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Failure of space spraying to eliminate dengue virus-infected *Aedes aegypti* may explain failure to prevent secondary cases in Southern Thailand

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Dengue is a febrile disease caused by a member of the Flaviviridae family called dengue virus (DENV). DENV is primarily transmitted to humans by blood ingestion of *Aedes (Ae.) aegypti*[1,2]. Space spraying is a vector control measure recommended by the World Health Organization (WHO) for eliminating vector mosquitos and preventing further infections in an epidemic setting[3]. Evidence of the effectiveness of space spraying in reducing vector mosquito populations and risk of contracting DENV is limited and inconclusive[4]. This study aimed to implement dengue virologic surveillance for assessing the effectiveness of space spraying in eliminating infected female *Ae. aegypti* over the course of spraying.

This study was approved by the Institutional Ethics Committee of the Faculty of Medicine (registration number: REC. 62-200-18-1) and the Animal Ethics Committee of the Faculty of Science (registration number: 2562-10-035), Prince of Songkla University, Thailand.

Adult *Ae. aegypti* samples were collected from households located within the targeted communities for space spraying in response to dengue cases in endemic areas in Songkhla, Thailand. The selected households consisted of index case households and surrounding households within the spraying area. Mosquito collection was conducted 5 times in each household (before, day of spraying, and 2, 4 and 6 days after spraying). Collected *Ae. aegypti* from each household on each collection day were placed into pools of 1 to 5 *Ae. aegypti* of the same sex for detection processes. RNA was extracted from the pooled samples, and were tested for DENV using a one-step real-time reverse transcription-polymerase chain reaction (RT-PCR).

The main outcome variable is the number of infected *Ae. aegypti* pooled samples. We used stratified jitter plots to identify the changes, characteristics, and distributional pattern of *Ae. aegypti* pooled samples over the course of spraying. Fisher's exact test or *Chi*-squared test was used to assess significant differences between/ among proportions, where appropriate. The level of significance in this study was set at P < 0.05.

The distribution and characteristics of female and male Ae. aegypti

pooled samples in 18 study houses are illustrated in Figure 1. Eight of 18 sampled households (44.44%), including one index case household, had at least 1 pool that consisted of at least 1 infected female *Ae. aegypti* from day 1 to day 7 after spraying. For male pools, the respective number was 9 of 18 households (50.00%). Table 1 summarizes the number of *Ae. aegypti* pooled samples and DENV prevalence by sex and serotype. The prevalence of DENV-2 (9.69%) was higher than DENV-3 (1.18%). The association between the 2 sexes and 3 serotypes was tested using Fisher's exact test. The *P* value was 0.424 indicating no evidence of an association between the sexes of *Ae. aegypti* and serotypes. Furthermore, the number of infected pooled samples in 5 collection days were 14, 4, 6, 10 and 12, while the total pooled samples were 90, 32, 80, 70 and 105. The was no association between the DENV prevalence and collection day, *Chi*-squared *P* value was 0.675.

This study showed that the circulation of DENV still remained after the implementation of space spraying as nearly half of the sample households contained at least 1 pool of active female *Ae. aegypti* with at least 1 positive DENV infection. In addition, half of the households contained at least 1 pool of male *Ae. aegypti* with at least 1 positive DENV infection which was unexpected, as male *Ae. aegypti* does not feed on human blood. Although males do not contribute to the direct transmission of DENV, this finding indicates possible transovarial or venereal transmission of the disease[5–8], which requires further investigation in order to determine the the

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Figure 1. Distribution and characteristics of Aedes aegypti pools in dengue endemic areas stratified by household. (A) Female; (B) Male.

Day of collection

(-ve)

DENV infection	Female pooled samples	Male pooled samples	Total pooled samples
	( <i>n</i> =222)	(n=201)	( <i>n</i> =423)
Negative	202 (90.99)	175 (87.06)	377 (89.13)
DENV-2	18 (8.11)	23 (11.44)	41 (9.69)
DENV-3	2 (0.90)	3 (1.49)	5 (1.18)

Fisher's exact test, P value = 0.424.

underlying mechanism, and together with persistent presence of infected *Ae. aegypti*, these factors may be a contributing cause of secondary dengue cases after space spraying.

# **Conflict of interest statement**

The authors declare there is no conflict of interest.

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## **Authors' contributions**

K.C., T.P. and V.C. conceptualized the study. K.C., T.P. and N.N.

performed data collection. Both K.C. and E.B.M. performed data entry and analysis. K.C. prepared the original draft of the manuscript. T.P., V.C., E.B.M. and N.N. reviewed and edited the manuscript. All authors contributed to the final version of the manuscript. V.C. supervised the project.

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