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Étude du potentiel des biomarqueurs sérologiques pour évaluer le risque de transmission de la dengue dans le nord-est de la Thaïlande

Bénédicte Fustec

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THÈSE POUR OBTENIR LE GRADE DE DOCTEUR DE L'UNIVERSITÉ DE MONTPELLIER

En Biologie-Santé

École doctorale CBS2

Unité de recherche "MIVEGEC: Maladies Infectieuses et Vecteurs: Ecologie, Génétique,
Evolution et Contrôle" UMR IRD 224-CNRS 5290-Université Montpellier

Étude du potentiel des biomarqueurs sérologiques pour évaluer le risque de transmission de la dengue dans le nord-est de la Thaïlande.

Présentée par Benedicte Fustec

Le 21 Décembre 2020

Sous la direction de Vincent CORBEL et Hans J. OVERGAARD

Devant le jury composé de

Mme. Nicole L. Achee, Professeur, University of Notre-Dame	Rapporteur
Mr. Lionel Almeras, CR, Université d'Aix-Marseille	Rapporteur
Mr. Jean-Pierre Dedet, Professeur Emérite Université de Montpellier	Président du jury
Mme. Audrey Lenhart, CR, United States Centers for Diseases Control and Prevention	Examineur
Mr. Richard Paul, CR, Global Health, Pasteur Institute	Examineur
Mr. Vincent Corbel, Directeur de recherche, HDR, IRD.	Directeur de thèse
Mr. Hans J. Overgaard, CR, University of Life Sciences Norway	Co-encadrant de thèse



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**Exploring the potential of serological biomarkers to assess the
risk of dengue transmission in north-Eastern Thailand.**

Defended by Benedicte Fustec

21st of December 2020

Under the supervision of Vincent CORBEL and Hans J. OVERGAARD

Before the jury composed of

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Contents

<u>LIST OF FIGURES</u>	<u>9</u>
<u>LIST OF TABLES</u>	<u>11</u>
<u>LIST OF ABBREVIATIONS</u>	<u>12</u>
<u>LIST OF PUBLICATIONS</u>	<u>16</u>
<u>LIST OF SCIENTIFIC COMMUNICATIONS</u>	<u>17</u>
<u>ACKNOWLEDGEMENTS</u>	<u>18</u>
<u>PREAMBLE.....</u>	<u>21</u>
<u>FIRST PART: GENERALITIES</u>	<u>24</u>
1. DENGUE DISEASE.....	24
1.2. Epidemiology.....	24
1.3. Viruses.....	26
1.4. Dengue distribution	28
1.4.1. Dengue in South East Asia.....	29
1.4.2. Dengue in Western Pacific region.....	30
1.4.3. Dengue in Americas	30
1.4.4. Dengue in Africa	31
1.4.5. Dengue in the Mediterranean region.....	31
1.5. Symptoms.....	32
1.6. Diagnostic.....	33
1.6.1. Epidemiological diagnostic	34

1.6.2. Laboratory diagnostic	34
1.6.2.1. Direct diagnosis	34
1.6.2.2. Indirect diagnosis	36
2. THE DENGUE VECTORS	39
2.1. Life cycle	40
2.2. Aedes vectors	41
2.2.1. Aedes aegypti	42
2.2.2. Aedes albopictus	43
3. GLOBAL STRATEGIES FOR DENGUE PREVENTION AND CONTROL	45
3.1. Vaccine	45
3.2. Treatment & prophylaxis	46
3.3. Dengue vector control	47
3.3.1. Integrated Aedes management strategies	48
3.3.1.1. Social mobilisation and community engagement	49
3.3.1.2. Larval control	51
3.3.1.3. Adult control	52
3.3.2. Alternative strategies for vector control	56
3.3.2.1. Wolbachia-based strategies	56
3.3.2.2. Genetically modified mosquitoes	57
3.3.2.3. Other tools currently under evaluation	57
3.3.3. Insecticide resistance	58
3.3.3.1. Global distribution	58
3.3.3.2. Mechanisms	60
3.3.3.3. Impact on vector control	61

4. METHODS AND INDICATORS FOR ASSESSING AND PREDICTING THE RISK OF DENGUE TRANSMISSION	61
4.1. Definition: concept of transmission	61
4.2. Epidemiological surveillance and it's limitations	64
4.3. Mathematical tools and their limitations	65
4.4. Entomology surveillance and it's limitations	66
4.4.1. Immature indices and their limitations	70
4.4.2. Adult indices and their limitations	71
4.5. Serological tools to estimate dengue transmission risk	71
4.5.1. Concept	71
4.5.2. Application of salivary biomarkers to Aedes transmitted diseases	73
<u>SECOND PART: CONTEXT OF THE THESIS</u>	76
1. CHALLENGES IN PREDICTING DENGUE TRANSMISSION AND OUTBREAKS IN THAILAND	76
2. THE DENGUE INDEX PROJECT	78
3. OBJECTIVES OF THE THESIS	79
3.1. Objective #1: Assessing the spatial and temporal dynamic of dengue in North-eastern Thailand	80
3.2. Objective #2: Addressing the complex relationship between <i>Aedes</i> vectors, dengue transmission and socio-economic factors	80
3.3. Objective #3: Assessing fine-scale variations in human exposure to <i>Aedes</i> mosquito bites using salivary biomarker during a vector control intervention	81
3.4. Objective #4: Evaluating the impact of vector control intervention on the selection of insecticide resistance in dengue vectors	81
4. STUDY DESIGN	82
4.1. Study area	82

4.2. Case control study.....	82
4.3. Cluster-randomized controlled trial	87
<u>THIRD PART: RESULTS OF THE THESIS</u>	<u>91</u>
CHAPTER 1. ASSESSING THE SPATIAL AND TEMPORAL PATTERNS OF DENGUE INCIDENCE IN NORTH-EASTERN THAILAND	91
Summary of the results:.....	92
CHAPTER 2: ADDRESSING THE COMPLEX RELATIONSHIPS BETWEEN Aedes VECTORS, SOCIO-ECONOMICS AND DENGUE TRANSMISSION IN NORTH-EASTERN THAILAND.....	95
Summary of the results:.....	95
CHAPTER 3: ASSESSING FINE SCALE VARIATIONS IN HUMAN EXPOSURE TO Aedes MOSQUITO BITES USING Aedes SALIVARY BIOMARKER DURING A RANDOMIZED VECTOR CONTROL INTERVENTION TRIAL.....	99
Summary of results:.....	100
CHAPTER 4: ASSESSING THE IMPACT OF VECTOR CONTROL INTERVENTION ON THE SELECTION OF INSECTICIDE RESISTANCE GENES IN DENGUE VECTORS.....	103
Summary of results:.....	103
<u>FOURTH PART: DISCUSSION AND PERSPECTIVES OF THE THESIS</u>	<u>107</u>
Challenges in assessing dengue transmission risk using conventional entomology indices	110
Potential of serological biomarkers for assessing Aedes-human relationships	112
Potential of serological biomarkers for assessing dengue transmission risk.....	113
Prospect of serological biomarkers for assessing vector control interventions.....	114
Barriers to dengue vector control in Thailand.....	115
<u>CONCLUSION</u>	<u>116</u>

REFERENCES.....117

RESUME DE LA THESE.....148

List of figures

Figure 1: Overlapping of global distribution of major mosquito borne diseases (malaria, dengue, chikungunya, Zika, yellow fever, Japanese encephalitis, lymphatic filariasis).....	23
Figure 2: Spread of dengue in the world since 1943 from Messina et al.	25
Figure 3: Dengue virus genome from Guzman et al.	27
Figure 4: Dengue virus envelope structure from Rey.....	28
Figure 5: Global distributions of dengue serotypes in 1970 and 2004 from Mackenzie et al.....	29
Figure 6: Dengue cases classification and level of severity, from WHO.....	33
Figure 7: Dengue illness course from WHO.....	34
Figure 8: NS1 positive Rapid Diagnostic Test.....	35
Figure 9: Time-line of immunoglobulins in primary and secondary dengue infection from WHO.....	36
Figure 10: Principle of hemagglutination assay for dengue diagnosis.....	37
Figure 11: Principle of MAC-ELISA experiment.....	38
Figure 12: IgG positive and IgM negative RDT.....	39
Figure 13: Aedes simplified life cycle from Biogents©.....	40
Figure 14: Aedes aegypti (left) and Aedes albopictus (right).....	42
Figure 15: Map showing the predicted distribution of <i>Aedes aegypti</i> from Kraemer et al.....	43
Figure 16: Map showing the predicted distribution of <i>Aedes albopictus</i> from Kraemer et al.....	44
Figure 17: Global Vector Control Response Framework.....	48
Figure 18: Conceptual framework of the IAM system from Roiz et al.....	49

Figure 19: Insecticide resistance against pyrethroid and organophosphate worldwide from Moyes et al.....	59
Figure 20: Dengue transmission cycles from Ahammad et al.	62
Figure 21: Human-vector relationships during arthropod-borne diseases from Sagna et al.	72
Figure 22: Amino-acid sequence of Nterm-34 kDa peptide.	73
Figure 23: Ab response to Nterm-34 kDa salivary peptide according to the season in children from Benin from Elanga et al.....	74
Figure 24: Number of monthly dengue cases reported in Thailand 2005-2015.	76
Figure 25: Number of dengue reported cases in Khon Kaen, Roi Et, Kalasin and Maha Sarakham provinces, in northeastern Thailand between 2005-2019.	78
Figure 26: Isan typical landscape.	82
Figure 27: Map and characteristics of study sites of the case-control study in northeastern Thailand.....	84
Figure 28: Flow diagram of case-control study design.	85
Figure 29: Map of the study sites of the RCT.....	88
Figure 30: Flow chart of the RCT study design.....	89
Figure 31: Mean monthly dengue incidence per 100,000 persons, from Phanitchat et al..	93
Figure 32: Mean dengue prevalence by sub-district, from Phanitchat et al.....	94
Figure 33: Immune response to <i>Aedes</i> salivary peptide Nterm-34 (Δ OD) in dengue case and control patients.	97
Figure 34: Seasonal variation of human IgG and A1c.....	100
Figure 35: Copy number variant in CCEAE3A gene in <i>Ae. aegypti</i> populations before and after PPF treatment.....	106

List of tables

Table 1: Dengue vaccines under development from Yauch and Shresta	46
Table 2: Strengths and limitations of larval control strategies from Roiz et al.	50
Table 3: Adult control strategies for Aedes-borne disease from Roiz et al.	53
Table 4: Strengths and weakness of entomological surveillance tools from Roiz et al. ...	67
Table 5: Variable definition for the case control study.	86
Table 6: Evolution of pyriproxyfen resistance in Aedes populations in different clusters of Khon Kaen following implementation of vector control intervention.	104

List of abbreviations

Ab: Antibody

ABTS: 2,2'-Azino-Bis (3-ethylbenzThiazoline 6-Sulfonic acid) di-ammonium

AchE: Acetylcholine esterase.

AGO: Autocidal Gravid Oviposition trap

AI: Adult *Aedes* Index

AI_in: Adult *Aedes* Index Indoor

AI_c: Adult Index at the cluster level

AIC: Akaike Information Criterion

ATSB: Attractive Toxic Sugar Baited

BI: Breteau Index

CCE: Carboxylesterase

CHIKV: chikungunya virus

CI: Container Index

CDC: Center for Disease Control

COMBI: Communication for Behavioral Impact

CNV: Copy Number Variation

CRISP-Cas9: Clustered Regularly Interspaced Short Palindromic Repeats associated protein 9.

CYP: Cytochrome P450

DALY: Disability-Adjusted Life Years

DDT: DichloroDiphenyl-Trichloroethane

DENV: Dengue virus

DF: Dengue Fever

DHF: Dengue Haemorrhagic fever

DNA: Desoxyribonucleic acid

DSS: Dengue Shock Syndrome

ELISA: Enzyme-Linked ImmunoSorbent Assay

ENSO: El Niño Southern Oscillation

GAT: Gravid Autocidal Trap

GMO: Genetically Modified Organism

GST: Glutathione-S-transferase

HI: House Index

HIA: Hemagglutination Inhibition Assay

IAM: Integrated *Aedes* Management

ICT: Immunochromatography

IgG: Immunoglobulin G

IgM: Immunoglobulin M

IGR: Insect Growth Regulator

IRS: Indoor Residual Spraying

IRM: Insecticide Resistance Management

ISS: Indoor Space Spray

IVM: Integrated Vector Management

JEV: Japanese Encephalitis Virus

KAP: Knowledge Attitude and Practice

Kdr: Knock down rate

KK: Khon Kaen

MAC-ELISA: immunoglobulin M Antibody Capture Enzyme-Linked ImmunoSorbent Assay

MEI: Mosquito Exposure Index

MET: Mosquito Electrocuting Trap

MoPH: Ministry of Public Health

NS: Non-structural

PCR: Polymerase Chain Reaction

PHI: Pupae per House Index

POC: Point of care

PPF: Pyriproxyfen

PPI: Pupae per Person Index

PNRT: Plaque Neutralization Reduction Test

PYR: Pyrethroid

ODPC: Office of Disease Prevention and Control

OP: Organophosphate

ORS: Outdoor Residual Spraying

RCT: Randomized Control Trial

RDT: Rapid Diagnostic Test

RE: Roi Et

RIDL: Release Insect with Dominant Lethality

RNA: Ribonucleic acid

RT-PCR: Retro Transcription Polymerase Chain Reaction

RR50: Resistance Ratio 50

SEA : South East Asia

SES: Socio-Economic Status

SIT: Sterile Insect Technic

SRRT: Surveillance Rapid Response Team

ULV: Ultra Low Volume

VBDU: Vector Borne Disease Unit

VGCR: Vector Global Control Response

VGSC: Voltage Gated Sodium Channel

WIN: Worldwide Insecticide resistance Network

WHO: World Health Organisation

WHO-TDR: World Health Organisation and special group for tropical diseases research.

WHO-VCAG: World Health Organisation

YFV: Yellow Fever Virus

ZIKV: Zika Virus

List of publications

1. **Fustec B**, Phanitchat T, Hoq M. I., Aromseree S., Pientong C., Thaewngiew K., Ekalaksananan T, Bangs MJ., Corbel V, Alexander N., Overgaard H. J. (2020). Complex relationships between Aedes vectors, socio-economics and dengue transmission— lessons learned from a case-control study in northeastern Thailand. *PLoS Negl Trop Dis.* 1;14(10). doi: 10.1371/journal.pntd.0008703.
2. **Fustec B**, Phanitchat T, Aromseree S, Pientong C, Thaewngiew K, Ekalaksananan T, Cerqueira D, Poinsignon A, Elguero E, Bangs M.J., Alexander N, Overgaard H.J, Corbel V. Assessing human exposure to Aedes mosquitoes by the use of Aedes salivary biomarker in a context of a vector control intervention: a randomized control trial in northeastern Thailand. (submitted to *Plos Negl Trop Dis.* in October 2020)
3. Phanitchat T, Zhao B, Haque U, Pientong C, Ekalaksananan T, Aromseree S, Thaewngiew K, **Fustec B**, Bangs MJ, Alexander N, Overgaard HJ. (2019). Spatial and temporal patterns of dengue incidence in northeastern Thailand 2006-2016. *BMC Infect Dis*, 19(1), 743. doi: 10.1186/s12879-019-4379-3.
4. Overgaard HJ, Pientong C, Thaewngiew K, Bangs MJ, Ekalaksananan T, Aromseree S, Phanitchat T, Phanthanawiboon S, **Fustec B**, Corbel V, Cerqueira D, Alexander N. (2018). Assessing dengue transmission risk and a vector control intervention using entomological and immunological indices in Thailand: study protocol for a cluster-randomized controlled trial. *Trials*, 19(1), 122. doi: 10.1186/s13063-018-2490-1. Corrected in *Trials*, in December 2018 19(1), 703. doi: 10.1186/s13063-018-3110-9

List of scientific communications

- **B. Fustec**, S. Aromseree, C. Pientong, K. Thaewnongiew, M. J. Bangs, N. Alexander, H. J. Overgaard, V. Corbel. Immunological and Entomological Indices to Evaluate the Risk of Dengue Transmission in Northeastern Thailand. *Joint International Tropical Medicine Meeting (JITMM), 6-8 December 2017, Amari Watergate, Bangkok, Thailand.* (POSTER)
- **B. Fustec**, H. J. Overgaard, T. Phanitchat, S. Aromseree, C. Pientong, K. Thaewnongiew, V. Corbel. Characterization of insecticide resistance in *Aedes aegypti* populations from Khon Kaen district, northeastern Thailand. *2nd Win International Conference 2018: Grand Copthorne Waterfront Hotel, 1-3 October 2018, Singapore.* (POSTER)
- **B. Fustec**, H. J. Overgaard, T. Phanitchat, S. Aromseree, C. Pientong, T. Ekalaksananan, K. Thaewnongiew, N. Alexander, M. J. Bangs, V. Corbel. *Aedes* abundance and dengue fever transmission, a difficult relationship: lessons from an ongoing case control study in northeastern Thailand. *The 6th International Forum for Surveillance and Control of Mosquitoes and Vector-borne Diseases (IFSCMVD) in Conjunction with 4th Meeting of the ASVEMC and 12th Conference of Medical and Veterinary Entomology of the Entomological Society of China, May 26-30, 2019, Xiamen, China.* (ORAL COMMUNICATION)
- **B. Fustec**, T. Phanitchat, S. Aromseree, C. Pientong, T. Ekalaksananan, K., Thaewnongiew, N. Alexander, M. J. Bangs, H.J. Overgaard, V. Corbel. Seasonal variations in immunological and entomological indices to estimate dengue vector infestation, a longitudinal study in Northeastern Thailand. *XXVI International Congress of Entomology, in Helsinki, Finland (ICE 2020), postpone to July 2021.* (ORAL COMMUNICATION)
- T. Phanitchat, C. Pientong, T. Ekalaksananan, S. Phanthanawiboon, S. Aromseree, **B. Fustec**, K. Thaewnongiew, M. J. Bangs, N. Alexander, H. J. Overgaard. Spatio-temporal analysis of dengue during 2006-2016 in Khon Kaen Province, Thailand. *10th Conference on Global Health and vaccination research (GLOBVAC), 14-15 March 2017, Trondheim, Norway.* (POSTER)
- H. J. Overgaard, C. Pientong, T. Ekalaksananan, S. Aromseree, T. Phanitchat, **B. Fustec**, K. Thaewnongiew, M. J. Bangs, V. Corbel, N. Alexander. Can Entomological and Immunological Indices Distinguish between Dengue Positive and Negative Households? A Prospective, Hospital-Based, Case-Control Study in Northeastern Thailand. *10th Conference on Global Health and vaccination research (GLOBVAC), 14-15 March 2017, Trondheim, Norway.* (POSTER)

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Preamble

Over 80% of the world's population lives in areas at risk of one or more of the seven major vector-borne diseases. Of these seven diseases, four are transmitted by mosquitoes of the genus *Aedes* (Golding et al. 2015) (Figure 1). During the last 10 years, infectious diseases caused by arthropod-borne viruses ("arboviruses"), including dengue (DENV), chikungunya (CHIKV), Zika (ZIKV) and yellow fever (YFV) viruses have been emerging throughout the world, driven by the two key mosquito vectors, *Aedes aegypti* and *Ae. albopictus* (Girard et al. 2020). The expansion of *Aedes*-borne diseases is attributed to factors that favour the dispersal and proliferation of *Aedes* mosquitoes as a result of climate change, global trade and unplanned urbanization, inefficient implementation of vector control programs, and a lack of community engagement and political will (Roiz et al. 2018). Efforts to address this increasingly urgent challenge have been recently boosted by a renewed focus on strengthening vector control, as witnessed at the May 2017 World Health Assembly, where the Global Vector Control Response (GVCR) received strong support from the member states (Organization 2017). The GVCR provides countries with high-level, strategic guidance to reduce the burden and threat of vector-borne diseases - including *Aedes*-borne diseases-, through effective, locally optimized and sustainable vector control. Despite this fresh impetus, many countries are still unprepared to address the challenge of *Aedes*-borne diseases, lack adequate guidance and tools to prevent the introduction, establishment and/or spread of both the mosquito vectors and the viruses (Roiz et al. 2018).

Substantial gaps exist in the surveillance systems for arboviral vectors, most notably in South East Asia and Latin America facing increasing arbovirus outbreaks (Weetman et al. 2018). *Aedes* borne diseases do not exhibit simple dynamic and outbreaks are particularly difficult to predict (Brady et al 2015). This raises concerns about the application of current outbreak guidelines and indicators for early warning and identification systems. Clearly, sensitive surveillance tools do not exist today, and most studies have failed to find good correlations between entomological indices and episodes of dengue (Bowman et al. 2014), and no entomological thresholds have proven effective in predicting *Aedes*-borne virus epidemics (Bowman et al. 2016, Reiner et al. 2016). Unfortunately, recent predictive models based on climatic conditions and urban growth suggest that both *Ae. aegypti* and *Ae. albopictus* are anticipated to continue expanding beyond their current distributions hence extending the risk of autochthonous transmission in new territories

(Kraemer et al. 2019). More cost-effective approaches and practical tools that can reliably measure real-time dengue transmission dynamics are needed to enable more accurate and useful predictions of incidence and outbreaks.

This thesis has been conducted in the framework of the DENGUE INDEX project funded by the Norway Research Council that aimed to develop practical and sensitive entomological and immunological indicators for dengue transmission that may be used to forecast dengue outbreaks. This thesis explores the determinants associated with dengue transmission risk in North-eastern Thailand using different approaches (entomology, immunology, virology) and design (retrospective study, case-control study and a randomized controlled trial). The first part of the thesis will present generalities related to dengue disease, the virus and the vectors and will review the main strategies actually deployed for the surveillance and control of the disease. The second part will present the context and the specific objectives of the thesis. The key findings will be resumed in the third part; The first chapter will describe the spatial and temporal dynamic of dengue incidence in North-eastern Thailand where the thesis has been carried out. The second chapter will discuss the complex relationships between dengue infection, vector infestation and human exposure risk to *Aedes* mosquito bites and will evaluate the accuracy of entomology and immunology indices to discriminate between dengue case and control (non-case) houses. The third chapter will investigate the close association between the levels of *Aedes* infestations and mosquito exposure risk as measured by the level of antibody response to *Aedes* salivary antigens to validate the use of salivary biomarkers as proxy for estimating “human-vector” contact and dengue transmission risk in the context of vector control intervention based on pyriproxyfen (a new Insect Growth regulator). The last chapter, which slightly differs from the three previous ones, will address the impact of the vector control intervention on the selection of insecticide resistance in order to guide vector control policies for dengue prevention. Altogether, the results presented in this thesis are expected to provide national authorities with more accurate information and tools for improving dengue surveillance and for monitoring and evaluation of vector control in Thailand and abroad. This thesis has led to 4 publications in peer review journals (including 2 as first authors) and 6 communications (4 poster and 2 lectures) at various symposium and international conferences.

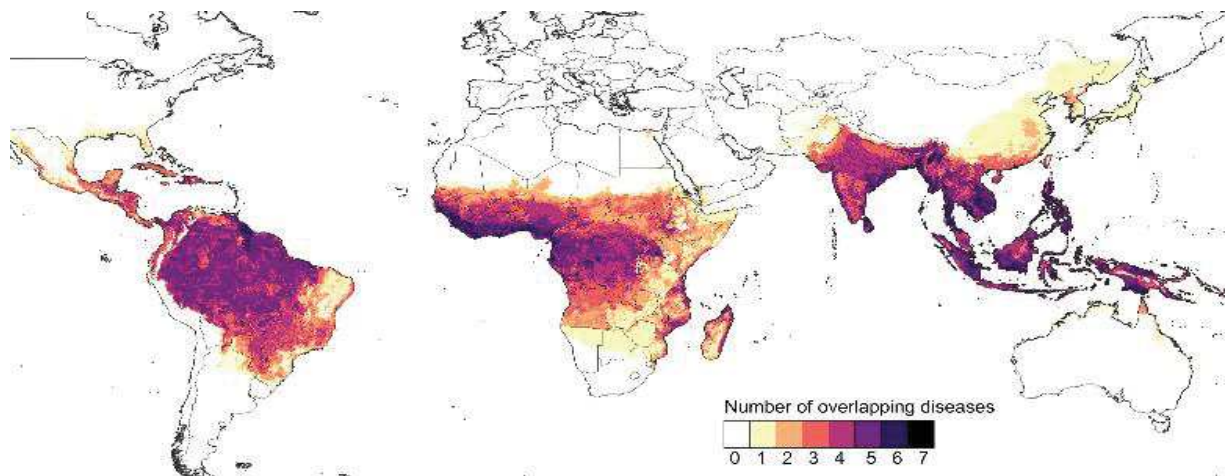


Figure 1: Overlapping of global distribution of major mosquito borne diseases (malaria, dengue, chikungunya, Zika, yellow fever, Japanese encephalitis, lymphatic filariasis)

First Part: Generalities

1. Dengue disease

Dengue is a viral vector-borne disease founded in tropical and subtropical area, caused by a *Flavivirus*, and transmitted by mosquito vectors, mainly *Aedes aegypti* and to a lesser extent *Aedes albopictus*. Dengue infection is characterized by a sudden feverish state, flu-like symptoms are very commonly observed, thus dengue fever is often called the “tropical-flu”. In some cases, dengue infection can induce plasma leakage which may result in massive haemorrhage and death.

1.2. Epidemiology

Dengue is an old viral vector-borne disease widespread through the tropical and sub-tropical regions. While dengue was suspected in Asia, America and Africa in the 1780’s, the first reports of dengue-like illness may be as older as the Chin dynasty (265 to 420 A.D.). However, the World War II set-up the perfect conditions for the spread of the dengue and other vector borne diseases. From local and sporadic outbreaks, countries started to demonstrate increased transmission and a new disease appeared in South East Asia (SEA), known as the dengue haemorrhagic fever (Gubler 1998). The first outbreak of dengue haemorrhagic fever was reported in Philippines in 1953. Within 30 years, dengue spread over the SEA region and was the first cause of hospitalization among children (World Health Organization 1986). Despite an interruption of dengue transmission in Americas between 1930 and 1977, granted by the massive use of DDT and the elimination of the mosquito vectors, *Aedes aegypti*, it re-invaded Latin America and dengue soared by 1980’s. Although there are few reports of dengue outbreaks in Africa before the 80’s, nowadays outbreaks are reported in more and more countries across the continent (Gubler 1998, Weetman et al. 2018). Most tropical and sub-tropical countries have now reported the circulation of the four DENV serotypes coupled with epidemic episodes (World Health Organization 2009). In early 2000’s World Health Organization (WHO) raised the alarm and urged member states to fight dengue, noticing the global expansion of the disease (Figure 2) (Messina et al. 2014).

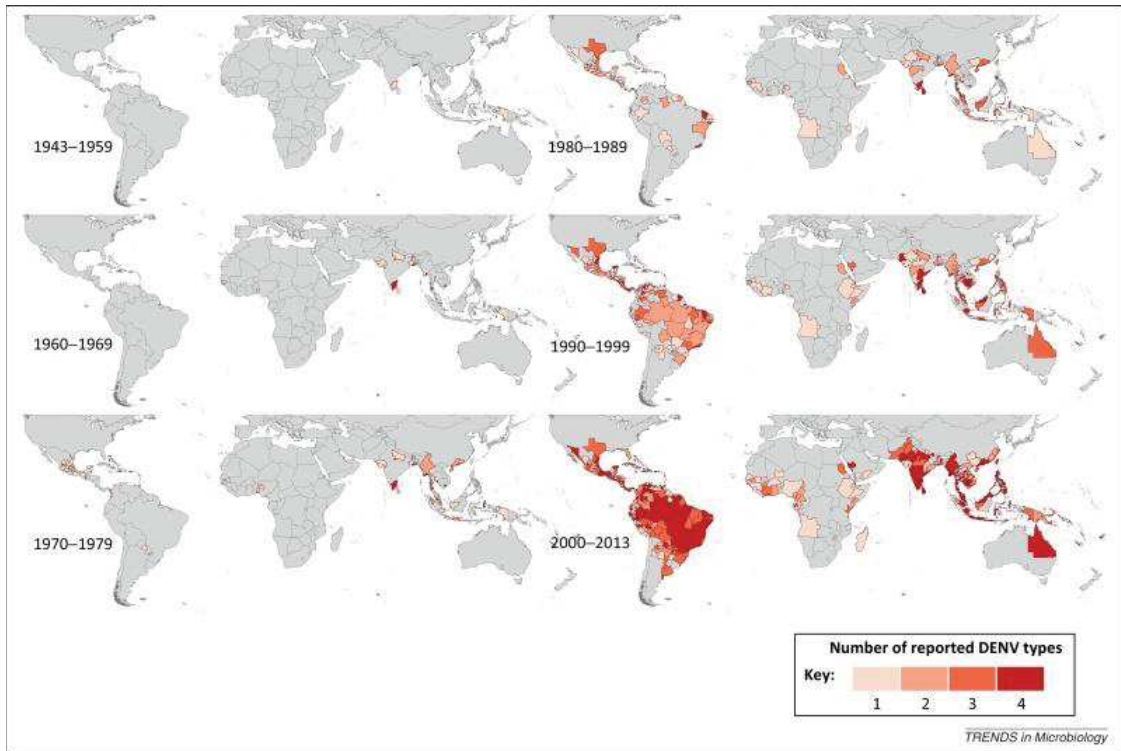


Figure 2: Spread of dengue in the world since 1943 from Messina et al.

Despite national and international surveillance, the actual distribution of dengue remains difficult to estimate due to an unknown proportion of asymptomatic cases (World Health Organization 1986, Endy et al. 2011, Duong et al. 2015b, Ten Bosch et al. 2018, Ly et al. 2019). Indeed, a study estimated the number of total infections to 390 million, with about 100 million of symptomatic cases (Bhatt et al. 2013). Another study evaluated that dengue fever is a threat in 128 countries and therefore considered that 3.9 billion of person are at risk of the disease (Brady et al. 2012). Moreover, due to the very broad distribution of the dengue vectors worldwide this mosquito transmitted disease might be a threat for even more people (Lambrechts et al. 2010).

Using reported cases and cost units for patients care, Shepard et al estimated 372 the disability-adjusted life years (DALYs) per million inhabitants in SEA, caused by dengue (Shepard et al. 2013). Another study in Northern Thailand accounting for both hospitalized and non-hospitalized febrile dengue showed that the DALYs lost due to dengue were 465.3 per million for this region (Anderson et al. 2007). The global burden of dengue relies also on the health coverage systems of the countries, where universal and affordable health system can reduce the economic

costs for those afflicted with dengue. For example, the global economic losses due to dengue have been estimated to be at least US\$ 9 billion per year (Bradshaw et al. 2016).

In most dengue endemic countries, cases occurred during all the year, yet the rainy season is associated with local or wider epidemic episode. Dengue epidemiology is characterized by seasonal peaks during the rainy season with major outbreaks every three to six years (van Panhuis et al. 2015, Churakov et al. 2019). Dengue epidemiology is also characterized by pluri-annual seasonal variations, with intra and inter-epidemic periods. Annual seasonality of dengue can be related to climatic factors, vector abundance and individual factors. Larger epidemic episodes are usually associated with changes in serotype distribution in a defined area. Global climatic changes foresaw more and more people at risk for dengue with an increase of the temperature and changes in rainfall patterns (Hales et al. 2002, Hii et al. 2012, Phaijoo et al. 2017). In addition, the phenomenon known as the El Niño Southern Oscillation (ENSO) is suspected to increase dengue transmission risk and to synchronize dengue outbreaks especially in SEA (Cummings et al. 2004, Huang et al. 2015, van Panhuis et al. 2015, Vincenti-Gonzalez et al. 2018). In addition, climate changes may contribute to extend the geographical distribution of both the mosquito vectors and the viruses. Global warming can contribute to increase dengue transmission risk by enhancing viral replication and by increasing the density, aggressiveness, survival, and reproduction rates of the mosquito vectors (Fan et al. 2014, Samuel et al. 2016).

Another key factor explaining the global expansion of dengue and other *Aedes*-arboviral diseases, is the increase of travels and exchange. Indeed, *Ae. albopictus* geographical expansion is very well correlated to the circulation of goods, tires trade and human movements (Hawley et al. 1987, Paupy et al. 2009). As a result, many countries have faced a resurgence/emergence of dengue cases due to a growing proportion of infected travellers returning home which can then facilitate local and autochthonous disease transmission if the vector is present (Wilder-Smith 2012, Jentes et al. 2016, Succo et al. 2016).

1.3. Viruses

The dengue virus is a positive sense single-stranded ribonucleic acid (RNA) of about 11 kbp, belonging to the genus *Flavivirus*, *Flaviviridae* family, to which other pathogens such as the YFV, ZIKV, and Japanese encephalitis virus (JEV) also belong. The disease is caused by four

genotypic distinct serotypes (DENV 1-4), however a fifth serotype was recently reported (Mustafa et al. 2015). Yet caution needs to be taken regarding this putative new serotype reported only in Malaysia as it may be a variant of the DENV-4 (Joob et al. 2016). The four characterised DENV serotypes share approximately 60% to 75% of the genome. The mature viral particle is about 50nm diameter and contains several copies of three structural proteins, host-derived membrane bilayer and a single copy of RNA. As shown in Figure 3, in addition to the three structural proteins (capsid C, precursor prM of membrane protein M, and the envelope E), the genome codes for 7 non-structural proteins (NS) (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5). Among all serotypes, different genotypes have been identified, demonstrating the great variability of dengue serotypes that can lead to increased viral fitness, infectivity and epidemic potential (Lambrechts et al. 2010, OhAinle et al. 2011). Moreover, intra-host diversity has been documented revealing an adaptation of the virus to the host's immune system (Kurosu 2011, OhAinle et al. 2011).

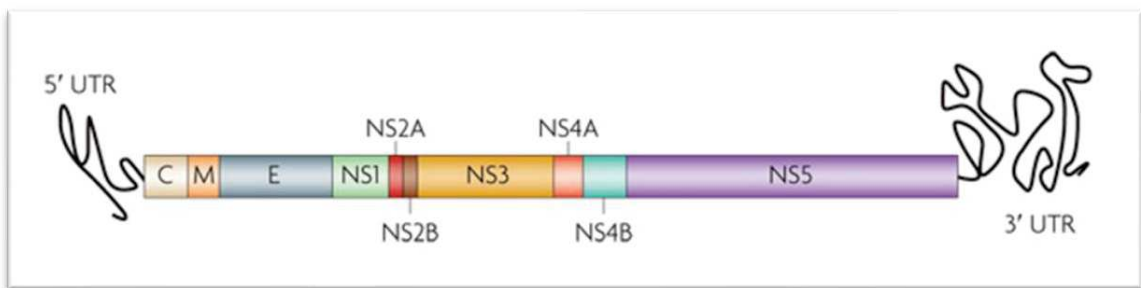


Figure 3: Dengue virus genome from Guzman et al. The dengue virus genome encodes three structural (capsid(C), membrane (M) and envelope (E)) and seven non-structural (NS1, NS2a/b, NS3, NS4a/b, NS5) proteins.

During dengue viral infection, DENV infected primarily the dendritic cells, however, DENV infection of macrophages and monocytes was demonstrated (Bente et al. 2006). Virus entry in the host cells is dependent of the fusion of the cell and viral membranes, which emphasize the crucial role of protein E in dengue infection (Alen et al. 2012). The envelope protein is composed of two sub-units organised in dimers as shows in Figure 4. In addition, glycoproteins on the surface of the virus envelope are responsible for the receptor-binding and membrane fusion. Following membrane fusion, viral RNA is released and is traduced into a polyprotein which will be divided in NS proteins. Non-structural proteins of DENV and their roles were well investigated (Zeidler

et al. 2017). The NS1 protein of dengue virus is considered responsible of the pathogenesis of dengue with a highly antigenic profile (Halstead 2019).

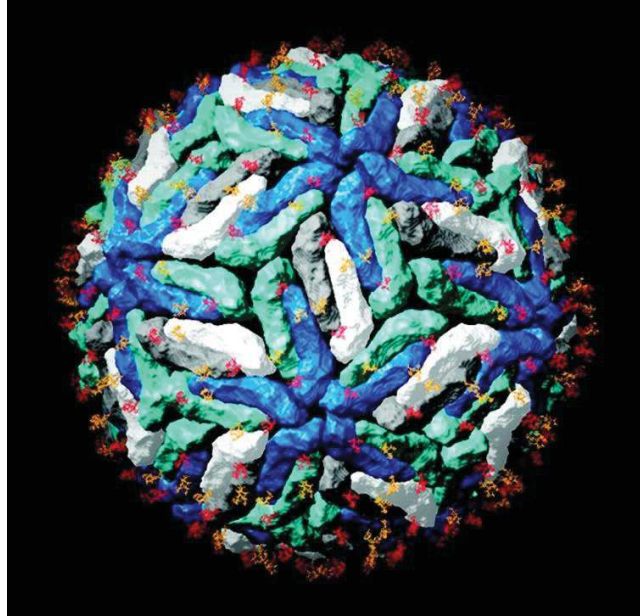


Figure 4: Dengue virus envelope structure from Rey. Dimers that lie at the icosahedral twofold axis in dark and light grey, and the dimers lying on local twofold axis in two shades of blue. Glycoproteins linked at the Asn-67 and -153 are shown as yellow and red sticks, respectively.

Genetic and proteinic differences between DENV serotypes induce specific humoral response in the host. Temporary cross-immunity between dengue serotypes have been reported (Anderson et al. 2014) while others showed that previous infections could induce a higher antibody response known as the antibody enhancement dependent, which leads to more severe dengue symptoms (Guzman et al. 2013, Soo et al. 2016). Therefore, secondary infections are suspected to lead to more severe dengue (Katzelnick et al. 2017, Khandia et al. 2018). This can be understood as an imperfect neutralization of the virus by the antibody produced during the previous infection, facilitating the entry of the virus in the host cells and leading to an increase in viral load and infectivity (Halstead 2015a, Khandia et al. 2018).

1.4. Dengue distribution

Dengue is widespread across sub-tropical and tropical areas threatening 3.9 billion people however, the different continents are not facing the same risk. While some regions are endemic for dengue and facing recurrent epidemic episodes, others reported dengue cases sporadically with

or without autochthonous (local) transmission. While in the 70's co-circulation of the four serotypes was exclusively reported in the SEA, they are now present in most continents with the exception of the middle east where only DENV-1 and DENV-2 were reported so far (Mackenzie et al. 2004) (Figure 5).

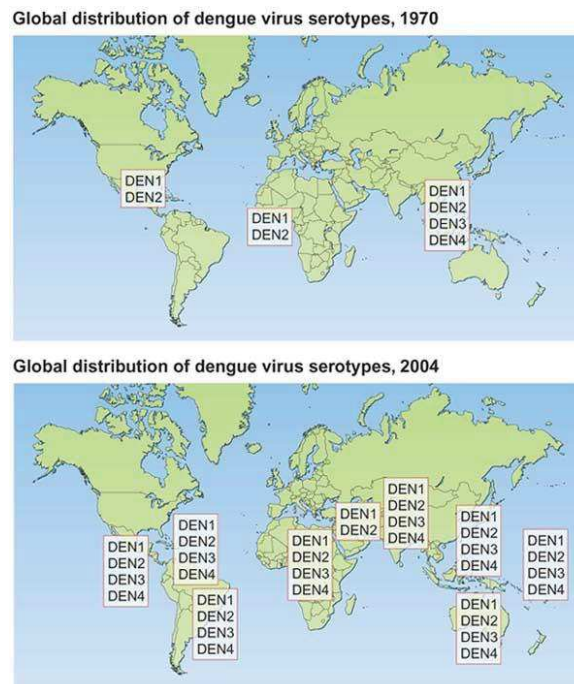


Figure 5: Global distributions of dengue serotypes in 1970 and 2004 from Mackenzie et al.

1.4.1. Dengue in South East Asia

According to the WHO, the South East Asia contribute for approximately 70% of dengue cases (Bhatt et al. 2013, World Health Organization. Regional Office for South-East 2018). Indeed, dengue is widespread in most SEA countries going from just sporadic cases (e.g., China) to hyper endemic transmission (e.g., Indonesia). Since 2000's, hundred thousand of dengue cases have been reported in Indonesia, Lao PDR, Myanmar, Timor-Lest and Thailand (World Health Organization 2009, Bravo et al. 2014, Bureau of Epidemiology et al. 2019). Changes in serotypes distribution, climatic and socio-demographic factors have resulted in major dengue outbreaks (Rodríguez-Barraquer et al. 2014, Woon et al. 2016). Recently, dengue incidence rose in India, Sri Lanka, and Bangladesh with major outbreaks reported in 2012-2013, 2016 and 2017 (Angel et al. 2009, World Health Organization 2009, Bhatia et al. 2013, Bodinayake et al. 2016, Telle et al. 2016, Guo et al. 2017, Uehara et al. 2017, Muraduzzaman et al. 2018, Agarwal et al. 2019). Additionally, dengue

re-emerged in Singapore after 35 years of effective control (Ooi et al. 2006, Bravo et al. 2014). Dengue is also present in several provinces of China, including Yunnan, Guangdong, and Guangxi (Zhang et al. 2014). Today, dengue was reported in all countries in the WHO South-East Asian region except in North Korea hence highlighting the global trend of disease expansion worldwide.

1.4.2. Dengue in Western Pacific region

Cambodia, Vietnam, Malaysia, Philippines are the most affected countries by dengue in the western pacific region. In addition, the dengue outbreak in 2008 in Cambodia indicated a rapid change in dengue epidemiology, with more rural transmission than previously observed (Huy et al. 2010). Moreover, dengue is spreading to the Pacific Islands such as Selangor (Malaysia), Fiji and Vanuatu, due to the re-introduction of DENV-3 serotype which had been absent for a decade (Getahun et al. 2019). Between 2008 and 2014, WHO reported a 2-fold increase in the number of dengue cases in the region, however, with a lower the fatality rate compared to previous years (Regional Committee for the Western 2016). Finally, dengue is also circulating sporadically in Australia (Queensland), with both imported and autochthonous cases, due to the presence of the very effective vector *Ae. aegypti* (Akter et al. 2019).

1.4.3. Dengue in Americas

For more than 30 years, dengue was absent from the Americas, as a result of the *Ae. aegypti* eradication campaign using DDT (1970-1980) (van den Berg et al. 2012, Epelboin et al. 2018). However, the discontinuation of vector control contributed to the re-invasion of *Ae. aegypti* in the early 80's (Guzman et al. 2003, Kotsakiozi et al. 2017). Following the vector (re) introduction DENV started to re-circulate in America, invading more and more countries (Teixeira et al. 2009b). In 2013, a major outbreak occurred in Latin America causing more than 2 million cases, including 38,000 severe dengue cases and 1,280 deaths (Pan America Health Organization 2020). Brazil was the most afflicted country with about 1.5 million cases reported (Nunes et al. 2019). Since then, recurrent dengue outbreaks occurred in this country causing about 1.6 and 3.1 million cases in 2015 and 2019, respectively (Nunes et al. 2019, Pan America Health Organization 2020). In the same time, Latin America was strongly also affected by other *Aedes*-borne diseases such as Zika outbreak causing >5 million of cases, mainly in Brazil. More recently yellow fever outbreaks were historically reported in Brazil (2000 human cases including 800 death during the 2016-2018) and the country has taken necessary actions to vaccinate the populations and keep travellers

informed and vaccinated prior to traveling to those areas (Zanotto et al. 2018, Dorigatti et al. 2019). Additionally, dengue is also circulating across the south of the USA and recent outbreaks occurred in Hawaii in 2015-2016 (Johnston et al. 2020) emphasizing the threat of emerging/imported cases and thus potential for local transmission and outbreaks where dengue is not endemic.

1.4.4. Dengue in Africa

Despite the presence of the native *Aedes aegypti* and the invasive *Ae. albopictus*, dengue has not been considered as a major public health threat in Africa until recently (Amarasinghe et al. 2011, Stoler et al. 2014). Evidence of dengue circulation in West Africa was recently highlighted by the abnormally high prevalence of dengue cases among returning travellers (Ninove et al. 2009, Fourié et al. 2020). There is a growing evidence that a non-negligible portion of fever cases were mis-diagnosed as malaria, while, dengue or other *Aedes*-borne diseases that shared the same symptomology, had not been investigated (Stoler et al. 2014). According to recent prediction models of incidence, Africa could share approximately 10-15% of the global dengue burden (World Health Organization 2009, Bhatt et al. 2013, Jaenisch et al. 2014). A recent meta-analysis on dengue seroprevalence and DENV presence demonstrated the high heterogeneity in dengue transmission risk between countries (Simo et al. 2019). Indeed, more and more countries faced dengue outbreaks such as Burkina-Faso (Ridde et al. 2016, Lim et al. 2019), Senegal (Faye et al. 2014, Gaye et al. 2019), Angola (Sessions et al. 2013, Hill et al. 2019), or Tanzania (Ward et al. 2017) and it's of primary importance to pursue the monitoring and diagnostic of DENV to have a more accurate estimate of dengue distribution and incidence in this part of the world.

1.4.5. Dengue in the Mediterranean region

Eastern Mediterranean region is facing regular dengue outbreaks such as Saudi Arabia, Yemen, Pakistan and Sudan (WHO/EMRO 2005, Ali et al. 2016, Ducheyne et al. 2018). Dengue in Western European region is sporadic, mainly due to imported cases from individuals traveling back from endemic countries (World Health Organization 2009). However, the introduction and establishment of the dengue vector *Ae. albopictus* in Europe and the Mediterranean basin set up favourable conditions for local transmission (Succo et al. 2016). For example, the chikungunya outbreak in Italy in 2007 and 2017, or several episodes of locally acquired dengue and chikungunya in France, Croatia and Spain, raise the possibility for the establishment of these pathogens in Europe (Calzolari 2016, Rezza 2016, Matusali et al. 2020). The increasing incidence of such

episodes demonstrates that Europe is not immune to mosquito-borne diseases, and that the continent is increasingly exposed to the threat of (re-) emerging pathogens. In addition, the presence of *Ae. aegypti* in Madeira Islands (Portugal) following its introduction in 2005, has led to recurrent epidemics that have affected thousands of people (Schaffner et al. 2014, Wilder-Smith A. 2014). Predictive models based on climatic conditions and urban growth suggest that *Ae. aegypti* is likely to establish in specific isolated regions in Europe such as southern Italy and Turkey, and may then contribute to the transmission in the future.

1.5.Symptoms

Dengue infection exhibits a broad range of symptoms, from no clinical symptoms or mild-fever, to severe haemorrhage or even deaths, which make the diagnosis difficult. Overall, the case-fatality rate of dengue fever is relatively low ($\approx 1\%$) despite a possible increase during outbreaks due to the public health structures overwhelming.

Prior 2009, dengue fever disease was classified into 3 categories: dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). In 2009, WHO re-evaluated the dengue classification to dengue with or without warning signs and severe dengue (Figure 6).

As shown in Figure 6, symptoms of dengue infection range from mild to acute fever, rashes, nausea, retro-orbital pain, arthralgia, leukopenia and positive tourniquet test. Some warning signs, which necessitate medical attention include rapid increased in haematocrit combined with a significant decrease in platelet count, mucosal bleeding, persistent vomiting, abdominal tenderness, or lethargy. Dengue infection, in some cases can evolve to more severe symptoms and to severe plasma leakage which might result in organ impairment and/or haemorrhagic syndrome (Halstead 2015b). Moreover, weakness and fatigue can persist for weeks which may increase dengue overall burden (Seet et al. 2007, Umakanth 2017).

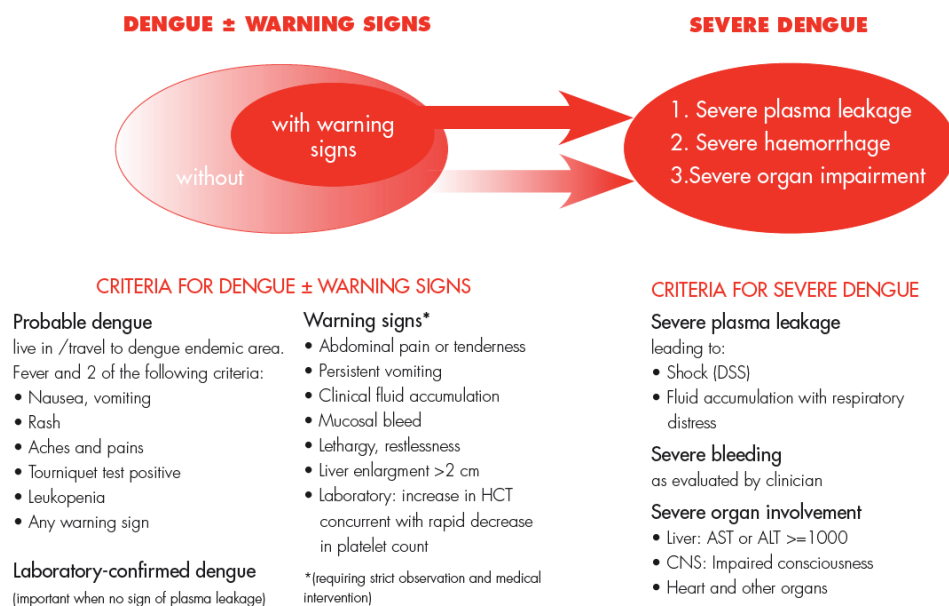


Figure 6: Dengue cases classification and level of severity, from WHO.

1.6. Diagnostic

To prevent dengue outbreaks, it is mandatory to detect cases early and accurately. In most dengue-endemic countries, dengue diagnosis is based on the presence of symptoms as described previously (see section 1.5). However, those symptoms can be encountered in many viral diseases and are not specific to dengue fever. In order to diagnose patients accurately, several techniques were developed. Due to the course of dengue illness and DENV viremia (Figure 7), direct diagnostic of dengue can be performed within the first days of illness (World Health Organization 2009). For late stages of dengue illness, indirect diagnosis will be preferred, using serological tools.

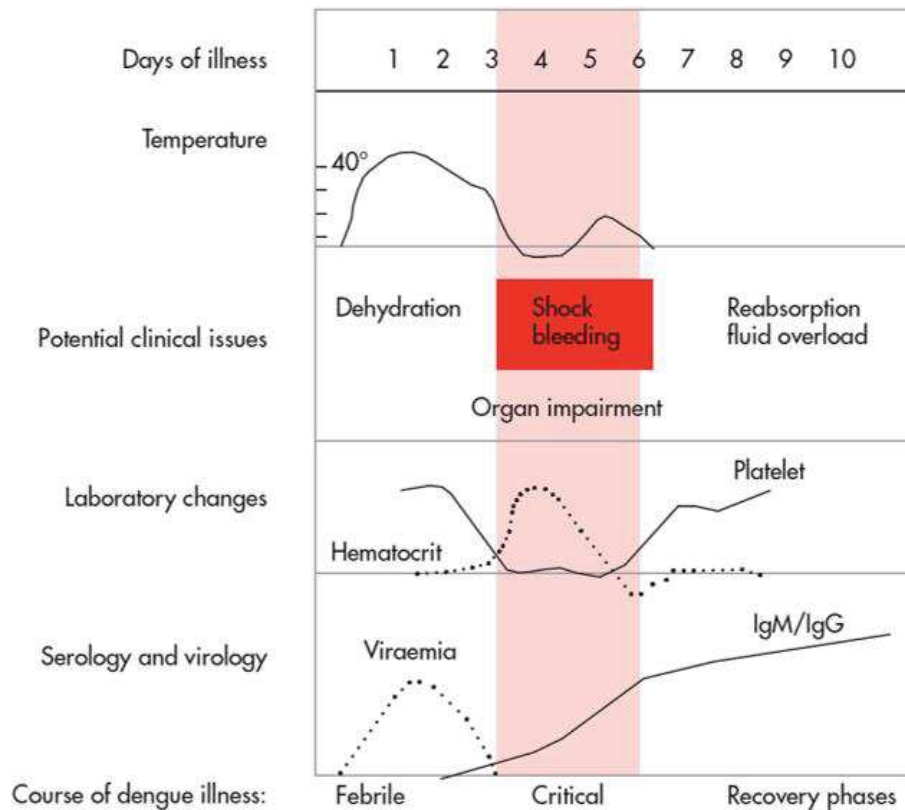


Figure 7: Dengue illness course from WHO.

1.6.1. Epidemiological diagnostic

Differential diagnosis, based on the clinical symptoms and laboratory analysis, is the first step for dengue diagnostic. During dengue illness course, thrombocytopenia, plasma leakage, joint pain and fever are typical. Any patients presenting at least two dengue symptoms (see section 1.4) are eligible for laboratory confirmation of dengue infection (World Health Organization 2009) (Figure 6).

1.6.2. Laboratory diagnostic

1.6.2.1. Direct diagnosis

Direct diagnostic of dengue, which can only be performed at early stage of disease, rely on virus detection, viral RNA detection or antigen detection. Virus detection is historically based on virus isolation by cell culture. Briefly, patient sera are incubated on susceptible cell lines, such as the C6/36 cell line from *Ae. albopictus* mosquitoes or Vero cells (from green monkey kidney cells),

and maintained for few days (Medina et al. 2012). This method, being highly specific, is the gold standard for dengue laboratory confirmation. However, virus isolation can only be performed at early stage of illness, and take time to get the result (usually between 3 to 10 days). Therefore, virus isolation is not the preferred method in case of emergency situation.

An alternative method had appeared in the 1990's with the development of reverse-transcription Polymerase Chain Reaction (RT-PCR), which allow the detection of DENV RNA in serum samples (Lanciotti et al. 1992). Recent progresses were made allowing a quicker and easier protocol for real-time DENV detection and serotype identification (Shu et al. 2003, Johnson et al. 2005, Chen et al. 2010). Serotype identification is not mandatory for patient care however, it can be useful for epidemiological surveillance purposes, such as changes in serotypes prevalence that can trigger outbreak.

The last method for a direct diagnostic of dengue is the detection of the non-structural protein-1 (NS1) of the DENV. The NS1 protein is produced by mammalian cells infected by DENV and induce a strong immune response. NS1 detection can be performed by ELISA or immunochromatography (ICT). The principle is to detect antigen-antibody complexes from patient sera. Since the first commercialization of kit for DENV NS1 detection by ELISA in 2006, several companies had developed their own tests, yet with variable specificities and sensibilities. The development of Rapid Diagnostic Test (RDT), based on the ICT of NS1 antigen, allowed to reduce the time needed for DENV diagnostic with a result in 5-15 min (Figure 8). Nowadays, RDTs targeting the NS1 antigen are strongly recommended by the WHO to guide dengue diagnosis (Teixeira et al. 2009a, World Health Organization 2009). However, a negative result of the NS1 detection is not sufficient to exclude dengue fever as the presence of NS1 protein, usually, does not last more than few days after the apparition of the symptoms. It is noteworthy to emphasize on the small window when direct diagnostic can be accurately used.

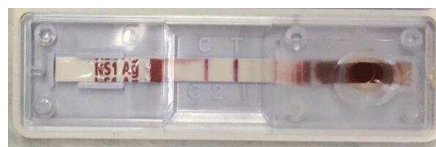


Figure 8: NS1 positive Rapid Diagnostic Test.

1.6.2.2. Indirect diagnosis

As a consequence of the broad symptoms and the difficulty to diagnose directly dengue infection, indirect diagnostic tools are commonly used in practice. One of the indirect diagnosis of dengue is based on the detection of specific antibody response against DENV (Salje et al. 2018). According to the course of dengue illness (Figure 7) and the time-line of antibody production (Figure 9), humoral response can be separated into two phases: the mid-early response with the production of IgM against dengue virus within few days after viral infection, and the later stage with the production of IgG, which confers the durable immunity against a given serotype. Therefore, the detection of IgM or IgG against DENV from blood or serum samples, and the comparison with the previous levels of immune response can provide information on seroconversion. Additionally, the IgM/IgG detection can provide information on the number of infection (i.e. primary or secondary infection). Indeed, the concomitantly presence of both type of immunoglobulin indicates secondary infections which may lead to more severe symptoms (Figure 9). There are several assays to detect immunoglobulins related to dengue infection (De Paula et al. 2004).

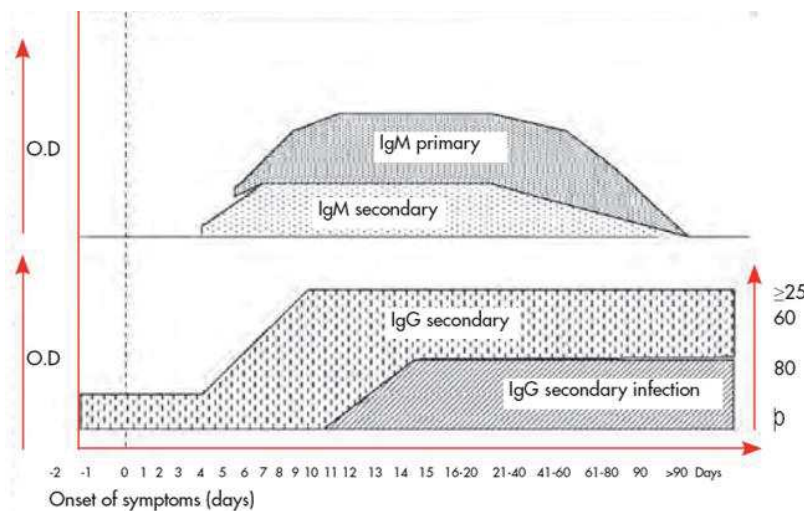


Figure 9: Time-line of immunoglobulins in primary and secondary dengue infection from WHO.

1.6.2.2.1. Plaque reduction neutralisation test

The plaque reduction neutralisation test (PRNT) has been developed to measure changes in the titers of neutralizing immunoglobulin against dengue virus (Peeling et al. 2010). The

principle of PRNT is to allow virus-antibody interactions and to measure the efficiency of antibodies to neutralize the virus (plaques). Briefly, virus-susceptible cells are cultured in a semi-solid media to avoid dispersal of virus progeny. Patients sera are incubated at various dilution prior mixing with constant amount of virus in order to maximize observation of plaques (local infection) which can be detected in various ways such as direct coloration of the cells (e.g., using neutral red or crystal violet) (World Health Organization 2007a, Timiryasova et al. 2013), or staining by using DENV-reactive antibodies (Roehrig et al. 2008). This assay is the gold standard to assess the level of neutralizing antibodies against the different DENV serotypes, however, PRNT is labour-intensive (e.g., approximately 5 to 7 days are required for plaques formation), requires BSL-2 laboratory facilities, and qualified staff for cell cultures and virus manipulation and cannot be performed in early dengue illness (World Health Organization 2009).

1.6.2.2.2. Hemagglutination inhibition assay

The hemagglutination inhibition assay (HIA) is also recommended by the WHO to confirm dengue infection. The principle of the HIA is summarized in Figure 10. In brief, patient serum is incubated with DENV antigens and later incubated with red blood cells. In the presence of DENV-antibody, the viral particles are neutralized which inhibits the hemagglutination of red blood cells. The results of the assay are reported as the lower dilution that inhibits hemagglutination. In addition, optimal HIA necessitate paired sera collections 7-10 days apart to ensure immunoglobulin presence.

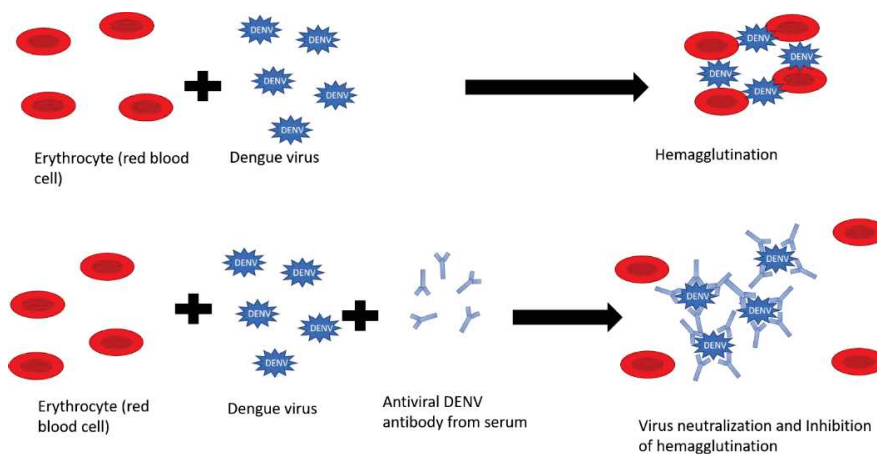


Figure 10: Principle of hemagglutination assay for dengue diagnosis.

1.6.2.2.3. IgM Capture-Enzyme Linked ImmunoSorbent Assay

The HIA method is however less and less used and is progressively replaced by IgM antibody Capture Enzyme linked ImmunoSorbent Assay (MAC-ELISA) (Matheus et al. 2005, Peeling et al. 2010, Lukman et al. 2016). The MAC-ELISA test is based on the qualitative detection of IgM-ELISA from patient paired sera and the differential level of IgM response. As summarized on Figure 11, patient sera are incubated on microplates coated with anti- μ chain of human IgM. During a second step, DENV antigens are allowed to bind on human DENV-specific fixed on the plate. Then, anti-DENV antibodies conjugated with enzyme are able to metabolize a colorless or light-colored substrate into a strong colored substrate. This color change is finally read by spectrophotometry. Nowadays, many commercial kits for DENV MAC-ELISA are available (PANbio®, Biorad®, Eurofins®, etc) and provide results within hours (Research et al. 2009, Andries et al. 2016, Lu et al. 2019). However, the sensitivity and specificity of those kits are highly variables (Hunsperger et al. 2009) and the results are often needed to be confirmed by PRNT (World Health Organization 2007a, Lu et al. 2019). Moreover, MAC-ELISA necessitates specific equipment (e.g., spectrophotometer, incubator), paired samples had been shown to increase sensitivity and specificity and need approximately four hours to get the results (Vázquez et al. 2003).

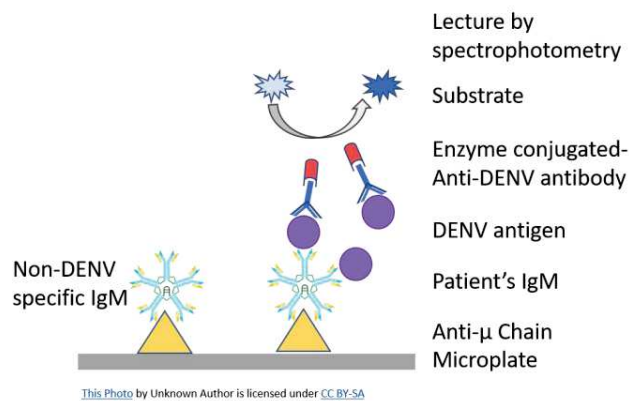


Figure 11: Principle of MAC-ELISA experiment.

1.6.2.2.4. Rapid Diagnostic Test

Finally, Rapid Diagnostic Test (RDT) targeting IgM and/or IgG were developed for dengue diagnostic. The principle of RDT is the migration of sample on a membrane and the detection of the target by immunochromatography test (ICT). Briefly, blood or sera sample are deposited into the cassette, then a specific buffer is added to allow migration of sample for few minutes (depending on the manufacturer's instruction). The results of the RDT are given in the form band indicating the presence of the target antigen and a control band indicating the validity of the test (Figure 12). The detection of IgM and IgG using RDT test allow the health officers to obtain results within few minutes (usually 5-30 min). However, those tests had been criticized regarding their lack of sensitivity and specificity (Hunsperger et al. 2009, Jang et al. 2019). Indeed, studies had risen the potential high rate of false negatives from RDT IgM/IgG, due either to the time course of dengue illness, or to the high antibody titers needed to trigger a visible band. Moreover, others had pointed the cross-reactivity of RDT IgM/IgG, with other *Aedes* -borne transmitted viruses, especially with CHIKV or ZIKV (Blacksell et al. 2011).



Figure 12: IgG positive and IgM negative RDT

2. The dengue vectors

Aedes aegypti (Linnaeus) (Diptera: Culicidae) and *Ae. albopictus* (Skuse) (Diptera: Culicidae) are mosquito species that can transmit several viruses to humans that cause diseases, such as dengue, Zika, chikungunya, and yellow fever. Over the last few decades, those diseases have spread rapidly partly due to the global expansion of the vectors. The distribution of *Aedes* mosquitoes is the widest ever recorded in history (Kraemer et al. 2015, Kraemer et al. 2019), and further research are need to better understand the causes and consequences of this rapid geographical expansion in order to propose more effective, durable and locally-adapted tools for vector control. In the following sections, I will describe current knowledge on *Aedes* vector

biology and ecology and provide new insight into the spatial distribution of *Aedes aegypti* and *Ae. albopictus* worldwide.

2.1. Life cycle

The life-cycle of *Aedes* includes aquatic and terrestrial stages (Figure 13) (Biogents 2020). The aquatic stage of *Aedes* development includes immatures stages, eggs, larvae and pupae. Only the adult stage is winged and terrestrial, and only the female is hematophagous.

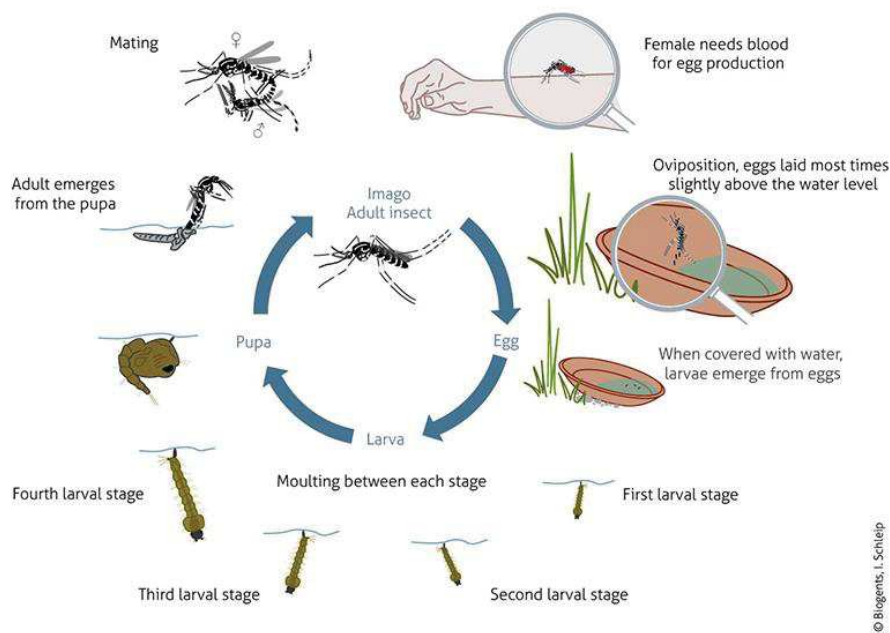


Figure 13: *Aedes* simplified life cycle from Biogents©.

Briefly, eggs are laid just above the water line in breeding sites (e.g., water storage containers, used tires, flower pot, etc) and they can survive to desiccation for several months (Rezende et al. 2008). Once eggs are hydrated, they hatch into first stage larvae (L1). Then the larvae will go through three supplementary stages (L2, L3, and L4). The larval stage lasts between 6-8 days in average, however, studies demonstrated that food stress can increase the duration of the larval stage nonetheless with consequences on the adult stage survival (Mitchell-Foster et al. 2012, Souza et al. 2019). After the larval stage, immatures *Aedes* transformed into pupae. The pupal stage lasts usually 24 to 48 hours after which adults will emerge. Males are usually the first to emerge while females emerge later. The duration of the aquatic stage of *Aedes* development is strongly dependent of both biotic (e.g. food availability, larval densities, competition between

species, predation) and abiotic factors such as the rainfall, the relative humidity and the temperature. Indeed, increased in mean temperature was related to a reduce development time (Scott et al. 2000b, Tun-Lin et al. 2000, Couret et al. 2014).

After emergence, adult mosquitoes will rest in shade places for 24 to 48 hours, in order to dry their cuticle, spread their wings and wait for their reproductive system to be functional. The male reproductive system needs in average 24 to 48 hours to be functional while it takes approximately 30-60 hours for the female reproductive system. Then, adult mosquitoes (males and females) will take their first sugar-meal, from flower nectar, which will be the only food source for male mosquitoes. Only the female needs to take a blood meal, rich in protein, for the egg maturation (Day et al. 1994, Styer et al. 2007). The mating occurs during flight and females usually mate only once shortly after emergence however, polyandry (i.e., mating with several males) was demonstrated in semi-field experiments (Helinski et al. 2012). Then the gonotrophic cycle starts with the host-seeking behaviour which is strongly related to anthropogenic environment, and ends with the oviposition. After blood meal, *Aedes* usually rests in shaded areas to complete eggs maturation. Depending on the amount of blood ingested, females *Aedes* will seek another host and/or will rest to digest the blood and mature the eggs. In average, *Aedes* produces around 100 eggs per clutch and about 4 to 5 batches in their life (Chadee et al. 2002, CDC 2020). Both *Ae. aegypti* and *Ae. albopictus* have a small flight range, then it follows that adults often stay close to their emerging site depending on the availability of breeding sites and (human) host to provide blood meal.

2.2. Aedes vectors

Although there are several “potential” *Aedes* dengue vectors, the field isolation of viruses and epidemiological evidence clearly show that *Ae. aegypti* (Figure 14) is responsible for the majority of dengue transmission (Gubler et al. 1997). The intrinsic ability of an arthropod to carry pathogens, ensure their multiplication and or development and to transmit the pathogens to a vertebrate host is defined as the vector competence. The vectorial capacity, which is the level of efficacy of the vector to transmit a pathogen, is highly dependent on abiotic factors such as the temperature, but also intrinsic characteristics of vector, virus and hosts (Liu-Helmersson et al. 2014). *Aedes aegypti* is the main vector of dengue due to its wide distribution, high vector

competence and vectorial capacity and its strongly anthropophilic behaviour (Macdonald 1956, Scott et al. 1993a, Scott et al. 1993b, Scott et al. 2000a, Gubler 2002, Carvalho et al. 2017).



Figure 14: *Aedes aegypti* (left) and *Aedes albopictus* (right).

2.2.1. *Aedes aegypti*

Aedes aegypti, originated from the African continent, is now present in tropical and subtropical area between latitude 35°N and 35°S (World Health Organization 2009) as shown in Figure 15 (Kraemer et al. 2015). *Aedes aegypti* lives close to humans and females bite during the daytime, both indoors and outdoors, often several times to have a complete oogenesis (Scott et al. 1993b). *Aedes aegypti* is found in urban and suburban settings and oviposits in any uncovered water containers such as vases, drums, and tanks for domestic water storage. *Aedes aegypti* is known to be well adapted to urban environment. Unplanned and increasing urbanization, poor waste management (e.g., plastic bottle, tires), or lack of piped-water favours *Ae. aegypti* proliferation (Gubler 2011) and dengue outbreaks. In laboratory conditions *Ae. aegypti* can live approximately 8 weeks (Degallier et al. 1988), however in field conditions, females *Aedes* are not expected to live longer than 10 to 35 days, and authors assume that *Ae. aegypti* in average make 3-5 gonotrophic cycle, three to five days apart, during their life (Goindin et al. 2015, Guzman et al. 2016). *Aedes aegypti* is a daytime feeder, with two peaks for host-seeking behaviour, the first one at dawn (6:00 to 8:00) and the second one at dusk (16:00-19:00). Because of the diurnal feeding behaviour, *Aedes aegypti* is often disturbed during the blood intake leading to multiple blood meals in a single gonotrophic cycle (Harrington et al. 2014). This has shown to increase the risk of pathogens transmission (Scott et al. 2012). In addition, *Aedes aegypti* is highly

anthropophilic, which means that humans are the preferred hosts for blood intake (McBride et al. 2014). *Aedes aegypti* biting behaviour is also highly endophilic and endophagic which means that this mosquito species usually rests and feed indoors (Scott et al. 2000b).

Once oogenesis is complete, *Aedes aegypti* females will seek for oviposition sites to lay their eggs. *Aedes aegypti* is well-adapted to urban environment and breed preferentially in man-made containers (e.g., flower pot, tires, drums, can, plastic bottles). In addition, the choice of the oviposition sites had been demonstrated to be related to the presence of larvae, the food availability but also the shape and colour of the container, and the sun exposure (OCDE 2018). Moreover, *Aedes aegypti* was shown to lay eggs in several water-holding containers, a behaviour known as the “skip-oviposition”, which contributes to maintain the population in case of unfavourable conditions (Abreu et al. 2015). This makes the elimination of larval breeding sites more challenging on the field.

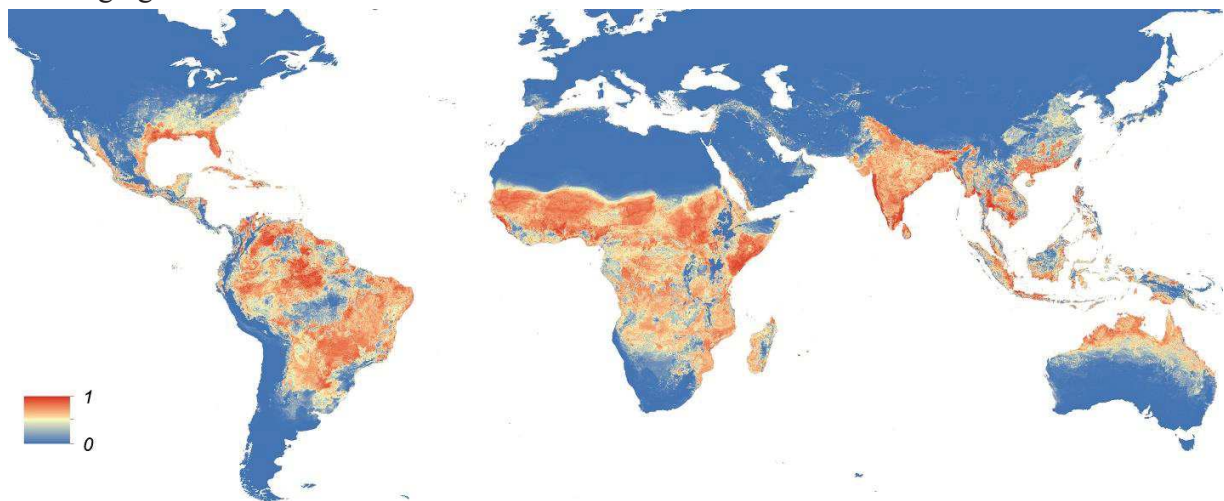


Figure 15: Map showing the predicted distribution of *Aedes aegypti* from Kraemer et al.

2.2.2. *Aedes albopictus*

Aedes albopictus, known as the Asian tiger mosquito, is considered as a secondary vector of dengue (Niyas et al. 2010, Paupy et al. 2010, McKenzie et al. 2019). Originated from South East Asia, *Ae. albopictus* has spread to every continent but Antarctica (Goubert et al. 2016), as shown in Figure 16. Unlike *Ae. aegypti*, *Ae. albopictus* can survive winter in temperate climate (Delatte et al. 2009, Brady et al. 2013). The global and rapid expansion of *Ae. albopictus* distribution worldwide can be related to its strong physiological and ecological plasticity, that allow a rapid adaptation to a broad range of habitats, but also to the increase of world trade,

especially related to the transport of used tires (Hawley et al. 1987). While *Ae. albopictus* was originally found in rural areas, the species recently adapted to suburban and urban areas (Paupy et al. 2009, Aida et al. 2011). *Aedes. albopictus* is an aggressive, opportunistic daytime feeder, that can bite humans and animals for blood meals (Kek et al. 2014). However, many studies demonstrated a preference for human hosts hence highlighting a strong anthropophilic behaviour of this species (Delatte et al. 2008, Paupy et al. 2009, Benelli et al. 2020). *Aedes albopictus* bites several times for complete oogenesis. In contrast to *Ae. aegypti*, *Ae. albopictus* is more often observed outdoors, either for host-seeking (exophagic behaviour) or for resting (exophilic behaviour) (Delatte et al. 2010). *Aedes albopictus* dwells in mostly in rural and suburban areas, lay eggs in water-filled artificial containers as used tires, flower pots, cans, but also in natural containers such as tree holes, or axil of orchid leaves, challenging breeding sites elimination (Paupy et al. 2010, World Health Organization 2011a).

Some studies showed that *Ae. albopictus* was as competent as *Ae. aegypti* for DENV-1 and DENV-3 but less competent for DENV-2 and DENV-4 (Christofferson 2015, Whitehorn et al. 2015). Moreover, *Ae. albopictus* can play a role in dengue transmission where *Ae. aegypti* is absent (e.g., La Reunion Island) (Delatte et al. 2008). Additionally, *Ae. albopictus* is a very competent vector for CHIKV and is considered as the primary vector of the disease (Paupy et al. 2009). *Aedes albopictus* is also competent for ZIKV (McKenzie et al. 2019). The global spread of *Ae. albopictus* worldwide and the increase of international travels foresaw an increasing part of the world's population at risk for *Aedes*-vector-borne diseases.

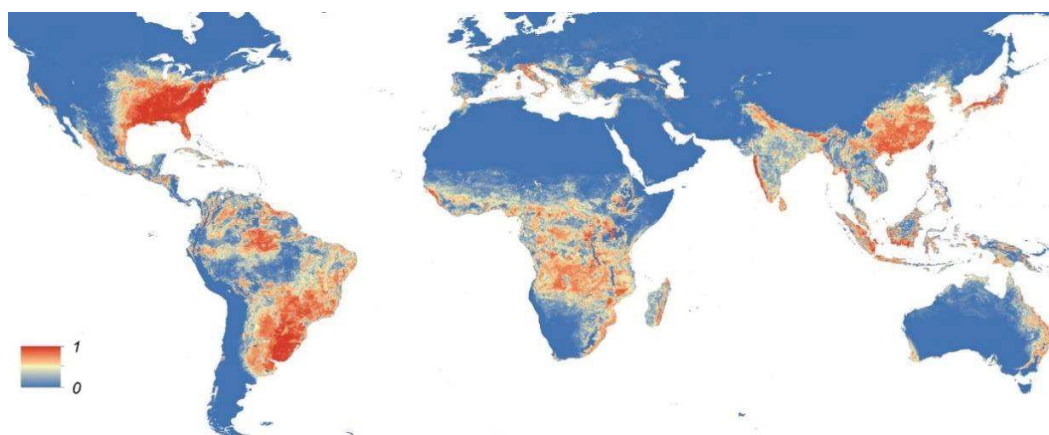


Figure 16: Map showing the predicted distribution of *Aedes albopictus* from Kraemer et al.

3. Global strategies for dengue prevention and control

The control of mosquito-borne diseases generally relies on four pillars: vaccination, chemo-prophylaxis, chemo-therapy and vector control. For dengue however, vaccination is limited (see section 3.1), and there is no preventive nor curative treatment (see section 3.2). Consequently, preventing or reducing dengue and other arboviral diseases caused by currently recognised or novel *Aedes*-borne viruses on a global scale continues to depend largely on controlling mosquito vector populations or interrupting human–vector contact (see section 3.3). A brief description of each strategy will be presented in the following sections.

3.1. Vaccine

The example of the YFV vaccine for disease control has shown the efficacy of this strategy, when deployed accurately, for *Aedes*-borne diseases. However, dengue vaccine development is far more complicated due to the complex and indeterminate immunoprotective and/or immunopathogenic response between the four serotypes (McArthur et al. 2013). Several vaccines for dengue are under clinical development (Yauch et al. 2014) (Table 1) but the Dengvaxia®, a tetravalent vaccine developed by Sanofi, has been licensed recently in more than ten dengue endemic countries including Brazil, the Philippines, Costa Rica, and Mexico. It has shown promising results in phase III of clinical trials (Capeding et al. 2014), but its efficacy was lower for serotype 1 (50.2%) and 2 (39.6%) than for serotype 3 (74.9%) and 4 (76.6%) (World Health Organization 2016a). After consultations, the manufacturer (Sanofi-Pasteur) and the WHO-Strategic Advisory Group of Experts on Immunization (SAGE) had warned on increased prevalence of severe cases in vaccinated children who never experienced dengue (World Health Organization 2016c, World Health Organization 2018). Therefore, this vaccine is recommended only for dengue endemic regions with high transmission and where seroprevalence is >50% to limit the risk to develop severe dengue in vaccinated-seronegative individuals (Flasche et al. 2019). Consequently, Sanofi does not recommend the use of Dengvaxia® in children <9 years old and in individuals who have not been previously infected with dengue. Despite that, the government of Philippines has recently suspended the distribution of dengue vaccine due to suspicious deaths (Flasche et al. 2019). The mitigate results obtained with the Dengvaxia®, might had reduced the enthusiasm for further vaccines development. However, vaccination should be considered as part

of global strategy including vector control and chemo-therapy and chemo-prophylaxis as developed in the following sections.

Table 1: Dengue vaccines under development from (Yauch and Shresta 2014)

Vaccine name	Vaccine type	Developer/ manufacturer	Progress
Dengvaxia (CYD-TDV)	Live attenuated vaccine	Sanofi Pasteur	Licensed
TAK-003 (DENVax)	Live attenuated vaccine + chimeric DENV2/DENV4	Mahidol university, Inviragen and Takeda	Phase III trial
TetraVax-DV TV003	Live attenuated vaccine	NIAID and Butantan	Phase III trial
LATV	Tetavalent, Live attenuated vaccine + chimeric	NIAID and Butantan	In vivo (Phase I-III)
DPIV	Inactivated virus tetavalent	GSK, Walter Reed Army Institute of Research, Fiocruz	In vivo (Phase I trial)
V180	Subunit vaccine	Merck	In vivo (Phase I trial)
TVDV	DNA vaccine	US AMRDC, WRAIR, NMRC Vical inc	Phase I trial
TLAV Prime/PIV boost	Heterologous prime/boost and reverse order		Phase I trial

3.2. Treatment & prophylaxis

Currently, there is no curative treatment for arboviral diseases. Patient care mainly aimed at reducing the symptoms by prescribing antipyretics (i.e., paracetamol), anti-nausea and pain killers. It is noteworthy to remind that nonsteroidal anti-inflammatory drugs (e.g., ibuprofen), aspirin and/or other salicylates should not be prescribed in case of suspected dengue fever because of their blood thinning effect leading to an increased risk of developing haemorrhage. In addition, patients presenting dengue cases with warning signs or severe dengue usually need fluid therapy (oral or intravenous) and/or blood or plasma transfusion to prevent severe organ hypoperfusion (World Health Organization 2009). Nevertheless, some drugs, already licensed for other diseases, are explored in dengue treatment, yet they failed in addressing strong clinical endpoints (Low et al. 2017). The absence of specific treatment against dengue emphasized the need for more effective strategies of prevention and control to prevent dengue outbreaks.

3.3. Dengue vector control

Considering the challenges mentioned above, dengue control and prevention depends essentially on controlling the vector mosquito and reducing human-vector contact (Guzman and Kouri 2003). Historically, vector control was initiated following the discovery of the implication of mosquitoes in pathogens transmission. In the United States, three strategies were successively used to control mosquito populations. The first strategy was based on mechanical control and was used between 1920-1940. The discovery of DDT opened a new path in vector control, and the chemical control strategy was used during the 1940-1970 period (Patterson 2016). The strong organization of the DDT campaigns, enabled the eradication of several diseases including malaria in Western Europe, dengue and Yellow fever in America. However, the acknowledgement of the harmful and carcinogenic effects of DDT led to its ban in the USA for vector control measure in the 1970's. Since then, USA and other countries have adopted integrated vector control strategies to maintain efficient and sustainable vector control intervention using the available tools. The GVCR recently endorsed by member states emphasizes on 4 pillars of action with 2 crucial elements: the reinforcement of vector surveillance and control capacities and capabilities; and the increase of fundamental and applied research and innovation in order to optimize vector control (Figure 17). The GVCR also underlines the critical factors necessary to achieve these objectives, such as country leadership, resource mobilization and coordination across sectors and diseases.

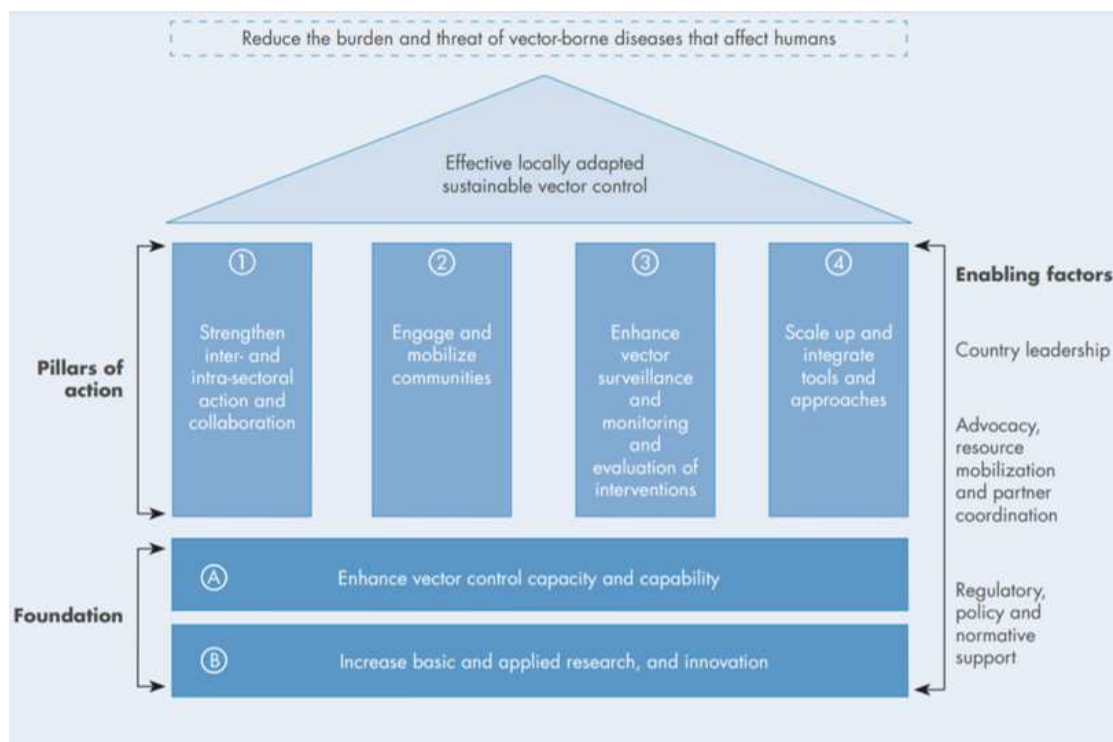


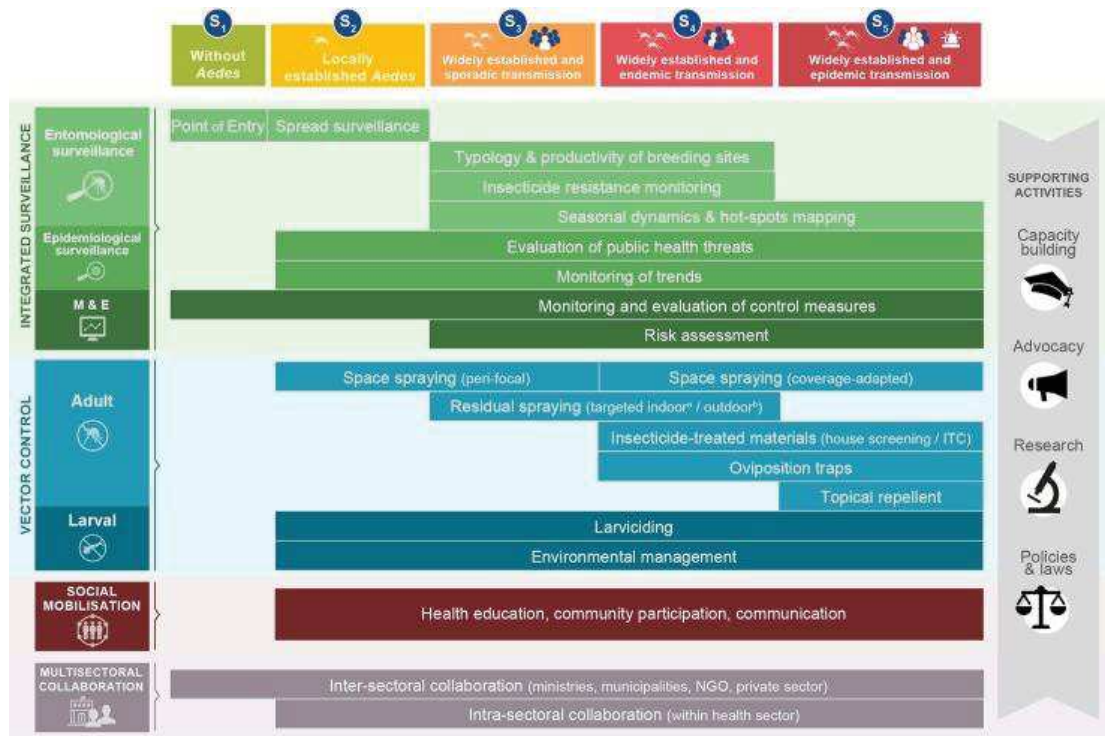
Figure 17: Global Vector Control Response Framework.

3.3.1. Integrated *Aedes* management strategies

The Worldwide Insecticide resistance Network (WIN), supported by the WHO, recently proposed a comprehensive framework (known as Integrated *Aedes* Management or IAM) based on available evidence to reduce the burden of *Aedes*-transmitted arboviruses (Figure 18). The originality of this framework is to propose, effective, integrated, community-based, locally adapted vector control strategies according to country capacity, levels of *Aedes* infestation and virus transmission risk so that countries may be better prepared for existing and emerging *Aedes*-borne

disease threats. A brief review of the vector control tools proposed in IAM framework, with their strength and weakness, will be presented in the following sections and in the Table 2 and Table 3.

Figure 18: Conceptual framework of the IAM system from Roiz et al.



3.3.1.1. Social mobilisation and community engagement

According to WHO, achieving sustainable vector control without community involvement might not be feasible (World Health Organization 2011a, Roiz et al. 2018). Community control is based on educational programs, social mobilization, and government policies. At the state or regional level, that imply educational programs and health communications (Roiz et al. 2018). Increasing knowledge on dengue prevention is mandatory to achieve this goal. Indeed, most the studies conducted worldwide on the knowledge, attitudes and practices (KAP) regarding dengue infection risk, showed that communities are quite aware of dengue symptoms yet, the relations between vector control, human behaviours and dengue risk remain poor (Brusich et al. 2015, Alyousefi et al. 2016, Kumaran et al. 2018). Inspiring behavioural changes through education or Communication for Behavioural Impact (COMBI) activities (Andersson et al. 2017), regarding self-implication in vector control and dengue prevention, is crucial to ensure sustainable vector control (Kumaran et al. 2018, Roiz et al. 2018).

Table 2: Strengths and limitations of larval control strategies from Roiz et al.

Stage/ scenario	Methodology	Type of intervention/ product	<u>Strength of evidence*</u>	Constraints/advantages	Specifications	References
Larval control for routine	Environmental management	Source reduction and educational outreach visits (door-to-door)	Epidemiological evidence (level 1) of community-based campaigns. Entomological evidence (level 3a and 3b).	Labour intensive. Larval development habitats need to be accurately identified. Must be done diligently and conscientiously and with access to a high number of dwellings	Requires a high level of education and community participation. Difficult to sustain over time. Need to characterize larval development habitats, including urban cryptic habitats. Essential to reduce mosquito larval development habitats in the long-term in private and public domains	(Heintze et al. 2007, Erlanger et al. 2008, Bartlett-Healy et al. 2011, Andersson et al. 2015, Bouzid et al. 2016, Bowman et al. 2016, Alvarado-Castro et al. 2017)
		Larviciding	Organophosphates (Temephos, Chlorpyrifos, Pirimiphos methyl, Fenthion)	Entomological evidence for Temephos (level 3b).	Affordable Not acceptable for treating drinking water containers and sources (except Temephos) Temephos resistance in several areas Regulatory constraints (e.g., OPs are not notified in the EU for mosquito control)	Cholinesterase inhibitors Different formulations (EC, GR) and application methods (manual or with hand sprayers)
	Insect growth regulators (pyriproxyfen, diflubenzuron, novaluron)		Epidemiological evidence for pyriproxyfen as part of community base (level 2b). Entomological evidence (level 3b).	More expensive Late acting effect (pupae) on juvenoids Acceptable for treating drinking water sources and containers Constraints for the treatment of cryptic breeding sites	Disruption of endocrine system for juvenoids (pyriproxyfen) and chitin synthesis inhibitor for ecdysoids (novaluron and diflubenzuron). Different formulations (WG, GR, DT) and application methods (manual or with hand sprayers)	(Bowman et al. 2016, Maoz et al. 2017)
	Bti		Entomological evidence (level 3a and 3b) for Bti.	No resistance Selective and safe Acceptable for treating drinking water sources and containers Low residual action in polluted habitats	Bacterial toxins targeting midgut epithelium cells Different formulations (WG, GR) and application methods (manual or with hand sprayers and fogging).	(Boyce et al. 2013, Faraji et al. 2016)
	Biological control	Fish (<i>Gambusia</i> , etc.)	Limited entomological evidence (level 3b) for fish.	Well accepted in several countries, needs a delivery mechanism and maintenance. Adequate for treating large and/or permanent mosquito habitats, not generally accepted for drinking water storage containers.	Predators of mosquito larvae (kill all stages). Controversial, harmful impacts of nonnative species, such as <i>Gambusia</i> .	(Kay et al. 2005, Han et al. 2015, Lazaro et al. 2015, Benelli et al. 2016, Faraji and Unlu 2016, Alvarado-Castro et al. 2017, Azevedo-Santos et al. 2017)
		Copepods (<i>Mesocyclops</i>)	Limited epidemiological (level 2b) and entomological evidence (level 3b) for copepods depending on settings.		Predators of mosquito larvae (kill young instar larvae).	

3.3.1.2. Larval control

Targeting the immature stages, has been the standard method to prevent adult *Aedes* emergence thus, to reduce adult density. The methods used for larval control had been described with regards to epidemiological outcomes by Roiz et al. as shown in Table 2. Yet, larval management should be considered as a part of a global strategy to improve its effectiveness and sustainability. Moreover, larval control must be carried routinely to achieve effective dengue vector control.

3.3.1.2.1. Environmental management

Environmental management is a key pillar of *Aedes* control and is recommended as part of IAM for all transmission settings and/or *Aedes* invasion stages (Roiz et al. 2018). It can be implemented at the community level for example to improve water supplies and waste management (Table 2). A pilot study in Merida, Mexico, demonstrated that involving communities by prompting plastic recycling and targeting the more at-risk population reduced *Aedes* density (Barrera-Perez et al. 2015). At the individual level, source reduction by covering the water storage containers, and removing water from flower pots, are efficient to reduce vector density. However, elimination of all potential breeding sites is challenging and time consuming as some are cryptic, especially for *Ae. albopictus*.

3.3.1.2.2. Chemical control

The use of chemicals remains the method of choice for dengue vector control. Larvicides are usually added into the water containers to prevent immature stage development (World Health Organization 2009). Yet, larvicides should be used with parsimony in order to extend life span of chemical insecticides and avoid resistance selection (Roiz et al. 2018). Only few larvicides are recommended by the WHO for the treatment of drinking water containers (i.e., temephos, metoprophene, pyriproxyfen and *Bacillus thuringiensis israelensis* –Bti) (World Health Organization 2009). Yet temephos, remains the most used insecticides globally for larval control due to its low cost, with the exception of Europe because OPs are not anymore registered for mosquito control (George et al. 2015). However, the rise of temephos resistance reinforce the needs for new insecticides for maintaining effective control of wild mosquito populations.

Pyriproxyfen (PPF) is an insect growth regulator (IGR), active against the pupal stage by preventing transformation into adult stage can also decrease in adult fertility and fecundity (World

Health Organization 2001). Pyriproxyfen presents also the advantage of being “disseminated” by adults in order to contaminate breeding sites and kill the larvae (Caputo et al. 2012, Chandel et al. 2016). Resistance against this compound has not yet been reported in *Aedes* mosquitoes (Del Rio-Galvan et al. 2016), nevertheless, a recent study has shown that PPF can be metabolized by mono-oxygenase P450 (Yunta et al. 2016) Other larvicides are recommended by the WHO such as pirimiphos-methyl, diflubenzuron, novaluron, and spinosad but are not widely deployed for larval control.

3.3.1.2.3. Biological control

Biological control is an environmentally sound and effective means of reducing or mitigating insect pests through the use of natural enemies (Eilenberg et al. 2001). Natural enemies of insects also known as biological control agents include predators, parasites, and pathogens (Benelli et al. 2016). Since the 19th century, the use of beneficial organisms had been recognized for the control of mosquitoes (Lamborn 1890). For instance, the use fish such as *Gambusia affinis* or other related species were successfully introduced into many countries to control mosquito larvae since the early 1900s (Legner 1995). Introduction of fish and copepods (i.e., crustaceans) in water filled containers showed to reduce the number of larvae per container in Vietnam (Tran et al. 2015) and Thailand (Chansang et al. 2004, Kittayapong et al. 2012). However, their use presents some limitations and epidemiological evidences had been lacking so far (Lazaro et al. 2015).

The *Bacillus thuringiensis israelensis* or *Bti*, is an entomopathogenic bacterium that induce the death of larvae when it is ingested, through a wide range of toxins targeting gut lining. Its larviciding activity was demonstrated in various insect species, including *Ae. aegypti* and no resistance had been demonstrated until now. However, despite entomological evidence of reduction of *Aedes* populations (Kittayapong et al. 2012), no robust epidemiological evidence had been reported (Roiz et al. 2018).

3.3.1.3. Adult control

The aim of adult control is to reduce the density of vectors and to minimize human-vector contact, to interrupt or reduce the risk of virus transmission. The current methods for *Aedes* vector control with their limitations were recently described by Roiz et al. and are summarized in Table 3.

Table 3: Adult control strategies for Aedes-borne disease from Roiz et al.

Stage/ scenario	Methodology	Type of intervention/ product	<u>Strength of evidence*</u>	Constraints/ advantages	Specifications	Ref
Adult control in emergency	Insecticide spraying	Space spraying (indoors, outdoors)	Epidemiological evidence for ISS based on observational studies (level 2b). Several entomological studies (level 3a and 3b) for ISS and OSS.	Insecticide resistance Low acceptability and limited sense of security in the community Poor persistence Regulatory and environmental constraints Needs skilled, experienced staff	Thermal fogging or cold fogging (ULV spray) using WHO- recommended insecticides Indoor house-to-house application using portable sprayer. Outdoor applications (i.e., vehicle- mounted fogger) if mosquitoes are exophilic and exophagic. Applications should be carried out at the right time, in the right place and according to prescribed instructions.	(Erlanger et al. 2008, Esu et al. 2010, World Health Organization 2011b, Stoddard et al. 2014, Bouzid et al. 2016, Bowman et al. 2016, Faraji and Unlu 2016, Samuel et al. 2017)
		Residual spraying (indoors or outdoors)	Epidemiological evidence of IRS (level 2a). Entomological evidence (level 3b) for IRS for <i>A. aegypti</i> and ORS for <i>A. albopictus</i> (level 3b).	Insecticide resistance Costly and time- consuming Requires high coverage Needs skilled, experienced staff	TIRS for indoor resting <i>Ae. aegypti</i> ORS on the vegetation Application by portable compression sprayers	(World Health Organization 2007b, Esu et al. 2010, Bowman et al. 2016, Faraji and Unlu 2016, Muzari et al. 2017, Samuel et al. 2017, Vazquez-Prokopec et al. 2017b)

Table 3: Adult control strategies for Aedes-borne disease from Roiz et al. (continued)

Stage/ scenario	Methodology	Type of intervention/ product	<u>Strength of evidence*</u>	Constraints/ advantages	Specifications	Ref
Adult control for routine and emergency	Mass trapping	Gravid traps (AGO or GAT)	Epidemiological evidence based on observational studies (level 2b). Entomological evidence (level 3b) for <i>A. aegypti</i> .	Low cost Possible to combine with community participation Sustainable, able to be reused for several seasons Individual-based action (requires high degree of compliance)	Need for a coverage of greater than 80% Use large autocidal gravid traps, as AGO or GAT, to maximise visual and olfactory attraction using grass or hay infusion DEET, the longest-lasting; IR3535 or picaridin, medium-long lasting protection; plant-derived oils (eucalyptus, citronella, or geranium), short-term (frequency of applications according to national legislation and/or manufacturer's recommendations)	(Lorenzi et al. 2016, Barrera et al. 2017, Johnson et al. 2017)
		Insecticide-treated materials (clothes, curtains, house screens, water container covers, etc.)	Epidemiological evidence for house screening (level 2b). Entomological evidence for ITCs, house screening, and water container covers (level 3a and 3b). No evidence for bed nets.	No residual activity Individual- and community-based action Residual activity with long-lasting technology Insecticide resistance Low protection against UV Degradation of insecticide	Clothes, curtains, and bed nets treated with WHO-recommended insecticides. Most evidence supports house screening for preventing dengue transmission	(Wilson et al. 2014, Bowman et al. 2016, Kittayapong et al. 2017)

3.3.1.3.1. Chemical control

Chemicals remains the most widely used method for targeting *Aedes* adult mosquitoes. Adult control is usually implemented using two families of insecticides, i.e., the organophosphates (OP) and the pyrethroids (PYR). Adult chemical vector control can be implemented using very limited number of molecules and type of applications as summarized in Table 3 (World Health Organization 2009, Roiz et al. 2018).

3.3.1.3.1.1. Space sprays

Space sprays using mainly PYR, are widely used in emergencies to rapidly kill the adult females and curtail the cycle of transmission. However, the evidence to support the deployment of this intervention is rather weak (Table 3). Indoor space spray (ISS) showed better results in controlling adult populations and dengue transmission, than outdoor space spray using either thermal fogging or cold fogging of Ultra Low Volume (ULV) against the highly endophilic *Ae. aegypti*. (Esu et al. 2010, Stoddard et al. 2014, Faraji and Unlu 2016).

To improve the sustainability of vector control insecticide residual treatment had been investigated (Table 3). Indoor residual spraying (IRS) has demonstrated strong evidence in reducing malaria burden, however, it remains so far, scarcely used for *Aedes*-borne disease control. Yet, study in Australia demonstrated the effectiveness of targeted IRS to prevent dengue in areas where *Ae. aegypti* is the sole vector (Vazquez-Prokopec et al. 2017b). Similarly, outdoors residual spraying (ORS) can be performed to control *Ae. albopictus*, by targeting the vegetation or external walls of habitations (Faraji and Unlu 2016). Nevertheless, IRS and ORS required qualified staff and must be repeated regularly in order to keep mosquito populations at low levels (Roiz et al. 2018).

3.3.1.3.1.2. Insecticide treated materials

Another strategy, taking advantage of the endophilic and anthropophilic behaviour of *Ae. aegypti*, is the use of insecticide-treated curtains, house screens and clothes to reduce adult population. However, insecticide-treated curtains were deployed in Mexico and Thailand with mitigated results (Wilson et al. 2014). While Kroeger et al. and Vanlerberghe et al. reported an entomological impact on the local vector population, Lenhart et al. found no evidence of effectiveness of insecticide-treated curtains for *Aedes* control (Kroeger et al. 2006, Lenhart et al. 2013, Vanlerberghe et al. 2013, Wilson et al. 2014). In addition, the use of insecticide-treated

uniforms was not associated with a reduction of dengue incidence in Thailand probably due to a rapid decline of insecticide efficacy after washing (Kittayapong et al. 2017).

It is worth to note that PYR are the gold standard for adult control but PYR resistance has been reported in many dengue endemic countries (Corbel et al. 2016, Moyes et al. 2017). The rise of PYR resistance foresaw a decline of efficiency of those class of adulticides for adult control and therefore, research on new tools and paradigms for mosquito control has become a priority.

3.3.2. *Alternative strategies for vector control*

Several tools and methods are under consideration by the World Health Organization Vector Control Advisory Group (WHO VCAG) and they have been the subjected of a recent review by (Achee et al. 2019). For instance, genetically modified mosquitoes and *Wolbachia*-infected mosquitoes for population suppression or population replacement have shown promising results in small-medium scale pilot trials (O'Connor et al. 2012, Indriani et al. 2020). The strength and weakness of the new tools and strategies will be briefly presented in the following sections.

3.3.2.1. *Wolbachia*-based strategies

Wolbachia is a bacterial endosymbiont originally found in *Culex* species and is naturally present in 60% of insect species, including *Ae. albopictus*, and is known to disturb host's reproduction, disrupt pathogen transmission, and prevent eggs from hatching by cytoplasmic incompatibility (Yen et al. 1971, Bourtzis et al. 2014, Farnesi et al. 2019). Population suppression strategy using *Wolbachia*-infected *Aedes* mosquitoes was shown to be species-specific (O'Connor et al. 2012, Mains et al. 2016) in California, Thailand, Singapore and Australia (Hoffmann et al. 2014, Tan et al. 2017, Kittayapong et al. 2019, Crawford et al. 2020). The population replacement strategy using strains of *Wolbachia*, seems also promising for interrupting DENV, CHIKV, and even ZIKV transmission, in particular the *wMel* strain, during field releases in Australia and Indonesia (Moreira et al. 2009, Aliota et al. 2016, Tan et al. 2017, Gonçalves et al. 2019, Indriani et al. 2020, Tantowijoyo et al. 2020). Although promising *Wolbachia* based strategies are challenging by several factors including a lack of community's adhesion, ethical and regulatory constraints and a lack of facilities and capacities for scaling-up the intervention (Lambrechts et al. 2015, Ritchie et al. 2017, Ritchie et al. 2018, Xue et al. 2018, Achee et al. 2019, Maïga et al. 2019, Crawford et al. 2020).

3.3.2.2. Genetically modified mosquitoes

Genetic control is defined as the genetic modification of the vectors by introducing genes, conferring a fitness-cost, that can interfere with the fertility, or prevent the emergence of adults, or confer resistance to a given pathogen (Thomas et al. 2000). Originally based on the sterile insect technique (SIT) which imply the release of insect males sterilized by irradiation (Achee et al. 2019, Kittayapong et al. 2019), advances in genetics, enable the release of insects of dominant lethality (RIDL) strategies, based on the insertion of a repressible dominant sex-specific lethal transgene spreading in the vector population (Alphey et al. 2013). The RIDL *Ae. aegypti* OX513A strain (Oxitec company), released in 2009 in Brazil, Panama, and Cayman Islands, has shown to reduce the wild *Ae. aegypti* population by >90% (Carvalho et al. 2015, Gorman et al. 2016). More recently, the development of Clustered Regularly Interspaced Short Palindromic Repeats associated protein 9 (CRISPR-Cas9) systems had enable designing gene drives that precisely cleave the DNA to insert a gene of interest that becomes heritable. While promising results has been shown for malaria vectors, only proof-of-principle was illustrated in *Ae. aegypti* (Achee et al. 2019, Li et al. 2020). Although these methods appear promising, they still need a strong evaluation of their ecological impact and community acceptance for mosquito releases, especially regarding the introduction of genetic modified organism in the environment (Achee et al. 2019). Moreover, these strategies are challenged by potential “re-emergence” of the native population and/or “replacement” by new invasive species (through migration and introduction) that may enable diseases transmission (Gorman et al. 2016).

3.3.2.3. Other tools currently under evaluation

In addition to the conventionally used methods mentioned above, a certain number of innovative vector control tools are under development and are currently examined by the WHO VCAG (Achee et al. 2019, Corbel et al. 2019). First, mosquito traps were modified to improve mosquito attractiveness and reduce vector populations by either killing the gravid females looking for oviposition sites or by eliminating the progeny. The gravid *Aedes* traps (GAT) developed by Biogents® and the autocidal gravidtraps (AGO) developed by the Centers for Disease Control and prevention (CDC) were demonstrated successful in medium scale field trials to control *Aedes* population in Latin America and Australia (Perich et al. 2003, Rapley et al. 2009, Wesson et al. 2012, Barrera et al. 2019, Montenegro et al. 2020). In addition, Attractive Toxic-Sugar Baited

(ATSB) were developed to attract both males and females and incite them to feed on “toxic” sugar meals applied on plants or used in bait station. So far, ATSB efficacy strongly relies on the attractiveness of the bait and the type of toxin used (e.g., spinosad, neonicotinoids, or fipronil) (Müller et al. 2005, Achee et al. 2019). In contrast to GMO and traps, ATSB can be challenged by insecticide resistance and by potential adverse effects of the toxin against non-targeted species and further investigations are needed to assess relevance for vector control. An alternative to chemical insecticides may come from natural compounds produced by fungi or by using the mosquitoes themselves to distribute the insecticide (i.e., pyriproxyfen) into cryptic breeding sites, known as the autodissemination strategy. Another strategy is to reduce the human-vector contact by using spatial repellent. Noteworthy other methods, not involving insecticides are explored such as the destruction of *Ae. aegypti* larvae using acoustic emission (Britch et al. 2016). Yet, these approaches are quite new and will need further research to ensure their efficiency and safety.

3.3.3. Insecticide resistance

Insecticide resistance is an increasing challenge for *Aedes*-borne disease prevention because dengue, Zika and chikungunya control strategies rely heavily on chemical control (see section 3.3). Moreover, few molecules are registered for vector control and the challenges to develop new chemicals for public health are extremely high. Thus, increasing insecticide resistance, enhanced by the massive use of pesticides in agriculture and public health, is now considered by the WHO as a major threat to dengue control and prevention worldwide (Corbel et al. 2016).

3.3.3.1. Global distribution

Insecticide resistance in *Ae. aegypti* and *Ae. albopictus* against the four main classes of insecticide (e.g., carbamates, organochlorine, OPs and PYRs) was reported in at least 57 countries, including South East Asia, the Americas and the Caribbean (Harris et al. 2010, Dusfour et al. 2011, Marcombe et al. 2012, Kasai et al. 2014, Corbel et al. 2017, Moyes et al. 2017, Marcombe et al. 2019).

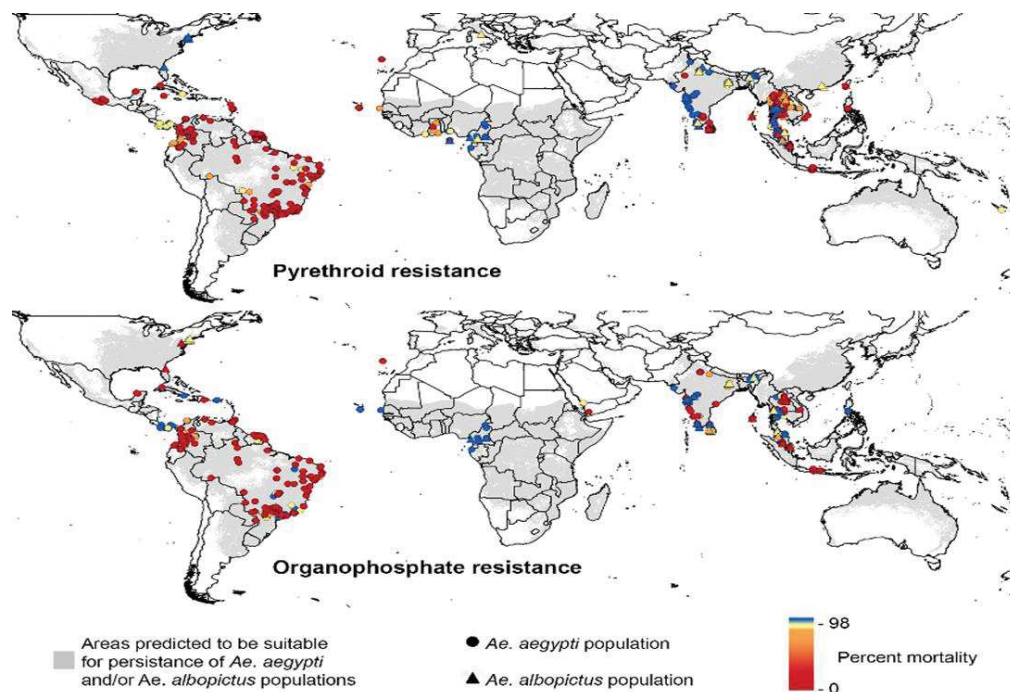


Figure 19: Insecticide resistance against pyrethroid and organophosphate worldwide from Moyes et al.

Evidence of reduced susceptibility to insecticides was recently reported in *Ae. albopictus* in Europe, including Italy, Greece and Spain (Bengoia et al. 2017, Moyes et al. 2017, Dusfour et al. 2019, Kasai et al. 2019, Pichler et al. 2019, Su et al. 2019), confirming the rapid spread of insecticide resistance across continents (Figure 19). The levels of resistance to PYRs and OPs is particularly high in Southeast Asia and Latin America where dengue transmission is also the greatest (Moyes et al. 2017). Temephos (OP) resistance has been reported worldwide (Jirakanjanakit et al. 2007, Strode et al. 2012) including Southeast Asia, Latin America (Ponlawat et al. 2005, Jirakanjanakit et al. 2007, Grisales et al. 2013) (Figure 20), and the Caribbean (Marcombe et al. 2012, Del Rio-Galvan et al. 2016). PYR resistance was reported in most of the dengue endemic areas such as Latin America (Dusfour et al. 2011, Maciel-de-Freitas et al. 2014), Southeast Asia (Li et al. 2015, Plernsub et al. 2016, Kasai et al. 2019, Marcombe et al. 2019) (Figure 19), Caribbean region (Marcombe et al. 2009, Bariami et al. 2012), Pacific region (Koou et al. 2014, Ishak et al. 2015) and Africa (Kamgang et al. 2011) yet, with variable patterns, frequency and mechanisms (Figure 19).

3.3.3.2. Mechanisms

Several studies on *Ae. aegypti* have highlighted a certain number of non-synonymous mutations in the Voltage Gated Sodium Channel (VGSC), known as kdr mutations, especially the S989P, V1016G, and F1534C conferring resistance to PYRs (Bregues et al. 2003, Saavedra-Rodriguez et al. 2007, Kasai et al. 2011, Yanola et al. 2011, Hirata et al. 2014). Moreover, the F1534S mutation in *Ae. albopictus* is associated with PYR resistance in many populations across the world (Chen et al. 2016, Xu et al. 2016). Recent findings suggested a reduction in AChE sensitivity to propoxur in *Ae. aegypti* populations from Trinidad and Tobago (Polson et al. 2011, Vontas et al. 2012), although no evidence for the presence of mutations in the acetylcholine esterase (AChE) gene was reported.

Metabolic resistance caused by changes in the patterns of enzyme's expression resulting in an enhanced insecticide detoxification system was reported in dengue vectors (Strode et al. 2008, Strode et al. 2012). Metabolic resistance is mainly due to three families of enzymes: cytochrome P450 mono-oxygenase (P450s or CYPs for genes), carboxylesterases (CCEs) and glutathione-S-transferases (GSTs) (Hemingway et al. 2004). While PYRs and OPs have different target and mode of action, their detoxification pathway can involve similar enzymes or class of enzymes such as P450s, CCEs, or GSTs (Dusfour et al. 2011, David et al. 2014, Grigoraki et al. 2016). For instance, the carboxylesterase 3 (CCE3A) was implicated in both resistance to OP and PYR in SEA and SEA regions. Consequently, some genes overexpressed in mosquito after exposure to OP can lead to a cross-resistance to PYR and vice versa.

Changes in enzymes expression can be caused by an up-regulation of gene transcription but also due to gene amplification resulting in multiple copies variants (CNV) of the genes coding for the enzymes (Bariami et al. 2012, Kasai et al. 2014, Faucon et al. 2015, Goindin et al. 2017, Cattel et al. 2020b). Duplications were demonstrated in several genes including CYPs, CCEs, and GSTs genes linked to PYR and OP resistance in *Ae. aegypti* in Latin America, Caribbean region, and Asia yet with different genetic profiles (Faucon et al. 2015, Faucon et al. 2017, Goindin et al. 2017, Cattel et al. 2020a, Cattel et al. 2020b).

While many studies focus on *Ae. aegypti*, there is also increasing evidence for the presence of insecticide resistance in the tiger mosquito *Ae. albopictus* (Kasai et al. 2019). Recently Ishak et al revealed that 10 genes were up-regulated in a pyrethroid-resistant population of *Ae. albopictus*

in Malaysia including five P450s (three CYP6, and two CYP9), two GSTs, one ABC transporter and two short-chain dehydrogenases were implicated (Ishak et al. 2016).

3.3.3.3. Impact on vector control

A potential consequence of insecticide resistance is the loss of effectiveness of vector control intervention (Marcombe et al. 2011). Despite increasing concern, the degree to which insecticide resistance compromises dengue control in the field remains largely unknown (Corbel et al. 2019). Recent studies performed in Latin America and Caribbean demonstrated the negative impact of insecticide resistance on vector control activities, targeting either larvae (Montella et al. 2007), or adults (Marcombe et al. 2011, Dusfour et al. 2015, Vazquez-Prokopec et al. 2017a). Gray et al also demonstrated that the use of household insecticide spray led to a selection of resistance mechanism such as *kdr*-alleles. Lessons learnt from the past suggest that monitoring the susceptibility level and changes in genotype frequency regularly allow to readjust vector control policies before vector control failure occur (Corbel et al. 2019). Nevertheless, further investigations are needed to address clearly the relationships between resistance mechanisms, *kdr* frequencies, and the impact of resistance on vector control interventions. This would help authorities to implement timely insecticide resistance management plan.

4. Methods and indicators for assessing and predicting the risk of dengue transmission

Dengue transmission remains complex to assess and even predict using actual tools and indicators. Indeed, dengue transmission risk is highly dependent on the interactions between *Aedes* vectors, human hosts, dengue viruses and the environment. This section will review the concept of dengue transmission, and will describe the actual epidemiological, mathematical, and entomological methods and indicators used to estimate dengue transmission risk with their limitations. Finally, the potential of new immunological tools for estimating “human-vector” contact will be discussed in relation to dengue transmission studies.

4.1. Definition: concept of transmission

Dengue virus is transmitted to human through an infected *Aedes* mosquito bites and mosquitoes can become infected while biting a human infected by dengue virus (Figure 20).

Complex interaction between the mosquito, the host and the vector are driven by key factors such as the intrinsic and extrinsic incubation periods. The intrinsic incubation period is the period when the pathogen (in our case, the dengue virus) is detectable in the (human) host's blood. Chan et al. reviewed 35 studies, published between 1903 and 2011, with relevant data on intrinsic incubation period (Chan et al. 2012) and they concluded that the most probable intrinsic incubation period is comprised between 3-10 days. However, virus can replicate in humans host only if the hosts never experienced this dengue serotype (see section 1.3 and 1.5). Thus, individuals can be infected multiple times by different serotypes. If the human host is already immune against dengue, the virus will not be able to replicate and the transmission will stop. Therefore, herd immunity, serotype distribution and circulation are decisive in estimating (and predicting) dengue transmission.

In addition, DENV transmission from human to human, by sexual transmission was recently reported in South Korea (Lee et al. 2019, Wilder-Smith 2019). Indeed, a woman contracted DENV after her partner previously got infected by dengue overseas. Nevertheless, such cases remain anecdotal, the *Aedes* mosquitoes being the principal vectors of the disease.

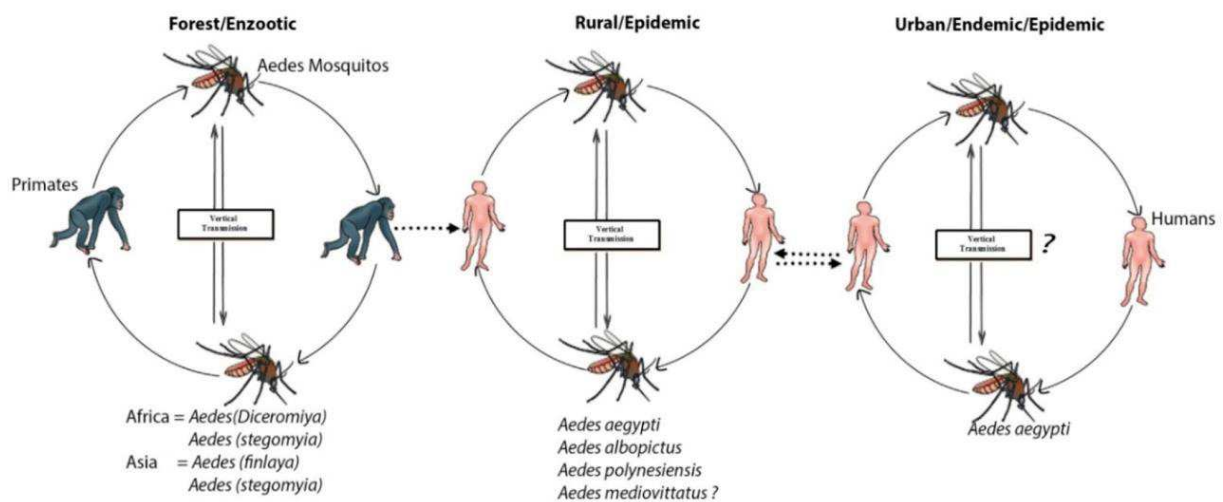


Figure 20: Dengue transmission cycles from Ahammad et al.

Once the virus is disseminated into the *Aedes* organism, it will remain until the mosquito dies. The extrinsic incubation period is defined as the period of time required, after ingestion of the virus during the blood-feeding process, for the vector to be able to transmit the pathogen (Fontaine et al. 2016). *Aedes* extrinsic incubation period for dengue virus, ranges from 8 to 12 days

(World Health Organization 2009), depending on temperature, mosquito populations, and virus serotypes (Whitehorn et al. 2015, Christofferson et al. 2016, Gloria-Soria et al. 2017). For instance, temperature about 29°C were shown to reduce the incubation period, producing more *Aedes* able to transmit the virus. Additionally, temperature above 32°C may reduce *Aedes* lifespan despite a quicker extrinsic incubation period (Christofferson and Mores 2016, Liu et al. 2017). On the other hand, temperature below 18°C strongly slow down viral “migration” thus preventing dissemination to the salivary glands. In addition, Gloria-Soria et al, demonstrated that the success of viral dissemination has variable influence depending on *Ae. aegypti* populations (Gloria-Soria et al. 2017).

In addition to the ability of transmitting dengue virus during their whole life, *Aedes* female can transmit the virus to their offspring (known as vertical transmission) (Castro et al. 2004, Arunachalam et al. 2008). Firstly demonstrated in laboratory conditions, vertical transmission of dengue virus in *Ae aegypti* and *Ae. albopictus* have been reported worldwide, particularly in Latin America and southeast Asia (Thenmozhi et al. 2007, Le Goff et al. 2011, Ferreira-de-Lima et al. 2018). Although experimental studies demonstrated that vertical transmission cannot be sustained after the fifth generation (Rohani et al. 2008, Sanchez-Vargas et al. 2018), vertical transmission of the viruses is a possible explanation for the persistence of dengue serotypes between epidemics (Ferreira-de-Lima and Lima-Camara 2018, Ferreira-de-Lima et al. 2020).

Nonetheless, dengue transmission is highly focal, and characterized by the occurrence of “hotspots”, *i.e.*, *area of higher risk or higher probability of disease incidence* (Martinez-Vega et al. 2015). *Aedes* mosquitoes are known to have small flight range (<100 meters), and usually rest near their sites of emergence. *Aedes aegypti* is often breed in people’s yard, thus somehow it can be considered as a “pet”-mosquito. The limited *Aedes* flight range explains the highly focal pattern of dengue transmission (Stoddard et al. 2013). Human movements, however, by moving between areas with relative dengue transmission risks, can introduce dengue virus into new areas/territories, whether by being dengue infected or by transporting infected vectors (e.g., eggs or adults). Therefore, the spread of dengue at large scale, such as countries, is mainly driven by human movement (Stoddard et al. 2013, Smith et al. 2014). Nonetheless, the presence of competent vectors is required to maintain the transmission.

Dengue and other *Aedes*-borne diseases transmission risk can be assessed through the use of multiple epidemiological, entomological, mathematical, and/or immunological surveillance tools and indicators each one having their strengths and weaknesses. This will be further discussed in the following sections.

4.2. Epidemiological surveillance and its limitations

Epidemiological surveillance is the cornerstone of the assessment of dengue transmission risk. Epidemiological surveillance aims to monitor dengue incidence trends in order to detect outbreaks in a timely manner and trigger interventions from public health stakeholders (World Health Organization 2009). Indicators used for epidemiological surveillance are based on dengue cases detection and serotype distributions.

Dengue case surveillance is based on case notification yet, only symptomatic cases from hospitals are accounted. Moreover, the difficult diagnosis of dengue based on symptoms lead to a non-negligible proportion of misdiagnosed patients (see section 1.5 and 1.6) which impoverish transmission risk assessment (Bhatt et al. 2013). Moreover, Duong et al. demonstrated that asymptomatic cases can transmit dengue virus to mosquitoes, and thus actively participate to dengue transmission (Duong et al. 2015a). Consequently, dengue transmission assessment is strongly challenged by asymptomatic and mild-symptomatic cases that would not seek medical attention or being misdiagnosed (see section 1.5). Moreover, the temporal disconnection between acquiring an infection to time of presenting illness and testing (i.e., identification of a case) may greatly affect attempts to link transmission with actual epidemiological conditions many days prior.

In addition, the possible multi-infections and immunities make outbreaks even more difficult to predict. Many the of biggest outbreaks were related to the sudden increase of prevalence of a serotype that was not circulating (i.e., introduction of a new serotype) or was circulating a lower prevalence during the past years (Mammen et al. 2008, Mondini et al. 2009, Getahun et al. 2019, Phanitchat et al. 2019). Better knowledge on serotype distribution and herd immunity dynamics would help to better predict and prevent dengue outbreaks by re-enforcing dengue vector prevention and control in a timely manner (Bhatia et al. 2013, Bhatt et al. 2013, Reich et al. 2016). Moreover, outbreak definition is subjective and many countries use their own definition (Brady et al. 2015). In most of dengue endemic countries, an outbreak is declared when the number of

hospitalized cases per week (or month) rises above the average of the number of cases in the previous years plus two standard deviation (World Health Organization 2009, Brady et al. 2015). Unfortunately, when the number of cases exceeds the threshold, it is often too late to implement effective vector control interventions and curtail the transmission. Additionally, most of dengue surveillance systems in endemic areas rely on hospital reports, thus missing mild symptomatic cases. Therefore, epidemiological surveillance must be combined to mathematical and entomological surveillance tools to better address transmission risk and prevent dengue outbreaks.

4.3. Mathematical tools and their limitations

Considering the complex relationships between, the vector, the human host, the virus and the environment, mathematical and computational tools were developed to predict transmission risk and prevent outbreak. Indeed, computational technologies enable complex calculation and take into account large datasets with many types of parameters such as case incidence, *Aedes* densities, but also climatic and demographic data or human movements. Predictive models of dengue incidence historically demonstrated variable accuracies in terms of dengue incidence and outbreak predictions (Ramadona et al. 2016, Olliaro et al. 2018, Johansson et al. 2019). The first model of dengue transmission risk (DENSIM) used mainly vectors characteristics and climatic factors (Focks et al. 1993b, Focks et al. 1995). Since then, numerous studies tried to predict dengue incidence using a combination of entomological, epidemiological and climatic factors (Morin et al. 2013, Lee et al. 2017, Johansson et al. 2019, Stolerman et al. 2019). Yet, models of dengue transmission using retrospective epidemiological and climatic data could demonstrate clear evidence of seasonal pattern of dengue transmission in South East Asia (van Panhuis et al. 2015). Recently, the WHO and the special group for tropical diseases research (WHO-TDR) developed a new adaptable model for dengue surveillance and outbreak response (World Health et al. 2017). Using retrospective country datasets, they could define and detect dengue outbreaks using probable/ hospitalised cases as the outbreak variable (defined in section 4.2), and then successfully predict these outbreaks using early changes in various entomological, meteorological and epidemiological “alarm” variables (World Health et al. 2017, Hussain-Alkhateeb et al. 2018). Although predictive models for dengue have been shown to be successful, particularly using climate and incidence data, the inconsistency of entomological data, highlighted the need for more reliable response for estimating dengue transmission risks (Bowman et al. 2016).

To conclude, despite the large number of dengue transmission models developed, they failed to be universally applicable. Indeed, the dengue transmission models developed for the Latin America are difficultly transposable to SEA and vice and versa (Johansson et al. 2016, Reich et al. 2016). In particular, integrating social (e.g., rent value or education level), demographic (e.g., population density or distance to urban habitats), and landscape (e.g., vegetation cover or type of urbanization) data in mathematical models could be helpful to better assess transmission risk and predict further spread and seasonal dynamics and could be less expensive than field studies. In addition, better understanding of the correlations between *Aedes* vectors and dengue transmission risk might improve mathematical models in assessing dengue transmission risk and thus would result in better predictions of dengue outbreaks.

4.4. Entomology surveillance and its limitations

Entomological surveillance is essential to provide information on the local vector population, especially the density and diversity of vector species, their vectorial capacity and their host seeking preferences (World Health Organization 2011b). Several entomological indices are used to assess the risk of transmission targeting different stages of the mosquito development, each presenting their strength and weakness (Table 4 for details).

Table 4: Strengths and weakness of entomological surveillance tools from Roiz et al.

Trap methodology	Index name	Index description	Formula	Unit	Target	Strengths/weaknesses	References
Ovitrap s	Ovitrap index (OI)	Average proportion of positive ovitraps	Positive ovitraps / No. of ovitraps examined in a given area per month/week/ fortnight	%	Eggs	Sensitive and economical method for detecting <i>Aedes</i> introduction and/or presence in large area (surveillance). Information not reliable for measuring <i>Aedes</i> density.	(European Centre for Disease Prevention and Control (ECDC) 2012, Flacio et al. 2015)
	Trap positivity index (TPI)	Proportion of positive traps	(Total no. of traps infested with eggs / Total traps) x 100	%	Eggs		
	Egg density index (EDI)	Ratio of no. of eggs/traps	Total no. of eggs / Total no. of traps	No. of eggs per trap	Eggs	Information not reliable for measuring <i>Aedes</i> density	
Larval indices	House index (HI), (also called premise index)	Proportion of houses positive for immature <i>Aedes</i>	(No. of houses infested / Total households) x 100	%	Pupae, larvae	Not reliable for measuring <i>Aedes</i> population level No information on the number of positive containers Does not take productivity into account Poor indication of adult production	(Focks 2004, European Centre for Disease Prevention and Control (ECDC) 2012, Bowman et al. 2016)
	Container index (CI)	Proportion of containers positive for immature <i>Aedes</i>	(No. of containers infested / Total containers inspected) x 100	%	Pupae, larvae	Relevant for focussing larval control efforts and for orienting educational messages Can provide data on larval development habitat characteristics	
	Breteau index (BI)	No. of <i>Aedes</i> -positive containers per 100 houses	(No. of containers infested / Total houses inspected) x 100	No. per 100 houses	Pupae, larvae	Does not take productivity into account Poor indication of adult production	

	Stegomyia index (SI)	Proportion of positive containers per population	No of positive containers per population) x1000	No. per 1000 people	Pupae, larvae	Not reliable for measuring <i>Aedes</i> population level, only a proxy of seasonal trends Does not take productivity into account Poor indication of adult production	
Pupal surveys	Pupae per person index (PPI)	No. of pupae per person	No. of pupae per household population	No. per person	Pupae	Useful indicator for planning source reduction and environmental management More relevant indicator (compared to larval indices) for estimating adult abundance and evaluating vector control interventions Labour intensive	(Focks 2004, Morrison et al. 2004, Romero-Vivas et al. 2006, Carrieri et al. 2011, European Centre for
	Pupa index (PI)	No. of pupae per house	No. of pupae / Total number of households inspected	%	Pupae	Same as above. Applicable to both public and private domains	Disease Prevention and Control (ECDC) 2012)
	Pupae per hectare index (PHI)	No. of pupae per hectare	No. of pupae per household area	No. per hectare	Pupae		
Adult surveys (BG-sentinel, other traps, human landing rates)	Adult trap index (ATI)	Average no. of adults per trap and per period	Total no. of females caught / Total no. of traps	No. per trap	Adults (indoors and outdoors)	Relevant for estimating relative abundance, seasonal dynamics and spatial distribution trends, and for evaluating vector control measures. Labour intensive and requires skilled staff More costly than other methods	(Silver et al. 2008, European Centre for Disease Prevention and Control (ECDC) 2012, Roiz et al. 2016)

Sticky trap surveys	Sticky trap index (STI)	No. of adults caught by the sticky trap per unit of time	(No of traps positive for <i>Aedes</i> sp./ Total no of inspected traps) x 100	%	Adults	Poor proxy of adult abundance. Useful for collecting gravid females. Easy to use, affordable, and can be deployed at large scale. Can be used to screen virus infections in mosquitoes	(Ritchie et al. 2003, Facchinelli et al. 2007 , Gama et al. 2007)
Adult surveys for viral detection	Vector infection index (VII)	Proportion of Infected females	(No. of virus-infected females / Total no. of females inspected)*100	%	Adults	Relevant for identifying the role of local species in virus transmission and/or for characterising viral strain. Costly, labour intensive, requires skilled staff and strong diagnostic capacity (RT-PCR).	(European Centre for Disease Prevention and Control (ECDC) 2012)
Adult surveys using human hosts	Human-baited double net (HDN) or mosquito electrocuting traps (MET)	Mean number of mosquito females per person per unit of time	No. of females collected per person per unit of time	No. mosquitoes collected per person per unit of time	Adults	Alternative techniques to the human landing catch (HLC) method for collecting anthropophilic mosquito species, and for measuring mosquito biting densities and mosquito behaviour. Labour-intensive, costly, and may pose logistic difficulties (e.g., access to power sources) Human-baited double traps are a good alternative to collecting outdoor mosquitoes. Mosquito electrocuting traps are a good exposure-free alternative to HLC. HDN and MET are well accepted and pose fewer ethical problems than HLC.	(Tangena et al. 2015, Govella et al. 2016)

4.4.1. *Immature indices and their limitations*

Dengue vector presence is more easily assessed through the presence of immature stages. Entomological indices based on larval presence such as the house index (HI), container index (CI), and Breteau index (BI) are commonly used. House index is calculated as a proportion of positive houses to immature stage of *Ae. aegypti*. Container index is the proportion of positive containers to immature stage of *Ae. aegypti* and the Breteau index is the proportion of containers infested by immature *Ae. aegypti* per 100 houses inspected. These indices remain widely used worldwide to assess dengue transmission risk. Indices thresholds were tentatively established to evaluate/estimate the risk of dengue transmission with $HI > 1\%$ and $BI > 5$ (Scott et al. 2003), but they failed to be universal (Table 4). Studies have shown that the BI can be suitable to identify areas at high risk of dengue transmission in regions where dengue incidence is low (Sanchez et al. 2006, Chang et al. 2015). However, in Singapore, the BI remains below the threshold while dengue transmission still occurs (Ooi et al. 2006). On the other hand, in Trinidad, the BI was systematically higher than the commonly accepted threshold of 5 irrespective of dengue status (Chadee 2009). Moreover, Chiaravalloti-Neto et al found no significant correlation between dengue incidence and any of immature indices (Chiaravalloti-Neto et al. 2015). Indeed, those indices are not representative of the adult vector density as immature stages present large mortality rates (Focks et al. 1993a). Therefore, most larval indices do not give information on the productivity of containers nor the actual *Aedes* adult production, thus might not be suitable for estimating dengue risk in high transmission settings (Roiz et al. 2018).

Focks et al (Focks et al. 2000, Focks 2004, Focks et al. 2006, Nathan et al. 2006) have proposed to use pupal indices to have a better assessment of the adult vector density. Pupae per person index (PPI) is calculated as the number of pupae per person in the household. Focks et al (Focks and Alexander 2006) showed that some containers produce more pupae and adults than others. They developed the “key-container” concept and suggested that focusing vector surveillance and control on these containers could significantly reduce dengue transmission. Unfortunately, pupae collections remain difficult to implement in routine basis because they are time-consuming and they require qualified entomological staff (Table 4). Moreover, the

correlation between pupal indices and dengue cases is heterogeneous across studies (Romero-Vivas et al. 2005, Favaro et al. 2013) and this would merit further investigations.

4.4.2. Adult indices and their limitations

Adult mosquito collections have been used to estimate the risk of dengue transmission (Lau et al. 2017, Parra et al. 2018), but they have also their weakness (Table 4). Several methods have been developed to sample adult *Aedes* such as sticky traps, gravitraps (see section 3.3.2.3), or mechanical batterie-driven aspirators (Vazquez-Prokopec et al. 2009). Recently, a new method inspired by the human landing catches was developed, the mosquito electrocuting trap (MET) and demonstrated similar results to the BG-sentinel traps (Ortega-López et al. 2020). The authors suggested a better assessment of host-seeking preferences and human biting rates using MET than BG-Sentinel traps as the latter method is less species-specific and is highly dependent on the types of lures (e.g., CO₂, hay infusion) used to attract mosquitoes (Bazin et al. 2018). Moreover, all adult collection methods are used as a proxy to estimate *Aedes* “density” but they cannot predict the real exposure time between the human host and the vector (Barnard et al. 2014). This information is yet crucial to identify population subsets at higher exposure risk to dengue vector bites and then virus transmission.

4.5. Serological tools to estimate dengue transmission risk

To counter the actual difficulties in estimating dengue transmission risk (see above), new serological tools relying on the human antibody response against arthropod salivary proteins were developed (Doucoure et al. 2015). These tools known as “salivary biomarkers” offer the opportunity to provide more direct and accurate estimation of the human exposure to vector bites, at both community and individual levels, and may then be used as proxy to estimate local disease transmission risk (Sagna et al. 2018). This section will describe the concept of serological biomarkers and how they can be used to predict *Aedes*-borne disease transmission risk.

4.5.1. Concept

Vector-borne diseases have in common to be caused by pathogens that are transmitted to vertebrate hosts (human or animal) through infective bite of the arthropod vector (Doucoure and Drame 2015, Sagna et al. 2018). During the blood feeding process arthropods inject saliva in order to facilitate the blood intake (Figure 21). Saliva of hematophagous insects is composed of

several proteins which modulate the immune response of the host and inhibits coagulation, in order to get a full blood meal. Interestingly, among this cocktail of molecules, some were shown to induce a specific immune response. Several studies have shown that the human antibody (Ab) response to arthropods bites such as ticks (Lane et al. 1999), *Phlebotomus* (Rohousova et al. 2005), and mosquitoes (Remoue et al. 2006, Poinsignon et al. 2008) can be used as relevant markers for assessing human exposure to insect bites and to estimate pathogens transmission risk (Ya-Umphan et al. 2017, Ya-Umphan et al. 2018). Patented biomarkers¹ have been developed to measure the levels of human exposure to *Anopheles* mosquito bites (Remoue et al. 2006, Poinsignon et al. 2008). Additionally, they have been successful to assess the effectiveness of vector control measures for malaria prevention (Drame et al. 2010a, Drame et al. 2013). More recently, salivary biomarker showed to be accurate enough to identify “hotspots” of vector abundance and malaria transmission (Poinsignon et al. 2009, Marie et al. 2015, Ya-Umphan et al. 2017, Ya-Umphan et al. 2018), and then represent promising tools for epidemiology studies.

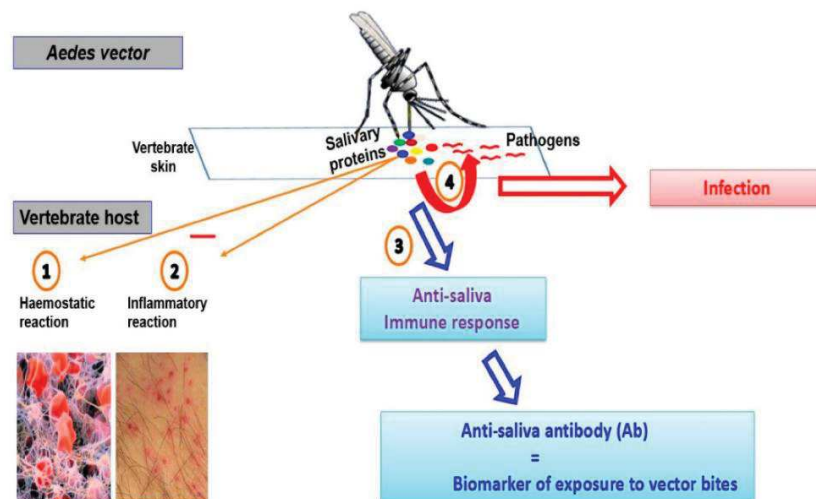


Figure 21: Human-vector relationships during arthropod-borne diseases from Sagna et al. During the bite, the vector (*Aedes*) injects its saliva in the human skin. Once in the skin, salivary proteins take the control of (1) the human hemostatic system by inhibiting the platelet activation and clotting mechanism, and (2) the inflammatory system. (3) The salivary proteins modulate the human immune response and promote the production of anti-salivary antibodies. (4) If ever the (*Aedes*) vector carries a pathogen, the salivary proteins contribute to its transmission into the human.

¹ Internationally patented by Remoue et al. 2011 (patent no. 2009.630 37 33 09).

4.5.2. Application of salivary biomarkers to *Aedes* transmitted diseases

Several studies have shown that the measurement of IgG response against salivary gland extracts from different *Aedes* species, such as *Ae. aegypti*, *Ae. polynesiensis*, *Ae. caspius* were reliable indicators of human-*Aedes* exposure in South-America (Doucoure et al. 2012, Londono-Renteria et al. 2013), Pacific Islands (Mathieu-Daude et al. 2018), Africa (Doucoure et al. 2014) and even Europe (Fontaine et al. 2011). However, the use salivary gland extracts presents some limitations because proteins amount varies between individuals and batches, and may lack of specificity (Sagna et al. 2018). Indeed, salivary extracts of *Anopheles*, *Culex*, and *Aedes* share similar proteins that can induce cross-reactivity. To solve these problems, a salivary peptide (named Nterm-34 kDa) was identified and designed as a specific biomarker of dengue vector mosquito bites (Wasinpiyamongkol et al. 2010, Elanga Ndille et al. 2012, Elanga Ndille et al. 2014) (Figure 22). Sequence alignment with the BLAST program on VectorBase showed no similarity with other mosquito species, indicating the Nterm-34 salivary peptide was specific to the *Aedes* genus (Elanga Ndille et al. 2012).

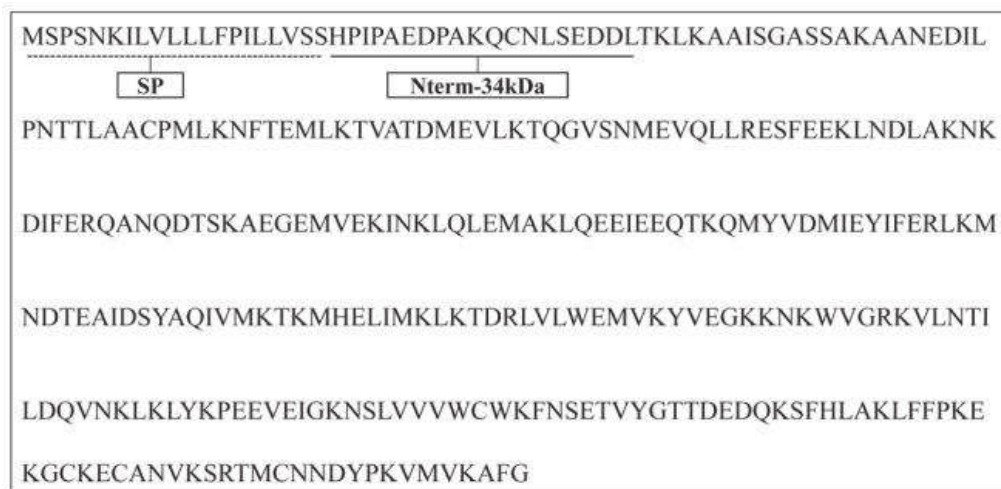


Figure 22: Amino-acid sequence of Nterm-34 kDa peptide.

Ideally, a reliable and accurate biomarker should be able to discriminate between un-exposed and exposed individuals, and should reflect the intensity of exposure (Sagna et al. 2018). This has been demonstrated by Elanga et al in Benin and Laos where individuals leaving in areas where both *Ae. aegypti* and *Ae. albopictus* are present had significantly higher levels of Ab response to the Nterm-34 kDa than unexposed individuals (e.g., living in the North of France). Moreover, the authors showed that the level of IgG response was positively correlated with the

density of *Ae. aegypti* (Elanga Ndille et al. 2012, Elanga Ndille et al. 2014) and varied according to the season (Boonklong et al. 2016) (Figure 23). More recently, the Nterm-34 kDa salivary peptide was used to assess the spatial distribution of *Aedes aegypti* in several urban districts of Senegal (Sagna et al. 2019). The authors demonstrated that the levels of Ab response against the *Aedes* salivary peptide varied according to the quality of sanitation services, i.e., with a lower Ab response in individuals living in districts with better sanitation compared to the one's living in poor sanitary areas. Finally, Yobo et al in Cote d'Ivoire showed that agricultural practices could also increase the risk of *Aedes* mosquito bites by providing suitable breeding habitats for *Aedes* mosquitoes all year round (Yobo et al. 2018).

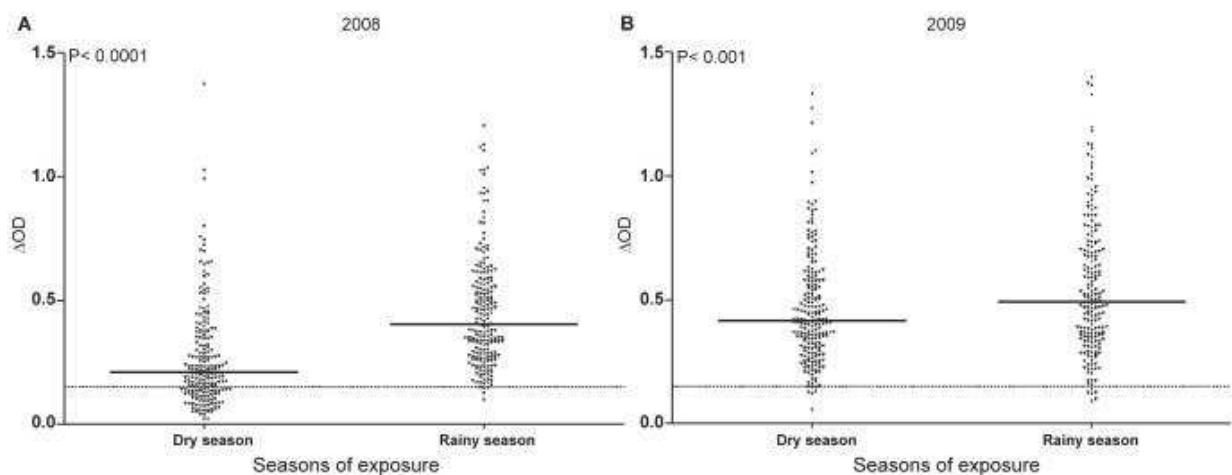


Figure 23: Ab response to Nterm-34 kDa salivary peptide according to the season in children from Benin from Elanga et al.

Overall, the results demonstrated the potential of the Nterm34 salivary peptide to assess variations in *Aedes* exposure risk in various entomological/social/demographical settings. Unfortunately, most of studies estimated the level of *Aedes* infestation using indirect proxy such as rainfall, immature indices, or urbanization levels which have some limitations to accurately address human-vector contact (see section 4.4.1 and 4.4.2 for details). The variations in vector abundance and Ab response to the Nterm-34kDa salivary peptide over time were rarely measured in those studies due to a lack of longitudinal follow-up of both entomology and immunological endpoints (Elanga Ndille et al. 2012, Elanga Ndille et al. 2014, Sagna et al. 2019). Moreover, human-vector exposure and Ab response to salivary peptide can be strongly influenced by individual characteristics and behaviour, such as age, sex, professional habits and/or presence of

vector control interventions as demonstrated previously with malaria vectors. Therefore, more evidence on the capability of the Nterm-34 kDa salivary peptide to assess small-scale variations in *Aedes* abundance and dengue transmission risk is needed to fully validate the Nterm-34 kDa peptide as a relevant serological biomarker for dengue epidemiology study.

Second part: Context of the thesis

1. Challenges in predicting dengue transmission and outbreaks in Thailand

Historically, Thailand has been supporting a vast proportion of the global dengue burden. From 20,000 to 140,000 cases of dengue are reported each year, hence leading to hospitalization and death among children <15 years old (Corbel et al. 2013, Limkittikul et al. 2014). The epidemiology is complex with periods of low and high dengue occurrence (Cummings et al. 2004) (Figure 24) with a peak during the rainy season (May-August) (Limkittikul et al. 2014, Phanitchat et al. 2019). The last major epidemics occurred in 2001-2002, 2010, 2013 and 2015 affecting more than 110,000 persons each. In Thailand the four dengue serotypes are circulating at various prevalence depending on years leading to various outbreak amplitude. During 2017-2018, the DENV-1 was the main serotype circulating among the population (60% prevalence) while the prevalence of DENV-4 was lower than previous years. DENV-3 was the most prevalent serotype between 2013 and 2015 accounting for approximately 30% of the dengue cases occurring at this period (Bureau of Epidemiology et al. 2011, Bureau of Epidemiology et al. 2015, Bureau of Epidemiology et al. 2016, Bureau of Epidemiology and Thailand 2019). The shifting pattern of DENV serotypes distribution remains so far unpredictable and can lead to large outbreaks.

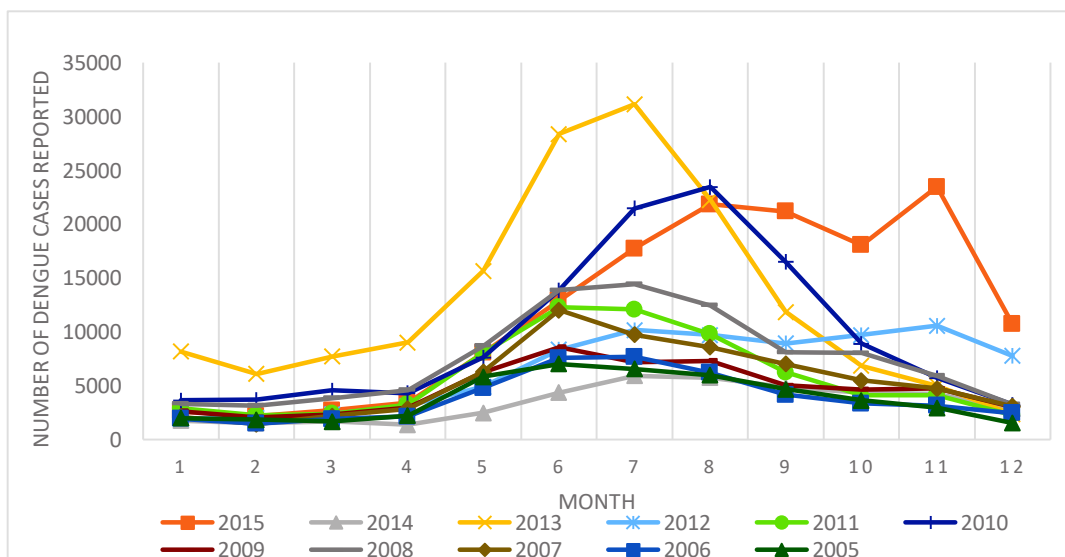


Figure 24: Number of monthly dengue cases reported in Thailand 2005-2015.

Despite the extensive use of epidemiological, entomological and climatic surveillance data for dengue prediction (see sections 4.2, 4.4, and 4.3 for more details), no universal threshold of

transmission risk could be yet established. More importantly, outbreaks continue to occur and the overall dengue incidence is increasing, despite efforts of national program to control the disease. Thailand has established entomological thresholds to assess higher risk of dengue transmission as $CI < 1\%$, $BI < 50$ and, $HI < 10\%$ (see section 4.4.1), however, there are no evidence of general adoption of these thresholds to predict any outbreaks. In addition, the decentralization of vector control has led to differences in Vector Borne Disease Unit (VBDO) leaderships and capacities resulting in varying efficacy in measuring entomology indices and dengue vector risk (Bhumiratana et al. 2014).

In addition, each region of Thailand has distinct patterns of dengue transmission. Using retrospective climatic and epidemiological data over the past 10 years Lauer et al. demonstrated that predictive models performed differently according to the provinces especially regarding the climatic data (Lauer et al. 2018). Moreover, they highlighted that interactions between climate variables and dengue incidence varies over time and space, thus model should take into account vector populations dynamic and their interactions with climatic factors to improve the prediction accuracy (Chumpu et al. 2019). Yet, several gaps exist in estimating dengue transmission risk and there is an urgent need to develop more cost-effective and practical tools that can reliably measure dengue transmission risk and prevent outbreaks.

The current thesis took place in the context of the DENGUE-INDEX Project that was developed to contribute to the development of early warning systems for dengue epidemics in Thailand. The project was supported by the Research Council of Norway and was conducted in North-eastern region of Thailand where dengue incidence is moderate (

Figure 25) but where several unpredictable outbreaks occurred in the last 5 years (see section 1 for details). More information about the objectives and study design of the project are briefly resume in the next section.

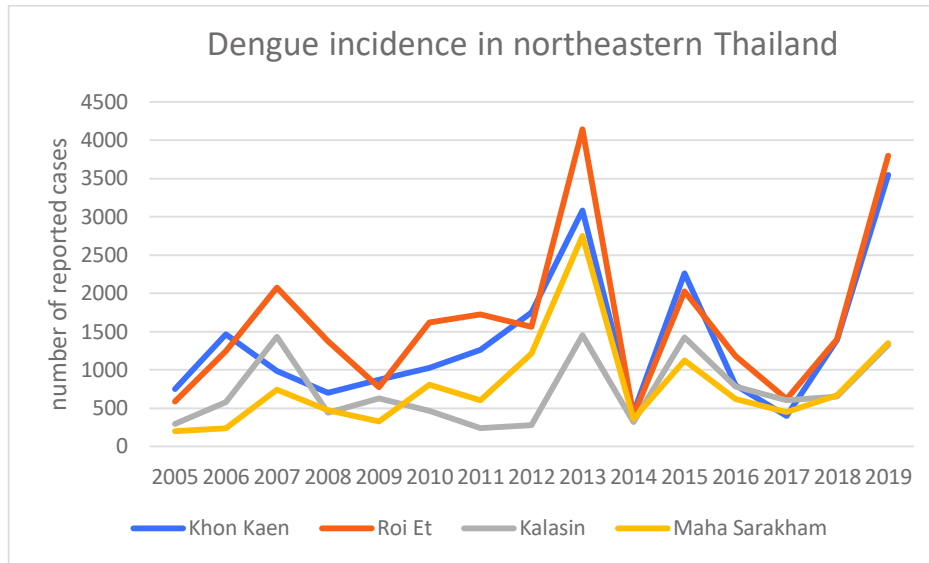


Figure 25: Number of dengue reported cases in Khon Kaen, Roi Et, Kalasin and Maha Sarakham provinces, in northeastern Thailand between 2005-2019.

2. The DENGUE INDEX project

The DENGUE INDEX project was conducted from June 2016 to August 2019 by five highly committed international and national institutions (Norwegian University of Life Science, Khon Kaen University, London School of Hygiene and Tropical Medicine, Office of Disease Prevention and Control region 7, and Institut de Recherche pour le Developpement) to fill knowledge on the fields of entomology, virology, immunology, and epidemiology. The project is relevant to international and national goals to control dengue, e.g., the Partnership for Dengue Control (www.controldengue.org), as well the new Sustainable Development Goals, particularly Goal 3 to ensure healthy lives and promote well-being for all (<https://sustainabledevelopment.un.org>). The intention of the project was to develop practical and sensitive entomological and immunological indicators for estimating dengue transmission risk. The project used an integrated approach using retrospective studies, observational case-control study and a cluster randomized controlled trial to achieve the goals. This project was setting up in north-eastern Thailand because unlike other regions, several gaps remained with regards to dengue epidemiology and its association with environmental, demographic, socio economics and entomology factors (Phanitchat et al. 2019).

The specific outcomes of this project were:

1. To assess the seasonal pattern of dengue transmission in North-eastern Thailand and to identify local clusters of symptomatic disease based on reported dengue cases (retrospective study)
2. To assess the accuracy of entomology and immunology indices in discriminating between dengue positive and negative households and identify potential cofactors as part of a Case Control study with passive detection (see details in section 4.1).
3. To assess the relationships between entomological and immunological indices and dengue incidence to develop dengue outbreak predictive models through a cohort longitudinal study (see details in section 4.3).
4. To assess the accuracy of various entomological, epidemiological and immunology indices in evaluating vector control intervention based on pyriproxyfen as part of a Cluster Randomized Control Trial (RCT) with active case detection (see details in section 4.3).

The project included a strong collaboration with the Ministry of Public Health (MoPH) through the involvement of local authorities. Moreover, dissemination and communication of findings to local communities, national authorities and regional and international stakeholders were provided throughout the lifetime of the project at local and stakeholder engagement meetings. The expected outcomes of the project were to provide national authorities solutions to better forecast epidemics and plan and execute appropriate and timely interventions. These were not only important for Thailand, but also for the whole Southeast Asia region and further afield.

3. Objectives of the thesis

This thesis was conducted in the framework of the DENGUE-INDEX project and aimed specifically to estimate the risk of dengue transmission in North-eastern Thailand using various entomological and serological indicators and to identify the main determinants associated with *Aedes* mosquito exposure using specific salivary biomarker. The thesis had four specific objectives as follows;

3.1.Objective #1: Assessing the spatial and temporal dynamic of dengue in North-eastern Thailand

This baseline study aimed to assess the seasonal patterns of dengue incidence in Khon Kaen province and to identify potential factors contributing to dengue dispersion at a fine-spatial resolution scale. To do so, we carried out a retrospective epidemiological study using monthly dengue incidence and climatic data at the sub-district level, to better understand dengue-climate relationships and to identify periods and areas at higher risk of dengue transmission. Data on dengue cases were retrieved from the national communicable disease surveillance system in Thailand. The association between monthly disease incidence and climate variations was analysed at the sub-district level using Bayesian poisson regression models while Local Indicators of Spatial Association (LISA) were used to identify significant “hotspots” (and “coldspots”) for dengue transmission. This study aimed to get a better picture of the dengue epidemiology in the study area and to get more reliable prediction models for future projections applied in early warning and response systems.

3.2.Objective #2: Addressing the complex relationship between *Aedes* vectors, dengue transmission and socio-economic factors

The objective was to identify entomological and immunological indices capable of discriminating between dengue case and control (non-case) houses in North-eastern Thailand. To do so, we conducted a case-control study (see section 4.1) to assess whether houses with and without dengue cases exhibited different “profiles” in terms of human exposure risk to *Aedes* mosquito bites, as measured by the levels of IgG response to salivary antigens and, *Aedes* infestation levels as measured by the presence and abundance of immature and adult stages. We assumed that people at higher risk of mosquito exposure risk (as measured by entomology and/or immunology outcomes) may be also at higher risk of dengue transmission. Finally, we assessed whether socio-economics, individual and household characteristics may represent additional “risk factors” for acquiring dengue infection.

3.3.Objective #3: Assessing fine-scale variations in human exposure to *Aedes* mosquito bites using salivary biomarker during a vector control intervention.

This study was conducted as part of a RCT (see section 4.3) aiming to evaluate the efficacy of a new vector control intervention for dengue prevention based on Pyriproxyfen (IGR). The objective was to assess the relationship between the intensity of the Ab response to the *Aedes* salivary peptide and the levels of *Aedes* infestation prior and after the deployment of the intervention. Risk factors associated with “*Aedes* exposure risk” including individual and household characteristics, human behaviour, and environmental factors were also explored. Finally, we investigated potential relationships between the intensity of Ab response to *Aedes* mosquito bites and DENV vector infectivity at the household levels. The idea was to generate robust evidence to validate the use of the *Aedes* salivary biomarker as proxy for estimating dengue transmission risk and evaluate vector control intervention in Thailand.

3.4.Objective #4: Evaluating the impact of vector control intervention on the selection of insecticide resistance in dengue vectors

This chapter, which slightly differs from the three previous ones, aimed to assess changes in insecticide resistance traits in local dengue vector populations following the deployment of the pyriproxyfen-based vector control intervention (see details in section 4.3). The rationale behind that study is that pyriproxyfen deployed in permanent breeding containers may select for insecticide resistance hence potentially impacting on the effectiveness of the intervention. To do so, we conducted mosquito collections to assess the levels and mechanisms of insecticide resistance through a combination of biological and molecular assays, prior, during and after the deployment of the intervention. Several candidate resistance markers were followed up for almost 2 years and correlation between resistance phenotype and genotype were addressed. This information is deemed important to determine possible causes of vector control failure and to guide vector control policies in Thailand.

The study design, including methods, randomization, endpoints and statistics are summarized in the following sections.

4. Study design

4.1. Study area

This study was conducted in the North-Eastern region of Thailand, mainly in Khon Kaen and Roi Et provinces but also in Maha Sarakham and Kalasin provinces. This region, known as a part of the Isan area, is on the Khorat Plateau (up to 187m of elevation) and crossed by the Chi river. The landscape is slightly hilly, with numerous swamps and the altitude varies between 90 and 180m above sea level (

Figure 26). Isan region is the third in terms of inhabitants yet, the region contributes only to ten percent of the national gross domestic product. The region is mostly rural with few densely populated urban city centers. The main sector of Isan economy is the agriculture, in particular sticky rice, yet since the 1970's trade and service sectors have increased due to the difficulties of farming (World Bank Group 2016) and rural-urban migration is common in this region of Thailand for multiple reasons including job opportunities, standard of living, better education, and health facilities (Katewongsa 2015). The total population of these four provinces is about 5 million inhabitants (National Statistical Office 2010).



Figure 26: Isan typical landscape.

4.2. Case control study

The first part of the study included a hospital-based prospective case control study conducted in the north-eastern region of Thailand (Fustec et al. 2020). The case control study was initially carried out in two provinces of north-eastern Thailand, Khon Kaen and Roi Et, and was

extended to two additional provinces, Kalasin and Maha Sarakham. A total of nineteen community district and sub-district hospitals in Khon Kaen, Roi Et, Kalasin and Maha Sarakham provinces were asked to participate (Figure 27). Hospitals were selected based on good clinical practices to detect dengue cases and willingness to participate in the study. The study collections began in June 2016 and was carried until sample size, calculated using data from Thomas et al. (Thomas et al. 2015), was reached in August 2019.

At the hospital, presumptive dengue cases were diagnosed using SD Duo Bioline RDT (Figure 28). Consenting dengue positive (cases) and negative patients (controls) were included in the study. Venous blood was taken for DENV detection and serotyping (Shu et al. 2003). Within the day of recruitment, entomological teams were mandated to visit each patient household to collect data on household characteristics, e.g., number of family members, sex, age, travel history, socioeconomic status, GPS position, etc. Additionally, entomological collections were performed at the houses of case and control patients, and in the four neighbouring houses. Collections included immature and adult stage of *Aedes*, captured using batterie-driven aspirators for 15 min each, indoors and outdoors. Furthermore, DENV infection was investigated among *Aedes* females using RT-qPCR (Lanciotti et al. 1992). Several entomological and immunological indices were used as described in Table 5. Entomological indices in patients' houses were distinguished from those at the neighbourhood level (i.e., patient's house + four surrounding houses, Table 5). The *Aedes*-specific immune response was evaluated in each case and control patient from dry blood spots by an indirect Enzyme-Linked Immunosorbent Assay (ELISA) using the Nterm-34kDa salivary peptide (Genepep, St Jean de Vedas, France) and was expressed as a differential optical density (ΔOD). The specific immune threshold (TR) was calculated by measuring the Ab response to the Nterm-34 salivary peptide from individuals with no known exposure history to *Ae. aegypti*. Thus, the mosquito exposure index (MEI) was defined as the sample-specific immune response to the salivary peptide (Table 5).

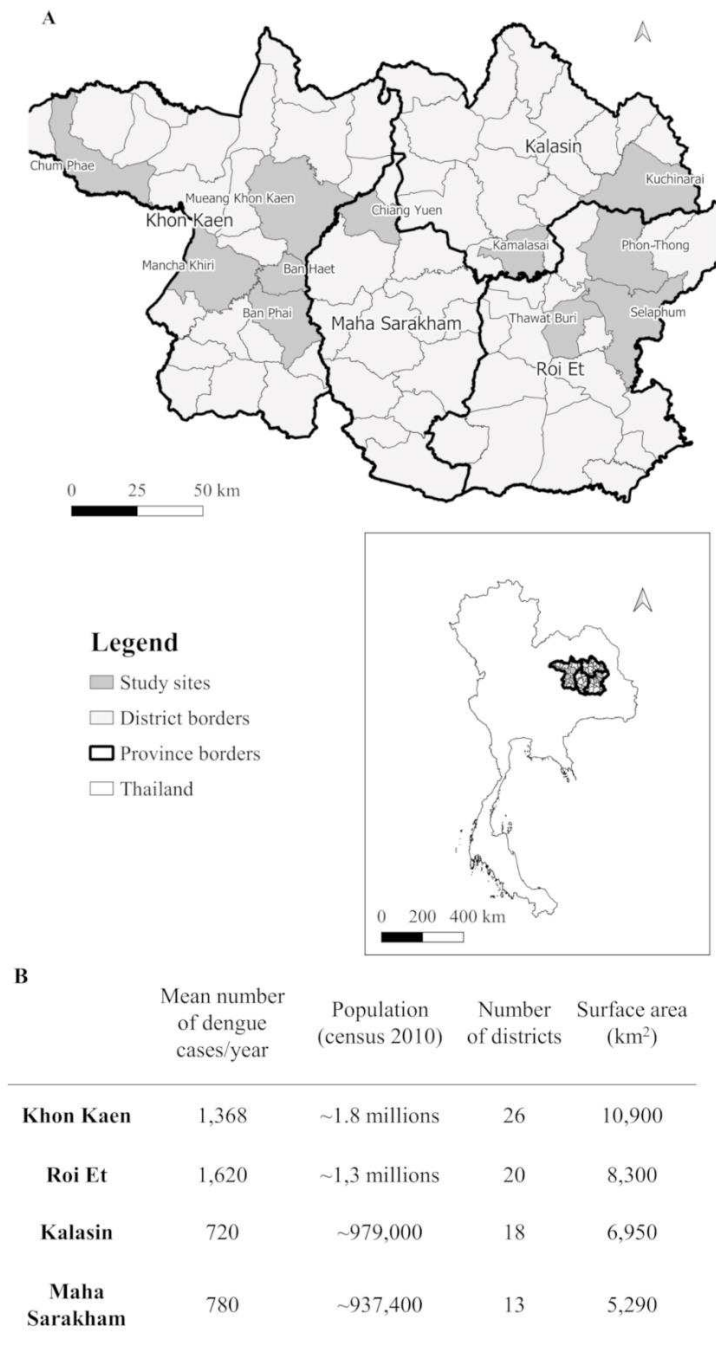


Figure 27: Map and characteristics of study sites of the case-control study in northeastern Thailand. A: Location of four provinces and study districts in north-eastern Thailand included in the case-control study. Map of study sites was built using QGis 3.10 software and shapefiles were obtained from the Humanitarian Data Exchange project (Humanitarian Data Exchange Project 2019) (CC BY 4.0). B: Study area characteristics, population and average number of dengue cases per year from 2005-2019 (National Statistical Office 2010).

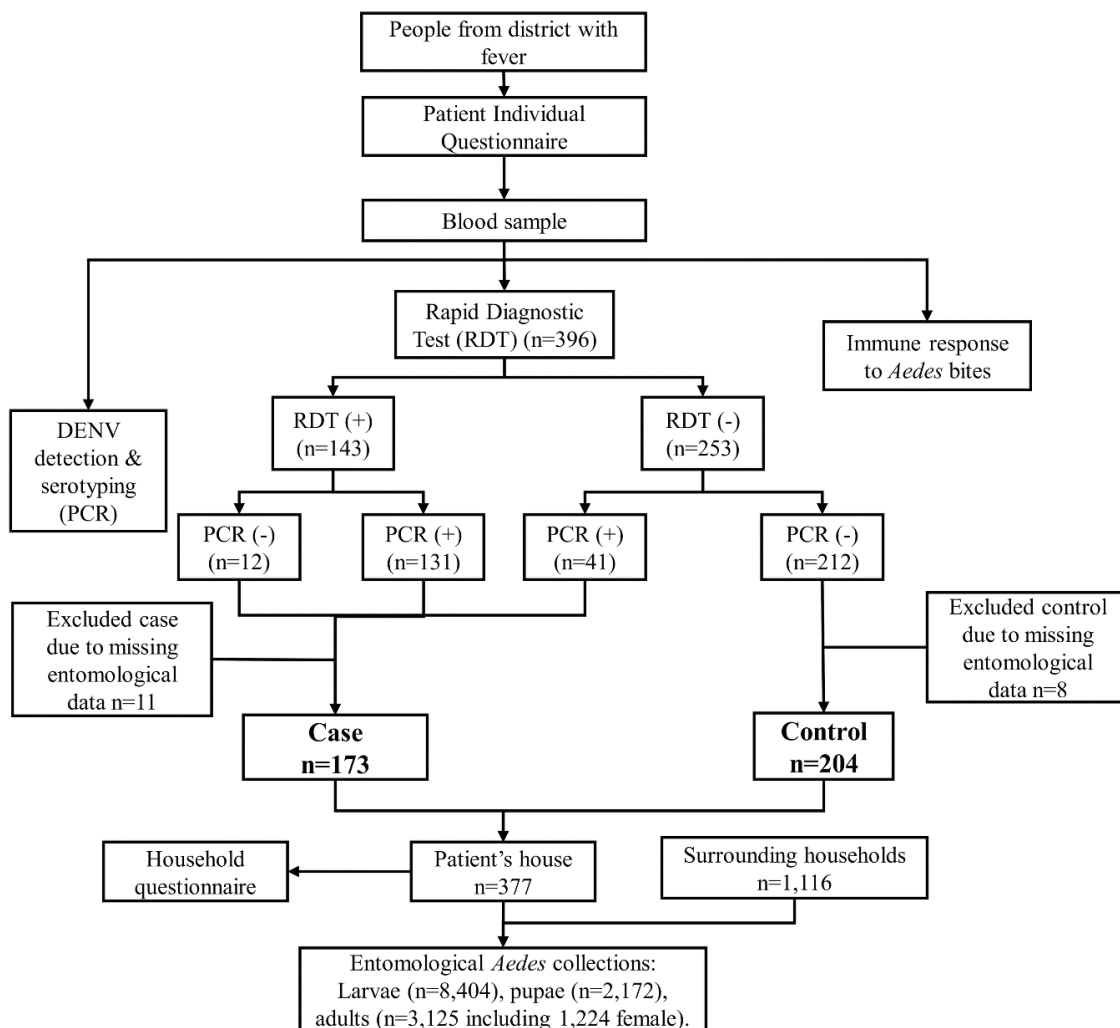


Figure 28: Flow diagram of case-control study design.

The data were analysed using R software version 3.5.1 (R Core Team, Vienna, Austria). The study population was analysed with descriptive statistics, and individuals' information and household characteristics were analysed with the dengue case occurrence as categorical variables using univariable logistic regression. The socio-economic status (SES) of each patient was calculated as a score based on the household questionnaire (e.g., assets, income) using principal component analysis (Vyas et al. 2006). Univariable binomial logistic regression was performed between each entomological and immunological index and dengue case/control status. Multivariable logistic regression was performed using all variables (i.e., individual characteristics, house characteristics, SES, entomological and immunological indices) with a statistically significant association ($p < 0.1$) with case/control status on the univariable analysis. Model selection

was based on backward/forward Akaike Information Criterion (AIC) selection with the selected model was the one with the lowest AIC.

This study was approved from the Khon Kaen University Ethics Committee (KKUEC, project number HE591099), the London School of Hygiene and Tropical Medicine Ethical Committee (LSHTM Ethics, project number 10534), and the Norwegian Regional Committees for Medical and Health Research Ethics (REC, no. 2016/357).

Table 5: Variable definition for the case control study.

Variable	Definition / Formula	<i>Aedes</i> life stage
Individual level		
MEI	MEI= $\Delta OD-TR$ (with TR=0.450) IgG response to Nterm 34 kDa salivary peptide	Adult
Household level (patient house)		
CI (%)	(No. positive container/ total no of wet container) x 100	Larvae & Pupae
AI	No. of adult female <i>Aedes</i> collected	Adult
AI _{in}	No. of adult female <i>Aedes</i> collected indoors (only)	Adult
AI ₊	Proportion of infected females <i>Aedes</i> in the patient house	Adult
PHI	No. of pupae collected at the patient house	Pupae
PPI	No. of pupae collected per person at the patient house	Pupae
Neighborhood level		
CI _n (%)	(No. positive container/ total no. of wet container) x 100	Larvae & Pupae
HI (%)	(No. positive house/ no. households visited) x 100	Larvae & Pupae
BI	No. positive container x100/ No. containers inspected / no. house visited	Larvae & Pupae
AI _n	No. of adult female <i>Aedes</i> collected/ no. households visited	Adult
AI _{n_in}	No of adult female <i>Aedes</i> collected indoor/ no. households visited	Adult
AI _{n+}	Proportion of infected adult female <i>Aedes</i> / no. houses visited	Adult
PHI _n	No. of pupae collected/ no. houses visited	Pupae

4.3. Cluster-randomized controlled trial

The full protocol was published in *Trials* (2018) 19:122, <https://doi.org/10.1186/s13063-018-2490-1>, **Assessing dengue transmission risk and a vector control intervention using entomological and immunological indices in Thailand: study protocol for a cluster randomized controlled trial.** Hans J. Overgaard, Chamsai Pientong, Kesorn Thaewngiew, Michael J. Bangs, Tipaya Ekalaksananan, Sirinart Aromseree, Thipruethai Phanitchat, Supranee Phanthanawiboon, **Benedicte Fustec**, Vincent Corbel, Dominique Cerqueira and Neal Alexander.

And corrected in *Trials* (2018), 19(1), 703. <https://doi.org/10.1186/s13063-018-3110-9>, **Correction to: Assessing dengue transmission risk and a vector control intervention using entomological and immunological indices in Thailand: study protocol for a cluster-randomized controlled trial.**

The second part of the study included a cohort cluster-randomized controlled trial (RCT) that was conducted in Khon Kaen and Roi Et cities. The complete and described protocol was published in *Trials* in February 2018 (see the section Publication4) and corrected in December 2018 (see the section Publication 4).

Briefly, the study started in September 2017 in KK city and October 2017 in RE city, and was conducted during 24-months in each city (Figure 29). The effectiveness of the vector control intervention was measured using immunological and entomological indices (see details in Overgaard et al 2018) including the abundance of *Aedes* adult female and vector infectivity and the intensity of the human Ab response to the *Aedes* salivary antigen. The effect of the intervention on the number of dengue cases was also assessed as a secondary outcome measure (paper under preparation). In addition, the entomological, immunological and climatic indices were used to develop predictive models of dengue transmission and outbreaks.

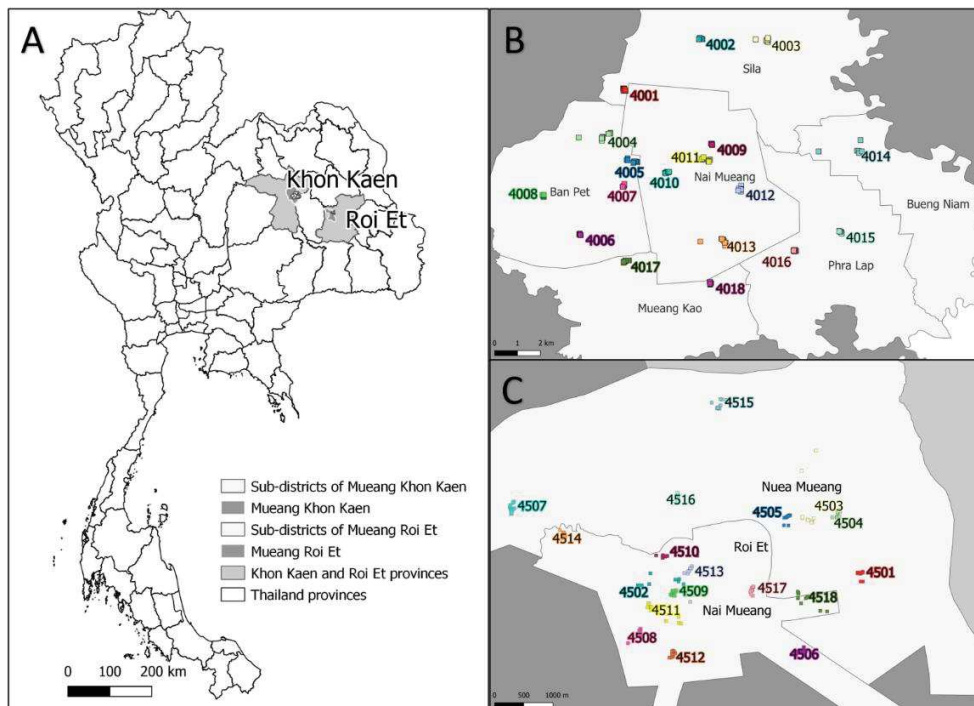


Figure 29: Map of the study sites of the RCT.

Sample size was calculated based on mosquito data from the case control study and the dengue incidence during the previous 10 years (2006-2015). A total of 18 clusters (city blocks) in each city were included with 10 HHs per cluster ($n=180$ HHs per city). Statistical methods for cluster-randomized trials were used to calculate this sample size (Hayes et al. 1999). Households were monitored weekly for presumptive dengue cases (fever cases) during 24 months using RDT by health volunteers. In addition, dried blood spots on filter paper were taken from each presumptive dengue case for immunological assays. In addition, entomological collections, including adult, pupae and larvae *Aedes*, were conducted in all households every four months (Figure 30). Concomitantly to the entomological investigation, dried blood spots were taken from selected inhabitants to assess the human exposure to mosquito bites. Additionally, entomological collections and dried blood spot collections were done every month in three sentinel HHs per cluster.

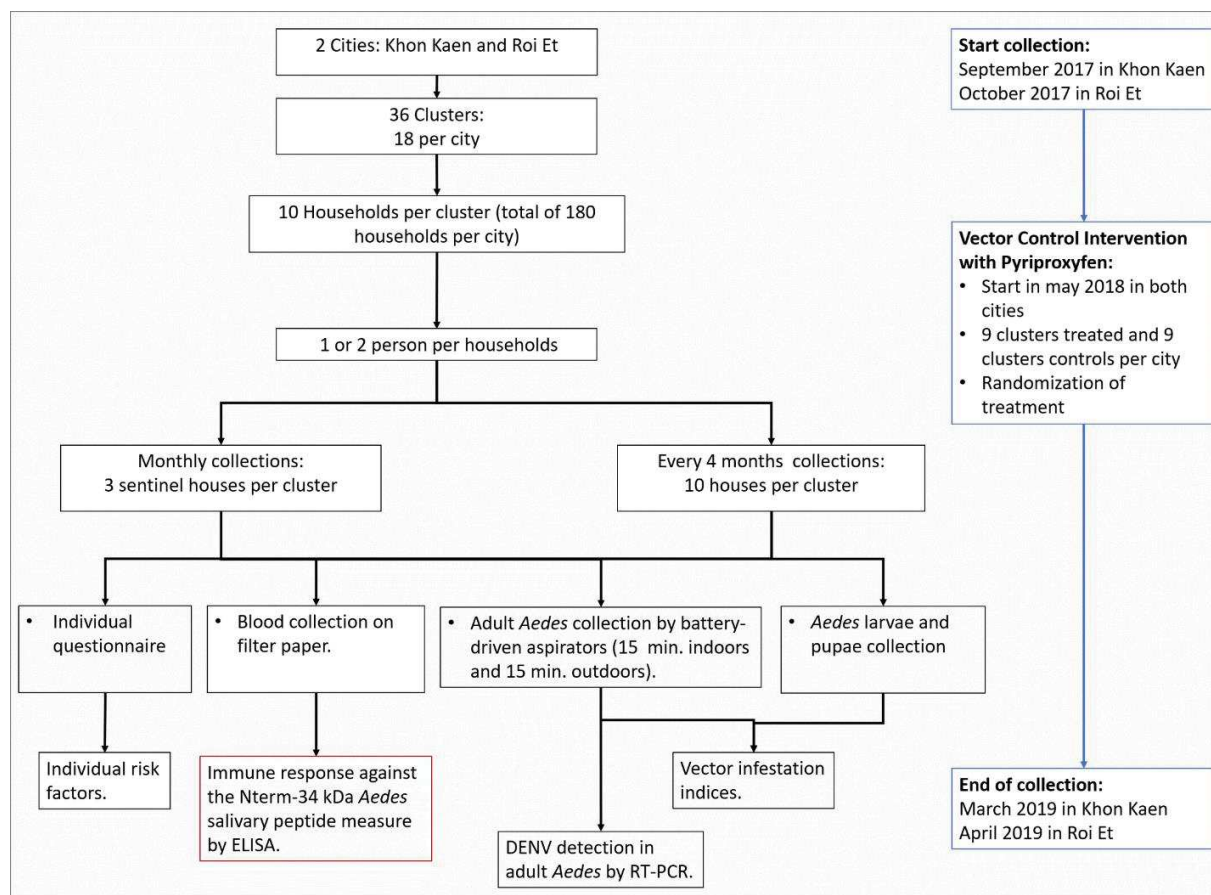


Figure 30: Flow chart of the RCT study design

After a 10 months baseline and just before the next rainy season, half of clusters in each city were randomly selected for a vector control intervention consisting of pyriproxyfen (applied as a 0.5% granule formulation) distributed every four months in permanent breeding habitats (targeted dose of 0.01 mg/L as per WHO recommendation). The other clusters remained as control and did not receive PPF intervention. Intervention and control clusters were followed-up for 14 additional months. Immunological index was calculated at the individual level and entomological indices were calculated for HH clusters all long the study. Impact of the trial was measured using logistic regression on adult *Aedes* index (AI) summarized by cluster using logistic negative binomial regression on the total number of *Aedes* collected and the total number of houses inspected. Additionally, dengue incidence in study households will be analyzed using negative binomial regression. Others entomological endpoints were analyzed the same way as the AI. Moreover, the study attempted to predict dengue incidence over time using entomological and immunological indices, by predicting the risk of a future outbreak, and by estimating associations

between dengue incidence and the indices. This trial was registered (ISRCTN, ISRCTN73606171) and approved by the Khon Kaen University Ethics Committee (KKUEC Record No. 4.4.01: 29/2017, Reference No. HE601221, 1 September 2017), the London School of Hygiene and Tropical Medicine Ethical Committee, UK (LSHTM Ethics Ref: 14275, 16 August 2017), and the Regional Committee for Medical and Health Research Ethics, Section B, South East Norway (REK Ethics ref: 2017/1826b, 03 March 2018).


Simultaneously, complementary mosquito collections were conducted as part of the RCT to evaluate the susceptibility levels of *Aedes* mosquitoes to the insecticide used (PPF) comparatively to the one's used in routine by the ODPC7 (temephos and deltamethrin at 0.5%). Briefly, larvae collections were performed in sentinel HHs, three times; at the beginning of the RCT (baseline), six months after the beginning of the vector control intervention and hence one-year post intervention to assess change in the levels and/or mechanisms of resistance over time. All collections included both treated and control clusters. Susceptibility status of *Ae. aegypti* populations was investigated using standard WHO susceptibility tests (World Health Organization 2005, World Health Organization 2013, World Health Organization 2016b). Additionally, relevant DNA markers (*kdr* mutations) and changes in CNV in genes associated with metabolic resistance were explored following protocols already established by Saavedra-Rodriguez et al (Saavedra-Rodriguez et al. 2007), Yanola et al (Yanola et al. 2011) and Cattell et al (Cattell et al. 2020b).

STUDY PROTOCOL

Open Access



Assessing dengue transmission risk and a vector control intervention using entomological and immunological indices in Thailand: study protocol for a cluster-randomized controlled trial

Hans J. Overgaard^{1*} , Chamsai Pientong^{2,3}, Kesorn Thaewngiew⁴, Michael J. Bangs^{5,6}, Tipaya Ekalaksananan^{2,3}, Sirinart Aromseree^{2,3}, Thipruethai Phanitchat², Supranee Phanthanawiboon^{2,3}, Benedicte Fustec^{2,7}, Vincent Corbel⁸, Dominique Cerqueira⁶ and Neal Alexander⁹

Abstract

Background: Dengue fever is the most common and widespread mosquito-borne arboviral disease in the world. There is a compelling need for cost-effective approaches and practical tools that can reliably measure real-time dengue transmission dynamics that enable more accurate and useful predictions of incidence and outbreaks. Sensitive surveillance tools do not exist today, and only a small handful of new control strategies are available. Vector control remains at the forefront for combating dengue transmission. However, the effectiveness of many current vector control interventions is fraught with inherent weaknesses. No single vector control method is effective enough to control both vector populations and disease transmission. Evaluations of novel larval and adult control interventions are needed.

Methods/design: A cluster-randomized controlled trial will be carried out between 2017 and 2019 in urban community clusters in Khon Kaen and Roi Et cities, northeastern Thailand. The effectiveness of a pyriproxyfen/spinosad combination treatment of permanent water storage containers will be evaluated on epidemiological and entomological outcomes, including dengue incidence, number of female adult dengue vectors infected or not infected with dengue virus (DENV), human exposure to *Aedes* mosquito bites, and several other indices. These indices will also be used to develop predictive models for dengue transmission and impending outbreaks. Epidemiological and entomological data will be collected continuously for 2 years, with the intervention implemented after 1 year.

(Continued on next page)

* Correspondence: hans.overgaard@nmbu.no

¹Norwegian University of Life Sciences, Ås, Norway

Full list of author information is available at the end of the article



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Discussion: The aims of the trial are to simultaneously evaluate the efficacy of an innovative dengue vector control intervention and developing predictive dengue models. Assessment of human exposure to mosquito bites by detecting antibodies generated against *Aedes* saliva proteins in human blood samples has, so far, not been applied in dengue epidemiological risk assessment and disease surveillance methodologies. Likewise, DENV detection in mosquitoes (adult and immature stages) has not been used in any practical way for routine disease surveillance strategies. The integration of multiple outcome measures will assist health authorities to better predict outbreaks for planning and applying focal and timely interventions. The trial outcomes will not only be important for Thailand, but also for the entire Southeast Asian region and further afield.

Trial registration: ISRCTN, [ISRCTN73606171](https://www.isrctn.com/ISRCTN73606171). Registered on 23 June 2017.

Keywords: Dengue monitoring, Entomology, Immunology, Dengue index, Risk assessment, Vector control

Background

Dengue fever is the most common and widespread arboviral disease in the world, with an estimated four billion people in at least 128 countries at risk of infection [1]. The exact global burden of dengue is not known, but there are estimates of about 390 million infections annually, of which only a minority (~ 25%) manifest clinically [2]. Yearly mortality figures of > 20,000 deaths have been reported [3]. Dengue fever and other arboviral diseases, such as Zika and chikungunya, are transmitted to humans primarily by *Aedes aegypti* and *Aedes albopictus* mosquitoes. There is currently no specific treatment for dengue and only recently has a vaccine been licensed, but it does not confer full protection for all virus serotypes [4]. Even with effective therapies and vaccines, vector control will likely remain important to curtail disease incidence and outbreaks. Perennial dengue incidence varies seasonally, and dengue outbreaks occur periodically in most endemic countries [5]. Infection of one of the four dengue virus serotypes (DENV1–4) typically confer lifelong protective homotypic (type-specific) immunity as well as production of more time-limited cross-reactive heterotypic neutralizing antibodies [6]; however, antibody-dependent enhancement may result in a second DENV serotype infection inducing a more severe clinical course [5].

For improved dengue control, reliable epidemic forecasting systems for early detection of temporal anomalies in disease incidence are needed, as well as more effective control strategies that affect both entomological and epidemiological endpoints. Sensitive surveillance tools do not exist today, and only a small handful of new control strategies are available [7–14]. For example, although temephos — the most commonly used chemical-based vector control method, used against immature mosquito stages — may be effective in reducing entomological indices, there is no evidence showing it reduces dengue transmission [7].

There is a compelling need to develop cost-effective approaches and practical tools that can reliably measure

real-time dengue transmission dynamics that enable more accurate and useful predictions of outbreaks. Currently, there is no universally accepted definition of what constitutes an outbreak [15]. This complicates the interpretation of early detection of cases that exceed expected normal seasonal variations. In many endemic countries, a dengue outbreak is declared when the number of reported cases during a specific time period (week or month) surpasses the historical average of the preceding 5 to 7 years above two standard deviations (SD), known as the *endemic channel* [5]. Outbreak definitions vary depending on how the historical average is calculated, which may involve, for example, the number of years used, type of mean (e.g., monthly or moving mean), whether or not outbreak years are included, how the critical threshold is calculated (e.g., ± 2 SD), and criteria used to define the outbreak (e.g., time above the threshold before a response is triggered) [15]. Ideally, when an outbreak alert has been triggered, standard vector control strategies should be implemented. However, current early warning systems and detection of outbreaks are usually neither accurate nor timely enough to initiate effective control interventions (outbreak response) to curb increased transmission after it has begun [11].

Various entomological indices are used to measure dengue vector infestation in and around structures (homes, buildings, etc.). However, these indices are seldom sensitive enough to precisely estimate dengue transmission risk or predict impending outbreaks [16, 17]. The *Stegomyia* indices, i.e., House index (HI, proportion of *Aedes* positive houses) and Container index (CI, proportion of *Aedes* positive containers) were developed nearly a century ago [18], followed by the Breteau index (BI, number of *Aedes* positive containers per 100 houses) [19]. These three measures are currently the most commonly used indices to assess dengue vector larval habitat infestations. They are relatively easy to measure, but are generally not correlated with disease incidence or outbreak risk [16]. In the 1990s, Focks et al. explored the use of pupal surveys as a potentially more

epidemiologically relevant index, correlating total pupal densities with resultant adult densities [20]. This led to further development of entomological thresholds using a pupal/demographic method, ambient temperature, and seroprevalence of dengue antibodies in the population [21]. As a result, container-specific, targeted source reduction was proposed by identifying the relative importance of major types of container habitats with high pupal productivity that contribute significantly to the transmission threshold [21]. However, it remains unclear if targeting only containers that are responsible for the vast majority, say 80–90%, of the pupal production [22–26] is sufficient to have an epidemiological impact on transmission. Other aquatic habitats may be important, such as unusual and cryptic sites, which are typically overlooked during vector control interventions [27, 28]. Furthermore, in many settings, such as in northeastern Thailand and southern Laos, as many as eight to ten of the most productive container types might only produce < 70% of all pupae [29]. Although there are perceived benefits to targeting only the most productive containers, such as reduced time and effort, they may not compensate for ignoring control of other less obvious breeding habitats. The difficulty in finding and effectively treating such cryptic sites can be addressed by using pyriproxyfen, a potent insect growth regulator, which can be transferred between habitats by female *Aedes* mosquitoes during oviposition, a strategy called auto-dissemination [30–32].

A recent study from Iquitos, Peru investigated the relationship between several indicators of *Ae. aegypti* abundance and DENV infection in humans using more than 8000 paired serological samples with corresponding entomological data [17]. The researchers found that indicators based on cross-sectional entomological surveillance, i.e., data from a single survey observation, are of little practical use. On the other hand, longitudinal-based, household-level entomological indicators using data from up to three yearly visits before a 6-month seroconversion period showed that the presence of adult female *Ae. aegypti* in a household increased the risk of DENV seroconversion by approximately 29% compared to households without mosquito vectors. The authors therefore challenged the assumption that most common *Ae. aegypti* indicators provide adequate proxies for DENV risk and transmission [17].

The general response by national dengue control programs to indications of increased disease transmission and possible outbreaks mainly consists of reactive vector control. Typically, control activities involve application of temephos (an organophosphate compound) to domestic water storage containers for larval control and/or peridomestic space spraying with an insecticide, most commonly a pyrethroid-based formulation, for adult

control. Although, these interventions may reduce vector populations dramatically, there is no evidence that they reduce dengue transmission substantially [7, 9]. Other possible options for vector control are community-based source reduction campaigns, application of bacteria-based larvicides, larvivorous fish, or copepods, or combinations of these approaches [8, 33–35]. Newer paradigms for *Aedes* vector control include microbial control of human pathogens in adult vectors, such as *Wolbachia* bacteria that shorten the lifespan of mosquitoes [36] and release of transgenic *Ae. aegypti* engineered to carry a dominant lethal gene that suppresses mosquito populations [37]. These novel approaches are currently not recommended for full-scale programmatic deployment by the World Health Organization (WHO) Vector Control Advisory Group, but rather implemented as carefully planned pilot interventions under operational conditions [38]. The effectiveness of many vector control interventions is fraught with inherent weaknesses, e.g., widespread insecticide resistance, quality of delivery, and other operational issues, such as availability and cost of insecticide, dedicated and trained personnel, and appropriate application equipment [39, 40].

The WHO Global Strategic Framework for integrated vector management (IVM) was released in 2004 and recommends a range of interventions, in combination, to increase impact [41]. This means there is no single vector control method that is effective enough to control both vector populations and disease transmission. Combinations of larval control interventions, such as mixtures of pyriproxyfen (an insect growth regulator) and spinosad (a biopesticide) have been evaluated. This combination reduced larval and pupal relative densities by 90% for at least 4 months in the French West Indies [42]. Pyriproxyfen, even in minute doses, can induce complete inhibition of adult emergence for several weeks after treatment [43]. Pyriproxyfen used alone and applied to storm drains in Colombia reduced dengue cases by 80% [44]. The benefit of using these two compounds in combination is that they have different modes of action and that pyriproxyfen targets the pupal stage while spinosad is active against larval stages. Both compounds have very low toxicity for humans and most other non-target fauna [45, 46]. The WHO draft on global vector control response for 2017–2030 [47] builds on the IVM approach but places stronger emphasis on enhancing human capacity and health education, increasing research and innovation by strengthening infrastructure, and increasing intersectoral and interdisciplinary action. The targets of the global response are to reduce mortality and incidence due to all vector-borne diseases globally relative to 2016 by at least 75% and 60%, respectively.

In view of the preceding discussion, this study aims to assess a specific vector control intervention and to

contribute to the development of a practical early warning system that can more accurately predict changes in dengue transmission and impending outbreaks. The trial will determine the efficacy of a pyriproxyfen/spinosad combination in water storage containers to reduce entomological risk indicators and dengue incidence. The hypothesis is that the study arm receiving the combination treatment in household water storage containers will have a lower density of adult female *Ae. aegypti* per house, both indoors and outdoors, compared to the study arm receiving an alternative intervention involving normal governmental action. Furthermore, the study aims to determine one or more entomological indices and an immunological index that best predicts dengue incidence for the study area.

Methods/design

Objectives

The specific objectives of this trial are to:

1. Assess the effect of periodically treating water storage containers with a pyriproxyfen/spinosad combination on entomological and epidemiological outcomes
2. Determine the most accurate and precise index or indices to predict variation in dengue incidence in time

Trial design

A stratified, cluster-randomized controlled trial is designed to study the effect of a vector control intervention in households located in pre-selected clusters in two urban areas in northeastern Thailand. Each cluster is randomized to one of two arms: intervention clusters receiving treatment of water containers with a pyriproxyfen/spinosad combination, and control clusters not receiving any intervention from this project. Control areas will rely on normal operational vector control interventions performed by the local public services. Randomization of arms is stratified by city (Khon Kaen and Roi Et, i.e., two levels). Stratification is done because there are potential differences between the two cities that may affect the outcomes (e.g., population size, and regional importance in terms of travel, commerce, services, health care, and education); therefore, stratification may reduce the residual statistical error when one compares the two arms. Including two cities may also alleviate problems of low incidence caused by the spatial and temporal variation in dengue transmission; i.e., one area functions as a backup if there are few cases in the other. Including two cities should also increase the generalizability of the trial.

A cluster design is considered the best option because the intervention is not performed on the individual level,

but is rather a spatial, area-wide approach involving treatment of containers in and immediately around each house and property in a study area (cluster). Within a household, it is not feasible to randomize some individuals to one intervention and other individuals to another. Furthermore, the entomological outcomes, both primary (Adult index) and secondary (e.g., Pupal index per person, and the Breteau index), will be estimated on a household level. We are using the larger clusters rather than single houses because (1) there may be mass (area) effects of the interventions, whereby entomological indices in each house may depend partly on the abundance of mosquitoes in neighboring ones, and (2) entire clusters having the same intervention more closely resembles how the intervention would be implemented, should it be scaled up.

Setting

The trial is carried out in two urban areas, Khon Kaen (N16.440236, E102.828272) and Roi Et (N16.055637, E103.652417) cities in northeastern Thailand (Fig. 1). Khon Kaen is the capital city of the province with the same name. The province has an area of ~10,900 km², divided into 26 districts and a population of 1,741,980 in the 2010 national census [48]. Khon Kaen district is the largest by area and population, with a population of around 400,000 over an area of 953.4 km² (population density 416 persons/km²). The district is divided into 17 sub-districts with 272 villages. In 2016, there was a population of 269,247 in six sub-districts that make up greater Khon Kaen (within the ring road) with a resident density of 2500/km² in the central parts. Roi Et is the capital city of Roi Et province and is divided into 20 districts covering a total area of 8300 km² with a population of 1,084,985 in 2010 [48]. The largest district is also called Roi Et, and it has ~160,000 inhabitants, covers an area of 493.6 km², and has a population density of approximately 311 inhabitants/km². There are 15 sub-districts and 195 villages in Roi Et district. The largest sub-district is Nai Mueang Roi Et municipality with a population of approximately 34,000 inhabitants. To delimit the study area, only villages completely within each city's primary access ring road are selected. We use the English word "village", although most are urban divisions. In Khon Kaen, there are 162 villages in six sub-districts within the ring road. In Roi Et, there are 56 villages in nine sub-districts within the ring road, although only 39 have clearly demarcated administrative boundaries.

Between 2006 and 2016, the total number of reported dengue cases (uncomplicated and severe categories) reported in Khon Kaen province was 15,195 (mean 1381 cases/year, range 439–3014), providing an incidence rate of 76.7 cases/100,000 population. In Khon Kaen district,

Table 1 Primary and secondary outcome measures and other entomological indices

Outcome	No.	Index abbreviation	Index name	Description	Unit	Frequency of data collection	Details
Primary outcome	1	AI	Adult index	Number of female adult <i>Ae. aegypti</i> and <i>Ae. albopictus</i> per house collected both indoors and outdoors	No./house	Once every 4 months in all households (HHs) and once every month in 3 HHs per cluster (the same ones each time)	Adult collections using a mechanical battery-powered aspirator for 30 min per house (indoors and outdoors)
Secondary outcomes	2	DIR	Dengue incidence rate	Number of confirmed dengue cases/ observation days of household populations	Rate	Weekly	VHVs detect fever cases. Hospital and project staff collect blood samples
	3	MEI	Mosquito exposure index	(1) Differential optical density for antibodies to <i>Ae. aegypti</i> saliva (2) Proportion above the immune threshold for this assay	(1) Number (2) %	Once every 4 months in all HHs and once every month in 3 HHs per cluster (the same HH as for the AI)	Recurring blood spots from two persons per HH taken on filter paper. IgG antibody response (positive or negative) to the <i>Ae. aegypti</i> Nterm-34 kDa salivary peptide
	4	IAI	Infected adult index	Number of DENV-infected adult female <i>Ae. aegypti</i> and <i>Ae. albopictus</i>	No./house	Once every 4 months in all HHs and once every month in 3 HHs per cluster	Based on adult mosquito collections indoors using a mechanical battery-powered aspirator for 15 min per house and DENV detection in individual mosquitoes
	5	ASTI	Adult sticky trap index	Total number of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> females collected by sticky traps per month	No./trap/month	7 consecutive days per month	Adult mosquitoes collected by sticky traps baited with hay infusion for 7 days every month
	6	PPI	Pupae per person index	Number of <i>Aedes</i> pupae per person	No./person	Once every 4 months in all HHs and once every month in 3 HHs per cluster	From immature collections. All pupae collected divided by the number of household participants
	7	BI	Breteau index	Number of <i>Aedes</i> positive containers per 100 houses	No./100 houses	Once every 4 months in all HHs and once every month in 3 HHs per cluster	From immature collections. Cluster-level result
	Stegomyia indices	8	HI	House index	Proportion of houses positive for immature <i>Aedes</i>	%	Once every 4 months in all HHs and once every month in 3 HHs per cluster
9		CI	Container index	Proportion of containers positive for immature <i>Aedes</i>	%	Once every 4 months in all HHs and once every month in 3 HHs per cluster	From immature collections. Cluster-level result
Pupal indices	10	IPPI	Infected pupae per person index	Number of DENV infected <i>Aedes</i> pupae per person	No./person	Once every 4 months in all HHs and once every month in 3 HHs per cluster	Based on PPI and DENV detection. All infected pupae collected divided by the number of household participants
	11	PHI	Pupae per house index	Number of pupae per house	No./house	Once every 4 months in all HHs and once every month in 3 HHs per cluster	Pupal collections
	12	IPHI	Infected pupae per house index	Number of DENV-infected <i>Aedes</i> pupae per house	No./house	Once every 4 months in all HHs and once every month in 3 HHs per cluster	Pupal collections and DENV detection
Adult indices	13	All	Adult indoor index	Number of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> females per house indoors	No./house	Once every 4 months in all HHs and once every month in 3 HHs per cluster	Adult collections indoors using a mechanical battery-powered aspirator for 15 min per house
	14	IAII	Infected adult indoor index	Number of DENV-infected <i>Ae. aegypti</i> and <i>Ae. albopictus</i> females per house indoors	No./house	Once every 4 months in all HHs and once every month in 3 HHs per cluster	Based on All and DENV detection

Table 1 Primary and secondary outcome measures and other entomological indices (*Continued*)

Outcome	No.	Index abbreviation	Index name	Description	Unit	Frequency of data collection	Details
	15	AOI	Adult outdoor index	Number of <i>Ae. aegypti</i> and <i>Ae. albopictus</i> females per house outdoors	No./house	Once every 4 months in all HHHs and once every month in 3 HHHs per cluster	Adult collections outdoors using a mechanical battery-powered aspirator for 15 min per house
	16	IAOI	Infected adult outdoor index	Number of DENV-infected <i>Ae. aegypti</i> and <i>Ae. albopictus</i> females per house outdoors	No./house	Once every 4 months in all HHHs and once every month in 3 HHHs per cluster	Based on AOI and DENV detection
	17	IASTI	Infected adult sticky trap index	Number of DENV-infected <i>Ae. aegypti</i> and <i>Ae. albopictus</i> females per sticky trap	No./trap/week	Once every 4 months in all HHHs and once every month in 3 HHHs per cluster	Based on ASTI and DENV detection
Premise index	18	PCI	Premise condition index	Degree of shade + condition of house + condition of yard	Number (min = 3, max 9)	Once every 4 months in all HHHs	Observation criteria [90]

VHV village health volunteer, IgG immunoglobulin G

clusters are split equally between the strata (Khon Kaen and Roi Et). To provide an equal number of clusters in each arm, one extra cluster is added per stratum, i.e., 18 clusters per stratum (city) and 9 clusters per arm in each stratum.

Eligibility criteria

Eligibility for participation in the trial is determined on four levels: (1) location or village, (2) cluster of houses within village, (3) households within cluster, and (4)

individuals within households (household residents) (Table 2).

Recruitment

The 162 villages in Khon Kaen and 39 villages in Roi Et located within the respective ring roads are the sampling frames for each stratum. In each stratum, villages are randomly sampled based on probability proportional to population size, i.e., the population of occupied houses (the target denominator of the primary endpoint). These

Table 2 Eligibility criteria by location, cluster, household, and individual

Level	Inclusion criteria	Exclusion criteria
Village	- Within ring roads of each city (stratum) - Populated residential areas	- Area < 0.125 km ² - Number of houses < 100 - Population < 300 - Coverage of residential area 70–80% (scattered housing) - Non-residential areas, e.g., agricultural fields, airports, industrial areas, commercial areas, (e.g., shopping malls), government offices, lakes, army camps, hospitals, and schools
Cluster	- All points of the cluster are at least 100 m from the nearest point of the village border	
Household	- Households that are permanently inhabited - Households that are built or re-populated during the study period	- Apartment buildings - Abandoned houses - Non-permanent households
Individual	- Individuals in households where household head has signed informed consent for household to participate in project - Self-reported fever within the last 7 days - Age ≥ 1 year old	- A travel history outside the village during the previous 7 days - Chronic disease, such as HIV/AIDS, or other health condition that preclude participation in the study - Apparent inability to give informed consent, e.g., due to mental disability

There is a distinction between being included in the final evaluation of endpoints and inclusion for receiving interventions. For example, abandoned houses and non-permanent household structures are not included in evaluation of endpoints, but they may be treated with an intervention if they are located within a radius of 100 m and as feasibly possible

selected villages will be randomly allocated between the arms (see the section on [Assignment of interventions](#) below).

Villages are normally much larger than the target cluster size (10 houses); therefore, to select a starting point for the house selection, a 50 × 50 m grid and a 100-m buffer zone inside of the village perimeter will be superimposed over each village map. The buffer zone of approximately 100 m on the inside of each village border is applied to reduce potential “contamination” (in-flying mosquitoes) from neighboring villages. A random grid cell is selected in each village and 10 houses nearest the centroid of that cell selected. This procedure is followed in each village in this manner as far as practically possible. For example, if a selected household does not want to participate in the study, a neighboring house will be selected.

Informational meetings are held at the sub-district and village administrative levels to provide information about the project and benefits to the communities. Household holders are visited and carefully informed about the study, and informed consent is obtained from the household head. A complete enumeration of all participants in the selected clusters will be completed with assistance from the local administration and village health volunteers (VHVs). This enumeration will be done three times during the study, allowing monitoring of potential participants’ discontinuation in the trial. Data on discontinuation, whether due to movement outside the study area or withdrawal of consent, are relevant for the secondary outcomes of DIR and MEI. Reasons for potential discontinuation will be monitored and taken into account in communication strategies to promote

retention. In addition, a minor monetary compensation for those who provide blood samples for the mosquito exposure study will promote participant retention to complete follow-up of individuals.

Interventions

Following approximately 10–12 months of baseline data collections, household interventions will begin in the selected intervention clusters (Fig. 2). The intervention specifically targets mosquito immature stages by applying a mixture of pyriproxyfen and spinosad to all permanent household containers, whether indoor or outdoor, found to contain water up to a 10-m perimeter from the house. Pyriproxyfen is an insect growth regulator (insect juvenile hormone analog) that is active against pupal stages, resulting in the inhibition of adult development (preventing emergence). It has low mammal toxicity and is recommended by WHO for vector control [52]. Spinosad is a natural insecticide produced by the soil bacterium *Saccharopolyspora spinosa*. It has a neurotoxic mode of action in insects, but with low mammal toxicity, and it is also recommended by WHO [52]. The doses recommended by WHO for *Aedes* immature mosquito control are 0.01 mg/L active ingredient (a.i.) pyriproxyfen (applied as a 0.5% granule formulation) and 0.1–0.5 mg/L a.i. spinosad (also a 0.5% granule formulation) [45, 53]. Both pyriproxyfen and spinosad have also been assessed and approved by WHO for use in drinking water containers [54, 55]. The reasons for selecting this novel intervention are that the combination of pyriproxyfen and spinosad has not yet been tested in a national dengue vector control program; that it should be effective, easy, and practical to use for national control authorities; and that its combined

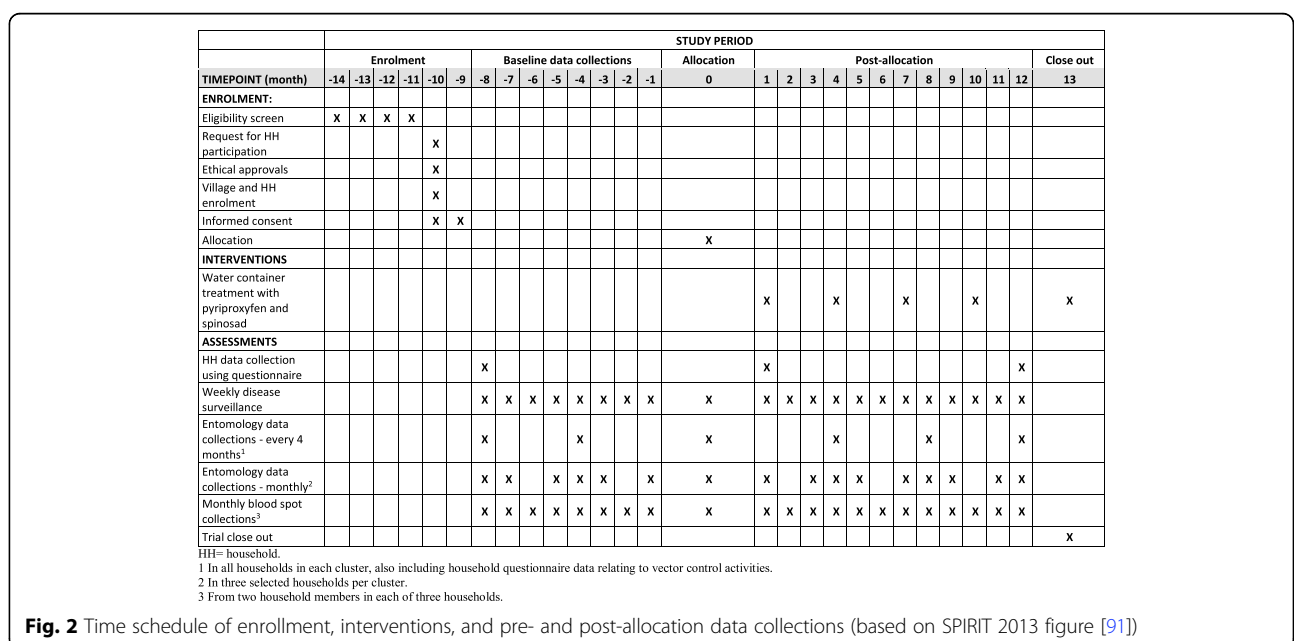


Fig. 2 Time schedule of enrollment, interventions, and pre- and post-allocation data collections (based on SPIRIT 2013 figure [91])

use reduces the risk of resistance development. The interventions will be implemented by project staff from the Ministry of Public Health, thereby ensuring adherence to intervention protocols.

The combination larvicide will be applied simultaneously to containers every 3 months. A buffer zone of approximately 100 m will be established around the selected intervention clusters. All selected households and other households inside this buffer zone will be treated. As far as feasibly possible, abandoned households, non-permanent households, non-occupied properties, and vacant lots inside the buffer zone will be treated in the same manner.

The households in the other half — the control arm clusters — will not receive any specific intervention initiated by the project. However, for ethical reasons, the comparator, i.e., the control arm, will receive normal governmental dengue control activities. Therefore, during the study period, both intervention and control clusters may be subjected to governmental action as part of the existing national dengue control program response. This may consist of space spraying with pyrethroids in and around a household where a dengue (index) case has been reported, including surrounding houses within a radius of 100 m from an index case. Additionally, larval control with temephos applied to household water-holding containers may occur. Larval control activities depend on the availability of staff, insecticides, and time. Although space spraying can be used in clusters of either arm (e.g., if a dengue case is detected), temephos will not be applied in the intervention clusters to avoid biased results and concerns from the public about potential negative effects on water quality. The pyriproxyfen/spinosad combination may be more effective than temephos, particularly since temephos resistance has been detected in *Ae. aegypti* in several sites in Thailand [56].

Assignment of interventions

Sequence generation and implementation

The assignment of intervention (allocation) and control to clusters will be accomplished by two open public lottery events, one in each city (Fig. 2). Allocation will be done several months after and independently from cluster recruitment (Fig. 2). The lottery events will be carried out just before the first intervention. Representatives from each respective sub-district and village, including householders, district village heads, VHVs, and sub-district hospitals, will be invited to attend. Information about dengue and the purpose of the project will be provided. The reasons for randomization, its procedures, and the concepts of intervention and control will be explained. Attendants will also have a chance to ask questions about dengue, vector control, health-seeking

behaviors, personal experiences of dengue, and specific details about the project.

Each of the two lotteries will be performed as follows. Small pieces of paper, of the same color and size and thus indistinguishable from one another, numbered from 1 to 18, will be folded and placed in small opaque envelopes and then placed in a bowl. Each number represents a cluster (village). A large screen with the numbered list of village names (from 1 to 18) will be shown above the bowl and visible to all. A person not involved in the study, and accepted by all participants, will be selected to make the draw. Two flip boards with large sheets of paper will be placed on either side of the bowl with the respective headings “Intervention” and “Control” (in Thai). The village on the first paper drawn will be assigned to the intervention arm, the village on the next paper drawn will be assigned to the control arm, and so on. Following the draw, the implications of being in either of the two arms will be discussed and the roles of participants, health volunteers, and sub-district hospital staff will be reviewed. By following this lottery scheme, the interventions are allocated at the same time as the sequence is generated, obviating the need for allocation concealment.

Blinding

This study is unblinded for both participants and data collectors because of the nature of the intervention and because it is neither practical nor financially feasible to obtain placebo (blank) granules of pyriproxyfen and spinosad to serve as a control. However, although knowledge of treatment allocation could affect mosquito endpoints (e.g., differential collection efforts), the MEI, which relies on the antibody response to *Ae. aegypti* saliva, should not be affected significantly. In terms of performance bias (i.e., systematic differences in care), dengue incidence is an endpoint, and dengue may initiate contact with health care personnel. However, the subsequent course of the episode does not affect any of the endpoints. In other words, care from health personnel will not affect the dengue diagnosis status, so performance bias should not be a concern.

Data collection

Household questionnaire

Following the consent (see more details later in the paper), the household head will be asked to complete a questionnaire on the normal number of people living in the house, their age and sex, and socioeconomic status, including observations of type and quality of house structure and facilities. The household questionnaire will be repeated *annually*. However, parts of the questionnaire relating to vector control activities will be carried out *every 4 months*.

Disease surveillance

VHVs will carry out *weekly* visits at participating households during the 24-month study period. At each visit, household members will be asked about any fever episodes during the preceding week. Body temperature, using an axilla (under-armpit) thermometer, will be measured by the VHVs in all subjects who have reported a recent or current fever. In order to include people who have a fever at times when the VHVs are not visiting, household participants will be asked to call the VHVs by telephone to inform them about this. In that case, the VHV will attempt to visit the house immediately and collect data and temperature from that person. If that is not possible, this person will be included in the next regular VHV visit. Subjects who have or have had a fever (i.e., irrespective of body temperature at the time of the visit) will be brought on the VHV's motorcycle or by other practical means to the collaborating sub-district hospital and offered a blood test using a commercial rapid diagnostic test kit (RDT: SD BIOLINE Dengue Duo Combo device, cat. no. 11FK46; Standard Diagnostic Inc., Suwon, Korea). This test is designed to detect dengue non-structural protein 1 (NS1) antigen and immunoglobulin M (IgM)/immunoglobulin G (IgG) antibodies. An additional 4-mL blood sample and blood spots will be taken for confirmation of DENV infection and serotype determination. All blood samples will be collected by a certified phlebotomist (or other qualified health staff) in accordance with national guidelines. All blood samples will be transferred to the Department of Microbiology, Khon Kaen University, where they will be processed for serum separation and transferred to a -80°C freezer to await further processing. RNA extraction will be performed using a QIAamp[®] Viral RNA Mini Kit (Qiagen, Hilden, Germany) on serum samples. Extracted RNA will be stored at -80°C for viral detection and sequencing. DENV will be confirmed by nucleic acid detection using reverse transcription polymerase chain reaction (RT-PCR) with DN-F and DN-R primers as described in Shu et al. [57]. Data on potential risk factors, such as patient's age, travel history, and previous dengue infection history, will be collected at time of blood sampling. Although not part of the outcome factors, tests for Zika [58] and chikungunya [59] infections will be performed using RT-PCR and sequencing for confirmation.

Inclusion criteria for individuals The inclusion criteria for individuals are as follows:

- Self-reported fever within the last 7 days
- Age ≥ 1 year old

Exclusion criteria for individuals The exclusion criteria for individuals are as follows:

- A continuous travel history outside the district during the last 7 days
- Diseases, such as HIV/AIDS or other health conditions, that preclude participation in the study, based on self-evaluation
- Apparent inability to give informed consent, e.g., due to mental disability or other incapacity, or lack of a legally authorized representative

Exposure to mosquito bites

To assess the level of exposure to *Aedes* bites, blood spots on filter paper will be taken from each person designated as a fever case (detected during the weekly visits) for immunological analysis. In addition, recurring blood spot collections will be taken from two additional individuals, ideally the same adult and child (5–14 years old) each time. These collections will be done *monthly* in each of three households per cluster and *every 4 months* in all households per cluster. Individuals will be selected based on their availability and willingness to participate over the full course of the study; ideally, they will be individuals who are present at home most of the time. Participants providing blood spots will receive a minor monetary compensation. As the immune background will be variable between individuals, the same individuals are needed to follow changes in their immune response to *Aedes* bites over time. Blood samples will be taken from people in their households by a certified phlebotomist (or other qualified health staff) using a finger prick. Two blood spots ($2 \times 75 \mu\text{L}$) will be placed on filter paper (Protein Card Saver 903[™]) and stored at 4°C until further analyses.

Blood samples will be eluted in phosphate-buffered saline (PBS) with 0.1% Tween for 24 h at 4°C and then stored at -20°C . The salivary peptide Nterm-34 kDa (Genepep, St Clement de Rivière, France) will be used as an *Aedes*-specific biomarker to quantify the immune response to *Ae. aegypti* mosquito bites by immunoassays [60]. Briefly, the peptide will be coated on a certified plate (MAXISORP[®]; Nunc, Roskilde, Denmark), and the blood samples will be incubated overnight at 4°C to allow specific IgG to bind to the salivary peptide. An anti-human IgG secondary antibody enzyme conjugate will be incubated to bind individual IgG attached to the biomarker. Substrate will be added for color development. The level of immune response will be assessed by measuring the absorbance after 120 min at 405 nm (Sunrise[™] spectrophotometer, Tecan, Männedorf, Switzerland). Each sample will be compared in duplicate wells and in a blank well (without antigen) to measure non-specific reactions. Individual results will be expressed as a differential optical density (ΔOD) value calculated as $\Delta\text{OD} = \text{OD}_x - \text{OD}_n$, where OD_x represents the mean of individual OD values in the

two wells containing antigen, and OD_n represents the OD value in the well without antigen. Specific anti-Nterm-34 kDa IgG response will be assayed in individuals who have not been exposed to *Ae. aegypti* mosquitoes to quantify the non-specific background antibody level and to calculate the specific immune threshold (TR) as follows:

$$TR = \text{mean}(\Delta OD_{\text{unexposed}}) + 3SD.$$

The main outcome for this immune response assay will be ΔOD , which is a continuous variable. In addition, a binary outcome will be calculated by considering an individual to be “exposed” if the ΔOD value is higher than the TR calculated from unexposed individuals.

Entomological collections

Mosquito collections will be carried out in all participating households every 4 months (Fig. 2). In addition, monthly collections will be done in the three sentinel households per cluster, using the same households as those used for the blood spot collections for logistical reasons. The following data will be recorded from each household: number of total containers (potential breeding sites, wet or dry), number of containers with water, number of mosquito positive and negative containers (any species), container type, and location (indoors/outdoors) using defined criteria. Mosquito larvae will be collected from all positive containers using a standard larval dipper to determine species composition (both *Ae. aegypti* and *Ae. albopictus* will be identified and recorded). Pupae will be collected using the pupal/demographic survey method [19] and the “five-sweep” net procedure for very large containers [61].

Adult mosquitoes will be collected for 15 min indoors (in living rooms, bedrooms, etc.) and 15 min outdoors (among man-made articles, vegetation, etc.) from each household using a Prokopack mechanical aspirator [62]. Adult mosquitoes will also be collected using stationary sticky lure gravid *Aedes* traps [63] placed in a location where mosquitoes are abundant (based on householders’ knowledge) at four selected households for 7 consecutive days every month. Specimens will be taken to a laboratory for sorting and identification using a stereomicroscope and morphological keys [64, 65]. Larvae (separated by species) and pupae (separated by species and sex) will be stored in absolute 99.5% ethanol in labeled 1.5-mL Eppendorf® tubes. Blood digestion status (fed or not fed) of female mosquitoes will be determined by external examination of abdomens. Adult mosquitoes (separated by species and sex) will be stored individually in absolute 99.5% ethanol in 1.5-mL labeled Eppendorf tubes. All specimens will be transported to Khon Kaen University and stored at -80°C until further processing.

Virus detection in mosquitoes

Virus detection will be performed on adults and pupae of *Ae. aegypti* and *Ae. albopictus*. The heads and abdomens of adult mosquitoes will be stored separately. Abdomens will be pooled using a pool size of 5–10 individual abdomens depending on abundance. Virus detection will first be performed on all pools; then, if positive, serotype detection will be done on individual mosquitoes (heads). As heads and abdomens cannot be separated in pupae, virus and serotype detection will be done on pools of whole bodies of pupae. The prevalence of infection in the pupal population, based on the proportion of positive pools, will be estimated using previously described methods [66, 67]. The total RNA will be isolated from mosquito specimens using Favorgen® reagent (FavorPrep™ Tissue Total RNA Mini Kit) following manufacturer instructions. The final solution will be stored at -80°C . DENV presence will be confirmed by real-time quantitative RT-PCR (qRT-PCR) conducted in the LightCycler® 480 Real-Time qPCR System using KAPA SYBR® FAST qPCR Master Mix (2X) Universal [68]. The Master Mix contains an optimized MgCl₂ concentration. Positive samples will be submitted to a second specific qPCR to determine the DENV serotype [69, 70].

Climate data

Climate data, including daily temperature, rainfall, and humidity data, will be collected from permanent weather stations located in Khon Kaen and Roi Et (Department of Meteorology of Thailand). Additionally, four rainfall gauges (three manual and one automatic) and eight temperature-humidity data loggers (iButtons Hygrochron Loggers, DS1923-F5) will be placed in each city at suitable locations to capture local variations.

Data management

Each participating household will be given a 6-digit identification number (indicating province, village, and household number) and an identification plate (with project name and ID number) attached in a secure location to the house. Each household member will also receive a unique ID number. Data from household questionnaires at household enrollment, entomological collections, blood spot sampling at households and hospitals (venipuncture for dengue positivity confirmation), and disease surveillance data by VHV will be collected on paper forms. Data will be securely stored in a password-protected central database. All hardcopy and electronic data will be placed in locked spaces or password-protected computers. Data management procedures will be detailed in specific

standard operating procedures and can be requested from the corresponding author.

Analysis

Index calculations

For all outcomes, baseline measurements will start in the second half of 2017. Post-intervention measurements will start in the second half of 2018. The following indices or rates will be used:

Adult index (AI). Number of adult female *Ae. aegypti* and *Ae. albopictus* per house (combined species) collected both indoors and outdoors for 15 min at each location (30 min total collection time), using a battery-driven mechanical aspirator. Collections will occur once every 4 months in all households and once every month in three repeat sentinel households per cluster

Dengue incidence rate (DIR). Number of confirmed dengue cases divided by observation days of household populations. All household members with a fever will be identified during weekly VHV visits in participating households. Confirmed dengue cases are those febrile patients with a rapid diagnostic test (RDT) positive for NS1, IgM, IgG, or combinations thereof and a subsequent positive laboratory RT-PCR

Mosquito exposure index (MEI). Δ OD in IgG antibodies to *Ae. aegypti* Nterm-34 kDa salivary peptide using immunoassays, within the sampling scheme described above. Also the proportion for whom this differential optical density is above the TR defined above

Infected adult index (IAI). Number of DENV-infected adult female *Ae. aegypti* and *Ae. albopictus* per house (combined species) collected both indoors and outdoors for 15 min each, using a battery-driven mechanical aspirator. DENV presence will be confirmed by real-time RT-PCR as described above

Adult sticky trap index (ASTI). Number of adult female *Ae. aegypti* and *Ae. albopictus* (combined species) collected each month using one sticky trap per house baited with an oviposition attractant hay infusion. Collections will be done in three selected households for 7 consecutive days per month

Pupae per person index (PPI). Total number of *Aedes* pupae collected in participating households divided by the number of persons in that household. Collections will be done once every 4 months in all households and once every month in three repeat sentinel households per cluster

Breteau index (BI). Number of immature *Aedes* positive containers per 100 houses measured at the cluster level. Collections will be done once every 4 months in all

households and once every month in three repeat sentinel households per cluster

Other indices are described in Table 1.

Analysis populations

At the cluster level, analysis will be by intention to treat, i.e., taking the trial arm as that to which each cluster was randomized. At the individual level, people will be taken to have the allocation of the arm in which they are resident at the time of any data contributed. There will be no intention-to-treat analysis, unless, for unforeseen reasons, the Technical Advisory Committee recommends that one be done. A flowchart showing numbers of clusters and average numbers of households per cluster over time will be constructed in accordance with Consolidated Standards of Reporting Trials (CONSORT) guidelines [71]. For the primary analysis, missing data may occur if complete clusters decline to continue in the trial. In this case, the cluster will still be included as long as any data on the primary outcome are available. This does introduce a risk of bias in estimating effectiveness, if loss of clusters is related to performance of the interventions.

Statistical methods

For the entomological endpoints, clustering will be taken into account by analyzing summary measures at the level of cluster. The MEI, expressed as a continuous variable, is a characteristic of individual people, not houses, and its main analysis will be by multivariable multilevel modeling with three levels: cluster, individuals within clusters, and measurements (time points) within individuals. As before, the exposure of main interest will be the arm of the trial (intervention versus control). Individual-level covariates will include age (5–14, 15–25, and > 25 years) and sex. Vector control intervention (i.e., arm of the trial) will be used as a covariate at cluster level. Other cluster-level covariates may include abiotic factors (such as rainfall, temperature, and relative humidity) and population density. An additional analysis of MEI will be by summary measures, as for the other endpoints.

For all analyses of summary measures, the arm of the trial will be the exposure of main interest and will be included in regression models as a dichotomous variable. Stratification will be represented by including a dichotomous variable for city. Finally, the baseline value of each outcome, summarized over the pre-intervention rounds, will be included as a categorical variable, with the expectation that this will reduce the residual error.

For each outcome variable, the response variable for the main analysis will be the aggregate value, for each cluster, of the post-baseline measurements. However, for the primary endpoint (AI), an additional analysis will

include the values at each post-baseline time point for each cluster, and will include an interaction between the arm of the trial and the time point, with the aim of identifying a possibly waning effect of the interventions.

Effect of intervention on primary outcome

For each cluster the total number of adult female *Ae. aegypti* and *Ae. albopictus* and the total number of house visits will be calculated. Taking these as summary measures, a negative binomial regression will be done with the number of mosquitoes as the outcome variable and number of house visits as the exposure (denominator) variable, i.e., with the logarithm of the number of houses as the offset. A logarithmic link function will be used. Hence, the exponential of the coefficient for arm will be the between-arm ratio in AI according to the response variable used.

Effect of intervention on secondary outcomes

Dengue incidence in study households will be analyzed using negative binomial regression. The response variable will be the number of dengue cases per cluster, and the exposure will be the person-time at risk. Hence, the analysis will yield rate ratios. Multilevel models will not be used for this outcome, since the number of cases may be too small for them to be fitted robustly. The total number of DENV-infected adult female *Ae. aegypti* and *Ae. albopictus*, i.e., the IAI, will be analyzed in the same way as the AI. The number of adult mosquitoes per sticky trap will be analyzed similarly to the AI, with the exposure variable being the number of traps. This analysis will yield ratios of the ASTI. Pupae per person (number of *Ae. aegypti* pupae/person) will be analyzed similarly to the AI. The denominator of the PPI is the number of persons present per cluster summed over time. For the BI (number of containers with *Ae. aegypti* immatures/100 houses) the denominator for each cluster is the number of house collections during the intervention period. For example, if the same houses are measured at all time points, the denominator is the number of houses times the number of time points.

Prediction

The study will attempt to predict dengue incidence over time using entomological and immunological indices based on repeated (monthly) field collections. This will be done in two ways: by predicting the risk of a future outbreak, and by estimating associations between dengue incidence and the indices.

Predict the risk of a future outbreak within a week or within a longer lead time According to the Ministry of Public Health, an outbreak is defined as the number of cases per week exceeding the median number of cases

during the last 3–5 years. We will have data on the stated indices 1 week every month, as opposed to every week, so approximately one quarter of the dengue case series data will be able to be used in this analysis.

Using logistic regression, we will develop a prediction rule for the outbreak status (i.e., outbreak or not) in a given week based on data on the indices and on climate, in the previous week or earlier. Climate variables will include rainfall amount and frequency and ambient maximum and minimum temperatures. We will also consider other variables related to housing type and socioeconomic status at the spatial level to be predicted. The aim of the analysis will be to obtain a rule with a high negative predictive value, i.e., with most of the negative predictions being borne out, and with few outbreaks being missed. We will also calculate other operating characteristics such as sensitivity and specificity. We will concentrate on trying to predict outbreaks from one week to the next, i.e., with a lag of 1 week, but will also assess rules for lags up to 4 weeks.

The accuracy of this prediction rule will be assessed by developing it on the majority of the data as a “training” dataset, then evaluating it on the remainder of the data as a “test” dataset. This reduces the tendency to overestimate the accuracy of prediction when the evaluation is done on the same dataset from which the rule was developed.

Estimate associations between dengue incidence and the indices

For this method, we will use Poisson regression and/or time series methods (e.g., autoregressive integrated moving average (ARIMA)) to relate the number of cases per week to our study indices and to climate. Again, the cases in one week will be modeled as a function of data from the previous week or earlier. The associations will be measured in terms of rate ratios or similar coefficients. This analysis will be done using both the incidence in the public health surveillance system and the incidence data from the current study. Associations identified in this analysis may be statistically significant but not of large enough magnitude to enable prediction of outbreak status in the previous section.

Harms

This study is deemed of minimal risk for the participants. Minimal risk is defined as the probability and magnitude of harm or discomfort anticipated in the research that are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests [72]. The vector control interventions, the pyriproxyfen and spinosad formulations, are recommended by WHO for use in disease vector control and in drinking water [46, 53–55]. Hence, adverse events

associated with the products are expected to be few. However, an adverse event, should one occur, will be registered by the community-based VHVs through the weekly visits to all households. The project information sheet, given to all participants, also contains contact telephone numbers of the principal investigators and the Khon Kaen University Ethical Committee should any questions or reservations arise. Any adverse event during the trial interventions or trial conduct will be discussed during weekly meetings of the research team at Khon Kaen University. Expedited decisions will be made as to whether any follow-up action is necessary. The opinion of the Technical Advisory Committee (see the following section) will be sought should there be adverse events believed possibly related to the interventions. Based on these considerations, no criteria have been set for discontinuing or modifying the interventions, nor have any trial stopping guidelines been deemed necessary.

Data monitoring

A Technical Advisory Committee consisting of three independent researchers assumes the role of a Data Monitoring Committee (DMC). The duties of the committee are to stay informed about the progress of the trial; provide advice to the research team when needed; assist in solving ethical issues and unforeseen or adverse events; and determine any potential termination of the trial. This committee is independent and will not benefit from the trial or otherwise influence the trial. The terms of reference of the Technical Advisory Committee can be accessed from the corresponding author. No interim analysis is planned.

Auditing

There will be no formal auditing of this trial.

Confidentiality

As described above, the personal information of enrolled participants will be stored in a safe website ensuring confidentiality before, during, and after the trial. Analysis and publication of the results will ensure that no identifiable information is released.

Ancillary and post-trial care

This trial is deemed of minimal risk to study participants. Therefore, there are no provisions for ancillary or post-trial care or for compensation to those who suffer harms from trial participation, beyond the existing Thai social security system.

Dissemination policy and access to data

Results from this trial will be published in open access, peer-reviewed journals. The presentation of the final results of this trial will follow the CONSORT 2010

statement and the extension to cluster-randomized trials [71] and, if needed, extensions on non-pharmacological interventions and pragmatic designs [73, 74]. This study protocol followed the recommendations of items to address in a clinical trial protocol (Additional file 1) and the minimum trial registration information of WHO (Additional file 2), in addition to what was registered in the primary ISRCTN registry. Access to the trial dataset will be made available upon publication of results. Access to data will also be archived and made available through the Norwegian University of Life Sciences and the Norwegian Centre for Research Data (<http://www.nsd.uib.no/nsd/english/>) after the project has officially ended. Results will be communicated to trial participants in easy-to-read local language pamphlets and through post-project dissemination events. Access to data collection forms can be requested from the corresponding author.

Discussion

This field trial has a novel combination of aims: to evaluate simultaneously the efficacy of an innovative dengue vector control intervention and to develop methods and indices to anticipate changes in dengue transmission and predict impending outbreaks. Such objectives are in harmony with recent published recommendations on global frameworks on vector control and contingency planning for dengue outbreaks [39, 47] as well as recommendations from several review papers on these topics [16, 75]. If successful, results from this study will provide important information on dengue vector control and contribute to the further development of early warning systems and deployment of effective responses to dengue outbreaks.

Currently, the primary vector control methods used by the majority of public-funded dengue control programs are treatment of water storage containers with a larvicide (commonly temephos) and/or peridomestic space spraying of insecticides. Additionally, source reduction practices through community-based clean-up campaigns are common vector control interventions. Although these standard interventions are recommended by WHO [5], there is currently no clear evidence that they have any demonstrable effect on reducing dengue transmission [7, 9, 33].

A systematic literature review on the effectiveness of temephos found that as a single community-based intervention it controlled larvae for 2–3 months, depending on study design, local circumstances, water turnover rates, and season [7]. However, temephos appears not to work well in combination with other interventions, possibly due to an inordinate trust in (or reliance on) its effectiveness when used alone, poor implementation and coverage, and low acceptability for its use in drinking

water [7]. The review concluded that many factors could influence the effectiveness of temephos, such as the degree of intervention coverage, quality of implementation and sustainability, how often treated water is exchanged, and characteristics and use of the target container itself.

A systematic review on the effectiveness of peridomestic space spraying (using pyrethroids, pyrethrins, or organophosphates) showed reductions in various entomological indices; however, the effect dissipated within a few days or weeks [9]. The authors concluded that the effectiveness of space spraying in reducing dengue transmission could not be confirmed and recommended more detailed research on its utility as a practical public health intervention. Container clean-up campaigns might be effective, although such interventions are often confounded by other simultaneous interventions, thus obscuring the effect of the source reduction campaign itself [75].

It appears that most current dengue vector control methods lack clear evidence of their effectiveness, which does not necessarily mean they are ineffective [75]. There have been few well-designed trials, and most have focused on measuring larval and pupal densities, which may not be epidemiologically reliable [16]. The current trial is therefore of great interest to the international dengue control community, as it will look at a much wider range of measures, including adult vector densities. Moreover, the novel use of a pyriproxyfen/spinosad combination in household water storage containers is a promising alternative to conventional vector control methods. Combining the two compounds in a large field trial under natural conditions has not yet been attempted. Furthermore, the two compounds complement each other in that one targets the mosquito larval stage (spinosad) and the other the pupal stage (pyriproxyfen); thus, they potentially provide long-term control in the environment and disease reduction [42, 44]. In Vietnam, pyriproxyfen used together with insecticide-treated covers of water storage containers successfully inhibited mosquito breeding for 5 months [76]. A small, simulated field trial using a pyriproxyfen/spinosad mixture reported that the mixture was effective for at least 8 months compared with 3 months for spinosad alone and 5 months for pyriproxyfen alone. In natural breeding sites the mixture remained effective for 4.5 months [42]. Both compounds are not toxic to humans or most non-target fauna [45, 46]. Pyriproxyfen also has an additional advantage in that it can be disseminated to other larval habitats by adult mosquitoes [30–32].

This trial is also designed to identify practical and sensitive entomological and immunological indicators for prediction of dengue transmission and increased risk for dengue outbreaks. The more accurate, timely, and site-specific the prediction, the greater the likelihood a control response would mitigate, if not prevent, the

outbreak from occurring. The originality of this trial is that virological and immunological methods are used in combination with standard entomological measures in both intervention and control clusters. The Peru study mentioned previously [17] investigated the relationship between indicators of mosquito abundance and DENV infection. Although, it is probably one of the most comprehensive studies to date, such abundance-based indicators are not likely to be sensitive enough to detect changes in intensity of transmission. A better indicator would be to monitor adult mosquitoes for dengue viral infection, similarly as is done to assess malaria transmission risk using the entomological inoculation rate. RDTs can be used as a simple method to detect DENV antigen in mosquitoes [77, 78]. New methods to monitor DENV-infected adult *Aedes* densities using various trapping designs and RDTs have been proposed as a new paradigm in *Aedes* surveillance [79, 80].

Another potentially promising indicator is to measure the exposure of people to mosquito bites using human antibody response to mosquito salivary protein. A recent study carried out along the Thai-Myanmar border areas demonstrated that levels of IgG response were positively associated with anopheline vector abundance and the entomological inoculation rate [81]. The antibody response to *Ae. aegypti* whole saliva has been shown to be a quantitative biomarker of human exposure in Africa and South America [82, 83]. More recently, a salivary peptide (Nterm-34 kDa) was identified as a specific *Aedes* biomarker [84, 85]. The IgG immune response to Nterm-34 kDa salivary peptide is not expected to last for more than 15–30 days; hence, it represents a relevant temporal biomarker to assess recent relative exposure of humans to *Aedes* bites [84]. This peptide was used successfully as a short-time indicator to evaluate vector control interventions against *Aedes* exposure in Réunion Island [86].

In this trial, DENV detection in adults and pupae will be assessed in relation to the number of recent and subsequent confirmed dengue incidents in humans in the same locality. Human exposure to *Aedes* bites measured by IgG antibody response will be examined for correlation with dengue cases. Data will be analyzed to include socioeconomic factors and influence of environmental and seasonal fluctuations, such as rainfall, relative humidity, and ambient temperature. These parameters and specific measures have so far not been fully integrated in epidemiological risk assessments and epidemic forecasting.

The permanent staff of local public health departments, sub-district hospitals, and VHVVs will collect data for all listed outcomes, thereby minimizing involvement of full-time trial project staff. This is intended, as much as possible, to allow national

authorities to emulate the project procedures in follow-on surveillance and intervention activities or adoption of these methods into routine vector control program activities. The exception to this is molecular-based assay confirmation of DENV in human blood and mosquitoes and human antibody response to *Ae. aegypti* salivary peptides; this testing will be conducted by project staff.

Study limitations

Several potential limiting factors may affect study outcomes. High spatial and temporal variation in dengue transmission dynamics may result in an insufficient number of incident infections to allow reliable associations between indices and dengue risk. This is why collections will be conducted over a 2-year period and in two urban areas to increase the potential of witnessing an upsurge in transmission as opposed to an interepidemic period. Nevertheless, the sample size required to detect significant differences in dengue incidence between the intervention and control arms was deemed unfeasibly large, thus relegating dengue incidence as a secondary outcome. Conversely, a dengue outbreak could likely overwhelm data collection systems used in the trial and further compel public health authorities to intervene with standard vector control interventions on a broad scale, thus potentially interfering with study outcomes. If outcome measures are substantially suppressed, this would negatively affect the power of the study.

Lastly, the proportion of asymptomatic (inapparent) and infectious persons in the study area may affect prediction outcomes, because they will not be captured by the data collection procedures used in the trial, although they may contribute to transmission [87]. Although the ratio of asymptomatic to symptomatic cases can be as high as 14:1 or higher, the epidemiological role of asymptomatic infections remains unclear [88].

Trial status

At the time of submission of this manuscript, the trial has enrolled village clusters, requested household participation, and started baseline data collections in Khon Kaen (but not yet in Roi Et). Recruitment of patients has not started.

Additional files

Additional file 1: SPIRIT checklist. (DOCX 53 kb)

Additional file 2: WHO Trial Registration Data Set (Version 1.3). (DOCX 21 kb)

Additional file 3: Consent forms. (DOCX 47 kb)

Abbreviations

Ae.: *Aedes*; AI: Adult index; ARIMA: Autoregressive integrated moving average; ASTI: Adult sticky trap index; BI: Breteau index; CI: Container index; DENV: Dengue virus; DIR: Dengue incidence rate; DMC: Data Monitoring Committee; HH: Household; HI: House index; IAI: Infected adult index;

IgG: Immunoglobulin G; IVM: Integrated vector management; kDa: kilodalton; KKUEC: Khon Kaen University Ethics Committee; MEI: Mosquito exposure index; NSD: Norwegian Centre for Research Data; OD: Optical density; Δ OD: Differential optical density; PBS: Phosphate-buffered saline; PPI: Pupae per person index; qPCR: Quantitative polymerase chain reaction; RDT: Rapid diagnostic test; RNA: Ribonucleic acid; RT-PCR: Reverse transcription polymerase chain reaction; SD: Standard deviation; TR: Immune threshold; USD: United States dollar; VHV: Village health volunteer; WHO: World Health Organization

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Availability of data and materials

The datasets generated and/or analyzed during this study will be made available in the NSD - Norwegian Centre for Research Data repository, <http://www.nsd.uib.no/nsd/english/>, or from the corresponding author on reasonable request.

Authors' contributions

HJO conceived, designed, and coordinated the study; wrote the first manuscript draft, reviewed and edited the manuscript; and secured funding for the project. CP, SA, TP, SP, BF, TE, and MJB developed protocols for disease surveillance and virological laboratory technical aspects. TP, SP, BF, and MJB developed protocols for entomological collections and technical aspects related to virus detection in mosquitoes. BF, DC, and VC developed protocols for exposure to mosquito bites. MJB provided substantial contributions to the study design and drafting and revision of the manuscript. KT contributed to the setting of the study. NA contributed to defining the objectives, the analysis plan, sample size calculations, and sampling methods. All authors contributed to the study design, and writing, reviewing, and editing of the manuscript, and read and approved the final version of the manuscript.

Ethics approval and consent to participate

This trial was approved by the Khon Kaen University Ethics Committee (KKUEC) (Record No. 4.4.01: 29/2017, Reference No. HE601221, 1 September 2017) and the London School of Hygiene and Tropical Medicine Ethical Committee, UK (LSHTM Ethics Ref: 14275, 16 August 2017). Ethical review of the Regional Committee for Medical and Health Research Ethics, Section B, South East Norway (REK) is ongoing. Any follow-on protocol amendments will be reviewed and approved by these committees. The ISRCTN trial registry will also be informed in a timely manner of such amendments. All subjects will be engaged in a process of documented informed consent, and assent where appropriate, before participating in any study activities (Additional file 3). These processes will be conducted by staff of the Office of Disease Prevention and Control in Khon Kaen supervised by Khon Kaen University. There will be three consent processes in this trial:

1. *Request for household participation in a research project.* Before starting the baseline data collections, households will be informed about the trial, and the household head will sign a consent form requesting his/her household to participate in the trial. Household agreement to participate means that its members may be subjected to individual request for participation, as stated in the following two points.
2. *Request for individual participation in a research project.* Individual consent, and assent if appropriate, to participate will be sought from each household member at the time when they have been identified as having a fever during the study observation period. Fever cases will be identified by the VHV's.
3. *Request for blood spot collections.* Consent (assent) will be sought from the two persons per household identified and selected for regular monthly blood spot collections each time blood spots are requested. These two individuals will be provided a small monetary compensation (50 baht = USD 1.50) for each blood sample.

Each of the three consent processes will take place at the households. Informed consent will be documented by means of a written signed and dated consent form after (1) a full description of the study on the information sheet is given to the participant, and (2) the procedures, advantages, disadvantages, and responsibilities and participant's rights to withdraw at any time have been discussed, clarified, and fully understood by the participant. All forms and questionnaires are translated from English into the Thai language and tested for accuracy and interpretation before use. Children will be given a chance to decide for themselves whether they want to participate or not without pressure or coercion from parents or project staff. The assent forms, adapted to the children's ability to read and write, provide a simple and general overview of what participation in the project entails. The children will also be informed about the project verbally in easy-to-understand language. Children will sign the forms before their parents or legal guardians sign. The assent form is for children 7–13 years old. Children 14–17 years old will use the same consent form as that for adults, which has space for both children and parents/guardians to sign. Although the age of majority in Thailand is 20 years, 18 is commonly used as an age of consent for treatment purposes [89]. All consent and assent forms explain the risks and benefits of the study. For participants who cannot read, the entire informed consent form will be read and explained by the project staff in the presence of a community witness. After consenting, these people will mark an inked thumb impression on the form, and the witness will be asked to sign it. Consent and assent forms approved and stamped by the KKUEC are used in accordance with the Ethics Committee's guidelines.

Consent for publication

All authors have consented to publication of this article.

Competing interests

The authors declare that they have no competing interests.

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Author details

¹Norwegian University of Life Sciences, Ås, Norway. ²Khon Kaen University, Khon Kaen, Thailand. ³HPV & EBV and Carcinogenesis Research Group, Khon Kaen University, Khon Kaen, Thailand. ⁴Office of Disease Prevention and Control, Region 7, Khon Kaen, Thailand. ⁵PT Freeport Indonesia/International SOS Indonesia, Kuala Kencana, Indonesia. ⁶Kasetsart University, Bangkok, Thailand. ⁷Université de Montpellier, Montpellier, France. ⁸Institut de Recherche pour le Développement (IRD), Maladies Infectieuses et Vecteurs, Ecologie, Génétique, Evolution et Contrôle (MIVEGEC, UM1-CNRS 5290-IRD 224), Montpellier, France. ⁹MRC Tropical Epidemiology Group, London, UK.

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References

- WHO. Dengue and severe dengue. Factsheet, updated April 2017. World Health Organization; 2017. <http://www.who.int/mediacentre/factsheets/fs117/en/>. Accessed 6 July 2017.
- Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. *Nature*. 2013;496(7446):504–7.
- WHO. Global Strategy for dengue prevention and control, 2012–2020. Geneva: World Health Organization; 2012. WHO/HTM/NTD/VEM/2012.5
- Hadinegoro SR, Arredondo-Garcia JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. *N Engl J Med*. 2015; 373(13):1195–206.
- WHO. Dengue — Guidelines for diagnosis, treatment, prevention and control. Geneva: World Health Organization; 2009.
- Sharp TM, Tomashek KM, Read JS, Margolis HS, Waterman SH. A new look at an old disease: recent insights into the global epidemiology of dengue. *Curr Epidemiol Rep*. 2017;4(1):11–21.
- George L, Lenhart A, Toledo J, Lazaro A, Han WW, Velayudhan R, et al. Community-effectiveness of temephos for dengue vector control: a systematic literature review. *PLoS Negl Trop Dis*. 2015;9(9):e0004006.
- Han WW, Lazaro A, McCall PJ, George L, Runge-Ranzinger S, Toledo J, et al. Efficacy and community effectiveness of larvivorous fish for dengue vector control. *Tropical Med Int Health*. 2015;20(9):1239–56.
- Esu E, Lenhart A, Smith L, Horstick O. Effectiveness of peridomestic space spraying with insecticide on dengue transmission; systematic review. *Tropical Med Int Health*. 2010;15(5):619–31.
- Horstick O, Runge-Ranzinger S, Nathan MB, Kroeger A. Dengue vector-control services: how do they work? A systematic literature review and country case studies. *Trans R Soc Trop Med Hyg*. 2010;104(6):379–86.
- Runge-Ranzinger S, Horstick O, Marx M, Kroeger A. What does dengue disease surveillance contribute to predicting and detecting outbreaks and describing trends? *Tropical Med Int Health*. 2008;13(8):1022–41.
- Runge-Ranzinger S, McCall PJ, Kroeger A, Horstick O. Dengue disease surveillance: an updated systematic literature review. *Tropical Med Int Health*. 2014;19(9):1116–60.
- Morrison AC, Zielinski-Gutierrez E, Scott TW, Rosenberg R. Defining challenges and proposing solutions for control of the virus vector *Aedes aegypti*. *PLoS Med*. 2008;5(3):e68.
- Achee NL, Gould F, Perkins TA, Reiner Jr RC, Morrison AC, Ritchie SA, et al. A critical assessment of vector control for dengue prevention. *PLoS Negl Trop Dis*. 2015;9(5):e0003655.
- Brady OJ, Smith DL, Scott TW, Hay SI. Dengue disease outbreak definitions are implicitly variable. *Epidemics*. 2015;11:92–102.
- Bowman LR, Runge-Ranzinger S, McCall PJ. Assessing the relationship between vector indices and dengue transmission: a systematic review of the evidence. *PLoS Negl Trop Dis*. 2014;8(5):e2848.
- Cromwell EA, Stoddard ST, Barker CM, Van Rie A, Messer WB, Meshnick SR, et al. The relationship between entomological indicators of *Aedes aegypti* abundance and dengue virus infection. *PLoS Negl Trop Dis*. 2017;11(3):e0005429.
- Connor ME, Monroe WM. *Stegomyia* indices and their value in yellow fever control. *Am J Trop Med Hyg*. 1923;1:9–19.
- Focks DA. A review of entomological sampling methods and indicators for dengue vectors. Geneva: Special Programme for Research and Training in Tropical Diseases (TDR) WHO; 2003. TDR/IDE/Den/03.1
- Focks DA, Chadee DD. Pupal survey: an epidemiologically significant surveillance method for *Aedes aegypti*: an example using data from Trinidad. *Am J Trop Med Hyg*. 1997;56(2):159–67.
- Focks DA, Brenner RJ, Hayes J, Daniels E. Transmission thresholds for dengue in terms of *Aedes aegypti* pupae per person with discussion of their utility in source reduction efforts. *Am J Trop Med Hyg*. 2000; 62(1):11–8.
- Edillo FE, Roble ND, Otero ND 2nd. The key breeding sites by pupal survey for dengue mosquito vectors, *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse), in Guba, Cebu City, Philippines. *Southeast Asian J Trop Med Public Health*. 2012;43(6):1365–74.
- Romero-Vivas C, Arango-Padilla P, Falconar A. Pupal-productivity surveys to identify the key container habitats of *Aedes aegypti* (L.) in Barranquilla, the principal seaport of Colombia. *Ann Trop Med Parasitol*. 2006;100:587–95.
- Midega JT, Nzovu J, Kahindi S, Sang RC, Mbogo C. Application of the pupal/demographic-survey methodology to identify the key container habitats of *Aedes aegypti* (L.) in Malindi district, Kenya. *Ann Trop Med Parasitol*. 2006; 100(Suppl 1):S61–s72.
- Lenhart AE, Castillo CE, Oviedo M, Villegas E. Use of the pupal/demographic-survey technique to identify the epidemiologically important types of containers producing *Aedes aegypti* (L.) in a dengue-endemic area of Venezuela. *Ann Trop Med Parasitol*. 2006;100:553–9.
- Wai KT, Arunachalam N, Tana S, Espino F, Kittayapong P, Abeyewickreme W, et al. Estimating dengue vector abundance in the wet and dry season: implications for targeted vector control in urban and peri-urban Asia. *Pathog Glob Health*. 2012;106(8):436–45.
- Barrera R, Amador M, Diaz A, Smith J, Munoz-Jordan JL, Rosario Y. Unusual productivity of *Aedes aegypti* in septic tanks and its implications for dengue control. *Med Vet Entomol*. 2008;22(1):62–9.
- Chitolina RF, Anjos FA, Lima TS, Castro EA, Costa-Ribeiro MC. Raw sewage as breeding site to *Aedes (Stegomyia) aegypti* (Diptera, culicidae). *Acta Trop*. 2016;164:290–6.
- Dada N, Vannavong N, Seidu R, Lenhart A, Stenstrom TA, Chareonviriyaphap T, et al. Relationship between *Aedes aegypti* production and occurrence of *Escherichia coli* in domestic water storage containers in rural and sub-urban villages in Thailand and Laos. *Acta Trop*. 2013;126(3):177–85.

30. Chandel K, Suman DS, Wang Y, Unlu I, Williges E, Williams GM, et al. Targeting a hidden enemy: pyriproxyfen autodissemination strategy for the control of the container mosquito *Aedes albopictus* in cryptic habitats. *PLoS Negl Trop Dis*. 2016;10(12):e0005235.
31. Caputo B, Ienco A, Cianci D, Pombi M, Petrarca V, Baseggio A, et al. The "auto-dissemination" approach: a novel concept to fight *Aedes albopictus* in urban areas. *PLoS Negl Trop Dis*. 2012;6(8):e1793.
32. Sihuincha M, Zamora-Perea E, Orellana-Rios W, Stancil JD, Lopez-Sifuentes V, Vidal-Ore C, et al. Potential use of pyriproxyfen for control of *Aedes aegypti* (Diptera: Culicidae) in Iquitos, Peru. *J Med Entomol*. 2005;42(4):620–30.
33. Heintze C, Velasco Garrido M, Kroeger A. What do community-based dengue control programmes achieve? A systematic review of published evaluations. *Trans R Soc Trop Med Hyg*. 2007;101(4):317–25.
34. Boyce R, Lenhart A, Kroeger A, Velayudhan R, Roberts B, Horstick O. *Bacillus thuringiensis israelensis* (Bti) for the control of dengue vectors: systematic literature review. *Tropical Med Int Health*. 2013;18(5):564–77.
35. Lazaro A, Han WW, Manrique-Saide P, George L, Velayudhan R, Toledo J, et al. Community effectiveness of copepods for dengue vector control: systematic review. *Tropical Med Int Health*. 2015;20(6):685–706.
36. McMeniman CJ, Lane RV, Cass BN, Fong AW, Sidhu M, Wang YF, et al. Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. *Science*. 2009;323(5910):141–4.
37. Harris AF, McKemey AR, Nimmo D, Curtis Z, Black I, Morgan SA, et al. Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. *Nat Biotechnol*. 2012;30(9):828–30.
38. WHO. Mosquito (vector) control emergency response and preparedness for Zika virus. World Health Organization; 2016. http://www.who.int/neglected_diseases/news/mosquito_vector_control_response/en/. Accessed 6 July 2017.
39. Runge-Ranzinger S, Kroeger A, Olliaro P, McCall PJ, Sanchez Tejada G, Lloyd LS, et al. Dengue contingency planning: from research to policy and practice. *PLoS Negl Trop Dis*. 2016;10(9):e0004916.
40. Ranson H, Burhani J, Lumjuan N, Black WC IV. Insecticide resistance in dengue vectors. *TropKAnet*. 2010;1:1–12.
41. WHO. Global strategic framework for integrated vector management. Geneva: World Health Organization; 2004. WHO/CDS/CPE/PVC/2004.10
42. Darriet F, Marcombe S, Etienne M, Yebakima A, Agnew P, Yp-Tcha MM, et al. Field evaluation of pyriproxyfen and spinosad mixture for the control of insecticide resistant *Aedes aegypti* in Martinique (French West Indies). *Parasit Vectors*. 2010;3:88.
43. Nayar JK, Ali A, Zaim M. Effectiveness and residual activity comparison of granular formulations of insect growth regulators pyriproxyfen and s-methoprene against Florida mosquitoes in laboratory and outdoor conditions. *J Am Mosq Control Assoc*. 2002;18(3):196–201.
44. Ocampo CB, Mina NJ, Carabali M, Alexander N, Osorio L. Reduction in dengue cases observed during mass control of *Aedes* (*Stegomyia*) in street catch basins in an endemic urban area in Colombia. *Acta Trop*. 2014;132:15–22.
45. WHO. Report of the 4th WHOPES Working Group meeting — IR3535, KBR3023, (RS)-methoprene 20% EC, pyriproxyfen 0.5% GR and lambda-cyhalothrin 2.5% CS. Geneva: World Health Organization; 2001. WHO/CDS/WHOPES/2001.2
46. WHO. Report of the 14th WHOPES working group meeting: WHO/HQ, Geneva, 11–15 April 2011: review of Spinosad® EC, Lifenet® LN, Magnet™ LN, Royal Sentry® LN, Yahe® LN. Geneva: World Health Organization; 2011. WHO/HTM/NTD/WHOPES/2011.7
47. WHO. Global vector control response 2017–2030. Geneva: World Health Organization; 2017. Background document to inform deliberations during the 70th session of the World Health Assembly (version 5.4)
48. NSO. The 2010 Population and Housing Census. National Statistical Office, Ministry of Information and Communication Technology; 2010. http://web.nso.go.th/en/census/poph/cen_poph_10.htm. Accessed 6 July 2017.
49. Hayes RJ, Bennett S. Simple sample size calculation for cluster-randomized trials. *Int J Epidemiol*. 1999;28(2):319–26.
50. Hemming K, Marsch J. A menu-driven facility for sample-size calculations in cluster randomized controlled trials. *Stata J*. 2013;13(1):114–35.
51. Alexander N. Review: analysis of parasite and other skewed counts. *Tropical Med Int Health*. 2012;17(6):684–93.
52. WHO. WHOPES-recommended compounds and formulations for control of mosquito larvae. Geneva: WHO Pesticides Evaluation Scheme (WHOPES); 2013. http://www.who.int/whopes/Mosquito_Larvicides_sep_2011.pdf. Accessed 14 June 2012.
53. WHO. Report of the 10th WHOPES Working Group meeting — Review of spinosad 0.5% GR and 12% SC, Lambda-cyhalothrin 10% CS, K-O Tab 1-2-3®, Interceptor®. Geneva: World Health Organization; 2007. WHO/CDS/NTD/WHOPES/2007.1
54. WHO. Pyriproxyfen in drinking-water: use for vector control in drinking-water sources and containers. Geneva: World Health Organization; 2008. Background document for development of WHO Guidelines for Drinking-water Quality. WHO/HSE/AMR/08.03/9
55. WHO. Spinosad DT in drinking-water: use for vector control in drinking-water sources and containers. Geneva: World Health Organization; 2010. Background document for development of WHO Guidelines for Drinking-water Quality. WHO/HSE/WSH/10.01/12
56. Chareonviriyaphap T, Bangs MJ, Suwonkerd W, Kongmee M, Corbel V, Ngoen-Klan R. Review of insecticide resistance and behavioral avoidance of vectors of human diseases in Thailand. *Parasit Vectors*. 2013;6:280.
57. Shu PY, Chang SF, Kuo YC, Yueh YY, Chien LJ, Sue CL, et al. Development of group- and serotype-specific one-step SYBR green I-based real-time reverse transcription-PCR assay for dengue virus. *J Clin Microbiol*. 2003;41(6):2408–16.
58. Corman VM, Rasche A, Baronti C, Aldabbagh S, Cadar D, Reusken CB, et al. Assay optimization for molecular detection of Zika virus. *Bull World Health Organ*. 2016;94(12):880–92.
59. Niyas KP, Abraham R, Unnikrishnan RN, Mathew T, Nair S, Manakkadan A, et al. Molecular characterization of Chikungunya virus isolates from clinical samples and adult *Aedes albopictus* mosquitoes emerged from larvae from Kerala, South India. *Virology*. 2010;7:189.
60. Elanga Ndille E, Doucoure S, Damien G, Mouchet F, Drame PM, Cornelie S, et al. First attempt to validate human IgG antibody response to Nterm-34kDa salivary peptide as biomarker for evaluating exposure to *Aedes aegypti* bites. *PLoS Negl Trop Dis*. 2012;6(11):e1905.
61. Knox TB, Yen NT, Nam VS, Gatton ML, Kay BH, Ryan PA. Critical evaluation of quantitative sampling methods for *Aedes aegypti* (Diptera: Culicidae) immatures in water storage containers in Vietnam. *J Med Entomol*. 2007;44(2):192–204.
62. Vazquez-Prokopec GM, Galvin WA, Kelly R, Kitron U. A new, cost-effective, battery-powered aspirator for adult mosquito collections. *J Med Entomol*. 2009;46(6):1256–9.
63. Heringer L, Johnson BJ, Filrig K, Oliveira BA, Silva RD, Townsend M, et al. Evaluation of alternative killing agents for *Aedes aegypti* (Diptera: Culicidae) in the gravid *Aedes* trap (GAT). *J Med Entomol*. 2016;53(4):873–9.
64. Bangs MJ, Focks DA. Abridged pupa identification key to the common container-breeding mosquitoes in urban Southeast Asia. *J Am Mosq Control Assoc*. 2006;22(3):565–72.
65. Rueda LM. Pictorial keys for the identification of mosquitoes (*Diptera: Culicidae*) associated with dengue virus transmission. *Zootaxa*. 2004;589:1–60.
66. Katholi CR, Toe L, Merriweather A, Unnasch TR. Determining the prevalence of *Onchocerca volvulus* infection in vector populations by polymerase chain reaction screening of pools of black flies. *J Infect Dis*. 1995;172(5):1414–7.
67. Gu W, Lampman R, Novak RJ. Assessment of arbovirus vector infection rates using variable size pooling. *Med Vet Entomol*. 2004;18(2):200–4.
68. Sim S, Aw PP, Wilm A, Teoh G, Hue KD, Nguyen NM, et al. Tracking dengue virus intra-host genetic diversity during human-to-mosquito transmission. *PLoS Negl Trop Dis*. 2015;9(9):e0004052.
69. Zandi K, Teoh B, Sam S, Wong P, Mustafa M, AbuBakar S. In vitro antiviral activity of Fisetin, Rutin and Naringenin against dengue virus type-2. *J Med Plant Res*. 2011;5(23):5534–9.
70. Seah CL, Chow VT, Tan HC, Can YC. Rapid, single-step RT-PCR typing of dengue viruses using five NS3 gene primers. *J Virol Methods*. 1995;51(2-3):193–200.
71. Campbell MK, Piaggio G, Elbourne DR, Altman DG. Consort 2010 statement: extension to cluster randomised trials. *BMJ*. 2012;345:e5661.
72. US DHHS. Code of Federal Regulations, 45 CFR 46, Public Welfare, Protection of Human Subjects. Washington, DC: Office for Human Research Protections, US Department of Health & Human Services; 2009.
73. Boutron I, Altman DG, Moher D, Schulz KF, Ravaut P. CONSORT statement for randomized trials of nonpharmacologic treatments: a 2017 update and a CONSORT extension for nonpharmacologic trial abstracts. *Ann Intern Med*. 2017;167(1):40–7.
74. Zwarenstein M, Treweek S, Gagnier JJ, Altman DG, Tunis S, Haynes B, et al. Improving the reporting of pragmatic trials: an extension of the CONSORT statement. *BMJ*. 2008;337:a2390.

75. Bowman LR, Donegan S, PJ MC. Is dengue vector control deficient in effectiveness or evidence? Systematic review and meta-analysis. *PLoS Negl Trop Dis*. 2016;10(3):e0004551.
76. Tsunoda T, Kawada H, Huynh TT, Luu LL, Le SH, Tran HN, et al. Field trial on a novel control method for the dengue vector, *Aedes aegypti* by the systematic use of Olyset(R) Net and pyriproxyfen in Southern Vietnam. *Parasit Vectors*. 2013;6:6.
77. Voge NV, Sanchez-Vargas I, Blair CD, Eisen L, Beaty BJ. Detection of dengue virus NS1 antigen in infected *Aedes aegypti* using a commercially available kit. *Am J Trop Med Hyg*. 2013;88(2):260–6.
78. Sylvestre G, Gandini M, de Araujo JM, Kubelka CF, Lourenco-de-Oliveira R, Maciel-de-Freitas R. Preliminary evaluation on the efficiency of the kit Platelia Dengue NS1 Ag-ELISA to detect dengue virus in dried *Aedes aegypti*: a potential tool to improve dengue surveillance. *Parasit Vectors*. 2014;7:155.
79. Lau SM, Chua TH, Sulaiman WY, Joanne S, Lim YA, Sekaran SD, et al. A new paradigm for *Aedes* spp. surveillance using gravid ovipositing sticky trap and NS1 antigen test kit. *Parasit Vectors*. 2017;10(1):151.
80. Lau SM, Vythilingam I, Doss JI, Sekaran SD, Chua TH, Wan Sulaiman WY, et al. Surveillance of adult *Aedes* mosquitoes in Selangor, Malaysia. *Tropical Med Int Health*. 2015;20(10):1271–80.
81. Ya-Umphun P, Cerqueira D, Parker DM, Cottrell G, Poinson A, Remoue F, et al. Use of an *Anopheles* salivary biomarker to assess malaria transmission risk along the Thailand-Myanmar border. *J Infect Dis*. 2017;215(3):396–404.
82. Doucoure S, Mouchet F, Cournil A, Le Goff G, Cornélie S, Roca Y, et al. Human antibody response to *Aedes aegypti* saliva in an urban population in Bolivia: a new biomarker of exposure to Dengue vector bites. *Am J Trop Med Hyg*. 2012;87(3):504–10.
83. Remoue F, Alix E, Cornélie S, Sokhna C, Cisse B, Doucoure S, et al. IgE and IgG4 antibody responses to *Aedes* saliva in African children. *Acta Trop*. 2007; 104(2-3):108–15.
84. Wasinpiyamongkol L, Patramool S, Luplertlop N, Surasombattapana P, Doucoure S, Mouchet F, et al. Blood-feeding and immunogenic *Aedes aegypti* saliva proteins. *Proteomics*. 2010;10(10):1906–16.
85. Ndille EE, Dubot-Peres A, Doucoure S, Mouchet F, Cornélie S, Sidavong B, et al. Human IgG antibody response to *Aedes aegypti* Nterm-34 kDa salivary peptide as an indicator to identify areas at high risk for dengue transmission: a retrospective study in urban settings of Vientiane city, Lao PDR. *Tropical Med Int Health*. 2014;19(5):576–80.
86. Elanga Ndille E, Doucoure S, Poinson A, Mouchet F, Cornélie S, D'Ortenzio E, et al. Human IgG antibody response to *Aedes* Nterm-34kDa salivary peptide, an epidemiological tool to assess vector control in chikungunya and dengue transmission area. *PLoS Negl Trop Dis*. 2016; 10(12):e0005109.
87. Duong V, Lambrechts L, Paul RE, Ly S, Lay RS, Long KC, et al. Asymptomatic humans transmit dengue virus to mosquitoes. *Proc Natl Acad Sci U S A*. 2015;112(47):14688–93.
88. Chastel C. Eventual role of asymptomatic cases of dengue for the introduction and spread of dengue viruses in non-endemic regions. *Front Physiol*. 2012;3:70.
89. Rose K, van den Anker JN, editors. *Guide to paediatric clinical research*. Basel: Karger; 2007.
90. Tun-Lin W, Kay BH, Barnes A. The Premise Condition Index: a tool for streamlining surveys of *Aedes aegypti*. *Am J Trop Med Hyg*. 1995;53(6):591–4.
91. Chan AW, Tetzlaff JM, Gotzsche PC, Altman DG, Mann H, Berlin JA, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ*. 2013;346:e7586.

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CORRECTION

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Correction to: Assessing dengue transmission risk and a vector control intervention using entomological and immunological indices in Thailand: study protocol for a cluster-randomized controlled trial

Hans J. Overgaard^{1*}, Chamsai Pientong^{2,3}, Kesorn Thaewongiew⁴, Michael J. Bangs^{5,6}, Tipaya Ekalaksananan^{2,3}, Sirinart Aromseree^{2,3}, Thipruethai Phanitchat², Supranee Phanthanawiboon^{2,3}, Benedicte Fustec^{2,7}, Vincent Corbel⁸, Dominique Cerqueira⁶ and Neal Alexander⁹

Correction to: *Trials* (2018) 19:122
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In the original publication [1], the first of two objectives was to “Assess the effect of periodically treating water storage containers with a pyriproxyfen/spinosad combination on entomological and epidemiological outcomes”. However, spinosad will not be a part of the intervention. The approval by the Food and Drug Administration of the Ministry of Public Health, Thailand to use spinosad in this research project was delayed and not in place when the interventions were supposed to begin. Therefore, a decision was made to exclude spinosad in the trial and only use pyriproxyfen. The correct version of objective 1 should now read “Assess the effect of periodically treating water storage containers with pyriproxyfen on entomological and epidemiological outcomes”. The second objective remains the same: “Determine the most accurate and precise index or indices to predict variation in dengue incidence in time”.

The exclusion spinosad in the trial has implications for several sections in the original article. The word spinosad appears 18 times in the main text of document; in the abstract, background, methods, and discussion. The reader should be aware of this important change when reading the article and its implications for the trial and outcomes.

Author details

¹Norwegian University of Life Sciences, Ås, Norway. ²Khon Kaen University, Khon Kaen, Thailand. ³HPV & EBV and Carcinogenesis Research Group, Khon Kaen University, Khon Kaen, Thailand. ⁴Office of Disease Prevention and Control, Region 7, Khon Kaen, Thailand. ⁵PT Freeport Indonesia/International SOS Indonesia, Kuala Kencana, Indonesia. ⁶Kasetsart University, Bangkok, Thailand. ⁷Université de Montpellier, Montpellier, France. ⁸Institut de Recherche pour le Développement (IRD), Maladies Infectieuses et Vecteurs, Ecologie, Génétique, Evolution et Contrôle (MIVEGEC, UM1-CNRS 5290-IRD 224), Montpellier, France. ⁹MRC Tropical Epidemiology Group, London, UK.

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1. Overgaard HJ, et al. Assessing dengue transmission risk and a vector control intervention using entomological and immunological indices in Thailand: study protocol for a cluster-randomized controlled trial. *Trials*. 2018;19:122. <https://doi.org/10.1186/s13063-018-2490-1>.

* Correspondence: hans.overgaard@nmbu.no

¹Norwegian University of Life Sciences, Ås, Norway

Full list of author information is available at the end of the article



Third part: Results of the thesis

Chapter 1. Assessing the spatial and temporal patterns of dengue incidence in North-eastern Thailand

The results from this study were published in *BMC Infectious Disease*, 19(1), 74 <https://doi.org/10.1186/s12879-019-4379-3> (2019). **Spatial and temporal patterns of dengue incidence in northeastern Thailand 2006-2016**. Phanitchat T, Zhao B, Haque U, Pientong C, Ekalaksananan T, Aromseree S, Thaewnongiew K, **Fustec B**, Bangs MJ, Alexander N, Overgaard HJ.

A retrospective epidemiological study using monthly dengue incidence at the sub-district level and climatic data was conducted by our team in order to better understand the spatial dengue-climate relationships at fine scale and to identify areas and periods at higher dengue transmission risk (Phanitchat et al. 2019) (see details in Phanitchat et al. 2019). The study was conducted in Khon Kaen province comprising 26 districts, 199 sub-districts and 2,139 villages. The province is primarily rural, with a few large urban centres. Dengue cases from the 1st of January 2006 to 31 December 2016 were retrieved from the MoPH and classified according to WHO dengue classification prior 2009 (i.e., DF, DHF, DSS). Meteorological data for the same period of time were downloaded from the data library of the International Research Institute for Climate and Society. For each sub-district, daily temperatures were aggregated to monthly average with a 0.2 x 0.2 degrees resolution, and daily rainfall was aggregated to monthly average with a 0.05 x 0.05 degrees resolution. Monthly data on dengue cases and climate (rainfall and temperature) from the study period were combined to visualize seasonal patterns and temporal trends. Bayesian Poisson model regression were used to assess associations with the number of monthly cases in 199 sub-districts. Population was used as the denominator in the model. For the main model the covariates were the population density per km², gender, mean age, mean rainfall, and minimum and maximum temperature. Population density was included in the regression model as a “proxy” for estimating the levels of urbanization. Conditional autoregressive structure was used as a random effect capturing the spatio-temporal autocorrelation. Local Indicators of Spatial Association (LISA) were used to identify “hotspots” of dengue (i.e., where incidence is higher than the

expected number given a random distribution of cases) and “coldspots”, and outliers of dengue incidence at the sub district level.

Summary of the results:

Over the 11-years period, >15,000 cases were reported, half of them being classified as severe dengue DHF/DSS. The highest incidence was recorded in 2013 with approximately 80 cases per 100,000 inhabitants. We demonstrated a shift over the last 10 years in case ages, with the age group 15-29 years old being the most affected by the disease. Our observation was consistent with a population age shift, potentially influenced by changes in birth and death rates. Similar trend in dengue infection pattern was observed in other countries in the SEA region (Limkittikul et al. 2014, Mohd-Zaki et al. 2014, Thomas et al. 2015, Alera et al. 2016).

Additionally, we showed that dengue incidence had a clear seasonal pattern with about 73% of the dengue cases occurring during the rainy season (Figure 31). Our findings showed a good correlation between dengue incidence and climatic factors, especially temperature and rainfall. Indeed, the rate ratio for maximum temperature was 1.055, implying 5.5% (95% CI 0.9–11.5%) increase in cases with an increase of 1 °C per month. The rate ratio for mean rainfall was 1.004, indicating that increasing rainfall by one unit (1 cm) per month would increase dengue incidence by about 0.4%. Other studies in Thailand and Timor Lest also demonstrated a strong correlation between dengue incidence and meteorological data (Wangdi et al. 2018). Although the dynamic of dengue incidence was clearly influenced by rainfall and temperature, our data show apparent spatial clustering of dengue cases associated with environmental parameters such as urbanization (Figure 32). Greater vulnerability to dengue infection has been previously observed in areas situated closer to urban centers (Tipayamongkhogul et al. 2011) and such neighboring effects have been related to similarities in human behavior, development infrastructure, and ecological surroundings. Spatial regression analysis suggests that other variables than urbanization may explain the differences in dengue incidence as half of dengue hotspots were found in rural areas located in the southwest of the province, hence corroborating the influence of other factors in dengue transmission. One speculation could be that the lakes and swamps that are common in this area may provide suitable humidity for mosquitoes to thrive, but this was not studied here.

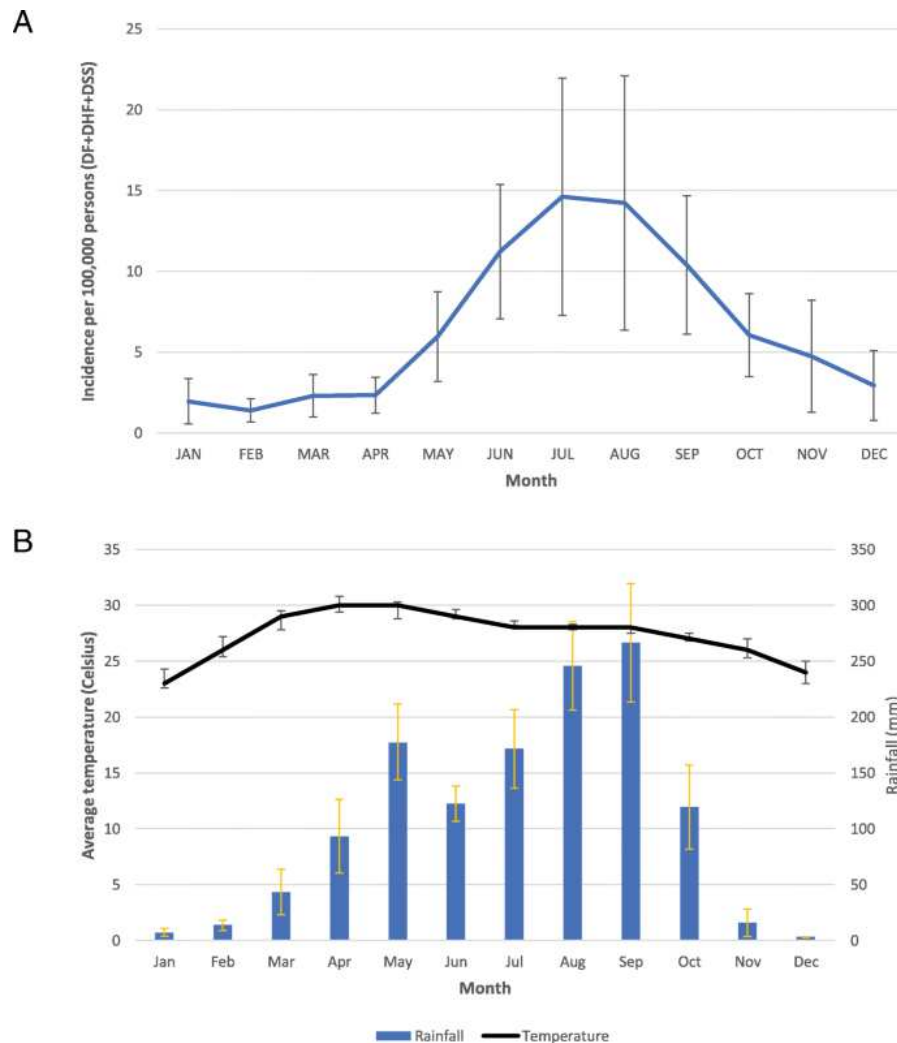


Figure 31: Mean monthly dengue incidence per 100,000 persons, from Phanitchat et al. (a) and monthly average of rainfall (bar) and temperature (line) (b) in Khon Kaen Province, Thailand, 2006–2016.

To conclude, this baseline study clearly showed the involvement of climatic factors on dengue transmission in the province. Spatial clustering of dengue cases was partly associated with urban areas closer to Khon Kaen city and rural areas in the southwest of the province. However, the current analysis was not able to detect a close proxy factors to quantify a relationship between urbanization and dengue incidence. This first study highlighted the need for further investigations on dengue-related risk factors in the study area in order to develop dengue early warning systems to guide vector control operations.

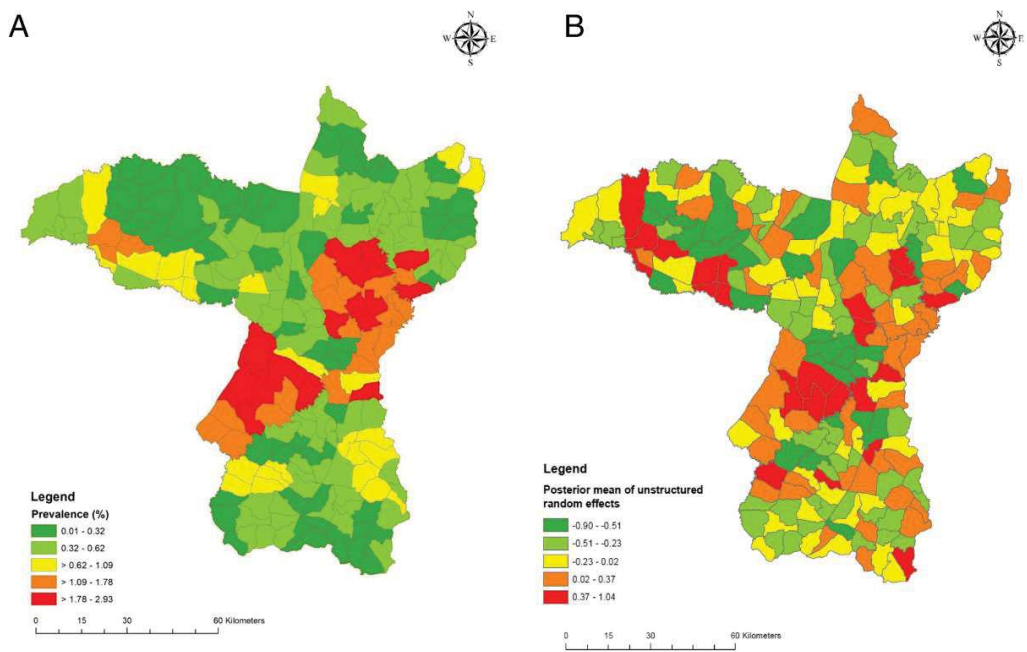



Figure 32: Mean dengue prevalence by sub-district, from Phanitchat et al. (a) and spatial distribution of the posterior means of random effects for dengue (b) in Khon Kaen Province, Thailand, 2006–2016

RESEARCH ARTICLE

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Spatial and temporal patterns of dengue incidence in northeastern Thailand 2006–2016

Thipruethai Phanitchat^{1,2}, Bingxin Zhao³, Ubydul Haque⁴, Chamsai Pientong^{1,5}, Tipaya Ekalaksananan^{1,5}, Sirinart Aromseree^{1,5}, Kesorn Thaewongiew⁶, Benedicte Fustec^{1,7}, Michael J. Bangs⁸, Neal Alexander⁹ and Hans J. Overgaard^{10*} 

Abstract

Background: Dengue, a viral disease transmitted by *Aedes* mosquitoes, is an important public health concern throughout Thailand. Climate variables are potential predictors of dengue transmission. Associations between climate variables and dengue have usually been performed on large-scale first-level national administrative divisions, i.e. provinces. Here we analyze data on a finer spatial resolution in one province, which is often more relevant for effective disease control design. The objective of this study was to investigate the effect of seasonal variations, monthly climate variability, and to identify local clusters of symptomatic disease at the sub-district level based on reported dengue cases.

Methods: Data on dengue cases were retrieved from the national communicable disease surveillance system in Thailand. Between 2006 and 2016, 15,167 cases were recorded in 199 sub-districts of Khon Kaen Province, northeastern Thailand. Descriptive analyses included demographic characteristics and temporal patterns of disease and climate variables. The association between monthly disease incidence and climate variations was analyzed at the sub-district level using Bayesian Poisson spatial regression. A hotspot analysis was used to assess the spatial patterns (clustered/dispersed/random) of dengue incidence.

Results: Dengue was predominant in the 5–14 year-old age group (51.1%). However, over time, dengue incidence in the older age groups (> 15 years) gradually increased and was the most affected group in 2013. Dengue outbreaks coincide with the rainy season. In the spatial regression model, maximum temperature was associated with higher incidence. The hotspot analysis showed clustering of cases around the urbanized area of Khon Kaen city and in rural areas in the southwestern portion of the province.

Conclusions: There was an increase in the number of reported dengue cases in older age groups over the study period. Dengue incidence was highly seasonal and positively associated with maximum ambient temperature. However, climatic variables did not explain all the spatial variation of dengue in the province. Further analyses are needed to clarify the detailed effects of urbanization and other potential environmental risk factors. These results provide useful information for ongoing prediction modeling and developing of dengue early warning systems to guide vector control operations.

Keywords: Dengue, Climate, Seasonal, Temperature, Rainfall, Thailand

* Correspondence: hans.overgaard@nmbu.no

¹⁰Faculty of Science and Technology, Norwegian University of Life Sciences, Ås, Norway

Full list of author information is available at the end of the article



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Background

The annual global burden of dengue is estimated at 390 million infections, of which 96 million present clinically [1]. Four closely related RNA viruses in the family *Flaviviridae* (DENV1 to DENV4) are responsible for dengue disease. They are transmitted by *Aedes* (primarily subgenus *Stegomyia*) mosquitoes, particularly *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) [2]. Dengue has developed from a sporadically occurring disease to a major and re-emerging global public health problem over recent decades causing substantial economic disruption and social burden in endemic areas in Asia, Africa, and the Americas. There is no effective treatment for dengue and vaccination, so far, offers only incomplete protection [3, 4]. Therefore, vector control remains the most important means of prevention [5]. Effective vaccine or not, vector control will remain the cornerstone of dengue control for years to come [3].

Due to increasing incidence and rapid geographical expansion, dengue is the most common vector-borne disease in Thailand [6]. From 2000 to 2011, the number of reported cases varied from 20,000 to 140,000 cases each year [7]. Both *Ae. aegypti* and *Ae. albopictus* are common species and widely distributed in Thailand [8]. All four serotypes co-circulated in each of the major outbreaks that occurred in 1958, 1987, 1998, 2001, 2013, and 2015 [9–14]. The highest incidence typically occurs in 13–24 year-old age group with case clustering seen predominately in urban areas [15]. Males represent the majority of reported dengue cases in several Asian countries [16]. A study in Singapore showed that men were more exposed to infected mosquitoes than women, during daytime hours, at the workplace or while travelling to and from work. A forceful public health policy in Singapore [17] has greatly reduced the number of mosquitoes in and around homes, potentially rendering the larger male labor force more exposed to mosquito bites during working hours [16, 18]. Other causes for these apparent gender differences could be different health seeking behaviors or male-female differences in disease severity [19]. In the Lao People's Democratic Republic male-female ratios in dengue cases varied between years and provinces [16]. We are not aware of similar spatio-temporal or socioeconomic differences in Thailand.

Thailand has adapted the dengue control strategy of the World Health Organization (WHO) [2], which consists of three main pillars: 1) patients diagnosed with dengue are required to avoid mosquito bites to prevent dengue transmission; 2) active community case detection of cases which do not result in clinical consultation; and 3) vector control, consisting of environmental management, source reduction, and chemical interventions using insecticide fogging against adult vectors and larvicides to control immature stages in containers [20]. Follow-up interventions

are conducted by health officers or village health volunteers [20]. To determine the most appropriate and feasible intervention or combination of interventions, health officers need to consider local environmental, resource, and contextual factors that may influence effectiveness [21].

Climate variables are predictors of dengue infection [4, 22, 23]. Seasonal variation in climate shows a strong relationship with *Ae. aegypti* abundance and historical dengue incidence [24]. Temperature affects population biology of *Aedes* mosquitoes [25]. Higher temperatures increase larval development [26] and rates of multiple feeding, but reduce mosquito size [27]. The extrinsic incubation period declines as temperature rises, thus increasing the proportion of infected vectors, and enhancing the transmission potential of the vector [27–29].

As ambient temperature increases, so does dengue epidemic potential, peaking at around 29 °C and then decreases [29]. In subtropical and tropical regions such as Thailand, with mean diel temperatures of 26 °C ($20\text{ °C} \leq T \leq 32\text{ °C}$), an increase in diurnal temperature range can enhance transmission [29]. An analysis of data from Thailand (1978–1997) showed the incidence of dengue hemorrhagic fever (DHF) was negatively associated with higher rainfall in the southern region of the country, but positively associated with elevated ambient temperatures in the central and northern regions [30]. Another study using provincial monthly dengue data from 1983 to 2001 concluded that the relationships between weather variables and dengue transmission are very complex in Thailand [31]. The study found that transmission occurs within a specific temperature range, but that changes in humidity within this range can amplify the transmission potential with 80% of dengue cases occurring at a mean temperature of between 27.0 and 29.5 °C and a mean relative humidity of >75%. They further found that large epidemics begin earlier, develop faster and can be predicted at a defined onset time. Non-linear modeling of more than 30 years (1982–2013) of monthly data by province in Thailand showed that inter-annual variations in rainfall and temperature with a lag time of one month can improve the explanation of dengue relative risk compared to a seasonal-spatial model [32]. The relationship between rainfall and dengue is complex, as it may create abundant breeding sites for the vector [33], but can also flush out sites if rain is too intense [33, 34]. Because household water storage may increase in the dry season, the resulting breeding habitats may weaken, or even reverse, the positive association between dengue and rainfall [35–39].

Spatio-temporal analysis can detect clusters of dengue disease and is useful for a better understanding of the dynamics of disease dispersion. Analysis of spatial and temporal variations is also useful in identifying high-risk

locations and times of higher transmission risk, which are important for disease surveillance and control [15, 40].

The above-mentioned research on climate and dengue focused on larger spatiotemporal scales, such as monthly dengue surveillance and climate records at the provincial level [31, 41, 42]. The current study is novel because it uses data on the lowest administrative level, the sub-district, in one province to understand fine-scale spatial dengue-climate relationships. This is useful for developing more reliable prediction models for future projections applied in early warning and response systems, thus ultimately improving timely control interventions.

We analyzed data on reported dengue cases in Khon Kaen Province, northeastern Thailand collected between 2006 and 2016 to 1) describe demographic characteristics and seasonal variations of dengue cases; 2) determine the potential impact of climate variability on dengue incidence; and 3) identify clusters of dengue cases at the sub-district level.

Methods

Study area

The study was conducted in Khon Kaen Province, an area of approximately 10,900 km² (16°25'12"N to 16°42'12"N and 102°49'48"E to 102°83'48"E). The province has 26 districts, 199 sub-districts, and 2139 villages. In 2010, the population was 1,767,601, of which 387,279 people lived in Mueang District that includes the provincial capital Khon Kaen (see Additional file 1). This province was selected as the study area because dengue is endemic with typical seasonal increases and occasional outbreaks. The province is primarily rural with a few large urban centers. Mueang District, the most densely populated area in the province, is a regional center for education, health, finance and commerce. The northern and southern parts of the district, along the major highway linking Bangkok with Lao People's Democratic Republic, are rapidly developing. The districts in the northwestern and southeastern parts of the province are rural and agricultural. Classification of urban and rural areas depends on population density. An urban area is defined as a municipality or town with a population over 100,000 and a population density above 300 persons per square kilometer [43]. The average minimum and maximum seasonal temperatures are 16.7°C (December–January) and 36.4°C (April–May). The monthly minimum and maximum rainfall vary from 0 mm (dry season: November–April) to 240 mm (wet season: May–October).

Data collection

The Office of Disease Prevention and Control, Region 7 Khon Kaen (ODPC7), Department of Disease Control,

Ministry of Public Health, Thailand provided data on the weekly number of reported dengue cases in Khon Kaen Province from 1 January 2006 to 31 December 2016. Dengue is a notifiable disease based on the National Communicable Disease Control Law, i.e., all government and private hospitals, clinics and other healthcare facilities must report all cases (confirmed and suspected) to the local health authority within 24 h of diagnosis [12]. Cases are recorded by degree of disease severity into one of three categories (at peak of illness): 1) dengue fever (DF), 2) dengue hemorrhagic fever (DHF), and 3) dengue shock syndrome (DSS), but the serotype is not recorded (or typically known except retrospectively). A patient is diagnosed with suspected DF when the following criteria are met and signs and symptoms are present: residence or recent travel to a dengue endemic area, acute fever accompanied by any two of the following: headache, myalgia, arthralgia, rash, positive tourniquet test and leucopenia, with no evidence of plasma leakage. DHF is recorded in patients with a temperature $\geq 38^\circ\text{C}$, petechiae, ecchymosis, or a positive tourniquet test, thrombocytopenia (platelets $< 100,000$ cells/mm³), and evidence of plasma leakage. DSS, the most severe disease manifestation, is defined as having the same signs and symptoms as DHF, but progressing to circulatory failure. The Provincial Health Offices enter patient data into the standardized Disease Surveillance Report (Report 506) for recording communicable diseases in Thailand. The form provides the patient's age, gender, house address, signs and symptoms, and date of medical consultation. DHF and DSS are based on both clinical symptoms and laboratory tests (usually complete blood count), and sometimes accompanied with a rapid diagnostic test (RDT); whereas, DF is seldom based on additional laboratory tests or by RDT.

Meteorological data from 1 January 2006 to 31 December 2016 were downloaded from the data library of the International Research Institute for Climate and Society [44], which contains specific climate data from different sources, such as The National Centers for Environmental Prediction (NCEP), Climate Forecast System Reanalysis (CFSR) [45], and Climate Hazards Group InfraRed Precipitation with Station data (CHIRPS) global rainfall datasets [46]. For each sub-district, daily temperatures (°C) were retrieved from NCEP and daily rainfall (mm) from CHIRPS. These data generated the monthly means used in the analysis (see Additional files 2 and 3). The spatial resolution of rainfall is 0.05×0.05 degrees (CHIRPS) and for temperature 0.2×0.2 degrees (NCEP CFSR v2, <https://rda.ucar.edu/datasets/ds094.1/>). A centroid was created for each sub-district. Rainfall and temperature data for each sub-district was determined based on the grid cell in which the centroid was located.

Analysis

Monthly data on dengue cases and climate (rainfall and temperature) from the study period were combined to visualize seasonal patterns and temporal trends. Dengue incidence was calculated using the monthly number of reported cases and sub-district population size in 2010 reflecting the mid-study denominator [47].

Bayesian Poisson regression models were used to assess associations with the number of monthly cases in 199 sub-districts. Population was used as the denominator in the model (i.e. log-population as an offset). The neighborhood relationship between the sub-districts were defined using their adjacency matrix; '1' for a pair of sub-districts sharing a border, otherwise '0'. Hence the following model was used:

$$Y_{ij} \sim \text{Poisson}(\mu_{ij})$$

$$\log(\mu_{ij}) = \log(P_i) + \theta_{ij}$$

$$\theta_{ij} = \alpha + \beta_k x_{ijk} + u_{ij},$$

where Y_{ij} is the observed mean number of cases for the i th sub-district in j th month ($i = 1, \dots, 199; j = 1, \dots, 12$), P_i is the sub-district population size, α is the intercept, and β_k is the regression coefficient for covariate k . For the main model, the covariates (x_k) were: population density per square kilometer; gender (proportion of males among the cases), mean age in years of the cases; mean rainfall; and minimum and maximum temperature. As a non-mechanistic way of measuring the seasonality of incidence, a second set of covariates was obtained by replacing three meteorological variables by sine and cosine terms with period 12 months. Finally, u_{ij} is the random effect that captures the spatio-temporal autocorrelation in response data Y_{ij} , whose variance depends on the adjacency matrix.

Conditional autoregressive (CAR) priors [48] structure were used on u_{ij} and for (α, β_k) , non-informative normal prior distributions was used. Flat and conjugate priors were specified for u_{ij} using inverse gamma distributions with shape and scale parameters equal to 0.001. Markov chain Monte Carlo simulation was used to estimate the model parameters, sampling 300,000 times, with the first 150,000 as the burn-in, and keeping the results from every tenth iteration. The "ST.CARar" function of the R statistical software package CARBayesST (www.r-project.org) was used to fit the model. Convergence was assessed by trace plots and checked by the convergence Z-score diagnostic function [49]. The Watanabe-Akaike Information Criterion (WAIC) was used as a measure of goodness of fit [50].

Local Indicators of Spatial Association (LISA) were used to identify significant hotspots, coldspots, and

outliers of dengue incidence at the sub-district level [51]. A hotspot is defined as an area that is surrounded by other high incidence areas, i.e. incidence is higher than the expected number given a random distribution of cases (so called high-high cluster). A coldspot is defined as an area surrounded by other low incidence areas (low-low cluster). Hotspot detection can be useful, even if the global pattern is not clustered. Moreover, case clusters that occur randomly can also have an influence on the spread of an infectious disease [52].

Results

General results

Dengue cases numbering 15,167 were reported over the 11-year period by all hospitals and clinics in Khon Kaen Province. Of these, there were 7461 dengue fever cases (49.2%) and 7706 severe dengue cases (50.8%), comprising both DHF and DSS. The demographic characteristics of patients are summarized in Table 1. Males represented the majority of patients (8057; 53.1%). Ages ranged from 4 months to 92 years old (median 13 years). The highest number of patients was in the 5–14 year-old age group (7758; 51.1%), followed by 15–29 years (5026; 33.1%) and 30–44 years (937; 6.2%). The proportion of older age groups (> 15 years), increased from nearly 20% of all cases in 2006 to more than 50% in 2016 (Fig. 1). The highest recorded disease incidence was in 2013, approximately 80 per 100,000 population (Fig. 2). Incidence was high during the rainy season (May–September), with July having the highest incidence (Fig. 3).

Table 1 Demographic characteristics of dengue reported cases in Khon Kaen Province, Thailand, 2006–2016

Characteristics	Number of cases	Percentage (%)
Gender		
Male	8057	53.1
Female	7110	46.9
Age group (years)		
< 1	72	0.5
1–<5	857	5.7
5–<15	7758	51.1
15–<30	5026	33.1
30–<45	937	6.2
45–<60	391	2.6
> 60	126	0.8
Diagnosis		
Dengue fever (DF)	7461	49.2
Dengue haemorrhagic fever (DHF)	7186	47.4
Dengue shock syndrome (DSS)	520	3.4

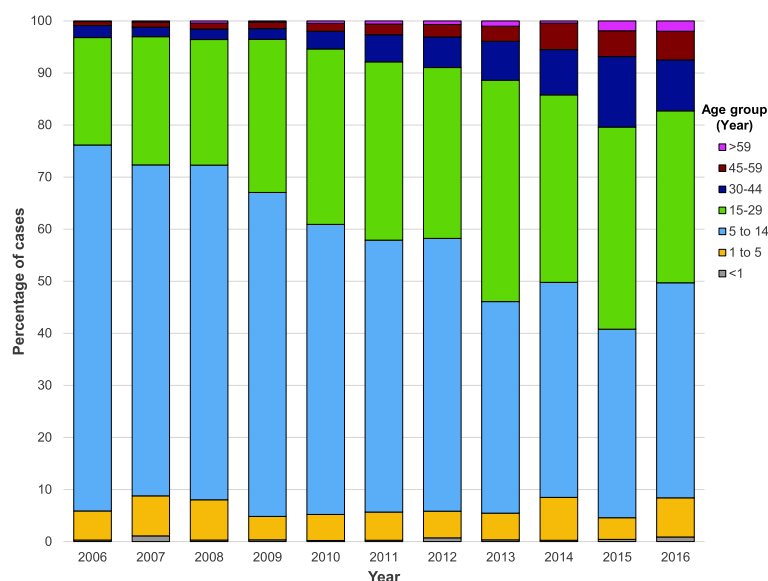


Fig. 1 Age distribution of reported dengue cases (DF, DHF and DSS) in Khon Kaen Province, Thailand, 2006–2016

Association between dengue cases and climatic factors

Mean rainfall and maximum temperature were positively associated with dengue incidence, and minimum temperature was negatively associated, in terms of their point estimates (Table 2). However, among the three 95% credible intervals (CIs), only the one for maximum temperature excluded 1 (null effect). The rate ratio for maximum temperature was 1.055, implying 5.5% (95% CI 0.9–11.5%) increase in cases with an increase of 1 °C per month. The range of this variable was from 30.7 °C to 44.9 °C. The rate ratio for mean rainfall was 1.004, indicating that increasing rainfall by one unit (1 cm) per month would increase dengue incidence by about 0.4%. The Watanabe-Akaike Information Criterion (WAIC) for this model was 10,028.75. For the model with two sinusoid terms replacing the three meteorological variables, the WAIC was very similar, at 10028.23. This sinusoid terms had a peak to trough rate ratio of 5.8, and a peak in mid-July, i.e. a roughly six-fold difference in fitted incidence from mid-July to mid-January.

The mean dengue incidence was high in the central northeastern sub-districts, around Khon Kaen city, and in the southwestern sub-districts of the province (red and orange in Fig. 4a). The distribution of the posterior means of the random effects (from the CAR model with meteorological variables) show some clustering, indicating that the variables in the model did not account fully for the spatial variation in the data (Fig. 4b). Posterior distribution plots are shown in Additional file 4. High clusters were present around Khon Kaen city and the southwestern portion of the province and low clusters were present in the northwestern area (Fig. 5), from the LISA analysis. When broken down by month, the

incidences show the same clustering patterns, especially during July–August (Additional file 5).

Discussion

The majority (~90%) of patients were below the age of 30 years. The trend during the study period showed that the proportion of dengue cases younger than 15 years declined from almost 80% in 2006 to below 50% in 2016. Dengue fever is generally more common in younger age groups [53], although there is evidence showing increasing incidence of more severe disease and outcomes among older age groups [54]. Our observations are also consistent with a population age shift, potentially influenced by demographic changes, such as the birth and death rates that show decreasing trends during 2011 and 2015 [55]. Thailand, in general, is undergoing a demographic transition where the proportion older adults are gradually increasing with an increase in median age of the general population. A higher proportion of adults will also increase the number of immune individuals (those with previous exposure to dengue virus) in the population, which might theoretically decrease the risk of dengue infection in younger people by providing alternative blood sources for infectious mosquitoes [56]. This age shift has also been observed in other Asian countries with a higher frequency of dengue cases among people 15 years of age and older [16]. Increases in disease incidence in older age groups may be explained by an increase in secondary infections and changes in circulating dengue virus serotypes [57], which have been shown to be important risk factors for severe clinical presentations [58–62].

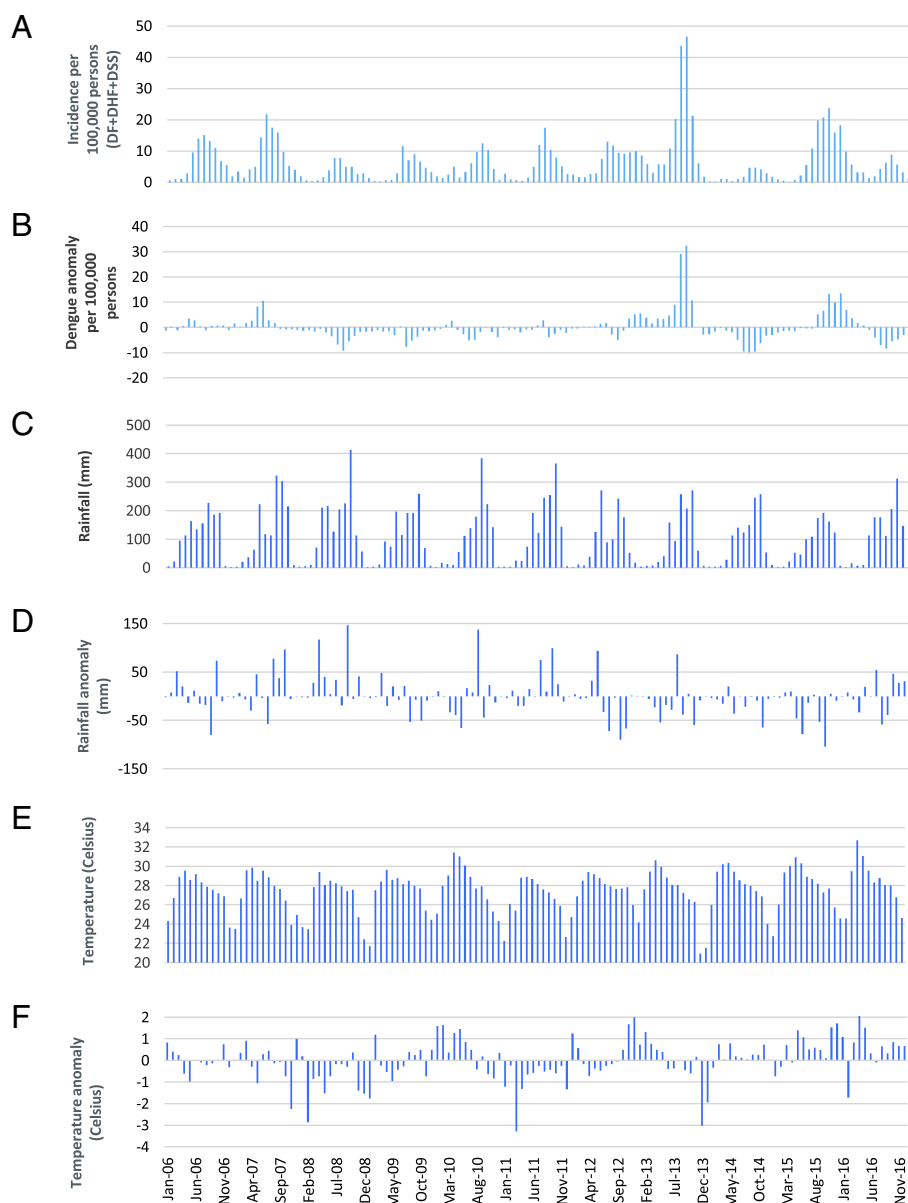


Fig. 2 Monthly dengue incidence (a), dengue anomaly (b), rainfall (c), rainfall anomaly (d), temperature (e) and temperature anomaly (f) in Khon Kaen Province, Thailand, 2006–2016. DF = dengue fever, DHF = dengue hemorrhagic fever, DSS = dengue shock syndrome

There were clear seasonal patterns of dengue incidence in Khon Kaen Province during the study period. Dengue occurs throughout the rainy season, with 73% of cases reported between May and September. Although maximum temperature was associated with higher incidence (Table 2), the model with meteorological covariates had similar performance (in terms of the WAIC) to a non-mechanistic model, which simply fitted a sinusoidal pattern with a period of 12 months. In our study, a 1 cm increase in monthly rainfall was associated with a 0.4% increase in dengue incidence. In Timor Leste, results from

similar modeling analyses showed a far larger effect: a 47% increase in incidence per 1 mm increase in annual rainfall [63]. Different climate patterns between Timor Leste and Thailand might explain these differences. Rainfall can affect the availability of mosquito larval habitats [34]. During rainy and dry periods of the year, permanent water containers are common in and around households; some located in toilet or bathroom spaces providing continuous year round mosquito production [35–39, 64]. Large water storage jars and tanks are the most commonly used containers in Thailand [64]. A study correlating rainfall

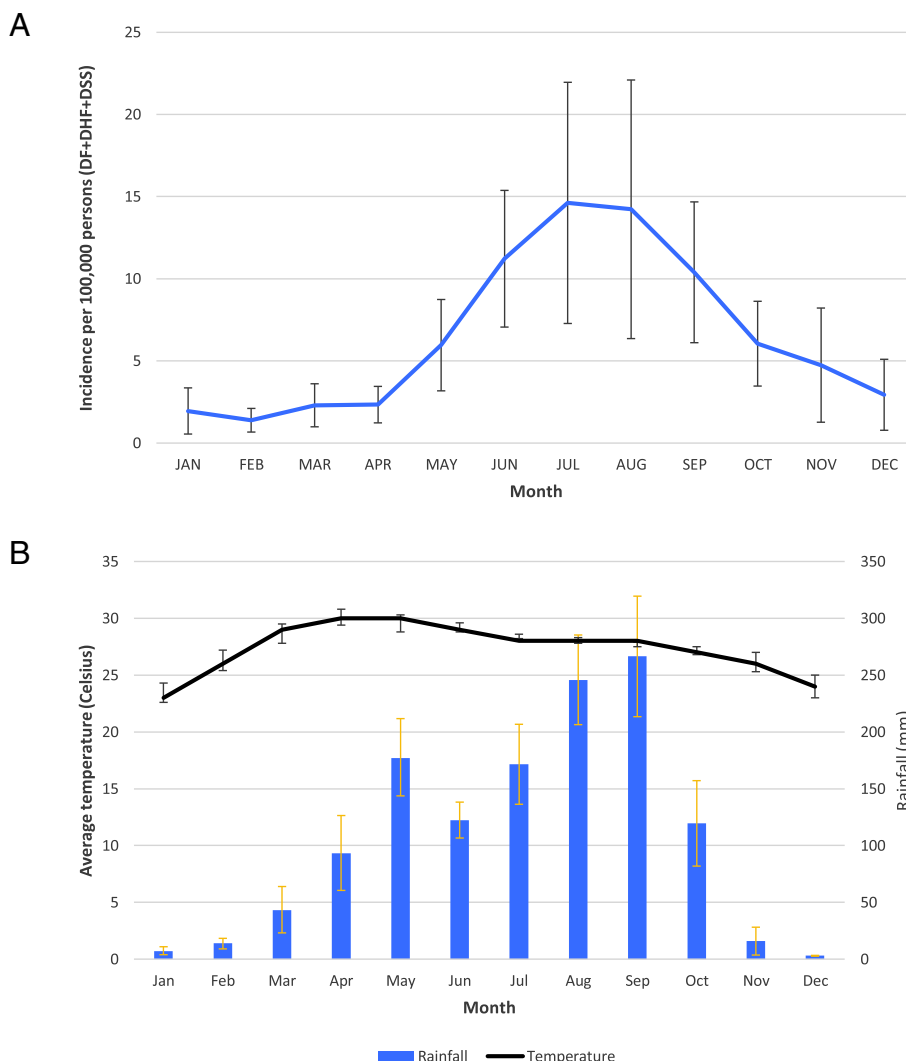


Fig. 3 Mean monthly dengue incidence per 100,000 persons (a) and monthly average of rainfall (bar) and temperature (line) (b) in Khon Kaen Province, Thailand, 2006–2016

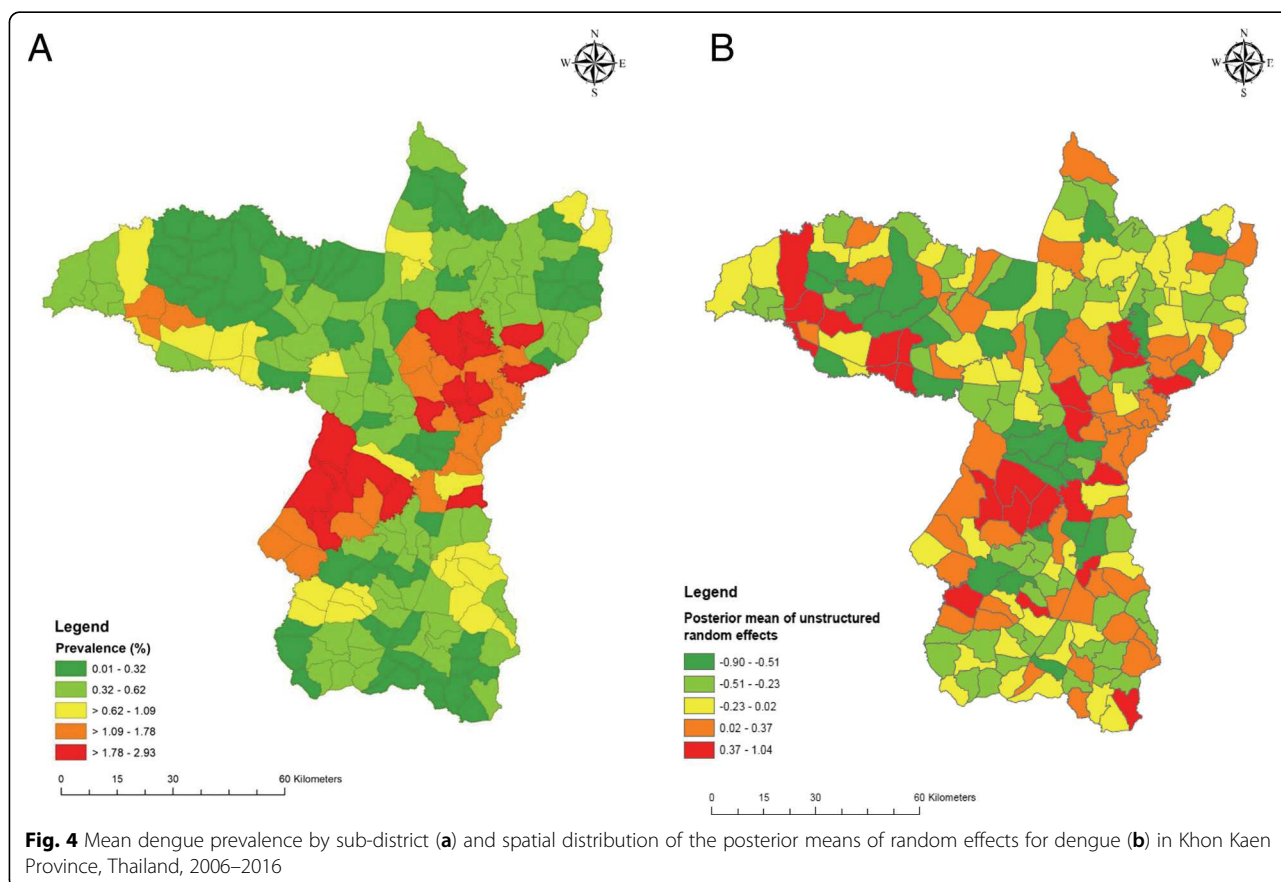
Table 2 Point estimates and 95% credible interval of the Bayesian Poisson regression model on number of all monthly dengue cases (DF, DHF and DSS) and covariates in Khon Kaen Province, Thailand, 2006–2016

Parameter	Rate ratios		
	Median	2.5%	97.5%
Mean monthly rainfall (cm)	1.004	0.990	1.017
Maximum temperature (°C)	1.055	1.009	1.115
Minimum temperature (°C)	0.958	0.927	1.024
Age (years)	0.990	0.985	0.994
Gender (proportion female ^a)	0.933	0.854	1.020
Density (thousands of people per km ²)	0.925	0.827	1.047

^aHence the rate ratio is for 100% female case composition relative to 100% male case composition

and clinical dengue cases in Thailand from 2002 to 2003 also found that the dengue incidence was closely related with rainfall [65].

Temperature is another primary environmental risk factor for dengue transmission. Sea surface temperature (SST) changes, generally related to periodic El Niño Southern Oscillation effects, and air temperature, having more direct short-term effects, have both been shown to influence dengue incidence [63, 66]. Dengue incidence increased by 19.4% with a 1 °C increase in SST and 2.6% with a 1 °C increase in weekly maximum temperature in the Texas-Mexico border region [66]. Another study found that a 1 °C monthly increase in mean ambient temperature, dengue incidence increased by 0.7% [63]. In our study, the rate ratio for maximum temperature was 1.055 per °C, within the range from 30.7 °C to 44.9 °C. Higher temperatures enhance viral replication in

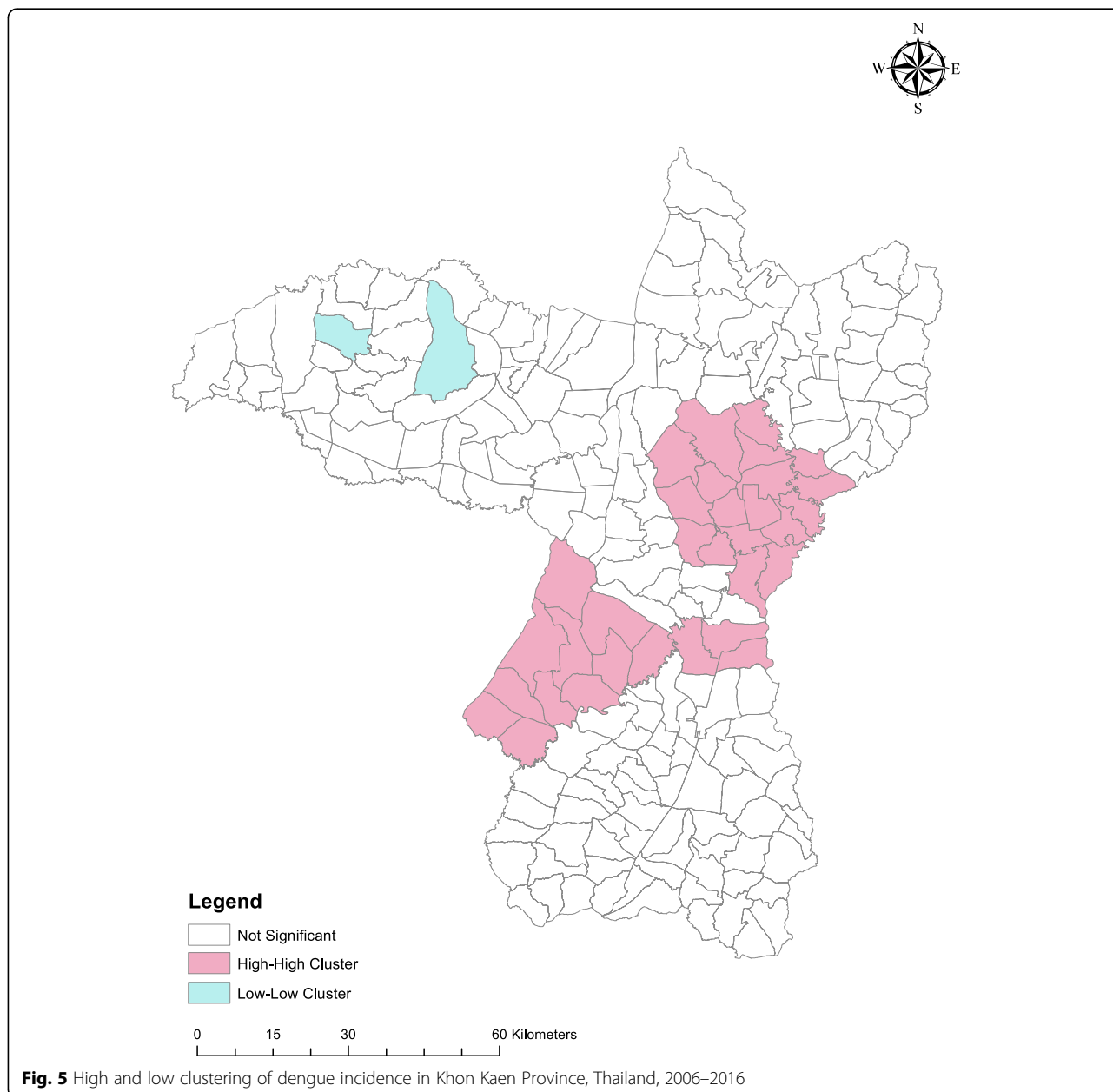


the vector mosquito in a shorter amount of time and thus increase transmission potential of dengue viruses. A study of the extrinsic incubation period (EIP) of dengue serotype 2 in *Aedes albopictus* found that the virus remained in the midgut at 18 °C but could disseminate and invade the salivary glands at temperatures between 23 °C and 32 °C [67], thereby showing higher temperatures produce a shorter EIP and greater transmission potential. The strong and consistent relationships between climate, particularly rainfall and temperature, and the number of dengue cases have been used to develop prediction models to implement more timely dengue control measures [68, 69]. Relationships between dengue transmission and climatic variables have been examined in numerous studies, as shown above, but the question remains how to use such relationships in predicting impending outbreaks and applying effective interventions in time to avert them. User-friendly tools, such as the operational guide on Early Warning and Response System developed with support from the WHO/TDR and the European Union [70], are needed and will be tested in forthcoming work in Khon Kaen Province.

The highest dengue incidence seen in this study occurred in two areas of the province: around Khon Kaen Mueang District in the northeast, and in Manchakhiri

and Khokphochai districts in the southwest. Mueang District includes the provincial capital and has the highest human population density, and in general, more conducive to dengue transmission. Manchakhiri and Khokphochai districts have lower population densities, but are, from our observations, seemingly similar to other districts in the province, i.e. vector species are present, larval habitats are plentiful, with a susceptible human population; therefore there must be other yet unexplored factors that support high dengue transmission in these two districts.

Although dengue incidence is influenced by rainfall and temperature, in our data there is no apparent spatial clustering of cases associated with the spatial variability in these environmental parameters. Rather, other factors such as urbanization are likely causes of the observed clustering effect [71]. However, population density, which was included in the regression model as a measure of urbanization, was not independently associated with dengue incidence. The residual spatial variation visible in Fig. 4b suggests that variables beyond those included in the spatial regression model are needed to explain differences in incidence between urban and rural subdistricts. Moreover, hotspots in more rural areas of southwestern Khon Kaen Province, further corroborate



the influence of factors other than urbanization driving transmission. We do not know of any specific reasons for why these rural areas should have elevated dengue prevalence. One speculation could be that the lakes and swamps that are common in this area may provide suitable humidity for mosquitoes to thrive, but this was not studied here. Large changes in population size over time will affect outcomes. However, during 2000 and 2015, the average annual population growth rate in Thailand was less than 0.5% [72], which might not have affected the results substantially. Rural-urban migration is common in Thailand, with people drawn by, for example, better education, job opportunities, health facilities,

standard of living, and wages [73]. Human movement is also an important factor in the dynamics of dengue transmission [74]. Adults are more likely to have greater mobility than younger age groups; therefore, to understand the circulation of the virus information on recent travel history and working conditions (location, time of work, etc.) is required. Elsewhere in Thailand, greater vulnerability to dengue infection has been observed in villages situated closer to urban centers [75]. Such neighboring effects are related to similarities in human behavior, development infrastructure, and ecological surroundings. Moreover, similar lifestyles and social interactions between neighboring areas are evident

between villages that share social and religious centers such as schools, temples, mosques and community halls [75]. Hence, the results presented here are generalizable to most of northern Thailand, Laos, and Cambodia, and potentially Vietnam and Myanmar as well, under similar epidemiological settings.

Data collected from national surveillance systems come with inherent limitations, including underreporting and misreporting of symptomatic cases as well as the absence of subclinical and asymptomatic infections [76]. Moreover, dengue cases are seldom laboratory confirmed or identified to serotype. Another limitation of this study is inaccuracy, albeit minor, of the population denominators within sub-districts, as these were taken as fixed values from a single census (2010). Lastly, the possibility of travel-related infections was not determined in this study, which would provide potential misclassification bias. Nationally, the importance of travel-related dengue would vary by locality based on mobility. Obviously, we cannot exclude the possibility that some dengue infections were acquired outside the study area, thus potentially affecting the analysis and conclusions. However, if the general travel patterns had not changed significantly over the 11-year observation period, the dengue disease trends reported in this study would remain valid.

Conclusion

We examined the epidemiology of dengue in Khon Kaen Province, Thailand between 2006 and 2016. There was an increase in older age groups reporting symptomatic dengue. Symptomatic dengue disease in people > 15 years of age is now more common than in children in this province, an observation that has been seen in other Asian countries. This study used monthly sub-district level data to show that rainfall and temperature have significant effects on dengue transmission in the province. Spatial clustering of cases is partly associated with urban areas closer to Khon Kaen city and rural areas in the southwest of the province. However, the current analysis was not able to detect a close proxy factor to quantify a relationship between urbanization and dengue incidence. The data set awaits further analysis for temporal patterns of infection for use in disease prediction modeling and developing dengue early warning systems to guide vector control operations.

Additional files

Additional file 1: Population density per sub-district, Khon Kaen province, Thailand, 2006 to 2016. Location of province in northeastern Thailand (inset). (PDF 128 kb)

Additional file 2: Average monthly temperature (°C) per sub-district, Khon Kaen province, Thailand, January to December 2006–2016. (PDF 51 kb)

Additional file 3: Average monthly rainfall (mm) per sub-district, Khon Kaen province, Thailand, January to December 2006–2016. (PDF 49 kb)

Additional file 4: Posterior distribution plots of A) Mean rainfall, B) Minimum temperature, C) Maximum temperature, D) Age, E) Gender, and F) Population density. (PDF 265 kb)

Additional file 5: Average monthly incidence of dengue (DF, DHF, and DSS) per 10,000 persons in Khon Kaen province, Thailand, January to December 2006–2016. (PDF 58 kb)

Abbreviations

CAR: Conditional autoregressive; DF: Dengue fever; DHF: Dengue hemorrhagic fever; DSS: Dengue shock syndrome; EIP: Extrinsic incubation period; ODPC7: Office of Disease Prevention and Control 7; SST: Sea surface temperature

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Consent for publications

Not applicable.

Authors' contributions

TP designed the study, performed data analysis, interpretation and drafted the manuscript. BZ and UH contributed to study design, analysis and writing. CP, TE, SA, BF, MJB and NA guided the research and reviewed the manuscript. KT provided dengue data and guided the research. HJO proposed the research, made substantial contributions to conception, design and coordination of the study, participated in interpretation of data and drafting the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study is part of the DENGUE-INDEX Project funded by the Research Council of Norway (Project no. 250443), which received ethical approval from Khon Kaen University Ethics Committee for Human Research (HE591099–20/04/2016 and HE601221–01/09/2017), the London School of Hygiene and Tropical Medicine Ethical Committee, UK (LSHTM Ethics Ref: 10534–19/04/2016 and LSHTM Ethics Ref: 14275–16/08/2017), and the Regional Committee for Medical and Health Research Ethics, South East Norway (2016/357–15/04/2016 and 2017/1826–13/03/2018). Permission to use these data was approved by the Ministry of Public Health, Thailand.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Microbiology, Khon Kaen University, Khon Kaen, Thailand. ²Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. ³Department of Biostatistics, University of North Carolina at Chapel Hill, Chapel Hill, USA. ⁴Department of Biostatistics and Epidemiology, University of North Texas Health Science Center, Fort Worth, TX, USA. ⁵HPV & EBV and Carcinogenesis Research Group, Khon Kaen University, Khon Kaen, Thailand. ⁶Department of Disease Control, Office of Disease Prevention and Control, Region 7 Khon Kaen, Ministry of Public Health, Khon Kaen, Thailand. ⁷Université de Montpellier, Montpellier, France. ⁸Public Health & Malaria Control, PT Freeport Indonesia, International SOS, Kuala Kencana, Papua, Indonesia. ⁹London School of Hygiene and Tropical Medicine, London, UK. ¹⁰Faculty of Science and Technology, Norwegian University of Life Sciences, Ås, Norway.

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References

- Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. *Nature*. 2013;496(7446):504–7.
- WHO. Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control: New Edition. edn. World Health Organization, Geneva; 2009.
- Christofferson RC, Mores CN. A role for vector control in dengue vaccine programs. *Vaccine*. 2015;33(50):7069–74.
- Descloux E, Mangeas M, Menkes CE, Lengaigne M, Leroy A, Tehei T, et al. Climate-based models for understanding and forecasting dengue epidemics. *PLoS Negl Trop Dis*. 2012;6(2):e1470.
- Wen TH, Lin NH, Chao DY, Hwang KP, Kan CC, Lin KC, et al. Spatial-temporal patterns of dengue in areas at risk of dengue hemorrhagic fever in Kaohsiung, Taiwan, 2002. *Int J Infect Dis*. 2010;14(4):e334–43.
- WHO. Vector-borne diseases (Dengue). World Health Organization. Available from: <http://www.who.int/mediacentre/factsheets/fs387/en/index2.html>.
- Limkittikul K, Brett J, L'Azou M. Epidemiological trends of dengue disease in Thailand (2000–2011): a systematic literature review. *PLoS Negl Trop Dis*. 2014;8(11):e3241.
- Ponlawat A, Scott JG, Harrington LC. Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* across Thailand. *J Med Entomol*. 2005;42(5):821–5.
- Halstead SB, Yamarat C. Recent epidemics of hemorrhagic fever in Thailand. Observations related to pathogenesis of a “new” dengue disease. *Am J Public Health Nations Health*. 1965;55:1386–95.
- Ministry of Public Health, Thailand. Epidemiology. Annual epidemiological surveillance report. Ministry of Public Health, Nonthaburi, Thailand. 1987.
- Ministry of Public Health, Thailand. Epidemiology. Annual epidemiological surveillance report. Ministry of Public Health, Nonthaburi, Thailand. 1998.
- Ministry of Public Health, Thailand. Epidemiology. Annual epidemiological surveillance report. Ministry of Public Health, Nonthaburi, Thailand. 2001.
- Ministry of Public Health, Thailand. Epidemiology. Annual epidemiological surveillance report. Ministry of Public Health, Nonthaburi, Thailand. 2013.
- Ministry of Public Health, Thailand. Annual epidemiological surveillance report. 2015. Ministry of Public Health, Nonthaburi, Thailand. Available from: <http://www.boe.moph.go.th/Annual/AESR2015/>.
- Jeefoo P, Tripathi NK, Souris M. Spatio-temporal diffusion pattern and hotspot detection of dengue in Chachoengsao province, Thailand. *Int J Environ Res Public Health*. 2011;8(1):51–74.
- Anker M, Arima Y. Male-female differences in the number of reported incident dengue fever cases in six Asian countries. *Western Pac Surveill Response J*. 2011;2(2):17–23.
- Ooi EE. Changing pattern of dengue transmission in Singapore. *Dengue Bulletin*. 2001;25.
- Ooi EE, Goh KT, Gubler DJ. Dengue prevention and 35 years of vector control in Singapore. *Emerg Infect Dis*. 2006;12(6):887–93.
- Yew YW, Ye T, Ang LW, Ng LC, Yap G, James L, et al. Seroepidemiology of dengue virus infection among adults in Singapore. *Ann Acad Med Singap*. 2009;38(8):667–75.
- Ministry of Public Health, Thailand. Reporting of Priority Diseases Guideline. Ministry of Public Health, Nonthaburi, Thailand. Available from: http://www.boe.moph.go.th/files/report/20121008_18818829.pdf.
- Thawillarp S. Evaluation of possible dengue outbreak detection methodologies for Thailand, which one should be implemented? Johns Hopkins University; 2017.
- Gould EA, Higgs S. Impact of climate change and other factors on emerging arbovirus diseases. *Trans R Soc Trop Med Hyg*. 2009;103(2):109–21.
- Sang S, Gu S, Bi P, Yang W, Yang Z, Xu L, et al. Predicting unprecedented dengue outbreak using imported cases and climatic factors in Guangzhou, 2014. *PLoS Negl Trop Dis*. 2015;9(5):e0003808.
- Hopp MJ, Foley JA. Worldwide fluctuations in dengue fever cases related to climate variability. *Clim Res*. 2003;25:85–94.
- Alto BW, Juliano SA. Temperature effects on the dynamics of *Aedes albopictus* (Diptera: Culicidae) populations in the laboratory. *J Med Entomol*. 2001;38(4):548–56.
- Rueda LM, Patel KJ, Axtell RC, Stinner RE. Temperature-dependent development and survival rates of *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol*. 1990;27(5):892–8.
- Scott TW, Amerasinghe PH, Morrison AC, Lorenz LH, Clark GG, Strickman D, et al. Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: blood feeding frequency. *J Med Entomol*. 2000;37(1):89–101.
- Rohani A, Wong YC, Zamre I, Lee HL, Zurainee MN. The effect of extrinsic incubation temperature on development of dengue serotype 2 and 4 viruses in *Aedes aegypti* (L). *Southeast Asian J Trop Med Public Health*. 2009;40(5):942–50.
- Liu-Helmersson J, Stenlund H, Wilder-Smith A, Rocklöv J. Vectorial capacity of *Aedes aegypti*: effects of temperature and implications for global dengue epidemic potential. *PLoS One*. 2014;9(3):e89783.
- Thammapalo S, Chongsuwatwong V, McNeil D, Geater A. The climatic factors influencing the occurrence of dengue hemorrhagic fever in Thailand. *Southeast Asian J Trop Med Public Health*. 2005;36(1):191–6.
- Campbell KM, Lin CD, Iamsirithaworn S, Scott TW. The complex relationship between weather and dengue virus transmission in Thailand. *Am J Trop Med Hyg*. 2013;89(6):1066–80.
- Lowe R, Cazelles B, Paul R, Rodo X. Quantifying the added value of climate information in a spatio-temporal dengue model. *Stoch Env Res Risk A*. 2016;30(8):2067–78.
- Rozilawati H, Zairi J, Adanan CR. Seasonal abundance of *Aedes albopictus* in selected urban and suburban areas in Penang. *Malaysia Trop Biomed*. 2007;24(1):83–94.
- Li CF, Lim TW, Han LL, Fang R. Rainfall, abundance of *Aedes aegypti* and dengue infection in Selangor, Malaysia. *Southeast Asian J Trop Med Public Health*. 1985;16(4):560–8.
- Lambdin BH, Schmaedick MA, McClintock S, Roberts J, Gurr NE, Marcos K, et al. Dry season production of filariasis and dengue vectors in American Samoa and comparison with wet season production. *Am J Trop Med Hyg*. 2009;81(6):1013–9.
- Bermudi PMM, Kowalski F, Menzato MM, Ferreira MDC, Passos W, Oku VJA, et al. *Aedes aegypti* breeding site in an underground rainwater reservoir: a warning. *Rev Saude Publica*. 2017;51:122.
- Serpa LL, Costa KV, Voltolini JC, Kakitani I. Seasonal variation of *Aedes aegypti* and *Aedes albopictus* in a city of southeastern Brazil. *Rev Saude Publica*. 2006;40(6):1101–5.
- Kittayapong P, Strickman D. Distribution of container-inhabiting *Aedes* larvae (Diptera: Culicidae) at a dengue focus in Thailand. *J Med Entomol*. 1993;30(3):601–6.
- Tonn RJ, Sheppard PM, Macdonald WW, Bang YH. Replicate surveys of larval habitats of *Aedes aegypti* in relation to dengue haemorrhagic fever in Bangkok. *Thailand Bull World Health Organ*. 1969;40(6):819–29.
- Sharma KD, Mahabir RS, Curtin KM, Sutherland JM, Agard JB, Chadee DD. Exploratory space-time analysis of dengue incidence in Trinidad: a retrospective study using travel hubs as dispersal points, 1998–2004. *Parasit Vectors*. 2014;7:341.
- Cummings DA, Irizarry RA, Huang NE, Endy TP, Nisalak A, Ungchusak K, Burke DS. Travelling waves in the occurrence of dengue haemorrhagic fever in Thailand. *Nature*. 2004;427(6972):344–7.
- van Panhuis WG, Choisy M, Xiong X, Chok NS, Akarasewi P, Iamsirithaworn S, et al. Region-wide synchrony and traveling waves of dengue across eight countries in Southeast Asia. *Proc Natl Acad Sci U S A*. 2015;112(42):13069–74.
- Lolekha S, Tanthiphabha W, Sornchai P, Kosuwan P, Sutra S, Warachit B, Chup-Upprakarn S, Hutagalung Y, Weil J, Bock HL. Effect of climatic factors and population density on varicella zoster virus epidemiology within a tropical country. *Am J Trop Med Hyg*. 2001;64(3–4):131–6.
- International Research Institute for Climate and Society: Climate Data Library. International Research Institute for Climate and Society. 2017.
- NCEP Climate Forecast System Reanalysis (CFSR) Selected Hourly Time-Series Products, January 1979 To December 2010. Research Data Archive at the National Center for Atmospheric Research, Computational and Information Systems Laboratory.
- Funk C, Peterson P, Landsfeld M, Pedreros D, Verdin J, Shukla S, et al. The climate hazards infrared precipitation with stations—a new environmental record for monitoring extremes. *Sci Data*. 2015;2:150066.
- National Statistical Office. The 2010 Population and housing census. Bangkok, Thailand 2010.
- Rushworth A, Lee D, Sarran C. An adaptive spatiotemporal smoothing model for estimating trends and step changes in disease risk. *J Royal Stat Soc Series C-Applied Stat*. 2017;66(1):141–57.
- Geweke J. In: Bernardo JM, Berger JO, Dawid AP, Smith AFM, editors. Evaluating the Accuracy of Sampling-Based Approaches to Calculating Posterior Moments. In *Bayesian Statistics 4*. Oxford: Clarendon Press; 1992.

50. Burnham KP, Anderson DR. Multimodel inference: understanding AIC and BIC in model selection. *Sociol Methods Res.* 2004;33(2):261–304.
51. Anselin L. Local indicators of spatial association-LISA. *Geogr Anal.* 1995;27:93–115.
52. Pessanha JE, Caiaffa WT, Almeida MC, Brandao ST, Proietti FA. Diffusion pattern and hotspot detection of dengue in Belo Horizonte, Minas Gerais. *Brazil J Trop Med.* 2012;2012:760951.
53. Rigau-Perez JG, Vorndam AV, Clark GG. The dengue and dengue hemorrhagic fever epidemic in Puerto Rico, 1994-1995. *Am J Trop Med Hyg.* 2001;64(1–2):67–74.
54. Guha-Sapir D, Schimmer B. Dengue fever: new paradigms for a changing epidemiology. *Emerg Themes Epidemiol.* 2005;2(1):1.
55. National Statistical Office. Population and Housing census (Births and Deaths by Sex: 2011–2015, Khon Kaen province). Khon Kaen, Thailand 2018.
56. Cummings DA, Iamsirithaworn S, Lessler JT, McDermott A, Prasanthong R, Nisalak A, et al. The impact of the demographic transition on dengue in Thailand: insights from a statistical analysis and mathematical modeling. *PLoS Med.* 2009;6(9):e1000139.
57. Kanakarathne N, Wahala WM, Messer WB, Tissera HA, Shahani A, Abeysinghe N, et al. Severe dengue epidemics in Sri Lanka, 2003-2006. *Emerg Infect Dis.* 2009;15(2):192–9.
58. Guzman MG, Kouri G. Dengue and dengue hemorrhagic fever in the Americas: lessons and challenges. *J Clin Virol.* 2003;27(1):1–13.
59. Deen JL, Harris E, Wills B, Balmaseda A, Hammond SN, Rocha C, et al. The WHO dengue classification and case definitions: time for a reassessment. *Lancet.* 2006;368(9530):170–3.
60. Karyanti MR, Uiterwaal CS, Kusriastuti R, Hadinegoro SR, Rovers MM, Heesterbeek H, et al. The changing incidence of dengue haemorrhagic fever in Indonesia: a 45-year registry-based analysis. *BMC Infect Dis.* 2014;14:412.
61. Guzman MG, Alvarez M, Halstead SB. Secondary infection as a risk factor for dengue hemorrhagic fever/dengue shock syndrome: an historical perspective and role of antibody-dependent enhancement of infection. *Arch Virol.* 2013;158(7):1445–59.
62. Mohd-Zaki AH, Brett J, Ismail E, L'Azou M. Epidemiology of dengue disease in Malaysia (2000-2012): a systematic literature review. *PLoS Negl Trop Dis.* 2014;8(11):e3159.
63. Wangdi K, Clements ACA, Du T, Nery SV. Spatial and temporal patterns of dengue infections in Timor-Leste, 2005-2013. *Parasit Vectors.* 2018;11(1):9.
64. Vannavong N, Seidu R, Stenstrom TA, Dada N, Overgaard HJ. Effects of socio-demographic characteristics and household water management on *Aedes aegypti* production in suburban and rural villages in Laos and Thailand. *Parasit Vectors.* 2017;10(1):170.
65. Wiwanitkit V. An observation on correlation between rainfall and the prevalence of clinical cases of dengue in Thailand. *J Vector Borne Dis.* 2006;43(2):73–6.
66. Brunkard JM, Cifuentes E, Rothenberg SJ. Assessing the roles of temperature, precipitation, and ENSO in dengue re-emergence on the Texas-Mexico border region. *Salud Publica Mex.* 2008;50(3):227–34.
67. Liu Z, Zhang Z, Lai Z, Zhou T, Jia Z, Gu J, et al. Temperature increase enhances *Aedes albopictus* competence to transmit dengue virus. *Front Microbiol.* 2017;8:2337.
68. Phung D, Talukder MR, Rutherford S, Chu C. A climate-based prediction model in the high-risk clusters of the Mekong Delta region, Vietnam: towards improving dengue prevention and control. *Tropical Med Int Health.* 2016;21(10):1324–33.
69. Karim MN, Munshi SU, Anwar N, Alam MS. Climatic factors influencing dengue cases in Dhaka city: a model for dengue prediction. *Indian J Med Res.* 2012;136(1):32–9.
70. WHO/TDR. Operational Guide: Early Warning and Response System (EWARS) for dengue outbreaks. Training in Tropical Diseases (TDR). World Health Organization, Special Programme for Research and Training in Tropical Diseases 2017;52.
71. Gubler DJ. Dengue, urbanization and globalization: the unholy trinity of the 21(st) century. *Trop Med Health.* 2011;39(4 Suppl):3–11.
72. United Nations, Department of Economic and Social Affairs, Population Division 2017. World Population Prospects: The 2017 Revision, custom data acquired via website: <https://population.un.org/wpp/DataQuery/>.
73. Katewongsa P. Benefits of rural-urban migration for migrants' better life: a case study in Nang Rong, Buriram Province. *Thailand Thammasat Review.* 2015;18(1):63–81.
74. Stoddard ST, Forshey BM, Morrison AC, Paz-Soldan VA, Vazquez-Prokopec GM, Astete H, Vilcarrromero S, Elder JP, Halsey ES, et al. House-to-house human movement drives dengue virus transmission. *Proc Natl Acad Sci U S A.* 2013;110(3):994–9.
75. Tipayamongkhogul M, Lisakulruk S. Socio-geographical factors in vulnerability to dengue in Thai villages: a spatial regression analysis. *Geospat Health.* 2011;5(2):191–8.
76. da Fonseca BA, Fonseca SN. Dengue virus infections. *Curr Opin Pediatr.* 2002;14(1):67–71.

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Chapter 2: Addressing the complex relationships between *Aedes* vectors, socio-economics and dengue transmission in North-eastern Thailand.

The results from this study were published in *PLOS Neglected Tropical Diseases*, 1;14(10), <https://doi.org/10.1371/journal.pntd.0008703>. (2020). **Complex relationships between *Aedes* vectors, socio-economics and dengue transmission-Lessons learned from a case-control study in northeastern Thailand.** Fustec B, Phanitchat T, Hoq MI, Aromseree S, Pientong C, Thaewongiew K, Ekalaksananan T, Bangs MJ, Corbel V, Alexander N, Overgaard HJ.

Our baseline study demonstrated that dengue distribution and dispersion in North-eastern Thailand was not only explained by climatic data, and that urbanization, human movements and entomological factors may partially explain the clustering effect observed (Phanitchat et al. 2019). Hence, we conducted a prospective hospital-based case control study (see section 4.1), to identify risk factors for dengue infections. The scope was to assess whether entomological and immunological indices could discriminate between dengue positive and negative households (see section 3.1).

Briefly, 377 individuals were recruited from the nineteen district and sub-district hospitals between June 2016 and August 2019. Dengue infection was detected by RDT targeting both dengue -NS1 and -IgM/IgG and confirmed by RT-qPCR allowing the identification of 173 recent dengue cases and 204 controls (0.85 case/control ratio). The participant ages ranged from 5 to 76 years with 190 (48%) females represented. Individual questionnaire and immature and adult *Aedes* entomological collections were performed in 377 patient houses and the 1,110 neighbouring surrounding households (mean of 3.94 houses per individual recruited). In addition, the levels of Ab response to Nterm-34 peptide could be measured in 368 patients. Socio-economic status, household and individual characteristics were analysed as additional risk factors for dengue infection (see details in section 4.1).

Summary of the results:

Our results showed that patient age was associated with higher odds of dengue. While dengue normally affects young children, we found that individuals aged between 10 and 25 years-old were at higher risk relative to those either younger or older. This is in agreement with our

baseline survey of dengue incidence in North-eastern Thailand (Phanitchat et al. 2019) and with other recent studies conducted in Thailand, Malaysia, and the Philippines (Limkittikul et al. 2014, Mohd-Zaki et al. 2014, Thomas et al. 2015, Alera et al. 2016). Although other studies had found a higher odds of dengue transmission in low-income family (Telle et al. 2016, Wijayanti et al. 2016a, Udayanga et al. 2018), no such association was found in the study area. However, we showed that household construction may play a role in dengue transmission risk, as individuals living in two-floor houses were at higher odds of dengue infections. Moreover, individuals who declared spending most of their time indoors were found at higher risk of dengue. Curiously, the presence of eave gaps in the house was negatively associated with dengue. Although counterintuitive, the apparent ‘protective’ effect of eave gaps might be due to increased air flow and ventilation inside the house hence creating exit routes for the vectors (von Seidlein et al. 2019).

Although not surprising, our study confirmed that traditional entomological indices were not good indicators of dengue transmission as they were statistically higher in the “control” houses than in “dengue case” houses. Indeed, vector infestation indices based on immature stages (HI, BI, and CI) were all negatively associated with dengue using univariable analysis (total containers inspected, 5,185 including 1,230 (23.7%) positive for immature *Aedes* stages). In other words, control households had more *Aedes* positive containers than case’s households. It is worth to mention that most of the inspected households (control and case) had immature indices values higher than the “outbreak-risk” thresholds setting up by the MoPH of Thailand (i.e., CI<1%, BI<50 and HI<10%) (Thai Ministry of Public Health 2013). Similarly, pupae indices (PPI and PHI) were not significantly different between case and control houses and even more *Aedes* adults were found in control households. Although surprising, this could be explained by higher vector control efforts following onset of dengue symptoms in the dengue “case” household which would have reduced vector infestation at the monitoring time point. This is corroborated by the positive association between dengue cases and the use of household insecticide products as declared by the head of the household in the questionnaire.

Nonetheless, our findings showed that the presence of DENV-infected *Ae. aegypti* in the households was positively associated with dengue infections ($p=0.018$). Indeed, the proportion of DENV-infected *Aedes* was higher in the patient houses ($\approx 8\%$) than the control houses (3%), hence suggesting that vector infectivity would be a more reliable indicator than vector abundance to

assess dengue transmission risk. Overall, about 13% of the selecting house's (including neighbourhood and patient house) had DENV-infected *Aedes* hence highlighting the hyperendemic situation of dengue in the study area.

Interestingly, individuals from the control group had higher level of Ab response to the Nterm-34 than individuals from the dengue case group, which corroborate entomology results (Figure 33). Our results suggest that individual with a higher Ab response to the salivary biomarker received more *Aedes* mosquito bites than lower immune responders (Elanga Ndille et al. 2012). This trend was more pronounced when considering the *Aedes* density indoor, hence suggesting a strong endophagic preference of the *Aedes* population in the study area. Nevertheless, neither the adult abundance in the patient household nor the level of human exposure to *Aedes* mosquito bites were correlated with dengue incidence. Interestingly, a positive and significant association was seen between the intensity Ab response to *Aedes* saliva and the presence of IgG response against dengue hence suggesting that patient's immunity may have biased the correlation between the mosquito exposure risk and dengue transmission. This highlights the fact that dengue virus transmission is complex and varies through time and space, and the relationship between vector density/aggressiveness and risk of human infection is not static nor linear. Moreover, the measure of Ab response to *Aedes* saliva reflects the overall exposure to *Aedes* bites in the previous 2-4 weeks and not necessarily at the time of virus transmission. Including all inhabitants from each house, irrespective to the dengue infection status, would have been useful to assess the differential exposure to *Aedes* bites in dengue case and control groups.

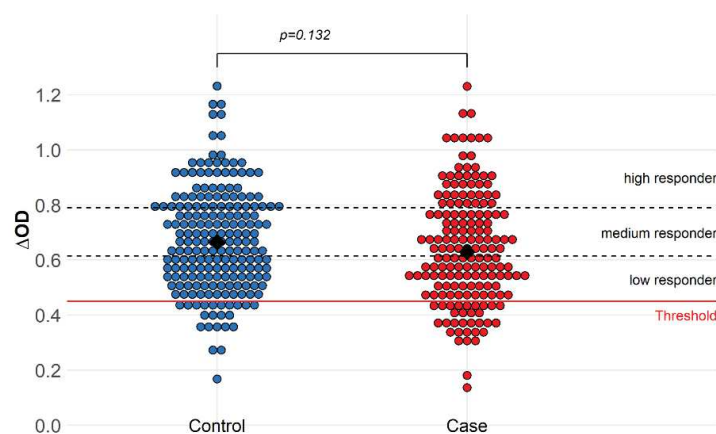


Figure 33: Immune response to *Aedes* salivary peptide Nterm-34 (Δ OD) in dengue case and control patients.

In conclusion, this first study highlights the complex relationship between *Aedes* vectors, socio-economic factors, and dengue transmission risk, and highlight the challenges to setting up accurate warning indicators for dengue prevention. A longitudinal randomized controlled study conducted as part of the DENGUE INDEX Project (see section 4.3) was then conducted to better evaluate the close relationship between the levels of human-*Aedes* contact, the levels of *Aedes* infestations, and dengue transmission risk in North-eastern Thailand. The main findings are described in the next chapter.

RESEARCH ARTICLE

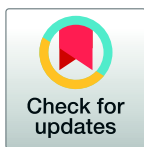
Complex relationships between *Aedes* vectors, socio-economics and dengue transmission—Lessons learned from a case-control study in northeastern Thailand

Benedicte Fustec^{1,2,3}, Thipruethai Phanitchat⁴, Mohammad Injamul Hoq⁵, Sirinart Aromseree^{2,6}, Chamsai Pientong^{2,6}, Kesorn Thaewngiew⁷, Tipaya Ekalaksananan^{2,6}, Michael J. Bangs^{8,9}, Vincent Corbel^{1,3}, Neal Alexander¹⁰, Hans J. Overgaard¹¹✉*

1 University of Montpellier, Montpellier, France, **2** Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand, **3** Institut de Recherche pour le Developpement, Montpellier, France, **4** Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, **5** School of Public Health, Epidemiology and Social Medicine at the Institute of Medicine, University of Gothenburg, Gothenburg, Sweden, **6** HPV & EBV and Carcinogenesis Research Group, Khon Kaen University, Khon Kaen, Thailand, **7** Office of Diseases Prevention and Control, Region, Khon Kaen, Thailand, **8** Public Health & Malaria Control, PT Freeport Indonesia/International SOS, Mimika, Papua, Indonesia, **9** Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand, **10** MRC Tropical Epidemiology Group, London School of Hygiene and Tropical Medicine, London, United Kingdom, **11** Norwegian University of Life Sciences, Ås, Norway

✉ These authors contributed equally to this work.

* hans.overgaard@nmbu.no



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Abstract

Background/Objectives

Dengue fever is an important public health concern in most tropical and subtropical countries, and its prevention and control rest on vector surveillance and control. However, many aspects of dengue epidemiology remain unclear; in particular, the relationship between *Aedes* vector abundance and dengue transmission risk. This study aims to identify entomological and immunological indices capable of discriminating between dengue case and control (non-case) houses, based on the assessment of candidate indices, as well as individual and household characteristics, as potential risk factors for acquiring dengue infection.

Methods

This prospective, hospital-based, case-control study was conducted in northeastern Thailand between June 2016 and August 2019. Immature and adult stage *Aedes* were collected at the houses of case and control patients, recruited from district hospitals, and at patients' neighboring houses. Blood samples were tested by RDT and PCR to detect dengue cases, and were processed with the Nterm-34 kDa salivary peptide to measure the human immune response to *Aedes* bites. Socioeconomic status, and other individual and household characteristics were analyzed as potential risk factors for dengue.

Norwegian Center for Research Data (NSD), a national archive and center for research data. Data can be accessed without specific permissions by filling in and submitting a Data Access Form at <https://nsd.no/nsd/english/order.html>, referring to the project ID no. NSD2864 and project name "Determination of entomological indices to assess dengue transmission, predict dengue outbreaks, and evaluate vector control interventions, 2019", short name: DENGUE-INDEX.

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Results

Study findings showed complex relationships between entomological indices and dengue risk. The presence of DENV-infected *Aedes* at the patient house was associated with 4.2-fold higher odds of dengue. On the other hand, *Aedes* presence (irrespective of infectious status) in the patient's house was negatively associated with dengue. In addition, the human immune response to *Aedes* bites, was higher in control than in case patients and *Aedes* adult abundance and immature indices were higher in control than in case houses at the household and the neighboring level. Multivariable analysis showed that children aged 10–14 years old and those aged 15–25 years old had respectively 4.5-fold and 2.9-fold higher odds of dengue infection than those older than 25 years.

Conclusion

DENV infection in female *Aedes* at the house level was positively associated with dengue infection, while adult *Aedes* presence in the household was negatively associated. This study highlights the potential benefit of monitoring dengue viruses in *Aedes* vectors. Our findings suggest that monitoring the presence of DENV-infected *Aedes* mosquitoes could be a better indicator of dengue risk than the traditional immature entomological indices.

Author summary

Dengue fever is a globally expanding arboviral disease, consisting of four distinct serotypes, transmitted primarily by synanthropic/peridomestic mosquitoes, *Aedes aegypti* and *Aedes albopictus*. Given the absence of specific treatment, and the incomplete protection provided by the currently available vaccine, vector surveillance and control remain the principal tool to prevent and control dengue transmission. However, vector surveillance through the monitoring of larval mosquito indices lacks consistency in addressing dengue risk. Surveillance based on pupal and adult stages is considered as more accurate to estimate dengue transmission risk, although monitoring is difficult to implement in routine. An alternative strategy is the use of the specific human antibody response to *Aedes* saliva to identify human exposure risk to *Aedes* bites. We conducted a hospital-based, case-control study in northeastern Thailand in order to identify risk factors for dengue infection using entomological and immunological indices, together with select individual and household characteristics. We found that people aged 10–25 years had significant higher odds of dengue than older adults (>25 years old). The presence of DENV-infected *Aedes* in the house was associated with 4.2-fold higher odds of dengue infection. Interestingly, *Aedes* adult abundance in the household was negatively associated with dengue revealing the complex role of *Aedes* density to dengue risk. This study highlights the potential benefit of monitoring dengue viruses in *Aedes* vectors to identify areas ("hot spots") and people ("hot pops") at higher risk of transmission.

Introduction

Dengue fever is a globally expanding mosquito-borne disease which threatens half the world's population [1]. Dengue virus (DENV) is transmitted by synanthropic *Aedes* mosquitoes, with

Aedes aegypti (L.) typically being the primary vector [2], and *Aedes albopictus* (Skuse) a secondary one [3]. The Southeast Asia region accounts for more than half of the reported dengue cases worldwide [2, 4, 5]. Thailand typically records more than 20,000 cases each year, with all four DENV serotypes circulating and both vector species spread throughout the country [6]. Although dengue incidence is highly seasonal, outbreaks are difficult to predict [7, 8]. Dengue virus transmission is highly efficient and it is assumed that only a few vector mosquitoes are sufficient to ensure transmission [9]. *Aedes aegypti* is particularly well adapted to urbanized environments and is a strongly anthropophilic diurnal blood feeder [10–13]. The absence of specific treatments for dengue and the incomplete protection offered by the currently available vaccine [14, 15], underscores the importance of vector surveillance and management as the principal strategy for dengue prevention and control [7, 16].

In Thailand, dengue prevention and control are mainly based on hospital case reporting and vector surveillance and control that are carried out collaboratively between hospitals and the Offices of Disease Prevention and Control (ODPC). When a dengue case is reported from hospital, a Surveillance and Rapid Response Team (SSRT) is mandated to carry out insecticide space spray (‘fogging’) within 100 meters of the case house within 24 hours of notice in order to interrupt transmission [17]. The reorganization of disease control operations in Thailand resulted in 76 provincial administrations being aggregated into 22 regional ODPCs [18]. The seventh regional ODPC includes four provinces: Khon Kaen, Roi Et, Maha Sarakham, and Kalasin with a total population of around 5 million. Northeastern Thailand is the third largest region in the country with regards to population size and land area, with an economy mainly based on agriculture.

In most dengue-endemic countries, vector surveillance usually consists of monitoring *Aedes* immature (larvae and pupae) stages present in natural and artificial breeding sites (larval habitats) in and near houses [19–21]. Vector presence and density are estimated by standardized indices such as the Breteau Index (BI), Container Index (CI), House Index (HI), and the Pupae per Person Index (PPI) [21–23]. Entomological measures as thresholds have been proposed to assess and estimate risk for use as early warning systems to predict dengue outbreaks [19, 22, 24]. In Thailand, vector density thresholds to estimate risk of dengue outbreaks occurrence have been set at $HI > 10$, $BI > 50$ and $CI > 1$ [25]. Additionally, vector control interventions are implemented to reduce vector abundance and prevent dengue transmission. However, numerous studies have failed to clearly link entomological indices to the risk of dengue transmission [7, 24, 26, 27]. Indeed, the larval stages (four successive instars) typically suffer high mortality during development to pupal stage, thus indices based only on their presence are generally poor indicators of the eventual adult vector density. Pupal indices (a stage with very low mortality) were proposed as a more accurate determination of actual adult production; however, pupal collections are far more challenging and time consuming to carry out [26, 28]. Adult collections can be performed via several devices such as gravidtraps, sticky traps, baited mechanical traps, and mouth or mechanical aspirators, but they only provide an imprecise estimation of the true vector density and do not reflect human-vector exposure.

Entomological collections for target *Aedes* species, of all kinds, are labor- and time-consuming, expensive, and contingent on access to the house being granted. However, estimating the human immune response to *Aedes* bites as a surrogate measure of bite exposure (intensity) might be less labor-intensive and more informative of relative ‘vector attack’ over time [29]. Upon initiating the blood feeding process, salivary gland proteins injected at the bite site induce a species-specific immune response by the host [30, 31]. These specific antibodies (against salivary proteins) have shown promising to measure seasonal variation of human exposure to mosquito bites [32–37] and to assess the effectiveness (i.e., reduction in biting) of vector control interventions [38].

The current study aims to identify risk factors for dengue transmission across four provinces in northeastern Thailand by comparing individuals with and without dengue in terms of i) their immune response to *Aedes* bites, ii) the presence and abundance of immature and adult *Aedes* in and close proximity around their houses, and iii) their individual and household characteristics. The first objective was to assess the accuracy of entomological and immunological indices to discriminate dengue positive and dengue negative households. We hypothesize that there will be more adult *Aedes* mosquitoes and a higher level of immune response to *Aedes* exposure (salivary proteins) in households with a recent dengue case compared to control (non-case) houses. The second objective was to assess whether socio-economics, household characteristics and entomological and immunological indices can be accurate predictors of dengue transmission risk.

Materials and methods

Study settings

This hospital-based case-control study was carried out in four provinces in northeastern Thailand (Fig 1) between June 2016 and August 2019. Ten district hospitals were included: Mancha Khiri, Chum Phae, Ban Phai, and Ban Haet districts in Khon Kaen Province; Selaphum, Phon Thong, Thawatburi districts in Roi Et Province; Kamalasai and Kuchinarai districts in Kalasin Province; and Chiang Yuen district in Maha Sarakham Province. Additionally, nine sub-district hospitals in Khon Kaen Muang district (Khon Kaen Province) were included. The four provinces cover approximately 31,440 km² with around 5 million inhabitants. Khon Kaen, Roi Et, Kalasin and Maha Sarakham provinces are divided in 26, 20, 18 and 13 districts, respectively (Fig 1). Over the previous 15 years, the region reported in average 4,488 dengue cases annually [39, 40]. A case-control design was chosen because it allowed the investigation of several risk factors concomitantly, it is effective for diseases with low incidence, and requires relatively, few study subjects.

Sample size

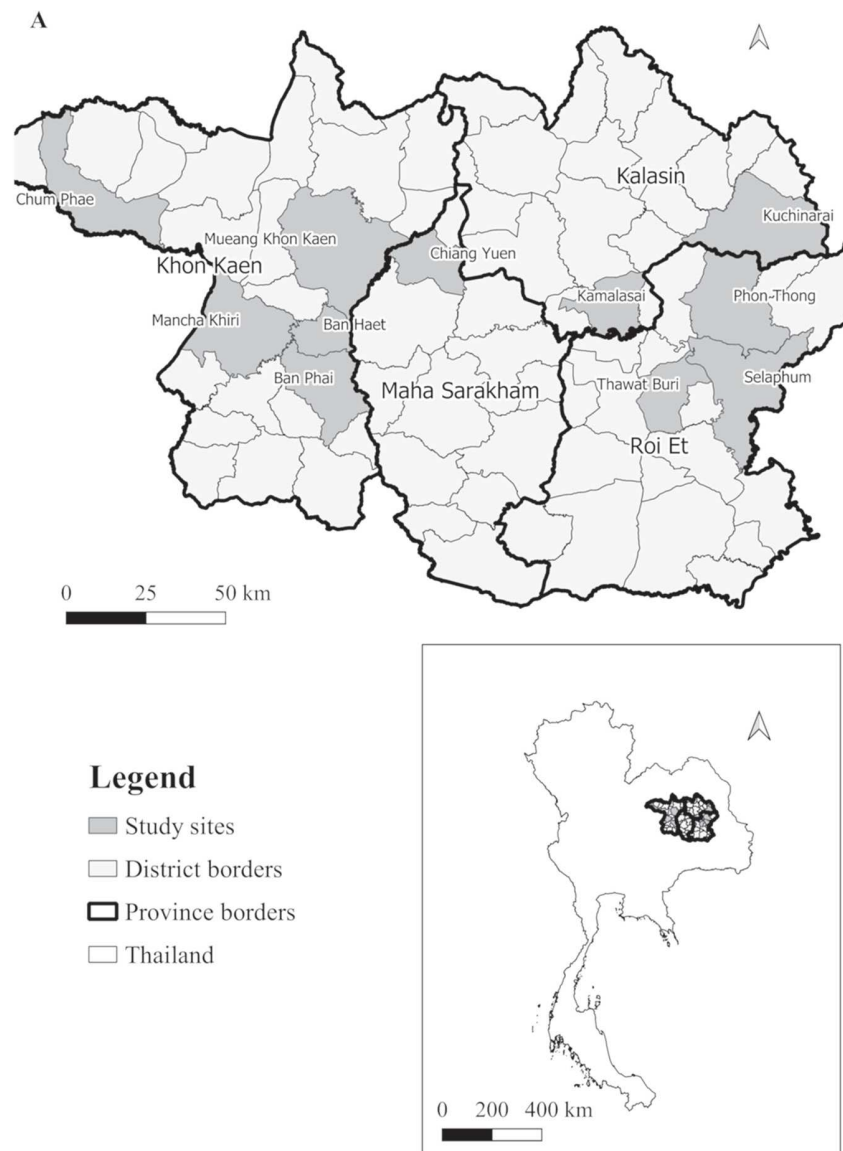
The study sample size was calculated using the unmatched case-control study module of OpenEpi, version 3 [42] with 90% power based on data from Thomas et al. [43]. Assuming a difference in DENV-infected female *Aedes* mosquitoes collected between dengue positive and dengue negative households, with an exposure of 10% of DENV-infected *Aedes* in the exposed group, and 1% of DENV-infected *Aedes* in the control group, the significance level was set at 5% (two-sided) and the ratio of control to case at 1. The result was a target sample size of 322 patients. To allow for a 15% loss at the household questionnaire stage, we increased the final sample to 370.

Patient recruitment

Patients presenting with dengue-like symptoms were recruited from the participating hospitals. Regarding Thai health services, public hospitals generally serve the communities in the districts and sub-districts in which they are located. Eligible patients with potential dengue infections were recruited based on presence of fever ($\geq 38^{\circ}\text{C}$), no recent travel history during the previous 7 days, and being older than five years-of-age.

Blood collections

A total of 6 mL of venous blood was drawn from each participant for the following three purposes (Fig 2):



B

	Mean number of dengue cases/year	Population (census 2010)	Number of districts	Surface area (km ²)
Khon Kaen	1,368	~1.8 millions	26	10,900
Roi Et	1,620	~1,3 millions	20	8,300
Kalasin	720	~979,000	18	6,950
Maha Sarakham	780	~937,400	13	5,290

Fig 1. Map and characteristics of study sites of the case-control study in northeastern Thailand. A: Location of four provinces and study districts in northeastern Thailand included in the case-control study. Map of study sites was built using QGIS 3.10 software and shapefiles were obtained from the Humanitarian Data Exchange project [41] under the Creative Commons Attribution International 4.0 license (CC BY 4.0). B: Study area characteristics, population and average number of dengue cases per year from 2005–2019 [39].

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1. Detect dengue non-structural protein 1 (NS1) and IgM / IgG antibodies using a Rapid Diagnostic Test (RDT) (SD BIOLINE Dengue Duo, Standard Diagnostics, Korea).
2. Determine the immune response to *Aedes* bites using two blood drops (approximately 75µL each) collected on protein saver cards 903 (Whatman, UK).
3. Confirm dengue infection by reverse transcription polymerase chain reaction (RT-PCR) (described below) and distinguish serotypes (not presented here) using 5.7 mL whole blood collected in heparin or EDTA tubes.

DENV confirmation in human samples and case definition

RNA was extracted from patients' blood for DENV screening, confirmation and serotyping by RT-PCR as described previously [44] and adapted to conventional PCR. According to the course of dengue illness, viremia usually drops after few days of fever, while antibody response is triggered within few days after the beginning of dengue symptoms [2]. Therefore, a positive sample

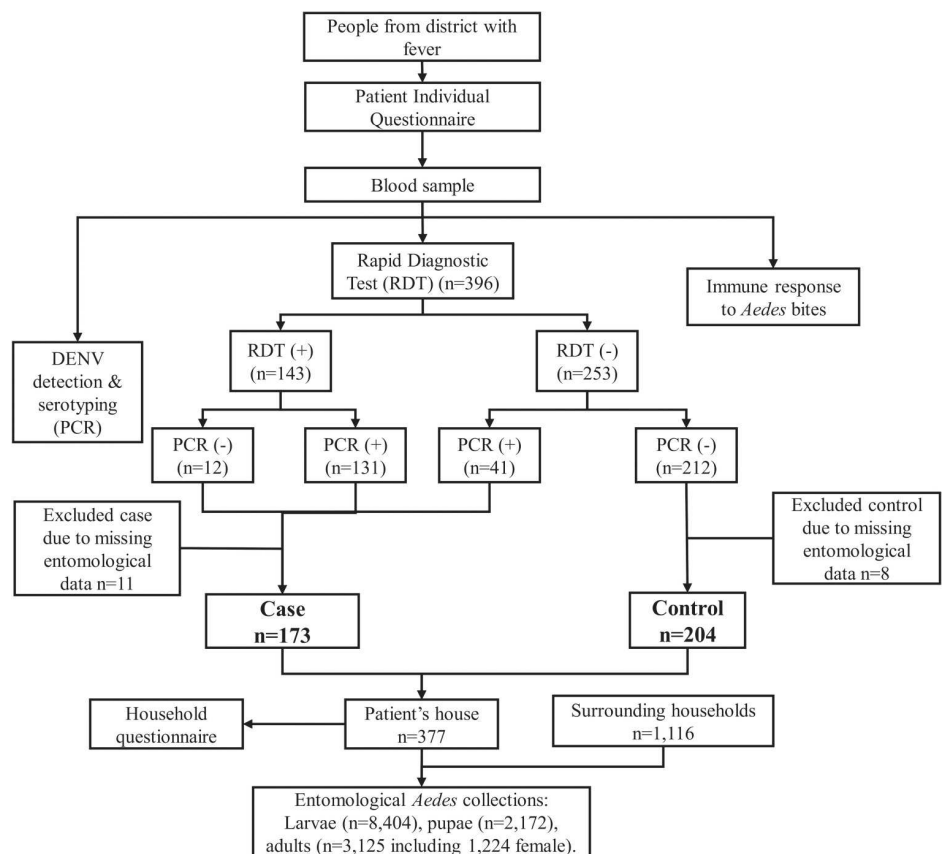


Fig 2. Flow diagram of case-control study design.

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for NS1 and/or IgM by RDT and/or positive for DENV by PCR was recorded a dengue case. A participant who was negative for both RDT and PCR or IgG-positive only was recorded a control (Fig 2). Hence the controls were selected on the basis of having an “imitation” disease with similar symptoms (e.g., fever) to dengue [45], a design method also known as ‘test-negative’ [46].

Individual characteristics

A questionnaire was used to collect information about each individual study case (positive and control). Patients were stratified into four age groups: 5–9 years-old; 10–14 years-old; 15–25 years-old; and > 25 years-old. History of previous dengue infections and vaccinations were recorded. Patients were asked about their main activities during weekdays and weekends (e.g., at home; at work away from home; at school; farming; other), as well as their typical resting/sleeping locations and habits (e.g., primarily indoor, outdoor, or equally indoor and outdoor). Travel history outside the resident district within the last three months was recorded and used as a binary variable.

Household characteristics

A questionnaire was used to collect data on house characteristics and socio-economic status, including monthly household income, possession of certain assets (e.g., TV, air conditioner, car, or motorbike), and source of drinking and non-drinking water. Observations on the house included the number of rooms, wall and ceiling construction material, and presence or absence of eaves gaps. Housing was differentiated between those having a family living on one or two floors; other types of living conditions, such as apartments, townhouses, or multiple families living in separate houses grouped together. Mosquito control methods used in the household were divided as follows: (1) larval control, (2) adult mosquito control, (3) both the preceding, and (4) no control. The Premise Index was estimated based on the general condition of the house, the surrounding yard area and degree of shade [47].

Entomological collections

Mosquito collections were systematically conducted in each patient house and in each of four surrounding houses. The total number of containers and those containing water were recorded at each household. A maximum of 20 third or fourth stage larval instars and all pupae were collected per container. Immature *Aedes* were identified to species using morphological keys [48, 49] and sex was determined for adults. Adult mosquitoes were collected using a battery-powered mechanical aspirator for 15 min indoors and 15 min outdoors in close proximity to house. Adults were identified to species and stored individually in 1.5mL micro-centrifuge tubes at -20°C until further analysis.

DENV detection in *Aedes* mosquito samples

Female *Aedes* were separated and labelled by location (indoors/outdoors; patient house/ surrounding house). Up to 15 adult female mosquito abdomens were pooled for RNA extraction and DENV detection. Retained head-thorax sections corresponding to positive pools were individually screened for DENV and serotyping by qRT-PCR using the protocol of Lanciotti et al. [50] with minor modifications to perform it in real-time.

Mosquito Exposure Index (MEI)

Aedes-specific immune response was evaluated in each case and control patient from dry blood spots by an indirect Enzyme-Linked Immunosorbent Assay (ELISA) using the Nterm-

34kDa salivary peptide (Genepep, St Jean de Vedas, France), an established marker of human exposure to *Aedes* salivary gland proteins [38, 51, 52]. Blood samples collected on filter paper were cut by a one cm diameter hole punch. Blood spots were eluted in 400 μ L Phosphate Buffer Saline (PBS)-0.1% Tween for 24h hours at 4°C before removing the filter paper. Eluates were stored at -20°C until further processing. Preliminary assays were conducted to adapt the protocol to the human population living in the study areas using individuals exposed and unexposed to *Aedes* mosquitoes (see below). Briefly, the salivary peptide was coated at 20 μ g/mL for 150 min at 37°C into Maxisorp plates (Nunc, Roskilde, Denmark). After washing with a solution of demineralized water plus 0.1% of Tween detergent, the protein-free blocking buffer (Pierce, Thermo Fisher, USA) was incubated for 1h at room temperature. Blood eluates diluted at 1:160 in PBS+1% Tween were incubated overnight at 4°C. Biotin-conjugated goat anti-human IgG (Invitrogen, Thermo Scientific, USA) was incubated at 1:6000 dilution for 1h30 at 37°C. Streptavidin HRP-conjugate was incubated for 1h at 37°C at 1:4000 dilution. Colorimetric reaction was performed using ABTS buffer (2,2'-azino-bis (3-ethylbenzthiazoline 6-sulfonic acid) di-ammonium) + 0.003% H₂O₂, and absorbance (optical density, OD) measured after 120 min at 405nm with Sunrise spectrophotometer (Tecan, Switzerland). Samples were assayed in duplicate and in a blank well (no antigen) to measure individual background and antibody response (Δ OD) expressed as:

$$\Delta OD = \text{mean } OD_{Ag+} - OD_{Ag-} \quad (1)$$

To quantify the non-specific immune reactions and calculate the immune threshold, anti-Nterm-34kDa IgG response was assayed from dried blood in individuals with no known exposure history to *Ae. aegypti* (i.e., blood samples from northern France collected between January and March 2016 to 2018, and Western Australia in October 2016). Specific immune threshold (TR) was defined as follows:

$$TR = \Delta OD_{\text{unexposed individuals}} + 3 SD_{\text{unexposed individuals}} \quad (2)$$

This value was calculated as 0.45. The MEI is the sample-specific immune response to the salivary peptide defined as:

$$MEI = \Delta OD - TR. \quad (3)$$

MEI was categorized into three classes: low, medium, and high responder. Samples with an Δ OD below the 0.45 TR, and therefore with a negative MEI value, were categorized as non-responders.

Entomological indices

Entomological indices in patients' houses were distinguished from those at the neighborhood level (i.e. patient's house + four surrounding houses, S1 Table). At the patient house level, the Container Index (CI) was calculated as the proportion of containers positive for immature *Aedes* among wet containers inspected. The Pupae per House Index (PHI) and the Pupae per Person Index (PPI) were calculated as the total number of pupae collected per house and the total number of pupae per person living in the patient's house, respectively. The female adult *Aedes* Index (AI) and the female *Aedes* indoor Index (AI_{in}) represent the number of female adult *Aedes* collected both indoors and outdoors and those collected only indoors, respectively. The female *Aedes* infected Index (AI₊) represent the proportion of all female sampled mosquitoes infected with DENV. At the neighborhood level, the House Index (HI) was calculated as the proportion of houses with immature *Aedes* and the Breteau Index (BI) as the number of *Aedes*-positive containers per 100 houses. The neighborhood Container Index (CI_n), Pupae per House Index (PHI_n),

female *Aedes* Index (AI_n), female Adult indoor Index (AI_{n_in}), and female *Aedes* infected Index (AI_{n+}) were calculated the same as described above, but at the neighborhood level.

Data analysis

Data analysis used R 3.5.1 software with the MASS, glm, and Rcmdr packages [53, 54]. Figures were designed using ggplot2 and ggpubr packages [55]. Map of study sites was built using QGIS 3.10 software and shapefiles were obtained from the Humanitarian Data Exchange project CC-BY 4.0 [41]. Distribution of indices was visualized by kernel density estimate. Vector control measures, household observations and Premise Index are categorical variables. The study population was analyzed with descriptive statistics, and individuals' information and household characteristics were analyzed with the dengue case occurrence as categorical variables using univariable logistic regression. The socio-economic status (SES) of each patient was calculated as a score based on the household questionnaire (e.g., assets, income) using principal component analysis [56]. A total of 16 items of durable household assets were used as proxies to estimate wealth status (S2 Table). The first principal component explained 17% of the variance. Based on this analysis, patients were categorized by tertiles of the first principal component in 'wealth' groups (high, intermediate, and low).

Univariable binomial logistic regression was performed between each entomological and immunological index and dengue case/control status. Multivariable logistic regression was performed using all variables (i.e. individual characteristics, house characteristics, SES, entomological and immunological indices) with a statistically significant association ($p < 0.1$) with case/control status on the univariable analysis. Only individuals with complete data for the variables of interest were kept for the multivariable analysis. Because of the overdispersion of the distributions of the entomological indices, they were transformed from continuous to categorical data of two groups: the null group (index value = 0) and the positive values (index value > 0). Model selection was based on backward/forward Akaike Information Criterion (AIC) selection. All variables were first included in the model and the selection was made by removing variables and/or then adding them (backward/forward selection). At each step, the AIC was calculated and the selected model was the one with the lowest AIC. Wald confidence intervals (95% CI) were calculated. Potential confounding variables of most interest were those which were plausibly associated with both entomological indices and risk of dengue, in particular socio-economic status and travel history.

Ethical statement

This study was approved from the Khon Kaen University Ethics Committee (KKUEC, project number HE591099), the London School of Hygiene and Tropical Medicine Ethical Committee (LSHTM Ethics, project number 10534), and the Norwegian Regional Committees for Medical and Health Research Ethics (REC, no. 2016/357). Each patient was fully informed about the study and, if agreeing to participate, provided signed informed consent. Patients 13–17 years old signed assent forms and their parents/guardians signed informed consent. Parents/guardians of patients 5–12 years old signed consent forms on the patient's behalf. For participating neighboring households, information about the study was given and signed consent for entomological collections was obtained before beginning sampling.

Results

Dengue cases, individual and household characteristics of the population

All 396 patients informed about the study agreed to participate and were recruited. Some were excluded from the analysis because of missing entomological and household data, mostly

because of limited capacity to follow-up multiple patients presenting at a facility on the same day (Fig 2). A total of 377 patients with complete entomological data were included in the final analysis, comprising 173 dengue cases and 204 controls (0.85 case/control ratio). The participant ages ranged from 5 to 76 years with 190 (48%) females represented (Table 1). Almost half of the dengue cases were between 10 and 14 years of age resulting in 4.28-fold higher odds for dengue infection than people aged greater than 25 years old ($p < 0.001$). Similarly, individuals aged between 15 and 25 years of age had 3.23-fold higher odds for dengue than individuals above 25 years ($p < 0.001$). The majority (60.4%) of the dengue case patients reported having lived in the respective district for more than ten years compared to 46% of the controls, yet there was no difference between the length of stay in the area and dengue risk ($p = 0.200$, $p = 0.356$ and $p = 0.975$ for a stay between 1 and 5 years, between 5 and 10 years and more than 10 years, respectively). Most of the study participants spent their time either at school or at home during the weekdays resulting in a lower odds of dengue for individuals working away from home or those at school compared to the people staying at home (OR: 0.48, 95% CI: 0.24–0.94, $p = 0.033$ and OR: 0.60, 95% CI: 0.37–0.97, $p = 0.035$, respectively). Working partly indoors and outdoors was associated with lower odds of dengue ($p = 0.045$) compared to working outdoors only. Although not statistically significant, there was a tendency for those working only indoors to have higher odds for dengue ($p = 0.085$). Travel outside the district in the previous three months was associated with lower odds of infection ($p = 0.031$).

Although there was no strong evidence of dengue transmission risk associated with SES, certain physical house characteristics were relevant. Living in a single family, two-floor house had increased odds compared to living in a single-floor house, while the presence of eaves gaps had lower odds than house lacking them (Table 1). The majority of households (80–90%) used some kind of vector control method(s), but these were not significantly associated with dengue risk ($p > 0.06$). In particular, adult mosquito control was more often used in case houses and was indicative of a higher odds of dengue (OR: 2.41, 95% CI: 0.95–6.18, $p = 0.065$), while a combination of larval and adult controls was more common in control houses, which showed a lower odds than houses using no vector control (OR: 0.52, 95% CI: 0.26–1.05, $p = 0.068$). Furthermore, insecticide applications to indoor wall surfaces (performed by vector control unit staff or private companies for dengue or pest control) was more common among controls than in the case group resulting in a lower odds of dengue in houses with sprayed walls in the last 12 months (OR: 0.54, 95% CI: 0.35–0.87, $p = 0.010$).

Mosquito exposure index

Only 10% ($n = 37$ of 368) of the tested individuals (cases and controls) were non-responders to the *Aedes* Nterm-34kDa salivary biomarker as their specific immune response was below the immune threshold TR (Fig 3). There was not significant difference in antibody response to *Aedes* salivary biomarker between case and control. Although not significant, being a medium or high responder to mosquito salivary antigens, surprisingly, tended to be negatively associated with dengue risk relative to non-responders (OR = 0.51, 95% CI: 0.24–1.10, $p = 0.08$, and OR = 0.50, 95% CI: 0.23–1.07, $p = 0.07$) (Table 2).

Entomological collections and indices

Entomological collections were carried in 1,487 households, of which 377 were patients houses and 1,110 surrounding houses (mean 3.94 houses per individual recruited). From 5,185 wet containers inspected, 1,230 (23.7%) were positive for immature *Aedes* stages, accounting for a total of 8,404 larval instars and 2,172 pupae. A total of 3,125 adult male and female *Aedes* were collected, the vast majority being *Ae. aegypti* (99.0%) and only 32 *Ae. albopictus* collected.

Table 1. Individual and household characteristics and their associations with dengue fever cases in northeastern Thailand, June 2016 and July 2019. Odds ratios (OR), obtained by logistic univariable regression, in bold text are significant ($p < 0.05$). Missing data by individual not included in the analysis.

		Case (n = 173)		Control (n = 204)		Total (n = 377)		OR (95% CI)	p-value
		n	(%)	n	(%)	n	(%)		
Province	Roi Et	45	(26.0)	47	(23.4)	92	(24.4)	Reference	0.835
	Khon Kaen	40	(23.1)	86	(42.2)	126	(33.4)	0.49 (0.27–0.84)	0.011
	Maha Sarakham	54	(31.2)	49	(24.0)	103	(27.3)	1.15 (0.65–2.02)	0.624
	Kalasin	34	(19.7)	22	(10.8)	56	(14.9)	1.61 (0.82–3.16)	0.164
Gender	Male	95	(54.9)	101	(49.5)	196	(52.0)	Reference	0.668
	Female	78	(45.1)	103	(50.5)	181	(48.0)	0.79 (0.53–1.19)	0.274
Age groups	More than 25 years old	21	(12.1)	55	(27.0)	76	(20.2)	Reference	<0.001
	15 to 25 years old	42	(24.3)	33	(16.2)	75	(19.9)	3.23 (1.64–6.36)	<0.001
	10 to 14 years old	85	(49.1)	52	(25.5)	137	(36.3)	4.28 (2.33–7.88)	<0.001
	5 to 9 years old	25	(14.5)	64	(31.4)	89	(23.6)	1.02 (0.51–2.02)	0.948
Lived in district	Less than 1 year	7	(4.05)	6	(2.94)	13	(3.45)	Reference	0.782
	Between 1 and 5 years	16	(9.25)	31	(15.2)	47	(12.5)	0.44 (0.12–1.53)	0.200
	Between 5 and 10 years	44	(25.4)	65	(31.9)	109	(28.9)	0.58 (0.18–1.84)	0.356
	More than 10 years	102	(60.0)	88	(43.1)	190	(50.4)	0.98 (0.32–3.03)	0.975
	(Missing)	4	(2.31)	14	(6.86)	18	(4.77)	-	-
Dengue diagnosed before	No	138	(79.8)	138	(67.7)	276	(73.2)	Reference	1
	Yes, this year	11	(6.36)	14	(6.86)	25	(6.63)	0.78 (0.34–1.79)	0.566
	Yes, last year	1	(0.58)	7	(3.43)	8	(2.12)	0.14 (0.01–1.18)	0.071
	Yes, before last year	19	(11.0)	32	(15.7)	51	(13.5)	0.59 (0.32–1.10)	0.097
	(Missing)	4	(2.31)	13	(6.37)	17	(4.51)	-	-
Spend week days	At home	60	(34.7)	44	(21.6)	104	(27.6)	Reference	0.118
	At work away from home	21	(12.1)	32	(15.7)	53	(14.1)	0.48 (0.24–0.94)	0.033
	At school/college/university	87	(50.3)	106	(52.0)	193	(51.2)	0.60 (0.37–0.97)	0.035
	At farm	0	(0.00)	2	(0.98)	2	(0.53)	-	0.981
	Other	1	(0.58)	3	(1.47)	4	(1.06)	0.24 (0.01–1.98)	0.229
	(Missing)	4	(2.31)	17	(8.33)	21	(5.57)	-	-
Spend week ends	At home	148	(85.6)	148	(72.6)	296	(78.5)	0.99 (0.79–1.25)	0.954
	At work away from home	14	(8.09)	23	(11.3)	37	(9.81)	0.61 (0.30–1.24)	0.172
	At school/college/university	3	(1.73)	6	(2.94)	9	(2.39)	0.50 (0.12–2.05)	0.338
	At farm	1	(0.58)	3	(1.47)	4	(1.06)	0.33 (0.03–3.26)	0.347
	Other	3	(1.73)	4	(1.96)	7	(1.86)	0.76 (0.17–3.43)	0.716
	(Missing)	4	(2.31)	20	(9.80)	24	(6.37)	-	-
Location of workplace	Outdoors	54	(31.2)	59	(28.9)	113	(30.0)	Reference	0.638
	Indoors	76	(43.9)	53	(26.0)	129	(34.2)	1.56 (0.94–2.60)	0.084
	Both indoors and outdoors	38	(22.0)	72	(35.3)	110	(29.2)	0.57 (0.34–0.99)	0.045
	(Missing)	5	(2.89)	20	(9.80)	25	(6.63)	-	-
Travel within the previous 3 months	No	156	(90.2)	162	(79.4)	318	(84.4)	Reference	0.695
	Yes	13	(7.51)	29	(14.2)	42	(11.1)	0.46 (0.23–0.93)	0.031
	(Missing)	4	(2.31)	13	(6.37)	17	(4.51)	-	-
Socio-economic status	High	54	(31.2)	64	(31.4)	118	(31.3)	Reference	0.358
	Intermediate	50	(28.9)	69	(33.8)	119	(31.6)	0.61 (0.36–1.02)	0.060
	Low	64	(37.0)	54	(26.5)	118	(31.3)	0.71 (0.43–1.19)	0.194
	(Missing)	5	(2.89)	17	(8.33)	23	(5.84)	-	-

(Continued)

Table 1. (Continued)

		Case (n = 173)		Control (n = 204)		Total (n = 377)		OR (95% CI)	p-value
		n	(%)	n	(%)	n	(%)		
Household type	One family, one floor	47	(27.2)	79	(38.7)	126	(33.4)	Reference	0.005
	One family, two floors	111	(64.2)	97	(47.6)	208	(55.2)	1.92 (1.22–3.02)	0.005
	Others	10	(5.78)	11	(5.39)	21	(5.57)	1.52 (0.60–3.87)	0.371
	(Missing)	5	(2.89)	17	(8.33)	22	(5.84)	-	-
Wall spray	No	127	(73.4)	117	(57.4)	244	(64.7)	Reference	0.565
	Yes	41	(23.7)	70	(34.3)	111	(29.4)	0.54 (0.35–0.87)	0.010
	(Missing)	5	(2.89)	17	(8.33)	22	(5.84)	-	-
Eaves gaps	No	112	(64.7)	97	(47.6)	209	(55.4)	Reference	0.334
	Yes	56	(32.4)	90	(44.1)	146	(38.7)	0.55 (0.36–0.84)	0.006
	(Missing)	5	(2.89)	17	(8.33)	22	(5.84)	-	-
Vector control	No	20	(11.6)	18	(8.82)	38	(10.1)	Reference	0.873
	Yes, against larvae	51	(29.5)	34	(16.7)	85	(22.6)	1.45 (0.56–1.97)	0.337
	Yes, against adult mosquito	28	(16.2)	11	(5.39)	39	(10.3)	2.41 (0.95–6.18)	0.065
	Yes, against both adult and larvae	69	(39.9)	124	(60.8)	193	(51.2)	0.52 (0.26–1.05)	0.068
	(Missing)	5	(2.89)	17	(8.33)	22	(5.84)	-	-

<https://doi.org/10.1371/journal.pntd.0008703.t001>

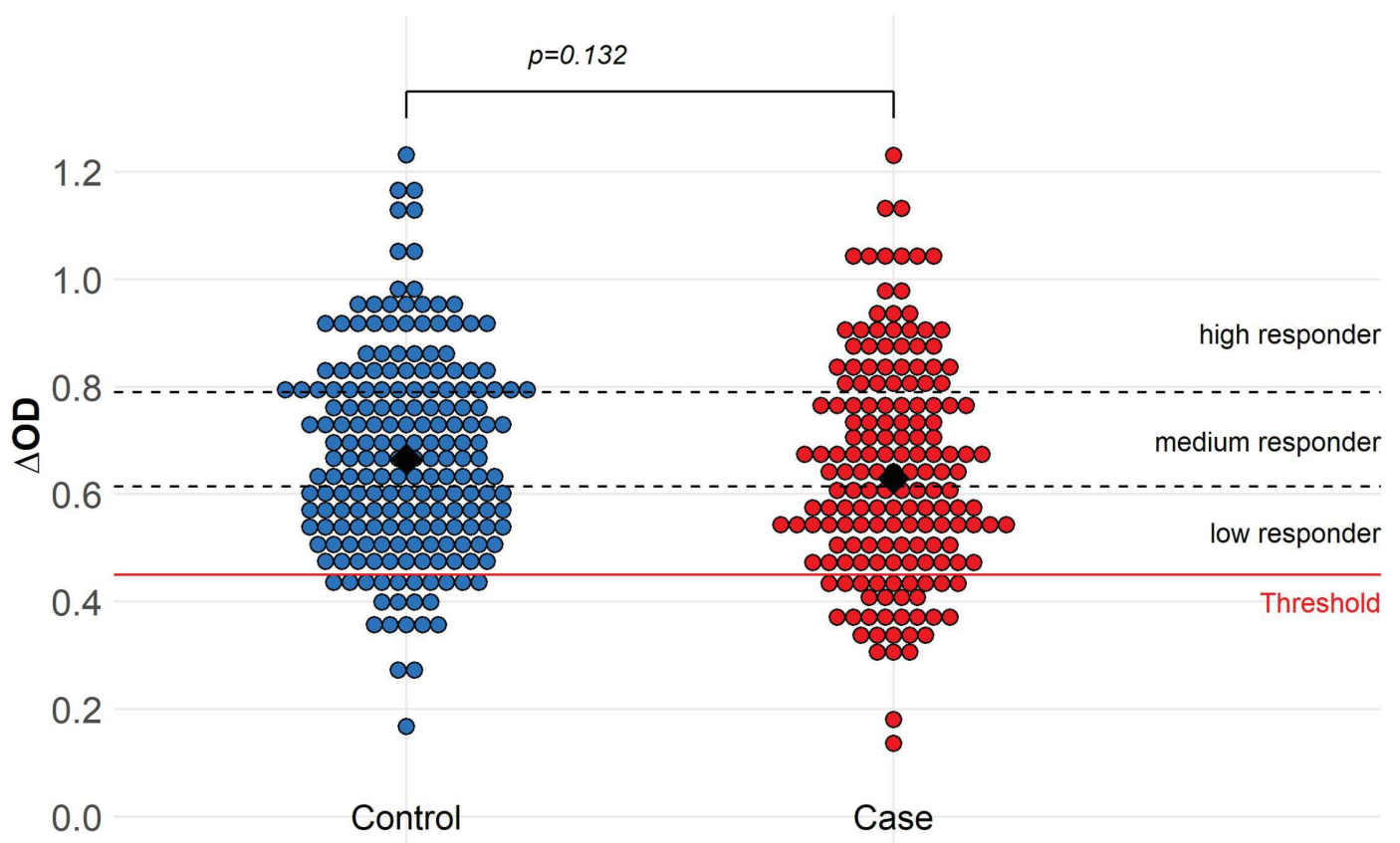


Fig 3. Immune response to *Aedes* saliva (Δ OD) in dengue case and control patients. The black diamonds represent the response medians. The dashed lines represent the limits of each group of intensity of response. The red line at 0.45 indicates the specific immune threshold TR defined from individuals not exposed to *Ae aegypti*.

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Table 2. Immunological and entomological indices and their associations with dengue fever cases in northeastern Thailand, June 2016 and June 2019. Odds ratios (OR) obtained by logistic univariable regression, and confidence intervals (95% CI) by Wald’s statistics. Odds ratios in bold are significant (p<0.05).

		Case% (n = 173)	Control% (n = 204)	OR	95% CI	p-values
Individual level						
MEI	Non-responder	12.7	7.35	Reference		
	Low responder	31.2	28.4	0.63	[0.30–1.35]	0.237
	Medium responder	26.6	29.9	0.51	[0.24–1.10]	0.086
	High responder	27.2	31.9	0.50	[0.23–1.07]	0.073
	(Not determined)	2.31	2.45	-	-	-
House level						
CI (%) (mean)		29.1	37.3	1.00	[0.99–1.00]	0.044
Aedes Index (AI)	0	61.3	48.5	Reference		
	>0	38.7	51.5	0.59	[0.39–0.89]	0.012
Aedes Index indoor (AI_in)	0	67.6	52.9	Reference		
	>0	32.4	47.1	0.53	[0.35–0.81]	0.003
Aedes Index infected (AI+)	0	91.9	96.6	Reference		
	>0	8.09	3.43	2.48	[0.97–6.28]	0.056
Pupae per House Index (PHI)	0	69.9	66.2	Reference		
	>0	30.1	33.8	0.83	[0.54–1.28]	0.397
Pupae per Person Index (PPI)	0	72.3	71.6	Reference		
	>0	27.7	28.4	0.95	[0.61–1.49]	0.824
Neighborhood level						
BI (mean)		68.6	93.4	0.99	[0.99–1.00]	<0.001
HI (%) (mean)		47.9	58.3	0.99	[0.98–1.00]	0.002
CI _n (%) (mean)		29.2	41.7	0.99	[0.98–1.00]	<0.001
Aedes Index (AI _n)	0	24.9	22.6	Reference		
	>0	75.1	77.4	0.87	[0.54–1.41]	0.581
Aedes Index indoor (AI _{n_in})	0	29.5	26.5	Reference		
	>0	70.5	73.5	0.86	[0.54–1.34]	0.498
Aedes Index _n infected (AI _{n+})	0	83.6	90.2	Reference		
	>0	16.2	9.8	1.77	[0.96–3.28]	0.067
Pupae per House Index (PHI _n)	0	41.6	38.7	Reference		
	>0	58.4	61.3	0.83	[0.54–1.28]	0.397

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Among the 1,224 females *Aedes* (39.2% of the total *Aedes* collected), 953 (77.8%) were collected indoors. Apart from the DENV-infected *Aedes* indices (AI+ and AI_{n+}), all entomological indices had higher values in control houses than in case houses (Table 2), regardless of including the patient house with or without the neighboring houses. The *Aedes* Index, AI, (which includes both indoor and outdoor adult collections) was positive (i.e., at least one *Aedes* collected) in 38.7% of the case houses and in 51.5% of the control houses (Fig 4A). Moreover, the presence of *Aedes* was associated with lower odds of dengue (OR: 0.59, 95% CI: 0.39–0.89, p = 0.012). The *Aedes* Index indoor, AI_{in} was positive in 38.4% and 47.1% of the case and control houses, respectively. Similar to the AI, a positive AI_{in} was also associated with lower odds of dengue (OR: 0.53, 95% CI: 0.35–0.81, p = 0.003). Only the female *Aedes* infected, AI+ appears to be associated with increased dengue odds (OR: 2.48, 95% CI: 0.97–6.28, p = 0.056). The pupal indices, PPI and PHI, were not significantly different between case and control houses. Accounting only for the patient’s house (excluding neighbors), the Container

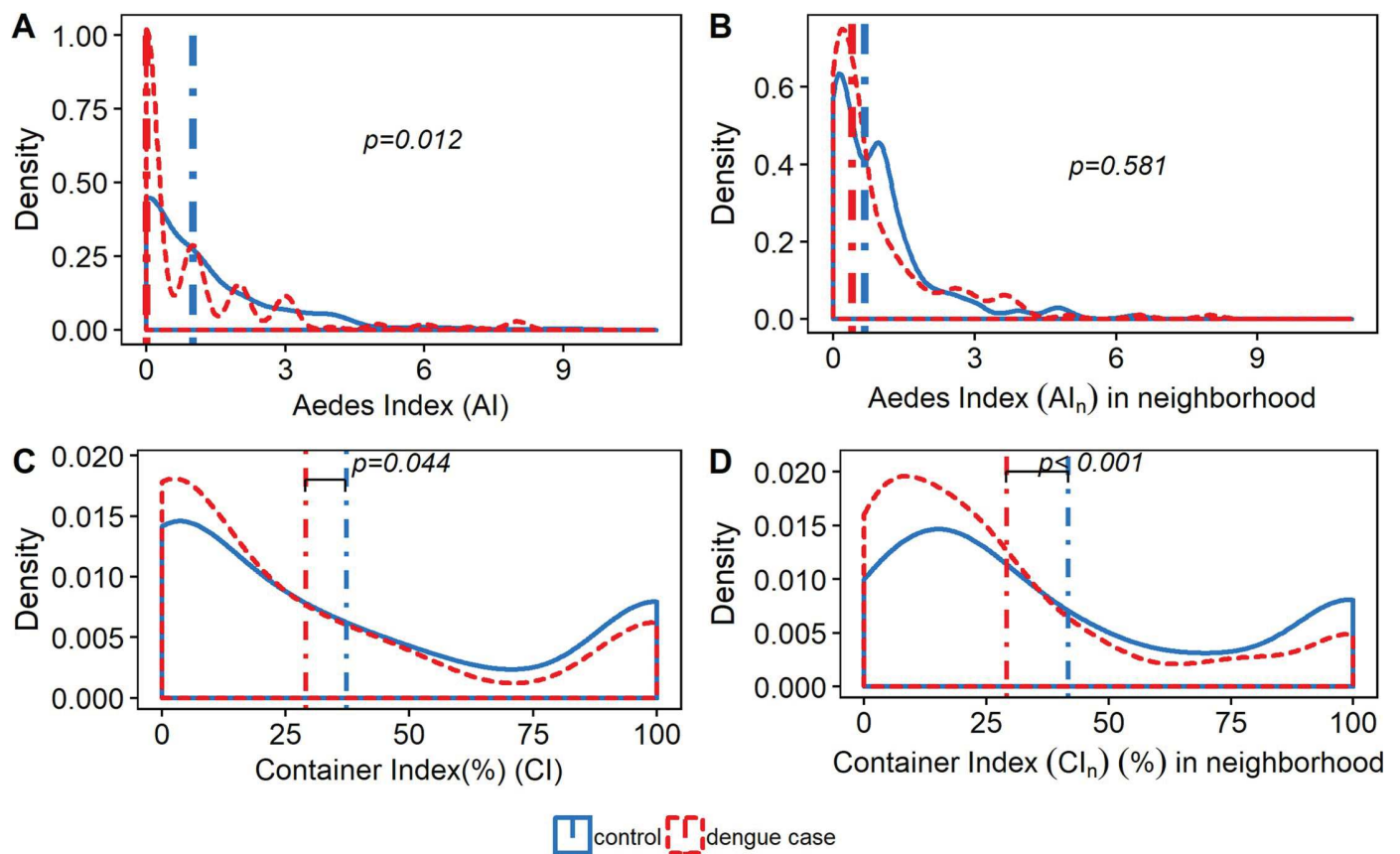


Fig 4. Distribution of adult and immature *Aedes* indices in dengue case (red line) and control (blue line) houses. Probability density distribution plots of *Aedes* Index (AI) at the patient house (A) and at the neighborhood level (including patient house) (B); and of the Container Index (CI) at the patient house (C) and at the neighborhood level (including patient house) (D). The blue and red vertical lines in A and B represent the median *Aedes* indices in control and dengue case house, respectively. The blue and red vertical lines in C and D represent the mean container indices in control and dengue case houses, respectively. P-values were calculated using univariable logistic regression.

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Index was associated with the case/control status of houses, with a higher CI in the control than in the case houses ($p = 0.044$) (Fig 4C).

Only the *Aedes* infected index, AI_{n+} of mosquitoes collected in neighborhoods appears to be associated with higher odds of having a dengue case in the patient house, although the association was not statistically significant (OR: 1.77, 95%CI: 0.96–3.28, $p = 0.067$). Larval indices, CI_n, BI and HI were negatively associated with dengue infections ($p < 0.001$, $p < 0.001$ and $p = 0.002$ respectively, Fig 4D). Likewise, the neighborhood adult *Aedes* indices (AI_n and AI_{n_in}) were higher in control households (Fig 4B). The presence of *Aedes* female (AI_n), the presence of female *Aedes* indoors (AI_{n_in}), or the presence of *Aedes* pupae (PHI_n) in the neighborhood were not significantly associated with dengue infection risk.

Multivariable analysis of dengue fever occurrence

Using multivariable analysis, only a few entomological indices at the house level, compared to individual and household characteristics, were associated with dengue risk (Table 3). Individuals aged between 10 and 14 years and between 15 and 25 years had a higher odds of dengue infection than older adults (OR: 4.45, 95% CI: 2.14–9.24, $p < 0.001$; OR: 2.88, 95% CI: 1.27–6.55, $p = 0.012$ respectively). Interestingly, younger children appeared to have similar odds as

Table 3. Multivariable analysis of risk factors associated with dengue. Odds ratios (OR) were calculated by multivariable logistic regression and confidence intervals calculated using Wald's statistics. Odds ratio in bold text were significant at $p < 0.05$.

		OR	95% CI	p-value
Age groups	> 25 years old	Reference		
	15 to 25 years old	2.88	[1.27–6.55]	0.012
	10 to 14 years old	4.45	[2.14–9.24]	<0.001
	5 to 9 years old	1.05	[0.36–2.37]	0.899
Location of workplace	Outdoors	Reference		
	Indoors	1.78	[0.94–3.36]	0.077
	Both indoors and outdoors	0.70	[0.36–1.35]	0.281
Travel within 3 months	No	Reference		
	Yes	0.48	[0.20–1.15]	0.101
Type of house	One floor, one family	Reference		
	Two floors, one family	2.11	[1.21–3.69]	0.009
	Other	2.07	[0.61–6.99]	0.242
Eaves gaps	No	Reference		
	Yes	0.40	[0.23–0.68]	0.001
Mosquito control	None	Reference		
	Yes, against larvae	1.13	[0.44–2.89]	0.800
	Yes, against adult	3.73	[1.19–11.7]	0.024
	Yes, against both larvae and adult	0.63	[0.27–1.44]	0.272
<i>Aedes</i> Index indoor (AI _{in})	0	Reference		
	>0	0.50	[0.28–0.87]	0.014
<i>Aedes</i> Index infected (AI ₊)	0	Reference		
	>0	4.20	[1.29–13.8]	0.018
Neighborhood level				
Container _n Index CI _n (per 10% increase)		0.93	[0.86–1.01]	0.089

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older adults, although with a wide confidence interval (OR: 1.05, 95% CI: 0.51–2.67, $p = 0.707$). Having an indoor workplace tended to higher odds than working outdoors (OR: 1.78, 95% CI: 0.94–3.36, $p = 0.077$). The type of house was also associated with dengue risk: living in a two-floor house had higher odds of dengue relative to a single floor dwelling (OR: 2.11, 95% CI: 1.21–3.69, $p = 0.009$). The presence of eaves gaps in the house was associated with lower odds of dengue (OR: 0.40, 95% CI: 0.23–0.68, $p < 0.001$). The application of adult vector control methods was associated with higher odds of dengue (OR: 3.73, 95% CI: 1.19–11.7, $p = 0.024$). The presence of adult female *Aedes* inside the patient's house was associated with lower odds of dengue (OR: 0.50, 95% CI: 0.19–0.73, $p = 0.003$). On the other hand, the presence of DENV-infected *Aedes* was associated with 4.20-fold higher odds of dengue infection compared to no infected mosquitoes present (OR: 4.20, 95% CI: 1.29–13.8, $p = 0.018$). In addition, the Container Index at the neighborhood level seemed associated with lower odds of dengue with OR of 0.93 per 10% increase (95% CI: 0.86–1.01, $p = 0.089$).

Discussion

In this hospital-based case-control study, we found that patient age, two-floor houses, application of adult vector control and the presence of DENV-infected *Aedes* were associated with higher odds of dengue. Interestingly, the presence of eave gaps in the house and the presence of female *Aedes* indoors were associated with lower odds of dengue. While dengue typically has had a greater impact on younger children, we found that individuals aged between 10 and

25 years-old were at higher risk relative to those either younger and older. This trend was also observed in several recent studies conducted in Thailand, Malaysia, and the Philippines [5, 43, 57, 58]. The increase in average age of infection may result from a change in demographic structure such as a decrease in birth rates or death rates [59, 60], leading to a lower proportion of naïve individuals or possibly a greater longevity of immune individuals in the population.

In northeastern Thailand, indoor workplaces are not always well protected against dengue mosquitoes, (e.g., shops lacking hard-wall storefronts, breeding container habitats within the building). *Aedes aegypti*, the main DENV vector in Thailand, is well adapted to human dwellings and their immediate surroundings. This day-biting mosquito typically feeds on multiple human hosts during each gonotrophic cycle, and usually rests indoors protected from more extreme outdoor elements [9]. This might explain the higher risk of dengue for individuals working indoors suggested in the current study. In contrast to other studies [61, 62], our results suggested that traveling outside the resident district during the previous three months was negatively associated with dengue risk (Table 1). Studies in Thailand have shown that dengue incidence is commonly spatially clustered [63, 64] and infection risk can be highly focal; thus moving out of the study areas might have exposed travelers to differential risks (higher or lower) of dengue transmission. Additional information to clarify areas traveled to, duration of trips, purpose, and the characterization of who is travelling might help resolve the negative association between dengue risk and travel seen in our study. Other individual characteristics were not informative for dengue risk using the multivariable model.

Our entomological findings showed that only the infected *Aedes* index at the household level (AI+) was positively associated with dengue infection, with more DENV positive females *Aedes* collected in case houses than in controls. A similar observation was found at the neighborhood level however not significant. In total, about 13% of the sampled neighboring households (including neighborhood and patient house) had DENV-positive female *Aedes*: 16% of the case neighboring households and about 10% of the control neighboring households. When focusing on the patient's houses specifically, approximately 3% of the control houses and 8% of the case houses had DENV-infected *Aedes*. The high proportion of DENV-infected *Aedes* demonstrates hyperendemicity conditions of dengue in northeastern Thailand [43]. In this study, determining the actual location of dengue case transmission is not possible. There is the possibility that the high proportion of DENV infected *Aedes* in case households was a result of DENV transmission from infected humans to the vectors present in the vicinity (i.e., not mosquito to human). For this study, vector infestation was measured only at the household level, thus recognizing that transmission could have happened elsewhere such as at schools or workplaces [65]. In Thailand, Ratanawong et al. [65] demonstrated the clustering of dengue cases among schools and among classrooms within schools, highlighting the importance of dengue transmission outside the home.

On the other hand, adult *Aedes* abundance in the household was negatively correlated with dengue with more *Aedes* found in control households than in houses with a recent dengue case. This counterintuitive association could be explained by potentially higher attention to mosquito control following onset of dengue symptoms in the case household, which would reduce vector infestation. Our results support this assumption as the associations between the *Aedes* Index indoor (AI_in) (Table 2), the mosquito control activities (Table 1) and the dengue risk were strengthened when adjusted for other variables (Table 3).

At the individual level, controls were more likely to have a high human immune response to *Aedes* salivary proteins than dengue cases, which correlate well with the higher abundance of *Aedes* adults in controls houses compared to case houses. This suggests that low responders actually received fewer *Aedes* bites than high responders, an observation previously shown in Benin [52]. Nevertheless, neither the adult abundance in the household nor the level of human

exposure to *Aedes* mosquito bites were correlated with higher transmission risk. This can be explained by the fact that dengue virus transmission is complex and varies through time and space, and the relationship between vector density/aggressiveness and risk of human infection is not static. In addition, antibody response to *Aedes* saliva was positively correlated with IgG dengue immunity (S3 Table). Altogether, our data suggested that individuals with high exposure to *Aedes* have less odds of being dengue positive than individuals with lower exposure. However, the association of dengue IgG and antibodies to *Aedes* saliva with recent dengue infection was not strong enough to remain in the final multivariable model. The results of this study should be viewed with caution as the immune response reflects the overall exposure to *Aedes* bites in the previous weeks and not necessarily at the time of transmission. Additional longitudinal studies, including all inhabitants from each house, irrespective of dengue infection status, might better assess the association between exposure to *Aedes* bites and risk of dengue.

As in other dengue endemic countries, vector surveillance in Thailand focuses on immature stages, in particular, the standard larval indices (HI, BI, and CI). While a positive association between dengue cases and entomological indices was found in Cuba and Trinidad [21, 23] this has not been universally seen elsewhere [66, 67]. In our study, vector infestation indices based on immature stages (HI, BI, CI, and CI_n) were all negatively associated statistically with dengue fever using univariable analysis. In other words, control households had more containers with immature *Aedes* than case households. However, this association was not statistically significant in the multivariable analysis except for CI_n. Moreover, most (~90%) of the inspected houses had wet containers at the household and nearly half of the houses were positive for immature *Aedes*. Furthermore, most of households sampled in this study had index values above the minimum thresholds for dengue outbreak risk set by the Thai Ministry of Public Health [25]. During the study, the northeastern region of Thailand also experienced very low dengue incidence compared to the previous decade [40, 68]. This study was conducted over a three-year period, thus capturing intra- and inter-epidemic dengue transmission in this northeastern region of Thailand. Dengue transmission in Thailand is highly seasonal with the highest incidence occurring during the wet season (May–October) [5]. This may account for the high proportion of houses with water-storage containers found positive for immature *Aedes*.

Other studies have found a higher risk of dengue transmission in poorer settings [69–71]. However, in our study, no such association was found (S4 Table). Nevertheless, household construction may play a role in transmission risk, wherein people living in two-floor houses appear to have had a greater risk for contracting dengue. Interestingly, in our study settings two-floor households were more commonly found among farmers (S5 Table). In addition, in rural two-floor houses, the lower one is often used for gatherings of family or community members, friends or neighbors [72], which may increase the risk of dengue [73]. The negative association between eaves gaps in houses and dengue risk appear counterintuitive (i.e., increased access for mosquitoes to enter a house). In central and southern Thailand, Brusich et al. [74] showed in rural settings, households with <25% eaves gaps have, overall, more mosquitoes indoors than those with 50% to 75% eaves gaps. Moreover, they reported that vector control activities were absent in houses with <25% eaves gaps and that bed nets were more systematically used in houses with >50% eaves gaps. However, the results from their study should be interpreted cautiously as it is based on few houses [74]. Nevertheless, the authors suggested that the presence of eaves gaps might result in a higher abundance of mosquitoes, which in turn, might induce more vector control activities by the household to reduce biting. However, in our study, no correlation was found between the presence of eaves gaps in the households and vector control methods used (S6 Table). Moreover, an apparent ‘protective’ effect by presence of eaves gaps on dengue risk might be explained by the location of productive breeding

sites. Indeed, if the majority of container habitats are located indoors, eaves gaps can represent exit routes for the vectors [75].

We identified two previous case-control studies of dengue with similar designs, i.e. both cases and controls recruited in health facilities, with controls being “test-negative”: one in Singapore [76] and another in Malaysia [46]. The Malaysian study included two sets of controls: one test-negative and the other being hospitalized (inpatient) with no suspicion of dengue (“traditional” control). In their analysis, no risk factors were identified in the test-negative controls, although the number of them was small (28). The authors suggest that test-negative studies could be subject to bias resulting from misclassification of dengue status due to imperfect diagnostic tests. In Singapore, the controls which were either DENV-PCR negative or had no evidence of seroconversion on follow-up, analysis found no associations between dengue risk and house construction, travel, working outdoors or indoors, or self-reported history of mosquito bites [76]. In the current study, misclassification of dengue infection is unlikely to be a major problem because all controls were PCR-negative and all but 12 (being RDT NS1 antigen and/or IgM positive only) of the 184 cases were DENV-PCR positive (Fig 1). However, we cannot rule out that our controls were infected with other *Aedes*-borne viruses such as chikungunya or Zika, and thereby biasing our assessment of the entomological risk factors. Chikungunya fever incidence was extremely low during the 2016–2017 period, with a total of 18 and 10 cases, in 2017 and 2016 respectively but increased to around 3600 cases in 2018, although the epidemic was centered in southern Thailand [40, 77, 78]. In addition, CHIKV was detected among eight patients out of 161 tested in the period 2016–2017 in our study participants [79]. Regarding Zika infection, a recent study demonstrated the circulation of the virus, at low incidence, in Thailand for years [80]. Indeed, the Bureau of Epidemiology of Thailand reported a cumulative number of 1,612 Zika cases for the period 2016–2017, while more than 118,000 dengue cases were reported during the same period [77, 78, 81]. Although potential dengue cases have similar febrile symptoms as potential controls (with other conditions), any difference in health-seeking behavior between them may have also biased the results [82]. Thailand has a universal health coverage program that allows people access to equitable and effective healthcare in primary care centers located in each subdistrict [83, 84]. Therefore, by recruiting patients at the main district hospitals, we feasibly captured a high proportion of the febrile patients, including children, living in the area.

Our study presented some further limitations in terms of generalizability. During the study period, dengue incidence was lower than expected, despite the high percentage of DENV-infected *Aedes* found in our study, the 173 cases were obtained only after extending the original study period and coverage area. This may suggest a high proportion of immune individuals. In Thailand, all four serotypes are endemic, dengue vector species are widespread, and a high percentage of DENV infected vectors may lead to a high proportion of dengue-immune individuals in the population, lessening dengue incidence. The relationship between entomological risk factors and dengue may vary according to the extent of serotype-specific immunity in the population and this, in turn, may vary between high and low incidence years and the predominant virus serotype(s) in circulation. Indeed, during 2017–2018, the main DENV serotype circulating among dengue cases was DENV-1, with an increased prevalence compared with the previous six years, while the prevalence of DENV-4 was lower than previous years. In addition, DENV-3 was the prevalent serotype between 2013 and 2015 accounting for approximately 30% of the dengue cases [40, 85–87]. As a result, caution is advised with drawing associations of risk with entomological thresholds as they depend on the immune status of the human population under study [22, 24, 88].

Another limitation is that we focused on household entomological indices, yet the transmission could have occurred in other locations and at other times, especially for many children

who spend most of their daytime hours at school. Including workplaces, schools and community centers where people gather might be helpful for understanding dengue transmission risk outside the household setting [65]. In this study, information on these other locations is limited and indirect. Most dengue case-control studies focused on the epidemiological risk factors associated with higher severity of dengue disease, while fewer have investigated the role of entomological factors. Moreover, the majority of those studies used immature *Aedes* indices to assess the infestation level (density) in the study area [61, 62]. Nevertheless, a study in Sao Paulo, Brazil demonstrated a strong association between numbers of female *Aedes* collected over a fortnight and dengue incidence [89]. Their findings were obtained after the re-introduction of DENV serotype 3, to which the majority of the population were susceptible, thus facilitating the assessment of entomological risk factors.

The retrospective case-control design means the temporal sequence of events cannot be determined with accuracy. In particular, entomological and immunological data were collected following patient recruitment. Indeed, symptoms of dengue fever can appear as quickly as a few days after DENV transmission (typically incubation period between 4–7, up to 14 days), delaying the recruitment of patients and therefore the entomological collections. This temporal disconnection between acquiring an infection to time of presenting illness and testing (i.e., identification of a case) may greatly affect attempts to link transmission with actual epidemiological conditions many days prior. Although speculative, the occurrence of a dengue case might plausibly prompt householders to reduce adult vector density, while the remaining mosquitoes may retain a higher prevalence of infection when the case is detected. A longitudinal, prospective study design might better assess the impact of entomological indices on dengue transmission risk in northeastern Thailand.

Our case-control study in northeastern Thailand highlights the complex relationship between *Aedes* vectors, socio-economic factors, and dengue transmission risk. The presence of DENV-infected *Aedes* was associated with higher odds of dengue infection. Our findings support the rationale of monitoring DENV in adult *Aedes* vectors resting in and near houses to assess risk of dengue transmission [90–92] and to develop early warning indicators for dengue outbreak prevention [93]. Although adult surveillance holds promise as an additional, if not more informative, *Aedes*-borne disease risk indicator, further work is needed investigating simple, inexpensive passive sampling tools to make this a feasible strategy. The results also suggest that monitoring dengue vector abundance alone, in particular immature-stage indices, may not be accurate enough to identify households at heightened risk of dengue infection.

Supporting information

S1 STROBE Statement. Checklist of items that should be included in reports of case-control studies.

(DOCX)

S1 Table. Variables definition.

(DOCX)

S2 Table. List of components used for the SES calculation according to Vyas and Kamaranayake, using varimax rotation.

(DOCX)

S3 Table. Association between antibody response to *Aedes* saliva in household inhabitants and presence of dengue IgG. Odds ratios obtained by logistic univariable regression and confidence intervals (95% CI) by Wald's statistics.

(DOCX)

S4 Table. Association between household characteristics with dengue risk on a subset of the dataset (n = 252 houses, including 153 controls and 99 dengue cases). Statistical analysis was conducted in R 3.5.1 software using logistic univariate regression and 95% confidence intervals (95% CI) were calculated using Wald statistics.
(DOCX)

S5 Table. Association between farming being the main source of income and household type in northeastern Thailand. Statistical analysis was conducted in R software 3.5.1 using a logistic binomial regression. 95% Confidence Intervals (95% CI) were calculated using Wald statistics. Odds ratio in bold are significant at $p < 0.05$.
(DOCX)

S6 Table. Differences in types of vector control activities in households with or without eaves gaps in northeastern Thailand, June 2016 and August 2019. Statistical analysis was conducted in R 3.5.1 software using chi-square (χ^2) test of independence for categorical variables.
(DOCX)

Author Contributions

Conceptualization: Michael J. Bangs, Neal Alexander, Hans J. Overgaard.

Data curation: Benedicte Fustec, Mohammad Injamul Hoq.

Formal analysis: Benedicte Fustec, Mohammad Injamul Hoq, Neal Alexander.

Funding acquisition: Hans J. Overgaard.

Investigation: Benedicte Fustec, Thipruethai Phanitchat, Sirinart Aromseree.

Methodology: Benedicte Fustec, Sirinart Aromseree, Chamsai Pientong, Tipaya Ekalaksananan, Michael J. Bangs, Hans J. Overgaard.

Project administration: Thipruethai Phanitchat, Chamsai Pientong, Kesorn Thaewngiew, Tipaya Ekalaksananan, Hans J. Overgaard.

Resources: Chamsai Pientong, Kesorn Thaewngiew, Tipaya Ekalaksananan.

Supervision: Benedicte Fustec, Michael J. Bangs, Vincent Corbel, Neal Alexander, Hans J. Overgaard.

Visualization: Neal Alexander, Hans J. Overgaard.

Writing – original draft: Benedicte Fustec, Michael J. Bangs, Neal Alexander, Hans J. Overgaard.

Writing – review & editing: Benedicte Fustec, Michael J. Bangs, Vincent Corbel, Neal Alexander, Hans J. Overgaard.

References

1. Bhatt S., et al., The global distribution and burden of dengue. *Nature*, 2013.
2. WHO, *Dengue guidelines for diagnosis, treatment, prevention and control* 2009, WHO: Geneva.
3. Gubler D.J. and Rosen L., Variation among geographic strains of *Aedes albopictus* in susceptibility to infection with dengue viruses. *Am J Trop Med Hyg*, 1976. 25(2): p. 318–25. <https://doi.org/10.4269/ajtmh.1976.25.318> PMID: [1259091](https://pubmed.ncbi.nlm.nih.gov/1259091/)

4. Bhatia R., Dash A.P., and Sunyoto T., Changing epidemiology of dengue in South-East Asia. *WHO South East Asia J Public Health*, 2013. 2(1): p. 23–27. <https://doi.org/10.4103/2224-3151.115830> PMID: [28612819](https://pubmed.ncbi.nlm.nih.gov/28612819/)
5. Limkittikul K., Brett J., and L'Azou M., Epidemiological trends of dengue disease in Thailand (2000–2011): a systematic literature review. *PLoS Negl Trop Dis*, 2014. 8(11): p. e3241. <https://doi.org/10.1371/journal.pntd.0003241> PMID: [25375766](https://pubmed.ncbi.nlm.nih.gov/25375766/)
6. Corbel V., et al., Challenges and prospects for dengue and malaria control in Thailand, *Southeast Asia. Trends Parasitol*, 2013. 29(12): p. 623–33. <https://doi.org/10.1016/j.pt.2013.09.007> PMID: [24215776](https://pubmed.ncbi.nlm.nih.gov/24215776/)
7. Ooi E.E., Goh K.T., and Gubler D.J., Dengue prevention and 35 years of vector control in Singapore. *Emerg Infect Dis*, 2006. 12(6): p. 887–93. <https://doi.org/10.3201/10.3201/eid1206.051210> PMID: [16707042](https://pubmed.ncbi.nlm.nih.gov/16707042/)
8. Phanitchat T., et al., Spatial and temporal patterns of dengue incidence in northeastern Thailand 2006–2016. *BMC Infect Dis*, 2019. 19(1): p. 743. <https://doi.org/10.1186/s12879-019-4379-3> PMID: [31443630](https://pubmed.ncbi.nlm.nih.gov/31443630/)
9. Scott T.W., et al., Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: population dynamics. *J Med Entomol*, 2000. 37(1): p. 77–88. <https://doi.org/10.1603/0022-2585-37.1.77> PMID: [15218910](https://pubmed.ncbi.nlm.nih.gov/15218910/)
10. Gubler D.J., *Aedes aegypti* and *Aedes aegypti*-borne disease control in the 1990s: top down or bottom up. Charles Franklin Craig Lecture. *Am J Trop Med Hyg*, 1989. 40(6): p. 571–8. <https://doi.org/10.4269/ajtmh.1989.40.571> PMID: [2472746](https://pubmed.ncbi.nlm.nih.gov/2472746/)
11. Gubler D.J., Surveillance for dengue and dengue hemorrhagic fever. *Bull Pan Am Health Organ*, 1989. 23(4): p. 397–404. PMID: [2611461](https://pubmed.ncbi.nlm.nih.gov/2611461/)
12. Scott T.W., et al., Blood-feeding patterns of *Aedes aegypti* (Diptera: Culicidae) collected in a rural Thai village. *J Med Entomol*, 1993. 30(5): p. 922–7. <https://doi.org/10.1093/jmedent/30.5.922> PMID: [8254642](https://pubmed.ncbi.nlm.nih.gov/8254642/)
13. Scott T.W., et al., Detection of multiple blood feeding in *Aedes aegypti* (Diptera: Culicidae) during a single gonotrophic cycle using a histologic technique. *J Med Entomol*, 1993. 30(1): p. 94–9. <https://doi.org/10.1093/jmedent/30.1.94> PMID: [8433350](https://pubmed.ncbi.nlm.nih.gov/8433350/)
14. da Silveira L.T.C., Tura B., and Santos M., Systematic review of dengue vaccine efficacy. *BMC Infect Dis*, 2019. 19(1): p. 750. <https://doi.org/10.1186/s12879-019-4369-5> PMID: [31455279](https://pubmed.ncbi.nlm.nih.gov/31455279/)
15. Rosa B.R., Cunha A., and Medronho R.A., Efficacy, immunogenicity and safety of a recombinant tetra-valent dengue vaccine (CYD-TDV) in children aged 2–17 years: systematic review and meta-analysis. *BMJ Open*, 2019. 9(3): p. e019368. <https://doi.org/10.1136/bmjopen-2017-019368> PMID: [30872537](https://pubmed.ncbi.nlm.nih.gov/30872537/)
16. Bowman L., Donegan S., and McCall P., Is dengue vector control deficient in effectiveness or evidence?: Systematic review and meta-analysis. *PLoS Negl Trop Dis*, 2016. 10(3).
17. Bureau of Emerging Infectious Disease, *Thailand's National Strategic Plan For Emerging Infectious Disease Preparedness, Prevention and Response 2013–2016*, M.o.P.H. Department of Disease Control, Editor. 2013, the War Veterans Organization of Thailand Under Royal Patronage of His Majesty the King: Nonthaburi, Thailand. p. 100.
18. Bhumiratana A., et al., Thailand momentum on policy and practice in local legislation on dengue vector control. *Interdiscip Perspect Infect Dis*, 2014. 2014: p. 217237. <https://doi.org/10.1155/2014/217237> PMID: [24799896](https://pubmed.ncbi.nlm.nih.gov/24799896/)
19. Chang F.S., et al., Re-assess vector indices threshold as an early warning tool for predicting dengue epidemic in a dengue non-endemic country. *PLoS Negl Trop Dis*, 2015. 9(9): p. e0004043. <https://doi.org/10.1371/journal.pntd.0004043> PMID: [26366874](https://pubmed.ncbi.nlm.nih.gov/26366874/)
20. Cromwell E.A., et al., The relationship between entomological indicators of *Aedes aegypti* abundance and dengue virus infection. *PLoS Negl Trop Dis*, 2017. 11(3): p. e0005429. <https://doi.org/10.1371/journal.pntd.0005429> PMID: [28333938](https://pubmed.ncbi.nlm.nih.gov/28333938/)
21. Chadee D.D., Dengue cases and *Aedes aegypti* indices in Trinidad, West Indies. *Acta Trop*, 2009. 112(2): p. 174–80. <https://doi.org/10.1016/j.actatropica.2009.07.017> PMID: [19632189](https://pubmed.ncbi.nlm.nih.gov/19632189/)
22. Focks D.A., et al., Transmission thresholds for dengue in terms of *Aedes aegypti* pupae per person with discussion of their utility in source reduction efforts. *Am J Trop Med Hyg*, 2000. 62(1): p. 11–8. PMID: [10761719](https://pubmed.ncbi.nlm.nih.gov/10761719/)
23. Sanchez L., et al., *Aedes aegypti* larval indices and risk for dengue epidemics. *Emerg Infect Dis*, 2006. 12(5): p. 800–6. <https://doi.org/10.3201/eid1205.050866> PMID: [16704841](https://pubmed.ncbi.nlm.nih.gov/16704841/)
24. Chiaravalloti-Neto F., et al., Assessment of the relationship between entomologic indicators of *Aedes aegypti* and the epidemic occurrence of dengue virus 3 in a susceptible population, Sao Jose do Rio Preto, Sao Paulo, Brazil. *Acta Trop*, 2015. 142: p. 167–77. <https://doi.org/10.1016/j.actatropica.2014.11.017> PMID: [25484110](https://pubmed.ncbi.nlm.nih.gov/25484110/)

25. Thai Ministry of Public Health, *Manual of assessment district for sustainable disease control 2013*, D.o.d. control, Editor. 2013, Ministry of Public Health of Thailand: Nonthaburi Province.
26. Romero-Vivas C.M. and Falconar A.K., Investigation of relationships between *Aedes aegypti* egg, larvae, pupae, and adult density indices where their main breeding sites were located indoors. *J Am Mosq Control Assoc*, 2005. 21(1): p. 15–21. [https://doi.org/10.2987/8756-971X\(2005\)21\[15:IORBAA\]2.0.CO;2](https://doi.org/10.2987/8756-971X(2005)21[15:IORBAA]2.0.CO;2) PMID: 15825756
27. Bowman L.R., Runge-Ranzinger S., and McCall P.J., Assessing the relationship between vector indices and dengue transmission: a systematic review of the evidence. *PLoS Negl Trop Dis*, 2014. 8(5): p. e2848. <https://doi.org/10.1371/journal.pntd.0002848> PMID: 24810901
28. Favaro E.A., et al., *Aedes aegypti* entomological indices in an endemic area for dengue in Sao Paulo State, Brazil. *Rev Saude Publica*, 2013. 47(3): p. 588–97. <https://doi.org/10.1590/s0034-8910.2013047004506> PMID: 24346572
29. Doucoure S. and Drame P.M., Salivary Biomarkers in the Control of Mosquito-Borne Diseases. *Insects*, 2015. 6(4): p. 961–76. <https://doi.org/10.3390/insects6040961> PMID: 26593952
30. Rohousova I., et al., Detection of species-specific antibody response of humans and mice bitten by sand flies. *Parasitology*, 2005. 130(Pt 5): p. 493–9. <https://doi.org/10.1017/s003118200400681x> PMID: 15991492
31. Poinsignon A., et al., Novel peptide marker corresponding to salivary protein gSG6 potentially identifies exposure to Anopheles bites. *PLoS ONE*, 2008. 3(6): p. e2472. <https://doi.org/10.1371/journal.pone.0002472> PMID: 18575604
32. Doucoure S., et al., Evaluation of the human IgG antibody response to *Aedes albopictus* saliva as a new specific biomarker of exposure to vector bites. *PLoS Negl Trop Dis*, 2012. 6(2): p. e1487. <https://doi.org/10.1371/journal.pntd.0001487> PMID: 22363823
33. Doucoure S., et al., Human antibody response to *Aedes aegypti* saliva in an urban population in Bolivia: a new biomarker of exposure to Dengue vector bites. *Am J Trop Med Hyg*, 2012. 87(3): p. 504–10. <https://doi.org/10.4269/ajtmh.2012.11-0477> PMID: 22848099
34. Drame P.M., et al., Human antibody responses to the Anopheles salivary gSG6-P1 peptide: a novel tool for evaluating the efficacy of ITNs in malaria vector control. *PLoS ONE*, 2010. 5(12): p. e15596. <https://doi.org/10.1371/journal.pone.0015596> PMID: 21179476
35. Remoue F., et al., Evaluation of the antibody response to Anopheles salivary antigens as a potential marker of risk of malaria. *Trans R Soc Trop Med Hyg*, 2006. 100(4): p. 363–70. <https://doi.org/10.1016/j.trstmh.2005.06.032> PMID: 16310235
36. Londono-Renteria B., et al., *Aedes aegypti* anti-salivary gland antibody concentration and dengue virus exposure history in healthy individuals living in an endemic area in Colombia. *Biomedica*, 2015. 35(4): p. 572–81. <https://doi.org/10.7705/biomedica.v35i4.2530> PMID: 26844447
37. Mathieu-Daude F., et al., Specific human antibody responses to *Aedes aegypti* and *Aedes polynesiensis* saliva: A new epidemiological tool to assess human exposure to disease vectors in the Pacific. *PLoS Negl Trop Dis*, 2018. 12(7): p. e0006660. <https://doi.org/10.1371/journal.pntd.0006660> PMID: 30040826
38. Elanga Ndille E., et al., Human IgG antibody response to *Aedes* Nterm-34kDa salivary peptide, an epidemiological tool to assess vector control in chikungunya and dengue transmission area. *PLoS Negl Trop Dis*, 2016. 10(12): p. e0005109. <https://doi.org/10.1371/journal.pntd.0005109> PMID: 27906987
39. National Statistical Office, *The 2010 Population and Housing Census.*, N.S. Office, Editor. 2010, National Statistical Office
40. Bureau of Epidemiology and M.o.P.H. Thailand, *Annual epidemiological surveillance report 2018*, Department of Disease Prevention, Editor. 2019. p. 808.
41. Humanitarian Data Exchange Project and Royal Thai Survey Department, *Thailand administrative boundaries common operational database*. 2019, United Nation Office for the Coordination of Humanitarian Affairs.
42. Dean A.G., Sullivan K.M., and Soe M.M. *OpenEpi: Open Source Epidemiologic Statistics for Public Health*. 2013/04/06 2020/04/10; Available from: www.OpenEpi.com.
43. Thomas S.J., et al., Improving dengue virus capture rates in humans and vectors in Kamphaeng Phet Province, Thailand, using an enhanced spatiotemporal surveillance strategy. *Am J Trop Med Hyg*, 2015. 93(1): p. 24–32. <https://doi.org/10.4269/ajtmh.14-0242> PMID: 25986580
44. Shu P.Y., et al., Development of group- and serotype-specific one-step SYBR green I-based real-time reverse transcription-PCR assay for dengue virus. *J Clin Microbiol*, 2003. 41(6): p. 2408–16. <https://doi.org/10.1128/jcm.41.6.2408-2416.2003> PMID: 12791857
45. Wacholder S., et al., Selection of controls in case-control studies. II. Types of controls. *Am J Epidemiol*, 1992. 135(9): p. 1029–41. <https://doi.org/10.1093/oxfordjournals.aje.a116397> PMID: 1595689

46. Nealon J., et al., Feasibility of case-control and test-negative designs to evaluate dengue vaccine effectiveness in Malaysia. *Vaccine*, 2019. 37(39): p. 5891–5898. <https://doi.org/10.1016/j.vaccine.2019.07.083> PMID: 31445770
47. Tun-Lin W., Kay B.H., and Barnes A., The Premise Condition Index: a tool for streamlining surveys of *Aedes aegypti*. *Am J Trop Med Hyg*, 1995. 53(6): p. 591–4. <https://doi.org/10.4269/ajtmh.1995.53.591> PMID: 8561259
48. Rueda L.M., *Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission*. Zootaxa Vol. 589. 2004, Auckland: Magnolia Press.
49. Bangs M.J. and Focks D.A., Abridged pupa identification key to the common container-breeding mosquitoes in urban Southeast Asia. *J Am Mosq Control Assoc*, 2006. 22(3): p. 565–72. [https://doi.org/10.2987/8756-971X\(2006\)22\[565:APIKTT\]2.0.CO;2](https://doi.org/10.2987/8756-971X(2006)22[565:APIKTT]2.0.CO;2) PMID: 17067066
50. Lanciotti R.S., et al., Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. *J Clin Microbiol*, 1992. 30(3): p. 545–51. <https://doi.org/10.1128/JCM.30.3.545-551.1992> PMID: 1372617
51. Elanga Ndille E., et al., Human IgG antibody response to *Aedes aegypti* Nterm-34 kDa salivary peptide as an indicator to identify areas at high risk for dengue transmission: a retrospective study in urban settings of Vientiane city, Lao PDR. *Trop Med Int Health*, 2014. 19(5): p. 576–80. <https://doi.org/10.1111/tmi.12280> PMID: 24641205
52. Elanga Ndille E., et al., First attempt to validate human IgG antibody response to Nterm-34kDa salivary peptide as biomarker for evaluating exposure to *Aedes aegypti* bites. *PLoS Negl Trop Dis*, 2012. 6(11): p. e1905. <https://doi.org/10.1371/journal.pntd.0001905> PMID: 23166852
53. Fox J., *The R Commander: A Basic Statistics Graphical User Interface to R*. *Journal of Statistical Software*, 2005. 14(9): p. 42.
54. Ripley B.D., V.W.N., *Modern Applied Statistics with S*. Fourth ed. 2002: Springer.
55. Wickham H., *ggplot2: Elegant Graphics for Data Analysis*. 2016: Springer-Verlag New York.
56. Vyas S. and Kumaranayake L., Constructing socio-economic status indices: how to use principal components analysis. *Health Policy Plan*, 2006. 21(6): p. 459–68. <https://doi.org/10.1093/heapol/czl029> PMID: 17030551
57. Alera M.T., et al., Incidence of dengue virus infection in adults and children in a prospective longitudinal cohort in the Philippines. *PLoS Negl Trop Dis*, 2016. 10(2): p. e0004337. <https://doi.org/10.1371/journal.pntd.0004337> PMID: 26845762
58. Mohd-Zaki A.H., et al., Epidemiology of dengue disease in Malaysia (2000–2012): a systematic literature review. *PLoS Negl Trop Dis*, 2014. 8(11): p. e3159. <https://doi.org/10.1371/journal.pntd.0003159> PMID: 25375211
59. Cummings D.A., et al., The impact of the demographic transition on dengue in Thailand: insights from a statistical analysis and mathematical modeling. *PLoS Med*, 2009. 6(9): p. e1000139. <https://doi.org/10.1371/journal.pmed.1000139> PMID: 19721696
60. Nisalak A., et al., Forty years of dengue surveillance at a tertiary pediatric hospital in Bangkok, Thailand, 1973–2012. *Am J Trop Med Hyg*, 2016. 94(6): p. 1342–1347. <https://doi.org/10.4269/ajtmh.15-0337> PMID: 27022151
61. Swain S., et al., Risk factors for dengue outbreaks in Odisha, India: A case-control study. *J Infect Public Health*, 2019.
62. Gibson G., et al., Conditions of the household and peridomicile and severe dengue: a case-control study in Brazil. *Infect Ecol Epidemiol*, 2014. 4.
63. Bhooniboonchoo P., et al., The spatial dynamics of dengue virus in Kamphaeng Phet, Thailand. *PLoS Negl Trop Dis*, 2014. 8(9): p. e3138. <https://doi.org/10.1371/journal.pntd.0003138> PMID: 25211127
64. Salje H., et al., Dengue diversity across spatial and temporal scales: Local structure and the effect of host population size. *Science*, 2017. 355(6331): p. 1302–1306. <https://doi.org/10.1126/science.aaj9384> PMID: 28336667
65. Ratanawong P., et al., Spatial Variations in Dengue Transmission in Schools in Thailand. *PLoS One*, 2016. 11(9): p. e0161895. <https://doi.org/10.1371/journal.pone.0161895> PMID: 27669170
66. Chadee D.D., Williams F.L., and Kitron U.D., Impact of vector control on a dengue fever outbreak in Trinidad, West Indies, in 1998. *Trop Med Int Health*, 2005. 10(8): p. 748–54. <https://doi.org/10.1111/j.1365-3156.2005.01449.x> PMID: 16045461
67. Pham H.V., et al., Ecological factors associated with dengue fever in a Central Highlands province, Vietnam. *BMC Infect Dis*, 2011. 11: p. 172. <https://doi.org/10.1186/1471-2334-11-172> PMID: 21679398
68. Bureau of Epidemiology, *National diseases surveillance*, Department of Disease Control, Editor. 2016. <https://doi.org/10.1080/19393210.2016.1170070> PMID: 27076265

69. Wijayanti S.P., et al., The Importance of socio-economic versus environmental risk factors for reported dengue cases in Java, Indonesia. *PLoS Negl Trop Dis*, 2016. 10(9): p. e0004964. <https://doi.org/10.1371/journal.pntd.0004964> PMID: 27603137
70. Telle O., et al., The spread of dengue in an endemic urban milieu-The case of Delhi, India. *PLoS One*, 2016. 11(1): p. e0146539. <https://doi.org/10.1371/journal.pone.0146539> PMID: 26808518
71. Udayanga L., et al., Comprehensive evaluation of demographic, socio-economic and other associated risk factors affecting the occurrence of dengue incidence among Colombo and Kandy Districts of Sri Lanka: a cross-sectional study. *Parasit Vectors*, 2018. 11(1): p. 478. <https://doi.org/10.1186/s13071-018-3060-9> PMID: 30143051
72. Nimsamer P. and Walliman N., Development of traditional house forms in riparian communities in Thailand. *Journal of the Siam Society*. 101: p. 49–63.
73. Stoddard S.T., et al., House-to-house human movement drives dengue virus transmission. *Proc Natl Acad Sci U S A*, 2013. 110(3): p. 994–9. <https://doi.org/10.1073/pnas.1213349110> PMID: 23277539
74. Brusich M., et al., Targeting educational campaigns for prevention of malaria and dengue fever: an assessment in Thailand. *Parasit Vectors*, 2015. 8: p. 43. <https://doi.org/10.1186/s13071-015-0653-4> PMID: 25612545
75. von Seidlein L., et al., Knowledge gaps in the construction of rural healthy homes: A research agenda for improved low-cost housing in hot-humid Africa. *PLoS Med*, 2019. 16(10): p. e1002909. <https://doi.org/10.1371/journal.pmed.1002909> PMID: 31600205
76. Yung C.F., et al., Epidemiological risk factors for adult dengue in Singapore: an 8-year nested test negative case control study. *BMC Infect Dis*, 2016. 16: p. 323. <https://doi.org/10.1186/s12879-016-1662-4> PMID: 27390842
77. Bureau of Epidemiology and M.o.P.H. Thailand, *Annual epidemiological surveillance report 2017*, M.o.P.H. Thailand, Editor. 2018, Ministry of Public Health Thailand, p. 765.
78. Bureau of Epidemiology and M.o.P.H. Thailand, *Annual epidemiological surveillance report 2016*, M.o.P.H. Thailand, Editor. 2017, Ministry of Public Health Thailand, p. 800.
79. Le B.C.T., et al., Interepidemic detection of chikungunya virus infection and transmission in northeastern Thailand. *Am J Trop Med Hyg*, 2020.
80. Ruchusatsawat K., et al., Long-term circulation of Zika virus in Thailand: an observational study. *Lancet Infect Dis*, 2019. 19(4): p. 439–446. [https://doi.org/10.1016/S1473-3099\(18\)30718-7](https://doi.org/10.1016/S1473-3099(18)30718-7) PMID: 30826189
81. Khongwicit S., et al., Zika virus in Thailand. *Microbes Infect*, 2018. 20(11–12): p. 670–675. <https://doi.org/10.1016/j.micinf.2018.01.007> PMID: 29477878
82. Jackson M.L. and Nelson J.C., The test-negative design for estimating influenza vaccine effectiveness. *Vaccine*, 2013. 31(17): p. 2165–8. <https://doi.org/10.1016/j.vaccine.2013.02.053> PMID: 23499601
83. Sumridetchkajorn K., et al., Universal health coverage and primary care, Thailand. *Bull World Health Organ*, 2019. 97(6): p. 415–422. <https://doi.org/10.2471/BLT.18.223693> PMID: 31210679
84. Tozan Y., et al., Household costs of hospitalized dengue illness in semi-rural Thailand. *PLoS Negl Trop Dis*, 2017. 11(9): p. e0005961. <https://doi.org/10.1371/journal.pntd.0005961> PMID: 28937986
85. Bureau of Epidemiology and Ministry of Public Health Thailand, *Annual epidemiological surveillance report 2015*, Ministry of Public Health Thailand, Editor. 2016, Ministry of Public Health Thailand, Nonthaburi. p. 1000.
86. Bureau of Epidemiology and Ministry of Public Health Thailand, *Annual epidemiological surveillance report 2010*, Ministry of Public Health Thailand, Editor. 2011, Ministry of Public Health Thailand, Nonthaburi.
87. Bureau of Epidemiology and M.o.P.H. Thailand, *Annual epidemiological surveillance report 2014*, M.o.P.H. Thailand, Editor. 2015, Ministry of Public Health Thailand, Nonthaburi.
88. Mondini A., et al., Spatio-temporal tracking and phylodynamics of an urban dengue 3 outbreak in Sao Paulo, Brazil. *PLoS Negl Trop Dis*, 2009. 3(5): p. e448. <https://doi.org/10.1371/journal.pntd.0000448> PMID: 19478848
89. Parra M.C.P., et al., Using adult *Aedes aegypti* females to predict areas at risk for dengue transmission: A spatial case-control study. *Acta Trop*, 2018. 182: p. 43–53. <https://doi.org/10.1016/j.actatropica.2018.02.018> PMID: 29462598
90. Lau S.M., et al., A new paradigm for *Aedes* spp. surveillance using gravid ovipositing sticky trap and NS1 antigen test kit. *Parasit Vectors*, 2017. 10(1): p. 151. <https://doi.org/10.1186/s13071-017-2091-y> PMID: 28327173
91. Voge N.V., et al., Detection of dengue virus NS1 antigen in infected *Aedes aegypti* using a commercially available kit. *Am J Trop Med Hyg*, 2013. 88(2): p. 260–6. <https://doi.org/10.4269/ajtmh.2012.12-0477> PMID: 23185074

92. Tan C.H., et al., Evaluation of the Dengue NS1 Ag Strip(R) for detection of dengue virus antigen in *Aedes aegypti* (Diptera: Culicidae). *Vector Borne Zoonotic Dis*, 2011. 11(6): p. 789–92. <https://doi.org/10.1089/vbz.2010.0028> PMID: [21395416](https://pubmed.ncbi.nlm.nih.gov/21395416/)
93. Liew J.W.K., et al., Gravid oviposition sticky trap and dengue non-structural 1 antigen test for early surveillance of dengue in multi-storey dwellings: study protocol of a cluster randomized controlled trial. *Infect Dis Poverty*, 2019. 8(1): p. 71. <https://doi.org/10.1186/s40249-019-0584-y> PMID: [31477185](https://pubmed.ncbi.nlm.nih.gov/31477185/)

Chapter 3: Assessing fine scale variations in human exposure to *Aedes* mosquito bites using *Aedes* salivary biomarker during a randomized vector control intervention trial.

The results from this study are currently under review for publication in *PLOS Neglected Tropical Diseases*: **Serological biomarker for assessing human exposure to *Aedes* mosquito bites during a randomized vector control intervention trial in northeastern Thailand.** **Fustec B**, Phanitchat T., Aromseree S., Pientong C., Thaewnongiew K., Ekalaksananan T., Cerqueira D., Poinsignon A., Elguero E, Bangs M. J., Alexander N., Overgaard H. J., Corbel V. (submitted in October 2020)

The case control study described previously highlighted the need for clarification about the relationships between *Aedes* infestation, *Aedes* exposure risk and dengue transmission. In addition, it was important to determine whether Ab response against the Nterm-34 salivary peptide may be a good proxy to assess small-scale variations in *Aedes* abundance where dengue is endemic (see section 4.5.2). To do so, we conducted a serology survey as part of the RCT in the two cities of RE and KK in north-eastern Thailand (see details in section 4.3). Individual factors such as, gender, age, occupations, as well as socio-economic, environmental, epidemiological and vector control intervention that could influence the Ab response to mosquito bites were explored. A cohort of 563 individuals were recruited among inhabitants of the RCT and followed up for serological and concomitant entomological surveys up to 19 months. Fever was recorded weekly to early detect dengue symptoms among study participants. More than 3,980 blood samples were collected on filter paper and analysed by ELISA. The level of Ab response to the Nterm-34 salivary peptide was used to develop a mosquito exposure index (MEI) reflecting the level of specific and individual IgG response to the *Aedes* salivary peptide. The relationships between the MEI and the *Aedes* indices as well as vector infectivity were assessed at both the household and cluster levels using multivariate a two-level mixed model (house, individual) with a one-month lag autoregressive correlation, assuming the antibody response persisted at detectable levels between two and six weeks (Orlandi-Pradines et al. 2007, Elanga Ndille et al. 2016)

Summary of results:

This longitudinal study demonstrated a high IgG seroprevalence rate among inhabitants from north-eastern Thailand with 57.3% and 60% of individuals being responders to the Nterm-34 salivary peptide in KK and RE, respectively. Moreover, in both cities, the IgG response increased few weeks after the peak of *Aedes* density (AIC) that occurred at the beginning of the rainy season (Figure 34). Additionally, the Ab response decreased from the cool season until the hot season while the mosquito densities dropped down after the rainy season and re-increased at the hot season. Our results hence corroborated previous findings in other transmission settings where higher Ab response against *Aedes* salivary antigens was observed with the occurrence of rainfall (Elanga Ndille et al. 2012, Yobo et al. 2018). Altogether, the results suggested a lagged positive association between *Aedes* abundance and human Ab response to *Aedes* bites.

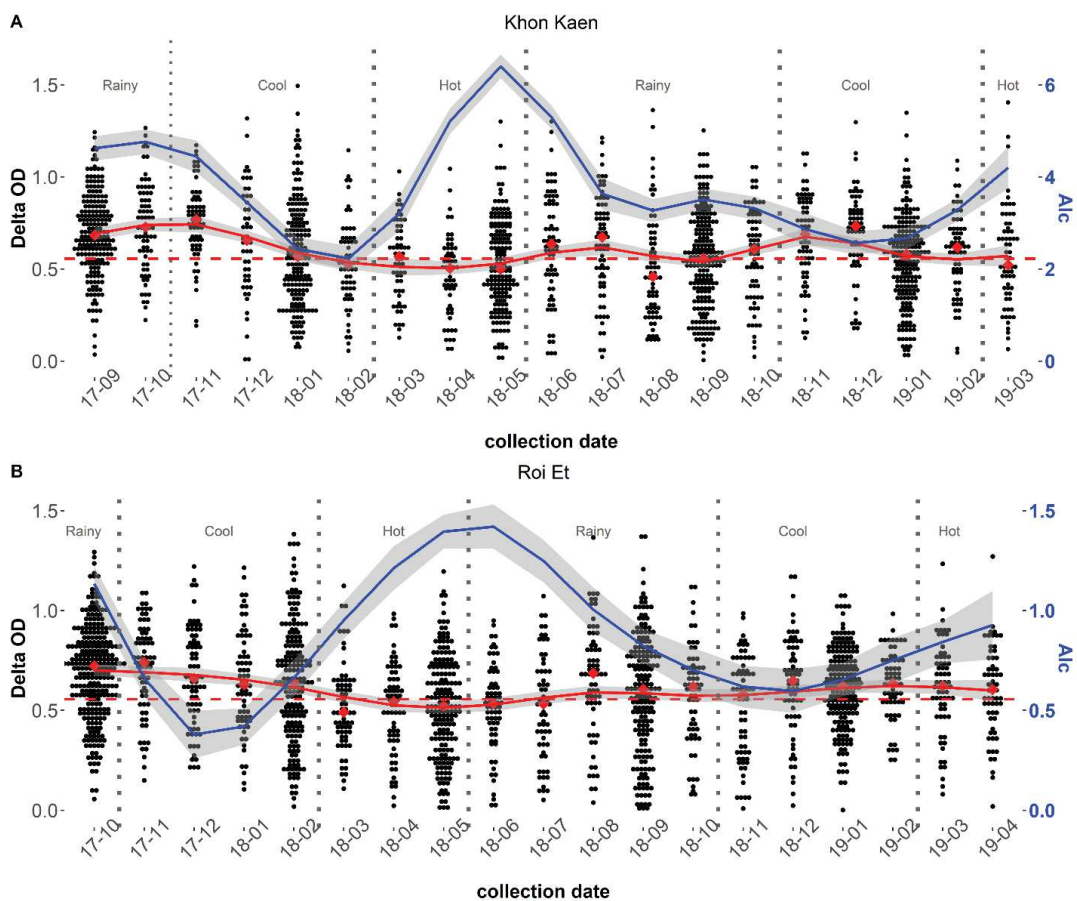


Figure 34: Seasonal variation of human IgG and AIC

Secondly, the multivariate analysis demonstrated for the first time a strong and positive dose-response association between the individual Ab response to Nterm-34 salivary peptide and the levels of *Aedes* abundance, especially when we consider the indoor *Aedes* density. In our study, a total 2,235 *Ae. aegypti* adult females were collected with the large majority being indoor (70% of the total). Therefore, the serological biomarker looks promising to detect small-scale variations in human exposure to *Aedes* mosquito bites. Although already shown for malaria vectors (Ya-Umphang et al. 2017), this is the first time we demonstrated such trend for dengue vectors.

Unfortunately, no clear relationships were observed between the intensity of Ab response to *Aedes* bites and vector infectivity (neither at the cluster level nor the household level). This confirms that dengue virus transmission is a complex affair that varies over time and space, and the relationship between vector density and virus transmission is not easily addressed through successive entomology surveys. Adversely, the relationship between human dengue infections and the intensity of the human Ab response to *Aedes* bites could not be addressed because no dengue cases were detected during the longitudinal cohort studies. Further analyses are on-going by the team to confirm the apparent lack of dengue infections during the study period.

Additionally, the multivariate analysis reveals that human-vector contact as measured by the MEI varied with individual characteristics such as gender and age, with older individuals being at higher risk of *Aedes* mosquito bites. Similarly, being a male was associated with higher *Aedes* exposure risk. Additionally, individuals spending most of their time indoors were associated with higher Ab response to salivary peptide hence confirming the strong endophagic preference of *Ae. aegypti* (Scott et al. 2000b).

Finally, our findings showed that the human IgG levels to the *Aedes* salivary antigen were significantly lower in the treated clusters compared to the control clusters (the one's having received 0.01 mg/L pyriproxyfen). Although speculative, these findings suggest that the PPF may have reduce *Aedes* densities under a certain threshold that was sufficient to reduce the human-*Aedes* contact in treated clusters compared to the control clusters. Unfortunately, the operational impact of PPF on dengue transmission is yet unknown. Complementary analyses are conducting by our team to fill this gap and to assess whether salivary biomarker may complement existing tools and indicators for monitoring and evaluation of vector control intervention.

To conclude, this study represents an important step toward the validation of using the *Aedes* salivary peptide Nterm-34kDa as a proxy measure to assess *Aedes* infestation levels and human-mosquito exposure risk in a dengue endemic area. Unfortunately, no dengue cases were detected during the follow-up, thus, the relationship between dengue transmission and *Aedes* exposure could not be addressed.

PLOS Neglected Tropical Diseases

Serological biomarker for assessing human exposure to Aedes mosquito bites during a randomized vector control intervention trial in northeastern Thailand

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Corresponding Author:	Benedicte Fustec Institut de recherche pour le developpement Montpellier Cedex 5, FRANCE
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Institut de recherche pour le developpement
Corresponding Author's Secondary Institution:	
First Author:	Benedicte Fustec
First Author Secondary Information:	
Order of Authors:	Benedicte Fustec Thipruethai Phanitchat Sirinart Aromseree Chamsai Pientong Kesorn Thaewnongiew Tipaya Ekalaksanan Dominique Ceirquera Anne Poinsignon Eric Elguero Micheal J. Bangs Neal Alexander Hans J. Overgaard Vincent Corbel
Order of Authors Secondary Information:	
Abstract:	Background: Aedes mosquitoes are vectors for several major arboviruses of public health concern including dengue viruses. The relationships between Aedes infestation and disease transmission are complex wherein the epidemiological dynamics can be difficult to discern because of lack of robust and sensitive indicators for predicting transmission risk. This study investigates the use of anti- Aedes saliva antibodies as a serological biomarker for Aedes mosquito bites to assess small scale variations in adult Aedes density and dengue virus (DENV) transmission risk in northeastern Thailand. Individual characteristics, behaviors/occupation and socio-demographics, climatic and epidemiological risk factors associated with human-mosquito exposure are also addressed.

	<p>Methods: The study was conducted within a randomized clustered control trial in Roi Et and Khon Kaen provinces over a consecutive 19 months period. Thirty-six (36) clusters were selected, each of ten houses. Serological and entomological surveys were conducted in all houses every four months and monthly in three sentinel households per cluster between September 2017 and April 2019 for blood spot collections and recording concurrent immature and adult <i>Aedes</i> indices. Additionally, the human exposure to <i>Aedes</i> mosquito bites (i.e., Mosquito Exposure Index or MEI) was estimated by ELISA measuring levels of human antibody response to the specific Nterm-34 kDa salivary antigen. The relationships between the MEI, vector infestation indices (adult and immature stages) and vector DENV infection were evaluated using a two-level (house and individual levels) mixed model analysis with one-month lag autoregressive correlation.</p> <p>Results: A strong positive relationship between the MEI and the intensity of adult <i>Aedes</i> infestation (difference in MEI mean of 0.091 $p < 0.0001$, and 0.131, $p < 0.0001$ for medium and high levels of infestation, respectively), particularly indoor densities (difference in mean MEI of 0.021, $p < 0.007$, 0.053, $p < 0.0001$ and 0.037, $p < 0.0001$ for low, medium and high levels of infestation, respectively) were found. The MEI was driven by individual characteristics, such as gender, age and occupation/behaviors, and varied according to climatic, seasonal factors and vector control intervention ($p < 0.05$). Nevertheless, the study did not demonstrate a clear correlation between MEI and the presence of DENV-infected <i>Aedes</i>.</p> <p>Conclusion: This study represents an important step toward the validation of the specific IgG response to the <i>Aedes</i> salivary peptide Nterm-34 kDa as a proxy measure for <i>Aedes</i> infestation levels and human-mosquito exposure risk in a dengue endemic setting. The use of IgG response to the Nterm-34 kDa peptide as a viable diagnostic tool for estimating dengue transmission requires further investigations and validation in other geographical and transmission settings.</p>
<p>Suggested Reviewers:</p>	<p>Berlin Londono-Renteira, PhD Assistant Professor, Kansas State University berlinlondo@yahoo.com She is immunologist and specialist in the development of mosquito salivary biomarkers. She has published several relevant papers on the use of salivary biomarker to evaluate human exposure to malaria and dengue vectors and to evaluate the efficacy of vector control tools.</p> <p>Jose M. C Ribeiro, MD, PhD National Institute of Allergy and Infectious Diseases Laboratory of Malaria and Vector Research jose.ribeiro@nih.gov Jose M.C. Ribeiro explores the biochemical and pharmacological diversity found in the salivary glands of blood feeding insects (especially mosquitoes) and ticks. He recently used vector salivary gland transcriptome analysis to discover new and interesting compounds and he has developed tools for bioinformatics analysis. He's has published 96 papers on salivary proteins on insect and has good understating on the potential applications of salivary biomarker for measuring the exposure risk to mosquito vectors.</p> <p>Lionel Almeras, PhD Researcher, Institut de recherche biomedicale des armees almeras.lionel@gmail.com Almeras L. is research fellow at University Aix-Marseille. His research centres on infectious diseases and modalities of their transmission. He has more than 15 years experience of working on serological markers of human exposure to vectors and pathogens (plasmodium) and in the identification of immunogenic proteins of pathogen and viral infections by using proteomics and transcriptomic approaches.</p> <p>Emmanuel ELANGA-NDILLE, PhD Researcher, Centre for Research in Infectious Diseases emmsdille@yahoo.fr Elanga Ndille E. is a medical entomologist in the Center for Research in Infectious Diseases, Yaounde, Cameroon. He has experiences in the developement and validation of biomarkers of human-vector contact. He has published several relevant papers on the use of salivary biomarker to evaluate human exposure to malaria and dengue vectors and to evaluate the efficacy of vector control tools.</p>
<p>Opposed Reviewers:</p>	

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<p>Describe where the data may be found in full sentences. If you are copying our sample text, replace any instances of XXX with the appropriate details.</p> <ul style="list-style-type: none"> • If the data are held or will be held in a 	<p>The data underlying the results presented in this paper are made publicly available following principles that data should be Findable, Accessible, Interoperable, and Reusable (FAIR, https://www.force11.org/group/fairgroup/fairprinciples) and regulations of the project owner, the Norwegian University of Life Sciences, that research data must be archived in approved national or international archives. The data used in this publication is archived at the Norwegian Center for Research Data (NSD), a national archive and center for research data. Data can be accessed without specific</p>

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<p>Additional data availability information:</p>	

9/5/2020

Micheal J. Turell
Editor at PLoS Neglected Tropical Diseases

Dear Editor,

‘Serological biomarker for assessing human exposure to *Aedes* mosquito bites during a randomized vector control intervention trial in northeastern Thailand’

We believe that the results in this manuscript, in particular the strong relation between the human antibody response against *Aedes* saliva and the *Aedes aegypti* densities, will be of great interest for the readers of *PLoS Neglected Tropical Diseases*.

Despite the increasing burden of dengue fever in tropical and sub-tropical areas, its prevention and control still target its *Aedes* mosquito vectors. However, the quantitative relationships between dengue infection risk and vector mosquito infestation remain unclear despite numerous indicators used to estimate transmission risk and predict dengue outbreaks. The aim of this study is to investigate the use of an *Aedes* salivary biomarker to assess the small-scale variation in human exposure to *Aedes* bites and the risk of dengue in the context of a vector control intervention in northeastern Thailand.

Several findings of this study highlight the complexity of the human dengue vector exposure. In particular, our study demonstrates a strong positive association between the level of resting adult *Aedes* infestation, especially indoors *Aedes* infestation, and the level of specific human antibody (Ab) response against *Ae. aegypti* salivary peptide.

This manuscript is the first combining both entomological and immunological endpoints investigating *Aedes* vectors and virus transmission. Additionally, risk factors associated with human-vector contact in terms of individual human characteristics and behavior, local vector control practices, and the prevailing environmental and climatic factors are addressed. First, our findings demonstrate that human Ab response against *Aedes* saliva is driven by individual characteristics (i.e. age, gender) and behavior, where staying indoors was associated with a higher Ab response against *Aedes* bites. In addition, our results suggest an impact of the intervention on the adult *Aedes* densities as intervention was associated with a lower Ab response to *Aedes* saliva. Nonetheless, no relationship between the Ab response to *Aedes* saliva and dengue transmission risk (i.e., vector infection) was demonstrated.

This study demonstrated the usefulness of the Ab response to assess heterogeneity in adult *Aedes* infestation indices and could assist public health authorities to better address disease transmission risk and the timeliness of effective vector control interventions.

Yours sincerely,

1 ***PLoS Neglected Tropical Diseases***

2 **Serological biomarker for assessing human exposure to *Aedes* mosquito**
3 **bites during a randomized vector control intervention trial in**
4 **northeastern Thailand**

5 Benedicte Fustec^{1,2,3*}, Thipruethai Phanitchat⁴, Sirinart Aromseree^{2,5}, Chamsai
6 Pientong^{2,5}, Kesorn Thaewongiew⁶, Tipaya Ekalaksananan^{2,5}, Dominique
7 Cerqueira⁸, Anne Poinsignon³, Eric Elguero³, Michael J. Bangs^{7,8}, Neal
8 Alexander⁹, Hans J. Overgaard¹⁰, Vincent Corbel^{1,3*}.

9 ¹Univ Montpellier, Montpellier, France; ² Khon Kaen University, Khon Kaen, Thailand; ³
10 MIVEGEC, Univ Montpellier, IRD, CNRS, Montpellier, France; ⁴ Department of Medical
11 Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok; ⁵ HPV & EBV and
12 Carcinogenesis Research Group, Khon Kaen University, Khon Kaen, Thailand; ⁶ Office of
13 Diseases Prevention and Control, Region 7, Khon Kaen, Thailand; ⁷ Department of
14 Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand; ⁸ Public Health
15 & Malaria Control, International SOS, Mimika, Papua, Indonesia; ⁹ London School of Hygiene
16 and Tropical Medicine, London, United Kingdom; ¹⁰ Norwegian University of Life Sciences,
17 Ås, Norway.

18 E-mail address:

19 BF: b.fustec@gmail.com

20 TP: thipruethai@gmail.com

21 SA: sirinart_a@kkumail.com

22 CP: chapie@kku.ac.th

23 KT: kesthaew@hotmail.com
24 DC: domi_cerqueira@hotmail.com
25 AP: anne.poinsignon@ird.fr
26 TE: tipeka@kku.ac.th
27 EE: eric.elguero@ird.fr
28 MJB: bangs_michael@yahoo.com
29 NA: neal.alexander@lshtm.ac.uk
30 HJO: hans.overgaard@nmbu.no
31 VC: vincent.corbel@ird.fr

32

33 *Corresponding authors

34

35 **ABSTRACT**

36 **Background:** *Aedes* mosquitoes are vectors for several major arboviruses of public health
37 concern including dengue viruses. The relationships between *Aedes* infestation and disease
38 transmission are complex wherein the epidemiological dynamics can be difficult to discern
39 because of a lack of robust and sensitive indicators for predicting transmission risk. This study
40 investigates the use of anti-*Aedes* saliva antibodies as a serological biomarker for *Aedes*
41 mosquito bites to assess small scale variations in adult *Aedes* density and dengue virus (DENV)
42 transmission risk in northeastern Thailand. Individual characteristics, behaviors/occupation
43 and socio-demographics, climatic and epidemiological risk factors associated with human-
44 mosquito exposure are also addressed.

45 **Methods:** The study was conducted within a randomized clustered control trial in Roi Et and
46 Khon Kaen provinces over a consecutive 19 months period. Thirty-six (36) clusters were
47 selected, each of ten houses. Serological and entomological surveys were conducted in all
48 houses every four months and monthly in three sentinel households per cluster between
49 September 2017 and April 2019 for blood spot collections and recording concurrent immature
50 and adult *Aedes* indices. Additionally, the human exposure to *Aedes* mosquito bites (i.e.,
51 Mosquito Exposure Index or MEI) was estimated by ELISA measuring levels of human
52 antibody response to the specific Nterm-34 kDa salivary antigen. The relationships between
53 the MEI, vector infestation indices (adult and immature stages) and vector DENV infection
54 were evaluated using a two-level (house and individual levels) mixed model analysis with one-
55 month lag autoregressive correlation.

56 **Results:** A strong positive relationship between the MEI and the intensity of adult *Aedes*
57 infestation (difference in MEI mean of 0.091 $p < 0.0001$, and 0.131, $p < 0.0001$ for medium and
58 high levels of infestation, respectively), particularly indoor densities (difference in mean MEI

59 of 0.021, $p < 0.007$, 0.053, $p < 0.0001$ and 0.037, $p < 0.0001$ for low, medium and high levels of
60 infestation, respectively) were found. The MEI was driven by individual characteristics, such
61 as gender, age and occupation/behaviors, and varied according to climatic, seasonal factors and
62 vector control intervention ($p < 0.05$). Nevertheless, the study did not demonstrate a clear
63 correlation between MEI and the presence of DENV-infected *Aedes*.

64 **Conclusion:** This study represents an important step toward the validation of the specific IgG
65 response to the *Aedes* salivary peptide Nterm-34kDa as a proxy measure for *Aedes* infestation
66 levels and human-mosquito exposure risk in a dengue endemic setting. The use of the IgG
67 response to the Nterm-34 kDa peptide as a viable diagnostic tool for estimating dengue
68 transmission requires further investigations and validation in other geographical and
69 transmission settings.

70 **Key words:** Thailand, *Aedes* mosquitoes, dengue transmission risk, human antibody response,
71 serological biomarker, salivary peptide Nterm-34kDa.

72 **Author summary:**

73 *Aedes* mosquitoes and the viruses they transmit are major public health concerns for over half
74 of the global human population. However, the quantitative relationships between virus
75 transmission and vector mosquito infestation remain unclear despite numerous indicators used
76 to estimate transmission risk and predict dengue outbreaks. The aim of this study is to
77 investigate the use of a salivary biomarker to assess the small-scale variation in human
78 exposure to *Aedes* bites and the risk of dengue infection in the context of a vector control
79 intervention in northeastern Thailand. A cohort of 539 persons visited every four months,
80 including 161 individuals visited monthly, were recruited for routine serological and
81 concurrent household entomological surveys during 19 consecutive months follow-up.
82 Antibody response to *Aedes* bites was measured by enzyme-linked immunosorbent assays to

83 assess the mosquito exposure index (MEI) and association with the *Aedes* adult and immature
84 abundance as well as the presence of dengue virus (DENV) in adult mosquitoes (transmission
85 risk). Additionally, the individual (cohort), climatic, and vector control intervention risk factors
86 associated with MEI are explored. This study demonstrates that the MEI was strongly related
87 to household adult *Aedes* density, particularly indoors resting mosquitoes. Additionally, the
88 MEI was influenced by individual characteristics (i.e., person age, gender, staying indoors),
89 and varied according to seasons and intervention. Nonetheless, no clear relationship between
90 MEI and dengue transmission risk (i.e., vector infection) was detected. This study
91 demonstrated the potential usefulness of the MEI to assess heterogeneity in adult *Aedes*
92 infestation indices that could assist public health authorities to rapidly identify mosquito “hot
93 spots” and the timeliness of effective vector control interventions.

94

95 **Introduction**

96 *Aedes aegypti* (L) and *Aedes albopictus* (Skuse) are vectors of important human viral pathogens
97 including dengue, yellow, chikungunya and Zika. In Southeast Asia, dengue fever is
98 widespread and accounts for around 70% of the total clinical dengue cases reported globally
99 [1, 2]. Since the first report of dengue infection in Thailand in 1949 [3], dengue incidence has
100 dramatically increased in line with expanding urbanization. With all four virus serotypes and
101 both major mosquito vectors present in the country around 20,000 cases are reported yearly
102 [4]. Despite an affordable, universal primary health coverage system and an organized,
103 nationwide dengue prevention program, the burden of dengue in Thailand is estimated to cost
104 the equivalent of at \$290 million (USD) each year [5].

105 In northeastern Thailand, dengue fever represents major public health concerns with
106 thousands of clinical cases each year [6]. To prevent secondary transmission in communities,
107 when a dengue case is detected, insecticide treatment using adult space spray is mandated
108 within 24 hours in attempt to rapidly eliminate virus-infected vectors, surrounding its home
109 setting [7]. In parallel, basic entomological surveillance is carried regularly by one of the 22
110 regional Offices of Diseases Prevention and Control (ODPCs) to monitor *Aedes* vector
111 infestations [7]. In Thailand standard entomological indices are used to estimate transmission
112 risk that guide the choice of vector control interventions [8]. While, some studies have shown
113 positive associations between various entomological indices and disease transmission risk [9,
114 10], other investigations have demonstrated only weak relationships [11-13]. Most of the
115 entomological indices used to monitor dengue vector infestations are based on measuring the
116 presence of immature mosquito life stages [14]. However, immature stages typically present
117 large mortality rates during development from egg to adult stage [15], thus larval indices do
118 not provide an accurate or concurrent temporal-spatial information on the ‘productivity’ of
119 containers regards actual *Aedes* adult production output [16]. Conversely, pupal indices have

120 been proposed to assess vector infestation with higher accuracy [17, 18] as pupae generally
121 present very low mortality up to adult emergence and thus more relevant to estimate container
122 productivity [19] and adult densities in a location [16]. Operationally, pupae collections remain
123 difficult to implement on a routine basis because they are time-consuming (generally all pupae
124 must be collected and counted) that requires additional entomological staff.

125 Adult mosquito collections have been used to estimate the risk of virus transmission
126 [19, 20], but they have also their limitations. Unlike malaria vector monitoring, human land-
127 catching cannot be performed to collect *Aedes* mosquitoes due to the inherent ethical
128 constraints and disease risks, as there is no preventive treatment nor effective vaccines for most
129 of *Aedes*-transmitted diseases/pathogens (except yellow fever virus). Moreover, *Aedes* adults
130 are most active during the day time, when most people are awake and can take some forms of
131 protection against bites. As a consequence, *Aedes* females are often interrupted in the course
132 of seeking a blood meal and can often feed on multiple hosts per gonotrophic cycle [21-23].
133 Other methods to sample adult *Aedes* include various versions of passive and active trapping
134 devices (e.g., gravitraps, sticky traps, mechanical battery-operated aspirators, and mosquito
135 electrocuting trap) [24], each presenting differing levels of efficiency [25]. However, they do
136 not measure the inter-individual heterogeneity of exposure influenced by human attraction
137 exerted on mosquitoes and individual host behaviors (e.g., use of personal protections).
138 Nevertheless, these capture methods are used as a proxy to estimate *Aedes* adult density in a
139 specific area but they are not representative of actual level of contact (biting) exposure between
140 human and vector [26]. This information is yet crucial to identify host population subsets at
141 higher risk of exposure to dengue vector bites and to better estimate virus transmission risk.

142 An alternative to direct entomological indices for estimating the human exposure to
143 mosquitoes is the measure of a host's antibody (Ab) response to mosquito saliva antigens [27-
144 29]. During blood feeding process, mosquito saliva is initially injected into human skin to

145 facilitate the blood intake and also acts as a vehicle for transmitting pathogens to the host [30].
146 Many salivary proteins are immunogenic and elicit an immune response including the
147 production of specific antibodies (Ab) that can be detected by simple analytic tools and
148 spectrophotometry [31-33]. Firstly developed for *Anopheles*, the vectors of malaria, so-called
149 biomarkers of exposure based on anti-saliva Ab response have been used successfully to
150 identify “hot spots” of vector presence and malaria transmission [34-36] along the Thailand-
151 Myanmar border [34, 37]. As far as *Aedes* genus is concerned, several other studies have shown
152 that IgG response to salivary gland extracts from different *Aedes* species, such as *Ae. aegypti*,
153 *Ae. polynesiensis*, *Ae. caspius* are reliable indicators of human-*Aedes* exposure in South-
154 America [38, 39], Pacific Islands [40], Africa [41] and Europe [31]. An *Ae. aegypti*-specific
155 salivary peptide (Nterm-34 kDa) has been identified and the human IgG response to the Nterm-
156 34 kDa antigen has shown good correlation with adult *Ae. aegypti* infestation indices in Benin
157 [42] and Laos [43]. More recently, the Nterm-34 kDa salivary peptide successfully investigated
158 the spatial heterogeneity of *Aedes* exposure in several urban districts of Senegal [44]. However,
159 most of these *Aedes* serological studies estimated vector infestation through “indirect”
160 (relative) indicators such as immature ‘*Stegomyia*’ (*Aedes*) indices and climatic factors, thus
161 were unlikely to represent more accurate adult infestation that which is directly associated with
162 virus transmission potential. Robust evidence of the relationships between the intensity of
163 human immune response to a specific salivary biomarker, *Aedes* adult abundance, and dengue
164 infective bite risk is needed to assess whether small scale variations in dengue transmission can
165 be detected using this immunological tool. This is particularly relevant for measuring the
166 impact of vector control interventions where entomological indices may lack the spatio-
167 temporal accuracy and sensitivity to demonstrate control effectiveness [16, 45, 46].

168 The primary objective of this study was to assess the relationship between the intensity
169 of the human IgG response to the Nterm-34kDa *Aedes* salivary peptide and selected

170 entomological indicators of vector infestation and dengue infection risk in northeastern
171 Thailand. This study took place within the context of a randomized controlled trial
172 implemented over a consecutive 19-month period to evaluate the efficacy of an insect growth
173 regulator tool for dengue transmission prevention [47, 48]. Additionally, risk factors associated
174 with human-vector contact in terms of individual human characteristics and behavior, local
175 vector control practices, and the prevailing seasonal and climatic factors were addressed. To
176 our knowledge, this is the first longitudinal study conducted to assess dengue transmission risk
177 using a serological *Aedes* salivary biomarker. Hopefully, these findings will assist national
178 authorities to improve the accuracy of dengue surveillance activities and contribute to
179 strengthening the monitoring and evaluation of vector control programs in Thailand and
180 elsewhere.

181

182 **Materials and Methods**

183 *Study sites*

184 The study was conducted in six sub-districts in the city of Khon Kaen (KK), Khon Kaen
185 Province, (N16.440236, E102.828272) and in two sub-districts within the city of Roi Et (RE),
186 Roi Et Province, (N16.055637, E103.652417), in northeastern Thailand (Fig. 1). In each city,
187 18 clusters of 10 households each were randomly selected for a total of 360 households under
188 19 months of follow-up.

189 **Fig. 1: Map of study sites.** (A) represents Thailand and the provinces of Roi Et and Khon
190 Kaen. (B) shows the location of the 18 clusters numbered from 4001 to 4018 in the city of
191 Khon Kaen (KK Mueang District). (C) shows the location of the 18 clusters numbered from
192 4501 to 4518 in the city of Roi Et (RE Mueang District).

193 ***Study design and settings***

194 This study was conducted within the framework of a randomized control intervention trial to
195 evaluate the efficacy of pyriproxyfen application (0.5% granule formulation) for dengue vector
196 control [47, 48]. The study was performed in Khon Kaen between September 2017 and March
197 2019 and between October 2017 and April 2019 in Roi Et (Fig. 2). All households were visited
198 every four months (except one time in RE between February 2018 and May 2018) to collect
199 indoor and outdoor container-breeding *Aedes* (both *Ae. aegypti* and *Ae. albopictus*) larvae,
200 pupae, adult resting mosquitoes, and blood samples from study volunteers living in randomly
201 selected households. In addition, three sentinel houses per cluster were visited monthly for
202 blood and entomological collections described previously. Following the initial 10 months of
203 baseline surveillance, the vector control intervention was distributed randomly in half of the
204 study clusters, in June 2018. The household selection in the cities and the randomization of the
205 intervention are described elsewhere [47, 48]. The vector control intervention was the
206 distribution of pyriproxyfen (0.5% granule formulation) into water-holding containers up to
207 0.01 mg/L active ingredient applied every four months in the treated clusters [47, 48].

208 **Fig. 2: Study design flow chart.** RT-PCR: Reverse Transcriptase Polymerase Chain Reaction

209 ***Individual volunteer characteristics***

210 In each participating household, at least one volunteer inhabitant was recruited in the study.
211 When possible, we tried to recruit inhabitants spending most of their time at home. To ensure
212 adequate representativeness of the entire target population, we recruited one adult and one child
213 per house when feasible. In addition, a pecuniary retribution (50THB) for blood sampling was
214 given to each participant. During each household visit, assigned trained Village Health
215 Volunteers (VHV) interviewed and collected blood of each participating house member.
216 Interview questions were relative to the general characteristics of the participant (i.e., age,
217 gender), occupation(s) during the weekdays and weekends (e.g., at home; at work away from

218 home; at school/college/university; at farm; others), in addition to normal activity and resting
219 habits (i.e., primarily indoor, outdoor or equally indoor and outdoor). The travelling history
220 within the previous 14 days and within the last three months was recorded.

221 ***Blood sample collections***

222 Blood samples (2 blood spots per participant, 10mm diameter each, approximately 150µl) were
223 collected at the fingertips of the inhabitants recruited in the study using sterile lancets [49] and
224 spotted on filter paper Protein Saver cards (Whatman, Maidstone, UK), air-dried, individually
225 placed in plastic sealable bags and stored at room temperature at the Office of Disease
226 Prevention and Control 7 (ODPC7) until delivery to Khon Kaen University (KKU) and stored
227 at 4°C.

228 ***Entomological collections***

229 At each household visit, the VHVs recorded the number of inhabitants in the household at the
230 time of the survey. Houses were inspected for adult and immature *Aedes* both indoor and
231 outside immediately surrounding the house. The total number of containers was recorded
232 together with the number of wet containers at each household. A maximum of 20 larvae
233 (preferably late stage instar) and all pupae were collected per infested container and stored in
234 absolute ethanol at the ODPC7. Immatures and adults were identified to species-level using
235 morphological keys [50, 51], and sex was determined for adults. *Aedes* adult collections were
236 performed using hand-held mechanical battery-powered aspirators [52] conducted 15 min each
237 both indoors and outdoors. Adults were stored individually in labelled 1.5mL microcentrifuge
238 tubes at -20°C and the house number and the location of collection (i.e., indoor/outdoor) was
239 recorded.

240 Entomological data were used to construct several indices as described in
241 Supplementary Table 1. At the cluster level, the Container Index (CI_c) was calculated as the
242 proportion of *Aedes* immature-positive containers per total wet containers inspected in all

243 visited households at the time of survey. The cluster-wide Breteau Index (BI_c) and the House
244 Index (HI_c) were calculated as the proportion of *Aedes* positive containers per 100 houses and
245 the proportion of positive households visited, respectively. The cluster-level pupal indices,
246 Pupae per House Index (PHI_c) and the Pupae per Person Index (PPI_c), represented the total
247 number of pupae collected per household and per inhabitants in each visited household,
248 respectively. The *Aedes* Index (AI_c) and the *Aedes* indoor Index (AI_{in_c}) at the cluster level
249 represented the total number of female *Aedes* collected per inspected houses and the total
250 number of female *Aedes* collected exclusively indoors, respectively.

251 ***Detection of dengue virus in adult mosquitoes***

252 The presence of dengue virus (DENV) in *Aedes* females was investigated in all captured adult
253 mosquitoes, by pooling up to 10 individual abdomens of female *Aedes* together for RNA
254 extraction and DENV detection by reverse transcriptase real-time polymerase chain reaction
255 (RT-qPCR) [49]. For positive pools, the head and thorax of the corresponding individual
256 mosquitoes were processed individually for DENV serotype detection according to Lanciotti
257 *et al* protocol and adapted by our team to be run on RT-qPCR [53]. The proportion of DENV
258 infected *Aedes* was calculated as the number of DENV infected individual *Aedes* divided by
259 the number of tested *Aedes* females per house (AI_{DENV+}) and per cluster (AI_c_{DENV+}),
260 respectively.

261 ***Climatic data***

262 The Meteorological Department of Thailand provided climatic data routinely recorded from
263 the meteorological stations located at the airport of each city [54]. Daily measures were used
264 to derive the minimum and maximum air temperatures ($^{\circ}C$), the percent relative humidity, and
265 the rainfall (mm) between January 2016 to January 2020. For analysis, the mean maximum and
266 minimum temperatures, mean percent relative humidity, and cumulative rainfall the previous

267 two weeks before entomological collections were used to account for an estimated time-lag
268 effect on vector population biology and transmission epidemiology.

269

270 ***Mosquito Exposure Index (MEI)***

271 The specific human IgG response to the *Aedes* Nterm-34kDa salivary peptide (Genepep, Saint
272 Jean de Védas, France) was measured by an enzyme-linked immunosorbent assay (ELISA) as
273 described previously [48, 55]. This secreted salivary peptide was selected because it exhibits
274 high antigenic properties and it is specific to *Aedes* genus, therefore allowing to specifically
275 measure the immune response to *Aedes* bites alone [42]. Briefly, for each individual sampled,
276 dried blood spots were cut using a one cm diameter hole punch and eluted in 400µl of
277 Phosphate Buffer Saline (PBS) for 24h at 4 °C. The resulting eluates were stored at -20°C until
278 further processing. 96-well Maxisorp micro-assay plates (Nunc, Roskilde, Denmark) were
279 coated with the salivary peptide diluted in PBS (20µg/mL) for 180 minutes at 37°C. Following
280 washing and blocking steps, the blood eluates were diluted at 1:160 in PBS containing 1% of
281 Tween20 (1%-PBST) and incubated overnight at 4°C. ELISA plates were incubated with goat
282 anti-human biotin-conjugated IgG (Invitrogen, Thermo Scientific, USA) diluted at 1:6000 in
283 1%- PBST for 90 min at 37°C, followed by streptavidin horseradish peroxidase (GE
284 Healthcare, Amersham Place, UK) diluted at 1:4000 in 1%-PBST for one hour at 37°C. The
285 colorimetric reaction was performed using ABTS buffer (2,2'-azino-bis (3-ethylbenzthiazoline
286 6-sulfonic acid) di-ammonium) + 0.003% H₂O₂ and absorbance (optical density, OD) was
287 measured after 120 min at 405nm with a Sunrise spectrophotometer (Tecan, Switzerland).
288 All samples were assayed in duplicate and in a blank well (no antigen) to measure individual
289 background and antibody response (ΔOD) expressed as:

290 (1)
$$\Delta OD = \text{mean} (OD_{Ag+}) - OD_{Ag-}$$

291 where "OD_{Ag+}" represents the OD value in the well with the salivary antigen and "OD_{Ag-}" the
292 OD value in the well without the antigen.

293 To quantify the non-specific immune reactions and calculate the immune threshold, anti-
294 Nterm-34kDa IgG response was assayed in individuals (n=16) with no known exposure history
295 to *Ae. aegypti* bites [56] (e.g., dry blood spots collected in northern France from January to
296 March 2016 to 2018, and in Western Australia in October 2016). The specific immune
297 threshold (TR) was defined as follows at 0.556.

$$298 \quad (2) \quad TR = \text{mean}(\Delta OD_{\text{unexposed individuals}}) + 3 SD_{\text{unexposed individuals}}$$

299 We also defined the Mosquito Exposure Index (MEI) for each participant as

$$300 \quad (3) \quad MEI = \Delta OD - TR .$$

301 The MEI represents the level of specific and individual IgG response to the *Aedes* salivary
302 peptide. Individuals with a ΔOD value above the TR, thus with a positive MEI, were classified
303 as "immune responders" (i.e., exposed to *Aedes*). Individuals with a ΔOD value equal or below
304 the TR, and therefore with a null or negative MEI value, were categorized as "non-responders"
305 (i.e., non-exposed to *Aedes*). Individuals with negative or null MEI were considered equally
306 having a null MEI as the background immune response cannot be addressed.

307 ***Analysis***

308 ***Covariates***

309 The human study population was stratified into five age groups: 5-19, 20-39, 40-59, 60-69,
310 and ≥ 70 years of age. Individual's characteristics were analyzed as categorical variables to
311 estimate their influence on the MEI. Overall travel history of each subject was used as a binary
312 variable. At the village level, adult *Aedes* indices recorded one-month before blood collection,
313 and immatures *Aedes* indices recorded at the time of survey were used. Additionally, the
314 pyriproxyfen intervention was used as a binary covariate. At the province level, the mean daily

315 maximum and minimum air temperatures, mean percent relative humidity, and the weekly
316 cumulative rainfall two weeks before collections were treated as covariates. The estimated 2-
317 week time-lag takes into account potential influence on vector population biology and
318 transmission epidemiology. Three general climatic seasons are defined according to the Thai
319 Meteorological Department [54] with 15-February to 14-May as the hot season, 15-May to 14-
320 October as the rainy (wet) season, and 15 October to 14-February as the cool season.

321 ***Statistical approach***

322 Data analysis was conducted using R software version 3.5.1 (R Core Team, Vienna, Austria)
323 and MASS, Rcmdr, nlme4, and lmerTest packages [57-59]. Figures were generated on R using
324 ggplot2 and ggpubr packages [60, 61]. Maps were built using QGIS software (version 3.10)
325 and shape files were obtained from the Humanitarian Data Exchange Project [62]. As the MEI
326 represents the specific exposition to *Ae. aegypti*, non-responder individuals were considered
327 with a null MEI, thus the MEI was considered as a positive continuous variable (i.e., $MEI \geq 0$).
328 The relation between MEI and entomological indices was explored using a multivariate 2-level
329 mixed model (house, individual) with a one-month lag autoregressive correlation, assuming
330 the antibody response persisted at detectable levels between two and six weeks [33, 63]. The
331 (1) *Aedes* adult index (2) *Aedes* adult indoor index, and (3) proportion of DENV-infected *Aedes*
332 at the cluster level were examined in three separate analyzes. A fourth analysis was conducted
333 with the proportion of DENV-infected *Aedes* at the household level to assess the heterogeneity
334 of dengue transmission risk between and within study clusters. To avoid the assumption of
335 linear relationships between antibody response to *Aedes* bites and entomological indices, risk
336 factors were categorized into categorical variables to represent the different levels of intensity.
337 Due to the over dispersion of mosquito numbers over time, immature stages and adult
338 entomological indices at the cluster level were categorized into four classes, the null value of
339 the index, and then following the terciles. The presence of DENV-infected *Aedes* was used as

340 a binary variable (0 or >0) due to the low number of sampled DENV-infected *Aedes*. All
341 analyzes were performed on individuals with complete data, while individuals with missing
342 data in covariates of interest were removed. Univariable analysis using a mixed model was
343 conducted with each covariate to identify adjustment factors related to immune response to
344 Nterm-34 kDa for all models. Multivariable mixed models were performed with all covariates
345 with a *p*-value set at < 0.2. Subsequently, models were adjusted by backward selection and
346 removing non-significant variables at *p*-value < 0.05.

347

348 ***Ethical considerations***

349 This trial was registered (ISRCTN, ISRCTN73606171) and approved by the Khon Kaen
350 University Ethics Committee (KKUEC Record No. 4.4.01: 29/2017, Reference No. HE601221,
351 1 September 2017), the London School of Hygiene and Tropical Medicine Ethical Committee,
352 UK (LSHTM Ethics Ref: 14275, 16 August 2017), and the Regional Committee for Medical
353 and Health Research Ethics, Section B, South East Norway (REK Ethics ref: 2017/1826b, 03
354 March 2018). Each participant was informed about the intent of the study and asked to
355 participate on a voluntary basis. In each household, the head of the house signed a consent form
356 to allow periodic entomological inspection inside and outside their residence. Additionally,
357 signed informed consent (or assent, if under 16 years old) were required each time blood
358 samples were taken.

359 **Results**

360 ***Population characteristics***

361 The studied population, 602 individuals (318 in KK and 284 in RE), were followed-up every
362 four months up to 19 months for an average of 3.5 visits per person (Table 1) producing a total
363 of 3,919 collected dried blood spot samples. Among the 602 individuals recruited, a sub-
364 sample of 92 and 71 individuals in KK and RE, respectively, were followed-up each month in

365 sentinel sites with an average of 14.7 visits per person. The majority of the cohort was female
 366 (65.3% and 69.0% in KK and RE, respectively). The median age of the cohort was 64 and 61
 367 years in KK and RE, respectively. The majority of the study cohort stayed most of the time at
 368 home during the weekdays and weekends (Table 2); although, in KK, about 30% of the cohort,
 369 mostly those of younger age, indicated spending some time in schools during the weekends. In
 370 KK, the vast majority of the individuals spent their weekdays indoors while in RE, about one
 371 fifth spent their weekdays both indoors and outdoors (near the location where they spend their
 372 time). Nevertheless, the behavioral trend was quite similar between KK and RE regarding
 373 daytime activities (e.g., indoor vs. outdoor locations). Most individuals were primarily
 374 sedentary with >95% declaring no travel in the previous 3 months before blood collections. At
 375 the time of the study, there was no evidence of incident (new) dengue infection; therefore,
 376 results presented herein is performed using entomological and immunological data only.

377 **Table 1: Population description and immunological status to Nterm-34 kDa salivary**
 378 **peptide.**

	Khon Kaen		Roi Et	
Population size, n individuals (no. dried blood spots)	318	(2003)	284	(1916)
Age in years, median (range of all participants)	64	(5-90)	61	(7-92)
Female proportion, % (no. females/total)	65.3	(1307/2003)	69.0	(1319/1916)
Dengue cases %, (no. cases/total)	0.00	(0/2003)	0.00	(0/1916)
Proportion of immune responder during the whole study, %, (no. responding/total)	All ages	57.3 (1148/2003)	60.0	(1150/1916)
	Age 5-19	46.7 (14/30)	53.8	(21/39)
	Age 20-39	48.9 (66/135)	64.7	(119/184)
	Age 40-59	58.9 (367/623)	60.2	(415/689)
	Age 60-69	58.2 (322/553)	54.0	(299/554)
	Age 70+	57.3 (379/662)	65.8	(296/450)

379

380 **Table 2: Individual participant characteristics, behavior and occupation.** (NA: Not
 381 available).

	Khon Kaen	Roi Et
--	------------------	---------------

		No. individuals=318		No. individuals=284	
Occupation weekdays, %, (no. answers/total)	Home	90.8	(1818/2003)	93.8	(1797/1916)
	Work away from home	7.19	(144/2003)	0.47	(9/1916)
	School/college/university	0.70	(14/2003)	0.68	(13/1916)
	Farm	1.10	(22/2003)	0.05	(1/1916)
	Other	0.10	(2/2003)	0.00	(0/1916)
	NA	0.15	(3/2003)	5.01	(96/1916)
Occupation weekends, %, (no. answers/total)	Home	69.3	(1388/2003)	94.2	(1805/1916)
	Work away from home	1.34	(27/2003)	0.05	(1/1916)
	School/college/university	29.3	(587/2003)	7.31	(14/1916)
	Farm	0.00	(0/2003)	0.05	(1/1916)
	Other	0.05	(1/2003)	0.00	(0/1916)
	NA	0.00	(0/2003)	4.96	(95/1916)
Location spent weekdays, %, (no. answers/total)	Indoor	94.6	(1895/2003)	67.4	(1291/1916)
	Outdoor	3.10	(64/2003)	0.68	(13/1916)
	Indoor and outdoor	2.00	(40/2003)	19.9	(382/1916)
	NA	0.20	(4/2003)	12.0	(230/1916)
Location spent weekends, %, (no. answers/total)	Indoor	46.0	(922/2003)	55.7	(1068/1916)
	Outdoor	0.50	(10/2003)	0.05	(1/1916)
	Indoor and outdoor	26.0	(521/2003)	18.5	(355/1916)
	NA	25.0	(550/2003)	25.7	(492/1916)
Travel in the last 14 days, %, (no. answers/total)	No	96.5	(1932/2003)	94.4	(1808/1916)
	Yes	3.54	(71/2003)	0.68	(13/1916)
	NA	0.00	(0/2003)	4.96	(95/1916)
Travel in the last 3 months, %, (no. answers/total)	No	95.3	(1909/2003)	91.6	(1756/1916)
	Yes	4.70	(94/2003)	3.390	(65/1916)
	NA	0.00	(0/2003)	4.96	(95/1916)
Travel overall during study, % (no. answers/total)	No	92.3	(1848/2003)	91.4	(1752/1916)
	Yes	7.70	(155/2003)	3.60	(69/1916)
	NA	0.00	(0/2003)	4.96	(95/1916)

382

383 ***Entomological collections and indices***

384 Overall, 2,235 resting adults female *Aedes* were captured, of which the vast majority, 1,772
385 (79.3%) were collected indoors (Table 3). *Aedes aegypti* was the overwhelmingly predominant
386 species identified (99.7%) compared to *Aedes albopictus* with only seven females *Ae.*
387 *albopictus* collected. In Khon Kaen, 1,397 females *Aedes* were collected during a combined

388 1,446 house visits, the large majority (77%) captured indoors (Table 3). Moreover, DENV
389 infection was detected among 16 females *Aedes* in KK. In Roi Et, 838 females *Aedes* were
390 sampled from 1,441 collections, of which 696 (83%) were collected indoors. Moreover, DENV
391 was detected among 14 females *Aedes* in RE. Additionally, 992 *Aedes* pupae (544 in KK and
392 448 in RE) were collected in the two cities. As with adult mosquitoes, *Ae. aegypti* pupae
393 represented the vast majority (95.7%) of collections. At the cluster level, the standard larval
394 indices (CI_c, HI, BI) indicated significantly higher *Aedes* infestation in Khon Kaen compared
395 to Roi Et with an average of 16.4% and 4.11% *Aedes* positive containers, respectively
396 (Supplementary Table S2). Similarly, the adult *Aedes* indices (AI_c and AI_{in_c}) were higher in
397 KK clusters than in RE, with an average of 3.7 and 1.0 *Aedes* in KK and 0.79 and 0.68 *Aedes*
398 in RE, respectively. Only the DENV-infected adult *Aedes* index (AI_c DENV+) was higher in
399 RE clusters than in KK with an average of 0.007 and 0.005 proportion of DENV positive *Aedes*
400 in RE and KK, respectively. The pupal indices were, however, slightly higher in RE than in
401 KK with 0.84 and 0.63 PHI_c and 0.26 and 0.19 PPI_c, respectively.

402 **Table 3: Entomological collection data and indices at household and cluster level**

	Khon Kaen			Roi Et		
	Houses	Visits	Total	Houses	Visits	Total
<i>Aedes</i> female collected	179	1446	1397	179	1441	838
<i>Aedes</i> female collected indoors	179	1446	1076	179	1441	696
<i>Aedes</i> pupae collected	179	1446	544	179	1441	448
Entomological indices						
	Mean	Std dev	Range	Mean	Std dev	Range
Household level						
Adult Index DENV+	0.005	0.049	[0-1]	0.005	0.057	[0-1]
Cluster level						
Container Index _c (CI _c) (%)	16.4	14.8	[0-100]	4.11	9.17	[0-66.7]
House Index _c (HI _c)(%)	45.5	33.8	[0-100]	12.9	21.3	[0-100]
Breteau Index _c (BI _c)	60.4	55.4	[0-300]	14.2	25.0	[0-137.5]
Pupae per House Index _c (PHI _c)	0.63	1.40	[0-10.7]	0.84	1.99	[0-10.7]
Pupae per Person Index _c (PPI _c)	0.19	0.45	[0-3.3]	0.26	0.72	[0-5.7]
Adult Index _c (AI _c)	3.71	2.42	[1-15]	0.79	0.84	[0-6]
Adult Index _{in_c} (AI _{in_c})	1.00	0.87	[0-5]	0.68	0.71	[0-4]
Adult Index _c DENV+	0.005	0.035	[0-0.33]	0.007	0.057	[0-0.67]

403

404 ***Spatial and seasonal variation in mosquito exposure and vector density***

405 During the study, 3,919 individual dried blood samples were collected and processed, including
406 2,003 and 1,916 in KK and RE, respectively. The seroprevalence rates for IgG reactivity were
407 57.3% and 60% in KK and RE, respectively, indicating that most individuals exhibited a
408 specific response to the Nterm-34kDa *Ae. aegypti* salivary peptide (Table 1). The proportion
409 of immune responders between combined RE and KK clusters was not statistically significant
410 (χ^2 p=0.08) (Supplementary Table S2).

411 In both cities, *Aedes* density (AI_c) strongly increased in May-June period corresponding to the
412 end of the hot season and the beginning of the rainy season (Fig. 3). Notably, the human IgG
413 response (Δ OD) increased a few weeks after the measured peak of mosquito density.
414 Additionally, the Δ OD decreased from the cool season until the hot season while the mosquito
415 densities were reduced during the rainy season with numbers rebounding during the hot season.
416 Collectively, the results indicated a lagged positive association between *Aedes* abundance and
417 human exposure to *Aedes* bites. Indeed, previous studies on malaria vectors showed that the
418 time-lag for human immune response was between three- to four- weeks after the vector bites
419 [64]. Additionally, univariate analysis of the intensity of MEI indicated a positive association
420 between the intensity of the human Ab response and the density of adult *Aedes* collected the
421 month before the blood spot collection (Supplementary Table S3).

422 **Fig. 3: Seasonal variations of the human IgG response to *Aedes* Nterm-34kDa salivary**
423 **biomarker and the adult density *Aedes* Index (AI_c), between September 2017 and April**
424 **2019 in Khon Kaen (A) and Roi Et (B) northeastern Thailand.** The dot plots represent the
425 individual IgG immune response to the *Aedes* salivary peptide Nterm-34 kDa (Δ OD). The red
426 diamonds represent the median response during each survey. The solid red lines represent the

427 means and the grey shaded areas represent the confidence interval of the IgG response to the
428 salivary biomarker. The red dashed horizontal lines represent the specific immune threshold
429 TR. The solid blue lines represent the means and the grey shaded areas represent the 95%
430 confidence interval respectively, for the AI_c at the cluster level.

431 ***Correlations between vector infestation, vector infectivity and human exposure risk to***
432 ***Aedes bites.***

433 Multivariate analysis was performed on a total of 539 individuals, with complete data,
434 including 378 individuals followed-up every four months, with an average number of 2.63
435 visits per person. Additionally, a sub-sample of 161 individuals, followed-up every month with
436 an average number of 12 visits per person were included in the analysis. The models showed a
437 strong positive correlation between the MEI and the *Aedes* adult density at the cluster level
438 when compared to the absence of *Aedes* for both the total adult AI_c (Figure 4 B and C, mean
439 difference in MEI 0.091, $p < 0.0001$, and 0.131, $p < 0.0001$ for medium and high level of
440 infestation, respectively) and the adult indoor density AI_{in_c} (Figure 4 A and C, difference in
441 mean MEI of 0.021, $p < 0.007$, 0.053, $p < 0.0001$ and 0.037, $p < 0.0001$ for low, medium and high
442 levels of infestation, respectively). There was a significant positive association between the
443 individual immune response and the three categories of *Aedes* intensity (low, medium and
444 high), compared with the reference (no *Aedes*), when considering adult mosquitoes collected
445 indoors ($p < 0.05$).

446

447 In contrast, no clear relationships were noted between MEI and vector DENV infection at the
448 cluster level (Table 4, $p = 0.671$) nor at the household level (Table 4, $p = 0.764$). Based on these
449 study findings, the intensity of the immune response to *Aedes* bite exposure was not associated
450 with a higher risk of being bitten by a DENV-infected vector (Table 4).

451 **Figure 4: Multivariate analysis of MEI, human immune response to the Nterm-34 kDa**
 452 **salivary. (A) Adult *Aedes* indoors index only multivariate model. (B) Adult *Aedes***
 453 **multivariate model. (C) Summary table of multivariate analysis of MEI.**

454

455 **Table 4: Multivariate mixed linear model of human immune response to Nterm-34kDa**
 456 ***Aedes* salivary peptide or MEI and the presence of DENV infected *Aedes* in the cluster.**

	Cluster level		Household level	
	Mean difference ^a	P	Mean difference ^a	P
DENV infected <i>Aedes</i>		0.003 ^b		0.050 ^b
0	Reference		Reference	
> 0	0.012	0.671	-0.015	0.764

Analyses were adjusted for age, gender, travel history, BI_c, PHI_c, season, and cluster variables, in addition to the other specified variables. The difference in mean MEI immune response in bold are significant at 0.05.

^a Defined as the difference between each class and the reference categories.

^b Likelihood ratio test to assess the global effect of the variable.

457

458 ***Demographic, social, operational and climatic factors associated with human exposure risk***
 459 ***to Aedes bites***

460 For both models exploring AI_c and AI_{inc}, using univariate analysis (Supplementary Table S3),
 461 all covariates (except “remain at home during the last 7 days”) were retained in the analysis.
 462 MEI differed according to age (p<0.0001), sex (p<0.0001), season (p=0.003), vector control
 463 intervention (p<0.0001) and human occupation (p<0.0001) (Figure 4). The 60-69 years old age
 464 group had higher levels of antibody response to *Aedes* bites compared to other classes (Figure
 465 4, p<0.001). Additionally, being male was associated with a higher risk of having had *Aedes*
 466 bites (p=0.003 and p<0.0001) in both models. Interestingly, people spending greater time
 467 preferentially indoors during weekdays had higher levels of IgG response to salivary peptide

468 than people spending time both indoors and outdoors (Figure 4, difference in MEI mean 0.036,
469 $p < 0.0001$ and 0.047, $p < 0.0001$ for total *Aedes* density and indoor *Aedes* density, respectively).

470 Several entomological indices of immature stages were significantly correlated to the
471 MEI. The Breteau Index was positively associated with IgG seroprevalence to the Nterm-34
472 kDa, although the strength of the association seemed to saturate at higher levels. Interestingly,
473 the Pupae per House Index (PHI_c) at the cluster level was negatively correlated with the MEI
474 (Figure 4, $p < 0.0001$). In both models, the presence of the trial vector control intervention was
475 associated with a decreased level of antibody response against *Aedes* bites (Figure 4, difference
476 in MEI mean -0.057 at $p < 0.0001$ and -0.068 at $p < 0.0001$ for the AI_c and the AI_{inc} models
477 respectively). Regarding climatic factors, the rainy season was positively associated with MEI
478 in both models.

479

480 **Discussion**

481 This study highlights a strong positive relationship between the intensity of human IgG
482 response against the *Aedes* salivary peptide Nterm-34kDa and adult *Aedes* population densities
483 in association with humans in northeastern Thailand. A clear gradient response between the
484 MEI and adult vector density indicated that individuals exhibiting higher antibody response to
485 the *Aedes* salivary peptide were located in areas with higher risk of potential dengue vector
486 bites. This was more evident with indoor infestations. This study corroborates previous work
487 [35-41] showing that the serological biomarker represents a promising surveillance tool to
488 assess small-scale variations in human exposure risk to *Aedes* bites in dengue endemic settings.
489 Although studied for malaria vectors [34], this is the first longitudinal study combining both
490 entomological and immunological endpoints investigating *Aedes* vectors and virus
491 transmission. Further investigations are needed to address the kinetics of human immune

492 response to *Aedes* salivary proteins, in particular the delay between bite exposure, and the
493 production and waning of IgG titers.

494 This study showed that the human-mosquito contact is influenced by human behavioral
495 characteristics, socio-demographic conditions, climatic factors, and trial vector control
496 interventions associated with dengue transmission risk as previously demonstrated [8, 19, 55].
497 The relationship between human dengue infections and the intensity of the human-antibody
498 response to *Aedes* bites could not be ascertained because incident dengue cases were not
499 detected in the study participants during the time of longitudinal follow-up. Further analysis is
500 on-going to confirm the observation of the apparent lack (or very low) transmission during the
501 study period (to be reported elsewhere). In a recent case-control study conducted in
502 northeastern Thailand (conducted by this study team), neither the adult mosquito abundance at
503 the household level nor the degree of human exposure to *Aedes* bites was correlated with a
504 higher odds of acquiring dengue infection [55]. Although consistent with some previous results
505 in Southeast Asia [43, 55], the small sample size of DENV-positive *Aedes* might explain the
506 lack of significance between human infection and vector density seen in this study. This
507 highlights dengue virus transmission is both a multi-factorial and a complex affair that varies
508 over time and space, and the relationship between vector density and virus transmission is
509 dynamic and thus might not be adequately or accurately characterized through standard
510 methods of entomological monitoring.

511 These findings show that the MEI was significantly associated with the season and
512 prevailing climatic factors. The proportion of immune responders to *Aedes* bites was higher
513 during the rainy season than the drier months of the year, corresponding to the period of greater
514 adult vector densities. This is probably explained by the dramatic increase in most
515 entomological indices during this period of the year where the number of suitable larval
516 habitats increases and adult survival (longevity) is presumably enhanced [15, 65]. Similar

517 results were reported in Benin, where the overall anti-saliva antibody response in children
518 increased during the rainy season [42]. A recent study in Cote d'Ivoire highlighted a strong
519 relationship between human mosquito exposure, season and agricultural practices [66].
520 Specific IgG responses remained high during both seasons in villages associated with intensive
521 agricultural compared to villages lacking agricultural practices. The authors suggest that the
522 presence of rubber and oil palm plantations, by providing a suitable environment for the
523 presence of *Aedes* vector species maintained a high level of human exposure to *Aedes* mosquito
524 bites regardless of annual seasonal changes.

525 Interestingly, the present study also suggests correlations between the MEI and *Aedes*
526 immature-based indices, although the association appeared weaker compared to adult
527 measures. The Breteau Index was associated with higher levels of antibody response against
528 *Aedes* bites but was not gradient-dependent. In contrast, the pupae per house index was
529 negatively associated with the MEI. This result might seem contradictory; however, that under
530 natural field conditions, larvae and pupae development rates are strongly influenced by climatic
531 factors, particularly ambient temperature and rainfall patterns, as well as density-dependent
532 factors of immature stages affecting resource competition [67-69]. Additionally, the presence
533 of larval stages in an aquatic habitat can inhibit further egg hatching [70]. Therefore, a decrease
534 in human immune response to *Aedes* bites could be the reflection of the cyclic fluctuations
535 between successive adult population densities influenced by site-specific immature mosquito
536 densities.

537 The MEI varied according to individual characteristics, such as gender, age, and
538 occupation. Interestingly, older people presented higher risk for mosquito bites than the
539 younger population. Similarly, being a male was associated with a higher exposure level to
540 *Aedes* bites. Similar results were found with *Anopheles* exposure and malaria transmission in
541 Thailand, where males were at higher risk than females, mainly due to differences in behavior

542 and occupational exposure [37]. Nevertheless, these results have to be viewed with caution as
543 the majority of the participants in the present study were female and the median age of the
544 cohort was 64 in KK and 61 in RE, which may have biased the outcomes. Indeed, the median
545 age of the cohort reflects the lack of representation of the younger population, which are
546 presumed more active (mobile) than older individuals. Our findings also showed that
547 individuals spending the majority of time indoors were associated with a higher exposure to
548 *Aedes* bites than those spending time more equally either indoors and out. An explanation is
549 that *Ae. aegypti* is a well-adapted species for resting and breeding inside dwellings, and is more
550 typically found indoors [22, 23]. This is also supported by the level of significance of human-
551 exposure risk using the *Aedes* indoor index. The risk of biting (i.e., transmission) inside a
552 dwelling appears particularly important and helps explain why insecticide-treated curtains and
553 targeted indoor residual spraying were highly effective against *Ae. aegypti* for the control and
554 prevention of dengue outbreaks in Mexico and Australia [46, 71].

555 This study suggests that the salivary biomarker is sensitive enough to detect small scale
556 variations in human exposure to *Aedes* bites over time, in particular during a vector control
557 intervention. The human IgG levels were significantly lower in treated clusters compared to
558 the control clusters. These findings would suggest an appreciable impact of pyriproxyfen
559 treatment on the density of *Aedes* adult populations. Similar results were observed in La
560 Réunion, where vector control intervention combining *Aedes* larval habitat source reduction
561 and insecticide space spray against adult mosquitoes was associated with a significant decrease
562 in human antibody response against *Ae. albopictus* bites [41, 63]. Investigations are on-going
563 in Thailand to assess the entomological and epidemiological impact of pyriproxyfen
564 intervention in the study area [48, 72].

565 This study represents an important step toward the validation of using the *Aedes*
566 salivary peptide Nterm-34kDa as a proxy measure to assess *Aedes* infestation levels and

567 human-mosquito exposure risk in a dengue endemic area. Although promising results are
568 described, the use of the Nterm-34 kDa as a surveillance indicator for estimating dengue
569 transmission risk requires further investigations including other geographical and transmission
570 settings.

571

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584 **Authors contributions**

585 **Conceptualization:** MJB NA HJO VC.

586 **Data curation:** BF EE.

587 **Formal analysis:** BF EE.

588 **Funding acquisition:** NA HJO.

589 **Investigation:** BF TP SA.

590 **Methodology:** BF SA CP TE DC AP EE MJB HJO VC.

591 **Project administration:** TP SA CP TE KT HJO.

592 **Resources:** CP TE KT.

593 **Supervision:** MJB NA HJO VC.

594 **Visualization:** BF VC.

595 **Writing – original draft:** BF VC.

596 **Writing – review & editing:** BF MJB AP NA HJO VC.

597

598 **Supporting information**

599 **Supplementary Table S1.** Defined variables

600 **Supplementary Table S2.** Comparison of proportion of immune responders and
601 entomological indices between Khon Kaen and Roi Et provinces using Chi square test and
602 ANOVA.

603 **Supplementary Table S3.** Univariate analysis of the human antibody response to the *Aedes*
604 salivary biomarker Nterm-34 kDa.

605

606 **References**

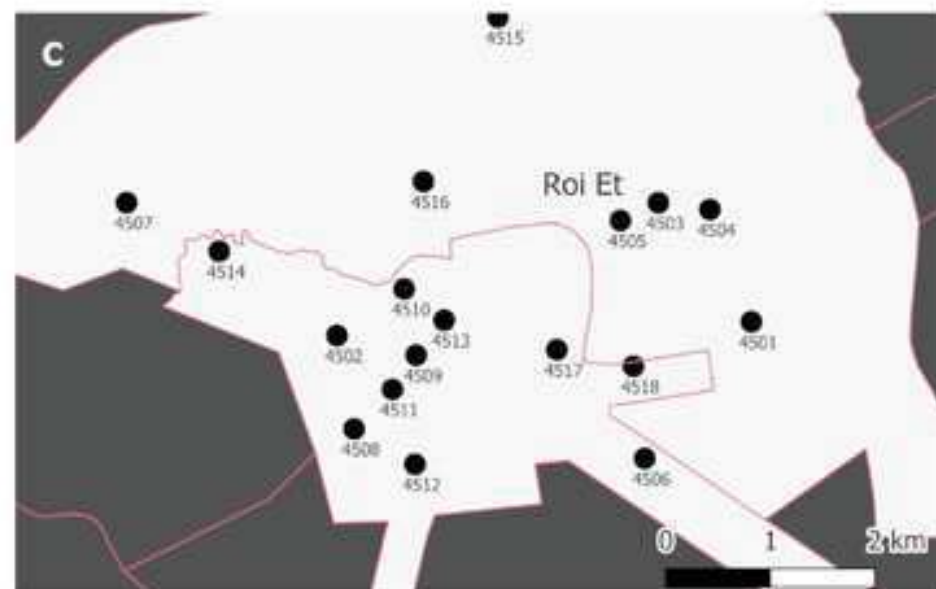
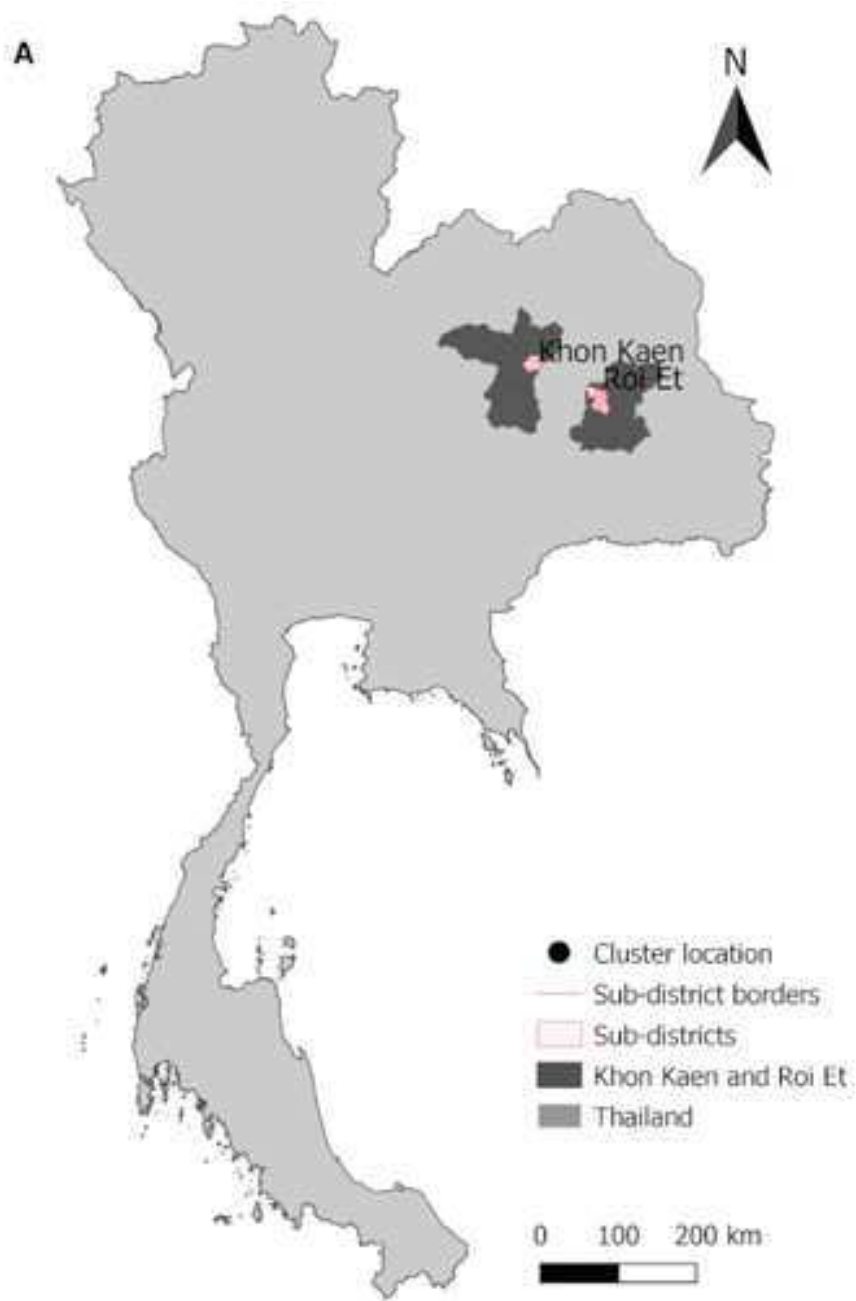
- 607 1. Bhatt, S., et al., *The global distribution and burden of dengue*. Nature, 2013.
- 608 2. World Health Organization, *Dengue guidelines for diagnosis, treatment, prevention and*
609 *control* 2009, WHO: Geneva.
- 610 3. Hammon, W.M., *Dengue hemorrhagic fever--do we know its cause?* Am J Trop Med Hyg,
611 1973. **22**(1): p. 82-91.
- 612 4. Bureau of Epidemiology and M.o.P.H. Thailand, *Annual epidemiological surveillance report*
613 *2018*, Department of Disease Prevention, Editor. 2019. p. 808.
- 614 5. Shepard, D.S., E.A. Undurraga, and Y.A. Halasa, *Economic and disease burden of dengue in*
615 *Southeast Asia*. PLoS Negl Trop Dis, 2013. **7**(2): p. e2055.
- 616 6. Limkittikul, K., J. Brett, and M. L'Azou, *Epidemiological trends of dengue disease in Thailand*
617 *(2000-2011): a systematic literature review*. PLoS Negl Trop Dis, 2014. **8**(11): p. e3241.
- 618 7. Bureau of Emerging Infectious Disease, *Thailand's National Strategic Plan For Emerging*
619 *Infectious Disease Preparedness, Prevention and Response 2013-2016*, M.o.P.H. Department
620 of Disease Control, Editor. 2013, the War Veterans Organization of Thailand Under Royal
621 Patronage of His Majesty the King: Nonthaburi, Thailand. p. 100.
- 622 8. Bowman, L.R., S. Runge-Ranzinger, and P.J. McCall, *Assessing the relationship between*
623 *vector indices and dengue transmission: a systematic review of the evidence*. PLoS Negl Trop
624 Dis, 2014. **8**(5): p. e2848.
- 625 9. Chadee, D.D., *Dengue cases and Aedes aegypti indices in Trinidad, West Indies*. Acta Trop,
626 2009. **112**(2): p. 174-80.
- 627 10. Sanchez, L., et al., *Aedes aegypti larval indices and risk for dengue epidemics*. Emerg Infect
628 Dis, 2006. **12**(5): p. 800-6.
- 629 11. Chadee, D.D., F.L. Williams, and U.D. Kitron, *Impact of vector control on a dengue fever*
630 *outbreak in Trinidad, West Indies, in 1998*. Trop Med Int Health, 2005. **10**(8): p. 748-54.
- 631 12. Ooi, E.E., K.T. Goh, and D.J. Gubler, *Dengue prevention and 35 years of vector control in*
632 *Singapore*. Emerg Infect Dis, 2006. **12**(6): p. 887-93.
- 633 13. Romero-Vivas, C.M. and A.K. Falconar, *Investigation of relationships between Aedes aegypti*
634 *egg, larvae, pupae, and adult density indices where their main breeding sites were located*
635 *indoors*. J Am Mosq Control Assoc, 2005. **21**(1): p. 15-21.
- 636 14. Focks, D., *A review of entomological sampling methods and indicators for dengue vectors*.
637 WHO-TDR, 2004.
- 638 15. Focks, D.A., et al., *Dynamic life table model for Aedes aegypti (Diptera: Culicidae): analysis of*
639 *the literature and model development*. J Med Entomol, 1993. **30**(6): p. 1003-17.
- 640 16. Roiz, D., et al., *Integrated Aedes management for the control of Aedes-borne diseases*. PLoS
641 Negl Trop Dis, 2018. **12**(12): p. e0006845.
- 642 17. Focks, D.A., et al., *Transmission thresholds for dengue in terms of Aedes aegypti pupae per*
643 *person with discussion of their utility in source reduction efforts*. Am J Trop Med Hyg, 2000.
644 **62**(1): p. 11-8.
- 645 18. Focks, D.A. and D.D. Chadee, *Pupal survey: an epidemiologically significant surveillance*
646 *method for Aedes aegypti: an example using data from Trinidad*. Am J Trop Med Hyg, 1997.
647 **56**(2): p. 159-67.
- 648 19. Parra, M.C.P., et al., *Using adult Aedes aegypti females to predict areas at risk for dengue*
649 *transmission: A spatial case-control study*. Acta Trop, 2018. **182**: p. 43-53.
- 650 20. Lau, S.M., et al., *A new paradigm for Aedes spp. surveillance using gravid ovipositing sticky*
651 *trap and NS1 antigen test kit*. Parasit Vectors, 2017. **10**(1): p. 151.
- 652 21. Harrington, L.C., et al., *Heterogeneous feeding patterns of the dengue vector, Aedes aegypti,*
653 *on individual human hosts in rural Thailand*. PLoS Negl Trop Dis, 2014. **8**(8): p. e3048.
- 654 22. Scott, T.W., et al., *Blood-feeding patterns of Aedes aegypti (Diptera: Culicidae) collected in a*
655 *rural Thai village*. J Med Entomol, 1993. **30**(5): p. 922-7.

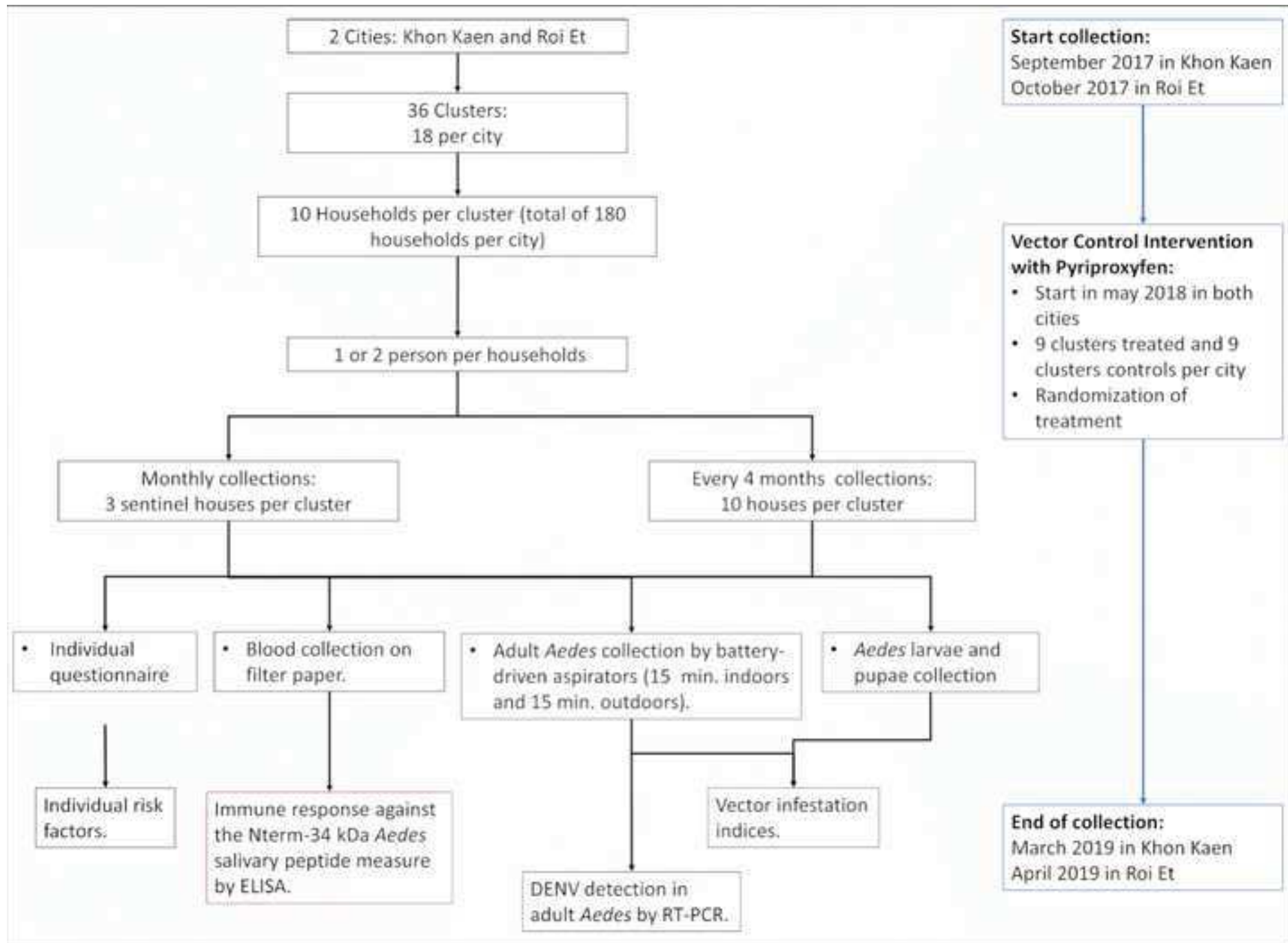
- 656 23. Scott, T.W., et al., *Detection of multiple blood feeding in Aedes aegypti (Diptera: Culicidae)*
657 *during a single gonotrophic cycle using a histologic technique.* J Med Entomol, 1993. **30**(1): p.
658 94-9.
- 659 24. Ortega-López, L.D., et al., *The mosquito electrocuting trap as an exposure-free method for*
660 *measuring human-biting rates by Aedes mosquito vectors.* Parasites & vectors, 2020. **13**(1):
661 p. 31-31.
- 662 25. Bazin, M. and C.R. Williams, *Mosquito traps for urban surveillance: collection efficacy and*
663 *potential for use by citizen scientists.* J Vector Ecol, 2018. **43**(1): p. 98-103.
- 664 26. Barnard, D.R., et al., *Measurement of landing mosquito density on humans.* Acta Trop, 2014.
665 **136**: p. 58-67.
- 666 27. Doucoure, S. and P.M. Drame, *Salivary biomarkers in the control of mosquito-borne diseases.*
667 *Insects*, 2015. **6**(4): p. 961-76.
- 668 28. Poinsignon, A., et al., *Novel peptide marker corresponding to salivary protein gSG6*
669 *potentially identifies exposure to Anopheles bites.* PLoS One, 2008. **3**(6): p. e2472.
- 670 29. Lombardo, F., et al., *The Anopheles gambiae salivary protein gSG6: an anopheline-specific*
671 *protein with a blood-feeding role.* Insect Biochem Mol Biol, 2009. **39**(7): p. 457-66.
- 672 30. Wasinpiyamongkol, L., et al., *Blood-feeding and immunogenic Aedes aegypti saliva proteins.*
673 *Proteomics*, 2010. **10**(10): p. 1906-16.
- 674 31. Fontaine, A., et al., *Relationship between exposure to vectors bites and antibody response to*
675 *mosquito salivary gland extracts.* PLoS ONE, 2011. **6**(12).
- 676 32. Remoue, F., et al., *Evaluation of the antibody response to Anopheles salivary antigens as a*
677 *potential marker of risk of malaria.* Trans R Soc Trop Med Hyg, 2006. **100**(4): p. 363-70.
- 678 33. Orlandi-Pradines, E., et al., *Antibody response against saliva antigens of Anopheles gambiae*
679 *and Aedes aegypti in travellers in tropical Africa.* Microbes Infect, 2007. **9**(12-13): p. 1454-62.
- 680 34. Ya-Umphon, P., et al., *Use of an Anopheles salivary biomarker to assess malaria transmission*
681 *risk along the Thailand-Myanmar border.* J Infect Dis, 2017. **215**(3): p. 396-404.
- 682 35. Poinsignon, A., et al., *Human IgG response to a salivary peptide, gSG6-P1, as a new immuno-*
683 *epidemiological tool for evaluating low-level exposure to Anopheles bites.* Malar J, 2009. **8**: p.
684 198.
- 685 36. Marie, A., et al., *The Anopheles gambiae cE5 salivary protein: a sensitive biomarker to*
686 *evaluate the efficacy of insecticide-treated nets in malaria vector control.* Microbes Infect,
687 2015. **17**(6): p. 409-16.
- 688 37. Ya-Umphon, P., et al., *Anopheles salivary biomarker as a proxy for estimating Plasmodium*
689 *falciparum malaria exposure on the Thailand-Myanmar border.* Am J Trop Med Hyg, 2018.
690 **99**(2): p. 350-356.
- 691 38. Londono-Renteria, B., et al., *Use of anti-Aedes aegypti salivary extract antibody*
692 *concentration to correlate risk of vector exposure and dengue transmission risk in Colombia.*
693 *PLoS One*, 2013. **8**(12): p. e81211.
- 694 39. Doucoure, S., et al., *Human antibody response to Aedes aegypti saliva in an urban*
695 *population in Bolivia: a new biomarker of exposure to dengue vector bites.* Am J Trop Med
696 Hyg, 2012. **87**(3): p. 504-10.
- 697 40. Mathieu-Daude, F., et al., *Specific human antibody responses to Aedes aegypti and Aedes*
698 *polynesiensis saliva: A new epidemiological tool to assess human exposure to disease vectors*
699 *in the Pacific.* PLoS Negl Trop Dis, 2018. **12**(7): p. e0006660.
- 700 41. Doucoure, S., et al., *Human antibody response to Aedes albopictus salivary proteins: a*
701 *potential biomarker to evaluate the efficacy of vector control in an area of chikungunya and*
702 *dengue virus transmission.* Biomed Res Int, 2014. **2014**: p. 746509.
- 703 42. Elanga Ndille, E., et al., *First attempt to validate human IgG antibody response to Nterm-*
704 *34kDa salivary peptide as biomarker for evaluating exposure to Aedes aegypti bites.* PLoS
705 Negl Trop Dis, 2012. **6**(11): p. e1905.

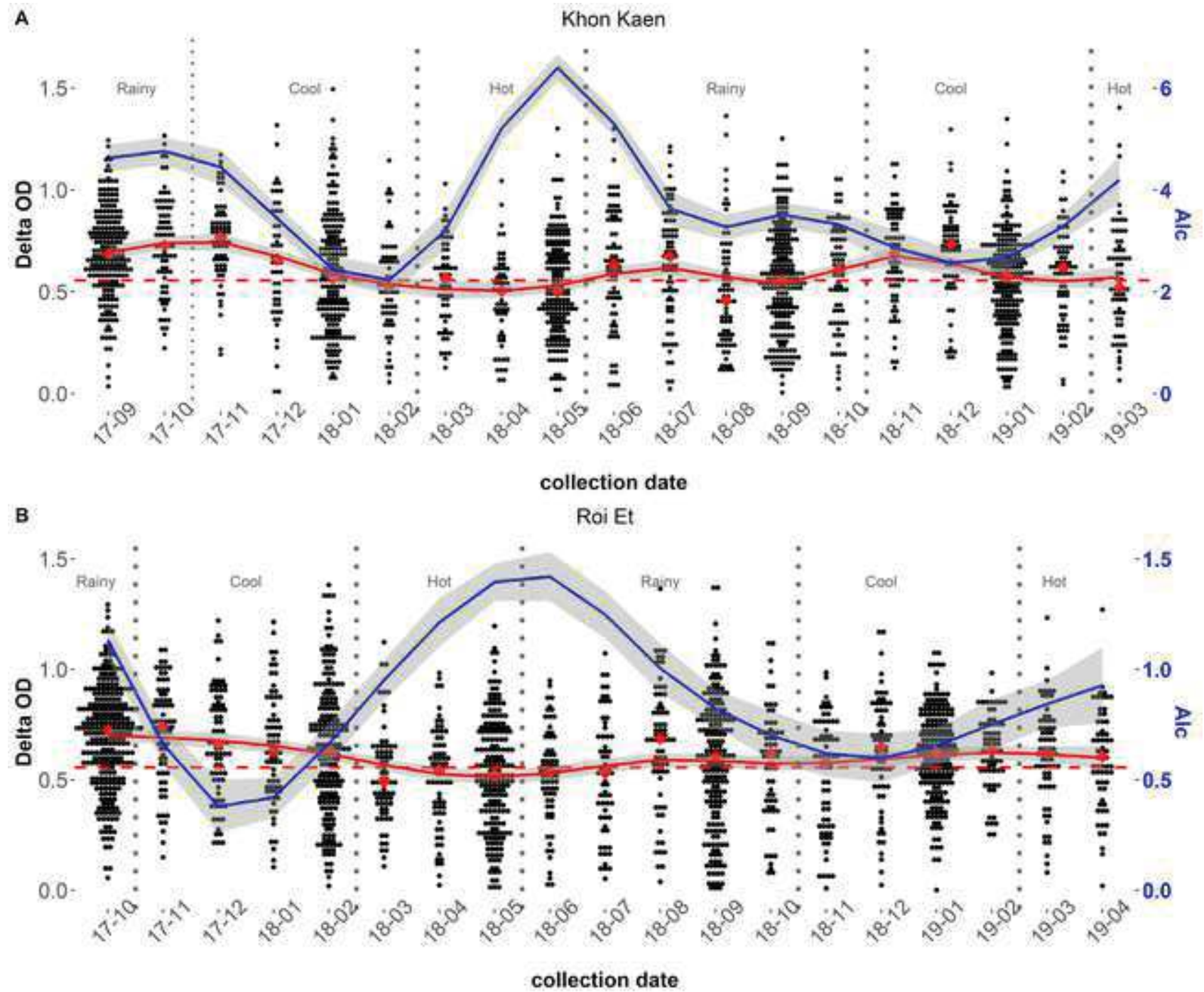
- 706 43. Elanga Ndille, E., et al., *Human IgG antibody response to Aedes aegypti Nterm-34 kDa*
707 *salivary peptide as an indicator to identify areas at high risk for dengue transmission: a*
708 *retrospective study in urban settings of Vientiane city, Lao PDR.* Trop Med Int Health, 2014.
709 **19**(5): p. 576-80.
- 710 44. Sagna, A.B., et al., *Spatial assessment of contact between humans and Anopheles and Aedes*
711 *mosquitoes in a medium-sized African urban setting, using salivary antibody-based*
712 *biomarkers.* J Infect Dis, 2019. **220**(7): p. 1199-1208.
- 713 45. Bowman, L., S. Donegan, and P. McCall, *Is dengue vector control deficient in effectiveness or*
714 *evidence?: Systematic review and meta-analysis.* PLoS Negl Trop Dis, 2016. **10**(3).
- 715 46. Erlanger, T.E., J. Keiser, and J. Utzinger, *Effect of dengue vector control interventions on*
716 *entomological parameters in developing countries: a systematic review and meta-analysis.*
717 Med Vet Entomol, 2008. **22**(3): p. 203-21.
- 718 47. Overgaard, H.J., et al., *Correction to: Assessing dengue transmission risk and a vector control*
719 *intervention using entomological and immunological indices in Thailand: study protocol for a*
720 *cluster-randomized controlled trial.* Trials, 2018. **19**(1): p. 703.
- 721 48. Overgaard, H.J., et al., *Assessing dengue transmission risk and a vector control intervention*
722 *using entomological and immunological indices in Thailand: study protocol for a cluster-*
723 *randomized controlled trial.* Trials, 2018. **19**(1): p. 122.
- 724 49. Roland, J.W. and G.P. Hooff, *State-of-the-art dried blood spot analysis: an overview of recent*
725 *advances and future trends.* Bioanalysis, 2013. **5**(17).
- 726 50. Rueda, L.M., *Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated*
727 *with dengue virus transmission.* Zootaxa Vol. 589. 2004, Auckland: Magnolia Press.
- 728 51. Bangs, M.J. and D.A. Focks, *Abridged pupa identification key to the common container-*
729 *breeding mosquitoes in urban Southeast Asia.* J Am Mosq Control Assoc, 2006. **22**(3): p. 565-
730 72.
- 731 52. Vazquez-Prokopec, G.M., et al., *A new, cost-effective, battery-powered aspirator for adult*
732 *mosquito collections.* J Med Entomol, 2009. **46**(6): p. 1256-9.
- 733 53. Lanciotti, R.S., et al., *Rapid detection and typing of dengue viruses from clinical samples by*
734 *using reverse transcriptase-polymerase chain reaction.* J Clin Microbiol, 1992. **30**(3): p. 545-
735 51.
- 736 54. Thai Meteorological Department. 19/02/2020]; Available from: <https://www.tmd.go.th/en/>.
- 737 55. Fustec, B., et al., *Complex relationships between Aedes vectors, socio-economics and dengue*
738 *transmission— lessons learned from a case-control study in northeastern Thailand.* PLoS Negl
739 Trop Dis, 2020.
- 740 56. Kraemer, M.U.G., et al., *The global distribution of the arbovirus vectors Aedes aegypti and*
741 *Ae. albopictus.* eLife, 2015. **4**: p. e08347-e08347.
- 742 57. Ripley, B.D. and W.N. Venables, *Modern applied statistics with S.* 2002, Springer: New York.
- 743 58. Fox, J., *The R Commander: a basic statistics graphical user interface to R.* Journal of
744 Statistical Software, 2005. **14**(9): p. 42.
- 745 59. Pinheiro, J., et al., *nlme: Linear and nonlinear mixed effect models.* 2020.
- 746 60. Wickham, H., *ggplot2: elegant graphics for data analysis.* 2016: Springer-Verlag New York.
- 747 61. Kassambara, A., *ggpubr: 'ggplot2' Based publication ready plots. R package version 0.2.*
748 2018.
- 749 62. Humanitarian Data Exchange Project, *Thailand administrative boundaries common*
750 *operational database.* 2019, United Nation Office for the Coordination of Humanitarian
751 Affairs.
- 752 63. Elanga Ndille, E., et al., *Human IgG antibody response to Aedes Nterm-34kDa salivary*
753 *peptide, an epidemiological tool to assess vector control in chikungunya and dengue*
754 *transmission area.* PLoS Negl Trop Dis, 2016. **10**(12): p. e0005109.

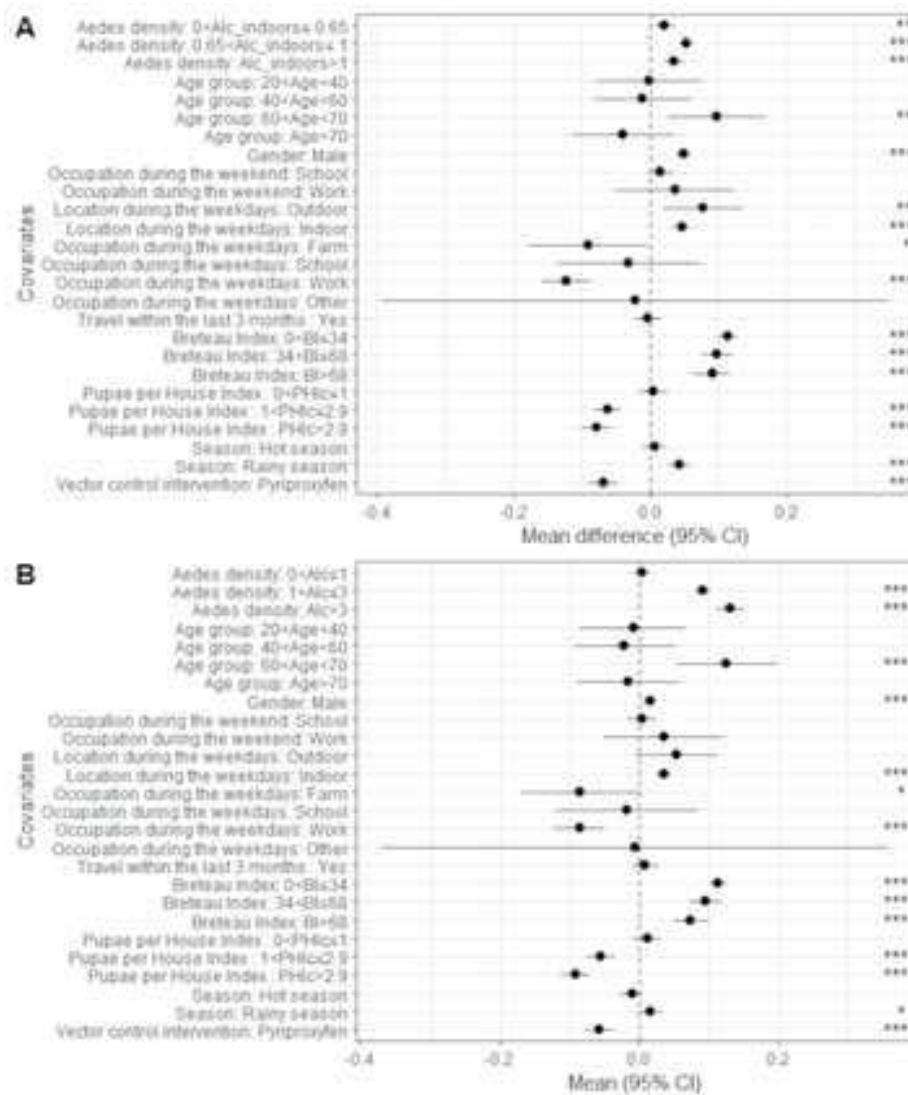
- 755 64. Drame, P.M., et al., *Human antibody responses to the Anopheles salivary gSG6-P1 peptide: a*
756 *novel tool for evaluating the efficacy of ITNs in malaria vector control*. PLoS One, 2010. **5**(12):
757 p. e15596.
- 758 65. Brady, O.J., et al., *Modelling adult Aedes aegypti and Aedes albopictus survival at different*
759 *temperatures in laboratory and field settings*. Parasit Vectors, 2013. **6**: p. 351.
- 760 66. Yobo, C.M., et al., *Evaluation of human exposure to Aedes bites in rubber and palm*
761 *cultivations using an immunoepidemiological biomarker*. Biomed Res Int, 2018. **2018**: p.
762 3572696.
- 763 67. Focks, D.A., et al., *Dynamic life table model for Aedes aegypti (Diptera: Culicidae): Simulation*
764 *results and validation*. Journal of Medical Entomology, 1993. **30**(6): p. 1018-1028.
- 765 68. Legros, M., et al., *Evaluation of location-specific predictions by a detailed simulation model of*
766 *Aedes aegypti populations*. PloS one, 2011. **6**(7): p. e22701-e22701.
- 767 69. Magori, K., et al., *Skeeter Buster: a stochastic, spatially explicit modeling tool for studying*
768 *Aedes aegypti population replacement and population suppression strategies*. PLoS
769 neglected tropical diseases, 2009. **3**(9): p. e508-e508.
- 770 70. Aznar, V., et al., *Modeling the complex hatching and development of Aedes aegypti in*
771 *temperate climates*. Ecological Modelling, 2013. **253**: p. 44-55.
- 772 71. Vazquez-Prokopec, G.M., et al., *Combining contact tracing with targeted indoor residual*
773 *spraying significantly reduces dengue transmission*. Sci Adv, 2017. **3**(2): p. e1602024.
- 774 72. Rochon, J., *Issues in adjusting for covariates arising post randomization in clinical trials*. Drug
775 Information Journal, 1999. **33**(4): p. 1219-1228.

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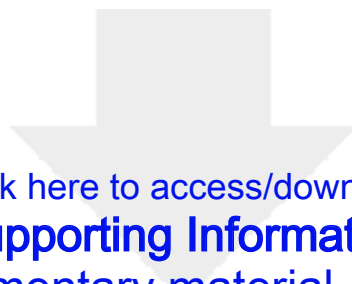
C	AI, (Indoors and outdoors)		AI, indoors only	
	Mean difference *	P	Mean difference *	P
Aedes density [†]	No Aedes	Reference	Reference	<0.001 [‡]
	Low	0.005	0.396	0.621
	Medium	0.093	<0.001	0.053
	High	0.131	<0.001	0.037
Age [‡]	Reference	<0.001 [‡]	Reference	<0.001 [‡]
	Age 5-19 y	-0.008	0.433	0.003
	Age 20-39 y	-0.021	0.572	0.009
	Age 40-59 y	0.126	0.001	0.192
Gender	Age 60-69 y	-0.016	0.676	0.079
	Age >70 y			0.713
Occupation during the weekdays	Female	Reference	Reference	0.259
	Male	0.036	0.003	0.048
Occupation during the weekends	Home	Reference	Reference	0.028
	Work away from home	-0.086	<0.001	-0.124
	School	-0.018	0.732	-0.031
	Farm	-0.086	0.050	-0.088
Location during weekdays	Other	-0.006	0.972	-0.018
	Home	Reference	Reference	0.003
	School	0.004	0.683	0.003
Travel abroad	Work	0.033	0.423	0.035
	Indoors and outdoors	Reference	Reference	<0.001 [‡]
	Indoors	0.036	<0.001	0.047
Biteau Index	Outdoors	0.055	0.023	0.077
	0	Reference	Reference	<0.001 [‡]
PHI	1-3	0.114	<0.001	0.114
	4-6	0.096	<0.001	0.098
	>6	0.074	<0.001	0.091
Season	0	Reference	Reference	<0.001 [‡]
	1-2	0.011	0.281	0.006
	3-4	-0.055	<0.001	-0.060
	5-6	-0.092	<0.001	-0.078
Vector control intervention	Hot season	Reference	Reference	<0.001 [‡]
	Rainy season	0.007	0.937	0.006
	Control	Reference	Reference	0.434
Vector control intervention	Pyriproxyfen	0.016	0.258	0.006
	Pyriproxyfen	0.016	0.254	0.041
Vector control intervention	Control	Reference	Reference	<0.001 [‡]
	Pyriproxyfen	-0.057	<0.001	-0.068

Analyses adjusted for rain, temperature maximum and cluster variables, in addition to the other specified variables. The difference in mean MEI is bold in significant at 0.05.

* Defined as the difference between each class and the reference category.

† Labelled zero test because the global effect of the variable.

‡ Adult density categories: 0-1, 1-3, and > 3 for AI, class, low, medium and high, respectively; 0-0.65, 0.66-1 and > 1 for AI_{in}, Aedes female collected indoors categories low, medium and high, respectively.



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Dear Editor and Reviewers of PLoS Neglected Tropical Diseases,

We really appreciated the effort and the time spend to improve our manuscript (PNTD-D-20-00965). We have modified the manuscript accordingly to the main comments and revisions asked. Please find below details concerning our answers, justifications and modifications to the points raised by each referee and the changes made in the text. Please note that the lines numbers refer to the clean version of the revised document.

Reviewer's Responses to Questions

Key Review Criteria Required for Acceptance?

As you describe the new analyses required for acceptance, please consider the following:

Methods

- Are the objectives of the study clearly articulated with a clear testable hypothesis stated?
- Is the study design appropriate to address the stated objectives?
- Is the population clearly described and appropriate for the hypothesis being tested?
- Is the sample size sufficient to ensure adequate power to address the hypothesis being tested?
- Were correct statistical analysis used to support conclusions?
- Are there concerns about ethical or regulatory requirements being met?

Reviewer #1: (No Response)

Reviewer #2: In general, the method is adequate.

I suggest specifying the study design.

In Figure 1, it is necessary to improve the quality of the image, as it is not possible to differentiate the color pattern from Figure 1 (A). Figure 2 needs a caption for the abbreviations presented.

We improved the resolution of the Figure 1 and added the missing caption for Figure 2.

Results

- Does the analysis presented match the analysis plan?
- Are the results clearly and completely presented?
- Are the figures (Tables, Images) of sufficient quality for clarity?

Reviewer #1: (No Response)

Reviewer #2: The results presentation was quite confusing.

The authors should describe the number of study participants cohesively.

For example:

Initially, in the descriptive analysis, the authors report 612 participants but, in Table 1 the total no. is not consistent with this sample. Data on mosquito exposure report 3,989 dried blood

samples, but, the text does not specify how many individuals are represented in these samples. Also, when presenting univariate and multivariate analyzes, the sample consists of 381 individuals.

We modified the Table 1 according to the reviewers' comments and clarified the repartition of the samples in the text at Lines 361-365. For the univariate and multivariate analysis, we clarified the total number of individuals used in the analysis, being 539 individuals including 378 followed-up every four months and 161 individuals followed-up once each month (Lines 433-436).

The confusion is also in the organization of the topics in the results section. Usually, unadjusted estimates are presented first and then adjusted estimates. The text would be more understandable if the authors followed this order in the presentation of the results.

In Table 1, both the title and the presentation of the results are quite confusing. The total number presented does not refer to 612 individuals. Also, the authors do not present the individuals' immunological status regarding the exposure biomarker.

The total number presented reflected the number of dried blood samples collected and analyzed from the cohort, however, individuals were visited several times (from 1 to 19 visits depending on households and persons).

Table 1 has been split into two tables, Table 1 presents the population studied, and Table 2 presents the results from the sociodemographic questionnaire.

The immunological status of individuals is presented in Table 1 as the proportion of immune responders according to location (province) and age groups.

We agree with the reviewers on the organization of the results. The first sections of the results present only descriptive statistics of the data without analysis. However, we had to present the unadjusted estimated in Supplementary material (S.Table 3), due to the large number of covariates analyzed. The univariate analysis was used mainly to screen the significant covariates to keep in the multivariate model. We assumed that the global message would be clearer by presenting the adjusted estimates in the main text while the unadjusted were presented as Supplementary material, therefore avoiding repetitions. Nonetheless, we modified the text on Lines 419-421 to mention the univariate analysis earlier in the text.

Conclusions

- Are the conclusions supported by the data presented?
- Are the limitations of analysis clearly described?
- Do the authors discuss how these data can be helpful to advance our understanding of the topic under study?
- Is public health relevance addressed?

Reviewer #1: (No Response)

Reviewer #2: Line 467: authors report that human-mosquito contact may be influenced by socioeconomic characteristics. I believe that the correct term is sociodemographic since the analysis did not include socioeconomic variables.

We replaced the word socio-economics with sociodemographic in the abstract and the text at Lines 43 and 494.

Besides, the authors cited three references when presenting the main results of the study, which needs to be corrected.

We modified the text presenting the main results, highlighting the similarities between our study and the cited studies at Lines 493-495.

Line 468: I suggest removing the word “unfortunately”.

We removed the word “unfortunately” at Line 496.

I believe that the text would benefit from the inclusion of a brief discussion on the differences observed in the analysis of the variables associated with the Aedes Index and Mosquito exposure index in the “outdoor and indoor” and “indoors only” models.

The application of the results in entomological surveillance could be further explored in this section.

We acknowledge the reviewers’ comments, yet, we focused our discussion on the model using “indoor only” as the association was strictly positive and significant for all levels of indoor *Aedes* infestation while in the “outdoor and indoor” model, the lower level of *Aedes* infestation was not significantly associated with a higher level of Ab response.

Editorial and Data Presentation Modifications?

Use this section for editorial suggestions as well as relatively minor modifications of existing data that would enhance clarity. If the only modifications needed are minor and/or editorial, you may wish to recommend “Minor Revision” or “Accept”.

Reviewer #1: (No Response)

Reviewer #2: Replace the socioeconomic term with sociodemographic in the abstract. The text and tables need formatting.

The text and tables were carefully reviewed and formatted accordingly.

Summary and General Comments

Use this section to provide overall comments, discuss strengths/weaknesses of the study, novelty, significance, general execution and scholarship. You may also include additional comments for the author, including concerns about dual publication, research ethics, or publication ethics. If requesting major revision, please articulate the new experiments that are needed.

Reviewer #1: Overall comments:

The authors present a study looking at a large suite of entomological indices for *Ae. aegypti* to

see how they compare to human immunological response to *Ae. aegypti* salivary proteins. This was an enjoyable study to read and an impressive amount of work went into this study. I have a few overall comments and specific comments for the authors to consider.

In the discussion the authors cite their own study (Fustec et al. 2020 PLOS NTD) which upon review looks very similar to the current study. The regions of Thailand and the time of sampling looks the same between that recent study and the current one but what I am not sure about is if these exact same households and human participants are included in both studies. If they are the same, the authors need to be more careful about articulating the difference between this other publication and the current one. It looks like the past compared the Mosquito Exposure Index to human dengue cases whereas the current one focuses on comparing MEI to a variety of the entomological indices. If these data originate from the same study, it would be important to introduce this context in the introduction section.

The data from the current study and the case-control study (Fustec et al. 2020 PLOS NTD) originated from two *different* studies, although they were conducted in the same area, they didn't involve the same districts; or the same households/individuals and they have different objectives.

The objective of the case-control study (Fustec et al. 2020 PLOS NTD) was to determine immunological, entomological and socioeconomic risk factors of dengue using passive hospital-based dengue detection. The objective of the current study was to determine the relationships between the intensity of the immune response to the *Aedes* salivary peptide, vector infestation indices (adult and immature stages) and vector DENV infection through longitudinal surveys (over 2 years). In the current study, dengue cases were investigated using active case detection.

On a similar note, this study appears to have taken place during an intervention with pyriproxyfen. My comment below observes a reference to this intervention in the discussion along with conclusions regarding the intervention success. I don't see any mention of the intervention in the methods or results. I suspect this represents a manuscript in prep that will focus on the results of the intervention. That is fine but the only potential conflict with this current study is if the intervention had an influence on the MEI results or any of the entomological indices. Does the longitudinal data associated with Figure 3 include households or communities in the intervention clusters? This could of course be a major artifact depending on the context of the intervention so the authors need to be more transparent about this throughout the MS.

Indeed, the results from the vector control intervention will be part of a separated publication and we cannot provide too much information in the current manuscript.

Figure 3 includes individuals from the intervention (treated) and the control clusters, however the current analysis aims to clarify the relationship between the intensity of the immune response to the Nterm-34 kDa salivary peptide and the *Aedes* density and also to individual characteristics, sociodemographic, climatic factors and vector control intervention.

The description of the intervention with pyriproxyfen was included in the Material and Method section at Lines 205-207. The influence of vector control intervention with pyriproxyfen was accounted in the models as a binary covariate at Lines 312-314.

The summary of results in the discussion concludes that there is a “strong positive relationship between the intensity of human IgG response against the Aedes salivary peptide Nterm-34kDa and adult Aedes infestation in northeastern Thailand.” My comments below show that it is currently difficult to see how ‘strong’ this really is. An additional multi-panel figure might help to show these differences better.

We removed Table 3 and included it into a multi-panel figure as Figure 4 presenting the mean difference in MEI according to each model.

Specific comments:

Ln. 370. What about the collection of mosquitoes in genera other than Aedes? The methods and results don't really discuss the ELISA's specificity to just *Ae. aegypti* versus antibodies to salivary proteins by other mosquitoes that feed on humans. I suspect the prior studies cited discuss this topic but it is an important one worth revisiting in the current study.

Indeed, other mosquito species were collected, mainly *Culex* spp.; however, the salivary peptide Nterm-34 kDa is specific to Aedes mosquito saliva (Elanga et al., 2012), therefore we do not expect a cross reaction with other mosquito species present in the study area. We emphasized on the Aedes-specificity of the Nterm-34kDa salivary peptide at Lines 273-275.

Ln. 417. In the results you are presenting the significance and mean difference in MEI for all the variables. Presenting these mean differences are hard for me to digest in terms of how MEI compares at the household or individual level. Although you already have 3 figures in the MS, only one is a data figure. Can you somehow present the results of table 3 (or supplemental table 3) as a figure? I see there are many significant relationships but the differences appear small so it would be nice to see these data in another way. I could see an additional multi-panel figure being added to main text or supplemental material.

We removed Table 3 and included it into a multi-panel figure presenting the mean difference in MEI according to each model (Figure 4).

Ln. 468-471. You state dengue cases were not detected during follow up. However, the Fustec et al. 2020 PLOS NTD reports many dengue cases during the 2016 to 2019 period. It looks like the current study is 2017 to 2019 so do these represent many of the same participants?

In Fustec et al. 2020 PLOS NTD, we reported some dengue [cases from hospital-based passive detection](#) whereas in the current study we investigated dengue cases using active case detection and fever measurement every week. Moreover, the FUSTEC et al. 2020 PLOS NTD study was conducted in 2016 and was extended until 2019 due to the low number of incident dengue cases reported/detected. Moreover, although the two studies were partly conducted in the same provinces, they did not include the same districts nor the same participants. Therefore, none of the individuals from the case control study (FUSTEC et al. PLOS NTD 2020) were included in the current longitudinal study presented here.

Ln. 526-528. You say IgG levels were significantly lower in treated clusters compared to control clusters which suggests the pyriproxyfen treatment reduced Aedes density. You are not citing these results in other studies and I don't see any information in the methods/results regarding

the intervention or an analysis involving the intervention. These statements in the discussion sound in appropriate as it sounds like you are referring to previously published work or unpublished work.

We added a sentence in the Material and Method section to describe the pyriproxyfen intervention (Lines 205-207). We also add a sentence in the analysis section to clarify how the intervention was accounted for in the analysis (Lines 313-314). However, the results from the intervention will be published elsewhere and we can't provide too much details on methods and result in this paper (entomology/epidemiology data).

Figure 3. If I understand Fig. 3 correctly, the blue line is the Adult number per household and the red line represents the IgG response to the salivary marker. The text says there is a delay between when the adult numbers peak and then when the immune response to *Ae. aegypti* feeding starts to increase. To me the variation in adult numbers is striking but the variation in immune response (or delta OD) is very subtle and hard to even notice a relationship with adult numbers. This putative lag is related to how long it takes for a person to develop these antibodies and then how long they persist (or at least are detectable). The authors don't appear to bring up this topic in the discussion so this would be important to include.

We added a sentence in the Result section of Figure 3 to introduce the time-lag between the bites and the production of antibodies (Lines 417-419) based on previous studies, mainly conducted on malaria vectors salivary peptide which showed that this time-lag was about three- to four-weeks (Drame et al. 2010). We added a sentence in the Discussion section to highlight the need of further research on the kinetics of IgG production and temporal waning (diminishing) antibody titres following *Aedes* bites (Ln. 490-492).

Supplemental Table 3. It looks like all the $<.0001$ should be <0.0001 .

We corrected the p -values in the supplementary Table S3.

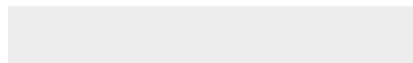
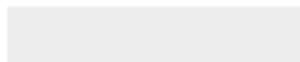
Reviewer #2: This is a study on the use of the biomarker of exposure to the mosquito bite and its relationship with entomological indicators and individual risk factors. The article is relevant and original. However, some changes to the text are necessary to improve clarity in the presentation of results and discussion.

We hope that the modifications made in the article will improve the global clarity of the results and the discussion for the reader.



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Revised Article with Changes Highlighted
Manuscript_revised_V4.docx



Chapter 4: Assessing the impact of vector control intervention on the selection of insecticide resistance genes in dengue vectors

In the previous chapter we have shown that the levels of individual response to the *Aedes* salivary biomarker were lower in the treated cluster compared to the control one's, hence suggesting potential reduction in *Aedes* density following the implementation of vector control. Although investigations are still on-going to assess the efficacy and residual activity of PPF in the study area, preliminary findings suggest however a lack of impact of the PPF on several entomological indices (Overgaard personal communication). Several operational factors could explain this outcome including an insufficient treatment coverage, inadequate frequency of application, inadequate dose or both. These assumptions are currently tested by the DENGUE INDEX team through complementary semi-field experimental studies. Another unaddressed explanation is the potential selection of PPF resistance/tolerance following the deployment of the insecticide in the treated area. Indeed, cross-resistance between PPF and PYRs has been detected previously and this may impede the benefit of using this new molecule for vector control in Thailand (see section for details 3.3.3.2).

Considering the above, we conducted a monitoring of insecticide resistance in the study area before, during and after the deployment of the intervention. Briefly, PPF was deployed in half of the clusters after ten months of follow up. The objective was to determine the baseline resistance status of *Aedes aegypti* to PPF and to conventionally used public health pesticides (baseline) and to assess change in the levels and frequency of relevant candidate markers after the deployment of the intervention. We assumed that the subsequent use of PPF in nine clusters of the study area (equivalent to 1,226 m²) may induce a selection pressure on resistance mechanisms already present in the population (see section 3.3.3.2). The main findings, that have not been published yet, are summarized in the following section.

Summary of results:

Among the eighteen clusters included in the RCT (see section 4.3), ten were randomly selected and followed up for the resistance survey (six clusters in the treated area and four clusters in the control area). Firstly, the study demonstrated the presence of high levels of insecticide resistance in *Ae. aegypti* populations in KK. Indeed, adult mortality ranged from 0% to 37.5% and

from 57% to 81% with the WHO discriminating concentrations of permethrin (0.25%) and deltamethrin (0.03%), respectively (World Health 2016). In addition, baseline susceptibility levels against various larvicides demonstrated high levels of resistance to temephos (with resistance ratio 50 (RR50) ranging from 1.8 to 28.4) in field populations compared to the susceptible reference colony (Bora). As expected, most of the *Ae. aegypti* populations were susceptible to PPF in baseline (i.e., prior intervention). After six months of treatment, PPF “tolerance” was reported in two clusters of the treated area (Table 6). More importantly, after one year of treatment, PPF resistance was reported in four cluster of the treated zone and in one cluster of the control zone (RR50 ranging from 0.33 in cluster 4018 to 22.3 in cluster 4003, and RR50 up to 16.98 in the control cluster 4017). Although baseline data were missing for some sites, our results suggest a rapid selection of PPF resistance in *Ae. aegypti* in Khon Kaen city following the introduction of PPF for vector control.

Table 6: Evolution of pyriproxyfen resistance in Aedes populations in different clusters of Khon Kaen following implementation of vector control intervention.

<i>Aedes</i> population	Treated/control clusters	After 6 months of PPF treatment		After 1year of PPF treatment	
		RR50	[95% CI]	RR50	[95% CI]
4002	Treated	0.01	[0.01-0.03]	3.66	[5.40-2.48]
4003	Treated	0.00	[0.00-0.00]	22.29	[30.3-16.4]
4007	Control	ND	ND	0.59	[0.60-0.58]
4008	Treated	ND	ND	11.13	[14.2-8.72]
4009	Control	ND	ND	2.34	[4.25-1.29]
4010	Control	0.01	[0.00-0.02]	1.03	[1.13-0.94]
4015	Treated	1.54	[1.55-1.54]	2.06	[2.45-1.72]
4016	Treated	ND	ND	12.80	[13.9-11.8]
4017	Control	ND	ND	16.98	[24.5-11.7]
4018	Treated	3.39	[0.65-17.6]	0.33	[0.34-0.32]

ND: not determined

The second part of the study aimed to address changes in the frequency of known insecticide resistance markers following the implementation of PPF. In ten clusters, about 30 individuals of adult *Ae. aegypti* were sampled before and after intervention, and were subjected to molecular assays for the detection of the V1016G and F1534C *kdr* mutations. Overall, the mutation 1016G was founded at low to medium frequencies (10-52%) except in one cluster where the 1016G

mutation was rare (3%). Similar results were observed with the 1534C *kdr* mutation, which was found at moderate prevalence (39-50%) among all clusters but one (<5% *kdr* frequency). Only few populations were at Hardy-Weinberg Equilibrium for the V1016G and 1534C mutations, hence suggesting strong selection pressure on resistant alleles. Surprisingly, no homozygote resistant individuals for the 1534C, neither double homozygote for 1016G and 1534C, were found among the mosquito populations, hence suggesting linkage disequilibrium between these two mutations. Overall, no significant changes in *kdr* resistant allele frequency were however reported after one year of PPF intervention.

Copy Number Variations (CNV) of six metabolic candidate genes related to insecticide resistance (GSTE2, CYP6-like CYP6Z8, CYP9J28, CYP6BB2 and CCEAE3A) and two domestic genes (CYP4D39 and RPS7) were investigated in seven mosquito pools per population (including Bora) before and after PPF intervention. Gene selection was based on previous studies which found significant correlation between CNV of these genes and PYR and/or OP resistance (Faucon et al. 2017, Cattel et al. 2020a). At baseline, the CCEAE3A gene displayed CNV comprised between 2 to 16 compared to the susceptible reference Bora strain. After one year of PPF treatment, CNV of CCEAE3A ranged from 1 and 8 (Figure 35), hence reflecting a decrease in CNV following PPF intervention. However, no duplications of CYP genes previously associated with higher detoxification of PYR or PPF were found before or after PPF intervention. Additionally, our results suggested a simple duplication event in GSTE2 gene in field populations compared to the Bora strain with no major changes in copy number over time. Overall, we showed that PPF did not significantly impact on CNV selection except for the CCEAE3A gene for which the number of copy variants decreased after treatment (i.e., 4002, 4003, 4008, 4015, 4016, 4018 populations) (Figure 35). The reduction in CCEAE3A copy number, which is associated to OP resistance, may reflect a strong fitness-cost associated to CCEAE3A in the absence treatment. The replacement of temephos (OP) by other larvicides with unrelated mode of action could then negatively impact on temephos resistance in the field.

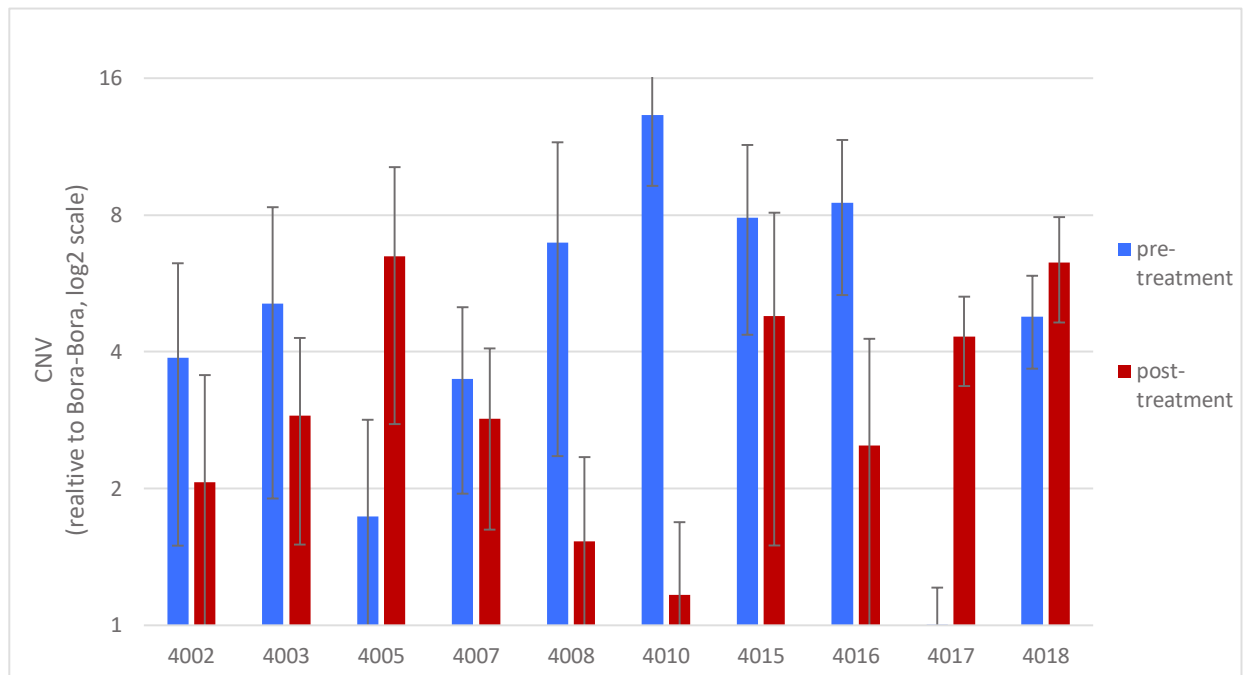


Figure 35: Copy number variant in CCEAE3A gene in *Ae. aegypti* populations before and after PPF treatment. The bars represent the average CNV for the CCEAE3A gene before (in blue) and after one year (in red) of PPF treatment in *Ae. aegypti* populations from treated (bold) and control clusters of the RCT conducted in north-eastern Thailand.

To conclude, *Ae. aegypti* populations from KK showed high levels of resistance to public health insecticides (PYR, OPs) that may reduce the efficacy of vector control interventions for dengue prevention. More worrying, our results demonstrated a rapid increase in PPF resistance (less than 1 year) after the deployment of the intervention that might partially explain the lack of entomological impact of PPF as deployed in the RCT. No clear association between CNVs and PPF resistance was however demonstrated hence indicating that the phenotypic resistance is probably caused by other (metabolic) genes than those investigated in the current study. Clearly further research is needed to assess the genetic causes of PPF resistance in KK to guide the choice of insecticides to use for vector control.

Fourth part: Discussion and perspectives of the thesis

In Thailand, dengue is a major cause of hospitalisations and deaths especially among children <15 years old. Dengue transmission occurs throughout the year yet, the rainy season is always associated with higher dengue incidence. Major dengue major occurs every three to six years (van Panhuis et al. 2015, Churakov et al. 2019) resulting in pluri-annual seasonal variations, with intra and inter-epidemic periods. Large epidemic episodes are generally caused by changes in serotype distribution in a given area but climatic factors, vector dynamic and individual characteristics also play a role in the virus circulation's dynamic. Due to highly seasonal and cyclical variations of serotypes, dengue outbreaks remain difficult to predict.

In north-eastern Thailand, a clear seasonal pattern of dengue incidence was observed with climatic factors, especially rainfall and temperature, explaining a significant part of dengue transmission risk (Phanitchat et al. 2019). Previous studies showed positive association between dengue and temperature and this can be explained by an increase in viral replication in the mosquito (hence, shortening the time needed for the vector to become infective) and enhanced vectorial capacity (Christofferson and Mores 2016, Liu et al. 2017). Although important, climatic variations did not explain the spatial clustering of dengue cases in Thailand. In KK and RE, we showed that dengue incidence was driven by other factors including individual age and levels of urbanization. Unfortunately, other factors such as human population movement and/or vector dynamics, such as seasonal changes in vector densities, that are known to influence the modalities of transmission were not explored due to the lack of relevant entomological data. For example, human movement is an important risk factor for dengue and rural-urban migration is common in Thailand, with people drawn by, for example, better education, job opportunities, health facilities, standard of living, and wages (Katewongsa 2015). Further information on human travel history and working conditions would have been required to address the impact of travel-related infection on dengue incidence in the study area. Our findings also highlighted the need to fill several knowledge gaps with regards to vector dynamic and socio economic and environmental factors that could contributed to the spatial clustering of cases dengue between rural and urban areas.

Nonetheless, such retrospective study comes with the inherent limitations of the data collection using passive case detection (from public and private health care centres, clinics, and hospitals) including underreporting and misreporting of symptomatic cases as well as the absence

of subclinical and asymptomatic infections. In Thailand, dengue diagnosis is mostly based on clinical signs, and only severe cases are subjected to laboratory diagnosis (see section 1.6). Consequently, only a small portion of dengue cases are submitted to dengue virus detection and/or serotyping. Thus, it is likely that a substantial part of dengue cases is ignored hence biasing epidemiology studies. Extensive use of RDT and systematic DENV serotyping in dengue-like symptoms patients would benefit surveillance systems to accurately diagnose dengue and provide real time information on the circulating serotypes. Considering dengue illness time-course, RDT should combine both NS1 and IgM/IgG detection to avoid exclusion of dengue cases.

As part of the national plan and strategies for dengue prevention and control through the Vector-Borne Disease Bureau regulations, when a dengue case is detected at the hospital and reported to the national dengue surveillance system (report 506), Surveillance and Rapid Response Teams (SRRT) are deployed in dengue-positive patient households to eliminate the vector. Since the early 1990's, Thailand has moved toward a decentralized model of vector-borne disease control resulting in the reorganization of disease control operations. Currently 76 provincial administrations being aggregated into 22 regional ODPCs are carrying routine surveillance and control operations with more or less success (Bhumiratana et al. 2014). Despite local and regional plan for dengue vector control and surveillance, the entomological thresholds defined by the MoPH, have not been generally adopted to address dengue transmission risk or to trigger vector control intervention. In addition, the decentralization of vector control has led to differences in VBDU leaderships and capacities resulting in varying efficacy in assessing dengue vector risk (Bhumiratana et al. 2014). Differences between/within provinces may lead to differences in vector surveillance and monitoring efficiency and in planning vector control interventions. While policies and strategies are still decided at the national level, the implementation of prevention and control remain under the authority of local VBUDs which may differ in terms of legislation and practices for disease prevention and control. Additionally, policies at the local level are challenged by the local administration, socio-political and socio-economic circumstances which differ between districts. For instance, the number of SRRT capacity is limited per province, thus they can intervene only for a restricted number of dengue cases, favouring the spread of the disease. Additionally, the resources allocated for vector control are planned according to the forecasts from MoPH and remaining chemicals for previous years (Suphanchaimat et al. 2019). Therefore, some

regions may lack of adequate human and financial resources to effectively control the vectors where dengue outbreak occur.

Recent efforts have been made to improve dengue surveillance and vector control through the WHO-GVCR which emphasises on the development of more cost-effective and practical tools for vector surveillance. Despite extensive research and numerous prediction models, no threshold and indicators could be clearly established to predict transmission risk and prevent outbreaks (Lauer et al. 2018, Phanitchat et al. 2019). In Thailand, dengue incidence is heterogeneous with distinct patterns of transmission within regions that makes dengue prediction particularly difficult to establish (Lauer et al. 2018, Phanitchat et al. 2019). Disease transmission is complex and involves many climatic, environmental and socio economical parameters that are rarely accounted in dengue transmission studies. For all these reasons, outbreaks continue to occur and the overall dengue incidence increased despite intensive efforts of national program to control the disease. More cost-effective approaches and practical tools that can reliably measure real-time dengue transmission dynamics are needed to enable more accurate and useful predictions of dengue incidence and outbreaks.

The current thesis was conducted in the framework of the DENGUE INDEX project that aimed to develop more practical and sensitive tools and indicators of dengue transmission risk in Thailand that may be used to forecast dengue outbreaks. The scope was to address the current challenges and limitations in dengue surveillance by exploring the potential of combined entomological and serological tools for assessing dengue transmission risk and to identify the determinants associated with human–*Aedes* relationships. This has been possible through the integration of multiple disciplines (entomology, immunology, virology, mathematics, etc), approaches (retrospective, case-control study, randomized controlled studies) and competences. The expected outcome was to validate the use of serological biomarkers of human exposure to *Aedes* bites as a proxy for estimating dengue transmission “hotspots” and “hot-pops” in North-eastern Thailand facing increasing outbreaks. The following sections will discuss the strength and weakness of entomology and immunology indicators for dengue epidemiology studies and provide guidance on how serological biomarkers could be further incorporated into national control programme for integrated vector surveillance. Potential applications of such serological

biomarkers of exposure to *Aedes* vector bites in the field of operational research, especially for evaluating vector control interventions are also discussed.

Challenges in assessing dengue transmission risk using conventional entomology indices

During the case control study conducted in north-eastern Thailand, we showed that the presence of DENV-infected *Ae. aegypti* in the households was positively associated with a higher risk of dengue. This confirms previous studies demonstrating good association between vector's infection and dengue transmission risk (Lau et al. 2017, Parra et al. 2018). In the study area, the prevalence of DENV-positive *Ae. aegypti* mosquitoes was high (13%), hence reflecting a high dengue transmission setting. Detection of dengue serotypes in mosquito vectors could be informative to detect the onset of an outbreak; if a serotype was absent for a long time, a larger part of the population will be naive to it, hence intensity of transmission may be greater when re introduced. Although vector infectivity might be a good proxy for assessing transmission risk, DENV detection in adult mosquitoes is rarely implemented in routine surveillance as it costly and time-consuming and it requires large sample size to get a robust estimate of virus circulation. Recent studies in Latin America showed that the monitoring of transovarial transmission of dengue virus in immature *Aedes* populations (known as xenomonitoring strategy) was a suitable strategy to enable rapid viral monitoring in areas of difficult access and to assess the progression of dengue disease (Arunachalam et al. 2008, Cruz et al. 2015, da Costa et al. 2017). Although promising, more investigations are needed to assess whether DENV xenomonitoring can enable timely identification of viral serotype's circulation and contribute to the development of more accurate predictive models and warning systems for preventing outbreaks.

Our entomology surveys showed however that the levels of *Aedes* infestation based on immature indices (HI, BI, and CI) were all negatively associated with dengue fever. Most of the inspected household (regardless dengue status) had water-holding containers positive for *Aedes* immature stages and entomology indices were higher than the “outbreak-risk” thresholds setting up by the MoPH of Thailand. The same was true with regards to adult abundance, where more *Aedes* were found in control households than in houses with a recent dengue case. Altogether these findings emphasized the lack of sensitivity of both immature and adult indices to predict dengue transmission risk and highlighted the need to quickly re-evaluate entomology thresholds for vector

surveillance and control. The lack of positive association between vector infestation (presence/abundance) and epidemiology outcomes (DENV infections) can be explained by several factors.

Firstly, we assumed that measurement of entomology indices may have been biased by vector control operations in case households following onset of dengue symptoms, which would have reduced vector infestation at the survey time point. However, mosquito collections at patient houses were conducted within 12 hours following patient inclusion, hence limiting the risk that SRRTs may have visited the household before our team. A higher use of household insecticide products in the case houses was however reported through the questionnaire survey, hence highlighting the possibility that some adult *Aedes* mosquitoes may have escaped the dwellings. Further investigations are needed to assess how individual protection measures may have impacted on vector density and thus dengue infections in the study area.

Another explanation for the lack of correlation between household-based entomological indices and dengue cases is the possibility that dengue transmission occurred in other locations than the household patients. Indeed, *Aedes* mosquitoes bite during the day, so people might be infected when they are in workplaces, schools and shops centers as previously demonstrated (Ratanawong et al. 2016). Information on human movement collected through social survey showed that only 27.6% of the patients declared staying at home during the weekdays (most of kids spending daytime at school), hence suggesting that a relatively high part of dengue transmission may have occurred elsewhere (Ratanawong et al. 2016). Several studies demonstrated that dengue transmission is mainly driven by human movements (Stoddard et al. 2013, Vazquez-Prokopec et al. 2013, Reiner et al. 2014) that may favor virus dispersion from high DENV transmission setting to low (non-immune) DENV transmission areas. Sensing human movements using GPS tracking could help to quickly identify the routes of virus circulation and trigger timely vector control response.

To conclude, despite the worldwide use of entomology endpoints for dengue surveillance, the correlation between entomological indices and dengue transmission risk remains unclear (Romero-Vivas and Falconar 2005, Bowman et al. 2014, Chang et al. 2015, Wijayanti et al. 2016b, Lau et al. 2017, Parra et al. 2018). Differences in study design, especially the spatial unit used to calculate entomological and epidemiological indices may also explain the lack of accuracy in

prediction's (Bowman et al. 2014). For example, a large spatial unit can mask hotspots of DENV transmission and/or vector abundance. Vector density, which varies itself over time, can be influenced by housing density and human movement, and so, the entomological collections have to be done quickly after clinical diagnosis (Chadee 2009). This would help reducing time-lag between entomology and epidemiology assessment hence improving prediction accuracies.

Potential of serological biomarkers for assessing *Aedes*-human relationships

Results from our randomized controlled trials in the two cities of RE and KK showed a strong and positive “dose-response” relationship between the *Aedes* abundance and the Ab response to the *Aedes* salivary biomarker, hence indicating that individuals exhibiting higher antibody response to the *Aedes* salivary peptide were also at higher risk of dengue vector bites. Overall, our study demonstrated that the intensity of Ab response varied according to the season, individual (gender, age, occupation) and household characteristics. Previous studies already demonstrated good correlations between the Ab response to *Aedes* salivary biomarker and entomology indices as well as with climatic and environmental factors (Elanga Ndille et al. 2012, Sagna et al. 2018, Yobo et al. 2018). Our study highlighted the potential of using *Aedes* salivary biomarker to assess fine scale variations of human-*Aedes* exposure that could be used to identify, target, and prioritize vector surveillance and control operations in areas with high risk of arbovirus transmission. This tool may be particularly relevant for invasive species such as *Aedes albopictus* that is currently invading new areas and territories, and for which timely identification of mosquito “hot-spots” could trigger locally adapted control response to prevent further establishment and spread.

In theory, serological surveys could be easier to implement in the field compared to entomology surveys as only a small amount of blood (< 1mL collected on filter papers as dried blood spots) could be sufficient to assess mosquito-exposure risk. Despite easy and cheap collection method, ELISA tests require highly qualified staff and specific equipment that is not easily achieved by national control programmes. A promising alternative to ELISA may come from the development of quantitative point-of-care (POC) test based on immunochromatography to enable a rapid and easy detection of IgG Ab response to *Aedes* salivary antigen. The development of POC device has been possible through public-private partnership including IRD and DIAG4ZOO (Montpellier, France) that has validated the “proof of concept”. Briefly, the

principle relies on colour lines that appear after applying a finger prick of blood to the test well. Although promising, the development of POC to stratify dengue vector exposure risk based on Ab response thresholds will require further research and validation using samples coming various entomological settings.

Potential of serological biomarkers for assessing dengue transmission risk

Although prosing for vector surveillance, serology biomarkers were initially designed to identify areas (hotspot) and people (hotpops) at high risk for vector-borne diseases (Sagna et al. 2018). Additionally, our serological longitudinal study demonstrated a strong and positive correlation between the intensity of individual immune response and *Aedes* densities. However, our case-control study conducted in North-eastern Thailand didn't show however significant association between the levels of Ab response to *Aedes* salivary peptide and dengue fever. This result suggests that *Aedes* salivary biomarker may be not accurate enough to assess spatio-temporal variations in disease transmission risk. Similar results were obtained by Elanga et in Vientiane, Lao PDR (Elanga Ndille et al. 2014) where no significant differences in the level of IgG response to the Nterm-34 kDa peptide was seen between DENV-positive and DENV-negative individuals using passive case detection. In this study however, only 45% of the studied population was "immune-responder" and the median IgG response for both groups (i.e., DENV positive and DENV negative) was below the immunological threshold hence reflecting a low *Aedes*-exposure area. In our study, individuals were however located in high *Aedes* exposure area as demonstrated by the high levels of entomological indices and the high seroprevalence to the Nterm-34 kDa peptide.

Several explanations can explain the lack of relationships between *Aedes*-exposure risk and dengue. First of all, in our case control study, the sample size was limited (368 dried spots were analysed) hence limiting the power of the analysis. Moreover, the retrospective case-control design means the temporal sequence of events cannot be determined with accuracy. In particular, immunological (and entomology) data were collected following patient recruitment. Indeed, symptoms of dengue fever can appear as quickly as a few days after DENV transmission (typically incubation period between 4-7 up to 14 days), delaying the recruitment of patients and therefore the entomological and serological surveys. Moreover, 3-4 weeks' time are generally needed to develop specific IgG Ab response to the salivary peptide hence the concordance between

serological outcomes and dengue infection is uncertain. This temporal disconnection between acquiring an infection to time of presenting illness and testing (i.e., identification of a case) may greatly affect attempts to link indicators of transmission with actual study design. Unfortunately, the relationship between dengue incidence and the intensity of the human-antibody response to *Aedes* bites could not be addressed through longitudinal follow up because no incident dengue cases were detected in household participants during the randomized controlled trial.

Finally, the role of acquired immunity against dengue fever in the lack of correlation between vector abundance or exposure and dengue infection cannot be ruled out. Indeed, the partial acquired immunity against one or more serotypes make the relationship between the vector risk and transmission even more complex to address, as only naïve individuals can be infected by a defined serotype. Thus, a non-negligible proportion of infective bites, may be not followed by dengue infections/fever in immune individuals. In our study, individuals with higher Ab response to *Aedes* mosquito bites had also higher odds of being positive for DENV-IgG. This suggests that individuals at high risk of *Aedes* bites were partially (or fully) protected against new dengue infections. Information's on herd immunity and serotype prevalence are keys to address the complex relationships between human-vector contact and dengue in high transmission settings. More evidence using robust experimental design will be needed to assess whether *Aedes* salivary biomarker can be used to identify foci of dengue transmission in Thailand and abroad.

Prospect of serological biomarkers for assessing vector control interventions

Salivary biomarkers showed promising results to evaluate the efficacy of vector control interventions, such as insecticide treated nets, for malaria control (Drame et al. 2010a, Drame et al. 2010b, Drame et al. 2013). Likewise, the Nterm-34 kDa salivary peptide proved to be a sensitive tool to evaluate the efficacy of an integrated approach combining environmental management and space sprays with PYR against *Ae. albopictus* in La Reunion (Elanga Ndille et al. 2016). The authors showed a significant reduction in the level of Ab response to the Nterm-34 salivary peptide two weeks post-treatment and the seroprevalence among the inhabitants remained low up to four weeks. In our randomized controlled study, we showed that the levels of IgG response to the Nterm-34 kDa were significantly lower in PPF-treated clusters compared to control clusters. Although speculative, these findings may reflect a reduction of vector densities after PPF treatment that would be sufficient to reduce human-*Aedes* contact in treated group compared to the control.

Unfortunately, the entomological impact of PPF is yet unknown and statistical analysis are under investigations by our team to fill this gap. Further evidence is needed to assess whether salivary biomarker may complement existing tools for monitoring and evaluation of vector control intervention. Serological biomarkers could be particularly relevant in areas where epidemiology studies cannot be implemented for operational and/or economic constraints.

Barriers to dengue vector control in Thailand

As seen previously, only SRRTs are mandated to eliminate the mosquito vector and interrupt dengue transmission. However, the same chemicals, especially temephos and deltamethrin, are used in routine for vector control operations in dengue-positive households but their efficacy to reduce or prevent dengue transmission has not been clearly demonstrated (Phuanukoonnon et al. 2005, Bowman et al. 2016). Chemical control is also challenged by the various operational constraints including community acceptance, lack of resources (e.g., expired insecticides, household aerosols) and the occurrence and spread of insecticide resistance to the main public health pesticides (Corbel et al. 2013, Suphanchaimat et al. 2019), hence representing an additional obstacle to dengue prevention. In our study, we showed strong levels of insecticide resistance against public health vector control insecticides in *Aedes* population from KK. Moreover, we demonstrated a rise of PPF resistance within one year after the implementation of PPF-intervention in treated clusters, hence indicating a strong selection pressure on resistance genes. The investigation of resistance mechanisms revealed a selection pressure on *kdr* mutations yet, without correlation with PPF application. Moreover, no CNVs in metabolic genes previously associated with PPF resistance were observed hence underlying that other metabolic genes may be involved. Interestingly, CNVs in CCE genes associated with OP resistance (Grigoraki et al. 2016, Moyes et al. 2017, Cattell et al. 2020b) were reduced following PPF intervention, hence suggesting that temephos resistance could be reverted in absence of temephos treatment. Unfortunately, the resistance levels to temephos couldn't be determined after 1 year-time due to a lack of samples for testing. If confirmed, this would be an excellent new for vector control with the scope to preserve the lifespan of existing public health pesticides for dengue prevention, as some genes might be involved in resistance against different insecticides.

To conclude, this study highlights the rationale of introducing alternative vector control methods as a part Integrated Vector Management (IVM) in Thailand and abroad. An alternative

would be to implement sequence or rotations of unrelated insecticides (minimum 3) to reduce the selection pressure on public health pesticides. Spinosad, a natural neurotoxic insecticide produced by the soil bacterium *Saccharopolyspora spinosa*, was initially selected together with PPF in the DENGUE INDEX project but we didn't receive approval by national authorities for further deployment. Insecticides still play a key role in the prevention and control of vector borne diseases and preserving insecticide susceptibility should be considered as a public good (Sternberg et al. 2018). As part of IRM, non-chemical-based control strategies should be privileged. The integration of alternative, safe and environmentally friendly methods relying on genetic or biological control of vectors should be encouraged to improve the control of resistant mosquitoes (Achee et al. 2019). Despite the great potential offer by new strategies for vector control to mitigate insecticide resistance, strong evidences are still missing for most. Further investigations are still needed to emphasize on the rationale of integrating these news strategies into IVM approaches.

Conclusion

The current thesis explored risk factors associated with *Aedes*-human exposure and dengue transmission risk in Thailand. Overall, we highlighted the complex relationships between *Aedes* abundance, vector infectivity, vector exposure and dengue infections in North-eastern Thailand facing recurrent and unpredictable outbreaks. We demonstrated the potential of using salivary biomarkers to assess fine scale variations in mosquito-exposure that could be further deployed as part of integrated vector surveillance. In contrast, we didn't show positive correlation between the levels of mosquito exposure risk (as measured by entomology and immunological indices) and dengue fever hence highlighting the limitations of existing tools for assessing dengue transmission risk. Several factors including human movements and habits, acquired immunity and presence of vector control partially explained that trend. Altogether, this thesis represents an important step toward the development of serological biomarkers for assessing *Aedes* exposure risk and to evaluate vector control intervention for dengue prevention. Further investigations are needed however to develop more sensible and accurate tools to measure real-time dengue transmission and predictive models of dengue outbreaks.

References

- Abreu, F. V., M. M. Morais, S. P. Ribeiro and E. Eiras Á (2015). "Influence of breeding site availability on the oviposition behaviour of *Aedes aegypti*." Mem Inst Oswaldo Cruz **110**(5): 669-676.
- Achee, N. L., J. P. Grieco, H. Vatandoost, G. Seixas, J. Pinto, L. Ching-Ng, A. J. Martins, W. Juntarajumnong, V. Corbel, C. Gouagna, J. P. David, J. G. Logan, J. Orsborne, E. Marois, G. J. Devine and J. Vontas (2019). "Alternative strategies for mosquito-borne arbovirus control." PLoS Negl Trop Dis **13**(1): e0006822.
- Agarwal, A., S. Gupta, T. Chincholkar, V. Singh, I. K. Umare, K. Ansari, S. Paliya, A. K. Yadav, R. Chowdhary, S. Purwar and D. Biswas (2019). "Co-circulation of dengue virus serotypes in Central India: Evidence of prolonged viremia in DENV-2." Infect Genet Evol **70**: 72-79.
- Aida, H. N., H. Dieng, T. Satho, A. T. Nurita, M. R. C. Salmah, F. Miake, B. Norasmah and A. H. Ahmad (2011). "The biology and demographic parameters of *Aedes albopictus* in northern peninsular Malaysia." Asian Pacific Journal of Tropical Biomedicine **1**(6): 472-477.
- Akter, R., S. Naish, M. Gatton, H. Bambrick, W. Hu and S. Tong (2019). "Spatial and temporal analysis of dengue infections in Queensland, Australia: Recent trend and perspectives." PLoS one **14**(7): e0220134-e0220134.
- Alen, M. M., K. Dallmeier, J. Balzarini, J. Neyts and D. Schols (2012). "Crucial role of the N-glycans on the viral E-envelope glycoprotein in DC-SIGN-mediated dengue virus infection." Antiviral Res **96**(3): 280-287.
- Alera, M. T., A. Srikiatkachorn, J. M. Velasco, I. A. Tac-An, C. B. Lago, H. E. Clapham, S. Fernandez, J. W. Levy, B. Thaisomboonsuk, C. Klungthong, L. R. Macareo, A. Nisalak, L. Hermann, D. Villa and I. K. Yoon (2016). "Incidence of dengue virus infection in adults and children in a prospective longitudinal cohort in the Philippines." PLoS Negl Trop Dis **10**(2): e0004337.
- Ali, A., H. Ahmad, M. Idrees, F. Zahir and I. Ali (2016). "Circulating serotypes of dengue virus and their incursion into non-endemic areas of Pakistan; a serious threat." Virol J **13**: 144.
- Aliota, M. T., S. A. Peinado, I. D. Velez and J. E. Osorio (2016). "The wMel strain of *Wolbachia* reduces transmission of zika virus by *Aedes aegypti*." Sci Rep **6**: 28792.
- Alphey, L., A. McKemey, D. Nimmo, M. Neira Oviedo, R. Lacroix, K. Matzen and C. Beech (2013). "Genetic control of *Aedes* mosquitoes." Pathog Glob Health **107**(4): 170-179.
- Alvarado-Castro, V., S. Paredes-Solís, E. Nava-Aguilera, A. Morales-Pérez, L. Alarcón-Morales, N. A. Balderas-Vargas and N. Andersson (2017). "Assessing the effects of interventions for *Aedes aegypti* control: systematic review and meta-analysis of cluster randomised controlled trials." BMC Public Health **17**(Suppl 1): 384.
- Alyousefi, T. A., R. Abdul-Ghani, M. A. Mahdy, S. M. Al-Eryani, A. M. Al-Mekhlafi, Y. A. Raja, S. A. Shah and J. C. Beier (2016). "A household-based survey of knowledge, attitudes and practices towards dengue fever among local urban communities in Taiz Governorate, Yemen." BMC Infect Dis **16**(1): 543.
- Amarasinghe, A., J. N. Kuritsk, G. W. Letson and H. S. Margolis (2011). "Dengue virus infection in Africa." Emerging infectious diseases **17**(8): 1349-1354.
- Anderson, K. B., S. Chunsuttiwat, A. Nisalak, M. P. Mammen, D. H. Libraty, A. L. Rothman, S. Green, D. W. Vaughn, F. A. Ennis and T. P. Endy (2007). "Burden of symptomatic dengue infection in children at primary school in Thailand: a prospective study." The Lancet **369**(9571): 1452-1459.

Anderson, K. B., R. V. Gibbons, D. A. T. Cummings, A. Nisalak, S. Green, D. H. Libraty, R. G. Jarman, A. Srikiatkachorn, M. P. Mammen, B. Darunee, I.-K. Yoon and T. P. Endy (2014). "A shorter time interval between first and second dengue infections is associated with protection from clinical illness in a school-based cohort in Thailand." *The Journal of Infectious Diseases* **209**(3): 360-368.

Andersson, N., J. Arostegui, E. Nava-Aguilera, E. Harris and R. J. Ledogar (2017). "Camino Verde (The Green Way): evidence-based community mobilisation for dengue control in Nicaragua and Mexico: feasibility study and study protocol for a randomised controlled trial." *BMC Public Health* **17**(Suppl 1): 407.

Andersson, N., E. Nava-Aguilera, J. Arosteguí, A. Morales-Perez, H. Suazo-Laguna, J. Legorreta-Soberanis, C. Hernandez-Alvarez, I. Fernandez-Salas, S. Paredes-Solís, A. Balmaseda, A. J. Cortés-Guzmán, R. Serrano de Los Santos, J. Coloma, R. J. Ledogar and E. Harris (2015). "Evidence based community mobilization for dengue prevention in Nicaragua and Mexico (Camino Verde, the Green Way): cluster randomized controlled trial." *BMJ* **351**: h3267.

Andries, A.-C., V. Duong, S. Ong, S. Ros, A. Sakuntabhai, P. Horwood, P. Dussart and P. Buchy (2016). "Evaluation of the performances of six commercial kits designed for dengue NS1 and anti-dengue IgM, IgG and IgA detection in urine and saliva clinical specimens." *BMC infectious diseases* **16**: 201-201.

Angel, B. and V. Joshi (2009). "Distribution of dengue virus types in *Aedes aegypti* in dengue endemic districts of Rajasthan, India." *Indian J Med Res* **129**(6): 665-668.

Arunachalam, N., S. C. Tewari, V. Thenmozhi, R. Rajendran, R. Paramasivan, R. Manavalan, K. Ayanar and B. K. Tyagi (2008). "Natural vertical transmission of dengue viruses by *Aedes aegypti* in Chennai, Tamil Nadu, India." *Indian J Med Res* **127**(4): 395-397.

Azevedo-Santos, V. M., J. R. Vitule, F. M. Pelicice, E. García-Berthou and D. Simberloff (2017). "Nonnative fish to control *Aedes* mosquitoes: a controversial, harmful tool." *BioScience* **67**(1): 84-90.

Bariami, V., C. M. Jones, R. Poupardin, J. Vontas and H. Ranson (2012). "Gene amplification, ABC transporters and cytochrome P450s: unraveling the molecular basis of pyrethroid resistance in the dengue vector, *Aedes aegypti*." *PLoS Negl Trop Dis* **6**(6): e1692.

Barnard, D. R., C. Z. Dickerson, K. Murugan, R. D. Xue, D. L. Kline and U. R. Bernier (2014). "Measurement of landing mosquito density on humans." *Acta Trop* **136**: 58-67.

Barrera-Perez, M. A., N. Pavia-Ruz, J. E. Mendoza-Mezquita, N. Torres-Arcila, R. Hernandez-Hernandez, F. Castro-Gamboa, E. Geded-Moreno, A. Cohuo-Rodriguez, A. Medina-Barreiro, E. Koyoc-Cardena, H. Gomez-Dantes, A. Kroeger, G. Vazquez-Prokopec and P. Manrique-Saide (2015). "Control of *Aedes aegypti* breeding sites with the program Recicla por tu bienestar in Merida, Mexico." *Salud Publica Mex* **57**(3): 201-210.

Barrera, R., V. Acevedo, G. E. Felix, R. R. Hemme, J. Vazquez, J. L. Munoz and M. Amador (2017). "Impact of autocidal gravid ovitraps on Chikungunya virus incidence in *Aedes aegypti* (Diptera: Culicidae) in areas with and without traps." *J Med Entomol* **54**(2): 387-395.

Barrera, R., A. Harris, R. R. Hemme, G. Felix, N. Nazario, J. L. Muñoz-Jordan, D. Rodriguez, J. Miranda, E. Soto, S. Martinez, K. Ryff, C. Perez, V. Acevedo, M. Amador and S. H. Waterman (2019). "Citywide control of *Aedes aegypti* (Diptera: Culicidae) during the 2016 zika epidemic by integrating community awareness, education, source reduction, larvicides, and mass mosquito trapping." *J Med Entomol* **56**(4): 1033-1046.

Bartlett-Healy, K., G. Hamilton, S. Healy, T. Crepeau, I. Unlu, A. Farajollahi, D. Fonseca, R. Gaugler, G. G. Clark and D. Strickman (2011). "Source reduction behavior as an independent measurement of the impact

of a public health education campaign in an integrated vector management program for the Asian tiger mosquito." Int J Environ Res Public Health **8**(5): 1358-1367.

Bazin, M. and C. R. Williams (2018). "Mosquito traps for urban surveillance: collection efficacy and potential for use by citizen scientists." J Vector Ecol **43**(1): 98-103.

Benelli, G., C. L. Jeffries and T. Walker (2016). "Biological control of mosquito vectors: past, present, and future." Insects **7**(4).

Benelli, G., A. B. B. Wilke and J. C. Beier (2020). "Aedes albopictus (Asian tiger mosquito)." Trends in Parasitology.

Bengoa, M., R. Eritja, S. Delacour, M. Miranda, A. Sureda and J. Lucientes (2017). "First data on resistance to pyrethroids in wild populations of Aedes albopictus from Spain." J Am Mosq Control Assoc **33**(3): 246-249.

Bente, D. A. and R. Rico-Hesse (2006). "Models of dengue virus infection." Drug discovery today. Disease models **3**(1): 97-103.

Bhatia, R., A. P. Dash and T. Sunyoto (2013). "Changing epidemiology of dengue in South-East Asia." WHO South East Asia J Public Health **2**(1): 23-27.

Bhatt, S., P. Gething, O. Brady, J. Messina, A. Farlow, C. Moyes, J. Drake, J. Brownstein, A. Hoen, O. Sankoh, M. Myers, D. George, T. Jaenisch, G. Wint, C. Simmons, T. Scott, J. Farrar and S. Hay (2013). "The global distribution and burden of dengue." Nature.

Bhumiratana, A., A. Intarapuk, S. Chujun, W. Kaewwaen, P. Sorosjinda-Nunthawarasilp and S. Koyadun (2014). "Thailand momentum on policy and practice in local legislation on dengue vector control." Interdiscip Perspect Infect Dis **2014**: 217237.

Biogents. (2020). "Aedes life cycle." Retrieved 01/09/2020, from <https://sea.biogents.com/life-cycle-aedes-mosquitoes/>.

Blacksell, S. D., R. G. Jarman, M. S. Bailey, A. Tanganuchitcharnchai, K. Jenjaroen, R. V. Gibbons, D. H. Paris, R. Premaratna, H. J. de Silva, D. G. Laloo and N. P. Day (2011). "Evaluation of six commercial point-of-care tests for diagnosis of acute dengue infections: the need for combining NS1 antigen and IgM/IgG antibody detection to achieve acceptable levels of accuracy." Clin Vaccine Immunol **18**(12): 2095-2101.

Bodinayake, C. K., L. G. Tillekeratne, A. Nagahawatte, V. Devasiri, W. Kodikara Arachichi, J. J. Strouse, O. M. Sessions, R. Kurukulasooriya, A. Uehara, S. Howe, X. M. Ong, S. Tan, A. Chow, P. Tummalapalli, A. D. De Silva, T. Østbye, C. W. Woods, D. J. Gubler and M. E. Reller (2016). "Emergence of epidemic dengue-1 virus in the southern province of Sri Lanka." PLoS neglected tropical diseases **10**(10): e0004995-e0004995.

Boonklong, O. and A. Bhumiratana (2016). "Seasonal and geographical variation of dengue vectors in Narathiwat, South Thailand." Can J Infect Dis Med Microbiol **2016**: 8062360.

Bourtzis, K., S. L. Dobson, Z. Xi, J. L. Rasgon, M. Calvitti, L. A. Moreira, H. C. Bossin, R. Moretti, L. A. Baton, G. L. Hughes, P. Mavingui and J. R. Gilles (2014). "Harnessing mosquito-Wolbachia symbiosis for vector and disease control." Acta Trop **132 Suppl**: S150-163.

Bouزيد, M., J. Brainard, L. Hooper and P. R. Hunter (2016). "Public health interventions for Aedes control in the time of zika virus- A meta-review on effectiveness of vector control strategies." PLoS Negl Trop Dis **10**(12): e0005176.

Bowman, L. R., S. Donegan and M. C. Pj (2016). "Is dengue vector control deficient in effectiveness or evidence? Systematic review and meta-analysis." PLoS Negl Trop Dis **10**.

Bowman, L. R., S. Runge-Ranzinger and P. J. McCall (2014). "Assessing the relationship between vector indices and dengue transmission: a systematic review of the evidence." PLoS Negl Trop Dis **8**(5): e2848.

Boyce, R., A. Lenhart, A. Kroeger, R. Velayudhan, B. Roberts and O. Horstick (2013). "Bacillus thuringiensis israelensis (Bti) for the control of dengue vectors: systematic literature review." Trop Med Int Health **18**(5): 564-577.

Bradshaw, C. J. A., B. Leroy, C. Bellard, D. Roiz, C. Albert, A. Fournier, M. Barbet-Massin, J.-M. Salles, F. Simard and F. Courchamp (2016). "Massive yet grossly underestimated global costs of invasive insects." Nature communications **7**: 12986-12986.

Brady, O., P. Gething, S. Bhatt, J. Messina, J. Brownstein, A. Hoen, C. Moyes, A. Farlow, T. Scott and S. Hay (2012). "Refining the global spatial limits of dengue virus transmission by evidence-based consensus." PLoS Negl Trop Dis.

Brady, O. J., M. A. Johansson, C. A. Guerra, S. Bhatt, N. Golding, D. M. Pigott, H. Delatte, M. G. Grech, P. T. Leisnham, R. Maciel-de-Freitas, L. M. Styer, D. L. Smith, T. W. Scott, P. W. Gething and S. I. Hay (2013). "Modelling adult Aedes aegypti and Aedes albopictus survival at different temperatures in laboratory and field settings." Parasit Vectors **6**: 351.

Brady, O. J., D. L. Smith, T. W. Scott and S. I. Hay (2015). "Dengue disease outbreak definitions are implicitly variable." Epidemics **11**: 92-102.

Bravo, L., V. G. Roque, J. Brett, R. Dizon and M. L'Azou (2014). "Epidemiology of dengue disease in the Philippines (2000-2011): a systematic literature review." PLoS Negl Trop Dis **8**(11): e3027.

Bregues, C., N. J. Hawkes, F. Chandre, L. McCarroll, S. Duchon, P. Guillet, S. Manguin, J. C. Morgan and J. Hemingway (2003). "Pyrethroid and DDT cross-resistance in Aedes aegypti is correlated with novel mutations in the voltage-gated sodium channel gene." Med Vet Entomol **17**(1): 87-94.

Britch, S., H. Nyberg, R. Aldridge, T. Swan and K. Linthicum (2016). "Acoustic control of mosquito larvae in artificial drinking water containers." Journal of the American Mosquito Control Association **32**: 341-344.

Brusich, M., J. Grieco, N. Penney, R. Tisgratog, W. Ritthison, T. Chareonviriyaphap and N. Achee (2015). "Targeting educational campaigns for prevention of malaria and dengue fever: an assessment in Thailand." Parasit Vectors **8**: 43.

Bureau of Epidemiology and Ministry of Public Health Thailand (2011). Annual epidemiological surveillance report 2010. Ministry of Public Health Thailand. Nonthaburi, Ministry of Public Health Thailand,.

Bureau of Epidemiology and Ministry of Public Health Thailand (2016). Annual epidemiological surveillance report 2015. Ministry of Public Health Thailand. Nonthaburi, Ministry of Public Health Thailand, : 1000.

Bureau of Epidemiology and M. o. P. H. Thailand (2015). Annual epidemiological surveillance report 2014. M. o. P. H. Thailand. Nonthaburi, Ministry of Public Health Thailand.

Bureau of Epidemiology and M. o. P. H. Thailand (2019). Annual epidemiological surveillance report 2018. Department of Disease Prevention: 808.

Calzolari, M. (2016). "Mosquito-borne diseases in Europe: An emerging public health threat." Reports in Parasitology **5**: 1.

Capeding, M. R., N. H. Tran, S. R. Hadinegoro, H. I. Ismail, T. Chotpitayasunondh, M. N. Chua, C. Q. Luong, K. Rusmil, D. N. Wirawan, R. Nallusamy, P. Pitisuttithum, U. Thisyakorn, I. K. Yoon, D. van der Vliet, E. Langevin, T. Laot, Y. Hutagalung, C. Frago, M. Boaz, T. A. Wartel, N. G. Tornieporth, M. Saville, A.

- Bouckenooghe and C. Y. D. S. Group (2014). "Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial." Lancet **384**(9951): 1358-1365.
- Caputo, B., A. Ienco, D. Cianci, M. Pombi, V. Petrarca and A. Baseggio (2012). "The "auto-dissemination" approach: a novel concept to fight *Aedes albopictus* in urban areas." PLoS Negl Trop Dis **6**.
- Carrieri, M., P. Angelini, C. Venturelli, B. Maccagnani and R. Bellini (2011). "*Aedes albopictus* (Diptera: Culicidae) population size survey in the 2007 Chikungunya outbreak area in Italy. I. Characterization of breeding sites and evaluation of sampling methodologies." J Med Entomol **48**(6): 1214-1225.
- Carvalho, D. O., A. R. McKemey, L. Garziera, R. Lacroix, C. A. Donnelly, L. Alphey, A. Malavasi and M. L. Capurro (2015). "Suppression of a field population of *Aedes aegypti* in Brazil by sustained release of transgenic male mosquitoes." PLoS Negl Trop Dis **9**(7): e0003864.
- Carvalho, F. D. and L. A. Moreira (2017). "Why is *Aedes aegypti* Linnaeus so successful as a species?" Neotropical Entomology **46**(3): 243-255.
- Castro, M. G., R. M. Nogueira, H. G. Schatzmayr, M. P. Miagostovich and R. Lourenco-de-Oliveira (2004). "Dengue virus detection by using reverse transcription-polymerase chain reaction in saliva and progeny of experimentally infected *Aedes albopictus* from Brazil." Mem Inst Oswaldo Cruz **99**(8): 809-814.
- Cattell, J., F. Faucon, B. Le Peron, S. Sherpa, M. Monchal, L. Grillet, T. Gaude, F. Laporte, I. Dusfour, S. Reynaud and J. P. David (2020a). "Combining genetic crosses and pool targeted DNA-seq for untangling genomic variations associated with resistance to multiple insecticides in the mosquito *Aedes aegypti*." Evol Appl **13**(2): 303-317.
- Cattell, J., C. Haberkorn, F. Laporte, T. Gaude, T. Cumer, J. Renaud, I. W. Sutherland, J. C. Hertz, J.-M. Bonneville, V. Arnaud, C. Noûs, B. Fustec, S. Boyer, S. Marcombe and J.-P. David (2020b). "A genomic amplification affecting a carboxylesterase gene cluster confers organophosphate resistance in the mosquito *Aedes aegypti*: from genomic characterization to high-throughput field detection." bioRxiv: 2020.2006.2008.139741.
- CDC. (2020). "Life cycle of *Aedes aegypti* and *Ae. albopictus* mosquitoes." Retrieved 10/09/2020, 2020, from <https://www.cdc.gov/mosquitoes/about/life-cycles/aedes.html>.
- Chadee, D. D. (2009). "Dengue cases and *Aedes aegypti* indices in Trinidad, West Indies." Acta Trop **112**(2): 174-180.
- Chadee, D. D., J. C. Beier and R. T. Mohammed (2002). "Fast and slow blood-feeding durations of *Aedes aegypti* mosquitoes in Trinidad." J Vector Ecol **27**(2): 172-177.
- Chan, M. and M. A. Johansson (2012). "The incubation periods of dengue viruses." PLoS One **7**(11): e50972.
- Chandel, K., D. S. Suman, Y. Wang, I. Unlu, E. Williges and G. M. Williams (2016). "Targeting a hidden enemy: pyriproxyfen autodissemination strategy for the control of the container mosquito *Aedes albopictus* in cryptic habitats." PLoS Negl Trop Dis **10**.
- Chang, F. S., Y. T. Tseng, P. S. Hsu, C. D. Chen, B. Lian le and D. Y. Chao (2015). "Re-assess vector indices threshold as an early warning tool for predicting dengue epidemic in a dengue non-endemic country." PLoS Negl Trop Dis **9**(9): e0004043.
- Chansang, U. R., A. Bhumiratana and P. Kittayapong (2004). "Combination of *Mesocyclops thermocyclopoides* and *Bacillus thuringiensis* var. *israelensis*: a better approach for the control of *Aedes aegypti* larvae in water containers." J Vector Ecol **29**(2): 218-226.

- Chen, C. F., P. Y. Shu, H. J. Teng, C. L. Su, J. W. Wu, J. H. Wang, T. H. Lin, J. H. Huang and H. S. Wu (2010). "Screening of dengue virus in field-caught *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) by one-step SYBR green-based reverse transcriptase-polymerase chain reaction assay during 2004-2007 in Southern Taiwan." Vector Borne Zoonotic Dis **10**(10): 1017-1025.
- Chen, H., K. Li, X. Wang, X. Yang, Y. Lin, F. Cai, W. Zhong, C. Lin, Z. Lin and Y. Ma (2016). "First identification of *kdr* allele F1534S in *VGSC* gene and its association with resistance to pyrethroid insecticides in *Aedes albopictus* populations from Haikou City, Hainan Island, China." Infect Dis Poverty **5**: 31.
- Chiaravalloti-Neto, F., M. Pereira, E. A. Favaro, M. R. Dibo, A. Mondini, A. L. Rodrigues-Junior, A. P. Chierotti and M. L. Nogueira (2015). "Assessment of the relationship between entomologic indicators of *Aedes aegypti* and the epidemic occurrence of dengue virus 3 in a susceptible population, Sao Jose do Rio Preto, Sao Paulo, Brazil." Acta Trop **142**: 167-177.
- Christofferson, R. C. (2015). "A Reevaluation of the role of *Aedes albopictus* in dengue transmission." J Infect Dis **212**(8): 1177-1179.
- Christofferson, R. C. and C. N. Mores (2016). "Potential for Extrinsic Incubation Temperature to Alter Interplay Between Transmission Potential and Mortality of Dengue-Infected *Aedes aegypti*." Environ Health Insights **10**: 119-123.
- Chumpu, R., N. Khamsemanan and C. Nattee (2019). "The association between dengue incidences and provincial-level weather variables in Thailand from 2001 to 2014." PLOS ONE **14**(12): e0226945.
- Churakov, M., C. J. Villabona-Arenas, M. U. G. Kraemer, H. Salje and S. Cauchemez (2019). "Spatio-temporal dynamics of dengue in Brazil: Seasonal travelling waves and determinants of regional synchrony." PLoS Negl Trop Dis **13**(4): e0007012.
- Corbel, V., N. L. Achee, F. Chandre, M. B. Coulibaly, I. Dusfour, D. M. Fonseca, J. Grieco, W. Juntarajumngong, A. Lenhart, A. J. Martins, C. Moyes, L. C. Ng, J. Pinto, K. Raghavendra, H. Vatandoost, J. Vontas, D. Weetman, F. Fouque, R. Velayudhan and J. P. David (2016). "Tracking insecticide resistance in mosquito vectors of arboviruses: The Worldwide Insecticide resistance Network (WIN)." PLoS Negl Trop Dis **10**(12): e0005054.
- Corbel, V., C. Durot, N. Achee, F. Chandre, M. Coulibaly, J.-P. David, G. Devine, I. Dusfour, D. Fonseca, J. Griego, W. Juntarajumngong, A. Lenhart, S. Kasai, A. Martins, C. Moyes, L. C. Ng, J. Pinto, J. Pompon, P. Müller and D. Weetman (2019). "Second WIN International Conference on "Integrated approaches and innovative tools for combating insecticide resistance in vectors of arboviruses", October 2018, Singapore." Parasites & Vectors **12**.
- Corbel, V., D. M. Fonseca, D. Weetman, J. Pinto, N. L. Achee, F. Chandre, M. B. Coulibaly, I. Dusfour, J. Grieco, W. Juntarajumngong, A. Lenhart, A. J. Martins, C. Moyes, L. C. Ng, K. Raghavendra, H. Vatandoost, J. Vontas, P. Muller, S. Kasai, F. Fouque, R. Velayudhan, C. Durot and J. P. David (2017). "International workshop on insecticide resistance in vectors of arboviruses, December 2016, Rio de Janeiro, Brazil." Parasit Vectors **10**(1): 278.
- Corbel, V., F. Nosten, K. Thanispong, C. Luxemburger, M. Kongmee and T. Chareonviriyaphap (2013). "Challenges and prospects for dengue and malaria control in Thailand, Southeast Asia." Trends Parasitol **29**(12): 623-633.
- Couret, J., E. Dotson and M. Q. Benedict (2014). "Temperature, larval diet, and density effects on development rate and survival of *Aedes aegypti* (Diptera: Culicidae)." PloS one **9**(2): e87468-e87468.

Crawford, J. E., D. W. Clarke, V. Criswell, M. Desnoyer, D. Cornel, B. Deegan, K. Gong, K. C. Hopkins, P. Howell, J. S. Hyde, J. Livni, C. Behling, R. Benza, W. Chen, K. L. Dobson, C. Eldershaw, D. Greeley, Y. Han, B. Hughes, E. Kakani, J. Karbowski, A. Kitchell, E. Lee, T. Lin, J. Liu, M. Lozano, W. MacDonald, J. W. Mains, M. Metlitz, S. N. Mitchell, D. Moore, J. R. Ohm, K. Parkes, A. Porshnikoff, C. Robuck, M. Sheridan, R. Sobecki, P. Smith, J. Stevenson, J. Sullivan, B. Wasson, A. M. Weakley, M. Wilhelm, J. Won, A. Yasunaga, W. C. Chan, J. Holeman, N. Snoad, L. Upson, T. Zha, S. L. Dobson, F. S. Mulligan, P. Massaro and B. J. White (2020). "Efficient production of male Wolbachia-infected *Aedes aegypti* mosquitoes enables large-scale suppression of wild populations." Nat Biotechnol **38**(4): 482-492.

Cruz, L. C., O. P. Serra, F. A. Leal-Santos, A. L. Ribeiro, R. D. Silhessarenko and M. A. Santos (2015). "Natural transovarial transmission of dengue virus 4 in *Aedes aegypti* from Cuiaba, State of Mato Grosso, Brazil." Rev Soc Bras Med Trop **48**(1): 18-25.

Cummings, D. A., R. A. Irizarry, N. E. Huang, T. P. Endy, A. Nisalak, K. Ungchusak and D. S. Burke (2004). "Travelling waves in the occurrence of dengue haemorrhagic fever in Thailand." Nature **427**(6972): 344-347.

da Costa, C. F., R. A. Dos Passos, J. B. P. Lima, R. A. Roque, V. de Souza Sampaio, T. B. Campolina, N. F. C. Secundino and P. F. P. Pimenta (2017). "Transovarial transmission of DENV in *Aedes aegypti* in the Amazon basin: a local model of xenomonitoring." Parasit Vectors **10**(1): 249.

David, J. P., F. Faucon, A. Chandor-Proust, R. Poupardin, M. A. Riaz, A. Bonin, V. Navratil and S. Reynaud (2014). "Comparative analysis of response to selection with three insecticides in the dengue mosquito *Aedes aegypti* using mRNA sequencing." BMC Genomics **15**: 174.

Day, J. F., J. D. Edman and T. W. Scott (1994). "Reproductive fitness and survivorship of *Aedes aegypti* (Diptera: Culicidae) maintained on blood, with field observations from Thailand." Journal of Medical Entomology **31**(4): 611-617.

De Paula, S. O. and B. A. L. d. Fonseca (2004). "Dengue: a review of the laboratory tests a clinician must know to achieve a correct diagnosis." Brazilian Journal of Infectious Diseases **8**: 390-398.

Degallier, N., J. P. Herve, A. P. Travassos da Rosa and G. C. Sa (1988). "[*Aedes aegypti* (L.): importance of its bioecology in the transmission of dengue and other arboviruses. I]." Bull Soc Pathol Exot Filiales **81**(1): 97-110.

Del Rio-Galvan, S. L., A. E. Flores, R. Barrera, B. Lopez-Monroy, G. Felix, M. Amador and G. Ponce-Garcia (2016). "Susceptibility to temephos and spinosad in *Aedes aegypti* (Diptera: Culicidae) from Puerto Rico." J Med Entomol **53**(5): 1211-1217.

Delatte, H., A. Desvars, A. Bouétard, S. Bord, G. Gimonneau, G. Vourc'h and D. Fontenille (2010). "Blood-feeding behavior of *Aedes albopictus*, a vector of Chikungunya on La Réunion." Vector Borne Zoonotic Dis **10**(3): 249-258.

Delatte, H., G. Gimonneau, A. Triboire and D. Fontenille (2009). "Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of *Aedes albopictus*, vector of chikungunya and dengue in the Indian Ocean." J Med Entomol **46**(1): 33-41.

Delatte, H., C. Paupy, J. S. Dehecq, J. Thiria, A. B. Failloux and D. Fontenille (2008). "*Aedes albopictus*, vector of chikungunya and dengue viruses in Reunion Island: biology and control." Parasite **15**(1): 3-13.

Dorigatti, I., S. Morrison, C. A. Donnelly, T. Garske, S. Bowden and A. Grills (2019). "Risk of yellow fever virus importation into the United States from Brazil, outbreak years 2016–2017 and 2017–2018." Scientific Reports **9**(1): 20420.

Doucoure, S. and P. M. Drame (2015). "Salivary biomarkers in the control of mosquito-borne diseases." Insects **6**(4): 961-976.

Doucoure, S., F. Mouchet, S. Cornelie, P. M. Drame, E. D'Ortenzio, J. S. DeHecq and F. Remoue (2014). "Human antibody response to *Aedes albopictus* salivary proteins: a potential biomarker to evaluate the efficacy of vector control in an area of chikungunya and dengue virus transmission." Biomed Res Int **2014**: 746509.

Doucoure, S., F. Mouchet, A. Cournil, G. Le Goff, S. Cornelie, Y. Roca, M. G. Giraldez, Z. B. Simon, R. Loayza, D. Misse, J. V. Flores, A. Walter, C. Rogier, J. P. Herve and F. Remoue (2012). "Human antibody response to *Aedes aegypti* saliva in an urban population in Bolivia: a new biomarker of exposure to dengue vector bites." Am J Trop Med Hyg **87**(3): 504-510.

Drame, P. M., A. Diallo, A. Poinsignon, O. Boussari, S. Dos Santos, V. Machault, R. Lalou, S. Cornelie, J. Y. LeHesran and F. Remoue (2013). "Evaluation of the effectiveness of malaria vector control measures in urban settings of Dakar by a specific *Anopheles* salivary biomarker." PLoS One **8**(6): e66354.

Drame, P. M., A. Poinsignon, P. Besnard, S. Cornelie, J. Le Mire, J. C. Toto, V. Foumane, M. A. Dos-Santos, M. Sembene, F. Fortes, F. Simondon, P. Carnevale and F. Remoue (2010a). "Human antibody responses to the *Anopheles* salivary gSG6-P1 peptide: a novel tool for evaluating the efficacy of ITNs in malaria vector control." PLoS One **5**(12): e15596.

Drame, P. M., A. Poinsignon, P. Besnard, J. Le Mire, M. A. Dos-Santos, C. S. Sow, S. Cornelie, V. Foumane, J. C. Toto, M. Sembene, D. Boulanger, F. Simondon, F. Fortes, P. Carnevale and F. Remoue (2010b). "Human antibody response to *Anopheles gambiae* saliva: an immuno-epidemiological biomarker to evaluate the efficacy of insecticide-treated nets in malaria vector control." Am J Trop Med Hyg **83**(1): 115-121.

Ducheyne, E., N. N. Tran Minh, N. Haddad, W. Bryssinckx, E. Buliva, F. Simard, M. R. Malik, J. Charlier, V. De Waele, O. Mahmoud, M. Mukhtar, A. Bouattour, A. Hussain, G. Hendrickx and D. Roiz (2018). "Current and future distribution of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in WHO Eastern Mediterranean Region." Int J Health Geogr **17**(1): 4.

Duong, V., L. Lambrechts, R. E. Paul, S. Ly, R. S. Lay and K. C. Long (2015a). "Asymptomatic humans transmit dengue virus to mosquitoes." Proc Natl Acad Sci U S A **112**.

Duong, V., L. Lambrechts, R. E. Paul, S. Ly, R. S. Lay, K. C. Long, R. Huy, A. Tarantola, T. W. Scott, A. Sakuntabhai and P. Buchy (2015b). "Asymptomatic humans transmit dengue virus to mosquitoes." Proc Natl Acad Sci U S A **112**(47): 14688-14693.

Dusfour, I., V. Thalmensy, P. Gaborit, J. Issaly, R. Carinci and R. Girod (2011). "Multiple insecticide resistance in *Aedes aegypti* (Diptera: Culicidae) populations compromises the effectiveness of dengue vector control in French Guiana." Mem Inst Oswaldo Cruz **106**(3): 346-352.

Dusfour, I., J. Vontas, J. P. David, D. Weetman, D. M. Fonseca, V. Corbel, K. Raghavendra, M. B. Coulibaly, A. J. Martins, S. Kasai and F. Chandre (2019). "Management of insecticide resistance in the major *Aedes* vectors of arboviruses: Advances and challenges." PLoS Negl Trop Dis **13**(10): e0007615.

Dusfour, I., P. Zorrilla, A. Guidez, J. Issaly, R. Girod, L. Guillaumot, C. Robello and C. Strode (2015). "Deltamethrin resistance mechanisms in *Aedes aegypti* populations from three french overseas territories worldwide." PLoS Negl Trop Dis **9**(11): e0004226.

Eilenberg, J., A. Hajek and C. Lomer (2001). "Suggestions for unifying the terminology in biological control." BioControl **46**(4): 387-400.

Elanga Ndille, E., S. Doucoure, G. Damien, F. Mouchet, P. M. Drame, S. Cornelié, H. Noukpo, S. Yamadjako, A. Djenontin, N. Moiroux, D. Misse, M. Akogbeto, V. Corbel, M. C. Henry, F. Chandre, T. Baldet and F. Remoue (2012). "First attempt to validate human IgG antibody response to Nterm-34kDa salivary peptide as biomarker for evaluating exposure to *Aedes aegypti* bites." *PLoS Negl Trop Dis* **6**(11): e1905.

Elanga Ndille, E., S. Doucoure, A. Poinsignon, F. Mouchet, S. Cornelié, E. D'Ortenzio, J. S. DeHecq and F. Remoue (2016). "Human IgG antibody response to *Aedes* Nterm-34kDa salivary peptide, an epidemiological tool to assess vector control in chikungunya and dengue transmission area." *PLoS Negl Trop Dis* **10**(12): e0005109.

Elanga Ndille, E., A. Dubot-Peres, S. Doucoure, F. Mouchet, S. Cornelié, B. Sidavong, F. Fournet and F. Remoue (2014). "Human IgG antibody response to *Aedes aegypti* Nterm-34 kDa salivary peptide as an indicator to identify areas at high risk for dengue transmission: a retrospective study in urban settings of Vientiane city, Lao PDR." *Trop Med Int Health* **19**(5): 576-580.

Endy, T. P., K. B. Anderson, A. Nisalak, I.-K. Yoon, S. Green, A. L. Rothman, S. J. Thomas, R. G. Jarman, D. H. Libraty and R. V. Gibbons (2011). "Determinants of inapparent and symptomatic dengue infection in a prospective study of primary school children in Kamphaeng Phet, Thailand." *PLoS neglected tropical diseases* **5**(3): e975-e975.

Epelboin, Y., S. C. Chaney, A. Guidez, N. Habchi-Hanriot, S. Talaga, L. Wang and I. Dusfour (2018). "Successes and failures of sixty years of vector control in French Guiana: what is the next step?" *Mem Inst Oswaldo Cruz* **113**(5): e170398.

Erlanger, T. E., J. Keiser and J. Utzinger (2008). "Effect of dengue vector control interventions on entomological parameters in developing countries: a systematic review and meta-analysis." *Med Vet Entomol* **22**(3): 203-221.

Esu, E., A. Lenhart, L. Smith and O. Horstick (2010). "Effectiveness of peridomestic space spraying with insecticide on dengue transmission; systematic review." *Tropical Med Int Health* **15**.

European Centre for Disease Prevention and Control (ECDC) (2012). Guidelines for the surveillance of invasive mosquitoes in Europe. Technical Report. Stockholm, ECDC.

Facchinelli, L., L. Valerio, M. Pombi, P. Reiter, C. Costantini and A. Della Torre (2007). "Development of a novel sticky trap for container-breeding mosquitoes and evaluation of its sampling properties to monitor urban populations of *Aedes albopictus*." *Med Vet Entomol* **21**(2): 183-195.

Fan, J., W. Wei, Z. Bai, C. Fan, S. Li, Q. Liu and K. Yang (2014). "A systematic review and meta-analysis of dengue risk with temperature change." *Int J Environ Res Public Health* **12**(1): 1-15.

Faraji, A. and I. Unlu (2016). "The eye of the tiger, the thrill of the fight: Effective larval and adult control measures against the asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae), in North America." *J Med Entomol* **53**(5): 1029-1047.

Farnesi, L. C., T. A. Belinato, J. S. M. Gestó, A. J. Martins, R. V. Bruno and L. A. Moreira (2019). "Embryonic development and egg viability of wMel-infected *Aedes aegypti*." *Parasit Vectors* **12**(1): 211.

Faucon, F., I. Dusfour, T. Gaude, V. Navratil, F. Boyer, F. Chandre, P. Sirisopa, K. Thanispong, W. Juntarajumnong, R. Poupardin, T. Chareonviriyaphap, R. Girod, V. Corbel, S. Reynaud and J. P. David (2015). "Identifying genomic changes associated with insecticide resistance in the dengue mosquito *Aedes aegypti* by deep targeted sequencing." *Genome Res* **25**(9): 1347-1359.

Faucon, F., T. Gaude, I. Dusfour, V. Navratil, V. Corbel, W. Juntarajumnong, R. Girod, R. Poupardin, F. Boyer, S. Reynaud and J. P. David (2017). "In the hunt for genomic markers of metabolic resistance to

pyrethroids in the mosquito *Aedes aegypti*: An integrated next-generation sequencing approach." PLoS Negl Trop Dis **11**(4): e0005526.

Favaro, E. A., M. R. Dibo, M. Pereira, A. P. Chierotti, A. L. Rodrigues-Junior and F. Chiaravalloti-Neto (2013). "Aedes aegypti entomological indices in an endemic area for dengue in Sao Paulo State, Brazil." Rev Saude Publica **47**(3): 588-597.

Faye, O., Y. Ba, O. Faye, C. Talla, D. Diallo, R. Chen, M. Mondo, R. Ba, E. Macondo, T. Siby, S. C. Weaver, M. Diallo and A. A. Sall (2014). "Urban epidemic of dengue virus serotype 3 infection, Senegal, 2009." Emerg Infect Dis **20**(3): 456-459.

Ferreira-de-Lima, V. H., P. d. S. Andrade, L. M. Thomazelli, M. T. Marrelli, P. R. Urbinatti, R. M. M. d. S. Almeida and T. N. Lima-Camara (2020). "Silent circulation of dengue virus in *Aedes albopictus* (Diptera: Culicidae) resulting from natural vertical transmission." Scientific Reports **10**(1): 3855.

Ferreira-de-Lima, V. H. and T. N. Lima-Camara (2018). "Natural vertical transmission of dengue virus in *Aedes aegypti* and *Aedes albopictus*: a systematic review." Parasit Vectors **11**(1): 77.

Flacio, E., L. Engeler, M. Tonolla, P. Lüthy and N. Patocchi (2015). "Strategies of a thirteen year surveillance programme on *Aedes albopictus* (*Stegomyia albopicta*) in southern Switzerland." Parasit Vectors **8**: 208.

Flasche, S., A. Wilder-Smith, J. Hombach and P. G. Smith (2019). "Estimating the proportion of vaccine-induced hospitalized dengue cases among Dengvaxia vaccinees in the Philippines." Wellcome open research **4**: 165-165.

Focks, D. (2004). "A review of entomological sampling methods and indicators for dengue vectors." WHO-TDR.

Focks, D. and N. Alexander (2006). "Multicountry study of *Aedes aegypti* pupal productivity survey methodology: findings and recommendations." WHO-TDR.

Focks, D. A., R. J. Brenner, J. Hayes and E. Daniels (2000). "Transmission thresholds for dengue in terms of *Aedes aegypti* pupae per person with discussion of their utility in source reduction efforts." Am J Trop Med Hyg **62**(1): 11-18.

Focks, D. A., E. Daniels, D. G. Haile and J. E. Keesling (1995). "A simulation model of the epidemiology of urban dengue fever: literature analysis, model development, preliminary validation, and samples of simulation results." Am J Trop Med Hyg **53**(5): 489-506.

Focks, D. A., D. G. Haile, E. Daniels and G. A. Mount (1993a). "Dynamic life table model for *Aedes aegypti* (Diptera: Culicidae): analysis of the literature and model development." J Med Entomol **30**(6): 1003-1017.

Focks, D. A., D. G. Haile, E. Daniels and G. A. Mount (1993b). "Dynamic life table model for *Aedes aegypti* (Diptera: Culicidae): Simulation results and validation." Journal of Medical Entomology **30**(6): 1018-1028.

Fontaine, A., D. Jiolle, I. Moltini-Conclois, S. Lequime and L. Lambrechts (2016). "Excretion of dengue virus RNA by *Aedes aegypti* allows non-destructive monitoring of viral dissemination in individual mosquitoes." Sci Rep **6**: 24885.

Fontaine, A., A. Pascual, E. Orlandi-Pradines, I. Diouf, F. Remoué, F. Pagès, T. Fusai, C. Rogier and L. Almeras (2011). "Relationship between exposure to vectors bites and antibody response to mosquito salivary gland extracts." PLoS ONE **6**(12).

Fourié, T., L. Luciani, S. Amrane, C. Zandotti, I. Leparç-Goffart, L. Ninove and A. Nougairède (2020). "Dengue virus type 1 infection in traveler returning from Benin to France, 2019." Emerg Infect Dis **26**(8): 1946-1949.

Fustec, B., T. Phanitchat, M. I. Hoq, S. Aromseree, C. Pientong, K. Thaewnongiew, T. Ekalaksananan, M. J. Bangs, V. Corbel, N. Alexander and H. J. Overgaard (2020). "Complex relationships between *Aedes* vectors, socio-economics and dengue transmission-Lessons learned from a case-control study in northeastern Thailand." *PLoS Negl Trop Dis* **14**(10): e0008703.

Gama, R. A., E. M. Silva, I. M. Silva, M. C. Resende and A. E. Eiras (2007). "Evaluation of the sticky MosquiTRAP for detecting *Aedes* (*Stegomyia*) *aegypti* (L.) (Diptera: Culicidae) during the dry season in Belo Horizonte, Minas Gerais, Brazil." *Neotrop Entomol* **36**(2): 294-302.

Gaye, A., E. Wang, N. Vasilakis, H. Guzman, D. Diallo, C. Talla, Y. Ba, I. Dia, S. C. Weaver and M. Diallo (2019). "Potential for sylvatic and urban *Aedes* mosquitoes from Senegal to transmit the new emerging dengue serotypes 1, 3 and 4 in West Africa." *PLoS Negl Trop Dis* **13**(2): e0007043.

George, L., A. Lenhart, J. Toledo, A. Lazaro, W. W. Han and R. Velayudhan (2015). "Community-effectiveness of temephos for dengue vector control: a systematic literature review." *PLoS Negl Trop Dis* **9**.

Getahun, A., A. Batikawai, D. Nand, S. Khan, A. Sahukhan and D. Faktaufon (2019). "Dengue in Fiji: epidemiology of the 2014 DENV-3 outbreak." *Western Pacific surveillance and response journal : WPSAR* **10**(2): 31-38.

Girard, M., C. B. Nelson, V. Picot and D. J. Gubler (2020). "Arboviruses: A global public health threat." *Vaccine* **38**(24): 3989-3994.

Gloria-Soria, A., P. M. Armstrong, J. R. Powell and P. E. Turner (2017). "Infection rate of *Aedes aegypti* mosquitoes with dengue virus depends on the interaction between temperature and mosquito genotype." *Proc Biol Sci* **284**(1864).

Goindin, D., C. Delannay, A. Gelasse, C. Ramdini, T. Gaude, F. Faucon, J. P. David, J. Gustave, A. Vega-Rua and F. Fouque (2017). "Levels of insecticide resistance to deltamethrin, malathion, and temephos, and associated mechanisms in *Aedes aegypti* mosquitoes from the Guadeloupe and Saint Martin islands (French West Indies)." *Infect Dis Poverty* **6**(1): 38.

Goindin, D., C. Delannay, C. Ramdini, J. Gustave and F. Fouque (2015). "Parity and longevity of *Aedes aegypti* according to temperatures in controlled conditions and consequences on dengue transmission risks." *PLoS One* **10**(8): e0135489.

Golding, N., A. L. Wilson, C. L. Moyes, J. Cano, D. M. Pigott, R. Velayudhan, S. J. Brooker, D. L. Smith, S. I. Hay and S. W. Lindsay (2015). "Integrating vector control across diseases." *BMC Med* **13**: 249.

Gonçalves, D. D. S., D. J. Hooker, Y. Dong, N. Baran, P. Kyrillos, I. Iturbe-Ormaetxe, C. P. Simmons and S. L. O'Neill (2019). "Detecting wMel Wolbachia in field-collected *Aedes aegypti* mosquitoes using loop-mediated isothermal amplification (LAMP)." *Parasit Vectors* **12**(1): 404.

Gorman, K., J. Young, L. Pineda, R. Márquez, N. Sosa, D. Bernal, R. Torres, Y. Soto, R. Lacroix, N. Naish, P. Kaiser, K. Tepedino, G. Philips, C. Kosmann and L. Cáceres (2016). "Short-term suppression of *Aedes aegypti* using genetic control does not facilitate *Aedes albopictus*." *Pest Manag Sci* **72**(3): 618-628.

Goubert, C., G. Minard, C. Vieira and M. Boulesteix (2016). "Population genetics of the Asian tiger mosquito *Aedes albopictus*, an invasive vector of human diseases." *Heredity (Edinb)* **117**(3): 125-134.

Govella, N. J., D. F. Maliti, A. T. Mlwale, J. P. Masallu, N. Mirzai, P. C. Johnson, H. M. Ferguson and G. F. Killeen (2016). "An improved mosquito electrocuting trap that safely reproduces epidemiologically relevant metrics of mosquito human-feeding behaviours as determined by human landing catch." *Malar J* **15**(1): 465.

- Grigoraki, L., V. Balabanidou, C. Meristoudis, A. Miridakis, H. Ranson, L. Swevers and J. Vontas (2016). "Functional and immunohistochemical characterization of CCEae3a, a carboxylesterase associated with temephos resistance in the major arbovirus vectors *Aedes aegypti* and *Ae. albopictus*." *Insect Biochem Mol Biol* **74**: 61-67.
- Grisales, N., R. Poupardin, S. Gomez, I. Fonseca-Gonzalez, H. Ranson and A. Lenhart (2013). "Temephos resistance in *Aedes aegypti* in Colombia compromises dengue vector control." *PLoS Negl Trop Dis* **7**(9): e2438.
- Gubler, D. J. (1998). "Dengue and dengue hemorrhagic fever." *Clinical microbiology reviews* **11**(3): 480-496.
- Gubler, D. J. (2002). "The global emergence/resurgence of arboviral diseases as public health problems." *Arch Med Res* **33**(4): 330-342.
- Gubler, D. J. (2011). "Dengue, Urbanization and Globalization: The Unholy Trinity of the 21(st) Century." *Tropical medicine and health* **39**(4 Suppl): 3-11.
- Gubler, D. J. and G. Kuno (1997). *Dengue and dengue hemorrhagic fever*. Wallingford, Oxon, UK ; New York, CAB International.
- Guo, C., Z. Zhou, Z. Wen, Y. Liu, C. Zeng, D. Xiao, M. Ou, Y. Han, S. Huang, D. Liu, X. Ye, X. Zou, J. Wu, H. Wang, E. Y. Zeng, C. Jing and G. Yang (2017). "Global epidemiology of dengue outbreaks in 1990-2015: A systematic review and meta-analysis." *Frontiers in cellular and infection microbiology* **7**: 317-317.
- Guzman, M., M. Alvarez and S. Halstead (2013). "Secondary infection as a risk factor for dengue hemorrhagic fever/dengue shock syndrome: an historical perspective and role of antibody-dependent enhancement of infection." *Arch Virol* **158**: 1445-14459.
- Guzman, M. G., D. J. Gubler, A. Izquierdo, E. Martinez and S. B. Halstead (2016). "Dengue infection." *Nature Reviews Disease Primers* **2**(1): 16055.
- Guzman, M. G. and G. Kouri (2003). "Dengue and dengue hemorrhagic fever in the Americas: lessons and challenges." *J Clin Virol* **27**(1): 1-13.
- Hales, S., N. de Wet, J. Maindonald and A. Woodward (2002). "Potential effect of population and climate changes on global distribution of dengue fever: an empirical model." *Lancet* **360**(9336): 830-834.
- Halstead, S. (2019). "Recent advances in understanding dengue." *F1000Research* **8**: F1000 Faculty Rev-1279.
- Halstead, S. B. (2015a). Dengue Antibody-Dependent Enhancement: knowns and unknowns. *Antibodies for Infectious Diseases*, American Society of Microbiology.
- Halstead, S. B. (2015b). "Pathogenesis of Dengue: Dawn of a New Era." *F1000Res* **4**.
- Han, W. W., A. Lazaro, P. J. McCall, L. George, S. Runge-Ranzinger, J. Toledo, R. Velayudhan and O. Horstick (2015). "Efficacy and community effectiveness of larvivorous fish for dengue vector control." *Trop Med Int Health* **20**(9): 1239-1256.
- Harrington, L. C., A. Fleisher, D. Ruiz-Moreno, F. Vermeulen, C. V. Wa, R. L. Poulson, J. D. Edman, J. M. Clark, J. W. Jones, S. Kitthawee and T. W. Scott (2014). "Heterogeneous feeding patterns of the dengue vector, *Aedes aegypti*, on individual human hosts in rural Thailand." *PLoS Negl Trop Dis* **8**(8): e3048.
- Harris, A. F., S. Rajatileka and H. Ranson (2010). "Pyrethroid resistance in *Aedes aegypti* from Grand Cayman." *Am J Trop Med Hyg* **83**(2): 277-284.

Hawley, W. A., P. Reiter, R. S. Copeland, C. B. Pumpuni and G. B. Craig (1987). "Aedes albopictus in North America: probable introduction in used tires from northern Asia." Science **236**(4805): 1114.

Hayes, R. J. and S. Bennett (1999). "Simple sample size calculation for cluster-randomized trials." Int J Epidemiol **28**(2): 319-326.

Heintze, C., M. Velasco Garrido and A. Kroeger (2007). "What do community-based dengue control programmes achieve? A systematic review of published evaluations." Trans R Soc Trop Med Hyg **101**(4): 317-325.

Helinski, M. E. H., L. Valerio, L. Facchinelli, T. W. Scott, J. Ramsey and L. C. Harrington (2012). "Evidence of polyandry for Aedes aegypti in semifield enclosures." The American journal of tropical medicine and hygiene **86**(4): 635-641.

Hemingway, J., N. J. Hawkes, L. McCarroll and H. Ranson (2004). "The molecular basis of insecticide resistance in mosquitoes." Insect Biochemistry and Molecular Biology **34**: 653-665.

Hii, Y. L., H. Zhu, N. Ng, L. C. Ng and J. Rocklöv (2012). "Forecast of dengue incidence using temperature and rainfall." PLOS Neglected Tropical Diseases **6**(11): e1908.

Hill, S. C., J. Neto de Vasconcelos, B. G. Granja, J. Theze, D. Jandondo, Z. Neto, M. Mirandela, C. D. S. Sebastiao, A. L. M. Candido, C. Clemente, S. Pereira da Silva, T. de Oliveira, O. G. Pybus, N. R. Faria and J. M. Afonso (2019). "Early genomic detection of cosmopolitan genotype of dengue virus serotype 2, Angola, 2018." Emerg Infect Dis **25**(4): 784-787.

Hirata, K., O. Komagata, K. Itokawa, A. Yamamoto, T. Tomita and S. Kasai (2014). "A single crossing-over event in voltage-sensitive Na⁺ channel genes may cause critical failure of dengue mosquito control by insecticides." PLoS Negl Trop Dis **8**(8): e3085.

Hoffmann, A. A., A. A. Goundar, S. A. Long, P. H. Johnson and S. A. Ritchie (2014). "Invasion of Wolbachia at the residential block level is associated with local abundance of Stegomyia aegypti, yellow fever mosquito, populations and property attributes." Medical and Veterinary Entomology **28**(S1): 90-97.

Huang, X., A. C. A. Clements, G. Williams, G. Devine, S. Tong and W. Hu (2015). "El Niño-Southern Oscillation, local weather and occurrences of dengue virus serotypes." Scientific reports **5**: 16806-16806.

Humanitarian Data Exchange Project (2019). Thailand administrative boundaries common operational database, United Nation Office for the Coordination of Humanitarian Affairs.

Hunsperger, E. A., S. Yoksan, P. Buchy, V. C. Nguyen, S. D. Sekaran, D. A. Enria, J. L. Pelegrino, S. Vázquez, H. Artsob, M. Drebot, D. J. Gubler, S. B. Halstead, M. G. Guzmán, H. S. Margolis, C. M. Nathanson, N. R. Rizzo Lic, K. E. Bessoff, S. Kliks and R. W. Peeling (2009). "Evaluation of commercially available anti-dengue virus immunoglobulin M tests." Emerg Infect Dis **15**(3): 436-440.

Hussain-Alkhateeb, L., A. Kroeger, P. Olliaro, J. Rocklöv, M. O. Sewe, G. Tejada, D. Benitez, B. Gill, S. L. Hakim, R. Gomes Carvalho, L. Bowman and M. Petzold (2018). "Early warning and response system (EWARS) for dengue outbreaks: Recent advancements towards widespread applications in critical settings." PLOS ONE **13**(5): e0196811.

Huy, R., P. Buchy, A. Conan, C. Ngan, S. Ong, R. Ali, V. Duong, S. Yit, S. Ung, V. Te, N. Chroeueng, N. C. Pheaktra, V. Uok and S. Vong (2010). "National dengue surveillance in Cambodia 1980-2008: epidemiological and virological trends and the impact of vector control." Bulletin of the World Health Organization **88**(9): 650-657.

Indriani, C., W. Tantowijoyo, E. Rancès, B. Andari, E. Prabowo, D. Yusdi, M. R. Ansari, D. S. Wardana, E. Supriyati, I. Nurhayati, I. Ernesia, S. Setyawan, I. Fitriana, E. Arguni, Y. Amelia, R. A. Ahmad, N. P. Jewell, S.

- M. Dufault, P. A. Ryan, B. R. Green, T. F. McAdam, S. L. O'Neill, S. K. Tanamas, C. P. Simmons, K. L. Anders and A. Utarini (2020). "Reduced dengue incidence following deployments of Wolbachia-infected *Aedes aegypti* in Yogyakarta, Indonesia: a quasi-experimental trial using controlled interrupted time series analysis." *Gates Open Res* **4**: 50.
- Ishak, I. H., Z. Jaal, H. Ranson and C. S. Wondji (2015). "Contrasting patterns of insecticide resistance and knockdown resistance (kdr) in the dengue vectors *Aedes aegypti* and *Aedes albopictus* from Malaysia." *Parasit Vectors* **8**: 181.
- Ishak, I. H., J. M. Riveron, S. S. Ibrahim, R. Stott, J. Longbottom, H. Irving and C. S. Wondji (2016). "The Cytochrome P450 gene CYP6P12 confers pyrethroid resistance in kdr-free Malaysian populations of the dengue vector *Aedes albopictus*." *Sci Rep* **6**: 24707.
- Jaenisch, T., T. Junghanss, B. Wills, O. J. Brady, I. Eckerle, A. Farlow, S. I. Hay, P. J. McCall, J. P. Messina, V. Ofula, A. A. Sall, A. Sakuntabhai, R. Velayudhan, G. R. W. Wint, H. Zeller, H. S. Margolis, O. Sankoh and G. Dengue in Africa Study (2014). "Dengue expansion in Africa-not recognized or not happening?" *Emerging infectious diseases* **20**(10): e140487.
- Jang, W. S., S. Y. Kwak, W. L. May, D. J. Yang, J. Nam and C. S. Lim (2019). "Comparative evaluation of three dengue duo rapid test kits to detect NS1, IgM, and IgG associated with acute dengue in children in Myanmar." *PLoS One* **14**(3): e0213451.
- Jentes, E. S., R. R. Lash, M. A. Johansson, T. M. Sharp, R. Henry, O. J. Brady, M. J. Sotir, S. I. Hay, H. S. Margolis and G. W. Brunette (2016). "Evidence-based risk assessment and communication: a new global dengue-risk map for travellers and clinicians." *J Travel Med* **23**(6).
- Jirakanjanakit, N., P. Rongnoparut, S. Saengtharapip, T. Chareonviriyaphap, S. Duchon, C. Bellec and S. Yoksan (2007). "Insecticide susceptible/resistance status in *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* (Diptera: Culicidae) in Thailand during 2003-2005." *J Econ Entomol* **100**(2): 545-550.
- Johansson, M. A., K. M. Apfeldorf, S. Dobson, J. Devita, A. L. Buczak, B. Baugher, L. J. Moniz, T. Bagley, S. M. Babin, E. Guven, T. K. Yamana, J. Shaman, T. Moschou, N. Lothian, A. Lane, G. Osborne, G. Jiang, L. C. Brooks, D. C. Farrow, S. Hyun, R. J. Tibshirani, R. Rosenfeld, J. Lessler, N. G. Reich, D. A. T. Cummings, S. A. Lauer, S. M. Moore, H. E. Clapham, R. Lowe, T. C. Bailey, M. García-Díez, M. S. Carvalho, X. Rodó, T. Sardar, R. Paul, E. L. Ray, K. Sakrejda, A. C. Brown, X. Meng, O. Osoba, R. Vardavas, D. Manheim, M. Moore, D. M. Rao, T. C. Porco, S. Ackley, F. Liu, L. Worden, M. Convertino, Y. Liu, A. Reddy, E. Ortiz, J. Rivero, H. Brito, A. Juarrero, L. R. Johnson, R. B. Gramacy, J. M. Cohen, E. A. Mordecai, C. C. Murdock, J. R. Rohr, S. J. Ryan, A. M. Stewart-Ibarra, D. P. Weikel, A. Jutla, R. Khan, M. Poultney, R. R. Colwell, B. Rivera-García, C. M. Barker, J. E. Bell, M. Biggerstaff, D. Swerdlow, Y. T.-R. L. Mier, B. M. Forshey, J. Trtanj, J. Asher, M. Clay, H. S. Margolis, A. M. Hebbeler, D. George and J. P. Chretien (2019). "An open challenge to advance probabilistic forecasting for dengue epidemics." *Proc Natl Acad Sci U S A* **116**(48): 24268-24274.
- Johansson, M. A., N. G. Reich, A. Hota, J. S. Brownstein and M. Santillana (2016). "Evaluating the performance of infectious disease forecasts: A comparison of climate-driven and seasonal dengue forecasts for Mexico." *Sci Rep* **6**: 33707.
- Johnson, B. J., S. A. Ritchie and D. M. Fonseca (2017). "The state of the art of lethal oviposition trap-based mass interventions for arboviral control." *Insects* **8**(1).
- Johnson, B. W., B. J. Russell and R. S. Lanciotti (2005). "Serotype-specific detection of dengue viruses in a fourplex real-time reverse transcriptase PCR assay." *Journal of clinical microbiology* **43**(10): 4977-4983.

- Johnston, D. I., M. A. Viray, J. M. Ushiroda, H. He, A. C. Whelen, R. H. Sciulli, G. Y. Kunimoto and Y. P. S (2020). "Investigation and response to an outbreak of dengue: Island of Hawaii, 2015-2016." Public Health Rep **135**(2): 230-237.
- Joob, B. and V. Wiwanitkit (2016). "Fifth serotype of dengue virus: What we should prepare for?" Medical journal, Armed Forces India **72**(2): 194-195.
- Kamgang, B., S. Marcombe, F. Chandre, E. Nchoutpouen, P. Nwane, J. Etang, V. Corbel and C. Paupy (2011). "Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* in Central Africa." Parasites & Vectors **4**(1): 79.
- Kasai, S., B. Caputo, T. Tsunoda, T. C. Cuong, Y. Maekawa, S. G. Lam-Phua, V. Pichler, K. Itokawa, K. Murota, O. Komagata, C. Yoshida, H. H. Chung, R. Bellini, Y. Tsuda, H. J. Teng, J. L. L. Filho, L. C. Alves, L. C. Ng, N. Minakawa, N. T. Yen, T. V. Phong, K. Sawabe and T. Tomita (2019). "First detection of a Vssc allele V1016G conferring a high level of insecticide resistance in *Aedes albopictus* collected from Europe (Italy) and Asia (Vietnam), 2016: a new emerging threat to controlling arboviral diseases." Euro Surveill **24**(5).
- Kasai, S., O. Komagata, K. Itokawa, T. Shono, L. C. Ng, M. Kobayashi and T. Tomita (2014). "Mechanisms of pyrethroid resistance in the dengue mosquito vector, *Aedes aegypti*: target site insensitivity, penetration, and metabolism." PLoS Negl Trop Dis **8**(6): e2948.
- Kasai, S., L. C. Ng, S. G. Lam-Phua, C. S. Tang, K. Itokawa, O. Komagata, M. Kobayashi and T. Tomita (2011). "First detection of a putative knockdown resistance gene in major mosquito vector, *Aedes albopictus*." Jpn J Infect Dis **64**(3): 217-221.
- Katewongsa, P. (2015). "Benefits of rural-urban migration for migrants' better life: A case study in Nang Rong, Buriram Province, Thailand." Thammasat Review **18**(1): 63-81.
- Katzelnick, L. C., L. Gresh, M. E. Halloran, J. C. Mercado, G. Kuan, A. Gordon, A. Balmaseda and E. Harris (2017). "Antibody-dependent enhancement of severe dengue disease in humans." Science (New York, N.Y.) **358**(6365): 929-932.
- Kay, B. and S. N. Vu (2005). "New strategy against *Aedes aegypti* in Vietnam." Lancet **365**(9459): 613-617.
- Kek, R., H. C. Hapuarachchi, C. Y. Chung, M. B. Humaidi, M. A. Razak, S. Chiang, C. Lee, C. H. Tan, G. Yap, C. S. Chong, K. S. Lee and L. C. Ng (2014). "Feeding host range of *Aedes albopictus* (Diptera: Culicidae) demonstrates its opportunistic host-seeking behavior in rural Singapore." J Med Entomol **51**(4): 880-884.
- Khandia, R., A. Munjal, K. Dhama, K. Karthik, R. Tiwari, Y. S. Malik, R. K. Singh and W. Chaicumpa (2018). "Modulation of dengue/zika Virus pathogenicity by Antibody-Dependent Enhancement and strategies to protect against enhancement in zika virus infection." Frontiers in immunology **9**: 597-597.
- Kittayapong, P., S. Ninphanomchai, W. Limohpasmanee, C. Chansang, U. Chansang and P. Mongkalagoon (2019). "Combined sterile insect technique and incompatible insect technique: The first proof-of-concept to suppress *Aedes aegypti* vector populations in semi-rural settings in Thailand." PLoS Negl Trop Dis **13**(10): e0007771.
- Kittayapong, P., P. Olanratmanee, P. Maskhao, P. Byass, J. Logan, Y. Tozan, V. Louis, D. J. Gubler and A. Wilder-Smith (2017). "Mitigating diseases transmitted by *Aedes* mosquitoes: A cluster-randomised trial of permethrin-impregnated school uniforms." PLoS Negl Trop Dis **11**(1): e0005197.
- Kittayapong, P., S. Thongyuan, P. Olanratmanee, W. Aumchareoun, S. Koyadun, R. Kittayapong and P. Butraporn (2012). "Application of eco-friendly tools and eco-bio-social strategies to control dengue vectors in urban and peri-urban settings in Thailand." Pathogens and Global Health **106**(8): 446-454.

- Koou, S. Y., C. S. Chong, I. Vythilingam, C. Y. Lee and L. C. Ng (2014). "Insecticide resistance and its underlying mechanisms in field populations of *Aedes aegypti* adults (Diptera: Culicidae) in Singapore." Parasit Vectors **7**: 471.
- Kotsakiozi, P., A. Gloria-Soria, A. Caccone, B. Evans, R. Schama, A. J. Martins and J. R. Powell (2017). "Tracking the return of *Aedes aegypti* to Brazil, the major vector of the dengue, chikungunya and zika viruses." PLoS Negl Trop Dis **11**(7): e0005653.
- Kraemer, M. U. G., R. C. Reiner, Jr., O. J. Brady, J. P. Messina, M. Gilbert, D. M. Pigott, D. Yi, K. Johnson, L. Earl, L. B. Marczak, S. Shirude, N. Davis Weaver, D. Bisanzio, T. A. Perkins, S. Lai, X. Lu, P. Jones, G. E. Coelho, R. G. Carvalho, W. Van Bortel, C. Marsboom, G. Hendrickx, F. Schaffner, C. G. Moore, H. H. Nax, L. Bengtsson, E. Wetter, A. J. Tatem, J. S. Brownstein, D. L. Smith, L. Lambrechts, S. Cauchemez, C. Linard, N. R. Faria, O. G. Pybus, T. W. Scott, Q. Liu, H. Yu, G. R. W. Wint, S. I. Hay and N. Golding (2019). "Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*." Nat Microbiol **4**(5): 854-863.
- Kraemer, M. U. G., M. E. Sinka, K. A. Duda, A. Q. N. Mylne, F. M. Shearer, C. M. Barker, C. G. Moore, R. G. Carvalho, G. E. Coelho, W. Van Bortel, G. Hendrickx, F. Schaffner, I. R. F. Elyazar, H.-J. Teng, O. J. Brady, J. P. Messina, D. M. Pigott, T. W. Scott, D. L. Smith, G. R. W. Wint, N. Golding and S. I. Hay (2015). "The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*." eLife **4**: e08347-e08347.
- Kroeger, A., A. Lenhart, M. Ochoa, E. Villegas, M. Levy, N. Alexander and P. J. McCall (2006). "Effective control of dengue vectors with curtains and water container covers treated with insecticide in Mexico and Venezuela: cluster randomised trials." BMJ **332**(7552): 1247-1252.
- Kumaran, E., D. Doum, V. Keo, L. Sokha, B. Sam, V. Chan, N. Alexander, J. Bradley, M. Liverani, D. B. Prasetyo, A. Rachmat, S. Lopes, J. Hii, L. Rithea, M. Shafique and J. Hustedt (2018). "Dengue knowledge, attitudes and practices and their impact on community-based vector control in rural Cambodia." PLoS Negl Trop Dis **12**(2): e0006268.
- Kurosu, T. (2011). "Quasispecies of dengue virus." Trop Med Health **39**(4 Suppl): 29-36.
- Lamborn, R. H. (1890). Dragon flies Vs. mosquitoes: Can the mosquito pest be mitigated? Studies in the life history of irritating insects, their natural enemies, and artificial checks, D. Appleton.
- Lambrechts, L., N. M. Fergusson, E. Harris, E. C. Holmes, E. A. McGraw, S. C. O'Neill, E. E. Ooi, S. A. Ritchie, P. A. Ryan, T. W. Scott, C. P. Simmons and S. C. Weaver (2015). "Assessing the epidemiological impact of Wolbachia deployment for dengue control." The Lancet Infectious Diseases **15**(7): 862-866.
- Lambrechts, L., T. W. Scott and D. J. Gubler (2010). "Consequences of the expanding global distribution of *Aedes albopictus* for dengue virus transmission." PLoS neglected tropical diseases **4**(5): e646-e646.
- Lanciotti, R. S., C. H. Calisher, D. J. Gubler, G. J. Chang and A. V. Vorndam (1992). "Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction." J Clin Microbiol **30**(3): 545-551.
- Lane, R. S., R. B. Moss, Y. P. Hsu, T. Wei, M. L. Mesirov and M. M. Kuo (1999). "Anti-arthropod saliva antibodies among residents of a community at high risk for Lyme disease in California." Am J Trop Med Hyg **61**(5): 850-859.
- Lau, S. M., T. H. Chua, W. Y. Sulaiman, S. Joanne, Y. A. Lim, S. D. Sekaran, K. Chinna, B. Venugopalan and I. Vythilingam (2017). "A new paradigm for *Aedes* spp. surveillance using gravid ovipositing sticky trap and NS1 antigen test kit." Parasit Vectors **10**(1): 151.
- Lauer, S. A., K. Sakrejda, E. L. Ray, L. T. Keegan, Q. Bi, P. Suangtho, S. Hinjoy, S. Iamsirithaworn, S. Suthachana, Y. Laosiritaworn, D. A. T. Cummings, J. Lessler and N. G. Reich (2018). "Prospective forecasts

of annual dengue hemorrhagic fever incidence in Thailand, 2010–2014." Proceedings of the National Academy of Sciences **115**(10): E2175.

Lazaro, A., W. W. Han, P. Manrique-Saide, L. George, R. Velayudhan, J. Toledo, S. Runge Ranzinger and O. Horstick (2015). "Community effectiveness of copepods for dengue vector control: systematic review." Trop Med Int Health **20**(6): 685-706.

Le Goff, G., J. Revollo, M. Guerra, M. Cruz, Z. Barja Simon, Y. Roca, J. Vargas Flores and J. P. Herve (2011). "Natural vertical transmission of dengue viruses by *Aedes aegypti* in Bolivia." Parasite **18**(3): 277-280.

Lee, C. and H. Lee (2019). "Probable female to male sexual transmission of dengue virus infection." Infectious Diseases **51**(2): 150-152.

Lee, J. S., M. Carabali, J. K. Lim, V. M. Herrera, I. Y. Park, L. Villar and A. Farlow (2017). "Early warning signal for dengue outbreaks and identification of high risk areas for dengue fever in Colombia using climate and non-climate datasets." BMC Infect Dis **17**(1): 480.

Legner, E. (1995). "Biological control of Diptera of medical and veterinary importance." Journal of Vector Ecology **20**(1): 59-120.

Lenhart, A., Y. Trongtokit, N. Alexander, C. Apiwathnasorn, W. Satimai, V. Vanlerberghe, P. Van der Stuyft and P. J. McCall (2013). "A cluster-randomized trial of insecticide-treated curtains for dengue vector control in Thailand." Am J Trop Med Hyg **88**(2): 254-259.

Li, C. X., P. E. Kaufman, R. D. Xue, M. H. Zhao, G. Wang, T. Yan, X. X. Guo, Y. M. Zhang, Y. D. Dong, D. Xing, H. D. Zhang and T. Y. Zhao (2015). "Relationship between insecticide resistance and kdr mutations in the dengue vector *Aedes aegypti* in Southern China." Parasit Vectors **8**: 325.

Li, M., T. Yang, N. P. Kandul, M. Bui, S. Gamez, R. Raban, J. Bennett, C. H. Sánchez, G. C. Lanzaro, H. Schmidt, Y. Lee, J. M. Marshall and O. S. Akbari (2020). "Development of a confinable gene drive system in the human disease vector *Aedes aegypti*." Elife **9**.

Lim, J. K., Y. Seydou, M. Carabali, A. Barro, D. L. Dahourou, K. S. Lee, T. Nikiema, S. Namkung, J. S. Lee, M. Y. Shin, E. Bonnet, T. Kagone, L. Kaba, T. Edwards, P. A. Somé, J. S. Yang, N. Alexander, I. K. Yoon and V. Ridde (2019). "Clinical and epidemiologic characteristics associated with dengue during and outside the 2016 outbreak identified in health facility-based surveillance in Ouagadougou, Burkina Faso." PLoS Negl Trop Dis **13**(12): e0007882.

Limkittikul, K., J. Brett and M. L'Azou (2014). "Epidemiological trends of dengue disease in Thailand (2000-2011): a systematic literature review." PLoS Negl Trop Dis **8**(11): e3241.

Liu-Helmersson, J., H. Stenlund, A. Wilder-Smith and J. Rocklöv (2014). "Vectorial capacity of *Aedes aegypti*: effects of temperature and implications for global dengue epidemic potential." PLoS One **9**(3): e89783.

Liu, Z., Z. Zhang, Z. Lai, T. Zhou, Z. Jia, J. Gu, K. Wu and X. G. Chen (2017). "Temperature increase enhances *Aedes albopictus* competence to transmit dengue virus." Front Microbiol **8**: 2337.

Londono-Renteria, B., J. C. Cardenas, L. D. Cardenas, R. C. Christofferson, D. M. Chisenhall, D. M. Wesson, M. K. McCracken, D. Carvajal and C. N. Mores (2013). "Use of anti-*Aedes aegypti* salivary extract antibody concentration to correlate risk of vector exposure and dengue transmission risk in Colombia." PLoS One **8**(12): e81211.

Lorenzi, O. D., C. Major, V. Acevedo, J. Perez-Padilla, A. Rivera, B. J. Biggerstaff, J. Munoz-Jordan, S. Waterman, R. Barrera and T. M. Sharp (2016). "Reduced incidence of chikungunya virus infection in

communities with ongoing *Aedes aegypti* mosquito trap intervention studies - Salinas and Guayama, Puerto Rico, November 2015-February 2016." MMWR Morb Mortal Wkly Rep **65**(18): 479-480.

Low, J. G. H., E. E. Ooi and S. G. Vasudevan (2017). "Current status of dengue therapeutics research and development." The Journal of Infectious Diseases **215**(suppl_2): S96-S102.

Lu, Y., O. Sengvilaipaseuth, A. Chanthongthip, O. Phonemixay, M. Vongsouvath, P. Phouminh, S. D. Blacksell, P. N. Newton and A. Dubot-Pérès (2019). "Comparison of two commercial ELISA kits for the detection of anti-dengue IgM for routine dengue diagnosis in Laos." Tropical medicine and infectious disease **4**(3): 111.

Lukman, N., G. Salim, H. Kosasih, N. H. Susanto, I. Parwati, S. Fitri, B. Alisjahbana, S. Widjaja and M. Williams (2016). "Comparison of the hemagglutination inhibition test and IgG ELISA in categorizing primary and secondary dengue infections based on the plaque reduction neutralization test." BioMed research international **2016**: 5253842-5253842.

Ly, S., C. Fortas, V. Duong, T. Benmarhnia, A. Sakuntabhai, R. Paul, R. Huy, S. Sorn, K. Nguon, S. Chan, S. Kimsan, S. Ong, K. S. Kim, S. Buoy, L. Voeung, P. Dussart, P. Buchy and A. Tarantola (2019). "Asymptomatic dengue virus infections, Cambodia, 2012-2013." Emerg Infect Dis **25**(7): 1354-1362.

Macdonald, W. W. (1956). "*Aedes aegypti* in Malaya. II. Larval and adult biology." Ann Trop Med Parasitol **50**(4): 399-414.

Maciel-de-Freitas, R., F. C. Avendanho, R. Santos, G. Sylvestre, S. C. Araujo, J. B. Lima, A. J. Martins, G. E. Coelho and D. Valle (2014). "Undesirable consequences of insecticide resistance following *Aedes aegypti* control activities due to a dengue outbreak." PLoS One **9**(3): e92424.

Mackenzie, J. S., D. J. Gubler and L. R. Petersen (2004). "Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses." Nature Medicine **10**(12): S98-S109.

Maïga, H., W. Mamai, N. S. Bimbilé Somda, A. Konczal, T. Wallner, G. S. Herranz, R. A. Herrero, H. Yamada and J. Bouyer (2019). "Reducing the cost and assessing the performance of a novel adult mass-rearing cage for the dengue, chikungunya, yellow fever and Zika vector, *Aedes aegypti* (Linnaeus)." PLoS Negl Trop Dis **13**(9): e0007775.

Mains, J. W., C. L. Brelsfoard, R. I. Rose and S. L. Dobson (2016). "Female adult *Aedes albopictus* suppression by *Wolbachia*-infected male mosquitoes." Sci Rep **6**: 33846.

Mammen, M. P., C. Pimgate, C. J. Koenraad, A. L. Rothman, J. Aldstadt, A. Nisalak, R. G. Jarman, J. W. Jones, A. Srikiatkachorn, C. A. Ypil-Butac, A. Getis, S. Thammaphalo, A. C. Morrison, D. H. Libraty, S. Green and T. W. Scott (2008). "Spatial and temporal clustering of dengue virus transmission in Thai villages." PLoS Med **5**(11): e205.

Maoz, D., T. Ward, M. Samuel, P. Müller, S. Runge-Ranzinger, J. Toledo, R. Boyce, R. Velayudhan and O. Horstick (2017). "Community effectiveness of pyriproxyfen as a dengue vector control method: A systematic review." PLoS Negl Trop Dis **11**(7): e0005651.

Marcombe, S., F. Darriet, M. Tolosa, P. Agnew, S. Duchon, M. Etienne, M. M. Yp Tcha, F. Chandre, V. Corbel and A. Yebakima (2011). "Pyrethroid resistance reduces the efficacy of space sprays for dengue control on the island of Martinique (Caribbean)." PLoS Negl Trop Dis **5**(6): e1202.

Marcombe, S., B. Fustec, J. Cattel, S. Chonephetsarath, P. Thammavong, N. Phommavanh, J. P. David, V. Corbel, I. W. Sutherland, J. C. Hertz and P. T. Brey (2019). "Distribution of insecticide resistance and mechanisms involved in the arbovirus vector *Aedes aegypti* in Laos and implication for vector control." PLoS Negl Trop Dis **13**(12): e0007852.

Marcombe, S., R. B. Mathieu, N. Pocquet, M. A. Riaz, R. Poupardin, S. Selior, F. Darriet, S. Reynaud, A. Yebakima, V. Corbel, J. P. David and F. Chandre (2012). "Insecticide resistance in the dengue vector *Aedes aegypti* from Martinique: distribution, mechanisms and relations with environmental factors." *PLoS One* **7**(2): e30989.

Marcombe, S., R. Poupardin, F. Darriet, S. Reynaud, J. Bonnet, C. Strobe, C. Brengues, A. Yebakima, H. Ranson, V. Corbel and J. P. David (2009). "Exploring the molecular basis of insecticide resistance in the dengue vector *Aedes aegypti*: a case study in Martinique Island (French West Indies)." *BMC Genomics* **10**: 494.

Marie, A., R. Ronca, A. Poinignon, F. Lombardo, P. M. Drame, S. Cornelié, P. Besnard, J. Le Mire, G. Fiorentino, F. Fortes, P. Carnevale, F. Remoue and B. Arca (2015). "The *Anopheles gambiae* cE5 salivary protein: a sensitive biomarker to evaluate the efficacy of insecticide-treated nets in malaria vector control." *Microbes Infect* **17**(6): 409-416.

Martinez-Vega, R. A., R. Danis-Lozano, F. A. Diaz-Quijano, J. Velasco-Hernandez, R. Santos-Luna, S. Roman-Perez, P. Kuri-Morales and J. Ramos-Castaneda (2015). "Peridomestic infection as a determining factor of dengue transmission." *PLoS Negl Trop Dis* **9**(12): e0004296.

Matheus, S., X. Deparis, B. Labeau, J. Lelarge, J. Morvan and P. Dussart (2005). "Use of four dengue virus antigens for determination of dengue immune status by enzyme-linked immunosorbent assay of immunoglobulin G avidity." *Journal of Clinical Microbiology* **43**(11): 5784.

Mathieu-Daude, F., A. Claverie, C. Plichart, D. Boulanger, F. A. Mphande and H. C. Bossin (2018). "Specific human antibody responses to *Aedes aegypti* and *Aedes polynesiensis* saliva: A new epidemiological tool to assess human exposure to disease vectors in the Pacific." *PLoS Negl Trop Dis* **12**(7): e0006660.

Matusali, G., F. Colavita, F. Carletti, E. Lalle, L. Bordi, F. Vairo, G. Ippolito, M. R. Capobianchi and C. Castilletti (2020). "Performance of rapid tests in the management of dengue fever imported cases in Lazio, Italy 2014-2019." *International Journal of Infectious Diseases* **99**: 193-198.

McArthur, M. A., M. B. Sztein and R. Edelman (2013). "Dengue vaccines: recent developments, ongoing challenges and current candidates." *Expert Rev Vaccines* **12**(8): 933-953.

McBride, C. S., F. Baier, A. B. Omondi, S. A. Spitzer, J. Lutomiah, R. Sang, R. Ignell and L. B. Vosshall (2014). "Evolution of mosquito preference for humans linked to an odorant receptor." *Nature* **515**(7526): 222-227.

McKenzie, B. A., A. E. Wilson and S. Zohdy (2019). "*Aedes albopictus* is a competent vector of Zika virus: A meta-analysis." *PLoS One* **14**(5): e0216794.

Medina, F., J. F. Medina, C. Colón, E. Vergne, G. A. Santiago and J. L. Muñoz-Jordán (2012). "Dengue virus: isolation, propagation, quantification, and storage." *Curr Protoc Microbiol* **Chapter 15**: Unit 15D 12.

Messina, J. P., O. J. Brady, T. W. Scott, C. Zou, D. M. Pigott, K. A. Duda, S. Bhatt, L. Katzelnick, R. E. Howes, K. E. Battle, C. P. Simmons and S. I. Hay (2014). "Global spread of dengue virus types: mapping the 70 year history." *Trends in microbiology* **22**(3): 138-146.

Mitchell-Foster, K., B. O. Ma, S. Warsame-Ali, C. Logan, M. E. Rau and C. Lowenberger (2012). "The influence of larval density, food stress, and parasitism on the bionomics of the dengue vector *Aedes aegypti* (Diptera: Culicidae): implications for integrated vector management." *J Vector Ecol* **37**(1): 221-229.

Mohd-Zaki, A. H., J. Brett, E. Ismail and M. L'Azou (2014). "Epidemiology of dengue disease in Malaysia (2000-2012): a systematic literature review." *PLoS Negl Trop Dis* **8**(11): e3159.

Mondini, A., R. V. de Moraes Bronzoni, S. H. Nunes, F. Chiaravalloti Neto, E. Massad, W. J. Alonso, E. S. Lazzaro, A. A. Ferraz, P. M. de Andrade Zanotto and M. L. Nogueira (2009). "Spatio-temporal tracking and phylodynamics of an urban dengue 3 outbreak in Sao Paulo, Brazil." *PLoS Negl Trop Dis* **3**(5): e448.

Montella, I. R., A. J. Martins, P. F. Viana-Medeiros, J. B. Lima, I. A. Braga and D. Valle (2007). "Insecticide resistance mechanisms of Brazilian *Aedes aegypti* populations from 2001 to 2004." *Am J Trop Med Hyg* **77**(3): 467-477.

Montenegro, D., L. Martinez, K. Tay, T. Hernandez, D. Noriega, L. Barbosa, J. Muñoz, H. Mateus, J. Daza, A. Teherán and J. D. Ramírez (2020). "Usefulness of autocidal gravid ovitraps for the surveillance and control of *Aedes (Stegomyia) aegypti* (Diptera: Culicidae) in eastern Colombia." *Med Vet Entomol*.

Moreira, L. A., I. Iturbe-Ormaetxe, J. A. Jeffery, G. Lu, A. T. Pyke, L. M. Hedges, B. C. Rocha, S. Hall-Mendelin, A. Day, M. Riegler, L. E. Hugo, K. N. Johnson, B. H. Kay, E. A. McGraw, A. F. van den Hurk, P. A. Ryan and S. L. O'Neill (2009). "A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and Plasmodium." *Cell* **139**(7): 1268-1278.

Morin, C. W., A. C. Comrie and K. Ernst (2013). "Climate and dengue transmission: evidence and implications." *Environ Health Perspect* **121**(11-12): 1264-1272.

Morrison, A. C., K. Gray, A. Getis, H. Astete, M. Sihuincha, D. Focks, D. Watts, J. D. Stancil, J. G. Olson, P. Blair and T. W. Scott (2004). "Temporal and geographic patterns of *Aedes aegypti* (Diptera: Culicidae) production in Iquitos, Peru." *J Med Entomol* **41**(6): 1123-1142.

Moyes, C. L., J. Vontas, A. J. Martins, L. C. Ng, S. Y. Koou, I. Dusfour, K. Raghavendra, J. Pinto, V. Corbel, J. P. David and D. Weetman (2017). "Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans." *PLoS Negl Trop Dis* **11**(7): e0005625.

Müller, G. and Y. Schlein (2005). "Plant tissues: the frugal diet of mosquitoes in adverse conditions." *Med Vet Entomol* **19**(4): 413-422.

Muraduzzaman, A. K. M., A. N. Alam, S. Sultana, M. Siddiqua, M. H. Khan, A. Akram, F. Haque, M. S. Flora and T. Shirin (2018). "Circulating dengue virus serotypes in Bangladesh from 2013 to 2016." *Virusdisease* **29**(3): 303-307.

Mustafa, M. S., V. Rasotgi, S. Jain and V. Gupta (2015). "Discovery of fifth serotype of dengue virus (DENV-5): A new public health dilemma in dengue control." *Medical journal, Armed Forces India* **71**(1): 67-70.

Muzari, M. O., G. Devine, J. Davis, B. Crunkhorn, A. van den Hurk, P. Whelan, R. Russell, J. Walker, P. Horne, G. Ehlers and S. Ritchie (2017). "Holding back the tiger: Successful control program protects Australia from *Aedes albopictus* expansion." *PLoS Negl Trop Dis* **11**(2): e0005286.

Nathan, M. B., D. A. Focks and A. Kroeger (2006). "Pupal/demographic surveys to inform dengue-vector control." *Ann Trop Med Parasitol* **100 Suppl 1**: S1-S3.

National Statistical Office (2010). The 2010 Population and Housing Census. N. S. Office, National Statistical Office

Ninove, L., P. Parola, C. Baronti, X. De Lamballerie, P. Gautret, B. Doudier and R. N. Charrel (2009). "Dengue virus type 3 infection in traveler returning from west Africa." *Emerg Infect Dis* **15**(11): 1871-1872.

Niyas, K. P., R. Abraham, R. N. Unnikrishnan, T. Mathew, S. Nair and A. Manakkadan (2010). "Molecular characterization of chikungunya virus isolates from clinical samples and adult *Aedes albopictus* mosquitoes emerged from larvae from Kerala, South India." *Virology* **7**.

Nunes, P. C. G., R. P. Dumas, J. C. Sánchez-Arcila, R. M. R. Nogueira, M. A. P. Horta and F. B. Dos Santos (2019). "30 years of fatal dengue cases in Brazil: a review." *BMC public health* **19**(1): 329-329.

O'Connor, L., C. Plichart, A. C. Sang, C. L. Brelsfoard, H. C. Bossin and S. L. Dobson (2012). "Open release of male mosquitoes infected with a wolbachia biopesticide: field performance and infection containment." *PLoS Negl Trop Dis* **6**(11): e1797.

OCDE (2018). *Safety Assessment of Transgenic Organisms in the Environment, Volume 8*.

OhAinle, M., A. Balmaseda, A. R. Macalalad, Y. Tellez, M. C. Zody, S. Saborío, A. Nuñez, N. J. Lennon, B. W. Birren, A. Gordon, M. R. Henn and E. Harris (2011). "Dynamics of dengue disease severity determined by the interplay between viral genetics and serotype-specific immunity." *Sci Transl Med* **3**(114): 114ra128.

Olliaro, P., F. Fouque, A. Kroeger, L. Bowman, R. Velayudhan, A. C. Santelli, D. Garcia, R. Skewes Ramm, L. H. Sulaiman, G. S. Tejada, F. C. Morales, E. Gozzer, C. B. Garrido, L. C. Quang, G. Gutierrez, Z. E. Yadon and S. Runge-Ranzinger (2018). "Improved tools and strategies for the prevention and control of arboviral diseases: A research-to-policy forum." *PLOS Neglected Tropical Diseases* **12**(2): e0005967.

Ooi, E. E., K. T. Goh and D. J. Gubler (2006). "Dengue prevention and 35 years of vector control in Singapore." *Emerg Infect Dis* **12**(6): 887-893.

Organization, W. H. (2017). "Global vector control response 2017–2030. Geneva: World Health Organization; 2017. Licence: CC BY-NC-SA 3.0 IGO."

Orlandi-Pradines, E., L. Almeras, L. Denis de Senneville, S. Barbe, F. Remoue, C. Villard, S. Cornelié, K. Penhoat, A. Pascual, C. Bourgoïn, D. Fontenille, J. Bonnet, N. Corre-Catelin, P. Reiter, F. Pages, D. Laffite, D. Boulanger, F. Simondon, B. Pradines, T. Fusai and C. Rogier (2007). "Antibody response against saliva antigens of *Anopheles gambiae* and *Aedes aegypti* in travellers in tropical Africa." *Microbes Infect* **9**(12-13): 1454-1462.

Ortega-López, L. D., E. Pondeville, A. Kohl, R. León, M. P. Betancourth, F. Almiré, S. Torres-Valencia, S. Saldarriaga, N. Mirzai and H. M. Ferguson (2020). "The mosquito electrocuting trap as an exposure-free method for measuring human-biting rates by *Aedes* mosquito vectors." *Parasites & vectors* **13**(1): 31-31.

Pan America Health Organization (2020). *Dengue*. W. H. Organisation.

Parra, M. C. P., E. A. Favaro, M. R. Dibo, A. Mondini, A. E. Eiras, E. G. Kroon, M. M. Teixeira, M. L. Nogueira and F. Chiaravalloti-Neto (2018). "Using adult *Aedes aegypti* females to predict areas at risk for dengue transmission: A spatial case-control study." *Acta Trop* **182**: 43-53.

Patterson, G. M. (2016). "Looking backward, looking forward: the long, torturous struggle with mosquitoes." *Insects* **7**(4): 56.

Paupy, C., H. Delatte, L. Bagny, V. Corbel and D. Fontenille (2009). "*Aedes albopictus*, an arbovirus vector: from the darkness to the light." *Microbes Infect* **11**(14-15): 1177-1185.

Paupy, C., B. Ollomo, B. Kamgang, S. Moutailler, D. Rousset, M. Demanou, J. P. Herve, E. Leroy and F. Simard (2010). "Comparative role of *Aedes albopictus* and *Aedes aegypti* in the emergence of Dengue and Chikungunya in central Africa." *Vector Borne Zoonotic Dis* **10**(3): 259-266.

Peeling, R. W., H. Artsob, J. L. Pelegrino, P. Buchy, M. J. Cardoso, S. Devi, D. A. Enria, J. Farrar, D. J. Gubler, M. G. Guzman, S. B. Halstead, E. Hunsperger, S. Kliks, H. S. Margolis, C. M. Nathanson, V. C. Nguyen, N. Rizzo, S. Vázquez and S. Yoksan (2010). "Evaluation of diagnostic tests: dengue." *Nature Reviews Microbiology* **8**(12): S30-S37.

- Perich, M. J., A. Kardec, I. A. Braga, I. F. Portal, R. Burge, B. C. Zeichner, W. A. Brogdon and R. A. Wirtz (2003). "Field evaluation of a lethal ovitrap against dengue vectors in Brazil." Med Vet Entomol **17**(2): 205-210.
- Phaijoo, G. R. and D. B. Gurung (2017). "Modeling impact of temperature and human movement on the persistence of dengue disease." Comput Math Methods Med **2017**: 1747134.
- Phanitchat, T., B. Zhao, U. Haque, C. Pientong, T. Ekalaksananan, S. Aromseree, K. Thaewongiew, B. Fustec, M. J. Bangs, N. Alexander and H. J. Overgaard (2019). "Spatial and temporal patterns of dengue incidence in northeastern Thailand 2006-2016." BMC Infect Dis **19**(1): 743.
- Phuanukoonnon, S., I. Mueller and J. H. Bryan (2005). "Effectiveness of dengue control practices in household water containers in Northeast Thailand." Trop Med Int Health **10**(8): 755-763.
- Pichler, V., C. Malandrucolo, P. Serini, R. Bellini, F. Severini, L. Toma, M. Di Luca, F. Montarsi, M. Ballardini, M. Manica, V. Petrarca, J. Vontas, S. Kasai, A. Della Torre and B. Caputo (2019). "Phenotypic and genotypic pyrethroid resistance of *Aedes albopictus*, with focus on the 2017 chikungunya outbreak in Italy." Pest Manag Sci **75**(10): 2642-2651.
- Plernsub, S., J. Saingamsook, J. Yanola, N. Lumjuan, P. Tippawangkosol, K. Sukontason, C. Walton and P. Somboon (2016). "Additive effect of knockdown resistance mutations, S989P, V1016G and F1534C, in a heterozygous genotype conferring pyrethroid resistance in *Aedes aegypti* in Thailand." Parasit Vectors **9**(1): 417.
- Poinsignon, A., S. Cornелиe, F. Ba, D. Boulanger, C. Sow, M. Rossignol, C. Sokhna, B. Cisse, F. Simondon and F. Remoue (2009). "Human IgG response to a salivary peptide, gSG6-P1, as a new immuno-epidemiological tool for evaluating low-level exposure to *Anopheles* bites." Malar J **8**: 198.
- Poinsignon, A., S. Cornелиe, M. Mestres-Simon, A. Lanfrancotti, M. Rossignol, D. Boulanger, B. Cisse, C. Sokhna, B. Arca, F. Simondon and F. Remoue (2008). "Novel peptide marker corresponding to salivary protein gSG6 potentially identifies exposure to *Anopheles* bites." PLoS One **3**(6): e2472.
- Polson, K. A., W. G. Brogdon, S. C. Rawlins and D. D. Chadee (2011). "Characterization of insecticide resistance in Trinidadian strains of *Aedes aegypti* mosquitoes." Acta Trop **117**(1): 31-38.
- Ponlawat, A., J. G. Scott and L. C. Harrington (2005). "Insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus* across Thailand." J Med Entomol **42**(5): 821-825.
- Ramadona, A. L., L. Lazuardi, Y. L. Hii, Å. Holmner, H. Kusnanto and J. Rocklöv (2016). "Prediction of Dengue Outbreaks Based on Disease Surveillance and Meteorological Data." PLoS One **11**(3): e0152688.
- Rapley, L. P., P. H. Johnson, C. R. Williams, R. M. Silcock, M. Larkman, S. A. Long, R. C. Russell and S. A. Ritchie (2009). "A lethal ovitrap-based mass trapping scheme for dengue control in Australia: II. Impact on populations of the mosquito *Aedes aegypti*." Med Vet Entomol **23**(4): 303-316.
- Ratanawong, P., P. Kittayapong, P. Olanratmanee, A. Wilder-Smith, P. Byass, Y. Tozan, P. Dambach, C. A. Quinonez and V. R. Louis (2016). "Spatial variations in dengue transmission in schools in Thailand." PLoS One **11**(9): e0161895.
- Regional Committee for the Western, P. (2016). *Dengue, Manila* : WHO Regional Office for the Western Pacific.
- Reich, N. G., S. A. Lauer, K. Sakrejda, S. Iamsirithaworn, S. Hinjoy, P. Suangtho, S. Suthachana, H. E. Clapham, H. Salje, D. A. Cummings and J. Lessler (2016). "Challenges in real-time prediction of infectious disease: A case study of dengue in Thailand." PLoS Negl Trop Dis **10**(6): e0004761.

Reiner, R. C., Jr., N. Achee, R. Barrera, T. R. Burkot, D. D. Chadee, G. J. Devine, T. Endy, D. Gubler, J. Hombach, I. Kleinschmidt, A. Lenhart, S. W. Lindsay, I. Longini, M. Mondy, A. C. Morrison, T. A. Perkins, G. Vazquez-Prokopec, P. Reiter, S. A. Ritchie, D. L. Smith, D. Strickman and T. W. Scott (2016). "Quantifying the epidemiological impact of vector control on dengue." *PLoS Negl Trop Dis* **10**(5): e0004588.

Reiner, R. C., Jr., S. T. Stoddard and T. W. Scott (2014). "Socially structured human movement shapes dengue transmission despite the diffusive effect of mosquito dispersal." *Epidemics* **6**: 30-36.

Remoue, F., B. Cisse, F. Ba, C. Sokhna, J. P. Herve, D. Boulanger and F. Simondon (2006). "Evaluation of the antibody response to Anopheles salivary antigens as a potential marker of risk of malaria." *Trans R Soc Trop Med Hyg* **100**(4): 363-370.

Research, U. N. U. W. B. W. S. P. f., D. Training in Tropical and I. Pediatric Dengue Vaccine (2009). Evaluation of commercially available anti-dengue virus immunoglobulin M tests. Geneva, World Health Organization.

Rezende, G. L., A. J. Martins, C. Gentile, L. C. Farnesi, M. Pelajo-Machado, A. A. Peixoto and D. Valle (2008). "Embryonic desiccation resistance in *Aedes aegypti*: presumptive role of the chitinized Serosal Cuticle." *BMC Developmental Biology* **8**(1): 82.

Rezza, G. (2016). "Dengue and other Aedes-borne viruses: a threat to Europe?" *Euro Surveill* **21**(21).

Ridde, V., I. Agier, E. Bonnet, M. Carabali, K. R. Dabire, F. Fournet, A. Ly, I. B. Meda and B. Parra (2016). "Presence of three dengue serotypes in Ouagadougou (Burkina Faso): research and public health implications." *Infect Dis Poverty* **5**: 23.

Ritchie, S. A. and B. J. Johnson (2017). "Advances in vector control science: Rear-and-release strategies show promise... but don't forget the basics." *The Journal of Infectious Diseases* **215**(suppl_2): S103-S108.

Ritchie, S. A., S. Long, A. Hart, C. E. Webb and R. C. Russell (2003). "An adulticidal sticky ovitrap for sampling container-breeding mosquitoes." *J Am Mosq Control Assoc* **19**(3): 235-242.

Ritchie, S. A., A. F. van den Hurk, M. J. Smout, K. M. Staunton and A. A. Hoffmann (2018). "Mission accomplished? We need a guide to the 'Post release' world of Wolbachia for Aedes-borne disease control." *Trends Parasitol* **34**(3): 217-226.

Rodríguez-Barraquer, I., R. Buathong, S. Iamsirithaworn, A. Nisalak, J. Lessler, R. G. Jarman, R. V. Gibbons and D. A. Cummings (2014). "Revisiting Rayong: shifting seroprofiles of dengue in Thailand and their implications for transmission and control." *Am J Epidemiol* **179**(3): 353-360.

Roehrig, J., J. Hombach and A. Barrett (2008). "Guidelines for plaque-reduction neutralization testing of human antibodies to dengue viruses." *Viral immunology* **21**: 123-132.

Rohani, A., I. Zamree, R. T. Joseph and H. L. Lee (2008). "Persistency of transovarial dengue virus in *Aedes aegypti* (Linn.)." *Southeast Asian J Trop Med Public Health* **39**(5): 813-816.

Rohousova, I., S. Ozensoy, Y. Ozbek and P. Volf (2005). "Detection of species-specific antibody response of humans and mice bitten by sand flies." *Parasitology* **130**(Pt 5): 493-499.

Roiz, D., S. Duperier, M. Roussel, P. Boussès, D. Fontenille, F. Simard and C. Paupy (2016). "Trapping the tiger: Efficacy of the novel BG-Sentinel 2 with several attractants and carbon dioxide for collecting *Aedes albopictus* (Diptera: Culicidae) in southern France." *J Med Entomol* **53**(2): 460-465.

Roiz, D., A. L. Wilson, T. W. Scott, D. M. Fonseca, F. Jourdain, P. Muller, R. Velayudhan and V. Corbel (2018). "Integrated Aedes management for the control of Aedes-borne diseases." *PLoS Negl Trop Dis* **12**(12): e0006845.

- Romero-Vivas, C., P. Arango-Padilla and A. Falconar (2006). "Pupal-productivity surveys to identify the key container habitats of *Aedes aegypti* (L.) in Barranquilla, the principal seaport of Colombia." *Ann Trop Med Parasitol* **100**.
- Romero-Vivas, C. M. and A. K. Falconar (2005). "Investigation of relationships between *Aedes aegypti* egg, larvae, pupae, and adult density indices where their main breeding sites were located indoors." *J Am Mosq Control Assoc* **21**(1): 15-21.
- Saavedra-Rodriguez, K., L. Urdaneta-Marquez, S. Rajatileka, M. Moulton, A. E. Flores, I. Fernandez-Salas, J. Bisset, P. J. McCall, M. J. Donnelly, H. Ranson, J. Hemingway and W. C. Black (2007). "A mutation in the voltage-gated channel gene associated with pyrethroid resistance in Latin American *Aedes aegypti*." *Insect Molecular Biology* **16**(6): 785-798.
- Sagna, A. B., D. Kassie, A. Couvray, A. M. Adja, E. Hermann, G. Riveau, G. Salem, F. Fournet and F. Remoue (2019). "Spatial assessment of contact between humans and *Anopheles* and *Aedes* mosquitoes in a medium-sized African urban setting, using salivary antibody-based biomarkers." *J Infect Dis* **220**(7): 1199-1208.
- Sagna, A. B., M. C. Yobo, E. Elanga Ndille and F. Remoue (2018). "New immuno-epidemiological biomarker of human exposure to *Aedes* vector bites: from concept to applications." *Trop Med Infect Dis* **3**(3).
- Salje, H., D. A. T. Cummings, I. Rodriguez-Barraquer, L. C. Katzelnick, J. Lessler, C. Klungthong, B. Thaisomboonsuk, A. Nisalak, A. Weg, D. Ellison, L. Macareo, I. K. Yoon, R. Jarman, S. Thomas, A. L. Rothman, T. Endy and S. Cauchemez (2018). "Reconstruction of antibody dynamics and infection histories to evaluate dengue risk." *Nature* **557**(7707): 719-723.
- Samuel, G. H., Z. N. Adelman and K. M. Myles (2016). "Temperature-dependent effects on the replication and transmission of arthropod-borne viruses in their insect hosts." *Curr Opin Insect Sci* **16**: 108-113.
- Samuel, M., D. Maoz, P. Manrique, T. Ward, S. Runge-Ranzinger, J. Toledo, R. Boyce and O. Horstick (2017). "Community effectiveness of indoor spraying as a dengue vector control method: A systematic review." *PLoS Negl Trop Dis* **11**(8): e0005837.
- Sanchez-Vargas, I., L. C. Harrington, J. B. Doty, W. C. t. Black and K. E. Olson (2018). "Demonstration of efficient vertical and venereal transmission of dengue virus type-2 in a genetically diverse laboratory strain of *Aedes aegypti*." *PLoS Negl Trop Dis* **12**(8): e0006754.
- Sanchez, L., V. Vanlerberghe, L. Alfonso, C. Marquetti Mdel, M. G. Guzman, J. Bisset and P. van der Stuyft (2006). "*Aedes aegypti* larval indices and risk for dengue epidemics." *Emerg Infect Dis* **12**(5): 800-806.
- Schaffner, F. and A. Mathis (2014). "Dengue and dengue vectors in the WHO European region: past, present, and scenarios for the future." *Lancet Infect Dis* **14**(12): 1271-1280.
- Scott, T. W., P. H. Amerasinghe, A. C. Morrison, L. H. Lorenz, G. G. Clark, D. Strickman, P. Kittayapong and J. D. Edman (2000a). "Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: blood feeding frequency." *J Med Entomol* **37**(1): 89-101.
- Scott, T. W., E. Chow, D. Strickman, P. Kittayapong, R. A. Wirtz, L. H. Lorenz and J. D. Edman (1993a). "Blood-feeding patterns of *Aedes aegypti* (Diptera: Culicidae) collected in a rural Thai village." *J Med Entomol* **30**(5): 922-927.
- Scott, T. W., G. G. Clark, L. H. Lorenz, P. H. Amerasinghe, P. Reiter and J. D. Edman (1993b). "Detection of multiple blood feeding in *Aedes aegypti* (Diptera: Culicidae) during a single gonotrophic cycle using a histologic technique." *J Med Entomol* **30**(1): 94-99.

- Scott, T. W. and A. C. Morrison (2003). *Aedes aegypti* density and the risk of dengue virus transmission. Ecological aspects for application of genetically modified mosquitoes. Dordrecht, The Netherlands, *FRONTIS*. **2**: 187-206.
- Scott, T. W., A. C. Morrison, L. H. Lorenz, G. G. Clark, D. Strickman, P. Kittayapong, H. Zhou and J. D. Edman (2000b). "Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: population dynamics." *J Med Entomol* **37**(1): 77-88.
- Scott, T. W. and W. Takken (2012). "Feeding strategies of anthropophilic mosquitoes result in increased risk of pathogen transmission." *Trends Parasitol* **28**(3): 114-121.
- Seet, R. C., A. M. Quek and E. C. Lim (2007). "Post-infectious fatigue syndrome in dengue infection." *J Clin Virol* **38**(1): 1-6.
- Sessions, O. M., K. Khan, Y. a. Hou, E. Meltzer, M. Quam, E. Schwartz, D. J. Gubler and A. Wilder-Smith (2013). "Exploring the origin and potential for spread of the 2013 dengue outbreak in Luanda, Angola." *Global health action* **6**: 21822-21822.
- Shepard, D. S., E. A. Undurraga and Y. A. Halasa (2013). "Economic and disease burden of dengue in Southeast Asia." *PLoS Negl Trop Dis* **7**(2): e2055.
- Shu, P. Y., S. F. Chang, Y. C. Kuo, Y. Y. Yueh, L. J. Chien, C. L. Sue, T. H. Lin and J. H. Huang (2003). "Development of group- and serotype-specific one-step SYBR green I-based real-time reverse transcription-PCR assay for dengue virus." *J Clin Microbiol* **41**(6): 2408-2416.
- Silver, J. B. and M. W. Service (2008). *Mosquito ecology: Field sampling methods*. Springer. Netherlands, Springer: 1473.
- Simo, F. B. N., J. J. Bigna, S. Kenmoe, M. S. Ndangang, E. Temfack, P. F. Moundipa and M. Demanou (2019). "Dengue virus infection in people residing in Africa: a systematic review and meta-analysis of prevalence studies." *Scientific reports* **9**(1): 13626-13626.
- Smith, D. L., T. A. Perkins, R. C. Reiner, Jr., C. M. Barker, T. Niu, L. F. Chaves, A. M. Ellis, D. B. George, A. Le Menach, J. R. Pulliam, D. Bisanzio, C. Buckee, C. Chiyaka, D. A. Cummings, A. J. Garcia, M. L. Gatton, P. W. Gething, D. M. Hartley, G. Johnston, E. Y. Klein, E. Michael, A. L. Lloyd, D. M. Pigott, W. K. Reisen, N. Ruktanonchai, B. K. Singh, J. Stoller, A. J. Tatem, U. Kitron, H. C. Godfray, J. M. Cohen, S. I. Hay and T. W. Scott (2014). "Recasting the theory of mosquito-borne pathogen transmission dynamics and control." *Trans R Soc Trop Med Hyg* **108**(4): 185-197.
- Soo, K. M., B. Khalid, S. M. Ching and H. Y. Chee (2016). "Meta-Analysis of Dengue Severity during Infection by Different Dengue Virus Serotypes in Primary and Secondary Infections." *PLoS One* **11**(5): e0154760.
- Souza, R. S., F. Virginio, T. I. S. Riback, L. Suesdek, J. B. Barufi and F. A. Genta (2019). "Microorganism-based larval diets affect mosquito development, size and nutritional reserves in the yellow fever mosquito *Aedes aegypti* (Diptera: Culicidae)." *Frontiers in Physiology* **10**: 152.
- Sternberg, E. D. and M. B. Thomas (2018). "Insights from agriculture for the management of insecticide resistance in disease vectors." *Evol Appl* **11**(4): 404-414.
- Stoddard, S. T., B. M. Forshey, A. C. Morrison, V. A. Paz-Soldan, G. M. Vazquez-Prokopec, H. Astete, R. C. Reiner, Jr., S. Vilcarrero, J. P. Elder, E. S. Halsey, T. J. Kochel, U. Kitron and T. W. Scott (2013). "House-to-house human movement drives dengue virus transmission." *Proc Natl Acad Sci U S A* **110**(3): 994-999.
- Stoddard, S. T., H. J. Wearing, R. C. Reiner Jr, A. C. Morrison, H. Astete, S. Vilcarrero, C. Alvarez, C. Ramal-Asayag, M. Sihuinchu, C. Rocha, E. S. Halsey, T. W. Scott, T. J. Kochel and B. M. Forshey (2014). "Long-term and seasonal dynamics of dengue in Iquitos, Peru." *PLoS Negl Trop Dis* **8**(7).

- Stoler, J., R. al Dashti, F. Anto, J. N. Fobil and G. A. Awandare (2014). "Deconstructing "malaria": West Africa as the next front for dengue fever surveillance and control." Acta Tropica **134**: 58-65.
- Stolerman, L. M., P. D. Maia and J. N. Kutz (2019). "Forecasting dengue fever in Brazil: An assessment of climate conditions." PLoS One **14**(8): e0220106.
- Strode, C., M. de Melo-Santos, T. Magalhaes, A. Araujo and C. Ayres (2012). "Expression profile of genes during resistance reversal in a temephos selected strain of the dengue vector, *Aedes aegypti*." PLoS One **7**(8): e39439.
- Styer, L. M., S. L. Minnick, A. K. Sun and T. W. Scott (2007). "Mortality and reproductive dynamics of *Aedes aegypti* (Diptera: Culicidae) fed human blood." Vector-Borne and Zoonotic Diseases **7**(1): 86-98.
- Su, X., Y. Guo, J. Deng, J. Xu, G. Zhou, T. Zhou, Y. Li, D. Zhong, L. Kong, X. Wang, M. Liu, K. Wu, G. Yan and X. G. Chen (2019). "Fast emerging insecticide resistance in *Aedes albopictus* in Guangzhou, China: Alarm to the dengue epidemic." PLoS Negl Trop Dis **13**(9): e0007665.
- Succo, T., I. Leparco-Goffart, J. B. Ferre, D. Roiz, B. Broche, M. Maquart, H. Noel, O. Catelinois, F. Entezam, D. Caire, F. Jourdain, I. Esteve-Moussion, A. Cochet, C. Paupy, C. Rousseau, M. C. Paty and F. Golliot (2016). "Autochthonous dengue outbreak in Nimes, South of France, July to September 2015." Euro Surveill. **21**.
- Suphanchaimat, R., P. Thammavijaya, P. Taweewigyakarn, P. Buahung, T. Boonchalermvichien, P. Pensuk, P. Niyamrattanakulsiri, M. F. Alikhan and Y. N. Aung (2019). "Systemic investigation of dengue incidence and control measures in Surin, Thailand, 2018." OSIR **12**(1): 15-23.
- Tan, C. H., P. J. Wong, M. I. Li, H. Yang, L. C. Ng and S. L. O'Neill (2017). "wMel limits zika and chikungunya virus infection in a Singapore Wolbachia-introgressed *Ae. aegypti* strain, wMel-Sg." PLoS Negl Trop Dis **11**(5): e0005496.
- Tangena, J. A., P. Thammavong, A. Hiscox, S. W. Lindsay and P. T. Brey (2015). "The human-baited double net trap: an alternative to human landing catches for collecting outdoor biting mosquitoes in Lao PDR." PLoS One **10**(9): e0138735.
- Tantowijoyo, W., B. Andari, E. Arguni, N. Budiwati, I. Nurhayati, I. Fitriana, I. Ernesia, E. W. Daniwijaya, E. Supriyati, D. H. Yusdiana, M. Victorius, D. S. Wardana, H. Ardiansyah, R. A. Ahmad, P. A. Ryan, C. P. Simmons, A. A. Hoffmann, E. Rancès, A. P. Turley, P. Johnson, A. Utarini and S. L. O'Neill (2020). "Stable establishment of wMel Wolbachia in *Aedes aegypti* populations in Yogyakarta, Indonesia." PLoS Negl Trop Dis **14**(4): e0008157.
- Teixeira, M. G. and M. L. Barreto (2009a). "Diagnosis and management of dengue." BMJ **339**: b4338.
- Teixeira, M. G., C. Costa Mda, F. Barreto and M. L. Barreto (2009b). "Dengue: twenty-five years since reemergence in Brazil." Cad Saude Publica **25 Suppl 1**: S7-18.
- Telle, O., A. Vaguet, N. K. Yadav, B. Lefebvre, A. Cebeillac, B. N. Nagpal, E. Daude and R. E. Paul (2016). "The spread of dengue in an endemic urban milieu-The case of Delhi, India." PLoS One **11**(1): e0146539.
- Ten Bosch, Q. A., H. E. Clapham, L. Lambrechts, V. Duong, P. Buchy, B. M. Althouse, A. L. Lloyd, L. A. Waller, A. C. Morrison, U. Kitron, G. M. Vazquez-Prokopec, T. W. Scott and T. A. Perkins (2018). "Contributions from the silent majority dominate dengue virus transmission." PLoS Pathog **14**(5): e1006965.
- Thai Ministry of Public Health (2013). Manual of assessment district for sustainable disease control 2013. D. o. d. control. Nonthaburi Province, Ministry of Public Health of Thailand.

- Thenmozhi, V., J. G. Hiriyan, S. C. Tewari, P. Philip Samuel, R. Paramasivan, R. Rajendran, T. R. Mani and B. K. Tyagi (2007). "Natural vertical transmission of dengue virus in *Aedes albopictus* (Diptera: Culicidae) in Kerala, a southern Indian state." Jpn J Infect Dis **60**(5): 245-249.
- Thomas, D. D., C. A. Donnelly, R. J. Wood and L. S. Alphey (2000). "Insect population control using a dominant, repressible, lethal genetic system." Science **287**(5462): 2474-2476.
- Thomas, S. J., J. Aldstadt, R. G. Jarman, D. Buddhari, I. K. Yoon, J. H. Richardson, A. Ponlawat, S. Iamsirithaworn, T. W. Scott, A. L. Rothman, R. V. Gibbons, L. Lambrechts and T. P. Endy (2015). "Improving dengue virus capture rates in humans and vectors in Kamphaeng Phet Province, Thailand, using an enhanced spatiotemporal surveillance strategy." Am J Trop Med Hyg **93**(1): 24-32.
- Timiryasova, T. M., M. I. Bonaparte, P. Luo, R. Zedar, B. T. Hu and S. W. Hildreth (2013). "Optimization and validation of a plaque reduction neutralization test for the detection of neutralizing antibodies to four serotypes of dengue virus used in support of dengue vaccine development." The American journal of tropical medicine and hygiene **88**(5): 962-970.
- Tipayamongkhogul, M. and S. Lisakulruk (2011). "Socio-geographical factors in vulnerability to dengue in Thai villages: a spatial regression analysis." Geospat Health **5**(2): 191-198.
- Tran, T. T., A. Olsen, E. Viennet and A. Sleigh (2015). "Social sustainability of *Mesocyclops* biological control for dengue in South Vietnam." Acta Trop **141**(Pt A): 54-59.
- Tun-Lin, W., T. R. Burkot and B. H. Kay (2000). "Effects of temperature and larval diet on development rates and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia." Med Vet Entomol **14**(1): 31-37.
- Udayanga, L., N. Gunathilaka, M. C. M. Iqbal, K. Lakmal, U. S. Amarasinghe and W. Abeyewickreme (2018). "Comprehensive evaluation of demographic, socio-economic and other associated risk factors affecting the occurrence of dengue incidence among Colombo and Kandy Districts of Sri Lanka: a cross-sectional study." Parasit Vectors **11**(1): 478.
- Uehara, A., H. A. Tissera, C. K. Bodinayake, A. Amarasinghe, A. Nagahawatte, L. G. Tillekeratne, J. Cui, M. E. Reller, P. Palihawadana, S. Gunasena, A. D. Desilva, A. Wilder-Smith, D. J. Gubler, C. W. Woods and O. M. Sessions (2017). "Analysis of dengue serotype 4 in Sri Lanka during the 2012-2013 dengue epidemic." The American journal of tropical medicine and hygiene **97**(1): 130-136.
- Umakanth, M. (2017). "Post dengue fatigue syndrome-Review." Saudi J. Med. Pharm. Sc **3**.
- van den Berg, H., M. Zaim, R. S. Yadav, A. Soares, B. Ameneshewa, A. Mnzava, J. Hii, A. P. Dash and M. Ejov (2012). "Global trends in the use of insecticides to control vector-borne diseases." Environ Health Perspect **120**(4): 577-582.
- van Panhuis, W. G., M. Choisy, X. Xiong, N. S. Chok, P. Akarasewi, S. Iamsirithaworn, S. K. Lam, C. K. Chong, F. C. Lam, B. Phommasak, P. Vongphrachanh, K. Bouaphanh, H. Rekol, N. T. Hien, P. Q. Thai, T. N. Duong, J.-H. Chuang, Y.-L. Liu, L.-C. Ng, Y. Shi, E. A. Tayag, V. G. Roque, Jr., L. L. Lee Suy, R. G. Jarman, R. V. Gibbons, J. M. S. Velasco, I.-K. Yoon, D. S. Burke and D. A. T. Cummings (2015). "Region-wide synchrony and traveling waves of dengue across eight countries in Southeast Asia." Proceedings of the National Academy of Sciences of the United States of America **112**(42): 13069-13074.
- Vanlerberghe, V., Y. Trongtokit, S. Jirarojwatana, R. Jirarojwatana, A. Lenhart, C. Apiwathnasorn, P. J. McCall and P. Van der Stuyft (2013). "Coverage-dependent effect of insecticide-treated curtains for dengue control in Thailand." Am J Trop Med Hyg **89**(1): 93-98.

- Vazquez-Prokopec, G. M., D. Bisanzio, S. T. Stoddard, V. Paz-Soldan, A. C. Morrison, J. P. Elder, J. Ramirez-Paredes, E. S. Halsey, T. J. Kochel, T. W. Scott and U. Kitron (2013). "Using GPS technology to quantify human mobility, dynamic contacts and infectious disease dynamics in a resource-poor urban environment." *PLoS One* **8**(4): e58802.
- Vazquez-Prokopec, G. M., W. A. Galvin, R. Kelly and U. Kitron (2009). "A new, cost-effective, battery-powered aspirator for adult mosquito collections." *J Med Entomol* **46**(6): 1256-1259.
- Vazquez-Prokopec, G. M., A. Medina-Barreiro, A. Che-Mendoza, F. Dzul-Manzanilla, F. Correa-Morales, G. Guillermo-May, W. Bibiano-Marin, V. Uc-Puc, E. Geded-Moreno, J. Vadillo-Sanchez, J. Palacio-Vargas, S. A. Ritchie, A. Lenhart and P. Manrique-Saide (2017a). "Deltamethrin resistance in *Aedes aegypti* results in treatment failure in Merida, Mexico." *PLoS Negl Trop Dis* **11**(6): e0005656.
- Vazquez-Prokopec, G. M., B. L. Montgomery, P. Horne, J. A. Clennon and S. A. Ritchie (2017b). "Combining contact tracing with targeted indoor residual spraying significantly reduces dengue transmission." *Sci Adv* **3**(2): e1602024.
- Vázquez, S., G. Lemos, M. Pupo, O. Ganzón, D. Palenzuela, A. Indart and M. G. Guzmán (2003). "Diagnosis of dengue virus infection by the visual and simple AuBioDOT immunoglobulin M capture system." *Clinical and diagnostic laboratory immunology* **10**(6): 1074-1077.
- Vincenti-Gonzalez, M. F., A. Tami, E. F. Lizarazo and M. E. Grillet (2018). "ENSO-driven climate variability promotes periodic major outbreaks of dengue in Venezuela." *Sci Rep* **8**(1): 5727.
- von Seidlein, L., H. Wood, O. S. Brittain, L. Tusting, A. Bednarz, S. Mshamu, C. Kahabuka, J. Deen, D. Bell, S. W. Lindsay and J. Knudsen (2019). "Knowledge gaps in the construction of rural healthy homes: A research agenda for improved low-cost housing in hot-humid Africa." *PLoS Med* **16**(10): e1002909.
- Vontas, J., E. Kioulos, N. Pavlidi, E. Morou, A. della Torre and H. Ranson (2012). "Insecticide resistance in the major dengue vectors *Aedes albopictus* and *Aedes aegypti*." *Pesticide Biochemistry and Physiology* **104**(2): 126-131.
- Vyas, S. and L. Kumaranayake (2006). "Constructing socio-economic status indices: how to use principal components analysis." *Health Policy Plan* **21**(6): 459-468.
- Wangdi, K., A. C. Clements, T. Du and S. V. Nery (2018). "Spatial and temporal patterns of dengue infections in Timor-Leste, 2005–2013." *Parasites & vectors* **11**(1): 9.
- Ward, T., M. Samuel, D. Maoz, S. Runge-Ranzinger, R. Boyce, J. Toledo, R. Velayudhan and O. Horstick (2017). "Dengue data and surveillance in Tanzania: a systematic literature review." *Trop Med Int Health* **22**(8): 960-970.
- Wasinpiyamongkol, L., S. Patramool, N. Luplertlop, P. Surasombatpattana, S. Doucoure, F. Mouchet, M. Seveno, F. Remoue, E. Demetere, J. P. Brizard, P. Jouin, D. G. Biron, F. Thomas and D. Misse (2010). "Blood-feeding and immunogenic *Aedes aegypti* saliva proteins." *Proteomics* **10**(10): 1906-1916.
- Weetman, D., B. Kamgang, A. Badolo, C. L. Moyes, F. M. Shearer, M. Coulibaly, J. Pinto, L. Lambrechts and P. J. McCall (2018). "Aedes mosquitoes and Aedes-borne arboviruses in Africa: Current and future threats." *International journal of environmental research and public health* **15**(2): 220.
- Wesson, D., A. Morrison, V. Paz Soldan, R. Moudy, K. Long, L. Ponnusamy, J. Mohler, H. Astete, L. Ayyash, E. Halsey, C. Schal, T. W. Scott and C. Apperson (2012). "Lethal ovitraps and dengue prevention: report from Iquitos, Peru." *International Journal of Infectious Diseases* **16**: e473.
- Whitehorn, J., D. T. Kien, N. M. Nguyen, H. L. Nguyen, P. P. Kyrylos, L. B. Carrington, C. N. Tran, N. T. Quyen, L. V. Thi, D. Le Thi, N. T. Truong, T. T. Luong, C. V. Nguyen, B. Wills, M. Wolbers and C. P. Simmons (2015).

"Comparative susceptibility of *Aedes albopictus* and *Aedes aegypti* to dengue virus infection after feeding on blood of viremic humans: Implications for public health." J Infect Dis **212**(8): 1182-1190.

WHO/EMRO (2005). Newsletters. R. O. f. t. E. M. World Health Organisation, Division of Communicable Disease Control. **6**: 7-8.

Wijayanti, S. P., T. Porphyre, M. Chase-Topping, S. M. Rainey, M. McFarlane, E. Schnettler, R. Biek and A. Kohl (2016a). "The Importance of socio-economic versus environmental risk factors for reported dengue cases in Java, Indonesia." PLoS Negl Trop Dis **10**(9): e0004964.

Wijayanti, S. P., S. Sunaryo, S. Suprihatin, M. McFarlane, S. M. Rainey, I. Dietrich, E. Schnettler, R. Biek and A. Kohl (2016b). "Dengue in Java, Indonesia: Relevance of mosquito indices as risk predictors." PLoS Negl Trop Dis **10**(3): e0004500.

Wilder-Smith, A. (2012). "Dengue infections in travellers." Paediatrics and international child health **32** Suppl 1(s1): 28-32.

Wilder-Smith, A. (2019). "Can dengue virus be sexually transmitted?" J Travel Med **26**(3).

Wilder-Smith A. , Q. M., Sessions O. , Rocklov J. , Liu-Helmersson J. , Franco L. , Khan K. (2014). "The 2012 dengue outbreak in Madeira: exploring the origins." Euro Surveill **19**(8).

Wilson, A. L., R. C. Dhiman, U. Kitron, T. W. Scott, H. van den Berg and S. W. Lindsay (2014). "Benefit of insecticide-treated nets, curtains and screening on vector borne diseases, excluding malaria: a systematic review and meta-analysis." PLoS Negl Trop Dis **8**(10): e3228.

Woon, Y. L., C. P. Hor, N. Hussin, A. Zakaria, P. P. Goh and W. K. Cheah (2016). "A Two-year review on epidemiology and clinical characteristics of dengue deaths in Malaysia, 2013-2014." PLOS Neglected Tropical Diseases **10**(5): e0004575.

World Bank Group (2016). Getting back on track: Reviving growth and securing prosperity for all: Thailand systematic country diagnosis. Washigton.

World Health, O. (2016). Monitoring and managing insecticide resistance in *Aedes* mosquito populations: interim guidance for entomologists. Geneva, World Health Organization.

World Health, O., U. N. U. W. B. W. S. P. f. Research and D. Training in Tropical (2017). Operational guide: Early Warning and Response System (EWARS) for dengue outbreaks. Geneva, World Health Organization.

World Health Organization (1986). Dengue hemorrhagic fever, diagnosis, treatment, and control Geneva, Switwerland, World Health Organisation.

World Health Organization (2001). Report of the fourth WHOPES working group meeting : WHO/HQ, Geneva, 4-5 December 2000 : review of : IR3535; KBR3023; (RS)-Methoprene 20% EC, Pyriproxyfen 0.5% GR; and Lambda-Cyhalothrin 2.5% CS. Geneva, World Health Organization.

World Health Organization (2005). Guidelines for laboratory and field testing of mosquito larvicides. Geneva, WHO.

World Health Organization (2007a). Guidelines for plaque reduction neutralization testing of human antibodies to dengue viruses. Geneva, World Health Organization.

World Health Organization (2007b). Manual for indoor residual spraying : application of residual sprays for vector control. Geneva, World Health Organization.

World Health Organization (2009). Dengue guidelines for diagnosis, treatment, prevention and control Geneva, WHO.

World Health Organization (2011a). Action against dengue: dengue day campaign across Asia. Geneva, WHO.

World Health Organization (2013). Test procedures for insecticide resistance monitoring in malaria vectors mosquitoes. Geneva, WHO.

World Health Organization (2016a). Background paper on dengue vaccines, WHO.

World Health Organization (2016b). Monitoring and managing insecticide resistance in Aedes mosquito populations. Interim guidance for entomologists Geneva, WHO: 11.

World Health Organization (2016c). "Weekly epidemiological report_ Dengue vaccine : WHO position paper." **30**.

World Health Organization (2018). "Weekly Epidemiological Record." Weekly Epidemiological Record **93**(36): 457-476.

World Health Organization, R. O. f. S.-E. A. (2011b). Comprehensive guideline for prevention and control of dengue and dengue haemorrhagic fever. Revised and expanded edition. New Delhi, WHO Regional Office for South-East Asia.

World Health Organization. Regional Office for South-East, A. (2018). "Dengue Bulletin, Vol-40."

Xu, J., M. Bonizzoni, D. Zhong, G. Zhou, S. Cai, Y. Li, X. Wang, E. Lo, R. Lee, R. Sheen, J. Duan, G. Yan and X. G. Chen (2016). "Multi-country survey revealed prevalent and novel F1534S mutation in Voltage-Gated Sodium Channel (VGSC) gene in Aedes albopictus." PLoS Negl Trop Dis **10**(5): e0004696.

Xue, L., X. Fang and J. M. Hyman (2018). "Comparing the effectiveness of different strains of Wolbachia for controlling chikungunya, dengue fever, and zika." PLoS Negl Trop Dis **12**(7): e0006666.

Ya-Umphon, P., D. Cerqueira, G. Cottrell, D. M. Parker, F. J. I. Fowkes, F. Nosten and V. Corbel (2018). "Anopheles salivary biomarker as a proxy for estimating Plasmodium falciparum malaria exposure on the Thailand-Myanmar border." Am J Trop Med Hyg **99**(2): 350-356.

Ya-Umphon, P., D. Cerqueira, D. M. Parker, G. Cottrell, A. Poinson, F. Remoue, C. Brengues, T. Chareonviriyaphap, F. Nosten and V. Corbel (2017). "Use of an Anopheles salivary biomarker to assess malaria transmission risk along the Thailand-Myanmar border." J Infect Dis **215**(3): 396-404.

Yanola, J., P. Somboon, C. Walton, W. Nachaiwieng, P. Somwang and L. A. Prapanthadara (2011). "High-throughput assays for detection of the F1534C mutation in the voltage-gated sodium channel gene in permethrin-resistant Aedes aegypti and the distribution of this mutation throughout Thailand." Trop Med Int Health **16**(4): 501-509.

Yauch, L. E. and S. Shresta (2014). "Dengue virus vaccine development." Adv Virus Res **88**: 315-372.

Yen, J. H. and A. R. Barr (1971). "New hypothesis of the cause of cytoplasmic incompatibility in Culex pipiens L." Nature **232**(5313): 657-658.

Yobo, C. M., C. A. M. Sadia-Kacou, M. A. Adja, E. Elanga-Ndille, A. B. Sagna, N. Guindo-Coulibaly, A. Poinson, F. Remoue and B. G. Koudou (2018). "Evaluation of human exposure to Aedes bites in rubber and palm cultivations using an immunoepidemiological biomarker." Biomed Res Int **2018**: 3572696.

Yunta, C., N. Grisales, S. Nasz, K. Hemmings, P. Pignatelli, M. Voice, H. Ranson and M. J. Paine (2016). "Pyriproxyfen is metabolized by P450s associated with pyrethroid resistance in An. gambiae." Insect Biochem Mol Biol **78**: 50-57.

Zanotto, P. M. d. A. and L. C. d. C. Leite (2018). "The challenges imposed by dengue, zika, and chikungunya to Brazil." Frontiers in Immunology **9**: 1964.

Zeidler, J. D., L. O. Fernandes-Siqueira, G. M. Barbosa and A. T. Da Poian (2017). "Non-canonical roles of dengue virus non-structural proteins." Viruses **9**(3): 42.

Zhang, H., Z. Li, S. Lai, A. Clements, L. Wang, W. Yin, H. Zhou, H. Yu, W. Hu and W. Yang (2014). "Evaluation of the performance of a dengue outbreak detection tool for China." PloS one **9**: e106144.

Résumé de la thèse

Plus de 80 % de la population mondiale vit dans des zones exposées à une ou plusieurs des sept principales maladies à transmission vectorielle. Sur ces sept maladies, quatre sont transmises par des moustiques du genre *Aedes* (Golding et al. 2015). Au cours des dix dernières années, des maladies infectieuses causées par des virus transmis par des arthropodes ("arboviroses"), notamment les virus de la dengue (DENV), du chikungunya (CHIKV), du Zika (ZIKV) et de la fièvre jaune (YFV), ont fait leur apparition dans le monde entier, sous l'impulsion des deux principaux moustiques vecteurs, *Aedes aegypti* et *Ae. albopictus* (Girard et al. 2020). L'expansion des maladies transmises par l'*Aedes* est attribuée à des facteurs qui favorisent la dispersion et la prolifération des moustiques *Aedes* en raison du changement climatique, du commerce mondial, de l'urbanisation non planifiée, de la mise en œuvre inefficace des programmes de contrôle des vecteurs, et du manque d'engagement communautaire et de volonté politique (Roiz et al. 2018). De plus, de nombreux pays ne sont toujours pas prêts à relever le défi des maladies à transmission vectorielle, et manquent d'orientations et d'outils adéquats pour prévenir l'introduction, l'établissement et/ou la propagation des moustiques vecteurs et des virus (Roiz et al. 2018). Par ailleurs, les systèmes de surveillance des vecteurs d'arboviroses actuels présentent d'importantes lacunes, notamment en Asie du Sud-Est et en Amérique latine, où les épidémies d'arboviroses sont en augmentation (Weetman et al. 2018). En effet, les maladies transmises par les *Aedes* ne présentent pas une dynamique simple et les flambées épidémiques sont particulièrement difficiles à prévoir (Brady et al. 2015). Cela suscite des inquiétudes concernant les systèmes de détection et d'alerte précoces des épidémies, en particulier quant à l'application de leurs lignes directrices et de leurs indicateurs actuels. Il n'existe pas aujourd'hui d'outils de surveillance sensibles, et la plupart des études n'ont pas réussi à démontrer de bonnes corrélations entre les indicateurs entomologiques et les épisodes de dengue (Bowman et al. 2014), et aucun seuil entomologique ne s'est avéré efficace pour prédire les épidémies de virus à *Aedes* (Bowman et al. 2016, Reiner et al. 2016). Malheureusement, de récents modèles prédictifs basés sur les conditions climatiques et la croissance urbaine suggèrent qu'*Ae. aegypti* et *Ae. albopictus* devraient continuer à s'étendre au-delà de leurs distributions actuelles, ce qui étendrait le risque de transmission autochtone à de nouveaux territoires (Kraemer et al. 2019). Des approches plus rentables et des outils pratiques capables de mesurer de manière fiable la dynamique de la transmission de la dengue en temps réel

sont nécessaires pour permettre des prévisions plus utiles et plus précises des épidémies et de l'incidence de la dengue.

Cette thèse a été menée dans le cadre du projet DENGUE INDEX, financé par le Conseil Norvégien de la Recherche, visant à mettre au point des indicateurs entomologiques et immunologiques de la transmission de la dengue sensibles et pratiques, pouvant être utilisés pour prédire les épidémies de dengue. Dans ce contexte, cette thèse a exploré les déterminants associés au risque de transmission de la dengue dans le nord-est de la Thaïlande en utilisant différentes approches (entomologie, immunologie, virologie) et conceptions (étude rétrospective, étude cas-témoins et un essai contrôlé randomisé), et à identifier les principaux déterminants associés à l'exposition aux moustiques *Aedes* en utilisant un biomarqueur sérologique spécifique. Ainsi, cette thèse avait les quatre objectifs spécifiques suivants : i) Évaluer la dynamique spatiale et temporelle de la dengue dans le nord-est de la Thaïlande ; ii) Aborder la relation complexe entre les vecteurs *Aedes*, la transmission de la dengue et les facteurs socio-économiques ; iii) Évaluer les variations à petite échelle de l'exposition humaine aux piqûres de moustiques *Aedes* à l'aide un biomarqueur salivaire au cours d'un essai randomisé d'intervention de contrôle vectoriel ; iv) le dernier objectif, qui diffère des précédents, visait à évaluer l'évolution des traits de résistance aux insecticides dans les populations locales de vecteurs de la dengue suite au déploiement de l'intervention de lutte antivectorielle à base de pyriproxifène (PPF).

La première partie décrit la dynamique spatiale et temporelle de l'incidence de la dengue dans le nord-est de la Thaïlande, où la thèse a été réalisée. La deuxième partie traite des relations complexes entre les infections de dengue, l'infestation vectorielle et le risque d'exposition humaine aux piqûres de moustiques *Aedes* et évalue la précision des indices entomologiques et immunologiques pour distinguer les maisons où la dengue est présente, des maisons témoins (non touchées). La troisième partie étudie l'étroite association entre les niveaux d'infestation d'*Aedes* et le risque d'exposition aux moustiques, mesuré par le niveau de réponse des anticorps à l'antigène salivaire d'*Aedes*, afin de valider l'utilisation de biomarqueurs salivaires comme approximation pour estimer le contact "homme-vecteur" et le risque de transmission de la dengue dans le contexte d'une intervention de contrôle vectoriel. La dernière partie, qui diffère légèrement des trois précédentes, aborde l'impact de l'intervention de contrôle des vecteurs sur la sélection de la

résistance aux insecticides afin d'orienter les stratégies de contrôles vectoriels pour la prévention de la dengue.

L'étude préliminaire visait à évaluer les tendances saisonnières de l'incidence de la dengue dans la province de Khon Kaen (incluant 199 sous-districts et 2 139 villages) et à identifier les facteurs potentiels contribuant à la dispersion de la dengue à une échelle de résolution spatiale fine. Pour ce faire, nous avons réalisé une étude épidémiologique rétrospective en utilisant l'incidence mensuelle de la dengue et les données climatiques au niveau des sous-districts, afin de mieux comprendre les relations entre la dengue et le climat et d'identifier les périodes et les zones à plus haut risque de transmission de la dengue. Les cas de dengue du 1er janvier 2006 au 31 décembre 2016 ont été extraits auprès du Ministère de la Santé Publique (MoPH) et classés selon la classification de la dengue de l'OMS pre-2009 (c'est-à-dire DF, DHF, DSS). Les données météorologiques pour la même période ont été téléchargées à partir de la bibliothèque de données de l'Institut international de recherche sur le climat et la société. La régression du modèle Bayésien de Poisson a été utilisée pour évaluer les associations entre l'incidence mensuelle de la dengue au niveau des sous-districts et les variations climatiques. La population a été utilisée comme dénominateur dans le modèle. Pour le modèle principal, les covariables étaient la densité de population par km², le sexe, l'âge moyen, les précipitations moyennes et les températures minimales et maximales. La densité de population a été incluse dans le modèle de régression en tant que "proxy" pour l'estimation des niveaux d'urbanisation. Une structure autorégressive conditionnelle a été utilisée comme un effet aléatoire capturant l'autocorrélation spatio-temporelle. De plus, les indicateurs locaux d'association spatiale (LISA) ont été utilisés pour identifier les "hotspots" de la dengue (c'est-à-dire lorsque l'incidence est plus élevée que le nombre attendu compte tenu d'une distribution aléatoire des cas) et les "coldspots" de l'incidence de la dengue à l'échelle des sous-districts.

Nous avons ainsi démontré un changement au cours des dix dernières années dans l'âge des cas, le groupe d'âge des 15-29 ans étant le plus touché par la maladie corroborant la tendance similaire dans le schéma d'infection de la dengue observée dans d'autres pays de la région SEA. En outre, nous avons montré que l'incidence de la dengue présentait un schéma saisonnier clair, avec environ 73% des cas de dengue survenant pendant la saison des pluies. Nos résultats ont montré une bonne corrélation entre l'incidence de la dengue et les facteurs climatiques, en

particulier la température et les précipitations. Bien que la dynamique de l'incidence de la dengue soit clairement influencée par les précipitations et la température, nos données montrent un regroupement spatial des cas de dengue associés à des paramètres environnementaux tels que l'urbanisation. L'analyse de régression spatiale suggère que d'autres variables que le niveau d'urbanisation, peuvent expliquer les différences d'incidence de la dengue, car la moitié des « hot-spots » de dengue ont été trouvés dans des zones rurales situées dans le sud-ouest de la province, corroborant ainsi l'influence d'autres facteurs dans la transmission de la dengue. Pour conclure, cette étude de base a clairement montré l'implication des facteurs climatiques sur la transmission de la dengue dans la province. Le regroupement spatial des cas de dengue a été en partie associé aux zones urbaines plus proches de la ville de Khon Kaen et aux zones rurales du sud-ouest de la province. Toutefois, l'analyse actuelle n'a pas permis de détecter un facteur de substitution proche pour quantifier une relation entre l'urbanisation et l'incidence de la dengue. Cette étude préliminaire a mis en évidence la nécessité d'approfondir les recherches sur les facteurs de risque liés à la dengue dans la zone d'étude afin de développer des systèmes d'alerte précoce de la dengue pour guider les opérations de contrôle des vecteurs.

Pour cela, nous avons mené une première étude prospective de cas-témoin en milieu hospitalier afin d'identifier les facteurs de risque des infections de dengue. Il s'agissait d'évaluer si les indicateurs entomologiques et immunologiques pouvaient faire la différence entre les maisons positives et négatives à la dengue. Au total, dix-neuf hôpitaux communautaires de district et de sous-district dans les provinces de Khon Kaen, Roi Et, Kalasin et Maha Sarakham ont été invités à participer à l'étude sur la base de leurs bonnes pratiques cliniques pour détecter les cas de dengue et de leur volonté de participer à l'étude. Les collectes de l'étude ont débuté en juin 2016 et se sont poursuivies jusqu'en août 2019. À l'hôpital, les cas présumés de dengue ont été diagnostiqués à l'aide du SD Duo Bioline RDT. Du sang a été prélevé pour la détection et le sérotypage du DENV (Shu et al. 2003). Le jour du recrutement, des équipes entomologiques ont été mandatées pour visiter chaque maison de patients afin de recueillir des données sur les caractéristiques de la maison, par exemple le nombre de membres de la famille, le sexe, l'âge, les antécédents de voyage, le statut socio-économique, la position GPS, etc. En outre, des collectes entomologiques ont été effectuées dans les maisons des patients cas et témoins, ainsi que dans les quatre maisons voisines. Les collectes ont porté sur les stades immatures et adultes *Aedes*, capturés à l'aide d'aspirateurs à batterie pendant 15 minutes, à l'intérieur et à l'extérieur. En outre, l'infection par le DENV a été

étudiée chez les femelles *Aedes* par RT-qPCR (Lanciotti et al. 1992). La réponse immunitaire spécifique à l'*Aedes* a été évaluée chez les patients cas et témoins à partir de papiers buvard de sang par un test indirect ELISA (Enzyme-Linked Immunosorbent Assay) utilisant le peptide salivaire Nterm-34kDa (Genepep, St Jean de Vedas, France), spécifique aux *Ae. aegypti*. L'indice d'exposition aux moustiques (MEI) a été défini comme la réponse immunitaire spécifique au peptide salivaire de chaque échantillon. L'analyse des facteurs de risque de la dengue a été analysée par régression logistique multivariée a été effectuée en utilisant toutes les variables (c'est-à-dire les caractéristiques individuelles, les caractéristiques de la maison, le statut socio-économique, les indices entomologiques et immunologiques) et le meilleur modèle a été sélectionné sur le critère d'information d'Akaike (AIC).

Nos résultats ont ainsi montré que l'âge du patient était associé à un risque plus élevé de dengue. Bien que la dengue touche normalement les jeunes enfants, nous avons constaté que les personnes de 10 à 25 ans étaient plus à risque que les personnes plus jeunes ou plus âgées. Bien que d'autres études aient constaté un risque plus élevé de transmission de la dengue dans les familles à faibles revenus (Telle et al. 2016, Wijayanti et al. 2016, Udayanga et al. 2018), aucune association de ce type n'a été trouvée dans notre étude. Cependant, nous avons montré que la construction des maisons peut jouer un rôle dans le risque de transmission de la dengue, car les personnes vivant dans des maisons à deux étages avaient un risque plus élevé d'infection par la dengue.

Bien que cela ne soit pas surprenant, notre étude a confirmé que les indices entomologiques traditionnels n'étaient pas de bons indicateurs de la transmission de la dengue, car ils étaient statistiquement plus élevés dans les maisons "témoins" que dans les maisons "cas" de dengue. En effet, les indices d'infestation par des vecteurs basés sur des stades immatures (HI, BI et CI) étaient tous associés négativement à la dengue en utilisant une analyse univariée. Il convient de mentionner que la plupart des foyers inspectés (témoins et cas) présentaient des indices d'immaturité supérieurs aux seuils de "risque d'épidémie" fixés par le MoPH (à savoir $CI < 1\%$, $BI < 50$ et $HI < 10\%$) (ministère thaïlandais de la santé publique 2013). De même, les indices de pupaison (PHI et PPI) n'étaient pas significativement différents entre les maisons de cas et les maisons témoins, tout comme un nombre encore plus important d'adultes *Aedes* a été trouvé dans les maisons témoins. Bien que surprenant, cela pourrait s'expliquer par des efforts plus importants

de lutte vectorielle après l'apparition des symptômes de la dengue dans les maisons "cas", ce qui aurait réduit l'infestation vectorielle au moment des enquêtes. Néanmoins, nos résultats ont montré que la présence d'*Ae. aegypti* infecté par le DENV dans les ménages était positivement associée aux infections de dengue ($p=0,018$). En effet, la proportion d'*Aedes* infectés par le DENV était plus élevée dans les maisons de patients ($\approx 8\%$) que dans les maisons témoins (3%), ce qui suggère que l'infectiosité des vecteurs serait un indicateur plus fiable que l'abondance vectorielle pour évaluer le risque de transmission de la dengue.

Il est intéressant de noter que les individus du groupe de contrôle présentaient un niveau de réponse anticorps (Ac) plus élevé au Nterm-34 que les individus du groupe de cas de dengue, ce qui corrobore les résultats de l'entomologie. Néanmoins, ni l'abondance d'adultes *Aedes* dans le foyer des patients, ni le niveau d'exposition humaine aux piqûres de moustiques *Aedes* n'ont été corrélés avec l'incidence de la dengue. Cela souligne le fait que la transmission du virus de la dengue est complexe et varie dans le temps et l'espace, et que la relation entre la densité/agressivité du vecteur et le risque d'infection humaine n'est ni statique ni linéaire.

En conclusion, cette première étude met en évidence la relation complexe entre les vecteurs *Aedes*, les facteurs socio-économiques et le risque de transmission de la dengue, et souligne les défis à relever pour mettre en place des indicateurs d'alerte précis pour la prévention de la dengue. Une étude longitudinale randomisée et contrôlée (RCT) menée dans le cadre du projet DENGUE INDEX a ensuite été réalisée pour mieux évaluer la relation étroite entre les niveaux de contact entre l'homme et l'*Aedes*, les niveaux d'infestation par l'*Aedes* et le risque de transmission de la dengue dans le nord-est de la Thaïlande.

Afin de déterminer si la réponse de l'Ac contre le peptide salivaire Nterm-34 pouvait être un bon indicateur pour évaluer les variations à petite échelle de l'abondance de l'*Aedes*, là où la dengue est endémique, nous avons donc mené une enquête sérologique dans le cadre du RCT dans les deux villes RE et KK du nord-est de la Thaïlande. Les facteurs individuels tels que le sexe, l'âge, la profession, ainsi que les interventions socio-économiques, environnementales, épidémiologiques et de contrôle des vecteurs qui pourraient influencer la réponse de l'anticorps aux piqûres de moustiques ont été étudiés.

Pour cette seconde étude, une cohorte de 563 individus a été recrutée parmi les habitants du RCT et a fait l'objet d'un suivi sérologique et entomologique concomitant pendant 19 mois. La

fièvre a été enregistrée chaque semaine pour détecter précocement les symptômes de la dengue chez les participants à l'étude. Des gouttes de sang séché ont été prélevées sur des habitants sélectionnés afin d'évaluer l'exposition humaine aux piqûres de moustiques. Parallèlement à l'enquête sérologique, des collectes entomologiques, comprenant les *Aedes* adultes, les pupes et les larves, ont été effectuées dans les 180 maisons tous les quatre mois. De plus, des collectes entomologiques et des collectes de tâches de sang séché ont été effectuées tous les mois dans trois maisons sentinelles par village. De plus, dans le cadre du RCT, une intervention de contrôle des vecteurs consistant en une distribution tous les quatre mois de PPF (appliqué sous forme de granules à 0,5 %) dans des gîtes larvaires permanents (dose cible de 0,01 mg/L selon la recommandation de l'OMS) a été initiée, après une période de référence de dix mois, dans la moitié des villages, sélectionnés aléatoirement (Overgaard et al 2018). Les villages contrôles n'ont pas bénéficié de l'intervention du PPF.

Plus de 3 980 échantillons de sang ont été prélevés sur papier buvard et analysés par ELISA. Le niveau de réponse Ac au peptide salivaire Nterm-34 a été utilisé pour développer un indice d'exposition des moustiques (MEI) reflétant le niveau de réponse IgG spécifique et individuelle au peptide salivaire *Aedes*. Les relations entre le MEI et les indices d'*Aedes* ainsi que l'infectiosité des vecteurs ont été évaluées au niveau des maisons et des villages à l'aide d'un modèle mixte multivarié à deux niveaux (maison, individu) avec une corrélation autorégressive avec un décalage d'un mois, en supposant que la réponse des anticorps persistait à des niveaux détectables entre deux et six semaines (Orlandi-Pradines et al. 2007, Elanga Ndille et al. 2016).

Cette étude longitudinale a démontré un taux de séroprévalence IgG élevé chez les habitants du nord-est de la Thaïlande, 57,3 % et 60 % des individus de KK et de RE respectivement, étant des répondeurs au peptide salivaire Nterm-34. De plus, dans ces deux villes, la réponse IgG a augmenté quelques semaines après le pic de densité d'*Aedes* (AIC) qui s'est produit au début de la saison des pluies. De plus, la réponse Ac a diminué entre la saison froide et la saison chaude, tandis que les densités de moustiques ont chuté après la saison des pluies et ont augmenté à nouveau pendant la saison chaude. Nos résultats ont donc corroboré les résultats précédents dans d'autres contextes de transmission où une réponse Ac plus élevée contre les antigènes salivaires de l'*Aedes* a été observée avec l'occurrence des pluies (Elanga Ndille et al. 2012, Yobo et al. 2018).

Dans l'ensemble, les résultats suggèrent une association positive décalée entre l'abondance de l'*Aedes* et la réponse de l'homme à la piqûre de l'*Aedes*.

Ensuite, l'analyse multivariée a démontré pour la première fois une association dose-réponse forte et positive entre la réponse individuelle de l'Ac au peptide salivaire Nterm-34 et les niveaux d'abondance de l'*Aedes*, en particulier si l'on considère la densité intérieure de l'*Aedes*. Dans notre étude, un total de 2 235 femelles adultes *Ae. aegypti* ont été collectées, la grande majorité d'entre elles (70 % du total) se trouvant à l'intérieur. Par conséquent, le biomarqueur sérologique semble prometteur pour détecter les petites variations de l'exposition humaine aux piqûres de moustiques *Aedes*. Bien que cela ait déjà été démontré pour les vecteurs du paludisme (Ya-Umphun et al. 2017), c'est la première fois que nous démontrons une telle tendance pour les vecteurs de la dengue.

Malheureusement, aucune relation claire n'a été observée entre l'intensité de la réponse de l'Ac aux piqûres de l'*Aedes* et l'infectiosité des vecteurs (ni au niveau des villages ni au niveau des maisons). Cela confirme que la transmission du virus de la dengue est une affaire complexe qui varie dans le temps et l'espace, et la relation entre la densité des vecteurs et la transmission du virus n'est pas facile à traiter par des enquêtes entomologiques successives. À l'inverse, la relation entre les infections de dengue humaine et l'intensité de la réponse Ac humaine aux piqûres d'*Aedes* n'a pas pu être traitée car aucun cas de dengue n'a été détecté au cours de l'étude longitudinale. Des analyses supplémentaires sont en cours par l'équipe du DENGUE INDEX pour confirmer l'apparente absence d'infection de dengue pendant la période d'étude.

Par ailleurs, l'analyse multivariée révèle que le contact entre les vecteurs et les humains, tel que mesuré par le MEI, varie en fonction de caractéristiques individuelles telles que le sexe et l'âge, les personnes plus âgées étant plus exposées au risque de piqûres d'*Aedes*. De même, le fait d'être un homme était associé à un risque d'exposition aux *Aedes* plus élevé. De plus, les personnes passant la plupart de leur temps à l'intérieur étaient associées à une réponse Ac plus élevée au peptide salivaire, confirmant ainsi la forte préférence endophagique des *Ae. aegypti* (Scott et al. 2000b).

Enfin, nos résultats ont montré que les niveaux d'IgG humaines de l'antigène salivaire d'*Aedes* étaient significativement plus faibles dans les villages traités (ayant reçu 0,01 mg/L de PPF) que dans les villages témoins. Bien que spéculatifs, ces résultats suggèrent que le PPF

pourrait avoir réduit les densités d'*Aedes* sous un certain seuil qui était suffisant pour réduire le contact humain-*Aedes* dans les villages traités par rapport aux groupes de contrôle. Malheureusement, l'impact opérationnel du PPF sur la transmission de la dengue est encore inconnu. Des analyses statistiques sont menées par notre équipe afin de combler cette lacune et d'évaluer si les biomarqueurs salivaires peuvent compléter les outils et indicateurs existants pour le suivi et l'évaluation de l'intervention de contrôle des vecteurs.

Ainsi, cette étude représente une étape importante vers la validation de l'utilisation du peptide salivaire d'*Aedes* Nterm-34kDa comme mesure de substitution pour évaluer les niveaux d'infestation par l'*Aedes* et le risque d'exposition de l'homme aux moustiques dans une zone d'endémie de la dengue. Malheureusement, aucun cas de dengue n'a été détecté au cours du suivi, de sorte que la relation entre la transmission de la dengue et l'exposition à l'*Aedes* n'a pas pu être examinée.

Dans l'étude longitudinale précédente, nous avons montré que les niveaux de réponse individuelle au biomarqueur salivaire de l'*Aedes* étaient plus faibles dans les villages traités que dans les villages témoins, ce qui suggère une réduction potentielle de la densité de l'*Aedes* suite à la mise en œuvre de la lutte antivectorielle. Bien que les recherches soient toujours en cours pour évaluer l'efficacité et l'activité résiduelle du PPF dans la zone d'étude, les résultats préliminaires suggèrent cependant un manque d'impact du PPF sur plusieurs indicateurs entomologiques (communication personnelle Overgaard). Plusieurs facteurs opérationnels pourraient expliquer ce résultat et certains sont actuellement testés par l'équipe DENGUE INDEX par le biais d'études complémentaires. Une autre explication non traitée est la sélection potentielle de la résistance/tolérance au PPF suite au déploiement de l'insecticide dans la zone traitée. En effet, des résistances croisées entre le PPF et les PYR ont été détectées précédemment, ce qui pourrait entraver l'utilisation de cette nouvelle molécule pour la lutte antivectorielle en Thaïlande. Compte tenu de ce qui précède, nous avons effectué un suivi de la résistance aux insecticides dans la zone d'étude avant, pendant et après le déploiement de l'intervention. L'objectif était de déterminer le statut de résistance de base de l'*Aedes aegypti* au PPF et aux pesticides de santé publique utilisés de manière conventionnelle (niveau de base) et d'évaluer les changements dans les niveaux et la fréquence des marqueurs candidats pertinents après le déploiement de l'intervention. Nous avons supposé que l'utilisation subséquente du PPF dans neuf villages de la zone d'étude (équivalent à 1

226 m2) pourrait induire une pression de sélection sur les mécanismes de résistance déjà présents dans la population.

Dans cette étude, nous avons montré de forts niveaux de résistance aux insecticides de lutte contre les vecteurs de santé publique dans la population d'*Aedes* de KK. De plus, nous avons démontré une sélection rapide de la résistance au PPF, dans l'année suivant la mise en œuvre de l'intervention PPF dans les villages traités. L'étude des mécanismes de résistance a révélé une pression de sélection sur les mutations du Kdr, sans corrélation avec l'application du PPF. Par ailleurs, aucune variation du nombre copie de gènes n'a été associée à la résistance au PPF, ce qui sous-tend que d'autres gènes de résistance métabolique pourraient être impliqués. Il est intéressant de noter que les CNV dans les gènes CCE associés à la résistance à l'OP (Grigoraki et al. 2016, Moyes et al. 2017) ont été réduits à la suite de l'intervention du PPF, ce qui suggère que la résistance au téméphos pourrait être inversée sans application de téméphos. Malheureusement, la sensibilité au téméphos n'a pas pu être déterminée après un an en raison du manque d'échantillons de moustiques pour les tests. Si cela se confirme, ce serait une excellente nouvelle pour la lutte contre les vecteurs, qui permettrait de préserver la durée de vie des pesticides de santé publique existants.

En conclusion, la présente thèse a exploré les facteurs de risques associés à l'exposition humaine à l'*Aedes* et le risque de transmission de la dengue en utilisant divers indicateurs épidémiologiques, entomologiques et immunologiques. Dans l'ensemble, nous avons mis en évidence les relations complexes entre l'abondance de l'*Aedes*, l'infectiosité des vecteurs, l'exposition des vecteurs et les infections de dengue dans le nord-est de la Thaïlande, confronté à des épidémies récurrentes et imprévisibles. Nous avons démontré le potentiel de l'utilisation de biomarqueurs salivaires pour évaluer les variations à petite échelle de l'exposition aux moustiques, qui pourraient être déployés dans le cadre d'une surveillance intégrée des vecteurs. En revanche, nous n'avons pas montré de corrélation positive entre les niveaux d'exposition aux moustiques (mesurés par des indices entomologiques et immunologiques) et la transmission de la dengue, ce qui met en évidence les limites des outils existants pour prédire le risque de transmission de la dengue. Plusieurs facteurs, dont les mouvements et les habitudes de l'homme, l'immunité acquise et la présence d'un contrôle vectoriel, expliquent en partie cette tendance. Dans l'ensemble, cette thèse représente une étape importante vers le développement de biomarqueurs sérologiques pour

évaluer le risque d'exposition à l'*Aedes* et pour évaluer l'intervention de contrôle des vecteurs pour la prévention de la dengue. Des recherches supplémentaires sont toutefois nécessaires pour développer des outils plus sensibles et plus précis pour mesurer en temps réel la transmission de la dengue et prévenir les épidémies.

Exploring the potential of serological biomarkers to assess the risk of dengue transmission in north-Eastern Thailand

In Thailand dengue epidemiology is seasonal and cyclical, yet outbreaks are particularly difficult to predict. Various epidemiological and entomological indices have been used for surveillance but they lack of reliability and accuracy for assessing dengue transmission risk. This thesis aims to develop more practical and sensitive tools and indicators of dengue transmission risk that may be used to forecast dengue outbreaks. A first retrospective epidemiological study showed that dengue incidence spatio-temporal pattern is strongly guided by climatic factors and urbanization. Serology surveys conducted through a randomized controlled trial evidenced a strong and positive “dose-response” association between *Aedes* adult abundance and the intensity of Ab response to *Aedes* salivary peptide, hence demonstrating the capacity of salivary biomarkers to assess fine-scale variations in *Aedes*-exposure risk. A case-control study conducted in the same area showed however that neither the level of *Aedes* infestation nor the intensity of Ab response to *Aedes* were good predictors of dengue and risk factors associated with dengue were age, house characteristics and the presence of DENV-infected *Aedes* at the patient house. This thesis highlighted the complex interactions between *Aedes* vectors, climatic and socioeconomic factors and dengue transmission risk in Thailand and discussed the implications for the development of more efficient warning indices to prevent outbreaks.

Key words: *Aedes*, dengue, Thailand, transmission, serology biomarkers, case-control study, RCT, human-vector contact, vector control.

Étude du potentiel des biomarqueurs sérologiques pour évaluer le risque de transmission de la dengue dans le nord-est de la Thaïlande.

En Thaïlande, l'épidémiologie de la dengue est saisonnière et cyclique, mais les épidémies sont particulièrement difficiles à prévoir. Divers indices épidémiologiques et entomologiques ont été utilisés pour la surveillance, mais ils manquent de fiabilité et de précision pour évaluer le risque de transmission de la dengue. Cette thèse vise à développer des outils et des indicateurs de risque de transmission de la dengue plus pratiques et plus sensibles qui peuvent être utilisés pour prévoir les épidémies de dengue. Une première étude épidémiologique rétrospective a montré que le schéma spatio-temporel de l'incidence de la dengue est fortement guidé par les facteurs climatiques et l'urbanisation. Des enquêtes sérologiques menées dans le cadre d'un essai contrôlé randomisé ont mis en évidence une association "dose-réponse" forte et positive entre l'abondance des adultes atteints d'*Aedes* et l'intensité de la réponse de l'Ab au peptide salivaire de l'*Aedes*, démontrant ainsi la capacité des biomarqueurs salivaires à évaluer les variations à petite échelle du risque d'exposition à l'*Aedes*. Une étude cas-témoins menée dans la même région a toutefois montré que ni le niveau d'infestation par l'*Aedes* ni l'intensité de la réponse de l'Ab à l'*Aedes* n'étaient de bons prédicteurs de la dengue et que les facteurs de risque associés à la dengue étaient l'âge, les caractéristiques de la maison et la présence d'*Aedes* infecté par le DENV au domicile du patient. Cette thèse a mis en évidence les interactions complexes entre les vecteurs *Aedes*, les facteurs climatiques et socio-économiques et le risque de transmission de la dengue en Thaïlande et a discuté des implications pour le développement d'indices d'alerte plus efficaces pour prévenir les épidémies.

Mots-clés : *Aedes*, dengue, Thaïlande, transmission, biomarqueurs sérologiques, étude cas-témoin, RCT, contact homme-vecteur, control vectoriel.