO, USA) and geo-coded into a Geographic Information System (GIS) database (CorporationArcMap™, version 9.1, ESRI, CA, USA).

Study Definitions

The authors recognize the potential confusion using terms such as, "index case," "contacts," and "cluster investigations." As the index case may not be the true first infection in space and time and the contact may not be a true infection resulting from DENV transmission from the initiating case we have attempted to more accurately define the relationships investigated in this study without making claims of causation. The hospitalized dengue cases serving as the initiator of community transmission investigations are referred to as the "initiating case," or "initiate". The enrolled individuals residing within the initiate's home or within 200 meters of the initiate are referred to as "associates" and if found to have DENV infection, as "associate cases". The combination of the initiate, associate and associate cases is referred to as a "spatiotemporal group."

Symptomatic DENV infection in either initiating or associated cases are defined as any febrile illness (reported or measured fever) paired with a confirmatory molecular or serologic assay run on the acute or acute and convalescent blood sample pair, respectively. Subclinical

DENV infections are defined as associated cases (all initiating cases were symptomatic and hospitalized) with a positive molecular or serologic result, but without reported or measured fever during the period between the acute and convalescent blood sample collection (0 to 14 days).

Subjects are serologically classified as having an "acute" DENV infection if their acute and/or convalescent blood sample pair (or day 0 and/or day 14 blood samples in associates) was dengue IgM positive; or, if IgM is negative, IgG is positive with rising titer. Subjects are classified as "recent" DENV infection if IgM was negative and IgG was positive with declining titer. These are further categorized as "primary" infection if the IgM to IgG ratio is ≥1·8 and "secondary" infection if the ratio is <1·8 [12].

Initiate Case Identification and Evaluation

KAVRU provides the KPPPH dengue diagnostic research assays for patients presenting with fever or history of fever and dengue-like symptoms as outlined in the World Health Organization (WHO) guidelines for the diagnosis and treatment of dengue [13]. Hospital staff members identified suspected dengue cases, completed and documented the informed consent process allowing the testing of a blood sample, and then sent the sample to KAVRU for RT-PCR testing. Virus RNA was extracted from human serum or mosquito suspension using Qiagen Viral RNA Extraction kits. Serotype-specific DNA fragments from each unknown sample were amplified by TaqDNA polymerase through RT-PCR performed at KAVRU following modifications to the Lanciotti protocol [14]. Suspected cases >6 months of age, that provided a sample collected within the prior 24 hours, and had detectable DENV RNA by RT-PCR were provided the opportunity for study enrollment as initiate cases. Following acquisition of informed consent, the acute blood sample was accessed and served as the baseline sample. Demographic and clinical laboratory information was collected. Initiate case house spatial coordinates were

recorded and geo-coded into a GIS database. Mosquitoes inside and outside of the initiate home were collected by aspiration and processed as described in preparation for DENV RNA detection RT-PCR [10]. In approximately 14 days a second blood sample was collected from the initiating case for serologic testing by in-house IgM/IgG EIA.

Associate Case Identification and Evaluation

Residents >6 months of age sharing the initiate household as their primary residence were provided the opportunity for study enrollment. Residents previously enrolled as an associate within the past 6 months were excluded in an attempt to improve geographic diversity and the likelihood of isolating DENV (i.e. reduce chances of recent infection and lingering homo- or heterotypic immunity). Following the informed consent process, clinical information and a blood sample were collected. In approximately 14 days a second blood sample was collected for serologic testing. General well-being of the associates was tracked by active surveillance (i.e., combination of self-reporting and outreach by staff via phone or daily home visit by village health worker over the 14 day period). If, between the day of enrollment and the day of convalescent blood sampling, the associate develops fever, a second acute blood sample was collected and the 14 day "clock" started again. Therefore, it was possible an associate might have two acute samples, one triggered as an associate of the initiating case (acute sample #1) and one triggered by the development of illness (acute sample #2), as well as one convalescent sample.

Individuals not residing within the same house as the initiate case, but living in a home within a 200m radius of the initiate case were also considered for enrollment as associates. If anyone within the home reported an active fever or history of fever (temperature ≥38ºC) within the past 7 days, all residents of the home >6 months of age were eligible for enrollment.

Following the informed consent process, residents had clinical and demographic information and an acute blood sample collected. Associates were followed and blood collected as detailed above.

House Mapping, Associate Case Home Identification, and Entomologic Sampling

GPS mapped initiate households were used to construct a digital map, enabling the team to precisely identify houses located within a 200m radius of the initiate case [10,15]. Study nurses visited households starting closest to the initiate house and moving in sequential fashion to the periphery of the area and back to the initiate house, then back out again along a different line until the circle was complete or 25 eligible associates were enrolled, whichever occurred first (Figure 1).

On day 1 of each initiate/associate case investigation, adult *Ae aegypti* were collected using standard backpack aspirators from inside and within the immediate vicinity of each potential associate's house. The end of each aspirator tube was fitted with a 1-pint cardboard cage. After completing the collection for each home, the cage was labeled and stored on dry ice for transportation to the laboratory where the chilled mosquitoes were examined, speciated, and processed for DENV detection. A thorough adult aspiration collection usually requires ~10-15 minutes per house. It was estimated that approximately 25% of the adult *Ae. aegypti* were captured in a single pass through the house (TW Scott, unpublished data). All specimens were identified to species by entomology field supervisors to ensure speciation accuracy and quality control. As previously mentioned, mosquitoes inside and outside of the initiate home were collected by aspiration and processed as described with serotype–specific DENV RNA detection RT-PCR performed on each individual mosquito [10].

Statistical Analyses

Data were analyzed using SPSS (SPSS for Windows version 19) and R (The R Project for Statistical Computing 2.12). Demographic, clinical, and laboratory parameters were analyzed at the initiate, associate, and house levels. Student's *t*-test and analysis of variance (ANOVA) were used to test for differences in continuous variables. Fisher's exact test and Pearson's chi-squared were used to examine associations between categorical variables.

Results

Enrollment of Initiates and Associates

Figure 2 depicts enrollment of initiates and associates and results of serologic and molecular testing for DENV infection. Approximately 49% of patients hospitalized with suspected dengue were PCR+ and of those 62% were enrolled in the study. Of the 4,345 households within a 200m radius of the 93 initiate homes, 124 (2.8%) had someone with active fever or history of fever; 115 of these households had volunteers who consented to enroll. Initiate and associate households contained 793 individuals meeting enrollment criteria, and 438 (55%) consented to enroll as associates. Complete serologic data was available on 93% of the enrolled associates and of these 21% had a positive dengue serology. Of the 86 associates with positive serology, 42% also were PCR+. All PCR+ associates had fever at the time of enrollment. Eleven associates who enrolled without fever developed fever following their first acute blood sampling; of these 8 were serology positive and 7 PCR+.

Of note logistic limitations guided enrollment on days with heavy dengue patient census accounting for the difference between the 149 PCR+ cases meeting inclusion criteria and the 93 ultimately enrolled. In these instances the study team randomly selected which initiate cases would lead to an associate case investigation using a random numbers table.

Characteristics of the Initiating Cases

240 Each of the 93 initiate cases was a hospitalized acute, PCR+ DENV infection (Table 1).
241 Infections with DENV-2 were most numerous followed by DENV-3 and then DENV-1; there
242 were no DENV-4 infections. Most cases were acute secondary DENV infections (85%).
243 Approximately half of all cases were DF (48%) and half DHF (52%); there were no deaths among
244 the initiates.
245 Associated Cases Available for Enrollment
246 The number of associate houses within 200m of an initiating case ranged from 1-232 with
247 a mean of 47.7 (SD 42 houses). The number of households enrolled ranged from 1-9 with a mean
248 of 2.2 (SD 1.5 households). For each spatiotemporal group there was a range of associates
249 enrolled from 0-18 people with a mean of 4.7 (SD 4.0 people).
250 Characteristics of the Associated Cases
251 The majority (79%) of 409 enrolled associates had no serologic evidence of infection,
252 20% had evidence of an acute DENV infection, and there was 1 case with evidence of JE
253 infection (IgM positive, no encephalitis; Table 1). There was complete concordance between the
254 DENV serotype of the initiating case and the DENV serotypes detected in associate cases in that
255 spatiotemporal unit. Nested PCR results among associates revealed that DENV-3 was the most
256 common infection followed by DENV-2 and then DENV-1.
257 There was a significant difference in the reporting of nausea between primary and
258 secondary DENV infections and significant variation in reporting of headache, rhinorrhea, cough,
259 and retro-orbital pain among the age groups (Tables 2 and 3). There was a statistically significant
260 difference in the probability of an associate experiencing a DENV infection based on the DENV
261 type infecting the initiate. DENV-3 infection in the initiate carried the highest probability of
262 associate infection (Table 4).

Spatial Distribution of Associate Case Households

Closer proximity of a household to the initiate was correlated with an increased associate household enrollment (Table 5). Households further from the initiate household had a lower rate of DENV infection among associate household residents (Table 6). This pattern of transmission was not gradual with distance. In an associate from a household with fever for example, the rate of infection was similarly high (around 0.3) within 120 meters and very low in households with fever beyond 120 meters. This would indicate that spatiotemporal transmission in this investigation was primarily limited to 120 meters and extending the radius from 100 to 200 meters did not substantially increase the efficiency of detecting dengue cases or viremia.

DENV Infection in *Ae. aegypti* Collected in the Homes of Human Dengue Cases

A total of 3,565 *Ae. aegypti* were collected from 4,438 households (i.e. all households). A total of 233 (6.5%) mosquitoes were from initiate households, of which 23 (9.9%) were DENV PCR+. There was a greater likelihood one or more collected mosquitoes was DENV PCR+ within the initiate house or a house closer to the initiate's house (Table 7).

There were 162 initiate households with at least one associate resident who had complete serology and 212 associate households with at least one associate with complete serology. Of the initiate households, female *Ae. aegypti* were collected in 54.9% and 58.5% of the associate households had female *Ae. aegypti* collected. Of the female mosquitoes collected in initiate households 29% of households had a DENV PCR+ mosquito while 6.5% of associate households had a DENV PCR+ mosquito. There was a higher likelihood of finding a DENV PCR+ mosquito in an initiate household (Table 8). Of the 31 initiate households with a DENV serology and an associate resident, 54.8% of the houses had female *Ae. aegypti* captured and

64.7% of these households had a DENV PCR+ mosquito. Of the 42 associate households with a DENV serology + associate, 57.1% of households had a female *Ae. aegypti* mosquito and one (4.1%) of these households had a DENV PCR+ mosquito. Despite a similar likelihood of finding female *Ae. aegypti* in households with a DENV+ serology resident, there was a higher likelihood of finding DENV PCR+ mosquitoes in the initiate household (Table 8). In total, there were 46 households with a DENV PCR+ mosquito and an associate residing with complete serology; 80.4% were initiate households (Table 8.). There was a high degree of concordance between the isolated DENV serotypes from mosquitoes within a cluster and the infecting serotype of the index case (Table 9). In year 2010, only one cluster (10-077) was found discordant from the mosquito (DENV-2) as compared to the index case (DENV-1). In 2012, one cluster (12-025) was found DENV-1 in index case while PCR results were DENV-2 and DENV-4 in 2 mosquito samples. The rest of the clusters were concordant.

Discussion

Our current study further characterizes the complex transmission dynamics and virus-vector-host interactions in a well characterized spatial area around an infected viremic inpatient in Central Thailand. We demonstrated a significant relationship between spatial proximity to the initiate case and likelihood of detecting DENV from associate cases and *Ae aegypti* with higher than anticipated virus detection from both human hosts and mosquito vectors. We propose that the sampling strategy described is valuable for ongoing surveillance of DENV transmission during and after field studies and the introduction of dengue vaccines.

The design of this study was built upon observations from previous prospective cohort studies of DENV transmission conducted with and without initiate case and associate

309 (contact/cluster) investigations [8-10,16,17]. Modifications were made to the study design in an
310 attempt to increase the detection of virus, symptomatic and subclinical associates of initiate cases,
311 and infected mosquito vectors. The result was a demographically diverse group of DENV
312 infected people representing a broad virologic, serologic, and clinical spectrum. Substantial virus
313 detection rates were observed in both human associate cases and mosquitoes.

314 The age range of initiate cases was surprisingly wide (2.6-56 years), and the mean age
315 was higher than expected at 18.7 years [18]. This observation is consistent with unpublished data
316 from the authors across additional dengue seasons and data from other sources. For example, in
317 2010, the Thailand MOPH reported that the highest case rate for DHF was in the 10-14 year age
318 group, and that the 5-9 and 15-24 year age groups had the second and third highest rates,
319 respectively (Thailand, Ministry of Public Health). These findings are in contrast to decades of
320 data where the mean age of hospitalized dengue was in the age range of 5-9 years [19-24]. One
321 explanation for the shift is that smaller birth cohorts had reduced number of susceptibles, thereby
322 impacting the force of infection and the time in a person's life when they acquire their first and
323 second DENV infection, the latter being more often associated with clinically significant disease
324 in Thailand [18].

325 We also enrolled associates across a wide age range (7 months – 94.2 years) with a mean
326 age almost double the initiate cases (31.4 years vs. 18.7 years). Serologic evidence of DENV
327 infection and symptomatic DENV infection was observed in a number of subjects aged 40 years
328 and older. Most of these were secondary (i.e., post-primary) DENV infections, but we also
329 detected primary DENV infections, defined serologically, in the 50-59 and 85+ year age groups.
330 Dengue occurs throughout the year with all four serotypes circulating with high rates of infection
331 and transmission (hyperendemic) in KPP. Pediatric cohort studies have demonstrated high

DENV infection attack rates (combined symptomatic and subclinical) in the range of 2.2% to 7.9% per year (average incidence 5.8%) over at least the preceding 10 years [9]. Based on these observations, it was assumed that lifelong KPP residents have experienced multiple DENV infections by their late 20's. A modified plaque reduction neutralization assay (single dilution neutralization assay, 1:30 dilution, 70% viral plaque reduction) completed on a cohort of children enrolled in a KPP prospective study from 1998 to 2002 revealed a gradually increasing prevalence of neutralizing antibodies to at least one DENV type from 45% among 4 year olds to 91% among 13 year olds (Yoon, IK, unpublished data) [10,16,25]. Our finding that all PCR+ associate cases had fever at the time of enrollment may also underscore the increasing infection burden in adults as they are more likely to experience symptomatic DENV infections compared to primary infection in children. Our observation of symptomatic dengue in older individuals merits reconsideration of traditional views on the force of infection in hyperendemic areas and the durability of homotypic and heterotypic immunity.

There was complete concordance between the infecting DENV serotypes in the initiate case and the corresponding associate cases. This finding supports the concept that DENV transmission is focal and serotype-conserved in space and time.[17,26] The authors' acknowledge that the assumption that each initiating case represents the true index (i.e., first) infection in each spatiotemporal group may be incorrect. Because most DENV infections are asymptomatic or not clinically severe enough to drive health care-seeking behavior it is possible the initiating case simply represents the first clinically overt infection in that defined geographic area.

The ratio of symptomatic to subclinical infection among primary infections was 1:0.2; the ratio in secondary infections was 1:0.4 and similar (1:0.4) among the 86 serologically positive

355 associates. These ratios are consistent with previous work by our group and others, which
356 reported symptomatic to subclinical ratios ranging from 1:0.9 to 1:18 [9,10,27-30]. The
357 differences in results are most likely associated with the study's surveillance focus in and around
358 symptomatic, PCR+ initiating cases. Previous studies conducted routine biologic sampling to
359 capture subclinical cases and cluster studies exploring houses with or without active or a recent
360 history of fever [9].

361 The probability of an associate becoming infected was associated with a number of factors
362 [31-33]. As mentioned above, a significant association existed between the DENV serotype
363 infecting the initiating case and the proportion of associates subsequently infected with the same
364 DENV serotype (p-value <0.001). Associates in samples initiated by a DENV-3 infection had a
365 30% chance of being serologically positive. The likelihood of infection was 19% for associates
366 in DENV-2 samples and 9% in DENV-1 samples. Taken by itself, this may point to viral
367 properties allowing for more efficient transmission, such as higher titer viremia in the human host
368 or mosquito vector, a longer duration of viremia increasing the potential window for transmission
369 to vectors and / or shorter incubation period in the mosquito. Another significant association was
370 between the distance a potential associate lived from an initiating case and the likelihood of
371 becoming infected (negative correlation, p-value <0.001). It is reasonable to assume the factors
372 culminating in an initiate infection (i.e., convergence of susceptible host and infected vector in
373 space and time) would also drive efficiency of transmission and associate infections until a
374 geographic barrier was introduced (i.e. next susceptible beyond the dispersal distance of the
375 vector) or other factors limited transmission (i.e., protective herd immunity of associates). We
376 did not define the relative roles of humans and mosquitoes in moving virus from one house to

another within clusters, although in general, humans tend to move more often and greater distances than *Ae. Aegypti* [31-33].

Approximately 29% of all households had female *Ae. aegypti* and 1.8% of the females collected were infected with DENV. Although initiating case houses and associate houses with a PCR+ contact had similar rates of female *Ae. aegypti* infestation (55% and 59%, respectively), the percentage of PCR+ mosquitoes was higher in the initiate case households (9.9%) compared to non-initiate households with and without a DENV positive contact, 1.2% and 1.1% respectively. There was a significant association (p-value < 0.001) between the distance of a home from the initiating case and the proportion of PCR+ mosquitoes found within the house. These observations are concordant with previous reports and intuitive; once a clinical case of dengue is identified there is a high likelihood of finding infected vector(s) co-residing in the same geographic location as the ill human.

Initiate cases represented the full spectrum of DENV infections clinically, serologically, and virologically. We consider the finding of more than 1 in 5 associates of an initiating case having serologic and, in some cases, clinical evidence of an acute DENV infection significant from an epidemiologic and transmission dynamics standpoint. These results represent the initial phase of a multi-year prospective study. The study design employed improved the efficiency of capturing DENV in associates and vector populations compared to previous efforts by focusing surveillance in areas and households where DENV transmission was most likely to be occurring. Our observations emphasize the focal spread of DENV and its spatial restriction associated with the mosquito vector and suggests that initiate cases were important in this study (ill and PCR+) in transmitting DENV to mosquitoes and thus to others living near the initiate. Previous studies support the observation that patient DENV viremia was a marker of human infectiousness and

blood meals containing high concentrations of DENV were positively associated with the prevalence of infectious mosquitoes.[34] Our results and study design may be useful when designing experiments to study fine scale patterns of DENV transmission and to increase detection of case contacts with inapparent infections.

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Conflicts of Interest

None of the authors on this manuscript have a conflict of interest with the manuscript and data presented.

Disclaimer

The opinions or assertions contained herein are the private views of the authors and are not to be construed as reflecting the official views of the United States Army, the United States Department of Defense, or the National Institutes of Health.

Key words: dengue; transmission; viremia, vector, flavivirus, arbovirus, epidemiology

Abbreviations: Analysis of variance, ANOVA; Armed Forces Research Institute of Medical Sciences, AFRIMS; Dengue virus DENV; Dengue hemorrhagic fever, DHF; Dengue shock syndrome, DSS; Deoxyribonucleic acid, DNA; Enzyme-linked immunosorbent assay, ELISA; Institutional Review Boards, IRB; Kamphaeng Phet, KPP; Kamphaeng Phet AFRIMS Virology Research Unit, KAVRU; Kamphaeng Phet Provincial Hospital, KPPPH; Polymerase chain reaction, PCR; Reverse transcriptase PCR, RT-PCR; Ribonucleic acid, RNA; State University of New York Upstate Medical Center, SUNY; Thai Ministry of Public Health, MOPH; University of Massachusetts Medical School, UMMS; University of California, Davis, UCD; Walter Reed Army Institute of Research, WRAIR; World Health Organization, WHO;

Author Contributions:

SJT- study design, study execution, data analysis, manuscript writing

JA- data analysis, manuscript writing

DT- study design, study execution, manuscript writing

IKY - study design, study execution, laboratory assays, data analysis, manuscript writing.

JR- study execution, entomologic data analysis, manuscript writing

AP- study execution, entomologic data analysis, manuscript writing

SI- - study design, study execution, data analysis, manuscript writing

TWS- - study design, study execution, data analysis, manuscript writing

ALR- study design, data interpretation, and editing manuscript

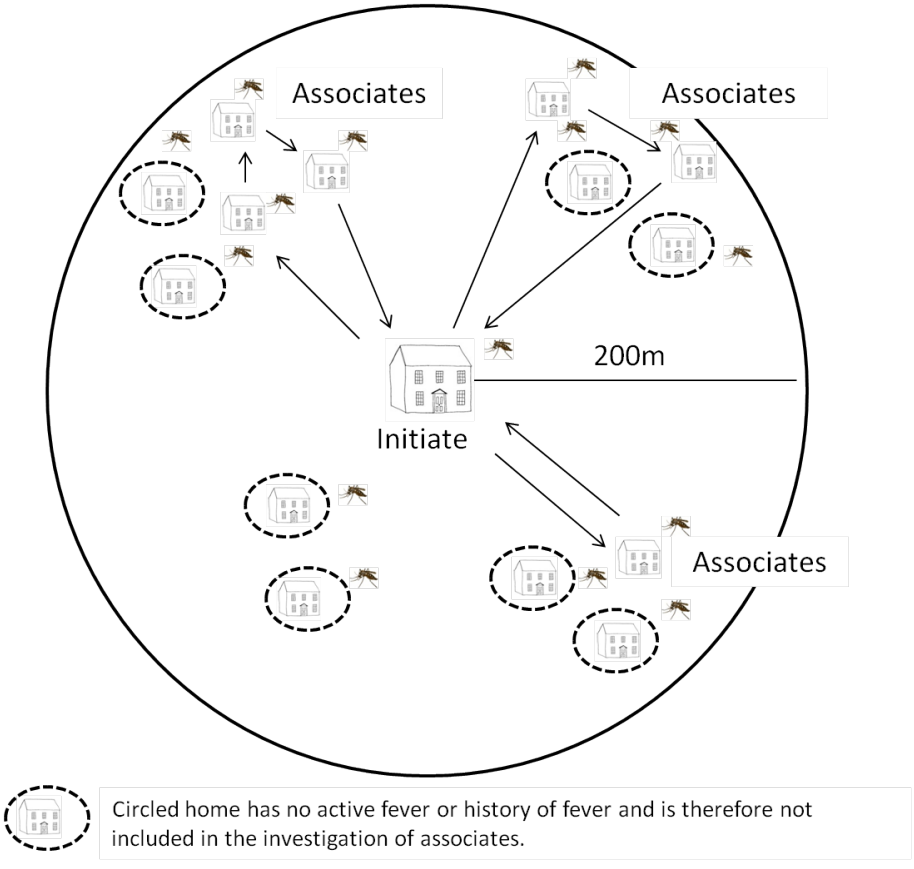
RVG – study design, study planning, interpreting results and editing manuscript

LL – study design, data interpretation, and editing manuscript

TPE- - study design, execution, data analysis, manuscript writing

Tables and Figures:

Figure 1. Method of identifying and enrolling associates around an initiating case. The clinical research team would begin at the initiate's home, identify a collection of homes within 200 meters of the initiate's home, identify homes with an occupant having active fever or history of fever within the past 7 days, and enroll associates only from those homes while bypassing homes without active fever or history of fever. The entomology research team, meanwhile, collected mosquitoes inside and outside the home from all homes within a 200-meter radius of the initiating case home regardless of fever history. Once investigations and enrollment of associates were completed in one grouping, the teams would return to the initiating case's home and then identify, using the aerial map, the next grouping of homes moving in a clockwise manner.

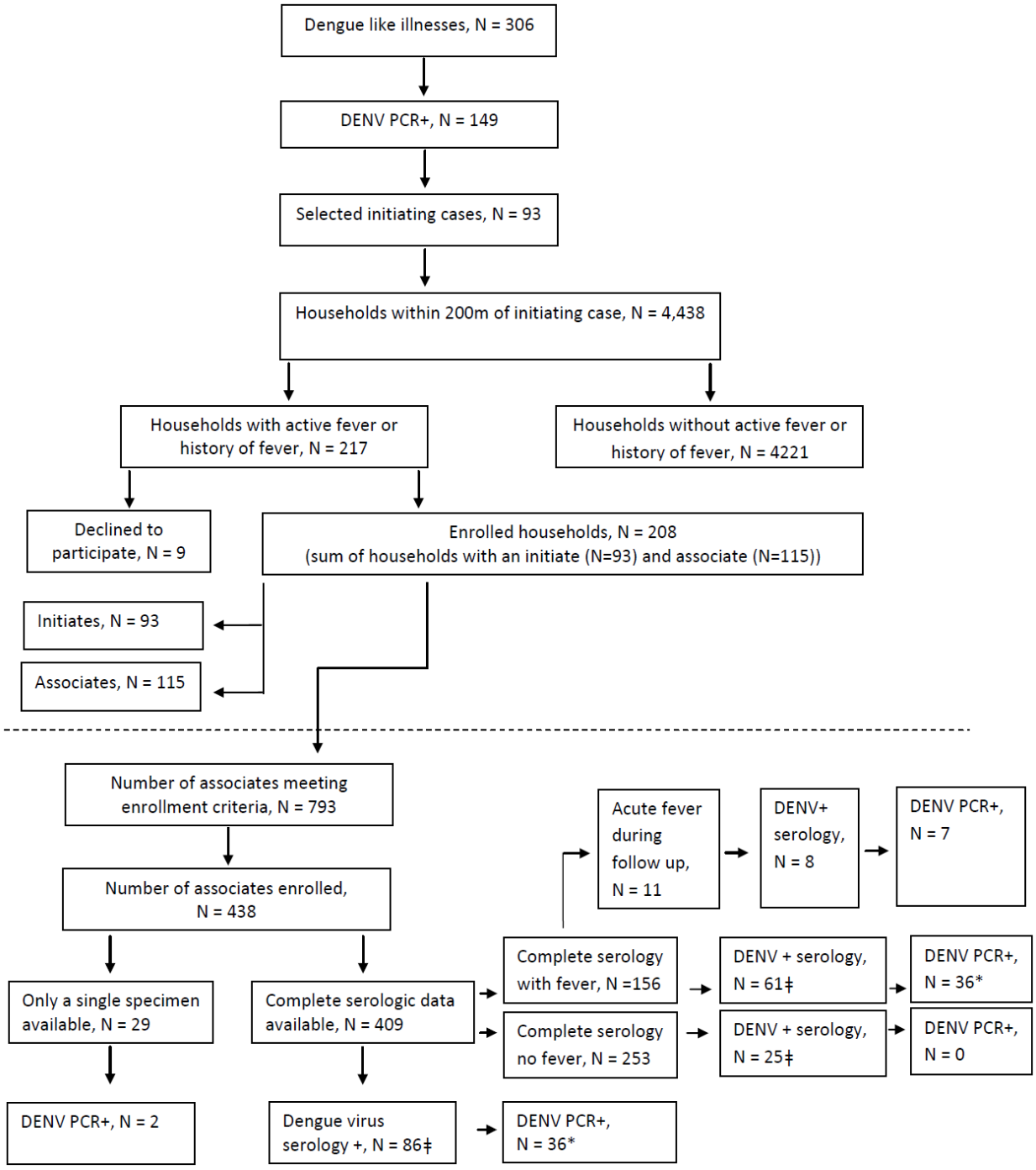


459 Table 1. Initiate and Associate Group Demographic Data and Infection Characterization.

		Initiate (N = 93)	Associate		
			Available Serology (N = 409)	Available Serology + Fever (N = 156)	Dengue + Serology (N = 86)
Age (years)					
	minimum	2.6	0.58	0.58	0.83
	maximum	56.0	94.2	77.0	82.0
	mean	18.7	31.4	20.1	23.1
Sex (%)					
	Female	48	54	57	55
	Male	52	46	43	45
Infecting DENV type (% of total typed cases)		Total case count = 93	Acute sample not available, no clinical symptoms.		Total case count = 86

	DENV-1	16			1
	DENV-2	60			16
	DENV-3	24			24
	DENV-4	0			0
	Not detected				58
Serology Results (% of total results)					
	Acute Primary	2	3	6	16
	Acute Secondary	85	17	25	80
	Recent Secondary	2	1	1	3
	No Serologic Diagnosis	8	79	67	
	JE Infection		0.2		

460 Figure 2. Flow Diagram of Initiate and Associate Enrollment and Testing Outcome



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462

463 Table 2. Symptom Complex in Primary vs. Secondary Associate Infections

Symptom	Primary (N=14)	Secondary (N=72)	p-value
Fever	11 (0.79)	50 (0.69)	0.749
Headache	5 (0.36)	37 (0.51)	0.384
Rhinorrhea	3 (0.21)	16 (0.22)	1.000
Anorexia	5 (0.36)	27 (0.38)	1.000
Cough	4 (0.29)	27 (0.38)	0.762
Nausea	0 (0.00)	25 (0.35)	0.008
Drowsiness	0 (0.00)	12 (0.17)	0.202
Muscle or Joint Pain	3 (0.21)	36 (0.50)	0.077
Abdominal Pain	2 (0.14)	15 (0.21)	0.727
Retro-orbital Pain	3 (0.21)	22 (0.31)	0.749
Rash	4 (0.29)	10 (0.14)	0.231
Diarrhea	2 (0.14)	14 (0.19)	1.000
Bleeding	0 (0.00)	5 (0.07)	0.586
Hospitalized	1 (0.07)	6 (0.05)	1.000

464 Table 3. Clinical Spectrum of EIA Positive Associates by Age Groups

Symptom	0-9 Years (N=22)	10-19 Years (N=31)	20-29 Years (N=10)	>30 Years (N=23)	p-value
Fever	18 (0.82)	24 (0.77)	8 (0.80)	11 (0.48)	0.052
Headache	6 (0.27)	20 (0.65)	7 (0.70)	9 (0.39)	0.021
Rhinorrhea	8 (0.36)	7 (0.23)	3 (0.30)	1 (0.04)	0.043
Anorexia	9 (0.41)	11 (0.35)	6 (0.60)	6 (0.26)	0.325
Cough	13 (0.59)	11 (0.35)	4 (0.40)	3 (0.13)	0.012
Nausea	4 (0.18)	12 (0.39)	5 (0.50)	4 (0.17)	0.101
Drowsiness	2 (0.09)	7 (0.23)	1 (0.10)	2 (0.09)	0.519
Muscle or Joint Pain	7 (0.32)	16 (0.52)	7 (0.70)	9 (0.39)	0.189
Abdominal Pain	3 (0.14)	8 (0.26)	2 (0.20)	4 (0.17)	0.741
Retro-orbital Pain	1 (0.05)	15 (0.48)	4 (0.40)	5 (0.22)	0.002
Rash	5 (0.23)	6 (0.19)	2 (0.20)	1 (0.04)	0.282
Diarrhea	2 (0.09)	8 (0.26)	3 (0.30)	3 (0.13)	0.285
Bleeding	2 (0.09)	2 (0.06)	1 (0.10)	0 (0.00)	0.440
Hospitalized	1 (0.05)	3 (0.10)	2 (0.20)	1 (0.04)	0.432

Table 4. Probability of Dengue Virus Infection in Associates According to the Initiating Case Infecting DENV Serotype.

	Infecting DENV of Initiate		
Serologic Diagnosis	DENV-1	DENV-2	DENV-3
EIA Negative	43	191	88
EIA Positive	4 (0.09)	44 (0.19)	38 (0.30)

Footnote: Numbers in parentheses are the proportions EIA positive. The infecting DENV type is taken from the initiating case. The differences are significant (Fisher's exact test, p-value = 0.003).

471 Table 5. Spatial Distribution of Households and Enrolled Households

472

		Distance from Initiate Household (meters)			
	>0-40	>40-80	>80-120	>120-160	>160-200
Total Households	539	814	971	1051	1063
Enrolled Households	103 (0.191)	53 (0.065)	32 (0.033)	13 (0.012)	7 (0.007)

473 Footnote: Numbers in parentheses indicate proportions of houses enrolled for each distance

474 category. Differences are significant, p-value < 0.001 by Fisher's exact test –.

475 Table 6. Spatial Distribution of Associates and Associates with DENV Infection by Serology

	Initiate Household	Distance from Initiate Household				
		>0-40	>40-80	>80-120	>120-160	>160-200
Total Associates with Available Serology	187	41	67	51	38	25
EIA Positive Associates	42 (0.225)	14 (0.341)	12 (0.179)	16 (0.314)	0 (0.000)	2 (0.080)

476 Footnote: Numbers in parentheses indicate proportions of associates that were EIA positive
477 within the initiate house and for each distance category. Differences are significant, p-value <
478 0.001 by Fisher's exact test.

479

480 Table 7. Spatial Distribution of Female *Ae. aegypti* within Houses and PCR+ Mosquitoes within

481 Houses

482

	Initiate Household	Distance to Initiate Household				
		>0-40	>40-80	>80-120	>120-160	>160-200
Total Mosquitoes	233	328	545	733	745	981
PCR+ Mosquitoes	23 (0.099)	10 (0.030)	7 (0.013)	13 (0.018)	7 (0.009)	3 (0.003)

483 Footnote: Numbers in parentheses indicate proportions of mosquitoes that were PCR+ for each

484 distance category. Differences are significant, p-value < 0.001 by Fisher's exact test.

485

Table 8. Associate Infection Rates by Infection Status of Female *Ae. aegypti* Captured in Households.

	Initiate Houses			Associate Houses		
	PCR Positive Female *Ae.aegypti*	Females Captured *Ae.aegypti*	No Females Captured *Ae.aegypti*	PCR Positive Females *Ae.aegypti*	Females Captured *Ae.aegypti*	No Females Captured *Ae.aegypti*
Total Associates with Available Serology	26	88	71	8	125	90
Serology Positive Associates	11 (0.423)	17 (0.193)	14 (0.197)	1 (0.125)	25 (0.200)	18 (0.200)

Footnote: Numbers in parentheses indicate proportions of associates that were serology positive within the initiate house and for each category.

Table Nine. Isolated DENV serotypes from clusters.

Year	2009-2010	2009-2012
Number of Ae. Samples	3545	9322
DENV-1	5	13
DENV-2	36	87
DENV-3	22	28
DENV-4	0	3

Year 2010, one cluster (10-077) was found discordant mosquito result (DENV-2) from the index case (DENV-1)

Year 2012, one cluster (12-025) was found DENV-1 in index case while PCR result were DENV-2 and DENV-4 in 2 mosquito samples.

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